Regioselective Glycosylations in Solution and on Soluble and Insoluble Polymeric Supports

Richard Geurtsen[a] and Geert-Jan Boons*[a]

Keywords: Glycosylation / Polymer support / Carbohydrates

A protected tetrasaccharide **13** derived from the mucin oligosaccharides surrounding oocytes of *Xenopus laevis*, was prepared by a two directional glycosylation methodology whereby an immobilized thioglycosyl donor **3** was coupled in solution in a regioselective manner with an acceptor **4** that has two hydroxyls of differing reactivity. High regioselectivity was achieved when MPEG was used as a polymeric support, whereas site-site reactions occurred when TentaGel® was employed for immobilization.

Introduction

Polymer-supported synthesis provides a powerful means by which to prepare oligopeptides, oligonucleotides and many small molecules.[1] Recent advances in oligosaccharide synthesis have also made it possible to prepare these compounds by polymeric-supported techniques, and in general two strategies can be considered.[2] In the first one, a glycosyl acceptor is immobilized to a polymer and then glycosylated with a glycosyl donor in solution. In the next step, a protecting group of the immobilized disaccharide is removed and the resulting acceptor glycosylated with a donor in solution. In an alternative strategy, a glycosyl donor is attached to the solid support and acceptors in solution added. We have introduced a more versatile synthetic strategy whereby an immobilized thioglycoside can act both as donor and acceptor.[3] In such a strategy, an immobilized saccharide can first act as a glycosyl acceptor and in the next glycosylation as a glycosyl donor. Alternatively, a two-directional approach can be conducted in such a way that the immobilized saccharide is first used as a glycosyl donor and in the next glycosylation step as a glycosyl acceptor.

Here, we report a novel two directional glycosylation methodology whereby an immobilized thioglycosyl donor is coupled in a regioselective manner with an acceptor in solution that has two hydroxyls of differing reactivity. The resulting disaccharide can then immediately be used as an acceptor in a subsequent coupling. While this type of regioselective glycosylation has been used successfully in classical solution-phase chemistry,[4] it has not been employed for polymer-supported oligosaccharide synthesis. In particular, it is important to establish how the attachment of a donor to a soluble or insoluble polymer affects the regioselectivity of a glycosylation. The new methodology was applied to the preparation of a core tetrasaccharide (Figure 1) found in the mucin-surrounding oocytes of the South African clawed toad, *Xenopus laevis*.[5] Multivalent interactions of the carbohydrates of this mucin with a lectin released after fertilization are critical in establishing a fertilization block to prevent poly-spermy.

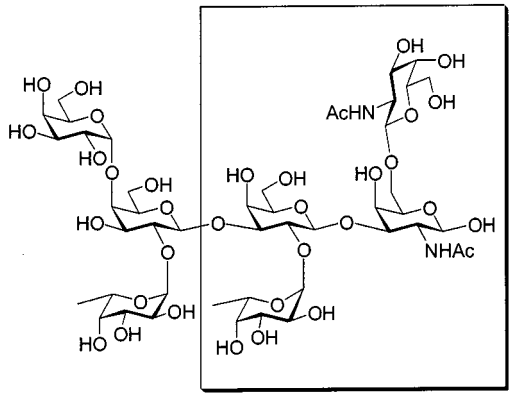


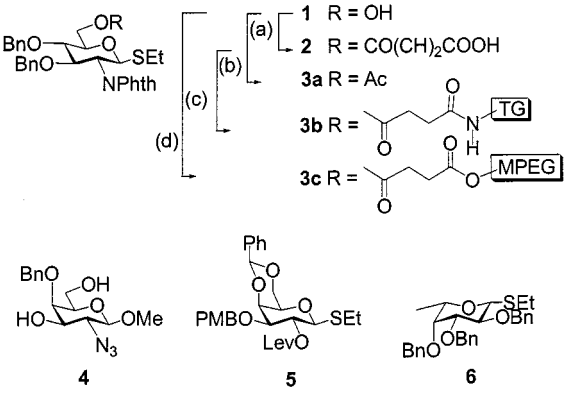
Figure 1. Tetrasaccharide fragment found in the mucin oligosaccharide surrounding *Xenopus laevis* oocytes

Results and Discussion

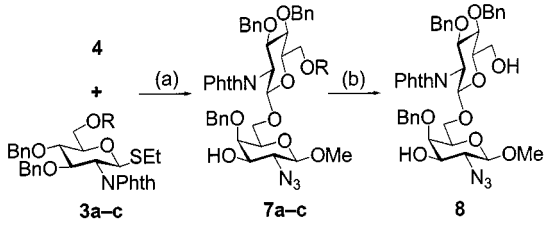
A key feature of the assembly of the target tetrasaccharide **13** was the preparation of the disaccharides **7a−c** by a regioselective coupling between the glycosyl donors **3a−c** and acceptor **4** (Scheme 1). Compounds **3a−c** were prepared from **1** (Scheme 2). Thus, acetylation of **1** with acetic

[a] Complex Carbohydrate Research Center, University of Georgia,
220 Riverbend Road, Athens, GA 30602, USA
Fax: (internat.) + 1-706/542-4412
E-mail: gjboons@ccrc.uga.edu

anhydride in pyridine gave reference glycosyl donor **3a** in a quantitative yield. Treatment of **1** with succinic anhydride afforded **2**, which was coupled with TentaGel® amino resin in the presence of benzotriazole-1-yloxytri-pyrrolidinophosphonium hexafluorophosphate (PyBOP) to give insoluble polymer-bound donor **3b**. Finally, **1** was coupled to succinoyl-modified methoxypolyethylene glycol (M_w = 5000) (MPEG) in the presence of DCC/DMAP [dicyclohexylcarbodiimide/4-(dimethylamino)pyridine] to give soluble polymer-bound **3c**. MPEG was chosen to take advantage of its solubility in many organic solvents.[6] However, the workup procedure involves precipitation of MPEG by addition of diethyl ether or *tert*-butyl methyl ether. Thus, excess of reagents and other side products can easily be removed by washing of the MPEG precipitate.



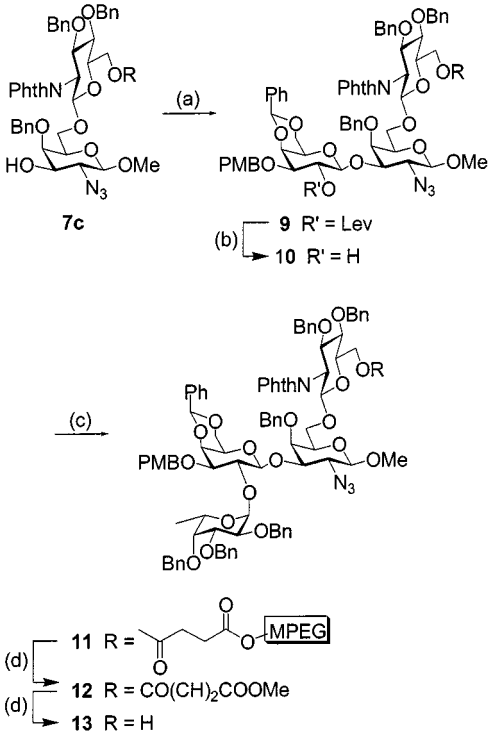
Scheme 1. Reagents and conditions: (a) succinic anhydride, DMAP, py; (b) Ac_2O, py; (c) TentaGel® amino resin, PyBOP, DIPEA, CH_2Cl_2; d) succinoyl-modified MPEG, DCC, DMAP, CH_2Cl_2



Scheme 2. Reagents and conditions: (a) NIS, TMSOTf; (b) NaOMe, MeOH, CH_2Cl_2

With the required glycosyl donors **3a**−**c** at hand, regioselective glycosylations with acceptor **4**,[7] which has free hydroxyls at C-3 and C-6, were examined (Scheme 1). *N*-iodosuccinimide (NIS)/trimethylsilyl trifluoromethanesulfonate (TMSOTf) mediated coupling[8] of TentaGel®-bound **3b** with five equivalents of **4** gave **7b**, which was cleaved from the polymeric support by treatment with NaOMe in methanol to give **8** exclusively as the β-anomer in an overall yield of 60% (based on **3b**). Apart from **8**, a small amount (3%) of a trisaccharide was isolated which arose from *bis*-glycosylation of **3b**.

Thus, after the formation of disaccharide **7b**, site-site glycosylation of **7b** with immobilized donor **3b** gave TentaGel®-bound trisaccharide, demonstrating that the polymer-bound saccharides are not site isolated and can react with each other. The amount of trisaccharide could be reduced by using a large excess (up to ten equivalents) of **4** or by employing the analogous 2-deoxy-2-phthalimido acceptor of **4**. However, the C-3 hydroxyl group of the latter glycosylation product was too sterically hindered to allow further glycosylations. Interestingly, a classical solution-phase glycosylation of reference donor **3a** with acceptor **4** also led, apart from the formation of disaccharide **7a** (65%), to the formation of a trisaccharide by-product (9%). It was anticipated that glycosylation of MPEG-bound donor **3c** with acceptor **4** would proceed with higher regioselectivity. In this case, trisaccharide formation would involve reaction of MPEG-bound disaccharide **7c** with MPEG-bound donor **3c**, which was expected to be disfavored due to severe steric hindrance between the two MPEG-bound derivatives. Indeed, NIS/TMSOTf-mediated coupling[8] of **3c** with **4** gave, after cleavage from MPEG, only disaccharide **8** (63%). Encouraged by these results, attention was focussed on the preparation of tetrasaccharide **13** using MPEG-supported **7c** (Scheme 3). Thus, coupling of **7c** with galactosyl donor **5** gave trisaccharide **9**, which was purified by precipitation with diethyl ether. The levulinic ester of **9** underwent neighboring group participation during glycosylation and therefore ensured stereospecific formation of the required β-glycoside. This ester-protecting group, however, could be selectively removed by treatment with hydrazine acetate[9] to give acceptor **10** and these conditions did not affect the base-sensitive linker. Next, immobilized acceptor **10** was coupled with fucosyl donor **6**[10] using iodonium dicollidine



Scheme 3. Reagents and conditions: (a) NIS, TMSOTf, **5**, CH_2Cl_2; (b) NH_2NH_2·HOAc, MeOH, CH_2Cl_2; (c) IDCP, **6**, Et_2O, CH_2Cl_2; (d) NaOMe, MeOH, CH_2Cl_2

perchlorate (IDCP)[11] as the promoter. This glycosylation was repeated to ensure complete conversion of the acceptor. After standard purification, the immobilized tetrasaccharide **11** was cleaved from the polymeric support by treatment with NaOMe in methanol to give **13**. Apart from tetrasaccharide **13**, a small quantity of the succinic acid derivative **12** was also isolated, which could be converted into **13** by a second treatment with NaOMe in methanol (42% overall yield). NMR spectroscopic and mass spectrometric analysis of **13** confirmed the structural integrity of the final compound.

Conclusion

In summary, glycosylations of glycosyl donors immobilized on MPEG with acceptors that have two hydroxyls of differing reactivities proceed with much higher regioselectivities than similar couplings under traditional solution-phase conditions or on insoluble polymers. The resulting coupling product can immediately be used as a glycosyl acceptor in a subsequent glycosylation without the need for protecting group manipulations. This strategy was successfully applied to the synthesis of a protected core tetrasaccharide derived from the jelly coat glycoprotein of *Xenopus laevis*. Currently, we are employing immobilized **9** for the assembly of the more complex heptasaccharide (Figure 1) by selective removal of the PMB protecting group followed by further glycosylations.

Experimental Section

General Methods: Chemicals were purchased from Aldrich, Acros, and Fluka and used without further purification unless otherwise noted. Molecular sieves were activated at 350 °C in vacuo for 3 h. All solvents were distilled from the appropriate drying agents. All the reactions were performed under anhydrous conditions and monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was effected by examination under UV light (254 nm) and by charring with a 10% sulfuric acid solution in methanol. Gravity column chromatography was performed on silica gel 60 (Merck, 70−230 mesh) and reactions were monitored by TLC. Size exclusion column chromatography was performed on Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden) with dichloromethane/methanol (1:1, v/v) as the eluent. Extracts were concentrated under reduced pressure at <40 °C (bath). All the ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Merc300 spectrometer, a Varian Inova500 spectrometer, or a Varian Inova600 spectrometer, each equipped with Sun workstations. For ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$, chemical shifts (δ) are given in ppm relative to solvent peaks (^{1}H: δ = 7.26; ^{13}C: δ = 77.0) as internal standard. Assignments were made by standard gCOSY and gHSQC experiments. Negative-ion matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using a 2,5-dihydroxybenzoic acid (gentisic acid) or 3,5-dimethoxy-4-hydrocynnaminic acid (sinapinic acid) matrix. Optical rotations were all measured in $CHCl_3$ on a JASCO P-1020 polarimeter and $[\alpha]_D$ values are given in units of deg cm^2 mg^{-1}.

Methyl 2-Azido-4-benzyl-2-deoxy-6-*O*-(6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranoside (7a): NIS (468 mg, 2.08 mmol) and TMSOTf (15 μL, 0.08 mmol) were added to a stirred mixture of compound **3a** (1.04 g, 1.81 mmol), compound **4** (629 mg, 2.03 mmol) and molecular sieves (4 Å, 4 g) in CH_2Cl_2 (30 mL). After stirring for 5 min at room temp., the reaction mixture was quenched with Et_3N (0.02 mL), diluted with CH_2Cl_2 (40 mL) and washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 98:2, v/v), followed by LH-20 gel filtration chromatography (CH_2Cl_2/MeOH, 1:1, v/v) to afford **7a** (972 mg, 65%) and the trisaccharide methyl 2-azido-4-benzyl-2-deoxy-3-*O*-(6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-*O*-(6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopy-ranosyl)-β-D-galactopyranoside (215 mg, 9%), both as a white glass. Compound **7a** was crystallized from Et_2O/hexane (1:1, v/v) and obtained as a white crystalline solid (m.p. 133.1 °C); R_f = 0.46 (CH_2Cl_2/acetone, 95:5, v/v). $[\alpha]_D^{26}$ = +22.7 (*c* = 1). MALDI-MS: *m/z* = 845.4 [M + Na]$^+$, 861.6 [M + K]$^+$. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.82−7.58 (m, 4 H, Phth), 7.42−7.25, 7.07−6.83 (m, 15 H, 3 $C_6H_5CH_2$), 5.17 (d, $J_{1',2'}$ = 8.5 Hz, 1 H, H-1′), 4.86 (d, J_{gem} = 10.9 Hz, 1 H, $C_6H_5CH_2$), 4.80 (d, J_{gem} = 11.8 Hz, 1 H, $C_6H_5CH_2$), 4.70 (d, J_{gem} = 11.5 Hz, 1 H, $C_6H_5CH_2$), 4.61 (d, J_{gem} = 10.9 Hz, 1 H, $C_6H_5CH_2$), 4.51 (d, J_{gem} = 11.5 Hz, 1 H, $C_6H_5CH_2$), 4.42 (d, J_{gem} = 12.1 Hz, 1 H, $C_6H_5CH_2$), 4.38−4.34 (m, $J_{2',3'}$ = 10.9 Hz, $J_{6'a,6'b}$ = 11.7 Hz, 2 H, H-3′, H-6′a), 4.23 (dd, $J_{5',6'b}$ = 3.2 Hz, 1 H, H-6′b), 4.14 (dd, 1 H, H-2′), 3.93−3.90 (m, $J_{6a,6b}$ = 10.0 Hz, 2 H, H-1, H-6a), 3.67 (m, 2 H, H-4′, H-5′), 3.60 (dd, 1 H, H-6b), 3.51 (d, $J_{3,4}$ = 3.3 Hz, 1 H, H-4), 3.34−3.31 (m, $J_{2,3}$ = 10.3 Hz, 2 H, H-2, H-5), 3.25 (s, 3 H, OCH_3), 3.17 (dd, 1 H, H-3), 1.98 (s, 3 H, CH_3CO). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 170.7 (CH_3CO), 137.9, 137.8, 137.5, 134.0, 128.7, 128.4, 128.2, 127.9, 127.6, 123.5 (3 $C_6H_5CH_2$), 131.6 (Cq, Phth), 103.0 (C-1), 98.2 (C-1′), 79.5, 79.4 (C-3′, C-4′/5′), 75.5, 75.3 (2 $C_6H_5CH_2$), 75.2 ($C_6H_5CH_2$, C-4), 73.4, 73.3 (C-4′/5′, C-2:5), 72.6 (C-3), 67.3 (C-6), 64.9 (C-2/5), 63.0 (C-6′), 56.8 (OCH_3), 56.1 (C-2′), 21.1 (CH_3CO). $C_{44}H_{46}N_4O_{12}$ (822.9): calcd. C 64.22, H 5.63, N 6.81; found C 64.35, H 5.66, N 6.78.

Methyl 2-Azido-4-benzyl-2-deoxy-3-*O*-(6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-*O*-(6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galacto-pyranoside: R_f = 0.62 (CH_2Cl_2/acetone, 95:5, v/v). $[\alpha]_D^{25}$ = +20.1 (*c* = 1). MALDI-MS: *m/z* = 1333.0 [M − N_2 + 2H + Na]$^+$, 1359.0 [M + Na]$^+$, 1374.7 [M + K]$^+$. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.84−7.60 (m, 8 H, 2 Phth), 7.40−7.24, 7.06−6.84 (2 m, 25 H, 5 $C_6H_5CH_2$), 5.21 (d, $J_{1',2'}$ = 8.5 Hz, 1 H, H-1′), 5.07 (d, $J_{1'',2''}$ = 8.5 Hz, 1 H, H-1′′), 4.87 (t, J_{gem} = 11.0 Hz, 2 H, $C_6H_5CH_2$), 4.82−4.77 (m, 3 H, $C_6H_5CH_2$), 4.65−4.60 (m, 2 H, $C_6H_5CH_2$), 4.46 (d, J_{gem} = 11.0 Hz, 2 H, $C_6H_5CH_2$), 4.41 (d, J_{gem} = 11.5 Hz, 1 H, $C_6H_5CH_2$), 4.46−4.41 (m, 1 H, H-6′a), 4.41 (dd, $J_{2',3'}$ = 10.5 Hz, 1 H, H-3′), 4.36 (dd, 1 H, H-6′′a, obscured by H-3′′), 4.35 (dd, $J_{2'',3''}$ = 10.5 Hz, 1 H, H-3′′), 4.28 (t, *J* = 4.0 Hz, 1 H, H-5′′), 4.25 (t, *J* = 4.0 Hz, 1 H, H-5′), 4.20 (dd, 1 H, H-2′), 4.08 (dd, 1 H, H-2′′), 3.77 (d, $J_{1,2}$ = 7.5 Hz, 1 H, H-1), 3.70−3.61 (m, 5 H, H-4′, H-4′′, H-4, H-6′b, H-6′′b), 3.50 (d, *J* = 5.0 Hz, 2 H, H-6a, H-6b), 3.26 (dd, $J_{2,3}$ = 10.5 Hz, 1 H, H-2), 3.2 (t, *J* = 5.5 Hz, 1 H, H-5), 3.07 (d, $J_{3,4}$ = 3.0 Hz, 1 H, H-3), 2.96 (s, 3 H, OCH_3), 2.03, 1.95 (2 s, 6 H, 2 CH_3CO). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 170.4, 170.3 (CH_3CO), 167.4 (*C*O Phth), 138.2, 137.7(2 ×), 137.4(2 ×), 133.6, 128.4−127.3, 123.1 (3 $C_6H_5CH_2$), 131.5 (Cq, Phth), 102.8 (C-1), 100.3 (C-1′), 98.0 (C-1′′), 81.8 (C-3), 79.3, 79.2, 78.8

(C-3′, C-3″, C-4/4′/4″), 75.0, 74.9, 74.4 ($C_6H_5CH_2$), 74.0 (C-4/4′/4″), 73.0, 72.9 (C-5, C-4/4′/4″), 68.8 (C-6), 62.7, 62.5 (C-6′, C-6″), 62.5, 62.4 (C-5′, C-5″, C-2), 56.0, 56.0, 55.8 (C-2′, C-2″, OCH_3), 20.9 (CH_3CO).

Methyl 2-Azido-4-benzyl-2-deoxy-6-*O*-(3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranoside (8). Method A: Diisopropylethylamine (0.35 mL, 2.0 mmol) and PyBop (480 mg, 0.92 mmol) were added to a suspension of compound **2** (574 mg, 0.91 mmol) and NovaSyn® amino resin HL (1.06 g, 0.46 mmol available amino groups) in CH_2Cl_2 (25 mL). After shaking for 14 h, a Kaiser test indicated the presence of free amines. An additional amount of **2** (465 mg, 0.73 mmol), PyBop (480 mg, 0.92 mmol) and diisopropylethylamine (0.35 mL, 2.0 mmol) was added and the reaction mixture was shaken for another 18 h after which the Kaiser test indicated completion of the reaction. The resin was washed with CH_2Cl_2 (3 × 50 mL), ethanol (3 × 50 mL) and CH_2Cl_2 (3 × 50 mL) and dried in vacuo to give **3b** (1.25 g, 94% of theoretical loading). Resin **3b** (103 mg, 35.6 μmol), **4** (43 mg, 139 μmol) and activated molecular sieves (0.2 g, 4 Å) were suspended in CH_2Cl_2 (2 mL). After shaking the suspension for 24 h, NIS (21 mg, 93 μmol) and TMSOTf (1.5 μL, 8.3 μmol) were added and the reaction mixture was stirred for 18 h under an atmosphere of dry argon. The beads were separated from molecular sieves and then washed with CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), and diethyl ether (3 × 10 mL). The beads were suspended in methanol/CH_2Cl_2 (2 mL, 1:1, v/v) and potassium *tert*-butoxide was added. After stirring for 18 h, the reaction mixture was diluted with CH_2Cl_2 (40 mL), filtered and the filtrate washed with H_2O (2 × 10 mL). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 5:95, v/v) to give **8** as a white glass (17.5 mg, 60% starting from **3b**).

Method B: DCC (1.5 g, 7.27 mmol) and DMAP (65 mg, 0.54 mmol) were added to a stirred solution of **2** (2.26 g, 3.57 mmol) and MPEG 5000 (8.9 g, 1.78 mmol) in CH_2Cl_2 (80 mL). After 18 h, the mixture was concentrated in vacuo and the residue dissolved in diethyl ether. The precipitate was filtered off and recrystallized from ethanol to give **3c** as a white powder (9.5 g). The loading was determined by treatment of a solution of **3c** (0.5 g) in methanol/CH_2Cl_2 (10 mL, 1:1, v/v) with sodium methoxide (20 mg). After stirring the reaction mixture for 18 h, acetic acid was added (1 drop) and the mixture concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (5 mL) and diethyl ether (50 mL) was added. The white precipitate was filtered off and the filtrate concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 99:1, v/v) to give **1** (28.9 mg, 60% loading). Integration of the methoxy signal of MPEG in the ^1H NMR and well-resolved saccharide signals confirmed the loading.

NIS (39 mg, 0.17 mmol) and TMSOTf (5 μL, 0.03 mmol) were added to a stirred mixture of compound **3c** (1.35 g, 0.14 mmol based on 60% loading), compound **4** (90 mg, 0.29 mmol) and molecular sieves (4 Å, 0.4 g) in CH_2Cl_2 (20 mL). After stirring for 5 min at room temp., the reaction mixture was quenched with Et_3N (0.02 mL), diluted with CH_2Cl_2 (40 mL), filtered through celite, and washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue dissolved in CH_2Cl_2 (3 mL). Addition of Et_2O (50 mL) afforded a white precipitate, which was filtered off and recrystallized from EtOH to give **7c** as a white solid. Part of this solid (509 mg) was dissolved in CH_2Cl_2/MeOH (10 mL, 1:9, v/v) and stirred with NaOMe (8 mg) for 5 h. The mixture was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (2 × 10 mL). The organic phase was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue was separated from MPEG by silica gel column chromatography (CH_2Cl_2/acetone, 5:95, v/v) to give **8** as a white glass (27 mg, 63% starting from **3c**); R_f = 0.39 (CH_2Cl_2/acetone, 92:8, v/v). $[\alpha]_D^{25}$ = +16.6 (*c* = 1). MALDI-MS: *m*/*z* = 777.4 [M − N_2 + 2H + Na]$^+$, 803.9 [M + Na]$^+$, 820.1 [M + K]$^+$. ^1H NMR ($CDCl_3$, 500 MHz): δ = 7.80−7.60 (m, 4 H, Phth), 7.38−7.29 (m), 7.00 (d), 6.91−6.83 (m, 15 H, 3 $C_6H_5CH_2$), 5.20 (d, $J_{1',2'}$ = 8.0 Hz, 1 H, H-1′), 4.88 (d, J_{gem} = 11.0 Hz, 1 H, $C_6H_5CH_2$), 4.81 (d, J_{gem} = 12.0 Hz, 1 H, $C_6H_5CH_2$), 4.72 (d, J_{gem} = 11.0 Hz, 1 H, $C_6H_5CH_2$), 4.71 (d, J_{gem} = 11.5 Hz, 1 H, $C_6H_5CH_2$), 4.59 (d, J_{gem} = 11.5 Hz, 1 H, $C_6H_5CH_2$), 4.45 (d, J_{gem} = 12.1 Hz, 1 H, $C_6H_5CH_2$), 4.36 (d, $J_{2',3'}$ = 10.5 Hz/11.0, $J_{3',4'}$ = 8.5 Hz/9.0 Hz, 1 H, H-3′), 4.11 (dd, $J_{2',3'}$ = 10.5 Hz, 1 H, H-2′), 3.95 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-1), 3.88 (dd, $J_{5,6a}$ = 7.0 Hz, $J_{6a,6b}$ = 10.0 Hz, 1 H, H-6a), 3.85 (dd, $J_{5',6'a}$ = 7.0 Hz, $J_{6'a,6'b}$ = 10.0 Hz, 1 H, H-6′a), 3.74−3.70 (m, 2 H, H-4′, H-6′b), 3.60 (dd, $J_{5,6b}$ = 6.5 Hz, $J_{6a,6b}$ = 10.0 Hz, 1 H, H-6b), 3.54−3.50 (m, 2 H, H-5′, H-4), 3.37 (dd, $J_{2,3}$ = 10.0 Hz, 1 H, H-2), 3.34 (t, 1 H, H-5), 3.26 (s, 3 H, OCH_3), 3.19 (m, 1 H, H-3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 137.8(2 ×), 137.7 (3 $C_6H_5CH_2$), 133.8, 123.2 (Phth), 131.4 (Cq, Phth), 128.5, 128.0, 127.80, 127.76, 127.3 (3 $C_6H_5CH_2$), 102.8 (C-1), 98.0 (C-1′), 79.2, 79.0 (C-4, C-3′) 75.4, 75.0 (C-4/5′), 75.2, 75.1, 74.9 (3 $C_6H_5CH_2$), 73.1 (C-2/5), 75.5 (C-3), 67.0 (C-6), 64.7 (C-2/5), 61.7 (C-6′), 56.6 (OCH_3), 56.0 (C-2′). $C_{42}H_{44}N_4O_{11}$ (780.8): calcd. C 64.61, H 5.68, N 7.81; found C 64.55, H 5.65, N 6.88.

Methyl 2-Azido-4-benzyl-2-deoxy-6-*O*-[6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-3-*O*-[2-*O*-(2,3,4-tri-*O*-benzyl-α-D-fucopyranosyl)-3-*O*-p-methoxy-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl]-β-D-galactopyranoside (13): NIS (41 mg, 0.18 mmol) and TMSOTf (1.5 μL, 0.01 mmol) were added to a stirred mixture of compound **7c** (504 mg, 0.05 mmol), compound **5** (70 mg, 0.11 mmol) and molecular sieves (4 Å, 0.2 g) in CH_2Cl_2 (15 mL). After stirring for 30 min at room temp., the reaction mixture was quenched with TEA (0.02 mL), diluted with CH_2Cl_2 (40 mL), filtered through celite, and the filtrate was washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue dissolved in CH_2Cl_2 (4 mL). Addition of Et_2O (50 mL) afforded a yellow precipitate, which was filtered off and recrystallized from EtOH to give **9** as a white/yellow solid (490 mg). Part of this solid (260 mg, 26 μmol) was dissolved in CH_2Cl_2 (10 mL) and stirred with a 0.8 M solution of NH_2NH_2·HOAc in MeOH (0.1 mL) for 18 h at room temp. The mixture was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (2 × 10 mL). The organic phase was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue dissolved in CH_2Cl_2 (3 mL). Addition of Et_2O (50 mL) afforded a white precipitate, which was filtered off and recrystallized from EtOH to give **10** as a white solid (215 mg).

IDCP (54 mg, 0.12 mmol) was added to a stirred mixture of compound **10** (215 mg, 21 μmol), **6** (26 mg, 54 μmol) and molecular sieves (4 Å, 0.3 g) in CH_2Cl_2/Et_2O (10 mL, 1:1, v/v). The mixture was stirred for 4 h at room temp., diluted with CH_2Cl_2 (40 mL), filtered through celite, and the filtrate was washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue dissolved in CH_2Cl_2 (2 mL). Addition of Et_2O (50 mL) afforded a white precipitate, which was collected by filtration. To ensure complete glycosylation the procedure was repeated again. The precipitate was recrystallized from EtOH to give **11** as

a white solid. Compound **11** was dissolved in CH_2Cl_2/MeOH (10 mL, 1:9, v/v) and stirred with NaOMe (5 mg) for 5 h. The mixture was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (2 × 10 mL). The organic phase was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and the residue was separated from MPEG by silica gel column chromatography (CH_2Cl_2/acetone, 95:5, v/v) to give **13** as a white glass (17 mg, 42% starting from **7c**); R_f = 0.41 (CH_2Cl_2/acetone, 92:8, v/v). $[\alpha]_D^{25}$ = +2.6 (c = 1). MALDI-MS: m/z = 1564.5 $[M - N_2 + 2H + Na]^+$, 1590.6 $[M + Na]^+$, 1606.8 $[M + K]^+$. ^1H NMR ($CDCl_3$, 500 MHz): δ = 7.74−7.58 (m, 4 H, Phth), 7.44−7.10 (m, 27 H), 6.98 (d, 2 H), 6.90−6.81 (m, 3 H) ($C_6H_5CHO_2$, 6 $C_6H_5CH_2$, $CH_3OC_6H_4CH_2$), 6.76 (d, 2 H, $CH_3OC_6H_4CH_2$), 5.58 (d, $J_{1'',2''}$ = 3.5 Hz, 1 H, H-1″), 5.39 (s, 1 H, $C_6H_5CHO_2$), 5.06 (d, $J_{1''',2'''}$ = 8.0 Hz, 1 H, H-1‴), 4.94 (d, J_{gem} = 11.5 Hz, 1 H, $C_6H_5CH_2$), 4.88 (d, J_{gem} = 11.5 Hz, 1 H, $C_6H_5CH_2$), 4.85 (d, J_{gem} = 11.0 Hz, 1 H, $C_6H_5CH_2$), 4.78 (d, J_{gem} = 12.0 Hz, 1 H, $C_6H_5CH_2$), 4.77 (d, J_{gem} = 11.0 Hz, 1 H, $C_6H_5CH_2$), 4.69 (t, J_{gem} = 12.0 Hz, 4 H, $C_6H_5CH_2$), 4.66 (d, $J_{1',2'}$ = 8.0 Hz, 1 H, H-1′), 4.60 (t, J_{gem} = 13.0 Hz, 2 H, $C_6H_5CH_2$), 4.56 (d, J_{gem} = 11.0 Hz, 1 H, $C_6H_5CH_2$), 4.52 (d, J_{gem} = 12.0 Hz, 1 H, $C_6H_5CH_2$), 4.45 (q, J = 6.0 Hz, 1 H, H-5″), 4.42 (d, J_{gem} = 12.0 Hz, 1 H, $C_6H_5CH_2$), 4.35 (dd, J = 8.5, J = 10.5 Hz, 1 H, H-3‴), 4.23 (m, 1 H, H-6a), 4.2 (dd, J = 8.0 Hz, J = 9.0 Hz, 1 H, H-2′), 4.07−3.98 (m, 5 H, H-2″, H-6b, H-2‴), 3.92 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-1), 3.86 (d, J = 2.5 Hz, 1 H), 3.81−3.77 (m, 3 H, H-6a), 3.76 (s, 3 H, $CH_3OC_6H_4CH_2$), 3.69−3.64 (m, 2 H, H-6b, H-4‴), 3.46−3.41 (m, 3 H, H-2, H-6a, H-5‴), 3.33 (br. s, 1 H), 3.28−3.22 (m, 3 H, H-3, H-6b), 2.97 (s, 3 H, OCH_3), 1.09 (d, J = 6.5 Hz, 3 H, CH_3 Fuc). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 167.7 (*C*O Phth), 159.2 (Cq, $CH_3OC_6H_4CH_2$), 138.9, 138.6, 138.0, 137.8 (Cq, 6 $C_6H_5CH_2$), 133.9 (Phth), 129.5−126.4 (6 $C_6H_5CH_2$, $CH_3OC_6H_4CH_2$), 113.9 ($CH_3OC_6H_4CH_2$), 103.3 (C-1′), 103.2 (C-1), 101.4 ($C_6H_5CHO_2$), 98.4 (C-1‴), 97.9 (C-1″), 79.9 (C-3), 79.1 (C-3‴), 73.0 (C-2′), 69.8 (C-6), 69.3 (C-6), 66.5 (C-5‴), 61.7 (C-6), 56.1 (C-2‴), 81.0, 79.8, 79.4, 78.2, 75.9, 75.3, 74.8, 74.2, 73.0, 66.5, 63.4 (C-2, C-2″, C-3′, C-3″, C-4, C-4′, C-4″, C-4‴, C-5, C-5′, C-5″), 75.2, 75.1, 75.0(4 ×), 74.8, 74.7, 73.0, 73.1, 72.9(2 ×), 70.8(2 ×) (6 $C_6H_5CH_2$, $CH_3OC_6H_4CH_2$), 56.0 (OCH_3), 55.4 ($CH_3OC_6H_4CH_2$), 16.8 (CH_3 Fuc). $C_{90}H_{94}N_4O_{21}$ (1567.7): calcd. C 68.95, H 6.04, N 3.57; found C 68.76, H 6.06, N 3.58.

Acknowledgments

This work was supported by the NIH Resource Center for Biomedical Complex Carbohydrates (P41-RR05351).

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Received November 16, 2001
[O01550]