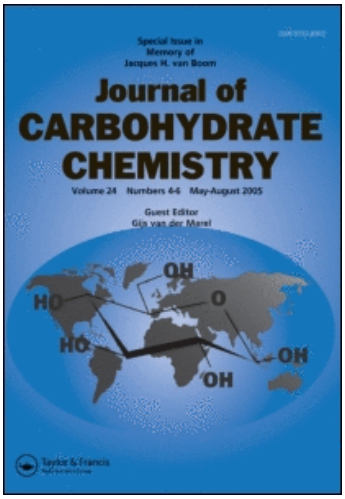

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Synthesis of Tri- and Disaccharide Fragments Related to the *O*-Antigen of Enteropathogenic *Escherichia Coli* O158

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Synthesis of Tri- and Disaccharide Fragments Related to the O-Antigen of Enteropathogenic *Escherichia Coli* O158

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Chemical synthesis of a tri- and a disaccharide related to the *O*-antigen of enteropathogenic *E. coli* O158 was achieved in high yield. In the disaccharide (**2**), one D-galactosamine unit is present in 1,2-*cis*-linked and the other is in the *trans*-orientation, and both of them were prepared from the same intermediate by tuning the reaction solvent. Yields were considerably high in all steps.

Keywords Trisaccharide; Disaccharide, *E. coli*; Glycosylation; Enteropathogenic

INTRODUCTION

Escherichia coli (*E. coli*) bacteria exist as harmless species to life-threatening microorganisms. In general, it is a nonpathogenic member of the human colonic flora. However, certain species of *E. coli* have acquired virulence factors and behave as an opportunistic pathogen responsible for several intestinal and urinary diseases in humans and animals. Three most frequent clinical syndromes caused by *E. coli* are (a) diarrhea, (b) urinary tract infections, and (c) sepsis and meningitis.[1] *E. coli* causing diarrhea are divided in six categories depending on the type of disease[2]: (1) enteropathogenic *E. coli* (EPEC), (2) enterotoxigenic *E. coli* (ETEC), (3) enteroinvasive *E. coli* (EIEC), (4)

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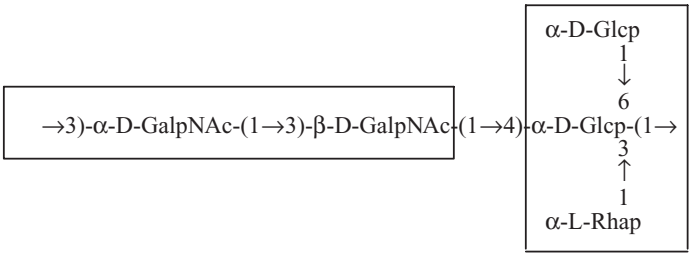


Figure 1: Structure of the pentasaccharide repeating unit of the *O*-antigen from the enteropathogenic *Escherichia coli* O158.

enterohaemorrhagic *E. coli* (EHEC), (5) enteroaggregative *E. coli* (EAEC), and (6) diffusely adherent *E. coli* (DAEC).

The enteropathogenic *E. coli* (EPEC) strains are known to be associated with various kinds of diarrhea in infants and are a cause of illness and death among children in the developing countries.[3] The *O*-antigen or endotoxins are the responsible virulence factor of the enteropathogenic *E. coli* strains, which mediate their interactions with the host at the initial stage of bacterial infection.[4] The structure of the pentasaccharide *O*-antigen of the enteropathogenic *E. coli* strain O158 has been reported by Datta et al. (Fig. 1).[5] The structure of the pentasaccharide repeating unit is unique in nature as it contains two D-glucopyranosyl and one L-rhamnopyranosyl moieties α-glycosidically linked and a disaccharide branch in which one D-galactosamine unit exists as α-glycosidically linked and the other one is β-glycosidically linked. In the recent past, chemical synthesis of immunodominant oligosaccharides has gained considerable interest.[6] In order to understand the relationship between structure and immunochemical specificity of the *O*-antigen, we decided to prepare a di- and a trisaccharide fragment related to the pentasaccharide repeating unit of the *O*-antigen of *E. coli* O158. We herein describe concise chemical synthesis of a tri- and a disaccharide (**1** and **2**) as their methyl glycoside and 2-(4-methoxyphenoxy) ethyl glycoside, respectively (Fig. 2).

RESULTS AND DISCUSSION

Trisaccharide **1** and disaccharide **2** were synthesized from a series of suitably protected monosaccharide intermediates by regio- and stereoselective glycosylations. The functionalized monosaccharide intermediates **3**,[7] **4**,[8] and **5**[9] were prepared from the commercially available monosaccharides using literature-reported reaction conditions. Iodonium ion promoted stereoselective glycosylation of compound **3** with thioglycoside derivative **4** in the presence of a *N*-iodosuccinimide (NIS)-trimethylsilyl trifluoromethanesulfonate (TMSOTf) combination,[10] which furnished disaccharide derivative **6** in 88% yield, which

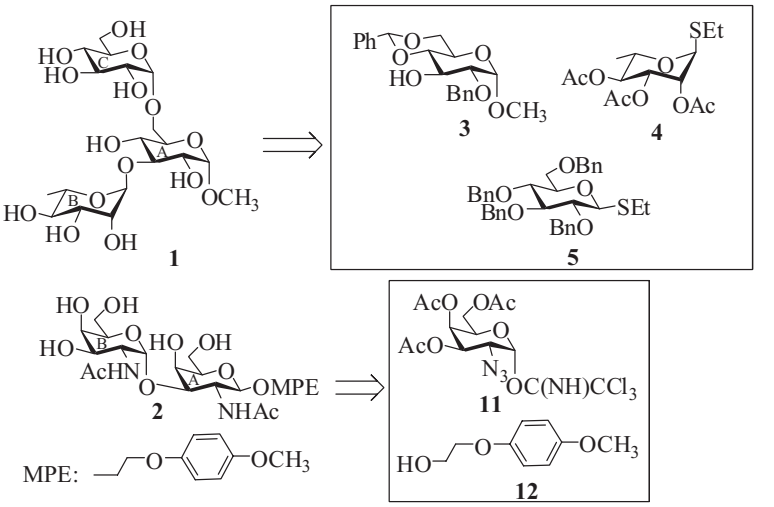
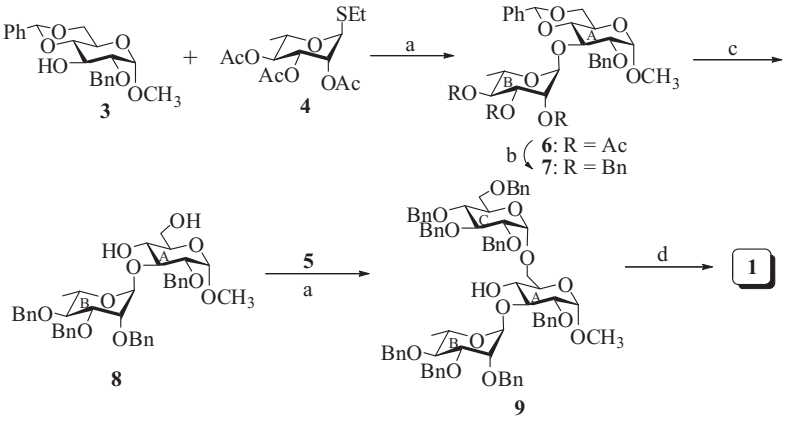


Figure 2: Structures of the synthesized tri- and disaccharide fragments corresponding to the *O*-antigen of enteropathogenic *Escherichia coli* O158.

was transformed to disaccharide derivative **7** under a one-pot deacetylation-benzylation[11] reaction condition in 90% yield. Removal of the benzylidene acetal[12] of compound **7** in the presence of $HClO_4$-SiO_2[13] resulted in the disaccharide diol derivative **8** in 79% yield. Regio- and stereoselective glycosylation of compound **8** with thioglycoside donor **5** in the presence of NIS-TMSOTf furnished trisaccharide derivative **9** in 80% yield. Appearance of a signal at δ 4.83 (d, $J = 3.8$ Hz) and 98.4 in the ^{1}H and ^{13}C NMR spectrum, respectively, confirmed the formation of compound **9** with the desired stereo outcome. Hydrogenation over Pearlman's catalyst furnished target trisaccharide **1** in 68%. It is worth mentioning that in compound **1**, all monosaccharide units are α-glycosidically linked (Scheme 1).

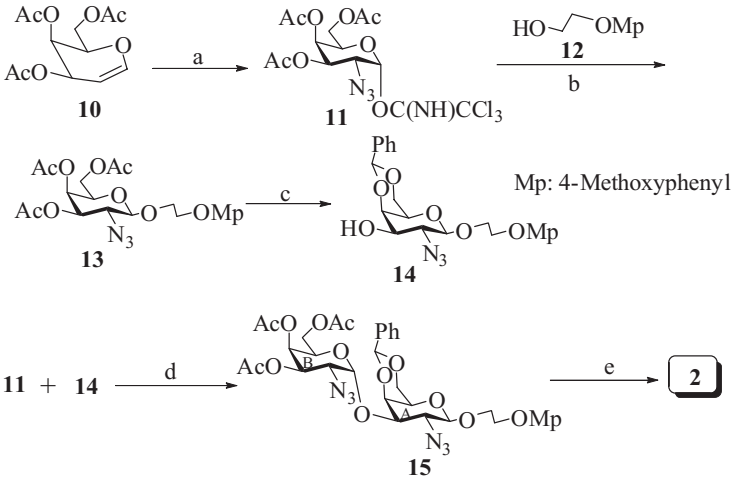
In a separate experiment, disaccharide **2** was synthesized as a 2-(4-methoxyphenoxy) ethyl glycoside (**2**). Following literature-reported protocol, 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl trichloroacetimidate (**11**)[14] was prepared from tri-*O*-acetyl-D-galactal (**10**) in three steps. Compound **11** was stereoselectively glycosylated with 2-(4-methoxyphenoxy) ethanol (**12**)[15] in the presence of TMSOTf in CH_3CN[16] to give compound **13** in 78% yield. Exclusive formation of 1,2-*trans* glycoside was achieved by exploiting the nitrile effect of the solvent. Sequential deacetylation and benzylidene acetal formation of compound **13** furnished compound **14** in 87% yield. Another α-selective glycosylation of compound **13** with glycosyl donor **11** in the presence of TMSOTf in methylene chloride[17] furnished disaccharide derivative **15** in 75% yield. Finally, hydrogenolysis[18] of disaccharide derivative **15**



Scheme 1: Reagents: (a) *N*-iodosuccinimide, TMSOTf, CH_2Cl_2, MS 4Å, −40°C, 1 h, 88% for **6** and 80% for **9**; (b) benzyl bromide, NaOH, TBAB, THF, rt, 6 h, 90%; (c) $HClO_4$-SiO_2, CH_3CN, H_2O, rt, 20 min, 79%; (d) H_2, 20% $Pd(OH)_2$-C, CH_3OH, rt, 24 h, 68%.

followed by *N*-acetylation and *O*-deacetylation afforded target disaccharide **2** as its 2-(4-methoxyphenoxy) ethyl glycoside in 72% yield (Scheme 2).

In summary, a tri- and a disaccharide related to the *O*-antigen of *E. coli* O158 were synthesized in excellent yield as methyl and 2-(4-methoxyphenoxy) ethyl glycoside, respectively. All glycosylation and protecting group manipulation steps were high yielding and reproducible for scale-up preparation. All



Scheme 2: Reagents: (a) Ref. (14); (b) TMSOTf, CH_3CN, −20°C, 1 h, 78%; (c) (i) 0.1 N CH_3ONa, CH_3OH, rt, 3 h; (ii) $PhCH(OCH_3)_2$, *p*-TsOH, CH_3CN, rt, 12 h, 87%; (d) TMSOTf, CH_2Cl_2, −20°C, 1 h, 75%; (e) (i) H_2, 20% $Pd(OH)_2$-C, CH_3OH, rt, 12 h; (ii) acetic anhydride, pyridine, rt, 2 h; (iii) 0.1 N CH_3ONa, CH_3OH, rt, 6 h, 72%.

monosaccharide moieties are α-linked in trisaccharide **1**. The disaccharide **2** was prepared from a single intermediate having a nonparticipating group at the C-2 position employing the solvent effect on the stereo outcome of the glycosylation.

EXPERIMENTAL

General Procedure

All reactions were monitored by thin layer chromatography using silica gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (2% $Ce(SO_4)_2$ in 2N H_2SO_4)-sprayed plates on a hot plate. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR, 2D COSY, and HMQC spectra were recorded on a Bruker Avance DRX 500 MHz using $CDCl_3$ and CD_3OD as solvents and TMS as internal reference unless stated otherwise. Chemical shift values are expressed in δ ppm. ESI-MS was recorded on a Micromass Quattro II triple quadrupole 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 were used in many reactions.

Methyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (6)

To a solution of compound **3** (1.5 g, 4.03 mmol) and compound **4** (1.6 g, 4.78 mmol) in anhydrous CH_2Cl_2 (20 mL) was added MS 4Å (2 g) and the reaction mixture was allowed to stir at rt under argon for 1 h. The reaction mixture was cooled to −40°C; to the cold reaction mixture were added *N*-iodosuccinimide (NIS; 1.3 g, 5.77 mmol) and TMSOTf (25 μL), and the reaction mixture was allowed to stir at the same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (100 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$, satd. $NaHCO_3$, and H_2O; dried (Na_2SO_4); and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (6:1) as eluant to give pure **6** (2.3 g, 88%). Yellow oil; IR (neat): 2937, 1748, 1373, 1248, 1224, 1137, 1088, 1048, 986, 750, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.46–7.25 (m, 10 H, Ar-H), 5.49 (s, 1 H, PhC*H*), 5.30–5.28 (m, 1 H, H-2_B), 5.24 (dd, J = 9.9 and 3.5 Hz, 1 H, H-3_B), 5.09 (br s, 1 H, H-1_B), 4.90 (t, J = 9.9 Hz, 1 H, H-4_B), 4.71 (d, J = 12 Hz, 1 H, PhCH_2), 4.56 (d, J = 12.1 Hz, 1 H, PhCH_2), 4.51 (d, J = 3.4 Hz, 1 H, H-1_A), 4.25–4.20 (m, 1 H, H-6_{aA}), 4.16–4.07 (m, 2 H, H-4_A and H-5_B), 3.79–3.76 (m, 1 H, H-5_A), 3.69–3.46 (m, 1 H, H-6_{bA}), 3.53–3.46 (m, 2 H, H-2_A and H-3_A), 3.35 (s, 3 H, OCH_3), 2.09 (s, 3 H, COCH_3), 1.98 (s, 3 H, COCH_3), 1.94 (s, 3 H, COCH_3),

0.75 (d, $J = 6.1$ Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9 (*C*OCH$_3$), 169.7 (*C*OCH$_3$), 169.5 (*C*OCH$_3$), 137.6–126.3 (Ar-C), 101.7 (Ph*C*H), 98.6 (C-1$_A$), 97.9 (C-1$_B$), 80.4 (C-2$_A$), 79.7, (Ph*C*H$_2$), 74.1 (C-5$_A$), 73.3, (C-3$_B$), 71.1, (C-3$_A$), 69.7 (C-4$_A$), 69.3 (C-2$_B$), 68.9 (C-4$_B$), 65.9, (C-5$_B$), 62.2 (C-6$_A$), 55.3 (O*C*H$_3$), 20.8 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 16.6 (C*C*H$_3$); ESI-MS: 667.2 [M+Na]$^+$; Anal. Calcd. for $C_{33}H_{40}O_{13}$ (644.25): C, 61.48; H, 6.25; found: C, 61.30; H, 6.50.

Methyl (2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-(1$\rightarrow$3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (7)

To a solution of compound **6** (2 g, 3.10 mmol) in THF (15 mL) were added powdered NaOH (1 g, 25 mmol), benzyl bromide (1.7 mL, 14.3 mmol), and Bu_4NBr (200 mg, 0.62 mmol) and the reaction mixture was allowed to stir briskly at rt for 6 h. The reaction mixture was poured into H_2O (300 mL) and extracted with CH_2Cl_2 (150 mL). The organic layer was washed with H_2O (200 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (8:1) to give pure **7** (2.2 g, 90%). Yellow oil; IR (neat): 3031, 2979, 2899, 2867, 1497, 1454, 1389, 1361, 1181, 1123, 1093, 1059, 1046, 1028, 995, 912, 750 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46–7.16 (m, 25 H, Ar-H), 5.45 (s, 1 H, PhC*H*), 5.17 (br s, 1 H, H-1$_B$), 4.86 (d, $J = 11.1$ Hz, 1 H, PhCH_2), 4.61–4.39 (m, 7 H, 3 PHCH_2 and H-1$_A$), 4.24–4.19 (m, 1 H, H-6$_{aA}$), 4.12 (t, $J = 9.3$ Hz, H-4$_A$), 4.01–3.97 (m, 1 H, H-5$_B$), 3.84–3.74 (m, 3 H, H-2$_B$, H-5$_A$, and H-6$_{bA}$), 3.65 (t, $J = 4.9$ Hz, 1 H, H-4$_B$), 3.53 (t, $J = 9.5$ Hz, 1 H, H-3$_A$), 3.41–3.36 (m, 2 H, H-2$_A$ and H-3$_B$), 3.35 (s, 3 H, OCH_3), 0.90 (d, $J = 6.1$ Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.0–126.3 (Ar-C), 101.6 (Ph*C*H), 98.6 (C-1$_A$), 98.4 (C-1$_B$), 80.7 (C-2$_A$), 80.4 (C-5$_A$), 80.1 (2 C, 2 Ph*C*H$_2$), 74.9 (2 C, 2 Ph*C*H$_2$), 74.4 (C-3$_B$), 72.8 (C-3$_A$), 72.1 (C-2$_B$), 72.0 (C-4$_A$), 68.9 (C-4$_B$), 67.7 (C-5$_B$), 62.6 (C-6$_A$), 55.5 (O*C*H$_3$), 17.4 (C*C*H$_3$); ESI-MS: 811.3 [M+Na]$^+$; Anal. Calcd. for $C_{48}H_{52}O_{10}$ (788.36): C, 73.08; H, 6.64; found: C, 72.86; H, 6.87.

Methyl (2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-(1$\rightarrow$3)-2-*O*-benzyl-α-D-glucopyranoside (8)

To a solution of compound **7** (2 g, 2.53 mmol) in CH_3CN-H_2O (50 mL, 9:1 v/v) was added $HClO_4$-SiO_2 (500 mg) and the reaction mixture was allowed to stir at rt for 20 min. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to give pure **8** (1.4 g, 79%). Yellow oil; IR (neat): 3364, 2922, 2857, 1497, 1454, 1370, 1207, 1121, 1060, 1027, 735 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.23 (m, 20 H, Ar-H), 5.04 (d, $J = 1.4$ Hz, 1 H, H-1$_B$), 4.94 (d, $J = 10.8$ Hz, 1 H, PhCH_2) 4.68 (d, $J = 12.3$ Hz, 1 H, PhCH_2), 4.64 (d, $J =$

10.8 Hz, 1 H, PhCH_2), 4.63 (d, J = 12.3 Hz, 1 H, PhCH_2) 4.59–4.53 (m, 3 H, PhCH_2), 4.51 (d, J = 3.6 Hz, 1 H, H-1_A), 4.39 (d, J = 12.1 Hz, 1 H, PhCH_2), 3.95–3.92 (m, 1 H, H-5_B), 3.86–3.64 (m, 2 H, H-2_B and H-4_B), 3.81–3.78 (m, 2 H, H-6_{aA} and H-3_B), 3.76–3.73 (m, 1 H, H-6_{bA}), 3.68 (t, J = 9.3 Hz, 1 H, H-4_A), 3.59–3.55 (m, 1 H, H-5_A), 3.40 (t, J = 9.1 Hz, 1 H, H-3_A), 3.35 (s, 3 H, OCH_3), 3.30 (dd, J = 9.5 and 3.6 Hz, 1 H, H-2_A), 1.34 (d, J = 6.1 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.8–127.9 (Ar-C), 100.7 (C-1_A), 98.4 (C-1_B) 85.4 (C-2_B), 80.5 (C-5_A), 79.6 (C-3_B), 78.0 (C-3_A), 75.8 (PhCH$_2$), 75.6 (C-2_A), 73.9 (PhCH$_2$), 73.0 (PhCH$_2$), 72.4 (PhCH$_2$), 71.1 (C-4_A), 70.8 (C-4_B), 69.8 (C-5_B), 62.9 (C-6_A), 55.6 (OCH$_3$), 18.4 (CCH$_3$); ESI-MS: 723.3 $[M+Na]^+$; Anal. Calcd. for $C_{41}H_{48}O_{10}$ (700.32): C, 70.27; H, 6.90; found: C, 70.04; H, 7.15.

Methyl (2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-(1→6)]-2-O-benzyl-α-D-glucopyranoside (9)

To a solution of compound **8** (1 g, 1.43 mmol) and compound **5** (1 g, 1.71 mmol) in anhydrous CH_2Cl_2 (10 mL) was added MS 4Å (1 g) and the reaction mixture was allowed to stir at rt for 1 h then cooled to −40°C. To the cold reaction mixture were added NIS (450 mg, 2 mmol) and TMSOTf (10 μL) and the reaction mixture was allowed to stir at the same temperature for 1 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (100 mL). The organic layer was successively washed with 5% $Na_2S_2O_3$, satd. $NaHCO_3$, and H_2O; dried (Na_2SO_4); and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure **9** (1.4 g, 80%). Yellow oil; IR (neat): 3064, 3030, 2919, 2870, 1730, 1496, 1454, 1362, 1216, 1061, 1028, 912, 753 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31–7.11 (m, 40 H, Ar-H), 5.03 (d, J = 1.4 Hz, 1 H, H-1_B), 5.0–4.81 (m, 3 H, PhCH_2), 4.83 (d, J = 3.8 Hz, 1 H, H-1_C), 4.88–4.76 (m, 3 H, PhCH_2), 4.68–4.57 (m, 4 H, PhCH_2), 4.56–4.50 (m, 6 H, H-1_A and PhCH_2), 4.49–4.47 (m, 1 H, PhCH_2), 4.44–4.33 (m, 1 H, PhCH_2), 4.06–4.03 (m, 1 H, H-6_{aC}), 3.98–3.88 (m, 1 H, H-5_B), 3.86–3.76 (m, 4 H, H-2_B, H-4_B, H-6_{bC} and H-5_C), 3.75–3.73 (m, 1 H, H-6_{aA}), 3.72–3.63 (m, 3 H, H-6_{bA}, H-3_B and H-4_C), 3.62–3.59 (m, 2 H, H-4_A and H-5_A), 3.58–3.56 (m, 1 H, H-3_C), 3.55–3.43 (m, 2 H, H-3_A and H-2_C), 3.30 (s, 3 H, OCH_3), 3.26 (dd, J = 9.5 and 3.6 Hz, 1 H, H-2_A), 1.3 (d, J = 6.1 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.0–127.8 (Ar-C), 104.4 (C-1_A), 100.8 (C-1_B), 98.4 (C-1_C), 85.1 (C-2_B), 82.7 (C-5_A), 80.5 (C-3_A), 79.7 (C-3_B), 78.2 (C-2_A), 78.1 (C-5_C), 76.1 (PhCH$_2$), 75.8 (PhCH$_2$), 75.7 (C-4_C), 75.4 (C-3_C), 75.3 (PhCH$_2$), 75.0 (PhCH$_2$), 73.9 (PhCH$_2$), 73.8 (2 PhCH$_2$), 73.1 (C-2_C), 73.0 (C-4_A), 72.4 (PhCH$_2$ and C-4_B), 70.8 (C-6_A), 70.5 (C-6_C), 70.1 (C-5_B), 55.6 (OCH$_3$), 18.4 (CCH$_3$); ESI-MS: 1245.5 $[M+Na]^+$; Anal. Calcd. for $C_{75}H_{82}O_{15}$ (1222.57): C, 73.63; H, 6.76; found: C, 73.44; H, 7.0.

Methyl (α-L-rhamnopyranosyl)-(1→3)-[α-D-glucopyranosyl)-(1→6)]-α-D-glucopyranoside (1)

To a solution of compound **9** (1 g, 0.82 mmol) in CH_3OH (10 mL) was added 20% $Pd(OH)_2$-C (200 mg) and the reaction mixture was allowed to stir at rt under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a Celite bed and concentrated under reduced pressure to give compound **1**, which was passed through a column of LH-20 Sephadex gel using CH_3OH-H_2O (8:1) as eluant to give pure **1** (280 mg, 68%). White powder; IR (KBr): ^{1}H NMR (500 MHz, CD_3OD): δ 4.97 (br s, 1 H, H-1_B), 4.69 (br s, 1 H, H-1_C), 4.39 (d, J = 3.4 Hz, 1 H, H-1_A), 4.08–4.06 (m, 1 H, H-3_B), 3.96–3.91 (m, 2 H, H-4_B and H-2_B), 3.84–3.80 (m, 1 H, H-6_{aC}), 3.77–3.73 (m, 1 H, H-5_B), 3.72–3.60 (m, 4 H, H-6_{abA}, H-4_A and H-4_C), 3.59–3.56 (m, 1 H, H-6_{bC}), 3.50–3.46 (m, 2 H, H-5_A and H-5_C), 3.42–3.36 (m, 2 H, H-2_A and H-2_C), 3.36 (s, 1 H, OCH_3), 3.34–3.28 (m, 1 H, H-3_C), 3.24 (t, J = 9.2 Hz, 1 H, H-3_C), 1.15 (d, J = 6.2 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 103.1 (C-1_A), 101.5 (C-1_B), 99.8 (C-1_C), 80.3 (C-4_A), 76.2 (C-2_A), 73.5 (C-3_A), 72.3 (C-2_C), 72.0 (C-5_B), 71.0 (C-4_C), 70.7 (C-2_B and C-4_B), 70.6 (C-5_C), 70.5 (C-3_C), 70.0 (C-5_A), 69.1 (C-3_B), 60.9 (C-6_A), 60.8 (C-6_C), 55.6 (OCH$_3$), 16.9 (CCH$_3$); ESI-MS: 525.1 $[M+Na]^+$; Anal. Calcd. for $C_{19}H_{34}O_{15}$ (502.19): C, 45.42; H, 6.82; found: C, 45.20; H, 7.10.

2-(4-Methoxyphenoxy) ethyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (13)

To a solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl trichloroacetimidate (**11**; 1 g, 2.1 mmol) in anhydrous CH_3CN (10 mL) was added 2-(4-methoxyphenoxy) ethanol (**12**; 550 mg, 3.27 mmol) and the solution was cooled to −20°C. To the cold reaction mixture was added TMSOTf (20 μL) and the reaction mixture was allowed to stir at the same temperature for 1 h. The reaction was quenched by addition of Et_3N (0.1 mL) and diluted with CH_2Cl_2 (100 mL). The organic layer was successively washed with satd. $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated to dryness. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure **13** (790 mg, 78%). Yellow oil; IR (neat): 3020, 2837, 2116, 1794, 1508, 1370, 1229, 1045, 827, 758 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.88–6.81 (m, 4 H, Ar-H), 5.33 (d, J = 3.2 Hz, 1 H, H-4), 4.79 (dd, J = 10.3 and 3.3 Hz, 1 H, H-3), 4.52 (d, J = 8.1 Hz, 1 H, H-1), 4.20–4.09 (m, 5 H, H-6_{ab}, OCH_{2ab}, and OCH_{2a}), 4.01–3.96 (m, 1 H, OCH_{2b}), 3.88–3.85 (m, 1 H, H-5), 3.76 (s, 3 H, OCH_3), 3.71 (dd, J = 10.9 Hz, 1 H, H-2), 2.16 (s, 3 H, COCH_3), 2.09 (s, 3 H, COCH_3), 2.03 (s, 3 H, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7 (COCH$_3$), 170.4 (COCH$_3$), 170.2 (COCH$_3$), 103.1 (C-1), 71.4 (C-4), 71.1 (C-3), 69.1 (C-5), 68.2 (OCH$_2$), 66.7 (OCH$_2$), 61.6 (C-2), 61.1 (C-6), 56.1 (OCH$_3$), 21.0 (3 C, 3

CO*C*H_3); ESI-MS: 504.1 $[M+Na]^+$; Anal. Calcd. for $C_{21}H_{27}N_3O_{10}$ (481.17): C, 52.39; H, 5.65; found: C, 52.20; H, 5.90.

2-(4-Methoxyphenoxy) ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-*β*-D-galactopyranoside (14)

A solution of compound **13** (700 mg, 1.45 mmol) in 0.1 M CH_3ONa (10 mL) was allowed to stir at rt for 3 h and neutralized with Amberlite IR 120 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure. To a solution of the deacetylated product in anhydrous CH_3CN (5 mL) was added benzaldehyde dimethylacetal (330 μL, 2.2 mmol) and *p*-TsOH (50 mg) and the reaction mixture was allowed to stir at rt for 12 h. The reaction was quenched with Et_3N (0.1 mL) and the solvents were removed under reduced pressure. The crude product was purified over SiO_2 using toluene-EtOAc (2:1) as eluant to give pure **14** (560 mg, 87%). Yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51–7.49 (m, 2 H, Ar-H), 7.37–7.35 (m, 3 H, Ar-H), 6.87 (d, J = 9.1 Hz, 2 H, Ar-H), 6.81 (d, J = 9.1 Hz, 2 H, Ar-H), 5.53 (s, 1 H, PhC*H*), 4.42 (d, J = 7.9 Hz, 1 H, H-1), 4.27 (dd, J = 12.5 and 1.3 Hz, 1 H, H-6_A), 4.23–4.19 (m, 1 H, OCH_{2a}), 4.15–4.12 (m, 3 H, OCH_{2ab} and H-4), 4.01 (dd, J = 12.5 and 1.7 Hz, 1 H, H-6_b), 3.97–3.93 (m, 1 H, OCH_{2b}), 3.74 (s, 3 H, OCH_3), 3.65 (dd, J = 10.8 Hz, 1 H, H-2), 3.53 (dd, J = 9.9 and 2.9 Hz, 1 H, H-3), 3.40–3.38 (m, 1 H, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.4–115.0 (Ar-C), 102.8 (Ph*C*H), 101.7 (C-1), 75.0 (C-5), 71.7 (C-4), 69.3 (C-3), 68.7 (O*C*H_2), 68.4 (O*C*H_2), 66.9 (C-6), 64.2 (C-2), 56.1 (O*C*H_3); ESI-MS: 466.1 $[M+Na]^+$; Anal. Calcd. for $C_{22}H_{25}N_3O_7$ (443.17): C, 59.59; H, 5.68; found: C, 59.41; H, 5.90.

2-(4-Methoxyphenoxy) ethyl (3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*α*-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-*β*-D-galactopyranoside (15)

A solution of compound **14** (500 mg, 1.13 mmol) and compound **11** (650 g, 1.37 mmol) in anhydrous CH_2Cl_2 (8 mL) was cooled to −20°C. To the cold reaction mixture was added TMSOTf (10 μL) and the reaction mixture was allowed to stir at the same temperature for 1 h. The reaction was quenched with Et_3N (0.1 mL) and diluted with CH_2Cl_2 (50 mL). The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure **15** (640 mg, 75%). Yellow oil; IR (neat): 3396, 2926, 1646, 1509, 1373, 1233, 1057, 823 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55–7.53 (m, 2 H, Ar-H), 7.35–7.33 (m, 3 H, Ar-H), 6.85 (d, J = 9.2 Hz, 2 H, Ar-H), 6.82 (d, J = 9.2 Hz, 2 H, Ar-H), 5.58 (s, 1 H, PhC*H*), 5.51 (d, J = 2.4 Hz, 1 H, H-4_B), 5.43 (dd, J = 11.3 and 3.3 Hz, 1 H, H-3_B), 5.21 (d, J = 3.6 Hz, 1 H, H-1_B), 4.53 (t, J = 6.5 Hz, 1 H, H-5_B), 4.49 (d, J = 8.0 Hz, 1 H, H-1_A), 4.31 (dd, J = 12.5 and 1.2 Hz, 1 H, H-6_{aA}), 4.27 (d,

J = 3.4 Hz, 1 H, H-4_A), 4.25–4.22 (m, 1 H, OCH_{2a}), 4.17–4.13 (m, 3 H, H-6_{abB} and OCH_{2a}), 4.09 (dd, J = 12.4 and 1.4 Hz, 1 H, H-6_{bA}), 4.06–4.01 (m, 1 H, OCH_{2b}), 4.00–3.96 (m, 1 H, OCH_{2b}), 3.90 (dd, J = 8.1 Hz, 1 H, H-2_A), 3.7 (s, 3 H, OCH_3), 3.66 (dd, J = 11.2 and 3.3 Hz, 1 H, H-2_B), 3.61 (dd, J = 10.5 and 3.6 Hz, 1 H, H-3_A), 3.42–3.39 (m, 1 H, H-5_A), 2.13, 2.03 (2 s, 9 H, 3 $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8 ($COCH_3$), 170.3 ($COCH_3$), 169.9 ($COCH_3$), 129.3–115.0 (Ar-C), 103.1 (C-1_A), 101.3 (Ph*C*H), 95.1 (C-1_B), 75.0 (C-3_A), 71.3 (C-4_A), 69.4 (C-6_A), 68.5 (C-6_B), 68.4 (C-4_B), 68.1 (C-3_B), 68.0 (C-5_B), 67.7 (C-5_A), 66.8 (OCH_2), 62.0 (OCH_2), 61.4 (C-2_A), 57.3 (C-2_B), 56.1 (OCH_3), 21.0 (3 C, 3 $COCH_3$); ESI-MS: 779.2 $[M+Na]^+$; Anal. Calcd. for $C_{34}H_{40}N_6O_{14}$ (756.26): C, 53.97; H, 5.33; found: C, 53.76; H, 5.55.

2-(4-Methoxyphenyl)-ethyl (2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1$\rightarrow$3)-2-acetamido-2-deoxy-β-D-galactopyranoside (2)

To a solution of compound **15** (600 mg, 0.79 mmol) in CH_3OH (5 mL) was added 20% $Pd(OH)_2$-C (100 mg) and the reaction mixture was allowed to stir under hydrogen at rt for 12 h. The reaction mixture was filtered through a Celite bed and concentrated. A solution of the crude product in acetic anhydride-pyridine (5 mL, 1:1 v/v) was kept at rt for 2 h and the solvents were removed under reduced pressure. A solution of the acetylated product in 0.1 M CH_3ONa in CH_3OH (5 mL) was allowed to stir at rt for 6 h and neutralized with Dowex 50W X8 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure to give **2**, which was further purified through a column of Sephadex LH-20 using CH_3OH as eluant (325 mg, 72%). White powder; IR (KBr): 2934, 2114, 1751, 1508, 1371, 1233, 1130, 1054, 823 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.87–6.81 (m, 4 H, Ar-H), 4.94 (d, J = 3.6 Hz, 1 H, H-1_B), 4.56 (d, J = 8.0 Hz, 1 H, H-1_A), 4.27 (d, J = 2.8 Hz, 1 H, H-4_A), 4.05–3.93 (m, 8 H, H-6_{abA}, H-6_{abB}, H-2_B, H-3_A and OCH_{2ab}), 3.85–3.69 (m, 4 H, H-3_B, H-4_B and OCH_{2ab}), 3.67 (s, 3 H, OCH_3), 3.58–3.49 (m, 3 H, H-5_A, H-5_B and H-2_A), 1.77, 1.46 (2 s, 6 H, 2 $NHCOCH_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.3 (2 $NHCOCH_3$), 129.6–127.1 (Ar-C), 101.7 (C-1_A), 94.6 (C-1_B), 71.9 (C-4_A), 71.7 (C-4_B), 69.4 (C-6_A), 68.6 (C-6_B), 68.4 (C-3_B), 68.3 (2 C, 2 OCH_2), 67.9 (C-3_A and C-5_B), 66.7 (C-5_A), 60.9 (C-2_A), 56.2 (OCH_3), 50.5 (C-2_B), 23.7 ($NHCOCH_3$), 23.3 ($NHCOCH_3$); ESI-MS: 597.2 $[M+Na]^+$; Anal. Calcd. for $C_{25}H_{38}N_2O_{13}$ (574.24): C, 52.26; H, 6.67; found: C, 52.02; H, 6.95.

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