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EXPEDIENT SYNTHESIS OF A SERIES OF *N*-ACETYLLACTOSAMINES

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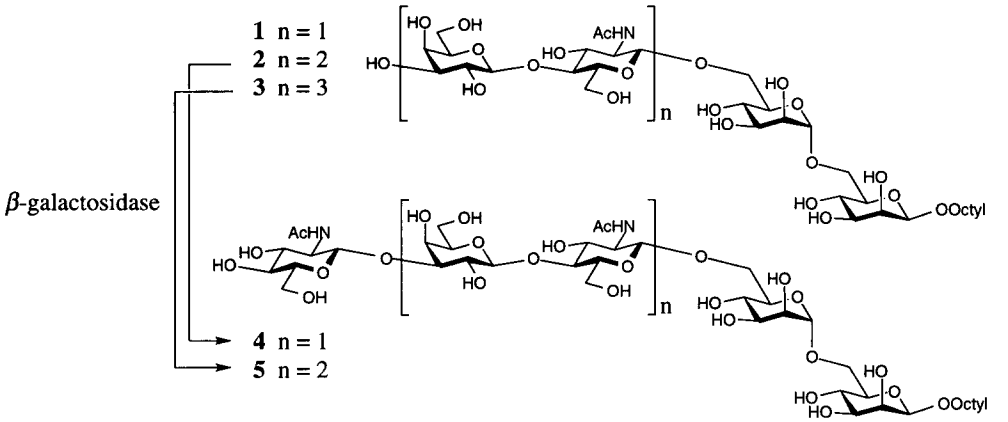
Abstract: A series of poly-*N*-acetyllactosamines representative of those found on complex *N*-glycans was synthesized for use in the kinetic characterization of recently cloned glycosyltransferases. The syntheses involved the iterative addition of a selectively protected *N*-acetyllactosaminyl donor to an octyl α-D-mannopyranosyl-1,6-β-D-mannopyranoside acceptor, followed by deprotection. In addition, non-reducing galactosyl residues were removed with β-galactosidase to furnish GlcNAc terminated compounds. In this manner tetra- to octasaccharides were efficiently produced.

Poly-*N*-acetyllactosamine-based oligosaccharides are widespread in higher organisms and influence many biological processes, notably cell- cell adhesion and immune response.[1] Composed of repeats of the disaccharide β-D-Gal-1,4-β-D-GlcNAc they make especially attractive synthetic targets as they can be readily transformed to a range of complex carbohydrates with commercially available enzymes.[2] Poly-*N*-acetyllactosamines are assembled by two glycosyltransferases, β1,4-galactosyltransferase and the β1,3-*N*-acetylglucosaminyltransferase (*i*-GlcNAc transferase). Interestingly, the average chain length of polylactosamines differs according to whether they occur on *N*-linked or *O*-linked glycans, the latter rarely consisting of more than two or three repeats.[3] In order to further understand lactosamine biosynthesis we have synthesized a series of oligolactosamines **1- 5** for use in enzyme kinetic studies.



Several methods for the synthesis of polylactosamines have been detailed previously, all of which utilize a protected lactosaminyl donor with an orthogonally removable protective group at O-3' of galactose.[4] Selective deprotection followed by glycosylation allows the stepwise addition of lactosamine units. Unfortunately, these methods often involve considerable protective group manipulation of lactosamine disaccharides, requiring a

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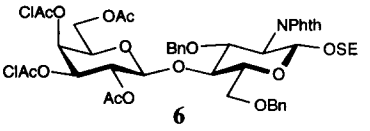
significant amount of labor. In this communication, we describe the synthesis of compounds containing up to three lactosamine repeats from a readily accessable lactosaminyl donor. In addition, we have synthesized two *N*-acetylglucosamine terminated structures through enzymatic removal of non-reducing galactose with β-galactosidase.



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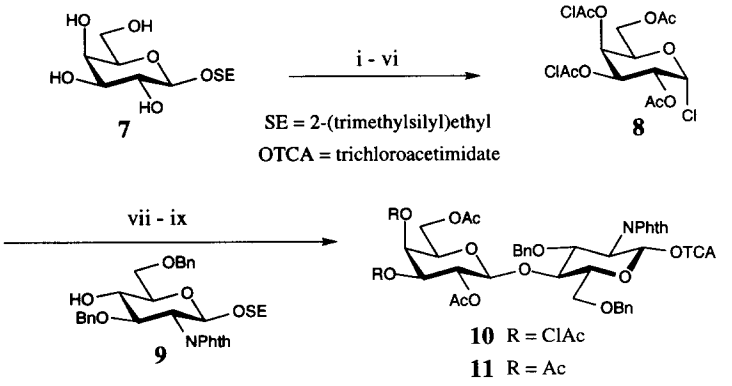
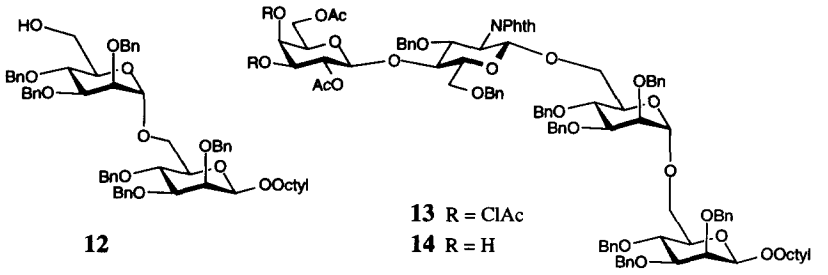
We sought to assemble a differentially protected lactosamine synthon from monosaccharide precursors in a minimum number of steps in order to improve somewhat on previous procedures. The disaccharide **6** was selected as a possible candidate in that the chloroacetyl and 2-trimethylsilylethyl (SE) groups are orthogonally removable, with the latter easily cleaved prior to conversion to the highly reactive trichloroacetimidate functionality. In fact, Nicolaou *et al.* successfully employed a similar disaccharide bearing a thiophenyl glycoside in the synthesis of trimeric Lewis^x.[5]



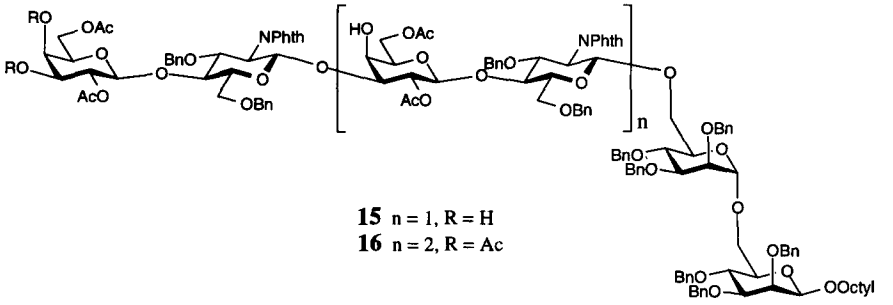
(i) 2,2-dimethoxypropane, CSA, 24 h; (ii) AcOH/EtOH, 1:4, 40°C; (iii) Ac_2O, pyridine; (iv) AcOH/H_2O, 4:1, 80°C; (v) $(ClAc)_2O$, pyridine; (vi) Cl_2CHOCH_3, $ZnCl_2$, 2 h; (vii) AgOTf, collidine, 4 Å mol. sieves, CH_2Cl_2, –20°C; (viii) TFA, CH_2Cl_2 ; (ix) Cl_3CCN, DBU, CH_2Cl_2, –20°C.

Scheme 1

Conversion of 2-trimethylsilylethyl β-D-galactopyranoside[6] **7** to the 3,4-di-*O*-chloroacetyl galactosyl chloride **8** was achieved in 6 steps and 65% overall yield (Scheme 1). Glycosylation of the glucosamine acceptor[6] **9** required over 2 equivalents of the chloride **8**, but gave the lactosamine **6** in 81% yield. Two step conversion to the trichloroacetimidate **10** was achieved in 75% yield giving the required bifunctional donor (Scheme 2). In a similar fashion, glycosylation of **9** with 2,3,4,6-tetra-*O*-acetyl-galactosyl chloride[7], followed by protective group manipulation, gave the imidate **11** (76% overall) which was ultimately used to introduce the terminal lactosamine unit as detailed below.



Treatment of the mannosyl acceptor[8] **12** with the imidate **10** in the presence of triethylsilyl triflate at –40°C gave the tetrasaccharide **13** in 62% yield. Removal of the chloroacetyl groups with thiourea and 2,6-lutidine gave the diol **14** in 81% yield following chromatography. Repetition of this two step cycle gave the hexasaccharide **15** in 56% overall yield. Finally, the triol **15** was glycosylated efficiently with the tetra-*O*-acetylated imidate **11** to furnish the octasaccharide **16** (63%).



Deprotection of the lactosamines **14**, **15** and **16** was achieved in four steps involving initial conversion to the *N*-acetyl derivatives through treatment with ethylenediamine in hot 1-butanol, followed by acetylation with Ac_2O in pyridine.[9] Removal of the remaining protecting groups (0.1 M NaOMe then H_2 over 10% Pd/C) gave the products **1**, **2**, and **3** in low to average yields (27-42%) following purification on LH-20 Sephadex. The two GlcNAc terminated compounds **4** and **5** were conveniently obtained from structures **2** and **3** through removal of non-reducing galactose by β-galactosidase (2 U per μmol) from *E. coli.* The reactions were typically performed on a 2 μmol scale. The products were characterized by ^{1}H NMR spectroscopy and high resolution FAB mass spectrometry.[10,11]

Compounds **1**- **5** were evaluated as substrates for the i- and I-GlcNAc transferases, as well as several β1,4-galactosyltransferases and were determined to be efficient primers for *in vitro* poly-*N*-acetyllactosamine biosynthesis. In contrast, poly-*N*-acetyllactosamine biosynthesis in *O*-linked glycans was found to become less efficient as chain length increased.[12]

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10. Partial ^{1}H NMR (300 MHz, CD_3OD) **1**; (500 MHz, D_2O) **2**,**3**,**4** and **5**. **1**; δ 4.80 (d, $J_{1',2'}$ = 1.5 Hz, H-1′), 4.50 (d, $J_{1'',2''}$ = 8.1 Hz, H-1″), 4.49 (s, H-1), 4.38 (d, $J_{1''',2'''}$ = 8.0 Hz, H-1‴), 1.98 (s, 3H, NH*Ac*). **2**; δ 4.86 (s, H-1′), 4.68 (d, J = 8.2 Hz, 1H) 4.64 (s, H-1), 4.55 (d, J = 8.0 Hz, 1H), 4.42-4.47 (m, 2H), 2.02, 2.00 (2s, 6H, NH*Ac*). **2**; δ 4.84 (s, H-1′), 4.62- 4.66 (m, 3H), 4.52 (d, J = 8.0 Hz, 1H), 4.39- 4.45 (m, 3H), 2.02, 1.99 (2s, 9H, NH*Ac*). **3**; δ 4.79 (d, $J_{1',2'}$ = 1.5 Hz, H-1′), 4.49 (d, $J_{1'',2''}$ = 8.1 Hz, H-1″), 4.48 (s, H-1), 2.00 (s, 3H, NH*Ac*). **4**; δ 4.84 (s, H-1′), 4.63 (s, H-1), 4.62 (d, J = 8.0 Hz, 1H), 4.52 (d, J = 8.2 Hz, 1H), 4.41 (d, J = 7.8 Hz, 1H), 2.00, 1.98 (2s, 6H, NH*Ac*). **5**; δ 4.84 (s, H-1′), 4.60- 4.65 (m, 3H), 4.52 (d, J = 8.0 Hz, 1H), 4.39- 4.44 (m, 2H), 2.01, 1.99 (2s, 9H, NH*Ac*).
11. *m/z* (hi-res FAB): **1**; 842.3633 [M+Na$^+$] calcd.; 842.3634. **2**; 1207.4945 [M+Na$^+$] calcd.; 1207.4955. **3**; 1572.6283 [M+Na$^+$] calcd.; 1572.6278. **4**; 1045.4427 [M+Na$^+$] calcd.; 1045.4427. **5**; 1410.5749 [M+Na$^+$] calcd.; 1410.5749.
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