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Simplifying Oligosaccharide Synthesis: Efficient Synthesis of Lactosamine and Siaylated Lactosamine Oligosaccharide Donors

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A practical sequence is described for converting d-glucosamine into peracetylated Gal(β-1,4)-GlcNTrc(β1-S)Ph and Neu5Ac(α-2,3)Gal(β-1,4)GlcNTrc(β1-S)Ph building blocks using a synthetic strategy based on chemoenzymatic oligosaccharide synthesis. The known trichloroethoxyxarnonyl, N-Troc, protecting group was selected as a suitable protecting group for both enzymatic and chemical reaction conditions. These oligosaccharide building blocks proved effective donors for the β-selective glycosylation of the unreactive OH-3 of a polymeric PEG-bound acceptor and for the axial OH-2 of a mannose acceptor in good yields. The resulting complex oligosaccharides are useful for vaccine and pharmaceutical applications.

Oligosaccharides play an important role in biological systems, such as cell–cell interaction, cell adhesion, and immunogenic recognition.1 To further understand and exploit the activities of oligosaccharides, access is required to natural and nonnatural oligosaccharides, yet the synthesis of reasonable quantities of complex oligosaccharides remains one of the most challenging areas of chemistry.2 One of the most efficient ways to synthesize oligosaccharides is to prepare di-, tri-, and higher oligosaccharide building blocks and then convergently assemble the large oligosaccharide from these building blocks.3 Often, these building blocks contain oligosaccharides with cis linkages or other “difficult” linkages such as those with 2-keto-acids such as sialic acids that frequently can only be formed in low yield, and the reactions often lead to complex mixtures that require careful separations.4 To assemble these building blocks, high-yielding glycosylation reactions that form trans linkages via neighboring group participation are typically used.5

N-Acetylated lactosamine (Gal(β-1,4)GlcNAc) and its siaylated (usually α2,3 or α2,6) extension are ubiquitous components of cell surface glycans.6 Thus, an efficient method to large-scale amounts of these compounds, as shown in Scheme 1, in a form suitable for further glycosylation chemistry is highly desirable. However, the available methods for the syntheses of lactosaminyl donors such as 1 are few in number, and all syntheses require many chemical steps with low overall yields.7 An alternative to the chemical procedures for oligosaccharide synthesis is given by protocols based on the use of glycosyltransferases for carbohydrate assembly since glycosyltransferases are highly stereo- and regioselective with regard to the glycoside bond formations, and no tedious protection/deprotection steps are required.8 On the other hand, with the recent emergence of a wide variety of cloned and expressed glycosyltransferases from bacterial sources, many enzymes are now available in synthetically useful quantities.9 Similarly, the requisite nucleotide donors are also becoming available in bulk at competitive prices.10 Following our earlier examples for the synthesis of oligosaccharides donors,8b,c we describe here a practical chemoenzymatic methodology to peracetylated Gal(β-1,4)GlcNTrc(β1-S)Ph 1b and Neu5Ac(α-2,3)Gal(β-1,4)GlcNTrc(β1-S)Ph 2b building block donors from 2-amino-2-deoxy-d-glucosamine 3. Their subsequent use for the efficient generation of complex oligosaccharides is also reported.

The key to this development is the choice of N-protecting group on the glucosamine since it must be capable of neighboring group participation to ensure high β-selectivity in the glycosylation reaction and stable to both enzymatic and chemical reaction conditions. N-
Phthalimide is an obvious choice since it is widely used in chemical glycosylation reactions.11 We started a program to prepare N-phthaloyl (N-Phth) lactosamine 1a and (α-2,3) sialylated lactosamine 2a building blocks from GlcNPhth/β1-SPh 5a (Scheme 2).12 Eventually, it was found that the GlcNPhth/β1-SPh 5a solubilized with a small amount of DMF (∼1% V/V) was a substrate for the available galactosyltransferase and sialyltransferase (see the Experimental Section) and after acetylation was turned into potential donor 8a; see Schemes 3 and 4. But, the overall yields were always low, even though TLC monitoring of the enzyme reactions suggested that all the substrates were consumed. The products showed (M + 18) + peaks in their MS spectra. Several stability studies at different pHs were monitored by 1H NMR and typically showed the disappearance of the anomeric proton peak (δ 5.69 ppm) of the substrate 5a and the appearance of a new anomeric peak (δ 5.06 ppm, see the Supporting Information). From these data, it was concluded that the N-Phth ring opened in water and that the opening is accelerated at high pH. The use of p-methoxybenzyl or even better p-nitrobenzyl glycosides of the N-Phth glucosamine minimized but did not eliminate this side reaction (not shown). With the small quantities of 8a available, several glycosylations with polymer-bound acceptors such as compound 10, that worked well with phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside as donor,13 failed or gave only low yields. Therefore, we decided to investigate other N-protecting groups.

2,5-Dimethylpyrrole (N-DMP) group14 is a stable and soluble N-protecting group in both chemical and enzymatic reaction conditions. However, the peracetylated N-DMP-protected thiophenyl and trichloroacetimidate glycoside as donors were not reactive with low nucleophilicity acceptors such as the unreactive HO-3 position of compound 10; see Scheme 5.

Further surveys of N-protecting groups led us to trichloroethoxycarbonyl (Troc) as a suitable protecting group, since the N-Troc group has proved to be useful with respect to facile introduction, deprotection, and β-selective glycosidation as well as increased reactivity of donors with a C-2 N-Troc group compared with N-Phth group.15 In addition, the N-Troc group is stable under a range of standard conditions used for chemical and enzymatic oligosaccharide synthesis, although it is sensitive to alcoholysis under basic conditions. The known thiophenyl glycoside of GlcNTroc 5b as substrate was readily prepared in a good yield in four steps from D-glucosamine 3 as shown in Scheme 2.16 Treatment with inexpensive UDP-glucose and a GalE-Glc/Gal epimerase fusion enzyme17 led to 89% conversion to the desired lactosamine derivative Gal(β1,4)GlcNTroc(β1-S)Ph 6b. This disaccharide 6b was easily acetylated with acetic

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**SCHEME 1**

![Chemical structures](image)

**SCHEME 2**

![Chemical structures](image)

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anhydride in the presence of pyridine to give a versatile lactosaminyl donor 1b in high yield. The $^1$H and $^{13}$C NMR data clearly indicated the new Gal(β-1,4)-linkage notably Gal H-4 $^1$H = 5.34 ppm $J_{3,4}$ = 2.7 Hz and Gal H-1 $^1$H = 4.48 ppm $J_{1,2}$ = 7.8 Hz. Further sialylation of the disaccharide 6b with a solution of CMP-Neu5Ac, which was generated with sialic acid and CTP from a CMP-synthetase at high pH (8.5),$^{19}$ and a sialyltransferase (FUS-01/2)$^{19}$ at low pH (6.0) generated Neu5Ac(α-2,3)-Gal(β-1,4)GlcNTroc(β-1-S)Ph 7b in nearly quantitative yield. Unlike our previous report,$^{15}$ in which the expensive nucleotide sugar donor CMP-Neu5Ac was generated in situ by a fusion enzyme, this time it was prepared using a CMP–Neu5Ac synthetase along with sialic acid and CTP. The supernatant after centrifugation was used without further purification. This procedure is more efficient since both the CMP-synthetase and sialyltransferase are used at their pH optimum (see above). This

\[ \text{(GaE-LgtB)} \]
means that less enzyme and less nucleotide sugar donor are needed for the same amount of product.

For the synthesis of final sialylated lactosamine donor 2b we treated compound 7b with acetic anhydride/pyridine at 45 °C to give a desired intra 1,2-lactone 8b which was indicated by $^1$H, $^{13}$C, and HMBC NMR spectra, notably the long range connectivity between the lactone C=O at $\delta^{13}$C = 163.12 ppm and Gal H-2 at $\delta^1$H = 4.68 ppm. Treatment of 1,2-lactone 8b with DMAP in dry methanol and then recrystallization with acetic anhydride/pyridine gave a complex mixture from which 2b was isolated as the main product in less than 10% yield. The sensitivity of the N-Troc group to methanol under basic condition may be the cause of this problem. A shorter alternative route was developed that involves esterification of compound 7b with an ion-exchange resin (H⁺ form) in methanol followed by acylation to give a reasonable yield (74%) of trisaccharide donor 2b accompanied by small amounts of lactone 9, as shown in Scheme 4. Lactone 9 is chromatographically and spectroscopically different from 8b but its definitive structural determination is in progress. It should be noted that the processing of compound 2b was greatly simplified by using acetic anhydride as the solvent and Sc(OTf)₃ as the acylation catalyst. This process is very simple and readily scalable as the product is isolated by aqueous acetylation catalyst. This process is very simple and readily scalable as the product is isolated by aqueous acetylation catalyst. This process is very simple and readily scalable as the product is isolated by aqueous acetylation catalyst.

To demonstrate the efficiency of these compounds lactosaminyl donor 1b was successfully reacted with the unreactive OH-3 of acceptor 10 which is bound to the polymer poly(ethylene glycol) (MEPG), CH₂OH(CH₂)ₙOH via the linker dioxyxylene [DOX, =CH₂-Ph₂-CH₃=O]- which was indicated by 1H, 13C, and HSQC, HMBC) of the GlcN-Troc anomeric proton δ 1H = 4.52 ppm, δ 13C = 153.71 ppm and Gal H-2 at δ 1H = 4.81 ppm, δ 13C = 75.11 ppm; see Scheme 5. This trisaccharide 11 is a model of a typical arm of an N-linked glycopeptide.

In conclusion, the large-scale synthesis of peracetylated Gal(β-1,4)GlcN-Troc(β1-S)Ph 1b and Neu5Ac(α-2,3)Gal(β-1,4)GlcN-Troc(β1-S)Ph 2b as effective building blocks was accomplished through enzymatic and chemical transformation of d-glucosamine by a short route with good yields. The key to this development was the choice of the N-Troc group since it is capable of neighboring group participation to ensure high β-selectivity in subsequent glycosylation reactions and stable to both enzymatic and chemical reaction conditions. Both building blocks have

1A Group B Streptococcus capsular polysaccharide of interest in our institute for vaccine development.23 Similarly, the trisaccharide donor 2b was reacted with the axial OH-2 of mannose acceptor 13 to yield tetrasaccharide 14 in 75% yield; see Scheme 6. The 1D and 2D NMR spectroscopy (gCOSY, HSQC, HMBC) of 14 indicated that the glycosidic linkage is a trans GlcN-Troc(β-1,2)-Manp linkage (chemical shift of the GlcN-Troc anomic proton is δ 1H = 4.81 ppm, δ 13C > 7.0 Hz). Standard one-step deprotection and N-acetylation of the N-Troc with Zn in acetic anhydride gave 15. Subsequently the O-benzyl groups were removed by hydrogenation over Pd/C and the acyl groups by transesterification followed by hydrolysis to yield 18 as shown in Scheme 6. The three anomers at GlcN δ 1H = 4.60 ppm J,δ = 8.0 Hz, Gal δ 1H = 4.56 ppm J,δ = 8.0 ppm J,δ = 1.0 Hz support the expected anomericities. Tetrasaccharide 18 is a model of a typical arm of an N-linked glycopeptide.24

In conclusion, the large-scale synthesis of peracetylated Gal(β-1,4)GlcN-Troc(β1-S)Ph 1b and Neu5Ac(α-2,3)Gal(β-1,4)GlcN-Troc(β1-S)Ph 2b as effective building blocks was accomplished through enzymatic and chemical transformation of d-glucosamine by a short route with good yields. The key to this development was the choice of the N-Troc group since it is capable of neighboring group participation to ensure high β-selectivity in subsequent glycosylation reactions and stable to both enzymatic and chemical reaction conditions. Both building blocks have
been used for the synthesis of oligosaccharides for vaccine and pharmaceutical applications. In principle, the synthetic strategy disclosed in this paper will be applicable to the synthesis of a variety of \( \text{di}^\text{4}\), \( \text{tri}^\text{1} \), and \( \text{oligosaccharide} \) donors. Further applications of this methodology are in progress and will be reported in due course.

**Experimental Section**

Optical rotations were obtained (\( \lambda = 589 \) nm) at 20 °C in a 10 cm 1 mL cell. NMR spectra were recorded on a 500 or 200 MHz instrument at 300 K. Chemical shifts were given in ppm relative to the signal of internal TMS or indirectly to solvent signals 7.26 (CDCl\( _3 \)) or 4.79 (D\( _2 \)O) for \( ^1H \) NMR spectra and to the solvent signals 77.0 (CDCl\( _3 \)) or 49.15 (internal methyl alcohol) for \( ^13C \) NMR spectra. All signal assignments were made by standard \( ^1H \)-\( ^1H \) COSY and \( ^1H \)-decoupled \( ^13C \)-COSY experiments. All chemicals for synthesis were purchased from commercial suppliers, and solvents were purified according to standard procedures. Silica gel (230–400 mesh) was used for flash chromatography. C\( _{18} \) silica gel (10% capped with TMS, 35–70 mesh) was used for reversed-phase chromatography.

**Enzymatic Synthesis of Phenyl \( \text{O-(1-β-D-Galactopyranosyl)} \)-(1-4)-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylaminolino)-β-D-glucopyranoside (6b).** A fusion enzyme UDP-glactosyltransferase (UTGase) and \( ^1H \) NMR (methanol-\( D_2 \)) afforded pure \( \text{C}_{24} \text{H}_{26} \text{O}_{14} \text{S} \) (\( \delta \text{12.5, 4.0 Hz, H-6} \)).

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20.62 × 2, 27.59 (10 × C, O==CCH)3; MALDI-MS calec for C41H124Cl2N14O28Na 1313.24, found m/z 1312.95 [M + Na+].

1b. 1H NMR (CDCl3) δ 7.16–7.36 (20.59, 10 H, Bz), 5.33 (dd, 1 H, J = 9.0, 4.5 Hz, H-1), 5.00 (s, 2 H, DOX, J = 12.5, 9.5 Hz, H-2), 4.67 (d, 1 H, J = 8.0, H-3), 4.61 (s, 3 H, OCH3, J = 136.66, 135.57, 133.51, 132.29, 129.79, 128.64, 128.47, 128.17, 128.10 (O==CPh), 124.97 (C-CH3), 101.11 (C-1H), 101.08 (C-1C), 54.97 (C-1C), 95.40 (C-2C), 72.20 (C-3), 75.80 (C-4), 74.08 (C-6CH2), 72.56 (C-5), 71.10 (2 × C, C-3, C-5), 71.36 (2 × C, C-2, C-5), 70.89 (C-3), 70.70 (C-5), 69.66 (OCH2CH2OAc), 69.46 (C-4), 69.12 (DOXCH2), 66.99 (C-4), 65.89 (DOXCH2), 62.67 (C-6), 60.75 (C-6C), 60.74 (C-6C), 65.35 (C-2), 20.11, 20.84, 20.72, 20.63 × 2, 20.60 × 2, 20.49 (8 × C, O==CCH3); MS (FAB) calec for C27H28O14SNa 684.46, found m/z 684.46 [M + Na+].

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Supporting Information Available: Experimental procedure and data for 8a,b, 10, and 18. 1H and 13C NMR and mass spectra of 1b, 5b,c, and 9. This material is available free of charge via the Internet at http://pubs.acs.org.