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Synthesis of 2'-Azidoethyl Trisaccharide, α-D-Gal-(1→2)-6d-α-D-*Altro*-Hepp-(1→3)-β-D-GlcNAc, an *O*-Antigenic Repeating Unit of *C. jejuni* O:23 and O:36

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A trisaccharide, the *O*-antigenic repeating unit of *C. jejuni* serotype O:23 and O:36, was synthesized as a 2'-azidoethyl glycoside by block addition of perbenzylated thiogalactoside donors to α-*altro*Hepp-(1→3)-GlcNPhth disaccharide acceptor in presence of IDCP promoter. The α-linked *altro*heptopyranoside moiety in the glycosyl acceptor was effectively prepared by *Swern* oxidation of α-*manno*hepp-(1→3)-GlcNPhth disaccharide followed by mild reduction with $NaCNBH_3$.

Key words: *Campylobacter jejuni*, *O*-Antigenic repeating unit, α-*Altro*Hepp-(1→3)-GlcNPhth disaccharide acceptor, 2'-Azidoethyl trisaccharide

INTRODUCTION

The *Campylobacter jejuni* (*C. jejuni*) is a leading cause of acute gastroenteritis in humans and is increasingly recognized for its association with neurological complications of the Miller-Fisher (MFS) and Guillain-Barre syndromes (GBS) (Moran *et al.*, 1996; Nam Shin *et al.*, 1998; Saleha *et al.*, 1998; Hanniffy *et al.*, 1999; Moran and Penner, 1999). According to the genetic and biochemical studies of a *C. jejuni* lipopolysaccharide (LPS) biosynthesis, the differences in chemical structure of high-molecular-weight (HMW) LPS may be the importance factor in the development of GBS (Karlyshev *et al.*, 2000).

The chemical structure of LPS from several *C. jejuni* serotypes was elucidated by Aspinall *et al.* (Aspinall *et al.*, 1992, 1992, 1993, 1993). Particularly, *C. jejuni* serotypes O:23 and O:36 were known to contain HMW LPS, which include trisaccharide repeating units with three unusual altroheptose variants. It was suggested that these heptose components must be related to serotypic discrimination, evading the immune response of the host, and permitting the infection to continue. In order to evaluate the immunological specificity and elucidate the role of *altro*-heptopyranosyl residues in serotypic differences, it is necessary to synthesis various oligosaccharides containing the repeating unit of *C. jejuni* serotypes O:23 and O:36. This report describes the formation of α-(1→3)-altroheptosyl linkage and the synthesis of 2'-azidoethyl glycoside of trisaccharide **1**, α-D-Gal-(1→2)-6-deoxy-α-D-*altro*Hepp-(1→3)-β-D-Glc*p*NAc, as one of the repeating units of *C. jejuni* serotype O:23 and O:36.

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MATERIALS AND METHODS

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 silical 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). ^1H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-LA 400 spectrometer on solutions in $CDCl_3$ with tetramethylsilane as the internal standard. Assignments were based on DEPT, COSY, and HMQC. 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 H_2O as a matrix.

Ethyl 1-thio-α-D-mannopyranoside (2)

Peracetylated mannose was obtained by acetic anhydride in pyridine at -15°C for 2 days from D-mannose (15.0 g, 83.3 mmol). To a solution of the crude product (28.9 g, 74.0 mmol, 89%, α:β=1.5/1) and EtSH (8.3 mL, 0.111 mol) in CH_2Cl_2 (100 mL) was added $BF_3{\cdot}Et_2O$ (3.74 mL, 29.6 mmol). After stirring 23 hours at room temperature, the solution was diluted with CH_2Cl_2, and washed with H_2O, aq $NaHCO_3$, and H_2O in sequence. The organic layer was dried and concentrated. Without further purification the residual syrup was treated with 25% NaOMe (2 mL) in MeOH (250 mL) for 1 h at room temperature. The reaction mixture was neutralized with Dowex 50 (H^+ form), filtered, and concentrated. Column chromatography (toluene-EtOAc-EtOH, 5:5:2) of the residual syrup gave **2** (12.58 g, 65%) having R_f 0.13 (toluene-EtOAc-EtOH, 5:5:2). ^{1}H-NMR ($CDCl_3$) δ 5.24 (s, 1 H, H-1), 3.90-3.63 (m, 6 H), 2.65 (m, 2 H, SCH_2CH_3), 1.28 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 86.0 (C-1), 74.8 (C-5), 73.7 (C-3), 73.2 (C-2), 68.9 (C-4), 62.8 (C-6), 25.7 (SCH_2CH_3), 15.2 (SCH_2CH_3).

Ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (3)

To a solution of **2** (12.58 g, 54.2 mmol) dissolved in DMF (84 mL), *p*-toluenesulfonic acid (53.6 mg, 0.282 mmol) and benzaldehyde dimethylacetal (8.13 mL, 54.2 mmol) were added and stirred for 1 h at 55°C under reduced pressure. Triethylamine (0.10 mL) was added to the reaction mixture and then evaporated to syrup. Crytallization from CH_2Cl_2-pet ether gave **3** (12.44 g, 80 %): mp 170-172°C; R_f 0.55 (toluene-EtOAc-EtOH, 5:5:2). ^{1}H-NMR ($CDCl_3$) δ 7.52-7.34 (m, 5 H, aromatic H), 5.56 (s, 1 H, C_6H_5CH), 5.34 (s, 1 H, H-1), 4.27-4.17 (m, 2 H), 4.08-3.78 (m, 4 H), 3.00 (d, 1 H, OH), 2.93 (d, 1 H, OH), 2.63 (m, 2 H, SCH_2CH_3), 1.30 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 137.1-126.3 (aromatic C), 102.3 (C_6H_5CH), 84.4 (C-1), 79.2 (C-4), 72.4 (C-2), 69.1 (C-6), 68.6 (C-3), 63.5 (C-5), 25.1 (SCH_2CH_3), 14.8 (SCH_2CH_3).

Ethyl 3-*O*-allyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (4)

A mixture of compound **3** (12.29 g, 39.5 mmol) and dibutyltin oxide (10.81 g, 43.4 mmol) in benzene (737 mL) was refluxed for 2 h. The reaction mixture was evaporated to about 490 mL and then cooled to room temperature. Then *tetra-n*-buthylammonium iodide (16.04 g, 43.4 mmol) and allyl bromide (8.54 mL, 98.7 mmol) were added. After stirring 18 h at 85°C, the reaction mixture was concentrated and diluted with CH_2Cl_2. The organic layer was washed with aq. $NaHCO_3$ and water, dried, and concentrated. The residual syrup was chromatographed on silica gel (toluene-EtOAc, 15:1) to give **4** (10.21 g, 74%) having R_f 0.33 (toluene-EtOAc, 5:3). ^{1}H-NMR ($CDCl_3$) δ 7.41-7.05 (m, 5 H, aromatic H), 5.78 (m ,1 H, $OCH_2CH{=}CH_2$), 5.47 (s, 1 H, C_6H_5CH), 5.23 (s, 1 H, H-1), 5.10 (m , 2 H, $OCH_2CH{=}CH_2$), 4.20-3.56 (m, 8 H), 3.16 (s, 1 H, OH), 2.45 (m, 2 H, SCH_2CH_3), 1.17 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 137.3-125.1 (aromatic C), 134.2 ($OCH_2CH{=}CH_2$), 117.3 ($OCH_2CH{=}CH_2$), 101.3 (C_6H_5CH), 84.2 (C-1), 78.8 (C-4), 75.2 (C-3), 71.6 ($OCH_2CH{=}CH_2$), 71.1 (C-2), 68.4 (C-6), 63.7 (C-5), 24.7 (SCH_2CH_3), 14.7 (SCH_2CH_3).

Ethyl 3-*O*-allyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (5)

Compound **4** (10.21 g, 29.0 mmol) in DMF (37 mL) was cooled at 0°C and then NaH (60% in mineral oil, 2.09 g) was added and stirred for 40 min. Then 4-methoxybenzyl chloride (5.93 mL, 43.5 mmol) was dropwised at 0°C. After stirring 2 h at room temperature, MeOH was added in order to destroy the excess hydride and evaporated. The residual syrup was diluted with CH_2Cl_2, washed with water, dried, and concentrated to a syrup, which was chromatographed on silica gel (toluene-EtOAc, 15:1) to give **5** (13.95 g, quantitatively) having R_f 0.26 (toluene-EtOAc, 15:1). ^{1}H-NMR ($CDCl_3$) δ 7.41-7.05 (m, 7 H, aromatic H), 6.89-6.86 (m, 2 H, $CH_3OC_6H_4$), 5.88 (m , 1 H, $OCH_2CH{=}CH_2$), 5.60 (s, 1 H, C_6H_5CH), 5.28 (m, 3 H, H-1, $OCH_2CH{=}CH_2$), 4.69 (m, 2 H, $C_6H_5CH_2$), 4.25-3.77 (m, 11 H), 2.56 (m, 2 H, SCH_2CH_3), 1.23 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 159.5-113.9 (aromatic C), 135.0 ($OCH_2CH{=}CH_2$), 116.9 ($OCH_2CH{=}CH_2$), 101.6 (C_6H_5CH), 83.8 (C-1), 79.4 (C-4), 77.7 (C-2), 76.2 (C-3), 72.9, 72.0 ($OCH_2CH{=}CH_2$, $CH_3OC_6H_4CH_2$), 68.8 (C-6), 64.8 (C-5), 55.4 (OCH3), 25.5 (SCH_2CH_3), 15.1 (SCH_2CH_3).

Ethyl 3-*O*-allyl-4-*O*-benzyl-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (6)

To a solution of **5** (13.4 g, 28.4 mmol) in Et_2O-CH_2Cl_2 (200 mL, 1/1) was added $LiAlH_4$ (1.51 g, 39.8 mmol) with stirring. A solution of $AlCl_3$ (5.84 g, 43.8 mmol) in dry Et_2O (97.5 mL) was dropwised at room temperature. The reaction mixture was stirred for 1 h. The excess hydride was quenched with successive addition of EtOAc and then brine. The mixture was diluted with Et_2O, and the insoluble materials were filtered off. The organic layer was washed with brine, dried, and concentrated. Column chromatograph (toluene-EtOAc, 15:1, followed by 5:1) of the residue gave **6** (7.49 g, 56%) having R_f 0.15 (toluene-EtOAc, 5:1). ^{1}H-NMR ($CDCl_3$) δ 7.25-7.06 (m, 7 H, aromatic H), 6.79 (d, 2 H, J = 8.52 Hz, $CH_3OC_6H_4$), 5.80 (m, 1 H, $OCH_2CH{=}CH_2$), 5.20 (s, 1 H, H-1), 5.24-5.08 (m, 2 H, $OCH_2CH{=}CH_2$), 4.85-4.54 (m, 4 H, $CH_3OC_6H_4CH_2$, $C_6H_5CH_2$),

3.95-3.63 (m, 11 H), 2.46 (m, 2 H, SCH_2CH_3), 2.14 (s, 1 H, OH), 1.13 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 159.2-113.6 (aromatic C), 134.6 ($OCH_2CH=CH_2$), 116.8 ($OCH_2CH=CH_2$), 82.1 (C-1), 79.8, 75.7, 74.8, 72.3, 75.0, 71.8, 70.8, 62.1 (C-6), 55.1 (OCH_3), 25.1 (SCH_2CH_3), 14.7 (SCH_2CH_3).

Ethyl 3-*O*-allyl-4-*O*-benzyl-6-cyano-6-deoxy-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (7)

Methanesulfonyl chloride (2.3 mL, 29.6 mmol) was added to a solution of compound **6** (7.0 g, 14.8 mmol) in pyridine (88 mL) at 0°C. The mixture was stirred for 4 h at room temperature. After addition of ice water to the reaction mixture, aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with aq. 2 N HCl, aq. $NaHCO_3$, and water successively, dried and then concentrated to syrup. Without further purification, to a stirred solution of residual syrup and 18-crown-6 (391 mg, 1.48 mmol) in DMSO (110 mL) was added KCN (3.85 g, 59.1 mmol) at 70°C. After stirring 4 h at 70°C, the solution was dissolved in CH_2Cl_2, washed with aq. 1 N HCl, aq. $NaHCO_3$, and water successively, dried and then concentrated to a syrup, which was chromatographed on silica gel (toluene-EtOAc, 20:1) to give **7** (5.82 g, 82%) having R_f 0.33 (toluene-EtOAc, 15:1). ^{1}H-NMR ($CDCl_3$) δ 7.42-7.22 (m, 7 H, aromatic H), 6.96 (d, 2 H, *J* = 7.84 Hz, $CH_3OC_6H_4$), 5.96 (m, 1 H, $OCH_2CH=CH_2$), 5.36 (s, 1 H, H-1), 5.39-5.25 (m, 2 H, $OCH_2CH=CH_2$), 5.05-4.65 (m, 4 H, $CH_3OC_6H_4CH_2$, $C_6H_5CH_2$), 4.23 (t, 1 H, H-4), 4.07 (m, 2 H), 3.92-3.77 (m, 6 H), 2.77-2.59 (m, 4 H), 1.32 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 159.3-113.7 (aromatic C), 134.3 ($OCH_2CH=CH_2$), 117.3, 117.2 (CN, $OCH_2CH=CH_2$), 81.9 (C-1), 79.7, 77.1, 75.4, 75.2, 71.7, 70.6, 67.9, 55.2 (OCH_3), 25.1 (SCH_2CH_3), 20.8 (C-6), 14.7 (SCH_2CH_3).

Ethyl 3-*O*-allyl-4-*O*-benzyl-6-deoxy-2-*O*-*p*-methoxybenzyl-1-thio-α-D-*manno*-heptopyranoside (8)

DIBAL (1 M in hexane, 50 mL) was added to a solution of compound **7** (5.18 g, 10.7 mmol) in THF (75 mL) at -78 °C under N_2 (g). After stirring 1 h at room temperature, TLC analysis showed that the 6-cyano-6-deoxy compound of R_f 0.33 disappeared and imine intermediate of R_f 0.23 (toluene-EtOAc, 15:1) appeared. MeOH (50 mL) was added in order to destroy the excess hydride at -30°C. After stirring 10 min, aq. 2 N HCl (50 mL) was added at -7 °C, stirred for 4 h, filtered, and washed with Et_2O. Combined filtrate was washed with 2 N HCl, aq. $NaHCO_3$, and water successively, dried and then concentrated. Without further purification, sodium borohydride (162 mg, 4.293 mmol) was added to the residual syrup in MeOH (75 mL) at 0°C. The solution was stirred for 1 h at room temperature and evaporated to dryness. The residue was dissolved in CH_2Cl_2, the organic layer was washed with water, dried, concentrated to a syrup, which was chromatographed (toluene-EtOAc, 5:1) to give **8** (4.13 g, 79%) having R_f 0.55 (toluene-EtOAc, 5:1). ^{1}H-NMR ($CDCl_3$) δ 7.40-7.22 (m, 7 H, aromatic H), 6.95 (d, 2 H, *J* = 8.8 Hz, $CH_3OC_6H_4$), 5.96 (m, 1 H, $OCH_2CH=CH_2$), 5.30 (s, 1 H, H-1), 5.39-5.23 (m, 2 H, $OCH_2CH=CH_2$), 5.03-4.65 (m, 4 H, $CH_3OC_6H_4CH_2$, $C_6H_5CH_2$), 4.17 (t, 1 H, H-4), 4.09 (d, 2 H, *J* = 4.16 Hz), 3.90-3.73 (m, 8 H), 2.60 (m, 2 H, SCH_2CH_3), 2.34 (s, 1 H, OH), 2.17 (m, 1 H, H-6a), 1.91 (m, 1 H, H-6b), 1.30 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 159.2-113.7 (aromatic C), 134.6 ($OCH_2CH=CH_2$), 117.0 ($OCH_2CH=CH_2$), 81.9 (C-1), 79.8, 78.3, 75.7, 75.2, 71.8, 70.8, 71.6, 60.9 (C-7), 55.2 (OCH_3), 33.7 (C-6), 25.2 (SCH_2CH_3), 14.7 (SCH_2CH_3).

Ethyl 3-*O*-allyl-7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-2-*O*-*p*-methoxybenzyl-1-thio-α-D-*manno*-heptopyranoside (9)

Benzoyl chloride (BzCl, 1.5 mL, 12.3 mmol) was added to a solution of compound **8** (4.0 g, 8.202 mmol) in pyridine (60 mL). After stirring 16 hours at room temperature, $NaHCO_3$ solution was added in order to destroy excess BzCl. The mixture was diluted with methylene chloride. The organic layer was washed with aq. 2 N HCl, aq. $NaHCO_3$, and water successively, dried and then concentrated, which was chromatographed on silica gel (toluene-EtOAc, 15:1) to give **9** (4.26 g, 88%) having R_f 0.43 (toluene-EtOAc, 5:1). ^{1}H-NMR ($CDCl_3$) δ 7.96-7.16 (m, 12 H, aromatic H), 6.80 (d, *J* = 7.8 Hz, 2 H, $CH_3OC_6H_4$), 5.81 (m, 1 H, $OCH_2CH=CH_2$), 5.18 (s, 1 H, H-1), 5.24-5.08 (m, 2 H, $OCH_2CH=CH_2$), 4.89-4.54 (m, 4 H), 4.34 (m, 2 H, H-7), 4.09 (t, 1 H, $J_{4,5}$ = 8.3 Hz, H-4), 3.94 (d, 2 H, *J* = 5.64 Hz, $OCH_2CH=CH_2$), 3.76 (s, 1 H, H-2), 3.69 (s, 3 H, OCH_3), 3.61 (m, 2 H, H-3, 5), 2.38 (m, 3 H, SCH_2CH_3, H-6a), 1.83 (m, 1 H, H-6b), 1.00 (t, 3 H, SCH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 166.3 (C=O), 159.1-113.6 (aromatic C), 134.6 ($OCH_2CH=CH_2$), 116.9 ($OCH_2CH=CH_2$), 81.7 (C-1), 80.0, 78.8 (C-3, 5), 75.5 (C-2), 75.2, 71.7 ($C_6H_5CH_2$, $CH_3OC_6H_4CH_2$), 70.7 ($OCH_2CH=CH_2$), 68.2 (C-4), 61.2 (C-7), 55.1 (OCH_3), 30.6 (C-6), 24.9 (SCH_2CH_3), 14.4 (SCH_2CH_3).

2'-Chloroethyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (10)

Benzaldehyde dimethylacetal (2.4 mL, 15.62 mmol) and *p*-toluenesulfonic acid (188 mg, 1.09 mmol) were added to a solution of 2'-chloroethyl 2-deoxy-2-*N*-phthalimido-α-D-glucopyranoside (5.20 mmol) in THF (30 mL). After stirring 16 h at room temperature, triethylamine (630 μL) was added. The reaction mixture was concentrated to a syrup, which was chromatographed on silica gel (toluene-EtOAc, 7:1) to give **10** (1.34 g, 57%) having R_f 0.83 (toluene-EtOAc-EtOH, 5:5:2). ^{1}H-NMR ($CDCl_3$) δ 7.66-

7.03 (m, 9 H, aromatic H), 5.41 (s, 1 H, C_6H_5CH), 5.13 (d, 1 H, $J_{1,2}$ = 8.56 Hz, H-1), 4.49 (t, 1 H, $J_{3,4}$ = 9.52 Hz, H-3), 4.21 (dd, 1 H, $J_{5,6a}$ = 4.16 Hz, $J_{6a,6b}$ = 10.52 Hz, H-6a), 4.10 (t, 1 H, $J_{2,3}$ = 9.52 Hz, H-2), 3.86 (m, 1 H, OCH_2CH_2Cl), 3.66 (t, 1 H, J = 9.88 Hz, H-5), 3.47 (m, 3 H), 3.29 (t, 1 H, J = 5.74 Hz, OCH_2CH_2Cl); ^{13}C-NMR ($CDCl_3$) δ 137.6-123.2 (aromatic C), 101.6 (C_6H_5CH), 99.0 (C-1), 81.8 (C-4), 69.6 (OCH_2CH_2Cl), 68.3 (C-6), 68.2 (C-3), 66.0 (C-5), 56.4 (C-2), 42.3 (OCH_2CH_2Cl).

2'-Chloroethyl *O*-(3-*O*-allyl-7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-2-*O*-*p*-methoxybenzyl-α-D-*manno*-heptopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (11)

A solution of **9** (3.08 g, 5.20 mmol) and **10** (1.82 g, 3.96 mmol) in CH_2Cl_2-Et_2O (2:5, v/v, 105 mL) was stirred with molecular sieve 5 Å (3 g) for 30 min at room temperature and then iodonium dicollidine perchlorate (IDCP; 5.56 g, 11.87 mmol) was added. After stirring 30 min at room temperature, the precipitate was filtered off through Celite-bed, and washed thoroughly 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, 40:1) of the residue gave **11** (2.59 g, 66%) having R_f 0.46 (toluene-EtOAc, 5:1). ^{1}H-NMR ($CDCl_3$) δ 8.02-6.67 (m, 23 H, aromatic H), 5.81 (m, 1 H, OCH_2CH=CH_2), 5.56 (s, 1 H, C_6H_5CH), 5.39 (d, 1 H, $J_{1,2}$ = 8.52 Hz, H-1), 5.29 (d, 1 H, $J_{1',2'}$ = 1.72 Hz, H-1'), 5.26-5.11 (m, 2H, OCH_2CH=CH_2), 4.66 (dd, 1 H, $J_{3,4}$ = 8.8 Hz, H-3), 4.74-4.29 (m, 5 H), 4.22 (dd, 1 H, $J_{2,3}$ = 10.48 Hz, H-2), 4.10 (t, 2 H, J = 7.44 Hz, H-7'), 3.89 (d, 2 H, J = 5.36 Hz, OCH_2CH=CH_2), 3.75 (s, 3 H, OCH_3), 4.04-3.62 (m, 6H), 3.47-3.41 (m, 4 H), 3.07-3.03 (m, 1 H, H-4'), 1.66 (m, 2 H, H-6'); ^{13}C-NMR ($CDCl_3$) δ 166.3 (C=O), 158.9-113.5 (aromatic C), 134.7 (OCH_2CH=CH_2), 116.5 (OCH_2CH=CH_2), 101.7 (C_6H_5CH), 99.0 (C-1), 98.0 (C-1'), 82.9 (C-4), 79.1, 77.2 (C-3', 5'), 74.4 ($C_6H_5CH_2$), 73.4 (C-2'), 72.2 (C-3), 71.5 ($CH_3OC_6H_4CH_2$), 70.6 (OCH_2CH=CH_2), 69.9 (OCH_2CH_2Cl), 68.7 (C-6), 65.8 (C-5), 61.8 (C-7'), 55.2 (OCH_3), 55.1 (C-2), 42.3 (OCH_2CH_2Cl), 30.6 (C-6').

2'-Chloroethyl *O*-(3-*O*-allyl-7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-2-*O*-*p*-methoxybenzyl-α-D-*manno*-heptopyranosyl)-(1→3)-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (1→2) and 2'-Chloroethyl *O*-(3-*O*-allyl-7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-α-D-*manno*-heptopyranosyl)-(1→3)-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (13)

A solution of **11** (838 mg, 0.847 mmol) in 70% acetic acid solution (40 mL) was stirred for 6 h at 65°C. The reaction mixture was cooled to room temperature, concentrated and coevaporated with toluene to dryness. Column chromatography (toluene-EtOAc, 15:1, followed by 5:3 and toluene-EtOAc-EtOH, 5:5:2) of the residue gave **12** (338 mg, 44%) having R_f 0.22 and **13** (311 mg, 41%) having R_f 0.03 (toluene-EtOAc, 5:3). ^{1}H-NMR ($CDCl_3$) for **12** δ 8.00-7.12 (m, 16 H, aromatic H), 6.87 (d, 2 H, J = 8.56 Hz, $CH_3OC_6H_4CH_2$), 5.80 (m, 1 H, OCH_2CH=CH_2), 5.25 (d, 1 H, $J_{1,2}$ = 8.32 Hz, H-1), 5.22-5.09 (m, 3 H), 4.66-4.37 (m, 5 H), 4.16-3.90 (m, 7 H), 3.81 (s, 3 H, OCH_3), 3.72-3.38 (m, 10 H), 1.66 (m, 2 H, H-6'); ^{13}C-NMR ($CDCl_3$) for **12** δ 166.4 (C=O), 159.4-113.8 (aromatic C), 134.7 (OCH_2CH=CH_2), 116.9 (OCH_2CH=CH_2), 98.6 (C-1'), 98.5 (C-1), 78.1, 77.9, 77.2, 75.4, 75.0, 73.4, 72.1 ($C_6H_5CH_2$, $CH_3OC_6H_4CH_2$), 72.4, 71.2 (OCH_2CH=CH_2), 70.2, 69.7 (OCH_2CH_2Cl), 62.6, 61.3 (C-6, 7'), 55.3 (OCH_3), 54.6 (C-2), 42.4 (OCH_2CH_2Cl), 30.9 (C-6'); ^{1}H-NMR ($CDCl_3$) for **13** δ 8.00-7.07 (m, 14 H, aromatic H), 5.86 (m, 1 H, OCH_2CH=CH_2), 5.42 (d, 1 H, $J_{1',2'}$ = 2.2 Hz, H-1'), 5.34 (d, 1 H, $J_{1,2}$ = 8.56 Hz, H-1), 5.19 (m, 2 H, OCH_2CH=CH_2), 4.66-4.35 (m, 3 H, H-3, $C_6H_5CH_2$), 4.21-3.67 (m, 10 H), 3.54-3.23 (m, 7 H), 1.77-1.62 (m, 2 H, H-6'); ^{13}C-NMR ($CDCl_3$) for **13** δ 168.5, 167.9 (NPhth C=O), 166.5 (C=O), 138.2-125.2 (aromatic C), 134.4 (OCH_2CH=CH_2), 117.3 (OCH_2CH=CH_2), 99.3 (C-1'), 98.4 (C-1), 78.7 (C-5), 76.6 (C-4'), 76.2 (C-3), 75.5 (C-3'), 73.8 ($C_6H_5CH_2$), 72.0 (C-4), 70.8 (OCH_2CH=CH_2), 69.6 (OCH_2CH_2Cl, C-5), 68.5 (C-2'), 61.7 (C-6), 61.6 (C-7'), 55.0 (C-2), 42.4 (OCH_2CH_2Cl), 30.3 (C-6').

2'-Chloroethyl *O*-(7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-2-*O*-*p*-methoxybenzyl-α-D-*manno*-heptopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (14)

Acetic anhydride (7 mL) was added to a solution of compound **12** (337 mg, 0.374 mmol) in pyridine (7 mL). After stirring 16 h at room temperature, the mixture was coevaporated with toluene to dryness. The residual syrup was employed in the next reaction without purification. Palladium chloride (39.8 mg, 0.225 mmol) was added to a solution of residual syrup in MeOH (17 mL) and the reaction mixture was stirred for 2 h at room temperature. The mixture was filtered through Celite-bed and concentrated. The crude product was purified by flash chromatography on a column of silica gel to yield **14** having R_f 0.43 (toluene-EtOAc, 5:3). ^{1}H-NMR ($CDCl_3$) δ 7.92-6.76 (m, 18 H, aromatic H), 5.19 (d, 1 H, $J_{1,2}$ = 8.56 Hz, H-1), 5.07 (t, 1 H, $J_{4,5}$ = 9.52 Hz, H-4), 4.75 (d, 1 H, $J_{1',2'}$ = 2.48 Hz, H-1'), 4.52-4.16 (m, 7 H), 4.05-3.59 (m, 10 H), 3.42-3.34 (m, 3 H), 3.24-3.17 (m, 2 H, H-4', H-5'), 2.01, 1.90 (each s, each 3 H, CH_3CO), 1.68-1.45 (m, 2 H, H-6'); ^{13}C-NMR ($CDCl_3$) δ 170.9, 169.2, 166.1 (C=O), 159.2-113.6 (aromatic C), 98.3 (C-1), 97.7 (C-1'), 78.1(C-4'), 77.4 (C-2'), 74.9 (C-5), 73.3, 72.3 ($C_6H_5CH_2$, $CH_3OC_6H_4CH_2$), 71.8 (C-3), 71.0 (C-4), 70.5 (C-3'), 69.6 (C-5'), 69.57 (OCH_2CH_2Cl), 61.8 (C-6), 61.1 (C-7'), 55.04 (OCH_3), 55.0 (C-2), 42.2 (OCH_2CH_2Cl), 29.7 (C-6'), 20.6, 20.59 (CH_3CO).

2'-Chloroethyl *O*-(7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-2-*O*-*p*-methoxybenzyl-α-D-*altro*-heptopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (15)

A solution of oxalyl chloride (117 μL, 1.37 mmol) in CH_2Cl_2 (1 mL) was cooled to -20°C, and DMSO (237 μL, 3.33 mmol) was dropwised. After stirring 15 min at -20°C, a solution of **14** (350 mg, 0.370 mmol) in CH_2Cl_2 (1.5 mL) was dropwised and stirred for 2 h at -20°C. After dropwise of triethylamine (960 L, 6.85 mmol), TLC analysis showed the conversion of R_f 0.43 for **14** into 0.56 for the 3'-ulose derivative (toluene-EtOAc, 5:3). After stirring 7 min at -20°C, water was added. The solution was diluted with CH_2Cl_2, and washed with 2 N HCl, aq. $NaHCO_3$, and water in sequence. The organic layer was dried and concentrated. The residual syrup was immediately reduced with sodium cyanoborohydride (1.16 g, 18.5 mmol) in DMF (2.3 mL)-MeOH (24 mL) and stirred for 5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and concentrated. The residual syrup was diluted with CH_2Cl_2 and washed with 2 N HCl, aq. $NaHCO_3$, and water in sequence. The organic layer was dried and concentrated. Column chromatography (toluene-EtOAc, 4:1, followed by 5:3) gave **14** (66.2 mg, 19%) and **15** (138 mg, 40% from **12**) having R_f 0.38 (toluene-EtOAc, 5:3). ^{1}H-NMR ($CDCl_3$) δ 7.96-6.77 (m, 18 H, aromatic H), 5.27 (d, 1 H, $J_{1,2}$ = 8.56 Hz, H-1), 5.11 (t, 1 H, $J_{4,5}$ = 9.52 Hz, H-4), 4.53 (d, 1 H, $J_{1',2'}$ = 4.64 Hz, H-1'), 4.49-4.01 (m, 10 H), 3.98-3.62 (m, 7 H), 3.43-3.28 (m, 5 H), 3.24-3.17 (m, 2 H, H-4', 5'), 2.05, 1.88 (each s, each 3 H, C*H*$_3$CO), 1.78-1.48 (m, 2 H, H-6'); ^{13}C-NMR ($CDCl_3$) δ 170.8, 169.5, 166.2 (C=O), 159.3-113.8 (aromatic C), 99.9 (C-1'), 98.5 (C-1), 77.8 (C-2'), 75.8 (C-5'), 75.7 (C-3), 73.5, 70.2 ($C_6H_5CH_2$, $CH_3OC_6H_4CH_2$), 72.0 (C-5), 71.0 (C-4), 69.7 (O*C*H$_2$CH$_2$Cl), 67.7 (C-3'), 67.6 (C-4'), 62.2 (C-7'), 61.4 (C-6), 55.2 (O*C*H$_3$, C-2), 42.2 (OCH$_2$*C*H$_2$Cl), 29.0 (C-6'), 20.8, 20.7 (*C*H$_3$CO).

2'-Chloroethyl *O*-(3-*O*-acetyl-7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-α-D-*altro*-heptopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (16)

Acetic anhydride (2 mL) was added to a solution of **15** (126 mg, 0.133 mmol) in pyridine (2 mL). After stirring 16 h at room temperature, the reaction mixture was coevaporated with toluene. To a solution of residual syrup in CH_2Cl_2-H_2O (2.8 mL, 18:1) was added DDQ (41.1 mg, 0.181 mmol) and the reaction mixture was stirred for 2 h at room temperature. The mixture was filtered through Celite-bed and the filtrate was washed with aq. $NaHCO_3$ and water, concentrated, and then chromatographed on silica gel (toluene-EtOAc, 5:1, followed by 5:3) to give **16** (86.7 mg, 83%) having R_f 0.36 (toluene-EtOAc, 5:3). ^{1}H-NMR ($CDCl_3$) δ 7.96-6.99 (m, 14 H, aromatic H), 5.29 (d, 1 H, $J_{1,2}$ = 8.52 Hz, H-1), 5.12 (t, 1 H, $J_{4,5}$ = 9.52 Hz, H-4), 4.75-3.91 (m, 10 H), 3.69-3.65 (m, 3 H), 3.43-3.40 (m, 3 H), 3.07 (m, 1 H), 2.04, 2.02, 1.94 (each s, each 3 H, C*H*$_3$CO), 1.54 (m, 2 H, H-6'); ^{13}C-NMR ($CDCl_3$) δ 170.8, 170.7, 170.6, 166.3 (C=O), 137.5-125.3 (aromatic C), 101.1 (C-1'), 98.6 (C-1), 77.4, 74.5, 71.8, 70.8 ($C_6H_5CH_2$), 70.7, 70.68, 70.3, 69.8 (O*C*H$_2$CH$_2$Cl), 68.8, 61.9, 61.5 (C-7', 6), 55.2 (C-2), 42.2 (OCH$_2$*C*H$_2$Cl), 29.3 (C-6'), 20.94, 20.9, 20.8 (*C*H$_3$CO).

2'-Chloroethyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→2)-(3-*O*-acetyl-7-*O*-benzoyl-4-*O*-benzyl-6-deoxy-β-D-*altro*-heptopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (18)

A solution of ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**17**) (42.2 mg, 0.0721 mmol) and **16** (47.6 mg, 0.0548 mmol) in CH_2Cl_2-Et_2O (2:5, v/v, 3.5 mL) was stirred with molecular sieve 5 (350 mg) for 30 min at room temperature and then iodonium dicollidine perchlorate (IDCP; 77.0 mg, 0.164 mmol) was added. After stirring 30 min at room temperature, the precipitate was filtered off through Celite-bed, and washed thoroughly with CH_2Cl_2. The combined filtrate was washed with 1 M $Na_2S_2O_3$, water successively, dried and concentrated. Column chromatography (toluene-EtOAc, 40:1) of the residue gave **18** (43.4 mg, 57%) having R_f 0.29 (toluene-EtOAc, 5:1). ^{1}H-NMR ($CDCl_3$) δ 7.88-6.72 (m, 34 H, aromatic H), 5.07 (t, 1 H, $J_{3',4'}$ = 3.78 Hz, H-3'), 5.02 (d, 1 H, $J_{1,2}$ = 8.56 Hz, H-1), 4.92-4.01 (m, 17 H), 3.93-3.29 (m, 15 H), 3.20 (m, 1 H, H-5), 1.99, 1.97, 1.96 (each s, each 3 H, C*H*$_3$CO), 1.52 (m, 1 H, H-6a'), 1.04 (m, 1 H, H-6b'); ^{13}C-NMR ($CDCl_3$) δ 170.9, 170.7, 169.7, 166.0 (C=O), 138.8-121.4 (aromatic C), 101.0 (C-1'), 99.6 (C-1"), 98.7 (C-1), 78.8, 78.0 (C-3), 76.0 (C-2"), 75.0 (C-2'), 74.6, 74.7, 73.4, 73.2, 72.6, 69.9 ($C_6H_5CH_2$), 71.8 (C-5), 71.5 (C-4'), 70.6 (C-4), 70.0, 69.7 (O*C*H$_2$CH$_2$Cl), 69.5 (C-6"), 66.8 (C-3'), 66.1, 62.1 (C-7'), 60.5 (C-6), 55.5 (C-2), 42.3 (OCH$_2$*C*H$_2$Cl), 28.9 (C-6'), 20.9, 20.7, 20.69 (*C*H$_3$CO).

2'-Azidoethyl *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1→2)-(3,4,7-tri-*O*-acetyl-6-deoxy-α-D-*altro*-heptopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (21)

Compound **18** (43.4 mg, 0.0312 mmol) in EtOAc-EtOH (1:2, 9 mL) was hydrogenated in the presence of 10% Pd/C for 26 h at room temperature. The reaction mixture was filtered over Celite-bed and concentrated to dryness. The residue was dissolved in pyridine (3 mL) and treated with acetic anhydride (3 mL). After stirring 16 h at room temperature, the mixture was coevaporated with toluene. The residue syrup was dissolved in DMF and treated with sodium azide (30.0 mg, 0.0466 mmol). After stirring 4 h at

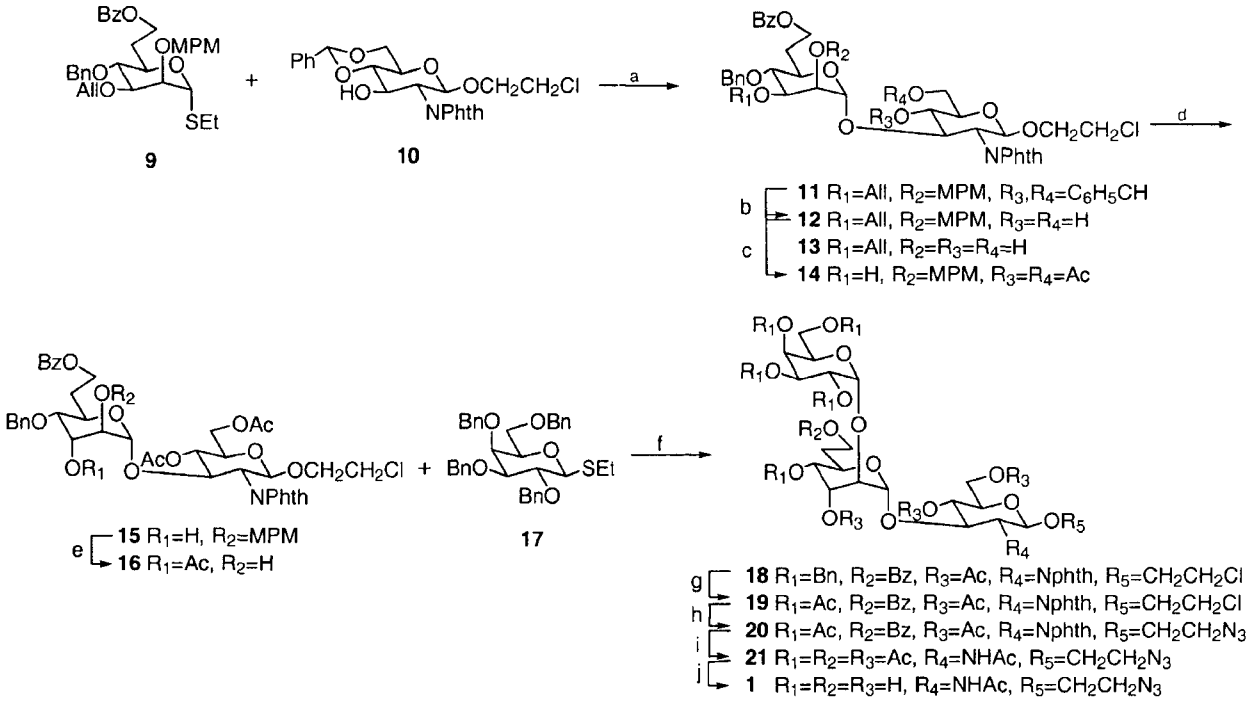
110°C, the mixture was cooled, evaporated, and then diluted in CH_2Cl_2. The organic layer was washed with water, dried, and concentrated. Without further purification, the residue was dissolved in EtOH (4 mL) and treated with hydrazine monohydrate (2 mL). After stirring for 1 h at 66°C, the mixture was coevaporated with toluene. Acetic anhydride (5 mL) was added to residual amine syrup in pyridine (5 mL). After stirring 16 h at room temperature, the mixture was coevaporated with toluene to dryness. Column chromatography (toluene-EtOAc-EtOH, 5:5:1) of the residue gave **21** (31.4 mg) having R_f 0.55 (toluene-EtOAc-EtOH, 5:5:2). ^{13}C-NMR ($CDCl_3$) δ 171.2, 171.0, 170.74, 170.7, 170.6, 170.5, 170.1, 169.8, 169.7, 169.6 (C=O), 100.1 (C-1'), 99.0 (C-1), 96.6 (C-1"), 77.5, 77.2, 73.6, 71.7, 70.9, 68.9, 68.8, 68.2, 67.8, 67.6, 67.0, 66.5, 62.5, 61.0, 59.9, 56.5 (C-2), 50.6 ($OCH_2CH_2N_3$), 29.3 (C-6'), 22.6, 22.3, 22.1, 20.9, 20.8, 20.7, 20.67, 20.6, 20.55, 20.5 (CH_3CO).

2'-Azidoethyl *O*-(α-D-galactopyranosyl)-(1→2)-(6-deoxy-α-D-*altro*-heptopyranosyl)-(1→3)-2-*N*-acetyl-β-D-glucopyranoside (1)

Compound **21** (25.0 mg) in MeOH (2 mL) was treated with 25% NaOMe (20 μL) for 1 h at room temperature. The reaction mixture was neutralized with Dowex 50 (H^+) resin, filtered, and concentrated. The crude product was treated to Bio-gel P2 column to give **1** (5.90 mg, 30% from **18**, 6 steps) having R_f 0.07 (*t*BuOH-EtOAc-AcOH-H_2O, 36:36:7:21). ^{1}H-NMR (D_2O) δ 5.42, 5.12 (each s, each 1 H, H-1' and H-1"), 4.63 (d, 1 H, $J_{1,2}$ = 8.32 Hz, H-1), 4.05-3.65 (m, 19 H), 3.52-3.41 (m, 4 H, H-4, H-5 and $OCH_2CH_2N_3$), 2.08 (s, 4 H), 1.82 (m, 1 H, H-6b'); ^{13}C-NMR (D_2O) δ 178.7 (C=O, NHAc), 103.7 (C-1), 101.8, 101.6 (C-1', C-1"), 81.9, 78.5, 78.4, 74.2, 73.6, 72.0, 71.96, 71.6 ($OCH_2CH_2N_3$), 71.0, 70.8, 70.5, 68.3, 64.1 (C-6), 63.3 (C-6"), 60.7 (C-7'), 56.8 (C-2), 53.2 ($OCH_2CH_2N_3$), 36.1 (C-6'), 25.3 (CH_3, NHAc); MALDI-TOF MS calcd. for $C_{23}H_{40}N_4O_{16}Na$ (M^++Na) and $C_{23}H_{40}N_4O_{16}K$ (M^++K) 651.234 and 667.208 found 652.081 and 669.078.

RESULTS AND DISCUSSION

Trisaccharide **1** can be divided into two building blocks, a perbenzylated thiogalactosyl donor **17** and the 6-deoxy-α-D-*altro*Hep*p*-(1→3)-β-D-GlcNphth disaccharide acceptor **16** (Scheme 1). Both donor and acceptor can be coupled by the glycosidation with IDCP promoter. Especially, the disaccharide acceptor **16** contains an unusual altroheptose and α-(1→3)-altroheptosyl linkage of which synthesis has not been reported yet as far as we know. Furthermore, stereoselective formation of α-altro glycosidic linkage was expected very difficult due to the 1, 3-syn diaxial stereoe-



Scheme 1. (a) IDCP, CH_2Cl_2-Et_2O (2/5), MS 5Å, 30 min, 66%. (b) 70% AcOH, 65°C, 6 h 44%. (c) Ac_2O, pyridine, rt, 16 h; $PdCl_2$, MeOH, rt, 2 h. (d) $(COCl)_2$-CH_2Cl_2, DMSO, -20°C, 2 h, TEA, 7 min; $NaCNBH_3$-DMF, MeOH, rt, 5 h, 40% from 12. (e) Ac_2O, pyridine, rt, 16 h; DDQ, CH_2Cl_2-H_2O (18/1), rt, 2 h, 83%. (f) IDCP, CH_2Cl_2-Et_2O (2/5), MS 5Å, rt, 30 min, 57%. (g) H_2, 10% Pd/C, EtOH/EtOAc (2/1), rt, 1 atm; Ac_2O, pyridine, rt, 16 h. (h) NaN_3, DMF, 110°C, 4 h. (i) N_2H_2:H_2O, EtOH, 70°C, 1 h; Ac_2O, pyridine, rt, 16 h. (j) NaOMe, MeOH, rt, 1 h, 30% from **18**.

lectronic repulsion of altroheptopyranoside ring system. Therefore, stereoselective formation of α-(1→3)-altroheptosyl linkage should be the most challengeable part in our synthesis. Direct α-glycosylation of altroheptose with various glycosidic acceptors under known glycosidic conditions failed because of low α-stereoselectivities and yield as expected. Finally, we got to the conclusion that α-(1→3)-altroheptosyl linkage could be made indirectly from α-(1→3)-mannoheptosyl derivatives by the epimerization of 3-OH of mannoheptose, and we believe this methodology must be the only and the best way for synthesizing α-(1→3)-altroheptosyl linkage. Therefore It was devised that the 6-deoxy-α-D-*altro*Hep*p*-(1→3)-β-D-GlcNphth disaccharide acceptor (**9**) could be synthesized from the 6-deoxy-α-D-*manno*Hep*p*-(1→3)-β-D-GlcNphth **14**. The mannosyl disaccharide **14** could be obtained by coupling of the 6-deoxy-*manno*heptopyranosyl donor **16** and an *N*-phthaloyl glucoside **10**.

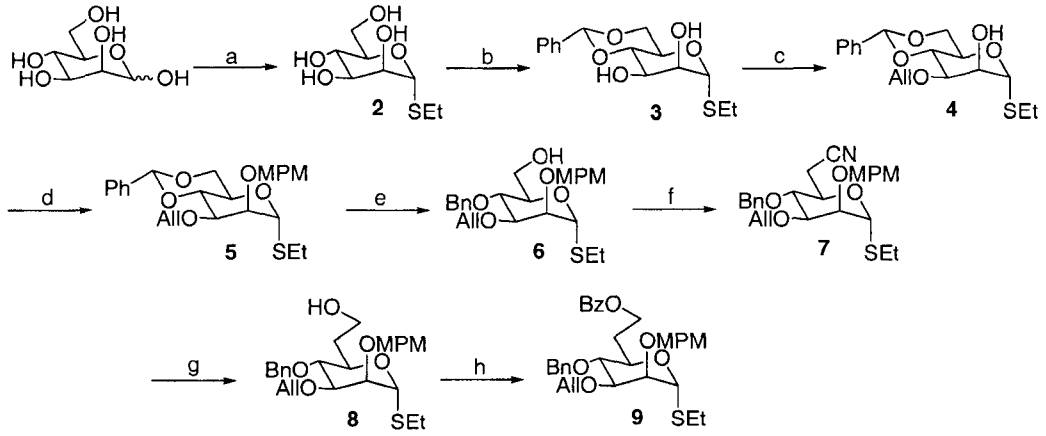
For the synthesis of 6-deoxy-*manno*heptopyranosyl donor **9**, ethyl 1-thio-α-D-mannoside **2** was prepared from successive treatments of mannose with acetic anhydride in pyridine, $BF_3{\cdot}OEt_2$ and ethanethiol in methylene chloride, and NaOMe in methanol in 65% overall yield. Subsequent treatments of compound **2** with benzaldehyde dimethylacetal and *p*-toluenesulfonic acid in DMF, dibutyltin oxide, tetra-*n*-butylammonium iodide and allyl bromide in benzene and finally *p*-methoxybenzyl chloride in DMF yielded the compound **5** in 59% overall yield from **2**. The reductive clevage of 4, 6-*O*-benzylidene compound **5** was performed with lithium aluminiumhydride-aluminium chloride in diethyl ether to give **6** in 56% yield. one carbon extension of 6-position in *manno*hexo-compound **6** to *manno*hepto-compound **8** was achieved by treatments of **6** with (i) 6-*O*-mesylation with methanesulfonyl chloride in pyridine and the followed by C-elongation *via* cyanide displacement with potassium cyanide and 18-crown-6 in dimethyl sulfoxide (Shin *et al.*, 1995) (82% yield); (ii) reduction to imine with dibuthyl aluminium hydride (DIBAL) in tetrahydrofuran and hydrolysis of imine to aldehyde with 2 N HCl; (iii) reduction to **8** with sodium borohydride in methanol in 79% yield. Benzoylation of **8** with benzoyl chloride in pyridine gave **9** (88% yield) (Scheme 2).

The glycosylation of the 6-deoxy-*manno*heptopyranosyl donor **9** with 2'-chloroethyl *N*-phthaloyl β-D-glucopyranoside acceptor **10** in the presence of IDCP promoter (Veeneman and van Boom, 1990) furnished disaccharide **11** in 66% yield. The formation of α-mannosidic linkage in **11** was confirmed by comparison of its H-1 and C-1 NMR spectrum data [5.29 ($J_{1,2}$ = 1.72 Hz) and 98.0 ppm] with the known values (Veeneman and van Boom, 1990).

Debenzylidenation of **11** with 70% acetic acid solution gave **12** in 44% yield, while side product **13** from concomitant cleavage of the *p*-methoxybenzyl ether linkage was also found in 41% yield. After acetylation of **12**, hydrogenolysis with $PdCl_2$ in methanol (Zhang and Magnusson, 1995) was performed to give **15**.

For the epimerization of 3'-OH in 6-deoxy-*manno*-heptopyranosyl donor **14**, sequential *Swern* oxidation (Mancuso and Swern, 1981) and reduction with $NaCNBH_3$ were performed to yield an epimeric mixture of α-*manno*-Hep*p*-(1→3)-GlcNPhth **14** and α-*altro*Hep*p*-(1→3)-GlcNPhth **15** in a 1:2 ratio. Undesired original precursor **14** was recovered and resubmitted to the oxidation-reduction protocol. Compound **15** was acetylated to protect its 3'-OH and then treated with DDQ to produce the disaccharide acceptor α-*altro*Hep*p*-(1→3)-GlcNPhth **16**, of which *altr*-*o*heptose has the free 2-OH.

The stereoselective glycosidation of perbenzylated



Scheme 2. (a) Ac_2O, pyridine, -15°C, 4 h, 0°C, 68 h; $BF_3{\cdot}Et_2O$, EtSH, CH_2Cl_2, rt, 23 h; NaOMe, MeOH, rt, 1 h, 65% from D-mannose. (b) $C_6H_5CH(OCH_3)_3$, DMF, *p*-TsOH, 55°C, 4 h, 80%. (c) Bu_2SnO, Benzene, Bu4NI, CH_2=$CHCH_2Br$, reflux, 18 h, 74%. (d) NaH, DMF, 4-MPMCl, rt, 3 h, quantitative. (e) $LiAlH_4$, $AlCl_3$, Et_2O-CH_2Cl_2, rt, 1 h, 56%. (f) MsCl, pyridine; KCN, 18-Crown-6, DMSO, 70°C, 3 h, 82%. (g) 1 M DIBAL-H, THF, -78°C, rt, 2 N HCl, 4 h; $NaBH_4$, MeOH, rt, 1 h, 79%. (h) BzCl, pyridine, rt, 16 h, 88%.

ethylthio β-D-galactopyranoside (**17**) with disaccharide acceptor **16** in the presence of IDCP afforded the trisaccharide **18** in 57% yield. Hydrogenolysis of the trisaccharide **18** with Pd/C-H_2 and subsequent acetylation gave **19**. Compound **19** was transformed to **20** having the 2'-azidoethyl linker arm (Schwartz *et al.*, 1985; Perez *et al.*, 1998), which make them suitable for linking to proteins by reduction of azide group. Hydrazinolysis (Schwartz *et al.*, 1985) and subsequent acetylation gave peracetylated trisaccharide **21**. Finally, the trisaccharide **21** was deacetylated under Zemplen condition to give the title compound **1** in 30% yield from **18**, and the product was characterized by ^{1}H-, ^{13}C-NMR and MALDI-TOF MS.

In summary, for the synthesis of trisaccharide containing α-linked *altro*heptopyranoside residue, direct glycosylations of *altro*heptopyranoside derivatives were proved to be unsatisfactory because of low stereoselectivities. However, epimerization of α-*manno*Hep*p*-(1→3)-GlcNPhth by sequential oxidation and reduction was efficiently applied to yield 6-deoxy-α-D-*altro*Hep*p*-(1→3)-β-D-GlcNphth disaccharide. Moreover, coupling with thiogalactosyl donor in the presence of IDCP promoter produced the title trisaccharide successfully.

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