JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

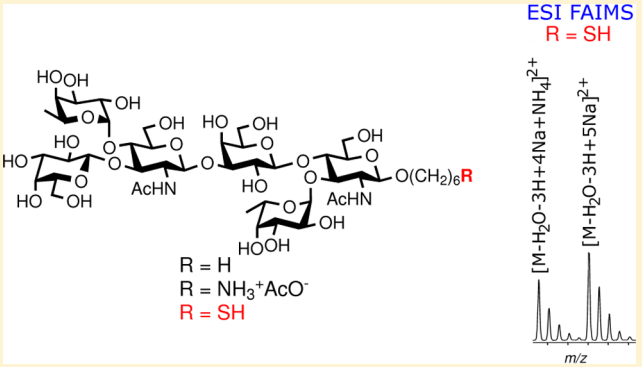
Synthesis of Tumor-Associated Le^{a}Le^x Hexasaccharides: Instability of a Thiol-Containing Oligosaccharide in Mass Spectrometry and Hypermetalation Detected by ESI FAIMS

Mickaël Guillemineau,[†] Jadwiga Lyczko, Wojciech Gabryelski, and France-Isabelle Auzanneau*

Department of Chemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada

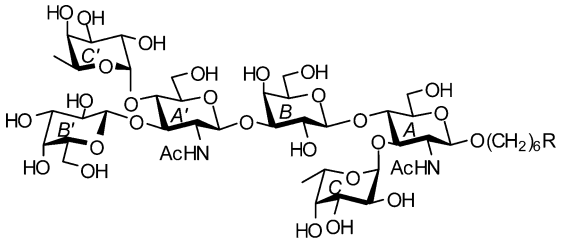
Supporting Information

ABSTRACT: We report the efficient synthesis of three analogues of the tumor-associated carbohydrate antigen Le^{a}Le^x. This hexasaccharide was prepared as a soluble inhibitor hexyl glycoside, as a 6-aminohexyl glycoside for conjugation to proteins, and as a 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 6-thiohexyl glycoside lost water and led to the formation of "hypermetalated" species which we propose are cyclic.



INTRODUCTION

Although not yet successful, the use of tumor-associated carbohydrate antigens (TACAs) to develop anticancer vaccines has led to intense research efforts in the past two decades.[1] Of particular interest to our group is the TACA Le^{a}Le^x which has been shown to be overexpressed 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^{a}Le^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 anticancer vaccine. To identify the smallest Le^{a}Le^x fragments that still display 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^{a}Le^x TACA. Hexyl glycoside **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. Thiohexyl glycoside **4** will be immobilized to a gold chip for the detection of antibodies by surface plasmon resonance (SPR).



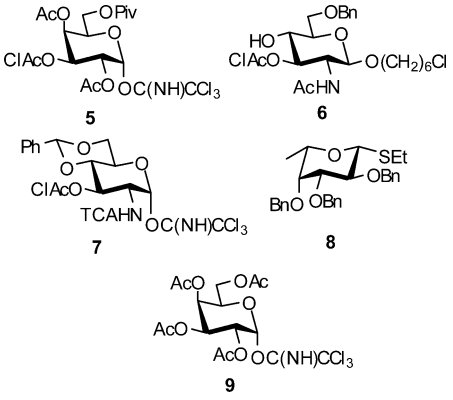
1 Le^{a}Le^x
2 R = H
3 R = $NH_3^+AcO^-$
4 R = SH

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^{a}Le^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

Received: May 27, 2015
Published: July 29, 2015

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

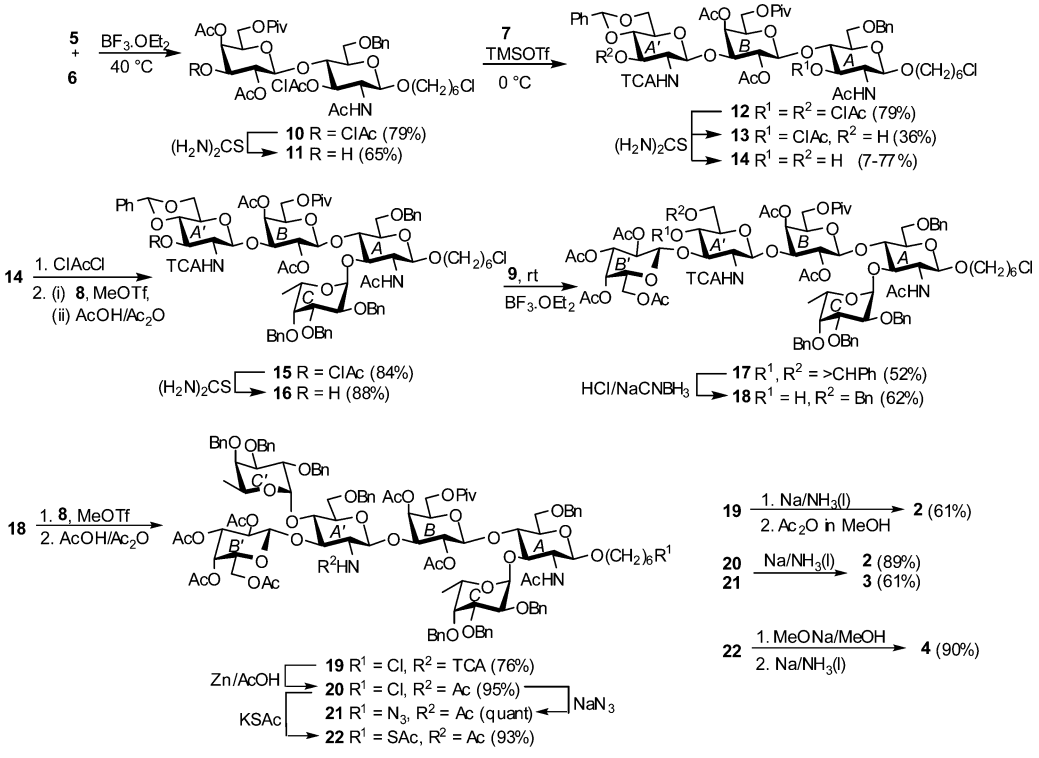
reactions involving the primary thiol and which led to the formation of dissociation species and species accommodating multiple sodium and ammonium ions. Using electrospray high-field asymmetric waveform ion mobility spectrometry (ESI FAIMS),[7] we were able to suppress the ion dissociation reactions and obtain high quality spectral data that were easily interpreted and suggested that the 6-thiohexyl glycoside **4** lost water and led to the formation of "hypermetalated" 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 in Scheme 1. Glycosylation of acceptor **6** with donor **5** (4 equiv) activated with $BF_3{\cdot}OEt_2$ (2 equiv) at 40 °C gave disaccharide **10** in very good yield (79%). Selective dechloroacetylation 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 ^{1}H 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-3A and O-3A′ in diol **14**, we reported that galactosylation at O-3A′ was mismatched while that at O-3A was matched.[13] Further studies are ongoing 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 dechloroacetylation at the nonreducing end O-3A′ of trisaccharide **12** using the conditions applied for the preparation of disaccharide **11**. 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, we revised the synthetic strategy, 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 characterized[13] diol **14** (77%). The diol was then first selectively chloroacetylated[13] 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 Ac_2O to convert any methyl imidate[14] into an *N*-acetyl group, and the desired tetrasaccharide **15** was isolated in 84% yield. Dechloroacetylation of tetrasaccharide **15** with excess thiourea gave the previously characterized[13] tetrasaccharide acceptor **16** in 88% yield, which was subjected to galactosylation with donor **9**. As anticipated,

Scheme 1. Synthesis of Hexasaccharides 2−4 from Building Blocks 5−9



DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

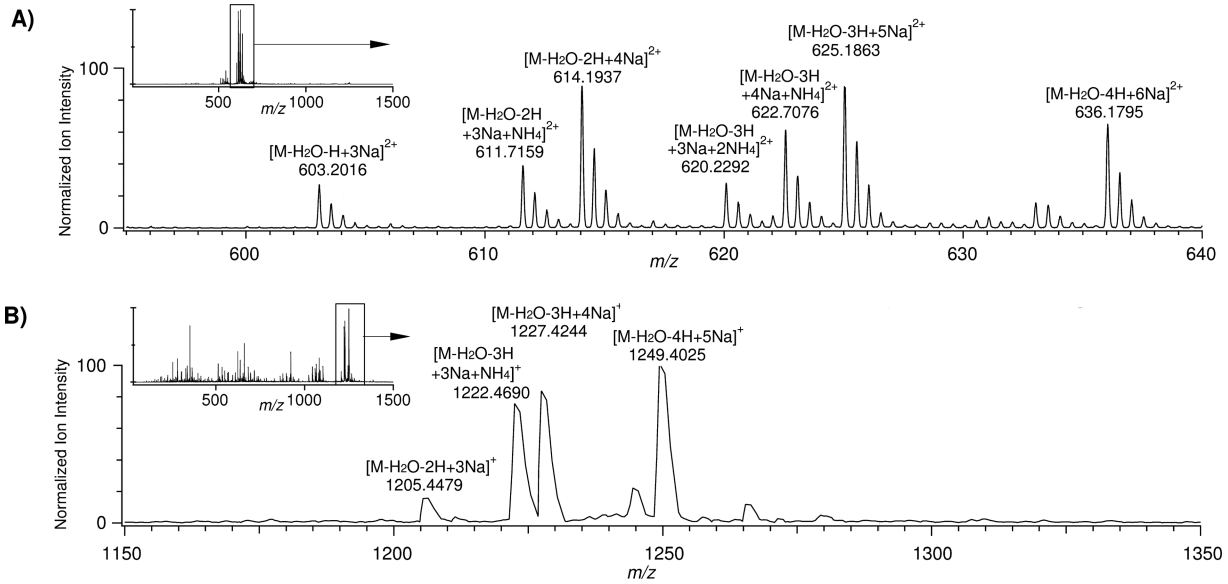


Figure 1. High resolution mass spectra recorded for 6-thiohexyl glycoside **4**. (A) Electrospray high-field asymmetric waveform ion mobility spectrometry high resolution mass spectrometry (ESI FAIMS HRMS); (B) electrospray high resolution mass spectrometry (ESI HRMS).

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 comigration of the products and degraded donor, the reaction was best monitored by RP-HPLC after quenching of an aliquot with NEt_3. 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 Ac_2O and gave the desired protected Le^aLe^x hexasaccharide **19** in excellent 76% yield. Reduction of the trichloroacetamide into an acetamide was achieved using 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 *N*-acetamido 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 workup 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** under 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 shown[9] that under dissolving metal conditions 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 subjected 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 ^{1}H 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 ^{1}H 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 SCH_2 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 ($M - H_2O$). Elemental ion compositions (determined from high resolution accurate mass measurements, see Supporting Information) showed that while two sodium ions provided

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

the +2 net ion charge, up to four additional sodium ions were also added. In addition to the sodium adducts, Figure 1A shows the formation of hypermetalated 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 hypermetalated 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 previously reported[9] 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 the 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**.

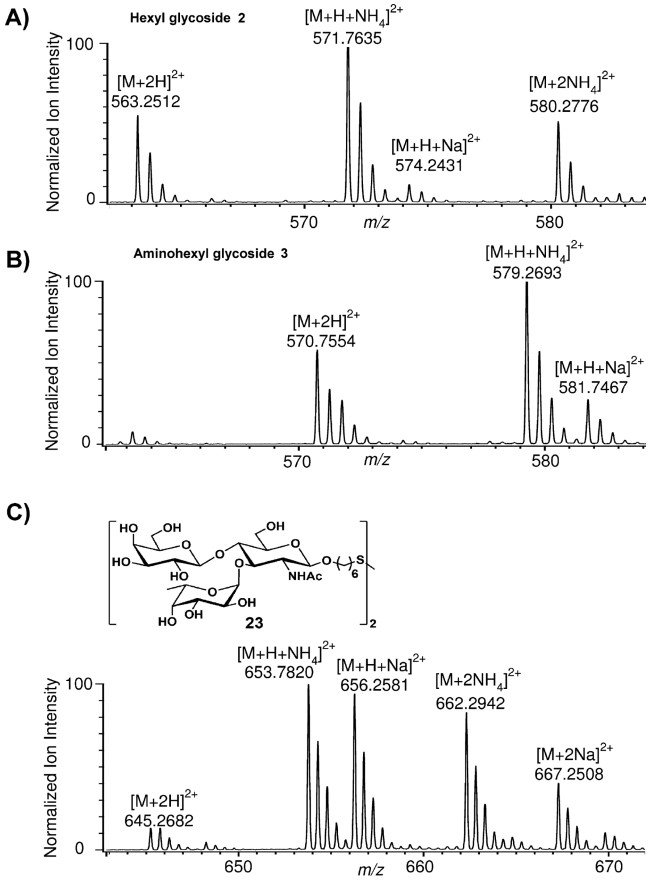


Figure 2. ESI FAIMS HRMS for (A) hexyl glycoside **2**; (B) aminohexyl glycoside **3**; (C) hexyl disulfide glycoside Le^x dimer **23**.

"Hypermetalation" of biomolecules in ESI MS was first named and described by Pope et al. 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 hypermetalation 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 reported[9] 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 hypermetalation 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

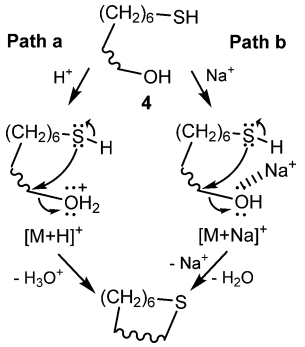


Figure 3. Proposed pathways leading to the loss of water in hexyl thiol glycoside **4**.

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). This cyclic species may trap sodium ions which are present in the sample solution at trace levels from the last synthetic step [$NH_3(l)$/Na] and undergo hypermetalation 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^{a}Le^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

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

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 alternating low and high electric fields generated in the gap between two closely spaced electrodes.[7c] Coupled with "soft" Q-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 spectrometry,[19] 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 hypermetalated through sodium exchange at the hydroxyl groups. While there are a few reports describing hypermetalation 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 under the same conditions gave barely detectable signals for hyperlithiated species.[18] We thus conclude that the formation of the cyclic ion may have favored hypermetalation 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 autosampler and pump from a nanoflow 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% CO_2 in N_2 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, 1.0, 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-D-galactopyranosyl)-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (10). A stirred solution of known[9] alcohol **6** (905 mg, 1.787 mmol) and known[8] galactosyl trichloroacetimidate **5** (4.07 g, 7.150 mmol, 4 equiv) in dry CH_2Cl_2 (45 mL) was heated to 40 °C under N_2. Freshly distilled $BF_3{\cdot}OEt_2$ (449 μL, 3.573 mmol, 2 equiv) was added to the mixture, which was stirred for 1 h at 40 °C and then quenched with NEt_3 (598 μL, 4.290 mmol, 2.4 equiv). The reaction was diluted with CH_2Cl_2 (55 mL) and washed with saturated aq $NaHCO_3$ (1 × 100 mL). The aqueous layer was re-extracted with CH_2Cl_2 (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. $[\alpha]_D$ −4.5 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 297 K): δ_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, C*H*HPh), 4.49 (d, *J* = 8.0 Hz, 1 H, H-1A), 4.39 (d, *J* = 12.0 Hz, 1 H, CH*H*Ph), 4.33 (d, *J* = 8.0 Hz, 1 H, H-1B), 4.09–3.98 (m, 4 H, H-6Bab, COCH_2Cl), 3.92–3.86 (m, 5 H, H-2A, H-4A, OC*H*HCH$_2$, COCH_2Cl), 3.70 (m, 2 H, H-6Aab), 3.54 (t, *J* = 6.7 Hz, 1 H, H-5B), 3.50 (t, *J* = 6.6 Hz, 2 H, CH_2Cl), 3.46 (m, 1 H, H-5A), 3.42 (m, 1 H, OCH*H*CH$_2$), 2.11, 1.95 (2 s, 6 H, 2 OCOCH_3), 1.92 (s, 3 H, NHCOCH_3), 1.73 (m, 2 H, O(CH$_2$)$_4$CH_2CH$_2$Cl), 1.55 (m, 2 H, OCH$_2$CH_2(CH$_2$)$_3$CH$_2$Cl), 1.41 (m, 2 H, O(CH$_2$)$_3$CH_2(CH$_2$)$_2$Cl), 1.32 (m, 2 H, O-(CH$_2$)$_2$CH_2(CH$_2$)$_3$Cl), 1.18 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 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 (*C*H$_2$Ph), 72.5 (C-3B), 70.5 (C-5B), 69.5 (OCH$_2$CH$_2$), 68.9 (C-2B), 67.3 (C-6A), 66.5 (C-4B), 60.6 (C-6B), 53.7 (C-2A), 45.0 (CH$_2$Cl), 40.9, 40.4 (COCH$_2$Cl), 38.8 (C(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), 23.3 (NHCOCH$_3$), 20.7, 20.6 (OCOCH$_3$). HRESIMS (*m*/*z*): [M + Na]$^+$ calcd for $C_{40}H_{56}Cl_3NO_{16}Na$ 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 and then allowed to come to rt. The reaction mixture was diluted with $CHCl_3$ (500 mL) and washed with HCl 2 N (1 × 600 mL). The aqueous layer was re-extracted with $CHCl_3$ (5 × 100 mL), and the combined organic layers were dried and concentrated. The product was purified by column chromatography ($CHCl_3$/MeOH, 20:1) to give alcohol **11** (4.04 g, 65%) pure as a yellow amorphous foam. $[\alpha]_D$ −3.1 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, 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-2B, C*H*HPh), 4.50 (d, *J* = 8.0 Hz, 1 H, H-1A), 4.43 (d, *J* = 12.0 Hz, 1 H, CH*H*Ph), 4.34 (d, *J* = 8.0 Hz, 1 H, H-1B), 4.13–3.98 (m, 4 H, H-6Bab, COCH_2Cl), 3.92–3.80 (m, 3 H, H-2A, H-4A, OC*H*HCH$_2$), 3.75 (m, 2 H, H-6Aab), 3.58–3.49 (m, 6 H, H-5A, H-3B, H-5B, OCH*H*CH$_2$, CH$_2$Cl), 2.48 (d, *J* = 6.0 Hz, 1 H, OH-3B), 2.14, 2.04 (2 s, 6 H, 2 OCOCH_3), 1.93 (s, 3 H, NHCOCH_3), 1.73 (m, 2 H, O(CH$_2$)$_4$CH_2CH$_2$Cl), 1.56 (m, 2 H, OCH$_2$CH_2(CH$_2$)$_3$CH$_2$Cl), 1.41 (m, 2 H, O(CH$_2$)$_3$CH_2(CH$_2$)$_2$Cl), 1.33 (m, 2 H, O-(CH$_2$)$_2$CH_2(CH$_2$)$_3$Cl), 1.19 (s, 9 H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 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 (CH$_2$Ph), 73.1 (C-2B), 71.5 (C-3B), 70.9 (C-5B), 69.5 (OCH$_2$CH$_2$), 69.4 (C-4B), 67.5 (C-6A), 61.4 (C-6B), 53.9 (C-2A), 45.0 (CH$_2$Cl), 40.9 (COCH$_2$Cl), 38.8 (C(CH$_3$)$_3$), 32.5 (O(CH$_2$)$_4$CH$_2$CH$_2$Cl), 29.3 (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), 23.4 (NHCOCH$_3$), 21.0, 20.7 (OCOCH$_3$). HRESIMS (*m*/*z*): [M + H]$^+$ calcd for $C_{38}H_{56}Cl_2NO_{15}$ 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-galactopyranosyl]-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (12). Disaccharide glycosyl acceptor **11** (40.0 mg, 0.04781 mmol) and known[10] 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 the mixture was cooled to 0 °C, freshly distilled TMSOTf (17.3 μL, 0.09562 mmol, 2

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073–8083

equiv) was added to the reaction, which was stirred at 0 °C for 40 min and then quenched with NEt_3 (16.0 μL, 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 aqueous layer was re-extracted with CH_2Cl_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). ^1H NMR (600 MHz, $CDCl_3$, 295 K): δ_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*H*Ph), 5.24 (d, *J* = 3.4 Hz, 1 H, H-4B), 5.07 (t, *J* = 9.1 Hz, 1 H, H-3A), 4.91 (dd, *J* = 8.3, 9.7 Hz, 1 H, H-2B), 4.73 (d, *J* = 8.0 Hz, 1 H, H-1A′), 4.69 (d, *J* = 12.1 Hz, 1 H, C*H*HPh), 4.42 (d, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.36 (d, *J* = 7.7 Hz, 1 H, H-1A), 4.31 (dd, *J* = 4.9, 10.4 Hz, 1 H, H-6Aa′), 4.27 (d, *J* = 8.0 Hz, 1 H, H-1B), 4.11−3.92 (m, 7 H, H-2A, H-6Bab, 2 $COCH_2Cl$), 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_2Cl), 3.40 (m, 1 H, H-5A), 3.35 (m, 1 H, $OCHHCH_2$), 2.04, 1.99 (2 s, 6 H, 2 $OCOCH_3$), 1.92 (s, 3 H, $NHCOCH_3$), 1.72 (m, 2 H, $O(CH_2)_4CH_2CH_2Cl$), 1.53 (m, 2 H, $OCH_2CH_2(CH_2)_3CH_2Cl$), 1.39 (m, 2 H, $O(CH_2)_3CH_2(CH_2)_2Cl$), 1.31 (m, 2 H, $O(CH_2)_2CH_2(CH_2)_3Cl$), 1.21 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$, 295 K): δ_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 (>CHPh) 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_2Ph), 72.3 (C-3A′), 71.0 (C-5B), 70.6 (C-2B), 69.5 (OCH_2CH_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 (CH_2Cl), 40.8, 40.5 ($COCH_2Cl$), 38.8 ($C(CH_3)_3$), 32.4 ($O(CH_2)_4CH_2CH_2Cl$), 29.2 ($OCH_2CH_2(CH_2)_3CH_2Cl$), 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 and then allowed to come to rt. The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with HCl 2 N (1 × 100 mL). The aqueous layer was re-extracted with $CHCl_3$ (5 × 25 mL), and the combined organic layers were dried and concentrated. Reversed-phase HPLC of the residue (CH_3CN/H_2O, 40:60 to 95:5, 30 min) 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 and then allowed to come to rt. The reaction mixture was diluted with $CHCl_3$ (150 mL) and washed with HCl 2 N (1 × 150 mL). The aqueous layer was re-extracted with $CHCl_3$ (5 × 20 mL), and the combined organic layers were dried and concentrated. The product was purified by column chromatography (CH_2Cl_2/MeOH, 20:1) to give diol **14** (338.2 mg, 77%) 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, CH_2Cl_2). ^1H NMR ($CDCl_3$, 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, >C*H*Ph), 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* = 12.1 Hz, 1 H, C*H*HPh), 4.44 (d, *J* = 7.7 Hz, 1 H, H-1A), 4.39 (d, *J* = 12.1 Hz, 1 H, C*H*HPh), 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, $COCH_2Cl$), 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, $OCHHCH_2$), 3.70 (t, *J* = 9.9 Hz, 1 H, H-6Ab′), 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′, CH_2Cl), 3.37−3.28 (m, 2 H, H-2A′, $OCHHCH_2$), 3.12 (br s, 1 H, OH-3A′), 2.03, 2.02 (2 s, 6 H, 2 $OCOCH_3$), 1.91 (s, 3 H, $NHCOCH_3$), 1.72 (m, 2 H, $O(CH_2)_4CH_2CH_2Cl$), 1.52 (m, 2 H, $OCH_2CH_2(CH_2)_3CH_2Cl$), 1.40 (m, 2 H, $O(CH_2)_3CH_2(CH_2)_2Cl$), 1.30 (m, 2 H, $O(CH_2)_2CH_2(CH_2)_3Cl$), 1.20 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$, 296 K): δ_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 (CCl_3), 81.3 (C-4A′), 76.4 (C-3B), 74.3 (C-5A), 74.1 (C-3A), 73.9 (C-4A), 73.6 (CH_2Ph), 71.1 (C-5B), 70.6 (C-2B), 69.4 (OCH_2CH_2), 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 (CH_2Cl), 40.8 ($COCH_2Cl$), 38.8 ($C(CH_3)_3$), 32.4 ($O(CH_2)_4CH_2CH_2Cl$), 29.2 ($OCH_2CH_2(CH_2)_3CH_2Cl$), 27.1 ($C(CH_3)_3$), 26.5 ($O(CH_2)_3CH_2(CH_2)_2Cl$), 25.1 ($O(CH_2)_2CH_2(CH_2)_3Cl$), 23.3 ($NHCOCH_3$), 21.1, 20.7 ($OCOCH_3$). HRESIMS(*m*/*z*): $[M + H]^+$ calcd for $C_{53}H_{70}Cl_5N_2O_{20}$ 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-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 (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 CH_2Cl_2 (280 mL) containing dry pyridine (225 μL, 2.79 mmol, 2 equiv) under N_2. The reaction was left at 0 °C for 2 h and then washed sequentially with HCl 2 N (1 × 100 mL) and saturated aq $NaHCO_3$ (1 × 100 mL). The aqueous layers were re-extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic layers were dried and concentrated to give the previously described[13] selectively chloroacetylated trisaccharide (1.72 g, quant) as a slightly yellowish amorphous foam. The trisaccharide (1.72 g, 1.397 mmol) and known[11] thioglycoside donor **8** (2.00 g, 4.191 mmol, 3 equiv) in dry CH_2Cl_2 (170 mL) containing freshly activated molecular sieves 4 Å (17 g) were stirred under N_2 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 and then quenched with NEt_3 (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 $NaHCO_3$ (1 × 300 mL). The aqueous 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 Ac_2O and AcOH (3:1, 100 mL), and the solution was stirred at rt for 75 min and then coconcentrated 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, CH_2Cl_2). ^1H NMR ($CDCl_3$, 600 MHz, 295 K): δ_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, >C*H*Ph), 5.46 (t, *J* = 10.3 Hz, 1 H, H-3A′), 5.24 (d, *J* = 3.1 Hz, 1 H, H-4B), 5.07 (d, *J* = 3.1 Hz, 1 H, H-1C), 4.98−4.91 (m, 2 H, H-2B, C*H*HPh), 4.85−4.77 (m, 3 H, H-1A, 2 C*H*HPh), 4.74−4.68 (m, 3 H, H-1A′, 2 C*H*HPH), 4.65 (d, *J* = 11.8 Hz, 1 H, C*H*HPh), 4.62 (d, *J* = 12.0 Hz, 1 H, C*H*HPh), 4.42−4.38 (m, 2 H, H-1B, C*H*HPh), 4.33 (dd, *J* = 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, $OCHHCH_2$), 3.64 (dd, *J* = 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, *J* = 6.7 Hz, 2 H, CH_2Cl), 3.35 (m, 1 H, $OCHHCH_2$), 2.02, 1.97 (2 s, 6 H, 2 $OCOCH_3$), 1.76 (s, 3 H, $NHCOCH_3$), 1.67 (m, 2 H, $O(CH_2)_4CH_2CH_2Cl$), 1.45 (m, 2 H, $OCH_2CH_2(CH_2)_3CH_2Cl$), 1.33 (m, 2 H, $O(CH_2)_3CH_2(CH_2)_2Cl$), 1.23 (m, 2 H, O-

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

$(CH_2)_2CH_2(CH_2)_3Cl$), 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): δ_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_2Ph), 74.2 (C-5A), 73.7 (C-4A), 73.5 (CH_2Ph) 73.2 (CH_2Ph), 72.9 (C-3A), 72.6 (CH_2Ph), 71.9 (C-3A′), 70.9 (C-2B), 70.7 (C-5B), 69.3 (OCH_2CH_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 (CH_2Cl), 40.4 ($COCH_2Cl$), 38.7 ($C(CH_3)_3$), 32.5 ($O(CH_2)_4CH_2CH_2Cl$), 29.2 ($OCH_2CH_2(CH_2)_3CH_2Cl$), 27.1 ($C(CH_3)_3$), 26.5 ($O(CH_2)_3CH_2(CH_2)_2Cl$), 25.2 ($O(CH_2)_2CH_2(CH_2)_3Cl$), 23.1 ($NHCOCH_3$), 21.1, 20.7 ($OCOCH_3$), 16.6 (C-6C). HRESIMS (m/z): $[M + H]^+$ calcd for $C_{80}H_{98}Cl_5N_2O_{24}$ 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 and then allowed to come to rt. The reaction mixture was diluted with $CHCl_3$ (200 mL) and washed with HCl 2 N (1 × 300 mL). The aqueous layer was re-extracted with $CHCl_3$ (5 × 60 mL), and the combined organic layers were dried and concentrated. The product was purified by column chromatography (CH_2Cl_2/MeOH, 20:1) to give the previously described[13] 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-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-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 (17). Freshly distilled $BF_3{\cdot}OEt_2$ (16 μL, 0.1273 mmol, 2 equiv) was added to a stirred solution of alcohol **16** (100 mg, 0.06365 mmol) and known[12] galactosyl trichloroacetimidate **9** (156.8 mg, 0.3182 mmol, 5 equiv) in dry CH_2Cl_2 (5 mL) at rt under N_2. The reaction was left to proceed for 1 h at rt and then quenched with NEt_3 (21.2 μL, 0.1528 mmol, 2.4 equiv). The solvent was evaporated and a column chromatography (EtOAc/hexanes, 1:1 and then 7:3) followed by a reverse-phase HPLC (CH_3CN/H_2O, 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, CH_2Cl_2). ^1H NMR ($CDCl_3$, 600 MHz, 295 K): δ_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, 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, 10.1 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′, OCHHCH$_2$), 3.57 (s, 1 H, H-4C), 3.55−3.44 (m, 6 H, H-2A, H-5A, H-5B, H-5A′, CH_2Cl), 3.35 (m, 1 H, OCHHCH$_2$), 3.24 (m, 1 H, H-2A′), 2.09, 2.02, 1.98, 1.93, 1.92, 1.76 (6 s, 18 H, 6 $OCOCH_3$), 1.74 (s, 3 H, $NHCOCH_3$), 1.67 (m, 2 H, $O(CH_2)_4CH_2CH_2Cl$), 1.45 (m, 2 H, $OCH_2CH_2(CH_2)_3CH_2Cl$), 1.33 (m, 2 H, $O(CH_2)_3CH_2(CH_2)_2Cl$), 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). ^{13}C NMR (125 MHz, $CDCl_3$, 295 K): δ_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 (CCl_3), 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 (CH_2Ph), 74.1 (C-5A), 73.7 (C-4A), 73.4 (CH_2Ph) 73.3 (CH_2Ph), 72.9 (C-3A), 72.6 (CH_2Ph), 70.9 (C-5B, C-3B′), 70.7 (C-5B′), 70.4 (C-2B), 69.3 (OCH_2CH_2), 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 (CH_2Cl), 38.7 ($C(CH_3)_3$), 32.5 ($O(CH_2)_4CH_2CH_2Cl$), 29.2 ($OCH_2CH_2(CH_2)_3CH_2Cl$), 27.1 ($C(CH_3)_3$), 26.6 ($O(CH_2)_3CH_2(CH_2)_2Cl$), 25.2 ($O(CH_2)_2CH_2(CH_2)_3Cl$), 23.1 ($NHCOCH_3$), 21.2, 20.8, 20.7, 20.6, 20.5 ($OCOCH_3$), 16.7 (C-6C). HRESIMS (m/z): $[M + NH_4]^+$ calcd for $C_{92}H_{118}Cl_4N_3O_{32}$ 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-β-D-galactopyranosyl)-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 (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), $NaCNBH_3$ (58.0 mg, 0.9225 mmol, 15 equiv), and methyl orange indicator (2 mg) was stirred under N_2 for 1 h at rt and cooled to 0 °C. A 2 M solution of HCl in Et_2O (461 μL, 0.9225 mmol, 15 equiv) was added dropwise 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 $NaCNBH_3$ (58.0 mg, 0.9225 mmol, 15 equiv) and 2 M solution of HCl in Et_2O (461 μL, 0.9225 mmol, 15 equiv) were added, and the reaction was left to proceed at rt for an additional 1 h and then 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 CH_2Cl_2 (30 mL) and washed with saturated aq $NaHCO_3$ (1 × 30 mL). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were dried and concentrated. Column chromatography (CH_2Cl_2/MeOH, 30:1) followed by RP HPLC (CH_3CN/H_2O, 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, CH_2Cl_2). ^1H NMR ($CDCl_3$, 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 Hz, 1 H, H-4B′), 5.33 (d, J = 3.5 Hz, 1 H, H-4B), 5.20 (dd, J = 8.0, 10.5 Hz, 1 H, H-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, CH_2Ph, CHHPh), 4.51 (d, J = 8.0 Hz, 1 H, H-1B′), 4 34 (d, J = 12.1 Hz, 1 H, CHHPh), 4.30−4.24 (m, 2 H, H-1B, H-5C), 4.18 (dd, J = 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′, OCHHCH$_2$), 3.58 (s, 1 H, H-4C), 3.53−3.43 (m, 6 H, H-5A, H-5B, H-4A′, H-5A′, CH_2Cl), 3.41−3.32 (m, 2 H, H-2A, OCHHCH$_2$), 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, $O(CH_2)_4CH_2CH_2Cl$), 1.46 (m, 2 H, $OCH_2CH_2(CH_2)_3CH_2Cl$), 1.33 (m, 2 H, $O(CH_2)_3CH_2(CH_2)_2Cl$), 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): δ_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_2Ph), 74.1 (C-5A), 73.6 (C-4A), 73.5 (CH_2Ph) 73.4 (CH_2Ph), 73.3 (CH_2Ph), 73.0 (C-3A), 72.5 (CH_2Ph), 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_2CH_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 (CH_2Cl), 38.7 ($C(CH_3)_3$), 32.5 ($O(CH_2)_4CH_2CH_2Cl$), 29.2 ($OCH_2CH_2(CH_2)_3CH_2Cl$), 27.1 ($C(CH_3)_3$), 26.6 (O-

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

$(CH_2)_3CH_2(CH_2)_2Cl$), 25.2 ($O(CH_2)_2CH_2(CH_2)_3Cl$), 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 $C_{92}H_{117}Cl_4N_2O_{32}$ 1901.634, found 1901.628.

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)-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 (19). A solution of the glycosyl acceptor **18** (32.6 mg, 0.01712 mmol) and known[11] thioglycoside donor **8** (40.9 mg, 0.08559 mmol, 5 equiv) in dry CH_2Cl_2 (2 mL) containing freshly activated molecular sieves 4 Å (100 mg) was stirred under N_2 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 and then quenched with NEt_3 (14.3 μL, 0.1027 mmol, 6 equiv) and filtered over Celite. The solids were washed with CH_2Cl_2 (3 × 10 mL), and the combined filtrate and washings were washed with saturated aq $NaHCO_3$ (1 × 15 mL). The aqueous 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 Ac_2O and AcOH (3:1, 2 mL), and the solution was stirred at rt for 16 h and then coconcentrated with toluene (3 × 3 mL). Column chromatography (EtOAc/hexanes, 4:6 then 1:1) followed by RP HPLC (CH_3CN/H_2O, 90:10 to 95:5, 30 min) of the residue gave hexasaccharide **19** (30.1 mg, 76%) pure as an amorphous white foam. $[\alpha]_D$ 46.0 (c 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$, 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 C*H*HPh), 4.87−4.77 (m, 6 H, H-1A, H-3B′, 4 C*H*HPh), 4.76−4.65 (m, 6 H, H-1A′, H-1B′, 4 C*H*HPh), 4.66−4.62 (m, 2 H, 2 C*H*HPh), 4.59 (d, J = 12.1 Hz, 1 H, C*H*HPh), 4.46 (m, 1 H, H-5C′), 4.41 (m, 2 H, CH_2Ph), 4 35 (d, J = 12.1 Hz, 1 H, C*H*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′, OC*H*HCH$_2$), 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, CH_2Cl), 3.35 (m, 1 H, OCH*H*CH$_2$), 2.04, 1.99, 1.92, 1.87, 1.85 (6 s, 18 H, 6 $OCOCH_3$), 1.74 (s, 3 H, $NHCOCH_3$), 1.67 (m, 2 H, $O(CH_2)_4CH_2CH_2Cl$), 1.45 (m, 2 H, $OCH_2CH_2(CH_2)_3CH_2Cl$), 1.33 (m, 2 H, $O(CH_2)_3CH_2(CH_2)_2Cl$), 1.26−1.21 (m, 5 H, H-6C′, $O(CH_2)_2CH_2(CH_2)_3Cl$), 1.16 (s, 9 H, $C(CH_3)_3$), 1.09 (d, J = 6.4 Hz, 3 H, H-6C). ^{13}C NMR (125 MHz, $CDCl_3$, 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 (CCl_3), 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 (CH_2Ph), 74.5 (C-3B), 74.5 (CH_2Ph), 74.4 (CH_2Ph), 74.0 (C-5A), 73.6 (C-4A), 73.5 (CH_2Ph), 73.2 (CH_2Ph), 72.9 (CH_2Ph), 72.8 (C-3A), 72.6 (CH_2Ph), 72.2 (CH_2Ph), 71.8 (C-4A′), 71.5 (C-2B), 71.2 (C-5B), 70.8 (C-3B′), 70.5 (C-5B′), 69.3 (OCH_2CH_2), 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 (CH_2Cl), 38.7 ($C(CH_3)_3$), 32.5 ($O(CH_2)_4CH_2CH_2Cl$), 29.2 ($OCH_2CH_2(CH_2)_3CH_2Cl$), 27.1 ($C(CH_3)_3$), 26.6 ($O(CH_2)_3CH_2(CH_2)_2Cl$), 25.2 ($O(CH_2)_2CH_2(CH_2)_3Cl$), 23.1 ($NHCOCH_3$), 21.2, 20.7, 20.6, 20.5 ($OCOCH_3$), 16.8 (C-6C′), 16.7 (C-6C). HRESIMS (m/z): $[M + H]^+$ calcd for $C_{119}H_{145}Cl_4N_2O_{36}$ 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)-2-acetamido-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 and 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 $NaHCO_3$ (1 × 60 mL). The aqueous layer was re-extracted with CH_2Cl_2 (3 × 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, CH_2Cl_2). ^1H NMR ($CDCl_3$, 600 MHz, 295 K): δ_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 C*H*HPh), 4.90−4.84 (m, 3 H, H-2B, H-3B′, 1 C*H*HPh), 4.83−4.76 (m, 4 H, H-1A′, 3 C*H*HPh), 4.75−4.71 (m, 2 H, 2 C*H*HPh), 4.70−4.60 (m, 6 H, H-1B′, H-5C′, 4 C*H*HPh), 4.45 (d, J = 12.1 Hz, 1 H, C*H*HPh), 4.41−4.33 (m, 4 H, H-5C, H-3A′, 2 C*H*HPh), 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′, OC*H*HCH$_2$), 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, CH_2Cl), 3.37 (m, 1 H, OCH*H*CH$_2$), 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 $OCOCH_3$), 1.93 (s, 3 H, $NHCOCH_3$), 1.88, 1.80 (2 s, 6 H, 2 $OCOCH_3$), 1.72−1.66 (m, 5 H, $NHCOCH_3$, $O(CH_2)_4CH_2CH_2Cl$), 1.48 (m, 2 H, $OCH_2CH_2(CH_2)_3CH_2Cl$), 1.36 (m, 2 H, $O(CH_2)_3CH_2(CH_2)_2Cl$), 1.29−1.22 (m, 5 H, H-6C′, $O(CH_2)_2CH_2(CH_2)_3Cl$), 1.17 (s, 9 H, $C(CH_3)_3$), 1.15 (d, J = 6.5 Hz, 3 H, H-6C). ^{13}C NMR (125 MHz, $CDCl_3$, 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, 127.8, 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 (CH_2Ph), 74.3 (CH_2Ph), 74.2 (C-5A), 74.0 (CH_2Ph), 73.9 (C-4A), 73.6 (CH_2Ph), 73.5 (CH_2Ph), 73.3 (C-3A), 73.2 (CH_2Ph), 72.4 (C-4A′, CH_2Ph), 72.2 (CH_2Ph), 71.2 (C-5B), 71.0 (C-2B, C-3B′), 70.3 (C-5B′), 69.4 (C-4B, OCH_2CH_2), 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 (CH_2Cl), 38.7 ($C(CH_3)_3$), 32.5 ($O(CH_2)_4CH_2CH_2Cl$), 29.2 ($OCH_2CH_2(CH_2)_3CH_2Cl$), 27.1 ($C(CH_3)_3$), 26.5 ($O(CH_2)_3CH_2(CH_2)_2Cl$), 25.1 ($O(CH_2)_2CH_2(CH_2)_3Cl$), 23.6, 23.1 ($NHCOCH_3$), 21.0, 20.8, 20.6, 20.5 ($OCOCH_3$), 16.8 (C-6C′), 16.7 (C-6C). HRESIMS (m/z): $[M + NH_4]^+$ calcd for $C_{119}H_{151}ClN_3O_{36}$ 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)-2-acetamido-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 (21). NaN_3 (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, and the residue was dissolved in CH_2Cl_2 (30 mL) and washed with water (2 × 30 mL). The aqueous layer was re-extracted with CH_2Cl_2 (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. $[\alpha]_D$ −46.5 (c 1.0, CH_2Cl_2). ^1H NMR ($CDCl_3$, 600 MHz, 295 K): δ_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 C*H*HPh), 4.90−4.84 (m, 3 H, H-2B, H-3B′, 1 C*H*HPh), 4.83−4.76 (m, 4 H, H-1A′, 3 C*H*HPh), 4.75−4.71 (m, 2 H, 2 C*H*HPh), 4.70−4.60 (m, 6 H, H-1B′, H-5C′, 4 C*H*HPh), 4.45 (d, J = 12.1 Hz, 1 H, C*H*HPh), 4.41−4.33 (m, 4 H, H-5C, H-3A′, 2 C*H*HPh), 4.31 (d, J = 8.2 Hz, 1 H, H-1B), 4.17−4.07 (m, 4 H, H-3A, H-2C, H-

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

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′, OC*H*HCH$_2$), 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, OCH*H*CH$_2$), 3.26 (br s, 1 H, H-2A), 3.20 (t, J = 6.9 Hz, 2 H, CH$_2$N$_3$), 3.06 (br s, 1 H, H-2A′), 2.06, 2.00, 1.94 (4 s, 12 H, 4 OCOCH$_3$), 1.93 (s, 3 H, NHCOC*H*$_3$), 1.88, 1.80 (2 s, 6 H, 2 OCOCH$_3$), 1.68 (s, 3 H, NHCOC*H*$_3$), 1.55−1.44 (m, 4 H, O(CH$_2$)$_4$C*H*$_2$CH$_2$N$_3$, OCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_2$N$_3$), 1.31−1.21 (m, 7 H, H-6C′, O(CH$_2$)$_3$C*H*$_2$(CH$_2$)$_2$N$_3$, O(CH$_2$)$_2$C*H*$_2$(CH$_2$)$_3$N$_3$), 1.17 (s, 9 H, C(CH$_3$)$_3$), 1.15 (d, J = 6.4 Hz, 3 H, H-6C). ^{13}C NMR (125 MHz, CDCl$_3$, 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 (*C*H$_2$Ph), 74.3 (*C*H$_2$Ph), 74.2 (C-5A), 74.0 (*C*H$_2$Ph), 73.9 (C-4A), 73.6 (*C*H$_2$Ph), 73.5 (*C*H$_2$Ph), 73.3 (C-3A), 73.2 (*C*H$_2$Ph), 72.4 (C-4A′, *C*H$_2$Ph), 72.2 (*C*H$_2$Ph), 71.2 (C-5B), 71.0 (C-2B, C-3B′), 70.3 (C-5B′), 69.4 (C-4B, OCH$_2$CH$_2$), 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 (*C*H$_2$N$_3$), 38.7 (*C*(CH$_3$)$_3$), 29.2 (OCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_2$N$_3$), 28.7 (O(CH$_2$)$_4$CH$_2$C*H*$_2$N$_3$), 27.1 (C(*C*H$_3$)$_3$), 26.4 (O(CH$_2$)$_3$C*H*$_2$(CH$_2$)$_2$N$_3$), 25.4 (O(CH$_2$)$_2$C*H*$_2$(CH$_2$)$_3$N$_3$), 23.5, 23.1 (NHCO*C*H$_3$), 21.0, 20.8, 20.6, 20.5 (OCO*C*H$_3$), 16.8 (C-6C′), 16.7 (C-6C). HRESIMS (m/z): [M + H]$^+$ calcd for C$_{119}$H$_{148}$N$_5$O$_{36}$ 2222.990, found 2222.987.

6-Acetylthiohexyl 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)-2-acetamido-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 (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 °C for 24 h. Workup 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. $[\alpha]_D$ −25.5 (c 0.9, CH$_2$Cl$_2$). ^{1}H NMR (CDCl$_3$, 600 MHz, 295 K): δ_H 7.42−7.40 (m, 2 H, Ar), 7.36−7.20 (m, 38 H, Ar), 5.78 (d, J = 7.3 Hz, 1 H, NHA), 5.51 (d, J = 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 C*H*HPh), 4.90−4.83 (m, 3 H, H-2B, H-3B′, 1 C*H*HPh), 4.82−4.75 (m, 4 H, H-1A′, 3 C*H*HPh), 4.74−4.70 (m, 2 H, 2 C*H*HPh), 4.69−4.60 (m, 6 H, H-1B′, H-5C′, 4 C*H*HPh), 4.44 (d, J = 12.1 Hz, 1 H, C*H*HPh), 4.41−4.32 (m, 4 H, H-5C, H-3A′, 2 C*H*HPh), 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′, OC*H*HCH$_2$), 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*H*CH$_2$), 3.22 (br s, 1 H, H-2A), 3.05 (br s, 1 H, H-2A′), 2.80 (t, J = 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, NHCOC*H*$_3$), 1.86, 1.79 (2 s, 6 H, 2 OCOCH$_3$), 1.67 (s, 3 H, NHCOC*H*$_3$), 1.52−1.43 (m, 4 H, O(CH$_2$)$_4$C*H*$_2$CH$_2$SAc, OCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_2$SAc), 1.30−1.21 (m, 7 H, H-6C′, O(CH$_2$)$_3$C*H*$_2$(CH$_2$)$_2$SAc, O(CH$_2$)$_2$C*H*$_2$(CH$_2$)$_3$SAc), 1.16 (s, 9 H, C(CH$_3$)$_3$), 1.13 (d, J = 6.5 Hz, 3 H, H-6C). ^{13}C NMR (125 MHz, CDCl$_3$, 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 (*C*H$_2$Ph), 74.3 (*C*H$_2$Ph), 74.2 (C-5A), 74.0 (*C*H$_2$Ph), 73.9 (C-4A), 73.6 (*C*H$_2$Ph), 73.5 (*C*H$_2$Ph), 73.3 (C-3A), 73.2 (*C*H$_2$Ph), 72.4 (C-4A′, *C*H$_2$Ph), 72.2 (*C*H$_2$Ph), 71.2 (C-5B), 71.0 (C-2B, C-3B′), 70.3 (C-5B′), 69.4 (C-4B, OCH$_2$CH$_2$), 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*(CH$_3$)$_3$), 30.6 (CH$_2$SAc), 29.4 (O(CH$_2$)$_4$CH$_2$CH$_2$SAc), 29.2 (OCH$_2$CH$_2$(CH$_2$)$_3$CH$_2$N$_3$), 28.4 (O(CH$_2$)$_3$CH$_2$(CH$_2$)$_2$N$_3$), 27.1 (C(*C*H$_3$)$_3$), 25.4 (O(CH$_2$)$_2$CH$_2$(CH$_2$)$_3$SAc), 23.6, 23.1 (NHCO*C*H$_3$), 21.1, 20.9, 20.7, 20.6, 20.5 (OCO*C*H$_3$), 16.8 (C-6C, C-6C′). HRESIMS (m/z): [M + H]$^+$ calcd for C$_{121}$H$_{151}$N$_2$O$_{37}$S 2255.9716, found 2255.9690.

***n*-Hexyl 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-D-galactopyranosyl}-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 Ac$_2$O (1 mL) and left at rt for 5 min, the solvent was evaporated, and the residue was dissolved in Milli-Q water and 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 μmol) was deprotected and purified as described above in method A, except that the remaining solution was neutralized with AcOH (500 μL) instead of Ac$_2$O. 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, H$_2$O). ^{1}H NMR (D$_2$O, 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), 4.67 (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′, OC*H*HCH$_2$), 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′, OCH*H*CH$_2$), 2.02, 2.01 (2 s, 6 H, 2 OCOCH$_3$), 1.52 (m, 2 H, OCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_3$), 1.33−1.23 (m, 6 H, OCH$_2$CH$_2$(C*H*$_2$)$_3$CH$_3$), 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(CH$_2$)$_5$C*H*$_3$). ^{13}C NMR (125 MHz, D$_2$O, 295 K): δ_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 (OCH$_2$CH$_2$), 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 (OCH$_2$CH$_2$(CH$_2$)$_3$CH$_3$), 33.5, 27.6, 24.8 (OCH$_2$CH$_2$(CH$_2$)$_3$CH$_3$), 25.0 (NHCOCH$_3$), 18.1 (C-6C′), 18.0 (C-6C), 16.1 (O(CH$_2$)$_5$CH$_3$). HRESIMS (m/z): [M + NH$_4$]$^+$ calcd for C$_{46}$H$_{84}$N$_3$O$_{29}$ 1142.519, found 1142.525.

6-Aminohexyl 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-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 μL) instead of Ac$_2$O. 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-Q water, and after repeated lyophilization from Milli-Q water, the deprotected hexasaccharide **3** (21.1 mg, 61%) was obtained as the acetate salt pure in the form of a white amorphous powder. $[\alpha]_D$ −64.5 (c 1.0, H$_2$O). ^{1}H NMR (D$_2$O, 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

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

(m, 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′, OC*H*HCH$_2$), 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′, OCH*H*CH$_2$), 2.97 (t, J = 7.5 Hz, 2 H, CH_2NH$_3^+$), 2.02, 2.01 (2 s, 6 H, 2 OCOCH_3), 1.91 (s, 3 H, $^-$OCOCH_3), 1.64 (m, 2 H, O(CH$_2$)$_4$CH_2CH$_2$NH$_3^+$), 1.54 (m, 2 H, OCH$_2$CH_2(CH$_2$)$_3$CH$_2$-NH$_3^+$), 1.40−1.31 (m, 4 H, O(CH$_2$)$_3$CH_2(CH$_2$)$_2$NH$_3^+$, O-(CH$_2$)$_2$CH_2(CH$_2$)$_3$NH$_3^+$), 1.17 (d, J = 6.6 Hz, 3 H, H-6C′), 1.14 (d, J = 6.6 Hz, 3 H, H-6C). ^{13}C NMR (125 MHz, D$_2$O, 295 K): δ_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 (OCH$_2$CH$_2$), 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 (CH$_2$NH$_3^+$), 31.1 (OCH$_2$CH$_2$(CH$_2$)$_3$CH$_2$NH$_3^+$), 29.4 (O(CH$_2$)$_4$CH$_2$CH$_2$NH$_3^+$), 28.0 (O(CH$_2$)$_3$CH$_2$(CH$_2$)$_2$NH$_3^+$), 27.4 (O(CH$_2$)$_2$CH$_2$(CH$_2$)$_3$NH$_3^+$), 25.0 (NHCOCH$_3$), 18.1 (C-6C′), 18.0 (C-6C). HRESIMS (m/z): [M + H]$^+$ calcd for C$_{46}$H$_{82}$N$_3$O$_{29}$ 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-D-galactopyranosyl}-β-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 and then deionized with Dowex 50 (H$^+$) resin. The resin was filtered off and 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 μL), 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. [α]$_D$ −27.5 (c 0.4, H$_2$O). ^{1}H NMR (D$_2$O, 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), 4.67 (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 = 9.7 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′, OC*H*HCH$_2$), 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′, OCH*H*CH$_2$), 2.89 (m, 2 H, CH_2S), 2.02 (2 s, 6 H, 2 OCOCH_3), 1.72 (m, 2 H, O(CH$_2$)$_4$CH_2CH$_2$S), 1.55 (m, 2 H, OCH$_2$CH_2(CH$_2$)$_3$-CH$_2$S), 1.42 (m, 2 H, O(CH$_2$)$_3$CH_2(CH$_2$)$_2$S), 1.33 (O-(CH$_2$)$_2$CH_2(CH$_2$)$_3$S), 1.17 (d, J = 6.6 Hz, 3 H, H-6C′), 1.14 (d, J = 6.6 Hz, 3 H, H-6C). ^{13}C NMR (125 MHz, D$_2$O, 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 (OCH$_2$CH$_2$), 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 (CH$_2$S), 31.0 (OCH$_2$CH$_2$(CH$_2$)$_3$CH$_2$S), 30.0 (O(CH$_2$)$_3$CH$_2$(CH$_2$)$_2$S), 27.3 (O(CH$_2$)$_2$CH$_2$(CH$_2$)$_3$S), 26.7 (O-(CH$_2$)$_4$CH$_2$CH$_2$S), 25.0 (NHCOCH$_3$), 18.1 (C-6C′), 18.0 (C-6C). ESI FAIMS HRMS (m/z): [M − H$_2$O − H + 3Na]$^{2+}$ calcd for C$_{46}$H$_{77}$N$_2$O$_{28}$SNa$_3$ 603.2033, found 603.2016.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01190.

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. ^{1}H and ^{13}C NMR spectra for compounds **10** and **11**. ^{1}H, COSY, ^{13}C, and HSQC NMR spectra for compounds **12**−**22** and **2**−**4** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: fauzanne@uoguelph.ca.

Present Address

†GL CHEMTEC, 1456 Wallace Road Oakville, Ontario L6L 2Y2 Canada.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Natural Science and Engineering Research Council of Canada for financial support of this work.

■ REFERENCES

(1) Hevey, R.; Ling, C.-C. *Future Med. Chem.* **2012**, *4*, 545−584.

(2) Mårtensson, S.; Due, C.; Pahlsson, P.; Nilsson, B.; Eriksson, H.; Zopf, D.; Olsson, L.; Lundblad, A. *Cancer Res.* **1988**, *48* (8), 2125−2131.

(3) (a) Lemieux, R. U.; Baker, D. A.; Weinstein, W. M.; Switzer, C. M. *Biochemistry* **1981**, *20* (1), 199−205. (b) Pettijohn, D. E.; Stranahan, P. L.; Due, C.; Rønne, E.; Sørensen, H. R.; Olsson, L. *Cancer Res.* **1987**, *47* (4), 1161−1169.

(4) (a) Pettijohn, D. E.; Pfenninger, O.; Brown, J.; Duke, R.; Olsson, L. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85* (3), 802−806. (b) Stranahan, P. L.; Howard, R. B.; Pfenninger, O.; Cowen, M. E.; Johnston, M. R.; Pettijohn, D. E. *Cancer Res.* **1992**, *52* (10), 2923−2930. (c) Battifora, H.; Sorensen, H. R.; Mehta, P.; Ahn, C.; Niland, J.; Hage, E.; Pettijohn, D. E.; Olsson, L. *Cancer* **1992**, *70*, 1867−1872. (d) Stroud, M. R.; Levery, S. B.; Martensson, S.; Salyan, M. E. K.; Clausen, H.; Hakomori, S. *Biochemistry* **1994**, *33* (35), 10672−10680.

(5) (a) Jackson, T. A.; Robertson, V.; Imberty, A.; Auzanneau, F.-I. *Bioorg. Med. Chem.* **2009**, *17* (4), 1514−1526. (b) Wang, A.; Auzanneau, F.-I. *Carbohydr. Res.* **2010**, *345* (9), 1216−1221. (c) Guillemineau, M.; Auzanneau, F.-I. *J. Org. Chem.* **2012**, *77* (20), 8864−8878.

(6) (a) Zhang, P.; Ng, K.; Ling, C. C. *Org. Biomol. Chem.* **2010**, *8* (1), 128−136. (b) Zhang, P.; Razi, N.; Eugenio, L.; Fentabil, M.; Kitova, E. N.; Klassen, J. S.; Bundle, D. R.; Ng, K. K. S.; Ling, C. C. *Chem. Commun.* **2011**, *47* (45), 12397−12399.

(7) (a) Buryakov, I. A.; Krylov, E. V.; Nazarov, E. G.; Rasulev, U. K. *Int. J. Mass Spectrom. Ion Processes* **1993**, *128* (3), 143−148. (b) Purves, R. W.; Guevremont, R.; Day, S.; Pipich, C. W.; Matyjaszczyk, M. S. *Rev. Sci. Instrum.* **1998**, *69* (12), 4094−4105. (c) Purves, R. W.; Guevremont, R. *Anal. Chem.* **1999**, *71* (13), 2346−2357. (d) Gabryelski, W.; Froese, K. L. *J. Am. Soc. Mass Spectrom.* **2003**, *14* (3), 265−277.

(8) Kuir, D.; Guillemineau, M.; Auzanneau, F.-I. *J. Org. Chem.* **2015**, *80*, 5004−5013.

(9) Wang, A.; Hendel, J.; Auzanneau, F.-I. *Beilstein J. Org. Chem.* **2010**, *6*, No. 10.3762/bjoc.6.17.

(10) Blatter, G.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *288*, 109−125.

(11) Lönn, H. *Carbohydr. Res.* **1985**, *139*, 105−113.

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083

(12) Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1983**, *7*, 1249−1256.
(13) Guillemineau, M.; Auzanneau, F.-I. *Carbohydr. Res.* **2012**, *357*, 132−138.
(14) (a) Liao, L.; Auzanneau, F.-I. *J. Org. Chem.* **2005**, *70* (16), 6265−6273. (b) Cheng, A.; Hendel, J. L.; Colangelo, K.; Bonin, M.; Auzanneau, F.-I. *J. Org. Chem.* **2008**, *73* (19), 7574−7579.
(15) (a) Ratner, D. M.; Adams, E. W.; Su, J.; O'Keefe, B. R.; Mrksich, M.; Seeberger, P. H. *ChemBioChem* **2004**, *5*, 379−382. (b) Hendel, J. L.; Auzanneau, F.-I. *Eur. J. Org. Chem.* **2011**, *34*, 6864−6876.
(16) Pope, R. M.; Shen, N.; Hofstadler, S. A.; Dearden, D. V. *Int. J. Mass Spectrom. Ion Processes* **1998**, *175*, 179−186.
(17) (a) Rodriquez, C. F.; Fournier, R.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. *Int. J. Mass Spectrom.* **1999**, *192*, 303−317. (b) Rodriquez, C. F.; Guo, X.; Shoeib, T.; Hopkinson, A. C.; Siu, K. W. M. *J. Am. Soc. Mass Spectrom.* **2000**, *11* (11), 967−975. (c) Grewal, R. N.; El Aribi, H.; Smith, J. C.; Rodriquez, C. F.; Hopkinson, A. C.; Siu, K. W. M. *Int. J. Mass Spectrom.* **2002**, *219* (1), 89−99.
(18) Madhusudanan, K. P. *J. Mass Spectrom.* **2003**, *38*, 409−416.
(19) Lyczko, J.; Beach, D. G.; Gabryelski, W. *J. Mass Spectrom.* **2015**, *50* (3), 463−469.

DOI: 10.1021/acs.joc.5b01190
J. Org. Chem. 2015, 80, 8073−8083