

Total synthesis of Le^A-LacNAc pentasaccharide as a ligand for *Clostridium difficile* toxin A†

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The toxins TcdA and TcdB produced by the human pathogen *Clostridium difficile* gain entrance to host epithelial cells by recognizing cell-surface carbohydrate ligands. Inhibiting the attachment of these toxins to host cells has been proposed to be a viable therapy to treat *C. difficile* infections. Glycan array screening previously revealed that the Le^A-LacNAc pentasaccharide binds strongly to TcdA. Here we report the efficient syntheses of the pentasaccharide and a structurally related tetrasaccharide motif. These compounds will be used to better define the carbohydrate-binding specificity of toxins from *C. difficile*, which will hopefully lead to the development of improved therapeutics.

Introduction

Clostridium difficile infection is one of the most common hospital-acquired diseases in the industrialized world, affecting hundreds of thousands of people in North America annually.¹ The clinical symptoms can range from mild to severe diarrhea, and in extreme cases pseudomembranous colitis.² The disease is linked to the use of broad-spectrum antibiotics that disrupt the normal microflora found in the colon.³ The resistance of *C. difficile* to commonly used antibiotics such as clindamycin and fluoroquinolones makes the colon vulnerable to colonization by these opportunistic pathogens when such antibiotics are administered systemically.⁴ Paradoxically, broad-spectrum antibiotics such as metronidazole and vancomycin remain the most effective and commonly used treatments of *C. difficile* infections, even though a side-effect of these treatments is the continued disruption of normal microbial populations in the colon. As a result, recurrent infections of *C. difficile* are unfortunately extremely common and troubling limitation of current treatments. To develop new and more effective treatments, we and others have undertaken molecular studies of virulence factors from *C. difficile*. Two of the most important virulence factors are the large toxin proteins TcdA and TcdB.^{5,6} These proteins bind to intestinal epithelial cells and specifically glucosylate a specific Thr residue in the Rho GTPases that are essential to the maintenance of the cellular cytoskeleton.^{7,8} Because the initial adhesion of toxins to host intestinal epithelial cells is mediated by carbohydrates,⁹ several novel approaches for treating *C. difficile* infections have been proposed based on the competitive inhibition of toxin binding to host epithelial cells^{10–12} Although a specific human receptor has yet to be

identified, the trisaccharide $\alpha\text{Gal}(1\rightarrow3)\beta\text{Gal}(1\rightarrow4)\beta\text{GlcNAc}$ was found to bind to toxin A with weak affinity and the crystal structure of the complex was solved.¹³ To define better the binding specificities of native toxin molecules, TcdA and TcdB, His₆-tagged recombinant proteins containing portions of the carboxyl-terminal carbohydrate-binding domains of TcdA and TcdB have been used by our group and others to screen the glycan microarray at the Consortium for Functional Glycomics (CFG).¹⁴ Several carbohydrate structures showed strong binding to TcdA, including **1** and **3** (Fig. 1). Compound **3** is a pentasaccharide containing a Lewis A motif linked to the O-3' position of a lactosamine disaccharide (Le^A-LacNAc) while the related tetrasaccharide contains a neolactosamine residue linked to the same lactosamine moiety. Here we report the chemical synthesis of two analogs **2** and **4**, which have the same glycan sequences compared to **1** and **3** respectively. A longer 6-aminohexyl linker was chosen to be the aglyconic group due to its higher flexibility when conjugating to scaffolds to generate potentially high affinity inhibitors using the multivalency concept.^{15,16}

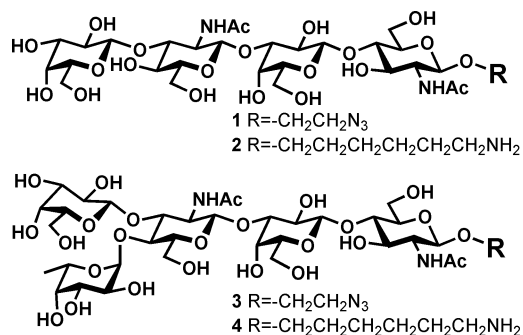


Fig. 1 Structures of ligands binds to toxin A from CFG screen experiments and synthetic targets.

Results and discussion

Since both compounds **2** and **4** have a LacNAc disaccharide fragment at the reducing end, a retrosynthetic analysis (Fig. 2)

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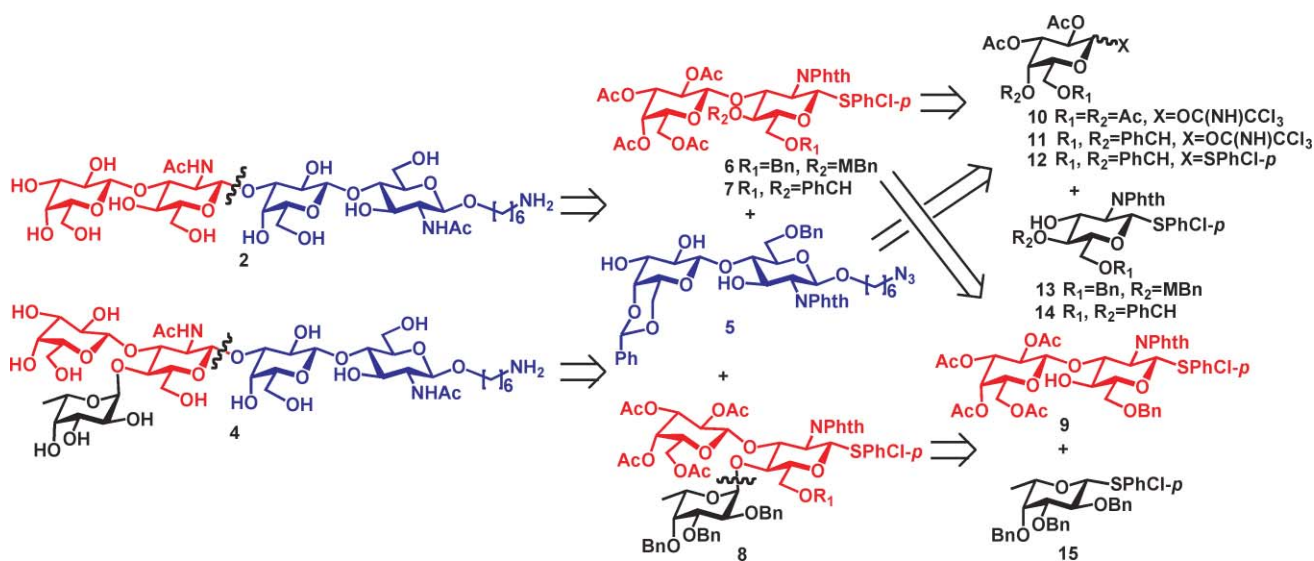
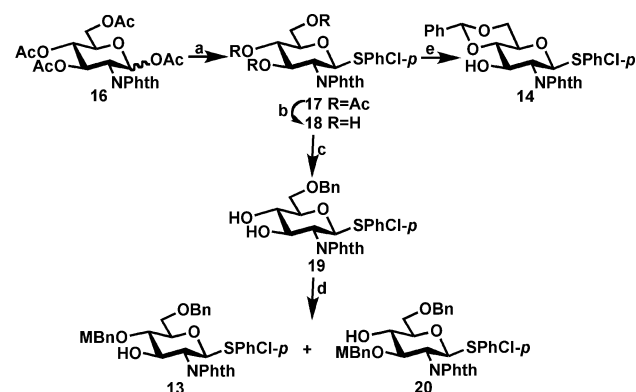


Fig. 2 Retrosynthetic analysis for both tetrasaccharide **2** and pentasaccharide **4**.

revealed that if a 2 + 2 or 3 + 2 disconnection could be respectively performed for tetrasaccharide **2** and pentasaccharide **4**, a disaccharide acceptor **5** could become a common intermediate for the synthesis of both saccharides. Although there are three hydroxyl groups presented in **5**, we estimated that the OH-3 of the galactose unit should be the most reactive, because the OH-2 of the same unit was known to be less reactive;¹⁷ the OH-3 of the glucosamine unit is sterically shielded by a phthalimido group at C-2 and galactosyl residue at O-4 thus less accessible for glycosylations. A neolactosamine donor is required for the synthesis of tetrasaccharide **2**. We decided to use thioglycosides as potential donors because of their versatility and remarkable stability towards many reaction conditions during functional group transformations. Donor **6** with the O-6 and O-4 of the GlcNAc residue orthogonally protected with a benzyl and a *p*-methoxybenzyl group respectively was the initial choice, as a selective removal of the *p*-methoxybenzyl group could generate a key alcohol **9**, required for the synthesis of a Lewis A trisaccharide donor **8** which is essential for the synthesis of pentasaccharide **4**. Alternatively, donor **7** with the O-6 and O-4 of the GlcNAc residue protected with a benzylidene could be used, as a regioselective opening of the benzylidene could also generate donor **9**. All disaccharides **5**–**7** could be prepared using galactosyl donors **10**,¹⁸ **11**¹⁷ or **12** and *N*-phthalimido protected thioglycosides **13** or **14** which can be used as either thioglycoside donor or acceptor. Fucosyl donor **15** was chosen to react with alcohol **9** to form the Lewis A¹⁹ trisaccharide donor **8**. The phthalic group was selected to be the protecting group of the 2-amino group of glucosamine²⁰ because of its excellent participating property that leads to the exclusive formation of β -glycosides; although the 2,2,2-trichloroethoxycarbonyl (Troc)^{19c,21} could also be used as a protecting group to give β -glycosides, a clear advantage of using phthalic group for the protection of amine is that all intermediates bearing the phthalimido group can be easily detected by charring with acid in thin-layer chromatography analysis; additionally, the phthalimido group exhibits higher stability in basic conditions compared to Troc group.²²

Scheme 1 showed the synthesis of *N*-phthalimido protected glucosamine thioglycosides **13** and **14**. The known compound **16**²³ was converted to **17** in large scale and high yield by the treatment of **16** with boron trifluoride etherate and *p*-chlorophenylthiol which is a preferred thiol because it is less malodorous compared to the more conventional ethanethiol or thiophenol. We found that if an *in situ* acetylation step was carried out after the reaction, we could convert the remaining thiol to the corresponding thioacetate thus essentially eliminating the unpleasant smell. In agreement with the literature,²⁴ the β -OAc of **16** was found to react much faster (4 h) than the corresponding α -OAc (up to 72 h). No chromatography was needed to isolate the thioglycoside **17** as a simple recrystallization from a mixture of ethyl acetate–hexane afforded the pure thioglycoside **17** in 76% yield, illustrating the advantage of using *p*-chlorophenylthiol as the aglycone. The subsequent removal of acetates was carried out using a milder Zemplén transesterification condition²² with a guanidine/guanidinium buffer as the deacetylation reagent in anhydrous methanol; compound **18** crashed out from the reaction media in pure form and in almost quantitative yield (98%). The more conventional condition (sodium methoxide

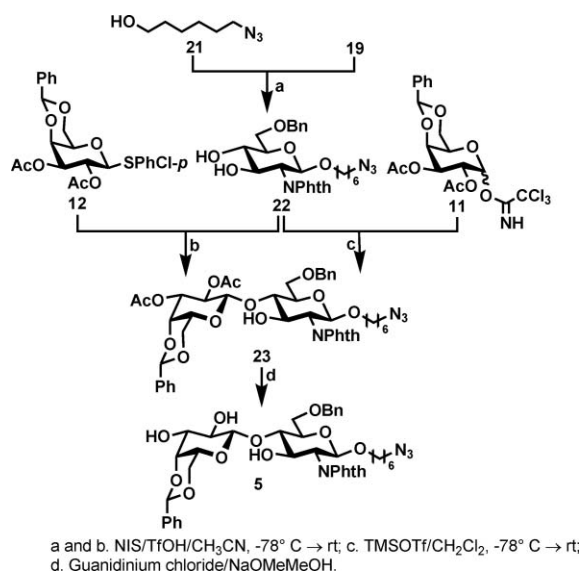


a. *p*-ClPhSH/BF₃·Et₂O/CH₂Cl₂; b. Guanidinium chloride/NaOMe/MeOH; c and d. (*n*-Bu)₂SnO/BnBr/toluene, 80 °C; e. PhCH(OMe)₂/CSA/CH₃CN.

Scheme 1 Syntheses of glucosaminyl donors/acceptors.

in methanol) was found to be less suitable as it resulted in a partial opening of the phthalimido group. To synthesize compound **14**, a transacetalation was carried out on triol **18** with benzaldehyde dimethyl acetal under the catalysis of camphorsulfonic acid to give the desired alcohol **14** in excellent yield (93%). To regioselectively protect the primary alcohol of **18**, the stannylidene acetal-mediated benzylation was found to be efficient; thus a solution of **18** with 1 equivalent of dibutyltin oxide in toluene was heated to reflux to form intermediate stannylidene acetal which allowed the installation of a benzyl group at *O*-6 in a regioselective manner to give compound **19** in 67% yield. To synthesize alcohol **13**, another dibutyltin oxide-mediated alkylation was carried out with *p*-methoxybenzyl (MBn) chloride; compound **13** was obtained in moderate yield (33%) together with the regioisomer **20** which has the MBn group installed at the *O*-3 position (30% yield).

The synthesis of the common disaccharide **5** (Scheme 2) began with the preparation of acceptor **22** which was obtained by coupling the 6-azidohexan-1-ol **21** with thioglycoside **19** under the activation of *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH).

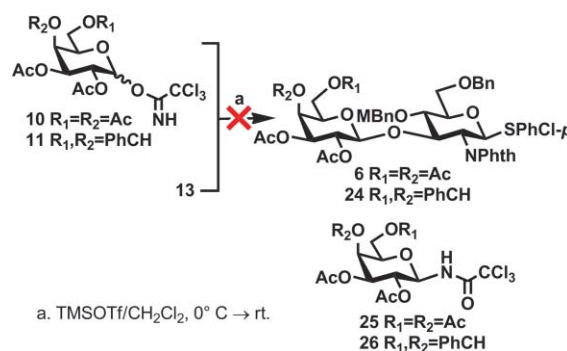


Scheme 2 Synthetic route to the common disaccharide acceptor **5**.

Despite the presence of two free hydroxyl groups in donor **19**, glycosylation proceeded in a remarkably smooth way to afford **22** in 85% yield. Clearly, the primary hydroxyl group of **21** exhibited sufficiently high reactivity to compete with all inter/intramolecular self-condensation side reactions. Coupling of **12** with **22** under the activation of NIS/TfOH condition gave the desired disaccharide **23** in moderate yields (47%). To improve the glycosylation efficiency, we switched the donor to imidate **11** to afford the desired disaccharide **23** in slightly improved yield (60%) under the catalysis of trimethylsilyl trifluoromethanesulfonate (TMSOTf) at -78° C. Finally, a selective deprotection of acetates was carried out using guanidine/guanidium buffer conditions to afford the triol **5** in high yield (84%).

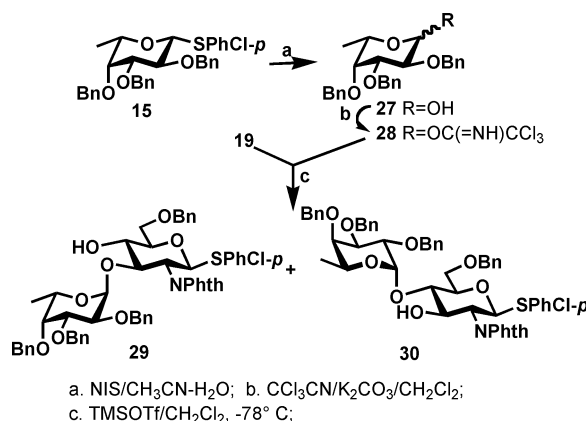
We next investigated the preparation of βGal(1→3)βGlcNPhth disaccharide donor **6** using the designed acceptor **13**. We first attempted the glycosylation with the peracetylated galactopy-

ranosyl imidate **10** in anhydrous dichloromethane (Scheme 3) under the catalysis of TMSOTf. By starting the reaction at 0° C and slowly warming the reaction up to room temperature, surprisingly, no desired glycoside **6** was formed even if we used a large excess amount of **10** (up to 3.0 equivalents). The major compound isolated from the reaction mixture was the unreacted acceptor **13** and a small amount of rearranged product **25** from donor. Attempts to change the reaction solvent to others such as anhydrous acetonitrile also failed. When we employed the more reactive imidate donor **11**, we were still unable to observe the formation of the desired disaccharide **24**. The imidate donor quickly rearranged to form the glycosyl amide **26** and the acceptor **13** was recovered.



Scheme 3 Attempts to prepare disaccharide **6** and **24** from **13**.

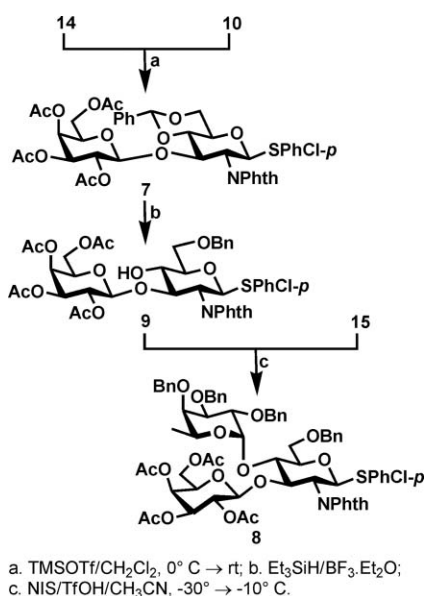
Previously, it was well-established²⁵ that if a tribenzylated *L*-fucopyranosyl residue is attached to the *O*-4 position of a *N*-phthalimido protected glucosamine acceptor, the OH-3 became inaccessible for glycosylations due to steric hindrance. Here the fact that we used a much smaller and electron-donating MBn group at *O*-4, and still could not glycosylate the OH-3 position, is surprising. In another experiment (Scheme 4), we reacted the analogous acceptor **19** which had no protecting group at *O*-4 with a reactive fucosyl imidate donor **28** (1.3 equivalents), we found that both OH-3 and OH-4 were reactive even at low temperature (-78° C); the OH-3 exhibited even higher reactivity than OH-4 as the corresponding disaccharide **29** and **30** were formed and



Scheme 4 Probing the sterically shielding effect on OH-3 of 2-phthalimido protected acceptor.

isolated in 52% and 27% yields respectively. We therefore concluded that the low reactivity of OH-3 in **13** was still due to the steric shielding from both the MBn group at *O*-4 and the 2-phthalimido group; probably both groups form a pocket which traps OH-3 and renders it unreactive towards the oxocarbenium intermediates formed from the glycosyl donors.

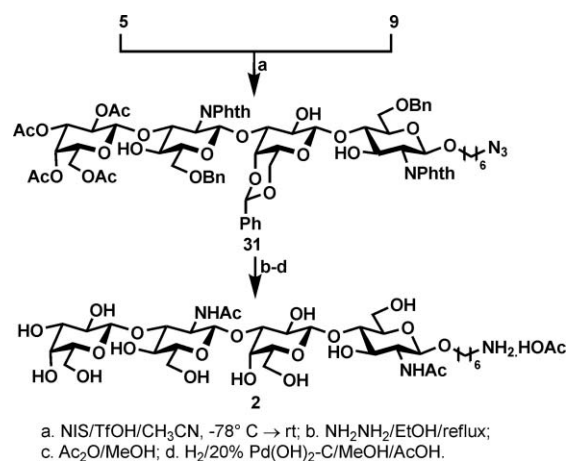
Based on the above probing results, the advantage of using 4,6-benzylidenedated **14** to synthesize the required disaccharide **9** became evident, because the cyclic benzylidene group provides simultaneous protection to both the *O*-4 and *O*-6 positions while making OH-3 available for reactions; most importantly the smaller cyclic acetal relieves steric congestion around OH-3, making it more accessible than OH-3 in **13**. Indeed, as shown in Scheme 5, when **14** was subjected to reaction with imidate **10** under the activation of TMSOTf, the desired disaccharide **7** was formed in a smooth manner and isolated in good yield (75%).



Scheme 5 Preparation of neolactosamine and Lewis A donor.

Using the triethylsilane²⁶ as the reducing agent under the catalysis of boron trifluoride etherate, the 4,6-benzylidene group in **7** was regioselectively opened to afford the expected disaccharide alcohol **9** in almost quantitative yield (98%). The free OH-4 in **9** should be much less steric hindered due to the higher rotational freedom of benzyl group attached to the *O*-6 position thus is readily available for α -fucosylation. We applied the armed-disarmed glycosylation principle by reacting the less reactive thioglycoside **9** (disarmed) with the more reactive thiofucoside **15** (armed) under the activation of NIS/TfOH to afford the desired Lewis A trisaccharide donor **8** in excellent yield (84%).

The success in preparing the requires donors **8** and **9** allowed us to proceed further to synthesize the target tetrasaccharide **2** and pentasaccharide **4**. As shown in Scheme 6, under the NIS/TfOH-promoted glycosylation conditions using triol **5** as the acceptor and thioglycoside **9** as the donor, the desired tetrasaccharide **31** was obtained in excellent yield (87%). As expected, despite the presence of three hydroxyl groups, the glycosylation proceeded in a remarkably regioselective manner to give only OH-3' glycosylated product. The regioselectivity of glycosylation was established by a



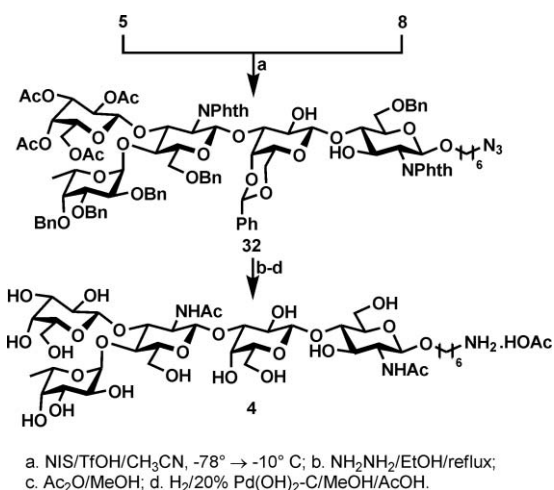
Scheme 6 Synthesis of tetrasaccharide **2**.

series of NMR experiments: 1D ¹H and 2D ¹H-¹H COSY NMR revealed a broad doublet (³*J* 1.9 Hz) at 2.83 ppm, which appeared to correlate with H-2', suggesting that it's the hydroxyl group (OH-2') attached to the C-2' position (the reducing end galactosyl unit). This was further confirmed by a selective 1D TOCSY experiment (see ESI[†]): we selectively excited the hydroxyl signal at 2.83 ppm, and observed that the magnetization was indeed transferred to the H-1', H-2', H-3' and H-4' protons (the reducing end galactosyl unit). These results confirmed that the glycosylation occurred at OH-3'. Next, the acetyl and *N*-phthalimido groups of **31** were concurrently removed in one step by the treatment with hydrazine hydrate in methanol under refluxing conditions. TLC revealed that the deprotection went very clean, only one spot was observed; the intermediate diamine was not isolated but subjected to a regioselective acetylation with acetic anhydride in methanol to provide the diamide which was subsequently debenzylated in 10% acetic acid-methanol under the catalysis of 20% Pd(OH)₂ on charcoal to give the targeted tetrasaccharide **2** in 48% overall yield after three steps. Compound **2** was isolated by passing through a C18 Sep-Pak cartridge using a gradient of MeOH-H₂O (0% → 10%) as the eluent.

Similarly, when the Lewis A donor **8** was reacted with **5** under similar conditions as for **9**, the pentasaccharide **32** was smoothly formed and isolated in very good yield (70%) and excellent regioselectivity (Scheme 7). Following the same deprotecting sequences as for **31**, the desired pentasaccharide **4** was obtained in 55% overall yield after three steps.

The structures of both compounds **2** and **4** were confirmed by 1D and 2D NMR experiments, and also by high resolution mass spectrometry (see ESI[†]).

In conclusion, we have presented a concise route to synthesize a pentasaccharide containing the Le^A-LacNAc sequence which has previously been shown to bind to TcdA from *C. difficile*. By applying the same strategy, a related tetrasaccharide was also prepared. Carefully choosing orthogonally protected donors and acceptors was found to be the key to the success of the syntheses and by effectively applying the armed-disarmed principle, we have significantly reduced the number of required steps thus improved the overall efficiency of the syntheses. These compounds are currently being used for binding and crystallographic studies, which will hopefully better define the molecular basis for



Scheme 7 Synthesis of pentasaccharide 4.

carbohydrate binding and ultimately lead to the development of novel therapeutics.

Experimental section

General methods

Optical rotations were determined in a 5 cm cell at 25 ± 2 °C. $[\alpha]_D$ values were given in units of 10^{-1} deg $\text{cm}^2 \text{g}^{-1}$. 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, Ontario). 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. The first order proton chemical shifts δ_{H} and δ_{C} were reported in δ (ppm) and referenced to either residual CHCl₃ (δ_{H} 7.24, δ_{C} 77.0, CDCl₃) or residual CD₂HOD (δ_{H} 3.30, δ_{C} 49.5, CD₃OD). ¹H and ¹³C NMR spectra were assigned with the assistance of GCOSY, GHSQC; for oligosaccharides with two galactose and two glucosamine residues, residues closer to the non-reducing end were defined with the “I” suffix (ex. Gal-I, GlcN-I) while the ones closer to the reducing end were defined with the “II” suffix (ex: Gal-II, 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 by voltage scan over a narrow range at a resolution of approximately 10 000 and recorded by the analytical services of the Department of Chemistry, University of Alberta.

Guanidine/guanidinium chloride solution

To a solution of guanidinium chloride (9.6 g, 100 mmol) in a mixture of anhydrous MeOH (900 mL) and CH₂Cl₂ (100 mL), was added a solution of NaOMe in anhydrous MeOH (1.5 M,

15 mL), and the resulting clear solution was kept at rt and used for subsequent de-*O*-acetylations.

***p*-Chlorophenyl 6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside (19).** A solution of the triol **18** (11.5 g, 26.4 mmol) and Bu₂SnO (7.9 g, 31.1 mmol, 1.2 equiv.) in anhydrous toluene (180 mL) was heated to reflux. With the help of a Dean–Stark, toluene (~120 mL) was removed and the mixture was allowed to cool to rt. To the remaining mixture, was added Bu₄Ni (425 mg, 0.1 equiv) and BnBr (9.2 mL, 77.45 mmol, 2.9 equiv), the mixture was heated to 80 °C for 24 h. The solution was cooled and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of MeOH–CH₂Cl₂ as eluent (0% → 2%) to afford pure **19** (3.95 g, 67.3%) as white solid (Found: C, 60.04; H, 4.85; N, 2.44%. C₂₇H₂₄NO₆ClS·H₂O requires C, 59.61; H, 4.82; N, 2.57%); $[\alpha]_D +16.9$ (*c* 0.7, CHCl₃); δ_{H} (400 MHz, CDCl₃) 7.88–7.74 (m, 2H, Phth), 7.74–7.65 (m, 2H, Phth), 7.46–7.05 (m, 9H, 5 × Bn + 4 × SPhCl), 5.52 (d, 1H, *J* 10.4 Hz, H-1), 4.58 (d, 1H, *J* 12.1 Hz, Bn), 4.54 (d, 1H, *J* 12.1 Hz, Bn), 4.30 (dd, 1H, *J* 9.3, 9.3 Hz, H-3), 4.15 (dd, 1H, *J* 10.3, 10.3 Hz, H-2), 3.82–3.71 (m, 2H, H-6a + H-6b), 3.70–3.57 (m, 2H, OH-4 + H-5), 3.53 (dd, 1H, *J* 8.8, 8.8 Hz, H-4); δ_{C} (100 MHz, CDCl₃) 168.00 (CO), 137.74, 134.24, 134.14, 133.90, 131.52, 130.54, 128.96, 128.47, 127.85, 127.73, 83.28 (C-1), 78.44 (C-5), 73.63 (C-4), 72.74 (C-2), 72.66 (C-3), 70.01 (C-6), 55.43 (C-2); *m/z* (ESI-HRMS) calcd for [C₂₇H₂₄NO₆ClS + Na]⁺ 548.0905, found 548.0903.

***p*-Chlorophenyl 6-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio- β -D-glucopyranoside (13) and *p*-Chlorophenyl 6-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-2-phthalimido-1-thio- β -D-glucopyranoside (20).** Diol **19** (500 mg, 0.95 mmol) was reacted with Bu₂SnO (314 mg, 1.24 mmol, 1.3 equiv.) and *p*-methoxybenzyl chloride (260 μ L, 1.90 mmol, 2.0 equiv) in anhydrous toluene (10 mL) similarly as **19**. Column chromatography on silica gel using a gradient of AcOEt – toluene as eluent (5% → 7%) afforded pure **13** (203.7 mg, 33.2% yield) and **20** (186 mg, 30.3% yield).

Data for **13** (Found: C, 65.47; H, 5.30; N, 2.02%. C₃₅H₃₂NO₇ClS requires C, 65.06; H, 4.99; N, 2.17%); $[\alpha]_D +13.5$ (*c* 0.7, CHCl₃); δ_{H} (400 MHz, CDCl₃) 7.92–7.78 (m, 2H, Phth), 7.78–7.68 (m, 2H, Phth), 7.45–7.29 (m, 7H, Ar), 7.21–7.12 (m, 4H, Ar), 6.84 (d, 2H, *J* 8.6 Hz, Ar), 5.54 (d, 1H, *J* 10.2 Hz, H-1), 4.70–4.56 (m, 4H, Bn), 4.42 (ddd, 1H, *J* 10.3, 8.7, 4.2 Hz, H-3), 4.20 (dd, 1H, *J* 10.3, 10.3 Hz, H-2), 3.88–3.79 (m, 2H, H-6a + H-6b), 3.78 (s, 3H, OMe), 3.67 (ddd, 1H, *J* 9.8, 3.9, 2.1 Hz, H-5), 3.57 (dd, 1H, *J* 9.5, 8.7 Hz, H-4), 2.25 (d, 1H, *J* 4.4 Hz, OH-3); δ_{C} (100 MHz, CDCl₃) 159.50, 138.13, 134.22, 134.20, 131.63, 130.41, 130.11, 129.64, 128.97, 128.45, 127.77, 127.75, 123.81, 114.08 (Ar), 83.06 (C-1), 79.28 (C-5), 78.72 (C-4), 74.37 (Bn or MBn), 73.51 (MBn or Bn), 72.73 (C-3), 68.88 (C-6), 55.57 (C-2), 55.26 (OMe–MBn); *m/z* (ESI-MS) calcd for [C₃₅H₃₂NO₇ClS + Na]⁺ 668.2, found 668.2.

Data for **20** (Found: C, 65.46; H, 5.25; N, 1.96%. C₃₅H₃₂NO₇ClS requires C, 65.06; H, 4.99; N, 2.17%); $[\alpha]_D +52.0$ (*c* 0.6, CHCl₃); δ_{H} (400 MHz, CDCl₃) 7.86–7.80 (m, 1H, Phth), 7.77–7.64 (m, 3H, Phth), 7.42–7.28 (m, 7H, Ar), 7.15 (d, 1H, *J* 8.5 Hz, Ar), 6.95 (d, 2H, *J* 8.6 Hz, Ar), 6.45 (d, 2H, *J* 8.6 Hz, Ar), 5.51 (d, 1H, *J* 10.1 Hz, H-1), 4.65 (d, 1H, *J* 12.1 Hz, Bn or MBn), 4.63 (d, 1H, *J* 11.8 Hz, MBn or Bn) 4.53 (d, 1H, *J* 11.8 Hz, MBn or Bn), 4.47 (d, 1H, *J* 12.1 Hz, Bn or MBn), 4.23 (dd, 1H, *J* 9.9,

8.1 Hz, H-3), 4.18 (dd, 1H, *J* 10.1, 10.1 Hz, H-2), 3.87–3.76 (m, 3H, H-4 + H-6a + H-6b), 3.69 (ddd, 1H, *J* 9.5, 4.8, 4.8 Hz, H-5), 3.63 (s, 3H, OMe–MBn), 2.90 (d, 1H, *J* 2.7 Hz, OH-4); δ_{C} (100 MHz, CDCl₃) 158.93, 137.68, 134.24, 134.11, 133.94, 133.91, 131.55, 130.38, 130.11, 129.64, 128.97, 128.54, 127.93, 127.77, 123.47, 123.21, 113.53 (Ar), 83.29 (C-1), 79.31 (C-3), 78.00 (C-5), 74.20 (Bn or MBn), 73.89 (C-4), 73.76 (MBn or Bn), 70.50 (C-6), 54.90 (OMe–MBn), 54.43 (C-2); *m/z* (ESI-MS) calcd for [C₃₃H₃₂NO₇SCl + Na]⁺ 668.2, found 668.2.

6-Azidoethyl 6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (22). Thioglycoside **19** (1.5 g, 2.85 mmol) and 6-azido-1-hexanol **21** (1.30 g, 9.08 mmol) were dissolved in anhydrous CH₂Cl₂ (7 mL) under Ar; crushed molecular sieves 4 Å (2.0 g) were added and the mixture was stirred at rt for 1 h. After cooling to –78° C, NIS (1.35 g, 5.7 mmol) was added; after stirring for another 5 mins, TfOH (34 μL) was added dropwise, and the temperature was allowed to slowly warm up to rt in 1 h. Et₃N (0.5 mL) was added and the reaction mixture was diluted with EtOAc (20 mL). The insoluble material was filtered off and washed with more EtOAc. The combined organic solution was washed with an 1 : 1 mixture of 10% aqueous Na₂S₂O₃ and 5% NaHCO₃ (2 × 20 mL), dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by column chromatography on silica gel using 10% acetone–toluene as eluent to afford the desired glycoside **22** (1.27 g, 85% yield) as a white solid (Found: C, 61.68; H, 6.47; N, 10.39%. C₂₇H₃₂N₄O₇ requires C, 61.82; H, 6.15; N, 10.68%); [α]_D²⁵ –25.2 (*c* 0.6, CHCl₃); δ_{H} (400 MHz, CDCl₃) 7.86–7.80 (m, 2H, Phth), 7.75–7.69 (m, 2H, Phth), 7.40–7.28 (m, 5H, Ph), 5.18 (d, 1H, *J* 8.4 Hz, H-1), 4.65 (d, 1H, *J* 12.1 Hz, Bn), 4.59 (d, 1H, *J* 12.0 Hz, Bn), 4.32 (dd, 1H, *J* 10.9, 7.6 Hz, H-3), 4.12 (dd, 1H, *J* 10.9, 8.4 Hz, H-2), 3.85–3.75 (m, 3H, H-6a + H-6b + OCHaHb), 3.67–3.56 (m, 2H, H-4 + H-5), 3.40 (ddd, 1H, *J* 9.8, 7.1, 6.0 Hz, OCHaHb), 3.03 (t, 2H, *J* 7.0, CH₂N₃), 1.51–1.33 (m, 2H, OCHaHbCH₂), 1.33–1.21 (m, 2H, CH₂CH₂N₃), 1.19–1.02 (m, 4H, 2 × CH₂-chain); δ_{C} (100 MHz, CDCl₃) 168.30 (CO), 137.63, 134.11, 131.66, 128.49, 127.87, 127.77, 123.36, 98.30 (C-1), 73.96 (C-5 or C-4), 73.76 (C-4 or C-5), 73.71 (CH₂Ph), 71.67 (C-3), 70.45 (C-6), 69.42 (OCHaHb), 56.30 (C-2), 51.12 (CH₂N₃), 29.09 (OCHaHbCH₂), 28.57 (CH₂CH₂N₃), 26.18 (CH₂-chain), 25.38 (CH₂-chain); *m/z* (ESI-HRMS) calcd for [C₂₇H₃₂N₄O₇ + Na]⁺ 547.2163, found 547.2165.

6-Azidoethyl 2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (23).

Method A. Thioglycoside **12** (1.028 g, 2.15 mmol) and diol **22** (563 mg, 1.07 mmol) were dissolved in anhydrous CH₂Cl₂ (6 mL) under Ar, and 4 Å molecular sieves (1.3 g) were added. After stirring at rt for 2 h, the mixture was cooled to –78° C, and NIS (532 mg, 2.25 mmol, 2.1 equiv.) was added. After stirring for another 5 min, TfOH (30 μL) was added dropwise, and the reaction was slowly warming up to rt within 2 h. Et₃N (1 mL) was added to quench the reaction. The reaction mixture was worked up as **22** and glycoside **23** (434 mg, 47% yield) was obtained by column chromatography on silica gel using 15% acetone–toluene as eluent.

Method B. A mixture of imidate **11**¹⁷ (1.659 g, 3.34 mmol) and diol **22** (700 mg, 1.34 mmol) was dissolved in anhydrous CH₂Cl₂

(6.5 mL) under Ar, and 4 Å molecular sieves (1.9 g) were added. After stirring at rt for 1 h, the mixture was cooled to –78° C, and TMSOTf (18 μL) was added dropwise, and the temperature of the reaction was slowly warmed up to rt. TLC (30% AcOEt–toluene) showed the all starting materials disappeared. The reaction was quenched by the addition of Et₃N (0.5 mL) and the mixture was filtered off. After removing the solvent under vacuum, the residue was purified by column chromatography on silica gel using 15% AcOEt–toluene as the eluent to give the desired disaccharide **23** (725.2 mg, 63.2% yield) as a white foam (Found: C, 61.40; H, 5.94; N, 6.16%. C₄₄H₅₀N₄O₁₄ requires C, 61.52; H, 5.87; N, 6.52%); [α]_D²⁵ +9.3 (*c* 0.6, CHCl₃); δ_{H} (400 MHz, CDCl₃) 7.95–7.78 (m, 2H, Phth), 7.78–7.63 (m, 2H, Phth), 7.53–7.30 (m, 10H, Ph + Bn), 5.44 (s, 1H, PhCH), 5.34 (dd, 1H, *J* 10.4, 8.0 Hz, H-2–Gal), 5.22 (d, 1H, *J* 8.5, H-1–GlcN), 4.86 (dd, 1H, *J* 10.4, 3.6 Hz, H-3–Gal), 4.77 (d, 1H, *J* 12.0, Bn), 4.53 (d, 1H, *J* 12.0, Bn), 4.50 (d, 1H, *J* 8.0, H-1–Gal), 4.44 (ddd, 1H, *J* 10.9, 8.3, 0.7 Hz, H-3–GlcN), 4.33 (dd, 1H, *J* 3.6, 0.7 Hz, H-4–Gal), 4.26 (dd, 1H, *J* 12.7, 1.3 Hz, H-6a–Gal), 4.18 (dd, 1H, *J* 10.9, 8.5 Hz, H-2–GlcN), 4.02 (d, 1H, *J* 0.6 Hz, OH-3–GlcN), 3.99 (dd, 1H, *J* 12.7, 1.6 Hz, H-6b–Gal), 3.84 (ddd, 1H, *J* 9.8, 6.3, 6.3 Hz, OCHaHb), 3.80–3.69 (m, 3H, H-6a–GlcN + H-6b–GlcN + H-4–GlcN), 3.65 (m, 1H, H-5–GlcN), 3.49 (m, 1H, H-5–Gal), 3.43 (ddd, *J* 9.7, 7.2, 5.7 Hz, OCHaHb), 3.04 (t, 2H, *J* 7.0 Hz, CH₂N₃), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.53–1.37 (m, 2H, OCHaHbCH₂), 1.36–1.21 (m, 2H, CH₂CH₂N₃), 1.21–1.04 (m, 4H, 2 × CH₂-chain); δ_{C} (100 MHz, CDCl₃) 170.58 (2 × CO), 168.91 (CO), 138.16, 137.22, 133.95, 131.80, 129.03, 128.47, 128.09, 127.86, 127.81, 126.28, 101.19 (PhCH), 100.97 (C-1–Gal), 98.32 (C-1–GlcN), 81.28 (C-4–GlcN), 74.26 (C-5–GlcN), 73.61 (Bn), 73.01 (C-4–Gal), 71.69 (C-3–Gal), 69.58 (C-3–GlcN), 69.45 (OCHaHb), 68.63 (C-2–Gal), 68.35 (C-6–Gal), 67.98 (C-6–GlcN), 66.59 (C-5–Gal), 56.05 (C-2–GlcN), 51.13 (CH₂N₃), 29.10 (OCHaHbCH₂), 28.58 (CH₂CH₂N₃), 26.20 (CH₂-chain), 25.41 (CH₂-chain), 20.78 (Ac), 20.74 (Ac); *m/z* (ESI-HRMS) calcd for [C₄₄H₅₀N₄O₁₄ + Na]⁺ 881.3216, found 881.3213.

6-Azidoethyl 4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-6-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (5).

Compound **23** (650 mg, 0.76 mmol) was dissolved in the above prepared guanidine/guanidinium chloride solution (5.0 mL), and the reaction was stirred at rt for 30 mins. CH₃CO₂H (2 mL) was added to quench the reaction, and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 50% AcOEt–toluene as the eluent to give the desired disaccharide **5** (493.4 mg, 84.1% yield) as a white solid (Found: C, 61.67; H, 6.31; N, 7.00%. C₄₀H₄₆N₄O₁₂ requires C, 62.01; H, 5.98; N, 7.23%); [α]_D²⁵ –47.5 (*c* 0.6, CHCl₃); δ_{H} (400 MHz, CDCl₃) 7.92–7.81 (m, 2H, Phth), 7.77–7.69 (m, 2H, Phth), 7.47–7.29 (m, 10H, Ar), 5.47 (s, 1H, PhCH), 5.24 (d, 1H, *J* 8.5 Hz, H-1–GlcN), 4.68 (d, 1H, *J* 12.1 Hz, Bn), 4.60 (d, 1H, *J* 12.1 Hz, Bn), 4.44 (dd, 1H, *J* 10.8, 8.2 Hz, H-3–GlcN), 4.32 (d, 1H, *J* 7.7 Hz, H-1–Gal), 4.25 (s, 1H, OH-3–GlcN), 4.24–4.14 (m, 2H, H-2–GlcN + H-6a–Gal), 4.13 (dd, 1H, *J* 3.6, ~1 Hz, H-4–Gal), 3.97 (dd, 1H, *J* 11.6, 1.0 Hz, H-6b–Gal), 3.91–3.80 (m, 3H, H-6a–GlcN + H-6b–GlcN + OCHaHb), 3.77–3.63 (m, 3H, H-4–GlcN + H-2–Gal + H-5–GlcN), 3.55 (ddd, 1H, *J* 9.2, 9.2, 3.7 Hz, H-3–Gal), 3.47–3.39 (m, 2H, H-5–Gal + OCHaHb), 3.17 (d, 1H, *J* 2.0 Hz, OH-2–Gal), 3.04 (t, 2H, *J* 7.0 Hz, CH₂N₃), 2.60

(d, 1H, *J* 8.9 Hz, OH-3-Gal), 1.54–1.35 (m, 2H, OCHaHbCH₂), 1.34–1.21 (m, 2H, CH₂CH₂N₃), 1.20–1.05 (m, 4H, 2 × CH₂-chain); δ_c (100 MHz, CDCl₃) 167.97 (CO), 137.83, 137.30, 134.02, 131.75, 129.22, 128.40, 128.20, 127.98, 127.78, 126.33, 103.93 (C-1-Gal), 101.31 (PhCH), 98.36 (C-1-GlcN), 83.71 (C-4-GlcN), 74.86 (C-4-Gal), 73.45 (C-5-GlcN), 73.43 (Bn), 72.67 (C-3-Gal), 71.45 (C-2-Gal), 69.80 (C-3-GlcN), 69.51 (OCHaHb), 68.64 (C-6-GlcN), 66.86 (C-6-Gal), 56.02 (C-2-GlcN), 51.13 (CH₂N₃), 29.11 (OCHaHbCH₂), 28.58 (CH₂CH₂N₃), 26.20 (CH₂-chain), 25.40 (CH₂-chain); *m/z* (ESI-HRMS) calcd for [C₄₀H₄₆N₄O₁₂ + Na]⁺ 797.3004, found 797.3005.

***p*-Chlorophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (7).** Imidate **10** (2.44 g, 4.95 mmol) and alcohol **14** (1.04 g, 1.98 mmol) were dissolved in anhydrous CH₂Cl₂ (10 mL) under Ar and 4 Å molecular sieves (2.5 g) were added. After stirring at rt for 1 h, TMSOTf (35 μL) was added dropwise. After 2 h, the reaction was neutralized with Et₃N (1.0 mL). The mixture was diluted with EtOAc (50 mL) and the insoluble molecular sieves were removed by filtration. After concentration, the residue was purified by chromatography on silica gel using a gradient of AcOEt–hexane (30% → 40%) to afford the desired disaccharide **7** (1.2 g, 75% yield) as a white solid (Found: C, 57.20; H, 5.08; N, 1.68%. C₄₁H₄₀NO₁₅SCl requires C, 57.64; H, 4.72; N, 1.64%); [α]_D²⁰ +33.3 (*c* 0.2, CHCl₃); δ_H (400 MHz, CDCl₃) 7.98–7.73 (m, 4H, Phth), 7.50–7.43 (m, 2H, Ph), 7.40–7.32 (m, 3H, Ph), 7.29 (d, 2H, *J* 8.5 Hz, SPhCl), 7.21 (d, 2H, *J* 8.5 Hz, SPhCl), 5.55 (s, 1H, PhCH), 5.51 (d, 1H, *J* 10.6 Hz, H-1-GlcN), 5.18 (dd, 1H, *J* 2.9, ~1.0 Hz, H-4-Gal), 4.96 (dd, 1H, *J* 10.3, 8.0 Hz, H-2-Gal), 4.76–4.68 (m, 2H, H-3-Gal + H-3-GlcN), 4.54 (d, 1H, *J* 8.0, H-1-Gal), 4.41–4.32 (m, 2H, H-2-GlcN + H-6a-GlcN), 4.02 (dd, 1H, *J* 11.0, 8.3 Hz, H-6a-Gal), 3.87–3.74 (m, 3H, H-6b-Gal + H-6b-GlcN + H-4-GlcN), 3.70 (ddd, 1H, *J* 9.6, 9.6, 4.6 Hz, H-5-GlcN), 3.45 (ddd, 1H, *J* 7.8, 6.2, ~1 Hz, H-5-Gal), 2.05 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.82 (s, 3H, Ac), 1.51 (s, 3H, Ac); δ_c (100 MHz, CDCl₃) 170.21 (CO), 170.00 (CO), 169.97 (CO), 168.81 (CO), 136.92, 134.73, 134.54, 129.45, 129.34, 129.13, 128.38, 126.04 (Ar), 101.50 (PhCH), 100.38 (C-1-Gal), 83.84 (C-1-GlcN), 80.69 (C-4-GlcN), 76.50 (C-3-Gal), 70.95 (C-3-GlcN), 70.60 (C-5-GlcN), 70.31 (C-5-Gal), 69.19 (C-2-Gal), 68.51 (C-6-GlcN), 66.59 (C-4-Gal), 60.74 (C-6-Gal), 54.28 (C-2-GlcN), 20.59 (Ac), 20.53 (Ac), 20.41 (Ac), 20.03 (Ac); *m/z* (ESI-HRMS) calcd for [C₄₁H₄₀NO₁₅SCl + Na]⁺ 876.1699, found 876.1695.

***p*-Chlorophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (9).** The disaccharide **7** (884 mg, 1.04 mmol) was dissolved in anhydrous CH₂Cl₂ (17 mL) and the mixture was cooled to 0 °C. After the addition of Et₃SiH (1.65 mL, 10.3 mmol), BF₃·Et₂O (239 μL, 1.9 mmol) was added dropwise. The reaction was continued at 0 °C for 1 h, and subsequently at rt for another 1 h. Et₃N (2.0 mL) was added to quench the reaction. After concentration, the mixture was purified by chromatography on silica gel using 35% AcOEt–hexane as eluent to give the desired disaccharide **9** (802 mg, 97.8% yield) as a white solid (Found: C, 57.24; H, 5.11; N, 1.63%. C₄₁H₄₂NO₁₅SCl requires C, 57.51; H, 4.94; N, 1.64%); [α]_D²⁰ +26.6 (*c* 1.2, CHCl₃); δ_H (400 MHz, CDCl₃) 7.92–7.86 (m, 2H, Phth), 7.84–7.78 (m, 2H, Phth), 7.40–7.29 (m, 7H, 5 × Bn + 2 × SPhCl), 7.10 (d, 2H, *J* 8.2 Hz, SPhCl), 5.39

(d, 1H, *J* 10.4 Hz, H-1-GlcN), 5.29 (dd, 1H, *J* 3.4, ~1.0 Hz, H-4-Gal), 5.13 (dd, 1H, *J* 10.4, 8.1 Hz, H-2-Gal), 4.78 (dd, 1H, *J* 10.5, 3.4 Hz, H-3-Gal), 4.62 (high order d, 1H, *J* 12.1 Hz, Bn), 4.58 (high order d, 1H, *J* 12.1 Hz, Bn), 4.48 (dd, 1H, *J* 10.2, 7.7 Hz, H-3-GlcN), 4.38 (d, 1H, *J* 8.0 Hz, H-1-Gal), 4.33 (dd, 1H, *J* 10.3, 10.3 Hz, H-2-GlcN), 4.12 (dd, 1H, *J* 12.0, 7.2 Hz, H-6a-Gal), 4.08 (dd, 1H, *J* 12.0, 6.8 Hz, H-6b-Gal), 3.98 (s, 1H, OH-4-GlcN), 3.97–3.89 (m, 2H, H-5-Gal + H-6a-GlcN), 3.75 (dd, 1H, *J* 10.6, 5.7 Hz, H-6b-GlcN), 3.72–3.60 (m, 2H, H-5-GlcN + H-4-GlcN), 2.12 (s, 3H, Ac), 2.04 (s, 4H, Ac), 1.87 (s, 3H, Ac), 1.53 (s, 3H, Ac); δ_c (100 MHz, CDCl₃) 170.32 (CO), 170.02 (CO), 169.90 (CO), 168.79 (CO), 168.42 (CO), 166.94 (CO), 138.27, 134.60, 134.11, 133.61, 131.36, 130.52, 128.96, 128.34, 127.58, 127.54, 123.81, 123.75 (Ar), 100.97 (C-1-Gal), 83.34 (C-1-GlcN), 82.88 (C-3-GlcN), 79.78 (C-5-GlcN), 73.47 (Bn), 71.19 (C-5-Gal), 70.76 (C-3-Gal), 69.64 (C-6-GlcN), 69.57 (C-4-GlcN), 68.46 (C-2-Gal), 66.75 (C-4-Gal), 61.43 (C-6-Gal), 53.84 (C-2-GlcN), 20.53 (Ac), 20.49 (Ac), 20.36 (Ac), 19.87 (Ac); *m/z* (ESI-HRMS) calcd for [C₄₁H₄₂NO₁₅SCl + Na]⁺ 878.1856, found 878.1854.

***p*-Chlorophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (8).** The disaccharide **9** (155.8 mg, 0.20 mmol) and thiofucoside **15** (331.5 mg, 0.59 mmol) were stirred with 4 Å molecular sieves (400 mg) in anhydrous CH₂Cl₂ (2 mL) under Ar for 1 h. After cooling to –30 °C, NIS (96.3 mg, 0.41 mmol, 2.1 equiv.) was added, and after 5 mins, TfOH (5.0 μL) was added dropwise. The reaction was slowly warmed up to –10 °C within 1 h, and quenched with Et₃N (0.5 mL). The reaction mixture was worked up similarly as **22** and compound **8** (187.2 mg) was obtained by column chromatography on silica gel using 30% EtOAc–hexane as eluent in 84% yield. Some starting material recovered (10 mg) was recovered. [α]_D²⁰ –16.8 (*c* 0.6, CHCl₃); δ_H (400 MHz, CDCl₃) 7.96–7.87 (m, 2H, Phth), 7.87–7.79 (m, 2H, Phth), 7.52–7.17 (m, 22H), 7.11 (d, 2H, *J* 8.1 Hz, SPhCl), 5.30 (d, 1H, *J* 10.4 Hz, H-1-GlcNAc), 5.21 (dd, 1H, *J* 2.7, 1.0 Hz, H-4-Gal), 5.16 (d, 1H, *J* 3.8 Hz, H-1-Fuc), 5.04–4.97 (m, 2H, Bn + H-2-Gal), 4.93 (d, 1H, *J* 11.6 Hz, Bn), 4.91–4.69 (m, 6H, H-3-Gal + H-5-Fuc + 4 × Bn), 4.45–4.35 (m, 3H, H-2-Fuc + 2 × Bn), 4.32 (dd, 1H, *J* 10.3, 10.3 Hz, H-2-GlcN), 4.28–4.16 (m, 3H, H-6a-Gal + H-3-GlcN + H-1-Gal), 4.04–3.93 (m, 4H, H-6b-Gal + H-6a-GlcN + H-3-Fuc + H-4-GlcN), 3.78 (br, 1H, H-4-Fuc), 3.74–3.59 (m, 3H, H-6b-GlcN + H-5-Gal + H-5-GlcN), 2.03 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.86 (s, 3H, Ac), 1.75 (s, 3H, Ac), 1.32 (d, 3H, *J* 6.5 Hz, H-6-Fuc); δ_c (100 MHz, CDCl₃) 170.20 (CO), 170.07 (CO), 169.90 (CO), 169.42 (CO), 138.91, 138.82, 138.47, 138.35, 135.01, 134.48, 134.44, 129.96, 129.12, 128.86, 128.61, 128.58, 128.52, 128.51, 128.44, 128.00, 127.81, 127.72, 127.68, 127.52, 127.14, 123.90 (Ar), 100.76 (C-1-Gal), 98.08 (C-1-Fuc), 83.20 (C-1-GlcN), 80.99 (C-4-GlcN), 80.18 (C-5-GlcN), 76.56 (C-4-Fuc), 75.85 (C-3-Gal), 75.72 (C-3-GlcN), 75.12 (Bn), 74.11 (Bn), 73.24 (Bn), 72.92 (C-3-Fuc), 72.41 (Bn), 71.19 (C-2-Fuc), 70.47 (C-5-Gal), 67.92 (C-2-Gal), 67.67 (C-6-GlcN), 66.58 (C-5-Fuc), 66.44 (C-4-Gal), 59.88 (C-6-Gal), 55.49 (C-2-GlcN), 20.74 (Ac), 20.61 (Ac), 20.59 (2 × Ac), 17.11 (C-6-Fuc); *m/z* (ESI-HRMS) calcd for [C₆₈H₇₀NO₁₉SCl + Na]⁺ 1294.3844, found 1294.3844.

6-Azidoethyl 2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl-(1 \rightarrow 3)-6-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl-(1 \rightarrow 3)-4,6-O-benzylidene- β -D-galactopyranosyl-(1 \rightarrow 4)-6-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (31). Compound **5** (82.7 mg, 0.11 mmol) and alcohol **9** (54 mg, 0.07 mmol) were dissolved in anhydrous CH_2Cl_2 (1.0 mL) under Ar, and 4 Å molecular sieves (200 mg) were added. After stirring at rt for 1 h, the mixture was cooled to -78°C , and NIS (41.3 mg, 0.17 mmol) was added. After another 5 mins, TfOH (3 μL) was added and the temperature was slowly warmed to -20°C within 1 h. The mixture was neutralized with Et_3N (0.5 mL). The mixture was worked up in a similar fashion as **22** to afford the desired tetrasaccharide **31** (89.9 mg, 86.8% yield) after column chromatography on silica gel using 35% EtOAc-hexane as eluent. $[\alpha]_{\text{D}} -16.4$ (c 0.7, CHCl_3); δ_{H} (600 MHz, CDCl_3) 7.89–7.79 (m, 2H, Phth), 7.79–7.73 (m, 2H, Phth), 7.68–7.74 (m, 2H, Phth), 7.67–7.62 (m, 2H, Phth), 7.42–7.28 (m, 10H, Bn), 5.39 (d, J 8.5 Hz, H-1-GlcN-I), 5.33 (s, 1H, PhCH), 5.30 (dd, 1H, J 2.9, \sim 1 Hz, H-4-Gal-I), 5.16 (d, 1H, J 8.4 Hz, H-1-GlcN-II), 5.14 (dd, 1H, J 8.1, 10.5 Hz, H-2-Gal-I), 4.81 (dd, 1H, J 3.5, 10.5 Hz, H-3-Gal-I), 4.63 (d, 1H, J 12.0 Hz, Bn), 4.59 (d, 1H, J 12.0 Hz, Bn), 4.56 (dd, 1H, J 10.7, 8.2 Hz, H-3-GlcN-I), 4.49 (d, 1H, J 12.2 Hz, Bn), 4.42 (d, 1H, J 8.1 Hz, H-1-Gal-I), 4.35 (d, 1H, J 12.5 Hz, Bn), 4.32 (dd, 1H, J 8.6, 10.8 Hz, H-2-GlcN-I), 4.29 (dd, 1H, J 8.4, 10.9 Hz, H-3-GlcN-II), 4.20 (br s, 1H, OH-3-GlcN-II), 3.91–4.18 (m, 11H, H-1-Gal-II + H-4-Gal-II + H-6a-GlcN-I + H-6b-GlcN-I + H-2-GlcN-II + H-6a-Gal-II + H-5-Gal-I + H-6a-GlcN-I + H-6b-GlcN-I + H-6a-Gal-I + OH-4-GlcN-I), 3.81–3.48 (m, 9H, OCHaHb + H-6b-Gal-II + H-6b-Gal-I + H-2-Gal-II + H-4-GlcN-I + H-5-GlcN-II + H-5-GlcN-I + H-3-Gal-II + H-4-GlcN-II), 3.37 (ddd, 1H, J 9.7, 6.5, 6.5 Hz, OCHaHb), 3.27 (m, 1H, H-5-Gal-II), 3.03 (t, 2H, J 7.0 Hz, CH_2N_3), 2.83 (br d, 1H, J 1.9 Hz, OH-2-Gal-II), 2.13 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.49–1.36 (m, 5H, OCHaHb CH_2 + Ac), 1.34–1.20 (m, 2H, $\text{CH}_2\text{CH}_2\text{N}_3$), 1.18–1.06 (m, 4H, $2 \times \text{CH}_2$); m/z (ESI-HRMS) calcd for $[\text{C}_{75}\text{H}_{83}\text{N}_5\text{O}_{27} + \text{Na}]^+$ 1508.5169, found 1508.5168.

6-Aminohexyl β -D-galactopyranosyl-(1 \rightarrow 3)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 3)- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-2-deoxy- β -D-glucopyranoside, acetic acid salt (2). A solution of tetrasaccharide **31** (83 mg, 0.056 mmol) was dissolved in EtOH (5.0 mL); a solution of hydrazine hydrate (70%, 0.8 mL) was added and the mixture was heated to reflux for 36 h. The solution was evaporated to dryness under vacuum. The residue was purified by chromatography on silica gel using 15% MeOH- CH_2Cl_2 as eluent to afford the intermediate amine (\sim 43 mg) which was redissolved in anhydrous MeOH (4.0 mL) and after cooling to 0°C , Ac_2O (0.5 mL) was added. After 1 h, the mixture was evaporated to dryness. The residue was dissolved in 10% AcOH-MeOH (4.0 mL), and hydrogenated with 20% Pd(OH) $_2$ on charcoal (20 mg) for 3 days at rt. The catalyst was removed by filtration and the solution was concentrated. The residue was dissolved in deionized H_2O , purified on a C18 Sep-Pak cartridge using a gradient of MeOH - H_2O (0% \rightarrow 5%) as eluent to give the desired tetrasaccharide **2** as a white solid after a lyophilization (24.6 mg, 48% yield for three steps). $[\alpha]_{\text{D}} -4.8$ (c 0.2, MeOH); δ_{H} (400 MHz, D_2O) 4.75 (d, 1H, J 8.3 Hz, H-1-GlcN-I), 4.54 (d, 1H, J 7.6 Hz, H-1-GlcN-II), 4.49 (d, 1H,

J 7.9 Hz, H-1-Gal-I), 4.47 (d, 1H, J 7.9 Hz, H-1-Gal-II), 4.18 (dd, 1H, J 3.3, 1.0 Hz, H-4-Gal-I), 4.01 (dd, 1H, J 12.3, 2.2 Hz, H-6a-Glc-II), 3.98–3.47 (m, 24H, H-2-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-6b-GlcN-II + H-2-Gal-I + H-3-Gal-I + H-5-Gal-I + H-6a-Gal-I + H-6b-Gal-I + H-2-Gal-II + H-3-Gal-II + H-4-Gal-II + H-5-Gal-II + H-6a-Gal-II + H-6b-Gal-II + OCHaHb + OCHaHb), 3.04–2.98 (m, 2H, CH_2NH_2), 2.05 (s, 6H, $2 \times \text{AcNH}$), 1.94 (s, 3H, $\text{CH}_3\text{CO}_2\text{H}$), 1.75–1.64 (m, 2H, OCHaHb CH_2), 1.64–1.54 (m, 2H, $\text{CH}_2\text{CH}_2\text{NH}_2$), 1.46–1.34 (m, 4H, $2 \times \text{CH}_2$); δ_{C} (100 MHz, D_2O) 174.96 (CO), 174.37 (CO), 103.48 (C-1-Gal-II), 102.90 (C-1-Gal-I), 102.56 (C-1-GlcN-II), 101.09 (C-1-GlcN-I), 82.09, 82.04, 78.54, 75.28, 75.18, 74.89, 74.75, 72.47, 72.42, 70.68, 70.40, 69.98, 68.52, 68.44, 68.27, 61.02, 60.94, 60.49, 60.08, 55.06 (C-2-GlcN-I), 54.69 (C-2-GlcN-II), 39.43 (CH_2NH_2), 28.34 (CH_2), 26.75 (CH_2), 25.24 (CH_2), 24.61 (CH_2), 22.22 (Ac), 22.17 (Ac); m/z (ESI-HRMS) calcd for $[\text{C}_{34}\text{H}_{61}\text{N}_5\text{O}_{21} + \text{H}]^+$ 848.3870, found 848.3871.

6-Azidoethyl 2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl-(1 \rightarrow 3)-[2,3,4-tri-O-benzyl- α -L-fucopyranosyl-(1 \rightarrow 4)]-6-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl-(1 \rightarrow 3)-4,6-O-benzylidene- β -D-galactopyranosyl-(1 \rightarrow 4)-6-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (32). A mixture containing the trisaccharide **8** (78.9 mg, 0.065 mmol), disaccharide **5** (50.3 mg, 0.065 mmol) and 4 Å molecular sieves (200 mg) in anhydrous CH_2Cl_2 (1 mL) was stirred under Ar for 2 h at rt. The temperature was cooled to -78°C , and NIS (35 mg, 0.15 mmol) was added followed by TfOH (4 μL). The temperature was slowly warmed up to -10°C within 1 h. Et_3N (0.5 mL) was added to neutralize the reaction. The mixture was worked up as **22** and purified by column chromatography on silica gel using a gradient of EtOAc-hexane (25% \rightarrow 30%) as eluent to afford the desired pentasaccharide **32** (84.9 mg, 69.6% yield). $[\alpha]_{\text{D}} -31.0$ (c 1.0, CHCl_3); δ_{H} (400 MHz, CDCl_3) 7.86–7.80 (m, 2H, Phth), 7.80–7.74 (m, 2H, Phth), 7.74–7.69 (m, 2H, Phth), 7.69–7.64 (m, 2H, Phth), 7.50–7.44 (m, 2H, Ar), 7.43–7.20 (m, 28H, Ar), 5.31 (s, 1H, PhCH), 5.31 (d, 1H, J 8.3 Hz, H-1-GlcN-I), 5.23 (dd, 1H, J 3.1, \sim 1 Hz, H-4-Gal-I), 5.16 (d, 1H, J 8.5 Hz, H-1-GlcN-II), 5.12 (d, 1H, J 3.7 Hz, H-1-Fuc), 5.06–4.96 (m, 2H, H-2-Gal-I + Bn), 4.93 (d, 1H, J 11.6 Hz, Bn), 4.91–4.82 (m, 3H, $2 \times \text{Bn} + \text{H-3-GlcN-I}$), 4.81–4.71 (m, 3H, $2 \times \text{Bn} + \text{H-5-Fuc}$), 4.51–4.44 (m, 2H, Bn + H-3-Gal-I), 4.40 (d, 1H, J 12.2 Hz, Bn), 4.38–4.18 (m, 7H, $2 \times \text{Bn} + \text{H-2-GlcN-I} + \text{H-3-GlcN-II} + \text{H-6a-Gal-I} + \text{H-2-Fuc} + \text{H-1-Gal-I}$), 4.15–3.89 (m, 8H, H-1-Gal-II + H-2-Gal-II + H-4-Gal-II + H-6a-Gal-II + H-6b-Gal-I + H-3-Fuc + H-4-GlcN-I + H-6a-GlcN-I), 3.83–3.63 (m, 8H, H-4-Fuc + H-6b-Gal-II + H-6b-GlcN-I + H-6a-GlcN-II + H-6b-GlcN-II + OCHaHb + H-5-Gal-I + H-5-GlcN-I), 3.62–3.49 (m, 3H, H-5-GlcN-II + H-2-Gal-II + H-4-GlcN-II) 3.45 (dd, 1H, J 9.8, 3.2 Hz, H-3-Gal-II), 3.38 (ddd, 1H, J 9.4, 6.7, 6.7 Hz, OCHaHb), 3.26 (m, 1H, H-5-Gal-II), 3.03 (t, 2H, J 7.0 Hz, CH_2N_3), 2.70 (d, 1H, J 1.7 Hz, OH-2-Gal-II), 2.00 (s, 6H, $2 \times \text{Ac}$), 1.85 (s, 3H, Ac), 1.76 (s, 3H, Ac), 1.51–1.34 (m, 2H, OCHaHb CH_2), 1.31 (d, 3H, J 6.5 Hz, H-6-Fuc), 1.29–1.20 (m, 2H, $\text{CH}_2\text{CH}_2\text{N}_3$), 1.20–1.02 (m, 4H, $2 \times \text{CH}_2$ -chain); δ_{C} (100 MHz, CDCl_3 , from HSQC) 134.16 (Ar), 133.79 (Ar), 128.13 - 126.06 (Ar), 123.11 (Ar), 122.98 (Ar), 104.04 (C-1-Gal-II), 100.29 (C-1-Gal-I), 100.02 (PhCH),

99.58 (C-1-GlcN-I), 98.17 (C-1-GlcN-II), 97.81 (C-1-Fuc), 83.70 (C-4-GlcN-II), 80.70 (C-4-GlcN-I), 78.94 (C-3-Gal-II), 76.30 (C-4-Fuc), 75.53 (C-2-Fuc), 75.32 (C-5-GlcN-II), 74.95 (Bn), 74.93 (C-4-Gal-II), 74.18 (C-3-GlcN-I), 73.80 (Bn), 72.88 (Bn), 72.81 (Bn), 72.81 (C-5-GlcN-II), 72.72 (C-3-Fuc), 71.99 (Bn), 70.81 (C-3-Gal-I), 70.26 (C-5-Gal-I), 69.57 (C-3-GlcN-II), 69.45 (OCHaHb), 69.35 (C-2-Gal-II), 69.20 (C-6-GlcN-II), 68.07 (C-6-Gal-II), 67.61 (C-6-GlcN-I), 67.41 (C-2-Gal-I), 66.53 (C-5-Gal-II), 66.09 (C-5-Fuc), 66.00 (C-4-Gal-I), 59.56 (C-6-Gal-I), 55.93 (C-2-GlcN-I), 55.47 (C-2-GlcN-II), 50.77 (CH₂N₃), 28.89 (OCHaHbCH₂), 28.24 (CH₂CH₂N₃), 25.59 (CH₂), 25.40 (CH₂), 20.42 (Ac), 20.27 (Ac), 20.23 (Ac), 20.03 (Ac), 16.40 (C-6-Fuc); *m/z* (ESI-HRMS) calcd for [C₁₀₂H₁₁₁N₅O₃₁ + Na]⁺ 1924.7155, found 1924.7172.

6-Aminohexyl β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside, acetic acid salt (4). Pentasaccharide **32** (25 mg, 0.013 mmol) was deprotected in a similar fashion as compound **2**. After a purification on a C18 Sep-Pak cartridge using a gradient of MeOH - H₂O (0% → 5%) as eluent, the desired pentasaccharide **4** was obtained as a white solid after lyophilization (7.6 mg, 55% yield). [α]_D +30.5 (*c* 0.2, MeOH); δ_H (400 MHz, D₂O) 5.07 (d, 1H, *J* 3.9 Hz, H-1-Fuc), 4.91 (dq, 1H, *J* 6.7, -1.0 Hz, H-5-Fuc), 4.75 (d, 1H, *J* 8.2 Hz, H-1-GlcN-I), 4.57 (d, 1H, *J* 7.4 Hz, H-1-GlcN-II), 4.55 (d, 1H, *J* 7.6 Hz, H-1-Gal-I), 4.51 (d, 1H, *J* 7.8 Hz, H-1-Gal-II), 4.21 (dd, 1H, *J* 3.2, 1.0 Hz, H-4-Gal-I), 4.13 (dd, 1H, *J* 9.7, 9.7 Hz, H-3-GlcN-I), 4.03 (dd, 1H, *J* 10.2, 1.8 Hz, H-6a-GlcN-I), 4.01–3.56 (m, 25H), 3.53 (dd, 1H, *J* 9.7, 7.6 Hz, H-2-Gal-I), 3.03 (t, 2H, *J* 7.5 Hz, CH₂NH₂), 2.08 (s, 6H, 2 × Ac), 1.96 (s, 3H, CH₃CO₂H), 1.75–1.66 (m, 2H, OCHaHbCH₂), 1.66–1.56 (m, 2H, CH₂CH₂NH₂), 1.48–1.34 (m, 4H, 2 × CH₂), 1.23 (d, 3H, *J* 6.6 Hz, H-6-Fuc); δ_C (100 MHz, D₂O) 174.76 (CO), 174.37 (CO), 102.91 (C-1-Gal-I or C-1-Gal-II), 102.85 (C-1-Gal-II or C-1-Gal-I), 102.63 (C-1-GlcN-I), 101.09 (C-1-GlcN-II), 98.01 (C-1-Fuc), 82.12, 78.52, 75.91, 75.22, 74.87, 74.82, 74.75, 72.42, 72.32, 72.11, 71.94, 70.50, 70.40, 69.93, 69.13, 68.34, 68.26, 67.78, 66.84, 63.49, 61.64, 60.94, 60.07, 59.60, 55.87 (C-2-GlcN-I), 55.06 (C-2-GlcN-II), 39.42 (CH₂NH₂), 28.33 (CH₂), 26.70 (CH₂), 25.23 (CH₂), 24.60 (CH₂), 22.28 (Ac), 22.16 (Ac), 15.35 (C-6-Fuc); *m/z* (ESI-HRMS) calcd for [C₄₀H₇₁N₃O₂₅ + H]⁺ 994.4449, found 994.4451.

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