Tetrahedron: *Asymmetry* 25 (2014) 632–636

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

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

Synthesis of a pentasaccharide repeating unit of the *O*-antigen of enteroadherent *Escherichia coli* O154 strain

Manas Jana [†], Abhijit Sau [†], Anup Kumar Misra [*]

Bose Institute, Division of Molecular Medicine, P-1/12, C.I.T. Scheme VII-M, Kolkata 700054, India

ARTICLE INFO

Article history:
Received 29 January 2014
Accepted 11 March 2014
Available online 4 April 2014

ABSTRACT

A straightforward linear synthetic strategy has been developed for the synthesis of the pentasaccharide repeating unit of the cell wall *O*-antigenic polysaccharide of enteroadherent *Escherichia coli* O154 strain. Newly developed glycosylation conditions using glycosyl trichloroacetimidate derivatives as glycosyl donors and nitrosyl tetrafluoroborate as the glycosylation activator have been used in all of the glycosylation reactions throughout the synthetic scheme. The stereochemical outcomes of the glycosylations were excellent and the yields were very good.

1. Introduction

Enteric infections such as diarrhea, gastroenteritis, colitis, and hemorrhagic uremic syndrome are serious concerns in developing countries, which are spread through contaminated food and water.[1–3] Most of the diarrheal outbreaks reported so far are associated with pathogenic *Escherichia coli* (*E. coli*) strains.[4,5] Although most *E. coli* strains are found in the human colonic flora as commensal microorganisms, certain strains acquire virulence factors and cause a number of enteric infections.[6] Diarrheal *E. coli* strains are classified in several classes based on the nature of their infections such as, enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroadhesive *E. coli* (EAEC) etc.[7] Bacterial cell wall *O*-polysaccharides are responsible for their pathogenic actions and play important roles in the initial stage of bacterial infection to the host. The emergence of multi-drug resistant bacterial strains poses extra challenges in drug discovery programs toward the development of antibacterial agents and their efficacy.[8] Since cell wall polysaccharides possess high antigenicity, several attempts have been made to develop antibacterial vaccine candidates using cell wall *O*-antigen polysaccharides.[9,10] Recently, Perepelov et al. reported on the structure of the pentasaccharide repeating unit of the cell wall *O*-antigen of enteroadherent *E. coli* O154 strain, which has been isolated from diarrheal patients from Brazil, Myanmar, and Japan.[11] The repeating unit consists of three L-rhamnose, one D-galactosamine, and one mannosamine units, which are alpha glycosidically linked. Since the oligosaccharide repeating unit of the polysaccharide shows similar antigenicity, it would be worthwhile developing a glycoconjugate derivative based on the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O154. In order to prepare glycoconjugate derivatives for biological evaluation, a significant quantity of oligosaccharide with adequate purity is required, which is practically inconvenient to isolate from natural sources. Hence, the development of a chemical synthetic strategy would be useful to gain access to a considerable amount of the required oligosaccharide with high purity. Therefore, a straightforward

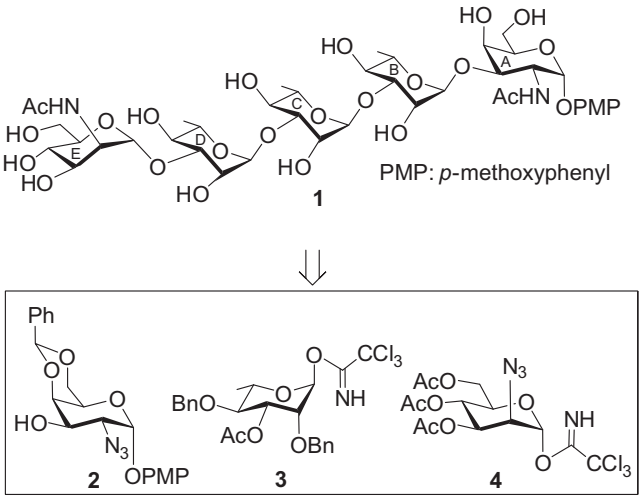


Figure 1. Structure of the synthesized pentasaccharide and its intermediates.

* Corresponding author. Tel.: +91 33 2569 3240; fax: +91 33 2355 3886.
E-mail address: akmisra69@gmail.com (A.K. Misra).
† Contributed equally.

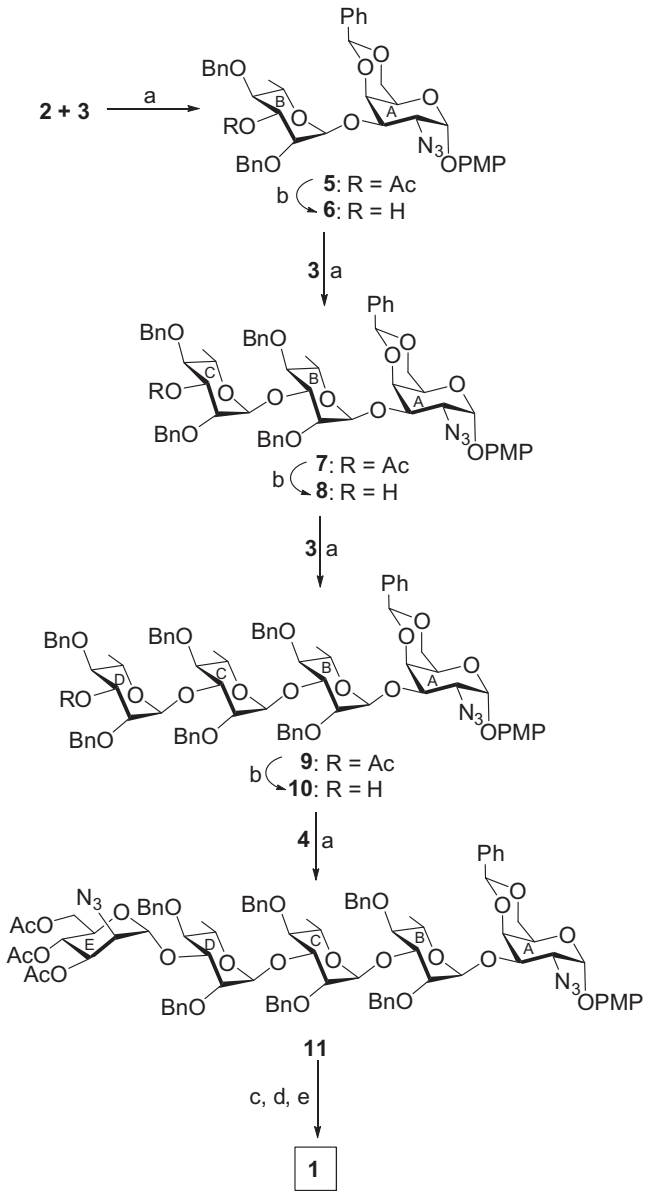
http://dx.doi.org/10.1016/j.tetasy.2014.03.007
0957-4166/

linear synthesis of the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O154 is presented herein (Fig. 1).

2. Results and discussion

The synthesis of target pentasaccharide **1** was achieved using a linear synthetic strategy involving sequential glycosylations of suitably functionalized monosaccharide intermediates **2**,[12] **3**,[13] and **4**,[14] which were prepared from the reducing sugars using a number of reported synthetic methodologies. Newly developed glycosylation conditions using glycosyl trichloroacetimidate derivatives as glycosyl donors and nitrosyl tetrafluoroborate ($NOBF_4$) as the glycosylation activator were used in all of glycosylation reactions throughout the synthetic scheme.[15] General glycosylation conditions were used in all of the glycosylation reactions and the minimum number of the protecting group manipulations are involved in the synthetic strategy. The single monosaccharide intermediate **3** was used several times during the progress of oligosaccharide formation, minimizing the overall number of reaction steps.

Stereoselective glycosylation of the 2-azido-D-galactosyl acceptor **2** with the L-rhamnosyl trichloroacetimidate donor **3** in the presence of $NOBF_4$[15] in Et_2O–CH_2Cl_2 mixed solvent furnished disaccharide derivative **5** in 78% yield. The formation of compound **5** was confirmed from spectroscopic analysis [signals at δ 5.53 (d, J = 3.5 Hz, H-1_A), 5.11 (d, J = 1.5 Hz, H-1_B) in the ^{1}H NMR and δ 100.8 (C-1_B), 97.7 (C-1_A) in the ^{13}C NMR spectra, respectively]. Treatment of compound **5** with sodium methoxide[16] resulted in the formation of disaccharide acceptor **6** in 99% yield, which was coupled with compound **3** in the presence of $NOBF_4$[15] in Et_2O–CH_2Cl_2 mixed solvent to give the trisaccharide derivative **7** in 76% yield. The stereoselective formation of compound **7** was supported by spectroscopic analysis [signals at δ 5.53 (d, J = 3.0 Hz, H-1_A), 5.12 (br s, H-1_B), 5.11 (br s, H-1_C) in the ^{1}H NMR and at δ 100.9 (C-1_B), 99.3 (C-1_C), 97.5 (C-1_A) in the ^{13}C NMR spectra, respectively]. Compound **7** was treated with sodium methoxide[16] to give the trisaccharide acceptor **8** in 97% yield, which was coupled again stereoselectively with compound **3** in the presence of $NOBF_4$[15] in Et_2O–CH_2Cl_2 mixed solvent to give tetrasaccharide derivative **9** in 74% yield. The formation of compound **9** was confirmed from spectroscopic analysis [signals at δ 5.51 (d, J = 3.0 Hz, H-1_A), 5.14 (br s, H-1_D), 5.12 (br s, H-1_B), 5.09 (br s, H-1_C) in the ^{1}H NMR and at δ 100.7 (C-1_B), 99.7 (C-1_D), 99.4 (C-1_C), 97.5 (C-1_A) in the ^{13}C NMR spectra, respectively]. Treatment of compound **9** with sodium methoxide[16] furnished the tetrasaccharide acceptor **10** in 94% yield, which was coupled with 2-azido-D-mannosyl trichloroacetimidate derivative **4** in the presence of $NOBF_4$[15] in Et_2O–CH_2Cl_2 mixed solvent to give pentasaccharide derivative **11** in 71% yield. The formation of compound **11** was confirmed from spectroscopic analysis [signals at δ 5.51 (d, J = 3.0 Hz, H-1_A), 5.16 (br s, H-1_B), 5.14 (br s, H-1_C), 5.13 (br s, H-1_D), 4.29 (br s, H-1_E) in the ^{1}H NMR and δ 100.6 (J_{C1-H1} = 171 Hz, C-1_B), 99.5 (J_{C1-H1} = 172 Hz, C-1_D), 99.0 (J_{C1-H1} = 171 Hz, C-1_C), 97.5 (J_{C1-H1} = 171 Hz, C-1_A), 93.2 (J_{C1-H1} = 172 Hz, C-1_E) in the ^{13}C NMR, respectively]. The formation of all of the alpha-glycosyl linkages in compound **11** was also confirmed using gated ^{1}H coupled ^{13}C NMR spectra.[17] The appearance of coupling constant (J_{C1-H1}) values 171 Hz, 172 Hz, 171 Hz, 171 Hz, and 172 Hz in the gated ^{1}H coupled ^{13}C NMR spectrum unambiguously confirmed the presence of five alpha linkages in compound **11**. Removal of the benzyl ethers and benzylidene acetal in compound **11**, as well as the reduction of the azido groups, were carried out using catalytic transfer hydrogenation conditions by treatment with triethylsilane in the presence of palladium over charcoal[18] to give a pentasaccharide diamine derivative, which was acetylated using acetic anhydride and pyridine to give a per-*O*-acetylated derivative. Finally, saponification of the acetylated pentasaccharide derivative using sodium methoxide[16] followed by purification using a Sephadex LH-20 column furnished pure pentasaccharide **1** in 66% overall yield. The structure of compound **1** was confirmed unambiguously from spectroscopic analysis [signals at δ 5.37 (d, J = 3.5 Hz, H-1_A), 4.98 (br s, H-1_C), 4.89 (br s, H-1_D), 4.88 (br s, H-1_E), 4.84 (br s, H-1_B) in the ^{1}H NMR and at δ 102.3 (C-1_C), 102.2 (C-1_B), 102.1 (C-1_D), 97.3 (C-1_A), 95.3 (C-1_E) in the ^{13}C NMR spectra, respectively] (Scheme 1).



Scheme 1. Reagents and conditions: (a) $NOBF_4$, Et_2O–CH_2Cl_2 (3:1), −15 °C, 40 min, 78% for compound **5**, 76% for compound **7**, 74% for compound **9** and 71% for compound **11**; (b) 0.1 M CH_3ONa, CH_3OH, room temperature, 1 h, 99% for compound **6**, 97% for compound **8**, 94% for compound **10**; (c) Et_3SiH, 10% Pd-C, CH_3OH/CH_2Cl_2 (2:1), room temperature, 10 h; (d) acetic anhydride, pyridine, room temperature, 1 h; (e) 0.1 M CH_3ONa, CH_3OH, room temperature, 3 h, overall 66%.

3. Conclusion

In conclusion, a pentasaccharide containing all alpha-glycosyl linkages corresponding to the repeating unit of the *O*-antigen of enteroadherent *E. coli* O154 strain was achieved in good yield using

similar functional group manipulations and glycosylation conditions involving $NOBF_4$ catalyzed activation of glycosyl trichloroacetimidate derivatives. The yields were excellent in all of the glycosylation reactions and intermediate functional group modification steps. Multiple uses of a single intermediate and similar reaction conditions reduced the total number of reaction steps. The target compound was prepared following a linear glycosylation approach.

4. Experimental

General: All 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 M H_2SO_4) sprayed plates in hot plate. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR spectra were recorded on Brucker Avance 500 MHz using $CDCl_3$ and D_2O as the solvents and TMS as the internal reference unless stated otherwise. Chemical shift values are expressed in δ ppm. MALDI-MS were recorded on a Brucker Daltronics mass spectrometer. Optical rotations were recorded in a Jasco P-2000 spectrometer. Commercially available grades of organic solvents of adequate purity are used in all reactions.

4.1. *p*-Methoxyphenyl (3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside 5

A solution of compound **2** (1.2 g, 3.0 mmol) and compound **3** (1.7 g, 3.20 mmol) in anhydrous Et_2O–CH_2Cl_2 (15 mL; 3:1; v/v) was cooled to −15 °C under argon and $NOBF_4$ (400 mg, 3.42 mmol) was added to it. The reaction mixture was allowed to stir at the same temperature for 40 min and poured into a satd. $NaHCO_3$ solution and extracted with CH_2Cl_2 (100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane–EtOAc (5:1) as eluant to give pure compound **5** (1.8 g, 78%). Colorless oil; $[\alpha]_D^{25}$ = +49 (*c* 1.0, $CHCl_3$); IR (neat): 3400, 3065, 3030, 2921, 2111, 1738, 1594, 1506, 1486, 1454, 1367, 1239, 1103, 1083, 1047, 999, 917, 893, 867, 828, 797, 755, 698, 666 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.52–6.81 (m, 19H, Ar-H), 5.54 (s, 1H, PhC*H*), 5.53 (d, *J* = 3.5 Hz, 1H, H-1_A), 5.24 (dd, *J* = 9.5, 3.5 Hz, 1H, H-3_B), 5.11 (d, *J* = 1.5 Hz, 1H, H-1_B), 4.70–4.57 (4d, *J* = 11.5 Hz each, 4H, 2PhCH_2), 4.45 (d, *J* = 3.0 Hz, 1H, H-4_A), 4.31 (dd, *J* = 9.5, 3.5 Hz, 1H, H-3_A), 4.26–4.24 (m, 1H, H-6_{aA}), 4.18–4.12 (m, 1H, H-5_B), 4.09 (dd, *J* = 9.5, 3.5 Hz, 1H, H-2_A), 4.05–4.01 (m, 2H, H-2_B, H-6_{bA}), 3.83 (br s, 1H, H-5_A), 3.77 (s, 3H, OCH_3), 3.61 (t, *J* = 9.5 Hz each, 1H, H-4_B), 1.92 (s, 3H, COCH_3), 1.29 (d, *J* = 6.5 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8 (*C*OCH$_3$), 156.4–114.7 (Ar-C), 100.9 (PhCH), 100.8 (C-1_B), 97.7 (C-1_A), 78.8 (C-4_B), 76.9 (C-3_A), 76.3 (C-4_A), 75.6 (C-2_B), 74.0 (PhCH_2), 73.3 (PhCH_2), 73.1 (C-3_B), 69.2 (C-6_A), 68.4 (C-5_B), 63.3 (C-5_A), 58.2 (C-2_A), 55.6 (OCH_3), 21.0 (CO*C*H$_3$), 18.1 (C*C*H$_3$); ESI-MS: 790.3 $[M+Na]^+$; Anal. Calcd for $C_{42}H_{45}N_3O_{11}$ (767.30): C, 65.70; H, 5.91; found: C, 65.24; H, 6.10.

4.2. *p*-Methoxyphenyl (2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside 6

A solution of compound **5** (1.6 g, 2.08 mmol) in 0.1 M CH_3ONa in CH_3OH (20 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered, and concentrated. The crude product was passed through a small pad of SiO_2 using hexane-EtOAc (1:1) as eluant to give pure compound **6** (1.5 g, 99%). Colorless oil; $[\alpha]_D^{25}$ = +64 (*c* 1.0, $CHCl_3$); IR (neat): 3434, 3065, 3010, 2930, 2111, 1606, 1507, 1454, 1399, 1367, 1341, 1241, 1215, 1173, 1106, 1083, 1047, 996, 868, 828, 797, 754, 698, 666 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.51–6.81 (m, 19H, Ar-H), 5.54 (s, 1H, PhC*H*), 5.53 (d, *J* = 3.5 Hz, 1H, H-1_A), 5.16 (br s, 1H, H-1_B), 4.88–4.61 (4d, *J* = 11.0 Hz each, 4H, 2 PhCH_2), 4.43 (d, *J* = 3.0 Hz, 1H, H-4_A), 4.29 (dd, *J* = 9.5, 3.5 Hz, 1H, H-3_A), 4.26–4.24 (m, 1H, H-6_{aA}), 4.09–4.00 (m, 4H, H-2_A, H-3_B, H-5_B, H-6_{bA}), 3.90–3.88 (m, 1H, H-2_B), 3.84–3.83 (m, 1H, H-5_A), 3.77 (s, 3H, OCH_3), 3.34 (t, *J* = 9.5 Hz each, 1H, H-4_B), 1.31 (d, *J* = 6.5 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.6–114.7 (Ar-C), 100.9 (PhCH), 100.1 (C-1_B), 97.7 (C-1_A), 81.7 (C-4_B), 78.7 (C-2_B), 76.5 (C-3_A), 75.6 (C-4_A), 74.3 (PhCH_2), 73.3 (PhCH_2), 71.3 (C-3_B), 69.2 (C-6_A), 68.1 (C-5_B), 63.3 (C-5_A), 58.4 (C-2_A), 55.6 (OCH_3), 18.1 (C*C*H$_3$); ESI-MS: 748.2 $[M+Na]^+$; Anal. Calcd for $C_{40}H_{43}N_3O_{10}$ (725.29): C, 66.19; H, 5.97; found: C, 66.00; H, 6.12.

4.3. *p*-Methoxyphenyl (3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside 7

A solution of compound **6** (1.4 g, 1.93 mmol) and compound **3** (1.1 g, 2.07 mmol) in anhydrous Et_2O–CH_2Cl_2 (15 mL; 3:1; v/v) was cooled to −15 °C under argon and $NOBF_4$ (260 mg, 2.22 mmol) was added to it. The reaction mixture was allowed to stir at same temperature for 40 min and poured into a satd. $NaHCO_3$ solution and extracted with CH_2Cl_2 (100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane–EtOAc (6:1) as eluant to give pure compound **7** (1.6 g, 76%). Colorless oil; $[\alpha]_D^{25}$ = +54 (*c* 1.0, $CHCl_3$); IR (neat): 3367, 3064, 3030, 2931, 2112, 1735, 1507, 1454, 1365, 1342, 1240, 1216, 1173, 1103, 1083, 1048, 999, 916, 893, 828, 796, 755, 698, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.55–6.81 (m, 29H, Ar-H), 5.55 (s, 1H, PhC*H*), 5.53 (d, *J* = 3.0 Hz, 1H, H-1_A), 5.27 (dd, *J* = 9.5, 3.0 Hz, 1H, H-3_C), 5.12 (br s, 1H, H-1_B), 5.11 (br s, 1H, H-1_C), 4.82–4.58 (6 d, *J* = 11.0 Hz each, 6H, PhCH_2), 4.47 (d, *J* = 2.5 Hz, 1H, H-4_A), 4.30 (dd, *J* = 9.5, 3.0 Hz, 1H, H-3_A), 4.27–4.24 (m, 2H, H-6_{aA}, PhCH_2), 4.15 (dd, *J* = 9.5, 3.0 Hz, 1H, H-3_B), 4.12–4.02 (m, 5H, H-2_A, H-5_B, H-5_C, H-6_{bA}, PhCH_2), 3.91–3.89 (m, 1H, H-2_B), 3.84–3.83 (m, 1H, H-5_A), 3.80–3.78 (m, 1H, H-2_C), 3.77 (s, 3H, OCH_3), 3.63 (t, *J* = 9.5 Hz each, 1H, H-4_C), 3.59 (t, *J* = 9.5 Hz each, 1H, H-4_B), 1.92 (s, 3H, COCH_3), 1.26 (d, *J* = 6.5 Hz, 3H, CCH_3), 1.22 (d, *J* = 6.5 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1 (*C*OCH$_3$), 156.2–114.7 (Ar-C), 101.0 (PhCH), 100.9 (C-1_B), 99.3 (C-1_C), 97.5 (C-1_A), 80.5 (C-4_B), 79.1 (C-4_C), 77.9 (C-2_B), 77.4 (C-2_C), 76.9 (2 C, C-3_A, C-3_B), 75.7 (C-4_A), 74.7 (PhCH_2), 74.2 (PhCH_2), 73.5 (C-3_C), 73.1 (PhCH_2), 72.4 (PhCH_2), 69.2 (C-6_A), 69.0 (C-5_C), 68.4 (C-5_B), 63.2 (C-5_A), 58.3 (C-2_A), 55.6 (OCH_3), 21.0 (CO*C*H$_3$), 18.0, 17.9 (2C*C*H$_3$); MALDI-MS: 1116.4 $[M+Na]^+$; Anal. Calcd for $C_{62}H_{67}N_3O_{15}$ (1093.45): C, 68.06; H, 6.17; found: C, 67.90; H, 6.32.

4.4. *p*-Methoxyphenyl (2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside 8

A solution of compound **7** (1.5 g, 1.37 mmol) in 0.1 M CH_3ONa in CH_3OH (20 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered, and concentrated. The crude product was passed through a small pad of SiO_2 using hexane-EtOAc (1:1) as eluant to give pure compound **8** (1.4 g, 97%). Colorless oil; $[\alpha]_D^{25}$ = +55 (*c* 1.0, $CHCl_3$); IR (neat): 3400, 3031, 2931, 2111, 1594, 1507, 1454, 1396, 1341, 1240, 1214, 1172, 1083, 1048, 998, 918,

828, 753, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.55-6.81 (m, 29H, Ar-H), 5.55 (s, 1H, PhC*H*), 5.52 (d, J = 3.0 Hz, 1H, H-1_A), 5.15 (br s, 1H, H-1_B), 5.10 (br s, 1H, H-1_C), 4.88–4.59 (6d, J = 11.0 Hz each, 6H, PhCH_2), 4.47 (d, J = 3.0 Hz, 1H, H-4_A), 4.28 (dd, J = 9.5, 3.0 Hz, 1H, H-3_A), 4.26–4.20 (m, 2H, H-6_{aA}, PhCH_2), 4.17 (dd, J = 9.5, 3.0 Hz, 1H, H-3_B), 4.15–4.09 (m, 2H, H-2_A, H-3_C), 4.05 (d, J = 11.5 Hz, 1H, PhCH_2), 3.98–3.91 (m, 2H, H-5_B, H-6_{bA}), 3.90–3.89 (m, 1H, H-2_B), 3.84–3.83 (m, 1H, H-5_A), 3.77 (s, 3H, OCH_3), 3.75–3.70 (m, 1H, H-5_C), 3.65–3.63 (m, 1H, H-2_C), 3.61 (t, J = 9.5 Hz each, 1H, H-4_C), 3.25 (t, J = 9.5 Hz each, 1H, H-4_B), 1.28 (d, J = 6.5 Hz, 3H, CCH_3), 1.22 (d, J = 6.5 Hz, 3H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.2–114.7 (Ar-C), 101.0 (PhCH), 100.8 (C-1_B), 98.7 (C-1_C), 97.5 (C-1_A), 82.2 (C-4_B), 80.7 (C-4_C), 79.2 (C-2_B), 78.0 (C-2_C), 77.4 (C-3_A), 76.9 (C-3_B), 75.7 (C-4_A), 74.9 (PhCH_2), 74.2 (PhCH_2), 73.1 (PhCH_2), 72.3 (PhCH_2), 71.5 (C-3_C), 69.2 (C-6_A), 69.0 (C-5_C), 67.8 (C-5_B), 63.2 (C-5_A), 58.3 (C-2_A), 55.6 (OCH_3), 18.0, 17.9 (2CCH_3); MALDI-MS: 1074.4 $[M+Na]^+$; Anal. Calcd for $C_{60}H_{65}N_3O_{14}$ (1051.44): C, 68.49; H, 6.23; found: C, 68.32; H, 6.42.

4.5. *p*-Methoxyphenyl (3-*O*-acetyl-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside 9

A solution of compound **8** (1.2 g, 1.14 mmol) and compound **3** (670 mg, 1.26 mmol) in anhydrous Et_2O–CH_2Cl_2 (10 mL; 3:1; v/v) was cooled to −15 °C under argon and $NOBF_4$ (160 mg, 1.37 mmol) was added to it. The reaction mixture was allowed to stir at same temperature for 40 min and then poured into a satd. $NaHCO_3$ solution and extracted with CH_2Cl_2 (100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane–EtOAc (6:1) as eluant to give pure compound **9** (1.2 g, 74%). Colorless oil; $[\alpha]_D^{25}$ = +71 (*c* 1.0, $CHCl_3$); IR (neat): 3368, 3030, 2926, 2112, 1737, 1596, 1486, 1454, 1366, 1342, 1239, 1214, 1101, 1048, 999, 918, 804, 755, 697, 666 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.56–6.75 (m, 39H, Ar-H), 5.54 (s, 1H, PhC*H*), 5.51 (d, J = 3.0 Hz, 1H, H-1_A), 5.25 (dd, J = 9.5, 3.0 Hz, 1H, H-3_D), 5.14 (br s, 1H, H-1_D), 5.12 (br s, 1H, H-1_B), 5.09 (br s, 1H, H-1_C), 4.80–4.57 (8 d, J = 11.0 Hz each, 8H, PhCH_2), 4.46 (d, J = 2.5 Hz, 1H, H-4_A), 4.32–4.24 (m, 5H, H-3_A, H-3_B, H-3_C, PhCH_2), 4.15–4.09 (m, 5H, H-2_A, H-5_B, H-6_{aA}, PhCH_2), 4.02 (d, J = 12.0 Hz, 1H, H-6_{bA}), 3.92–3.91 (m, 1H, H-2_B), 3.84 (s, 3H, OCH_3), 3.82–3.77 (m, 4H, H-2_D, H-5_A, H-5_C, H-5_D), 3.74–3.73 (m, 1H, H-2_C), 3.60–3.56 (m, 3H, H-4_B, H-4_C, H-4_D), 1.91 (s, 3H, COCH_3), 1.26, 1.20, 1.18 (3 d, J = 6.5 Hz each, 9H, 3CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (*C*OCH$_3$), 155.2–111.2 (Ar-C), 101.0 (PhCH), 100.7 (C-1_B), 99.7 (C-1_D), 99.4 (C-1_C), 97.5 (C-1_A), 80.6 (C-4_B), 80.5 (C-4_C), 79.0 (C-4_D), 78.6 (2 C, C-2_B, C-2_C), 78.3 (C-2_D), 78.0 (C-3_D), 77.4 (C-3_A), 76.7 (C-3_B), 75.5 (C-4_A), 74.6 (PhCH_2), 74.5 (PhCH_2), 74.3 (PhCH_2), 73.5 (C-3_C), 73.0 (PhCH_2), 72.6 (PhCH_2), 72.1 (PhCH_2), 69.1 (C-6_A), 69.0 (C-5_D), 68.9 (C-5_C), 68.2 (C-5_B), 63.4 (C-5_A), 58.2 (C-2_A), 56.8 (OCH_3), 21.0 (COCH_3), 18.1, 18.0, 17.8 (3CCH_3); MALDI-MS: 1442.4 $[M+Na]^+$; Anal. Calcd for $C_{82}H_{89}N_3O_{19}$ (1419.60): C, 69.33; H, 6.31; found: C, 69.15; H, 6.50.

4.6. *p*-Methoxyphenyl (2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-L-galactopyranoside 10

A solution of compound **9** (1.1 g, 0.77 mmol) in 0.1 M CH_3ONa in CH_3OH (15 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered, and concentrated. The crude product was passed through a small pad of SiO_2 using hexane–EtOAc (1:1) as eluant to give pure compound **10** (1 g, 94%). Colorless oil; $[\alpha]_D^{25}$ = +115 (*c* 1.0, $CHCl_3$); IR (neat): 3551, 3088, 3064, 3030, 2972, 2932, 2112, 1953, 1877, 1811, 1729, 1596, 1486, 1454, 1394, 1366, 1342, 1316, 1269, 1214, 1173, 1083, 1048, 999, 918, 837, 803, 754, 697, 667, 618, 586 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.55–6.74 (m, 39H, Ar-H), 5.54 (s, 1H, PhC*H*), 5.51 (d, J = 3.0 Hz, 1H, H-1_A), 5.14–5.12 (3br s, 3H, H-1_B, H-1_C, H-1_D), 4.86–4.58 (8 d, J = 11.0 Hz each, 8H, PhCH_2), 4.46 (d, J = 2.5 Hz, 1H, H-4_A), 4.30–4.22 (m, 5H, H-3_A, H-3_B, H-3_C, PhCH_2), 4.15–4.02 (m, 7H, H-2_A, H-3_D, H-5_B, H-6_{abA}, PhCH_2), 3.97–3.85 (m, 2H, H-2_B, H-3_D), 3.83 (s, 3H, OCH_3), 3.80–3.79 (m, 1H, H-2_D), 3.78–3.77 (m, 1H, H-5_A), 3.76–3.70 (m, 2H, H-5_C, H-5_D), 3.64–3.55 (m, 3H, H-2_C, H-4_B, H-4_C), 3.26 (t, J = 9.5 Hz each, 1H, H-4_D), 1.26–1.18 (3 d, J = 6.5 Hz each, 9H, 3CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.6–111.2 (Ar-C), 101.0 (PhCH), 100.7 (C-1_B), 99.4 (C-1_D), 98.9 (C-1_C), 97.5 (C-1_A), 82.2 (C-4_D), 80.8 (C-4_B), 80.4 (C-4_C), 79.1 (2C, C-2_B, C-2_C), 78.6 (C-2_D), 78.4 (C-3_D), 78.0 (C-3_A), 77.4 (C-4_A), 75.5 (C-3_B), 74.6 (PhCH_2), 74.5 (PhCH_2), 74.3 (PhCH_2), 73.0 (PhCH_2), 72.4 (PhCH_2), 72.1 (PhCH_2), 71.5 (C-3_C), 69.2 (C-6_A), 69.0 (C-5_D), 68.9 (C-5_C), 67.6 (C-5_B), 63.4 (C-5_A), 58.2 (C-2_A), 56.8 (OCH_3), 18.1, 18.0, 17.9 (3CCH_3); MALDI-MS: 1400.5 $[M+Na]^+$; Anal. Calcd for $C_{80}H_{87}N_3O_{18}$ (1377.59): C, 69.70; H, 6.36; found: C, 69.53; H, 6.54.

4.7. *p*-Methoxyphenyl (3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside 11

A solution of compound **10** (900 mg, 0.65 mmol) and compound **4** (340 mg, 0.71 mmol) in anhydrous Et_2O–CH_2Cl_2 (10 mL; 3:1; v/v) was cooled to −15 °C under argon and $NOBF_4$ (90 mg, 0.77 mmol) was added to it. The reaction mixture was allowed to stir at same temperature for 40 min and then poured into a satd. $NaHCO_3$ solution and extracted with CH_2Cl_2 (100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane–EtOAc (5:1) as eluant to give pure compound **11** (780 mg, 71%). Colorless oil; $[\alpha]_D^{25}$ = +44 (*c* 1.0, $CHCl_3$); IR (neat): 3368, 2927, 2110, 1748, 1587, 1507, 1454, 1367, 1219, 1080, 1047, 918, 828, 752, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.55–6.78 (m, 39H, Ar-H), 5.55 (s, 1H, PhC*H*), 5.51 (d, J = 3.0 Hz, 1H, H-1_A), 5.28 (dd, J = 9.5, 3.0 Hz, 1H, H-3_E), 5.18 (t, J = 9.5 Hz each, 1H, H-4_E), 5.16 (br s, 1H, H-1_B), 5.14 (br s, 1H, H-1_C), 5.13 (br s, 1H, H-1_D), 4.85–4.63 (8 d, J = 11.0 Hz each, 8H, PhCH_2), 4.47 (d, J = 11.0 Hz, 1H, PhCH_2), 4.46 (d, J = 2.5 Hz, 1H, H-4_A), 4.29 (br s, 1H, H-1_E), 4.28–4.22 (m, 3H, H-3_A, PhCH_2), 4.18–4.07 (m, 5H, H-2_A, H-3_B, H-5_B, H-6_{aA}, PhCH_2), 4.05 (d, J = 12.0 Hz, 1H, H-6_{bA}), 4.00–3.92 (m, 4H, H-2_B, H-2_C, H-3_C, H-3_D), 3.87–3.84 (m, 1H, H-6_{aE}), 3.83 (s, 3H, OCH_3), 3.81–3.78 (m, 3H, H-5_A, H-5_C, H-5_E), 3.75–3.72 (m, 1H, H-5_D), 3.65–3.59 (m, 3H, H-2_D, H-4_B, H-6_{bE}), 3.52 (t, J = 9.5 Hz each, 1H, H-4_C), 3.50 (t, J = 9.5 Hz each, 1H, H-4_D), 3.46–3.44 (m, 1H, H-2_E), 2.09, 1.86, 1.83 (3s, 9H, 3COCH_3), 1.27, 1.23, 1.21 (3 d, J = 6.5 Hz each, 9H, 3CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6, 170.2, 170.0 (*C*OCH$_3$), 156.2–111.2 (Ar-C), 101.0 (PhCH), 100.6 (J_{C1-H1} = 171 Hz, C-1_B), 99.5 (J_{C1-H1} = 172 Hz, C-1_D), 99.0 (J_{C1-H1} = 171 Hz, C-1_C), 97.5 (J_{C1-H1} = 171 Hz, C-1_A), 93.2 (J_{C1-H1} = 172 Hz, C-1_E), 80.4 (3 C, C-4_B, C-4_C, C-4_D), 79.4 (C-2_B), 79.0 (C-2_C), 78.5 (C-2_D), 78.4 (C-3_D), 77.5 (C-3_A), 75.5 (2C, C-3_B, C-4_A), 75.1 (PhCH_2), 74.5 (PhCH_2), 74.3 (PhCH_2), 73.6 (C-3_C), 73.0 (2C, C-3_C, PhCH_2), 72.1 (PhCH_2), 71.9 (PhCH_2), 71.2 (C-3_E), 69.1 (C-6_A), 69.0 (C-5_E), 68.8 (C-5_D), 68.5 (C-5_C), 68.1 (C-5_B), 65.3 (C-4_E), 63.4 (C-5_A), 61.3 (C-6_E), 61.2 (C-2_E), 58.2 (C-2_A), 56.8 (OCH_3), 20.5 (3C, 3COCH_3), 18.1, 18.0, 17.9 (3CCH_3); MALDI-MS: 1713.6 $[M+Na]^+$; Anal. Calcd for $C_{92}H_{102}N_6O_{25}$ (1690.68): C, 65.31; H, 6.08; found: C, 65.15; H, 6.30.

4.8. *p*-Methoxyphenyl (2-acetamido-2-deoxy-α-D-mannopyranosyl)-(1→3)-(α-L-rhamnopyranosyl)-(1→3)-(α-L-rhamnopyranosyl)-(1→3)-(α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside 1

To a solution of compound **11** (700 mg, 0.41 mmol) and 10% Pd-C (150 mg) in CH_3OH–CH_2Cl_2 (10 mL, 2:1; v/v) was added dropwise Et_3SiH (3 mL, 18.78 mmol) over 30 min and the reaction mixture was allowed to stir at room temperature for 10 h. The reaction mixture was then filtered through a Celite® bed, washed with CH_3OH (50 mL), and the combined filtrate was concentrated under reduced pressure. A solution of the crude product in acetic anhydride (5 mL) and pyridine (5 mL) was kept at room temperature for 1 h and evaporated to dryness under reduced pressure. A solution of the acetylated product in 0.1 M CH_3ONa (15 mL) was stirred at room temperature for 3 h. The reaction mixture was neutralized using Dowex 50W X8 (H^+) resin, filtered, and concentrated to give the product, which was passed through a Sephadex® LH-20 column using CH_3OH–H_2O (3:1) as the eluent to give pure compound **1** (260 mg, 66%). White powder; $[\alpha]_D^{25}$ = 130 (*c* 1.0, H_2O); IR (KBr): 3434, 2936, 2851, 1721, 1657, 1590, 1400, 1056, 996 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 7.00 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.86 (d, *J* = 9.0 Hz, 2H, Ar-H), 5.37 (d, *J* = 3.5 Hz, 1H, H-1_A), 4.98 (br s, 1H, H-1_C), 4.89 (br s, 1H, H-1_D), 4.88 (br s, 1H, H-1_E), 4.84 (br s, 1H, H-1_B), 4.40 (dd, *J* = 10.5, 3.0 Hz, 1H, H-2_A), 4.28 (d, *J* = 3.5 Hz, 1H, H-2_E), 4.17–4.16 (m, 1H, H-2_D), 4.05–3.99 (m, 5H, H-2_B, H-2_C, H-3_A, H-3_E, H-4_A), 3.89–3.85 (m, 1H, H-5_E), 3.82–3.74 (m, 7H, H-3_B, H-3_C, H-3_D, H-5_A, H-5_B, H-5_C, H-5_D), 3.72–3.70 (m, 2H, H-6_{abA}), 3.68 (s, 3H, OC*H*$_3$), 3.62–3.59 (m, 2H, H-6_{abE}), 3.57 (t, *J* = 9.5 Hz each, 1H, H-4_E), 3.47–3.42 (m, 3H, H-4_B, H-4_C, H-4_D), 1.96, 1.95 (2s, 6H, 2 COC*H*$_3$), 1.20–1.19 (m, 9H, 3CC*H*$_3$); ^{13}C NMR (125 MHz, D_2O): δ 174.8, 174.2 (2COC*H*$_3$), 154.6–115.0 (Ar-C), 102.3 (C-1_C), 102.2 (C-1_B), 102.1 (C-1_D), 97.3 (C-1_A), 95.3 (C-1_E), 78.4 (C-3_C), 77.9 (C-3_B), 76.7 (C-3_A), 74.5 (C-3_D), 72.2 (C-5_E), 71.7 (C-5_A), 71.3 (C-4_D), 71.2 (C-2_C), 70.3 (C-4_C), 70.2 (C-2_B), 69.9 (C-4_B), 69.4 (C-3_E), 69.2 (C-5_B), 69.1 (C-5_D), 68.9 (C-5_C), 68.4 (C-4_A), 66.4 (C-4_E), 66.1 (C-2_D), 60.9 (C-6_A), 60.2 (C-6_E), 55.8 (OCH_3), 52.8 (C-2_E), 48.5 (C-2_A), 21.9, 21.8 (2COC*H*$_3$), 16.7 (3C, 3CC*H*$_3$); ESI-MS: 991.3 $[M+Na]^+$; Anal. Calcd for $C_{41}H_{64}N_2O_{24}$ (968.38): C, 50.82; H, 6.66; found: C, 50.65; H, 6.40.

Acknowledgements

M.J. and A.S. thank CSIR, New Delhi for providing Senior Research Fellowships. This work was supported by DST, New Delhi (No. SR/S1/OC-83/2010).

References

1. Hunter, P. R. *Waterborne Disease: Epidemiology and Ecology*; Wiley Chester: New York, 1997. pp 45–50.
2. Thapar, N.; Sanderson, I. R. *Lancet* **2004**, *363*, 641–653.
3. Wright, J.; Grundy, S.; Conroy, R. *J. Trop. Med. Int. Health* **2004**, *9*, 106–117.
4. Presterl, E.; Zwick, R. H.; Reichmann, S.; Aichelburg, A.; Winkler, S.; Kremsner, P. G. *Am. J. Trop. Med. Hyg.* **2003**, *69*, 406–410.
5. Okeke, I. N.; Lamikanra, A.; Steinruck, H.; Kaper, J. B. *J. Clin. Microbiol.* **2000**, *38*, 7–12.
6. Weintraub, A. *J. Med. Microbiol.* **2007**, *56*, 4–8.
7. Nataro, J. P.; Kaper, J. B. *Clin. Microbiol. Rev.* **1998**, *11*, 142–201.
8. Levy, S. B. *Adv. Drug Deliv. Rev.* **2005**, *57*, 1446–1450.
9. Roy, R. *Drug Discovery Today: Technol.* **2004**, *1*, 327–336. and references cited therein.
10. Doores, K. J.; Gamblin, D. P.; Davis, B. G. *Chem. Eur. J.* **2006**, *12*, 656–665.
11. Perepelov, A. V.; Wang, Q.; Senchenkova, S. N.; Feng, L.; Shashkov, A. S.; Wang, L.; Knirel, Y. A. *Carbohydr. Res.* **2013**, *379*, 51–54.
12. Mukherjee, C.; Misra, A. K. *Tetrahedron: Asymmetry* **2008**, *19*, 2746–2751.
13. Kerékgyártó, J.; Szurmai, Z.; Lipták, A. *Carbohydr. Res.* **1993**, *245*, 65–80.
14. Briner, K.; Vasella, A. *Helv. Chim. Acta* **1987**, *70*, 1341–1356.
15. Sau, A.; Santra, A.; Misra, A. K. *Synlett* **2012**, 2341–2348.
16. Zemplen, G. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1254–1266.
17. Bock, K.; Pedersen, C. J. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.
18. Santra, A.; Ghosh, T.; Misra, A. K. *Beilstein J. Org. Chem.* **2013**, *9*, 74–78.