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Synthesis of Rationally Designed Tetrasaccharides for Crystallographic and Binding Studies with *Clostridium difficile* Toxins and Unexpected Partial N-methylations during Catalytic Hydrogenation of Azides in Methanol

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Abstract:

A β-GlcNAc-Le\textsuperscript{X} tetrasaccharides and another α-Gal-Le\textsuperscript{X} analog have been synthesized for studying interaction with toxins produced by the human pathogen *Clostridium difficile*. Le\textsuperscript{X} based-trisaccharides bearing either a 2-azidoethyl or a 6-azidohexyl aglycone have been employed in the total synthesis to afford the desired tetrasaccharides. Interestingly, during the final catalytic hydrogenation step to remove benzyl, benzylidene protecting groups and simultaneously reduce azide functionality, partial N-methylations were observed. The N-methylations appear to be a general issue with catalytic hydrogenation of azides in methanol.

**Key Words:** Oligosaccharide synthesis, glycosylation, catalytic hydrogenolysis, azide reduction, N-methylation, *Clostridium difficile* toxins.
1. Introduction

*Clostridium difficile* infection (CDI) constitutes one of the most common hospital-acquired infections in the industrialized world. In North America alone, hundreds of thousands of cases occur annually.\(^1\) CDI typically arises following the use of antibiotics which disrupt the normal colonic microflora\(^2\) thus providing opportunities for *C. difficile* to colonize; once established, the infection can be difficult to treat, often leading to persistent, recurring gastrointestinal infections. The symptoms of CDI include mild to severe diarrhea, and in more severe cases, pseudomembranous colitis can occur. At the molecular level, the pathogenesis of *C. difficile* infection is linked to two large, structurally related protein toxins, TcdA and TcdB\(^3,4\) that recognize epithelial glycans.\(^5\) The binding of the toxins to glycans on the epithelial cell surface leads to cell entry via receptor-mediated endocytosis. Both TcdA and TcdB can function as enzymes to glucosylate a specific Thr residue in the Rho GTPases which are responsible for regulating many aspects of intracellular events and for the maintenance of the cellular cytoskeleton. The glycosylation disrupts GTPase-dependent signalling pathways, leading to changes in the
cytoskeleton that can ultimately result in cell death and inflammation.\textsuperscript{6,7} To improve the treatment of CDI, several novel approaches have been proposed to block toxin binding to host epithelial cells.\textsuperscript{8,9,10} One of the major challenges to the development of toxin-specific inhibitors is understanding the molecular basis of toxin-glycan recognition. X-ray crystallography and binding studies have revealed the molecular structural nature of TcdA interactions with the trisaccharide $\alpha$-Gal(1\(\rightarrow\)3)$\beta$-Gal(1\(\rightarrow\)4)$\beta$-GlcNAc.\textsuperscript{11,12} The binding interactions for related oligosaccharides, including a more complex pentasaccharide (supposedly Le\textsuperscript{A}-LacNAc, 1, Figure 1) have been identified to bind to TcdA with better affinity than the above trisaccharide.\textsuperscript{13} Through detailed synthetic studies\textsuperscript{14,15} and X-ray crystallography, we have later corrected the true carbohydrate sequence of the leading pentasaccharide to be the neoLacNAc-Le$^X$ (2, Figure 1). The erroneous structural assignment was due to the unexpected regiospecificity of the fucosylation reaction catalyzed by the recombinant human $\alpha$-(1,3)/$\alpha$-(1,4)-transferase (FUT III). Compound 1 indeed can bind to TcdA but with an affinity ca \(\sim\)5 times lower than 2. Our preliminary X-ray crystallographic studies revealed that the non-reducing end galactopyranosyl residue of the pentasaccharide 2 (shaded) does not interact with TcdA, as it appears to be projecting into bulk solvent (Figure 2). We thus hypothesized that removing the non-interacting terminal galactosyl residue could yield a tetrasaccharide with improved binding by reducing solvation energy. Compound 3 with a 2-aminoethyl linker was thus designed to be a synthetic target ($\beta$-GlcNAc-Le$^X$). Furthermore, we also became interested in the related tetrasaccharide 4 ($\alpha$-Gal-Le$^X$), because the $\alpha$-galactopyranosyl residue linked to the O3-position of nonreducing end galactosyl...
residue of Le$^x$ generates a tetrasaccharide with a different topology. Glycan microarray screens have also indicated that $\alpha$-Gal-Le$^x$ can bind to TcdA with modest binding affinity. Here we report the synthesis of these two tetrasaccharides for further crystallographic and binding studies.

Figure 1. The structures of two previously synthesized pentasaccharides (1 and 2) that bind to TcdA and two new tetrasaccharides (3 and 4) derived from the Le$^x$ trisaccharide.
2. Results and Discussion

The synthesis of fully protected tetrasaccharide 11 and 16 started from the previously available Lewis X donor 5 and acceptor 12 (Scheme 1). The Lewis X donor 5 has the $p$-chlorophenylthio as a convenient aglycone with adequate reactivity. The installation of a participating 2-phthalimido group on the glucosamine residue ensured the desired $\beta$-selectivity. Thus, to prepare compound 11 (Scheme 1, A), a glycosylation of Lewis X thioglycoside 5 (donor) with 2-chloroethanol 6 (acceptor, 2 equivalents) was carried in anhydrous dichloromethane using N-iodosuccinimide (NIS)/triflic acid as a promoting reagent; the temperature of the reaction was slowly warmed up to room temperature from -78 °C to afford the desired glycoside 7 in high yield (87%). The $O$-acetates on O2- and O3-positions of the galactopyranosyl residue of the trisaccharide

Figure 2. Schematic representation of the previously synthesized pentasaccharide 2 bound to TcdA, with the non-terminal galactopyranosyl residue projecting into the bulk of water.
were then removed. To prevent the ring opening of cyclic imide and also reduce chances of nucleophilic side reactions or β-elimination on the 2-chloroethyl group, a milder transesterification condition was carried out using a methanolic guanidine/guanidium solution; such a condition was reported\textsuperscript{16} to be milder enough to preserve the integrity of the sensitive $N$-2,2,2-trichloroethoxycarbonyl ($N$-Troc) group. Indeed, the $O$-transesterifications were found to be complete after 30 minutes to afford the desired diol 8 in excellent yield (96%). Neither the $N$-phthalimido nor the chloride group was affected. In the $^{13}$C NMR spectrum (DEPTQ), a methylene carbon peak was observed at 42.5 ppm, which was assigned to be the methylene group that had the chloride attached to. A subsequent glycosylation of the trisaccharide acceptor 8 with 3,4,6-tri-$O$-acetyl-2-$N$-phthalimido-protected thioglucoside donor 9\textsuperscript{14} (3 equivalents) was then carried out using similar activation conditions as above; this reaction was found to work well by starting from -50 °C and subsequently letting the temperature warm up to -10 °C. The desired tetrasaccharide 10 was obtained in very good yield (85%) after a column chromatography on silica gel using 35% ethyl acetate – toluene as the eluent. Conforming with previous reports,\textsuperscript{17,14,15} the OH-3 of the β-galactopyranosyl residue is less sterically hindered, thus more accessible to be glycosylated. The chloride was then substituted by sodium azide in $N,N$-dimethylformamide at 90 °C for 48 hours to afford the fully protected tetrasaccharide 11 in excellent yield (91%). The evidence of azide substitution was provided by electrospray high resolution mass spectrometry (ESI HRMS) as well as by $^{13}$C NMR. For example, a methylene carbon peak at 50.5 ppm was observed, which corresponds to the typical -CH$_2$N$_3$ group that is present in the expected product 11; in the ESI HRMS, an $m/z$ at 1574.5424 was observed, which corresponds to the expected
sodium adduct of the molecular ion of compound 11: [C_{83}H_{85}N_{25}O_{25} + Na]^+ (expected m/z: 1574.5426).

Scheme 1: synthesis of fully protected β-GlcNAc-LeX tetrasaccharide 11 and α-Gal-LeX 16 from LeX trisaccharide donor 5 or from LeX trisaccharide acceptor 12.
To prepare the fully protected α-Gal-Le\textsuperscript{X} tetrasaccharide 16, we started from the Lewis X trisaccharide acceptor 12. To install the desired α-galactopyranosyl linkage, we first attempted to use the per-O-benzylated β-thiogalactopyranoside 13\textsuperscript{18}, which also contain the p-chlorophenylthio group. The reaction was carried out in -78 °C initially in anhydrous dichloromethane using NIS / triflic acid as the promoter and the temperature was allowed to warm up to -50 °C; unfortunately, the glycosylation gave a complex mixture, and we were unable to isolate the desired tetrasaccharide 15. The unsuccessful glycosylation could be the result of incompatible reactivities between the donor and acceptor. It is well known that the per-O-benzylated β-thioglycoside donors are usually very reactive, thus the donor 13 could have decomposed too quickly before reacting with the less reactive acceptor 12. We thus chose to use another β-thiogalactopyranosyl donor 14\textsuperscript{18} which possessed a 4,6-O-benzylidene acetal group and 2,3-O-benzyl groups; it is known that introduction of 4,6-O-benzylidene group usually reduces the reactivity of the donor. As expected, the glycosylation of donor 14 with acceptor 12 proceeded better at the -78 to -30 °C temperature range; after 45 minutes, the desired tetrasaccharide 16 containing an α-galactopyranoside linkage was isolated in pure form in modest yield (43% yield) by column chromatography; additionally, a small amount of impure tetrasaccharide 16 that was contaminated with the corresponding β-galactosylated anomer (not shown) was also obtained (~18% yield). The structure of tetrasaccharide 16 was confirmed by \textsuperscript{1}H-NMR spectrum combined with \textsuperscript{1}H-\textsuperscript{13}C HSQC correlation spectrum. For example, the anomeric proton of newly formed α-galactopyranosyl residue was observed at 4.72 ppm ($J_{1,2} = 3.8$ Hz) while the other α-anomeric proton of the
fucopyranosyl residue was found at 5.21 ppm ($J_{1,2} = 2.9$ Hz); additionally, the other two β-anomeric protons were observed at 5.05 ppm (β-GlcN, $J_{1,2} = 8.4$ Hz) and 4.58 ppm (β-Gal, $J_{1,2} = 2.9$ Hz), respectively. The ESI HRMS also revealed a peak at $m/z$ 1643.6774 which corresponds to the [C$_{94}$H$_{100}$N$_{4}$O$_{21}$ + Na]$^+$ adduct of compound 16 (calculated: 1643.6772).

With both fully protected tetrasaccharide 11 and 16 in hand, we next proceeded to their stepwise deprotection. A similar deprotection sequence was carried out for both tetrasaccharides. In the case of compound 11, the $N$-phthalimido group was removed first by using 1,2-ethylenediamine as a reagent in $n$-butanol at 100 °C; the three O-acettes were presumably removed at the same time. After 24 hours, a complete acetylation of the obtained intermediate was carried out at 50 °C using an excess amount of acetic anhydride in pyridine to afford a fully acetylated intermediate which was subsequently transesterified using Zemplén conditions; this permitted a selective removal of all the O-acettes. The obtained intermediate was purified by column chromatography (without extensive characterizations) and directly subjected to a catalytic hydrogenation in methanol (contain a few drops of acetic acid) using palladium hydroxide on charcoal (20%) as a catalyst; the benzyl, benzylidene groups should be removed and the azide functionality on the aglycone should be simultaneously reduced to a primary amine. The final compound was purified by reverse-phase chromatography on a C18 Sep-Pak cartridge using a gradient of methanol-water (0-5%). For compound 16, the full deprotection followed a similar reaction sequence with some minor differences. For example, the removal of $N$-phthalimido group was carried out in refluxing ethanol using hydrazine hydrate as a reagent; this was followed by a regioselective $N$-acytelylation using
acetic anhydride in a 3:1 (v/v) mixture of methanol – dichloromethane solution. The obtained intermediate was then purified by column chromatography on silica gel and the obtained intermediate was finally deprotected by catalytic hydrogenation; the terminal azido group on the hexyl aglycone was also expected to be reduced to primary amine, isolated in the acetic acid salt form.

**Scheme 2:** Full deprotection of fully protected β-GlcNAc-LeX tetrasaccharide 11 and α-Gal-LeX 16.

The $^1$H NMR spectra of fully deprotected compounds from compounds 11 and 16 are illustrated in Figure 3. Clearly, each of them constituted a tetrasaccharide with the correct anomic configurations. For example, in the spectrum of compounds obtained from deprotection of 11, three β-anomeric protons were observed at 4.60 ppm ($J = 8.4$
Hz), 4.51 ppm ($J = 8.3$ Hz) and 4.36 ppm ($J = 7.8$ Hz), which are assigned to be the anomic proton of non-reducing end β-GlcNAc (I), βGal, and reducing end β-GlcNAc (II) residue, respectively; in addition, the anomic proton of the most acid-labile α-Fuc residue was observed at 5.02 ppm ($J = 4.0$ Hz); moreover, the H6 protons of αFuc residue was observed as a doublet at 1.08 ppm, and the H5 proton of the same residue was observed at ~4.75 ppm (underneath the solvent peak, HOD, typical for LeX). In the spectrum of compounds obtained from deprotection of 16, two β-anomeric protons were observed at 4.41 ppm (overlapped, $J = 8.4$ Hz), which are assigned to be the anomic protons of β-Gal and β-GlcNAc residue, respectively. Furthermore, two α-anomeric protons were observed at 5.03 ppm ($J = 3.9$ Hz) and 5.00 ppm ($J = 4.0$ Hz), which were assigned to be the anomic protons of non-reducing end terminal α-Gal and α-Fuc residue, respectively; for H6 and the H5 proton of the αFuc residue, same features are observed as above.
Figure 3. The $^1$H NMR spectra of fully deprotected products from compounds 11 and 16 (D$_2$O, 400 MHz); partial N-methylations were observed in both cases.

However, when the aglycone regions of both tetrasaccharides were carefully examined, it became evident to us that none of two deprotections gave a single compound. For example, the methylene protons beside the amine functionality of products obtained 11 had an unusually complex patterns and were observed between 3.30-3.06 ppm regions. Normally, we expected these two protons to be diastereotopic and each of them should appear as a doublet of doublets of doublets (ddd). Additionally, we observed two singlets at 2.83 ppm and 2.65 ppm. These could suggest partial N-methylations of formed primary amine. Similar complications were observed for the deprotection of tetrasaccharide 16. For example, the methylene protons beside the amine functionality of the hexyl aglycone were expected to be a triplet with an integration of two protons. However, we observed two sets of peaks at 3.01 ppm and 2.87 ppm – each of them were integrated to be less than 2 protons; while the set of peaks at 2.87 ppm appeared to be a normal triplet, the set of peaks at 3.01 ppm appeared to be more complex. Moreover, two singlets at 2.78 ppm and 2.61 ppm were also observed. These patterns also suggested the obtained products to be also partially N-methylated.

The more affirmative proofs were finally obtained from the ESI HRMS spectra of both products. As can be seen in Figure 4, in the products obtained from the deprotection of compound 11, three tetrasaccharides were observed: the presence of the expected tetrasaccharide 3 was confirmed by the m/z’s at 776.3321 [C$_{30}$H$_{53}$N$_{3}$O$_{20}$ + H]$^+$ (expected: 776.3295); 789.3119 [C$_{30}$H$_{53}$N$_{3}$O$_{20}$ + Na]$^+$ (expected: 789.3115). Additionally, the
presence of an \(N\)-methylated tetrasaccharide 17 and an \(N,\,N\)-dimethylated tetrasaccharide 18 were also confirmed by their respective molecular ion peaks: \(m/z\) for 17: 790.3466 \([C_{31}H_{55}N_3O_{20} + H]^+\) (expected: 790.3452); \(m/z\) for 18: 804.3709 \([C_{32}H_{57}N_3O_{20} + Na]^+\) (expected: 804.3608); for both compounds 17 and 18, the molecular ions of their sodium adducts were also observed (see Figure 4).

Similarly, in the isolated products from the deprotection of compound 16 (Figure 4), we also observed three tetrasaccharides which correspond to the expected tetrasaccharide 4: \(m/z\): 791.3671 \([C_{32}H_{58}N_2O_{20} + H]^+\) (expected: 791.3656); \(N\)-methylated tetrasaccharide 19: 805.3823 \([C_{33}H_{50}N_2O_{20} + H]^+\) (expected: 805.3812); \(N,\,N\)-dimethylated tetrasaccharide 20: \(m/z\): 819.3979 \([C_{34}H_{62}N_2O_{20} + H]^+\) (expected: 819.3969).
Figure 4. ESI HRMS spectra of products obtained from the deprotections of compound 11 and 16. In each case, the expected tetrasaccharides (3 and 4) were obtained. In addition, an N-methylated tetrasaccharides (17 and 19) and an N,N-dimethylated tetrasaccharides (18 and 20) were obtained, as a result of partial N-methylation on the intermediate amines.

From the mass spectra of both tetrasaccharides, it is also interesting to note that the N,N-dimethylated tetrasaccharide was formed as a majority. This could be explained
by the higher nucleophilicity of the \( N \)-methylated secondary amine intermediate, which tends to react faster during the subsequent \( N \)-alkylation.

The origin of \( N \)-methylations could be attributed to the presence of trace amount of formaldehyde in methanol, which reacts instantly with the primary amine formed from reduction of azido functionality in each case to form an imine intermediate that was subsequently hydrogenated. It is important to note that the methanol used in our experiments was redistilled over magnesium methoxide and stored under argon. When and how the formaldehyde was formed remained puzzling to us. To confirm if other azides could suffer similar \( N \)-methylation problems, we used 11-azidoundecan-1-ol\(^{19}\) as a substrate to carry out the hydrogenation in undistilled methanol, using either palladium (II) hydroxide (20%) on charcoal or palladium (5%) on charcoal as a catalyst. As expected, after hydrogenation, the 11-aminoundecan-1-ol product was formed as the major product in both conditions. However, ESI HRMS also confirmed both the \( N \)-mono- and \( N,N \)-dimethylated side products were also formed in small amounts (0.5 - 5%) from both hydrogenation conditions, suggesting the consistent presence of formaldehyde in the both reaction conditions.

3. Conclusions

We have successfully completed the synthesis for two tetrasaccharides that respectively contain the \( \beta \)-GlcNAc-Le\(^X\) and \( \alpha \)-Gal-Le\(^X\) sequences for studying the binding with \( C. \) difficile toxins. During the final reduction of the azido functionality to a primary amine, partial \( N \)-methylations were observed, which provided the \( N \)-methylated and \( N,N \)-dimethylated derivatives. Based on our earlier crystallographic studies of the
glycan binding sites in TcdA, these N-methyl groups should not affect the binding significantly, as they are not part of essential glycan binding sites. We also observed partial N-methylations during the catalytic hydrogenation of organic azides; this appears to have a wide implication in bioorganic chemistry especially in carbohydrate chemistry, as the azide functionality is commonly used as a masked amine group for future bioconjugation. We are currently carrying out a systematic study on the origin of formaldehyde.

4. Experimental Section

General methods

Optical rotations were determined in a 5 cm cell at 25 ± 2°C. [α]D values are given in units of 10⁻¹ deg cm² g⁻¹. Analytical TLC was performed on Silica Gel 60-F₂₅₄ (Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 5% sulfuric acid in water or with a ceric ammonium molybdate dip. All commercial reagents were used as supplied unless otherwise stated. Column chromatography was performed on Silica Gel 60 (Silicycle, Quebec). Molecular sieves were stored in an oven at 100°C and flame-dried under vacuum before use. Organic solutions from extractions were dried with anhydrous Na₂SO₄ prior to concentration under vacuum at < 40°C (bath). ¹H NMR spectra were recorded at 300 and 400 MHz on Bruker spectrometers at 298 K. The first order proton chemical shifts δH and δC were reported in δ (ppm) and referenced to either residual CHCl₃ (δH 7.24, δC 77.0, CDCl₃) or external acetone (δH 2.225, δC 30.4). ¹H and ¹³C NMR spectra were assigned with the assistance of GCOSY, GHSQC; for oligosaccharides with two glucosamine residues, residues closer to the non-reducing end
were defined with the "I" suffix (ex. GlcN_I) while the ones closer to the reducing end were defined with the "II" suffix (ex: GlcN_II). Microanalyses and Electrospray Ionization (ESI) Mass Spectrometry were performed by the analytical services of the Department of Chemistry, University of Calgary. For high resolution mass determination, spectra were obtained using an Agilent 6520 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS.

2-Chloroethyl 2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-benzyl-α-L-fucopyranosyl–(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7).

To a solution of trisaccharide 5\textsuperscript{14} (347 mg, 0.271 mmol) and 2-chloroethanol 6 (37 µL, 0.54 mmol) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (5 mL), was added molecular sieves 4 Å (500 mg), and the mixture was stirred for 1 hr at room temperature. The mixture was cooled to -78 °C, and N-iodosuccinimide (65 mg, 0.27 mmol) was added; a solution of saturated triflic acid in CH\textsubscript{2}Cl\textsubscript{2} (110 µL) was added dropwise, and the reaction was allowed to warm up to room temperature. After 1 hr, the reaction was quenched with the addition of Et\textsubscript{3}N (0.5 mL), and mixture was filtered off, and the solid washed with EtOAc (40 mL). The organic solution was extracted with a 1:1 mixture of 5% aqueous NaHCO\textsubscript{3} and 5% Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} solutions (40 mL), dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 30% EtOAc – hexane to afford the desired trisaccharide 7 (287 mg, yield 87%). R\textsubscript{f} 0.19 (40% EtOAc – hexane). [α]\textsubscript{D} -36° (c 0.4, CHCl\textsubscript{3}). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 7.68 (m, 4H, Phth), 7.53 (m, 2H, Ar), 7.48 – 7.40 (m, 14H, Ar), 7.40 – 7.07 (m, 14H, Ar), 7.00 (m, 4H, Ar).
Ar), 6.91 (m, 2H, Ar), 5.54 (s, 1H, PhCH), 5.35 (dd, $J = 10.2$, 8.4 Hz, 1H, H-2_Gal), 5.10 (d, $J = 8.6$ Hz, 1H, H-1_GlcN), 4.87 (d, $J = 12.1$ Hz, 1H, H_Bn), 4.82 – 4.65 (m, 5H, H-1_Fuc + H-5_Fuc + H-1_Gal + H-3_Gal + H-3_GlcN), 4.55 (s, 2H, 2 × H_Bn), 4.51 (d, $J = 11.9$ Hz, 1H, H_Bn), 4.48 – 4.34 (m, 3H, H-2_GlcN + H_Bn + H-6a_Gal), 4.30 - 4.16 (m, 3H, H-4_Gal + H-4_GlcN + H_Bn), 4.08 – 3.96 (m, 4H, H_Bn + H6b_Gal + H6a_GlcN + OCHaCHbCHcHdCl), 3.92 – 3.81 (m, 2H, H-3_Fuc + H-6b_GlcN), 3.67 – 3.58 (m, 2H, H_Bn + H-2_Fuc), 3.55 (m, 1H, H-5_GlcN), 3.48 – 3.36 (m, 3H, OCHaC_HbC_HcHdCl), 3.14 (dd, $J = 1.6$, < 1 Hz, 1H, H-4_Fuc), 3.07 (m, 1H, H-5_Gal), 2.14 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.13 (d, $J = 6.4$ Hz, 3H, H-6_Fuc). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 170.65 (Ac), 168.70 (Ac), 139.49, 139.44, 138.24, 138.01, 137.64, 134.05, 128.88, 128.59, 128.15, 128.04, 127.96, 127.88, 127.78, 127.73, 127.57, 127.33, 126.95, 126.74, 125.81, 123.54 (Ar), 99.68 (PhCH), 99.62 (C-1_Gal), 98.70 (C-1_GlcN), 97.56 (C-1_Fuc), 78.96 (C-3_Fuc), 78.87(C-4_Fuc), 75.53 (C-5_GlcN), 74.85(C-4_GlcN), 74.76 (Bn), 73.78 (H-2_Fuc), 73.47 (Bn), 73.27(C-4_Gal), 72.81 (Bn), 72.17 (C-3_Gal), 71.46 (C-3_GlcN), 71.38 (Bn), 69.41(OCHaCHb), 68.93(C-6_Gal), 68.72 (C-2_Gal), 67.70 (C-6_GlcN), 66.37 (C-5_Fuc), 66.29 (C-5_Gal), 56.26 (C-2_GlcN), 42.40 (CHaCHbCl), 20.87 (Ac), 20.82 (Ac), 16.05 (C-6_Fuc). ESI-HRMS: calcd $m/z$ for [C$_{67}$H$_{70}$ClNO$_{18}$ + Na]$^+$ 1234.4174; found 1234.4194.

2-Chloroethyl 4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-benzyl-α-L-fucopyranosyl–(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside

(8).
Compound 7 (55 mg, 0.045 mmol) was dissolved in a methanolic solution of guanidine/guanidinium chloride\(^\text{16}\) (2.0 mL), and the reaction was stirred at room temperature for 30 minutes. CH\(_3\)CO\(_2\)H (~0.5 mL) was added to quench the reaction, and the mixture was concentrated under reduced pressure. The mixture was dissolved in EtOAc (15 mL), and washed with a 10% brine solutions (10 mL), dried over anhydrous Na\(_2\)SO\(_4\), and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 50% AcOEt – hexane as the eluent to give the desired trisaccharide diol 8 (49 mg, 96% yield) as a white solid. \([\alpha]_D\) -24\(^\circ\) (c 1.1, CHCl\(_3\)).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.69 (m, 4H, Phth), 7.49 (m, 2H, Ar), 7.43 – 7.09 (m, 19H, Ar), 7.03 (m, 4H, Ar), 6.94 (m, 2H, Ar), 5.57 (s, 1H, PhCH), 5.12 (d, \(J = 8.4\) Hz, 1H, H-1_GlcN), 4.85 – 4.65 (m, 4H, H_Bn + H-1_Fuc + H-5_Fuc + H-3_GlcN), 4.58 (m, 4H, H-1_Gal + 3 \times H_Bn), 4.45 (dd, \(J = 8.6, 10.5\) Hz, H-2_GlcN), 4.41 (d, \(J = 12.0\) Hz, 1H, H_Bn), 4.32 (dd, \(J = 12.4, \sim 1\) Hz, 1H, H-6a_Gal), 4.28 – 4.17 (m, 3H, H-4_GlcN + 2 \times H_Bn), 4.12 (dd, \(J = 11.2, 2.9\) Hz, 1H, H-6a_GlcN), 4.07 (dd, \(J = 3.6, \sim 1\) Hz, 1H, H-4_Gal), 4.04 (ddd, \(J = 5.1, 5.1, 11.3\) Hz, 1H, OCH\(_2\)HbCHcHdCl), 3.98 (dd, \(J = 1.7, 12.4\) Hz, 1H, H-6b_Gal), 3.93 – 3.85 (m, 2H, H-3_Fuc + H-6b_GlcN), 3.71 (m, 1H, H-5_GlcN), 3.69 – 3.59 (m, 4H, H-2_Fuc + H-2_Gal + Bn + OCH\(_2\)HbCHcHdCl), 3.51 – 3.39 (m, 3H, OCH\(_2\)HbCHcHdCl + H-3_Gal), 3.27 (dd, \(J = 2.2, \sim 1\) Hz, 1H, H-4_Fuc), 3.07 (m, 1H, H-5_Gal), 2.96 (br, 1H, OH-2_Gal), 2.53 (d, \(J = 8.4\) Hz, 1H, OH-3_Gal), 1.02 (d, \(J = 6.6\) Hz, 3H, H-6_Fuc). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 139.27, 138.24, 138.20, 137.68, 134.03, 131.85, 128.97, 128.43, 128.17, 128.11, 128.02, 127.87, 127.84, 127.68, 127.66, 127.62, 127.36, 127.06, 126.93, 125.82, 123.46 (Ar), 101.33(C-1_Gal), 100.11 (PhCH), 98.74 (C-1_GlcN), 97.91 (C-1_Gal), 78.99 (C-3_Fuc), 78.56 (C-4_Fuc),
75.41 (C-4_GlcN), 75.31 (C-5_GlcN), 75.17 (C-4_Gal), 74.78 (Bn), 73.91 (C-2_Fuc), 73.15 (Bn), 72.85 (Bn), 72.83 (C-3_Gal), 72.66 (C-3_GlcN), 71.89 (Bn), 71.60 (C-2_Gal), 69.45 (OCHaHb), 69.23 (C-6_Gal), 68.36 (C-6_GlcN), 66.56 (C-5_Gal), 66.41 (C-5_Fuc), 56.27 (C-2_GlcN), 42.49 (CHcHdCl), 16.44 (C-6_Fuc). ESI-HRMS: calcd m/z for [C_{63}H_{66}ClNO_{16} + Na]^+ 1150.3962; found 1150.3969.


To a solution of trisaccharide diol 8 (70 mg, 0.062 mmol) and thioglycoside 9 (105 mg, 0.186 mmol) in anhydrous CH₂Cl₂ (1.5 mL), was added molecular sieves 4 Å (300 mg), and the mixture was stirred for 1 hr at room temperature. The mixture was cooled to -50 °C, and N-iodosuccinimide (44 mg, 0.186 mmol) was added; a solution of saturated triflic acid in CH₂Cl₂ (37 µL) was added dropwise, and the reaction was allowed to warm up to -10 °C. After 2 hr, the reaction was quenched with the addition of Et₃N (0.3 mL), and mixture was filtered off, and the solid washed with EtOAc (25 mL). The organic solution was extracted with a 1:1 mixture of 5% aqueous NaHCO₃ and 5% Na₂S₂O₃ solutions (15 mL), dried over anhydrous Na₂SO₄, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 35% EtOAc – toluene to afford the desired tetrasaccharide 10 (82 mg, yield 85%). R₇ 0.15 (35% EtOAc – toluene). [α]D -41° (c 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.90 – 7.79 (m, 2H, Phth), 7.76 – 7.57 (m, 6H, Phth), 7.49 – 7.32 (m, 7H, Ar), 7.30 – 7.06 (m, 13H, Ar), 7.05 – 6.91 (m,
4H, Ar), 6.85 (m, 2H, Ar), 5.93 (dd, J = 10.8, 9.1 Hz, 1H, H-3\_GlcN\_I), 5.84 (d, J = 8.4 Hz, 1H, H-1\_GlcN\_I), 5.49 (s, 1H, PhCH), 5.22 (dd, J = 9.6, 9.6 Hz, 1H, H-4\_GlcN\_I), 5.05 (d, J = 8.6 Hz, 1H, H-1\_GlcN\_II), 4.77 (d, J = 12.3 Hz, 1H, H\_Bn), 4.67 – 3.91 (m, 21H, H-3\_GlcN\_II + H-1\_Fuc + 6 \times H\_Bn + H-5\_Fuc + H-2\_GlcN\_I + H-2\_GlcN\_II + H-1\_Gal + H-6a\_GlcN\_I + H-6b\_GlcN\_I + H-4\_GlcN\_II + H-6a\_Gal + H-4\_Gal + H-6b\_Gal + O\_HcHbCHcHdCl + H-6a\_GlcN\_II + H-5\_GlcN\_I), 3.86 – 3.76 (m, 2H, H-3\_Fuc + H-6b\_GlcN\_II), 3.68 – 3.36 (m, 7H, H-5\_GlcN\_II + O\_HcHbCHcHdCl), 3.31 (d, J = 11.2 Hz, 1H, Bn), 2.98 (m, 2H, H-4\_Fuc + H-5\_Gal), 2.45 (br, 1H, OH-2\_Gal), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.91 (s, 3H, Ac), 0.49 (d, J = 6.4 Hz, 3H, H-6\_Fuc). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 170.63, 170.14, 169.49 (Ac), 139.44, 139.31, 138.24, 138.21, 137.95, 134.36, 133.96, 128.51, 128.43, 128.04, 127.96, 127.89, 127.77, 127.73, 127.69, 127.48, 127.26, 126.95, 126.92, 126.78, 125.84, 123.46 (Ar), 100.82 (C-1\_Gal), 99.68 (PhCH), 98.68 (C-1\_GlcN\_II), 98.56 (C-1\_GlcN\_I), 98.01 (C-1\_Fuc), 78.88, 78.48, 78.10, 75.36, 75.21, 74.57, 74.47, 73.52, 73.14, 72.74, 72.51, 71.90, 71.52, 70.98, 70.50, 69.34, 69.16, 69.03, 68.23, 66.56, 66.11, 61.92 (C-6\_GlcN\_I), 56.16 (C-2\_GlcN\_II), 54.74 (C-2\_GlcN\_I), 42.42 (CHcHdCl), 20.75 (Ac), 20.65 (Ac), 20.51(Ac), 15.75 (C-6\_Fuc). ESI-HRMS: calcd m/z for [C$_{83}$H$_{85}$ClN$_2$O$_{25}$ + Na]$^+$ 1567.5022; found 1567.5014.

2-Azidoethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (11).
Tretrasaccharide **10** (70 mg, 0.045 mmol) was dissolved in anhydrous DMF (2 mL), and sodium azide (15 mg, 0.23 mmol) was added, and the mixture was heated to 90 °C for 48 hrs. The mixture was diluted with EtOAc (30 mL), and extracted with a solution 10% brine (2 ×15 mL), dried over anhydrous Na$_2$SO$_4$, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 30% EtOAc – toluene to afford the desired azide **11** (64 mg, yield 91%). R$_f$ 0.13 (35% EtOAc – toluene). [α]$_D$ -44.3° (c 0.4, CHCl$_3$). δ 7.88 – 7.79 (m, 2H, Phth), 7.63 (m, 6H, Phth), 7.47 – 7.32 (m, 7H, Ar), 7.30 – 7.13 (m, 12H, Ar), 7.10 (m, 1H, Ar), 7.02 (m, 2H, Ar), 6.96 (m, 2H, Ar), 6.85 (m, 2H, Ar), 5.94 (dd, $J$ = 10.9, 9.1 Hz, 1H, H-3_GlcN_I), 5.82 (d, $J$ = 8.4 Hz, 1H, H-1_GlcN_I), 5.49 (s, 1H, PhCH), 5.21 (dd, $J$ = 9.2, 10.0 Hz, 1H, H-4_GlcN_I), 5.07 (d, $J$ = 8.6 Hz, 1H, H-1_GlcN_II), 4.78 (d, $J$ = 12.1 Hz, 1H, H_Bn), 4.65 – 3.87 (m, 21H, H-3_GlcN_II + H-1_Fuc + 6 × H_Bn + H-5_Fuc + H-2_GlcN_I + H-2_GlcN_II + H-1_Gal + H-6a_GlcN_I + H-6b_GlcN_I + H-4_GlcN_II + H-6a_Gal + H-4_Gal + H-6b_Gal + OCHAhbCHcHdN$_3$ + H-6a_GlcN_II + H-5_GlcN_I), 3.86 – 3.75 (m, 2H, H-3_Fuc + H-6b_GlcN_II), 3.63 – 3.43 (m, 5H, H-5_GlcN_II + OCHAhbCHcHdCl + H-3_Gal + H-2_Fuc + H-2_Gal), 3.37 – 3.25 (m, 2H, Bn + OCHAhbCHcHdN$_3$), 3.15 (ddd, $J$ = 3.5, 4.6, 13.2 Hz, OCHAhbCHcHdN$_3$), 2.99 (m, 2H, H-4_Fuc + H-5_Gal), 2.31 (d, $J$ = 1.5 Hz, 1H, OH-2_Gal), 2.07 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.91 (s, 3H, Ac), 0.49 (d, $J$ = 6.4 Hz, 3H, H-6_Fuc). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 170.63, 170.14, 169.49 (Ac), 139.43, 139.29, 138.24, 138.23, 137.95, 134.35, 133.93, 128.51, 128.42, 128.03, 127.95, 127.85, 127.77, 127.73, 127.69, 127.50, 127.27, 126.95, 126.90, 126.79, 125.85, 123.44 (Ar), 100.80 (C-1_Gal), 99.69 (PhCH), 98.58 (C-1_GlcN_I), 98.45 (C-1_GlcN_II), 98.10 (C-1_Fuc), 78.88, 78.49, 78.13, 75.42, 75.21,
73.54, 73.17, 72.76, 71.89, 71.54, 70.96, 70.50, 69.17, 69.03, 68.26, 67.84, 66.56, 66.14, 
61.91(C-6_GlcN_I), 56.08 (C-2_GlcN_II), 54.73(C-2_GlcN_II), 50.45 (CHcHdN₃), 
20.74 (Ac), 20.65(Ac), 20.51(Ac), 15.75 (C-6_Fuc). ESI-HRMS: calcd m/z for 
[C₈₃H₈₅N₅O₂₅ + Na]⁺ 1574.5426; found 1574.5424.

6-Azidoethyl 4,6-O-benzylidene-2,3-di-O-benzyl-α-D-galactopyranosyl-(1→3)-4,6-
O-benzylidene-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-O-benzyl-α-L-fucopyranosyl–
(1→3)]-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (18).

To a solution of the previously reported trisaccharide diol 12 (50 mg, 0.042 mmol) and 
thioglycoside 14 (72 mg, 0.12 mmol) in anhydrous CH₂Cl₂ (1.5 mL), was added 
molecular sieves 4 Å (150 mg), and the mixture was stirred for 1 hr at room temperature. 
The mixture was cooled to -78 °C, and N-iodosuccinimide (30 mg, 0.12 mmol) was 
added; a solution of saturated triflic acid in CH₂Cl₂ (20 µL) was added dropwise, and the 
reaction was allowed to warm up to -30 °C. After 45 minutes, the reaction was quenched 
with the addition of Et₃N (0.2 mL). The mixture was filtered off, and the solid washed 
with EtOAc (20 mL). The organic solution was extracted with a 1:1 mixture of 5% 
aqueous NaHCO₃ and 5% Na₂S₂O₃ solutions (15 mL), dried over anhydrous Na₂SO₄, and 
evaporated under reduced pressure. The residue was purified by column chromatography 
on silica gel using a gradient of EtOAc – toluene (10 → 15%) as an eluent to afford the 
desired tetrasaccharide 16 (29 mg, yield 43%) in pure form. Further elution gave 
tetrosaccharide 16 that is contaminated with the β-anomer (12 mg, 18%). Rf 0.48 (30% 
EtOAc – toluene). [α]D +8.1° (c 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.84 - 7.60 
(m, 4H, Phth), 7.54 – 7.48 (m, 4H, Ar), 7.44 – 7.09 (m, 30H, Ar), 7.08 – 6.96 (m, 4H,
Ar), 6.92 (m, 2H, Ar), 5.53 (s, 1H, PhCH), 5.49 (s, 1H, PhCH), 5.21 (d, $J = 2.9$ Hz, 1H, H-1_Fuc), 5.05 (d, $J = 8.5$ Hz, 1H, H-1_GlcN), 4.84 – 4.64 (m, 7H, H-5_Fuc + H-3_GlcN + H-1_αGal + 4 × H_Bn), 4.61 – 4.54 (m, 3H, 2 × Bn + H-1_βGal), 4.50 (d, $J = 12.1$ Hz, 1H, Bn), 4.46 – 4.37 (m, 2H, H-2_GlcN + Bn), 4.37 – 3.78 (m, 17H + H-4_GlcN + H-6a_GlcN + H-6b_GlcN + H-2_Fuc + H-3_Fuc + H-2_βGal + H-4_βGal + H-6a_βGal + H-6b_βGal + H-4_αGal + H-5_αGal + H-6a_αGal + H-6b_αGal + OCHaHa_Hexyl + 3 × H_Bn), 3.71 (m, 1H, H-5_GlcN), 3.62 (dd, $J = 10.4$, 3.7 Hz, 1H, H-2_αGal), 3.57 (d, $J = 11.2$ Hz, 1H, Bn), 3.43 (dd, $J = 9.7$, 3.5 Hz, 1H, H-3_βGal), 3.35 (ddd, $J = 10.2$, 7.8, 5.7 Hz, 1H, OCHaHa_Hexyl), 3.32 (br, 1H, OH-2_βGal), 3.22 (dd, $J = 2.0$, < 1 Hz, 1H, H-4_Fuc), 3.00 (m, 3H, CH$_2$N$_3$ + H-5_βGal), 1.49 – 1.00 (11H, m, 4 × CH$_2$_Hexyl + H-6_Fuc). ESI-HRMS: calcd m/z for [C$_{94}$H$_{100}$N$_4$O$_{21}$ + Na]$^+$ 1643.6772; found 1643.6774.

2-Aminoethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl–(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, acetic acid salt (3); 2-N-methylaminoethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl–(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, acetic acid salt (17); 2-N,N-dimethylaminoethyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl–(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, acetic acid salt (18)
A solution of tetrasaccharide 11 (50 mg, 0.032 mmol) was dissolved in n-butanol (3.0 mL); 1,2-ethylenediamine (700 µL) was added and the mixture was heated to 100 °C for 24 h. The solution was evaporated to dryness under vacuum. The residue was redissolved in anhydrous pyridine (2.0 mL) and acetic anhydride (1 mL) was added; and the mixture was stirred at 50° C overnight. The mixture was concentrated under reduced pressure and co-evaporated with toluene (3 times). The obtained residue was dissolved in anhydrous methanol, and treated with a solution of methanolic NaOMe (1.5 M, 100 µL). After stirring at room temperature for 1 hr, the solution was neutralized with AcOH, and concentrated to dryness. The residue was purified by column-chromatography on silica gel using a gradient of MeOH-CH₂Cl₂ (4 - 5%) to afford an intermediate, which was directly subjected to a catalytic hydrogenation in MeOH (4.0 mL) containing 2 drops of acetic acid, using 20% Pd(OH)₂ on charcoal (20 mg) as a catalyst. After stirring for 2 days, the catalyst was removed by filtration and the solution was concentrated. The residue was dissolved in deionized H₂O, purified on a C18 Sep-Pak cartridge using a gradient of MeOH - H₂O (0% - 5%) as eluent to give the tetrasaccharide 3, 17 and 18 as an inseparable mixture (31.2% : 17.3% : 51.5%) which was lyophilized (11.8 mg, ~47% yield for three steps). ¹H NMR (400 MHz, D₂O) δ 5.02 (d, J = 4.0 Hz, 1H, H-1_Fuc), 4.73 (m, 1H, H-5_Fuc), 4.60 (d, J = 8.4 Hz, 1H, H-1_GlcN_I), 4.51 (d, J = 8.3 Hz, 1H, H-1_Gal), 4.36 (d, J = 7.8 Hz, 1H, H-1_GlcN_II), 4.09 - 3.06 (m, 23.8H, H-2_Fuc + H-3_Fuc + H-4_GlcN_I + H-3_GlcN_I + H-4_GlcN_I + H-5_GlcN_I + H-6a_GlcN_I + H-6b_GlcN_I + H-2_GlcN_II + H-3_GlcN_II + H-4_GlcN_II + H-5_GlcN_II + H-6a_GlcN_II + H-6b_GlcN_II + H-2_βGal + H-3_βGal + H-4_βGal + H-5_βGal + H-6a_βGal + H-6b_βGal + OCHa_ethyl + OCHb_ethyl + CHcHdNMe₂_ethyl

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6-Aminohexyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl–(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, acetic acid salt (4); 6-N-Methylaminohexyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl–(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, acetic acid salt (19); 6-N,N-dimethylaminohexyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl–(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside, acetic acid salt (20).

A solution of tetrasaccharide 16 (51 mg, 0.031 mmol) was dissolved in ethanol (4.0 mL); a solution of hydrazine hydrate (800 µL) was added and the mixture was refluxed overnight. The solution was concentrated to dryness under high vacuum. The residue was dissolved in a mixture of methanol – CH₂Cl₂ (v/v 3:1, 4 mL), and acetic anhydride (0.2 mL) was added. After stirring for 2 hrs at room temperature, the solution was concentrated under reduced pressure, and co-evaporated with toluene (2 times) to afford a
residue which was purified by column-chromatography on silica gel using 60% ethyl acetate-toluene as an eluent. The obtained intermediate was hydrogenated in MeOH (3.0 mL) containing 2 drops of acetic acid, using 20% Pd(OH)$_2$ on charcoal (20 mg) as a catalyst. After 2 days, the catalyst was removed by filtration and the solution was concentrated. The residue was dissolved in deionized H$_2$O, purified on a C18 Sep-Pak cartridge using a gradient of MeOH - H$_2$O (0% → 5%) as eluent to give the tetrasaccharide 4, 19 and 20 as an inseparable mixture (23.6% : 5.8% : 70.6%) which was lyophilized (14.7 mg, ~58% yield for three steps). $^1$H NMR (400 MHz, D$_2$O) δ 5.03 (d, $J$ = 3.9 Hz, 1H, H-1_αGal), 5.00 (d, $J$ = 4.0 Hz, 1H, H-1_Fuc), 4.73 (m, 1H, H-5_Fuc), 4.41 (m, 2H, H-1_GlcN + H-1_βGal), 4.08 (ddd, 1H, $J$ = 6.5 Hz, 6.5 Hz, < 1 Hz, H-5_αGal), 4.04 (dd, $J$ = 2.9 Hz, 1H, H-4_βGal), 3.91 – 3.44 (m, 2H, H-4_αGal + H-6a_GlcN), 3.89 – 3.42 (m, 19H, H-2_Fuc + H-3_Fuc + H-4_Fuc + H-2_GlcN + H-3_GlcN + H-4_GlcN + H-5_GlcN + H-6b_GlcN + H-2_βGal + H-3_βGal + H-5_βGal + H-6a_βGal + H-6b_βGal + H-2_αGal + H-3_αGal + H-6a_αGal + H-6b_αGal + OCHA_hexyl + OCHb_hexyl), 3.02 (m, 1.4H, CH$_2$N), 2.89 (m, 1.2H, CH$_2$N), 2.76 (s, 3.4H, N-CH$_3$), 2.61 (s, 0.3H, N-CH$_3$), 1.93 (s, 3H, NHAc), 1.82 (s, 3H, HOAc), 1.59 (m, 2H, CH$_2$_hexyl), 1.44 (m, 2H, CH$_2$_hexyl), 1.30 – 1.20 (m, 4H, 2 × CH$_2$_hexyl), 1.06 (d, $J$ = 6.8 Hz, 3H, H-6_Fuc). ESI-HRMS for 4: calcd $m/z$ for [C$_{32}$H$_{58}$N$_2$O$_{20}$ + H]$^+$ 791.3656; found 791.3671. ESI-HRMS for 19: calcd $m/z$ for [C$_{33}$H$_{60}$N$_2$O$_{20}$ + H]$^+$ 805.3812; found 805.3823. ESI-HRMS for 20: calcd $m/z$ for [C$_{34}$H$_{62}$N$_2$O$_{20}$ + H]$^+$ 819.3969; found 819.3979.

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6. References


7 For a review, see: Jank, T.; Giesemann, T.; Aktories, K. Glycobiology 2007, 17, 15R–22R.


