Efficient Synthesis of Two Sialylated Tetrasaccharides Found in Goat Milk

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Abstract: Two regioisomeric sialylated tetrasaccharides found in goat milk have been obtained in excellent yield by a concise synthesis using a common disaccharide intermediate. Regioselective glycosylations and a minimal number of protecting-group manipulations are the key features of this synthetic strategy.

Key words: oligosaccharides, glycosylations, sialic acid, goat milk, tetrasaccharides

Mammalian milk is an excellent source of complex oligosaccharide antigens and bifidus factors for breastfed newborns. To date, more than 130 different oligosaccharide structures have been isolated and characterized from different mammalian milks.[1] For a long time, the biological significance of a large number of complex oligosaccharides found in milk were not properly studied because it was thought that they are not directly related to nutrition and were treated as byproducts formed during the synthesis of milk. Recently, it has been demonstrated that milk oligosaccharides have strong potential to inhibit pathogenic microbial adhesion to the host cell surface, which is the initial step of bacterial infections.[2] Oligosaccharides found in human milk can inhibit the adhesion of *Streptococcus pneumoniae* and *Haemophilus influenzae* to human pharyngeal or buccal epithelial cells and *Escherichia coli* adhesion to uroepithelial cells.[2,3] A number of glycoproteins or glycolipids that function as tumor-associated antigens are found in human milk.[4] In addition, milk oligosaccharides may function as prebiotics, promoting the growth of benign microorganisms, such as *Bifidobacterium bifidus*, within the lower gastrointestinal tract, and inhibiting the proliferation of pathogenic organisms.[5] In spite of several investigations, the physiological role of the milk-derived oligosaccharides is not yet completely established. Different types of milk have been analyzed for their oligosaccharide structures that have immunological properties.[6] Recently, it has been reported that goat milk contains two regioisomeric sialylated tetrasaccharides.[7] Although these oligosaccharides can be isolated from the natural sources, it is essential to synthesize them chemically to provide larger quantities for their proper bioevaluations. We report herein the concise chemical synthesis of two sialylated tetrasaccharides found in goat milk (Figure 1).

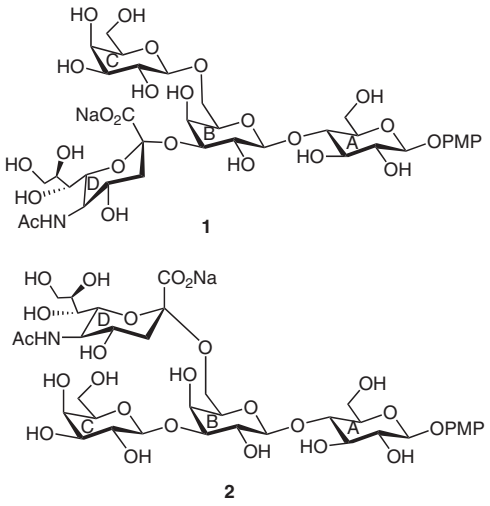

Figure 1 Structures of two synthesized sialylated tetrasaccharides as their 4-methoxyphenyl glycosides found in goat milk

Two regioisomeric sialylated tetrasaccharides **1** and **2** (Figure 1) were synthesized from a common disaccharide derivative **8** in a very concise manner (Scheme 1 and Scheme 2). For this purpose, a number of suitably protected monosaccharide synthons **3**,[8] **4**,[9] **5**,[10] **6**,[11] and **7**[12] (Figure 2) were prepared from naturally available monosaccharides according to methodologies reported in the literature.

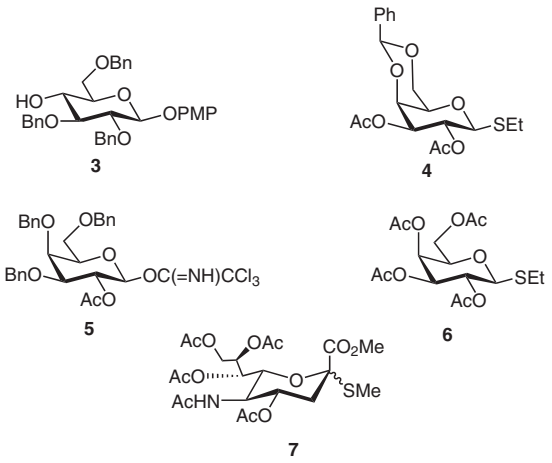

Figure 2 Suitably protected monosaccharide intermediates used for the preparation of compounds **1** and **2**

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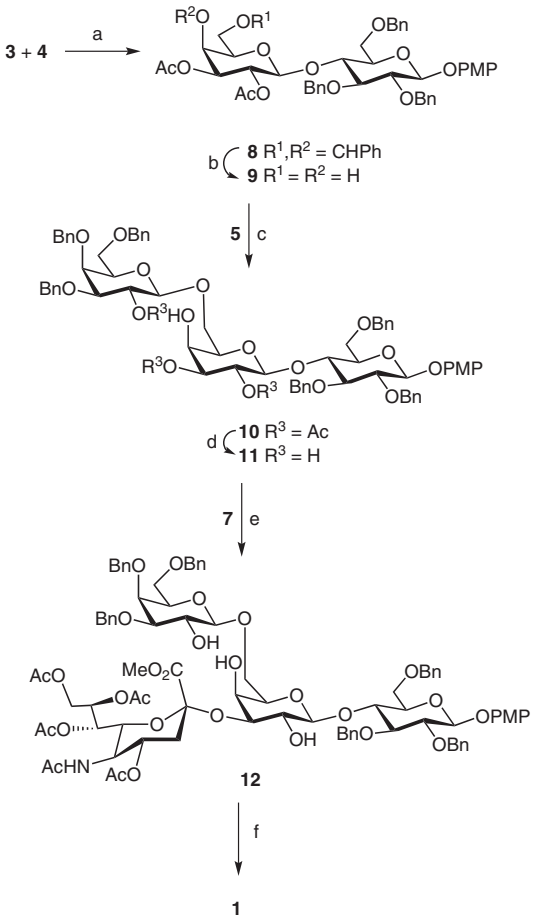
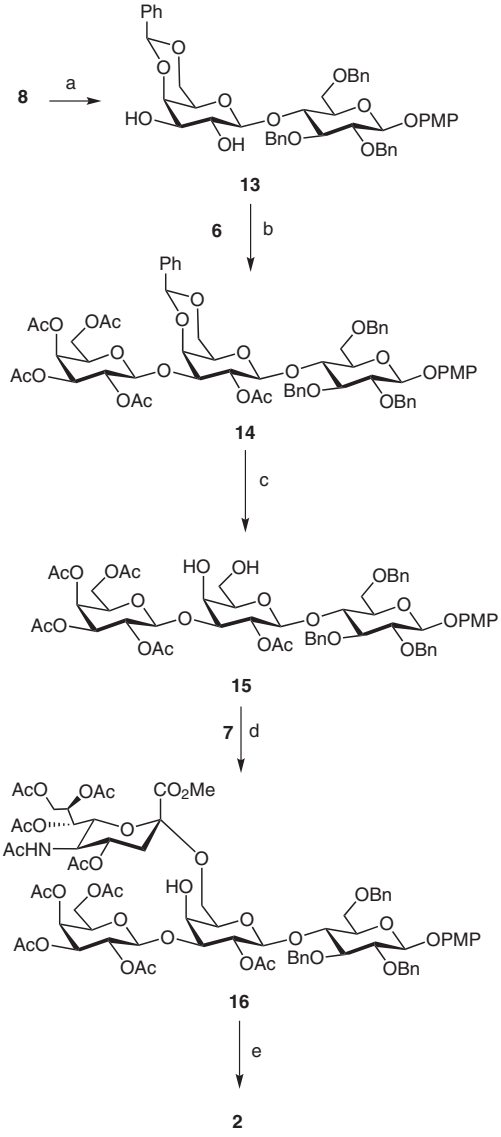


Scheme 1 *Reagents and conditions*: (a) NIS, TMSOTf, 4 Å MS, CH_2Cl_2, –30 °C, 45 min, 92%; (b) $HClO_4{\cdot}SiO_2$, MeCN–H_2O (9:1), r.t., 30 min, 95%; (c) **5**, TMSOTf, 4 Å MS, CH_2Cl_2, –40 °C, 1 h, 82%; (d) 0.1 M NaOMe, MeOH, r.t., 4 h, 100%; (e) **7**, NIS, TfOH, 3 Å MS, MeCN–CH_2Cl_2 (5:1), –30 °C, 20 h, 50%; (f) 1. H_2, 20% $Pd(OH)_2$/C, MeOH, r.t., 24 h; 2. 0.1 M NaOMe, MeOH, r.t., 12 h, then H_2O (5 drops), r.t., 6 h, 70%.



Scheme 2 *Reagents and conditions*: (a) 0.1 M NaOMe, MeOH, r.t., 2 h, 100%; (b) 1. **6**, NIS, TMSOTf, CH_2Cl_2, 4 Å MS, –50 °C, 45 min; 2. Ac_2O, py, r.t., 2 h, 74% (2 steps); (c) $HClO_4{\cdot}SiO_2$, MeCN–H_2O (9:1), r.t., 30 min, 92%; (d) **7**, NIS, TfOH, 3 Å MS, MeCN–CH_2Cl_2 (5:1), –30 °C, 20 h, 67%; (e) 1. H_2, 20% $Pd(OH)_2$/C, MeOH, r.t., 24 h; 2. 0.1 M NaOMe, MeOH, r.t., 12 h, then H_2O (5 drops), r.t., 6 h, 72%.

Condensation of monosaccharide acceptor **3** with thioglycoside donor **4** in the presence of a combination of *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate[13] furnished the disaccharide derivative **8** in 92% yield; this was the common starting point for the synthesis of both **1** (Scheme 1) and **2** (Scheme 2). The presence of signals at δ = 5.46 (s, PhC*H*), 4.86 (d, *J* = 7.4 Hz, H-1_A), and 4.70 (d, *J* = 8.1 Hz, H-1_B) in the ^{1}H NMR and at δ = 102.8 (C-1_A), 101.0 (Ph*C*H), and 100.8 (C-1_B) in the ^{13}C NMR spectra confirmed its formation.

Removal of the benzylidene acetal from compound **8** by use of perchloric acid supported on silica[14] at room temperature resulted in the formation of disaccharide diol derivative **9** in 95% yield (Scheme 1). Regioselective glycosylation of compound **9** with D-galactose-derived trichloroacetimidate derivative **5** in the presence of trimethylsilyl trifluoromethanesulfonate[15] afforded expected trisaccharide derivative **10** in 82% yield. Presence of ^{1}H NMR signals at δ = 4.86 (d, *J* = 8.0 Hz, H-1_A), 4.61 (d, *J* = 3.9 Hz, H-1_B), and 4.30 (d, *J* = 8.0 Hz, H-1_C) and ^{13}C NMR signals at δ = 102.9 (C-1_A), 101.3 (C-1_C), and 100.2 (C-1_B) supported its formation. Compound **10** was deacetylated with sodium methoxide, to furnish trisaccharide triol acceptor **11** in quantitative yield, to be used for glycosylation with the sialic acid derivative. Stereo- and regioselective glycosylation[16] of sialic acid derived thioglycoside donor **7** with compound **11** in the presence of the *N*-iodosuccinimide–trifluoromethanesulfonic acid combination[13] in a mixed solvent (MeCN–CH_2Cl_2, 5:1) furnished sialylated tetrasaccharide derivative **12** in 50% yield. Hydrogenolysis of tetrasaccharide derivative **12** over Pearlman's catalyst[17] followed by conventional saponification furnished tetrasaccharide **1** as its sodium salt and 4-methoxyphenyl glycoside in 80% overall yield; it was purified over Sephadex LH-20 gel (MeOH–H_2O, 4:1)

(Scheme 1). The presence of signals at δ = 4.79 (d, *J* = 7.3 Hz, H-1_A), 4.42 (d, *J* = 7.9 Hz, H-1_B), 4.28 (d, *J* = 7.0 Hz, H-1_C), 2.74 (dd, *J* = 12.1, 3.7 Hz, 1 H, H-$3e_D$), and 1.87 (t, *J* = 11.8 Hz, 1 H, H-$3a_D$) in the ^{1}H NMR and at δ = 104.3 (C-2_D), 104.2 (2 C, C-1_C, C-1_B), and 101.9 (C-1_A) in the ^{13}C NMR spectra confirmed the formation of compound **1**.

For the synthesis of compound **2** (Scheme 2), disaccharide derivative **8** was deacetylated with sodium methoxide to afford disaccharide diol acceptor **13** in quantitative yield. Compound **13** was regioselectively glycosylated with thioglycoside **6** under *N*-iodosuccinimide–trimethylsilyl trifluoromethanesulfonate catalysis; conventional acetylation followed, to furnish trisaccharide derivative **14** in 74% overall yield. Presence of signals at δ = 4.80 (d, *J* = 7.5 Hz, H-1_A), 4.62 (d, *J* = 7.9 Hz, H-1_B), and 4.58 (d, *J* = 8.0 Hz, H-1_C) in the ^{1}H NMR and at δ = 102.7 (C-1_A), 101.6 (C-1_B), and 100.9 (C-1_C) in the ^{13}C NMR spectra confirmed its formation. Removal of the benzylidene acetal from compound **14** was achieved by use of perchloric acid supported on silica at room temperature; this gave trisaccharide diol derivative **15** in 92% yield. Stereo- and regioselective glycosylation of trisaccharide acceptor **15** with sialic acid thioglycoside derivative **7** in the presence of the *N*-iodosuccinimide–trifluoromethanesulfonic acid combination in a mixed solvent (MeCN–CH_2Cl_2, 5:1) furnished 6-*O*-α-sialylated tetrasaccharide derivative **16** in 67% yield. Hydrogenolysis of tetrasaccharide derivative **16** over Pearlman's catalyst followed by conventional saponification furnished tetrasaccharide **2** as its sodium salt and 4-methoxyphenyl glycoside in 78% overall yield; it was purified over Sephadex LH-20 gel (MeOH–H_2O, 4:1) (Scheme 2). The presence of signals at δ = 4.79 (d, *J* = 7.3 Hz, H-1_A), 4.47 (d, *J* = 7.3 Hz, H-1_B), 4.41 (d, *J* = 7.5 Hz, H-1_C), 2.73 (dd, *J* = 12.3, 4.2 Hz, 1 H, H-$3e_D$), and 1.75 (t, *J* = 11.9 Hz, 1 H, H-$3a_D$) in the ^{1}H NMR and at δ = 105.3 (C-1_B), 103.7 (C-1_C), 101.9 (C-1_A), 99.5 (C-2_D) in the ^{13}C NMR spectra supported the formation of compound **2**.

In summary, efficient syntheses of two regioisomers of sialylated tetrasaccharides **1** and **2** found in goat milk were achieved in excellent yield. A common disaccharide derivative has been used as the starting point for the preparation of both tetrasaccharides in a minimal number of steps. Both tetrasaccharides contain the 4-methoxyphenyl group as a temporary anomeric protecting group, which can be removed by standard procedures for the preparation of glycoconjugates.

All the reactions were monitored by TLC on plates coated with silica gel. The TLC spots were visualized by warming the TLC plates sprayed with 2% $Ce(SO_4)_2$ in 2 N H_2SO_4 on a hot plate. Silica gel (230–400 mesh) was used for column chromatography. ^{1}H and ^{13}C NMR, 2D COSY, and HSQC spectra were recorded on a Bruker Avance DPX 300 MHz spectrometer; $CDCl_3$ and D_2O were used as solvents and TMS as internal reference, unless stated otherwise. ESI-MS was carried out on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. Elementary analysis was carried out on a Carlo ERBA-1108 analyzer. Optical rotations were measured at 25 °C on a Rudolf Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity were used in many reactions.

4-Methoxyphenyl (2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8)

To a soln of **3** (5 g, 9 mmol) and thioglycoside donor **4** (4.3 g, 10.8 mmol) in CH_2Cl_2 (50 mL) was added 4 Å MS (5 g), and the mixture was allowed to stir at r.t. under argon for 30 min. The mixture was cooled to –30 °C and NIS (3.2 g, 14 mmol) and TMSOTf (30 μL) were added. After stirring of the mixture at the same temperature for 45 min, it was filtered through a Celite bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (100 mL), sat. $NaHCO_3$ (100 mL), and H_2O (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was purified by chromatography (silica gel, hexane–EtOAc, 5:1); this gave pure **8**.

Yield: 7.4 g (92%); R_f = 0.3 (hexane–EtOAc, 3:1); white solid; mp 139–41 °C; $[\alpha]_D^{25}$ –4.2 (*c* 1.5, $CHCl_3$).

IR (KBr): 2915, 2871, 1740, 1506, 1454, 1374, 1230, 1061, 736, 698 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.49–7.19 (m, 20 H, Ar-H), 7.02 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.81 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.46 (s, 1 H, PhC*H*), 5.33 (dd, *J* = 10.3, 8.1 Hz, 1 H, H-2_B), 5.12 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 5.02 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.86 (d, *J* = 7.4 Hz, 1 H, H-1_A), 4.84–4.73 (m, 4 H, H-3_B, 3 PhCH_2), 4.70 (d, *J* = 8.1 Hz, 1 H, H-1_B), 4.53 (d, *J* = 11.9 Hz, 1 H, PhCH_2), 4.25 (d, *J* = 3.4 Hz, 1 H, H-4_B), 4.18 (d, *J* = 12.4 Hz, 1 H, H-$6a_B$), 4.00 (t, *J* = 9.0 Hz, 1 H, H-3_A), 3.87–3.75 (m, 3 H, H-$6b_B$, H-$6ab_A$), 3.79 (s, 3 H, OCH_3), 3.72–3.66 (m, 3 H, H-2_A, H-5_A, H-5_B), 3.52–3.46 (m, 1 H, H-4_A), 2.09, 2.00 (2 s, 6 H, 2 COCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.6, 168.9 (2 *C*OCH$_3$), 155.3–114.5 (Ar-C), 102.8 (C-1_A), 101.0 (Ph*C*H), 100.8 (C-1_B), 82.9 (C-2_A), 81.7 (C-5_A), 77.4 (C-3_A), 75.6 (Ph*C*H$_2$), 75.0 (3 C, PH*C*H$_2$, C-4_A, C-5_B), 73.6 (Ph*C*H$_2$), 73.3 (C-4_B), 72.2 (C-3_B), 69.4 (C-2_B), 68.6 (C-6_B), 68.0 (C-6_A), 55.6 (O*C*H$_3$), 20.8 (2 C, *C*OCH_3).

ESI-MS: *m*/*z* = 913.2 [M + Na]$^+$.

Anal. Calcd for $C_{51}H_{54}O_{14}$ (890.35): C, 68.75; H, 6.11. Found: C, 68.58; H, 6.30.

4-Methoxyphenyl (2,3-Di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9)

$HClO_4$·SiO_2 (500 mg) was added to a soln of **8** (2.5 g, 2.8 mmol) in MeCN–H_2O (9:1; 50 mL), and the mixture was allowed to stir at r.t. for 30 min. It was then filtered through a Celite bed and evaporated to dryness. The crude product was purified through a short pad of silica gel (hexane–EtOAc, 1:1); this gave pure **9**.

Yield: 2.2 g (95%); R_f = 0.2 (hexane–EtOAc, 1:2); white solid; mp 156–58 °C; $[\alpha]_D^{25}$ –31.4 (*c* 1.5, $CHCl_3$).

IR (KBr): 3432, 2870, 1743, 1508, 1455, 1373, 1229, 1059, 751, 698 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43–7.29 (m, 15 H, Ar-H), 7.05 (d, *J* = 9.1 Hz, 2 H, Ar-H), 6.81 (d, *J* = 9.2 Hz, 2 H, Ar-H), 5.26 (dd, *J* = 10.1, 8.0 Hz, 1 H, H-2_B), 5.02 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.98 (d, *J* = 11.2 Hz, 1 H, PhCH_2), 4.85–4.79 (m, 3 H, H-1_A, PhCH_2), 4.75 (d, *J* = 10.4, 3.6 Hz, 1 H, H-3_B), 4.70 (d, *J* = 11.9 Hz, 1 H, PhCH_2), 4.58 (d, *J* = 8.0 Hz, 1 H, H-1_B), 4.52 (d, *J* = 11.9 Hz, 1 H, PhCH_2), 4.03–3.97 (m, 2 H, H-4_B, H-3_A), 3.73 (s, 3 H, OCH_3), 3.75–3.65 (m, 2 H, H-$6ab_A$), 3.63–3.60 (m, 2 H, H-2_A, H-5_A), 3.57–3.54 (m, 2 H, H-$6ab_B$), 3.52–3.48 (m, 1 H, H-4_A), 3.25–3.22 (m, 1 H, H-5_B), 2.09, 1.99 (2 s, 6 H, 2 COCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 169.3 (2 *C*OCH$_3$), 155.3–114.5 (Ar-C), 102.7 (C-1_A), 100.3 (C-1_B), 82.5 (C-2_A), 81.3 (C-5_A),

76.4 (C-3_A), 75.3 (C-3_B), 74.9 (PhCH$_2$), 74.8 (PhCH$_2$), 73.7 (C-4_A), 73.5 (2 C, PhCH$_2$, C-5_B), 69.4 (C-2_B), 68.1 (C-4_B), 67.8 (C-6_A), 62.1 (C-6_B), 55.5 (OCH$_3$), 20.7 (2 C, COCH$_3$).

ESI-MS: m/z = 825.1 [M + Na]$^+$.

Anal. Calcd for $C_{44}H_{50}O_{14}$ (802.32): C, 65.82; H, 6.28. Found: C, 65.60; H, 6.50.

4-Methoxyphenyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→6)-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10)

A soln of **9** (2 g, 2.5 mmol) and **5** (2 g, 3.2 mmol) in anhyd CH_2Cl_2 (25 mL) was cooled to –50 °C. TMSOTf (100 μL) was added, and the mixture was stirred at –40 °C for 1 h. It was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed with sat. aq $NaHCO_3$ (100 mL) and H_2O (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by chromatography (silica gel, hexane–EtOAc, 2:1); this gave pure **10**.

Yield: 2.6 g (82%); R_f = 0.4 (hexane–EtOAc, 1:2); white solid; mp 176–78 °C; $[\alpha]_D^{25}$ –17.3 (*c* 1.5, $CHCl_3$).

IR (KBr): 3465, 2874, 1747, 1506, 1371, 1230, 1063, 747, 698 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43–7.17 (m, 30 H, Ar-H), 7.00 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.80 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.28 (t, *J* = 9.0 Hz, 1 H, H-2_C), 5.21 (t, 9.1 Hz, 1 H, H-2_B), 4.97 (d, *J* = 11.1 Hz, 2 H, PhCH_2), 4.90 (d, *J* = 12.1 Hz, 1 H, PhCH_2), 4.86 (d, *J* = 8.0 Hz, 1 H, H-1_A), 4.84–4.80 (m, 1 H, H-3_B), 4.79 (d, *J* = 11.8 Hz, 1 H, PhCH_2), 4.78 (d, *J* = 12.0 Hz, 1 H, PhCH_2), 4.71 (d, *J* = 12.0 Hz, 1 H, PhCH_2), 4.61 (d, *J* = 3.9 Hz, 1 H, H-1_B), 4.58–4.54 (d, *J* = 12.0 Hz, 3 H, PhCH_2), 4.49–4.39 (m, 3 H, PhCH_2), 4.30 (d, *J* = 8.0 Hz, 1 H, H-1_C), 4.03–3.98 (m, 2 H, H-4_B, H-3_A), 3.85 (br s, 1 H, H-4_C), 3.79 (s, 3 H, OCH_3), 3.76–3.73 (m, 2 H, H-6ab$_A$), 3.71–3.59 (m, 4 H, H-6ab$_B$, H-2_A, H-5_C), 3.55–3.39 (m, 5 H, H-6ab$_C$, H-5_A, H-3_C, H-4_A), 3.32–3.26 (m, 1 H, H-5_B), 2.09, 2.05, 1.99 (3 s, 9 H, 3 COCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.8, 169.5, 169.1 (3 *C*OCH$_3$), 155.3–114.5 (Ar-C), 102.9 (C-1_A), 101.3 (C-1_C), 100.2 (C-1_B), 82.8 (C-2_A), 81.7 (C-5_C), 80.7 (C-5_A), 76.2 (C-3_A), 75.7 (PhCH$_2$), 74.9 (C-3_C), 74.8 (PhCH$_2$), 74.4 (PhCH$_2$), 73.5 (2 C, PhCH$_2$), 73.4 (C-4_A), 73.1 (2 C, C-3_B, C-5_B), 72.6 (C-4_C), 72.1 (PhCH$_2$), 71.2 (C-2_C), 70.4 (C-2_B), 68.3 (C-6_C), 67.9 (C-6_A), 66.5 (2 C, C-6_B, C-4_B), 55.5 (OCH$_3$), 21.0, 20.8, 20.7 (3 C, 3 CO*C*H$_3$).

ESI-MS: m/z = 1299.4 [M + Na]$^+$.

Anal. Calcd for $C_{73}H_{80}O_{20}$ (1276.52): C, 68.64; H, 6.31. Found: C, 68.45; H, 6.55.

4-Methoxyphenyl (3,4,6-Tri-*O*-benzyl-β-D-galactopyranosyl)-(1→6)-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11)

A soln of **10** (2.5 g, 2 mmol) in 0.1 M NaOMe in MeOH (mL) was allowed to stir at r.t. for 4 h and was then neutralized with Amberlite-IR 120 (H$^+$) resin. The mixture was filtered and then evaporated to dryness; this gave the crude product, which was passed through a short column of silica gel (toluene–EtOAc, 1:1); this gave pure **11**.

Yield: 2.3 g (100%); R_f = 0.3 (toluene–EtOAc, 1:2); white solid; mp 168–69 °C; $[\alpha]_D^{25}$ +3.6 (*c* 1.5, $CHCl_3$).

IR (KBr): 3449, 3020, 2926, 2364, 1505, 1216, 1064, 763, 669 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37–7.17 (m, 30 H, Ar-H), 6.98 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.78 (d, *J* = 9.0 Hz, 2 H, Ar-H), 4.97 (d, *J* = 11.2 Hz, 1 H, PhCH_2), 4.92 (d, *J* = 11.0 Hz, 1 H PhCH_2), 4.85 (d, *J* = 4.86 Hz, 1 H, H-1_A), 4.82–4.77 (m, 3 H, PhCH_2), 4.71–4.63 (m, 3 H, PhCH_2), 4.54 (d, *J* = 12.1 Hz, 1 H, PhCH_2), 4.53 (d, *J* = 11.7 Hz, 1 H, PhCH_2), 4.42 (d, *J* = 12.4 Hz, 1 H, PhCH_2), 4.41 (d, *J* = 6.3 Hz, 1 H, H-1_B), 4.38 (d, *J* = 11.8 Hz, 1 H, PhCH_2), 4.07 (d, *J* = 7.7 Hz, 1 H, H-1_C), 4.06–4.02 (m, 1 H, H-3_A), 3.99–3.82 (m, 4 H, H-6ab$_A$, H-2_C, H-6a$_B$), 3.78–3.76 (m, 1 H, H-4_B), 3.75 (s, 3 H, OCH_3), 3.68–3.66 (m, 2 H, H-2_A, H-2_B), 3.62–3.58 (m, 1 H, H-6b$_B$), 3.56–3.49 (m, 3 H, H-4_C, H-3_B, H-6a$_C$), 3.46–3.44 (m, 1 H, H-6b$_C$), 3.41–3.32 (m, 4 H, H-3_C, H-5_C, H-4_A, H-5_A), 3.30–3.28 (m, 1 H, H-5_B).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.2–114.5 (Ar-C), 103.5 (C-1_C), 102.8 (C-1_A), 102.7 (C-1_B), 83.0 (C-2_A), 82.2 (C-5_A), 81.7 (C-5_C), 76.2 (C-3_A), 75.5 (PhCH$_2$), 75.0 (C-4_C), 74.7 (PhCH$_2$), 74.6 (PhCH$_2$), 73.5 (2 C, C-4_A, C-3_C), 73.4 (2 C, 2PhCH$_2$), 73.3 (C-5_B), 73.1 (C-3_B), 72.7 (PhCH$_2$), 72.1 (C-2_B), 71.2 (C-2_C), 68.4 (C-6_C), 68.3 (C-6_B), 68.1 (C-4_B), 67.8 (C-6_A), 55.4 (OCH$_3$).

ESI-MS: m/z = 1168.2 [M + NH$_4$]$^+$.

Anal. Calcd for $C_{67}H_{74}O_{17}$ (1150.49): C, 69.90; H, 6.48. Found: C, 69.70; H, 6.72.

4-Methoxyphenyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-d-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-[(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→6)]-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12)

To a soln of **11** (1 g, 0.87 mmol) and thioglycoside donor **7** (0.9 g, 1.7 mmol) in anhyd MeCN–CH_2Cl_2 (5:1; 20 mL) was added 3 Å MS (2 g), and the mixture was allowed to stir at r.t. under argon for 30 min. The mixture was cooled to –30 °C and NIS (500 mg, 2.3 mmol) and TMSOTf (15 μL) were added. After the mixture had stirred at the same temperature for 20 h, it was filtered through a Celite bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (100 mL), sat. aq $NaHCO_3$ (100 mL), and H_2O (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was purified by chromatography (silica gel, toluene–EtOAc, 1:2); this gave pure **12**.

Yield: 650 mg (50%); R_f = 0.2 (toluene–EtOAc, 1:3); white solid; mp 127–29 °C; $[\alpha]_D^{25}$ +8.4 (*c* 1.5, $CHCl_3$).

IR (KBr): 3020, 2925, 2855, 2364, 1742, 1506, 1370, 1217, 1062, 765, 669 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43–7.22 (m, 30 H, Ar-H), 7.01 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.77 (d, *J* = 9.1 Hz, 2 H, Ar-H), 5.46–5.44 (m, 1 H, H-8_D), 5.35–5.29 (m, 1 H, H-7_D), 5.04 (d, *J* = 12.1 Hz, 1 H, PhCH_2), 4.98 (d, *J* = 12.5 Hz, 1 H, PhCH_2), 4.88 (d, *J* = 5.0 Hz, 1 H, H-4_D), 4.85 (d, *J* = 8.6 Hz, 1 H, H-1_A), 4.82 (d, *J* = 10.3 Hz, 1 H, PhCH_2), 4.79 (d, *J* = 10.6 Hz, 1 H, PhCH_2), 4.72–4.62 (m, 4 H, PhCH_2), 4.60 (d, *J* = 4.4 Hz, 1 H, H-1_B), 4.54 (d, *J* = 11.4 Hz, 1 H, PhCH_2), 4.52 (d, *J* = 11.2 Hz, 1 H, PhCH_2), 4.49 (d, *J* = 11.8 Hz, 1 H, PhCH_2), 4.39 (d, *J* = 11.8 Hz., 1 H, PhCH_2), 4.28 (dd, *J* = 10.1, 3.9 Hz, 1 H, H-9a$_D$), 4.18–3.97 (m, 7 H, H-1_C, H-4_B, H-9b$_D$, H-5_D, H-6_D, H-3_A, H-3_B), 3.87–3.78 (m, 4 H, H-2_C, H-2_B, H-6ab$_A$), 3.75–3.69 (m, 3 H, H-2_A, H-4_C, H-6a$_B$), 3.73 (s, 3 H, OCH_3), 3.66–3.64 (m, 1 H, H-6b$_B$), 3.63 (s, 3 H, COOCH_3), 3.56–3.53 (m, 2 H, H-4_A, H-6a$_C$), 3.50–3.45 (m, 2 H, H-3_C, H-6b$_C$), 3.37–3.29 (m, 3 H, H-5_A, H-5_B, H-5_C), 2.71 (dd, *J* = 12.3, 4.5 Hz, 1 H, H-3e$_D$), 2.10–1.89 (5 s, 15 H, 5 COCH_3), 2.00–1.98 (m, 1 H, H-3a$_D$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8, 170.4, 170.3, 169.9 (2 C), 168.1 (5 *C*OCH$_3$, *C*OOCH$_3$), 155.0–114.4 (Ar-C), 103.4 (C-1_C), 102.5 (C-1_A), 102.3 (C-1_B), 97.5 (C-2_D), 82.9 (C-2_A), 82.1 (C-5_A), 81.6 (C-5_C), 76.8 (C-3_A), 76.4 (C-4_B), 76.3 (PhCH$_2$), 75.6 (C-3_C), 75.1 (C-3_B), 74.8 (PhCH$_2$), 74.6 (PhCH$_2$), 73.4 (PhCH$_2$), 73.2 (C-4_A), 73.1 (C-5_B), 73.0 (PhCH$_2$), 72.6 (2 C, PhCH$_2$, C-6_D), 71.2 (C-2_B), 69.9 (C-2_C), 68.7 (C-6_C), 68.5 (C-8_D), 68.3 (C-6_B), 68.2 (C-4_D), 67.5 (C-6_A), 66.9 (2 C, C-7_D, C-4_C), 62.1 (C-9_D), 55.5 (OCH$_3$), 53.0 (COOCH$_3$), 49.3 (C-5_D), 37.7 (C-3_D), 23.0 (NHCOOCH$_3$), 21.1, 20.7, 20.6, 20.5 (4 COCH$_3$).

ESI-MS: m/z = 1646.5 [M + Na]$^+$.

Anal. Calcd for $C_{87}H_{101}NO_{29}$ (1623.64): C, 64.31; H, 6.27; found: C, 64.12; H, 6.50.

4-Methoxyphenyl (4,6-*O*-Benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13)

A soln of **8** (3 g, 3.4 mmol) in 0.1 M NaOMe in MeOH (100 mL) was allowed to stir at r.t. for 2 h and then neutralized with Amberlite-IR 120 (H^+) resin. The mixture was filtered and evaporated to dryness to give the crude product, which was passed through a short column of silica gel (hexane–EtOAc, 2:1); this gave pure **13**.

Yield: 2.73 g (100%); R_f = 0.3 (hexane–EtOAc, 1:1); yellow oil; $[\alpha]_D^{25}$ –25.3 (*c* 1.5, $CHCl_3$).

IR (neat): 3416, 2919, 2851, 2362, 1734, 1505, 1459, 1218, 1059, 766, 698 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.48–7.23 (m, 20 H, Ar-H), 7.02 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.82 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.46 (s, 1 H, PhC*H*), 5.10 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 5.03 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.95 (d, *J* = 11.1 Hz, 1 H, PhCH_2), 4.88 (d, *J* = 7.3Hz, 1 H, H-1_A), 4.83 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.71 (d, *J* = 12.1 Hz, 1 H, PhCH_2), 4.59 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.56 (d, *J* = 7.5 Hz, 1 H, H-1_B), 4.11–4.01 (m, 4 H, H-3_A, H-4_B, H-$6a_A$, H-$6a_B$), 3.89–3.85 (m, 2 H, H-$6b_A$, H-$6b_B$), 3.78 (s, 3 H, OCH_3), 3.76–3.72 (m, 3 H, H-2_A, H-5_A, H-5_B), 3.69–3.56 (m, 2 H, H-2_B, H-4_A), 3.48–3.39 (m, 1 H, H-3_B).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.3–114.5 (Ar-C), 103.4 (C-1_B), 102.9 (C-1_A), 101.2 (Ph*C*H), 83.4 (C-2_A), 81.8 (C-5_A), 77.5 (C-3_A), 75.5 (Ph*C*H_2), 75.2 (C-4_B), 75.0 (Ph*C*H_2), 74.7 (C-2_B), 73.3 (2 C, Ph*C*H_2, C-4_A), 72.8 (C-3_B), 72.0 (C-5_B), 68.8 (C-6_A), 68.2 (C-6_B), 55.4 (O*C*H_3).

ESI-MS: *m*/*z* = 829.3 $[M + Na]^+$.

Anal. Calcd for $C_{47}H_{50}O_{12}$ (806.33): C, 69.96; H, 6.25. Found: C, 69.75; H, 6.50.

4-Methoxyphenyl (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (14)

To a soln of **13** (2 g, 2.5 mmol) and thioglycoside donor **6** (1.1 g, 2.7 mmol) in CH_2Cl_2 (40 mL) was added 4 Å MS (5 g), and the mixture was allowed to stir at r.t. under argon for 30 min. The mixture was cooled to –50 °C and NIS (790 mg, 3.5 mmol) and TMSOTf (10 μL) were added. After the mixture had stirred at the same temperature for 30 min, it was filtered through a Celite bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (100 mL), sat. aq $NaHCO_3$ (100 mL), and H_2O (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was acetylated with Ac_2O (5 mL) and py (5 mL) at r.t. The acetylated crude mass was purified by chromatography (silica gel, hexane–EtOAc, 3:1); this gave pure **14**.

Yield: 2.2 g (74%); R_f = 0.4 (hexane–EtOAc, 1:1); white solid; mp 130–32 °C; $[\alpha]_D^{25}$ –3.7 (*c* 1.5, $CHCl_3$).

IR (KBr): 3465, 2871, 1751, 1507, 1370, 1226, 1061, 751, 699 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.50–7.15 (m, 20 H, Ar-H), 6.98 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.77 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.50 (s, 1 H, PhC*H*), 5.35–5.34 (m, 1 H, H-4_C), 5.29–5.23 (m, 1 H, H-2_B), 5.15 (dd, *J* = 10.4, 8.0 Hz, 1 H, H-2_C), 5.06 (d, *J* = 11.1 Hz, 1 H, PhCH_2), 4.98 (d, *J* = 11.1 Hz, 1 H, PhCH_2), 4.93 (t, *J* = 5.3 Hz, 1 H, H-3_C), 4.82 (d, *J* = 11.1 Hz, 1 H, PhCH_2), 4.80 (d, *J* = 7.5 Hz, 1 H, H-1_A), 4.78–4.71 (2 d, *J* = 11.8 Hz, 2 H, PhCH_2), 4.62 (d, *J* = 7.9 Hz, 1 H, H-1_B), 4.58 (d, *J* = 8.0 Hz, 1 H, H-1_C), 4.51 (d, *J* = 11.9 Hz, 1 H, PhCH_2), 4.24–4.08 (m, 4 H, H-$6ab_C$, H-$6a_A$, H-4_B), 3.97–3.87 (m, 3 H, H-3_A, H-$6b_A$, H-5_C), 3.82–3.72 (m, 3 H, H-$6ab_B$, H-5_A), 3.75 (s, 3 H, OCH_3), 3.69–3.64 (m, 2 H, H-5_B, H-2_A), 3.60 (dd, *J* = 10.3, 3.5 Hz, 1 H, H-3_B), 3.47–3.44 (m, 1 H, H-4_A), 2.19, 2.06, 2.05, 2.04, 1.99 (5 s, 15 H, 5 COCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 170.0, 169.9, 168.9, 168.5 (5 *C*OCH$_3$), 155.3–114.5 (Ar-C), 102.7 (C-1_A), 101.6 (C-1_B), 100.9 (C-1_C), 100.6 (Ph*C*H), 82.9 (C-2_A), 81.6 (C-5_B), 77.3 (C-3_A), 77.2 (C-3_B), 75.7 (C-4_B), 75.5 (Ph*C*H_2), 75.1 (C-4_A), 74.9 (Ph*C*H_2), 73.6 (Ph*C*H_2), 70.9 (C-2_B), 70.8 (C-3_C), 70.7 (C-5_C), 68.4 (2 C, C-2_C, C-5_A), 67.9 (C-6_B), 66.8 (C-4_C), 61.1 (2 C, C-6_A, C-6_C), 55.4 (O*C*H_3), 21.0, 20.6 (2 C), 20.5 (2 C) (5 CO*C*H_3).

ESI-MS: *m*/*z* = 1201.3 $[M + Na]^+$.

Anal. Calcd for $C_{63}H_{70}O_{22}$ (1178.43): C, 64.17; H, 5.98. Found: C, 64.0; H, 6.20.

4-Methoxyphenyl (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-(2-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (15)

$HClO_4$·SiO_2 (500 mg) was added to a soln of **14** (2 g, 1.7 mmol) in MeCN–H_2O (9:1; 50 mL), and the mixture was allowed to stir at r.t. for 30 min. It was then filtered through a Celite bed and evaporated to dryness. The crude product was purified through a short pad of silica gel (toluene–EtOAc, 1:1); this gave pure **15**.

Yield: 1.7 g (92%); R_f = 0.4 (toluene–EtOAc, 1:2); colorless oil; $[\alpha]_D^{25}$ –12.3 (*c* 1.5, $CHCl_3$).

IR (neat): 3482, 2920, 2361, 1749, 1506, 1370, 1225, 1062, 755 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.25 (m, 15 H, Ar-H), 6.98 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.78 (d, *J* = 9.0 Hz, 2 H, Ar-H), 5.35–5.34 (m, 1 H, H-4_C), 5.19–5.12 (m, 2 H, H-2_B, H-2_C), 4.99–4.94 (m, 3 H, H-3_C, 2 PhCH_2), 4.81 (d, *J* = 8.1 Hz, 1 H, H-1_A), 4.78 (d, *J* = 10.6 Hz, 1 H, PhCH_2), 4.76 (d, *J* = 12.3 Hz, 1 H, PhCH_2), 4.73 (d, *J* = 12.0 Hz, 1 H, PhCH_2), 4.53 (d, *J* = 8.0 Hz, 1 H, H-1_B), 4.49 (d, *J* = 11.2 Hz, 1 H, PhCH_2), 4.46 (d, *J* = 7.8 Hz, 1 H, H-1_C), 4.19–4.05 (m, 2 H, H-$6ab_C$), 3.94–3.89 (m, 3 H, H-4_B, H-5_C, H-3_A), 3.77 (s, 3 H, OCH_3), 3.75–3.68 (m, 2 H, H-$6ab_A$), 3.67–3.58 (m, 3 H, H-5_B, H-$6a_B$, H-2_A), 3.55–3.44 (m, 3 H, H-3_B, H-$6b_B$, H-4_A), 3.32–3.28 (m, 1 H, H-5_A), 2.16, 2.06, 1.98 (3 s, 15 H, 5 COCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.2, 170.0, 169.9, 169.0, 168.6 (5 *C*OCH$_3$), 155.3–114.5 (Ar-C), 102.8 (C-1_A), 101.5 (C-1_B), 100.3 (C-1_C), 82.4 (C-2_A), 81.3 (C-5_B), 80.1 (C-3_B), 76.6 (C-3_A), 75.5 (Ph*C*H_2), 75.1 (C-4_A), 74.9 (Ph*C*H_2), 74.6 (C-5_A), 73.6 (Ph*C*H_2), 71.1 (C-2_C), 70.9 (C-3_C), 70.6 (C-4_B), 68.7 (C-5_C), 68.5 (C-2_B), 67.9 (C-6_A), 66.9 (C-4_C), 61.6 (C-6_B), 61.3 (C-6_C), 55.5 (O*C*H_3), 20.8 (2 C), 20.6, 20.5, 20.4 (5 CO*C*H_3).

ESI-MS: *m*/*z* = 1113.2 $[M + Na]^+$.

Anal. Calcd for $C_{56}H_{66}O_{22}$ (1090.40): C, 61.64; H, 6.10. Found: C, 61.46; H, 6.32.

4-Methoxyphenyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)]-(2-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16)

To a soln of **15** (1 g, 0.92 mmol) and thioglycoside donor **7** (960 mg, 1.8 mmol) in anhyd MeCN–CH_2Cl_2 (5:1; 15 mL) was added 3 Å MS (2 g), and the mixture was allowed to stir at r.t. under argon for 30 min. The mixture was cooled to –30 °C and NIS (525 mg, 2.3 mmol) and TMSOTf (10 μL) were added. After the mixture had stirred at the same temperature for 3 h, it was filtered through a Celite bed and washed with CH_2Cl_2 (100 mL). The organic layer was washed with 5% $Na_2S_2O_3$ (100 mL), sat. aq $NaHCO_3$ (100 mL), and H_2O (100 mL) in succession, dried (Na_2SO_4), and evaporated to dryness. The crude mass was purified by chromatography (silica gel, toluene–EtOAc, 1:2); this gave pure **16**.

Yield: 940 mg (67%); R_f = 0.3 (toluene–EtOAc, 1:3); white solid; mp 142–44 °C; $[\alpha]_D^{25}$ –11.4 (*c* 1.5, $CHCl_3$).

IR (KBr): 3486, 2925, 1748, 1507, 1371, 1226, 1064, 741, 699 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.24 (m, 15 H, Ar-H), 7.00 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.78 (d, *J* = 9.1 Hz, 2 H, Ar-H), 5.39–5.32 (m, 3 H, H-7_D, H-8_D, H-4_C), 5.26–5.15 (m, 3 H, H-2_C, H-2_B, H-6_D), 5.02–4.93 (m, 3 H, H-3_C, H-4_D, PhCH_2), 4.86 (d, *J* = 7.7 Hz, 1 H, H-1_A), 4.85 (d, *J* = 11.3 Hz, 1 H, PhCH_2), 4.77 (d, *J* = 11.1 Hz, 1 H, PhCH_2), 4.75 (d, *J* = 11.0 Hz, 1 H, PhCH_2), 4.69 (d, *J* = 8.2 Hz, 1 H, H-1_B), 4.65 (d, *J* = 8.0 Hz, 1 H, H-5_D), 4.57 (d, *J* = 8.0 Hz, 1 H, H-1_C), 4.53 (d, *J* = 12.7 Hz, 1 H, PhCH_2), 4.35–4.25 (m, 1 H, H-$6a_C$), 4.24–4.18 (m, 1 H, H-$6a_B$), 4.13–4.04 (m, 4 H, H-5_C, H-$9a_D$, H-$6b_C$, H-$6b_B$), 3.95–3.90 (m, 3 H, H-4_B, H-5_A, H-$9b_D$), 3.82–3.72 (m, 2 H, H-$6ab_A$), 3.76 (s, 6 H, OCH_3 and COOCH_3), 3.71–3.62 (m, 2 H, H-5_B, H-2_A), 3.58–3.51 (m, 2 H, H-3_B, H-4_A), 3.38 (t, *J* = 7.0 Hz, 1 H, H-3_A), 2.55 (dd, *J* = 12.1, 4.4 Hz, 1 H, H-$3e_D$), 2.16–1.88 (10 s, 30 H, 10 COCH_3), 1.99–1.89 (m, 1 H, H-$3a_D$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 170.7, 170.5, 170.3 (2 C), 170.2, 170.1, 170.0, 169.3, 168.9, 167.9 (9 *C*OCH$_3$, *C*OOCH$_3$, NH*C*OCH$_3$), 155.3–114.5 (Ar-C), 102.6 (C-1_A), 101.5 (C-1_C), 100.0 (C-1_B), 99.0 (C-2_D), 82.4 (C-2_A), 81.7 (C-5_B), 79.5 (C-3_B), 76.4 (C-5_A), 74.9 (C-4_A), 74.8 (2 C, 2 Ph*C*H$_2$), 73.7 (C-4_B), 73.6 (Ph*C*H$_2$), 72.9 (C-5_C), 72.3 (C-3_A), 71.4 (C-2_B), 71.3 (C-2_C), 70.8 (2 C, C-3_C, C-4_D), 69.3 (C-4_C), 69.1 (C-6_D), 68.5 (C-9_D), 67.6 (C-7_D), 66.8 (C-8_D), 62.4 (C-6_C), 62.1 (C-6_B), 60.9 (C-6_A), 55.6 (O*C*H$_3$), 52.9 (COO*C*H$_3$), 49.4 (C-5_D), 37.0 (C-3_D), 22.7 (NHCO*C*H$_3$), 21.1, 21.0, 20.9, 20.8, 20.7, 20.6 (2 C), 20.5, 20.3 (9 CO*C*H$_3$).

ESI-MS: *m*/*z* = 1581.1 $[M + NH_4]^+$.

Anal. Calcd for $C_{76}H_{93}NO_{34}$ (1563.56): C, 58.34; H, 5.99. Found: C, 58.13; H, 6.25.

4-Methoxyphenyl (Sodium 5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-[(β-D-galactopyranosyl)-(1→6)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (1)

To a soln of the tetrasaccharide derivative **12** (500 mg, 0.33 mmol) in MeOH (20 mL) was added 20% $Pd(OH)_2$/C (400 mg), and the mixture was allowed to stir at r.t. for 24 h under a positive pressure of H_2. The mixture was filtered through a Celite bed and concentrated under reduced pressure. The crude mass was dissolved in 0.1 M NaOMe in MeOH (30 mL), and the mixture was allowed to stir at r.t. for 12 h; then a few drops of distilled H_2O was added, and the mixture was allowed to stir for 6 h. The mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered, and evaporated to dryness and again passed through a short pad of Dowex 50W X8 (Na^+) resin. The crude product was purified by being passed through a column of Sephadex-LH-20 (MeOH–H_2O, 4:1); this gave tetrasaccharide **1** as its sodium salt.

Yield: 215 mg (70%); R_f = 0.2 (MeCN–MeOH–H_2O, 4:1:0.5); white powder; $[\alpha]_D^{25}$ –9.3 (*c* 1.1, H_2O).

IR (KBr): 3443, 3021, 1637, 1216, 767, 669 cm^{-1}.

^1H NMR (300 MHz, D_2O): δ = 7.01 (d, *J* = 9.0 Hz, 2 H, Ar-H), 6.80 (d, *J* = 9.0 Hz, 2 H, Ar-H), 4.79 (d, *J* = 7.3 Hz, 1 H, H-1_A), 4.42 (d, *J* = 7.9 Hz, 1 H, H-1_B), 4.28 (d, *J* = 7.0 Hz, 1 H, H-1_C), 4.06–3.96 (m, 2 H, H-8_D, H-7_D), 3.92–3.75 (m, 9 H, H-$_{4D}$, H-$9a_D$, H-6_D, H-5_D, H-3_A, H-$6ab_B$, H-$6ab_A$), 3.72–3.65 (m, 3 H, H-4_B, H-$6ab_C$), 3.71 (s, 3 H, OCH_3), 3.65–3.55 (m, 5 H, H-2_B, H-$9b_D$, H-3_B, H-3_C, H-4_C), 3.52–3.43 (m, 6 H, H-2_A, H-2_C, H-5_B, H-5_A, H-5_C, H-4_A), 2.74 (dd, *J* = 12.1, 3.7 Hz, 1 H, H-$3e_D$), 1.97 (s, 3 H, NHCOCH_3), 1.87 (t, *J* = 11.8 Hz, 1 H, H-$3a_D$).

^{13}C NMR (75 MHz, D_2O): δ = 174.0 (*C*OONa), 155.6–114.5 (Ar-C), 104.3 (C-2_D), 104.2 (2 C, C-1_C, C-1_B), 101.9 (C-1_A), 81.2 (C-5_A), 76.5 (C-7_D), 75.5 (2 C, C-2_A, C-2_C), 75.2 (C-4_A), 74.3 (2 C, C-4_C, C-5_B), 73.6 (C-5_C), 73.4 (C-4_D), 71.6 (2 C, C-3_A, C-3_C), 69.5 (C-8_D), 69.3 (C-6_D), 69.0 (C-2_B), 68.8 (C-6_A), 68.4 (C-3_B), 67.7 (C-4_B), 63.7 (C-9_D), 61.5 (C-6_B), 61.0 (C-6_C), 55.1 (O*C*H$_3$), 52.7 (C-5_D), 39.9 (C-3_D), 22.6 (NHCO*C*H$_3$).

ESI-MS: *m*/*z* = 924.1 $[M + 1]^+$.

Anal. Calcd for $C_{36}H_{54}NNaO_{25}$ (923.28): C, 46.81; H, 5.89. Found: C, 46.60; H, 6.15.

4-Methoxyphenyl (Sodium 5-acetamido-3,5-dideoxy-D-glycero-α-d-galacto-2-nonulopyranosylonate)-(2→6)-[(β-D-galactopyranosyl)-(1→3)]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (2)

To a soln of the tetrasaccharide derivative **16** (600 mg, 0.38 mmol) in MeOH (20 mL) was added 20% $Pd(OH)_2$/C (400 mg), and the mixture was allowed to stir at r.t. for 24 h under a positive pressure of H_2. The mixture was filtered through a Celite bed and concentrated under reduced pressure. The crude mass was dissolved in 0.1 M NaOMe in MeOH (30 mL), and the mixture was allowed to stir at r.t. for 12 h; then a few drops of distilled H_2O was added, and the mixture was allowed to stir for 6 h. The mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered, and evaporated to dryness, before it was again passed through a short pad of Dowex 50W X8 (Na^+) resin. The crude product was purified by being passed through a column of Sephadex-LH-20 (MeOH–H_2O, 4:1); this gave tetrasaccharide **2** as its sodium salt.

Yield: 250 mg (72%); R_f = 0.3 (MeCN–MeOH–H_2O, 4:1:0.5); white powder; $[\alpha]_D^{25}$ –8.7 (*c* 1.1, H_2O).

IR (KBr): 3021, 2926, 2401, 1216, 767, 670 cm^{-1}.

^1H NMR (300 MHz, D_2O): δ = 7.01 (d, *J* = 8.9 Hz, 2 H, Ar-H), 6.80 (d, *J* = 8.9 Hz, 2 H, Ar-H), 4.79 (d, *J* = 7.3 Hz, 1 H, H-1_A), 4.47 (d, *J* = 7.3 Hz, 1 H, H-1_B), 4.41 (d, *J* = 7.5 Hz, 1 H, H-1_C), 4.14–4.05 (m, 3 H, H-8_D, H-7_D, H-$9a_D$), 3.91–3.76 (m, 7 H, H-4_D, H-5_D, H-4_B, H-6_D, H-$6ab_A$, H-$9b_D$), 3.75–3.67 (m, 3 H, H-2_C, H-$6ab_C$), 3.71 (s, 3 H, OCH_3), 3.64–3.57 (m, 6 H, H-3_A, H-2_B, H-5_B, H-3_C, H-$6ab_B$), 3.55–3.44 (m, 6 H, H-2_A, H-4_A, H-5_A, H-3_B, H-4_C, H-5_C), 2.73 (dd, *J* = 12.3, 4.2 Hz, 1 H, H-$3e_D$), 1.96 (s, 3 H, NHCOCH_3), 1.75 (t, *J* = 11.9 Hz, 1 H, H-$3a_D$).

^{13}C NMR (75 MHz, D_2O): δ = 174.4 (*C*OONa), 173.9 (NH*C*OCH$_3$), 155.6–114.5 (Ar-C), 105.3 (C-1_B), 103.7 (C-1_C), 101.9 (C-1_A), 99.5 (C-2_D), 83.4 (C-2_C), 80.5 (C-5_B), 75.7 (C-2_B), 75.2 (2 C, C-3_A, C-3_C), 74.1 (C-4_A), 73.7 (C-5_A), 73.5 (2 C, C-3_B, C-4_C), 71.9 (2 C, C-4_D, C-6_D), 70.4 (C-4_B), 69.3 (C-8_D), 69.2 (C-7_D), 68.8 (C-5_C), 68.3 (C-2_A), 63.6 (C-9_D), 63.1 (C-6_B), 61.5 (C-6_C), 60.9 (C-6_A), 55.1 (O*C*H$_3$), 52.6 (C-5_D), 40.8 (C-3_D), 21.9 (NHCO*C*H$_3$).

ESI-MS: *m*/*z* = 924.2 $[M + 1]^+$.

Anal. Calcd for $C_{36}H_{54}NNaO_{25}$ (923.28): C, 46.81; H, 5.89. Found: C, 46.58; H, 6.18.

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