

Synthesis of GlcNAc β -(1 \rightarrow 3)-Galp- α -(1 \rightarrow 2)-6-deoxy-*altro*Hepp- α -(1 \rightarrow O-propyl), an O-Antigenic Repeating Unit from *C. jejuni* O:23 and O:36

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A trisaccharide, GlcNAc β -(1 \rightarrow 3)-Galp- α -(1 \rightarrow 2)-6-deoxy-*altro*Hepp- α -(1 \rightarrow O-propyl), as an O-antigenic repeating unit of *C. jejuni* serotype O:23 and O:36 was synthesized. Coupling of the GlcNPhth-(1 \rightarrow 3)-Gal disaccharide donor with allyl 6-deoxy-*altro*Hep acceptor in the presence of iodonium dicollidine perchlorate (IDCP) promoter afforded the α -galactosidic trisaccharide with high stereoselectivities. Subsequent deacetalation, dephthaloylation, N-acetylation, and hydrogenolytic debenzoylation furnished the title compound.

Key Words : Altroheptose, Trisaccharide, *Campylobacter jejuni*, Antigenic repeating unit

Introduction

Campylobacter jejuni (*C. jejuni*) is a group of gram-negative bacteria that cause human gastroenteritis¹⁻³ and their importance as pathogens is being recognized due to their possible relation to neuropathy known as the Guillain-Barre syndrome (GBS) and Miller-Fisher Syndrome (MFS).^{4,5} In previous investigation of serotyping system developed by Penner, their serological differentiation relates to the structures of lipopolysaccharides (LPSs) from *C. jejuni*.¹ Investigations on their chemical structures by Aspinall *et al.*,⁶⁻⁹ showed that *C. jejuni* have low-molecular weight LPSs bearing N-acetylneuraminic acid residues and a minority of serotypes (O:23 and O:36) possess both low-molecular weight LPSs and high-molecular weight LPSs. Serotypes O:23 and O:36 are cross-reacting and their core regions of LPS are the indistinguishable but O-glycan structures are varied from batch to batch. O-Antigens, a potential basis of the serological classification of *C. jejuni*, consisted of trisaccharide repeating units of four closely-related types, in which the variable heptose residues differed in the presence or absence of oxygenation at C-6 and methylation at O-3

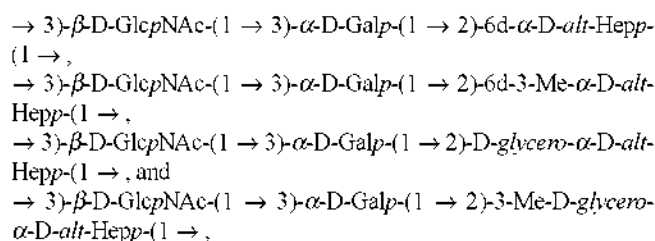


Figure 1. Chemical structures of O-antigens from *Campylobacter jejuni* O:23 and O:36.

(Fig. 1). Differences in ability to vary the proportions of heptose components may constitute a basis for serotypic discrimination, or evading the immune response of the host, which is permitting infection to continue.

In order to evaluate the immunological specificity and elucidate the role of *altro*heptopyranosyl residues in serotypic differences, it was necessary to synthesize various oligosaccharides having different reading frame with their size. This report describes the synthetic approach for trisaccharide **1** containing *altro*heptose which is rarely found in nature as its corresponding propyl glycoside, β -D-GlcpNAc-(1 \rightarrow 3)- α -D-Galp-(1 \rightarrow 2)-6-deoxy- α -D-*altro*Hepp-(1 \rightarrow O-propyl (Scheme 1).

Results and Discussion

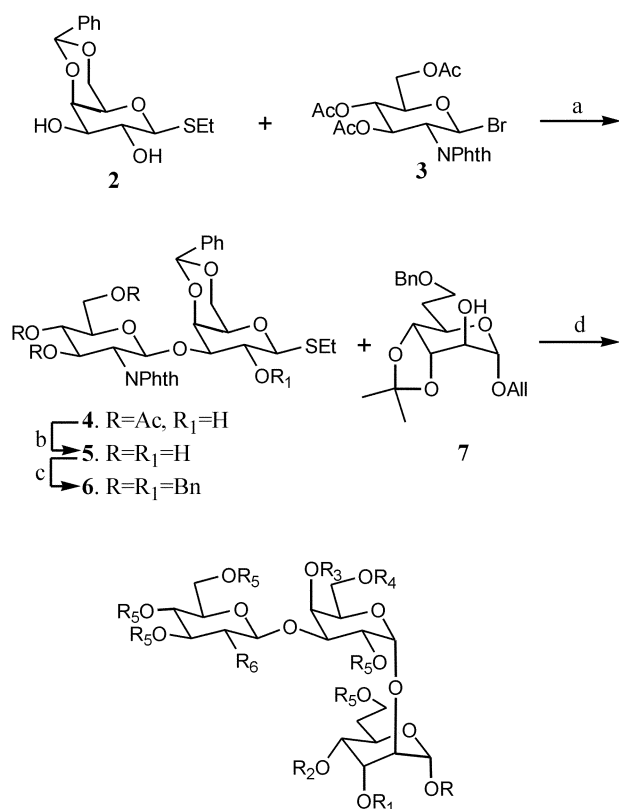
Trisaccharide **1** can be divided into two synthetic building blocks, a benzylated glycosyl donor **6** and allyl 6-deoxy- α -D-*altro*Hepp acceptor **7**.¹⁰ The benzylated thio-galactosyl donor **6** was obtained by coupling of an N-phthaloyl glucosaminyl bromide **3** and a thio-galactopyranoside **2**. The monosaccharide units **2** and **3** were synthesized as described earlier.^{11,12}

Regio and stereoselective glycosylation of **2** with corresponding bromide **3** in the presence of silver triflate^{12,13} as a promoter, s-collidine as an acid acceptor, and molecular sieve (4 Å) in CH₂Cl₂ at -25 °C gave GlcNPhth- β -(1 \rightarrow 3)-Gal-SEt **4** as one product in 60% yield. Subsequent deacetylation of **4** with 0.1 N sodium methoxide in methanol afforded **5**, and perbenzylation of **5** with benzyl bromide and sodium hydride in DMF gave **6** in 88% and 32% yield, respectively. It is noteworthy that benzylation proceeded sluggishly due to the phthalimido N-protecting group.

Because of the rarity of *altro*heptose in nature, its glycosidation has not been reported yet. Therefore, before attempting the 1,2-cis-glycosidation between disaccharide donor **6** and acceptor **7** employing armed thioglycosides-

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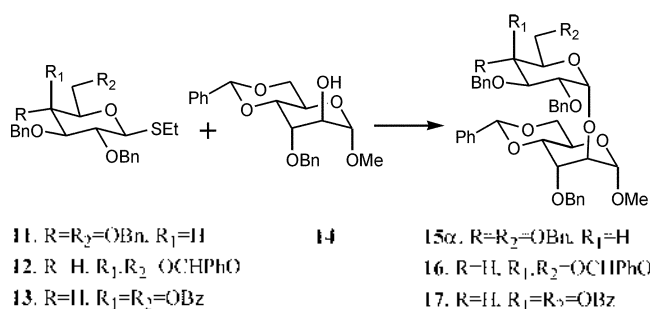
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Scheme 1. (a) AgOTf-toluene, collidine, MS 4A, -25 °C, 30 min, 60%; (b) 0.1 M NaOMe-MeOH, MeOH, rt, 2 h, 88%; (c) BnBr, NaH, DMF, rt, 2 h, 32%; (d) IDCP, CH₂Cl₂-Et₂O, MS 5A, rt, 2 h, 60%; (e) aq. HOAc, 50 °C, 7 h, 69%; (f) NH₂NH₂·H₂O, EtOH, 70 °C, 4 h and then Ac₂O, CH₂Cl₂-MeOH, rt, 2 h, 71%; (g) Pd/C-H₂, EtOH-AcOH, rt, 3 days, 70%.

IDCP promoter,^{14,15} it was necessary to investigate the effects of remote OH protecting groups to the anomeric center not only on the reactivity of thiogalactoside donors but also on the α -stereoselectivity. For the purpose, IDCP promoted glycosidations of methyl 3-*O*-benzyl-4,6-*O*-benzylidene- α -D-altropyranoside (**14**)¹⁶ acceptor were examined with various thioglucoside donors **11**, **12**, and **13** containing different OH protecting groups (Scheme 2). It was found α -stereoselectivity of 1,2-glycosidation was highly dependent on the nature of OH blocking groups remote to the anomeric center of galactosyl donor molecules. Glycosidation of **14** with perbenzylated thio- β -D-glucopyranoside **11** afforded α - and β -disaccharides **15 α** and **15 β** in 58% and 10% yield. Coupling of **14** with **12** and **13** provided only α -Gal-Alt disaccharides **16** and **17** in 65% and 26% yield, respectively. This result clearly shows that perbenzylated glycosyl donor is not imperative for the IDCP mediated 1,2-cisglycosidation of thiogalactoside.

It is also interesting to note that a galactosyl donor having 4,6-*O*-benzylidene group shows the best result in terms of α -



Scheme 2. General glycosidation reaction condition: IDCP, MS 5A, CH₂Cl₂-Et₂O, rt.

stereoselectivity and yield, while the presence of benzoyl esters in a glycosyl donor shows the lowest yield.

Therefore, 4,6-*O*-benzylidene protected GlcNPhth-Gal glycosyl donor **6** was chosen for stereocontrolled α -glycosidation with allyl 6-deoxy- α -D-altroHep glycosyl acceptor **7**. The coupling reaction was performed in the presence of IDCP at room temperature for 2 hours and the desired α -glycosylated trisaccharide **8** was obtained as one product in 60% yield. Successive treatment of compound **8** with 70% aqueous acetic acid, hydrazine hydrate in ethanol followed by Ac₂O in CH₂Cl₂-methanol, and finally Pd/C-H₂ (g) in ethanol yielded the title compound **1**.

In summary, the first synthesis of trisaccharide repeating unit of *C. jejuni* serotypes O:23 and O:36 containing 6-deoxy- α -D-altroheptopyranoside was efficiently achieved by stereospecific glycosidation between GlcNPhth-Gal disaccharide donor having 4,6-*O*-benzylidene group and allyl 6-deoxy- α -D-altroHep acceptor.

For the further studies, azido or amino group would be introduced into the allyl group of **8** to prepare the trisaccharide which has a linker arm for the conjugation with proteins.

Experimental Section

General. Organic solvents were dried and purified before use. Concentrations were performed under reduced pressure at below 40 °C. Thin layer chromatography (tlc) was performed using precoated silica gel plates (60F-254, E. Merck) and the spots were detected by charring with 5% sulfuric acid in ethanol. Column chromatography was performed on silica gel (E. Merck, Art 9385, 230-400 mesh in the flash mode). Optical rotations were measured with a PerkinElmer 241 polarimeter, using a 10 cm, 1 mL cell. IR spectra were recorded on Mattson 3000 FT-IR spectrometer using thin film on KBr plates. ¹H and ¹³C NMR spectra were recorded with a Varian VXR-200 or JEOL JNM-LA 400 spectrometer on solutions in CDCl₃ with tetramethylsilane as the internal standard. FAB mass spectra were obtained on a JEOL JMS-AX505WA instrument using glycerol as a matrix. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) spectra were obtained on a Voyager-DE™ mass spectrometer using 2,4-dihydroxybenzoic acid (DHB) in H₂O as a matrix. Melting points were determined

with an Edmund Buhlen 7400 SPA-1 and are uncorrected.

Ethyl 4,6-O-benzylidene-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-1-thio- β -D-galactopyranoside (4). The suspension of **3**¹¹ (2.4 g, 4.8 mmol), 4,6-O-benzylidene derivative **2**¹² (1.65 g, 5.27 mmole), and molecular sieve 4 Å in methylene chloride (30 mL) was cooled to -25 °C. Silver triflate (1.85 g, 7.2 mmole) in toluene (24 mL) and 2,4,6-trimethylpyridine (0.76 mL, 5.75 mmole) were added. After stirring 30 min at -25 °C, methylene chloride was added, and the reaction mixture was filtered through Celite bed. The organic layer was washed with 1 M sodium thiosulfate solution, 1 M sulfuric acid, and sodium hydrogencarbonate solution. The collected organic layer was dried and concentrated. Column chromatography (toluene/EtOAc, 5/3) of the residue gave disaccharide **4** (2.12 g, 60%) having R_f 0.2 (toluene/EtOAc, 5/3). $[\alpha]_D^{20}$ (c 1.3, methylene chloride): ¹H NMR (CDCl₃) for **4** δ 7.83-7.65 (m, 4H, NPhth aromatic H), 7.45-7.25 (m, 5H, aromatic H), 5.82 (dd, 1H, H-3'), 5.76 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1'), 5.50 (s, 1H, PhCH), 5.21 (dd, 1H, H-4'), 3.41 (s, 1H, 2-OH), 2.72-2.52 (m, 2H, SCH₂CH₃), 2.05, 2.00, and 1.85 (3 s, each 3H, CH₃CO₂), 1.18 (t, 3H, SCH₂CH₃); ¹³C NMR (CDCl₃) for **4** δ 170.8, 170.1, 169.5, 167.9 (C=O), 138.0, 134.5, 128.8, 128.1, 126.1, 123.7 (NPhth, CHPh aromatic C), 100.9 (CHPh), 99.3 (C-1'), 85.5 (C-1), 81.3, 75.8, 71.95, 70.8, 70.2, 69.3, 69.2, 67.7, 61.8 (C-6'), 54.8 (C-2'), 23.2 (SCH₂CH₃), 20.8, 20.7, and 20.5 (CH₃CO₂), 15.2 (SCH₂CH₃). FABMS Anal. Calcd. for C₃₅H₃₉NO₁₄S 729.75, found m/z 729.

Ethyl 4,6-O-benzylidene-3-O-(2-deoxy-2-phthalimido- β -D-glucopyranosyl)-1-thio- β -D-galactopyranoside (5). Methanolic sodium methoxide (0.1 M, 8.2 mL) was added to a solution of **4** (2.93 g, 4.01 mmole) in methanol (95 mL). The reaction mixture was stirred for 2 hours at room temperature, then neutralized by an ion-exchange resin (Dowex-50, H⁺ form), and concentrated. Column chromatography (toluene/EtOAc, 1/1) of the residue gave **5** (2.14 g, 88%) having R_f 0.24 (toluene/EtOAc/EtOH, 5/5/2), mp 152-154 °C (ether): ¹H NMR (MeOH-d₄) δ 7.90-7.74 (m, 4H, NPhth aromatic H), 7.58-7.30 (m, 5H, PhCH), 5.58 (s, 1H, PhCH), 5.54 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1'), 4.46-3.30 (m, 12H), 2.74-2.54 (m, 2H, SCH₂CH₃), 1.23 (t, 3H, SCH₂CH₃); ¹³C NMR (MeOH-d₄) δ 168.9 (NPhth C=O), 138.9-123.2 (Aromatic C), 100.9 (PhCH), 100.5 (C-1'), 86.1 (C-1), 81.7, 77.3, 76.7, 71.6, 70.3, 69.3, 68.3, 62.0 (C-6'), 57.6 (C-2'), 23.4 (SCH₂CH₃), 14.6 (SCH₂CH₃). FABMS Anal. Calcd. for C₂₉H₃₃NO₁₁S 603.64, found m/z 604.

Ethyl 2-O-benzyl-4,6-O-benzylidene-3-O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-1-thio- β -D-galactopyranoside (6). A solution of compound **5** (1.36 g, 2.25 mmole) in dry DMF (43 mL) was cooled to 0 °C, and sodium hydride (486 mg, 20.3 mmole, 60% dispersion in oil) was added over 30 min. After stirring 1 hour at room temperature, benzyl bromide (1.18 mL, 9.9 mmole) was added and stirred for 2 hours at room temperature. The mixture was cooled to 0 °C and methanol was added slowly in order to destroy the excess of sodium hydride, and then the mixture was partitioned between toluene and water. The

organic layer was washed with water, dried, and concentrated. Column chromatography (toluene/EtOAc, 10/1) of the residue gave **6** (695 mg, 32%) having R_f 0.4 (toluene/EtOAc, 5/1): ¹H NMR (CDCl₃) δ 7.56-6.81 (m, 29H, aromatic H), 5.49 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1'), 5.43 (s, 1H, PhCH), 5.05-4.13 (m, 13 H), 3.81-3.61 (m, 7H), 3.26 (m, 1H), 2.76-2.52 (m, 2H, SCH₂CH₃), 1.16 (t, 3H, SCH₂CH₃); ¹³C NMR (CDCl₃) δ 167.4 (Nphth C=O), 138.4-123.0 (aromatic C), 100.6 (PhCH), 99.1 (C-1'), 84.1 (C-1), 82.5, 79.7, 79.3, 75.7, 75.4, 74.9, 74.7, 74.6, 74.5, 73.3, 69.6, 69.5, 69.0, 23.5 (SCH₂CH₃), 14.8 (SCH₂CH₃). FABMS Anal. Calcd. for C₅₇H₅₇NO₁₁S 964.02, found m/z 962.

Allyl O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 \rightarrow 3)-O-(2-O-benzyl-4,6-O-benzylidene- α -D-galactopyranosyl)-(1 \rightarrow 2)-7-O-benzyl-3,4-O-isopropylidene-6-deoxy- α -D-*altro*-heptopyranoside (8). A solution of **6** (766 mg, 0.79 mmole) and allyl 7-O-benzyl-3,4-O-isopropylidene-6-deoxy- α -D-*altro*-heptopyranoside¹⁰ (**7**, 220 mg, 0.608 mmole) in methylene chloride-diethyl ether (2 : 5, v/v, 49 mL) was stirred with powdered molecular sieve 5 Å for 30 min at room temperature, and then IDCP (1.1 g, 2.37 mmole) was added. After stirring 2 hours at room temperature, the reaction mixture was filtered and diluted with methylene chloride. The organic layer was washed with 1 M sodium thiosulfate solution and water, dried over magnesium sulfate, and concentrated. Column chromatography (toluene/EtOAc, 7/1) of the residue gave **8** (460 mg, 60%) having R_f 0.32 (toluene/EtOAc, 5/1): ¹H NMR (CDCl₃) δ 7.62-6.84 (m, 34H, aromatic H), 5.9-5.7 (m, 1H, CH=CH₂), 5.45 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1'), 5.43 (s, 1H, PhCH), 5.31 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1'), 5.24-5.1 (m, 2H, CH=CH₂), 4.9-3.54 (m, 3 H), 2.15-1.96, 1.86-1.67 (2m, each 1H, H-6a, 6b), 1.37, 1.23 (=C(CH₃)₂); ¹³C NMR (CDCl₃) δ 167.8 (NPhth C=O), 138.4-123.0 (aromatic C), 134.1, 133.5 (NPhth aromatic C), 131.5 (CH=CH₂), 123.0 (NPhth aromatic C), 116.6 (CH=CH₂), 110.2 (C(CH₃)₂), 99.3 (C-1'), 98.7 (C-1), 97.1 (C-1'), 79.7, 79.3, 77.3, 76.7, 74.9, 74.7, 74.5, 73.8, 73.4, 72.9, 72.3, 69.5, 69.2, 68.3, 68.0, 66.3, 62.3, 56.2 (C-2'), 34.0 (C-6), 27.3, 25.0 (C(CH₃)₂). FABMS Anal. Calcd. for C₇₅H₇₉NO₁₇ 1266.27, found m/z 1267.

Allyl O-(3,4,6-tri-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 \rightarrow 3)-O-(2-O-benzyl- α -D-galactopyranosyl)-(1 \rightarrow 2)-7-O-benzyl-6-deoxy- α -D-*altro*-heptopyranoside (9). A solution of **8** (104 mg, 0.082 mmole) in 70% acetic acid solution (10 mL) was stirred for 7 hours at 50 °C. The reaction mixture was concentrated and coevaporated with toluene. Column chromatography (toluene/EtOAc, 5/3) of the residue gave **9** (63 mg, 69%) having R_f 0.13 (toluene/EtOAc, 1/1): ¹H NMR (CDCl₃) δ 7.62-6.84 (m, 29H, aromatic H), 5.78-5.74 (m, 1H, CH=CH₂), 5.33 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1'), 5.18-5.13 (m, 2H, CH=CH₂), 4.88-3.46 (m, 32H), 2.28-2.19, 1.78-1.71 (2m, each 1H, H-6a, 6b); ¹³C NMR (CDCl₃) δ 168.0 (NPhth C=O), 138.2-123.2 (aromatic C), 133.8, 132.9 (NPhth aromatic C), 131.3 (CH=CH₂), 123.2 (NPhth aromatic C), 118.2 (CH=CH₂), 99.1, 99.0 (C-1, 1'), 97.3 (C-1'), 80.0, 79.5, 79.1, 77.1, 75.1, 74.9, 74.7, 73.9, 73.4, 73.1, 73.0, 69.9, 69.4, 69.0, 68.7, 68.3, 68.2, 66.4,

65.9, 63.1, 55.8 (C-2"), 32.0 (C-6).

Propyl O-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2-O-benzyl-α-D-galactopyranosyl)-(1→2)-7-O-benzyl-6-deoxy-α-D-altro-heptopyranoside (10). Hydrazine monohydrate (57 μL, 1.16 mmole) was added to a solution of **9** (24.5 mg, 0.0215 mole) in ethanol (2 mL). After refluxing 4 hours, the reaction mixture was concentrated and coevaporated with toluene. The residue was dissolved in methylene chloride-methanol (1/1, v/v, 2 mL) and treated with acetic anhydride (0.5 mL) at room temperature. After stirring 24 hours, the reaction mixture was concentrated and coevaporated with toluene. Column chromatography (toluene/EtOAc, 5/1) of the residue gave **10** (14 mg, 71%) having R_f 0.36 (toluene/EtOAc/EtOH, 5/5/1); IR (KBr) 3270 cm^{-1} (acetamide NH), 1650 cm^{-1} (acetamide C=O); ^1H NMR (CDCl_3) δ 4.87 (s, 1H, $J_{1,2} < 1$ Hz, H-1), 4.84-4.79 (m, 3H), 4.71 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1"), 4.65-4.50 (m, 6H), 4.14-4.13 (m, 1H), 3.90-3.56 (m, 16H), 3.32-3.29 (m, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$), 2.29-2.25, 1.86-1.71 (2m, each 1H, H-6, 6'), 1.62 (s, 3H, NHCOCH_3), 1.57-1.52 (m, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$), 0.86 (t, 3H, $\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$). ^{13}C NMR (CDCl_3) δ 170.7 (NHAc C=O), 138.2-127.6 (aromatic C), 101.6 (C-1"), 98.6 (C-1'), 98.2 (C-1), 81.1, 79.3, 78.4, 76.1, 74.9, 74.7, 74.6, 74.56, 73.4, 73.3, 73.0, 70.2, 69.7, 69.69, 69.2, 68.8, 68.4, 66.5 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$), 65.6, 63.2 (C-6'), 55.8 (C-2"), 32.1 (C-6), 23.3 (CO_2NHCH_3), 22.5, and 10.5 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$).

Propyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-α-D-galactopyranosyl-(1→2)-6-deoxy-α-D-altro-heptopyranoside (1). Compound **10** (21.9 mg, 0.019 mmol) in EtOH (1 mL) and acetic acid (0.5 mL) was hydrogenated in the presence of 10% Pd/C for 3 days at room temperature. The reaction mixture was filtered over Celite-bed and concentrated to dryness. Celite column chromatograph (water-gradient EtOH) of the residue gave **1** (8.1 mg, 70%) having R_f 0.35 (BuOH/EtOAc/HAc/H₂O, 37/37/7/21), $[\alpha]_D^{25} +58.6^\circ$ (c 0.6, H₂O); ^1H NMR (D_2O) δ 4.92, 4.73 (2s, 2H, H-1, 1'), 4.51 (dd, 1H), 4.02 (s, 1H), 3.88-3.47 (m, 16H), 3.41-3.27 (m, 4H), 1.95-1.85 (m, 1H, H-6), 1.86 (s, 3H, NHCOCH_3),

1.61-1.52 (m, 1H, H-6'), 1.43 (m, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$), 0.72 (t, 3H, $\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$); ^{13}C NMR (D_2O) δ 175.9 (NHAc C=O), 103.7 (C-1"), 99.97, 99.2 (C-1, C-1'), 79.8, 76.7, 74.7, 72.1, 71.2, 70.8, 70.2, 69.2, 69.1, 69.08, 68.2, 66.5, 62.1, 61.5, 59.2 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$), 56.7 (C-2"), 34.2 (C-6), 23.2 (CO_2NHCH_3), 22.9, and 10.8 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{O}$). MALDI-TOF MS Anal. Calcd. for $\text{C}_{24}\text{H}_{43}\text{NO}_{16}\text{Na}$ ($\text{M}^+ + \text{Na}$) and ($\text{M}^+ + \text{K}$) 624.6 and 640.7, found m/z 624.3 and 640.3.

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