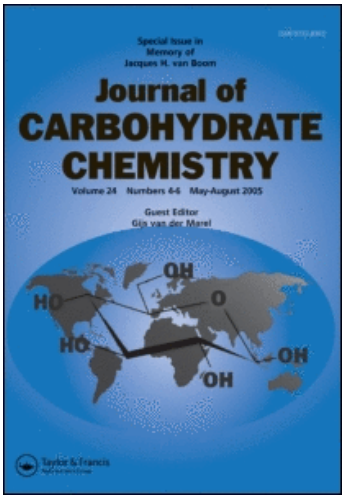

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Synthesis of the Trisaccharide Repeating Unit of the O-Antigen Related to the Enterohemorrhagic *Escherichia coli* Type O26:H

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ABSTRACT

L-Fucose was converted to the 2-azido-2-deoxy-L-fucose derivative, which together with the monosaccharide synthons prepared from L-rhamnose and D-glucosamine hydrochloride were utilized for the synthesis of the *p*-ethoxyphenyl glycoside of the trisaccharide repeating unit of the antigen from enterohemorrhagic *Escherichia coli* type O26:H.

Key Words: Synthesis; *Escherichia coli* type O26:H; Trisaccharide.

INTRODUCTION

Escherichia coli play an important role in maintaining intestinal physiology. Within this species,[1] however, there are fully pathogenic strains that cause distinct syndromes of diarrheal disease. Of the four major categories of diarrheagenic *E. coli*, enterohemorrhagic *E. coli* is one of the most virulent types of pathogen. The

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structures (**I**) of the O-antigen of enterohemorrhagic *E. coli* 026:H has already been reported.[2]

$$\rightarrow 4)-\alpha-\text{L}-\text{Fuc}p\text{NAc}-(1\rightarrow 3)-\beta-\text{D}-\text{Glc}p\text{NAc}-(1\rightarrow 3)-\alpha-\text{L}-\text{Rha}p-(1\rightarrow$$

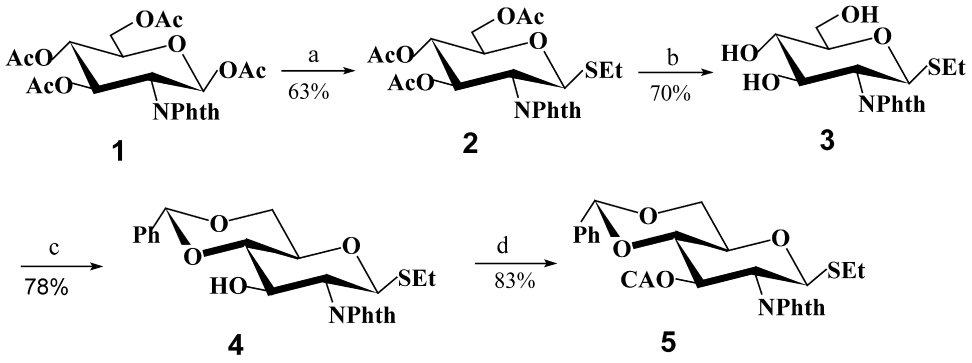
I

The sugar moieties α-L-Rha*p*, α-L-Fuc*p*NAc and β-D-GlcpNAc may be responsible[3] individually or collectively for the immunogenicity of the native antigen. It is therefore pertinent to synthesize the complex oligosaccharides related to the repeating unit of this antigen. They can act as inhibitors and can also be attached to a solid support or a protein carrier for utilization as immunoadsorbent and artificial antigens (vaccines), respectively. Carbohydrate-based antibacterial vaccines are among the most successful carbohydrate pharmaceuticals.[4] As a part of our programme to determine the relationship between the structure and the immunological specificity of the carbohydrate moieties, our primary aim in this communication is to synthesize the trisaccharide repeating unit of the antigen from *E. coli* O26.

RESULTS AND DISCUSSION

Our strategy is to synthesize the target trisaccharide from the three monosaccharide synthons, namely ethyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-phthalimido-1-thio-β-D-glucopyranoside (**5**), 4-methoxyphenyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**11**) and 2-azido-3,4-di-*O*-acetyl-2-deoxy-β-galactopyranosyl trichloroacetimidate (**15**).

For the synthesis of **5**, 1,3,4,5-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**1**)[5] was converted into the ethyl 1-thioglycoside (**2**).[6] Removal of the *O*-acetyl groups of **2** followed by treatment of the product with α,α-dimethoxytoluene[7] in the presence of *p*-toluenesulfonic acid (*p*-TsOH) in acetonitrile gave the benzylidine derivative **4**. Chloroacetylation[8] of **4** with chloroacetic anhydride and triethylamine gave **5** (Scheme 1) in the form of fine crystals.



Scheme 1. (a) EtSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2; (b) NaOMe, MeOH; (c) α,α-dimethoxytoluene, *p*-TsOH, CH_3CN; (d) $(ClCH_2CO)_2O$, Et_3N, CH_2CH_2.

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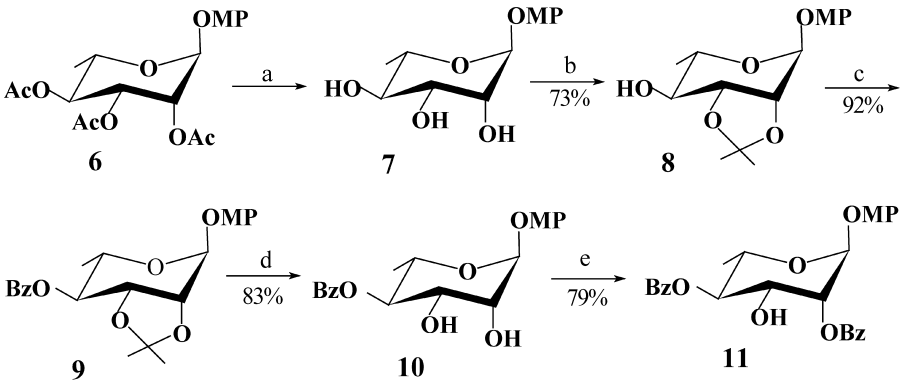
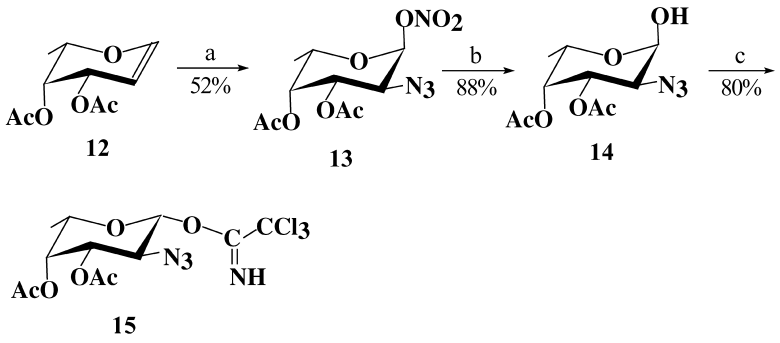


Scheme 2. (a) NaOMe, MeOH; (b) 2,2-Dimethoxypropane, *p*-TsOH, DMF; (c) Benzoyl chloride, pyr; (d) 80% AcOH, 80°C; (e) i. Trimethylorthobenzoate, camphor-10-sulfonic acid, CH_2Cl_2; ii. 80% AcOH, rt.

In another experiment, 4-methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (**6**) was prepared according to the known method[9] starting from L-rhamnose. The product was conventionally deacetylated and then treated with 2,2-dimethoxypropane[10] in the presence of *p*-TsOH. The resulting 2,3-*O*-isopropylidine derivative (**8**) was benzoylated to give the 4-*O*-benzoyl derivative **9**. Opening of the isopropylidine ring with 80% acetic acid, treatment of the product **10** with trimethyl orthobenzoate[11] and camphor-10-sulfonic acid followed by mild hydrolysis afforded the acceptor **11** (Scheme 2).

In a separate experiment, 3,4-di-*O*-acetyl-L-fucal (**12**) was prepared according to the standard method[12] from L-fucose. Azidonitration[13] of **12** followed by treatment[14] of the product (**13**) with sodium nitrite in dioxane-water (20:1) at 80°C gave the reducing 2-azido compound **14** which on treatment[15,16] with trichloroacetonitrile and potassium carbonate in dichloroethane afforded the β-trichloroacetimidate **15** (Scheme 3).



Scheme 3. (a) CAN, NaN_3; (b) $NaNO_2$, 1:20 H_2O-dioxane, 80°C; (c) Cl_3CCN, K_2CO_3.

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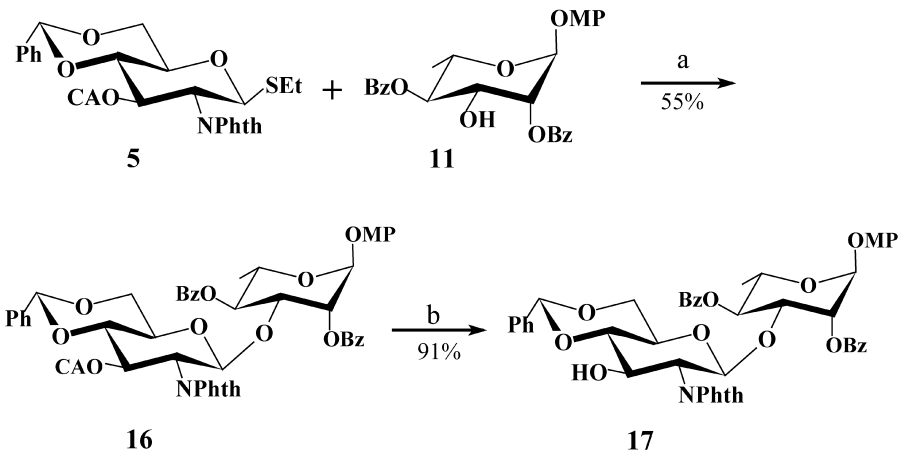
The thioglycoside donor **5** was allowed to react with the acceptor **11** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[17,18] in dichloromethane to afford the disaccharide derivative **16** in 55% yield (Scheme 4). The compound **16** was characterized by its signals at δ 3.70 ($ClCH_2CO$), 1.02 (CCH_3) in the ^1H NMR spectrum and at δ165.6, 165.1 (2*C*OPh), 163.9 (*C*OCH_2Cl), 100.6 (*C*HPh), 97.9 (C-1^{II}), 95.3 (C-1^{I}), 54.3 (O*C*H_3), 53.9 (C-2^{II}), 39.2 (CO*C*H_2Cl), and 16.5 (C*C*H_3) in its ^{13}C NMR spectrum. The chloroacetyl group of **16** was removed[19] with thiourea in the presence of excess sodium bicarbonate to give the acceptor **17** (Scheme 4) which was characterized from its NMR signals for benzylidene, $CHCH_3$, OMe and two anomeric protons and carbons.

The disaccharide acceptor **17** was then allowed to react[20] with the trichloroacetimidate donor **15** in the presence of triethysilyl trifluoromethanesulfonate to give the trisaccharide derivative **18** as crystals in 62% isolated yield (Scheme 5). The formation of α-glycoside results from employing the nonparticipating azido group in the 2-position of **15**. The compound **18** was characterized from the signals for benzylidene, OMe, two acetyl groups, two $CHCH_3$ and three anomeric protons and carbons in its ^1H and ^{13}C NMR spectra.

Compound **18** was hydrogenolyzed with hydrogen and 10% Pd/C in the presence of acetic anhydride, conditions under which the azido group was converted[21] into an acetamido, with simultaneous removal of the benzylidene moiety to give **19**. Treatment of **19** with ethylenediamine in 1-butanol[22] followed by acetic anhydride/pyridine afforded the peracetate derivative **20** which could be purified by column chromatography. Compound **20** was characterized by the presence of OMe, three anomeric protons and carbons, two $CHCH_3$ and the appearance of two $NHCOCH_3$ groups in its NMR spectra. Conventional deacetylation of the acetate **20** gave the desired target trisaccharide repeating unit **21** of the antigen from *E. coli* O26. The final compound **21** was characterized from its NMR signals for two $CHCH_3$, OMe, two $NHCOCH_3$ and three anomeric protons and carbons.



Scheme 4. (a) NIS-TfOH; (b) Thiourea, $NaHCO_3$, 2:1 MeOH-CH_2Cl_2.

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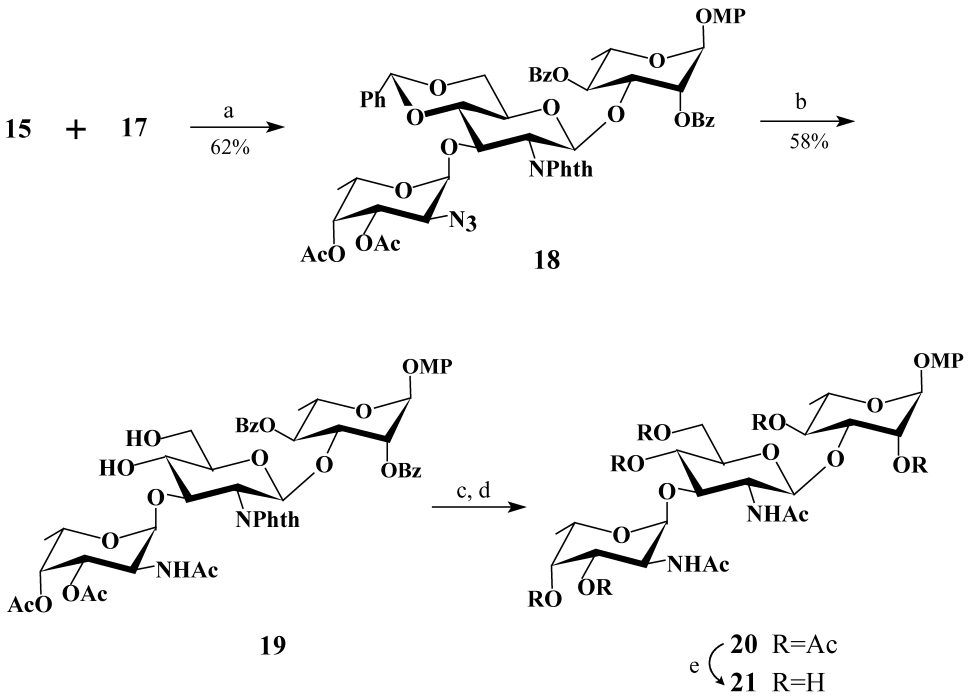

Scheme 5. (a) TESOTf, dichloroethane, −30°C, 30 min; (b) 10% Pd on charcoal, Ac_2O, EtOH; (c) i. Ethylenediamine, 1-butanol, 90°C, 20 h; (d) Ac_2O, Pyr; (e) 0.5 N NaOMe, MeOH.

EXPERIMENTAL

General. All reactions were monitored by TLC on silica gel G (E. Merck). Column chromatography was performed on 100–200 mesh silica gel (SRL, India). All solvents were distilled and/or dried before use and all evaporations were conducted below 50°C under reduced pressure unless stated otherwise. Optical rotations were measured with a Perkin Elmer model 241 MC polarimeter. The ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 Spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard unless otherwise mentioned. Melting points were determined on a paraffin oil bath and are uncorrected.

Ethyl 3,4,5-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2). Ethane thiol (5 mL, 67.6 mmol) and $BF_3.OEt_2$ (10 mL, 78.9 mmol) were added to a solution of 1,3,4,5-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**1**)[5] (10.6 g, 22.2 mmol) in CH_2Cl_2 (86 mL) with stirring. The reaction was monitored with TLC and after 22 h the solution was washed with water, saturated $NaHCO_3$ and water in succession, dried (Na_2SO_4) and concentrated to give **2** (6.2 g, 62.5%) in the form of fine crystals: mp 116°C (EtOAc-hexane); $[\alpha]_D^{25}$ + 43.07 (*c* 1.7, $CHCl_3$). ^1H NMR: δ 7.80, 7.68 (2m, 4H, phthalimido protons), 5.76 (t, 1H, J = 9.9 Hz, H-3), 5.41 (d,1H, $J_{1,2}$ = 10.5 Hz, H-1), 5.11 (t,1H, J = 9.6 Hz, H-4), 4.33 (t, 1H, J = 10.5

Hz, H-2), 4.25, 4.11 (2m, 2H, H-6); 3.82 (m, 1H, H-5), 2.61 (m, 2H, SCH_2CH_3) 2.04, 1.96, 1.79 (3s, 9H, $3OCOCH_3$), 1.15 (t, 2H, J = 7.2 Hz, SCH_2CH_3).

Ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3). Compound **2** (3 g, 6.7 mmol) was deacetylated in the usual way with 0.05 N sodium methoxide in methanol (25 mL) for 3 h. Column chromatography (EtOAc) of the product gave pure **3** (1.49 g, 69.5%) as an amorphous solid $[\alpha]_D^{25} + 2.87$ (*c* 15.7, $CHCl_3$).

Anal. Calcd for $C_{16}H_{19}O_6NS$: C, 54.38; H, 5.42. Found: 54.25, H, 5.21.

Ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4). A solution of compound 3 (3 g, 9.4 mmol) in acetonitrile (40 mL) was stirred at room temperature with α,α-dimethoxytoluene (2 mL, 13.1 mmol) and MS 3Å (4 g) for 30 min. *p*-Toluenesulfonic acid (*p*-TsOH, 300 mg) was then added and stirring was continued overnight. TLC showed about 80% conversion. The reaction was quenched with Et_3N and the solution was concentrated to a syrup. Column chromatography (5:I toluene-EtOAc) gave compound 4 (3.2 g, 77.5%) as white foam. $[\alpha]_D^{25} - 4.98°$ (*c* 1.2, $CHCl_3$). ^1H NMR: δ 7.82, 7.76 (2m, 4 protons of phthalimido), 7.45—7.31 (m, other aromatic protons), 5.51 (s, C*H*Ph), 5.36 (d, J = 10.5 Hz, H-1), 4.62 (t, J = 10.0 Hz, H-3), 4.35 (m, H-4), 4.28 (t, J = 10.4 Hz, H-2), 3.76, 3.66, 3.56 (m, 3H, H-5, H-6), 2.63 (m, SCH_2CH_3), 1.13 (t, J = 7.4 Hz, SCH_2CH_3).

Anal. Calcd for $C_{23}H_{23}O_6NS$: C, 62.57; H, 5.25. Found: 62.74, H, 5.31.

Ethyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (5). To a solution of compound **4** (317 mg, 0.72 mmol) in dichloromethane (3 mL) at 0°C, triethylamine (0.5 mL, 5 eq) and chloroacetic anhydride (0.36 g, 2.16 mmol) were added and the mixture was stirred for 3 h when TLC showed completion of the reaction. The reaction mixture was then diluted with CH_2Cl_2, washed with water, saturated $NaHCO_3$ solution and water in succession and dried (Na_2SO_4). The organic layer was concentrated and the syrupy product on column chromatography with 7:1 toluene-EtOAc gave **7** (310 mg, 83%) which crystallized from ethyl ether: mp 153–155°C; $[\alpha]_D^{25} - 8.87°$ (*c* 1.6, $CHCl_3$). ^1H NMR: δ 7.84, 7.74 (2m, 4 H, phthalimido protons), 7.73-7.15 (other aromatic protons), 6.01(t, $J_{2,3} = J_{3,4} = 9$ Hz, H-3), 5.57 (d, $J_{1,2} = 10.7$ Hz, H-1), 5.35 (s, 1H, C*H*Ph) 4.45 (m, 1H, H-2), 4.23 (m, 1H, H-2), 3.71 (s, 2 H, $COCH_2Cl$), 2.49 (m, SCH_2CH_3), 1.03 (t, J = 7.4 Hz, SCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 167.1 ($COCH_2Cl$), 137.1 – 124.1 (aromatic carbons), 102.1 (C_6H_5CH), 82.2 (C-1), 79.5, 72.6, 70.9, 69.0, 54.3 (C-2) 40.7 ($ClCH_2CO$), 24.8 (SCH_2CH_3), and 15.3 (SCH_2CH_3).

Anal. Calcd for $C_{25}H_{24}O_7NS$: C, 57.97; H, 4.67. Found: C, 57.79, H, 4.81.

4-Methoxyphenyl 2,3,4-tetra-*O*-acetyl-α-L-rhamnopyranoside (6). The compound **6** was prepared from L-rhamnose tetraacetate in 72% yield according to the known[9] method; $[\alpha]_D - 65.0°$ (*c* 1.4, $CHCl_3$). ^1H NMR: δ 6.95, 6.75 (2d, 4H, aromatic protons), 5.43 (dd, 1H, $J_{2,3} = 3.5$ Hz, $J_{3,4} = 10.0$ Hz, H-3), 5.35 (dd, 1H, $J_{2,3} = 3.5$ Hz, $J_{1,2} = 1.8$ Hz, H-2), 5.27 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1), 5.07 (t, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 3.95 (m, 1H, H-5), 3.7 (s, 3H, OCH_3), 2.11, 1.99, 1.95 (3s, 9H, $3COCH_3$), 1.14 (d, 1H, J = 6.2 Hz, H-6).

Anal. Calcd for $C_{17}H_{21}O_7$: C, 63.54; H, 6.59. Found: C, 63.83, H, 6.70.

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4-Methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (8). Compound **6** (4 g, 10.1 mmol) was de-*O*-acetylated as described for compound **5** to give 4-methoxyphenyl α-L-rhamnopyranoside (**7**, 2.58 g, 94.5%). To a solution of **7** (5 g, 18.5 mmol) dissolved in DMF (20 mL), 2,2-dimethoxypropane (32 mL) and *p*-TsOH (250 mg) were added and the mixture was stirred at room temperature for 5 h. The reaction was then quenched with NEt_3 and the solvents were removed under reduced pressure. Column chromatography with 4:1 toluene-EtOAc gave **8** (4.2g, 73%); mp 62°C (Et_2O-pet ether); $[\alpha]_D - 49.7°$ (*c* 1.1, $CHCl_3$). ^1H NMR signals at δ 7.0–6.8 (m, 4H, aromatic protons), 5.62 (bs, 1H, H-1), 4.36 (d, $J_{2,3}$ = 5.7 Hz, H-2), 4.24 (t, $J_{2,3}$ = 5.7 Hz, H-3), 3.78 (m, 1H, H-4), 3.76 (s, O-CH_3), 3.49 (dd, 1H, J = 1.8 Hz, J = 7.5 Hz, H-5), 1.57 (2s, 6H, CMe_2),1.26 (d, J = 6.3 Hz,C-CH_3).

Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.14. Found: C, 61.79; H, 7.31.

4-Methoxyphenyl 4-*O*-benzoyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (9). Compound **8** (4.7 g, 15.2 mmol) was dissolved in pyridine (25 mL) and benzoyl chloride (4.4 mL) was added with a syringe while cooling the reaction mixture at 0°C. The reaction mixture was then stirred at room temperature for 4 h when TLC (3:1 toluene-Et_2O) showed completion of the reaction. Water (1.2 mL) was added to decompose the excess benzoyl chloride and the mixture was concentrated and co-evaporated thrice with toluene. The product was then dissolved in CH_2Cl_2 (50 mL) and the solution was washed with water, dried (Na_2SO_4) and concentrated to a syrup. Column chromatography with 3:1 toluene-Et_2O gave pure **9** (5.8 g, 92%) which crystallized from hot ethanol (mp 88–90°C). $[\alpha]_D^{25} - 1.03°$ (*c* 1.6, $CHCl_3$); ^1H NMR: δ 8.21–6.83 (m, 9H, aromatic protons), 5.68 (s, 1H, H-2) 5.19 (dd, 1H, $J_{3,4}$ = 7.5 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 4.51 (dd, 1H, $J_{3,4}$ = 7.8 Hz, $J_{2,3}$ = 5.4 Hz, H-3), 4.42 (d, 1-H, $J_{2,3}$ = 5.4 Hz, H-2), 4.04 (m,1H, H-5), 3.78 (s, 3H, OCH_3) 1.66, 1.40 (2s, 6H, CMe_2), 1.18 (d, J = 6.3 Hz, H-6).

Anal. Calcd for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32. Found: C, 66.47, H, 6.21.

4-Methoxyphenyl 4-*O*-benzoyl-α-L-rhamnopyranoside (10). Compound **9** (5 g, 12.1 mmol) was stirred with 80% AcOH (35 mL) at 80°C for 2 h, when TLC showed one major spot. The reaction mixture was concentrated to a syrup which on column chromatography with 3:1 toluene-EtoAc gave **10** (3.77 g, 83.5%) as amorphous solid. $[\alpha]_D^{25} - 101.8°$ (*c* 1.6, $CHCl_3$).

Anal. Calcd for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 64.39, H, 6.11.

4-Methoxyphenyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (11). Compound **10** (2.65 g, 7.09 mmol) in dry CH_2Cl_2 (27 mL) was treated with trimethyl orthobenzoate (6 mL) and (±) camphor-10-sulfonic acid (catalytic amount) with stirring. After 15 min TLC (3:1 toluene-EtOAc) showed a single faster moving spot and the reaction was quenched by adding a few drops of NEt_3. The solution was concentrated to a syrup and treated with 80% aqueous acetic acid (52 mL) at room temperature for 30 min. The solution was then concentrated and the resulting syrupy product was purified by column chromatography with 3:1 toluene-EtOAc to afford compound **11** (2.68 g) which crystallized from hot ethanol: mp 112°C; $[\alpha]_D - 18.1°$ (*c* 2.8, $CHCl_3$). ^1H NMR: δ 8.10–6.83 (m, aromatic protons, 14 H), 5.58 (bs, 1H, H-1), 5.56 (m, 1H, H-2), 5.34 (t, J = 9.8 Hz, H-4), 4.52 (dd, $J_{2,3}$ = 3.26 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 4.23 (m,

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1H, H-5), 3.78 (s , 3H, OCH_3), 1.32 (d, J = 6.2 Hz, CCH_3), ^{13}C NMR δ: 167.5, 166.4 (2 $COCH_3$), 155.7–115.1 (aromatic carbons), 97.0 (C-1), 75.9, 73.5, 69.3. 67.3, 56.1 (OCH_3), 18.1 (CCH_3).

Anal. Calcd for $C_{27}H_{26}O_8$: C, 67.78; H, 5.48. Found: C, 67.94, H, 5.31.

2-Azido-3,4-di-*O*-acetyl-2-deoxy-α-L-fucopyranosyl nitrate (13). To a solution of **12** (800 mg, 3.75 mmol) in acetonitrile (20 mL) at −15°C was added sodium azide (0.36 gm, 5.53 mmol) and ceric ammonium nitrate (7.4 g, 13.5 mmol). The suspension was vigorously stirred for 10 h when TLC showed completion of the reaction. The mixture was diluted with cold ethyl ether and washed with ice-water. The organic layer was concentrated and the resulting syrupy product was column chromatographed (5:1 toluene-EtOAc) to afford compound **13** (620 mg, 52%) which crystallized from ether (mp 114–116°C); $[\alpha]_D^{25} - 126.7°$ (*c* 0.8, $CHCl_3$). ^1H NMR: δ 6.32 (d,1H, $J_{1,2}$ = 4.2 Hz, H-1), 5.35 (m, I H, H-4), 5.28 (dd,1H, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 4.31 (m, 1H, H-5), 4.09 (dd, 1H, $J_{1,2}$ = 4.16 Hz, $J_{2,3}$ = 11.2 Hz, H-2), 2.19, 2.07 (2s, 6H, $2COCH_3$), 1.22 (d, J = 6.4 Hz, CCH_3); I.R: 2131 cm^{-1} (N_3).

Anal. Calcd for $C_{10}H_{14}O_8N_4$: C, 41.96; H, 4.93. Found: C, 42.02, H, 4.77.

3,4-Di-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranose (14). To a solution of compound **13** (90 mg, 283 mmol) in 20:1 dioxane-water (2.1 mL), sodium nitrite (42.6 mg, 0.62 mmol) was added. The mixture was stirred at 80°C for 20 h when TLC showed only a trace of the starting compound. Water (25 mL) was added and the mixture was extracted with CH_2Cl_2 (15 mL × 3). The extract was dried (Na_2SO_4), filtered and the filtrate was concentrated to dryness. Column chromatography (3:1 toluene-EtOAc) gave **14** (60 mg, 88%) as a syrup.

Anal. Calcd for $C_{10}H_{15}O_6N_3$: C, 43.95; H, 5.53. Found: C, 44.12, H, 5.72.

2-Azido-3,4-di-*O*-acetyl-2-deoxy-α-L-fucopyranosyl trichloroacetimidate (15). To a solution of **14** (68 mg, 0.25 mmol) in dichloroethane (1 mL), potassium carbonate (91 mg, .68 mmol) was added followed by the addition of trichloroacetonitrile (0.13 mL, 1.2 mmol) with vigorous stirring under N_2. The reaction was complete in 3.5 h (TLC) after which the mixture was filtered through Celite and washed with CH_2Cl_2 (5 mL). The filtrate was concentrated to afford **15** (83.5 mg, 80%) as a syrup. ^1H NMR: δ 8.76 (s, 1H, C = N*H*), 5.69 (d, 1H, J = 8.4 Hz, H-1), 5.25 (d, 1H, $J_{3,4}$ = 3.27 Hz H-4), 4.89 (dd, 1H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 3.99 (m, 1H, H-2), 3.89 (m, 1H, H-5), 2.18, 2.07 (2s, 6H, $2COCH_3$), 1.25 (d, 3H, J = 6.33 Hz, CCH_3).

4-Methoxyphenyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (16). To a solution of the donor **5** (390 mg, 0.75 mmol) and the acceptor **11** (300 mg, 0.63 mmol) in CH_2Cl_2 (12 mL), 4Å MS (3 g) were added and the mixture was stirred overnight at room temperature under N_2. The mixture was then cooled to −25°C and NIS (203 mg, 0.90 mmol) was added. After 15 min TfOH (9.5 μL, 0.11 mmol) was introduced and stirring was continued. After 45 min the acceptor was completely consumed, and the reaction mixture was filtered through a Celite bed and washed with

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CH_2Cl_2. The combined filtrate and washings were washed successively with 5% $Na_2S_2O_3$ solution, aqueous saturated $NaHCO_3$ and water, dried (Na_2SO_4), concentrated and purified by column chromatography with 12:1 toluene-Et_2O to afford the disaccharide **16** as a foam (300 mg, 54.9%); $[\alpha]_D^{25} - 5.5°$ (*c* 0.9, $CHCl_3$). ^{1}H NMR: δ 8.08–6.75 (23 H, aromatic protons), 5.74 (m, 1H, H-3^{II}), 5.60 (d, $J_{1,2}$ = 8.1 Hz, H-1^{II}), 5.57 (m, 1H, H-4^{I}), 5.34 (bs, 1H, C*H*Ph), 4.44 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.4 Hz, H-3^{I}), 4.33 (m, 1H, H-4^{II}), 4.18 (dd, 1H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 10.2 Hz, H-2^{II}), 3.97 (m, 1H, H-5^{I}), 3.70 (s, 2H, ClCH_2CO), 3.68 (s, 3H, OCH_3), 3.60 (m, 1H, H-6^{II}), 3.56 (m, 2H, H-5^{II}), 1.02 (d, $J_{5,6}$ = 6.6 Hz, H-6^{I}). ^{13}C NMR: δ 165.6,165.1 (2O*C*OPh), 163.9 (ClCH$_2$*C*O), 154.2–113.7 (aromatic carbons), 100.6 (*C*HPh), 97.9 (C-1^{II}), 95.3 (C-1^{I}), 77.6, 74.9, 71.4, 71.0, 70.3, 67.4, 65.9, 64.9, 54.3 (O*C*H$_3$), 53.9 (C-2^{II}), 39.2 (Cl*C*H$_2$CO), 16.5 (C-6^{I}).

Anal. Calcd for $C_{50}H_{44}O_{15}NCl$: C, 64.27; H, 4.75. Found: C, 64.42, H, 4.87.

4-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (17). Thiourea was added to a solution of **16** (40 mg, 0.046 mmol) in 2:1 CH_3OH-CH_2Cl_2 (0.9 mL) and excess $NaHCO_3$ (80 mg) was added. The mixture was stirred at 25°C for 1 h (TLC) and the solution was concentrated. The residue was treated with CH_2Cl_2 and washed with water, dried (Na_2SO_4) and concentrated. Column chromatography (5:1 toluene-EtOAc) of the residue afforded compound **17** (32 mg, 91%) as a foam; $[\alpha]_D^{25} - 25.9°$ (*c* 1.0, $CHCl_3$). ^{1}H NMR: δ 8.15–6.82 (23H, aromatic protons), 5.63 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, H-2^{I}), 5.54 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1^{I}), 5.48 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1^{II}), 5.43 (s, 1H, C*H*Ph), 5.42 (t, 1H, J = 9.7 Hz, H-4^{I}), 4.51 (m, 1H, H-3^{I}), 4.47 (m, 1H, H-3^{II}), 4.33 (m, 1H, H-4^{II}), 4.14 (dd, 1H, $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = 10.5 Hz, H-2^{II}), 4.04 (m, 1H, H-5^{II}), 3.77 (s, 2H, ClCH_2CO), 3.76 (s, 3H, OCH_3), 3.58 (m, 2H, H-6^{II}), 3.41 (m, 1H, H-5^{II}), 1.1 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6^{I}). ^{13}C NMR: δ 166.6, 165.3 (2 CO of OBz), 155.6–115.1 (aromatic carbons), 102.2 (*C*HPh), 99.7 (C-1^{II}), 96.8 ((C-1^{I}), 82.2, 76.1, 72.9, 72.6, 68.85, 68.7, 67.3, 66.4, 56.8 (C-2^{II}), 56.1 (O*C*H$_3$), 17.9 (C-6^{I}).

Anal. Calcd for $C_{48}H_{43}O_{14}N$: C, 67.20; H, 5.05. Found: C, 67.02, H, 5.17.

4-Methoxyphenyl 2-azido-3,4-di-*O*-acetyl-2-deoxy-α-L-fucopyranosyl-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (18). A solution of **17** (210 mg, 0.27 mmol) and **15** (260 mg, 0.63 mmol) in CH_2Cl_2 (3 mL) was stirred in the presence of MS 4Å (1 g) under Ar for 1 h. The mixture was then cooled to − 30°C and a solution of TESOTf (6 μL, 0.027 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise. The reaction was allowed to proceed at − 30°C for 30 min when TLC showed the disappearance of the donor **22**. The reaction was quenched with the addition of Et_3N and the mixture was filtered through a Celite bed. The filtrate was concentrated and the syrupy product was purified by column chromatography with10:1 toluene-Et_2O to afford **18** (175 mg, 62.2%) which crystallized as fine needles: mp 148°C (ethanol); $[\alpha]_D^{25} - 29.4°$ (*c* 1, $CHCl_3$). ^{1}H NMR: δ 8.15–6.83 (aromatic protons), 5.60 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.5 Hz, H-2^{I}), 5.54 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1^{III}), 5.42 (s, 2H, H-1^{I}, C*H*Ph), 5.40 (d, 1H, J = 8.6 Hz, H-1^{II}), 4.99 (bs, 1H, H-4^{III}), 4.96 (m, 1H, H-4^{I}), 4.37 (dd, 1H,

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J = 4.0 Hz, J = 9.8 Hz, H-2^{II}), 4.22 (dd, 1H, J = 10.4 Hz, J = 8.4 Hz, H-4^{II}), 3.78 (s, 3H, OCH_3), 3.33 (m, 1H, H-5^{II}), 1.95, 1.86 (2s, 6H, $2COCH_3$), 1.08 (d, 3H, J = 6.3 Hz, H-6^{I}), 0.33 (d, 3H, J = 6.6 Hz, H-6^{III}). ^{13}C NMR: δ 169.2, 168.5 (CO of 2OAc), 165.2, 163.9 (CO of 2 OBz), 101.3 (*C*HPh), 98.3 (C-1^{II}), 97.6, 95.3 (C-1^{I}, C-1^{III}), 79.4, 74.1, 71.6, 71.1, 69.5, 68.3, 65.9, 65.4, 64.0 (C-2^{III}), 56.6 (C-2^{II}), 54.6 (O*C*H_3), 19.5, 19.4 ($2CO$*C*H_3), 16.5 (C-6^{I}), 13.7 (C-6^{III}).

Anal. Calcd for $C_{58}H_{56}O_{19}N_4$: C, 63.86; H, 5.27. Found: C, 64.02, H, 5.40.

4-Methoxyphenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-L-fucopyranosyl-(1 → 3)-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (19). A solution of **18** (50 mg, 0.05 mmole) in aldehyde free ethanol (4 mL) containing acetic anhydride (0.2 mL) was stirred with 10% Pd on charcoal under hydrogen for three days when all the starting material was transformed into a slower moving compound as observed in the TLC (EtOAc). The mixture was filtered through a Celite bed, the filtrate was concentrated to a syrupy product which on column chromatography with 3:1 EtOAc-toluene gave pure **19** (27 mg, 58%); $[\alpha]_D^{25}$ −46.1 (*c* 0.9, $CHCl_3$). ^{1}H NMR: δ 8.17–6.82 (18H, aromatic protons), 5.92 (d, IH, J = 3.6 Hz, N-H), 5.49 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1^{II}), 5.43 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1^{I}), 5.39 (t, 1H, J = 9.8 Hz, H-4^{I}), 5.12 (d, 1H, J = 2.3 Hz, H-2^{I}), 4.94 (m, 1H, H-4^{II}), 4.67 (d, 1H, J = 3.7 Hz, H-1^{III}), 4.44 (dd, 1H, J = 3.7 Hz, H-2^{II}), 2.08, 1.87 (2s, 6H, $2OCOCH_3$), 1.1 (d, 1H, J = 2.1 Hz, H-6^{I}), 1.08 (d, 1H,J = 2.4 Hz, H-6^{III}). ^{13}C NMR: δ 170.8, 170.5 (2 *C*OPh), 169.8, 167.0 (2 *C*OCH_3) 164.8 (NH*C*OCH_3), 155.2, 149.9, 134.1, 133.7, 132.9, 130.6, 130.2, 129.5, 129.0, 128.7, 128.3, 128.2, 117.7, 114.7 (aromatic carbons), 98.9 (C-1^{II}), 98.7 (C-1^{III}), 96.4 (C-1^{I}), 81.2, 76.4, 76.0, 72.5, 71.9, 70.1, 70.0, 88.3, 66.9, 66.4, 62.0, 55.7 (O*C*H_3), 55.0 (C-2^{II}), 47.7 (C-2^{III}), 22.10, 20.64, 20.62 ($3CO$*C*H_3), 17.56 (C-6^{1}), 15.96 (C-6^{III}).

Anal. Calcd for $C_{53}H_{56}O_{20}N_2$: C, 61.15; H, 5.42. Found: C, 61.32, H, 5.49.

4-Methoxyphenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-L-fucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 3)-2,4-di-*O*-acetyl-α-L-rhamnopyranoside (20). Ethylenediamine (0.5 mL) was added to a solution of compound **19** (27 mg, 0.026 mmol) in 1-butanol (2.5 mL) under argon. The solution was stirred for 20 h at 90°C when TLC (EtOAc) indicated completion of the reaction. The solvents were evaporated and the residue was coevaporated twice with toluene. The product was treated with pyridine (0.5 mL) and Ac_2O (0.5 mL) for 20 h when TLC (EtOAc) showed one major spot. The reaction mixture was concentrated under reduced pressure followed by coevaporation with toluene to remove trace reagents. Column chromatography (3:1 EtOAc-toluene) then gave compound **20** (12 mg, 50% overall); $[\alpha]_D$ −48.6° (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 6.97, 6.82 (2d, 4H, J = 9 Hz, aromatic protons of 4-methoxyphenyl), 5.95 (d, IH, J = 7.3 Hz, NH), 5.82 (d, IH, J = 9.1 Hz, NH), 5.34 (bs, 2H, H-1^{I}, H-1^{III}), 5.17 (m, 1H, H-2^{I}), 5.12 (d, 1H, J = 7.9 Hz, H-1^{II}), 4.89 (dd, 1H, J = 9.5 Hz, J = 9.3 Hz, H-4^{I}), 2.17, 2.12, 2.09, 2.07, 2.01, 2.00 (8s, 24H, $6OCOCH_3$, 2 $NHCOCH_3$), 1.16 (d, 3H, J = 6.2 Hz, H-6^{I}), 1.10 (d, 3H, J = 6.4 Hz, H-6^{III}). ^{13}C NMR: δ 170.2, 170.1, 170.0, 169.7, 169.6, 169.3, 169.2, 168.9 (8 *C*OCH_3), 154.3, 148.9, 116.8, 113.7 (aromatic carbons), 98.4 (C-1^{II}), 96.6 (C-1^{III}), 95.4 (C-1^{I}), 77.1, 74.0, 73.5, 71.6, 70.9, 70.7, 69.6, 69.4, 67.3, 65.8, 64.8, 61.4 (C-6^{II}),

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57.5 (C-2^{II}), 54.6 (O*C*H_3), 47.3 (C-2^{III}), 28.7, 22.6, 22.3, 21.7, 20.1, 20.0, 19.8, 19.7 (8 CO*C*H_3), 16.5 (C-6^{I}), 14.4 (C-6^{III}).

Anal Calcd for $C_{41}H_{56}O_{21}N_2$: C, 53.94%; H, 6.18%. Found C, 54.1; H, 6.35.

4-Methoxyphenyl 2-acetamido-2-deoxy-α-L-fucopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside (21). The acetate **20** (16.0 mg, 0.0175 mmol) was treated with 0.05 M NaOMe in methanol (5 mL) as described in the case of compound **3**. The deacetylated product was dissolved in water and filtered through Sep-Pak C-18 cartridge and concentrated to dryness to afford pure **21** (10.6 mg, 91.4%); $[\alpha]_D^{25} - 90.7°$ (*c* 0.7, water). ^{1}H NMR: δ 6.99, 6.87 (2d, 4H, J = 8.8 Hz, aromatic protons of 4-methoxyphenyl), 5.31 (s, IH, H-1^{I}), 4.92 (d, 1H, J = 3.8 Hz, H-1^{III}), 4.68 (bs, 1H, H-2^{I}), 4.56 (d, 1H, J = 8.4 Hz), 4.25 (m, 1H, H-4^{III}), 3.99 (dd, J = 3.8 Hz, J = 11.1 Hz, H-2^{III}), 3.86 (dd. 1H, J = 2.9 Hz, J = 9.8 Hz, H-3^{I}), 3.69 (s, 3H, O*C*H_3), 3.60 (t, 1H, J = 8.9 Hz, H-2^{II}), 1.93, 1.86 (2s, 6H, 2 NHCOCH_3), 1.10, 1.07 (2d, 6H, J = 6.2 Hz, H-6^{I}, H-6^{III}). ^{13}C NMR: δ 174.80, 174.77 (2NH*C*OCH$_3$), 155.22, 149.75, 119.36, 115.60 (aromatic carbons), 103.40 (C-1^{II}), 99.30 (C-1^{III}), 98.17 (C-1^{I}), 80.50, 78.89, 76.01, 71.60, 71.28, 70.17, 68.74, 68.16, 67.28, 60.93 (C-6^{II}), 56.27 (C-2^{II}), 56.11 (O*C*H$_3$), 49.91 (C-2^{III}), 22.69, 22.58 (2NHCO*C*H_3), 16.97 (C-6^{I}), 15.72 (C-6^{III}).

Anal. Calcd for $C_{29}H_{44}O_{15}N_2$: C, 52.72%; H, 6.71%. Found C, 52.51; H, 6.95.

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