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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($\beta$-1,4)-GlcNTroc($\beta$1-S)Ph and Neu5Ac($\alpha$-2,3)Gal($\beta$-1,4)GlcNTroc($\beta$1-S)Ph building blocks using a synthetic strategy based on chemoenzymatic oligosaccharide synthesis. The known trichloroethoxycarbonyl, *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 $\beta$-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($\beta$-1,4)GlcNAc) and its sialylated (usually $\alpha$2,3 or $\alpha$2,6) extension are ubiquitous components of cell surface glycans.[6] Thus, an efficient method to large-scale amounts of these compounds, as shown in Scheme 1, in a form suitable for further glycosylation chemistry is highly desirable. However, the available methods for the syntheses of lactosaminyl donors such as **1** are few in number, and all syntheses require many chemical steps with low overall yields.[7] An alternative to the chemical procedures for oligosaccharide synthesis is given by protocols based on the use of glycosyltransferases for carbohydrate assemblage since glycosyltransferases are highly stereo- and regioselective with regard to the glycoside bond formations, and no tedious protection/deprotection steps are required.[8] On the other hand, with the recent emergence of a wide variety of cloned and expressed glycosyltransferases from bacterial sources, many enzymes are now available in synthetically useful quantities.[9] Similarly, the requisite nucleotide donors are also becoming available in bulk at competitive prices.[10] Following our earlier examples for the synthesis of oligosaccharides donors,[8b,c] we describe here a practical chemoenzymatic methodology to peracetylated Gal($\beta$-1,4)GlcNTroc($\beta$1-S)Ph **1b** and Neu5Ac-($\alpha$-2,3)Gal($\beta$-1,4)GlcNTroc($\beta$1-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 $\beta$-selectivity in the glycosylation reaction and stable to both enzymatic and chemical reaction conditions. *N*-

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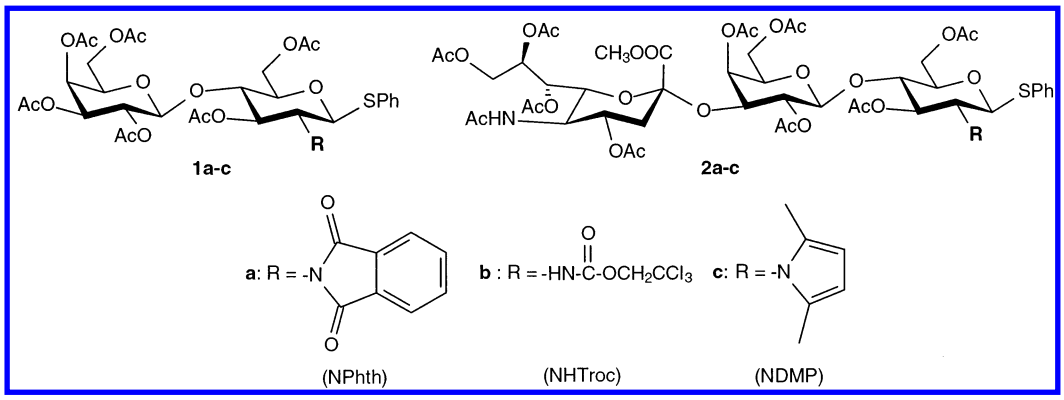
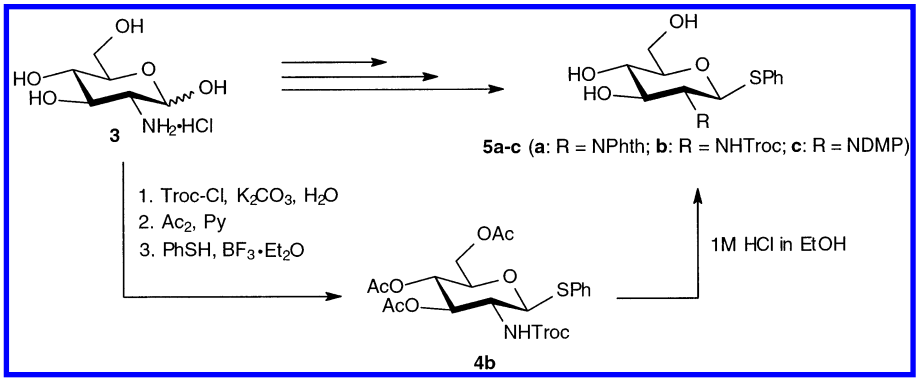
**SCHEME 1**




**SCHEME 2**




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$\beta$1-SPh **5a** (Scheme 2).[12] Eventually, it was found that the GlcNPhth$\beta$1-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 $^1$H NMR and typically showed the disappearance of the anomeric proton peak ($\delta$ 5.69 ppm) of the substrate **5a** and the appearance of a new anomeric peak ($\delta$ 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-$\beta$-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) group[14] 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 $\beta$-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 enzyme[17] led to 89% conversion to the desired lactosamine derivative Gal($\beta$-1,4)GlcNTroc($\beta$1-S)Ph **6b**. This disaccharide **6b** was easily acetylated with acetic

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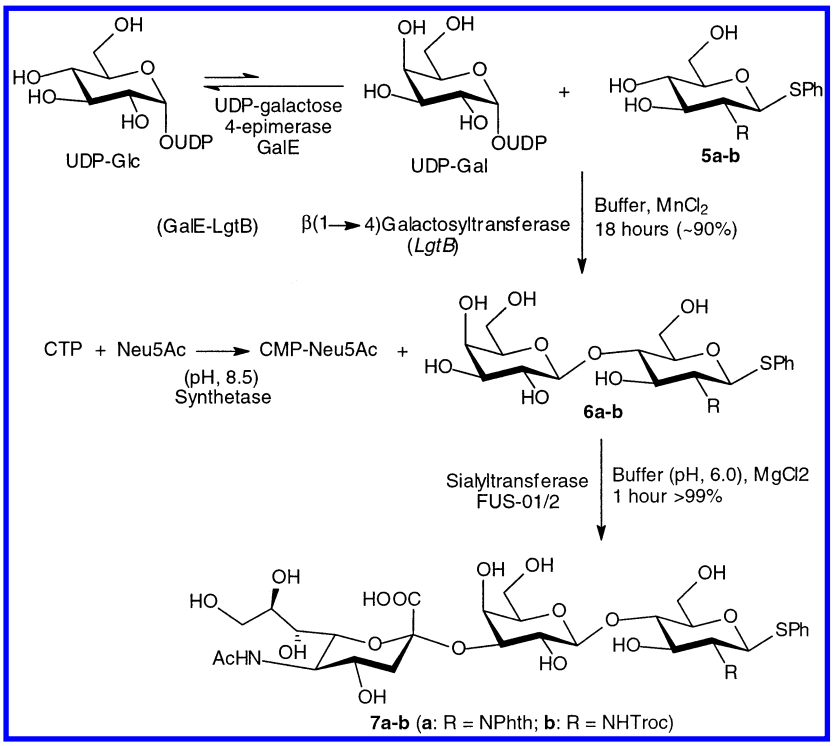
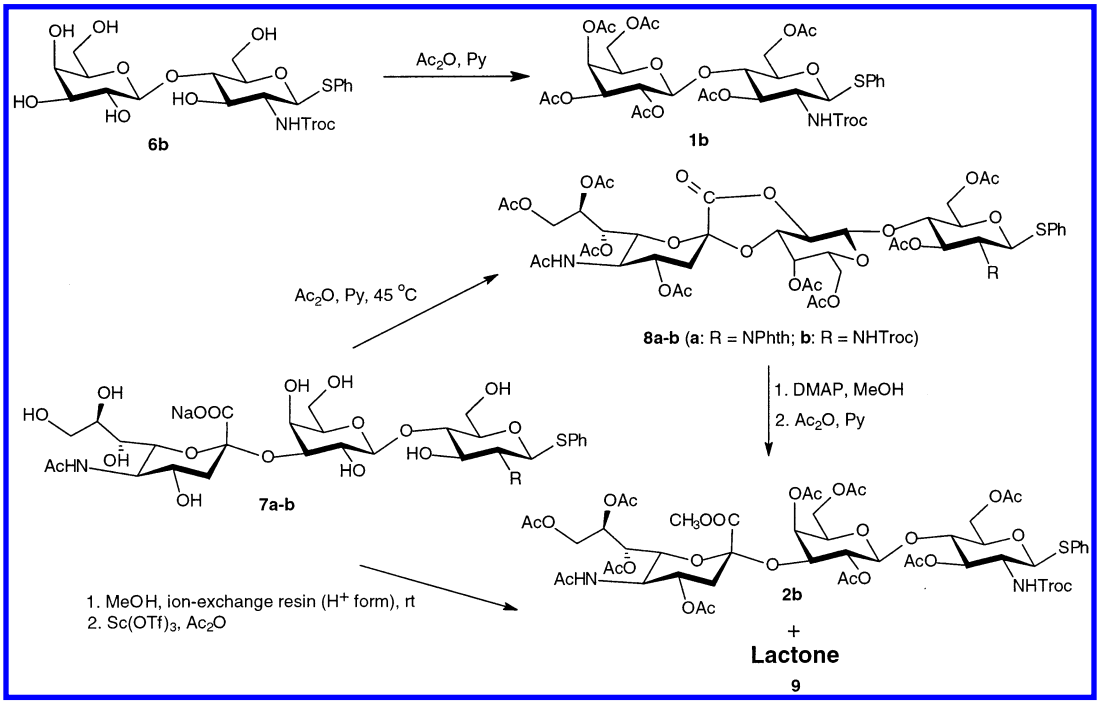
**SCHEME 3**




**SCHEME 4**




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($\beta$-1,4)-linkage notably Gal H-4 $\delta$ $^1$H = 5.34 ppm $J_{3,4}$ = 2.7 Hz and Gal H-1 $\delta$ $^1$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),[18] and a sialyltransferase (FUS-01/2)[19] at low pH (6.0) generated Neu5Ac($\alpha$-2,3)-Gal($\beta$-1,4)GlcNTroc($\beta$1-S)Ph **7b** in nearly quantitative yield. Unlike our previous report[17] 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

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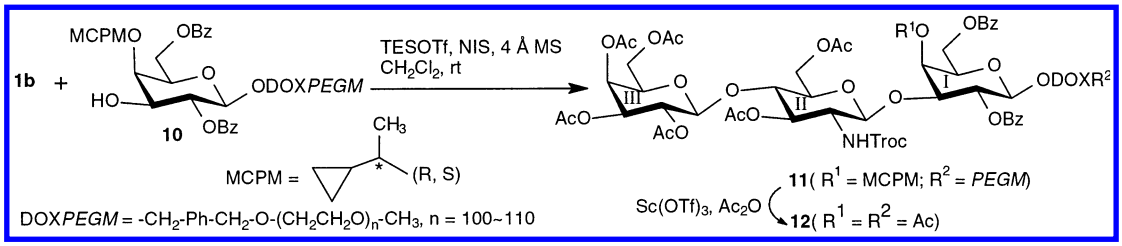
**SCHEME 5**

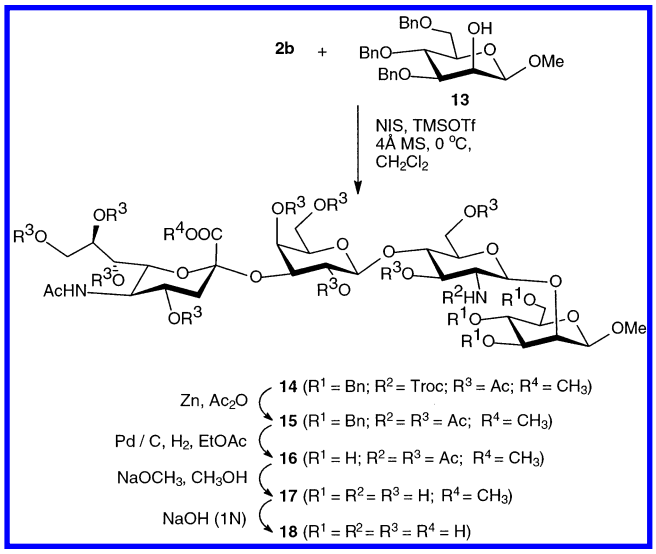



means that less enzyme and less nucleotide sugar donor are needed for the same amount of product.

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 reacetylation with acetic anhydride/pyridine[8c] 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 acetylation 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 acetylation catalyst. This process is very simple and readily scalable as the product is isolated by aqueous organic solvent extractions followed by flash silica gel chromatography.

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) (MPEG), $CH_3O(CH_2CH_2O)_n$-H via the linker dioxyxylene [DOX, $-(O)CH_2PhCH_2(O)-$][20] to yield the $\beta$-linked trisaccharide **11**. The small electron donating 1-methyl 1′-cyclopropylmethyl, MCPM, protecting group was used to activate the O-3.[21] The successful glycosylation with **1b** but not with **1a** perhaps reflects the 30-fold greater reactivity of *N*-Troc versus *N*-Phth reported for monosaccharide donors.[22] Donors **2a**, **2b**, and **2c** are all 3,4,6-triacetyl protected and therefore deactivated and thus require strong promoters. After $Sc(OTf)_3$-mediated cleavage, the trisaccharide **12** was isolated without affecting the *N*-Troc group as evidenced by the C=O $\delta$ $^{13}$C = 153.71 ppm and the C=OOC$H_2$ $\delta$ $^1$H = 4.52 and 4.28 ppm, $J$ = 12.7 Hz; see Scheme 5. This trisaccharide $\beta$-**12** is part of the type

**SCHEME 6**




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 GlcNTroc($\beta$-1,2)-Man*p* linkage (chemical shift of the GlcN-Troc anomeric proton is $\delta$ $^1$H = 4.81 ppm, $J$ > 7.0 Hz). Standard one-step deprotection and N-acetylation of the *N*-Troc with Zn in acetic anhydride gave **15**.[15a] 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 anomerics at GlcN $\delta$ $^1$H = 4.60 ppm $J_{1,2}$ = 8.0 Hz, Gal $\delta$ $^1$H = 4.56 ppm $J_{1,2}$ = 8.0 Hz, and Man $\delta$ $^1$H = 4.80 ppm $J_{1,2}$ = 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($\beta$-1,4)GlcNTroc($\beta$1-S)Ph **1b** and Neu5Ac(α-2,3)Gal-($\beta$-1,4)GlcNTroc($\beta$1-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 $\beta$-selectivity in subsequent glycosylation reactions and stable to both enzymatic and chemical reaction conditions. Both building blocks have

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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 di-, tri-, and 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 ($D_2O$) for $^1$H NMR spectra and to the solvent signals 77.0 ($CDCl_3$) or 49.15 (internal methyl alcohol) for $^{13}$C NMR spectra. All signal assignments were made by standard $^1$H−$^1$H-COSY and $^1$H-decoupled $^{13}$C−$^1$H-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 *O*-(*β*-D-Galactopyranosyl)-(1→4)-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranoside (6b)**. A fusion enzyme UDP-galactose 4-epimerase/*β*(1→ 4)galactosyltransferase (*GalE-LgtB*, 26.4 units, 24 mL) was added to a solution of 3.2 mM acceptor **5b** (1.0 g), 15 mM $MnCl_2$ (105 mL of 100 mM), and 1.28 mM UDP-Glc (6.0 mL of 150 mM) in 50 mM HEPES buffer (35 mL of 1 M, pH 7.4). The reaction was performed at 37 °C for a total of 20 h while two additional portions of 1.28 mM UDP-Glc (6.0 mL of 150 mM) were added in 30 min intervals. The formation of **6b** ($R_f$ 0.43) was monitored by TLC ($MeOH-CHCl_3-0.5\%$ $CaCl_2$ 40:50:10). Chromatography of the crude reaction mixture on a $C_{18}$ reversed-phase column (water−MeOH 1:1) afforded pure **6b** (1.22 g, 89%): $[\alpha]_D$ −5.6 (*c* 0.4, $CH_3OH$); $^1$H NMR (methanol-$d_4$) $\delta$ 7.52−7.55 (m, 2 H, Ph), 7.26−7.35 (m, 3 H, Ph), 4.91 (d, 1 H, $J$ = 12.0 Hz, 1/2 $CH_2CCl_3$), 4.82 (d, 1 H, $J$ = 10.5 Hz, H-1$^{GlcN}$), 4.76 (d, 1 H, $J$ = 12.0 Hz, 1/2 $CH_2CCl_3$), 4.42 (d, 1 H, $J$ = 7.5 Hz, H-1$^{Gal}$), 3.95 (dd, 1 H, $J$ = 12.5, 2.5 Hz, H-6$^{GlcN}$), 3.88 (dd, 1 H, $J$ = 12.5, 4.0 Hz, H-6$^{GlcN}$), 3.83 (d, 1 H, $J$ = 3.0 Hz, H-4$^{Gal}$), 3.80 (dd, 1 H, $J$ = 11.5, 7.5 Hz, H-6$^{Gal}$), 3.70 (dd, 1 H, $J$ = 11.5, 4.0 Hz, H-6$^{Gal}$), 3.66−3.69 (m, 2 H, H-4$^{GlcN}$, H-3$^{GlcN}$), 3.59−3.62 (m, 1 H, H-5$^{Gal}$), 3.57 (t, 1 H, $J$ = 9.5 Hz, H-2$^{GlcN}$), 3.55 (brt, 1 H, $J$ = 8.5 Hz, H-2$^{Gal}$), 3.50 (dd, 1 H, $J$ = 9.5, 3.0 Hz, H-3$^{Gal}$), 3.46 (brs, 1 H, H-5$^{GlcN}$); $^{13}$C NMR (methanol-$d_4$) $\delta$ 157.49 (*O*=*C*$OCH_2CCl_3$), 136.33, 133.43, 130.72, 129.28 (6 × C, Ph), 105.79 (C-1$^{Gal}$), 98.02 ($CCl_3$), 89.56 (C-1$^{GlcN}$), 81.49 (C-5$^{GlcN}$), 81.18 (C-4$^{GlcN}$), 78.03 (C-5$^{Gal}$), 76.42 (2 × C, $CH_2CCl_3$, C-3$^{GlcN}$), 75.66 (C-3$^{Gal}$), 73.48 (C-2$^{Gal}$), 71.22 (C-4$^{Gal}$), 63.47 (C-6$^{Gal}$), 62.81 (C-6$^{GlcN}$), 58.62 (C-2$^{GlcN}$); MS (FAB) calcd for $C_{21}H_{28}Cl_3NO_{11}S$ 607.0, found *m/z* 607.1 [$M^+$].

**Phenyl *O*-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(*β*-D-galactopyranosyl)-(1→4)-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranoside (7b).** The reaction was performed in a total volume of 400 mL, and the following reagents were added sequentially: 2.2 mM acceptor **6b** (550 mg, 0.90 mmol), 50 mM (2-(*N*-morpholino)ethanesulfonic acid) hydrate (MES, 40 mL of 0.5 M, pH 6.0), 100 mM $MgCl_2$ (40 mL of 1 M), and 3.0 mM CMP-NeuNAc (60 mL of 20 mM). The reaction was allowed to proceed at 37 °C after the addition of the sialyltransferase (FUS-01/2, 150 units, 7.5 mL). The reaction progress, i.e., the formation of material having $R_f$ 0.15, was monitored by TLC ($MeOH-CHCl_3-0.5\%$ $CaCl_2$ 40:50:10). After a total reaction time of 2 h, the crude product was chromatographed ($C_{18}$ reversed-phase column; elution with water and then 65:35 water−MeOH) to yield pure trisaccharide **7b** (700 mg, 94%): $[\alpha]_D$ −24.2 (*c* 0.1, $H_2O$); $^1$H NMR ($D_2O$) $\delta$ 7.54−7.57 (m, 2 H, Ph), 7.39−7.44 (m, 3 H, Ph), 4.96 (d, 1 H, $J$ = 12.5 Hz, 1/2 $CH_2CCl_3$), 4.95 (d, 1 H, $J$ = 10.5 Hz, H-1$^{GlcN}$), 4.75 (d, 1 H, $J$ = 12.0 Hz, 1/2 $CH_2CCl_3$), 4.57 (d, 1 H, $J$ = 8.0 Hz, H-1$^{Gal}$), 4.13 (dd, 1 H, $J$ = 10.0, 2.5 Hz, H-3$^{Gal}$), 4.00 (dd, 1 H, $J$ = 12.0 Hz, $J$ < 1.0 Hz, H-6$^{GlcN}$), 3.97 (d, 1 H, $J$ = 2.5 Hz, H-4$^{Gal}$), 3.88−3.92 (m, 2 H, H-8$^{Neu}$, H-9$^{Neu}$), 3.87 (dd, 1 H, $J$ = 12.5 Hz, $J$ = 4.5 Hz, H-6$^{GlcN}$), 3.86 (t, 1 H, $J$ = 10.0 Hz, H-5$^{Neu}$), 3.78 (t, 1 H, $J$ = 8.5 Hz, H-4$^{GlcN}$), 3.76 (t, 1 H, $J$ = 9.5 Hz, H-3$^{GlcN}$), 3.71−3.75 (m, 3 H, 2 × H-6$^{Gal}$, H-5$^{Gal}$), 3.70 (td, 1 H, $J$ = 11.5, 4.5 Hz, H-4$^{Neu}$), 3.67 (m, 1 H, H-9$^{Neu}$), 3.65 (dd, 1 H, $J$ = 10.5, 1.5 Hz, H-6$^{Neu}$), 3.62−3.64 (brs, 1 H, H-5$^{GlcN}$), 3.61 (t, 1 H, $J$ = 8.0 Hz, H-2$^{GlcN}$), 3.60 (d, 1 H, $J$ = 8.0 Hz, H-7$^{Neu}$), 3.58 (dd, 1 H, $J$ = 10.0, 8.0 Hz, H-2$^{Gal}$), 2.77 (dd, 1 H, $J$ = 12.5, 5.0 Hz, H-3$_{eq}^{Neu}$), 2.04 (s, 3 H, O=C$CH_3$), 1.81 (t, 1 H, $J$ = 12.5 Hz, H-3$_{ax}^{Neu}$); $^{13}$C NMR ($D_2O$) $\delta$ 175.71, 174.57 (C-1$^{Neu}$, *O*=*C*$CH_3$), 157.16 (*O*=*C*$OCH_2CCl_3$), 132.96, 132.21, 130.04, 128.83 (6 × C, Ph), 103.24 (C-1$^{Gal}$), 100.50 (C-2$^{Neu}$), 95.63 ($CCl_3$), 87.59 (C-1$^{GlcN}$), 79.57 (C-5$^{GlcN}$), 78.63 (C-4$^{GlcN}$), 76.17 (C-3$^{Gal}$), 75.88 (C-5$^{Gal}$), 74.95 ($CH_2CCl_3$), 74.48 (C-3$^{GlcN}$), 73.57 (C-6$^{Neu}$), 72.46 (C-8$^{Neu}$), 70.07 (C-2$^{Gal}$), 69.03 (C-4$^{Neu}$), 68.78 (C-7$^{Neu}$), 68.18 (C-4$^{Gal}$), 63.27 (C-9$^{Neu}$), 61.74 (C-6$^{Gal}$), 60.80 (C-6$^{GlcN}$), 56.78 (C-2$^{GlcN}$), 52.37 (C-5$^{Neu}$), 40.32 (C-3$^{Neu}$), 22.72 (O=C$CH_3$); MS (FAB) calcd for $C_{32}H_{45}Cl_3N_2O_{19}S$ 921.1, found *m/z* 921.1 [$M^+$].

**Phenyl *O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)- (2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-2-deoxy-3,6-di-*O*-acetyl-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranoside (2b).** A solution of **7b** (280 mg, 0.34 mmol) in 20 mL of dry methanol was stirred under argon with pretreated DOWEX500Wx8-200 resin ($H^+$ form) (4 g) at room temperature for 16 h. The resin was removed by filtration. The filtrate was concentrated, and the residue was coevaporated with toluene (3 × 20 mL) and dried under high vacuum to give the methyl ester of **7b**. To a solution of the crude methyl ester in acetic anhydride (10 mL) was added scandium triflate (15 mg, 0.03 mmol), and the reaction mixture was stirred at rt for 2 h under argon. The reaction was quenched with $NaHCO_3$ (10 mg, 0.12 mmol), and product was extracted with $CH_2Cl_2$ (100 mL) three times. The combined organic layer was washed with aqueous $NaHCO_3$, brine, and water successively, dried over $Na_2SO_4$, and evaporated. The residue was purified by silica gel chromatography (ethyl acetate−methanol 98:2) to yield pure **2b** (305 mg, 74%) and lactone **9** (10 mg, 2%). **2b**: $[\alpha]_D$ −5.1 (*c* 0.5, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.49 (m, 2 H, Ph), 7.29 (m, 3 H, Ph), 5.54 (ddd, 1 H, $J$ = 9.2, 5.6, 2.8 Hz, H-8$^{Neu}$), 5.39 (dd, 1 H, $J$ = 9.2 , 2.8 Hz, H-7$^{Neu}$), 5.25 (d, 1 H, $J$ = 9.6 Hz, NH$^{GlcN}$), 5.11 (t, 1 H, $J$ = 9.6 Hz, H-3$^{GlcN}$), 5.08 (d, 1 H, $J$ = 10.0 Hz, NH$^{Neu}$), 4.93 (dd, 1 H, $J$ = 10.4, 8.0 Hz, H-2$^{Gal}$), 4.88 (td, 1 H, $J$ = 11.2, 4.8 Hz, H-4$^{Neu}$), 4.87 (d, 1 H, $J$ = 3.6 Hz, H-4$^{Gal}$), 4.80 (d, 1 H, $J$ = 12.4 Hz, 1/2 $CH_2CCl_3$), 4.72 (d, 1 H, $J$ = 12.4 Hz, 1/2 $CH_2CCl_3$), 4.69 (d, 1 H, $J$ = 8.0 Hz, H-1$^{Gal}$), 4.68 (d, 1 H, $J$ = 10.4 Hz, H-1$^{GlcN}$), 4.52 (dd, 1 H, $J$ = 10.0, 3.2 Hz, H-3$^{Gal}$), 4.50 (dd, 1 H, $J$ = 11.6, 2.0 Hz, H-6$^{GlcN}$), 4.42 (dd, 1 H, $J$ = 12.4, 2.8 Hz, H-9$^{Neu}$), 4.19 (dd, 1 H, $J$ = 12.0, 6.4 Hz, H-6$^{GlcN}$), 4.04 (t, 1 H, $J$ = 10.0 Hz, H-5$^{Neu}$), 4.00 (m, 2 H, 2 × H-6$^{Gal}$), 3.98 (dd, 1 H, $J$ = 12.4, 5.6 Hz, H-9$^{Neu}$), 3.85 (m, 1 H, H-5$^{Gal}$), 3.84 (s, 3 H, COO$CH_3$), 3.81 (m, 1 H, H-4$^{GlcN}$), 3.78 (t, 1 H, $J$ = 10.0 Hz, H-2$^{GlcN}$), 3.64 (m, 1 H, H-5$^{GlcN}$), 3.63 (dd, 1 H, $J$ = 10.8, 2.8 Hz, H-6$^{Neu}$), 2.58 (dd, 1 H, $J$ = 12.8, 4.8 Hz, H-3$_{eq}^{Neu}$), 2.24, 2.16, 2.09, 2.09, 2.08, 2.05, 2.05, 2.04, 2.00, 1.85 (s, 30 H, O=C$CH_3$), 1.81 (t, 1 H, $J$ = 12.5 Hz, H-3$_{ax}^{Neu}$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.88, 170.65, 170.56, 170.41, 170.39, 170.32, 170.31, 170.26, 170.14, 169.59 (10 × C, *O*=*C*$CH_3$), 167.91 (C-1$^{Neu}$), 154.20 (*O*=*C*$OCH_2CCl_3$), 132.38, 131.71, 128.86, 127.96 (6 × C, Ph), 101.01 (C-1$^{Gal}$), 96.74 (C-2$^{Neu}$), 95.28 ($CCl_3$), 87.10 (C-1$^{GlcN}$), 77.25 (C-5$^{GlcN}$), 76.25 (C-4$^{GlcN}$), 74.52 ($CH_2CCl_3$), 73.97 (C-3$^{GlcN}$), 72.01 (C-6$^{Neu}$), 71.28 (C-3$^{Gal}$), 70.53 (C-5$^{Gal}$), 69.80 (C-2$^{Gal}$), 69.28 (C-4$^{Neu}$), 67.78 (C-8$^{Neu}$), 67.28 (C-4$^{Gal}$), 66.88 (C-7$^{Neu}$), 62.54 (C-6$^{GlcN}$), 62.21 (C-9$^{Neu}$), 61.53 (C-6$^{Gal}$), 55.17 (C-2$^{GlcN}$), 53.12 (COO$CH_3$), 49.06 (C-5$^{Neu}$), 37.35 (C-3$^{Neu}$), 23.14, 21.49, 20.89, 20.78, 20.74 × 3,

20.62 × 2, 20.59 (10 × C, O=C*CH*$_3$); MALDI-MS calcd for $C_{51}H_{65}Cl_3N_2O_{28}Na$ 1313.24, found *m/z* 1312.95 [M + $Na^+$].

**(4-*O*-Acetoxymethyl)benzyl *O*-(2,4,6-Tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2-deoxy-3,6-di-*O*-acetyl-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranosyl]-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside (12).** The PEG-bound acceptor **10** (310 mg, 0.055 mmol), lactosaminyl donor **1b** (70 mg, 0.083 mmol), and powdered molecular sieves 4 Å were dried at 40 °C in vacuo overnight. After being cooled to rt, the mixture was dissolved in $CH_2Cl_2$ under an atmosphere of argon and NIS (37 mg, 0.165 mmol) followed by TESOTf (7.45 $\mu$L, 0.033 mmol) were added. After the mixture was stirred for 2 h, the reaction was quenched with *N*,*N*-diisopropylethylamine and the polymer was precipitated with *tert*-butylmethyl ether (TBME, 150 mL). The precipitate was recovered by filtration, after rinsing with TBME followed by diethyl ether, and reprecipitated from absolute EtOH (60 mL). The resulting solid was filtered,and after washing with cold EtOH and ethyl ether it was dissolved in $CH_2Cl_2$, filtered, and concentrated. The residue (320 mg) containing 27% of PEGM bound trisaccharide **11** was dissolved in $CH_2Cl_2$ (3 mL) and cooled in an ice bath under an atmosphere of argon, and acetic anhydride (3 mL) followed by $Sc(OTf)_3$ (19 mg, 0.04 mmol) were added. The mixture was warmed to rt and allowed to stir overnight. The polymer was precipitated with TBME (100 mL) and filtered. The combined filtrates were concentrated to dryness. The residue was purified by preparative TLC (ethyl acetate−hexane 1:1) to yield pure **12** (10.2 mg, 55%): $[\alpha]_D$ +7.6 (*c* 0.3, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 7.25−8.25 (m, 10 H, Bz), 7.10 (brs, 4 H, DOX), 5.53 (brd, 1 H, H-4$^{I}$), 5.49 (dd, 1 H, *J* = 10.0, 8.1 Hz, H-2$^{I}$), 5.32 (brd, 1 H, H-4$^{III}$), 5.06 (dd, 1 H, *J* = 10.5, 7.8 Hz, H-2$^{III}$), 5.01 (m, 2 H, NH, H-3$^{II}$), 5.00 (s, 2 H, DOX*CH*$_2$OAc), 4.94 (dd, 1 H, *J* = 10.5, 3.4 Hz, H-3$^{III}$), 4.83 (d, 1 H, *J* = 12.7 Hz, 1/2 O*CH*$_2$-DOX), 4.65 (d, 1 H, *J* = 12.9, 9.0 Hz, H-6$^{III}$), 4.61 (d, 1 H, *J* = 12.7 Hz, 1/2 O*CH*$_2$DOX), 4.53 (d, 1 H, *J* = 8.1 Hz, H-1$^{I}$), 4.50 (m, 4 H, H-1$^{II}$, H-1$^{III}$, H-6$^{I}$, 1/2 *CH*$_2$CCl$_3$), 4.39 (dd, 1 H, *J* = 11.6, 5.5 Hz, H-6$^{I}$), 4.25 (d, 1 H, *J* = 12.0 Hz, 1/2 *CH*$_2$CCl$_3$), 4.06 (brs, 2 H, 2 × H-6$^{III}$), 3.99 (brt, H-5$^{I}$), 3.94 (dd, 1 H, *J* = 12.7, 4.9 Hz, H-6$^{II}$), 3.92 (dd, 1 H, *J* = 10.0, 3.4 Hz, H-3$^{I}$), 3.83 (brt, 1 H, H-5$^{III}$), 3.73 (dd, 1 H, *J* = 9.2, 9.2 Hz, H-4$^{II}$), 3.43 (m, 2 H, H-2$^{II}$, H-5$^{II}$), 2.19, 2.12, 2.10, 2.04, 2.04, 2.02, 1.97, 1.95 (s, 24 H, O=C*CH*$_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.81, 170.44, 170.33, 170.10 × 2, 170.08 × 2, 169.08 (*O*=*C*CH$_3$), 166.19, 164.82 (*O*=*C*Ph), 153.71 (*O*=*C*OCH$_2$CCl$_3$), 136.66, 135.57, 133.51, 133.29, 129.79, 128.64, 128.47, 128.17, 128.10 (O=CPh, DOX), 101.11 (C-1$^{III}$), 101.08 (C-1$^{II}$), 98.97 (C-1$^{I}$), 95.40 ($CCl_3$), 77.20 (C-3$^{I}$), 75.80 (C-4$^{II}$), 74.08 (*CH*$_2$CCl$_3$), 72.66 (C-5$^{II}$), 71.40 (2 × C, C-3$^{II}$, C-5$^{I}$), 71.36 (2 × C, C-2$^{I}$, C-5$^{III}$), 70.89 (C-3$^{III}$), 70.70 (C-5$^{III}$), 69.66 (O*CH*$_2$DOX), 69.46 (C-4$^{I}$), 69.12 (C-2$^{III}$), 66.59 (C-4$^{III}$), 65.89 (DOX*CH*$_2$OAc), 62.67 (C-6$^{I}$), 60.75 (C-6$^{III}$), 60.74 (C-6$^{II}$), 56.35 (C-2$^{II}$), 21.00, 20.84, 20.72, 20.63 × 2, 20.60 × 2, 20.49 (8 × C, O=C*CH*$_3$); MS (FAB) calcd for $C_{59}H_{66}Cl_3NO_{28}Na$ 1364.3, found *m/z* 1364.2 [$M^+$].

**Methyl *O*-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl)-(1→4)-*O*-[2-deoxy-3,6-di-*O*-acetyl-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranosyl]-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (14).** The mannose acceptor **13** (18 mg, 0.038 mmol), trisaccharide **2b** (50 mg, 0.04 mmol), and powdered molecular sieves 4 Å were dried at 40 °C in vacuo overnight. The mixture was dissolved in $CH_2Cl_2$ (2 mL) under an atmosphere of argon at 0 °C, and NIS (18 mg, 0.08 mmol) followed by TESOTf (6.75 $\mu$L, 0.03 mmol) were added. After the mixture was stirred for 2 h, the reaction was quenched with triethylamine and product was extracted with $CH_2Cl_2$ (100 mL) three times. The combined organic layer was washed with brine and water, dried over $Na_2SO_4$, and evaporated. The residue was purified by silica gel chromatography (ethyl acetate/methanol, 98:2) to yield pure **14** (50 mg, 80%): $^1$H NMR ($CDCl_3$) $\delta$ 7.16−7.36 (m, 15 H, Ph), 5.53 (ddd, 1 H, *J* = 9.0, 6.5, 2.5 Hz, H-8$^{Neu}$), 5.40 (dd, 1 H, *J* = 9.2, 3.0 Hz, H-7$^{Neu}$), 5.31 (t, 1 H, *J* = 9.5 Hz, H-3$^{GlcN}$), 5.08 (d, 2 H, *J* = 10.0 Hz, NH$^{GlcN}$, NH$^{Neu}$), 4.93 (dd, 1 H, *J* = 10.5, 8.5 Hz, H-2$^{Gal}$), 4.88 (d, 1 H, *J* = 2.5 Hz, H-4$^{Gal}$), 4.87 (td, 1 H, *J* = 10.5, 5.0 Hz, H-4$^{Neu}$), 4.86 (d, 1 H, *J* = 12.5 Hz, 1/2 *CH*$_2$CCl$_3$), 4.81 (br, 1 H, *J* > 7.0 Hz, H-1$^{GlcN}$), 4.76 (d, 1 H, *J* = 11.5 Hz, 1/2 *CH*$_2$-Ph), 4.67 (d, 1 H, *J* = 8.0 Hz, H-1$^{Gal}$), 4.66 (s, 1 H, H-1$^{Man}$), 4.60 (d, 1 H, *J* = 14.0 Hz, 1/2 *CH*$_2$Ph), 4.47−4.56 (m, 7 H, 2 × *CH*$_2$Ph, H-3$^{Gal}$, H-6$^{GlcN}$, 1/2 *CH*$_2$CCl$_3$), 4.43 (dd, 1 H, *J* = 12.5, 2.5 Hz, H-9$^{Neu}$), 4.16 (dd, 1 H, *J* = 12.0, 6.5 Hz, H-6$^{GlcN}$), 4.11 (brs, 1 H, H-2$^{Man}$), 3.98−4.04 (m, 4 H, H-5$^{Neu}$, 2 × H-6$^{Gal}$, H-9$^{Neu}$), 3.89 (dd, 1 H, *J* = 9.0, 2.5 Hz, H-3$^{Man}$), 3.80−3.88 (m, 2 H, H-4$^{Man}$, H-4$^{GlcN}$), 3.84 (s, 3 H, COO*CH*$_3$), 3.61−3.73 (m, 5 H, 2 × H-6$^{Man}$, H-5$^{Man}$, H-6$^{Neu}$, H-5$^{GlcN}$), 3.48 (dd, 1 H, *J* = 12.5, 7.5 Hz, H-2$^{GlcN}$), 3.32 (s, 3 H, $OCH_3$), 2.72 (dd, 1 H, *J* = 12.5, 4.5 Hz, H-3$_{eq}^{Neu}$), 2.23, 2.19, 2.16, 2.08, 2.06, 2.05, 2.04, 2.00, 1.98, 1.85 (s, 30 H, O=C*CH*$_3$), 1.68 (t, 1 H, *J* = 12.5 Hz, H-3$_{ax}^{Neu}$); $^{13}$C NMR ($CDCl_3$) $\delta$ 170.87, 170.62, 170.55, 170.37, 170.31, 170.30, 170.21, 170.05, 169.61, 169.52 (10 × C, *O*=*C*CH$_3$), 167.94 (C-1$^{Neu}$), 153.69 (*O* = COCH$_2$CCl$_3$), 138.45, 138.20, 128.31, 128.30, 128.28, 127.94, 127.92, 127.63, 127.54, 127.44, 127.41 (18 × C, $CH_2$Ph), 100.93 (C-1$^{Gal}$), 99.30 (C-1$^{GlcN}$), 98.45 (C-1$^{Man}$), 96.75 (C-2$^{Neu}$), 95.52 ($CCl_3$), 78.19 (C-3$^{Man}$), 76.43 (C-4$^{GlcN}$), 74.93 (*CH*$_2$CCl$_3$), 74.55 (C-4$^{Man}$), 74.30 (C-2$^{Man}$), 73.16 (2 × C, *CH*$_2$Ph), 72.90 (C-5$^{GlcN}$), 72.05 (C-6$^{Neu}$), 72.04 (C-3$^{GlcN}$), 71.75 (C-5$^{Man}$), 71.26 (C-3$^{Gal}$), 71.25 (*CH*$_2$Ph), 70.46 (C-5$^{Gal}$), 69.88 (C-2$^{Gal}$), 69.29 (2 × C, C-6$^{Man}$, C-4$^{Neu}$), 67.84 (C-8$^{Neu}$), 67.30 (C-4$^{Gal}$), 66.88 (C-7$^{Neu}$), 62.64 (C-6$^{GlcN}$), 62.16 (C-9$^{Neu}$), 61.59 (C-6$^{Gal}$), 56.35 (C-2$^{GlcN}$), 54.76 (O*CH*$_3$), 53.13 (COO*CH*$_3$), 49.11 (C-5$^{Neu}$), 37.38 (C-3$^{Neu}$), 23.16, 21.50, 20.90, 20.82, 20.75 × 2, 20.69, 20.68, 20.67, 20.62 (10 × C, O=C*CH*$_3$); MALDI-MS calcd for $C_{73}H_{91}Cl_3N_2O_{34}Na$ 1669.46, found *m/z* 1669.01 [M + $Na^+$].

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**Supporting Information Available:** Experimental procedure and data for **8a**,**b**, **10**, and **18**. $^1$H and $^{13}$C 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.

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