Synthesis of the Trifucosylated *N*-Linked Hexasaccharide of a Glycoprotein from *Haemonchus contortus*

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The synthesis of a hexasaccharide from the inner part of an *N*-linked oligosaccharide portion of a glycoprotein from *Haemonchus contortus* is described. This hexasaccharide contains a novel fucosylation pattern with three α-linked fucosyl groups at *O*-3, *O*-6 and *O*-3′ of the two *N*-acetylglucosamine residues. In the synthesis two consecutive regioselective glycosylations were performed, thereby limiting protecting group manipulations, to yield a trisaccharide with β-(1→4) linkages. Subsequent trifucosylation in one step, followed by inversion of configuration via a displacement reaction at *C*-2′′ of the terminal glucosyl residue produced the resulting β-mannosyl linkage. The 2-acetamido-2-deoxy-glucosylamine residue was capped by an *N*-acetyl group and the hexasaccharide was deprotected.

Introduction

Recently, a structural study of *N*-linked oligosaccharides from the nematode *Haemonchus contortus* revealed a novel core structure not previously encountered in any eukaryotic glycoprotein.[1] The core trisaccharide structure is conserved in mammalian, plant and invertebrate *N*-linked glycoproteins, while the fucosyl substitution is different. In mammalian glycoproteins, fucose is linked to *O*-6 of the GlcNAc residue at the reducing end, whereas in plants the linkage is to *O*-3. Invertebrates can produce core structures with both *O*-3 and *O*-6 linked fucoses on the same GlcNAc residue.[2] The structural study conducted by Haslam et al.[1] revealed a unique substitution pattern, with two fucoses linked to both *O*-3 and *O*-6 on the GlcNAc residue at the reducing end, and a fucose linked to *O*-3′ on the distal GlcNAc residue (Figure 1).

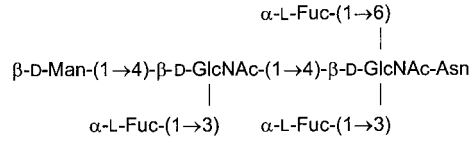

Figure 1. An *N*-glycan core structure present on a glycoprotein from *Haemonchus contortus*

Fucosylation at the 3-position of the GlcNAc residue proximal to the polypeptide chain makes the *N*-linked glycan resistant towards *N*-glycosidase F.[3] We report here a synthesis of the highly fucosyl-substituted core region of an *N*-linked hexasaccharide substituting a *Haemonchus contortus* glycoprotein. In particular, we describe the synthesis of a hexasaccharide derivative having an *N*-acetyl group instead of an asparagine residue.

Results and Discussion

Disconnection of the target hexasaccharide reveals three major synthetic steps: construction of a trisaccharide core, trifucosylation and conversion of a terminal β-glucopyranoside into a β-mannopyranoside. To minimize the protecting group manipulations in the synthesis of the hexasaccharide, we decided to employ a strategy based on matched-mismatched donor-acceptor pairs for construction of the trisaccharide core.[4−6] In the synthesis of oligosaccharides where the core trisaccharide has been a central part of the structure several methods exist for the formation of β-mannosyl linkages. These include, inter alia, direct β-mannosylation with an insoluble silver salt,[7] *p*-methoxybenzyl-assisted internal aglycon delivery,[8,9] inversion reactions of a β-linked galactosyl residue[10] and inversion of the configuration at *C*-2 of a β-glucosyl residue by displacement of an equatorially positioned leaving group.[11] In our synthesis we have chosen the latter approach.

The monosaccharide building blocks **2**−**4** were designed to fit the synthetic plan (Scheme 1).

Diol **2** was prepared from **6**[12] by *O*-deacetylation with guanidine/guanidinium nitrate[13] (G/GHNO$_3$) followed by acetalization to form **7**, which was then reductively opened to generate the acceptor with two free hydroxyl groups. The distal GlcNAc residue **3**, which was to be used first as a glycosyl donor and then as the terminal part of the acceptor in the following step, was made from **8**[14] by *O*-acetylation.

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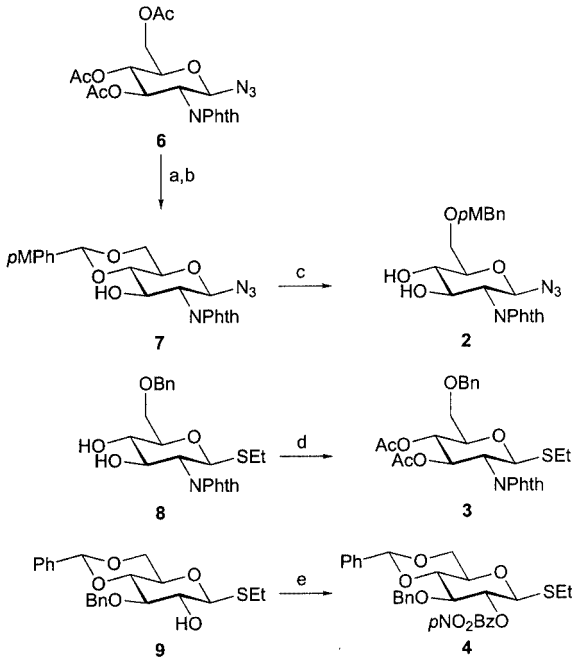
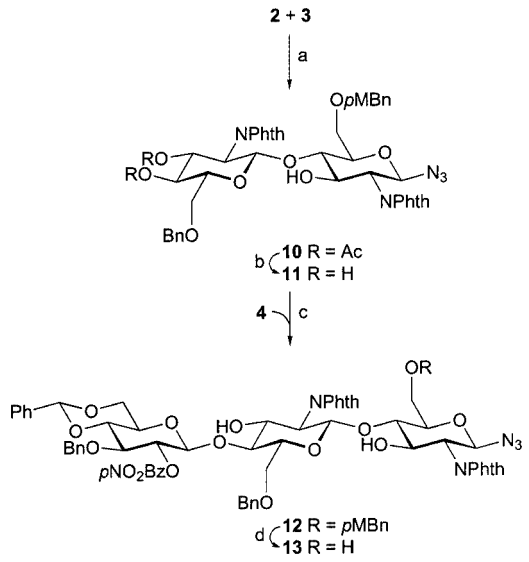


Scheme 1. Reagents and conditions: (a) G/$GHNO_3$ (excess), MeOH, room temp.; (b) *p*-methoxybenzaldehyde dimethyl acetal (1.5 equiv.), *p*-TsOH, acetonitrile, 30 min, 50 °C; 71% over two steps; (c) $NaCNBH_3$, TFA, DMF, 3 Å mol. sieves, 18 h, 0 °C → room temp., 61%; (d) Ac_2O (3 equiv.), DMAP (4 equiv.), CH_2Cl_2, 10 min, room temp., 93%; (e) *p*-NO_2BzCl (1.3 equiv.), DMAP (2 equiv.), CH_2Cl_2, 15 min, room temp., 95%



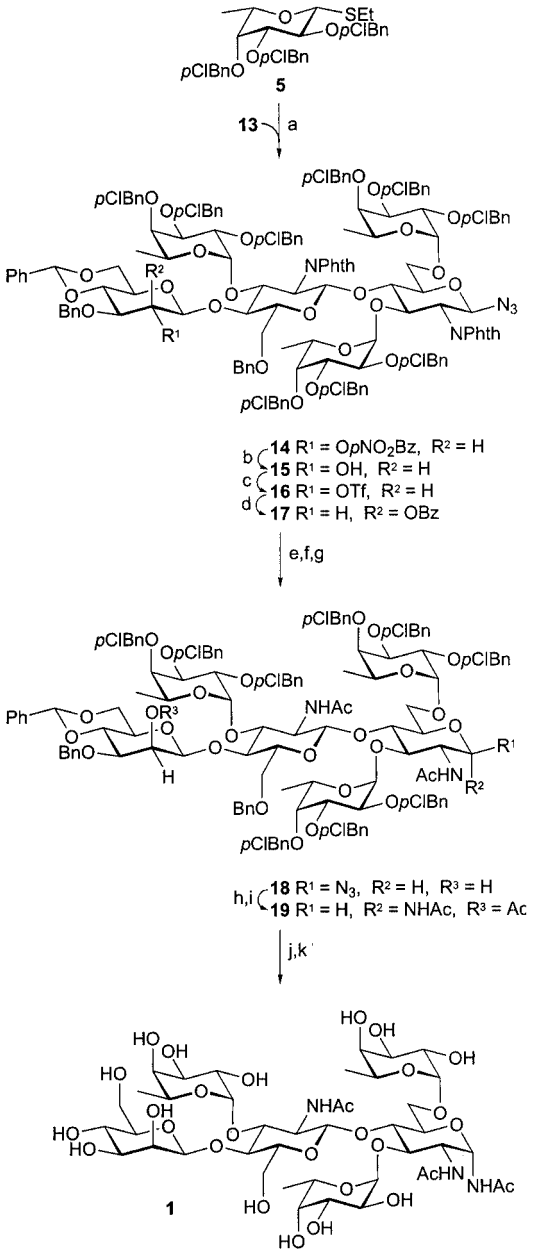
Scheme 2. Reagents and conditions: (a) **2** (1 equiv.) ,**3** (1.2 equiv.), NIS (1.2 equiv.), AgOTf (0.33 equiv.), CH_2Cl_2, 4 Å mol. sieves, 80%; (b) G/$GHNO_3$ (excess), MeOH, 90%; (c) **11** (1 equiv.), **4** (1.1 equiv.), NIS (1.2 equiv.), AgOTf (0.48 equiv.), 4 Å mol. sieves, CH_2Cl_2, 69%; (d) DDQ (1.2 equiv.), CH_2Cl_2, 89%

The β-directing glucosyl donor **4** was made from **9**[15] by *O*-acylation. The *p*-nitrobenzoyl group at *O*-2 in donor **4** was anticipated to be easier to remove than an acetyl or benzoyl group, in the presence of the phthalimido functionalities.

The formation of the trisaccharide core (Scheme 2) started with a regioselective glycosylation between donor **3** and acceptor **2** providing disaccharide **10** in 80% yield. Treatment of **10** with G/$GHNO_3$ facilitated the removal of the acetates without affecting the phthaloyl groups, affording disaccharide **11** in 90% yield. The newly formed disaccharide acceptor was employed in the second regioselective glycosylation using donor **4** to give trisaccharide **12** in 69% yield, together with 17% of a tetrasaccharide. In order to proceed with the trifucosylation the *p*-methoxybenzyl group then had to be removed. Treating **12** with DDQ[16] gave trisaccharide **13** in 89% yield. Initially, ethyl 2,3,4-tri-*O*-benzylthio-β-L-fucopyranoside was chosen as a glycosyl donor. However, in the coupling reaction a good deal of hydrolysis product was formed and a large excess of donor was required for product formation. The use of *p*-chlorobenzyl protecting groups has been reported to work well for fucosyl groups,[17] and compound **5** was therefore used. The trifucosylation was performed under halide ion assisted conditions,[18] to provide hexasaccharide **14** in 62% (Scheme 3).

The formation of the β-mannoside started with the deprotection of the *p*-nitrobenzoyl group, again employing G/$GHNO_3$. The transformation was readily performed in 5 h and provided compound **15** in 86% yield. Triflation of the free hydroxyl group followed by treatment with tetrabutylammonium benzoate was chosen for the inversion of configuration at *C*-2′′ of the glucosyl residue. Preliminary displacement reactions at the disaccharide level resulted in formation of β-mannoside products in greater than 80% yields. This finding agrees well with the recently described synthesis of an asparagine-linked heptasaccharide representing the basic structure of *N*-glycans, where a similar yield was obtained.[19] However, when the same reaction conditions were applied to **16** the resulting β-mannoside **17** was isolated in a moderate yield of 51%. That a β-mannoside had been formed was evident by ^1H NMR spectroscopy, where signals belonging to the same spin system in **17** were observed, inter alia, at $\delta_H = 5.61$ ($J_{H2,H3} = 3.4$ Hz) and $\delta_H = 3.09$ (ddd, $J_{H5,H6a} = 4.9$ Hz, $J_{H5,H4} = J_{H5,H6b} = 9.4$ Hz), assigned to H2 and H5 in the β-mannopyranosyl group, respectively. The phthaloyl groups were then removed by the action of ethylenediamine,[20] followed by re-*N*,*O*-acetylation and removal of the *O*-acetyl group under Zemplen conditions, to give **18** in 69% yield.

There are several ways to transform an anomeric azido group to the corresponding *N*-acetyl group.[21] In the present case compound **18** was treated with 1,3-propanedithiol[22] followed by *N*- and *O*-acetylation. The resulting product **19** was isolated in 71% yield. However, it was obvious from the ^{13}C chemical shift at $\delta = 47.0$ that the α-anomeric configuration had been formed (vide infra) compared to $\delta = 53.4$ of *C*-2 in 1,2-bisacetamido-3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-glucopyranose.[23] Deprotection of the benzyl and *p*-chlorobenzyl groups together with the 4,6-*O*-benzylidene group was performed by hydrogenolysis using a catalytic amount of $Pd(OH)_2$. After *O*-deacetylation and purification by gel permeation chromatography compound **1** was isolated in 83% yield. The anomeric region of the ^1H,^{13}C-HSQC spectrum of **1** is shown in Figure 2. That



Scheme 3. Reagents and conditions: (a) **5** (10 equiv.), Br_2 (11 equiv.), **13**, Et_4NBr (0.5 equiv.), CH_2Cl_2/DMF 2:1, 4 Å mol. sieves, 48 h, 62%; (b) G/$GHNO_3$ (excess), CH_2Cl_2, 86%; (c) pyridine (15 equiv.), Tf_2O (8 equiv.); (d) QOBz (4 equiv.), 51% over two steps; (e) ethylenediamine, *n*-butyl alcohol, 18 h, 90 °C; (f) Ac_2O/pyridine; (g) NaOMe/MeOH, 30 min, 69% over three steps; (h) 1,3-propanedithiol (20 equiv.), diisopropyl ethyl amine, 4 h; (i) Ac_2O/pyridine; (j) $Pd(OH)_2$, 100 psi, 15 h; (k) NaOMe/MeOH, 83% over three steps

the 2-acetamido-*N*-acetyl-2-deoxy-D-glucopyranosylamine in **1** indeed has the α-anomeric configuration of the *N*-acetyl-glucopyranosylamine is evident from the $J_{H-1,H-2}$ value of 3.5 Hz; the β-anomer should have a significantly larger value of approximately 9.8 Hz.[24,25] ^1H or ^{13}C NMR chemical shifts of anomeric atoms are also useful indicators of the anomeric configuration,[26,27] whereas $J_{H-1,C-1}$ values differ only slightly.[28]

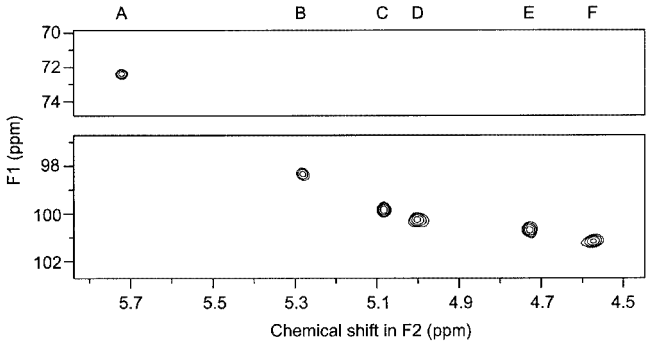


Figure 2. The anomeric region of the $^1H,^{13}C$-HSQC spectrum of **1**: (A) α-GlcNAc; (B-D) α-Fuc; (E) β-Man; (F) β-GlcNAc

Future studies will utilize and extend the synthetic route presented here, which, from a limited number of protecting group manipulations, provides the highly fucosylated oligosaccharide. Furthermore, the unique fucosyl substitution pattern on the core structure may affect its conformational properties. These issues are well-suited for investigation by solution state NMR spectroscopy.

Experimental Section

General: CH_2Cl_2 was distilled from CaH_2 prior to use. Concentrations were performed under reduced pressure at temperatures of less than 40 °C (bath). NMR spectra were recorded at 30 °C for solutions in $CDCl_3$, or at 27 °C in D_2O using Varian 300, 400 and 600 spectrometers and standard pulse sequences. Chemical shifts are reported in ppm relative to internal $SiMe_4$ (δ_H = 0.00, δ_C = 0.00) or internal sodium 4,4-dimethyl-4-sila(2,2,3,3-D_4)pentanoate (δ_H = 0.00). High Resolution Fast Atom Bombardment Mass Spectrometry (HR-FABMS) was performed in the positive mode at a resolution of 10 000 on a JEOL SX-102 using triethylene glycol or 3-nitrobenzyl alcohol as a matrix.

6-*O*-*p*-Methoxybenzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide (2): Compound **6** (9.99 g, 21.7 mmol) was suspended in MeOH (50 mL) and a solution of G/$GHNO_3$ (250 mL) was added. When TLC ($CHCl_3$/MeOH 7:1) showed complete reaction, the mixture was neutralized with acetic acid, evaporated and the crude residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH 7:1). The purified glycosyl azide was suspended in acetonitrile (25 mL) and *p*-methoxybenzaldehyde dimethyl acetal (5.94 g, 32.6 mmol) was added together with a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture was stirred at 50 °C for 30 min, after which time the reaction was quenched with triethylamine. Evaporation of the solvent and purification by column chromatography (silica gel, toluene/EtOAc 2:1) gave derivative **7** (6.98 g) in 71% yield. ^{13}C NMR ($CDCl_3$): δ = 55.3, 56.0, 68.2, 68.3, 81.7, 86.2, 101.9, 113.7, 123.6−137.8, 160.3, 167.9, 168.1.

A cooled (0 °C) solution of trifluoroacetic acid (25.5 mL, 332 mmol) in DMF (25 mL) was added dropwise during 30 min to a cooled mixture of $NaCNBH_3$ (8.29 g, 132 mmol) and 3 Å molecular sieves in DMF (25 mL). The mixture was then allowed to stir for 15 min. A solution of **7** (6.02 g, 13.3 mmol) in DMF was then added to this mixture during 30 min. The reaction mixture was allowed to reach room temperature, and stirring was continued for 18 h. The reaction was quenched with triethylamine, filtered

through celite, diluted with toluene and washed with water. The organic layer was dried ($NaSO_4$), filtered, concentrated and purified by column chromatography (silica gel, toluene/acetone 2:1), to give **2** (3.67 g, 61%). ^{13}C NMR ($CDCl_3$): δ = 55.3, 55.7, 69.4, 71.4, 73.2, 73.5, 76.1, 85.7, 114.0, 123.6−134.3, 159.5, 166.1. HR-FABMS: $[M + Na]^+$ *m*/*z* calcd. for $C_{22}H_{22}O_7N_4Na$ 477.1386, found 477.1391.

Ethyl 3,4-Di-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3): Compound **8** (5.99 g, 13.5 mmol) and 4-dimethylaminopyridine (DMAP) (8.26 g, 67.6 mmol) were mixed together in CH_2Cl_2 (25 mL) and acetic anhydride (3.84 mL, 40.6 mmol) was then added. After 10 min the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with water, 1 M HCl and saturated aqueous $NaHCO_3$. Flash column chromatography (silica gel, toluene/EtOAc 7:1) gave **3** (6.62 g, 93%). ^{13}C NMR ($CDCl_3$): δ = 15.4, 20.9, 21.1, 54.1, 69.4, 70.1, 72.0, 73.8, 77.7, 81.2, 123.8−137.9, 167.7, 168.0, 169.6, 170.2. FABMS: $[M + Na]^+$ *m*/*z* = 550.1.

Ethyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*p*-nitrobenzoyl-1-thio-β-D-glucopyranoside (4): Compound **9** (3.0 g, 7.5 mmol) and DMAP (1.8 g, 15 mmol) were mixed together in CH_2Cl_2 (25 mL) and *p*-nitrobenzoyl chloride (1.8 g, 9.7 mmol) was then added. After 15 min the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with water, 1 M HCl and saturated aqueous $NaHCO_3$. Flash column chromatography (silica gel, toluene/EtOAc 7:1) gave **4** (3.9 g, 95%). ^{13}C NMR ($CDCl_3$): δ = 15.3, 24.5, 68.9, 71.0, 72.9, 74.5, 79.2, 82.0, 84.3, 101.5, 123.6−131.1, 135.2, 137.2, 137.8, 150.7, 163.4. FABMS: $[M + H]^+$ *m*/*z* = 552.2.

6-*O*-Benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-*O*-*p*-methoxybenzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide (11): Compounds **2** (720 mg, 1.58 mmol) and **3** (1.00 g, 1.89 mmol) were mixed together with 4 Å molecular sieves (2 g) in CH_2Cl_2 (30 mL) and cooled to −45 °C. *N*-iodosuccinimide (NIS) (512 mg, 2.27 mmol) and AgOTf (195 mg, 0.76 mmol) were then added. When TLC indicated complete reaction, triethylamine was added. The reaction mixture was filtered through celite, diluted with CH_2Cl_2, washed with $Na_2S_2O_3$, dried, filtered and concentrated. Column chromatography (silica gel, toluene/EtOAc 3:1) gave **10** (1.17 g, 80%). ^{13}C NMR ($CDCl_3$): δ = 20.4, 20.6, 54.8, 55.3, 55.4, 67.3, 68.8, 69.5, 69.9, 70.6, 72.6, 72.8, 73.5, 76.2, 81.6, 85.5, 98.7, 113.6, 123.7−137.0, 159.1, 167.5, 169.6, 170.0. FABMS: $[M + Na]^+$ *m*/*z* = 942.1.

$G/GHNO_3$ (50 mL) was added to a solution of **10** (1.20 g, 1.30 mmol) in CH_2Cl_2 (10 mL). The reaction was complete within 1 h. The reaction mixture was then neutralized with acetic acid, concentrated and gave **11** (980 mg, 90%) after purification by column chromatography (silica gel, toluene/EtOAc 1:1). ^{13}C NMR ($CDCl_3$): δ = 55.3, 55.5, 56.4, 67.6, 69.7, 69.8, 71.4, 72.5, 72.6, 73.5, 74.2, 76.2, 81.3, 85.5, 99.0, 113.7, 123.6−137.3, 159.1, 168.2. FABMS: $[M + Na]^+$ *m*/*z* = 858.2.

3-*O*-Benzyl-4,6-*O*-benzylidene-2-*p*-nitrobenzoyl-β-D-glucopyranosyl-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-*O*-*p*-methoxybenzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide (12): Compounds **4** (690 mg, 1.25 mmol) and **11** (950 mg, 1.13 mmol) were mixed together with 4 Å molecular sieves (2 g) in CH_2Cl_2 (20 mL) and cooled to −45 °C. NIS (310 mg, 1.38 mmol) and AgOTf (141 mg, 0.549 mmol) were then added. After 45 min TLC indicated complete reaction and triethylamine was added. The reaction mixture was filtered through celite, diluted with CH_2Cl_2 washed with $Na_2S_2O_3$, dried, filtered and concentrated. Column chromatography (silica gel, toluene/EtOAc 5:1) gave **12** (1.04 g, 69%). ^{13}C NMR ($CDCl_3$): δ = 55.3, 55.4, 55.8, 66.5, 67.4, 68.0, 68.1, 69.3, 69.7, 72.5, 72.9, 73.0, 73.2, 74.0, 76.1, 81.3, 81.4, 82.1, 85.4, 98.8, 101.4, 102.2, 113.6, 123.4−137.5, 159.1, 164.7, 167.8. FABMS: $[M + Na]^+$ *m*/*z* = 1347.3.

3-*O*-Benzyl-4,6-*O*-benzylidene-2-*p*-nitrobenzoyl-β-D-glucopyranosyl-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide (13): DDQ (214 mg, 0.941 mmol) was added to a solution of **12** (1.04 g, 0.785 mmol) in CH_2Cl_2/H_2O (20:1, 21 mL). After 2.5 h the reaction was complete. The reaction mixture was then extracted with saturated aqueous $NaHCO_3$, and the organic layer was dried ($NaSO_4$), filtered and concentrated. The residue was purified by chromatography (silica gel, toluene/EtOAc 3:1) to give **13** (840 mg, 89%). ^{13}C NMR ($CDCl_3$): δ = 55.5, 55.6, 60.5, 66.5, 67.9, 68.1, 69.5, 69.6, 73.3, 73.6, 74.0, 74.1, 76.3, 80.9, 81.1, 81.4, 85.8, 99.2, 101.4, 101.5, 123.5−137.5, 150.7, 163.0, 167.4, 168.1. FABMS: $[M + Na]^+$ *m*/*z* = 1227.2.

3-*O*-Benzyl-4,6-*O*-benzylidene-2-*p*-nitrobenzoyl-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→6)]-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide (14): The ethyl thiofucoside donor **5** (2.90 g, 4.99 mmol) was dissolved in CH_2Cl_2, the solution cooled to 0 °C and bromine was added. After 15 min the reaction was quenched with cyclohexene (1 mL) and concentrated. The generated fucosyl halide was dissolved in 3 mL of CH_2Cl_2 and 1.5 mL of dry DMF, and transferred to a 25 mL flask containing trisaccharide **13** (600 mg, 0.499 mmol), 4 Å molecular sieves (2 g) and tetraethylammonium bromide (0.4 g). The mixture was stirred for 48 h, diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$, dried, concentrated and purified by column chromatography (silica gel, (boiling range 40−60 °C)/petroleum ether/EtOAc 3:1) to give **14** (855 mg, 62%). ^{13}C NMR ($CDCl_3$): δ = 16.3, 16.4, 16.9, 55.7, 56.5, 64.4, 66.1, 66.4, 67.0, 67.4, 68.1, 68.5, 71.7−79.7, 81.8, 85.1, 95.7, 97.2, 97.2, 97.4, 98.9, 101.3, 123.3−137.6, 150.4, 162.6, 167.4, 167.9, 168.8.

3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→6)]-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide (15): Compound **14** (800 mg, 0.289 mmol) was dissolved in 5 mL of CH_2Cl_2 and 25 mL of $G/GHNO_3$ was added. The target compound **15** was formed within 2 h. The reaction mixture was neutralized with HOAc, concentrated and purified by column chromatography (silica gel, petroleum ether/EtOAc 2:1) to give **15** (651 mg, 86%). ^{13}C NMR ($CDCl_3$): δ = 16.4, 16.5, 16.8, 55.8, 56.4, 64.5, 66.1, 66.6, 67.2, 68.1, 68.5, 71.5−79.1, 80.5, 81.0, 85.2, 95.6, 96.8, 97.0, 97.3, 101.0, 101.6, 123.3−138.1, 167.0, 167.5, 168.7. FABMS: $[M + Cs]^+$ *m*/*z* = 2748.5.

2-*O*-Benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→6)]-2-deoxy-2-phthalimido-β-D-glucopyranosyl Azide (17): Dry pyridine (0.277 mL, 3.44 mmol) was added to CH_2Cl_2 (2 mL) and cooled to 0 °C in an ice bath. Trifluoromethanesulfonic anhydride (0.308 mL, 1.83 mmol) was then added slowly to this solution. The resulting mixture was allowed to stir for 10 min and compound **15** (600 mg, 0.229 mmol) dissolved

in CH_2Cl_2 was added. The reaction mixture was allowed to reach room temperature and stirring was continued for 10 h when TLC indicated complete formation of the triflate **16**. The mixture was transferred to a separating funnel and extracted with saturated aqueous $NaHCO_3$, the organic layer was dried (Na_2SO_4), filtered, evaporated and co-evaporated with toluene. The crude residue was dissolved in toluene and tetrabutylammonium benzoate (333 mg, 0.918 mmol) was added. The reaction mixture was refluxed for 8 h. The reaction mixture was allowed to attain room temperature, concentrated and the residue was purified by column chromatography (silica gel, toluene/EtOAc 12:1) to give **17** (319 mg, 51%). ^{13}C NMR ($CDCl_3$): δ = 16.3, 16.8, 55.9, 56.7, 64.0, 66.0, 66.1, 66.7, 67.3, 67.5, 68.5, 72.3−79.4, 81.7, 85.0, 95.9, 96.7, 97.2, 97.4, 99.5, 101.0, 123.4−139.2, 164.0, 167.2, 167.9, 168.6.

3-*O*-Benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl Azide (18): *n*-Butyl alcohol (7 mL) and ethylenediamine (1.5 mL) were added to hexasaccharide **17** (300 mg, 0.110 mmol). The reaction mixture was heated to 90 °C and stirring was maintained for 18 h. The solvents were evaporated and co-evaporated twice from toluene and ethanol, respectively. The residue was dissolved in pyridine (10 mL) and Ac_2O (5 mL) was added. When the reaction was complete (TLC, toluene/acetone 2:1) the reaction solvents were evaporated and dissolved in 0.1 M NaOMe in MeOH, and stirred for 30 min, concentrated and purified by column chromatography (silica gel, toluene/acetone 2:1) to give **18** (185 mg, 69%). ^{13}C NMR ($CDCl_3$): δ = 16.8, 17.1, 22.9, 23.2, 51.0, 54.3, 66.5, 67.2, 67.3, 68.3, 68.4, 69.1, 69.5, 71.2−79.6, 88.3, 96.5, 98.2, 99.0, 99.8, 100.0, 101.5, 125.8−137.9, 169.9, 171.0.

2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→3)]-[2,3,4-tri-*O*-*p*-chlorobenzyl-α-L-fucopyranosyl-(1→6)]-2-acetamido-*N*-acetyl-2-deoxy-α-D-glucopyranosylamine (19): Compound **18** (150 mg, 0.615 mmol) was dissolved in CH_2Cl_2/MeOH (1:10). 1,3-Propanedithiol (20 equiv.) and diisopropyl ethyl amine were subsequently added. After 4 h no starting material was detected by TLC ($CHCl_3$/MeOH 9:1). The reaction mixture was concentrated, and dissolved in pyridine (7 mL) and Ac_2O (4 mL). When acetylation was complete, the mixture was concentrated and purified by flash column chromatography (silica gel, toluene/acetone 1:1) to give compound **19** (109 mg, 71%). ^{13}C NMR ($CDCl_3$): δ = 16.8, 17.1, 21.1, 23.0, 23.3, 23.5, 47.0, 54.6, 65.7−79.3, 96.4, 97.5, 98.1, 98.9, 100.9, 101.4, 125.1−137.4, 169.1, 170.2, 170.8, 171.9. FABMS: $[M + Na]^+$ *m/z* = 2520.5.

β-D-Mannopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido2-deoxy-β-D-glucopyranosyl)-(1→4)-[α-L-fucopyranosyl-(1→3)][α-L-fucopyranosyl-(1→6)]-2-acetamido-*N*-acetyl-2-deoxy-α-D-glucopyranosylamine (1): Compound **19** (58 mg, 0.020 mmol) was dissolved in a mixture of EtOAc/EtOH (1:10, 10 mL). A catalytic amount of $Pd(OH)_2$ was added and hydrogenolysis was conducted at 100 psi for 15 h after which time the material was filtered. Evaporation of the solvent and subsequent treatment of the residue with 0.1 M NaOMe in MeOH gave compound **1** (21 mg, 83%) after gel filtration (Bio-Gel P-2, water 1% *n*-butyl alcohol). Selected ^1H/^{13}C NMR (D_2O): δ = 5.72/72.3 ($J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz, $J_{H\text{-}1,C\text{-}1}$ = 160 Hz), 5.28/98.3 ($J_{H\text{-}1,H\text{-}2}$ = 4.0 Hz, $J_{H\text{-}1,C\text{-}1}$ = 172 Hz), 5.09/99.8 ($J_{H\text{-}1,H\text{-}2}$ = 3.9 Hz, $J_{H\text{-}1,C\text{-}1}$ = 171 Hz), 5.01/100.2 ($J_{H\text{-}1,H\text{-}2}$ = 4.0 Hz, $J_{H\text{-}1,C\text{-}1}$ = 172 Hz), 4.73/100.7 ($J_{H\text{-}1,C\text{-}1}$ = 162 Hz), 4.57/101.1 ($J_{H\text{-}1,C\text{-}1}$ = 165 Hz), 4.56/68.2, 4.30/68.3, 4.24/50.3, 3.51/67.9, 3.36/77.6, 2.12 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 1.26 (d, $J_{H\text{-}5,H\text{-}6}$ = 6.6 Hz, 3 H), 1.23 (d, $J_{H\text{-}5,H\text{-}6}$ = 6.6 Hz, 3 H), 1.18 (d, $J_{H\text{-}5,H\text{-}6}$ = 6.6 Hz, 3 H). HR-FABMS: $[M + H]^+$ *m/z* calcd. for $C_{42}H_{72}O_{28}N_3$ 1066.4302, found 1066.4330.

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[1] S. M. Haslam, G. C. Coles, E. A. Munn, T. S. Smith, H. F. Smith, H. R. Morris, A. Dell, *J. Biol. Chem.* **1996**, *271*, 30561−30570.
[2] V. Kubelka, F. Altmann, E. Staudacher, V. Tretter, L. März, K. Hård, J. P. Kamerling, J. F. G. Vliegenthart, *Eur. J. Biochem.* **1993**, *213*, 1193−1204.
[3] V. Tretter, F. Altmann, L. März, *Eur. J. Biochem.* **1991**, *199*, 647−652.
[4] N. M. Spijker, C. A. A. van Boeckel, *Angew. Chem.* **1991**, *103*, 179−182; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 180−183.
[5] J. Zhang, A. Otter, D. R. Bundle, *Bioorg. Med. Chem.* **1996**, *4*, 1989−2001.
[6] Z. Gan, S. Cao, Q. Wu, R. Roy, *J. Carbohydr. Chem.* **1999**, *18*, 755−773.
[7] Z.-W. Guo, Y. Ito, Y. Nakahara, T. Ogawa, *Carbohydr. Res.* **1998**, *306*, 539−544.
[8] Y. Ohnishi, H. Ando, T. Kawai, Y. Nakahara, Y. Ito, *Carbohydr. Res.* **2000**, *328*, 263−276.
[9] J. Seifert, M. Lergenmüller, Y. Ito, *Angew. Chem.* **2000**, *112*, 541−544; *Angew. Chem. Int. Ed.* **2000**, *39*, 531−534.
[10] I. Matsuo, M. Isomura, K. Ajisaka, *J. Carbohydr. Chem.* **1999**, *18*, 841−850.
[11] S. Weiler, R. R. Schmidt, *Tetrahedron Lett.* **1998**, *39*, 2299−2302.
[12] L. Szilágyi, Z. Györgydeák, *Carbohydr. Res.* **1985**, *143*, 21−41.
[13] U. Ellervik, G. Magnusson, *Tetrahedron Lett.* **1997**, *38*, 1627−1628.
[14] Y.-M. Zhang, A. Brodzky, P. Sinaÿ, G. Saint-Marcoux, B. Perly, *Tetrahedron: Asymmetry* **1995**, *6*, 1195−1216.
[15] H. M. Zuurmond, P. A. M. van der Klein, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1993**, *49*, 6501−6514.
[16] Y. Oikawa, T. Tanaka, K. Horita, T. Yoshioka, O. Yonemitsu, *Tetrahedron Lett.* **1984**, *25*, 5393−5396.
[17] S. Nilsson, H. Lönn, T. Norberg, *Glycoconjugate J.* **1989**, *6*, 21−34.
[18] R. U. Lemieux, K. B. Hendriks, R. V. Stick, K. James, *J. Am. Chem. Soc.* **1975**, *97*, 4056−4062.
[19] M. V. Chiesa, R. R. Schmidt, *Eur. J. Org. Chem.* **2000**, 3541−3554.
[20] O. Kanie, S. C. Crawley, M. M. Palcic, O. Hindsgaul, *Carbohydr. Res.* **1993**, *243*, 139−164.
[21] T. Takeda, A. Utsuno, N. Okamoto, Y. Ogihara, S. Shibata, *Carbohydr. Res.* **1990**, *207*, 71−79.
[22] H. Bayley, D. N. Standring, J. R. Knowles, *Tetrahedron Lett.* **1978**, *39*, 3633−3634.
[23] E. Meinjohanns, M. Meldal, T. Jensen, O. Werdelin, L. Galli-Stampino, S. Mouritsen, K. Bock, *J. Chem. Soc., Perkin Trans. 1* **1997**, 871−884.
[24] A. J. Ratcliffe, B. Fraser-Reid, *J. Chem. Soc., Perkin Trans. 1* **1990**, 747−750.
[25] J. J. Kaylor, J. M. Risley, *Carbohydr. Res.* **2001**, *331*, 439−444.
[26] B. Paul, R. J. Bernacki, W. Korytnyk, *Carbohydr. Res.* **1980**, *80*, 99−115.
[27] S. Shibata, H. Nakanishi, *Carbohydr. Res.* **1980**, *86*, 316−320.
[28] L. Öhberg, *Doctoral dissertation*, Stockholm University, **1998**.

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