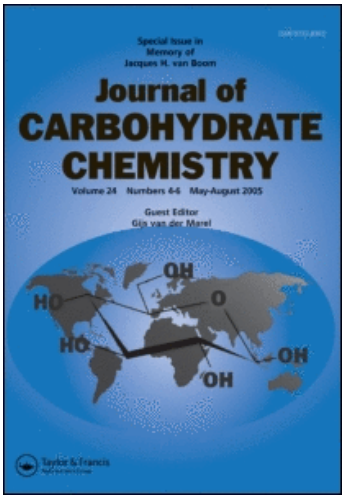

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SYNTHESIS OF A MONOFUCOSYL HEPTASACCHARIDE CORRESPONDING TO A TUMOR-ASSOCIATED GLYCOLIPID DEFINED BY MONOCLONAL ANTIBODY ACFH-18.

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ABSTRACT

p-Trifluoroacetamidophenylethyl *O*-β-D-galactopyranosyl-(1→4)-*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-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside (13), which is a derivative of a tumor-associated glycolipid defined by monoclonal antibody ACFH-18, was synthesized from thioglycoside intermediates. Suitably protected disaccharide derivatives of β-D-Galp-(1→4)-*O*-β-D-GlcNp were used as building blocks. These were stepwise coupled to give a linear hexasaccharide, which after selective deblocking was monofucosylated and deprotected to give 13.

INTRODUCTION

The monoclonal antibody ACFH-18, obtained from immunization of mice with human gastric cancer cells, defines a series of glycolipids found in gastrointestinal adenocarcinoma cells. The antibody showed the strongest binding to the monofucosyl glycolipid shown in Figure 1[1].

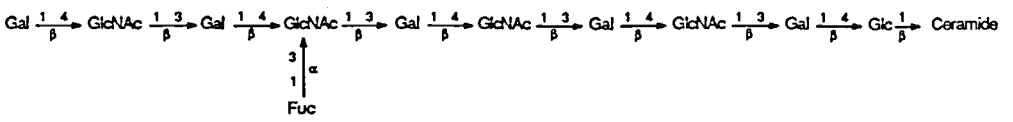


Figure 1

Synthesis of tumor-associated carbohydrate structures are of importance since they are potentially useful in cancer diagnosis and treatment[2,3]. We have earlier reported on synthesis of di- and trimeric Lewis X derivatives[4,5]. Synthesis of other related structures have also been published[6,7,8,9]. We now report on synthesis of compound 13, which is a partial structure of the glycolipid shown in Figure 1.

RESULTS AND DISCUSSION

The synthesis was based on the same strategy as reported in our synthesis of di- and trimeric Lewis X derivatives.[4,5]

The strategy was to synthesize the disaccharide 3, which has a thioethyl group on C-1 and a chloroacetyl group on O-3′. The thioethyl group in 3 was converted into a *p*-nitrophenylethyl group and the chloroacetyl group was removed to give the acceptor disaccharide 5, which was coupled with ethyl 4-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside[4] to give the linear tetrasaccharide 6. Compound 6 was after selective deblocking glycosylated with 3, giving a linear hexasaccharide 8, which after removal of the *p*-methoxybenzyl group was fucosylated giving the heptasaccharide 10. Compound 10 was then deblocked to give the target structure 13. The following steps were performed:

Ethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[10] was benzylated using benzyl bromide and sodium hydride in *N,N*-dimethylformamide, giving compound 1 in 71% yield. The 4,6-benzylidene acetal in 1 was opened by treatment with sodium cyanoborohydride and HCl-diethyl ether in tetrahydrofuran, giving the 4-OH compound 2 in 88% yield.

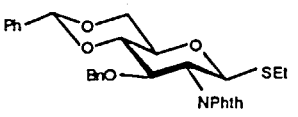
The glycosyl acceptor 2 was coupled with 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-galactopyranosyl bromide[4] in the presence of silver triflate in

dichloromethane, giving the disaccharide **3** in 89% yield. The thioethyl group in **3** was converted into a *p*-nitrophenylethyl group by treatment with *p*-nitrophenylethyl alcohol in dichloromethane, using methyl triflate[11] as the glycosidation promoter and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as the acid acceptor, giving **4** in 72% yield. Treatment of **4** with hydrazine acetate in ethyl acetate-methanol (1:1) removed the chloroacetyl group selectively, giving the OH-3 compound **5** in 95% yield. Glycosidation of **5** with ethyl 4-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside[4], using dimethyl-(methylthio)sulfonium triflate (DMTST)[12] as the promoter and DTBMP as the acid acceptor, gave the tetrasaccharide **6** in 88% yield. The chloroacetyl group in **6** was removed by treatment with hydrazine acetate giving the OH-3 compound **7** in 82% yield. Glycosidation of **7** with **3** using DMTST as the promoter and DTBMP as the acid acceptor, gave the hexasaccharide **8** in 77% yield.

The *p*-methoxybenzyl group in **8** was removed by treatment with ceric ammonium nitrate (CAN) in water-dichloromethane to give compound **9** in 73% yield. Fucosylation of **9** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[10], using silver triflate-collidine as the promoter, gave the heptasaccharide **10** in 83% yield. The phthalimido groups and the *O*-acetyl groups in **10** were removed by refluxing with hydrazine acetate in 2:3 toluene-ethanol giving free amino groups which were *N*-acetylated with acetic anhydride in 1:1 dichloromethane-methanol giving compound **11** in 77% yield. Reduction of the nitro group in **11** by treatment with aluminum amalgam, followed by trifluoroacetylation and then treatment with methanolic sodium methoxide gave compound **12** in 88% yield. Hydrogenolysis of **12** over Pd/C gave **13** in 83% yield.

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed under reduced pressure at < 40° (bath). Optical rotations were recorded for 0.3-0.8% 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



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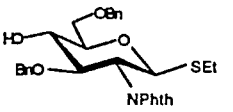

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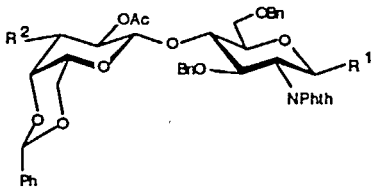
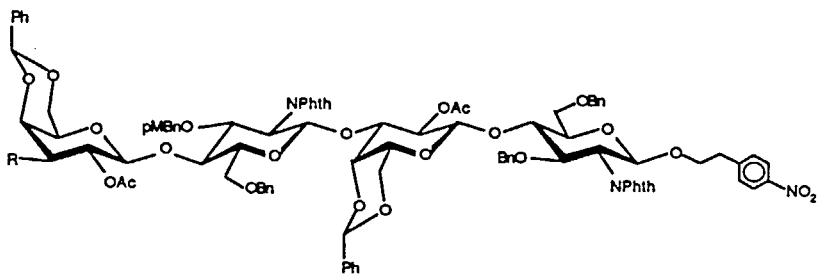
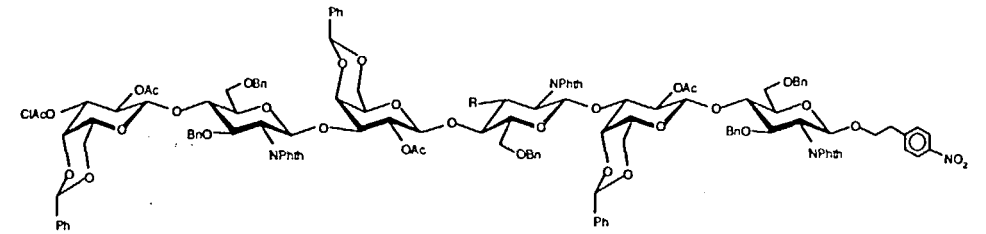


3 R^1 = SEt, R^2 = OClAc
4 R^1 = $O(CH_2)_2$-Ph-pNO_2, R^2 = OClAc
5 R^1 = $O(CH_2)_2$-Ph-pNO_2, R^2 = OH

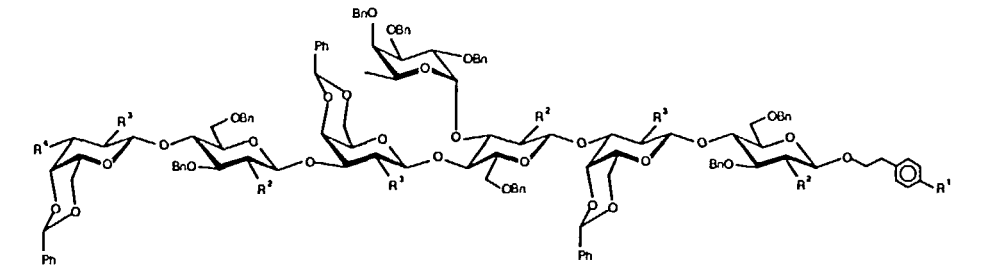


6 R = OClAc
7 R = OH

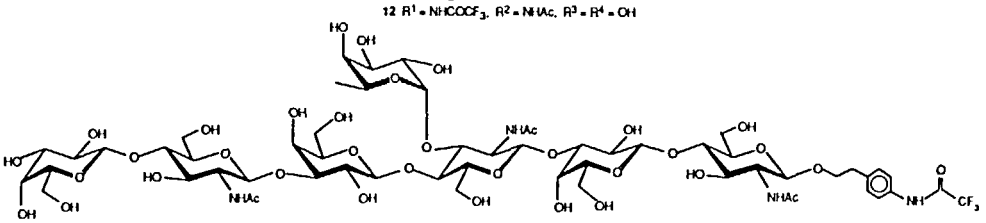
in agreement with the postulated structures, and only selected data are reported. For some compounds ^1H shift values and coupling constants (values in parentheses) are given in table form. In these tables the sugar residues are given as GlcNA, GlcNB, GlcNC, GalA, GalB, GalC, and Fuc where A,B and C 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 (3Å or 4Å, Fluka) were desiccated at 300 °C



8 R = OpMBn
9 R = OH



10 $R^1 = NO_2$, R^2 = NPhth, R^3 = OAc, R^4 = OClAc
11 $R^1 = NO_2$, R^2 = NHAc, $R^3 = R^4$ = OH
12 R^1 = NHCOCF$_3$, R^2 = NHAc, $R^3 = R^4$ = OH



13

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. The FAB-MS spectrum was recorded with a VG ZAB-SE mass spectrometer. The primary beam consisted of xenon atoms with a maximum energy of 8 KeV. The sample was dissolved in thioglycerol and positive ions were extracted and accelerated over a potential of 10 kV.

Ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (1). — A solution of ethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[10] (12.9 g, 29.2 mmol) and benzyl bromide (7.0 mL,

59 mmol) in dry *N,N*-dimethyl formamide (130 mL) was added dropwise to 60% sodium hydride (2.3 g, 58 mmol) at 0 °C under nitrogen. The reaction mixture was stirred overnight at room temperature. Acetic anhydride (10 mL) was added and after 2 h the mixture was partitioned between toluene and aqueous sodium hydrogencarbonate. The organic layer was washed with water, dried and concentrated. The product was purified by column chromatography (petroleum ether-ethyl acetate, 3:1) to yield 1 (11.0 g, 20.7 mmol, 71%) $[\alpha]_{578}$ +66° (*c* 0.6, chloroform), R_F 0.8 (toluene-ethyl acetate, 3:1). NMR data : ^{13}C, δ 14.9 (Me ethyl), 23.9 (CH_2S), 54.6 (C-2), 68.6-81.7 (C-3,4,5,6), 82.9 (C-1), 101.1 (PhCH), 123.2-137.8 (aromatic C), 167.3, 167.6 (C=O phthalimido); ^1H, δ 3.71 (m, H-5), 3.83 (dd, $J_{3,4}$=8.8 Hz, $J_{4,5}$=9.0 Hz, H-4), 4.30 (dd, $J_{1,2}$=10.5 Hz, $J_{2,3}$=10.0 Hz, H-2), 4.42 (dd, H-3), 5.34 (d, H-1).

Ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2). — Diethyl ether saturated with hydrogen chloride was added, at room temperature, to a stirred mixture of compound 1 (9.30 g, 17.5 mmol), sodium cyanoborohydride (6.60 g, 105 mmol) and molecular sieves (3Å) in tetrahydrofuran (300 mL) until the mixture was acidic (as determined with indicator paper). The reaction mixture was stirred at room temperature for 20 min, then filtered through Celite, washed with water, dried and concentrated. The product was purified by column chromatography (petroleum ether-ethyl acetate, 3:1) and then crystallized from ethyl acetate-petroleum ether to give pure 2 (8.25 g, 15.5 mmol, 88%) having mp 111-112 °C, $[\alpha]_{578}$ +46° (*c* 0.6, chloroform), R_F 0.58 (toluene-ethyl acetate, 3:1). NMR data: ^{13}C, δ 14.9 (Me ethyl), 24.0 (CH_2S), 54.4 (C-2), 70.9-79.5 (C-3,4,5,6), 81.2 (C-1), 123.3-138.1 (aromatic C), 167.5, 168.1 (C=O phthalimido); ^1H, δ 3.00 (d, $J_{OH,4}$=2.4 Hz, OH), 3.67 (m, H-5), 3.76 (dd, $J_{5,6a}$=5.1 Hz, $J_{6a,6b}$=10.1 Hz, H-6a), 3.82 (m, $J_{3,4}$=8.4 Hz, H-4), 3.85 (dd, $J_{5,6b}$=4.8 Hz, H-6b), 4.22 (dd, $J_{1,2}$=10.4 Hz, $J_{2,3}$=10.1 Hz, H-2), 4.27 (dd, H-3), 5.27 (d, H-1).

Anal. Calcd. for $C_{30}H_{31}NO_6S$: C, 67.5; H, 5.9; N, 2.6; S, 6.0. Found: C, 67.7; H, 5.9; N, 2.6; S, 5.8.

Ethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3). — A dry solution of silver triflate (1.30 g, 5.05 mmol) in toluene (15 mL) was added to a stirred mixture of 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-galactopyranosyl bromide[4] (1.52 g, 3.37 mmol), 2 (1.50 g, 2.81 mmol), and molecular sieves (4Å) 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 of the residue gave **3** (2.25 g, 2.49 mmol, 89%), $[\alpha]_{578}$ +49° (*c* 0.7, chloroform), R_F 0.47 (toluene-ethyl acetate, 3:1). NMR data: ^{13}C, δ 14.9 (Me ethyl), 20.8 (Me acetyl), 23.8 (CH_2S), 40.6 (CH_2Cl), 54.7 (C-2), 65.9-79.1 (C-3,4,5,6, C-2′,3′,4′,5′,6′), 81.0 (C-1), 100.1 (C-1′), 101.0 (PhCH), 123.2-138.5 (aromatic C), 167.0 (C=O chloroacetyl), 167.5, 167.7 (C=O phthalimido), 169.0 (C=O acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcN	5.21 (8.4)	4.24 (10.4)	4.31 (8.5)	4.14	3.56
Gal	4.65 (8.0)	5.36 (10.4)	4.81 (3.6)	4.30 (1.7)	3.21

Anal. Calcd. for $C_{47}H_{48}NO_{13}SCl$: C, 62.5; H, 5.4; N, 1.5. Found: C, 62.5; H, 5.4; N, 1.5.

***p*-Nitrophenylethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4).** — Methyl triflate (1.0 mL, 9.5 mmol) was added at room temperature to a stirred mixture of **3** (1.42 g, 1.58 mmol), *p*-nitrophenylethyl alcohol (0.53 g, 3.2 mmol), and molecular sieves (4Å) in dry dichloromethane (50 mL). The reaction was left overnight and triethylamine (2 mL) was then added. After 1 h the mixture was filtered through Celite and concentrated. Column chromatography of the residue gave **4** (1.15 g, 1.14 mmol, 72%), having $[\alpha]_{578}$ +17° (*c* 0.6, chloroform), R_F 0.33 (toluene-ethyl acetate, 3:1). NMR data: ^{13}C, δ 20.8 (Me acetyl), 35.3 (CH_2pNO_2Ph), 40.6 (CH_2Cl), 55.4 (C-2), 65.9-78.3 (C,3,4,5,6, C-2′,3′,4′,5′,6′), 98.2 (C-1), 100.2 (C-1′), 101.0 (PhCH), 122.8-138.5 (aromatic C), 146.7 (aromatic C pNO_2Ph), 167.0 (C=O chloroacetyl), 168.9 (C=O acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcN	5.01 (8.4)	4.07	4.15 (8.3)	4.08	3.50
Gal	4.53 (8.0)	5.32 (10.4)	4.77 (3.7)	4.28	3.20

Anal. Calcd. for $C_{53}H_{51}N_2O_{16}Cl$: C, 63.2; H, 5.1; N, 2.8. Found: C, 63.2; H, 5.0; N, 2.6.

***p*-Nitrophenylethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5).** — Hydra-

zine acetate (1.10 g, 12 mmol) dissolved in methanol (40 mL) was added to a stirred solution of 4 (1.37 g, 1.36 mmol) in dichloromethane-ethyl acetate (1:1, 80 mL) at room temperature. The reaction was stirred for 2 h and then concentrated. The residue was partitioned between dichloromethane and water. The dichloromethane layer was dried and concentrated. Column chromatography gave 5 (1.20 g, 1.29 mmol, 95%), $[\alpha]_{578}$ -11° (*c* 0.4, chloroform), R_F 0.47 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 21.1 (Me acetyl), 35.4 (CH_2pNO_2Ph), 55.4 (C-2), 66.2-78.5 (C-3,4,5,6, C-2′,3′,4′,5′,6′), 98.2 (C-1), 100.3 (C-1′), 101.4 (PhCH), 122.9-138.5 (aromatic C), 146.8 (aromatic C *p*-NO_2Ph), 167.3, 167.7 (C=O phthalimido), 170.2 (C=O acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcN	5.02 (8.4)	4.08 (10.6)	4.16 (8.2)	4.06	3.54
Gal	4.52 (8.0)	5.04 (10.0)	3.47 (3.7)	4.09	3.20

Anal. Calcd. for $C_{51}H_{50}N_2O_{15}$: C, 65.8; H, 5.4; N, 3.0. Found: C, 65.5; H, 5.4; N, 2.9.

***p*-Nitrophenylethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-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)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (6).** — A solution of DMTST (1.01 g, 3.91 mmol) in dry dichloromethane (5 mL) was added to a stirred mixture of ethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside[4] (910 mg, 976 μmol), 5 (606 mg, 651 μmol), DTBMP (1.21 g, 5.85 mmol) and molecular sieves (4Å) in dichloromethane (20 mL) at 0 °C under nitrogen. The mixture was stirred for 2 h at room temperature and then triethylamine (2 mL) was added. After 30 min at room temperature the mixture was filtered through Celite and concentrated. Column chromatography (chloroform-ethyl acetate 1:1) followed by precipitation from dichloromethane-isooctane gave 6 (1.04 g, 576 μmol, 88%) having $[\alpha]_{578}$ -12° (*c* 0.8, chloroform), R_F 0.53 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.3, 20.9 (2 Me acetyl), 35.3 (CH_2pNO_2Ph), 40.6 (CH_2Cl), 54.7 (MeO), 55.3, 55.6 (C-2 GlcNA, C-2 GlcNB), 66.0-78.7 (C ring) 98.1 (C-1 GlcNA), 99.1 (C-1 GlcNB), 100.4, 100.6 (C-1 GalA, C-1 GalB), 100.7, 101.2 (2 PhCH), 113.1 (aromatic C *p*-methoxybenzyl), 122.9-138.6 (aromatic C), 146.7 (aromatic C pNO_2Ph), 158.4, (aromatic C *p*-methoxybenzyl), 167.1 (C=O chloroacetyl), 168.4, 169.0 (2 C=O acetyl);

^{1}H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.93 (8.4)	3.98	4.01	3.94	3.34
GlcNB	5.24 (8.2)	4.15 (10.8)	4.26 (8.2)	4.01 (9.8)	3.64
GalA	4.28 (8.0)	5.00 (10.1)	3.46 (3.7)	4.18 (1.6)	3.06
GalB	4.64 (8.0)	5.38 (10.3)	4.88 (3.7)	4.32 (1.6)	3.29

Anal. Calcd. for $C_{97}H_{94}N_3O_{29}Cl$: C, 64.7; H, 5.3; N, 2.3. Found: C, 64.8; H, 5.3; N, 2.3.

***p*-Nitrophenylethyl *O*-(2-*O*-acetyl-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)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7).** — Hydrazine acetate (276 mg, 3.0 mmol) in methanol (10 mL) was added to a stirred solution of **6** (800 mg, 444 μmol) in dichloromethane-ethyl acetate (1:1, 20 mL) at room temperature. The reaction was stirred for 2 h and then concentrated. The residue was partitioned between dichloromethane and water. The dichloromethane layer was dried and concentrated. Precipitation from dichloromethane-isooctane gave **7** (628 mg, 364 μmol, 82%), $[\alpha]_{578}$ -32° (*c* 0.6, chloroform), R_F 0.22 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.3, 21.1 (2 Me acetyl), 35.3 (CH_2pNO_2Ph), 54.7, 55.3, 55.6 (C-2 GlcNA, C-2 GlcNB, MeO), 98.1 (C-1 GlcNA), 99.1 (C-1 GlcNB), 100.51, 100.54 (C-1 GalA, C-1 GalB), 100.7, 101.5 (2 PhCH), 113.1 (aromatic C *p*-methoxybenzyl), 122.9-138.5 (aromatic C), 146.7 (aromatic C pNO_2Ph), 158.4, (aromatic C *p*-methoxybenzyl), 168.4, 170.2 (2 C=O acetyl).

***p*-Nitrophenylethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-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)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8).** — A solution of DMTST (675 mg, 2.61 mmol) in dry dichloromethane (10 mL) was added to a stirred mixture of **7** (563 mg, 326 μmol), **3** (588 mg, 652 μmol), DTBMP (676 mg, 2.61 mmol) and molecular sieves (4Å) 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 using chloroform-ethyl acetate (1:1) as the packing-solvent and chloroform-dichloromethane-ethyl acetate (1:1:1) as the eluent gave 8 (644 mg, 251 μmol, 77%) having $[\alpha]_{578}$ -23° (*c* 0.8, chloroform), R_F 0.44 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.2, 20.3, 20.8 (3 Me acetyl), 35.2 (CH_2pNO_2Ph), 40.6 (CH_2Cl), 54.7 (MeO), 55.3, 55.4 , 55.5 (3 C-2 GlcN), 98.0 (C-1 GlcNA), 99.0, 100.37, 100.44, 100.56, 100.64, 100.7, 101.0 (5 C-1 and 3 PhCH), 112.9 (aromatic C *p*-methoxybenzyl), 122.8-138.5 (aromatic C), 146.7 (aromatic C pNO_2Ph), 158.3, (aromatic C *p*-methoxybenzyl), 167.0 (C=O) chloroacetyl), 168.3, 168.5, 169.0 (3 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 (8.3)	ND	ND	3.89	3.32
GlcNB	5.16 (8.2)	4.07 (10.8)	ND	3.82	3.48
GlcNC	5.27 (8.1)	4.20 (10.6)	4.28 (8.0)	4.03	3.63
GalA	4.21 (8.0)	4.97 (10.0)	3.37	4.12	3.01
GalB	4.38 (8.0)	5.06 (10.1)	3.59 (3.5)	4.22	3.14
GalC	4.67 (8.0)	5.38 (10.1)	4.88 (3.7)	4.31 (1.6)	3.27

Anal. Calcd. for $C_{140}H_{135}N_4O_{41}Cl$: C, 65.5; H, 5.3; N, 2.2. Found: C, 65.4; H, 5.3; N, 2.2.

***p*-Nitrophenylethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-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)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9).** — CAN (590 mg, 1.08 mmol) in acetonitrile (15 mL) was added to a stirred solution of 8 (460 mg, 179 μmol) in dichloromethane (25 mL) saturated with water at 0 °C. After 2 h at this temperature the reaction was complete and the organic layer was washed with aqueous sodium hydrogencarbonate and water, dried and concentrated. Column chromatography (chloroform-dichloromethane-ethyl acetate, 1:1:1) gave 9 (375 mg, 131 μmol, 73%), having $[\alpha]_{578}$ -26° (*c* 0.7, chloroform), R_F 0.40 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.3, 20.4, 20.8 (3 Me acetyl), 35.3 (CH_2 pNO_2Ph), 40.6 (CH_2Cl), 55.3, 55.5, 55.6 (C-2 GlcNA, C-2 GlcNB, C-2 GlcNC), 98.1, 98.9, 99.1, 100.4, 100.46, 100.52, 100.7, 101.1, 101.5 (6 C-1 and 3 PhCH), 122.9-138.6 (aromatic C), 146.7 (aromatic C pNO_2Ph), 167.1 (C=O) chloroacetyl), 168.4, 168.5, 169.0 (3 C=O acetyl).

p-Nitrophenylethyl *O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-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)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10). — A solution of silver triflate (250 mg, 127 μmol) and 2,4,6-trimethylpyridine (130 μL, 978 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[10] (316 mg, 637 μmol), **9** (327 mg, 127 μmol) and molecular sieves (4Å) 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, 2:1) of the residue gave **10** (302 mg, 106 μmol, 83%) having $[\alpha]_{578}$ -63° (*c* 0.7, chloroform), R_F 0.58 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 15.6 (C-6 Fuc), 20.30, 20.34, 20.8 (3 Me acetyl), 35.3 (CH_2 *p*NO_2Ph), 40.6 (CH_2Cl), 55.3 55.7, 56.1 (3 C-2 GlcN), 97.6 (C-1 Fuc), 98.1 (C-1 GlcNA), 99.0, 99.7, 99.8, 100.4, 100.5, 100.6. 101.1 (5 C-1 Gal and 3 PhCH), 122.9-139.6 (aromatic C), 146.7 (aromatic C *p*NO_2Ph), 167.1 (C=O chloroacetyl), 168.0, 168.4, 169.0 (3 C=O acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.91 (8.3)	3.98	3.85	3.76	3.49
GlcNB	5.10 (8.1)	4.35 (10.8)	4.50 (8.6)	3.98	3.43
GlcNC	5.25 (8.2)	4.23 (10.8)	4.34 (8.3)	4.00	3.63
GalA	4.24 (7.9)	4.96 (10.0)	3.43 (3.7)	4.14	3.05
GalB	4.41 (8.1)	4.98 (10.0)	3.54 (3.7)	4.18 (1.5)	3.02
GalC	4.64 (7.9)	ND (10.3)	4.85 (3.7)	4.28 (1.6)	3.23
Fuc	4.51 (3.7)	3.48 (10.4)	3.76 (3.2)	3.00	ND

p-Nitrophenylethyl *O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(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-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyrano-

side (11). — Hydrazine acetate (460 mg, 5.0 mmol) was added to a mixture of 10 (206 mg, 72 μmol) in toluene-ethanol (1:1, 10 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 0.27 (toluene-ethyl acetate-methanol, 3:5:1), was concentrated. The residue was dissolved in dichloromethane-methanol (1:1, 10 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 (toluene-ethyl acetate-methanol, 5:5:1) gave **11** (133 mg, 56 μmol, 77%) having R_F 0.37 (toluene-ethyl acetate-methanol, 3:5:1). NMR data ($CDCl_3$-CD_3OD, 1:1): ^{13}C, δ 16.3 (C-6 Fuc), 22.9, 23.1, 23.4 (3 Me *N*-acetyl), 36.2 (CH_2 *p*NO_2Ph), 66.7, 66.9, 67.0 (3 C-2 GlcN), 97.7 (C-1 Fuc), 100.2, 101.3, 101.5, 101.7, 102.1, 102.6, 102.7, 103.5, 103.8 (6 C-1 and 3 PhCH), 123.7-139.8 (aromatic C), 147.9 (aromatic C *p*NO_2Ph), 172.0, 172.7, 173.1 (3 C=O *N*-acetyl).

***p*-Trifluoracetamidophenylethyl *O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(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-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (12).** — A solution of **11** (114 mg, 48 μmol) in tetrahydrofuran-water (9:1, 10 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 0.31 (toluene-ethyl acetate-methanol, 3:5:1), was dissolved in dichloromethane (5 mL) and treated with pyridine (120 μL, 1.5 mmol) and trifluoroacetic anhydride (100 μL, 0.72 mmol) at 0 °C. After 1 h at room temperature, the reaction mixture was treated with sodium methoxide in methanol, neutralized with acetic acid and concentrated. Column chromatography (toluene-ethyl acetate-methanol, 5:5:1) yielded **12** (103 mg, 42 μmol, 88%) having R_F 0.39 (toluene-ethyl acetate-methanol, 3:5:1). NMR data (Acetone d-6): ^{13}C, δ 16.9 (C-6 Fuc), 23.47, 23.54, 23.9 (3 Me *N*-acetyl), 36.1 (CH_2*p*$NHCOCF_3$Ph), 97.6 (C-1 Fuc), 100.4, 101.3, 101.5, 102.0, 103.0, 103.1, 103.3, 104.2, 104.5 (6 C-1 and 3 PhCH), 170.9, 171.7 (3 C=O *N*-acetyl).

***p*-Trifluoracetamidophenylethyl *O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[3-*O*-(α-L-fucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranoside (13).** — A solution of **12** (90 mg, 37 μmol) in a mixture of ethyl acetate-acetic acid-water (12:3:2) was hydrogenated over Pd/C at 400 kPa overnight, then filtered and concentrated. The

residue was purified on a Biogel P-2 column, using water as eluent, giving compound **13** (45 mg, 31 μmol, 83%) having $[\alpha]_{578}$ -31° (*c* 0.3, water), R_F 0.36 (ethyl acetate-acetic acid-methanol-water, 4:3:3:2). NMR data (D_2O; Me_2CO, δ_H=2.225; δ_C=31.1): ^{13}C, δ 15.9 (C-6 Fuc), 22.6, 22.8, 22.9 (3 Me *N*-acetyl), 35.0 (CH_2 ethyl), 55.5 (C-2 GlcNA), 55.8 (C-2 GlcNC), 56.6 (C-2 GlcNB), 60.3, 60.5, 60.7, 61.6, 61.7, 62.1 (3 C-6 GlcN, 3 C-6 Gal), 67.4 (C-5 Fuc), 68.3 (C-2 Fuc), 68.9 (C-4 GalA, C-4 GalB), 69.2 (C-4 GalC), 70.6 (C-2 GalA), 70.9 (CH_2 ethyl), 71.1 (C-2 GalB), 71.6 (C-2 GalC), 72.5 (C-4 Fuc), 73.2 (C-3 GalC), 73.4 (C-4 GlcNB), 75.4 (C-3 GlcNB), 78.8, 79.1 (C-4 GlcNA, C-4 GlcNC), 82.3 (C-3 GalB), 82.7 (C-3 GalA), 99.4 (C-1 Fuc), 101.6 (C-1 GlcNA), 102.4 (C-1 GalB), 103.2 (C-1 GlcNB), 103.4 (C-1 GlcNC), 103.5 (C-1 GalA, C-1 GalC), 122.9, 130.3, 133.5, 138.6 (aromatic C), 174.9, 175.4, 175.5 (3 C=O *N*-acetyl); ^1H NMR data are shown in the following table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.46	3.63	ND	3.66	ND
GlcNB	4.71 (8.5)	3.95	3.85	3.94	ND
GlcNC	4.70 (8.5)	3.79	ND	3.75	ND
GalA	4.43 (7.8)	3.56 (10.0)	3.70 (3.3)	4.15 (1.1)	ND
GalB	4.44 (7.8)	3.52 (9.9)	3.71 (3.3)	4.10 (1.4)	ND
GalC	4.48 (7.8)	3.54 (10.0)	3.67 (3.3)	3.93 (1.1)	ND
Fuc	5.12 (4.0)	3.69 (10.4)	3.89 (3.2)	3.78	4.81

Positive ion FAB-MS showed an M+H ion at m/z 1475 and an M+Na ion at m/z 1497.

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