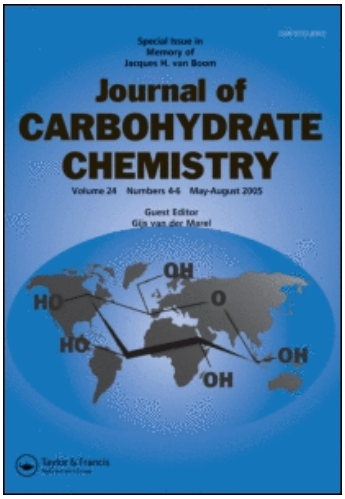

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Synthesis of a Trifucosyl Ley Heptasaccharide Corresponding to a Tumor-Associated Glycolipid

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SYNTHESIS OF A TRIFUCOSYL Le^y HEPTASACCHARIDE CORRESPONDING TO A TUMOR-ASSOCIATED GLYCOLIPID.

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ABSTRACT

The trifucosyl Le^y derivative, 2-(*p*-trifluoracetamidophenyl)ethyl *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, was synthesized from thioglycoside building blocks. A two plus three condensation gave a linear pentasaccharide derivative which was difucosylated and deprotected to give the target structure.

INTRODUCTION

In a report by Hakomori *et al.*[1] a series of glycolipids from human colonic adenocarcinoma cells, defined by the monoclonal antibody AH6, were isolated and characterized. One major component, common in all studied cases, was identified as the glycolipid shown in Figure 1. Its absence or low abundance in extracts from normal tissues indicated that the structure was tumor related.

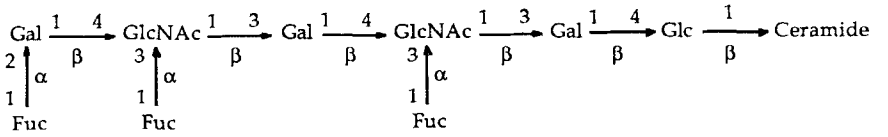


Figure 1

As part of a program aimed at synthesizing tumor-associated carbohydrate derivatives, we have earlier reported on synthesis of structures containing the Le^x-determinant.[2-5] Reports have also been published by Lönn,[6] Ogawa[7,8] and Nicolaou.[9] Syntheses of compounds having the Le^y-determinant have also been described.[10-13]

We now report on the synthesis of the heptasaccharide **13**, containing the Le^x- and the Le^y-determinant, which is a partial structure of the glycolipid in Figure 1.

RESULTS AND DISCUSSION

The synthesis was performed using the same kind of strategy as in the syntheses of the di- and trimeric Le^x derivatives.[2,4] Our initial strategy was to synthesize a suitably protected type 2 chain,[14,15] which after selective deblocking was intended for trifucosylation. However, in practice it turned out to be easier to monofucosylate the disaccharide **5** in the 2′-position and then condense the obtained trisaccharide **6** with the disaccharide **7**.[2] The obtained pentasaccharide **8** was then difucosylated giving the protected heptasaccharide **10**, which was deblocked giving the target compound **13**. The following steps were performed:

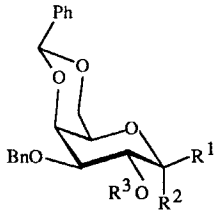
Ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[16] was selectively benzylated in the 3-position using benzyl bromide and sodium hydride giving **1** in 48% yield. Acetylation of **1** using acetyl chloride and pyridine gave compound **2** in 98% yield. Treatment of **2** with bromine gave the bromo sugar **3**, which was used directly in glycosidation with ethyl 6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside.[2] The coupling reaction was performed using silver triflate as promoter and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as acid acceptor, giving the disaccharide **4** in 84% yield.

Deacetylation of **4** with sodium methoxide in dichloromethane-methanol gave the 2′-OH compound **5** in 80% yield. Glycosidation of **5** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide,[17] using silver triflate-collidine as promoter, gave the trisaccharide **6** in 95% yield.

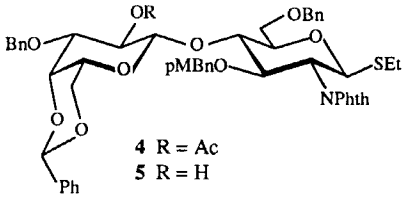
Glycosidation of 2-(*p*-nitrophenyl)ethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside**7** with compound **6** using dimethyl(methylthio)sulfonium triflate (DMTST)[18] as promoter and DTBMP as acid acceptor gave the pentasaccharide **8** in 80% yield.

The two *p*-methoxybenzyl groups in **8** were removed by ceric ammonium nitrate (CAN)-oxidation giving the diol **9** in 72% yield. Difucosylation of **9** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[17] promoted by silver triflate-collidine gave the heptasaccharide **10** in 81% yield.

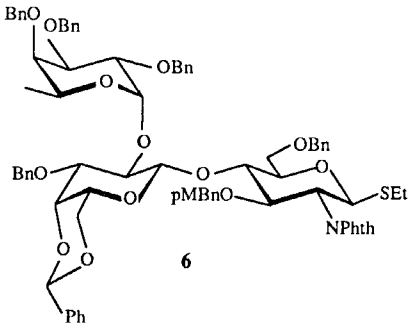
The phthalimido groups and the *O*-acetyl group in **10** were removed by refluxing with hydrazine acetate in toluene-ethanol 1:1. The obtained amino groups were *N*-acetylated using acetic anhydride in dichloromethane-methanol 1:1 giving compound **11** in 84% yield. Reduction of the nitro group in **11** with aluminum amalgam, followed by subsequent treatment with trifluoroacetic anhydride and methanolic sodium methoxide gave compound **12** in 75% yield. Hydrogenolysis of **12** over Pd/C gave the target compound **13** in 91% yield.



1 R^1 = SEt, R^2 = H, R^3 = H
2 R^1 = SEt, R^2 = H, R^3 = Ac
3 R^1 = H, R^2 = Br, R^3 = Ac



4 R = Ac
5 R = H



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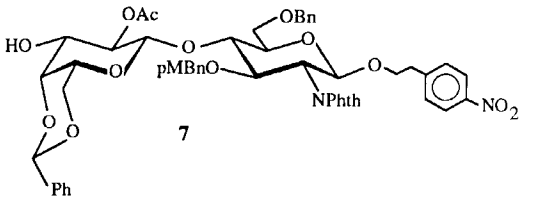

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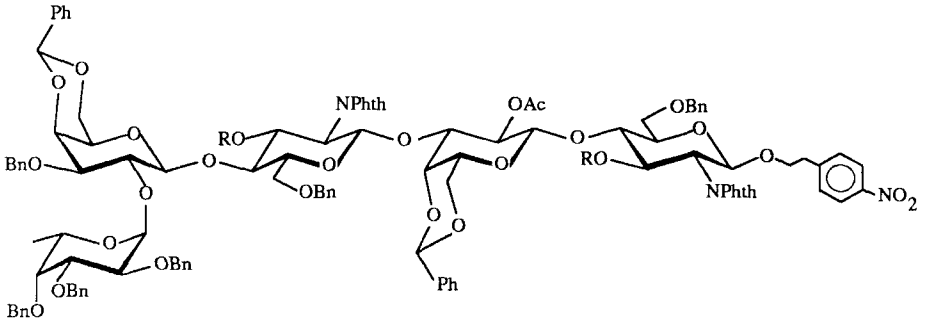
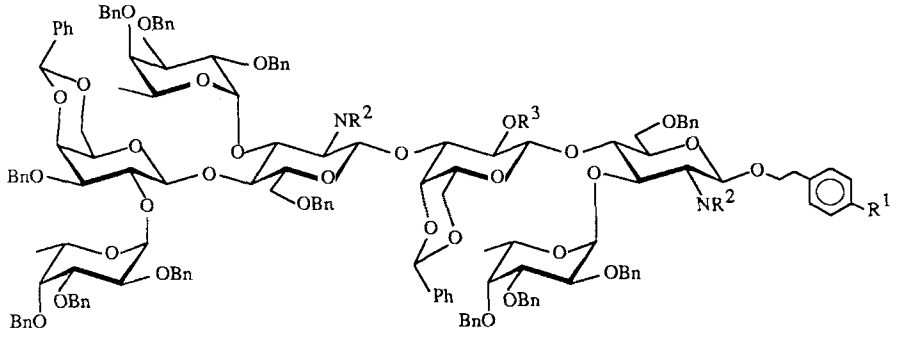
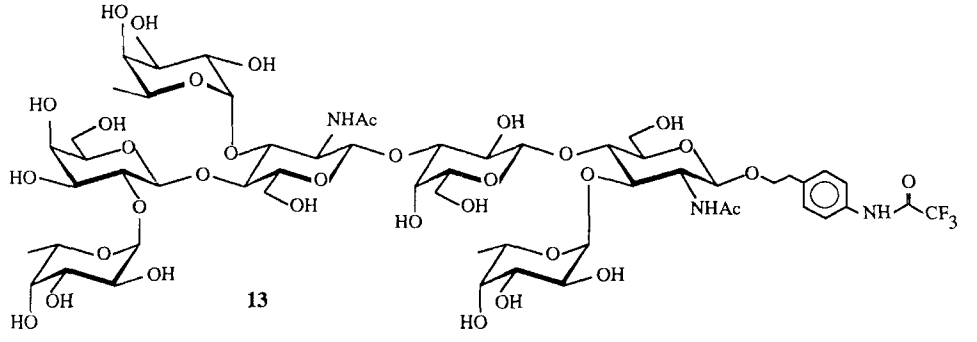


8 R = pMBn
9 R = H



10 $R^1 = NO_2$, R^2 = Phth, R^3 = Ac
11 $R^1 = NO_2$, R^2 = HAc, R^3 = H
12 $R^1 = NHCOCF_3$, R^2 = HAc, R^3 = H



13

EXPERIMENTAL

General methods. Melting points are corrected. Concentrations were performed under reduced pressure at < 40 °C (bath). Optical rotations were recorded for 0.3-1.0% solutions at room temperature (22-25 °C) using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25 °C for solutions in $CDCl_3$, using JEOL GX-270 and Bruker AM 500 MHz instruments, and chemical shifts are given in ppm relative to internal tetramethylsilane, unless otherwise stated. All ^1H assignments were based on 2D experiments. NMR spectra recorded for all new compounds, were in agreement with the postulated structures, and only selected data are reported. For some compounds ^1H shift values and coupling constants (values in parentheses) are given in table form. In these tables the sugar residues are given as GlcNA, GlcNB, GalA, GalB, FucA, FucB1 and FucB2, where A and B designations are arbitrary. TLC was performed on Silica Gel F_{254} HPTLC (Merck) with detection by UV and/or by charring with sulfuric acid. Column chromatography was performed on silica gel (Matrex Silica Si 60A, 35-70μ, Amicon). Organic solutions were dried over magnesium sulfate. Molecular sieves (4Å, Fluka) were desiccated at 300 °C overnight. Elemental analyses were not obtained for some amorphous compounds. These were purified by column chromatography and the purity was ascertained by HPTLC (in two different systems) and by NMR spectroscopy.

Ethyl 3-*O*-Benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (1). Sodium hydride (450 mg, 80%, 15 mmol) was added to a solution of ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[16] (4.60 g, 14.7 mmol) and benzyl bromide (1.4 mL, 12 mmol) in dry *N,N*-dimethylformamide (130 mL). The reaction mixture was stirred at room temperature and after 1 h the mixture was partitioned between toluene and aqueous sodium hydrogencarbonate. The organic layer was washed with water, dried and concentrated. Crystallization from ethyl acetate-isooctane gave **1** (2.28 g, 5.66 mmol, 48%), $[\alpha]_{578}$ +9° (*c* 0.4, chloroform), R_F 0.45 (toluene-ethyl acetate 2:1), mp 158-159 °C, NMR data: ^{13}C, δ 15.3 (Me ethyl), 23.0 (CH_2S), 68.0-80.3 (C-2, 3, 4, 5, 6), 85.3 (C-1), 101.2 (PhCH), 126.4-138.1 (aromatic C); ^1H, δ 3.42 (m, H-5), 3.50 (dd, $J_{2,3}$=9.4 Hz, $J_{3,4}$=3.4 Hz, H-3), 3.98 (dd, $J_{5,6a}$=1.7 Hz, $J_{6a,6b}$=12.5 Hz, H-6a), 4.07 (m, $J_{1,2}$=9.4 Hz, $J_{OH,2}$=1.7 Hz, H-2), 4.19 (dd, H-4), 4.32 (dd, $J_{5,6b}$=1.6 Hz, H-6b), 4.35 (d, H-1).

Anal. Calcd for $C_{22}H_{26}O_5S$: C, 65.6; H, 6.5. Found: C, 65.5; H, 6.4.

Ethyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (2). Acetyl chloride (1.5 mL, 21 mmol) was added to a solution of **1** (4.38 g, 10.9 mmol) and pyridine (5 mL) in dichloromethane (50 mL) at 0 °C. The reaction mixture was

stirred at room temperature and after 1 h the mixture was washed with aqueous sodium hydrogencarbonate and water, dried and concentrated. Column chromatography (petroleum ether-ethyl acetate, 5:2) of the residue gave **2** (4.75 g, 10.7 mmol; 98%). Crystallization from ethyl acetate-isooctane gave material having mp 166-167 °C, $[\alpha]_{578}$ +12° (*c* 0.5, chloroform), R_F 0.67 (toluene-ethyl acetate, 2:1), NMR data: ^{13}C, δ 14.9 (Me ethyl), 21.2 (Me acetyl), 22.7 (CH_2S), 68.2-78.6 (C-2, 3, 4, 5, 6), 85.3 (C-1), 101.5 (PhCH), 126.6-138.2 (aromatic C), 169.7 (C=O acetyl); ^1H, δ 3.41 (m, H-5), 3.60 (dd, $J_{2,3}$=9.6 Hz, $J_{3,4}$=3.4 Hz, H-3), 3.98 (dd, $J_{5,6a}$=1.7 Hz, $J_{6a,6b}$=12.4 Hz, H-6a), 4.21 (dd, $J_{4,5}$=0.7 Hz, H-4), 4.33 (dd, $J_{5,6b}$=1.7 Hz, H-6b), 4.36 (d, $J_{1,2}$=10.0 Hz, H-1), 5.46 (dd, $J_{2,3}$=9.6 Hz, H-2).

Anal. Calcd for $C_{24}H_{28}O_6S$: C, 64.8; H, 6.4. Found: C, 64.6; H, 6.1;

2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranosyl bromide (**3**). Bromine (120 μL, 2.3 mmol) was added to a solution of **2** (1.03 g, 2.3 mmol) in dichloromethane (50 mL) at 0 °C. The reaction mixture was stirred at room temperature and when TLC (R_F 0.80 toluene-ethyl acetate, 2:1) indicated complete reaction, cyclohexene (1mL) was added and **3** was used without further purification. NMR data: ^{13}C, δ 20.9 (Me acetyl), 67.4-74.2 (C-2, 3, 4, 5, 6), 91.5 (C-1), 101.0 (PhCH), 126.3-138.0 (aromatic C), 170.0 (C=O acetyl).

Ethyl *O*-(2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (**4**). A dry solution of silver triflate (600 mg, 2.3 mmol) in toluene (15 mL) was added to a stirred mixture of **3** (1.07 g, 2.3 mmol), ethyl 6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside[2] (800 mg, 1.4 mmol), DTBMP (290 mg, 1.4 mmol) and molecular sieves in dry dichloromethane (50 mL) at -30 °C under nitrogen. After 10 min at this temperature, collidine (2 mL) and sodium thiosulfate (10%, 25 mL) were added, and the reaction mixture was allowed to attain room temperature. The mixture was filtered through Celite, and the organic layer was washed with water, dried and concentrated. Column chromatography (toluene-ethyl acetate, 4:1) of the residue gave **4** (1.13 g, 1.2 mmol, 84%), $[\alpha]_{578}$ +51° (*c* 1, chloroform), R_F 0.48 (toluene-ethyl acetate, 2:1). NMR data: ^{13}C, δ 15.0 (Me ethyl), 21.2 (Me acetyl), 23.9 (CH_2S), 54.8, 54.9 (C-2, *p*-methoxybenzyl), 66.4-79.4 (C ring), 81.1 (C-1), 100.7 (C-1′), 101.4 (PhCH), 113.1 (aromatic C *p*-methoxybenzyl), 123.1-138.4 (aromatic C), 158.5 (aromatic C *p*-methoxybenzyl), 167.6 (C=O phthalimido), 169.2 (C=O acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcN	5.22 (10.2)	4.17 (10.3)	4.29 (8.1)	4.08 (10.3)	3.55
Gal	4.56 (8.0)	5.34 (10.0)	3.40 (3.6)	4.09	3.10

Anal. Calcd for $C_{53}H_{55}NO_{13}S$: C, 67.3; H, 5.9; N, 1.5. Found: C, 67.1; H, 5.9; N, 1.4.

Ethyl *O*-(3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (5). Methanolic sodium methoxide (1 mL, 0.5M) was added to a solution of **4** (556 mg, 590 μmol) in dichloromethane (10 mL). The reaction mixture was stirred at room temperature for 15 min, then neutralized with Dowex 50 (H^+) resin, and concentrated. Column chromatography (toluene-ethyl acetate, 2:1) of the residue gave **5** (425 mg, 470 μmol, 80%), R_F 0.45 (toluene-ethyl acetate, 2:1). NMR data: ^{13}C, δ 15.0 (Me ethyl), 23.8 (CH_2S), 54.8, 55.0 (C-2 GlcN, *p*-methoxybenzyl), 66.5-79.3 (C ring), 81.2 (C-1), 101.2 (PhCH), 103.2 (C-1′), 113.2 (aromatic C *p*-methoxybenzyl), 123.1-138.3 (aromatic C), 158.5 (aromatic C *p*-methoxybenzyl), 167.6, 168.0 (C=O phthalimido).

Ethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (6). A solution of silver triflate (160 mg, 620 μmol) and collidine (60 μL, 450 μmol) in dichloromethane-toluene (3:2, 5 mL) was added to a stirred mixture of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[17] (450 mg, 900 μmol), **5** (410 mg, 450 μmol) and molecular sieves in dry dichloromethane (20 mL) at -30 °C under nitrogen. Stirring was continued for 10 min, then aqueous sodium thiosulfate (10%, 5 mL) was added, and the mixture was allowed to attain room temperature. Dichloromethane (10 mL) was added and the mixture was filtered through Celite. The organic layer was washed with water, dried and concentrated. Column chromatography (toluene-ethyl acetate, 4:1) of the residue gave **6** (569 mg, 430 μmol, 95%) having $[\alpha]_{578}$ -33° (*c* 0.5, chloroform), R_F 0.58 (toluene-ethyl acetate, 2:1). NMR data: ^{13}C, δ 15.1 (Me ethyl), 16.9 (C-6 Fuc), 23.8 (CH_2S), 54.9 (C-2 GlcN, *p*-methoxybenzyl), 66.4-81.5 (C ring, C-1 GlcN), 97.5 (C-1 Fuc), 100.8 (C-1 Gal), 101.1 (PhCH), 113.2 (aromatic C *p*-methoxybenzyl), 123.1-139.1 (aromatic C), 158.5 (aromatic C *p*-methoxybenzyl), 167.6 (C=O phthalimido), 168.0 (C=O acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcN	5.19 (10.4)	ND	ND	3.78	3.41
Gal	4.54 (7.8)	4.15	3.61 (3.8)	4.04	3.08
Fuc	5.64 (3.7)	4.04 (10.4)	3.86 (2.8)	3.74	4.34

Anal. Calcd for $C_{78}H_{81}NO_{16}S$: C, 70.9; H, 6.2; N, 1.1 Found: C, 70.7; H, 6.0; N, 1.0

2-(*p*-Nitrophenyl)ethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside (8). A solution of DMTST (150 mg, 580 μmol) in dry dichloromethane (1 mL) was added to a stirred mixture of **7**[2] (238 mg, 250 μmol), **6** (479 mg, 360 μmol), DTBMP (100 mg, 480 μmol) and molecular sieves in dichloromethane (20 mL) at 0 °C under nitrogen. The mixture was stirred for 2 h at room temperature and then triethylamine (1 mL) was added. After 30 min at room temperature the mixture was filtered through Celite and concentrated. Column chromatography (toluene-ethyl acetate, 1:1) followed by precipitation from dichloromethane-isooctane gave **8** (440 mg, 200 μmol, 80%) having $[\alpha]_{578}$ -36° (*c* 0.5, chloroform), R_F 0.50 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 17.1 (C-6 Fuc), 20.4 (Me acetyl), 35.5 ($CH_2Ph_pNO_2$), 54.8, 54.9 (2 *p*-methoxybenzyl), 55.5, 55.6 (C-2 GlcNA, C-2 GlcNB), 66.0-78.7 (C ring, $CH_2CH_2Ph_pNO_2$), 97.6, 98.3, 99.7, 100.7, 100.9, 100.9, 101.2 (5 C-1 and 2 PhCH), 113.1, 113.2 (2 aromatic C *p*-methoxybenzyl), 123.0-139.0 (aromatic C), 146.9 (aromatic C $_pNO_2Ph$), 158.5 (aromatic C *p*-methoxybenzyl), 168.4, (C=O acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.93 (8.4)	ND	ND	ND	ND
GlcNB	5.22 (8.1)	4.18 (9.7)	4.26 (10.0)	4.01 (8.4)	ND
GalA	4.29 (8.0)	4.99 (10.2)	3.44 (3.8)	4.21	3.06
GalB	4.55 (7.8)	4.20 (9.5)	3.70 (3.9)	4.09	3.19
Fuc	5.64 (3.4)	4.06 (10.0)	3.91 (2.8)	3.79	4.38

Anal. Calcd for $C_{128}H_{127}N_3O_{32}$: C, 69.3; H, 5.8; N, 1.9. Found: C, 69.2; H, 5.6 N, 1.9.

2-(*p*-Nitrophenyl)ethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9). CAN (175 mg, 320 μmol) in acetonitrile (5 mL) was added to a stirred solution of **8** (235 mg, 110 μmol) in dichloromethane saturated with water (25 mL) at 0 °C. After 16 h at room temperature the reaction mixture was partitioned between dichloromethane and aqueous sodium hydrogencarbonate. The organic layer was washed

with water, dried and concentrated. Column chromatography (toluene-ethyl acetate, 3:2) gave **9** (152 mg, 77 μmol, 72%) having R_F 0.47 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 16.9 (C-6 Fuc), 20.4 (Me acetyl), 35.3 (CH_2Ph*p*NO_2), 55.7, 56.0 (C-2 GlcNA, C-2 GlcNB), 66.6-81.2 (C ring, CH_2CH_2Ph*p*NO_2), 97.6, 98.2, 99.3, 100.6, 100.8, 101.4, 101.6 (5 C-1 and 2 PhCH), 123.0-138.9 (aromatic C), 146.9 (aromatic C *p*NO_2Ph), 167.9 (C=O phthalimido), 168.5 (C=O acetyl).

2-(*p*-Nitrophenyl)ethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-[6-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside (10). A solution of silver triflate (70 mg, 270 μmol) and collidine (35 μL, 260 μmol) in dichloromethane-toluene (3:2, 5 mL) was added to a stirred mixture of 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide[17] (135 mg, 270 μmol), **9** (133 mg, 68 μmol) and molecular sieves in dry dichloromethane (20 mL) at -30 °C under nitrogen. Stirring was continued for 10 min, then aqueous sodium thiosulfate (10%, 5 mL) was added, and the mixture was allowed to attain room temperature. Dichloromethane (10 mL) was added and the mixture was filtered through Celite. The organic layer was washed with water, dried and concentrated. Column chromatography (toluene-ethyl acetate, 4:1) of the residue gave **10** (153 mg, 54 μmol, 81%) having $[\alpha]_{578}$ -89° (*c* 0.5, chloroform), R_F 0.63 (toluene-ethyl acetate, 2:1). NMR data: ^{13}C, δ 15.6, 16.1, 16.5 (3 C-6 Fuc), 20.1 (Me acetyl), 35.3 (CH_2Ph*p*NO_2), 55.9, 56.3 (C-2 GlcNA, C-2 GlcNB), 66.3-80.9 (C ring, CH_2CH_2Ph*p*NO_2), 97.6, 97.9, 98.1, 98.3, 99.5, 99.6, 99.7, 99.7 100.3 (7 C-1 and 2 PhCH), 122.9-139.5 (aromatic C), 146.6 (aromatic C *p*NO_2Ph), 167.9 (C=O acetyl);
^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.88 (8.6)	4.25 (10.9)	4.38 (8.9)	4.04	3.29
GlcNB	5.14 (8.1)	4.49 (10.5)	4.64 (8.9)	4.12	3.52
GalA	4.41 (8.2)	4.94 (10.1)	3.45 (3.9)	4.18	3.02
GalB	4.55 (8.0)	4.12 (10.2)	3.66 (3.9)	4.06	3.01
FucA	4.60 (3.8)	3.58 (10.4)	3.84	3.02	4.77
FucB1	4.42 (4.0)	3.46 (10.5)	3.76	2.98	4.54
FucB2	5.66 (3.9)	4.11 (10.2)	3.86 (3.7)	3.78	4.38

Anal. Calcd for $C_{166}H_{167}N_3O_{38}$: C, 70.9; H, 6.0; N, 1.5 Found: C, 71.0; H, 6.3; N, 1.4

2-(*p*-Nitrophenyl)ethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside (11). Hydrazine acetate (110 mg, 1.20 mmol) was added to a mixture of **10** (55 mg, 20 μmol) in toluene-ethanol (1:1, 5 mL). The mixture was refluxed overnight and then concentrated. The residue was partitioned between dichloromethane and water. The organic layer, which contained the product, having R_F 0.19 (chloroform-acetone 4:1), was concentrated. The residue was dissolved in dichloromethane-methanol (1:1, 5 mL) and treated with acetic anhydride (1 mL) at room temperature. After 2 h the reaction mixture was concentrated and co-evaporated with ethanol. Column chromatography (chloroform-acetone, 4:1) gave **11** (43 mg, 17 μmol, 84%) having R_F 0.25 (toluene-ethyl acetate 1:2). NMR data : ^{13}C, δ 23.2, 23.3 (2 Me *N*-acetyl), 97.7, 98.1, 99.3, 99.4, 99.9, 100.0, 100.2, 101.0, 101.8 (7 C-1 and 2 PhCH), 170.2, 171.6 (2 C=O *N*-acetyl).

2-(*p*-Trifluoracetamidophenyl)ethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranosyl]-(1→3)-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside (12). A solution of **11** (51 mg, 20 μmol) in tetrahydrofuran-water (9:1, 5 mL) was treated with aluminum amalgam at room temperature for 4 h. The mixture was filtered through Celite and concentrated. The residue, having R_F 0.45 (chloroform-acetone, 2:1), was dissolved in dichloromethane (5 mL) and treated with pyridine (50 μL, 620 μmol) and trifluoroacetic anhydride (20 μL, 40 μmol) at 0 °C. After 1 h at room temperature, the reaction mixture was treated with sodium methoxide (1 mL, 0.1 M) in methanol for 10 minutes, neutralized with acetic acid and concentrated. Column chromatography (chloroform-acetone 4:1) yielded **12** (39 mg, 15 μmol, 75%) having R_F 0.34 (toluene-ethyl acetate, 1:2). NMR data: ^{13}C, δ 16.2, 16.4, 16.6 (3 C-6 Fuc), 23.3, 23.3 (2 Me *N*-acetyl), 35.6 ($CH_2PhpNHCOCF_3$), 97.7, 98.0, 99.3, 99.5, 99.96, 100.05, 100.2, 101.0, 101.8 (7 C-1 and 2 PhCH), 115.9 (q, J=288 Hz, CF_3C=O), 154.8 (q, J=37 Hz, CF_3C=O), 170.4, 171.7 (2 C=O *N*-acetyl).

2-(*p*-Trifluoracetamidophenyl)ethyl *O*-α-L-Fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-*O*-2-acetamido-2-deoxy-β-D-

glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (13). A solution of **12** (26.5 mg, 10 μmol) in ethyl acetate-acetic acid-water (12:3:2) was hydrogenolyzed over Pd/C at 400 kPa overnight, then filtered and concentrated. The residue was purified on a Biogel P-2 column, using water containing 1% *n*-butanol as eluent, giving compound **13** (12.7 mg, 9.1 μmol, 91%) having $[\alpha]_{578}$ -62° (*c* 0.3, water), R_F 0.43 (ethyl acetate-acetic acid-methanol-water, 4:3:3:2). NMR data (D_2O; Me_2CO, δ_H=2.225; external TMS δ_C=0): ^{13}C, δ 16.0, 16.2, 16.2 (C-6 Fuc), 22.8, 23.1 (2 Me *N*-acetyl), 35.2 ($CH_2PhpNHCOCF_3$), 56.4, 56.9 (2 C-2 GlcN), 99.4, 99.5, 100.2, 101.0, 101.6, 102.6, 103.2 (7 C-1), 117.4, 123.1, 130.4, 138.7 (aromatic C), 174.8, 175.5 (2 C=O *N*-acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.48 (8.0)	3.81	3.77 (9.4)	3.88 (10.2)	3.55
GlcNB	4.71 (8.4)	3.95	3.84 (9.3)	3.94 (10.4)	3.44
GalA	4.42 (7.9)	3.49 (10.0)	3.68 (3.2)	4.08	3.60
GalB	4.51 (7.8)	3.65	3.86	3.88	ND
FucA	5.01 (4.0)	3.65 (10.0)	3.85 (3.6)	3.76	4.78
FucB1	5.12 (4.0)	3.70 (10.4)	3.92 (3.3)	3.81	4.88 (6.6)
FucB2	5.28 (3.4)	3.80	ND	3.82	4.24 (6.8)

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