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Note

Synthesis of Le^{a}Le^x oligosaccharide fragments and efficient one-step deprotection

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ABSTRACT

We describe here the synthesis of two oligosaccharide fragments of the tumor associated carbohydrate antigen Le^{a}Le^x. While the linear lacto-*N*-triose I: β-D-Gal*p*-(1→4)-β-D-GlcNAc*p*-(1→3)-β-D-Gal*p*-OMe is a known compound, this is the first reported preparation of the branched tetrasaccharide β-D-GlcNAc*p*-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-GlcNAc*p*-OMe. Our synthetic schemes involved using an *N*-trichloroacetylated trichloroacetimidate glucosaminyl donor activated with excess TMSOTf at 0 °C for glycosylation at O-3 of galactosyl residues and that of trichloroacetimidate galactosyl donors activated with excess $BF_3{\cdot}OEt_2$ to glycosylate either O-3 or O-4 of glucosamine residues. The fucosylation at O-3 of the glucosamine acceptor was accomplished using a thiofucoside donor activated with copper(II) bromide and tetrabutylammonium bromide. Thus, syntheses of the protected tri- and tetrasaccharides were achieved easily and efficiently using known building blocks. Of particular interest, we also report that these protected oligosaccharides were submitted to dissolving metal conditions (Na–NH_3) to provide in one single step the corresponding deprotected compounds. Under these conditions all protecting groups (*O*-acyl, benzylidene, benzyl, and *N*-trichloroacetyl) were efficiently cleaved. The work-up procedure for such reactions usually involves quenching with excess methanol and then neutralization with acetic acid. In our work the neutralization was carried out using acetic anhydride rather than acetic acid to ensure N-acetylation of the glucosamine residue. Both fully deprotected compounds were then simply purified and desalted by gel permeation chromatography on a Biogel P2 column eluted with water.

The hexasaccharide Le^{a}Le^x has been shown to be over-expressed at the surface of squamous carcinoma cells and as such has been identified as a Tumor Associated Carbohydrate Antigen (TACA).[1] Our research efforts aim at discovering epitopes displayed by this antigen at the surface of tumor cells to eventually develop an anti-cancer vaccine based on this hexasaccharide and that would no-longer display the Le^a trisaccharide also largely expressed on non-cancerous cells. In this context, we have recently described the synthesis of the trisaccharide fragment α-L-Fuc*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-OMe and established through molecular mechanics and NMR experiments that it was highly flexible. We describe here the synthesis of two additional fragments of the Le^{a}Le^x hexasaccharide: the so-called[3] lacto-*N*-triose I trisaccharide (**2**) and the branched fucosylated tetrasaccharide fragment **3** (Chart 1).

Lacto-*N*-triose I was first isolated following partial hydrolysis of the human milk tetrasaccharide lacto-*N*-tetraose[3] and was later purified by Morgan and co-workers from a partial acid-hydrolysate of blood group substances.[4] While the synthesis of trisaccharide **2** has, in turn, been reported by Matta and co-workers,[5] to the best of our knowledge, we are reporting here the first chemical synthesis of tetrasaccharide **3**. The synthetic approach that Matta and co-workers followed for the preparation of trisaccharide **2** relied on the use of an oxazoline glycosyl donor to form the β-D-GlcNAc*p*-(1→3)-D-Gal*p* glycosidic bond. In our syntheses of tri- and tetrasaccharides **2** and **3** we chose to use the more reactive *N*-trichloroacetylated trichloroacetimidate glycosyl donor **4** introduced by Blatter et al.[6]

Thus, the known[5] trisaccharide **2** was prepared in five steps (Scheme 1) from the known monosaccharide building blocks **4**,[6] **5**,[7] and **8**.[8] Under activation with 1 equiv of TMSOTf at 0 °C coupling of acceptor **5** with donor **4** (2.5 equiv) was achieved in 70% yield. The disaccharide **6** was then converted in two steps to the acceptor **7**. Applying conditions that were used previously in our group for a similar disaccharide,[2] the acetyl groups were first selectively removed by methanolysis at room temperature using a 1 M solution of HCl in MeOH (AcCl–MeOH). The resulting crude triol was then selectively protected at positions 4 and 6 by reaction with benzaldehyde dimethylacetal (3 equiv) under camphorsulfonic acid catalysis in acetonitrile at 50 °C. Acceptor **7** was isolated in an acceptable 56% yield over the two steps and was, in turn, glycosylated with the galactosyl donor **8**. Coupling of donor **8** (5 equiv) with acceptor **7** was carried out at room temperature in

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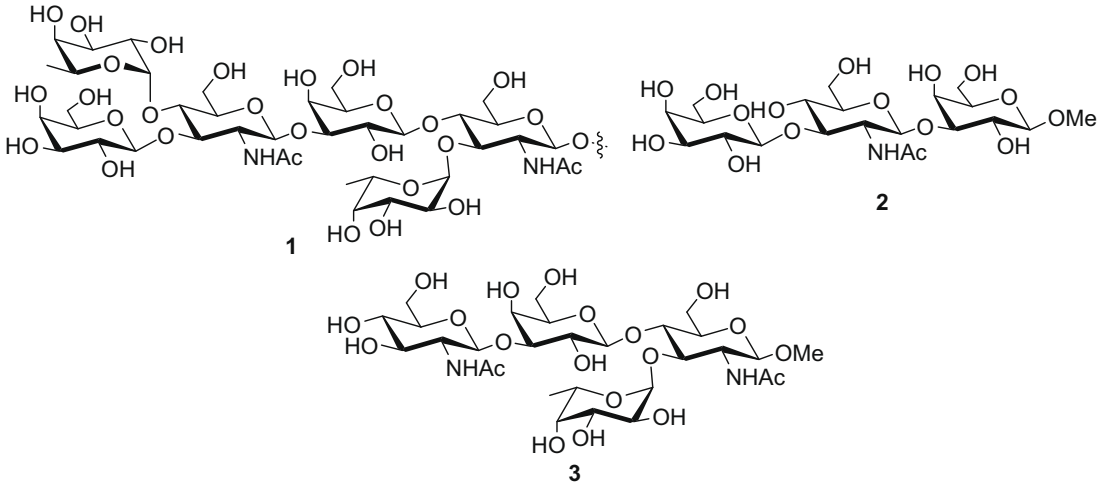


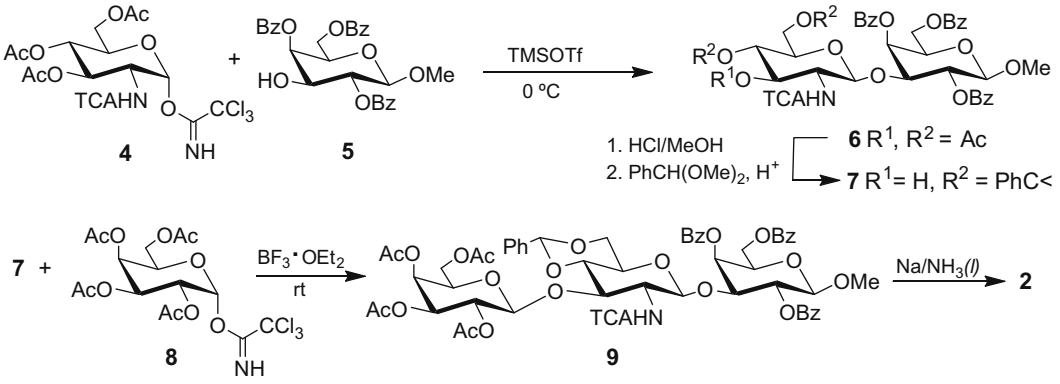
Chart 1.

dichloromethane under promotion by 2 equiv of $BF_3{\cdot}OEt_2$. Flash column chromatography followed by centrifugal chromatography of the impure fractions gave the protected trisaccharide **9** in 64% yield.

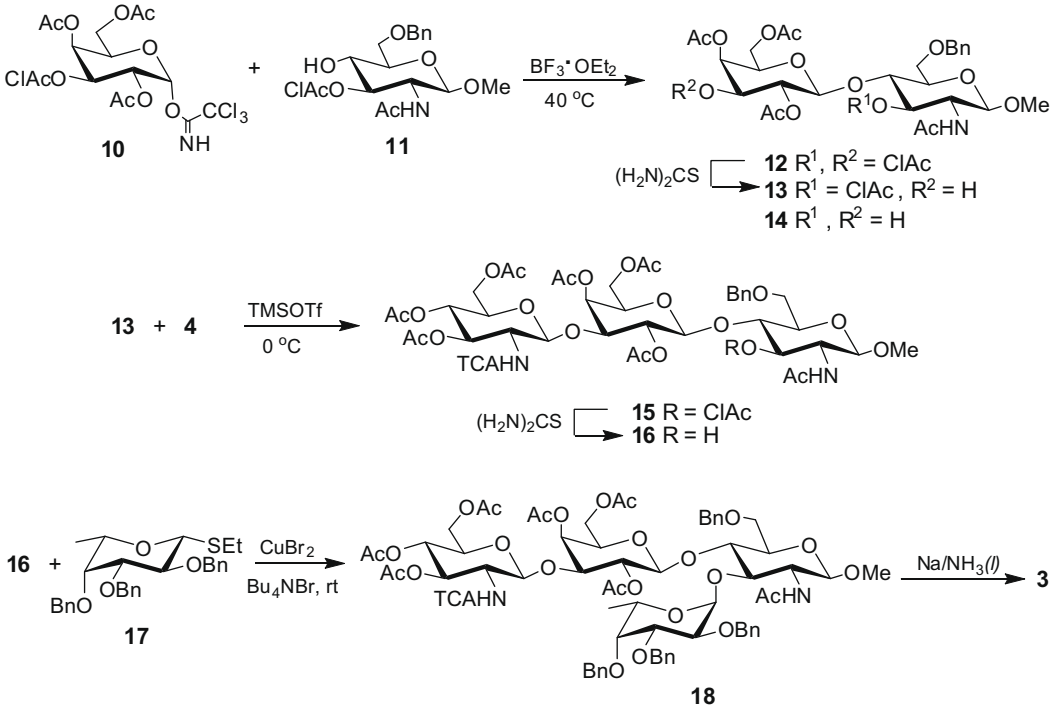
In our quest for an efficient one-step deprotection procedure, we investigated the removal of all protecting groups from trisaccharide **9** using sodium–liquid ammonia in THF at −78 °C (Birch reduction conditions). Indeed, expanding on the work by Seeberger and co-workers,[9] we have reported that Birch reduction conditions led not only to the easy removal of *O*-benzyl and *O*-acyl groups but also to the concurrent reduction of 6-chlorohexyl glycosides to the corresponding hexyl glycosides.[10] Thus, we postulated that under these conditions the *O*-acyl groups would be removed, the benzylidene group reductively cleaved, and that the *N*-trichloroacetyl group would be either reduced to the corresponding acetamido or removed to give the corresponding free amine. Because it is more likely that, under such basic conditions, the *N*-trichloroacetyl group would be removed rather than de-chlorinated, the work-up procedure was modified to ensure N-acetylation of the resulting free amine. Thus, after quenching of the reaction with methanol and evaporation of the liquid ammonia at room temperature, the resulting mixture was treated with an excess acetic anhydride (1.3 equiv to the sodium added) rather than neutralized with acetic acid. After concentration, the deprotected trisaccharide **2** did not require silica gel chromatography and was obtained pure and free of salts in 55% yield after gel permeation chromatography (2 × Biogel P2, 100 cm × 1 cm, water). The NMR data recorded for trisaccharide **2** were in total agreement with the data reported for lacto-*N*-triose I (Scheme 2).[5]

The trisaccharide **3** was prepared in six steps from the donor **10**[10] and acceptor **11**,[11] which we have reported previously and from the known donors **4**[6] and **17**.[12] The coupling of acceptor **11** with trichloroacetimidate donor **10** was carried out under conditions that we have established for the efficient glycosylation at the poorly reactive O-4 position of N-acetylated glucosamine glycosyl acceptors.[11,13] Thus, donor **10** (5 equiv) was activated with 2 equiv of $BF_3{\cdot}OEt_2$ and was allowed to react with acceptor **11** for 1 h at 40 °C. Upon flash chromatography (EtOAc and hexanes, 8:2) the desired disaccharide **12** was obtained in 60% yield (estimated by ^{1}H NMR) contaminated with unreacted acceptor **11** in a 3:1 ratio. Centrifugal chromatography of this mixture using a $CHCl_3$–MeOH (20:1) eluent provided the pure desired disaccharide **12** in 54% yield.

This mediocre yield was in sharp contrast with the ~90% yield that we have reported for the glycosylation of acceptor **11** with α-trichloroacetimidate peracetylated glucopyranose under the same coupling conditions.[11] The observation that some acceptor remained unreacted suggests that galactosylation at O-4 of acceptor **11** is more difficult to achieve than its glucosylation. Indeed, other galactosylations at O-4 of *N*-acetylglucosamine acceptors that we have recently described[10] using the same coupling conditions also



Scheme 1. Synthesis of trisaccharide **2**.



Scheme 2. Synthesis of tetrasaccharide **3**.

led to maximum yields around ~70%. Observing that the chloroacetyl group at O-3′ of disaccharide **12** was less sterically hindered than that at O-3, we reasoned that it should be possible to selectively remove it using controlled reaction conditions to give acceptor **13**. Indeed, best yields of acceptor **13** were obtained applying conditions established by Naruto et al.,[14] that is, treating disaccharide **12** with thiourea (1.3 equiv) and sodium bicarbonate (20 mg/mL) in ethanol at 70 °C (4 h). Under these conditions, the desired acceptor **13** was isolated in 65% yield while the diol **14** was obtained in 13% yield.

Disaccharide acceptor **13** was, in turn, glycosylated with the *N*-trichloroacetylated trichloroacetimidate donor **4**. This coupling gave best results when it was carried out in dichloromethane using a large excess of TMSOTf (2 equiv) at 0 °C for 1 h. Under these conditions, trisaccharide **15** was isolated in 74% yield after two chromatographic purification steps ($CHCl_3$–MeOH, 20:1; then EtOAc–hexanes, 9:1). It is worth mentioning that lower concentrations of TMSOTf, as well as using 2 equiv of $BF_3{\cdot}OEt_2$ at 40 °C, did not lead to acceptable yields of the desired trisaccharide but to the rapid formation of the corresponding known[6] oxazoline, which was then unreactive as a glycosyl donor under these conditions.

Removal of the O-3 chloroacetate from trisaccharide **15** was accomplished easily using thiourea in a pyridine–ethanol mixture at 65 °C and gave trisaccharide acceptor **16** in 76%. Finally, coupling of acceptor **16** with fucosyl donor **17** proceeded well under activation with copper(II) bromide and tertabutylammonium bromide at room temperature and the desired protected tetrasaccharide was isolated in 76% yield. The α configuration of the newly formed fucosidic bond was confirmed by ^{1}H NMR using the coupling constant measured between H-1 and H-2 of the fucosyl residue (3.7 Hz). The tetrasaccharide was, in turn, submitted to dissolving metal conditions [Na−NH_3(*l*)] as described above for the one-step deprotection of trisaccharide **9**. Expecting that these conditions would lead to the removal of all protecting groups including that of the trichloroacetamido, the reaction was quenched with methanol and after evaporation of the excess ammonia the mixture was treated with acetic anhydride to neutralize the sodium methoxide formed and to acetylate the C-2‴ free amine. The desired deprotected tetrasaccharide **3** was then purified and desalted by passing twice on a gel permeation column (Biogel P2) eluted with water and was isolated in 72% yield.

In conclusion, syntheses of the protected tri- and tetrasaccharides were achieved easily and efficiently via the assembly of known building blocks. These protected oligosaccharides were submitted to dissolving metal conditions (Na–NH_3) and provided in one single step after N-acetylating work-up the corresponding deprotected compounds. Both fully deprotected compounds were then simply purified and desalted by gel permeation chromatography on a Biogel P2 column eluted with water and will be used in molecular modeling and binding experiments.

1. Experimental

1.1. General methods

^{1}H (600.13, 400.13 or 300.13 MHz) and ^{13}C NMR (150, 100 or 75 MHz) spectra were recorded at 300 K for solution in $CDCl_3$ (internal standard, for ^{1}H residual $CHCl_3$ δ 7.24; for ^{13}C $CDCl_3$ δ 77.0) or D_2O [external standard 3-(trimethylsilyl)-propionic acid-d_4, sodium salt (TSP) for ^{1}H δ 0.00, for ^{13}C δ 0.00]. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in parts per million (ppm). Coupling constants (*J*) are reported in hertz (Hz). Chemical shifts and coupling constants were obtained from a first-order analysis of one-dimensional spectra. Assignments of proton and carbon resonances were based on two dimensional ^{1}H–^{1}H and ^{13}C–^{1}H correlation experiments. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broadened (br). Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F_{254} precoated plates (250 μm) with a fluorescent indicator, visualized under UV, and charred with 10% sulfuric acid in ethanol. Compounds were purified by flash chromatography

with Silica Gel 60 (230–400 mesh) unless otherwise stated. Solvents were distilled and dried according to standard procedures,[15] and organic solutions were dried over Na_2SO_4 and concentrated below 40 °C, under reduced pressure. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded by the analytical services of the McMaster Regional Center for Mass Spectrometry, Hamilton, Ontario.

1.2. Methyl 3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (6)

Acceptor **5** (500 mg, 0.99 mmol) and donor **4** (1.47 g, 2.47 mmol, 2.5 equiv) were dissolved in anhyd CH_2Cl_2 (30 mL) under N_2 and the solution was cooled down to 0 °C. Freshly distilled TMSOTf (180 μL, 0.99 mmol, 1.0 equiv) was added, the mixture was stirred at 0 °C for 1 h and the reaction was quenched by the addition of Et_3N (138 μL, 0.99 mmol). The solvent was evaporated and flash chromatography of the residue (EtOAc–hexanes, 2:3) gave the pure disaccharide **6** (660 mg, 70%) as a yellowish foam. $[\alpha]_D$ +25 (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.96–8.11, 7.35–7.60 (15H, Ar), 6.59 (d, 1H, *J* = 8.1 Hz, NH), 5.81 (br d, 1H, *J* = 3.4 Hz, H-4), 5.55 (dd, 1H, *J* = 7.7, 9.8 Hz, H-2), 5.29 (dd, 1H, *J* = 9.2, 10.6 Hz, H-3′), 5.01 (d, 1H, *J* = 8.2 Hz, H-1′), 4.96 (t, 1H, *J* = 9.6 Hz, H-4′), 4.54 (d, 1H, *J* = 7.7 Hz, H-1), 4.41–4.53 (m, 2H, H-6a, H-6b), 4.24 (dd, 1H, *J* = 3.4, 9.8 Hz, H-3), 4.19 (dd, 1H, *J* = 2.4, 12.3 Hz, H-6a′), 4.03–4.16 (m, 2H, H-5, H-6b′), 3.53–3.68 (m, 2H, H-2′, H-5′), 3.45 (OCH_3), 1.95, 1.94, 1.86 (3s, 9H, $CH_3CO \times 3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.69, 170.22, 169.25, 166.16, 165.52, 165.07, 161.56 (C=O), 133.43, 133.22, 133.13, 130.07, 129.87, 129.64, 129.61, 129.46, 129.37, 128.53, 128.42, 128.34 (Ar), 102.10 (C-1), 99.26 (C-1′), 91.64 (CCl_3), 76.53 (C-3), 71.87, 71.67, 71.45 (C-2, C-5, C-5′), 70.55 (C-3′), 69.85 (C-4), 68.24 (C-4′), 62.95 (C-6), 61.34 (C-6′), 56.79 (OCH_3), 56.49 (C-2′), 20.57, 20.49, 20.37 ($CH_3CO \times 3$). HRESIMS calcd for $C_{42}H_{46}Cl_3N_2O_{17}$ $[M+NH_4]^+$: 955.1862, found:955.1829.

1.3. Methyl 3-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido -β-D-glucopyranosyl)2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (7)

AcCl (1 mL) was added to a solution of disaccharide **6** (444 mg, 0.473 mmol) in anhyd MeOH (12.5 mL) and the reaction mixture was stirred under N_2 at rt for 12 h. $NaHCO_3$was added to the reaction mixture and once gas evolution had ceased the solids were filtered off and rinsed with MeOH. The combined filtrate and washings were concentrated and the residue was dried under high vacuum. Benzaldehyde dimethyl acetal (213 μL, 3.0 equiv) and CSA (80 mg) were added to a suspension of the crude triol in anhyd MeCN (8 mL) stirred under N_2 at 50 °C. The reaction was allowed to proceed for 30 min at 50 °C and was quenched by the addition of Et_3N (50 μL). Solvents were evaporated and flash chromatography of the residue (EtOAc–hexanes, 1:4) gave pure benzylidene acetal **7** (238 mg, 56% over two steps) as a yellowish glass. $[\alpha]_D$ +13 (*c* 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.98–8.15, 7.30–7.62 (20H, Ar), 6.76 (d, 1H, *J* = 6.4 Hz, NH), 5.81 (d, 1H, *J* = 2.9 Hz, H-4), 5.59 (dd, 1H, *J* = 7.8, 9.8 Hz, H-2), 5.44 (s, 1H, PhC*H*), 5.13 (d, 1H, *J* = 8.1 Hz, H-1′), 4.56 (d, 1H, *J* = 7.8 Hz, H-1), 4.46–4.53 (m, 2H, H-6a, H-6b), 4.30 (dd, 1H, *J* = 4.3, 10.4 Hz, H-6a′), 4.20–4.27 (m, 2H, H-3, H-3′), 4.13 (t, 1H, *J* = 6.2 Hz, H-5), 3.53–3.62 (m, 1H, H-6b′), 3.49 (s, 3H, OCH_3), 3.34–3.45 (m, 2H, H-4′, H-5′), 3.16–3.23 (m, 1H, H-2′).[13]C NMR (100 MHz, $CDCl_3$): δ 166.20, 165.63, 165.34, 162.41 (C=O), 136.82, 133.52, 133.42, 133.25, 130.11, 129.95, 129.73, 129.66, 129.47, 129.41, 129.30, 128.61, 128.56, 128.44, 128.38, 128.32, 126.25, 126.21 (Ar), 102.20 (C-1), 101.77 (PhCH), 98.93 (C-1′), 91.80 (CCl_3), 81.11 (C-4′), 76.53 (C-3), 71.60 (C-2, C-5), 71.67, 71.45 (C-5′), 69.83 (C-4), 68.89 (C-3′), 68.28 (C-6′), 66.13 (C-5′), 62.76 (C-6), 60.04 (C-2′), 56.98 (OCH_3). HRESIMS calcd for $C_{43}H_{44}Cl_3N_2O_{14}$ $[M+NH_4]^+$: 917.1858, found: 917.1873.

1.4. Methyl 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]-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (9)

$BF_3{\cdot}OEt_2$ (44 μL, 0.36 mmol, 2.0 equiv) was added at rt to a solution stirred under N_2 of disaccharide acceptor **7** (161 mg, 0.179 mmol) and donor **8** (439 mg, 0.891 mmol, 5.0 equiv) in anhyd CH_2Cl_2 (8 mL). The reaction mixture was stirred for 30 min and the reaction quenched by the addition of Et_3N (50 μL). The solvent was evaporated and flash chromatography of the residue (EtOAc–hexanes, 3:7) followed by centrifugal chromatography (EtOAc–hexanes, 3:7) of the impure fractions gave pure trisaccharide **9** (140 mg, 64%) as a yellowish amorphous glass. $[\alpha]_D$ +21(*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 8.09–8.15, 7.98–8.06, 7.52–7.63, 7.39–7.50, 7.30–7.38 (20H, Ar), 6.83 (d, 1H, *J* = 6.8 Hz, NH), 5.81 (d, 1H, *J* = 2.8 Hz, H-4), 5.54 (dd, 1H, *J* = 7.8, 9.9 Hz, H-2), 5.44 (s, 1H, PhC*H*), 5.30 (d, 1H, *J* = 8.1 Hz, H-1′), 5.21 (dd, 1H, *J* = 0.6, 3.3 Hz, H-4″), 5.50 (dd, 1H, *J* = 8.0, 10.4 Hz, H-2″), 4.81 (dd, 1H, *J* = 3.4, 10.4 Hz, H-3″), 4.59 (d, 1H, *J* = 8.0 Hz, H-1″), 4.53 (d, 1H, *J* = 7.7 Hz, H-1), 4.46–4.58 (m, 3H, H-6a, H-6b, H-3′), 4.21–4.32 (m, 2H, H-3, H-6a′), 4.09–4.14 (m, 1H, H-5), 3.97 (dd, 1H, *J* = 6.7, 11.3 Hz, H-6a″), 3.82 (dd, 1H, *J* = 6.8, 11.2 Hz, H-6b″), 3.53–3.63 (m, 3H, H-4′, H-6b′, H-5″), 3.42–3.51 (m, 4H, H-5′, OCH_3), 3.10–3.17 (m, 1H, H-2′), 2.04, 1.90, 1.89, 1.70 (4s, 12H, $CH_3CO \times 4$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.21, 170.11, 170.00, 169.25, 166.14, 165.56, 165.10, 161.83 (C=O), 136.86, 133.50, 133.36, 133.24, 130.05, 129.94, 129.69, 129.62, 129.59, 129.40, 129.29, 128.57, 128.49, 128.42, 128.26, 126.21 (Ar), 102.33 (C-1), 101.36 (PhCH), 98.79 (C-1″), 98.36 (C-1′), 91.72 (CCl_3), 78.24 (C-4′), 76.46 (C-3), 74.83 (C-3′), 71.50 (C-5), 71.19 (C-2), 70.90 (C-3″), 70.52 (C-5″), 69.93 (C-4), 68.77 (C-2″), 68.31 (C-6′), 66.79 (C-4″), 66.05 (C-5′), 62.70 (C-6), 61.15 (C-6″), 59.48 (C-2′), 56.98 (OCH_3), 20.57, 20.53, 20.46 ($CH_3CO \times 4$). HRESIMS calcd for $C_{57}H_{62}Cl_3N_2O_{23}$ $[M+NH_4]^+$: 1247.2809, found: 1247.2798.

1.5. Methyl 3-*O*-[2-deoxy-2-acetamido-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (2)

Trisaccharide **9** (20 mg, 0.016 mmol) was dissolved in THF (5 mL) and liquid ammonia (20 mL) was condensed into the reaction flask at −78 °C. Sodium (83 mg, 3.6 mmol) was added into the reaction mixture which turned deep blue. After 50 min at −78 °C, the reaction was quenched by the addition of MeOH (5 mL) and the ammonia was allowed to evaporate at rt. Ac_2O (445μL, 4.7 mmol) was added to the remaining solution, the solvent was evaporated and the residue was submitted to gel permeation chromatography on a Biogel P2 column eluted with water. The desired compound was obtained contaminated with residual acetate salts and submitted again to chromatography on Biogel P2 (water) to give the known[5] trisaccharide **2** (5 mg, 55%), which was isolated as a white amorphous powder upon freeze-drying. $[\alpha]_D$ +9 (*c* 0.3, MeOH). ^1H NMR (600 MHz, D_2O): δ 4.72 (d, 1H, *J* = 8.4 Hz, H-1″), 4.42 (d, 1H, *J* = 7.7 Hz, H-1′), 4.28 (d, 1H, *J* = 8.0 Hz, H-1), 4.12 (d, 1H, *J* = 3.2 Hz, H-4), 3.85–3.90 (m, 3H, H-2′, H-6a′, H-4″), 3.65–3.82 (m, 9H, H-3, H-5, H-6a, H-6b, H-3′, H-6b′, H-5″, H-6a″, H-6b″), 3.62 (dd, 1H, *J* = 3.4, 9.9 Hz, H-3″), 3.44–3.57 (m, 7H, H-2, H-4′, H-5′, H-2″, OCH_3), 2.0 (s, 3H, CH_3CO). ^{13}C NMR (150 MHz, D_2O): δ 174.97 (C=O), 103.83 (C-1′), 103.44 (C-1″), 102.38 (C-1), 82.18, 82.05 (C-3, C-3′), 75.23, 75.15, 74.67 (C-5, C-5′, C-5″), 72.41 (C-3″), 70.63 (C-2″), 69.69 (C-2), 68.47, 68.41, 68.30 (C-4, C-4′, C-4″), 60.98, 60.86, 60.45 (C-6, C-6′, C-6″), 57.15 (OCH_3), 54.71 (C-2′), 22.19 (CH_3CO).

1.6. Methyl 2-acetamido-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-chloroacetyl-β-D-galactopyranosyl)-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (12)

$BF_3{\cdot}Et_2O$ (220 μL, 1.75 mmol, 2.0 equiv) was added to a solution stirred at 40 °C of acceptor **11** (350 mg, 0.87 mmol) and donor **10** (2.3 g, 4.4 mmol, 5.0 equiv) in anhyd CH_2Cl_2 (20 mL). The reaction mixture was stirred at 40 °C for 1 h and the reaction quenched by the addition of Et_3N (250 μL). The solvent was evaporated and flash chromatography of the residue (EtOAc–hexanes, 4:1) gave a mixture of disaccharide **12** and unreacted acceptor **11** (484 mg, **12**:**11** = 3:1 by NMR integration, 60%). Further purification by centrifugal chromatography ($CHCl_3$–MeOH, 20:1) gave the pure disaccharide **12** as a white amorphous solid (362 mg, 54%). $[\alpha]_D$ −8 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25–7.40 (m, 5H, Ar), 5.79 (d, 1H, *J* = 9.3 Hz, NH), 5.23 (d, 1H, *J* = 3.3 Hz, H-4′), 5.10 (dd, 1H, *J* = 8.9, 10.3 Hz, H-3), 4.95 (dd, 1H, *J* = 8.0, 10.3 Hz, H-2′), 4.80 (dd, 1H, *J* = 3.5, 10.4 Hz, H-3′), 4.74 (d, 1H, *J* = 12.1 Hz, PhC*H*$_2$), 4.34–4.45 (m, 3H, H-1, H-1′, PhC*H*$_2$), 4.00–4.13 (m, 4H, H-6a′, H-6b′, $ClCH_2CO$), 3.88–4.00 (m, 4H, H-2, H-4, $ClCH_2CO$), 3.66–3.75 (m, 2H, H-6a, H-6b), 3.55–3.62 (m, 1H, H-5′), 3.40–3.50 (m, 4H, H-5, OCH_3), 2.10, 2.05, 1.93, 1.92 (4s, 12H, $CH_3CO \times 4$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.39, 170.34, 170.27, 168.85, 167.38, 166.55 (C═O), 137.59, 128.63, 128.18, 128.13 (Ar), 101.79 (C-1), 99.97 (C-1′), 74.60, 74.36 (C-3, C-4, C-5), 73.64 (PhC*H*$_2$), 72.52 (C-3′), 70.41 (C-5′), 68.74 (C-2′), 67.12 (C-6), 66.60 (C-4′), 60.86 (C-6′), 56.63 (OCH_3), 53.33 (C-2), 40.80, 40.33 ($ClCH_2CO \times 2$), 23.30, 20.64, 20.60 ($CH_3CO \times 4$). HRESIMS calcd for $C_{32}H_{42}Cl_2NO_{16}$ $[M+H]^+$: 766.1881, found: 766.1821.

1.7. Methyl 2-acetamido-4-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (13) and methyl2-acetamido-4-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (14)

Thiourea (47 mg, 0.617 mmol, 1.3 equiv) and $NaHCO_3$ (260 mg, 20 mg/mL) were added to a stirred solution of disaccharide **12** (365 mg, 0.476 mmol) in EtOH (13 mL). The reaction mixture was stirred at 70 °C for 4 h, solids were filtered off, rinsed with EtOH and the combined filtrate and washing were concentrated. Flash chromatography of the residue ($CHCl_3$–MeOH, 20:1) gave the desired acceptor **13** isolated as a white amorphous solid (213 mg, 65%) followed by diol **14** also obtained as a white amorphous solid (37 mg, 13%).

1.7.1. Analytical data for 13

$[\alpha]_D$ −4 (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.37 (m, 5H, Ar), 5.97 (d, 1H, *J* = 9.3 Hz, NH), 5.18 (d, 1H, *J* = 3.4 Hz, H-4′), 5.10 (dd, 1H, *J* = 9.0, 10.0 Hz, H-3), 4.67–4.77 (m, 2H, H-2′, PhC*H*H), 4.45 (d, 1H, *J* = 12.1 Hz, PhCH*H*), 4.37 (d, 1H, *J* = 8.2 Hz, H-1), 4.35 (d, 1H, *J* = 8.0 Hz, H-1′), 3.95–4.33 (m, 5H, H-2, H-6a′, H-6b′, $ClCH_2CO$), 3.90 (t, 1H, *J* = 9.2 Hz, H-4), 3.70–3.78 (m, 2H, H-6a, H-6b), 3.53–3.62 (m, 1H, H-3′, H-5′), 3.41–3.51 (m, 4H, H-5, OCH_3), 2.80–2.88 (br, 1H, OH), 2.11, 2.06, 2.01, 1.92 (4s, 12H, $CH_3CO \times 4$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.82, 170.77, 170.44, 167.51 (C═O), 137.76, 128.50, 127.92, 127.86 (Ar), 101.85 (C-1), 100.02 (C-1′), 74.66, 74.55, 74.41 (C-3, C-4, C-5), 73.52 (PhC*H*$_2$), 72.73 (C-2′), 70.99, 70.83 (C-3′, C-5′), 69.50 (C-4′), 67.31 (C-6), 61.66 (C-6′), 56.59 (OCH_3), 53.29 (C-2), 40.89 ($ClCH_2CO$), 23.23, 20.93, 20.70, 20.69 ($CH_3CO \times 4$). HRESIMS calcd for $C_{30}H_{41}ClNO_{15}$ $[M+H]^+$: 690.2165, found: 690.2175.

1.7.2. Analytical data for 14

$[\alpha]_D$ +12 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.40 (m, 5H, Ar), 5.97 (d, 1H, *J* = 8.3 Hz, NH), 5.23 (d, 1H, *J* = 3.4 Hz, H-4′), 4.92 (dd, 1H, *J* = 8.1, 9.9 Hz, H-2′), 4.67 (d, 1H, *J* = 12.1 Hz, PhC*H*H), 4.56 (d, 1H, *J* = 8.2 Hz, H-1), 4.50 (d, 1H, *J* = 12.1 Hz, PhCH*H*), 4.43 (d, 1H, *J* = 8.1 Hz, H-1′), 3.97–4.17 (m, 2H, H-6a′, H-6b′), 3.78-3.91 (m, 2H, H-4, H-5′), 3.63–3.77 (m, 3H, H-6a, H-6b, H-3′), 3.55–3.63 (m, 1H, H-3), 3.46 (s, 3H, OCH_3), 3.41–3.52 (m, 2H, H-2, H-5), 2.13, 2.03, 1.97 (3s, 12H, $CH_3CO \times 4$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.75, 170.59 (C═O), 138.10, 128.40, 127.72, 127.63 (Ar), 101.12 (C-1, C-1′), 81.27 (C-3), 73.98 (C-5), 73.53 (PhC*H*$_2$), 72.31 (C-2′), 71.81 (C-4), 71.42 (C-5′), 70.93 (C-3′), 69.49 (C-4′), 6.13 (C-6), 61.88 (C-6′), 56.56, 56.41 (OCH_3, C-2), 23.48, 20.87, 20.68, 20.52 ($CH_3CO \times 4$). HRESIMS calcd for $C_{28}H_{40}NO_{14}$ $[M+H]^+$: 614.2449, found: 614.2424.

1.8. Methyl 2-acetamido-3-*O*-[2,4,6-tri-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-6-*O*-benzyl-3-*O*-chloroacetyl-2-deoxy-β-D-glucopyranoside (15)

Freshly distilled TMSOTf (26 μL, 0.14 mmol, 2.0 equiv) was added to a solution of disaccharide acceptor **13** (50 mg, 0.072 mmol) and donor **4** (216 mg, 0.363 mmol, 5.0 equiv) stirred at 0 °C under N_2 in anhyd CH_2Cl_2 (3.5 mL). The reaction mixture was stirred at 0 °C for 1 h, the reaction quenched by the addition of Et_3N (20 μL, 0.14 mmol) and the solvent evaporated. Flash chromatography ($CHCl_3$–MeOH, 20:1) of the residue followed by further chromatography (EtOAc–hexanes, 9:1) of the fractions containing the desired product gave pure trisaccharide **15** (60 mg, 74%) as a white amorphous solid. $[\alpha]_D$ +11 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.40 (m, 5H, Ar), 6.90 (d, 1H, *J* = 8.2 Hz, NH), 5.88 (d, 1H, *J* = 9.4 Hz, NH), 5.39 (dd, 1H, *J* = 9.2, 10.7 Hz, H-3″), 5.29 (d, 1H, *J* = 3.3 Hz, H-4′), 5.51–5.11 (m, 2H, H-3, H-4″), 4.90 (dd, 1H, *J* = 8.1, 10.1 Hz, H-2′), 4.88 (d, 1H, *J* = 8.0 Hz, H-1″), 4.72 (d, 1H, *J* = 12.1 Hz, PhC*H*H), 4.46 (d, 1H, *J* = 12.1 Hz, PhCH*H*), 4.34 (d, 1H, *J* = 7.7 Hz, H-1), 4.29 (d, 1H, *J* = 8.0 Hz, H-1′), 4.28–4.34 (m, 1H, H-6a″), 3.94–4.16 (m, 6H, H-2, H-6a′, H-6b′, H-6b″, $ClCH_2CO$), 3.89 (t, 1H, *J* = 8.6 Hz, H-4), 3.65-3.78 (m, 4H, H-6a, H-6b, H-3′, H-5″), 3.54–3.65 (m, 2H, H-5′, H-2″), 3.40–3.53 (m, 4H, H-5, OCH_3), 2.08, 2.07, 2.00, 1.97, 1.96, 1.94 (6s, 21H, $CH_3CO \times 7$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.72, 170.65, 170.53, 170.45, 169.95, 169.37, 168.99, 167.49, 161.79 (C═O), 137.89, 128.58, 128.02, 127.97 (Ar), 101.88 (C-1), 100.24 (C-1′), 99.27 (C-1″), 92.14 (CCl_3), 75.84 (C-3′), 74.61, 74.46, 74.09 (C-3, C-4, C-5), 73.66 (PhC*H*$_2$), 72.04 (C-5″), 71.15 (C-5′), 70.76 (C-2′), 70.48 (C-3″), 68.76 (C-4′), 68.37 (C-4″), 67.48 (C-6), 61.96 (C-6′), 61.14 (C-6″), 56.80, 56.67 (C-2″, OCH_3), 52.85 (C-2), 40.83 ($ClCH_2CO$), 23.31, 21.09, 20.77, 20.73, 20.62, 20.57 ($CH_3CO \times 7$). HRESIMS calcd for $C_{44}H_{57}Cl_4N_2O_{23}$ $[M+H]^+$:1121.2106, found: 1121.2057.

1.9. Methyl 2-acetamido-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-6-*O*benzyl-2-deoxy-β-D-glucopyranoside (16)

Thiourea (120 mg, 1.58 mmol, 9 equiv) was added to a solution of trisaccharide **15** (200 mg, 0.178 mmol) in a mixture of pyridine and EtOH (2:1, 10 mL). The reaction mixture was warmed up to 65 °C and stirred for 12 h. The solvent was evaporated and the residue was co-concentrated with toluene (10 mL × 2), dissolved in CH_2Cl_2 (20 mL) and washed successively with 8% HCl (10 mL), satd aq $NaHCO_3$ (10 mL) and brine (10 mL). The aqueous phases were re-extracted with CH_2Cl_2 and the combined organic phases were dried and concentrated. Flash chromatography of the residue ($CHCl_3$–MeOH, 20:1→9:1) gave pure trisaccharide acceptor **16** (142 mg, 76%) as a white amorphous solid. $[\alpha]_D$ +21 (*c* 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.40 (m, 5H, Ar), 7.03 (d, 1H, *J* = 8.3 Hz, NH), 5.65 (d, 1H, *J* = 8.2 Hz, NH), 5.30–5.38 (m, 2H, H-4′, H-3″), 5.00–5.14 (m, 2H, H-2′, H-4″), 4.89 (d, 1H, *J* = 8.0 Hz,

H-1″), 4.67 (d, 1H, J = 12.2 Hz, PhC*H*H), 4.44–4.54 (m, 2H, H-1, PhCH*H*), 4.38 (d, 1H, J = 8.1 Hz, H-1′), 4.27–4.34 (m, 1H, H-6a″), 4.05–4.20 (m, 3H, H-6a′, H-6b″, OH), 3.94–4.04 (m, 1H, H-6b′), 3.77–3.88 (m, 3H, H-3, H-3′, H-5′), 3.51–3.74 (m, 6H, H-2, H-4, H-6a, H-6b, H-2″, H-5″), 3.40–3.50 (m, 4H, H-5, OCH_3), 2.10, 2.08, 2.03, 2.00, 1.98, 1.97 (6s, 21H, $CH_3CO \times 7$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.71, 170.58, 169.96, 169.30, 169.13, 161.81 (C=O), 138.25, 128.40, 127.67 (Ar), 101.30 (C-1, C-1′), 99.37 (C-1″), 92.12 (CCl_3), 81.15 (C-4), 75.69 (C-3′), 74.12 (C-5), 73.58 ($PhCH_2$), 72.10, 71.58 (C-3, C-5′, C-5″), 70.73 (C-3″), 70.32 (C-2′), 68.56 (C-4′), 68.19 (C-4″), 68.09 (C-6), 62.18 (C-6′), 61.06 (C-6″), 56.62, 56.54 (C-2″, OCH_3), 55.97 (C-2), 23.58, 21.08, 20.77, 20.66, 20.58, 20.54 ($CH_3CO \times 7$). HRESIMS calcd for $C_{42}H_{56}Cl_3N_2O_{22}$ $[M+H]^+$: 1045.2390, found: 1045.2360.

1.10. Methyl 2-acetamido-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-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)

Trisaccharide acceptor **16** (34 mg, 0.032 mmol) and fucosyl donor **17** (47 mg, 0.097 mmol, 3.0 equiv) were dissolved in a mixture of CH_2Cl_2 and DMF (1:1, 2 mL) containing activated powdered MS 4 Å (100 mg). The mixture was stirred at rt for 30 min, Cu(II) Br_2 (22 mg, 0.098 mmol, 3.0 equiv) followed by n-Bu_4NBr (33 mg, 0.099 mmol, 3.1 equiv) were added and the reaction was allowed to proceed under stirring for 20 h at rt. The solids were filtered off on Celite® and washed with CH_2Cl_2 (5 mL). The combined filtrate and washing were diluted with CH_2Cl_2 (50 mL) and washed successively with brine (15 mL) and satd aq $NaHCO_3$(15 mL × 6). The aqueous phases were re-extracted with CH_2Cl_2 (50 mL) and the combined organic phases were dried and concentrated. Flash chromatography of the residue (EtOAc–hexanes, 1:1 to $CHCl_3$–MeOH, 20:1) gave pure tetrasaccharide **18** (34 mg, 73%) as a colorless oil. $[\alpha]_D$ −18 (c 0.9, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 7.24–7.44 (m, 20H, Ar), 6.78 (d, 1H, J = 8.3 Hz, NH), 6.68 (d, 1H, J = 8.2 Hz, NH), 5.36–5.41 (m, 2H, H-4″, H-3‴), 5.17 (d, 1H, J = 3.7 Hz, H-1′), 5.12 (t, 1H, J = 9.6 Hz, H-4‴), 5.02 (dd, 1H, J = 7.9, 9.9 Hz, H-2″), 4.97 (d, 1H, J = 11.8 Hz, PhC*H*$_2$), 4.90 (d, 1H, J = 8.1 Hz, H-1‴), 4.70–4.83 (m, 4H, PhC*H*$_2$), 4.65–4.70 (m, 2H, H-1, PhC*H*$_2$), 4.58 (d, 1H, J = 12.0 Hz, PhC*H*$_2$), 4.40–4.45 (m, 2H, H-1″, PhC*H*$_2$), 4.38 (dd, 1H, J = 2.4, 12.3 Hz, H-6a‴), 4.16 (dd, 1H, J = 4.0, 12.3 Hz, H-6b‴), 4.09–4.14 (m, 3H, H-2′, H-5′, H-6a″), 4.06 (t, 1H, J = 5.6 Hz, H-4), 4.01 (dd, 1H, J = 6.4, 11.4 Hz, H-6b″), 3.89–3.93 (m, 2H, H-3, H-6a), 3.86 (dd, 1H, J = 2.6, 10.1 Hz, H-3′), 3.75–3.83 (m, 3H, H-2, H-6b, H-3″), 3.63–3.73 (m, 4H, H-5, H-5″, H-2‴, H-5‴), 3.60–3.62 (m, 1H, H-4′), 3.33 (s, 3H, OCH_3), 2.13, 2.06, 2.05, 2.03, 2.02, 1.87 (6s, 21H, $CH_3CO \times 7$), 1.13 (d, 3H, J = 6.5 Hz, H-6′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.63, 170.57, 170.33, 170.23, 169.62, 169.42, 169.28, 161.74 (C=O), 138.86, 138.82, 138.49, 138.07, 128.43, 128.34, 128.30, 128.27, 128.16, 127.77, 127.65, 127.58, 127.56, 127.44, 127.33, 127.25, 125.48 (Ar), 100.76 (C-1), 99.43, 99.39 (C-1″, C-1‴), 96.01 (C-1′), 92.02 (CCl_3), 79.45 (C-3′), 77.14 (C-4′), 76.25 (C-2′), 75.05 (C-3″), 74.46 ($PhCH_2$), 73.94 (C-5), 73.49 (C-3), 73.34, 72.84, 72.71 ($PhCH_2 \times 3$), 72.05 (C-4, C-5‴), 71.05 (C-5″), 70.77 (C-2″), 70.54 (C-3‴), 69.06 (C-6), 68.53 (C-4″), 68.20 (C-4‴), 66.67 (C-5′), 61.22 (C-6″), 61.14 (C-6‴), 56.59 (C-2‴), 56.39 (OCH_3), 23.11, 21.12, 20.73, 20.69, 20.61, 20.62, 20.49 ($CH_3CO \times 7$), 16.57 (C-6′). HRESIMS calcd for $C_{69}H_{84}Cl_3N_2O_{26}$ $[M+H]^+$: 1461.4378, found: 1461.4384.

1.11. Methyl 2-acetamido-2-deoxy-3-*O*-α-L-fucopyranosyl-4-*O*-[3-*O* -(2-deoxy-2-acetamido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (3)

Tetrasaccharide **18** (42 mg, 0.027 mmol) was deprotected as described above Section 1.5 for the preparation of trisaccharide **2**. Purification by gel permeation chromatography (2 × Biogel P2, eluted with water) gave pure tetrasaccharide **3** (16 mg, 72%) which was obtained as an amorphous white powder upon freeze-drying. $[\alpha]_D$ −18 (c 0.5, MeOH). ^1H NMR (600 MHz, D_2O): δ 5.07 (d, 1H, J = 4.0 Hz, H-1′), 4.77–4.83 (m, 1H, H-5′), 4.66 (d, 1H, J = 8.4 Hz, H-1‴), 4.45 (d, 1H, J = 8.2 Hz, H-1), 4.41 (d, 1H, J = 7.9 Hz, H-1″), 4.07 (d, 1H, J = 3.0 Hz, H-4″), 3.96–4.01 (m, 1H, H-6a), 3.79–3.92 (m, 6H, H-2, H-3, H-6b, H-3′, H-3‴, H-6a‴), 3.62–3.78 (m, 7H, H-2′, H-4′, H-3″, H-6a″, H-6b″, H-2‴, H-6b‴), 3.39-3.61 (m, 9H, H-4, H-5, H-2″, H-5″, H-4‴, H-5‴, OCH_3), 2.01, 2.00 (2s, 6H, $CH_3CO \times 2$), 1.13 (d, 3H, J = 6.6 Hz, H-6′). ^{13}C NMR (150 MHz, D_2O): δ 174.89, 174.42 (C=O), 102.77 (C-1‴), 101.79, 101.72 (C-1, C-1″), 98.74 (C-1′), 81.50 (C-3″), 75.60, 75.33, 74.98, 74.43, 73.49 (C-3, C-4, C-5, C-4‴, C-5‴), 73.14 (C-3‴), 71.83 (C-4′), 70.51 (C-5″), 69.68 (C-2″), 69.18 (C-3′), 68.24 (C-4″), 67.61 (C-2′), 66.69 (C-5′), 61.42 (C-6″), 60.45 (C-6‴), 59.74 (C-6), 57.11 (OCH_3), 55.61 (C-2, C-2‴), 22.19 and 22.12 ($CH_3CO \times 2$), 15.24 (C-6′). HRESIMS calcd for $C_{29}H_{50}N_2NaO_{20}$ $[M+Na]^+$: 769.2855, found:769.2845.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.03.038.

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