Synthesis of the 2'-Azidoethyl Trisaccharide, 6d-*altro*Hep*p*-GlcNAc-Gal Hapten, an *O*-Antigenic Repeating Unit of *Campylobacter jejuni* Serotypes O:23 and O:36

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A trisaccharide, 6d-*Altro*-Hep*p*α(1→3) GlcNAcβ(1→3) Galα(1→*O*$CH_2CH_2N_3$, as an *O*-antigenic repeating unit of *Campylobacter jejuni* serotypes O:23 and O:36, was synthesized. Coupling of the 6d-*altro*-Hep*p*α (1→3) GlcNAcβ(1→SEt donor with Galα(1→*O*CH_2CH_2Cl acceptor in the presence of NIS-TfOH promoter afforded the trisaccharide having the β(1→3) Gal linkage. β-Stereospecificity and the desired regioselectivity for the 3-OH Gal are obtained. Subsequent hydrogenation, acetylation, azide displacement, hydrazinolysis, *N*-acetylation, and finally deacetylation furnished the title trisaccharide hapten for further glycoconjugation.

Key Words : *Campylobacter jejuni*, Trisaccharide, 6d-*altro*Hep*p*-GlcNAc-Gal hapten, *O*-Antigenic repeating unit

Introduction

Campylobacter jejuni, a species of curved, motile and Gram-negative bacilli, is recognized as one of the commonest causes of infective diarrhea and acute bacterial enteritis. Campylobacter infection has been reported to be associated with neuropathy known as Guillain-Berre syndrome (GBS) and Miller-Fisher syndrome (MFS).[1,2]

Investigations on their chemical structures by Aspinall *et al.*,[3-6] showed that *C. jejuni* serotypes O:23 and O:36 possess high-molecular weight LPS *O*-glycans which are cross-reacting. The *O*-glycans, a potential basis of the serological classification of *C. jejuni* are consisted of trisaccharide repeating units having unusual *altro*heptose residues which vary in the presence or absence of oxygenation at C-6 and methylation at *O*-3 from batch to batch. The structural difference in the heptose components may constitute a basis for serotypic discrimination, or evading the immune response of the host. In order to elucidate the role of *altro*heptose residues and evaluate the different reading frames in the immunological specificity, it was necessary to synthesize various oligosaccharides containing the repeating unit of the *C. jejuni* serotypes O:23 and O:36.[6-9] Three trisaccharides of different reading frames with the repeating sequence have been synthesized.[10,11] Herein, we wish to describe a synthesis of the trisaccharide, 6d-*Altro*-Hep*p*α (1 → 3) GlcNAcβ (1 → 3) Galα, in the form of its 2'-azidoethyl glycosides (Figure 1). The azide group was introduced to the trisaccharide as a linker to a peptide, which would enable to evaluate the trisaccharide as an immunogen.

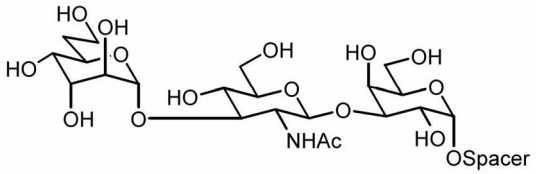

Figure 1. *O*-Antigenic Polysaccharide of *Campylobacter jejuni* O:23 and O:36.

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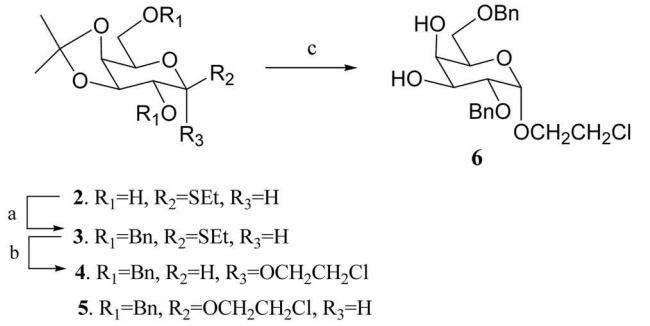
Results and Discussion

For the synthesis of the desired trisaccharide **1**, the two building blocks, 6d-*altro*-Hep*p*α(1 → 3) GlcNPhthβ(1 → SEt donor **7** and Galα (1 → *O*CH_2CH_2Cl acceptor **6**, were first synthesized, as shown in Schemes 1 and 2.

The 6d-*altro*-Hep*p* α(1 → 3) GlcNPhth **7** has been prepared from 6d-*manno*-Hep*p* α(1 → 3) GlcNPhth by *Swern* oxidation[12] and subsequent reduction as described previously.[10] For the synthesis of the Gal acceptor **6**, ethylthio 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside **3** was prepared by acetonation of ethylthio β-D-galactopyranoside with dimethoxypropane and 4-toluenesulfonic acid in THF (73% yield), and the subsequent benzylation with benzyl bromide in DMF (a quantitative yield). The fully protected ethylthio β-D-galactopyranoside **3** was coupled to 2-chloroethanol in the presence of IDCP[10-13] giving the α- and β-2'-chloroethyl galactoside **4** and **5** in ratio of 5.1/1 in 65% yield. The use of NIS-TfOH[14,15] promoter decreased the stereoselectivity of α/β to 1.7/1, while increasing the coupling yield to 94%. The 3,4-*O*-isopropylidene group in **4** was deacetonated with a catalytic amount of *p*-toluenesulfonic acid and gave the 2'-chloroethyl galactoside donor **6** which has free 3- and 4-OH groups (Scheme 1).

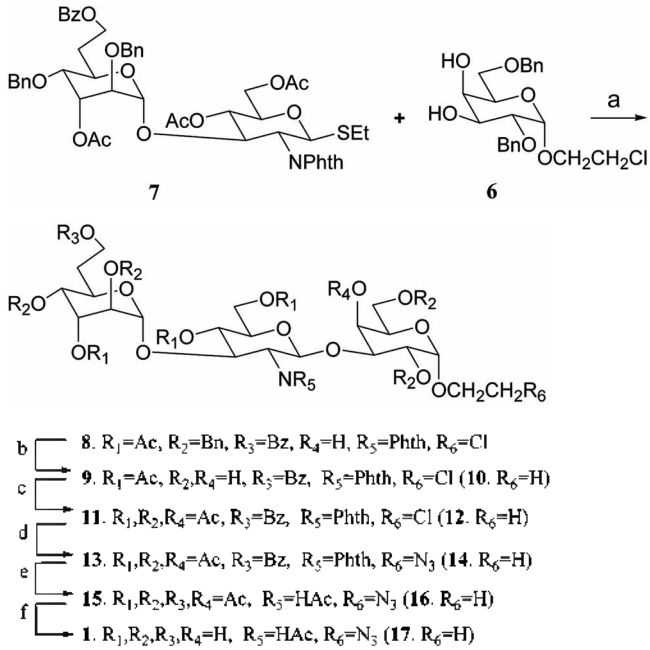
The coupling of the *altro*Hep-GlcNPhth disaccharide donor **7** and Gal acceptor **6** in the presence of NIS-TfOH[14,15] afforded the trisaccharide, 2'-chloroethyl *O*-(3-*O*-acetyl-7-*O*-benzoyl-2,4-di-*O*-benzyl-6-deoxy-α-D-*altro*heptopyranosyl)-(1 → 3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-α-D-glucopyranosyl)-(1 → 3)-2,6-di-*O*-benzyl-β-D-galactopyranoside **8** in 64% yield. The trisaccharide **8** showed the desired β-stereospecificity and the regioselectivity for the 3-OH group of the Gal acceptor. After coupling, the downfield shift of



Scheme 1. Synthesis of the acceptor **6**: (a) 1) NaH, DMF, 0 °C, 40 min, 2) BnBr, rt, 1h, quntitative; (b) 2-chloroethanol, IDCP, CH_2Cl_2-Et_2O (2/5, v/v), MS5Å, rt, 30 min, 54%; (c) *p*-TsOH, MeOH, 65 °C, 40 min, 91%.

the C-3 value of the galactosyl residue from 69.1 to 79.8 ppm has been observed, while the C-4 value remained at 68.9 ppm in the ^{13}C-NMR spectrum. This confirms the formation of the 1 → 3 linked *β*-galactosyl trisaccharide (Scheme 2). The formation of the 1 → 4 linked *β*-galactosyl trisaccharide was also observed in its ^{13}C-NMR spectrum. The 1 → 4 linked trisaccharide has not been identified further. Such a regioselectivity was not achieved in the previous synthesis. *i.e.*, in the coupling of an *α*-Gal acceptor having free 2-OH and 3-OH groups. where 1 → 2 and 1 → 3 linked trisaccharides were obtained in a 1:1 ratio.[15,17]

The trisaccharide 6d-*altro*Hep-GlcNPhth-Gal-*O*CH_2CH_2Cl **8** was hydrogenated with H_2, Pd/C and then acetylated to give **11**. During the hydrogenation dechlorination accompanied and gave ethyl glycoside **12** as a minor product. The trisaccharide **11** and its side product **12** were not separated, but



Scheme 2. (a) Nis-TfOH, CH_2Cl_2, MS4Å, 0 °C, 5 min, 64%; (b) H_2, 10% Pd/C, EtOH/EtOAc (2/1, v/v), rt, 60 h; (c) Ac_2O, Py, rt, 16 h; (d) NaN_3, DMF, 110 °C, rt 4 h; (e) 1) N_2H_4·H_2O, EtOH, 70 °C, 1h, 2) Ac_2O, Py, rt, 16 h, 64% (from **8**, 5 steps); (f) NaOMe, MeOH, rt, 3 h, 35% (from **8**, 6 steps).

the carbon signals at 42.6 and 14.0 ppm show the presence of the chloroethyl glycoside **11** and ethyl glycoside **12** in a 5:2 ratio. This was also confirmed by the mass spectrum of the trisaccharides. The MALDI-TOF MS peaks of the produced trisaccharides showed at m/z values of 1171.91 $(M+Na)^+$ for the chloroethyl glycoside **11** and at 1138 $(M_1+Na)^+$ for the ethyl glycoside **12**. The sequential reactions of an azide displacement with NaN_3 in DMF. a hydrazinolysis[18,19] with N_2H_4·H_2O in EtOH and an acetylation transformed the mixture of **11** and **12** into the corresponding peracetylated mixture, 2'-azidoethyl *O*-(2,3,4,7-tetra-*O*-acetyl-6-deoxy-*α*-D-*altro*heptopyranosyl)-(1 → 3)-(4,6-di-*O*-acetyl-2-deoxy-2-*N*-acetyl-*β*-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-acetyl-*α*-D-galactopyranoside **15** and 2'-ethyl glycoside **16**. The Zemplin de-*O*-acetylation[20] of the mixture **15** and **16** with NaOMe in MeOH gave the desired final trisaccharide. 2'-azidoethyl *O*-(6-deoxy-*α*-D-*altro*heptopyranosyl)-(1 → 3)-(2-deoxy-2-*N*-acetyl-*β*-D-glucopyranosyl)-(1 → 3)-*α*-D-galactopyranoside **1** and 2'-ethyl glycoside **17**, as a white solid which was then purified using a Bio-gel P2 column. The overall yield after the six deprotection steps is in a 35%. The MALDI-TOF MS spectrum of compound **1** (MW 628.6) showed a peak at 651.422 $(M+Na)^+$. The presence of the side product **17**, 6d-*altro*Hep-GlcNAc-Gal-*O*CH_2CH_3 (MW 587.57), was also showed as an additional peak at m/z value of 610.466 $(M+Na)^+$. It is surprising that dechlorination took place during the hydrogenolysis of the trisaccharide **8**, since it was not observed in the previous synthesis, *i.e.*, the hydrogenolysis of another trisaccharide analogue, Gal-6d-*altro*Hep-GlcNPhth-*O*CH_2CH_2Cl.[10] Even the debenzylation was found to be much slower for **8** (6d-*altro*Hep-GlcNPhth-Gal trisaccharide, 60 h) than for the analogue (Gal-6d-*altro*Hep-GlcNPhth trisaccharide, 26 h). The longer reaction time for debenzylation and the concomitant dechlorination seems to ascribe to the steric environmental differences between the two trisaccharides. The 2'-azidoethyl trisaccharide can be linked to a peptide (or a protein) for glycoconjugation.

Experimental

General. ^{1}H and ^{13}C spectra were recorded on JEOL JNM-LA 400 (400 MHz) using $CDCl_3$ or D_2O and chemical shifts are reported in parts per million (*δ*) downfield from tetramethylsilane as internal standard. Assignments were based on DEPT, COSY, and HMQC. Matrix associated laser desorption time-of-flight (MALDI-TOF) spectra were recorded on Voyger Biospectrometry workstation with a 337 nm nitrogen laser and a 1.2 m linear mass analyzer (PerSeptive Biosystems, Framingham, MA). TLC was performed on Merck pre-coated $60F_{254}$ plates. Column chromatography was performed on silica gel (Merck, Art 9385 230-400 mesh in the flash mode). All the anhydrous solvents were distilled over CaH_2 or P_2O_5 or Na/benzophenone prior to the reaction.

Ethylthio 3,4-*O*-isopropylidene-*β*-D-galactopyranoside (2). To a solution of ethylthio *β*-D-galactopyranoside (0.6902 g, 2.972 mmol) dissolved in THF (15 mL), *p*-toluenesulfonic

acid (0.0896 g, 0.5201 mmol) and 2,2-dimethoxypropane (0.73 mL, 5.944 mmol) were added and stirred for 2.5 hr at room temperature. Triethylamine (0.15 mL) was added to the reaction mixture and evaporated to a syrup, which was chromatographed on silica gel (toluene-EtOAc, 5:3, followed by 1:1 and toluene-EtOAc-EtOH, 5:5:2) to give **2** (0.5722 g, 73%) having R_f 0.56 (toluene-EtOAc-EtOH, 5:5:2). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.30 (d, $J_{1,2}$ = 10 Hz, 1H, H-1), 4.21 (dd, $J_{4,5}$ = 1.96 Hz, 1H, H-4), 4.10 (t, $J_{3,4}$ = 5.60 Hz, 1H, H-3), 3.97-3.79 (m, 3H, H-6a, H-5, & H-6b), 3.56 (d, $J_{2,3}$ = 7.32 Hz, 1H, H-2), 3.32 (s, 1H, OH), 2.94 (s, 1H, OH), 2.80-2.71 (m, 2H, SCH_2), 1.52 (s, 3H, $SCH_2C\underline{H}_3$), 1.35-1.30 (m, 6H, $C(C\underline{H}_3)_2$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 110.0 ($\underline{C}(CH_3)_2$), 85.0 (C-1), 79.0 (C-3), 76.8 (C-5), 73.6 (C-4), 71.8 (C-2), 62.0 (C-6), 27.9 & 26.1 ($C(\underline{C}H_3)_2$), 24.1 ($S\underline{C}H_2$), 15.0 ($SCH_2\underline{C}H_3$).

Ethylthio 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (3). Compound **2** (0.728 g, 0.0275 mmol) in DMF (21 mL) was cooled at 0 °C and NaH (60% in mineral oil, 0.7933 g) was added and stirred for 40 min. Then benzyl bromide (1.64 mL, 0.1377 mmol) was added dropwise at 0 °C and stirred for 1 hr at room temperature and then concentrated. The residual syrup was diluted with CH_2Cl_2, washed with water, dried and concentrated to a syrup, which was chromatographyed on silica gel (toluene-EtOAc, 50:1) to give **3** (1.224 g, quantitative) having R_f 0.38 (toluene-EtOAc, 15:1). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.41-7.21 (m, 10H, aromatic H), 4.84-4.47 (m, 4H, $C_6H_5C\underline{H}_2$), 4.40 (d, $J_{1,2}$ = 11Hz, 1H, H-1), 4.16-4.12 (m, 2H, H-4 & H-3), 3.86-3.83 (m, 1H, H-5), 3.74-3.73 (m, 2H, H-6), 3.46-3.42 (dd, $J_{2,3}$ = 6.36 Hz, 1H, H-2), 2.64 (m, 2H, $SC\underline{H}_2$), 1.40 (s, 3H, $SCH_2C\underline{H}_3$), 1.31-1.26 (m, 6H, $C(C\underline{H}_3)_2$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 138.1-127.3 (aromatic C), 109.6 ($\underline{C}(CH_3)_2$), 83.5 (C-1), 79.4 (C-3), 78.9 (C-2), 75.4 (C-5), 73.7 (C-4), 71.8 ($C_6H_5\underline{C}H_2$*2), 69.4 (C-6), 27.7 & 26.1($C(\underline{C}H_3)_2$), 24.4 ($S\underline{C}H_2$), 14.8 ($SCH_2\underline{C}H_3$).

2'-Chloroethyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (4) & 2'-chloroethyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (5). i) Employing IDCP promoter. A solution of **3** (0.3499 g, 0.787 mmol) and 2-chloroethanol (53 μL, 0.7873 mmol) in CH_2Cl_2-Et_2O (2:5, v/v, 28 mL) was stirred with freshly powdered MS 5 Å (2 g) for 30 min at room temperature and then iodonium dicollidine perchlorate (IDCP; 1.105 g, 2.361 mmol) was added. After stirring 30 min at room temperature, the precipitate was filtered off through celite-bed, and washed with CH_2Cl_2. The combined filtrate was washed with 1 M $Na_2S_2O_3$ and water, dried and then concentrated. Column chromatography (toluene-EtOAc, 15:1) of the residue gave α, **4** (0.1976 g, 54%) and β, **5** (0.0387 g, 11%) having R_f 0.59 for **4** and 0.52 for **5** (toluene-EtOAc, 5:1).

ii) Employing NIS-TfOH promoter. A solution of **3** (7.52 g, 0.0163 mol) and 2-chloroethanol (1.65 mL, 0.0245 mol) in CH_2Cl_2 (72 mL) was stirred with freshly powdered MS 4 (2 g) for 30 min at room temperature and then cooled to 0 °C. To the cooled mixture was added, with stirring, *N*-iodosuccinimide (NIS; 9.1731 g, 0.0408 mol) and trifluoromethane-sulfonic acid (TfOH; 453 μL, 0.0051 mmol)/CH_2Cl_2 (5 mL). The reaction mixture was stirred for 10 min at 0 °C. The precipitate was filtered through celite-bed, and washed with CH_2Cl_2. The combined filtrate was washed with 1 M $Na_2S_2O_3$, water, $NaHCO_3$, and water, dried and then concentrated, which was separated on silica gel (toluene-EtOAc, 15:1) to give α, **4** (4.486 g, 59%) and β, **5** (2.597 g, 34%). ^{1}H-NMR ($CDCl_3$, 400 MHz) for **4** δ 7.38-7.24 (m, 10H, aromatic H), 4.83-4.52 (m, 4H, $C_6H_5C\underline{H}_2$*2), 4.82 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 4.35 (dd, $J_{3,4}$ = 5.6 Hz, 1H, H-3), 4.32-4.28 (m, 1H, H-5), 4.20 (dd, $J_{4,5}$ = 2.44 Hz, 1H, H-4), 3.89-3.66 (m, 6H, $OC\underline{H}_2CH_2Cl$, $C\underline{H}_2Cl$, H-6), 3.53 (dd, $J_{2,3}$ = 7.58 Hz, 1H, H-2), 1.39 & 1.33 (each s, 6H, $C(C\underline{H}_3)_2$); ^{13}C-NMR ($CDCl_3$, 100 MHz) for **4** δ 138.2-127.5 (aromatic C), 109.2 ($\underline{C}(CH_3)_2$), 97.4 (C-1), 76.3 (C-2), 75.6 (C-3), 73.6 (C-4), 73.3 & 72.5 ($C_6H_5\underline{C}H_2$*2), 69.4 (C-6), 68.4 ($O\underline{C}H_2CH_2Cl$), 66.9 (C-5), 42.6 ($\underline{C}H_2Cl$), 28.0 & 26.3 ($C(\underline{C}H_3)_2$); ^{1}H-NMR ($CDCl_3$, 400 MHz) for **5** δ 7.41-7.25 (m, 10H, aromatic H), 4.88-4.53 (m, 4H, $C_6H_5C\underline{H}_2$*2), 4.36 (d, $J_{1,2}$ = 8.04 Hz, 1H, H-1), 4.13 (m, 3H), 3.91 (t, 1H), 3.83-3.67 (m, 5H, $OC\underline{H}_2CH_2Cl$, $C\underline{H}_2Cl$), 3.40 (dd, $J_{2,3}$ = 6.36 Hz, 1H, H-2), 1.35 & 1.32 (each s, 6H, $C(C\underline{H}_3)_2$); ^{13}C-NMR ($CDCl_3$, 100 MHz) for **5** δ 138.2-127.5 (aromatic C), 109.9 ($\underline{C}(CH_3)_2$), 103.1 (C-1), 79.2, 78.8, 73.7, 73.6 & 73.5 ($C_6H_5\underline{C}H_2$*2), 72.2, 69.6 ($O\underline{C}H_2CH_2Cl$), 69.5 (C-6), 42.6 ($\underline{C}H_2Cl$), 27.7 & 26.3 ($C(\underline{C}H_3)_2$).

2'-Chloroethyl 2,6-di-*O*-benzyl-α-D-galactopyranoside (6). To a solution of **4** (2.414 g, 5.214 mmol) in MeOH (100 mL) was added *p*-toluenesulfonic acid (0.1984 g, 1.043 mmol) with stirring for 40 min at 65 °C. Triethylamine (0.3 ml) was added to the reaction mixture and evaporated to dryness. Column chromatography (toluene-EtOAc, 5:3) of the residue gave **6** (2.035 g, 91%) having R_f 0.30 (toluene-EtOAc, 5:3). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.38-7.26 (m, 10H, aromatic H), 4.85 (d, $J_{1,2}$ = 3.68 Hz, 1H, H-1), 4.71-4.56 (m, 4H, $C_6H_5C\underline{H}_2$*2), 4.04-3.97 (m, 3H, H-4, H-5, & H-3), 3.85-3.63 (m, 7H, $OC\underline{H}_2CH_2Cl$, $C\underline{H}_2Cl$, H-6, & H-2), 3.00 (d, J = 1.24 Hz, 1H, OH), 2.77 (d, J = 3.64 Hz, 1H, OH); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 138.0-127.6 (aromatic C), 97.2 (C-1), 76.6 (C-2), 73.5 & 72.8 ($C_6H_5\underline{C}H_2$*2), 69.8 (C-6), 69.7 (C-4), 69.1 (C-3), 68.8 (C-5), 68.3 ($O\underline{C}H_2CH_2Cl$), 42.8 ($\underline{C}H_2Cl$).

2'-Chloroethyl *O*-(3-*O*-acetyl-7-*O*-benzoyl-2,4-di-*O*-benzyl-6-deoxy-α-D-*altro*heptopyranosyl)-(1$\rightarrow$3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1$\rightarrow$3)-2,6-di-*O*-benzyl-α-D-galactopyranoside (8). A solution of ethyl *O*-(3-*O*-acetyl-7-*O*-benzoyl-2,4-di-*O*-benzyl-6-deoxy-α-D-*altro*heptopyranosyl)-(13)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **7**[10] (0.22 g, 0.234 mmol) and **6** (0.2017 g, 0.468 mmol) in CH_2Cl_2 (10 mL) was stirred with freshly powdered MS 4 (1 g) for 30 min at room temperature and then cooled to 0 °C. To the cooled mixture were added, with stirring, *N*-iodosuccinimide (NIS; 0.1316 g, 0.585 mmol) and trifluoromethanesulfonic acid (TfOH; 6.6 μL, 0.0748 mmol)/CH_2Cl_2 (0.6 mL). The reaction mixture was stirred for 5 min at 0 °C. The precipitate was filtered through celite-bed, and washed with CH_2Cl_2. The

combined filtrate was washed with 1 M $Na_2S_2O_3$, water, $NaHCO_3$, and water, dried and then concentrated, which was chromato-graphed on silica gel (toluene-EtOAc, 4:1) to give **8** (0.196 g, 64%) having R_f 0.55 (toluene-EtOAc, 5:3, v/v). ^{1}H-NMR ($CDCl_3$, 400 MHz) for **7** δ 7.95-6.97 (m, 19H, aromatic H), 5.29 (d, $J_{1,2}$ = 10.48 Hz, 1H, H-1), 5.05 (t, $J_{4,5}$ = 9.64 Hz, 1H, H-4), 4.95 (dd, $J_{3',4'}$ = 2.72 Hz, 1H, H-3'), 4.58-3.96 (m, 9H, H-1', $C_6H_5C\underline{H}_2$*2, H-7' & H-6), 4.49 (t, $J_{3,4}$ = 9.64 Hz, 1H, H-3), 4.28 (t, $J_{2,3}$ = 10.28 Hz, 1H, H-2), 3.67-3.62 (m, 1H, H-5), 3.48 (dd, $J_{2',3'}$ = 6.7 Hz, 1H, H-2'), 3.38-3.33 (m, 2H, H-5' & H-4'), 2.66-2.54 (m, 2H, SCH_2), 2.01, 1.86 & 1.76 (each s, 9H, CH_3*3), 1.76 (m, 1H, H-6a'), 1.51-1.46 (m, 1H, H-6b'), 1.13 (t, 3H, $SCH_2C\underline{H}_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz) for **7** δ 170.62, 170.59 & 169.4 (C=O, Ac), 168.4 & 167.2 (C=O, NPhth), 166.1 (C=O, Bz), 137.9-122.9 (aromatic C), 100.5 (C-1'), 81.3 (C-1), 77.5 (C-3), 76.4 (C-2'), 76.0 (C-5), 73.5 (C-4'), 73.7 & 70.5 ($C_6H_5\underline{C}H_2$*2), 70.9 (C-4), 68.2 (C-3' & C-5'), 62.5 (C-6), 61.2 (C-7'), 54.5 (C-2), 28.8 (C-6'), 24.1 (SCH_2), 20.7 & 20.4 (CH_3*3), 14.8 ($SCH_2\underline{C}H_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) for **8** δ 7.94-6.95 (m, 29H, aromatic H), 5.38 (d, $J_{1',2'}$ = 8.52 Hz, 1H, H-1'), 5.01 (t, $J_{4',5'}$ = 9.52 Hz, 1H, H-4'), 4.96 (d, $J_{3'',4''}$ = 5.36 Hz, 1H, H-3''), 4.57-3.93 (m, 20H, H-1'', H-3', $C_6H_5C\underline{H}_2$*4, H-1, H-2', H-4, $OC\underline{H}_2CH_2Cl$, H-5, H-7'' & H-6'), 3.88 (dd, $J_{3,4}$ = 3.16 Hz, 1H, H-3), 3.68-3.46 (m, 7H, H-5', H-2, H-6, CH_2Cl & H-2''), 3.38 (s, 2H, H-5'' & H-4''), 2.62 (s, 1H, 4-OH), 1.92, 1.88 & 1.77 (each s, 9H, CH_3*3), 1.77 (m, 1H, H-6a''), 1.46 (m, 1H, H-6b''); ^{13}C-NMR ($CDCl_3$, 100 MHz) for **8** δ 170.7, 170.6 & 169.5 (C=O, Ac), 168.5 & 167.5 (C=O, NPhth), 166.2 (C=O, Bz), 138.3-123.2 (aromatic C), 100.6 (C-1''), 98.9 (C-1'), 98.0 (C-1), 79.8 (C-3), 76.7 (C-3'), 76.3 (C-2''), 74.4 (C-2), 73.4 (C-4''), 73.6, 73.4, 73.3 & 70.5 ($C_6H_5\underline{C}H_2$*4), 71.9 (C-5'), 70.9 (C-4'), 69.4 (C-6), 68.9 (C-4), 68.4 (C-5), 68.3 ($\underline{C}H_2CH_2Cl$), 68.0 (C-3'' & C-5''), 62.3 (C-6'), 61.1 (C-7''), 55.7 (C-2'), 42.5 (CH_2Cl), 28.9 (C-6''), 20.8, 20.6 & 20.5 (CH_3*3).

2'-Azidoethyl *O*-(2,3,4,7-tetra-*O*-acetyl-6-deoxy-α-D-*altro*-heptopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranoside (15) & 2'-ethyl *O*-(2,3,4,7-tetra-*O*-acetyl-6-deoxy-α-D-*altro*heptopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranoside (16). Compound **8** (0.0846 g, 0.0646 mmol) in EtOAc-EtOH (1:2, 12 mL) was hydrogenated in the presence of 10% Pd/C for 60 hr at room temperature. The reaction mixture was filtered over celite-bed and concentrated to dryness. The residue was dissolved in pyridine (5 mL) and treated with acetic anhydride (5 mL). After stirring for 16 hr at room temperature, the mixture was concentrated. The syrupy mixture (**11** & **12**) was dissolved in DMF (5 mL) stirred with sodium azide (0.0063 g, 0.0967 mmol) at 110 °C for 4 hr, the mixture was cooled, evaporated and then diluted in CH_2Cl_2. The organic layer was washed with water, dried, and concentrated. Without further purification, the residue was dissolved in EtOH (5 mL) and treated with hydrazine monohydrate (98%, 3 mL). After stirring for 1 hr at 72 °C, the mixture was coevaporated with toluene. Acetic anhydride (7 mL) was added and then dried after stirring for 16 hr at room temperature. Column chromatography (toluene-EtOAc-EtOH, 5:5:1) of the residue gave mixture **15** and **16** (0.0414 g, 64% from **8**, 5 steps) having R_f 0.48 (toluene-EtOAc-EtOH, 5:5:2, v/v/v). ^{1}H-NMR ($CDCl_3$, 400 MHz) for the mixture of **11** and **12** δ 7.94-7.11 (m, 9H, aromatic H), 5.36 (dd, $J_{4,5}$ = 7.66 Hz, 1H, H-4), 5.23 (d, $J_{1',2'}$ = 8.52 Hz, 1H, H-1'), 5.05 (t, $J_{4',5'}$ = 8.78 Hz, 1H, H-4'), 4.96 (m, 1H, H-3''), 4.87 (d, $J_{1,2}$ = 3.65 Hz, 1H, H-1), 4.80 (dd, $J_{4'',5''}$ = 7.2 Hz, $J_{3'',4''}$ = 3.44 Hz, 1H, H-4''), 4.73-4.69 (m, 2H, H-2'' & H-2), 4.54 (m, 1H, H-1''), 4.49 (t, $J_{3',4'}$ = 9.76 Hz, 1H, H-3), 4.20-3.54 (m, 15H, H-6', H-2', H-3, H-5, H-6, H-7'', H-5', H-5'', OCH_2 & CH_2Cl), 2.06, 2.05, 2.03, 2.02, 2.00, 1.99, 1.98 & 1.97 (each s, 24H, CH_3*8), 1.57 (m, 1H, H-6a''), 1.43 (m, 1H, H-6b''), 1.11 (t, 1H, $CH_2C\underline{H}_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz) for the mixture of **11** and **12** δ 170.8, 170.5, 170.46, 170.3, 169.9, 169.6, 169.4, & 169.2 (C=O*8, Ac), 168.6 & 167.0 (C=O, NPhth), 166.1 (C=O, Bz), 134.0-122.8 (aromatic C), 99.2 & 99.1 (C-1''), 98.1 & 98.0 (C-1'), 96.1 & 95.5 (C-1), 78.1 (C-3'), 71.9 (C-5'), 71.6 (C-3), 70.4 (C-4'), 70.2 (C-2), 69.8 (C-4), 69.1 (C-2''), 68.5 (OCH_2), 68.0 (C-4''), 67.2 (C-3''), 67.0 (C-5''), 66.7 (C-5), 62.5 (C-6), 61.7 (C-6'), 60.3 (C-7''), 55.8 (C-2'), 42.6 (CH_2Cl), 29.6 (C-6''), 20.74, 20.7, 20.64, 20.61, 20.55, 20.51, 20.5, 20.3 (CH_3, Ac), 14.0 ($CH_2\underline{C}H_3$); MALDI-TOF MS 1171.91 & 1138 [(M+Na) & (M_1+Na)]; ^{13}C-NMR ($CDCl_3$, 100 MHz) for the mixture of **15** and **16** δ 172.3, 171.8, 171.7, 171.4, 171.2, 171.1, 170.8, 170.5, 170.3 & 170.0 (C=O*10, Ac & NHAc), 100.2 & 100.0 (C-1'), 99.39 & 99.35 (C-1''), 96.6 & 96.1 (C-1), 79.0, 72.7, 71.8, 71.0, 70.8, 70.0, 69.6, 68.6, 67.6, 67.57 (OCH_2), 67.1, 66.3, 63.0, 62.3, 60.5, 57.5 (C-2'), 50.7 (CH_2N_3), 32.3 (C-6''), 26.5 (CH_3, NHAc), 21.1, 20.92, 20.9, 20.86, 20.84, 20.8, 20.75, 20.7 & 20.6 (CH_3*9, Ac), 15.0 ($CH_2\underline{C}H_3$); IR 2110.26 cm^{-1} (N_3), 1739.90 (C=O); MALDI-TOF MS 1029.25 & 988.242 [(M+Na) & (M_1+Na)].

2'-Azidoethyl *O*-(6-deoxy-α-D-*altro*heptopyranosyl)-(13)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→3)-α-D-galactopyranoside (1) & 2'-ethyl *O*-(6-deoxy-α-D-*altro*heptopyranosyl)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→3)-α-D-galactopyranoside (17). A mixture of compound **15** and **16** (0.0192 g, 0.019 mmol) was treated with 25% NaOMe (0.01 mL) in MeOH (1 mL) for 3 hr at room temperature. The reaction mixture was neutralized with Dowex 50 (H^+) resin, filtered, and concentrated to a solid (**1** & **17**). The crude product was treated to Bio-gel P2 column to give a mixture **1** and **17** (0.0042 g, 35% from **8**, 6 steps) having R_f 0.07 (*t*BuOH-EtOAc-AcOH-H_2O, 36:36:7:21). ^{13}C-NMR (D_2O, 100 MHz) for **1** and **17** δ 177.8 (C=O), 105.2 (C-1''), 102.9 (C-1'), 101.4 (C-1), 82.5, 82.0, 78.3, 73.6, 73.24, 73.2, 73.0, 72.8, 71.9, 71.4, 70.0, 69.4 (OCH_2), 64.0, 63.1 & 60.8 (C-6, C-6' & C-7''), 57.1 (C-2'), 53.3 (CH_2N_3), 35.4 (C-6''), 25.3 (CH_3, NHAc), 17.0 ($OCH_2\underline{C}H_3$); MALDI-TOF MS 651.422 & 610.466 [100, (M+Na) & 40, (M_1+Na)].

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