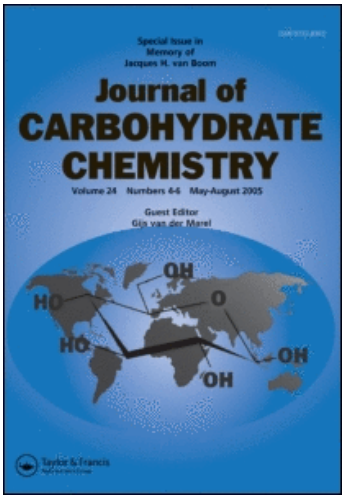

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Synthesis of the Trisaccharide Repeat of the *O*-Antigen of *Rhanella Aquatilis* 1-95

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Synthesis of the Trisaccharide Repeat of the *O*-Antigen of *Rhanella Aquatilis* 1-95

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A branched trisaccharide containing D-fucose and D-galactofuranose moieties corresponding to the repeating unit of the *O*-antigen of *Rahnella aquatilis* 1–95 has been synthesized from the monosaccharide intermediates in a five-step synthesis in an overall 38% yield. All glycosylation steps and protecting group functionalization steps are high yielding.

Keywords Trisaccharide; *Rahnella aquatilis*; Glycosylation; D-fucose; 2-Aminoethyl; D-Galactofuranose.

INTRODUCTION

Rahnella aquatilis belongs to the family of Enterobacteriaceae, which are found in soil and water.[1] Although formerly *R. aquatilis* was assigned to the *Enterobacter* agglomerans biogroup, later it was recognized as a distinct species.[2] *R. aquatilis* is an opportunistic pathogen responsible for a number of pathogenic infections in the gastrointestinal and urinary tracts and in the respiratory and cardiovascular systems.[3] In the pathogenesis of diseases caused by Gram-negative bacteria, endotoxin (lipopolysaccharide) plays a significant role.[4] Recently, for the first time, the structure of the *O*-antigen of *R. aquatilis* strain 1–95 was reported by Zdorovenko et al. (Fig. 1).[4] The trisaccharide repeating unit of the *O*-antigen is branched in nature and contains uncommon monosaccharide units such as D-fucose and D-galactofuranose moieties.

Preparation of the glycoconjugate vaccines against infectious diseases is considered an important area in medicinal research.[5] The prime requirement for designing a glycoconjugate vaccine candidate is to have pure

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α-D-Gal*p*

1

↓

2

→3)-β-D-Gal*f*-(1→3)-α-D-Fuc*p*-(1→

Figure 1: Structure of the repeating unit of the *O*-antigen of *Rahnella aquatilis* 1–95.

oligosaccharides or polysaccharides at hand. The limited availability of pure oligosaccharide fragments of a particular polysaccharide isolated from the natural sources cannot meet the requirement for their extensive biological studies. Concise chemical synthesis can provide access to larger quantities of pure oligosaccharides and several analogs thereof. As part of our program directed toward the synthesis of bacterial *O*-antigen fragments for use in glycoconjugate preparation,[6] we describe a concise chemical synthesis of a branched trisaccharide repeating unit of the *O*-antigen of *R. aquatilis* 1–95 as its 2-aminoethyl glycoside (**1**, Fig. 2). The 2-aminoethyl group serves as a linker for the conjugation to a carrier protein.

RESULTS AND DISCUSSION

In order to synthesize the target trisaccharide **1** having a branched D-fucopyranosyl moiety, three suitably protected monosaccharide intermediates (**2**, **3**, and **4**, Fig. 2) have been prepared. Starting from 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**),[7] D-fucose derivative **6**[8] was prepared in 90% yield following Lerner's procedure.[8] Compound **6** was converted to 2-azidoethyl 3,4-*O*-isopropylidene-α-D-fucopyranoside (**2**) following a set of reactions involving hydrolytic removal of isopropylidene acetal, Fischer

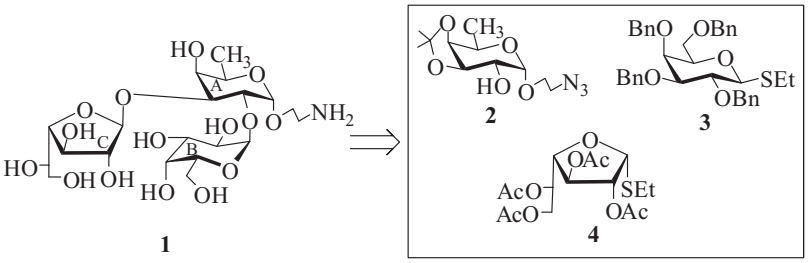
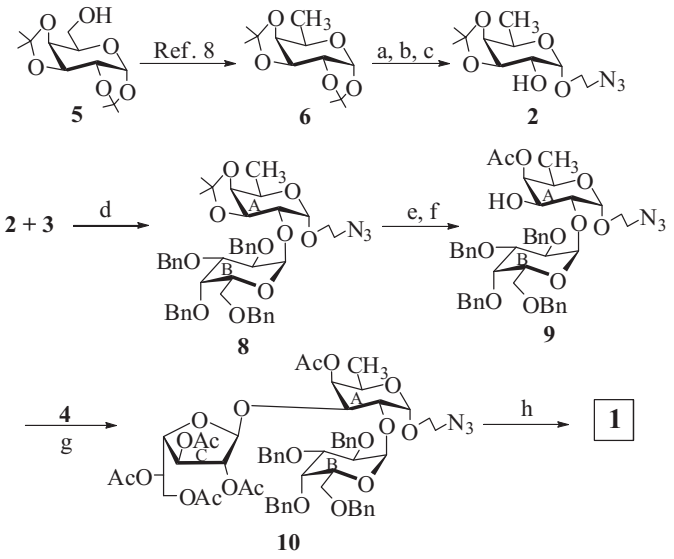


Figure 2: Structure of the target trisaccharide **1** and of the monosaccharide building blocks **2**, **3,** and **4**.

glycosylation with 2-azido ethanol in the presence of $HClO_4$-SiO_2, [9] and isopropylidenation in 80% overall yield. Compound **3**[10] was synthesized following literature-reported reaction conditions. Ethyl 2,3,5,6-tetra-*O*-acetyl-1-thio-α-D-galactofuranoside (**4**)[11] was prepared from D-galactose diethyldithioacetal (**7**) following the report of Wolfrom et al.[11] Stereoselective glycosylation of compound **2** with thioethyl glycoside **3** in the presence of a combination of copper(II) bromide-tetrabutylammonium bromide (TBAB)[12] in a mixed solvent (1,2-dichloroethane-DMF; 5:1, v/v) at room temperature furnished disaccharide derivative **8** in 76% yield. Appearance of a signature peak at δ 5.02 (br s, H-1_B) in the ^{1}H NMR spectrum confirmed the stereoselective formation of compound **8**. Removal of the isopropylidene acetal followed by selective acetylation of the axial hydroxyl group through orthoester formation[13] gave disaccharide acceptor **9** in 88% yield. Iodonium ion promoted stereoselective glycosylation of compound **9** with D-galactofuranose thioglycoside donor **4** in the presence of *N*-iodosuccinimide, and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[14,15] combination furnished trisaccharide derivative **10** in 82% yield. Appearance of signals at δ 5.34 (br s, H-1_C), 5.07 (d, J = 3.2 Hz, H-1_B), and 5.00 (d, J = 3.2 Hz, H-1_A) in the ^{1}H NMR spectrum and δ 107.3 (C-1_C), 96.9 (C-1_A), and 95.3 (C-1_B) in the ^{13}C NMR spectrum supported the formation of compound **10**. As the value of 1,2-coupling constant of the β-D-galactofuranosyl linkage is very low,[16] sometimes it appears as a broad singlet. The formation of β-D-galactofuranosyl linkage can be explained by considering the neighboring participating *O*-acetyl group at the C-2 position of thioglycoside donor **4**. Saponification of compound **10** followed by hydrogenolysis[17] furnished target trisaccharide **1** as its 2-aminoethyl glycoside in 70% yield (Sch. 1). Appearance of anomeric signals at δ 5.16 (br s, 1 H, H-1_C), 5.01 (br s, 1 H, H-1_B), and 5.00 (br s, 1 H, H-1_A) in the ^{1}H NMR and at δ 107.8 (C-1_C), 94.5 (C-1_A), and 94.0 (C-1_B) in the ^{13}C NMR confirmed the formation of trisaccharide **1**. Anomeric signals of **1** were compared with those of the native polysaccharide [δ 5.25 (br s, α-D-Fucp), 5.11 (br s, α-D-Galp), and 5.23 (br s, β-D-Galf) in ^{1}H NMR and δ 110.3, 97.4, and 96.8 in ^{13}C NMR spectra].[4] The signals of the native polysaccharide have been shifted from compound **1** and may be due to the polysaccharide structure.

In summary, a concise synthesis of a branched trisaccharide repeating unit of the *O*-antigen of *R. aquatilis* 1–95 as its 2-aminoethyl glycoside was achieved in 38% yield starting from the three monosaccharide building blocks **2**, **3**, and **4**. The key features of the synthesis are the preparation of the D-fucose precursor from D-galactose and its subsequent glycosylation with 2-azido ethanol, as well as glycosylation of the D-galactofuranose residue. It is noteworthy that D-fucose and D-galactose moieties are linked through 1,2-*cis* linkages. The trisaccharide **1** can be conjugated to a carrier protein using the amino function of its 2-aminoethyl aglycon.



Scheme 1: Reagents: (a) 80% aq. AcOH, 80°C, 6 h; (b) 2-azidoethanol, $HClO_4$-SiO_2, 80°C, 50 h; (c) 2,2-dimethoxypropane, acetone, rt, 2 h, 80%; (d) Cu(II)Br_2, TBAB, MS 4Å, 1,2-$(CH_2Cl)_2$-DMF, rt, 48 h, 76%; (e) 80% aq. AcOH, 80°C, 2 h; (f) $CH_3C(OC_2H_5)_3$, *p*-TsOH, DMF, rt, 2 h; 80% aq. AcOH, rt, 30 min, 88%; (g) *N*-iodosuccinimide, TMSOTf, CH_2Cl_2, MS 4Å, −40°C, 1 h, 82%; (h) (i) CH_3ONa, CH_3OH, rt, 5 h; (ii) 20% $Pd(OH)_2$-C, CH_3OH-AcOH-H_2O, rt, 12 h, 70%.

EXPERIMENTAL

General Procedure

All reactions were monitored by thin layer chromatography over 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 Bruker Avance DRX 500 MHz using $CDCl_3$ and D_2O as solvents and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. ESI-MS were recorded on a Micromass Quattro II triple quadrupole mass spectrometer. Elementary analysis was carried out on 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.

2-Azidoethyl 3,4-O-isopropylidene-α-D-fucopyranoside (2)

A solution of compound **6** (4 g, 16.4 mmol) in 80% aq. AcOH (100 mL) was allowed to stir at 80°C for 6 h and evaporated and coevaporated with toluene

(3 × 50 mL) under reduced pressure. To a solution of the crude product in 2-azidoethanol (15 mL) was added $HClO_4$-SiO_2 (500 mg) and the reaction mixture was allowed to stir at 80°C for 50 h. The reaction mixture was cooled to rt and the solvents were removed under reduced pressure. To a solution of the crude reaction mixture in anhydrous acetone (30 mL) was added 2,2-dimethoxypropane (5 mL, 40.6 mmol) and it was allowed to stir at rt for 2 h. The reaction mixture was neutralized with Et_3N (1 mL), filtered, and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **2** (3.6 g, 80%). Yellow oil; $[\alpha]_D^{25}$ +5 (*c* 1.6, $CHCl_3$); IR (neat): 3450, 2989, 2933, 2877, 2107, 1738, 1635, 1456, 1381, 1345, 1292, 1245, 1218, 1180, 1155, 1130, 1072, 992, 921, 800, 756, 687 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 4.86 (d, J = 3.8 Hz, 1 H, H-1), 4.24 (t, J = 6.3 Hz, 1 H, H-2), 4.19–4.17 (m, 1 H, H-5), 4.08 (dd, J = 2.3, 6.1 Hz, 1 H, H-3), 4.00–3.96 (m, 2 H, OCH_2), 3.83 (dd, J = 3.9, 6.1 Hz, 1 H, H-4), 3.72–3.68 (m, 2 H, OCH_2), 3.50–3.39 (m, 2 H, N-CH_2), 1.51 (s, 3 H, CH_3), 1.35 (s, 3 H, CCH_3), 1.31 (d, J = 6.0 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 109.7 ($C(CH_3)_2$), 98.1 (C-1), 76.2 (C-5), 75.9 (C-3), 69.5 (C-4), 67.6 (C-2), 64.8 (OCH_2), 51.1 (NCH_2), 28.1 (CH_3), 26.2 (CH_3), 16.7 (CCH_3); ESI-MS: 296.1 $[M+Na]^+$; Anal. Calcd. for $C_{11}H_{19}N_3O_5$ (273.13): C, 48.34; H, 7.01; Found: C, 48.15; H, 7.25.

2-Azidoethyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→2)-3,4-*O*-isopropylidene-α-D-fucopyranoside (8)

To a solution of compound **2** (2.5 g, 9.15 mmol) and compound **3** (6.4 g, 10.94 mmol) in anhydrous 1,2-$(CH_2Cl)_2$-DMF (30 mL, 5:1 v/v) were added MS 4Å (5 g), $CuBr_2$ (3.6 g, 16.12 mmol), and TBAB (700 mg, 2.17 mmol) and the reaction mixture was allowed to stir at rt under argon for 48 h. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (150 mL). The organic layer was successively washed with 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 (5:1) as eluant to give pure compound **8** (5.5 g, 76%). Yellow oil; $[\alpha]_D^{25}$ +2.28 (*c* 1.6, $CHCl_3$); IR (neat): 3062, 3030, 2979, 2927, 2873, 2144, 2105, 1606, 1480, 1454, 1355, 1339, 1299, 1209, 1158, 1109, 1089, 1068, 1049, 989, 967, 863, 751, 727, 702, 632 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.36–7.22 (m, 20 H, Ar-H), 5.02 (br s, 1 H, H-1_B), 4.94 (d, J = 11.3 Hz, 1 H, PhCH_2), 4.82–4.78 (m, 3 H, PhCH_2, H-1_A), 4.74 (d, J = 11.7 Hz, 1 H, PhCH_2), 4.64 (d, J = 11.7 Hz, 1 H, PhCH_2), 4.57 (d, J = 11.3 Hz, 1 H, PhCH_2), 4.43 (d, J = 11.9 Hz, 1 H, PhCH_2), 4.40 (d, J = 11.9 Hz, 1 H, PhCH_2), 4.30 (dd, J = 5.6, 7.7 Hz, 1 H, H-5_B), 4.26–4.23 (m, 1 H, H-3_A), 4.19–4.18 (m, 1 H, H-5_A), 4.09–4.07 (m, 3 H, H-2_B, H-3_B, H-4_B), 4.03 (dd, J = 2.4, 5.4 Hz, 1 H, H-4_A), 3.77–3.71 (m, 2 H, H-2_A, OCH_{2a}), 3.6

(t, J = 8.6 Hz, 1 H, OCH_{2b}), 3.48–3.43 (m, 2 H, H-6_{abB}), 3.31–3.26 (m, 1 H, NCH_{2a}), 3.05–3.00 (m, 1 H, NCH_{2b}), 1.39 (s, 3 H, CH_3), 1.32 (d, J = 6.6 Hz, 3 H, CCH_3), 1.27 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 128.8–127.8 (Ar-C), 109.1 ($C(CH_3)_2$), 97.1 (C-1_A), 96.5 (C-1_B), 79.2 (C-3_B), 76.6 (C-2_B), 76.4 (C-4_B), 75.3 (PhCH_2), 75.2 (C-2_A), 74.8 (C-3_A), 74.5 (C-4_A), 73.8 (PhCH_2), 73.4 (PhCH_2), 73.0 (PhCH_2), 69.4 (C-5_B), 68.5 (C-6_B), 68.0 (OCH_2), 64.2 (C-5_A), 50.8 (N-CH_2), 28.5, 26.8 (2 CH_3), 16.7 (CCH_3); ESI-MS: 818.3 $[M+Na]^+$; Anal. Calcd. for $C_{45}H_{53}N_3O_{10}$ (795.37): C, 67.91; H, 6.71; Found: C, 67.73; H, 6.95.

2-Azidoethyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→2)-4-*O*-acetyl-α-D-fucopyranoside (9)

A solution of compound **8** (5 g, 6.28 mmol) in 80% aq. AcOH (100 mL) was allowed to stir at 80°C for 2 h and the solvents were evaporated and coevaporated under reduced pressure. To a solution of the crude product in anhydrous DMF (10 mL) was added $CH_3C(OC_2H_5)_3$ (7 mL, 38.18 mmol) followed by *p*-TsOH (200 mg) and the reaction mixture was allowed to stir at rt for 2 h concentrated under reduced pressure. The crude product was dissolved in 80% aq. AcOH (50 mL) and the reaction mixture was allowed to stir at rt for 30 min. The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to give pure compound **9** (4.4 g, 88%). Yellow oil; $[\alpha]_D^{25}$ +8.5 (*c* 1.6, $CHCl_3$); IR (neat): 3414, 3019, 2923, 2400, 2109, 1740, 1454, 1373, 1215, 1094, 1047, 928, 760, 699, 669, cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33–7.25 (m, 20 H, Ar-H), 5.25 (d, J = 2.8 Hz, 1 H, H-4_A), 4.99 (d, J = 3.7 Hz, 1 H, H-1_B), 4.91 (d, J = 11.6 Hz, 1 H, PhCH_2), 4.88 (d, J = 3.6 Hz, 1 H, H-1_A), 4.83 (d, J = 11.7 Hz, 1 H, PhCH_2), 4.79 (d, J = 11.9 Hz, 1 H, PhCH_2), 4.74 (d, J = 11.9 Hz, 1 H, PhCH_2), 4.64 (d, J = 11.9 Hz, 1 H, PhCH_2), 4.54 (d, J = 11.6 Hz, 1 H, PhCH_2), 4.47 (d, J = 11.9 Hz, 1 H, PhCH_2), 4.37 (d, J = 12.0 Hz, 1 H, PhCH_2), 4.21 (dd, J = 6.5, 12.1 Hz, 1 H, H-5_B), 4.11–4.05 (m, 3 H, H-3_A, H-2_B, H-5_A), 3.99 (dd, J = 2.7, 10.1 Hz, 1 H, H-3_B), 3.8 (d, J = 1.8 Hz, 1 H, H-4_B), 3.77–3.73 (m, 2 H, H-2_A, H-6_{aB}), 3.55–3.51 (m, 3 H, H-6_{bB}, OCH_{2a}, OH), 3.36–3.30 (m, 1 H, OCH_{2b}), 3.28–3.26 (m, 1 H, NCH_{2a}), 3.19–3.17 (m, 1 H, NCH_{2b}), 2.06 (s, 3 H, COC$H_{3)}$, 1.11 (d, J = 6.6 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.2 (COCH$_3$), 139.0–127.9 (Ar-C), 98.7 (2 C, C-1_A, C-1_B), 79.0 (C-2_A), 78.8 (C-3_A), 76.7 (C-5_A), 75.2 (C-4_B), 75.0 (PhCH_2), 73.9 (PhCH_2), 73.8 (PhCH_2), 73.5 (C-4_A), 73.2 (PhCH_2), 70.7 (C-5_B), 70.0 (C-6_B), 68.2 (OCH_2), 67.3 (C-3_B), 65.8 (C-2_B), 50.9 (NCH_2), 21.2 (COCH$_3$), 16.6 (CCH_3); ESI-MS: 820.3 $[M+Na]^+$; Anal. Calcd. for $C_{44}H_{51}N_3O_{11}$ (797.35): C, 66.23; H, 6.44; Found: C, 66.05; H, 6.68.

2-Azidoethyl (2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→2)]-4-*O*-acetyl-α-D-fucopyranoside (10)

To a solution of compound **9** (2 g, 2.5 mmol) and compound **4** (1.2 g, 3 mmol) in anhydrous CH_2Cl_2 (15 mL) was added MS 4Å (2 g) and the reaction mixture was allowed to stir under argon at rt for 1 h and then cooled to -40°C. To the cold reaction mixture were added *N*-iodosuccinimide (800 mg, 3.55 mmol) and TMSOTf (15 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% aq. $Na_2S_2O_3$, H_2O, dried (Na_2SO_4), and evaporated to dryness. The crude product was purified over SiO_2 using hexane-EtOAc (4:1) as eluant to give pure compound **10** (2.3 g, 82%). Yellow oil; $[\alpha]_D^{25}$ +5.85 (*c* 1.6, $CHCl_3$); IR (neat): 3020, 2926, 2857, 2401, 2108, 1744, 1496, 1454, 1372, 1217, 1054, 980, 758, 698, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.33–7.25 (m, 20 H, Ar-H), 5.43 (m, 1 H, H-5_C), 5.34 (br s, 1 H, H-1_C), 5.18 (d, J = 2.3 Hz, 1 H, H-4_A), 5.09 (d, J = 1.4 Hz, 1 H, H-2_C), 5.07 (d, J = 3.2 Hz, 1 H, H-1_B), 5.0 (d, J = 3.2 Hz, 1 H, H-1_A), 4.92 (d, J = 11.4 Hz, 1 H, PhCH_2), 4.91 (s, 1 H, H-3_C), 4.80–4.78 (m, 3 H, 3 PhCH_2), 4.68 (d, J = 11.7 Hz, 1 H, PhCH_2), 4.53 (d, J = 11.4 Hz, 1 H, PhCH_2), 4.50 (d, J = 12.1 Hz, 1 H, PhCH_2), 4.45 (d, J = 12.1 Hz, 1 H, PhCH_2), 4.39–4.35 (m, 2 H, H-4_B, OCH_{2a}), 4.28–4.21 (m, 3 H, OCH_{2b}, H-3_B, H-3_A), 4.17–4.14 (m, 3 H, H-2_A, H-5_A, H-5_B), 4.06–4.04 (m, 2 H, H-2_B, H-4_C), 3.74–3.72 (m, 1 H, H-6_{aC}), 3.56–3.54 (m, 1 H, H-6_{abB}), 3.49–3.47 (m, 1 H, H-6_{bC}), 3.29–3.28 (m, 1 H, NCH_{2a}), 3.14–3.11 (m, 1 H, NCH_{2b}), 2.11, 2.06, 2.01, 1.98, 1.96 (5 s, 15 H, 5 *C*OCH_3), 1.1 (d, J = 6.6 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.0, 170.8, 170.4 (2 C), 169.7 (5 *C*OCH$_3$), 139.4–127.6 (Ar-C), 107.3 (C-1_C), 96.9 (C-1_A), 95.3 (C-1_B), 81.4 (C-2_C), 81.2 (C-4_B), 79.5 (C-2_B), 77.5 (C-3_C), 76.5 (C-4_C), 75.7 (C-2_A), 75.2 (Ph*C*H$_2$), 73.9 (C-5_A), 73.5 (2 C, 2 Ph*C*H$_2$), 73.0 (Ph*C*H$_2$), 72.9 (C-4_A), 71.3 (C-5_B), 70.2 (C-3_B), 69.8 (2 C, C-3_A, C-5_C), 67.5 (C-6_B), 65.6 (C-6_C), 62.8 (O*C*H$_2$), 21.2, 21.1, 21.0 (2 C), 20.9 (5 CO*C*H$_3$), 16.3 (C*C*H$_3$); ESI-MS: 1150.5 $[M+Na]^+$; Anal. Calcd. for $C_{58}H_{69}N_3O_{20}$ (1127.45): C, 61.75; H, 6.16; Found: C, 61.57; H, 6.40.

2-Aminoethyl (β-D-galactofuranosyl)-(1→3)-[(α-D-galactopyranosyl)-(1→2)]-α-D-fucopyranoside (1)

A solution of compound **10** (1.5 g, 1.33 mmol) in 0.1 M CH_3ONa in CH_3OH (50 mL) was allowed to stir at rt for 5 h and neutralized with Dowex 50W X8 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure. To a solution of the crude product in CH_3OH-H_2O-AcOH (20 mL, 10:1:0.1, v/v) was added 20% $Pd(OH)_2$-C (100 mg) and the reaction mixture was allowed to stir under a positive pressure of hydrogen at rt for 12 h. The

reaction mixture was filtered through a Celite bed and evaporated to dryness to give compound **1**, which was further purified over Sephadex LH-20 column using CH_3OH-H_2O (5:1) as eluant (490 mg, 70%). White powder; $[\alpha]_D^{25}$ +6.24 (*c* 1.6, CH_3OH); IR (KBr): 3406, 2936, 2107, 1639, 1433, 1380, 1244, 1073, 1032, 977, cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.16 (br s, 1 H, H-1_C), 5.01 (br s, 1 H, H-1_B), 5.00 (br s, 1 H, H-1_A), 4.12–4.04 (m, 4 H, H-2_A, H-2_C, H-3_B, H-3_C), 4.01–3.97 (m, 3 H, H-2_B, H-3_A, H-2_A), 3.94 (br s, 1 H, H-4_C), 3.84 (d, J = 2.6 Hz, 1 H, H-5_B), 3.80–3.78 (m, 2 H, H-4_B, H-5_A), 3.77–3.70 (m, 4 H, H-5_C, H-6_{aC}, OCH_2), 3.64–3.60 (m, 2 H, H-6_{abB}), 3.52–3.47 (m, 2 H, NCH_{2a}, H-6_{bc}), 3.33–3.28 (m, 1 H, NCH_{2b}), 1.21 (d, J = 6.1 Hz, 3 H, CCH_3); ^{13}C NMR (125 MHz, CD_3OD): δ 107.8 (C-1_C), 94.5 (C-1_A), 94.0 (C-1_B), 81.9 (C-3_C), 80.0 (C-2_C), 76.1 (C-3_A), 73.6 (C-4_C), 70.3 (C-5_B), 69.3 (2 C, C-2_A, C-5_A), 69.0 (C-3_B), 68.3 (C-4_B), 67.7 (C-4_A), 67.0 (C-5_C), 64.4 (C-6_C), 64.0 (C-2_B), 61.1 (C-6_B), 59.4 (OCH_2), 45.3 (NCH_2), 13.3 (CCH_3); Anal. Calcd. for $C_{20}H_{37}NO_{15}$ (531.22): C, 45.20; H, 7.02; found: C, 44.95; H, 7.33.

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