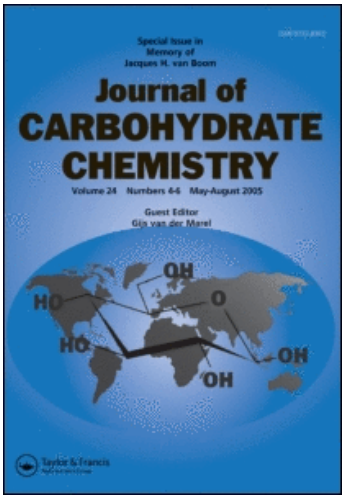

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis of Carbohydrate Derivatives Corresponding to a Tumor-Associated Glycolipid: A Trimeric Lewis X Nonasaccharide and a Trimeric *N*-Acetyl Lactosamine Hexasaccharide

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To cite this Article Nilsson, Marianne and Norberg, Thomas(1989) 'Synthesis of Carbohydrate Derivatives Corresponding to a Tumor-Associated Glycolipid: A Trimeric Lewis X Nonasaccharide and a Trimeric *N*-Acetyl Lactosamine Hexasaccharide', Journal of Carbohydrate Chemistry, 8: 4, 613 — 627
To link to this Article: DOI: 10.1080/07328308908048021
URL: http://dx.doi.org/10.1080/07328308908048021

J. CARBOHYDRATE CHEMISTRY, 8(4), 613-627 (1989)

SYNTHESIS OF CARBOHYDRATE DERIVATIVES CORRESPONDING TO A TUMOR-ASSOCIATED GLYCOLIPID: A TRIMERIC LEWIS X NONASACCHARIDE AND A TRIMERIC N-ACETYL LACTOSAMINE HEXASACCHARIDE

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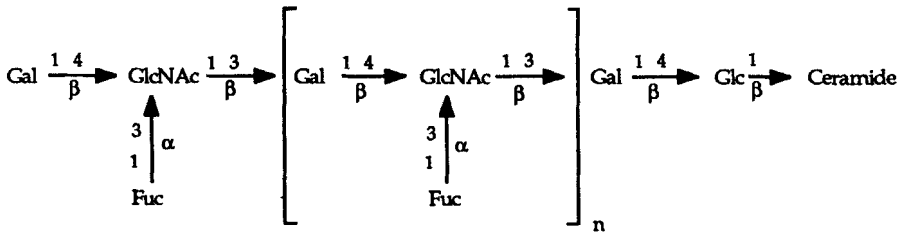
Received January 11, 1989 - Final Form May 19, 1989

ABSTRACT

A trimeric Lewis X derivative, *p*-trifluoracetamidophenylethyl *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*-[3-*O*-(α-L-fucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-3-*O*-(α-L-fucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside corresponding to the tumor-associated glycolipid in Figure 1 (n=2), and a trimeric derivative of *N*-acetyl lactosamine, *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*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside, were synthesised from thioglycoside building blocks. The synthesis was based on intermediates earlier reported.

INTRODUCTION

Isolation and characterization of glycolipids from adenocarcinoma tissue having the general structure shown in Figure 1, was reported in 1984 by Hakomori



n = 1 or 2.

FIG. 1

et. al.[2] These structures were regarded as tumor-associated since they were not present in any appreciable extent in corresponding normal tissues. The interest in the carbohydrate part of these glycolipids[3] is due to their potential usefulness in cancer diagnosis and treatment.

As part of a program aimed at synthesizing tumor-associated carbohydrate structures, we have earlier synthesized a dimeric Lewis X hexasaccharide derivative.[1] Other derivatives related to the structure in Figure 1 have also been reported.[4,5,6] We now report the synthesis of the nonasaccharide **12** and the linear trimer of *N*-acetyllactosamine **7**, both carrying a *p*-trifluoracetamidophenylethyl linking arm that makes possible attachment to proteins, lipids or solid matrixes via the corresponding isothiocyanate derivative.[7] Synthesis of di-, tri- and tetrameric *N*-acetyllactosamine derivatives has been reported earlier by Alais and Veyrieres.[8,9]

RESULTS AND DISCUSSION

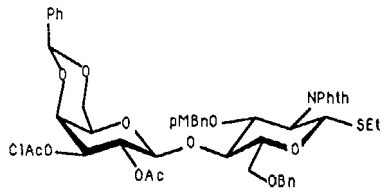
The synthesis was based on building blocks used in synthesis of the dimeric Lewis X hexasaccharide[1] derivative. Our strategy was to use the disaccharide ethyl 4-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (**1**)[1] and the suitable protected tetrasaccharide **2**[1]. Compound **2** was after selective

deblocking glycosylated with **1**, giving a linear hexasaccharide **4**, which after deprotection gave the trimeric *N*-acetyllactosamine derivative **7**. Selective removal of the *p*-methoxybenzyl groups in **4** gave a triol hexasaccharide, which was trifucosylated. The obtained nonasaccharide was then deblocked to give the target structure **12**. The following steps were performed:

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)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside)[1] (**2**) was treated with hydrazine acetate giving the OH-3 compound **3** in 95% yield. Glycosidation of **3** 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 (**1**)[1], using dimethyl(methylthio)sulfonium triflate (DMTST)[10] as the promoter and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as the acid acceptor, gave the hexasaccharide **4** in 75% yield. The phthalimido groups and the *O*-acetyl groups in **4** 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 **5** in 72% yield. Reduction of the nitro group in **5** by treatment with aluminum amalgam, followed by trifluoroacetylation and then treatment with methanolic sodium methoxide gave compound **6** in 65% yield. Hydrogenolysis of **6** over Pd/C gave the deprotected *N*-acetyllactosamine derivative **7** in 79% yield.

The three *p*-methoxybenzyl groups in **4** were removed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in water-dichloromethane to give the triol **8** in 87% yield. Trifucosylation of **8** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[11], using silver triflate-collidine as promotor, gave the nonasaccharide **9** in 68% yield. No mono- or difucosylated compounds were observed in the purified product (NMR, HPTLC).

Dephthalimidization and *N*-acetylation of **9**, in the same way as described above, gave **10** in 82% yield. Reduction of the nitro group in **10**, followed by trifluoroacetylation and deacylation gave **11** in 81% yield. Finally, **11** was hydrogenolyzed over Pd/C giving the deblocked nonasaccharide **12** in 91% yield.



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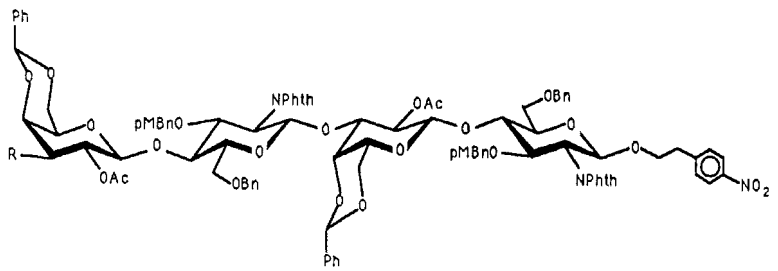
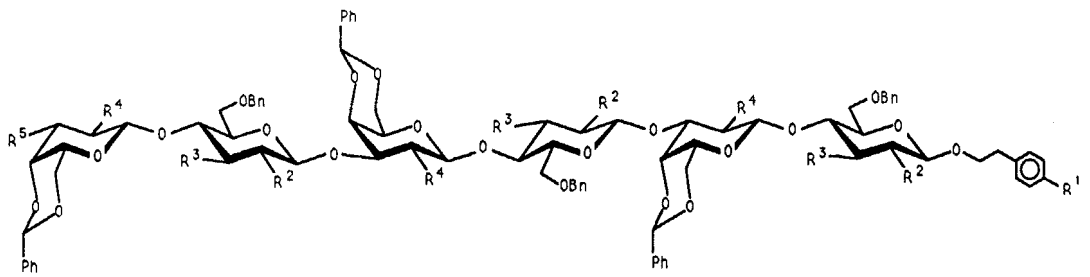


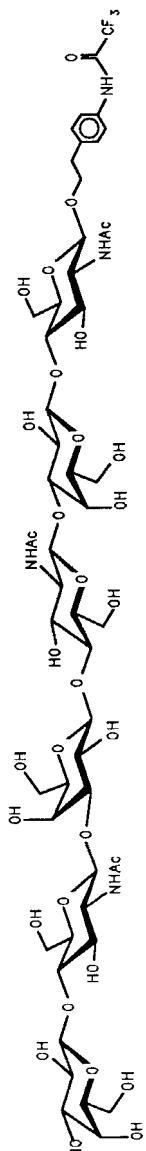
2 R = OClAc
3 R = OH



4 $R^1 = NO_2$, R^2 = NPhth, R^3 = OpMBn, R^4 = OAc, R^5 = OClAc
5 $R^1 = NO_2$, R^2 = NHAc, R^3 = OpMBn, $R^4 = R^5$ = OH
6 $R^1 = NHCOCF_3$, R^2 = NHAc, R^3 = OpMBn, $R^4 = R^5$ = OH
8 $R^1 = NO_2$, R^2 = NPhth, R^3 = OH, R^4 = OAc, R^5 = OClAc

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed under reduced pressure at < 40 °C (bath). Optical rotations were recorded for 0.4-1.0% 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



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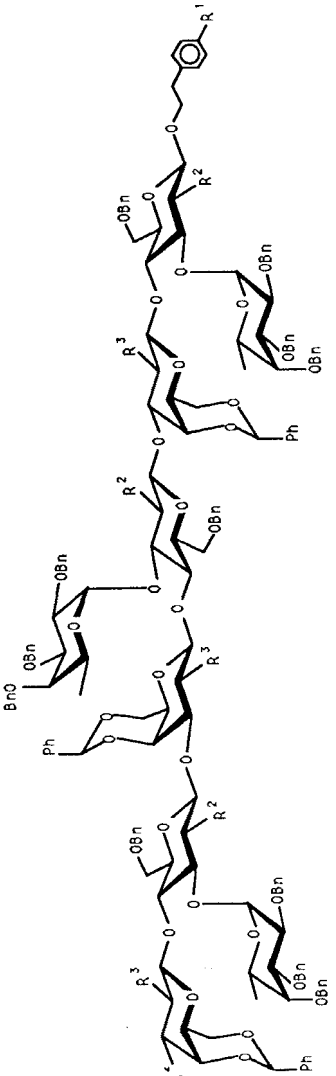
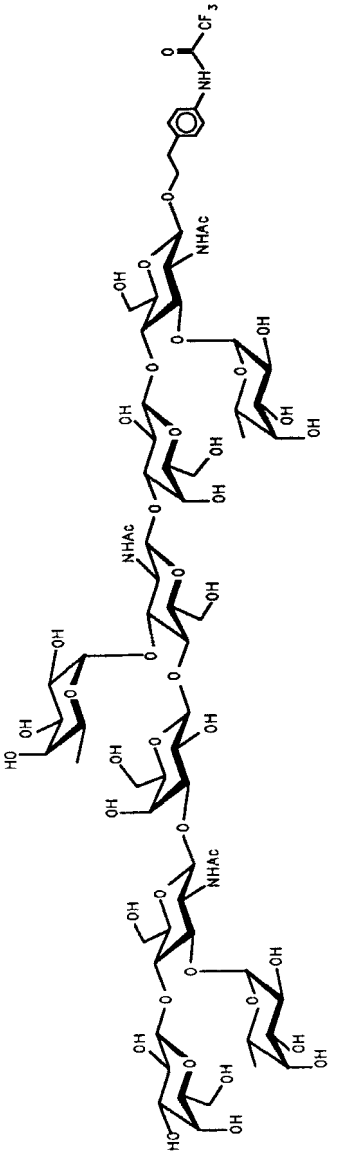


9 $R^1 = NO_2$, R^2 = NPhth, R^3 = OAc, R^4 = OClAc
10 $R^1 = NO_2$, R^2 = NHAc, $R^3 = R^4$ = OH
11 $R^1 = NHCOCF_3$, R^2 = NHAc, $R^3 = R^4$ = OH



12

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, FucA, FucB and FucC where A,B and C designations are arbitrary. TLC was performed on Silica Gel F_{254} (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. Hydrazine acetate was prepared by mixing hydrazine hydrate (10 mL, 0.2 mmol) and acetic acid (11 mL, 0.2 mmol) in methanol (20 mL) at room temperature. Crystallization was obtained by addition of diethyl ether (30 mL) followed by storage at -20 °C overnight. Elemental analyses were not obtained for some amorphous compounds. These were purified by column chromatography and the purity was ascertained by TLC and by NMR spectroscopy. The FAB-MS spectra were recorded with a VG ZAB-SE mass spectrometer. The primary beam consisted of xenon atoms with a maximum energy of 8 KeV. The samples were dissolved in thioglycerol and positive ions were extracted and accelerated over a potential of 10 kV.

***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)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside)** (3). — Hydrazine acetate (0.55 g, 6.0 mmol) in methanol (20 mL) was added to a stirred solution of *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)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside) (2)[1] (1.18 g, 0.64 mmol) in dichloromethane-ethyl acetate (1:1, 40 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 3 (1.07 g, 0.61 mmol, 95%), $[\alpha]_{578}$ -30° (*c* 0.6, chloroform), R_F 0.39 (toluene-ethyl acetate, 1:2). NMR data: ^{13}C, δ 20.3, 21.1 (2 Me acetyl), 35.3 (CH_2 *p*NO_2Ph), 54.66, 54.74 (2 MeO), 55.4, 55.6 (C-2 GlcNA, C-2 GlcNB), 98.1 (C-1 GlcNA),

99.2 (C-1 GlcNB), 100.5 (C-1 GalA, C-1 GalB), 100.7, 101.5 (2 PhCH), 112.9, 113.1 (2 aromatic C *p*-methoxybenzyl), 122.5-138.1 (aromatic C), 146.7 (aromatic C pNO_2Ph), 158.3, 158.5 (2 aromatic C *p*-methoxybenzyl), 168.4, 170.2 (2 C=O acetyl); ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.93 (8.2)	ND	ND	3.90	3.35
GlcNB	5.25 (8.2)	4.15 (10.7)	4.27 (8.1)	3.99	3.68
GalA	4.26 (8.1)	4.99 (10.1)	3.46 (3.6)	4.20	3.72
GalB	4.60 (8.1)	5.09 (9.8)	3.59	4.13	3.95

Anal. Calcd for $C_{96}H_{95}N_3O_{29}$: C, 65.7; H, 5.5; N, 2.4. Found: C, 65.3; H, 5.5; N, 2.3.

***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)-*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)-*O*-(6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside) (4).** — A solution of DMTST (1.15 g, 4.5 mmol) in dry dichloromethane (10 mL) was added to a stirred mixture of **3** (516 mg, 0.29 mmol), 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 (**1**)[1] (764 mg, 0.82 mmol), DTBMP (930 mg, 4.5 mmol) and molecular sieves (4Å) in dichloromethane 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 **4** (580 mg, 0.22 mmol, 75%) having $[\alpha]_{578}$ -21° (*c* 0.6, chloroform), R_F 0.62 (toluene-ethyl acetate, 1:2). NMR data: ^{13}C, δ 20.2, 20.4, 20.9 (3 Me acetyl), 35.3 (CH_2 pNO_2Ph), 40.6 (CH_2Cl), 54.6, 54.71, 54.74 (3 MeO), 55.35, 55.45 (C-2 GlcNA, C-2 GlcNB), 55.55 (C-2 GlcNC), 66.0 (C-5 GalC), 66.3 (C-5 GalA), 66.4 (C-5 GalB), 69.2 (C-2 Gal C), 70.4 C-2 GalA), 70.6 (C-2 Gal C), 74.6 (C-3 GalC), 74.9 (C-5 GlcNC), 74.97 (C-5 GlcNA), 75.03 (C-5 GlcNB), 77.7 (C-3 GalB),

77.8 (C-3 GalA), 98.1 (C-1 GlcNA), 99.09, (C-1 GlcNB), 99.11 (C-1 GlcNC), 100.4 (C-1 GalC), 100.5 (C-1 GalA), 100.66, 100.71 (2 PhCH), 100.8 (C-1 GalB), 101.2 (PhCH), 112.9,113.0, 113.1 (3 aromatic C *p*-methoxybenzyl), 122.8-138.0 (aromatic C), 146.7 (aromatic C pNO_2Ph), 158.26, 158.30, 158.4 (3 aromatic C *p*-methoxybenzyl), 167.1 (C=O) chloroacetyl), 168.4, 168.5, 169.0 (3 C=O acetyl); ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.91 (8.0)	ND	ND	3.87	3.32
GlcNB	5.15 (7.9)	ND	4.08	3.82	3.49
GlcNC	5.27 (8.1)	4.15 (10.6)	4.25 (8.0)	4.02 (9.8)	3.63
GalA	4.21 (8.0)	4.94 (10.1)	3.38	4.13	3.01
GalB	4.36 (8.0)	5.06 (10.0)	3.57 (3.4)	4.21	3.14
GalC	4.64 (8.1)	5.38 (10.2)	4.88 (3.6)	4.31 (1.5)	3.30

FAB-MS showed a molecular ion cluster centered around m/z 2265, as predicted by isotope calculation.

Anal. Calcd for $C_{142}H_{139}ClN_4O_{43}$: C, 65.0; H, 5.4; N, 2.1. Found: C, 64.2; H, 5.2; N, 2.2.

***p*-Nitrophenylethyl *O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-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*-*p*-methoxybenzyl-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*-*p*-methoxybenzyl-β-D-glucopyranoside) (5).** — Hydrazine acetate (690 mg, 7.5 mmol) was added to a mixture of **4** (135 mg, 51 μmol) in toluene-ethanol (2:3, 15 mL). The mixture was refluxed overnight and then concentrated. The product was dissolved in dichloromethane-ethanol 1:1, washed with water and concentrated. The residue, having R_F 0.58 (ethyl acetate-methanol-water, 20:3:2), was then 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 **5** (80 mg, 37 μmol, 72%) having R_F 0.64 (ethyl acetate-methanol-water, 20:3:2). NMR data ($CDCl_3$-CD_3OD, 1:1): ^{13}C, δ 23.0, 23.1 (3 Me N-acetyl), 36.1 (CH_2

pNO_2Ph), 101.5, 101.8, 102.7, 102.8, 103.6, 103.7, 103.8 (6 C-1 and 3 PhCH), 114.0 (3 aromatic C *p*-methoxybenzyl), 147.0 (aromatic C *p*NO_2Ph), 159.6, 159.7 (3 aromatic C *p*-methoxybenzyl), 172.1, 172.9 (3 C=O N-acetyl).

***p*-Trifluoracetamidophenylethyl *O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-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*-*p*-methoxybenzyl-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*-*p*-methoxybenzyl-β-D-glucopyranoside) (6).** — A solution of **5** (61 mg, 28 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 0.60 (ethyl acetate-methanol-water, 20:3:2), was dissolved in dichloromethane (10 mL) and treated with pyridine (64 μL, 790 μmol) and trifluoroacetic anhydride (55 μL, 400 μmol) at 0 °C. After 1 h at room temperature, the reaction mixture was treated with sodium methoxide in methanol for 10 min, neutralized with acetic acid and concentrated. Column chromatography (toluene-ethyl acetate-methanol, 5:5:1) yielded **6** (40 mg, 18 μmol, 65%) having R_F 0.66 (ethyl acetate-methanol-water, 20:3:2). NMR data ($CDCl_3$-CD_3OD, 1:1): ^{13}C, δ 22.95, 23.12, 23.15 (3 Me N-acetyl), 35.9 (CH_2 ethyl), 101.52, 101.77, 102.61, 103.48, 103.66, 103.73, 103.9 (6 C-1 and 3 PhCH), 113.9, 114.0 (3 aromatic C *p*-methoxybenzyl), 159.58, 159.61 (3 aromatic C *p*-methoxybenzyl), 172.2, 172.77, 172.80 (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*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (7).** — A solution of **6** (39 mg, 18 μmol) in a mixture of ethyl acetate-acetic acid-ethanol-water (10:3:3:2) 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 the eluent, giving compound **7** (19 mg, 14 μmol, 79%) having $[\alpha]_{578}$ 7° (*c* 0.3, water), R_F 0.40 (ethyl acetate-acetic acid-methanol-water, 4:3:3:2). The purity and homogeneity of the material was confirmed by HPTLC and gel filtration. Only a single band was detected on HPTLC (two different systems) and a single band was collected from gel filtration, using a refractometer as detector. Furthermore, the NMR spectra required a high degree (> 90%) of purity. NMR data

(D_2O; Me_2CO, δ_H=2.225; δ_C=23.2): ^{13}C, δ 22.8, 23.0 (3 Me N-acetyl), 35.2 (CH_2 ethyl), 55.7, 56.0 (3 C-2 GlcN), 60.7, 60.9, 61.77, 61.84 (3 C-6 GlcN, 3 C-6 Gal), 69.1, 69.4, 70.8, 71.0, 71.8, 73.0, 73.2, 73.3, 75.4, 75.5, 75.7, 76.2 (ring C), 79.0, 79.3 (3 C-4 GlcN), 82.9, (C-3 Gal, C-3 GalB), 101.7 (C-1 GlcNA), 103.6, 103.7 (3 C-1 Gal, C-1 GlcNB, C-1 GlcNC), 123.1, 130.4, 133.7, 138.8 (aromatic C), 175.0, 175.7 (3 C=O N-acetyl); ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4
GlcNA	4.46	3.66	ND	ND
GlcNB	4.70 (8.5)	3.81	ND	ND
GlcNC	4.71 (8.5)	3.81	ND	ND
GalA	4.44 (7.9)	3.57 (10.0)	3.71 (3.2)	4.15
GalB	4.47 (7.8)	3.58 (10.0)	3.73 (3.2)	4.16
GalC	4.48 (7.8)	3.54 (9.8)	3.67 (3.4)	3.93 (1.1)

Positive ion FAB-MS showed an M+H ion at m/z 1329 and an M+Na ion at m/z 1351.

***p*-Nitrophenylethyl *O*-(2-O-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-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)-*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)-*O*-(6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside) (8).** — DDQ (150 mg, 660 μmol) was added to a stirred solution of **4** (289 mg, 110 μmol) in dichloromethane (15 mL) saturated with water. After 3 h at room 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 **8** (218 mg, 96 μmol, 87%), having $[\alpha]_{578}$ -56° (*c* 0.6, chloroform), R_F 0.54 (toluene-ethyl acetate, 1:2). NMR data: ^{13}C, δ 20.2, 20.3, 20.8 (3 Me acetyl), 35.4 (CH_2 *p*NO_2Ph), 40.6 (CH_2Cl), 98.0, 98.99, 99.01, 100.4, 100.5, 100.9, 101.1, 101.4 (6 C-1 and 3 PhCH), 122.8-138.1 (aromatic C), 146.8 (aromatic C *p*NO_2Ph), 167.0 (C=O) chloroacetyl), 168.4, 168.5, 169.0 (3 C=O acetyl)

FAB-MS showed a molecular ion cluster centered around m/z 2625, as predicted by isotope calculation.

Anal. Calcd for $C_{118}H_{115}ClN_4O_{40}$: C, 62.6; H, 5.1; N, 2.5. Found: C, 61.8; H, 5.0; N, 2.3

***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*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-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*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-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*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-phthalimido-β-D-glucopyranoside]** (9). — A solution of silver triflate (817 mg, 3.2 mmol) and 2,4,6-trimethylpyridine (423 μL, 3.2 mmol) in dichloromethane-toluene (3:2, 5 mL) was added to a stirred mixture of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[11] (1.32 g, 2.7 mmol), **8** (400 mg, 0.18 mmol) and molecular sieves (4Å) in dry dichloromethane (10 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 **9** (422 mg, 0.12 mmol, 68%) having $[\alpha]_{578}$ -104° (*c* 0.5, chloroform), R_F 0.73 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 15.4, 16.1 (C-6 FucA, C-6 FucB, C-6 FucC), 20.2, 20.4, 20.8 (3 Me acetyl), 35.2 (CH_2 *p*NO_2Ph), 40.6 (CH_2Cl), 55.9 (C-2 GlcNA), 56.3 (C-2 GlcNB), 56.4 (C-2 GlcNC), 66.2 (C-5 GalC, C-5 FucA), 66.3 (C-5 FucB, C-5 FucC), 66.5 (C-5 GalA), 66.7 (C-5 GalB), 68.5 (C-2 GalC), 70.3 (C-2 GalA), 70.6 (C-2 GalB), 71.3 (C-3 GlcNC), 71.4 (C-3 GlcNB), 71.5 (C-3 GlcNA), 73.0 (C-4 GalC), 73.7 (C-3 GalC, C-2 FucA, C-2 FucB, C-2 FucC), 74.3 (C-4 GlcNA), 74.7 (C-4 GlcNB), 75.3 (C-4 GlcNC), 75.4 (C-5 GlcNC, C-4 GalA), 75.6 (C-5 GlcNA, C-5 GlcNB, C-4 GalB), 76.3 (C-3 GalB), 76.6 (C-3 GalA), 78.8 (C-3 FucA, C-3 FucB, C-3 FucC, C-4 FucA, C-4 FucC), 79.0 (C-4 FucB), 97.5 (C-1 FucA), 97.6 (C-1 FucB, C-1 FucC), 98.0 (C-1 GlcNA), 98.9 (C-1 GlcNB, C-1 GlcNC), 99.5 (C-1 GalA, C-1 GalC), 99.7 (C-1 GalB, 3 PhCH), 122.9-139.6 (aromatic C), 146.6 (aromatic C *p*NO_2Ph), 167.1 (C=O chloroacetyl), 167.9, 168.1, 168.7 (3 C=O acetyl); ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.86 (8.2)	4.24 (10.7)	4.37 (8.8)	4.01 (10.3)	3.29
GlcNB	5.06 (8.1)	4.38 (10.8)	4.52 (8.8)	3.96 (10.1)	3.42
GlcNC	5.19 (8.2)	4.46 (10.7)	4.70 (8.9)	4.11	3.58
GalA	4.32 (7.9)	4.84 (10.0)	3.41 (3.7)	4.11	2.95
GalB	4.41 (8.0)	4.92 (9.9)	3.60 (4.0)	4.19	3.08
GalC	4.68 (8.0)	5.36 (10.0)	4.81 (3.8)	4.28 (1.6)	3.12
FucA	4.40 (3.8)	3.45 (10.6)	3.75 (3.3)	2.95	4.51
FucB	4.54 (3.7)	3.51 (10.6)	3.77 (3.1)	3.00	4.55
FucC	4.65 (3.8)	3.58 (10.6)	3.82 (3.5)	3.11	4.71

_p_-Nitrophenylethyl _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)-_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)-_O_-[2-acetamido-6-_O_-benzyl-2-deoxy-3-_O_-(2,3,4-tri-_O_-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside] (10). — Hydrazine acetate (690 mg, 7.5 mmol) was added to a mixture of **9** (197 mg, 56 μmol) in toluene-ethanol (2:3, 15 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 0.20 (toluene-ethyl acetate-methanol, 5:5:1), 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 **10** (140 mg, 45 μmol, 82%) having R_F 0.27 (toluene-ethyl acetate-methanol, 5:5:1). NMR data: ^{13}C, δ 16.2, 16.4 (C-6 FucA, C-6 FucB, C-6 FucC), 23.0, 23.18, 23.22 (3 Me N-acetyl), 35.7 (CH_2 _p_NO_2Ph), 66.35, 66.39, 66.45 (C-2 GlcNA, C-2 GlcNB, C-2 GlcNC), 97.8, 98.2 (C-1 FucA, C-1 FucB, C-1 FucC), 99.3, 99.8, 100.9, 101.1, 101.8, (6 C-1 and 3 PhCH), 123.2-139.4 (aromatic C), 147.2 (aromatic C _p_NO_2Ph), 170.3, 170.4 (3 C=O N-acetyl).

_p_-Trifluoracetamidophenylethyl _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)-*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)-*O*-[2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside] (11). — A solution of **10** (125 mg, 41 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 0.22 (toluene-ethyl acetate-methanol, 5:5:1), was dissolved in dichloromethane (5 mL) and treated with pyridine (65 μL, 810 μmol) and trifluoroacetic anhydride (56 μl, 400 μmol) at 0 °C. After 1 h at room temperature, the reaction mixture was treated with sodium methoxide in methanol for 10 min, neutralized with acetic acid and concentrated. Column chromatography (toluene-ethyl acetate-methanol, 5:5:1) yielded **11** (104 mg, 33 μmol, 81%) having R_F 0.26 (toluene-ethyl acetate-methanol, 5:5:1). NMR data: ^{13}C, δ 16.2, 16.4 (C-6 FucA, C-6 FucB, C-6 FucC), 22.9, 23.0, 23.1 (3 Me N-acetyl), 35.3 (CH_2 *p*NO_2Ph), 97.7-101.8 (9 C-1 and 3 PhCH), 170.6, 171.4 (3 C=O N-acetyl).

***p*-Trifluoracetamidophenylethyl *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*-[3-*O*-(α-L-fucopyranosyl)-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-glucopyranoside (12).** — A solution of **11** (104 mg, 33 μmol) in a mixture of ethyl acetate-ethanol-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 **12** (54 mg, 31 μmol, 91%) having $[\alpha]_{578}$ -73° (*c* 0.5, water), R_F 0.30 (ethyl acetate-acetic acid-methanol-water, 2:3:3:2). The purity and homogeneity of the material was confirmed by HPTLC and gel filtration. Only a single band was detected on HPTLC (two different systems) and a single band was collected from gel filtration, using a refractometer as detector. Furthermore, the NMR spectra required a high degree (> 90%) of purity. NMR data (D_2O; Me_2CO, δ_H=2.225; δ_C=23.2): ^{13}C, δ 16.12, 16.15, 16.18 (3 C-6 Fuc), 22.9, 23.1 (3 Me *N*-acetyl), 35.3 (CH_2 ethyl), 56.4 (C-2 GlcNA), 56.8 (C-2 GlcNB, C-2 GlcNC), 60.5, 60.7, 62.28, 62.30, 62.35 (3 C-6 GlcN, 3 C-6 Gal), 67.6 (3 C-5 Fuc), 68.5 (C-2 FucA), 68.6, 68.7 (C-2 FucB, C-2 FucC), 69.1 (C-4 GalA, C-4 GalB), 69.2 (C-4 GalC), 70.1 (3 C-3 Fuc), 71.1 (CH_2 ethyl), 71.4 (C-2 GalA, C-2 GalB), 71.9 (C-2 GalC), 72.7 (3 C-4 Fuc), 73.4 (C-3 GalC), 73.7,

73.9 (3 C-4 GlcN), 75.6 (C-3 GlcNB, C-3 GlcNC), 75.8 (C-3 GlcNA), 82.5 (C-3 GalA, C-3 GalB), 99.5 (C-1 FucC), 99.6 (C-1 FucA, C-1 FucB), 101.6 (C-1 GlcNA), 102.6 (3 C-1 Gal), 103.3 (C-1 GlcNB, C-1 GlcNC), 123.2, 130.5, 133.7, 138.8 (aromatic C), 174.4, 174.8 (3 C=O N-acetyl); ^{1}H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
GlcNA	4.49	3.80	3.76	3.87	ND
GlcNB	4.70 (8.5)	3.95 (10.2)	3.87	3.95	ND
GlcNC	4.70 (8.5)	3.97 (10.4)	3.85	3.97	ND
GalA	4.42 (7.8)	3.49 (9.9)	3.68 (3.4)	4.08 (1.4)	ND
GalB	4.44 (7.9)	3.51 (9.9)	3.69 (3.4)	4.09 (1.4)	ND
GalC	4.47 (7.8)	3.50 (9.9)	3.66 (3.3)	3.89 (1.5)	ND
FucA	5.01 (4.0)	3.64 (10.2)	3.85 (3.5)	3.75 (1.7)	4.78 (6.6)
FucB	5.12 (3.9)	3.68 (10.1)	3.89 (3.4)	3.77 (1.9)	4.81 (6.6)
FucC	5.13 (3.9)	3.69 (10.1)	3.90 (3.4)	3.79 (1.9)	4.83 (6.6)

Positive ion FAB-MS showed an M+H ion at m/z 1768 and an M+Na ion at m/z 1790.

ACKNOWLEDGEMENTS

We thank Mr Stefan Strömberg (Analytical Department, BioCarb AB) for recording FAB mass spectra and Mr Gunnar Grönberg (Analytical Department, BioCarb AB) for recording the 500 MHz NMR spectra.

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