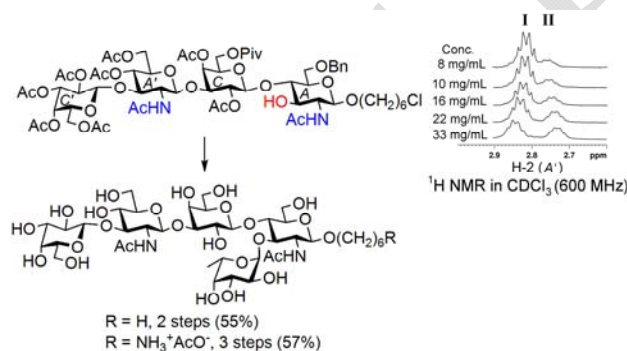


Aggregation of a Tetrasaccharide Acceptor Observed by NMR: Synthesis of Pentasaccharide Fragments of the Le^aLe^x Tumor-Associated Hexasaccharide Antigen

Deng Kuir, Mickaël Guillemineau, France-Isabelle Auzanneau*

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

fauzanne@uoguelph.ca



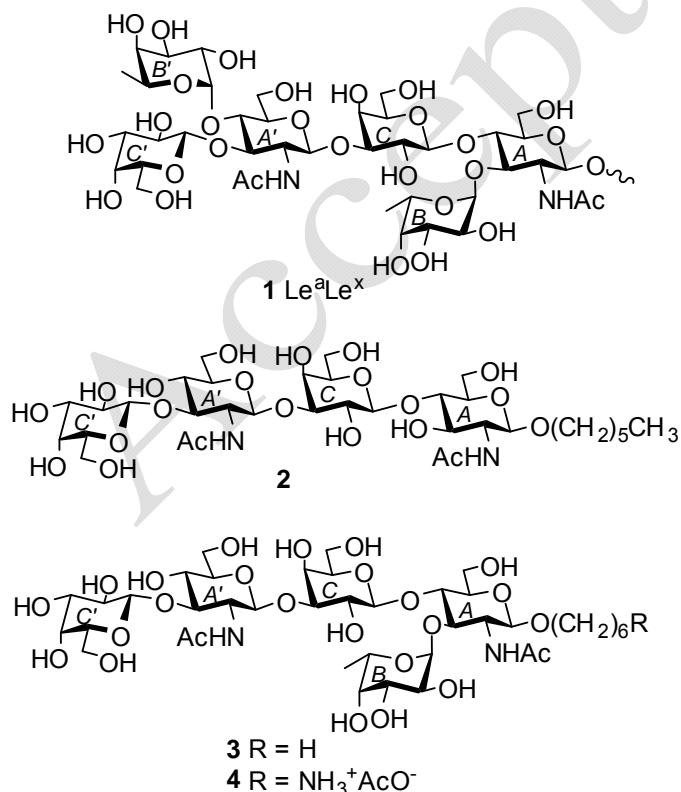
Abstract

We report the synthesis of a tetrasaccharide and two pentasaccharide fragments of the Le^aLe^x tumor associated carbohydrate antigen α -L-Fuc-(1→4)-[β -D-Gal-(1→3)]- β -D-GlcNAc-(1→3)- β -D-Gal-(1→4)-[α -L-Fuc-(1→3)]- β -D-GlcNAc-(1→OR). The choice of protecting groups permitted a one-step global deprotection (Na/NH_{3(l)}). The protected chlorohexyl glycoside pentasaccharide was the precursor to the hexyl glycoside, to be used as a soluble inhibitor, and the aminohexyl glycoside analogue, to be conjugated to proteins for surface immobilization and immunization experiments. We observed that a linear tetrasaccharide that contained two *N*-acetylglucosamine residues and a free OH group gave two distinct sets of ¹H NMR signals when the data was acquired in deuterated chloroform. Data acquisition at variable concentrations and variable temperatures suggest that the

second set of NMR signals results from aggregation of the tetrasaccharide driven by the formation of intermolecular H-bonds involving the NHAc. While the formation of intra- and intermolecular H-bonds involving *N*-acetylglucosamine residues has been reported in non-H-bonding solvents, this is, to our knowledge, the first time that these lead to the appearance of two distinct sets of signals in the NMR spectra. This aggregation, may explain the lack of reactivity observed when attempting to glycosylate such acceptor using non H-bonding solvents such as dichloromethane.

Introduction

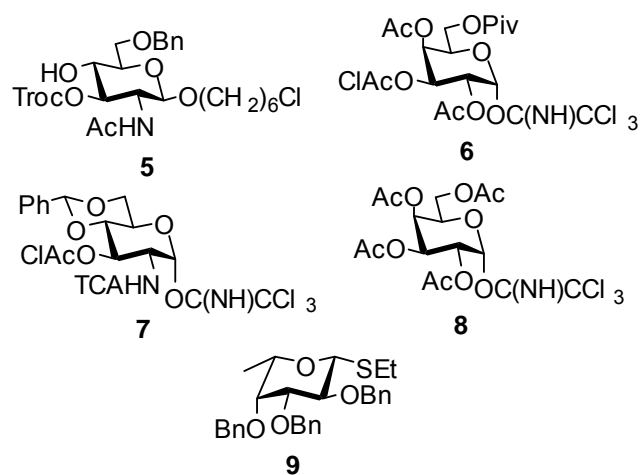
The development of anti-cancer vaccines that specifically target tumor-associated carbohydrate antigens (TACAs) has been the focus of intensive research for the past three decades. While not yet successful, advances have been made that have been summarized in multiple reviews.¹ The TACA Le^aLe^x hexasaccharide (**1**) is such a TACA which is associated with lung cancer and particularly squamous lung carcinoma (SLC).²



However, while Le^aLe^x is overexpressed on SLC cells, the Le^a trisaccharide displayed at the non-reducing end of this hexasaccharide is expressed at the surface of numerous non-cancerous cells.^{2a, 3} Interestingly, immunization of mice with SLC cells allowed cloning of an antibody (MAb 43-9F) which was shown to specifically recognize Le^aLe^x while it only weakly bound to the Le^a trisaccharide.^{2a-c} Such findings support that the Le^aLe^x TACA may display immuno-relevant internal epitopes that do not involve the Le^a trisaccharide and which, if identified, may be used for the development of an anti-SLC vaccine. We have been actively attempting to identify a fragment of the Le^aLe^x hexasaccharide that would no longer carry the non-reducing end Le^a trisaccharide but possibly retain the internal epitopes identified by mAb 43-9F. We recently reported⁴ the efficient preparation of tetra and pentasaccharide fragments that lacked the non-reducing end galactosyl residue (*C'*), here we described the preparation of the last two tetra and pentasaccharide fragments **2** and **3** missing to our panel and that do not carry the non-reducing end fucosyl residue (*B'*). The pentasaccharide was prepared as the hexyl glycoside **3** for immunochemistry and the aminohexyl glycoside **4** for conjugation to carrier proteins. The synthetic strategy that we followed was designed to avoid the challenging deprotection steps faced in our previous study.⁴ Thus, the final compounds were obtained in good yields from the protected intermediates following one single deprotection step using dissolving metal conditions [Na/NH₃(*l*)].⁵ Of particular interest, we report the observation that a tetrasaccharide intermediate gave two distinct sets of ¹H NMR chemical shifts when the spectrum was recorded for a solution in deuterated chloroform. Temperature and concentration dependence NMR studies indicated that the second set of NMR signals likely resulted from aggregation of the oligosaccharide driven the formation of intermolecular H-bond involving the *N*-acetyl groups.

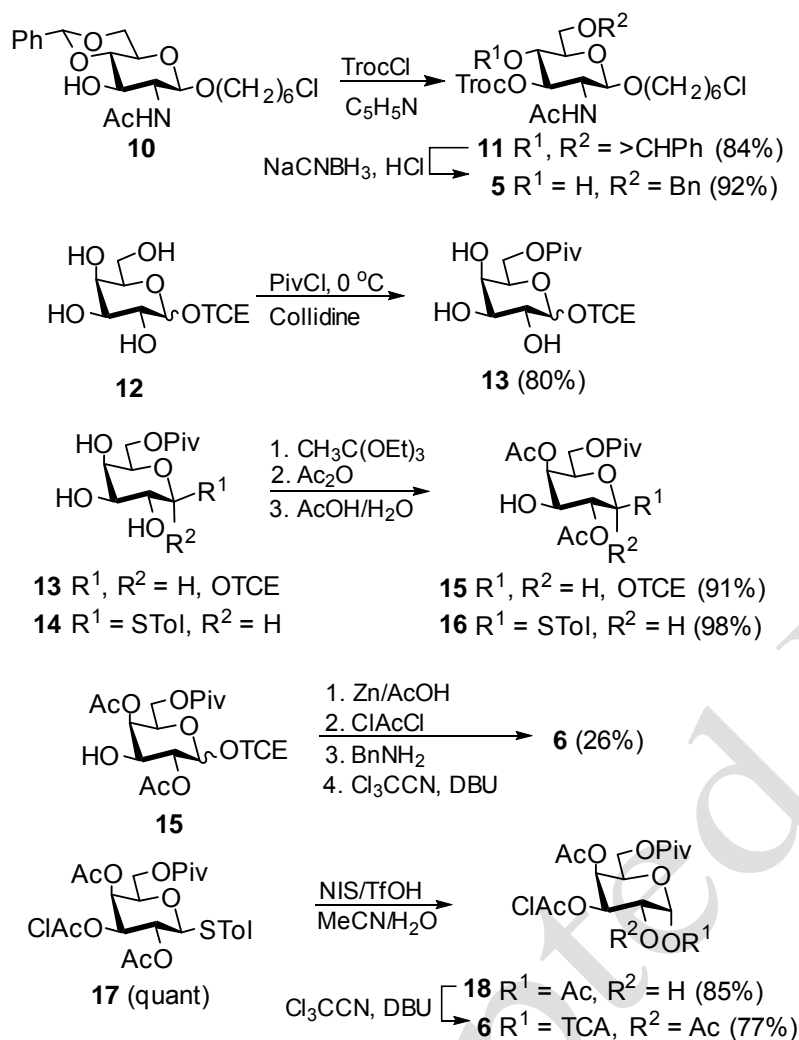
Results and Discussion

The tetra- penta- saccharides were assembled efficiently using novel chlorohexyl acceptor **5** and galactosyl donor **6**, as well as the known donors **7**⁶, **8**⁷ and **9**⁸.



Acceptor **5** was prepared smoothly (77% overall) in two steps from the known^{5b} monosaccharide **10** (Scheme 1).

SCHEME 1. Synthesis of Donor 5 and Acceptor 6

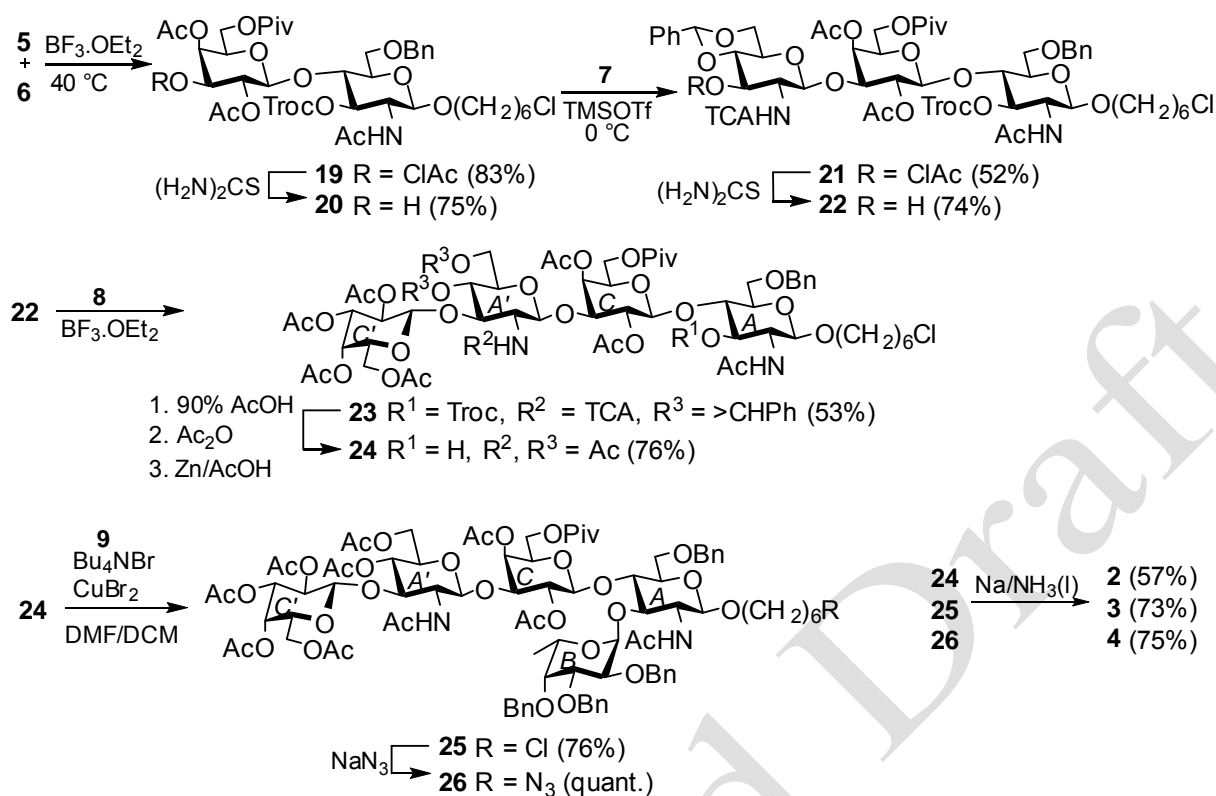


Treatment with trichloroethoxycarbonyl chloride led to the Troc-protected intermediate **11** which was submitted to reductive opening of the benzylidene acetal ($\text{NaCNBH}_3\text{-HCl}$) yielding the desired acceptor **5**. To facilitate the one step full deprotection of the final compounds under dissolving metal conditions, we elected to prepare galactosyl donor **6** that carries a pivaloyl group at O-6. Indeed, we have demonstrated⁴ that the use of a silyl group at this position was not compatible with this one-step deprotection scheme. Donor **6** was prepared from the known⁹ anomeric mixture of trichloroethyl glycoside **12** or from the known¹⁰ thiotolyl glycoside **14** (Scheme 1). Glycoside **12** was selectively pivaloylated at O-6 to give triol **13**, which was converted in three steps to alcohol **15**: introduction of an 3,4-*O*-orthoacetate, in situ O-2 acetylation followed by the acid catalyzed opening of the orthoester to the O-4 acetate. Alcohol **15** was then converted to donor **6** in moderate yield (26% from

15 or 24% from **13**) over four steps: removal of the trichloroethyl group (65%), di-chloroacetylation at O-1 and O-3 (69%), selective removal of the anomeric chloroacetate (quant) then conversion of the resulting hemiacetal to the trichloroacetimidate donor (57%). The alternative preparation of donor **6** from triol **14** provided donor **6** in a much higher overall yield (64% from **14**). Conversion of triol **14** to alcohol **16** was achieved in 98% yield following the same sequence of steps described above to prepare alcohol **15** from triol **13**. In turn, chloroacetylation at O-3 of **16** gave tolyl glycoside **17** quantitatively, which was submitted to hydrolytic conditions aimed at removing the thiotolyl group. As expected,^{4, 5d, 11} these conditions led to migration of the acetate at O-2 and isolation of alcohol **18** in 85% yield. In turn, treatment of alcohol **18** with trichloroacetonitrile and DBU gave as previously reported on similar analogues,^{4, 5d, 11} the desired donor **6** in 77% yield.

With the monosaccharide building blocks in hand the straightforward assembly of tetrasaccharide **23** began with the glycosylation of acceptor **5** with 1.8 equivalents of donor **6** under activation with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2 equiv) at 40 °C (Scheme 2).^{4, 5d, 12} Under these conditions, the desired disaccharide (**19**) was obtained in excellent yields and subsequently converted to acceptor **20** by treatment with excess thiourea at 55 °C. Glycosylation of acceptor **20** with trichloroacetimidate **7** (3 equiv) under activation with TMSOTf (1.8 equiv) at 0–10 °C then gave trisaccharide **21** (52%), which was converted to acceptor **22** by treatment with thiourea (12 equiv, 70°C). Galactosylation of acceptor **22** with donor **8** (6 equiv) was best achieved under $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2.4 equiv) activation maintaining the temperature at 0 °C for 3 h then allowing the reaction to run at room temperature for an additional hour.

SCHEME 2. Synthesis of Final Compounds 2–4



Despite the large excess of donor **8**, tetrasaccharide **23** was only isolated in a disappointing 53% yield while some unreacted acceptor was also recovered (12%). We attribute this yield to the known¹³ poor match between the non-reducing end O-3 position in acceptor **22** and a β -D-galactosyl donor. Tetrasaccharide **23** was then converted in 3 steps to acceptor **24**. When we first attempted the combined removal of the O-3 trichloroethoxycarbonyl group and reduction of the *N*-trichloroacetate using Zn in acetic acid, we observed the concurrent partial loss of the benzylidene group. Thus, the benzylidene group was first removed with aqueous acetic acid, the resulting diol acetylated and the acetylated intermediate treated with a large excess of Zn in acetic acid at 50 °C to remove the Troc group and reduce the trichloroacetamido to an *N*-acetyl group.

Interestingly while a product homogenous by TLC was isolated in 76% yield, ¹H NMR in CDCl₃ (~22 mg/mL, 16 mM) at 295 K showed two similar but distinct sets of signals (**I** and **II**, Figure 1a) that integrated for a ratio 7 to 3. Selective 1D-TOCSY experiments (Figure 1, b–f) allowed assignment of a large number of proton signals for each of **I** and **II** that are listed in Table 1.

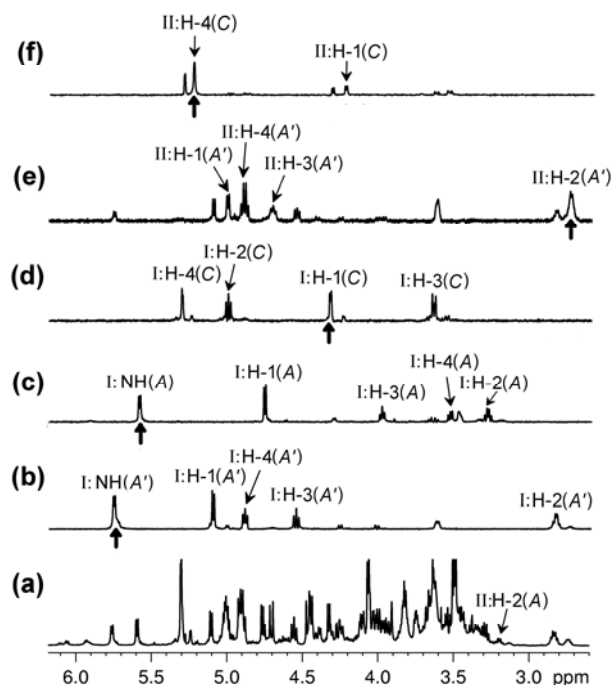


FIGURE 1. ^1H NMR And 1D Selective TOCSY Experiments (600 MHz) for Tetrasaccharide 24 in CDCl_3 (16 mM) at 295 K. (a) ^1H NMR; (b–f) TOCSYs, the signal irradiated is indicated with a bold arrow (\uparrow).

The vicinal coupling constants measured for the ring signals (Table 1) supported $^4\text{C}_1$ chair conformations for the galactose and *N*-acetylglucosamine rings in both **I** and **II**. Thus, we concluded that unlike our previous observation¹⁴ for protected branched trisaccharides, there was no evidence of ring distortion that would explain the appearance of two conformations in the NMR spectrum.

TABLE 1: ^1H NMR (600 MHz) Data Recorded for **I** and **II** in CDCl_3 at 16 mM and 295 K.

	δ (ppm) (m, J Hz) ^a	δ (ppm) (m, J Hz) ^a	$\Delta(\delta)$ ^b (ppm)
	I	II	
NH(A)	5.595	^c	
H-1(A)	4.765	4.306	0.459
H-2(A)	3.286	3.196	0.090
H-1(C)	4.326 (d, 7.6)	4.238 (d, 7.5)	0.088
H-4(C)	5.303	5.24	0.063

	(bd, ~2)	(bs, ~2)	
H-1(A')	5.104	5.011	0.093
	(d, 8.0)	(d, 7.7)	
H-2(A')	2.831	2.740	0.091
H-3(A')	4.555	4.712	-0.157
	(t, 10)	(t, 10)	
H-4(A')	4.894	4.910	-0.016
	(t, 9.2)	(t, 9.3)	
H-5(A')	3.618	3.624	-0.006
NH(A')	5.761	^c	
H-1(C')	4.443	4.386	0.057

^aMultiplicity observed in TOCSY, coupling constant measured in Hz. ^bChemical shift difference for given hydrogens in **I** and **II**: $\delta(\mathbf{I}) - \delta(\mathbf{II})$. ^cWas not observed.

Interestingly, as can be seen on Figure 1e, irradiation of the signal corresponding to H-2 (A') of **II** at 2.74 ppm, did not give transfer to the signal found for NH(A') at 5.76 ppm, while irradiation of the later (Figure 1b) showed correlation to the signal assigned to H-2 (A') of **I** at 2.83 ppm. Thus, suspecting that H-bonding involving NH(A') may still result in these two sets of chemical shifts, we acquired NMR data for tetrasaccharide **24** in the H-bonding solvent CD₃OD. Indeed, as can be seen in the HSQC experiments shown on Figure 2, the anomeric region showed two sets of 4 cross-peaks when the spectrum was acquired in the non H-bonding CDCl₃, but simplified to one single set of 4 signals (two are overlapping) when the spectrum was acquired for a solution in CD₃OD.

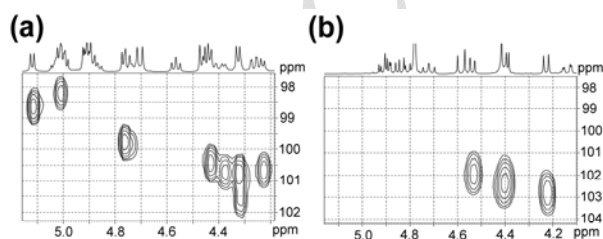


FIGURE 2. HSQC (600 MHz) Anomeric Regions Recorded for Tetrasaccharide 24 in (a) CDCl₃ and (b) CD₃OD.

To further understand the occurrence of this second set of signals in CDCl₃, we recorded ¹H NMR spectra at various concentrations: 6–22 mM (at 295 K) and temperatures 220–320 K (at 16 mM). We

show on Figure 3 the variation in chemical shifts measured for NH(*A*) and NH(*A'*) in **I** with increasing concentrations and increasing temperature.

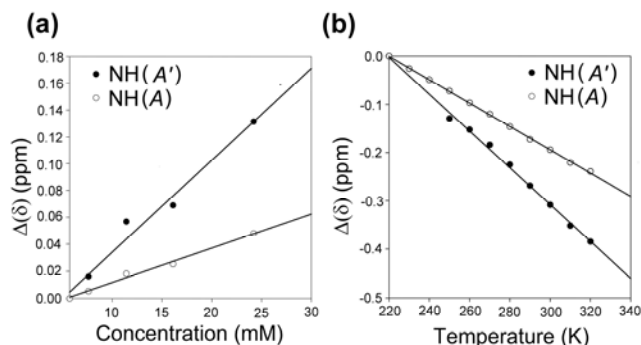


FIGURE 3. Concentration (a) and Temperature (b) Dependence of δ (NH-*A*) and δ (NH-*A'*) Measured at 600 MHz for Tetrasaccharide 24 in CDCl_3 . (a) Temperature: 295 K; (b) Concentration: 16 mM.

The linear positive dependence of the NH chemical shifts with concentration: +6.9 ppb for NH(*A'*); +2.5 ppb for NH(*A*), and their linear negative dependence with temperature: -3.8 ppb for NH(*A'*); -2.4 ppb for NH(*A*), indicate that NH(*A*) and NH(*A'*) in **I** are involved in the formation of intermolecular H-bonds.¹⁵ Figure 4 shows the signals observed for H-2(*A'*) in **I** and **II** with increasing concentration (Figure 4a, top to bottom) and decreasing temperature (Figure 4b, top to bottom).

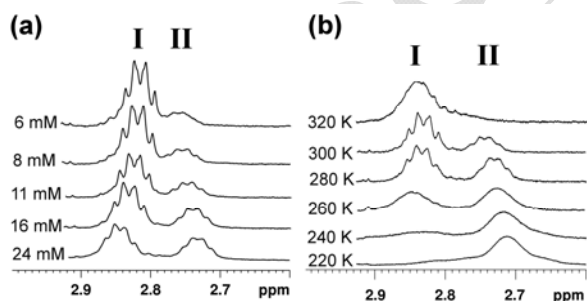


FIGURE 4. ^1H NMR (600 MHz) for H-2(*A'*) in **I and **II** Recorded for Tetrasaccharide 24 in CDCl_3 . (a) Increasing Concentrations at 295 K; (b) Decreasing Temperatures at 16 mM.**

As can be seen, the relative amount of **II** increased with increasing concentrations and decreasing temperatures suggesting that self-association of the tetrasaccharide led to this second set of chemical

shifts.^{15c} Thus, as H-bonds involving the NHAc in **I** become increasingly stronger with increasing concentrations or decreasing temperature, the relative proportion of **II** increases concurrently. Therefore, we propose that much like what is observed in peptides,^{15a, b} intermolecular H-bonds involving the NH groups and most particularly NH(A') in **I**, lead to self-association of tetrasaccharide **24** and appearance of a second set of NMR signal (**II**) that likely results from an aggregated form.

Given this tendency to aggregate in non H-bonding solvent, it was not surprising that fucosylation of tetrasaccharide **24** using donor **9** activated with NIS/triflic acid in dichloromethane was unsuccessful. In contrast, glycosylation in a mixture of DMF and dichloromethane using 6 equivalents of donor **9** (6 equiv) activated with copper (II) bromide and tetrabutylammonium bromide at room temperature gave the desired pentasaccharide **25** in 76% yield. Treatment of chlorohexyl glycoside **25** with sodium azide in DMF at 80 °C promptly afforded the 6-azidohexyl analogue **26** in quantitative yield. Interestingly, NMR in CDCl₃, did not show evidence of aggregation for the pentasaccharides. This observation may indicate that either the bulky fucosyl residue prevents the formation of inter-molecular H-bonds that would promote aggregation of the pentasaccharides, or that the C-3(A) hydroxyl group now fucosylated in the pentasaccharides had a role in stabilizing the formation of the aggregate **II** in tetrasaccharide **24**.

Single step deprotection of tetrasaccharide **24** and pentasaccharides **25** and **26** was easily achieved under dissolving metal conditions [Na/NH₃(l) in THF, -78 °C] and gave the corresponding final compounds **2–4**. Hexyl glycosides **2** and **3** were obtained pure and salt-free in 57% and 73% yield, respectively after purification on a Biogel P2 column eluted with milli-Q water. Aminohexyl glycoside **4** was obtained (75%) as the ammonium acetate salt after purification on Biogel P2 column eluted with 0.05 M ammonium acetate and repeated freeze-drying from milli-Q water. The structure

and purity of the final compounds was confirmed by NMR and MS spectroscopy, the analytical data recorded for pentasaccharide **4** was in accordance with that previously reported Ling and co.¹⁶

Conclusion

Building on our experience,⁴ we report here the convergent synthesis of a tetrasaccharide and two pentasaccharide fragments of the Le^aLe^x tumor associated hexasaccharide. Carefully choosing the protecting groups allowed for an easy one step total deprotection of the intermediate protected oligosaccharide under dissolving metal conditions. This strategy differed from that of Ling and co. who have reported block syntheses of pentasaccharide **4** and an analogue of tetrasaccharide **2** using phthalimido groups at C-2 of the glucosamine residues and multi-step deprotection schemes.¹⁶⁻¹⁷ Of particular interest is our discovery that the linear tetrasaccharide **24** that carries two *N*-acetyl groups showed a tendency to self-associate in non H-bonding solvent such as chloroform. While self-association has been suggested in *N*-acetyl allosamine and glucosamine monosaccharides,^{15c, d} this is, to our knowledge, the first time that two distinct sets of NMR signals are observed as a result of self-association likely leading to aggregation. Since the conditions that we used (~ 15 mg in ~ 0.7 mL of CDCl₃ at 295 K) to record the first set of NMR data are standard conditions to record ¹H NMR data in synthetic carbohydrate chemistry, our observation that these can lead to aggregation of *N*-acetylglucosamine-containing linear oligosaccharides is particularly noteworthy. Indeed, the appearance of a second set of NMR signals could be attributed mistakenly to the presence of multiple compounds in the sample. To avoid such error, acquisition of NMR data using a hydrogen bonding solvent such as deuterated methanol that will prevent self-association and aggregation is best.

Experimental Section

6-Chlorohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-trichloroethoxycarbonyl- β -D-glucopyranoside (11). 2,2,2-Trichloroethyl chloroformate (3.8 mL, 2 equiv) was added dropwise

to a suspension of benzylidene acetal **10**^{5b} (7.6 g, 164 mmol) in CH₂Cl₂ (180 mL) containing anhyd pyridine (7.2 mL) and stirred under N₂. The reaction was stirred at rt for 1.5 h, diluted in CH₂Cl₂ (300 mL) and sequentially washed with 2 N HCl (2 × 400 mL) and satd aq NaHCO₃ (2 × 400 mL). The aq layers were extracted with CH₂Cl₂ (2 × 50 mL) and the combined organic layers were dried and concentrated. The residue was purified by column chromatography (EtOAc:hexanes, 4:6) to give compound **11** (9.01 g, 84%) pure as a white amorphous solid. [α]_D -29.0 (*c* 1.0, MeOH). ¹H NMR (400 MHz, CDCl₃, 295 K): δ _H 7.43–7.33 (m, 5 H, Ar), 5.61 (d, *J* = 8.2 Hz, 1 H, NH), 5.46 (s, 1 H, >CHPh), 5.36 (t, *J* = 10.0 Hz, 1 H, H-3), 4.85 (d, *J* = 8.3 Hz, 1 H, H-1), 4.84 (d, *J* = 11.9, 1 H, COCHHCCl₃), 4.67 (d, *J* = 10.9, 1 H, COCHHCCl₃), 4.30 (dd, *J* = 5.0, 10.5 Hz, 1 H, H-6a), 3.88–3.62 (m, 4 H, H-2, H-4, H-6b, OCHHCH₂), 3.61–3.40 (m, 4 H, H-5, CH₂Cl, OCHHCH₂), 1.90 (s, 3 H, NHCOCH₃), 1.68, 1.51, 1.37, 1.28 (4 m, 4 × 2 H, OCH₂(CH₂)₄CH₂Cl). ¹³C NMR (100 MHz, CDCl₃, 295 K): δ _C 170.2, 153.9 (C=O), 136.7 (quat Ar), 129.1, 128.2, 126.1 (Ar), 101.4 (>CHPh), 100.7 (C-1), 94.4 (CCl₃), 78.8 (C-4), 76.8 (CH₂CCl₃), 75.9 (C-3), 70.0 (OCH₂CH₂), 68.6 (C-6), 65.9 (C-5), 56.0 (C-2), 45.0 (CH₂Cl), 32.4, 29.2, 26.4, 25.1 (OCH₂(CH₂)₄CH₂Cl), 23.3 (NHCOCH₃). HRESIMS *m/z*: [M+H]⁺ Calcd for C₂₄H₃₂NO₈Cl₄ 602.0882; Found 602.0877.

6-Chlorohexyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-trichloroethoxycarbonyl- β -D-glucopyranoside (5). A solution of benzylidene acetal **11** (6.84 g, 11.3 mmol) in anhyd THF (300 mL) containing freshly activated 3 Å molecular sieves (30 g), NaCNBH₃ (13.5 g, 18 equiv) and methyl orange indicator (~20 mg) was stirred under N₂ at 0 °C for 30 min. HCl gas was bubbled through the reaction mixture until the solution turned pink (less than 5 min) and the reaction was then allowed to proceed under stirring at rt for 17 h. Solids were filtered off over Celite®, washed thoroughly with THF and the combined filtrate and washings were concentrated. The oily residue was dissolved in CH₂Cl₂ (400 mL), washed with satd aq NaHCO₃ (2 × 400 mL) and the aq layers were re-extracted with CH₂Cl₂ (2 × 200 mL). The combined organic phases were dried, concentrated

and column chromatography of the residue (CH₂Cl₂: MeOH, 20:1) gave acceptor **5** (6.34 g, 92%) pure as a yellowish amorphous solid. $[\alpha]_D -16.2$ (*c* 1.0, MeOH). ¹H NMR (400 MHz, CDCl₃, 295 K): δ_H 7.40–7.25 (m, 5 H, Ar), 5.52 (s, 1 H, NH), 5.13 (dd, *J* = 3.8, 10.6 Hz, 1 H, H-3), 4.82 (d, *J* = 11.9 Hz, 1 H, COCHHCCl₃), 4.73 (d, *J* = 8.2 Hz, 1 H, H-1), 4.64 (d, *J* = 11.9 Hz, 1 H, COCHHCCl₃), 4.63–4.50 (m, 2 H, CH₂Ph), 3.68–3.53 (m, 4 H, H-4, H-6ab, OCHHCH₂), 3.68–3.53 (m, 2 H, H-2, H-5), 3.53–3.40 (m, 3 H, CH₂Cl, OCHHCH₂), 1.88 (s, 3 H, NHCOCH₃), 1.68, 1.50, 1.37, 1.31 (4 m, 4 × 2 H, OCH₂(CH₂)₄CH₂Cl). ¹³C NMR (100 MHz, CDCl₃, 295 K): δ_C 170.6, 168.4 (C=O), 137.5 (quat Ar), 128.4, 127.9, 127.7 (Ar), 100.6 (C-1), 94.4 (CCl₃), 77.4 (C-3), 73.8 (C-5, CH₂Ph), 70.6 (C-4), 70.2 (C-6), 69.4 (OCH₂CH₂), 54.0 (C-2), 45.0 (CH₂Cl), 32.4, 29.2, 26.4, 25.1 (OCH₂(CH₂)₄CH₂Cl), 23.2 (NHCOCH₃). HRESIMS *m/z*: [M+H]⁺ Calcd for C₂₄H₃₄NO₈Cl₄ 604.1039; Found 604.1016.

Trichloroethyl 6-O-pivaloyl-(α,β)-D-galactopyranoside (13). Pivaloyl chloride (4.75 mL, 1.5 equiv) was added to a solution of known⁹ **12** (8.28 g, 26.6 mmol) in anhyd CH₂Cl₂ (140 mL) containing collidine (17 mL) and stirred under N₂ at 0 °C. The reaction was stirred at 0 °C for 24 h, more PivCl (0.95 mL, 0.3 equiv) was added and the reaction was left under stirring at 0 °C for another 20 h. MeOH (2 mL) was added to the mixture which was then diluted with CH₂Cl₂ (160 mL) and washed with 2 N HCl (400 mL). The aqueous layer was extracted with CH₂Cl₂ (2 × 50 mL) and the combined organic phases were dried and concentrated. The residue was purified by chromatography (CH₂Cl₂: MeOH, 20:1 then 12:1) to give the anomeric mixture ($\alpha:\beta$ = 9:1, assessed by NMR) of pivaloate **13** (8.39 g, 80%) as a white amorphous foam. ¹H NMR (400 MHz, CDCl₃, 295 K): δ_H for the α anomer 5.18 (d, *J* = 3.4 Hz, 1 H, H-1), 4.38 (dd, *J* = 5.4, 11.6 Hz, 1 H, H-6a), 4.27 (d, *J* = 11.4 Hz, 1 H, CHHCCl₃), 4.22 (dd, *J* = 7.2, 11.6 Hz, 1 H, H-6b), 4.12–4.04 (m, 2 H, CHHCCl₃, H-5), 3.97 (bd, *J* = 1.6 Hz, H-4), 3.92–3.83 (m, 2 H, H-2, H-3), 1.20 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 295 K): δ_C for the α anomer 178.5 (C=O), 98.9 (C-1), 95.9 (CCl₃), 79.0

(CH₂CCl₃), 70.4 (C-3), 69.7 (C-2), 69.4 (C-5), 68.3 (H-4), 63.7 (C-6), 38.8 (C(CH₃)₃), 27.2 (C(CH₃)₃). HRESIMS m/z: [M+Na]⁺ Calcd for C₁₃H₂₁O₇Cl₃Na 417.0250; Found 417.0243.

Trichloroethyl 2,4-di-O-acetyl-6-O-pivaloyl-(α,β)-D-galactopyranoside (15).

Triethylorthoacetate (12.5 mL, 4 equiv) and CSA (320 mg, 0.08 equiv) were added to a solution of compound **13** (7.0 g, 17.7 mmol) in anhyd MeCN (200 mL) stirred under N₂. The solution was stirred at rt for 15 min, anhyd pyridine and Ac₂O (41 mL, 25 equiv) were then added, and the mixture was heated to 50°C for 2 h. The mixture was co-concentrated with PhMe (3 × ~200 mL) and the resulting oily residue was left under high vacuum for 30 min. It was dissolved in a mixture of AcOH and H₂O (8:2, 100 mL), stirred for 30 min, then diluted with CH₂Cl₂ (200 mL) and washed sequentially with satd aq NaHCO₃ (200 mL) and HCl 2 N (2 × 200 mL). The aq layers were re-extracted with CH₂Cl₂ (2 × 100 mL) and the combined organic phases were dried and concentrated to give the corresponding anomeric mixture ($\alpha:\beta = 9:1$, assessed by NMR) of diacetate **15** (7.7 g, 91 %) as a slightly yellowish foam. ¹H NMR (400 MHz, CDCl₃, 295 K): δ_{H} for the α anomer 5.41 (m, 2 H, H-1, H-4), 4.95 (dd, $J = 3.6, 10.4$, 1 H, H-3), 4.33 (m, 1 H, H-2), 4.27 (m, 1 H, H-5), 4.21 (d, $J = 11.6$ Hz, 1 H, CHHCCl₃), 4.15–4.03 (m, 3 H, H-6ab, CHHCCl₃), 2.17, 2.11 (2 s, 6 H, 2 OCOCH₃), 1.19 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 295 K): δ_{C} for the α anomer 177.9, 171.0, 170.9 (C=O), 96.7 (C-1), 96.0 (CCl₃), 79.2 (CH₂CCl₃), 70.9 (C-3), 70.2 (C-2), 67.8 (C-5), 66.3 (C-4), 62.1 (C-6), 38.7 (C(CH₃)₃), 27.0 (C(CH₃)₃), 20.8, 20.7 (OCOCH₃). HRESIMS m/z: [M+H]⁺ Calcd for C₁₇H₂₆Cl₃O₉ 479.0642; Found 479.0633.

***p*-Tolyl 2,4-di-O-acetyl-6-O-pivaloyl- β -1-thio-D-galactopyranoside (16).**

Triethylorthoacetate (53.4 mL, 4 equiv) and CSA (1.35 g, 0.08 equiv) were added to a solution of known¹⁰ triol **14** (27.0 g, 72.87 mmol) in anhyd MeCN (700 mL) under N₂. The solution was stirred at rt for 15 min, anhyd pyridine (236 mL, 40 equiv) and Ac₂O (138 mL, 20 equiv) were then added, and the mixture was heated to 50°C for 2 h. The mixture was co-concentrated with PhMe (3 × 150

mL), and the resulting oily residue was left under high vacuum overnight. It was dissolved in a mixture of AcOH and H₂O (8:2, 250 mL), stirred for 10 min, then diluted with CH₂Cl₂ (300 mL) and washed sequentially with satd aq NaHCO₃ (2 × 500 mL) and HCl 2 N (2 × 500 mL). The aq layers were re-extracted with CH₂Cl₂ (2 × 150 mL) and the combined organic layers were dried and concentrated to give alcohol **16** (32.5 g, 98%) pure as a white amorphous glass. $[\alpha]_D^{+15.5}$ (c 1.0, MeOH). ¹H NMR (600 MHz, CDCl₃, 296 K): δ_H 7.37 (d, J = 8.0 Hz, 2 H, Ar), 7.09 (d, J = 7.9 Hz, 2 H, Ar), 5.31 (d, J = 2.8 Hz, 1 H, H-4), 4.97 (t, J = 9.8 Hz, 1 H, H-2), 4.61 (d, J = 10.0 Hz, 1 H, H-1), 4.12 (m, 2 H, H-6ab), 3.88–3.81 (m, 2 H, H-3, H-5), 2.35 (d, J = 6.0 Hz, 1 H, OH-3), 2.32 (s, 3 H, CH₃ tolyl), 2.15, 2.13 (2 s, 6 H, 2 OCOCH₃), 1.16 (s, 9 H, C(CH₃)₃). ¹³C NMR (125 MHz, CDCl₃, 296 K): δ_C 178.0, 170.9 (C=O), 138.3 (quat Ar), 132.8 (Ar), 129.7 (Ar), 129.0 (quat Ar), 86.9 (C-1), 74.7 (C-5), 72.4 (C-3), 70.8 (C-2), 69.9 (C-4), 62.0 (C-6), 38.7 (C(CH₃)₃), 27.0 (C(CH₃)₃), 21.1 (CH₃ tolyl), 21.0, 20.7 (OCOCH₃). HRESIMS m/z: [M+K]⁺ Calcd for C₂₂H₃₀O₈SK 493.1298; Found 493.1294.

***p*-Tolyl 2,4-di-*O*-acetyl-3-*O*-chloroacetyl-6-*O*-pivaloyl- β -1-thio-D-galactopyranoside (**17**).**

Chloroacetyl chloride (11.4 mL, 2 equiv) was added slowly to a solution of alcohol **16** (32.5 g, 71.50 mmol) in anhyd CH₂Cl₂ (500 mL) containing anhyd pyridine (23 mL, 4 equiv) and stirred at rt under N₂. The mixture was stirred for 15 min, diluted with CH₂Cl₂ (100 mL), and washed sequentially with HCl 2 N (2 × 400 mL) and satd aq NaHCO₃ (2 × 400 mL). The organic layer was dried and concentrated to give the chloroacetate **17** (38 g, quant) pure as a yellow amorphous glass. $[\alpha]_D^{+20.0}$ (c 1.0, CH₂Cl₂). ¹H NMR (600 MHz, CDCl₃, 296 K): δ_H 7.37 (d, J = 8.1 Hz, 2 H, Ar), 7.10 (d, J = 7.9 Hz, 2 H, Ar), 5.37 (dd, J = 0.7, 3.2 Hz, 1 H, H-4), 5.22 (t, J = 10.0 Hz, 1 H, H-2), 5.09 (dd, J = 3.3, 10.0 Hz, 1 H, H-3), 4.66 (d, J = 10.0 Hz, 1 H, H-1), 4.15 (dd, J = 6.9, 11.3 Hz, 1 H, H-6a), 4.10 (dd, J = 6.7, 11.2 Hz, 1 H, H-6b), 3.94–3.90 (m, 3 H, H-5, COCH₂Cl), 2.32 (s, 3 H, CH₃ tolyl), 2.09, 2.08 (2 s, 6 H, 2 OCOCH₃), 1.14 (s, 9 H, C(CH₃)₃). ¹³C NMR (151 MHz, CDCl₃, 296 K): δ_C 177.9, 170.3,

169.4, 166.6 (C=O), 138.5 (quat Ar), 133.0 (Ar), 129.7 (Ar), 128.6 (quat Ar), 87.2 (C-1), 74.2 (C-5), 73.7 (C-3), 67.0 (C-2, C-4), 61.3 (C-6), 40.4 (COCH₂Cl), 38.7 (C(CH₃)₃), 27.0 (C(CH₃)₃), 21.1 (CH₃ tolyl), 20.8, 20.6 (OCOCH₃). HRESIMS m/z: [M+Na]⁺ Calcd for C₂₄H₃₁ClO₉SNa 553.1275; Found 553.1284.

1,4-Di-O-acetyl-3-O-chloroacetyl-6-O-pivaloyl- α -D-galactopyranose (18). NIS (18.63 g, 1.1 equiv) and TfOH (633 μ L, 0.1 equiv) were added to a solution of chloroacetate **17** (37.96 g, 71.50 mmol) in a mixture of MeCN (600 mL) and H₂O (8.75 mL) at rt. The reaction was allowed to proceed under stirring for 10 min at rt, quenched with NEt₃ (2.49 mL, 0.25 equiv) and the mixture was concentrated. The residue was dissolved in CH₂Cl₂ (500 mL) and was washed with a 20% w/w solution of aq Na₂S₂O₃ (1 \times 600 mL). The aq layer was re-extracted with CH₂Cl₂ (2 \times 150 mL) and the combined organic layers were dried and concentrated. The product was purified by column chromatography (EtOAc:hexanes, 4:6) to give alcohol **18** (25.82 g, 85%) pure as an amorphous glass. [α]_D +117.2 (*c* 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃, 296 K): δ _H 6.29 (d, *J* = 3.9 Hz, 1 H, H-1), 5.42 (dd, *J* = 1.1, 3.2 Hz, 1 H, H-4), 5.24 (dd, *J* = 3.3, 10.5 Hz, 1 H, H-3), 4.27 (t, *J* = 7.4 Hz, 1 H, H-5), 4.20 (m, 1 H, H-2), 4.10–4.03 (m, 4 H, H-6ab, COCH₂Cl), 2.17, 2.12 (2 s, 6 H, 2 OCOCH₃), 1.95 (d, *J* = 7.9 Hz, 1 H, OH-2), 1.14 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 296 K): δ _C 177.9, 170.1, 169.3, 167.0 (C=O), 91.7 (C-1), 72.2 (C-3), 68.5 (C-5), 67.1 (C-4), 65.8 (C-2), 60.8 (C-6), 40.6 (COCH₂Cl), 38.7 (C(CH₃)₃), 27.0 (C(CH₃)₃), 20.9, 20.6 (OCOCH₃). HRESIMS m/z: [M+Na]⁺ Calcd for C₁₇H₂₅ClO₁₀Na 447.1034; Found 447.1019.

2,4-Di-O-acetyl-3-O-chloroacetyl-6-O-pivaloyl- α -D-galactopyranosyl trichloroacetimidate (6).

Method A from 15: Sodium acetate (28 g, 10 equiv) and activated Zn powder (34 g, 15 equiv) were added to a solution of glycoside **15** (17.12 g, 35.7 mmol) in AcOH (225 mL) and the reaction

mixture was stirred at rt for 32 h. Solids were filtered off over a bed of Celite® which was washed thoroughly with CH₂Cl₂ (800 mL). The combined filtrate and washing were poured slowly into ice cold satd aq NaHCO₃ (800 mL) and the resulting two layers were separated. The aq layer was re-extracted with CH₂Cl₂ (3 × 300 mL) and the combined organic layers were dried and concentrated. Column chromatography (CH₂Cl₂:MeOH, 20:1) of the residue gave the corresponding hemiacetal diol (8.02 g, 65%) which was engaged directly in the next step. Pyridine (18 mL, 10 equiv) and chloroacetyl chloride (7.1 mL, 4 equiv) were added to a solution of the diol (7.63 g, 21.9 mmol) in anhyd CH₂Cl₂ (126 mL). The reaction mixture was stirred at rt for 30 min and co-concentrated with toluene (3 × 50 mL). The residue was dissolved in CH₂Cl₂ (300 mL) and washed with 2 N HCl (2 × 300 mL). The aq layers were re-extracted with CH₂Cl₂ (2 × 50 mL) and the combined organic layers were dried and concentrated. Column chromatography (EtOAc:hexanes, 2:8, then 3:7) gave the corresponding di-chloroacetate (7.63 g) in 69% yield from the diol, (45% from **15**). Benzylamine (1.7 mL, 1 equiv) was added to a stirred solution of the dichloroacetate (7.53 g, 15.0 mmol) in anhyd THF (180 mL) and the solution was stirred 9 h at rt. More benzylamine (0.8 mL, 0.5 equiv) was added, the solution was stirred for another 4 h at rt and then diluted with H₂O (250 mL). The mixture was transferred to a separatory funnel and the product was extracted with CH₂Cl₂ (3 × 300 mL). The organic layers were washed with brine (600 mL), combined, dried and concentrated. Column chromatography (EtOAc:hexanes, 3:7) of the residue gave the corresponding hemiacetal which was used immediately in the next step. Trichloroacetonitrile (6.5 mL, 3 equiv) was added to a solution of the hemiacetal in anhyd CH₂Cl₂ (150 mL) stirred under N₂ at rt. DBU (400 μL, 0.25 equiv) was then slowly added and the mixture was stirred at rt for 7.5 h. More DBU (0.08 equiv) and trichloroacetonitrile (1 equiv) were added and the reaction was allowed to proceed for another 3 h. The reaction mixture was then concentrated and a column chromatography of the residue (EtOAc:hexanes, 3:7 containing 0.1% NEt₃) gave trichloroacetimidate **6** (4.91 g, 57% from the

dichloroacetate, 26% from **15**) pure as a slightly yellowish amorphous foam.

Method B from 18: Trichloroacetonitrile (18.3 mL, 3 equiv) was added to a solution of alcohol **19** (25.82 g, 60.8 mmol) in anhyd CH₂Cl₂ (600 mL) stirred at rt under N₂. DBU (2.27 mL, 0.25 equiv) was then added slowly and the mixture was stirred at rt for 4 h. The reaction mixture was concentrated and column chromatography, as described above, gave trichloroacetimidate **6** (26.61 g, 77%) pure as a slightly yellowish amorphous foam.

Analytical data for 6. [α]_D +89.0 (*c* 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃, 296 K): δ _H 8.65 (s, 1 H, NH), 6.58 (d, *J* = 3.6 Hz, 1 H, H-1), 5.51 (dd, *J* = 1.0, 3.2 Hz, 1 H, H-4), 5.47 (dd, *J* = 3.2, 10.7 Hz, 1 H, H-3), 5.36 (dd, *J* = 3.6, 10.7 Hz, 1 H, H-2), 4.42 (t, *J* = 6.8 Hz, 1 H, H-5), 4.10 (d, *J* = 6.7 Hz, 2 H, H-6ab), 3.97 (s, 2 H, COCH₂Cl), 2.13, 2.00 (2 s, 6 H, 2 OCOCH₃), 1.12 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 296 K): δ _C 177.7, 170.2, 169.9, 166.4 (C=O), 160.8 (C=N), 93.3 (C-1), 90.7 (CCl₃), 69.3 (C-3), 69.0 (C-5), 67.2 (C-4), 66.6 (C-2), 61.2 (C-6), 40.4 (COCH₂Cl), 38.7 (C(CH₃)₃), 27.0 (C(CH₃)₃), 20.6, 20.5 (OCOCH₃). HRESIMS *m/z*: [M+Na]⁺ Calcd for C₁₉H₂₅Cl₄NO₁₀Na 590.0130; Found 590.0106.

6-Chlorohexyl 2-acetamido-4-O-(2,4-di-O-acetyl-3-O-chloroacetyl-6-O-pivaloyl- β -D-galactopyranosyl)-6-O-benzyl-2-deoxy-3-O-trichloroethoxycarbonyl- β -D-glucopyranoside (19).

A solution of alcohol **5** (1.12 g, 1.85 mmol) and galactosyl trichloroacetimidate **6** (2.05 g, 2 equiv) in anhydrous CH₂Cl₂ (45 mL) was heated to 40 °C. BF₃.OEt₂ (490 μ L, 2 equiv) was added and the reaction mixture was stirred for 1.5 h at 40 °C. The reaction was quenched with NEt₃ (651 μ L, 2.4 equiv), the mixture diluted with CH₂Cl₂ (60 mL) and washed with satd aq NaHCO₃ (100 mL). The aq layer was re-extracted with CH₂Cl₂ (3 \times 15 mL) and the organic layers were combined, dried and concentrated. Column chromatography (EtOAc:hexanes, 3:7) of the residue gave disaccharide **19** (1.56 g, 83%) pure as a white amorphous solid. [α]_D -7.6 (*c* 1.0, MeOH). ¹H NMR (400 MHz,

CDCl₃, 295 K): δ_{H} 7.41–7.29 (m, 5 H, Ar), 5.58 (d, $J = 8.4$ Hz, 1 H, NH), 5.16–5.24 (m, 2 H, H-3, H-4'), 5.04 (dd, $J = 8.4, 10.4$ Hz, 1 H, H-2'), 4.89 (d, $J = 11.5$ Hz, 1H, CHHCCl₃), 4.86 (dd, $J = 3.5, 10.4$ Hz, H-3'), 4.77 (d, $J = 12.1$ Hz, 1 H, CHHPh), 4.71 (d, $J = 7.4$ Hz, 1 H, H-1), 4.67 (d, $J = 11.8$ Hz, 1 H, CHHCCl₃), 4.43 (d, $J = 12.1$ Hz, 1 H, CHHPh), 4.41 (d, $J = 8.0$ Hz, 1 H, H-1'), 4.05 (m, 2 H, H-6'ab), 3.94 (t, $J = 8.9$ Hz, 1 H, H-4), 3.92 (s, 2 H, COCH₂Cl), 3.83 (m, 1 H, OCHHCH₂), 3.72 (m, 1 H, H-6ab), 3.69–3.60 (m, 2 H, H-2, H-5'), 3.54–3.47 (m, H-5, CH₂Cl), 3.43, (m, 1 H, OCHHCH₂), 2.11, 1.95, 1.90 (3 s, 9 H, 3 COCH₃), 1.74, 1.56, 1.41, 1.33 (4 m, 4 \times 2 H, OCH₂(CH₂)₄CH₂Cl), 1.15 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 295 K): δ_{C} 177.7, 170.3, 170.1, 169.2, 166.5, 153.8 (C=O), 137.7 (quat Ar), 128.6, 128.1, 128.0 (Ar), 100.3 (C-1), 100.0 (C-1'), 94.4 (CCl₃) 77.2 (C-3), 76.8 (CH₂CCl₃) 75.2 (C-4), 74.1 (C-5), 73.7 (CH₂Ph), 72.6 (C-3'), 70.3 (C-5'), 69.5 (OCH₂CH₂), 68.7 (C-2'), 67.4 (C-6), 66.4 (C-4'), 60.2 (C-6'), 55.0 (C-2), 45.0 (CH₂Cl), 40.3 (COCH₂Cl), 38.6 (C(CH₃)₃), 32.4, 29.2, 26.4, 25.1 (OCH₂(CH₂)₄CH₂Cl), 27.1 (C(CH₃)₃), 23.3, 20.6 (2 \times COCH₃). HRESIMS m/z: [M+H]⁺ Calcd for C₄₁H₅₇Cl₅NO₁₇ 1010.2069; Found 1010.2063 .

6-Chlorohexyl 2-acetamido-4-O-(2,4-di-O-acetyl-6-O-pivaloyl- β -D-galactopyranosyl)-6-O-benzyl-2-deoxy-3-O-trichloroethoxycarbonyl- β -D-glucopyranoside (20). Thiourea (450 mg, 5 equiv) was added to a solution of disaccharide **19** (1.12 g, 1.11 mmol) in a mixture EtOH:pyridine (1:1, 12 mL) stirred at 55 °C. The reaction was allowed to proceed at 55 °C for 4 then the mixture was co-concentrated with toluene (2 \times 15 mL). The residue was dissolved in CH₂Cl₂ (100 mL) and washed with 2 N HCl (100 mL). The aq layer was re-extracted with CH₂Cl₂ (3 \times 40 mL), and the combined organic layers were dried and concentrated. Chromatography (EtOAc:hexanes, 1:1) of the residue gave disaccharide acceptor **20** (780 mg, 75%) pure as a white amorphous foam. $[\alpha]_{\text{D}} -1.7$ (*c* 0.3, MeOH). ¹H NMR (400 MHz, CDCl₃, 295 K): δ_{H} 7.38–7.25 (m, 5 H, Ar), 5.64 (m, 1 H, NH), 5.22–5.15 (m, 2 H, H-3, H-4'), 4.89 (d, $J = 11.9$, CHHCCl₃), 4.79 (dd, $J = 8.0, 10.0$ Hz, 1 H, H-2'), 4.70 (m, 2 H, H-1, CHHPh), 4.63 (d, $J = 12.0$ Hz, CHHCCl₃), 4.47 (d, $J = 12.1$, 1 H, CHHPh), 4.42

(d, $J = 8.0$ Hz, H-1'), 4.03 (m, 2 H, H-6'ab), 3.94 (t, $J = 8.9$ Hz, 1 H, H-4), 3.83 (m, 1H, OCHHCH₂), 3.75 (m, 2 H, H-6ab), 3.70–3.58 (m, 3 H, H-2, H-3', H-5'), 3.57–3.48 (m, 3 H, H-5, CH₂Cl), 3.45 (m, 1 H, OCHHCH₂), 2.39 (m, 1 H, OH), 2.15, 2.03, 1.89 (3 s, 9 H, 3 COCH₃), 1.74, 1.57, 1.42, 1.32 (4 m, 4 × 2 H, OCH₂(CH₂)₄CH₂Cl), 1.17 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 295 K): δ_C 177.8, 170.9, 170.7, 170.0, 153.7 (C=O), 137.9 (quat Ar), 128.4, 127.9, 127.8 (Ar), 100.3 (C-1), 100.1, (C-1'), 94.4 (CCl₃), 77.2 (C-3), 76.8 (CH₂CCl₃), 75.3 (C-4), 74.3 (C-5), 73.6 (CH₂Ph), 72.8 (C-2'), 71.4 (C-3') 70.7 (C-5'), 69.5 (OCH₂CH₂), 69.2 (C-4'), 67.6 (C-6), 60.9 (C-6'), 54.9 (C-2), 45.0 (CH₂Cl), 38.7 (C(CH₃)₃), 32.4, 29.2, 26.5, 25.2 (OCH₂(CH₂)₄CH₂Cl), 27.0 (C(CH₃)₃), 23.3, 20.9, 20.8 (COCH₃). HRESIMS m/z: [M+H]⁺ Calcd for C₃₉H₅₆NO₁₆Cl₄ 934.2353; Found 934.2335.

6-Chlorohexyl 2-acetamido-4-O-[2,4-di-O-acetyl-3-O-(4,6-O-benzylidene-3-O-chloroacetyl-2-deoxy-2-trichloroacetamido- β -D-glucopyranosyl)-6-O-pivaloyl- β -D-galactopyranosyl]-6-O-benzyl-2-deoxy-3-O-trichloroethoxycarbonyl- β -D-glucopyranoside (21).

A solution of alcohol **20** (750 mg, 0.801 mmol) and known⁶ trichloroacetimidate donor **7** (1.65 g, 3 equiv) in anhyd CH₂Cl₂ (35 mL) containing freshly activated MS 4 Å (1.75 g) was stirred at 0 °C for 1 h. TMSOTf (315 μ L, 1.8 equiv) was then added and the reaction mixture was stirred at 0 °C for 2.5 h, allowed to slowly reach 10 °C and the reaction was quenched with triethylamine (300 μ L). Solids were filtered off on Celite[®] and washed with CH₂Cl₂ (~100 mL). The filtrates were combined and washed with satd aq NaHCO₃ (300 mL). The aq layer was extracted with CH₂Cl₂ (2 × ~150 mL) and the combined organic layers were dried and concentrated. Chromatography (EtOAc:hexanes 3:7 → 4:6) gave pure trisaccharide **21** (590 mg, 52%) as an amorphous foam. $[\alpha]_D -14.5$ (c 0.2, MeOH). ¹H NMR (400 MHz, CDCl₃, 295 K): δ_H 7.42–7.28 (m, 10 H, Ar), 6.72 (d, $J = 8.7$ Hz, 1 H, NH''), 5.69 (d, $J = 8.7$ Hz, 1 H, NH), 5.50 (s, 1 H, CHPh), 5.47 (t, $J = 9.7$ Hz, 1 H, H-3''), 5.28 (bd, $J = 3.9$ Hz, H-4'), 5.09 (dd, $J = 8.0, 8.7$ Hz, 1 H, H-3), 4.98 (dd, $J = 8.1$ Hz, 10.1 Hz, 1 H, H-2'), 4.86 (d, $J = 11.9$ Hz, 1 H, CHHCCl₃), 4.79 (d, $J = 8.0$ Hz, H-1''), 4.70 (d, $J = 12.0$ Hz, 1 H, CHHPh), 4.66 (d, $J =$

11.9 Hz, 1 H, $CHHCCl_3$), 4.61 (d, $J = 7.1$ Hz, 1 H, H-1), 4.46 (d, $J = 12.1$, $CHHPh$), 4.37 (d, $J = 8.0$ Hz, 1 H, H-1'), 4.34 (dd, $J = 5.0, 10.5$ Hz, 1 H, H-6a'), 4.06–3.97 (m, 4 H, H-6'ab, $COCH_2Cl$), 3.94 (t, $J = 8.0$ Hz, 1 H, H-4), 3.86–3.63 (m, 9 H, H-2, H-6ab, H-3', H-5', H-6a', H-2'', H-4'', $OCHHCH_2$), 3.57 – 3.46 (m, 4 H, H-5, H-5'', CH_2Cl), 4.41 (m, 1H, $OCHHCH_2$), 2.12, 2.00, 1.91 (s, 9 H, 3 $COCH_3$), 1.73, 1.55, 1.40, 1.32 (4 m, 4×2 H, $OCH_2(CH_2)_4CH_2Cl$), 1.19 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 295 K): δ_C 177.8, 170.0, 169.9, 169.1, 167.5, 162.1, 153.6 (C=O), 138.0, 136.5 (quat Ar), 129.3, 128.6, 128.5, 128.3, 128.0, 127.9, 127.8, 126.0 (Ar), 101.3 ($>CHPh$), 100.4 (C-1'), 100.2 (C-1, C-1''), 94.4 (CCl_3''), 92.1 (CCl_3), 78.1 (C-4''), 76.9 (C-3', OCH_2CCl_3), 75.5 (C-3'), 74.5 (C-4), 74.3 (C-5'), 73.7 (CH_2Ph), 72.2 (C-3''), 70.8 (C-5), 70.7 (C-2'), 69.4 (OCH_2CH_2), 68.6 (C-4'), 68.2 (C-6''), 67.8 (C-6), 66.1 (C-5''), 61.0 (C-6'), 56.6 (C-2''), 53.6 (C-2), 45.0 (CH_2Cl), 40.4 ($COCH_2Cl$), 38.7 ($C(CH_3)_3$), 32.4, 29.2, 26.5, 25.2 ($OCH_2(CH_2)_4CH_2Cl$), 27.0 ($C(CH_3)_3$), 23.2, 21.1, 20.8 ($COCH_3$). HRESIMS m/z : $[M+H]^+$ Calcd for $C_{56}H_{71}N_2O_{22}Cl_8$ 1403.2007; Found 1403.1968.

6-Chlorohexyl 2-acetamido-4-O-[2,4-di-O-acetyl-3-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido- β -D-glucopyranosyl)-6-O-pivaloyl- β -D-galactopyranosyl]-6-O-benzyl-2-deoxy-3-O-trichloroethoxycarbonyl- β -D-glucopyranoside (22). A solution of trisaccharide **21** (1.0 g, 0.71 mmol) in EtOH/pyridine (1:1, 34 mL) was heated to 70 °C. Thiourea (640 mg, 12 equiv) was then added and the reaction mixture was stirred for 6 h at 70 °C. The cold reaction mixture was co-concentrated with toluene (2×20 mL) and the residue dissolved in CH_2Cl_2 (~100 mL) was filtered through Celite[®]. The solids were washed with CH_2Cl_2 (~100 mL) and the combined filtrates were concentrated. Column chromatography on the residue (EtOAc:hexanes, 1:1) gave trisaccharide acceptor **22** (700 mg, 74%) as a colorless foam. $[\alpha]_D -5.0$ (c 0.6, CH_2Cl_2). 1H NMR (400 MHz, $CDCl_3$, 295 K): δ_H 7.48–7.28 (m, 10 H, Ar), 6.89 (d, $J = 6.9$ Hz, 1 H, NH''), 5.67 (d, $J = 8.2$ Hz, 1 H, NH), 5.53 (s, $CHPh$), 5.30 (d, $J = 3.4$ Hz, H-4'), 5.11 (m, 2 H, H-3, H-1''), 4.99 (dd, $J = 8.2, 9.9$ Hz, 1

H, H-2'), 4.85 (d, $J = 11.8$ Hz, 1 H, $CHHCCl_3$), 4.73–4.60 (m, 3 H, H-1, $CHHPh$, $CHHCCl_3$), 4.46 (d, $J = 12.1$ Hz, $CHHPh$), 4.40–4.30 (m, 3 H, H-1', H-3'', H-6''a), 4.06–3.87 (m, 3 H, H-4, H-6'ab), 3.84–3.67 (m, 6 H, H-2, H-6ab, H-3', H-6''b, $OCHHCH_2$), 3.64 (m, 1 H, H-5'), 3.56–3.45 (m, 5 H, H-5, H-4'', H-5'', CH_2Cl), 3.41 (m, 1 H, $OCHHCH_2$), 3.22 (m, 1 H, H-2'') 2.10, 2.00, 1.91 (3 s, 9 H, 3 $COCH_3$), 1.73, 1.55, 1.41, 1.33 (4 m, 4×2 H, $OCH_2(CH_2)_4CH_2Cl$), 1.18 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$, 295 K): δ_C 177.8, 170.0, 169.7, 169.4, 162.2, 153.6 (C=O), 138.0, 136.8 (quat Ar), 129.3, 128.5, 128.3, 127.9, 127.8, 126.2 (Ar), 101.8 ($>CHPh$), 100.5 (C-1''), 100.1 (C-1), 99.3 (C-1'), 94.3, 92.3 (CCl_3), 81.2 (C-4''), 76.8 (CH_2CCl_3 , C-3), 75.9 (C-3'), 74.5 (C-4), 74.2 (C-5), 73.6 (CH_2Ph), 70.9 (C-5'), 70.7 (C-2'), 69.4 (OCH_2CH_2), 68.9 (C-4'), 68.6 (C-3''), 68.3 (C-6''), 67.8 (C-6), 66.0 (C-5''), 61.0 (C-6'), 59.9 (C-2''), 53.8 (C-2), 45.0 (CH_2Cl), 40.4 ($COCH_2Cl$), 38.6 ($C(CH_3)_3$), 32.4, 29.2, 26.5, 25.1 ($OCH_2(CH_2)_4CH_2Cl$), 27.0 ($C(CH_3)_3$), 23.2, 21.1, 20.8 ($COCH_3$). HRESIMS m/z : $[M+H]^+$ Calcd for $C_{54}H_{70}O_{21}N_2Cl_7$ 1327.2291; Found 1327.2339.

6-Chlorohexyl 2-acetamido-4-O-{2,4-di-O-acetyl-3-O-[3-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido- β -D-glucopyranosyl]-6-O-pivaloyl- β -D-galactopyranosyl}-6-O-benzyl-2-deoxy-3-O-trichloroethoxycarbonyl- β -D-glucopyranoside (23). $BF_3 \cdot OEt_2$ (45 μ L, 2.4 equiv) was added at to 0 °C to a solution of alcohol **22** (200 mg, 0.150 mmol) and trichloroacetimidate donor **8** (440 mg, 6 equiv) in anhyd CH_2Cl_2 (10 mL) stirred under N_2 . The mixture was stirred 3 h at 0 °C then 1 h at rt and the reaction quenched with triethylamine (60 μ L). The reaction mixture was diluted with CH_2Cl_2 (80 mL) and washed with satd aq $NaHCO_3$ (1 \times 80 mL). The aq layer was re-extracted with CH_2Cl_2 (2 \times 30 mL) and the combined organic phases dried and concentrated. Chromatography (EtOAc:hexanes, 1:1), followed by RP-HPLC purification ($CH_3CN:H_2O$, 1:1 \rightarrow 8.5:1.5 over 60 min) gave tetrasaccharide **23** (132 mg, 53%) pure as a white amorphous solid as well as some unreacted trisaccharide acceptor **22** (23 mg, 11%). $[\alpha]_D -13.3$ (c 0.3, MeOH). 1H NMR (600 MHz, $CDCl_3$, 295 K): δ_H 7.47–7.43, 7.38–7.28 (2 m,

10 H, Ar), 7.03 (d, $J = 7.02$ Hz, 1 H, NHA'), 5.70 (d, $J = 8.6$ Hz, 1 H, NHA), 5.53 (s, 1 H, >CHPh), 5.29 (bs, 2 H, H-4C, H-4C'), 5.20 (d, $J = 7.8$ Hz, H-1A'), 5.14–5.09 (m, 2 H, H-3A, H-2C'), 4.95 (dd, $J = 8.0, 10.1$ Hz, 1 H, H-2C), 4.90 (dd, $J = 3.4, 10.4$ Hz, 1 H, H-3C'), 4.85 (d, $J = 11.9$ Hz, 1 H, CHHCCL₃), 4.74 (d, $J = 7.9$ Hz, 1 H, H-1C'), 4.69 (d, $J = 12.1$ Hz, 1 H, CHCHPh), 4.65 (d, $J = 11.8$ Hz, 1 H, CHHCCL₃), 4.64 (d, $J = 7.0$ Hz, 1 H, H-1A), 4.51 (t, $J = 9.5$, 1 H, H-3A'), 4.46 (d, $J = 12.1$, CHHPh), 4.34 (d, $J = 8$ Hz, 1 H, H-1C), 4.34 (dd, $J = 4.9, 10.5$ Hz, 1 H, H-6A'), 4.08 (dd, $J = 6.5, 11.3$ Hz, 1 H, H-6Ca or H-6C'a), 4.02 (dd, $J = 7.3, 11.2$ Hz, 1 H, H-6Cb or H-6C'b), 4.00–3.94 (m, 2 H, H-6Cb, H-6C'b), 3.91 (t, $J = 7.9$ Hz, 1 H, H-4A), 3.82–3.68 (m, 8 H, H-2A, H-6Aab, H-3C, H-5C or H-5C', H-6A'a, OCHHCH₂), 3.63 (bt, $J = 7.1$, 1 H, H-5C or H-5C'), 3.55–3.47 (m, 4 H, H-5A', H-5A', CH₂Cl), 3.40 (m, 1 H, OCHHCH₂), 3.25 (m, 1 H, H-2A'), 2.10, 2.09, 2.08, 2.00, 1.99, 1.92, 1.91, 1.77 (7 s, 21 H, 7 COCH₃), 1.73, 1.53, 1.40, 1.33 (4 m, 4 × 2 H, OCH₂(CH₂)₄CH₂Cl), 1.18 (s, 9 H, C(CH₃)₃). ¹³C NMR (151 MHz, CDCl₃, 295 K): δ_C 177.7, 170.3, 170.1, 170.0, 169.6, 169.5, 169.4, 169.3, 161.9, 153.5 (C=O), 138.0, 136.8 (quat Ar), 129.4, 128.5, 128.3, 127.8, 126.1 (Ar), 101.5 (>CHPh) 100.6 (C-1C), 100.0 (C-1A), 98.9 (C-1A'), 98.5 (C-1C'), 94.4, 92.179 (CCl₃), 78.1 (C-4A'), 76.8 (C-3A, CH₂CCl₃), 75.7 (C-3C), 75.0 (C-3A'), 74.5 (C-4A), 74.2 (C-5A or C-5A'), 73.6 (CH₂Ph), 70.9 (C-3A'), 70.7, 70.5 (C-5C, C-5C'), 69.4 (OCH₂CH₂), 68.9 (C-4C'), 68.8 (C-2C'), 68.4, 67.8 (C-6A, C-6A'), 66.8 (C-4C), 66.2 (C-5A or C-5A'), 61.3, 60.9 (C-6C, C-6C'), 59.2 (C-2A'), 53.8 (C-2A), 45.0 (CH₂Cl), 38.7 (C(CH₃)₃), 32.4, 29.2, 26.5, 25.2 (OCH₂(CH₂)₄CH₂Cl), 27.1 (C(CH₃)₃), 23.3, 21.2, 20.9, 20.7, 20.5, 20.4 (COCH₃). HRESIMS m/z: [M+NH₄]⁺ Calcd for C₆₈H₉₁O₃₀N₃Cl₇ 1674.3507; Found 1674.3457.

6-Chlorohexyl 2-acetamido-4-O-{3-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-2-deoxy- β -D-glucopyranosyl]-2,4-di-O-acetyl-6-O-pivaloyl- β -D-galactopyranosyl}-6-O-benzyl-2-deoxy- β -D-glucopyranoside (24). A solution of tetrasaccharide **23** (123 mg, 0.0740 mmol) in 90% AcOH (10 mL) was stirred at 80 °C for 2.5 h and co-concentrated

with toluene (3×10 mL). The residue was dried under high vacuum for 30 min, dissolved in a 1:1 mixture of Ac_2O and pyridine (12 mL) and stirred 1 h at rt. It was then co-concentrated with toluene (3×10 mL), diluted in CH_2Cl_2 (80 mL) and washed with 2 N HCl (1×80 mL). The aq layer was re-extracted with CH_2Cl_2 (2×20 mL) and the combined organic phases were dried and concentrated. The residue was dried overnight under high vacuum overnight and dissolved in AcOH (5 mL). Freshly activated Zn powder (556 mg) was then added and the reaction was sonicated at 50°C for 4 h. Additional Zn powder (100 equiv) was added and the reaction was left at 50°C under sonication for 7 h then left stirring overnight at 50°C . Solids were filtered off over Celite[®] and rinsed with MeOH (100 mL). The combined filtrate and washing were concentrated and the dry residue was dissolved in AcOH (4 mL). Zn powder (100 equiv) was added and the mixture was left at 50°C under sonication for another 4 h. Solids were filtered off over Celite[®], washed with MeOH (100 mL) and the combined filtrate and washing were concentrated. The oily residue was dissolved in CH_2Cl_2 (60 mL), washed with 2 N HCl (1×60 mL) and the aq layer was re-extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried and concentrated and column chromatography of the residue (CH_2Cl_2 :MeOH, 45:1) gave tetrasaccharide acceptor **24** (78 mg, 76%) pure as a white amorphous solid. $[\alpha]_{\text{D}} -4.0$ (c 0.5, MeOH). ^1H NMR (400 MHz, CD_3OD , 296 K): δ_{H} 7.93, 7.78 (2 d, $J = 7.93$ and 7.78 Hz, partly exchanged 0.2 and 0.4 H, 2 NH), 7.29–7.17 (m, 5 H, Ar), 5.26 (bd, $J = 3.5$ Hz, 1 H, H-4C), 5.22 (dd, $J = 0.6$ 3.5 Hz, 1 H, H-4C'), 4.91 (dd, $J = 3.5$, 10.3 Hz, 1 H, H-3C'), 4.88 (dd, $J = 8.2$, 10.1 Hz, 1 H, H-2C), 4.82 (dd, $J = 7.8$, 10.3 Hz, 1 H, H-2C'), 4.72 (t, $J = 9.6$ Hz, H-4A'), 4.58 (d, $J = 12.0$ Hz, 1 H, *CHHPh*), 4.54 (d, $J = 7.8$ Hz, 1 H, H-1C'), 4.44 – 4.38 (m, 3 H, *CHHPh*, H-1 A or H-1A', H-1C, *CHHPh*), 4.23 (d, $J = 8.4$ Hz, 1 H, H-1A or H-1A'), 4.14 (dd, $J = 2.4$, 12.4 Hz, 1 H, H-6A'a), 4.08–3.97 (m, 4 H, H-6A'b, H-6Ca, H-6C'a, H-6Cb or H-6C'b), 3.92–3.77 (m, 4 H, H-3A', H-5C, H-5C', H-6Cb or H-6C'b), 3.75–3.66 (m, 2 H, H-3C, *OCHHCH}_2*), 3.64–3.49 (m, 5 H, H-2A, H-6Aab, H-2A', H-5A'), 3.48–3.40 (m, 4 H, H-4A, H-5A, CH_2Cl), 3.38–3.30

(m, 2 H, H-3A, OCHHCH₂), 2.00, 1.99, 1.95, 1.94, 1.92, 1.90, 1.84, 1.82, 1.79 (10 s, 30 H, 10 COCH₃), 1.62, 1.44, 1.28 (3 m, 2 × 2 H and 4 H, OCH₂(CH₂)₄CH₂Cl), 1.05 (s, 9 H, C(CH₃)₃). ¹³C NMR (100 MHz, CD₃OD, 296 K): δ_C 179.8, 173.5, 172.8, 172.2, 172.1, 171.9, 171.7, 171.6, 171.1 (C=O), 139.9 (quat Ar), 129.8, 129.4, 129.1 (Ar), 102.9 (C-1C'), 102.3 (C-1c and C-1A or C-1A'), 102.1 (C-1A or C-1A'), 81.5 (C-4A), 78.4 (C-3A') 78.2 (C-3C), 75.7 (C-3A), 74.8 (CH₂Ph), 73.9 (C-5A), 73.1, 73.0 (C-5A', C-5C'), 72.7 (C-3C'), 71.8, 71.8 (C, 2C, C-5C), 71.3 (C-4C), 70.7 (OCH₂CH₂), 70.6 (C-2C'), 70.2 (C-4A'). 69.5 (C-6A), 68.8 (C-4C'), 63.6 (C-6C or C-6C'), 63.1 (C-6A'), 62.6 (C-6C or C-6C'), 56.8 (C-2A, C-2A'), 45.9 (CH₂Cl), 40.0 (C(CH₃)₃), 33.9, 30.6, 27.8, 26.6 (OCH₂(CH₂)₄CH₂Cl), 27.7 (C(CH₃)₃), 23.5, 23.2, 21.4, 21.3, 21.1, 21.0, 20.9, 20.8, 20.7 (COCH₃). HRESIMS m/z: [M+H]⁺ Calcd for C₆₂H₉₀N₂O₃₀Cl 1377.5267; Found 1377.5254.

6-Chlorohexyl 2-acetamido-4-O-{3-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-2,4-di-O-acetyl-6-O-pivaloyl-β-D-galactopyranosyl}-6-O-benzyl-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranoside (25). A solution of tetrasaccharide acceptor **24** (27 mg, 0.020 mmol), donor **9** (55 mg, 6 equiv) in CH₂Cl₂/DMF (3 mL, 1:1) containing molecular sieve 4 Å (300 mg) was stirred at rt under N₂ for 3 h. CuBr₂ (31 mg, 7 equiv) and Bu₄NBr (46 mg, 7 equiv) were added and the reaction mixture was stirred at rt for 20 h. Solids were filtered off on Celite[®] and washed with CH₂Cl₂ (50 mL). The combined filtrate and washings were washed with brine (1 × 30 mL) and satd aq NaHCO₃ (6 × 30 mL). The aq layers were re-extracted with CH₂Cl₂ (2 × 10 mL) and the combined organic phases were dried and concentrated. Chromatography (CH₂Cl₂:MeOH, 100:1, then 50:1) of the residue gave pentasaccharide **25** (26.8 mg, 76%) pure as a white amorphous solid. [α]_D -7.7 (c 0.4, MeOH). ¹H NMR (600 MHz, CDCl₃, 297 K): δ_H 7.40–7.20 (m, 20 H, Ar), 5.76 (bd, *J* = 7.0 Hz, 1 H, NHA), 5.67 (bd, *J* = 6.8 Hz, 1 H, NHA'), 5.32 (bd, *J* = 3.5 Hz, 1 H, H-4C'), 5.26 (bd, *J* = 3.7 Hz, 1 H, H-4C), 5.07 (d, *J* = 8.1 Hz, 1 H, H-1A'), 5.05–4.99 (m, 2 H, H-1B, H-2C'), 4.96–4.86 (m, 6 H,

H-1A, H-2C, H-4A', H-3C', 2 × CHHPh), 4.82–4.62 (m, 5 H, 5 × CHHPh), 4.57 (t, $J = 10.1$ Hz, 1 H, H-3A'), 4.45 (d, $J = 7.9$ Hz, H-1C'), 4.43–4.37 (m, 3 H, H-5B, H-1C, CHHPh), 4.23 (dd, $J = 2.4$ Hz, 12.3 Hz, 1 H, H-6A'a), 4.14 (t, $J = 8.4$ Hz, 1 H, H-3A), 4.12–4.00 (m, 5 H, H-2B, H-6Ca, H-6A'a, H-6C'ab), 3.94–3.87 (m, 2 H, H-3B, H-6Cb), 3.86–3.70 (m, 5 H, H-4A, H-6Aab, H-5C', OCHHCH₂), 3.67–3.59 (m, 2 H, H-4B, H-5A'), 3.54 (bt, $J = 6.5$ Hz, 1 H, H-5C), 3.50–3.43 (m, 4 H, H-5A, H-3C, CH₂Cl), 3.37 (m, 1 H, OCHHCH₂), 3.22 (m, 1 H, H-2A), 2.78 (m, 1 H, H-2A'), 2.12, 2.04_s, 2.04, 2.03, 2.02, 2.01, 1.95, 1.94, 1.87, 1.68 (10 s, 10 × 3 H, 10 COCH₃), 1.69, 1.47, 1.35, 1.27 (4 m, 4 × 2 H, OCH₂(CH₂)₄CH₂Cl), 1.17 (s, 9 H, C(CH₃)₃), 1.14 (d, $J = 6.5$ Hz, 3 H, H-6B). ¹³C NMR (151 MHz, CDCl₃, 297 K): δ_c 177.7, 171.2, 170.7, 170.4, 170.2, 169.5, 169.4, 168.9 (C=O), 139.1, 138.9, 138.6, 138.0 (quat Ar), 128.5, 128.3, 128.2, 128.1, 127.9, 127.6, 127.4, 127.1 (Ar), 100.6 (C-1C'), 99.4 (C-1C), 99.3 (C-1A), 99.0 (C-1A'), 97.4 (C-1B), 80.1 (C-3B), 77.2 (C-4B), 76.6 (C-5A or C-3C and C-2B), 75.8 (C-3A'), 74.4 (CH₂Ph), 74.4 (C-5A or C-3C), 74.1 (C-4A), 73.7, 73.6 (CH₂Ph), 73.4 (C-3A), 72.5 (CH₂Ph), 71.8 (C-5A'), 71.0 (C-5C), 71.0, 70.4, 68.9 (C-2C, C-4A', C-3C'), 70.6 (C-5C'), 69.5 (OCH₂), 69.3 (C-4C, C-2C'), 68.3 (C-6A), 66.8 (C-4C'), 66.3 (C-5B), 62.1 (C-6A'), 61.2 (C-6C), 60.9 (C-6C'), 58.8 (C-2A'), 57.1 (C-2A), 45.0 (CH₂Cl), 38.7 (C(CH₃)₃), 32.5, 29.2, 26.6, 25.2 (OCH₂(CH₂)₄CH₂Cl), 27.1 (C(CH₃)₃), 23.7, 23.2, 21.0, 20.9, 20.8, 20.7, 20.5 (COCH₃), 16.8 (C-6B). HRESIMS m/z : [M+H]⁺ Calcd for C₈₉H₁₁₈O₃₄N₂Cl 1793.7255; Found 1793.7242.

6-Azidohexyl 2-acetamido-4-O-{3-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-2-deoxy- β -D-glucopyranosyl]-2,4-di-O-acetyl-6-O-pivaloyl- β -D-galactopyranosyl}-6-O-benzyl-3-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-2-deoxy- β -D-glucopyranoside (26). NaN₃ (10.6 mg, 10 equiv) was added to a solution of chlorohexyl glycoside **25** (29 mg, 0.016 mmol) in anhyd DMF (2 mL). The reaction was stirred at 80 °C for 22 h and the solution was concentrated. The residue was dissolved in CH₂Cl₂ (25 mL) and washed with H₂O (2 ×

25 mL). The aq layer was re- extracted with CH₂Cl₂ (4 × 10 mL) and the combined organic phases were dried and concentrated to give azidohexyl **26** (29 mg, quant) pure as a white amorphous foam. $[\alpha]_D -20.5$ (*c* 0.2, MeOH). ¹H NMR (400 MHz, CDCl₃, 296 K): δ_H 7.41–7.19 (m, 20 H, Ar), 5.75 (d, *J* = 7.2 Hz, 1 H, NHA), 5.72 (d, *J* = 6.9 Hz, 1 H, NHA'), 5.31 (dd, *J* = 0.7, 3.4 Hz, 1 H, H-4C'), 5.25 (bd, *J* = 3.5 Hz, 1 H, H-4C), 5.06 (d, *J* = 8.0 Hz, 1 H, H-1A'), 5.01 (m, 2 H, H-1B, H-2C'), 4.97–4.82 (m, 6 H, H-1A, H-2C, H-4A', H-3C', 2 × CHHPh), 4.82–4.63 (m, 5 H, 5 × CHHPh), 4.57 (t, *J* = 10.1 Hz, 1 H, H-3A'), 4.45–4.35 (m, 4 H, H-5B, H-1C, H-1C', CHHPh), 4.22 (dd, *J* = 2.5 Hz, 12.2 Hz, 1 H, H-6A'a), 4.15 (t, *J* = 8.0 Hz, 1 H, H-3A), 4.12–3.95 (m, 5 H, H-2B, H-6Ca, H-6A'a, H-6C'ab), 3.95–3.69 (m, 7 H, H-4A, H-6Aab, H-3B, H-6Cb, H-5C', OCHHCH₂), 3.67–3.59 (m, 2 H, H-4B, H-5A'), 3.52 (bt, *J* = 6.7 Hz, 1 H, H-5C), 3.47–3.40 (m, 2 H, H-5A, H-3C), 3.37 (m, 1 H, OCHHCH₂), 3.21–3.15 (m, 3 H, H-2A, CH₂N₃), 2.77 (m, 1 H, H-2A'), 2.13, 2.04₅, 2.04, 2.03, 2.02, 2.01, 1.95, 1.94, 1.85, 1.65 (10 s, 10 × 3 H, 10 COCH₃), 1.50, 1.26 (2 m, 4 H and 4 H, OCH₂(CH₂)₄CH₂Cl), 1.17 (s, 9 H, C(CH₃)₃), 1.13 (d, *J* = 6.4 Hz, 3 H, H-6B). ¹³C NMR (100 MHz, CDCl₃, 296 K): δ_C 177.7, 171.2, 170.8, 170.4, 170.3, 170.2, 169.6, 169.4, 169.0, 168.9 (C=O), 139.0, 138.8, 138.6, 138.0 (quat Ar), 128.8, 128.6, 128.5, 128.4, 127.4, 128.2 (Ar), 100.6 (C-1C'), 99.4 (C-1C), 99.3 (C-1A), 98.9 (C-1A'), 97.5 (C-1B), 80.1 (C-3B), 77.1 (C-4B), 76.7 (C-3C), 76.6 (C-2B), 75.8 (C-3A'), 74.4 (C-5A), 74.3 (CH₂Ph), 74.1 (C-4A), 73.7, 73.6 (CH₂Ph), 73.3 (C-3A), 72.4 (CH₂Ph), 71.7 (C-5A'), 71.0 (C-5C), 71.0 (C-3C'), 70.6 (C-4A'), 70.5 (C-5C'), 69.5 (OCH₂), 69.4, 69.3 (C-4C, C-2C'), 68.9 (C-2C), 68.1 (C-6A), 66.8 (C-4C'), 66.2 (C-5B), 62.1 (C-6A'), 61.2, 60.9 (C-6C, C-6C'), 58.8 (C-2A'), 57.3 (C-2A), 55.3 (CH₂N₃), 38.7 (C(CH₃)₃), 29.7, 28.7, 26.3, 25.4 (OCH₂(CH₂)₄CH₂N₃), 27.0 (C(CH₃)₃), 23.7, 23.2, 21.0, 20.9, 20.8, 20.7, 20.5 (COCH₃), 16.8 (C-6B). HRESIMS *m/z*: [M+H]⁺ Calcd for C₈₉H₁₁₈N₅O₃₄ 1800.7658; Found 1800.7677.

n-Hexyl 2-acetamido-2-deoxy-4-O-{3-O-[2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside (2) Liquid

ammonia (~25 mL) was condensed in a 50 mL two-necked flask at $-78\text{ }^{\circ}\text{C}$. Sodium (50.0 mg, 2.174 mmol) was then added. A solution of the tetrasaccharide **24** (41 mg, 0.032 mmol) in anhydrous THF (5 mL) was added to the reaction flask and the reaction mixture stirred at $-78\text{ }^{\circ}\text{C}$ for 1 h. The reaction was quenched with MeOH (5 mL) and the ammonia was allowed to evaporate at rt for 3 h. The methanolic solution was neutralized with AcOH (500 μL), the solvent evaporated and the residue dissolved in milli-Q water was passed through a Biogel[®] P2 column (100 \times 1 cm) eluted with milli-Q water. Upon freeze-drying hexyl glycoside **2** (15 mg, 57 %) was obtained pure as an amorphous powder. $[\alpha]_{\text{D}} -36.5$ (c 0.2, H_2O). ^1H NMR (D_2O , 600 MHz, 295 K): δ_{H} 4.73 (d, $J = 8.5$ Hz, 1 H, H-1A'), 4.53 (d, $J = 7.7$ Hz, 1 H, H-1A), 4.47 (d, $J = 7.9$ Hz, 1 H, H-1C), 4.45 (d, $J = 7.8$ Hz, 1H, H-1C'), 4.16 (bd, $J = 3.2$ Hz, H-4C), 3.99 (dd, $J = 2.04, 12.3$ Hz, 1 H, H-6Aa), 3.94–3.87 (m, 4 H, H-2A', H-6A'a, H-4C', OCHHCH₂), 3.85–3.68 (m, 13 H, H-2A, H-3A, H-4A, H-6Ab, H-3C, H-5C, H-6Cab, H-3A', H-6A'b, H-5C', H-6C'ab), 3.65 (dd, $J = 3.4, 10.0$ Hz, H-3C'), 3.62–3.65 (m, 4 H, H-5A, H-2C, H-4A', OCHHCH₂), 3.53 (dd, $J = 7.8, 9.9$ Hz, 1 H, H-2C'), 3.49 (m, 1 H, H-5A'), 2.03 (s, 6 H, 2 COCH₃), 1.52, 1.3 (2 m, 2 H and 6 H, OCH₂(CH₂)₄CH₃), 0.88 (t, $J = 6.9$ Hz, 3 H, O(CH₂)₅CH₃). ^{13}C NMR (D_2O , 151 MHz, 295 K): δ_{C} 177.8, 177.2 (C=O), 106.3 (C-1C'), 105.7 (C-1C), 105.4 (C-1A'), 103.9 (C-1A), 84.6 (C-3C, C-3A'), 81.3 (C-4A), 78.1, 78.00 (C-5C, C-5C'), 77.7 (C-5A'), 77.5 (C-5A), 75.3 (C-3A, C-3C'), 73.5 (C-2C'), 73.4 (OCH₂CH₂), 72.8, 71.4 (C-2C, C-4A'), 71.2 (C-4C'), 71.1 (C-4C), 63.9, 63.8 (C-6C, C-6C'), 63.3 (C-6A'), 62.9 (C-6A), 57.9 (C-2A), 57.3 (C-2A'), 33.5, 31.3, 27.6, 24.9 (OCH₂(CH₂)₄CH₃), 25.1, 25.0 (COCH₃), 16.2 ((CH₂)₅CH₃). HRESIMS m/z : $[\text{M}+\text{Na}]^+$ Calcd for C₃₄H₆₀N₂O₂₁Na 855.3586; Found 855.3609.

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

Pentasaccharide **25** (25 mg, 0.014 mmol) was deprotected as described above for the preparation of tetrasaccharide **2**. Biogel[®] P2 column (100 \times 1 cm) chromatography eluted with Milli-Q water gave

pentasaccharide **3** (9.9 mg, 73 %) pure as a white amorphous powder. $[\alpha]_D -2.5$ (*c* 0.2, H₂O). ¹H NMR (D₂O, 600 MHz, 295 K): δ_H 5.10 (d, *J* = 4.0 Hz, 1 H, H-1B), 4.81 (m, 1 H, H-5B), 4.73 (d, *J* = 8.1 Hz, 1 H, H-1A'), 4.53 (bd, *J* = 8.1 Hz, 1 H, H-1A), 4.45 (2 d, *J* = 7.7 Hz and 7.8 Hz, 2 H, H-1C and H-1C'), 4.10 (d, *J* = 3.2 Hz, 1 H, H-4C), 3.99 (dd, *J* = 1.9, 12.3 Hz, 1 H, H-6Aa), 3.95–3.67 (m, 19 H, H-2A, H-3A, H-4A, H-6Ab, H-2B, H-3B, H-4B, H-3C, H-5C or H-5C', H-6Cab, H-2A', H-3A', H-6A'ab, H-4C', H-6C'ab, OCHHCH₂), 3.65 (dd, *J* = 3.3, 9.9 Hz, 1 H, H-3C'), 3.61–3.56 (m, 4 H, H-5A, H-4A', H-5C or H-5C', OCHHCH₂), 3.55–3.50 (m, 2 H, H-2C, H-2C'), 3.48 (m, 1 H, H-5A'), 2.03 (s, 6 H, 2 COCH₃), 1.54, 1.29 (2 m, 2 H and 6 H, OCH₂(CH₂)₄CH₃), 1.15 (d, *J* = 6.5 Hz, 1 H, H-6B), 0.87 (t, *J* = 6.9 Hz, 3 H, O(CH₂)₅CH₃). ¹³C NMR (151 MHz, D₂O, 295 K): δ_C 177.8, 177.0 (C=O), 106.3 (C-1C), 105.4 (C-1C'), 104.6 (C-1A'), 103.8 (C-1A), 101.6 (C-1B), 84.8 (C-3A'), 84.4 (C-3C), 78.2, 78.1, 78.0, 77.8, 77.3 (C-3A, C-5A, C-5C, C-5A', C-5C'), 75.9, 75.3, 74.7 (C-4C', C-4B, C-3C'), 73.5 (OCH₂CH₂), 73.5, 73.4 (C-2C, C-2C'), 72.0, 71.4, 71.2, 71.1, 70.5 (C-4A, C-2B, C-3B, C-4C, C-4A'), 69.5 (C-5B), 64.3, 63.9, 63.3, 62.6 (C-6A, C-6C, C-6A', C-6C'), 58.7 (C-2A), 57.5 (C-2A'), 33.5, 31.4, 27.6, 24.9 (OCH₂(CH₂)₄CH₃), 25.1, 25.0 (COCH₃), 18.1 (C-6B), 16.2 (O(CH₂)₅CH₃). HRESIMS *m/z*: [M+Na]⁺ Calcd for C₄₀H₇₀N₂O₂₅Na 1001.4165; Found 1001.4164.

6-Aminohexyl 2-acetamido-2-deoxy-3-O- α -L-fucopyranosyl-4-O-{3-O-[2-acetamido-2-deoxy-3-O-(β -D-galactopyranosyl)- β -D-glucopyranosyl]- β -D-galactopyranosyl]- β -D-glucopyranoside (4). Azidohexyl pentasaccharide **26** (25 mg, 0.014 mmol) was deprotected as described above for the preparation of tetrasaccharide **2**. Biogel[®] P2 column (100 × 1 cm) chromatography eluted with aq ammonium acetate (0.05 M) gave aminohexyl pentasaccharide **4** (11 mg, 75 %) pure as a greyish powder. $[\alpha]_D -39$ (*c* 0.2, H₂O). ¹H NMR (D₂O, 600 MHz, 295 K): δ_H 5.10 (d, *J* = 3.9 Hz, 1 H, H-1B), 4.82 (m, 1 H, H-5B), 4.73 (d, *J* = 8.5 Hz, 1 H, H-1A'), 4.53 (bd, *J* = 8.2 Hz, 1 H, H-1A), 4.45 (d, *J* = 7.7 Hz, 1 H, H-1C), 4.44 (d, *J* = 7.8 Hz, 1 H, H-1C'), 4.10 (bd, *J* =

3.2 Hz, 1 H, H-4C), 3.99 (bd, $J = 12.2$ Hz, 1 H, H-6Aa), 3.94–3.67 (m, 19 H, H-2A, H-3A, H-4A, H-6Ab, H-2B, H-3B, H-4B, H-3C, H-5C or H-5C', H-6Cab, H-2A', H-3A', H-6A'ab, H-4C', H-6C'ab, OCHHCH₂), 3.65 (dd, $J = 3.6, 10.1$ Hz, 1 H, H-3C'), 3.61–3.56 (m, 4 H, H-5A, H-4A', H-5C or H-5C', OCHHCH₂), 3.56–3.50 (m, 2 H, H-2C, H-2C'), 3.48 (m, 1 H, H-5A'), 2.99 (t, $J = 7.4$ Hz, 2 H, O(CH₂)₅CH₂NH₂), 2.03 (s, 6 H, 2 COCH₃), 1.66, 1.56, 1.37 (3 m, 2 × 2 H and 4 H, OCH₂(CH₂)₄CH₂NH₂), 1.15 (d, $J = 6.6$ Hz, 1 H, H-6B). ¹³C NMR (151 MHz, D₂O, 295 K): δ_C 177.8, 177.0 (C=O), 106.3 (C-1C), 105.4 (C-1C'), 104.6 (C-1A'), 103.8 (C-1A), 101.6 (C-1B), 84.8 (C-3A'), 84.4 (C-3C), 78.2, 78.1, 78.0, 77.8, 77.3 (C-3A, C-5A, C-5C, C-5A', C-5C'), 75.9 (C-4C'), 75.3 (C-3C'), 74.7 (C-4B), 73.5, 73.4 (C-2C, C-2C'), 73.3 (OCH₂CH₂), 72.0 (C-3B), 71.4, 71.2, 71.1, 70.5 (C-2B, C-4C, C-4A', C-4A), 69.5 (C-5B), 64.3, 63.9, 63.3 (C-6C, C-6A', C-6C'), 62.6 (C-6A'), 58.6 (C-2A), 57.5 (C-2A'), 42.2 (CH₂(CH₂)CH₂NH₂), 31.2, 29.5, 28.1, 27.5 (OCH₂(CH₂)₄CH₂NH₂), 25.1, 25.0 (COCH₃), 18.1 (C-6B). HRESIMS m/z : [M-H]⁻ Calcd for C₄₀H₇₀N₃O₂₅ 992.4293; Found 992.4295.

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

Supporting Information Available. General Experimental Procedures, ¹H and ¹³C NMR spectra for all new compounds are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (a) Buskas, T.; Thompson, P.; Boons, G. J.; *Chem. Commun.* **2009**, (36), 5335–5349; (b) Astronomo, R. D.; Burton, D. R.; *Nat. Rev. Drug Discov.* **2010**, 9 (4), 308–324; (c) Morelli, L.; Poletti, L.; Lay, L.; *Eur. J. Org. Chem.* **2011**, (29), 5723–5777; (d) Hevey, R.; Ling, C. C.; *Future*

Med. Chem. **2012**, *4* (4), 545–584; (e) Liu, C.-C.; Ye, X.-S.; *Glycoconjugate J.* **2012**, *29* (5), 259–271.

2. (a) Pettijohn, D. E.; Stranahan, P. L.; Due, C.; Rønne, E.; Sørensen, H. R.; Olsson, L.; *Cancer Res.* **1987**, *47* (4), 1161-1169; (b) Pettijohn, D. E.; Pfenninger, O.; Brown, J.; Duke, R.; Olsson, L.; *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85* (3), 802–806; (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) Stranahan, P. L.; Howard, R. B.; Pfenninger, O.; Cowen, M. E.; Johnston, M. R.; Pettijohn, D. E.; *Cancer Res.* **1992**, *52* (10), 2923–2930; (e) Stroud, M. R.; Levery, S. B.; Martensson, S.; Salyan, M. E. K.; Clausen, H.; Hakomori, S.; *Biochemistry* **1994**, *33* (35), 10672–10680; (f) Stranahan, P. L.; Laroe, J.; McCombs, R.; Goldsmith, A.; Rahim, I.; Overland, M.; Pettijohn, D. E.; *Glycoconjugate J.* **1996**, *13*, 741–747; (g) Stranahan, P. L.; Laroe, J.; McCombs, R.; Rahim, I.; Kuhn, C. W.; Pettijohn, D. E.; *Oncol. Rep.* **1998**, *5*, 235–239.
3. Lemieux, R. U.; Baker, D. A.; Weinstein, W. M.; Switzer, C. M.; *Biochemistry* **1981**, *20* (1), 199–205.
4. Guillemineau, M.; Auzanneau, F. I.; *J. Org. Chem.* **2012**, *77* (20), 8864–8878.
5. (a) Ratner, D. M.; Adams, E. W.; Su, J.; O'Keefe, B. R.; Mrksich, M.; Seeberger, P. H.; *ChemBioChem* **2004**, *5*, 379–382; (b) Wang, A.; Auzanneau, F.-I.; *Carbohydr. Res.* **2010**, *345* (9), 1216–1221; (c) Wang, A.; Hendel, J.; Auzanneau, F.-I.; *Beilstein J. Org. Chem.* **2010**, *6*, doi 10.3762/bjoc.6.17; (d) Hendel, J. L.; Auzanneau, F.-I.; *Eur. J. Org. Chem.* **2011**, (34), 6864–6876.
6. Blatter, G.; Jacquinet, J.-C.; *Carbohydr. Res.* **1996**, *288*, 109–125.

7. (a) Schmidt, R. R.; Stumpp, M.; *Liebigs Ann. Chem.* **1983**, *7*, 1249-1256; (b) Amvam-Zollo, P. H.; Sinay, P.; *Carbohydr. Res.* **1986**, *150*, 199-212; (c) Toepfer, A.; Schmidt, R. R.; *J. Carbohydr. Chem.* **1993**, *12*, 809-822.
8. Lönn, H.; *Carbohydr. Res.* **1985**, *139*, 105-113.
9. Risbood, P. A.; Reed III, L. A.; Goodman, L.; *Carbohydr. Res.* **1981**, *88* (2), 245-251.
10. Liu, C.-Y.; Chen, H.-L.; Ko, C.-M.; Chen, C.-T.; *Tetrahedron* **2011**, *67*, 872-876.
11. Yu, H.; Ensley, H. E.; *Tetrahedron Lett.* **2003**, *44* (52), 9363-9366.
12. (a) Hendel, J. L.; Cheng, A.; Auzanneau, F.-I.; *Carbohydr. Res.* **2008**, *343*, 2914-2923; (b) Hendel, J. L.; Wang, J. W.; Jackson, T. A.; Hardmeier, K.; De Los Santos, R.; Auzanneau, F. -I.; *J. Org. Chem.* **2009**, *74* (21), 8321-8331.
13. Guillemineau, M.; Auzanneau, F.-I.; *Carbohydr. Res.* **2012**, *357*, 132-138.
14. Liao, L.; Robertson, V.; Auzanneau, F.-I.; *Carbohydr. Res.* **2005**, *340* (18), 2826-2832.
15. (a) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C.; *J. Am. Chem. Soc.* **1980**, *102* (23), 7048-7050; (b) Gellman, S. H.; Dado, G. P.; Liang, G. B.; Adams, B. R.; *J. Am. Chem. Soc.* **1991**, *113* (4), 1164-1173; (c) Fowler, P.; Bernet, B.; Vasella, A.; *Helv. Chim. Acta* **1996**, *79* (1), 269-287; (d) Vasella, A.; Witzig, C.; *Helv. Chim. Acta* **1995**, *78* (8), 1971-1982.
16. 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.
17. Zhang, P.; Ng, K.; Ling, C. C.; *Org. Biomol. Chem.* **2010**, *8* (1), 128-136.

Accepted Draft