Synthesis of Tumor-Associated Le^aLe^x hexasaccharides: Instability of a Thiol-containing Oligosaccharide in Mass Spectrometry and Hypermetalation detected by ESI FAIMS.

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Abstract

We report the efficient synthesis of three analogues of the tumor associated carbohydrate antigen Le^aLe^x. This hexasaccharide was prepared as a soluble inhibitor hexyl glycoside, a 6aminohexyl glycoside for conjugation to proteins, and as 6-thiohexyl glycoside for immobilization to a gold surface. These three analogues were obtained from a common hexasaccharide intermediate and isolated pure following efficient deprotection reactions that involved metal dissolving conditions. While all other intermediates and analogues gave the expected molecular ions in ESI HRMS, the 6-thiohexyl glycoside final compound gave a complex spectrum in which no signal matched the molecular ion. Using ESI FAIMS HRMS, we were able to prevent ion dissociation reactions and obtained high quality spectral data. The ions detected could be characterized unambiguously from their accurate masses and gave insight into the behavior of the thiohexyl analogue in the gas phase. These results indicate that the 6thiohexyl glycoside lost water and led to the formation of "hyper-metalated" species which we propose are cyclic.

Introduction

Although not yet successful, the use of Tumor Associated Carbohydrate Antigens (TACAs) to develop anti-cancer vaccines has led to intense research efforts in the past two decades.¹ Of particular interest to our group is the TACA Le^aLe^x which has been shown to be over-expressed on the surface of squamous lung carcinoma (SLC) cells glycoproteins as well as secreted soluble mucins from SLC cells.² While the nonreducing end Le^a trisaccharide is also displayed by normal cells,³ monoclonal antibody (mAb) 43-9F raised in mice against human SLC cells was shown to recognize preferentially Le^aLe^x over Le^a and to recognize an epitope primarily displayed on SLC cells and associated with the tumorigenicity of these cells.^{2, 3b, 4} Thus, we are exploring the possibility of using the epitope recognized by mAb 43-9F to develop an anti-cancer vaccine. To identify the smallest Le^aLe^x fragments that still displays this epitope, we have already chemically prepared numerous fragments of the natural hexasaccharide using stepwise approaches and attempting to limit the deprotection steps leading to the desired final compounds.⁵ We report here the efficient and convergent chemical synthesis of analogues 2-4 of the Le^aLe^x TACA. Hexylglycoside 2 will be used as soluble inhibitor in competitive ELISA studies while aminohexyl glycoside 3 will be conjugated to tetanus toxoid for immunizations and BSA for immobilization on ELISA plates. Thiohexylglycoside 4 will be immobilized to a gold chip for the detection of antibodies by surface plasmon resonance (SPR).

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To the best of our knowledge, while fragments of the TACA were also prepared in Ling's laboratory,⁶ only one enzymatic synthesis of the Le^aLe^x glycosphingolipid has been reported in the literature by Hakomori.^{4d} Interestingly, while we confirmed the structure of analogues **2** and **3** easily by ESI HRMS, data for the 6-thiohexyl glycoside **4** did not show the expected molecular ion. Indeed, the results presented here suggest that the hexyl-6-thiol aglycone in hexasaccharide **4** gave a molecular ion which was too labile to be detected. Interpretation of the data obtained from electrospray high resolution mass spectrometry (ESI HRMS) was complicated by gas phase reactions involving the primary thiol and which led to the formation of dissociation species and species accommodating multiple sodium and ammonium ions. Using electrospray high-field asymmetric waveform ion mobility spectrometry (ESI FAIMS),⁷ we were able to suppress the ion dissociation reactions and obtain high quality spectral data that was easily interpreted and suggested that the 6-thiohexyl glycoside **4** lost water and led to the formation of "hyper-metalated" cyclic species. Interestingly, ESI FAIMS HRMS on analogues **2** and **3** did not lead to either loss of water nor hypermetalation.

Results and Discussion

The rapid assembly of the $Le^{a}Le^{x}$ hexasaccharide employed known monosaccharide building blocks 5^{8} , 6^{9} , 7^{10} , 8^{11} and 9^{12} and is shown on Scheme 1.





SCHEME 1. Synthesis of hexasaccharides 2–4 from building blocks 5–9.

Glycosylation of acceptor **6** with donor **5** (4 equiv) activated with BF₃·OEt₂ (2 equiv) at 40 °C gave disaccharide **10** in very good yield (79%). Selective de-chloroacetylation at O-3' of disaccharide **10** was achieved in 65% yield using the conditions that we have established previously on a similar analogue^{5c} (1.2 equiv of thiourea at 55 °C). The position of the free hydroxyl group in acceptor **11** was confirmed by the upfield shift of H-3', from 4.79 ppm in **10** to 3.54 ppm in the ¹H NMR spectrum. Acceptor **11** was then glycosylated with 3 equiv of trichloroacetimidate donor **7** activated at 0 °C with TMSOTf (2 equiv) and the desired trisaccharide **12** was obtained in 79%. While studying the reactivity of O-3*A* and O-3*A*' in diol **14**, we have reported that galactosylation at O-3*A*' was mismatched while that at O-3*A* was matched.¹³ Further studies are on-going in our laboratory to explain these results but we expected

that the introduction of the galactosyl B' would require harsh reaction conditions which may lead to the loss of a fucosyl unit if it was introduced first at O-3A. Thus, we attempted the selective de-chloroacetylation at the non-reducing end O-3A' of trisaccharide 12 using the conditions applied for the preparation of disaccharide 11. While after 7 h analytical RP-HPLC showed 80% conversion to acceptor 13 with only 12% of diol 14 and 8% of starting material, chromatography of the crude mixture gave the desired trisaccharide 13 in only 36% yield, while diol 14 was obtained in 7% yield. Faced with this poor yield, the synthetic strategy was revised and fucosylation at the reducing end was scheduled to occur before the galactosylation at O-3A'. Both chloroacetates were removed (excess thiourea) to give the previously characterized¹³ diol 14 (77%). The diol was then first selectively chloroacetylated¹³ at the reducing end O-3A', and the resulting acceptor was fucosylated at O-3A with donor 8 (3 equiv) activated by MeOTf (5 equiv). The crude mixture was treated with 25% AcOH in Ac₂O to convert any methyl imidate¹⁴ into N-acetyl group and the desired tetrasaccharide 15 was isolated in 84% yield. Dechloroacetylation of tetrasaccharide 15 with an excess thiourea gave the previously characterized¹³ tetrasaccharide acceptor 16 in 88% yield, which was submitted to galactosylation with donor 9. As anticipated harsh reaction conditions were required to obtain 17: excess $BF_3 \cdot OEt_2$ (2 equiv) at room temperature and 5 equiv of donor 9. Due to co-migration of the products and degraded donor, the reaction was best monitored by RP-HPLC after quenching of an aliquot with NEt₃. One hour of reaction was found to be ideal to obtain the best yield of desired tetrasaccharide 17 (52%) while avoiding excessive degradation and loss of the fucosyl unit, however unreacted acceptor 16 was also recovered in 20% yield. Regioselective opening of the benzylidene acetal in pentasaccharide 17 then gave pentasaccharide acceptor 18 in 62% yield. Fucosylation of 18 with 5 equiv of donor 9 activated with MeOTf was followed by

treatment of the crude mixture with 25% AcOH in Ac₂O and gave the desired protected Le^aLe^x hexasaccharide **19** in excellent 76% yield. Reduction of the trichloroacetamide into an acetamide was achieved using with zinc in acetic acid under sonication at 50 °C for 7 h and gave hexasaccharide 20 in excellent yield. Portions of the 6-chlorohexyl glycoside 20 were then converted to the 6-azidohexyl and 6-acetylthiohexyl glycosides 21 and 22 through the nucleophilic displacement of the chlorine atom using excess sodium azide or potassium thioacetate, respectively, in DMF at 80 °C. The 6-azidohexyl glycoside 21 was obtained quantitatively while the 6-acetylthiohexyl glycoside 22 was isolated in 93% yield. Hexyl hexasaccharide 2 was then prepared from either the trichloroacetamido intermediate 19 or the Nacetamido hexasaccharide 20. Full deprotection of hexasaccharide 20 using dissolving metal conditions led to reduction of the chlorohexyl to an hexyl chain and removal of all protecting groups including the trichloracetamido at C-2A' giving the free amine. $^{5b, c, 9, 15}$ The free amine was acetylated through work up of the reaction mixture with acetic anhydride and hexasaccharide 2 was isolated pure in 61% yield after chromatography on a Biogel® P2 column eluted with milli-Q water. Alternatively dissolving metal conditions applied to chlorohexyl glycoside 20 gave the desired hexasaccharide 2 in 89% yield. Deprotection of the 6-azidohexyl glycoside 21 in the same conditions gave the 6-aminohexyl glycoside hexasaccharide 3 as the ammonium acetate salt in 61% yield after purification on a Biogel® P2 column eluted with 0.05 M ammonium acetate and repeated freeze-drying. We have shown⁹ that under dissolving metal condition an S-acetyl hexyl chain underwent desulfurization to give the corresponding hexyl glycoside. Thus hexasaccharide 22 first underwent Zemplèn deacetylation and the crude reaction product was then submitted to dissolving metal conditions to give the 6-thiohexyl hexasaccharide 4 in 90% yield after purification on a Biogel® P2 column eluted with milli-Q water.

High resolution mass spectrometry along with ¹H NMR spectroscopy that showed a triplet at 0.85 ppm corresponding to the hexyl methyl group, confirmed the structure of the hexyl glycoside **2**. Similarly, the structure of aminohexyl glycoside **3** was confirmed by HRMS and by the presence of a triplet at 2.97 ppm corresponding to the terminal $CH_2NH_3^+$ in the ¹H NMR. In contrast, while by analogy to hexasaccharides **2** and **3** NMR spectroscopy unequivocally supported the structure and purity of thiol **4**, no molecular ion could be seen in HRMS when the sample was analyzed at three different facilities. Interestingly, the chemical shift measured for the SCH₂ appeared 0.19 ppm lower field than that (2.70 pm) of the analogue hexyl disulfide glycoside Le^x dimer (see **23** Figure 2) that we have reported.⁹ We observed that this triplet at 2.89 ppm matched that of a minor compound formed when preparing the Le^x analogue and that we assumed to be the corresponding hexyl thiol glycoside. We thus concluded that hexasaccharide **4** was likely a thiol rather than a disulfide dimer.

Figure 1 shows the results from the high resolution mass spectrometry (HRMS) analysis of 6-thiohexyl glycoside **4** using two different techniques. As can be seen on the inserted full spectra, electrospray-field asymmetric waveform ion mobility spectrometry (ESI FAIMS) high resolution mass spectrometry^{7d} gave a simple spectrum (Figure 1A, insert) with excellent signal-to-noise ratio. The expanded spectrum (Figure 1A) shows the detection of several major doubly charged ions formed from 6-thiohexyl glycoside **4** after loss of water (M–H₂O). Elemental ion compositions (determined from high resolution accurate mass measurements, see supporting information) showed that while two sodium ions provided the +2 net ion charge, up to four additional sodium ions were also added.



FIGURE 1. High resolution mass spectra recorded for 6-thiohexyl glycoside 4. A) Electrospray high-field asymmetric waveform ion mobility spectrometry high resolution mass spectrometry (ESI FAIMS HRMS); B) electrospray high resolution mass spectrometry (ESI HRMS).

In addition to the sodium adducts, Figure 1A shows the formation of hyper-metalated ammonium adducts giving signals to the left of the major sodium adduct ions. The conventional ESI HRMS analysis of 6-thiohexyl glycoside 4 (Figure 1B, insert) gave a much more complex spectrum than the ESI FAIMS HRMS with over 100 major ions detected. The many ions present can be attributed to dissociation products of labile doubly and singly charged sodium adducts of the glycoside and the spectral interpretation is complicated by the relatively poor signal-to-noise ratio and overlapping ions of similar m/z. However, we were able to find singly charged ions of hyper-metalated species that had also lost water and were in agreement with the results given by the ESI FAIMS HRMS for doubly charged species (Figure 1B).

To investigate the impact of the thiol group in hexasaccharide 4 on the formation of the ions observed by ESI FAIMS HRMS, we also studied the hexyl and aminohexyl hexasaccharides 2 and 3 as well as the previsouly reported⁹ hexyl disulfide glycoside Le^x dimer (23). The full ESI

FAIMS spectra are given in the supporting information while expanded regions showing the most abundant ions that were detected in these experiments are given in Figure 2. Only doubly charged ions were detected that were identified from elemental ion composition (see supporting information) as proton, ammonium or sodium adduct, as well as mixed proton/sodium or ammonium adducts (Figure 2). Interestingly, none of the compounds underwent loss of water and no hypemetalated species were seen for either the hexyl or amino glycosides **2** and **3**, or the disulfide dimer **23**.



FIGURE 2. ESI FAIMS HRMS for A) hexyl glycoside 2; B) aminohexylglycoside 3; and hexyl disulfide glycoside Le^x dimer 23.

"Hyper-metalation" of biomolecules in ESI MS was first named and described by Pope et al.

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for the cyclic peptide Gramicidin S which was shown to add four or more sodium in the +2 charge state.¹⁶ The authors attributed the formation of these adducts to the displacement of protons from the Gramicidin S amide nitrogen atoms leading to salts and leaving the net charge of the ion unchanged. While, there have been several other reports on the hyper-metalation of peptides,¹⁷ we could only find one such report involving carbohydrates. Indeed, Madhusuanan reported multiple lithium exchanges while studying cyclodextrins by ESI MS under lithium cationization.¹⁸ In our study, the fact that no molecular ion was detected for hexyl thiol **4** while we encountered no such issue when analyzing hexasaccharides 2 and 3 or the reported⁹ analogue hexyl disulfide glycoside Le^x dimer 23, suggests that the hexyl thiol chain played a major role in the loss of water and ions being formed. Since cyclic species such as cylcodextrins or Gramicidin S seem to be more prone to hyper-metalation through the trapping and intramolecular coordination of metal ions, we propose that the molecular ion generated from hexyl thiol 4 underwent cyclization involving the thiol group and leading to the concomitant loss of a water molecule. This may have been following protonation (Figure 3, path a) or adduction of a sodium ion on a hydroxyl group (Figure 3, path b) forming the molecular ion and subsequent nucleophilic attack by the thiol displayed at the end of a long and flexible hexyl chain (Figure 3).



FIGURE 3. Proposed pathways leading to the loss of water in hexyl thiol glycoside 4

This cyclic species may trap sodium ions which are present in the sample solution at trace levels from the last synthetic step $[NH_3(1)/Na]$ and undergo hyper-metalation through proton displacement from the hydroxyl groups and intramolecular coordination of sodium ions.

Conclusion

 We are reporting the first fully chemical preparation of three analogues of the TACA Le^aLe^x. Building on our experience, the synthetic scheme proved straightforward efficient and convergent and the final intermediates were easily deprotected using dissolving metal conditions. Most interestingly, we have observed that the hexasaccharide bearing a long hexyl 6-thiol aglycone at the reducing end did not give the expected molecular ion in ESI HRMS and underwent considerable ion dissociation. Using the more recent ESI FAIMS HRMS technique proved to be invaluable. FAIMS is a gas-phase atmospheric pressure separation technique,^{7a} which was developed for use with MS.^{7b} It operates between the ESI source and the MS detection behaving as an ion filter that separates ions according to their differential ion mobility in

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alternating low and high electric fields generated in the gap between two closely spaced electrodes.^{7c} Coupled with "soft" O-TOF mass spectrometry, it is capable of detecting extremely labile ions with minimal fragmentation. Mass calibration was carried out using formaldehyde, as described recently for use with "soft" Q-TOF mass spectrometry¹⁹ and it appeared that all ions detected for compounds 2-4 and 23 were doubly charged. Hexyl glycoside 2, aminohexyl glycoside 3, and hexyl disulfide analogue 23 gave stable doubly charged ions through protonation, or adduction of ammonium or sodium. In contrast, and based on the ions that could be unequivocally assigned through their accurate masses, we observed that the molecular ion formed from the 6-thiohexyl glycoside 4 was highly unstable. We propose that loss of a water equivalent occurred in the gas phase with the concomitant formation of cyclic species involving the nucleophilic thiol present at the end of the long hexyl chain. The resulting cyclic structure then became hyper-metalated through sodium exchange at the hydroxyl groups. While there are a few reports describing hyper-metalation of peptides during ESI HRMS,¹⁶⁻¹⁷ it is rarely observed during the analysis of oligosaccharides. Indeed, while multiple lithium exchange has been observed during ESI MS of cyclodextrins under lithium cationization, open-ended maltohexoses treated in the same conditions gave barely detectable signals for hyper-lithiated species.¹⁸ We thus conclude that the formation of the cyclic ion may have favored hyper-metalation through the efficient coordination of the sodium ions by the hydroxyl groups brought in closer proximity.

Experimental Section

Electrospray high-field asymmetric waveform ion mobility spectrometry high resolution mass spectrometry (ESI FAIMS HRMS) on hexasaccharides 2–4 and disulfide 23. A ~10 μM solution of the compound to be analyzed was prepared using methanol/water (9/1 v/v) containing 0.1 mM ammonium acetate. The solution was infused into a nanospray source at a flow rate of 400 nL/min using the auto-sampler and pump from a nano-flow capillary UPLC system. Ionization was carried out in positive mode with a spray voltage of 5000 V. The sample was analyzed using an FAIMS analyzer with 20% CO₂ in N₂ as a carrier and desolvation gas and a dispersion voltage of 4000 V. Detection was carried out using a Q-TOF mass spectrometer. Ion transmission conditions at low ion kinetic energy were optimized in order to minimize ion dissociation during the analysis. The sample cone voltage, extraction cone voltage, and collision energy voltage were set at 20 V, 1.0 V and 4 V, respectively. After acquisition, data were summed, smoothed two times using the Savitzky-Golay method and then centered. The mass calibration was carried out using formaldehyde, as described recently for use with "soft" Q-TOF mass spectrometry.¹⁹ Mass accuracies were <5 ppm, and were sufficient to determine the elemental composition of the product ions presented. The product ions are listed in the Supporting Information.

6-Chlorohexyl 2-acetamido-4-*O*-(2,4-di-*O*-acetyl-3-*O*-chloroacetyl-6-*O*-pivaloyl-β-Dgalactopyranosyl)-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (10). A stirred solution of known⁹ alcohol 6 (905 mg, 1.787 mmol) and known⁸ galactosyl trichloroacetimidate 5 (4.07 g, 7.150 mmol, 4 equiv) in dry CH₂Cl₂ (45 mL) was heated to 40 °C under N₂. Freshly distilled BF₃·OEt₂ (449 µL, 3.573 mmol, 2 equiv) was added to the mixture, which was stirred for 1 h at 40 °C, then quenched with NEt₃ (598 µL, 4.290 mmol, 2.4 equiv). The reaction was diluted with CH₂Cl₂ (55 mL) and washed with saturated aq NaHCO₃ (1 × 100 mL). The aq layer was re-extracted with CH₂Cl₂ (2 × 30 mL) and the combined organic layers were dried and concentrated. The product was purified by column chromatography (EtOAc/hexanes, 1:1) to give the disaccharide **10** (1.29 g, 79%) pure as a slightly yellowish amorphous foam. [*α*]_D –4.5 (*c* 1.0,

CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃, 297 K): $\delta_{\rm H}$ 7.40–7.30 (m, 5 H, Ar), 5.65 (d, J = 9.1 Hz, 1 H, NHA), 5.19 (d, J = 3.3 Hz, 1 H, H-4B), 5.14 (dd, J = 9.0, 10.2 Hz, 1 H, H-3A), 4.94 (dd, J =8.0, 10.3 Hz, 1 H, H-2B), 4.79 (dd, J = 3.5, 10.3 Hz, 1 H, H-3B), 4.75 (d, J = 12.0 Hz, 1 H, CHHPh), 4.49 (d, J = 8.0 Hz, 1 H, H-1A), 4.39 (d, J = 12.0 Hz, 1 H, CHHPh), 4.33 (d, J = 8.0 Hz, 1 H, H-1B), 4.09–3.98 (m, 4 H, H-6Bab, COCH₂Cl), 3.92–3.86 (m, 5 H, H-2A, H-4A, OCHHCH₂, COCH₂Cl), 3.70 (m, 2 H, H-6Aab), 3.54 (t, J = 6.7 Hz, 1 H, H-5B), 3.50 (t, J = 6.6Hz, 2 H, CH₂Cl), 3.46 (m, 1 H, H-5A), 3.42 (m, 1 H, OCH*H*CH₂), 2.11, 1.95 (2 s, 6 H, 2 OCOCH₃), 1.92 (s, 3 H, NHCOCH₃), 1.73 (m, 2 H, O(CH₂)₄CH₂CH₂Cl), 1.55 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂Cl), 1.41 (m, 2 H, O(CH₂)₃CH₂(CH₂)₂Cl), 1.32 (m, 2 H, O(CH₂)₂CH₂(CH₂)₃Cl), 1.18 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 297 K): δ_C 177.8, 170.4, 170.2, 169.1, 167.5, 166.6 (C=O), 137.7 (quat Ar), 128.7, 128.2 (Ar), 100.9 (C-1A), 99.9 (C-1B), 74.4 (C-3A), 74.3 (C-4A), 74.2 (C-5A), 73.7 (CH₂Ph), 72.5 (C-3B), 70.5 (C-5B), 69.5 (OCH₂CH₂), 68.9 (C-2B), 67.3 (C-6A), 66.5 (C-4B), 60.6 (C-6B), 53.7 (C-2A), 45.0 (CH₂Cl), 40.9, 40.4 (COCH₂Cl), 38.8 (C(CH₃)₃), 32.5 (O(CH₂)₄CH₂CH₂Cl), 29.2 (OCH₂CH₂(CH₂)₃CH₂Cl), 27.1 (C(CH₃)₃), 26.5 (O(CH₂)₃CH₂(CH₂)₂Cl), 25.2 (O(CH₂)₂CH₂(CH₂)₃Cl), 23.3 (NHCOCH₃), 20.7, 20.6 (OCOCH₃). HRESIMS (*m/z*): [M+Na]⁺ calcd for C₄₀H₅₆Cl₃NO₁₆Na 934.2562, found 934.2585.

6-Chlorohexyl 2-acetamido-4-O-(2,4-di-O-acetyl-6-O-pivaloyl-β-D-

galactopyranosyl)-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (11).

Disaccharide **10** (6.75 g, 7.392 mmol) was dissolved in a mixture of pyridine and EtOH (2:1, 180 mL), thiourea (675.2 mg, 8.870 mmol, 1.2 equiv) was added and the solution was heated to 55 °C for 4 h, then allowed to come to rt. The reaction mixture was diluted with CHCl₃ (500 mL) and washed with HCl 2 N (1×600 mL). The aq layer was re-extracted with CHCl₃ (5×100 mL)

and the combined organic layers were dried and concentrated. The product was purified by column chromatography (CHCl₃/MeOH, 20:1) to give alcohol 11 (4.04 g, 65%) pure as a vellow amorphous foam. $[\alpha]_D = -3.1$ (c 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃, 296 K): δ_H 7.38–7.27 (m, 5 H, Ar), 5.57 (d, J = 9.0 Hz, 1 H, NHA), 5.18-5.12 (m, 2 H, H-3A, H-4B), 4.76-4.68 (m, 2 H, H-3A, H-4B)H, H-2B, CHHPh), 4.50 (d, J = 8.0 Hz, 1 H, H-1A), 4.43 (d, J = 12.0 Hz, 1 H, CHHPh), 4.34 (d, J = 8.0 Hz, 1 H, H-1B), 4.13–3.98 (m, 4 H, H-6Bab, COCH₂Cl), 3.92–3.80 (m, 3 H, H-2A, H-4A, OCHHCH₂), 3.75 (m, 2 H, H-6Aab), 3.58–3.49 (m, 6 H, H-5A, H-3B, H-5B, OCHHCH₂), CH_2CI), 2.48 (d, J = 6.0 Hz, 1 H, OH-3B), 2.14, 2.04 (2 s, 6 H, 2 OCOCH₃), 1.93 (s, 3 H, NHCOCH₃), 1.73 (m, 2 H, O(CH₂)₄CH₂CH₂Cl), 1.56 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂Cl), 1.41 (m, 2 H, O(CH₂)₃CH₂(CH₂)₂Cl), 1.33 (m, 2 H, O(CH₂)₂CH₂(CH₂)₃Cl), 1.19 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 296 K): δ_C 177.9, 170.9, 170.8, 170.1, 167.4 (C=O), 137.9 (quat Ar), 128.6, 128.0, 127.9 (Ar), 101.0 (C-1A), 99.9 (C-1B), 74.5 (C-3A), 74.5 (C-4A), 74.4 (C-5A), 73.6 (CH₂Ph), 73.1 (C-2B), 71.5 (C-3B), 70.9 (C-5B), 69.5 (OCH₂CH₂), 69.4 (C-4B), 67.5 (C-6A), 61.4 (C-6B), 53.9 (C-2A), 45.0 (CH₂Cl), 40.9 (COCH₂Cl), 38.8 (C(CH₃)₃), 32.5 (O(CH₂)₄CH₂CH₂Cl), 29.3 (OCH₂CH₂(CH₂)₃CH₂Cl), 27.1 (C(CH₃)₃), 26.5 (O(CH₂)₃CH₂(CH₂)₂Cl), 25.2 (O(CH₂)₂CH₂(CH₂)₃Cl), 23.4 (NHCOCH₃), 21.0, 20.7 (OCOCH₃). HRESIMS (m/z): $[M+H]^+$ calcd for C₃₈H₅₆Cl₂NO₁₅ 836.3027, found 836.3003.

6-Chlorohexyl 2-acetamido-4-*O*-[2,4-di-*O*-acetyl-6-*O*-pivaloyl-3-*O*-(4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-glactopyranosyl]-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (12). Disaccharide glycosyl acceptor 11 (40.0 mg, 0.04781 mmol) and known¹⁰ glycosyl donor 7 (90.8 mg, 0.1434 mmol, 3 equiv) were dissolved in dry CH_2Cl_2 (2.5 mL) under N_2 . After cooling the mixture to 0 °C, freshly distilled TMSOTf (17.3 µL, 0.09562 mmol, 2 equiv) was added to the

reaction, which was stirred at 0 °C for 40 min, then quenched with NEt ₃ (16.0 μ L, 0.1147 mmol,
2.4 equiv). The reaction was diluted with CH_2Cl_2 (15 mL) and washed with saturated aq
NaHCO ₃ (1 × 15 mL). The aq layer was re-extracted with CH ₂ Cl ₂ (3 × 10 mL) and the combined
organic layers were dried and concentrated. The product was purified by column
chromatography (EtOAc/hexanes, 1:1) to give trisaccharide 12 (49.2 mg, 79%) pure as a yellow
amorphous foam. $[\alpha]_D = 8.0 (c \ 1.0, CH_2Cl_2)$. ¹ H NMR (600 MHz, CDCl ₃ , 295 K): $\delta_H 7.40 = 7.27$
(m, 10 H, Ar), 7.19 (d, J = 8.8 Hz, 1 H, NHA'), 5.95 (d, J = 9.2 Hz, 1 H, NHA), 5.52 (t, J = 9.9
Hz, 1 H, H-3A'), 5.47 (s, 1 H, >C <i>H</i> Ph), 5.24 (d, <i>J</i> = 3.4 Hz, 1 H, H-4B), 5.07 (t, <i>J</i> = 9.1 Hz, 1 H,
H-3A), 4.91 (dd, <i>J</i> = 8.3, 9.7 Hz, 1 H, H-2B), 4.73 (d, <i>J</i> = 8.0 Hz, 1 H, H-1A'), 4.69 (d, <i>J</i> = 12.1
Hz, 1 H, C <i>H</i> HPh), 4.42 (d, <i>J</i> = 12.0 Hz, 1 H, C <i>H</i> HPh), 4.36 (d, <i>J</i> = 7.7 Hz, 1 H, H-1A), 4.31 (dd,
<i>J</i> = 4.9, 10.4 Hz, 1 H, H-6Aa'), 4.27 (d, <i>J</i> = 8.0 Hz, 1 H, H-1B), 4.11–3.92 (m, 7 H, H-2A, H-
6Bab, 2 COCH ₂ Cl), 3.87–3.80 (m, 2 H, H-4A, H-2A'), 3.78 (m, 1 H, OCHHCH ₂), 3.72–3.66 (m,
2 H, H-4A', H-6Ab'), 3.64 (m, 2 H, H-6ab), 3.59–3.54 (m, 2 H, H-3B, H-5B), 3.52–3.47 (m, 3
H, H-5A', CH ₂ Cl), 3.40 (m, 1 H, H-5A), 3.35 (m, 1 H, OCH <i>H</i> CH ₂), 2.04, 1.99 (2 s, 6 H, 2
OCOCH ₃), 1.92 (s, 3 H, NHCOCH ₃), 1.72 (m, 2 H, O(CH ₂) ₄ CH ₂ CH ₂ Cl), 1.53 (m, 2 H,
OCH ₂ CH ₂ (CH ₂) ₃ CH ₂ Cl), 1.39 (m, 2 H, O(CH ₂) ₃ CH ₂ (CH ₂) ₂ Cl), 1.31 (m, 2 H,
O(CH ₂) ₂ CH ₂ (CH ₂) ₃ Cl), 1.21 (s, 9 H, C(CH ₃) ₃). ¹³ C NMR (125 MHz, CDCl ₃ , 295 K): $\delta_{\rm C}$ 178.0,
170.1, 170.0, 169.0, 167.6, 167.5, 162.1 (C=O), 137.8, 136.5 (quat Ar), 129.2, 128.5, 128.3,
128.0, 125.9 (Ar), 101.2 (> <i>C</i> HPh) 101.0 (C-1A), 100.2 (C-1A'), 100.1 (C-1B), 92.1 (CCl ₃), 78.2
(C-4A'), 76.2 (C-3B), 74.4 (C-5A), 74.3 (C-3A), 74.1 (C-4A), 73.6 (CH ₂ Ph), 72.3 (C-3A'), 71.0
(C-5B), 70.6 (C-2B), 69.5 (OCH ₂ CH ₂), 68.6 (C-4B), 68.2 (C-6A'), 67.3 (C-6A), 66.0 (C-5A'),
61.6 (C-6B), 56.6 (C-2A'), 52.8 (C-2A), 45.0 (<i>C</i> H ₂ Cl), 40.8, 40.5 (CO <i>C</i> H ₂ Cl), 38.8 (<i>C</i> (CH ₃) ₃),
32.4 (O(CH ₂) ₄ CH ₂ CH ₂ Cl), 29.2 (OCH ₂ CH ₂ (CH ₂) ₃ CH ₂ Cl), 27.0 (C(CH ₃) ₃), 26.5

 $(O(CH_2)_3CH_2(CH_2)_2Cl)$, 25.2 $(O(CH_2)_2CH_2(CH_2)_3Cl)$, 23.2 $(NHCOCH_3)$, 21.1, 20.7 $(OCOCH_3)$. HRESIMS (m/z): $[M+H]^+$ calcd for $C_{55}H_{71}Cl_6N_2O_{21}$ 1305.2680, found 1305.2687.

6-Chlorohexyl 2-acetamido-4-*O*-[2,4-di-*O*-acetyl-6-*O*-pivaloyl-3-*O*-(4,6-*O*benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-6-*O*benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (13) and 6-chlorohexyl 2-acetamido-4-*O*-[2,4-di-*O*-acetyl-6-*O*-pivaloyl-3-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (14).

Method A: Trisaccharide **12** (514.9 mg, 0.394 mmol) was dissolved in a mixture of pyridine and EtOH (2:1, 21 mL), thiourea (36 mg, 0.473 mmol, 1.2 equiv) was added and the solution was heated to 55 °C for 7 h, then allowed to come to rt. The reaction mixture was diluted with CHCl₃ (100 mL) and washed with HCl 2 N (1 × 100 mL). The aq layer was re-extracted with CHCl₃ (5 × 25 mL) and the combined organic layers were dried and concentrated. Reversed-phase HPLC of the residue (CH₃CN/H₂O, 40:60 to 95:5, 30 minutes) gave trisaccharide **13** (178.8 mg, 36%) pure as a white amorphous foam and trisaccharide **14** (33 mg, 7%) pure as a slightly yellowish amorphous foam. Analytical data for diol **14** were reported previously.¹³

Method B: Trisaccharide **12** (500 mg, 0.382 mmol) was dissolved in a mixture of pyridine and EtOH (1:1, 40 mL), thiourea (291 mg, 3.823 mmol, 10 equiv) was added and the solution was heated to 70 °C for 19 h, then allowed to come to rt. The reaction mixture was diluted with CHCl₃ (150 mL) and washed with HCl 2 N (1 × 150 mL). The aq layer was re-extracted with CHCl₃ (5 × 20 mL) and the combined organic layers were dried and concentrated. The product was purified by column chromatography (CH₂Cl₂/MeOH, 20:1) to give diol **14** (338.2 mg, 77%)

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pure as a slightly yellowish amorphous foam. Analytical data for diol **14** were reported previously.¹³

Analytical data for 13: $[\alpha]_D - 7.2$ (c 1.0, CH₂Cl₂). ¹H NMR (CDCl₃, 600 MHz, 296 K): δ_H 7.46– 7.42 (m, 2 H, Ar), 7.39–7.28 (m, 8 H, Ar), 7.20 (d, J = 7.2 Hz, 1 H, NHA'), 5.74 (d, J = 9.0 Hz, 1 H, NHA), 5.51 (s, 1 H, >CHPh), 5.26 (d, J = 3.4 Hz, 1 H, H-4B), 5.11 (t, J = 9.1 Hz, 1 H, H-3A), 5.06 (d, J = 8.0 Hz, 1 H, H-1A'), 4.92 (dd, J = 8.2, 9.8 Hz, 1 H, H-2B), 4.70 (d, J = 12.1Hz, 1 H, CHHPh), 4.44 (d, J = 7.7 Hz, 1 H, H-1A), 4.39 (d, J = 12.1 Hz, 1 H, CHHPh), 4.34– 4.28 (m, 2 H, H-3A', H6Aa'), 4.20 (d, J = 8.0 Hz, 1 H, H-1B), 4.12-4.01 (m, 3 H, H-6Ba, H-6Ba) $COCH_2CI$), 3.92 (dd, J = 8.1, 11.5 Hz, 1 H, H-6Bb), 3.87–3.80 (m, 2 H, H-2A, H-4A), 3.77 (m, 1 H, OCHHCH₂), 3.70 (t, J = 9.9 Hz, 1 H, H-6Ab²), 3.66 (m, 2 H, H-6Aab), 3.61 (dd, J = 3.4, 10.0 Hz, 1 H, H-3B), 3.54–3.40 (m, 6 H, H-5A, H-5B, H-4A', H-5A', CH₂Cl), 3.37–3.28 (m, 2 H, H-2A', OCH*H*CH₂), 3.12 (br s, 1 H, OH-3A'), 2.03, 2.02 (2 s, 6 H, 2 OCOCH₃), 1.91 (s, 3 H, NHCOCH₃), 1.72 (m, 2 H, O(CH₂)₄CH₂CH₂Cl), 1.52 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂Cl), 1.40 (m, 2 H, O(CH₂)₃CH₂(CH₂)₂Cl), 1.30 (m, 2 H, O(CH₂)₂CH₂(CH₂)₃Cl), 1.20 (s, 9 H, C(CH₃)₃). ¹³C NMR (125 MHz, CDCl₃, 296 K): $\delta_{\rm C}$ 178.0, 170.2, 170.0, 169.2, 167.4, 162.2 (C=O), 137.8, 136.9 (quat Ar), 129.3, 128.6, 128.3, 128.1, 126.2 (Ar), 101.8 (>CHPh), 100.8 (C-1A), 100.1 (C-1B), 99.5 (C-1A'), 92.4 (CCl₃), 81.3 (C-4A'), 76.4 (C-3B), 74.3 (C-5A), 74.1 (C-3A), 73.9 (C-4A), 73.6 (CH₂Ph), 71.1 (C-5B), 70.6 (C-2B), 69.4 (OCH₂CH₂), 68.9 (C-4B), 68.6 (C-3A'), 68.4 (C-6A'), 67.3 (C-6A), 66.0 (C-5A'), 61.6 (C-6B), 59.8 (C-2A'), 53.4 (C-2A), 45.0 (CH₂Cl), 40.8 (COCH₂Cl), 38.8 (C(CH₃)₃), 32.4 (O(CH₂)₄CH₂CH₂Cl), 29.2 (OCH₂CH₂(CH₂)₃CH₂Cl), 27.1 (C(CH₃)₃), 26.5 (O(CH₂)₃CH₂(CH₂)₂Cl), 25.1 (O(CH₂)₂CH₂(CH₂)₃Cl), 23.3 (NHCOCH₃), 21.1, 20.7 (OCOCH₃). HRESIMS(m/z): $[M + H]^+$ calcd for C₅₃H₇₀Cl₅N₂O₂₀ 1229.2965, found 1229.2960.

6-Chlorohexyl 2-acetamido-4-O-[2,4-di-O-acetyl-6-O-pivaloyl-3-O-(4,6-O-

benzylidene-3-O-chloroacetyl-2-deoxy-2-trichloroacetamido-B-D-glucopyranosyl)-B-Dgalactopyranosyl]-6-O-benzyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-Dglucopyranoside (15). Chloroacetyl chloride (111 µL, 1.394 mmol, 1 equiv) was slowly added at 0 °C to a stirred solution of the trisaccharide 14 (1.61 g, 1.394 mmol) in anhyd CH₂Cl₂ (280 mL) containing dry pyridine (225 µL, 2.79 mmol, 2 equiv) under N₂. The reaction was left at 0 °C for 2 h, then washed sequentially with HCl 2 N ($1 \times 100 \text{ mL}$) and saturated ag NaHCO₃ ($1 \times$ 100 mL). The aq layers were re-extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were dried and concentrated to give the previously described¹³ selectively chloroacetylated trisaccharide (1.72 g, quant.) as a slightly yellowish amorphous foam. The trisaccharide (1.72 g, 1.397 mmol) and known¹¹ thioglycoside donor 8 (2.00 g, 4.191 mmol, 3 equiv) in dry CH₂Cl₂ (170 mL) containing freshly activated molecular sieves 4 Å (17 g) were stirred under N₂ for 3 h at rt. MeOTf (790 µL, 6.984 mmol, 5 equiv) was added to the reaction mixture, which was stirred at rt for 30 min, then guenched with NEt₃ (1.17 mL, 8.381 mmol, 6 equiv) and filtered over Celite[®]. The solids were washed with CH_2Cl_2 (4 × 50 mL) and the combined filtrate and washings were washed with saturated aq NaHCO₃ (1×300 mL). The aq layer was re-extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were dried and concentrated. The resulting residue was dissolved in a mixture of Ac₂O and AcOH (3:1, 100 mL) and the solution was stirred at rt for 75 min, then co-concentrated with toluene (3×80 mL). The product was purified by column chromatography (EtOAc/hexanes, 4:6 then 1:1) to give tetrasaccharide 15 (1.94 g, 84%) pure as white amorphous glass. $[\alpha]_D$ –55.2 (c 1.0, CH₂Cl₂). ¹H NMR (CDCl₃, 600 MHz, 295 K): $\delta_{\rm H}$ 7.43–7.39 (m, 2 H, Ar), 7.37–7.20 (m, 23 H, Ar), 6.85 (d, J = 8.8 Hz, 1 H, NHA'), 5.92 (d, J = 7.6 Hz, 1 H, NHA), 5.50 (s, 1 H, >CHPh), 5.46 (t, J = 10.3

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Hz, 1 H, H-3A'), 5.24 (d, <i>J</i> = 3.1 Hz, 1 H, H-4B), 5.07 (d, <i>J</i> = 3.1 Hz, 1 H, H-1C), 4.98–4.91 (m,
2 H, H-2B, CHHPh), 4.85–4.77 (m, 3 H, H-1A, 2 CHHPh), 4.74–4.68 (m, 3 H, H-1A', 2
CHHPH), 4.65 (d, J = 11.8 Hz, 1 H, CHHPh), 4.62 (d, J = 12.0 Hz, 1 H, CHHPh), 4.42–4.38 (m,
2 H, H-1B, C <i>H</i> HPh), 4.33 (dd, <i>J</i> = 4.9, 10.4 Hz, 1 H, H-6Aa'), 4.23 (m, 1 H, H-5C), 4.11–3.98
(m, 5 H, H-3A, H-6Ba, H-2C, $COCH_2Cl$), 3.94 (dd, $J = 6.8$, 11.0 Hz, 1 H, H-6Bb), 3.89-3.72
(m, 7 H, H-4A, H-6Aab, H-3C, H-2A', H-4A', H-6Ab'), 3.68 (m, 1 H, OC <i>H</i> HCH ₂), 3.64 (dd, <i>J</i>
= 3.5, 10.0 Hz, 1 H, H-3B), 3.61–3.56 (m, 2 H, H-5B, H-4C), 3.55–3.48 (m, 3 H, H-2A, H-5A,
H-5A'), 3.45 (t, <i>J</i> = 6.7 Hz, 2 H, CH ₂ Cl), 3.35 (m, 1 H, OCH <i>H</i> CH ₂), 2.02, 1.97 (2 s, 6 H, 2
OCOCH ₃), 1.76 (s, 3 H, NHCOCH ₃), 1.67 (m, 2 H, O(CH ₂) ₄ CH ₂ CH ₂ Cl), 1.45 (m, 2 H,
OCH ₂ CH ₂ (CH ₂) ₃ CH ₂ Cl), 1.33 (m, 2 H, O(CH ₂) ₃ CH ₂ (CH ₂) ₂ Cl), 1.23 (m, 2 H,
$O(CH_2)_2CH_2(CH_2)_3Cl)$, 1.18 (s, 9 H, $C(CH_3)_3$), 1.11 (d, $J = 6.4$ Hz, 3 H, H-6C). ¹³ C NMR (125)
MHz, CDCl ₃ , 295 K): <i>δ</i> _C 177.7, 170.1, 169.7, 169.1, 167.4, 162.1 (C=O), 138.9, 138.5, 138.1,
136.5 (quat Ar), 129.3, 128.4, 128.3, 128.2, 127.8, 127.6, 127.5, 127.3, 127.0, 126.0 (Ar), 101.4
(>CHPh), 100.1 (C-1A'), 99.5 (C-1A), 99.3 (C-1B), 96.8 (C-1C), 92.0 (CCl ₃), 79.8 (C-3C), 78.1
(C-4A'), 77.2 (C-4C), 76.6 (C-2C), 75.1 (C-3B), 74.4 (CH ₂ Ph), 74.2 (C-5A), 73.7 (C-4A), 73.5
(CH ₂ Ph) 73.2 (CH ₂ Ph), 72.9 (C-3A), 72.6 (CH ₂ Ph), 71.9 (C-3A'), 70.9 (C-2B), 70.7 (C-5B),
69.3 (OCH ₂ CH ₂), 68.7 (C-6A), 68.6 (C-4B), 68.2 (C-6A'), 66.5 (C-5C), 66.3 (C-5A'), 61.0 (C-
6B), 56.6 (C-2A'), 54.6 (C-2A), 45.0 (<i>C</i> H ₂ Cl), 40.4 (CO <i>C</i> H ₂ Cl), 38.7 (<i>C</i> (CH ₃) ₃), 32.5
(O(CH ₂) ₄ CH ₂ CH ₂ Cl), 29.2 (OCH ₂ CH ₂ (CH ₂) ₃ CH ₂ Cl), 27.1 (C(CH ₃) ₃), 26.5
(O(CH ₂) ₃ CH ₂ (CH ₂) ₂ Cl), 25.2 (O(CH ₂) ₂ CH ₂ (CH ₂) ₃ Cl), 23.1 (NHCOCH ₃), 21.1, 20.7 (OCOCH ₃),
16.6 (C-6C). HRESIMS (m/z) : $[M + H]^+$ calcd for C ₈₀ H ₉₈ Cl ₅ N ₂ O ₂₄ 1645.495, found 1645.490.

6-Chlorohexyl 2-acetamido-4-*O*-[2,4-di-*O*-acetyl-6-*O*-pivaloyl-3-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-6-*O*-

benzyl-3-*O***-(2,3,4-tri-***O***-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (16).** Tetrasaccharide **15** (1.94 g, 1.177 mmol) was dissolved in a mixture of pyridine and EtOH (1:1, 100 mL), thiourea (896 mg, 11.77 mmol, 10 equiv) was added and the solution was heated to 70 °C for 7 h, then allowed to come to rt. The reaction mixture was diluted with CHCl₃ (200 mL) and washed with HCl 2 N (1 × 300 mL). The aq layer was re-extracted with CHCl₃ (5 × 60 mL) and the combined organic layers were dried and concentrated. The product was purified by column chromatography (CH₂Cl₂/MeOH, 20:1) to give the previously described¹³ alcohol **16** (1.62 g, 88%) pure as a white amorphous foam.

6-Chlorohexyl 2-acetamido-4-O-{2,4-di-O-acetyl-6-O-pivaloyl-3-O-[4,6-Obenzylidene-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2trichloroacetamido-β-D-glucopyranosyl]-β-D-galactopyranosyl}-6-O-benzyl-3-O-(2,3,4-tri-**O-benzyl-\alpha-L-fucopyranosyl)-2-deoxy-\beta-D-glucopyranoside (17).** Freshly distilled BF₃·OEt₂ $(16 \,\mu\text{L}, 0.1273 \,\text{mmol}, 2 \,\text{equiv})$ was added to a stirred solution of alcohol **16** (100 mg, 0.06365) mmol) and known¹² galactosyl trichloroacetimidate 9 (156.8 mg, 0.3182 mmol, 5 equiv) in dry CH_2Cl_2 (5 mL) at rt under N₂. The reaction was left to proceed for 1 h at rt, then quenched with NEt₃ (21.2 μ L, 0.1528 mmol, 2.4 equiv). The solvent was evaporated and a column chromatography (EtOAc/hexanes, 1:1 then 7:3) followed by a reverse-phase HPLC (CH₃CN/H₂O, 75:25 to 85:15, 30 min) of the residue gave recovered glycosyl acceptor 16 (20.1 mg, 20%) and pentasaccharide 17 (62.5 mg, 52%) pure as an amorphous colorless glass. $[\alpha]_D$ – 29.0 (c 1.0, CH₂Cl₂). ¹H NMR (CDCl₃, 600 MHz, 295 K): $\delta_{\rm H}$ 7.47–7.43 (m, 2 H, Ar), 7.38–7.22 (m, 23 H, Ar), 6.98 (d, J = 7.0 Hz, 1 H, NHA'), 5.90 (d, J = 7.6 Hz, 1 H, NHA), 5.53 (s, 1 H, >CHPh), 5.29 (d, J = 2.8 Hz, 1 H, H-4B'), 5.26 (d, J = 3.4 Hz, 1 H, H-4B), 5.19 (d, J = 7.8 Hz, 1 H, H-1A'), 5.12 (dd, J = 7.9, 10.3 Hz, 1 H, H-2B'), 5.06 (d, J = 3.7 Hz, 1 H, H-1C), 4.96–4.92

(m, 2 H, H-2B, CHHPh), 4.90 (dd, J = 3.4, 10.4 Hz, 1 H, H-3B'), 4.83 (d, J = 11.9 Hz, 1 H, H-3B')CHHPh), 4.82–4.78 (m, 2 H, H-1A, CHHPh), 4.75–4.68 (m, 3 H, H-1B', 2 CHHPH), 4.66 (d, J = 11.9 Hz, 1 H, CHHPh), 4.60 (d, J = 12.1 Hz, 1 H, CHHPh), 4.52 (t, J = 9.7 Hz, 1 H, H-3A'), 4.40-4.36 (m, 2 H, H-1B, CHHPh), 4.33 (dd, J = 4.9, 10.6 Hz, 1 H, H-6Aa'), 4.23 (m, 1 H, H-5C), 4.11-4.05 (m, 3 H, H-3A, H-2C, H-6Ba'), 4.03 (dd, J = 7.0, 11.3 Hz, 1 H, H-6Ba), 3.97 (dd, J = 6.9, 11.3 Hz, 1 H, H-6Bb'), 3.91 (dd, J = 6.8, 11.1 Hz, 1 H, H-6Bb), 3.86 (dd, J = 2.5, 11.1 Hz, 1 Hz, 11.1 Hz, 11 H10.1 Hz, 1 H, H-3C), 3.84–3.80 (m, 2 H, H-4A, H-6Aa), 3.79–3.64 (m, 6 H, H-3B, H-6Ab, H-4A', H-6Ab', H-5B', OCHHCH₂), 3.57 (s, 1 H, H-4C), 3.55–3.44 (m, 6 H, H-2A, H-5A, H-5B, H-5A', CH₂Cl), 3.35 (m, 1 H, OCH*H*CH₂), 3.24 (m, 1 H, H-2A'), 2.09, 2.02, 1.98, 1.93, 1.92, 1.76 (6 s, 18 H, 6 OCOCH₃), 1.74 (s, 3 H, NHCOCH₃), 1.67 (m, 2 H, O(CH₂)₄CH₂CH₂Cl), 1.45 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂Cl), 1.33 (m, 2 H, O(CH₂)₃CH₂(CH₂)₂Cl), 1.23 (m, 2 H, $O(CH_2)_2CH_2(CH_2)_3Cl)$, 1.18 (s, 9 H, $C(CH_3)_3$), 1.11 (d, J = 6.5 Hz, 3 H, H-6C). ¹³C NMR (125) MHz, CDCl₃, 295 K): $\delta_{\rm C}$ 177.6, 170.3, 170.1, 169.4, 161.9 (C=O), 139.0, 138.9, 138.6, 138.1, 136.8 (quat Ar), 129.4, 128.5, 128.4, 128.3, 128.2, 127.8, 127.6, 127.5, 127.4, 127.1, 126.2 (Ar), 101.6 (>CHPh), 99.5 (C-1A, C-1B), 99.0 (C-1A'), 98.5 (C-1B'), 96.9 (C-1C), 92.2 (CCl₃), 79.8 (C-3C), 78.1 (C-4A'), 77.1 (C-4C), 76.6 (C-2C), 75.9 (C-3B), 75.0 (C-3A'), 74.4 (CH₂Ph), 74.1 (C-5A), 73.7 (C-4A), 73.4 (CH₂Ph) 73.3 (CH₂Ph), 72.9 (C-3A), 72.6 (CH₂Ph), 70.9 (C-5B, C-3B'), 70.7 (C-5B'), 70.4 (C-2B), 69.3 (OCH₂CH₂), 69.1 (C-4B), 68.8 (C-2B'), 68.7 (C-6A), 68.4 (C-6A'), 66.8 (C-4B'), 66.5 (C-5C), 66.2 (C-5A'), 61.4 (C-6B'), 60.9 (C-6B), 59.1 (C-2A'), 54.7 (C-2A), 45.0 (CH₂Cl), 38.7 (C(CH₃)₃), 32.5 (O(CH₂)₄CH₂CH₂Cl), 29.2 (OCH₂CH₂(CH₂)₃CH₂Cl), 27.1 (C(CH₃)₃), 26.6 (O(CH₂)₃CH₂(CH₂)₂Cl), 25.2 (O(CH₂)₂CH₂(CH₂)₃Cl), 23.1 (NHCOCH₃), 21.2, 20.8, 20.7, 20.6, 20.5 (OCOCH₃), 16.7 (C-6C). HRESIMS (m/z): $[M + NH_4]^+$ calcd for C₉₂H₁₁₈Cl₄N₃O₃₂ 1916.6453, found 1916.6445.

6-Chlorohexyl 2-acetamido-4-O-{2,4-di-O-acetyl-6-O-pivaloyl-3-O-[6-O-benzyl-3-O-

(2,3,4,6-tetra-O-acetyl-B-D-galactopyranosyl)-2-deoxy-2-trichloroacetamido-B-Dglucopyranosyl]-β-D-galactopyranosyl}-6-O-benzyl-3-O-(2.3,4-tri-O-benzyl-α-Lfucopyranosyl)-2-deoxy- β -D-glucopyranoside (18). A solution of the benzylidene acetal 17 (117 mg, 0.06150 mmol) in dry THF (6 mL) containing freshly activated molecular sieves 3 Å (900 mg), NaCNBH₃ (58.0 mg, 0.9225 mmol, 15 equiv) and methyl orange indicator (2 mg) was stirred under N₂ for 1 h at rt and cooled to 0 °C. A 2 M solution of HCl in Et₂O (461 µL, 0.9225 mmol, 15 equiv) was added drop wise to the reaction mixture at 0 °C until the methyl orange indicator turned pink, remained as such for 10 min, and $H_2(g)$ was no longer generated. The reaction was stirred at rt for 2.5 h, more NaCNBH₃ (58.0 mg, 0.9225 mmol, 15 equiv) and 2 M solution of HCl in Et₂O (461 µL, 0.9225 mmol, 15 equiv) were added and the reaction was left to proceed at rt for an additional hour, then was filtered over Celite[®]. The solids were washed with THF (3×15 mL) and the combined filtrate and washings were concentrated. The residue was dissolved in CH₂Cl₂ (30 mL) and washed with saturated ag NaHCO₃ (1×30 mL). The ag layer was re-extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic layers were dried and concentrated. Column chromatography (CH₂Cl₂/MeOH, 30:1) followed by RP HPLC (CH₃CN/H₂O, 75:25 to 85:15, 30 min) of the residue gave alcohol **18** (73.0 mg, 62%) pure as an amorphous white foam. $[\alpha]_D = 24.2$ (c 1.0, CH₂Cl₂). ¹H NMR (CDCl₃, 600 MHz, 295 K): δ_H 7.36–7.20 (m, 25 H, Ar), 6.86 (d, J = 7.2 Hz, 1 H, NHA'), 5.89 (d, J = 7.4 Hz, 1 H, NHA), 5.35 $(d, J = 3.2 \text{ Hz}, 1 \text{ H}, \text{H-4B}^2), 5.33 (d, J = 3.5 \text{ Hz}, 1 \text{ H}, \text{H-4B}), 5.20 (dd, J = 8.0, 10.5 \text{ Hz}, 1 \text{ H}, \text{H-4B})$ 2B'), 5.04 (d, J = 3.7 Hz, 1 H, H-1C), 4.97–4.83 (m, 6 H, H-1A, H-2B, H-1A', H-3B', 2 CHHPh), 4.79 (d, J = 11.8 Hz, 1 H, CHHPh), 4.71 (d, J = 11.8 Hz, 1 H, CHHPh), 4.67 (d, J = 11.9 Hz, 1 H, CHHPh), 4.64 (d, J = 12.5 Hz, 1 H, CHHPh), 4.62–4.57 (m, 3 H, CH₂Ph, CHHPh),

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4.51 (d, <i>J</i> = 8.0 Hz, 1 H, H-1B'), 4 34 (d, <i>J</i> = 12.1 Hz, 1 H, C <i>H</i> HPh), 4.30–4.24 (m, 2 H, H-1B,
H-5C), 4.18 (dd, <i>J</i> = 7.6, 10.0 Hz, 1 H, H-3A'), 4.12–4.03 (m, 4 H, H-3A, H-2C, H-6Bab'), 4.01
(dd, J = 5.9, 11.3 Hz, 1 H, H-6Ba), 3.95 (t, J = 6.5 Hz, 1 H, H-5B'), 3.90-3.79 (m, 3 H, H-6Bb),
H-3C, H-6Aa'), 3.81–3.76 (m, 2 H, H-4A, H-6Aa), 3.75–3.63 (m, 5 H, H-3B, H-6Ab, OH-4A',
H-6Ab', OC <i>H</i> HCH ₂), 3.58 (s, 1 H, H-4C), 3.53–3.43 (m, 6 H, H-5A, H-5B, H-4A', H-5A',
CH ₂ Cl), 3.41–3.32 (m, 2 H, H-2A, OCH <i>H</i> CH ₂), 3.21 (m, 1 H, H-2A'), 2.14, 2.06, 2.02, 2.00,
1.95, 1.89 (6 s, 18 H, 6 OCOCH ₃), 1.72 (s, 3 H, NHCOCH ₃), 1.67 (m, 2 H, O(CH ₂) ₄ CH ₂ CH ₂ Cl),
1.46 (m, 2 H, OCH ₂ CH ₂ (CH ₂) ₃ CH ₂ Cl), 1.33 (m, 2 H, O(CH ₂) ₃ CH ₂ (CH ₂) ₂ Cl), 1.23 (m, 2 H,
$O(CH_2)_2CH_2(CH_2)_3Cl)$, 1.17 (s, 9 H, $C(CH_3)_3$), 1.11 (d, $J = 6.4$ Hz, 3 H, H-6C). ¹³ C NMR (125
MHz, CDCl ₃ , 295 K): <i>δ</i> _C 177.6, 170.4, 170.1, 170.0, 169.5, 169.4, 161.8 (C=O), 139.0, 138.9,
138.6, 138.2, 137.8 (quat Ar), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.6, 127.5, 127.4,
127.3 (Ar), 100.7 (C-1B'), 99.5 (C-1A), 99.3 (C-1B), 98.3 (C-1A'), 97.0 (C-1C), 92.1 (CCl ₃),
81.4 (C-3A'), 79.9 (C-3C), 77.1 (C-4C), 76.6 (C-2C), 75.4 (C-5A'), 75.1 (C-3B), 74.4 (CH ₂ Ph),
74.1 (C-5A), 73.6 (C-4A), 73.5 (CH ₂ Ph) 73.4 (CH ₂ Ph), 73.3 (CH ₂ Ph), 73.0 (C-3A), 72.5
(CH ₂ Ph), 71.1 (C-5B, C-5B'), 70.9 (C-2B), 70.7 (C-3B'), 69.7 (C-6A'), 69.4 (C-4B), 69.3
(OCH ₂ CH ₂), 69.2 (C-4A'), 68.5 (C-6A, C-2B'), 66.7 (C-4B'), 66.4 (C-5C), 61.6 (C-6B), 61.2
(C-6B'), 58.5 (C-2A'), 55.1 (C-2A), 45.0 (<i>C</i> H ₂ Cl), 38.7 (<i>C</i> (CH ₃) ₃), 32.5 (O(CH ₂) ₄ CH ₂ CH ₂ Cl),
29.2 (OCH ₂ CH ₂ (CH ₂) ₃ CH ₂ Cl), 27.1 (C(CH ₃) ₃), 26.6 (O(CH ₂) ₃ CH ₂ (CH ₂) ₂ Cl), 25.2
(O(CH ₂) ₂ CH ₂ (CH ₂) ₃ Cl), 23.1 (NHCOCH ₃), 21.3, 20.9, 20.8, 20.6, 20.5 (OCOCH ₃), 16.7 (C-6C).
HRESIMS (m/z) : $[M + H]^+$ calcd for C ₉₂ H ₁₁₇ Cl ₄ N ₂ O ₃₂ 1901.634, found 1901.628.

 $\label{eq:2.4.4} 6-Chlorohexyl 2-acetamido-4-O-\{2,4-di-O-acetyl-6-O-pivaloyl-3-O-[6-O-benzyl-3-O-(2,3,4,6-tetra-O-acetyl-\beta-D-galactopyranosyl)-4-O-(2,3,4-tri-O-benzyl-\alpha-L-fucopyranosyl)-2-deoxy-2-trichloroacetamido-\beta-D-glucopyranosyl]-\beta-D-galactopyranosyl}-6-O-benzyl-3-O-(2,3,4-tri-O-benzyl-3-O-benzyl-3-O-benzyl-3-O-(2,3,4-tri-O-benzyl-3-O-b$

(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (19). A solution of the glycosyl acceptor **18** (32.6 mg, 0.01712 mmol) and known¹¹ thioglycoside donor **8** (40.9 mg, 0.08559 mmol, 5 equiv) in dry CH₂Cl₂ (2 mL) containing freshly activated molecular sieves 4 Å (100 mg) was stirred under N₂ for 1 h 30 min at rt. MeOTf (9.7 µL, 0.08559 mmol, 5 equiv) was added to the reaction mixture, which was stirred at rt for 40 min, then guenched with NEt₃ (14.3 μ L, 0.1027 mmol, 6 equiv) and filtered over Celite[®]. The solids were washed with CH₂Cl₂ (3 × 10 mL) and the combined filtrate and washings were washed with saturated aq NaHCO₃ (1×15 mL). The aq layer was re-extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were dried and concentrated. The resulting residue was dissolved in a mixture of Ac₂O and AcOH (3:1, 2 mL) and the solution was stirred at rt for 16 h, then co-concentrated with toluene $(3 \times 3 \text{ mL})$. Column chromatography (EtOAc/hexanes, 4:6 then 1:1) followed by RP HPLC $(CH_3CN/H_2O, 90:10 \text{ to } 95:5, 30 \text{ min})$ of the residue gave hexasaccharide **19** (30.1 mg, 76%) pure as an amorphous white foam. $[\alpha]_D$ –46.0 (c 1.0, CH₂Cl₂). ¹H NMR (CDCl₃, 600 MHz, 295 K): δ_H 7.41–7.39 (m, 2 H, Ar), 7.36–7.20 (m, 38 H, Ar), 6.72 (d, J = 8.2 Hz, 1 H, NHA'), 5.92 (d, J =7.4 Hz, 1 H, NHA), 5.32 (d, J = 3.2 Hz, 1 H, H-4B'), 5.20 (d, J = 3.6 Hz, 1 H, H-4B), 5.08 (dd, J = 8.2, 10.4 Hz, 1 H, H-2B'), 5.06 (d, J = 3.7 Hz, 1 H, H-1C), 5.03 (d, J = 3.7 Hz, 1 H, H-1C'),4.97-4.90 (m, 3 H, H-2B, 2 CHHPh), 4.87-4.77 (m, 6 H, H-1A, H-3B', 4 CHHPh), 4.76-4.65 (m, 6 H, H-1A', H-1B', 4 CHHPh), 4.66-4.62 (m, 2 H, 2 CHHPh), 4.59 (d, J = 12.1 Hz, 1 H, 1)*CH*HPh), 4.46 (m, 1 H, H-5C'), 4.41 (m, 2 H, CH₂Ph), 4 35 (d, *J* = 12.1 Hz, 1 H, *CH*HPh), 4.29 (d, J = 8.0 Hz, 1 H, H-1B), 4.22 (m, 1 H, H-5C), 4.14–3.98 (m, 7 H, H-3A, H-6Ba, H-2C, H-3A', H-6Bab', H-2C'), 3.96 (t, J = 7.9 Hz, 1 H, H-4A'), 3.90–3.85 (m, 3 H, H-6Bb, H-3C, H-3C'), 3.83–3.78 (m, 3 H, H-4A, H-6Aa, H-5B'), 3.77 (dd, J = 4.0, 10.7 Hz, 1 H, H-6Aa'), 3.74– 3.62 (m, 5 H, H-3B, H-6Ab, H-6Ab', H-4C', OCHHCH₂), 3.60–3.52 (m, 3 H, H-4C, H-2A', H-

 5A'), 3.50 (m, 1 H, H-5A), 3.48–3.43 (m, 4 H, H-2A, H-5B, CH₂Cl), 3.35 (m, 1 H, OCHHCH₂), 2.04, 1.99, 1.92, 1.87, 1.85 (6 s, 18 H, 6 OCOCH₃), 1.74 (s, 3 H, NHCOCH₃), 1.67 (m, 2 H, O(CH₂)₄CH₂CH₂Cl), 1.45 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂Cl), 1.33 (m, 2 H, O(CH₂)₃CH₂(CH₂)₂Cl), 1.26–1.21 (m, 5 H, H-6C', O(CH₂)₂CH₂(CH₂)₃Cl), 1.16 (s, 9 H, $C(CH_3)_3$, 1.09 (d, J = 6.4 Hz, 3 H, H-6C). ¹³C NMR (125 MHz, CDCl₃, 295 K): δ_C 177.7, 170.1, 169.9, 169.8, 169.7, 169.6, 169.4, 161.3 (C=O), 139.0, 138.9, 138.5, 138.2, 138.1, 137.9 (quat Ar), 128.5, 128.4, 128.3, 128.2, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 127.0 (Ar), 100.0 (C-1B'), 99.5 (C-1A), 99.2 (C-1B), 98.6 (C-1A'), 97.3 (C-1C'), 96.8 (C-1C), 92.3 (CCl₃), 80.5 (C-3C'), 79.8 (C-3C), 77.2 (C-4C), 76.6 (C-2C), 76.4 (C-3A', C-4C'), 76.6 (C-5A', C-2C'), 74.7 (CH₂Ph), 74.5 (C-3B), 74.5 (CH₂Ph), 74.4 (CH₂Ph), 74.0 (C-5A), 73.6 (C-4A), 73.5 (CH₂Ph), 73.2 (CH₂Ph), 72.9 (CH₂Ph), 72.8 (C-3A), 72.6 (CH₂Ph), 72.2 (CH₂Ph), 71.8 (C-4A'), 71.5 (C-2B), 71.2 (C-5B), 70.8 (C-3B'), 70.5 (C-5B'), 69.3 (OCH₂CH₂), 68.7 (C-6A), 68.6 (C-4B), 68.1 (C-2B'), 67.8 (C-6A'), 66.7 (C-4B'), 66.5 (C-5C, C-5C'), 61.6 (C-6B), 60.1 (C-6B'), 58.2 (C-2A'), 54.5 (C-2A), 45.0 (CH₂Cl), 38.7 (C(CH₃)₃), 32.5 (O(CH₂)₄CH₂CH₂Cl), 29.2 (OCH₂CH₂(CH₂)₃CH₂Cl), 27.1 (C(CH₃)₃), 26.6 (O(CH₂)₃CH₂(CH₂)₂Cl), 25.2 (O(CH₂)₂CH₂(CH₂)₃Cl), 23.1 (NHCOCH₃), 21.2, 20.7, 20.6, 20.5 (OCOCH₃), 16.8 (C-6C'), 16.7 (C-6C). HRESIMS (m/z): $[M + H]^+$ calcd for C₁₁₉H₁₄₅Cl₄N₂O₃₆ 2317.833, found 2317.827.

6-Chlorohexyl 2-acetamido-4-*O*-{2,4-di-*O*-acetyl-6-*O*-pivaloyl-3-*O*-[6-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2acetamido-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranosyl}-6-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (20). To a solution of hexasaccharide 19 (62.6 mg, 0.02698 mmol) dissolved in AcOH (3.0 mL) was added freshly activated Zn (176 mg, 2.698 mmol, 100 equiv). The reaction mixture was heated to 50 °C under

sonication for 4 h, more Zn (176 mg, 2.698 mmol, 100 equiv) was added and the reaction was left to proceed at 50 °C under sonication for an additional 3 h, then filtered over Celite[®]. The solids were washed with CH_2Cl_2 (2 × 50 mL) and the combined filtrate and washings were washed with saturated aq NaHCO₃ (1 \times 60 mL). The aq layer was re-extracted with CH₂Cl₂ (3 \times 30 mL) and the combined organic layers were dried and concentrated to give N-acetamide 20 (56.8 mg, 95%) pure as a white amorphous foam. $[\alpha]_D$ –41.8 (*c* 1.0, CH₂Cl₂). ¹H NMR (CDCl₃, 600 MHz, 295 K): $\delta_{\rm H}$ 7.44–7.41 (m, 2 H, Ar), 7.38–7.21 (m, 38 H, Ar), 5.79 (d, J = 7.1 Hz, 1 H, NHA), 5.57 (d, J = 7.3 Hz, 1 H, NHA'), 5.33 (d, J = 3.2 Hz, 1 H, H-4B'), 5.25 (d, J = 3.6 Hz, 1 H, H-4B), 5.09–5.02 (m, 3 H, H-1C, H-2B', H-1C'), 4.98–4.91 (m, 4 H, H-1A, 3 CHHPh), 4.90-4.84 (m, 3 H, H-2B, H-3B', 1 CHHPh), 4.83-4.76 (m, 4 H, H-1A', 3 CHHPh), 4.75-4.71 (m, 2 H, 2 CHHPh), 4.70–4.60 (m, 6 H, H-1B', H-5C', 4 CHHPh), 4.45 (d, J = 12.1 Hz, 1 H, CHHPh), 4.41–4.33 (m, 4 H, H-5C, H-3A', 2 CHHPh), 4.31 (d, J = 8.2 Hz, 1 H, H-1B), 4.17– 4.07 (m, 4 H, H-3A, H-2C, H-6Ba', H-2C'), 4.05–3.98 (m, 2 H, H-6Ba, H-6Bb'), 3.94–3.90 (m, 2 H, H-3C, H-3C'), 3.89–3.79 (m, 5 H, H-4A, H-6Bb, H-4A', H-6Aa', H-5B'), 3.78–3.71 (m, 4 H, H-6Aab, H-4C', OCHHCH₂), 3.67 (m, 1 H, H-6Ab'), 3.61 (s, 1 H, H-4C), 3.57 (m, 1 H, H-5A'), 3.50–3.42 (m, 5 H, H-5A, H-3B, H-5B, CH₂Cl), 3.37 (m, 1 H, OCHHCH₂), 3.25 (br s, 1 H, H-2A), 3.06 (br s, 1 H, H-2A'), 2.06, 2.00, 1.94 (4 s, 12 H, 4 OCOCH₃), 1.93 (s, 3 H, NHCOCH₃), 1.88, 1.80 (2 s, 6 H, 2 OCOCH₃), 1.72–1.66 (m, 5 H, NHCOCH₃), O(CH₂)₄CH₂CH₂Cl), 1.48 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂Cl), 1.36 (m, 2 H, O(CH₂)₃CH₂(CH₂)₂Cl), 1.29–1.22 (m, 5 H, H-6C', O(CH₂)₂CH₂(CH₂)₃Cl), 1.17 (s, 9 H, C(CH₃)₃), 1.15 (d, J = 6.5 Hz, 3 H, H-6C). ¹³C NMR (125 MHz, CDCl₃, 295 K): $\delta_{\rm C}$ 177.7, 170.5, 170.1, 170.0, 169.8, 169.6, 169.4, 169.0 (C=O), 139.0, 138.8, 138.6, 138.5, 138.3, 137.8 (quat Ar), 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.6, 127.5, 127.4, 127.3, 127.0 (Ar), 100.3 (C-

1B'), 99.4 (C-1A'), 99.3 (C-1A), 99.2 (C-1B), 97.4 (C-1C'), 97.3 (C-1C), 80.6 (C-3C'), 80.0 (C-3C), 77.1 (C-4C), 76.7 (C-2C), 76.3 (C-4C'), 75.9 (C-3B, C-3A'), 75.6 (C-2C'), 75.3 (C-5A'), 74.7 (CH₂Ph), 74.3 (CH₂Ph), 74.2 (C-5A), 74.0 (CH₂Ph), 73.9 (C-4A), 73.6 (CH₂Ph), 73.5 (CH₂Ph), 73.3 (C-3A), 73.2 (CH₂Ph), 72.4 (C-4A', CH₂Ph), 72.2 (CH₂Ph), 71.2 (C-5B), 71.0 (C-2B, C-3B'), 70.3 (C-5B'), 69.4 (C-4B, OCH₂CH₂), 68.5 (C-2B'), 68.1 (C-6A, C-6A'), 66.6 (C-4B'), 66.2 (C-5C, C-5C'), 61.8 (C-6B), 60.0 (C-6B'), 57.8 (C-2A'), 56.4 (C-2A), 45.0 (CH₂Cl), 38.7 (C(CH₃)₃), 32.5 (O(CH₂)₄CH₂CH₂Cl), 29.2 (OCH₂CH₂(CH₂)₃CH₂Cl), 27.1 (C(CH₃)₃), 26.5 (O(CH₂)₃CH₂(CH₂)₂Cl), 25.1 (O(CH₂)₂CH₂(CH₂)₃Cl), 23.6, 23.1 (NHCOCH₃), 21.0, 20.8, 20.6, 20.5 (OCOCH₃), 16.8 (C-6C'), 16.7 (C-6C). HRESIMS (*m*/*z*): [M + NH₄]⁺ calcd for $C_{119}H_{151}CIN_3O_{36}$ 2232.977, found 2232.968.

6-Azidohexyl 2-acetamido-4-*O*-{2,4-di-*O*-acetyl-6-*O*-pivaloyl-3-*O*-[6-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2acetamido-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranoside (21). NaN₃ (17.6 mg, 0.2706 mmol, 10 equiv) was added to a solution of the hexasaccharide 20 (60 mg, 0.02706 mmol) in DMF (6 mL) and the reaction mixture was heated to 80 °C for 20 h. The solvent was evaporated, the residue was dissolved in CH₂Cl₂ (30 mL) and washed with water (2 × 30 mL). The aq layer was re-extracted with CH₂Cl₂ (3 × 10 mL) and the combined organic layers were dried and concentrated to give the azido 21 (60.2 mg, quant) pure as a white foam. [*α*]_D –46.5 (*c* 1.0, CH₂Cl₂). ¹H NMR (CDCl₃, 600 MHz, 295 K): $\delta_{\rm H}$ 7.44–7.41 (m, 2 H, Ar), 7.38–7.21 (m, 38 H, Ar), 5.79 (d, *J* = 7.2 Hz, 1 H, NHA), 5.56 (d, *J* = 7.3 Hz, 1 H, NHA'), 5.33 (d, *J* = 3.2 Hz, 1 H, H-4B'), 5.25 (d, *J* = 3.6 Hz, 1 H, H-4B), 5.09–5.02 (m, 3 H, H-1C, H-2B', H-1C'), 4.98–4.91 (m, 4 H, H-1A, 3 CHHPh), 4.90–4.84 (m, 3 H, H-2B, H-3B', 1 CHHPh), 4.83–4.76 (m, 4 H, H-

1A', 3 CHHPh), 4.75–4.71 (m, 2 H, 2 CHHPh), 4.70–4.60 (m, 6 H, H-1B', H-5C', 4 CHHPh), 4.45 (d, J = 12.1 Hz, 1 H, CHHPh), 4.41–4.33 (m, 4 H, H-5C, H-3A', 2 CHHPh), 4.31 (d, J = 8.2Hz, 1 H, H-1B), 4.17–4.07 (m, 4 H, H-3A, H-2C, H-6Ba', H-2C'), 4.05–3.98 (m, 2 H, H-6Ba, H-6Bb'), 3.94–3.90 (m, 2 H, H-3C, H-3C'), 3.89–3.79 (m, 5 H, H-4A, H-6Bb, H-4A', H-6Aa', H-5B'), 3.78–3.71 (m, 4 H, H-6Aab, H-4C', OCHHCH₂), 3.67 (m, 1 H, H-6Ab'), 3.61 (s, 1 H, H-4C), 3.57 (m, 1 H, H-5A'), 3.50–3.42 (m, 3 H, H-5A, H-3B, H-5B), 3.37 (m, 1 H, OCHHCH₂), 3.26 (br s, 1 H, H-2A), 3.20 (t, J = 6.9 Hz, 2 H, CH₂N₃), 3.06 (br s, 1 H, H-2A'), 2.06, 2.00, 1.94 (4 s, 12 H, 4 OCOCH₃), 1.93 (s, 3 H, NHCOCH₃), 1.88, 1.80 (2 s, 6 H, 2 OCOCH₃), 1.68 (s, 3 H, NHCOCH₃), 1.55–1.44 (m, 4 H, O(CH₂)₄CH₂CH₂N₃, OCH₂CH₂(CH₂)₃CH₂N₃), 1.31–1.21 (m, 7 H, H-6C', O(CH₂)₃CH₂(CH₂)₂N₃, O(CH₂)₂CH₂(CH₂)₃N₃), 1.17 (s, 9 H, C(CH₃)₃), 1.15 (d, J =6.4 Hz, 3 H, H-6C). ¹³C NMR (125 MHz, CDCl₃, 295 K): δ_C 177.7, 170.5, 170.1, 170.0, 169.8, 169.6, 169.4, 169.0 (C=O), 139.0, 138.8, 138.6, 138.5, 138.3, 137.8 (quat Ar), 128.5, 128.4, 128.3, 128.2, 128.0, 127.7, 127.6, 127.5, 127.4, 127.3, 127.0 (Ar), 100.3 (C-1B'), 99.4 (C-1A'), 99.3 (C-1A), 99.2 (C-1B), 97.4 (C-1C'), 97.3 (C-1C), 80.6 (C-3C'), 80.0 (C-3C), 77.1 (C-4C), 76.7 (C-2C), 76.3 (C-4C'), 75.9 (C-3B, C-3A'), 75.6 (C-2C'), 75.3 (C-5A'), 74.7 (CH₂Ph), 74.3 (CH₂Ph), 74.2 (C-5A), 74.0 (CH₂Ph), 73.9 (C-4A), 73.6 (CH₂Ph), 73.5 (CH₂Ph), 73.3 (C-3A), 73.2 (CH₂Ph), 72.4 (C-4A', CH₂Ph), 72.2 (CH₂Ph), 71.2 (C-5B), 71.0 (C-2B, C-3B'), 70.3 (C-5B'), 69.4 (C-4B, OCH₂CH₂), 68.5 (C-2B'), 68.1 (C-6A, C-6A'), 66.6 (C-4B'), 66.2 (C-5C, C-5C'), 61.8 (C-6B), 60.0 (C-6B'), 57.8 (C-2A'), 56.4 (C-2A), 51.3 (CH₂N₃), 38.7 (C(CH₃)₃), 29.2 (OCH₂CH₂(CH₂)₃CH₂N₃), 28.7 (O(CH₂)₄CH₂CH₂N₃), 27.1 (C(CH₃)₃), 26.4 (O(CH₂)₃CH₂(CH₂)₂N₃), 25.4 (O(CH₂)₂CH₂(CH₂)₃N₃), 23.5, 23.1 (NHCOCH₃), 21.0, 20.8, 20.6, 20.5 (OCOCH₃), 16.8 (C-6C'), 16.7 (C-6C). HRESIMS (m/z): $[M + H]^+$ calcd for C₁₁₉H₁₄₈N₅O₃₆

2222.990, found 2222.987.

6-Acetylthiohexyl 2-acetamido-4-0-{2,4-di-0-acetyl-6-0-pivaloyl-3-0-[6-0-benzyl-3-
O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-4-O-(2,3,4-tri-O-benzyl-α-L-
fucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-β-D-galactopyranosyl}-6- <i>O</i> -
benzyl-3- <i>O</i> -(2,3,4-tri- <i>O</i> -benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (22). KSAc
(4.9 mg, 0.04285 mmol, 10 equiv) was added to a solution of the hexasaccharide 20 (9.5 mg,
0.004285 mmol) in DMF (0.5 mL) and the reaction mixture was heated to 80 $^\circ$ C for 24 h. Work
up as described above for compound 21 followed by a column chromatography of the residue
(CH ₂ Cl ₂ /MeOH, 30:1) gave the thioacetate 22 (9.0 mg, 93%) pure as a brown amorphous glass.
$[\alpha]_{\rm D}$ –25.5 (<i>c</i> 0.9, CH ₂ Cl ₂). ¹ H NMR (CDCl ₃ , 600 MHz, 295 K): $\delta_{\rm H}$ 7.42–7.40 (m, 2 H, Ar),
7.36–7.20 (m, 38 H, Ar), 5.78 (d, <i>J</i> = 7.3 Hz, 1 H, NHA), 5.51 (d, <i>J</i> = 7.4 Hz, 1 H, NHA'), 5.31
(d, J = 3.4 Hz, 1 H, H-4B'), 5.23 (d, J = 3.6 Hz, 1 H, H-4B), 5.08–5.02 (m, 3 H, H-1C, H-2B',
H-1C'), 4.97–4.91 (m, 4 H, H-1A, 3 CHHPh), 4.90–4.83 (m, 3 H, H-2B, H-3B', 1 CHHPh),
4.82–4.75 (m, 4 H, H-1A', 3 CHHPh), 4.74–4.70 (m, 2 H, 2 CHHPh), 4.69–4.60 (m, 6 H, H-1B',
H-5C', 4 C <i>H</i> HPh), 4.44 (d, <i>J</i> = 12.1 Hz, 1 H, C <i>H</i> HPh), 4.41–4.32 (m, 4 H, H-5C, H-3A', 2
CHHPh), 4.29 (d, J = 8.2 Hz, 1 H, H-1B), 4.16–4.10 (m, 3 H, H-3A, H-6Ba', H-2C'), 4.08 (dd, J
= 3.8, 10.0 Hz, 1 H, H-2C), 4.04–3.96 (m, 2 H, H-6Ba, H-6Bb'), 3.93–3.88 (m, 2 H, H-3C, H-
3C'), 3.87–3.77 (m, 5 H, H-4A, H-6Bb, H-4A', H-6Aa', H-5B'), 3.76–3.69 (m, 4 H, H-6Aab, H-
4C', OCHHCH ₂), 3.66 (m, 1 H, H-6Ab'), 3.60 (s, 1 H, H-4C), 3.56 (m, 1 H, H-5A'), 3.48–3.39
(m, 3 H, H-5A, H-3B, H-5B), 3.35 (m, 1 H, OCH <i>H</i> CH ₂), 3.22 (br s, 1 H, H-2A), 3.05 (br s, 1 H,
H-2A'), 2.80 (t, <i>J</i> = 7.4 Hz, 2 H, CH ₂ SAc), 2.29 (s, 3 H, SCOCH ₃), 2.05, 1.99, 1.98, 1.93 (4 s, 12
H, 4 OCOCH ₃), 1.92 (s, 3 H, NHCOCH ₃), 1.86, 1.79 (2 s, 6 H, 2 OCOCH ₃), 1.67 (s, 3 H,
NHCOCH ₃), 1.52–1.43 (m, 4 H, O(CH ₂) ₄ CH ₂ CH ₂ SAc, OCH ₂ CH ₂ (CH ₂) ₃ CH ₂ SAc), 1.30–1.21
(m, 7 H, H-6C', O(CH ₂) ₃ CH ₂ (CH ₂) ₂ SAc, O(CH ₂) ₂ CH ₂ (CH ₂) ₃ SAc), 1.16 (s, 9 H, C(CH ₃) ₃), 1.13

 (d, J = 6.5 Hz, 3 H, H-6C). ¹³C NMR (125 MHz, CDCl₃, 295 K): δ_{C} 196.0, 177.7, 170.6, 170.2, 170.1, 170.0, 169.8, 169.6, 169.4, 169.0 (C=O), 139.1, 138.9, 138.7, 138.6, 138.4, 138.3, 137.8 (quat Ar), 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.6, 127.5, 127.4, 127.3, 127.0 (Ar), 100.3 (C-1B'), 99.4 (C-1A'), 99.3 (C-1A, C-1B), 97.4 (C-1C'), 97.3 (C-1C), 80.7 (C-3C'), 80.1 (C-3C), 77.1 (C-4C), 76.7 (C-2C), 76.3 (C-4C'), 75.9 (C-3B, C-3A'), 75.6 (C-2C'), 75.3 (C-5A'), 74.7 (CH₂Ph), 74.3 (CH₂Ph), 74.2 (C-5A), 74.0 (CH₂Ph), 73.9 (C-4A), 73.6 (CH₂Ph), 73.5 (CH₂Ph), 73.3 (C-3A), 73.2 (CH₂Ph), 72.4 (C-4A', CH₂Ph), 72.2 (CH₂Ph), 71.2 (C-5B), 71.0 (C-2B, C-3B'), 70.3 (C-5B'), 69.4 (C-4B, OCH₂CH₂), 68.5 (C-2B'), 68.1 (C-6A, C-6A'), 66.6 (C-4B'), 66.3 (C-5C'), 66.2 (C-5C), 61.8 (C-6B), 60.0 (C-6B'), 57.8 (C-2A'), 56.4 (C-2A), 38.7 (C(CH₃)₃), 30.6 (CH₂SAc), 29.4 (O(CH₂)₄CH₂CH₂SAc), 29.2 (OCH₂CH₂(CH₂)₃CH₂N₃), 28.4 (O(CH₂)₃CH₂(CH₂)₂N₃), 27.1 (C(CH₃)₃), 25.4 (O(CH₂)₂CH₂(CH₂)₃SAc), 23.6, 23.1 (NHCOCH₃), 21.1, 20.9, 20.7, 20.6, 20.5 (OCOCH₃), 16.8 (C-6C, C-6C'). HRESIMS (*m*/*z*): [M + H]⁺ calcd for C₁₂₁H₁₅₁N₂O₃₇S 2255.9716, found 2255.9690.

n-Hexyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-{3-*O*-[2-acetamido-2deoxy-4-*O*-(α-L-fucopyranosyl)-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-Dgalactopyranosyl}-β-D-glucopyranoside (2).

Method A : Liquid ammonia (25 mL) was condensed into a flask at -78 °C and a piece of sodium (60.0 mg, 2.609 mmol, 158 equiv) was added. A solution of the hexasaccharide **19** (38.3 mg, 16.50 µmol) in dry THF (5 mL) was added to the deep blue solution and the mixture was stirred for 1 h at -78 °C. The reaction was quenched with MeOH (5 mL) and the ammonia was allowed to evaporate at rt for 3 h. The remaining solution was treated with Ac₂O (1 mL) and left at rt for 5 min, the solvent was evaporated and the residue was dissolved in milli-Q water and

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passed through a Biogel P2 size exclusion column (100×1 cm) eluted with Milli-Q water. After lyophilization, the hexyl glycoside **2** (11.3 mg, 61%) was obtained pure as a white amorphous powder.

Method B: Hexasaccharide 20 (11.0 mg, 4.961 μ mol) was deprotected and purified as described above in method A, except that the remaining solution was neutralized with AcOH (500 μ L) instead of Ac₂O. The hexyl glycoside 2 (5.0 mg, 89%) was obtained pure as a white amorphous powder.

Analytical data for 2: $[\alpha]_D$ –49.0 (c 1.0, H₂O). ¹H NMR (D₂O, 600 MHz, 295 K): δ_H 5.08 (d, J = 4.0 Hz, 1 H, H-1C), 5.02 (d, J = 4.0 Hz, 1 H, H-1C'), 4.87 (m, 1 H, H-5C'), 4.80 (m, 1 H, H-5C'), 4.80 (m, 1 H, H-1C'), 4.80 (m, 1 H, H-1C'1 H, H-5C), 4.67 (d, J = 8.5 Hz, 1 H, H-1A'), 4.53–4.48 (m, 2 H, H-1A, H-1B'), 4.42 (d, J = 7.9 Hz, 1 H, H-1B), 4.08 (d, J = 3.2 Hz, 1 H, H-4B), 4.06 (t, J = 9.9 Hz, 1 H, H-3A'), 3.99–3.79 (m, 12 H, H-2A, H-3A, H-4A, H-6Aab, H-3C, H-2A', H-6Aab', H-4B', H-3C', OCHHCH₂), 3.80-3.65 (m, 10 H, H-3B, H-6Bab, H-2C, H-4C, H-4A', H-6Bab', H-2C', H-4C'), 3.61 (dd, J = 3.3, 9.8 Hz, 1 H, H-3B'), 3.59–3.40 (m, 7 H, H-5A, H-2B, H-5B, H-5A', H-2B', H-5B', OCHHCH₂), 2.02, 2.01 (2 s, 6 H, 2 OCOCH₃), 1.52 (m, 2 H, OCH₂CH₂(CH₂)₃CH₃), 1.33–1.23 (m, 6 H, $OCH_2CH_2(CH_2)_3CH_3$, 1.17 (d, J = 6.6 Hz, 3 H, H-6C'), 1.14 (d, J = 6.6 Hz, 3 H, H-6C), 0.85 (t, J = 6.6 Hz, 3 H, O(CH₂)₅CH₃). ¹³C NMR (125 MHz, D₂O, 295 K): $\delta_{\rm C}$ 177.5, 176.9 (C=O), 105.6 (C-1B'), 105.3 (C-1A'), 104.5 (C-1B), 103.7 (C-1A), 101.5 (C-1C), 100.8 (C-1C'), 84.4 (C-3B), 78.7 (C-3A'), 78.1 (C-5B), 77.9 (C-5B'), 77.7 (C-3A), 77.6 (C-5A'), 77.2 (C-5A), 75.8 (C-4A), 75.0 (C-3B'), 74.8 (C-4A'), 74.7 (C-4C'), 74.6 (C-4C), 73.4 (OCH₂CH₂), 73.2 (C-2B, C-2B'), 71.9 (C-3C, C-3C'), 71.1 (C-4B'), 71.0 (C-4B), 70.5 (C-2C'), 70.4 (C-2C), 69.6 (C-5C'), 69.5 (C-5C), 64.4 (C-6B'), 64.2 (C-6B), 62.5 (C-6A), 62.3 (C-6A'), 58.6 (C-2A, C-2A'), 31.3

(OCH₂CH₂(CH₂)₃CH₃), 33.5, 27.6, 24.8 (OCH₂CH₂(CH₂)₃CH₃), 25.0 (NHCOCH₃), 18.1 (C-6C'), 18.0 (C-6C), 16.1 (O(CH₂)₅CH₃). HRESIMS (m/z): [M + NH₄]⁺ calcd for C₄₆H₈₄N₃O₂₉ 1142.519, found 1142.525.

6-Aminohexyl 2-acetamido-2-deoxy-3-O-(α-L-fucopyranosyl)-4-O-{3-O-[2acetamido-2-deoxy-4-O-(α-L-fucopyranosyl)-3-O-(β-D-galactopyranosyl)-β-Dglucopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside (3). Hexasaccharide 21 (60.0 mg, 0.02698 mmol) was deprotected as described above for the preparation of 2 method A, except that the remaining solution was neutralized with AcOH (500 μ L) instead of Ac₂O. After concentration, the residue was dissolved in 0.05 M ammonium acetate in Milli-Q and passed twice through a Biogel P2 size exclusion column (100×1 cm) eluted with 0.05 M ammonium acetate in Milli-O water and after repeated lyophilization from Milli-O water, the deprotected hexasaccharide 3 (21.1 mg, 61%) was obtained as the acetate salt pure in the form of a white amorphous powder. $[\alpha]_{\rm D}$ -64.5 (c 1.0, H₂O). ¹H NMR (D₂O, 600 MHz, 295 K): $\delta_{\rm H}$ 5.08 (d, J = 4.0 Hz, 1 H, H-1C), 5.02 (d, J = 4.0 Hz, 1 H, H-1C), 4.87 (m, 1 H, H-5C), 4.80 (m,4.67 (d, J = 8.5 Hz, 1 H, H-1A'), 4.53–4.48 (m, 2 H, H-1A, H-1B'), 4.42 (d, J = 7.9 Hz, 1 H, H-1B), 4.08 (d, J = 3.2 Hz, 1 H, H-4B), 4.06 (t, J = 9.9 Hz, 1 H, H-3A'), 3.99–3.79 (m, 12 H, H-2A, H-3A, H-4A, H-6Aab, H-3C, H-2A', H-6Aab', H-4B', H-3C', OCHHCH₂), 3.80–3.65 (m, 10 H, H-3B, H-6Bab, H-2C, H-4C, H-4A', H-6Bab', H-2C', H-4C'), 3.61 (dd, J = 3.3, 9.8 Hz, 1 H, H-3B'), 3.59–3.40 (m, 7 H, H-5A, H-2B, H-5B, H-5A', H-2B', H-5B', OCHHCH₂), 2.97 (t, J $= 7.5 \text{ Hz}, 2 \text{ H}, CH_2 \text{NH}_3^+), 2.02, 2.01 (2 \text{ s}, 6 \text{ H}, 2 \text{ OCOCH}_3), 1.91 (\text{s}, 3 \text{ H}, \text{OCOCH}_3), 1.64 (\text{m}, 2 \text{ COCH}_3), 1.64 (\text$ H, O(CH₂)₄CH₂CH₂NH₃⁺), 1.54 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂NH₃⁺), 1.40–1.31 (m, 4 H, $O(CH_2)_3CH_2(CH_2)_2NH_3^+$, $O(CH_2)_2CH_2(CH_2)_3NH_3^+$), 1.17 (d, J = 6.6 Hz, 3 H, H-6C'), 1.14 = 6.6 Hz, 3 H, H-6C). ¹³C NMR (125 MHz, D₂O, 295 K): $\delta_{\rm C}$ 177.5, 176.9 (C=O), 105.6 (C-1B'),

105.3 (C-1A'), 104.5 (C-1B), 103.7 (C-1A), 101.5 (C-1C), 100.8 (C-1C'), 84.4 (C-3B), 78.7 (C-3A'), 78.1 (C-5B), 77.9 (C-5B'), 77.7 (C-3A), 77.6 (C-5A'), 77.2 (C-5A), 75.8 (C-4A), 75.0 (C-3B'), 74.8 (C-4A'), 74.7 (C-4C'), 74.6 (C-4C), 73.3 (OCH₂CH₂), 73.2 (C-2B, C-2B'), 71.9 (C-3C, C-3C'), 71.1 (C-4B'), 71.0 (C-4B), 70.5 (C-2C'), 70.4 (C-2C), 69.6 (C-5C'), 69.5 (C-5C), 64.4 (C-6B'), 64.2 (C-6B), 62.5 (C-6A), 62.3 (C-6A'), 58.6 (C-2A, C-2A'), 42.1 (CH₂NH₃⁺), 31.1 (OCH₂CH₂(CH₂)₃CH₂NH₃⁺), 29.4 (O(CH₂)₄CH₂CH₂NH₃⁺), 28.0 (O(CH₂)₃CH₂(CH₂)₂NH₃⁺), 27.4 (O(CH₂)₂CH₂(CH₂)₃NH₃⁺), 25.0 (NHCOCH₃), 18.1 (C-6C'), 18.0 (C-6C). HRESIMS (m/z): [M + H]⁺ calcd for C₄₆H₈₂N₃O₂₉ 1140.503, found 1140.502.

6-Thiohexyl [2-acetamido-2-deoxy-3-O-(α-L-fucopyranosyl)-4-O-{3-O-[2-acetamido-2-deoxy-4-O-(α -L-fucopyranosyl)-3-O-(β -D-galactopyranosyl)- β -D-glucopyranosyl]- β -Dgalactopyranosyl}-B-D-glucopyranoside (4). A solution of thioacetate 22 (7.9 mg, 3.500 µmol) in 0.25 M NaOMe in MeOH (2 mL) was stirred at rt for 2 h 30 min, then deionized with Dowex 50 (H⁺) resin. The resin was filtered off, washed with MeOH (4 × 5 mL) and the combined filtrate and washings were concentrated. The residue was dissolved in dry THF (3 mL) and was added to a deep blue solution of liquid ammonia (25 mL), condensed into a flask at -78 °C, containing a piece of sodium (60.0 mg, 2.609 mmol, 745 equiv). The mixture was stirred for 1 h at -78 °C, the reaction was quenched with MeOH (5 mL) and the ammonia was allowed to evaporate at rt for 3 h. The remaining solution was neutralized with AcOH (500 μ L), the solvent was evaporated and the residue was dissolved in Milli-Q and passed through a Biogel P2 size exclusion column (100×1 cm) eluted with Milli-Q water. After lyophilization, glycoside 4 (3.6 mg, 90%) was obtained pure as a white amorphous powder. $[\alpha]_D = 27.5$ (c 0.4, H₂O). ¹H NMR $(D_2O, 600 \text{ MHz}, 295 \text{ K})$: $\delta_H 5.08 \text{ (d}, J = 4.0 \text{ Hz}, 1 \text{ H}, \text{H-1C}), 5.02 \text{ (d}, J = 4.0 \text{ Hz}, 1 \text{ H}, \text{H-1C}),$ 4.87 (m, 1 H, H-5C'), 4.80 (m, 1 H, H-5C), 4.67 (d, J = 8.5 Hz, 1 H, H-1A'), 4.53-4.48 (m, 2 H, J)

H-1A, H-1B'), 4.42 (d, J = 7.9 Hz, 1 H, H-1B), 4.08 (d, J = 3.2 Hz, 1 H, H-4B), 4.06 (t, J = 9.7Hz, 1 H, H-3A'), 3.99–3.79 (m, 12 H, H-2A, H-3A, H-4A, H-6Aab, H-3C, H-2A', H-6Aab', H-4B', H-3C', OCHHCH₂), 3.80-3.65 (m, 10 H, H-3B, H-6Bab, H-2C, H-4C, H-4A', H-6Bab', H-2C', H-4C'), 3.61 (dd, J = 3.3, 9.8 Hz, 1 H, H-3B'), 3.59–3.40 (m, 7 H, H-5A, H-2B, H-5B, H-5A', H-2B', H-5B', OCHHCH₂), 2.89 (m, 2 H, CH₂S), 2.02 (2 s, 6 H, 2 OCOCH₃), 1.72 (m, 2 H, O(CH₂)₄CH₂CH₂S), 1.55 (m, 2 H, OCH₂CH₂(CH₂)₃CH₂S), 1.42 (m, 2 H, O(CH₂)₃CH₂(CH₂)₂S), $1.33 (O(CH_2)_2CH_2(CH_2)_3S), 1.17 (d, J = 6.6 Hz, 3 H, H-6C'), 1.14 (d, J = 6.6 Hz, 3 H, H-6C).$ ¹³C NMR (125 MHz, D₂O, 295 K): δ_C 177.5, 177.0 (C=O), 105.6 (C-1B'), 105.3 (C-1A'), 104.5 (C-1B), 103.7 (C-1A), 101.5 (C-1C), 100.8 (C-1C'), 84.4 (C-3B), 78.7 (C-3A'), 78.1 (C-5B), 77.9 (C-5B'), 77.7 (C-3A), 77.6 (C-5A'), 77.2 (C-5A), 75.8 (C-4A), 75.0 (C-3B'), 74.8 (C-4A'), 74.7 (C-4C'), 74.6 (C-4C), 73.3 (C-2B, C-2B'), 73.2 (OCH₂CH₂), 71.9 (C-3C, C-3C'), 71.1 (C-4B'), 71.0 (C-4B), 70.5 (C-2C'), 70.4 (C-2C), 69.6 (C-5C'), 69.4 (C-5C), 64.4 (C-6B'), 64.2 (C-6B), 62.5 (C-6A), 62.3 (C-6A'), 58.6 (C-2A, C-2A'), 53.7 (CH₂S), 31.0 (OCH₂CH₂(CH₂)₃CH₂S), 30.0 (O(CH₂)₃CH₂(CH₂)₂S), 27.3 (O(CH₂)₂CH₂(CH₂)₃S), 26.7 (O(CH₂)₄CH₂CH₂S), 25.0 (NHCOCH₃), 18.1 (C-6C'), 18.0 (C-6C). ESI FAIMS HRMS (*m/z*): $[M-H_2O-H + 3Na]^{2+}$ calcd for C₄₆H₇₇N₂O₂₈SNa₃ 603.2033, found 603.2016.

ASSOCIATED CONTENT

 Supporting Information Available. Tables 1-4: listing of ions detected by ESI FAIMS HRMS for compounds 2–4, 23. , Full spectra ESI FAIMS HRMS for compounds 2–4, 23; full spectra ESI HRMS for compounds 2–4. General Experimental Procedures, ¹H and ¹³C NMR spectra for compounds 10 and 11; ¹H, COSY, ¹³C and HSQC NMR spectra for compounds 12–22 and 2–4. This material is available free of charge via the Internet at <u>http://pubs.acs.org</u>.

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Notes

The authors declare no competing financial interest.

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