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Efficient Synthesis of Lactosaminylated Core-2 *O*-glycans

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Abstract—A series of lactosaminylated oligosaccharides found in mucin type *O*-glycans was synthesized using a generalized block strategy. The synthesis involved the addition of a protected lactosamine donor to a partially protected T-disaccharide derivative. The nonreducing galactose residues of the deblocked oligosaccharide products could be removed by β-galactosidase from jack bean to produce the corresponding GlcNAc terminated compounds. A series of tri- to hexasaccharides was thus efficiently produced.

Serine and threonine glycosylation of proteins occurs in the secretory pathway of all eukaryotic cells.[1] The modification of serine or threonine residues on proteins by addition of a GalNAc residue results in *O*-linked oligosaccharides or *O*-glycans, which can lead to mucin type molecules. Mucins are defined as cell surface or secreted glycoproteins with large numbers of clustered *O*-glycans. Though the structures of mucin-type *O*-glycans are extremely heterogeneous, they have been classified as core-1, -2, -3, and so on, according to the branching pattern at the α-GalNAc residue.[2] Mucin-type glycoproteins carrying poly-*N*-acetyllactosamine oligosaccharides have been demonstrated on a variety of tumor cells.[3] Poly-*N*-acetyllactosamine chains may be modified further by sialylation and fucosylation to produce selectin ligands.[4] Poly-*N*-acetyllactosamine can be formed on core-2 branched oligosaccharides by the sequential tandem action of β-(1,4)-galactosyltransferase IV and β-(1,3)-*N*-acetylglucosaminyltransferase (*i*-GlcNAc transferase). Biological studies have shown that, unlike the *N*-glycans, core-2 *O*-glycans rarely consist of more than two or three *N*-acetyllactosamine repeats.[5] We therefore undertook the synthesis of the oligosaccharides **1**–**4**, which represent biosynthetic intermediates in the synthesis of short core-2 polylactosamine chains. These compounds are for use in kinetic studies on recently cloned glycosyltransferases.

Several reports on the synthesis of polylactosamine-derived sequences have appeared in which a lactosamine donor with an orthogonally protected OH-3 group of galactose was utilized,[6] a strategy requiring significant protecting group manipulation. In continuation of our efforts to increase the availability of biologically important carbohydrate molecules, we here used a differentially protected lactosamine donor (**8**) made from monosaccharide precursors in a minimum number of steps. This disaccharide was then employed as the glycosyl donor in a general block synthetic glycosylation strategy to furnish oligolactosaminylated core-2 *O*-glycans (Fig. 1).

Ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**6**), prepared from ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5**)[7] in four steps, was coupled with 2,6-di-*O*-acetyl-3,4-di-*O*-chloroacetyl-α-D-galactopyranosyl chloride (**7**)[6] in the presence of AgOTf to give the required bifunctional disaccharide donor (**8**) in 73% yield (Scheme 1).

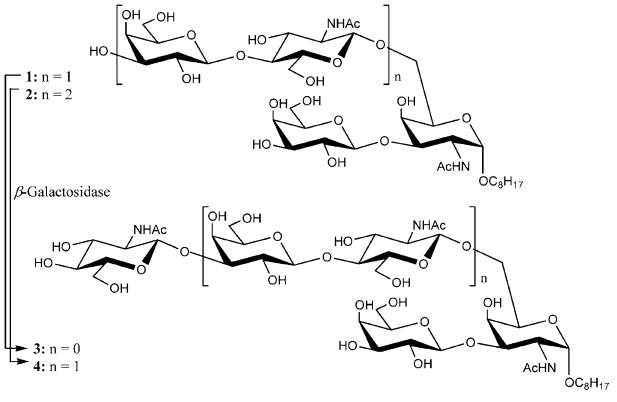

Figure 1. Lactosaminylated core-2 *O*-glycans.

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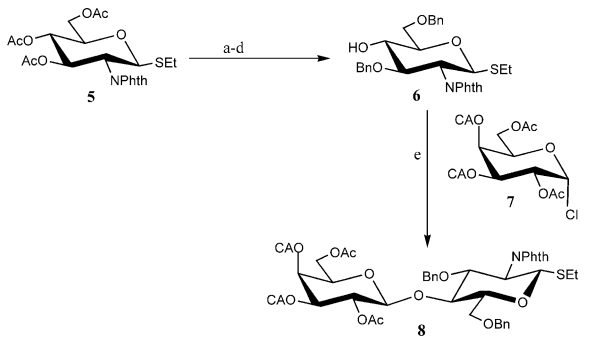
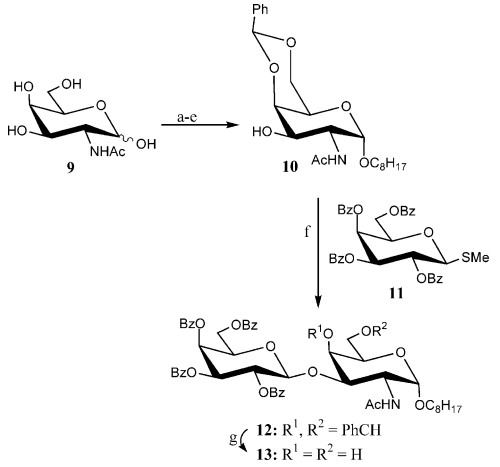


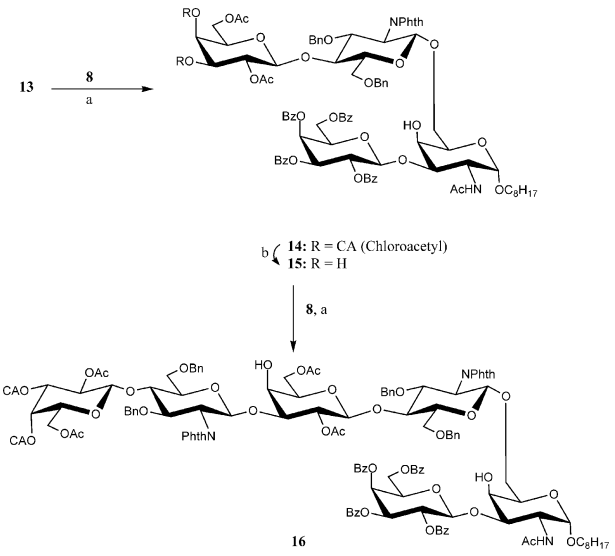
Scheme 1. (a) 0.02 M MeONa, MeOH, rt, 20 min, 90%; (b) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, rt, 87%; (c) BnBr, NaH, DMF, 0 °C, 2 h, 74%; (d) $NaBH_3CN$, HCl–Et_2O, MS-3 Å, 0–5 °C, 1 h, 76%; (e) AgOTf, 2,4,6-collidine, CH_2Cl_2, MS-4 Å, −20 to 0 °C, 5 h, 73%.



Scheme 2. (a) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, rt, 5 h, 72%; (b) BzCl, Pyr, CH_2Cl_2, −25 °C, 2.5 h, 70%; (c) CCl_3CN, DBU, CH_2Cl_2, −10 °C, 80%; (d) 1-octanol, $BF_3{\cdot}OEt_2$, THF, MS-3 Å, 76%; (e) 0.5 M MeONa, MeOH, rt, 12 h, quant; (f) DMTST, CH_2Cl_2, MS-4 Å, 0 °C to rt, 16 h, 68%; (g) 80% aq AcOH, 80 °C, 2 h, quant.

Octyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**10**) was prepared from commercially available *N*-acetyl-D-galactosamine (**9**) in five steps, taking advantage of the presence of the 4,6-*O*-acetal in the molecule and a reported solvent effect to induce α-glycosylation.[8] The suitably protected T-disaccharide acceptor **12** was prepared by coupling of methyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**11**) with **10** in a dimethyl(methylthio)sulfonium triflate (DMTST)[9] promoted reaction (68%).[10] The disaccharide diol **13** was obtained on benzylidene cleavage under acidic conditions (Scheme 2).

Reaction of the disaccharide donor **8** with the disaccharide acceptor **13** in the presence of DMTST gave the tetrasaccharide **14** in 61% yield. De-chloroacetylation of **14** using 'hydrazinedithiocarbonate'[11] in 2,6-lutidine–HOAc (3:1) then furnished the tetrasaccharide triol acceptor **15** in 74% yield after chromatographic purification. DMTST catalyzed glycosylation of **15** with the donor **8** gave the hexasaccharide derivative **16** (54%), which could in principle be further extended by the above mentioned two-step sequence (Scheme 3). Conventional deprotection of **15** and **16** was achieved in four steps involving de-phthaloylation (ethylenediamine



Scheme 3. (a) DMTST, CH_2Cl_2, MS-4 Å, −10 °C, 12 h, 61%; (b) 'hydrazinedithiocarbonate', 2,6-lutidine–AcOH (3:1), rt, 30 min, 74%.

in hot 1-butanol), *N*- and *O*-acetylation (Ac_2O/Pyr), *O*-deacetylation (0.1 M NaOMe/MeOH) and catalytic hydrogenolysis [H_2 over 20% $Pd(OH)_2$/C] giving an overall yield of 56% for **1** and 49% for **2** after purification on LH-20 Sephadex.

The two required GlcNAc terminated oligosaccharides **3** and **4** (Fig. 1) were conveniently obtained on a 5 mg scale from **1** and **2** by β-galactosidase[12] from jack beans.

The structures of all the products were supported by their NMR[13] and mass spectral data.[14] Compounds **1**–**4** are being evaluated in a kinetic study of the *i*- and *I*-GlcNAc transferases as well as several β-(1,4)-galactosyltransferases. Preliminary results indicate that the biosynthesis of polylactosamines in *O*-glycans becomes less efficient as chain length is increased. Using **1**–**4** and other synthetic acceptors and several recombinant β-(1,4)-galactosyltransferases, it appears that β-(1,4)-galactosyltransferase IV together with β-(1,3)-*N*-acetylglucosaminyltransferase is capable of synthesizing poly-*N*-acetyllactosamines in core-2 branched oligosaccharides and the efficiency decreases dramatically with chain length. Detailed results will be reported elsewhere.

Acknowledgements

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10. General procedure for glycosylation: a mixture of **11**, **8**, or **5** (1.2 mmol), acceptor sugars **10**, **13**, or **15** (1.0 mmol), MS-4 Å (4 g) and DMTST (4.8 mmol) in CH_2Cl_2 (20 mL) was stirred at −10 or 0 °C for 8 to 12 h. Dilution with CH_2Cl_2 was followed by filtration through Celite. The organic layer was washed with satd $NaHCO_3$, water, dried (Na_2SO_4) and concentrated. Column chromatography on SiO_2 using toluene–EtOAc as solvent gave the pure glycosylation products.
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12. Typical procedure for enzymatic digestion: to a solution of compound **1** (1 mg, 1.16 μmol) in 190 μL of 50 mM sodium citrate buffer was added β-galactosidase (EC 3.2.1.23; jack bean; 1 U, 10 μL) and the solution was kept at 37°C for 24 h. The reaction mixture was applied to a C_{18}-SepPak cartridge which was washed with water. Product was eluted with methanol. The methanolic fraction was concentrated and further purified on Sephadex LH-20 to give **3**.
13. Partial NMR (500 MHz; D_2O): ^{1}H NMR: The following common signals for the octyl aglycon were observed in D_2O soln: δ 1.60–1.40 (2H, OCH_2CH_2), 1.40–1.10 (10H, $OCH_2CH_2(CH_2)_5CH_3$), 0.85 (t, 3H, octyl CH_3). H-1 indicates the anomeric proton of the GalNAc residue, H-1′ the anomeric proton of the Gal residue linked to O-3 of the GalNAc and H-1″ the anomeric proton of the GlcNAc residue linked to O-6 of the GalNAc and onwards. **1**: δ 4.83 (d, $J_{1,2}=3.6$ Hz, 1H, H-1), 4.52 (d, $J_{1'',2''}=7.8$ Hz, 1H, H-1″), 4.42 (2d, $J_{1',2'}=7.8$ Hz and $J_{1''',2'''}=7.8$ Hz, 2H, H-1′ and H-1‴), 4.26 (dd, 1H, H-3), 4.17 (bs, 1H, H-4), 2.0 and 2.02 (2s, each 3H, 2 NHAc); ^{13}C NMR: δ 105.4, 103.7, 102.2 and 97.5. **2**: δ 4.86 (d, $J_{1,2}=3.5$ Hz, 1H, H-1), 4.71 (d, $J_{1'',2''}=7.5$ Hz, 1H, H-1″), 4.53 (d, $J_{1'''',2''''}=8.0$ Hz, 1H, H-1⁗), 4.47 (3d, $J=7.5$ Hz each, 3H, H-1′, H-1‴ and H-1⁗), 4.30 (dd, 1H, H-3), 4.19 (bs, 1H, H-4), 4.13 (bs, 1h, H-4‴), 1.99, 2.0 and 2.02 (3s, each 3H, 3 NHAc; ^{13}C NMR: δ 105.3, 103.5, 103.5, 103.4, 102.1 and 97.3. **3**: δ 4.86 (d, $J_{1,2}=3.0$ Hz, 1H, H-1), 4.52 (d, $J_{1',2'}=8.5$ Hz, 1H, H-1′), 4.46 (d, $J_{1'',2''}=8.0$ Hz, 1H, H-1″), 4.30 (dd, 1H, H-3), 4.21 (bs, 1H, H-4), 2.02 and 2.01 (2s, each 3H, 2 NHAc); ^{13}C NMR: δ 105.3, 102.1, 97.2. **4**: δ 4.86 (d, $J_{1,2}=3.5$ Hz, 1H, H-1), 4.70 (d, $J_{1'',2''}=9.0$ Hz, 1H, H-1″), 4.54 (d, $J_{1'''',2''''}=8.0$ Hz, 1H, H-1⁗), 4.47 (2d, $J=7.5$ Hz each, 2H, H-1′ and H-1‴), 4.30 (dd, 1H, H-3), 4.20 (bs, 1H, H-4), 4.14 (bs, 1H, H-4‴), 1.99, 2.0 and 2.02 (3s, each 3H, 3 NHAc); ^{13}C NMR: δ 105.2, 103.4 (2×C), 102.0, 97.2.
14. HRMS: **1**: calcd for $C_{36}H_{64}O_{21}N_2$ ($M+Na^+$) 883.3894; found 883.3910. **2**: calcd for $C_{50}H_{87}O_{31}N_3$ ($M+Na^+$) 1248.5216; found 1248.5165. **3**: calcd for $C_{30}H_{54}O_{16}N_2$ ($M+Na^+$) 721.3366; found 721.3365. **4**: calcd for $C_{44}H_{77}O_{26}N_3$ ($M+Na^+$) 1086.4687; found 1086.4701.