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Human milk oligosaccharides: an enzymatic protection step simplifies the synthesis of 3′- and 6′-*O*-sialyllactose and their analogues

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Abstract

We describe a chemo-enzymatic synthesis of 3′- and 6′-*O*-sialyllactose, two trisaccharides occurring in the 'acidic fraction' of the human milk oligosaccharides and endowed with potential antiadhesive activity. The key step is the highly regioselective 6′-*O*-acylation of benzyllactoside, which gave access to suitably protected lactose building blocks to be used as acceptors in the sialylation reaction. Moreover, the synthesis of the carboxymethyl and sulfo analogues of the title compounds is reported. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Human milk oligosaccharides; Enzymatic 6′-*O*-acylation; Benzyllactoside

1. Introduction

The oligosaccharide fraction is the third most abundant solid constituent (between 12 and 20 g/L) of human milk and consists of more than one hundred different structures. Despite many investigations[1–3] that have been reported on the subject, the physiological role of these compounds is not completely understood. The fact that human milk oligosaccharides bear structural homology to cell-surface glycoconjugates used as receptors by pathogens may suggest their implication in protecting breast-fed infants from infections. Concerning this issue, evidence has emerged that these oligosaccharides are able to inhibit the adhesion of *Streptococcus pneumoniae* and *Haemophilus influenzae* to human pharingeal or buccal epithelial cells.[2] Moreover, the fucosylated fraction of small human milk oligosaccharides inhibits the *Escherichia coli* adhesion to uroepithelial cells.[3]

In this study, we turned our attention to sialylated human milk oligosaccharides. It has been recently demonstrated[4] that whey glycoproteins, containing *N*-acetylneuraminic acid, exert a potential inhibitory effect on S-fimbriae mediated adhesion of *E. coli*.

In order to assess the antiadhesive properties of selected oligosaccharides present in human milk, we describe herein the synthesis of 3′- and 6′-*O*-sialyllactoses and their corresponding simplified analogues where the sialic acid unit is replaced by a negatively charged group such as a sulfate and a carboxymethyl group. Sulfate groups may serve as an effective substituent for sialic acid[5] in some biological systems and 3′-*O*-sulfo-lactose has recently been found as an oligosaccharide present in dog milk.[6] Moreover the carboxymethyl group in analogues of sialyl Lewis X was shown to be useful for mimicking the negative charge of sialic acid.[7] In previous papers, we reported on the use of lipase catalysed acylation as a tool to prepare useful building blocks for oligosaccharide synthesis.[8–11] In the present paper, the enzymatically catalysed 6′-*O*-acylation of lactose has been exploited for designing a versatile chemo-enzymatically protected lactose building block useful for the synthesis of both 3′- and 6′-*O*-sialyllactoses.

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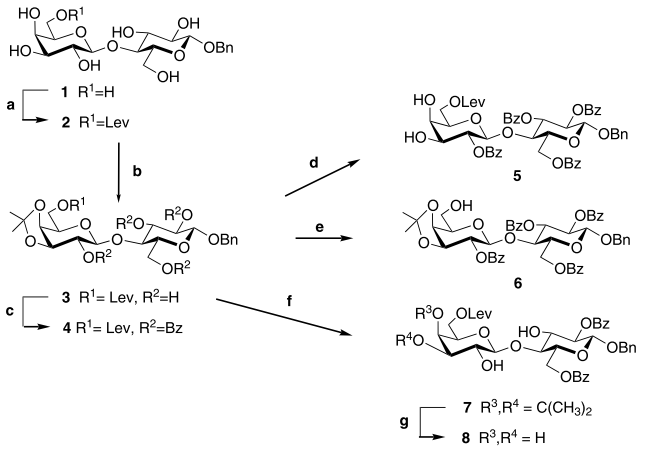


Scheme 1. Reagents and conditions: (a) trifluoroethyl levulinate, *C. antarctica* lipase, THF, 40 °C, 4 days, 83%; (b) acetone, CSA, Sikkon, refl, 4 h, 78%; (c) BzCl, Py, DMAP, CH_2Cl_2, 0 °C → rt, 48 h, 80%; (d) TFA (60%), CH_2Cl_2, 0 °C → rt, 1 h, 85%; (e) $AcONH_2NH_3$, EtOH–Et_2O, rt, 20 min, 97%; (f) BzCN, TEA, THF, −25 °C, 17 h, 50%; (g) TFA (60%), CH_2Cl_2, 0 °C → rt, 1.5 h, 92%.

2. Results and discussion

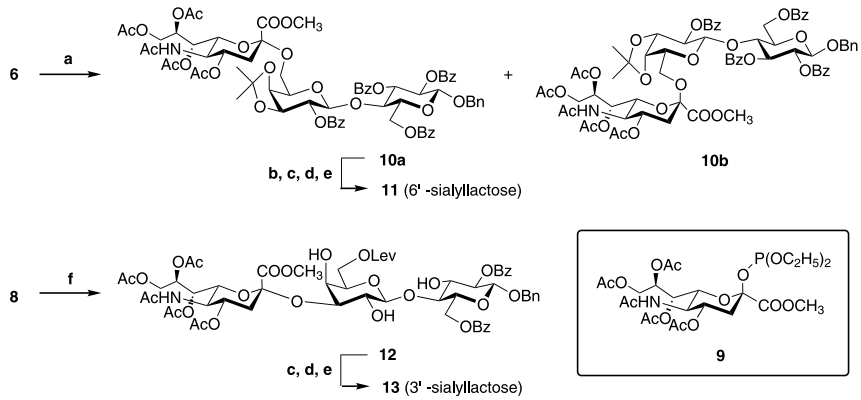
The selective protection of the 6′-OH position of benzyllactoside **1**[12] with a levulinoyl group was achieved in high yield through an enzymatic acylation[11] catalysed by *Candida antarctica* lipase in THF (Scheme 1). The subsequent protection of 3′,4′-positions of **2** as isopropylidene acetal afforded compound **3**. Full benzoylation of **3** gave the versatile building block **4** useful for the synthesis of the target compounds. In fact, orthogonal deprotection of isopropylidene or levulinoyl groups afforded acceptors **5** and **6**, respectively (Scheme 1), which were separately submitted to sialylation using sialyl phospite **9**[13] as a donor (Scheme 2).

Glycosylation of acceptor **6** in acetonitrile at −40 °C with trimethylsilyltrifluoromethansulfonate (TMSOTf) as a promoter, afforded the 6′-*O*-sialyllactoside **10a**/**b** as a 2:1 α/β mixture in 58% overall yield (Scheme 2). Even though selectivity was not high, the two isomers were completely separated by MP chromatography. Anomeric configuration of the newly formed glycosidic linkage for **10a** and **10b** was determined by comparison of NMR data of the two compounds. In accordance with the known ^{1}H NMR empirical rules,[14–16] δ H-3″eq (α) = 2.73 > δ H-3″eq (β) = 2.61, δ H-4″ (α) = 4.52 < δ H-4″ (β) = 5.49, $J_{7'',8''}$ (α) = 7.9 Hz > $J_{7'',8''}$ (β) = 3.1 Hz.

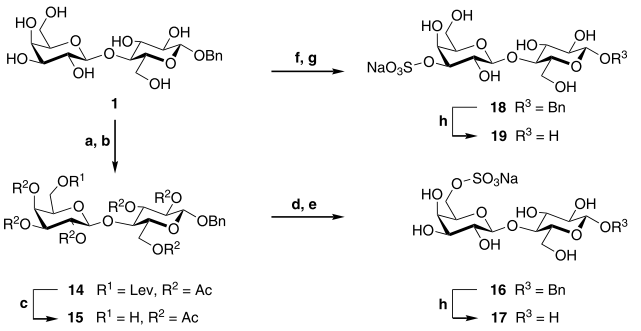
Disappointingly, sialylation of acceptor **5** resulted in a complex mixture of products from which most of the acceptor was recovered unreacted and the supposed 3′-*O*-sialyllactose derivative was isolated in a very poor yield. These poor results might be due to the steric hindrance and to the presence of electron withdrawing groups close to the reactive centre. Therefore, a new and more reactive acceptor **8** was synthesised through selective benzoylation[17] of **3** followed by acidic hydrolysis of the isopropylidene acetal (Scheme 1).

Sialylation of this new acceptor in acetonitrile–THF at −44 °C in the presence of TMSOTf as a promoter, afforded the 3′-*O*-sialyllactoside **12** in 51% yield (Scheme 2). This result is in line with that reported by Lönn et al.,[18] who used a very similar acceptor employing a glycosyl xanthate as the donor. The (2″ → 3′) glycosylation regiochemistry was ascertained from the observation of COSY cross-peaks between OH-2′ (δ 3.17) and H-2′ (δ 3.75) and OH-4′ (δ 2.38) and H-4′ (δ 3.63). Moreover, the HMBC[19] experiment showed the presence of the expected cross-peak between H-3′ (δ 4.09) and the quaternary carbon C-2″ (δ 97.6). The α configuration of the newly formed glycosidic linkage was determined on the basis of the occurrence of the Neu5Ac H-4 signal at δ 4.97, as well as the $J_{7'',8''}$ = 8.4 Hz; these data are in accordance with the reported trend for similar compounds.[18,20]

The synthesis of 6′-*O*- and 3′-*O*-sulfated analogues of the corresponding sialyllactoses was based on two different strategies. Lactoside **15** was obtained in three



Scheme 2. Reagents and conditions: (a) **9** (1.7 equiv), TMSOTf, CH_3CN, −40 °C, 1 h, 58%, α/β 2:1; (b) TFA (60%), CH_2Cl_2; (c) MeOH, MeONa; (d) NaOH, water, (e) H_2, Pd/C, H_2O; (f) **9** (2 equiv), TMSOTf, CH_3CN–THF, −44 °C, 2 h, 51%.

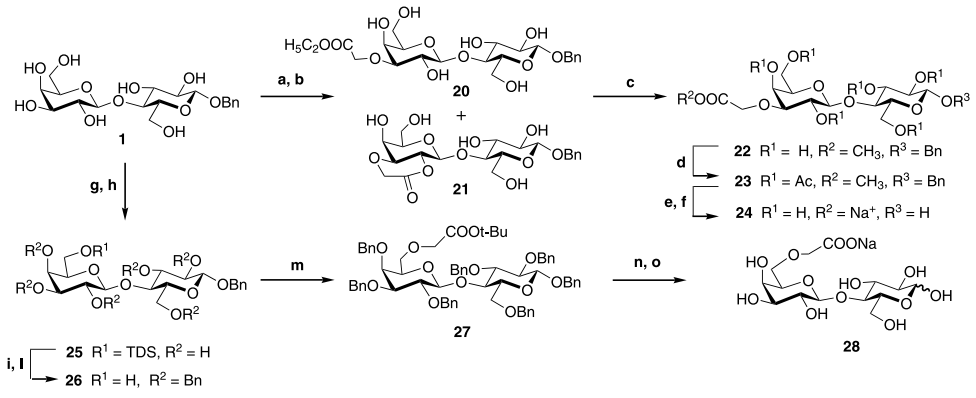


Scheme 3. Reagents and conditions: (a) trifluoroethyl levulinate, *C. antarctica* lipase, THF, 40 °C, 4 days, 83%; (b) Ac_2O, Py, 80%; (c) $AcONH_2NH_3$, EtOH–Et_2O, rt, 2 h, 98%; (d) SO_3NMe_3 1.5 equiv, DMF, 60 °C, 2 days; (e) MeONa, MeOH, rt, 81%; (f) Bu_2SnO (1.1 equiv), MeOH, refl, 16 h; (g) SO_3NMe_3 (2 equiv), DMF, rt, 2 days, 75%; (h) H_2, Pd/C, MeOH–water, rt.

steps from benzyllactoside **1** through enzymatic 6′-*O*-levulinoylation, followed by acetylation (→**14**) and removal of the levulinoyl group with hydrazinium acetate (Scheme 3). The sulfation of **15** (SO_3NMe_3 complex), followed by deacetylation, provided the 6′-*O*-sulfated lactoside **16**. The 3′-*O*-sulfated lactoside **18** was obtained in a one pot procedure,[21] by treatment of benzyllactoside **1** with Bu_2SnO and SO_3NMe_3 complex (Scheme 3).

The same approach was applied to the synthesis of 3′-*O*-(alkoxycarbonyl) methyllactoside (Scheme 4). In this case, the alkylation of the stannylene acetal obtained from benzyllactoside **1** produced compound **20** and the corresponding lactone **21**. This mixture was treated without separation with sodium methoxide (→**22**), followed by acetylation to afford pure compound **23** in good yield. The acetylation step was required in order to better purify the compound. The introduction of the (alkoxycarbonyl) methyl group at 6′-position was first attempted by treatment of **15** with ethyl bromoacetate in the presence of Ag_2O and TBAI. Disappointingly, a complex mixture of products was obtained, probably derived from acyl migration and/or hydrolysis. Much better results were achieved on the benzylated compound **26**,[22] which was synthesised in three steps from benzyllactoside **1** through regioselective 6′-*O*-silylation,[23] which afforded compound **25**, followed by full benzylation and final 6′-*O*-desilylation. Thus, alkylation of **26** with *tert*-butyl-bromoacetate[24] afforded compound **27** in almost quantitative yield (Scheme 4).

Finally, compounds **10a**, **12**, **16**, **18**, **23** and **27** were conventionally deprotected to afford the title compounds **11**, **13**, **17**, **19**, **24**, and **28**, respectively. Full deprotection of compound **10a** was achieved by acidic hydrolysis of the isopropylidene group, followed by Zemplén deacylation, saponification of the methylester with NaOH, and hydrogenolysis of the anomeric benzyl group, affording the 6′-*O*-sialyllactose **11** (Scheme 2). NMR data for **11** are in agreement with those previously reported for the natural compound.[25] Compound **12** was converted into the known compound **13** by Zemplén deacylation, saponification with NaOH, and hydrogenolysis of the anomeric benzyl ether. NMR data for **13** are consistent with those reported for the natural compound[25] and by Ogawa.[20] Sulfated lactose derivatives **17** and **19** were quantitatively obtained from **16** and **18** by hydrogenolysis of the anomeric benzyl ether. Zemplén deacylation of **23**, followed by one-pot saponification with NaOH and hydrogenolysis, afforded compound **24**, whereas **28** was obtained from **27** by acidic hydrolysis of the *tert*-butyl ester followed by hydrogenolysis of the remaining benzyl groups. Final desalting of the deprotected compounds was performed by gel permeation chromatography through a Sephadex G10 column, followed by freeze-drying.



Scheme 4. Reagents and conditions: (a) Bu_2SnO 1.1 equiv, MeOH, refl, 16 h; (b) $BrCH_2COOEt$ 5 equiv, TBAI, DMF, 40 °C, 48 h; (c) MeONa, MeOH, rt, 24 h; (d) Ac_2O, Py, rt, 24 h, 56% from **1**; (e) MeONa, MeOH, rt, 16 h, then water, 20 h; (f) H_2, Pd/C, 91% from **23**; (g) Bu_2SnO 1.1 equiv, MeOH, refl, 16 h; (h) TDSCl 1.1 equiv, THF, rt, 18 h, 76%; (i) BnBr, NaH, DMF, rt, 16 h; (l) TBAF, THF, 0 °C→rt, 6 h, 66% from **25**; (m) $BrCH_2COOtBu$, NaOH, $(Bu)_4NHSO_4$, CH_2Cl_2, 4 h, 96%; (n) TFA (10%), CH_2Cl_2, 30 min; (o) H_2, $Pd(OH)_2$/C, 72 h, 95%.

Results of antiadhesion tests and BIACORE analysis will be reported elsewhere.

3. Experimental

General methods.—^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 200, Bruker AC 300, Bruker Avance 400, Bruker Am 500 and Bruker 600 DRX spectrometers. The HMBC spectra were acquired using 128 scans per series in 1K × 256W data points and optimised for the $^3J_{C-H}$ of 8 and 4 Hz. Melting points were determined with a Büchi apparatus and are not corrected. Optical rotations were measured at rt with a Perkin–Elmer 241 polarimeter. TLC was carried out on E. Merck Silica-Gel 60 F_{254} plates (0.25 mm thickness), and spots were visualised by spraying with a solution containing H_2SO_4 (31 mL), ammonium molybdate (21 g) and $Ce(SO_4)_2$ (1 g) in 500 mL water, followed by heating at 110 °C for 5 min. Column chromatography was performed by the flash procedure using E. Merck Silica-Gel 60 (230–400 mesh). Elemental analyses were performed using the Carlo–Erba elemental analyser 1108. In the description of the ^{13}C spectra, signals corresponding to aromatic carbons were omitted. Lipase from *C. antarctica* was purchased from Roche Diagnostic (Chirazyme® L-2, c.f. C2 lyo). Solvents were dried by standard procedures.

Benzyl 6-O-levulinoyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**2**).—Benzyl β-D-lactoside (**1**)[12] (700 mg, 1.62 mmol) was suspended in dry THF (70 mL). Trifluoroethyl levulinate[8] (22 g, 113 mmol) and *C. antarctica* lipase (2.1 g) were added, the suspension was shaken for 4 days at 40 °C and monitored by TLC (8:1.5:0.5 EtOAc–MeOH–water). The enzyme was filtered, and the solvent was removed under diminished pressure. Purification by flash chromatography (10:1 EtOAc–MeOH) afforded compound **2** as a white foam (713 mg, 83%). Optical rotation value and NMR data are in agreement with those reported by us in a previous publication.[8]

Benzyl 3,4-O-isopropylidene-6-O-levulinoyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**3**).—Compound **2** (53 mg, 0.1 mmol) was dissolved in acetone (3 mL) in an inert atmosphere, sikkon (180 mg) was added and the mixture was stirred for 30 min. Then a catalytic amount of camphorsulfonic acid (CSA) was added and the suspension was refluxed for 4 h while monitoring the reaction by TLC (9.5:0.5 EtOAc–MeOH). After cooling to rt, the mixture was neutralised with TEA, sikkon was filtered off and the solvent was removed under diminished pressure. Purification by flash chromatography (9:1 EtOAc–MeOH) afforded compound **3** as a white foam (44 mg, 78%): $[\alpha]_D^{20}$ +9.8° (*c* 1.7, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.35–7.20 (m, 5 H, H_{Ar}), 4.87 (d, 1 H, *J* 11.8 Hz, C*H*HPh), 4.64 (d, 1 H, C*H*HPh), 4.42 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.37 (dd, 1 H, $J_{6'a,6'b}$ 12.0, $J_{6'a,5'}$ 3.2 Hz, H-6′a), 4.33 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.26 (dd, 1 H, $J_{6'b,5'}$ 8.6 Hz, H-6′b), 4.12–4.05 (m, 3 H, H-4′, H-3′, H-3), 3.85 (m, 2 H, H-6a, H-6b), 3.64–3.38 (m, 5 H, H-2, H-4, H-5, H-2′, H-5′), 2.77–2.73 (m, 2 H, CH_2 lev.), 2.62–2.58 (m, 2 H, CH_2 lev.), 2.20 (s, 3 H, CH_3 lev.), 1.49 (s, 3 H, CH_3 isoprop.), 1.30 (s, 3 H, CH_3 isoprop.); ^{13}C NMR ($CDCl_3$, 75.44 MHz): δ 207.0 (CO lev.), 172.6 (COO lev.), 110.6 (Cq isoprop.), 103.0, 101.8 (C-1, C-1′), 81.7, 79.3, 74.9, 74.3, 73.7, 73.2, 73.1, 71.5 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 71.3 (*C*H_2Ph), 63.4, 62.2 (C-6, C-6′), 37.8 (*C*H_2COCH$_3$ lev.), 28.0 (CH_3 lev.), 27.9 (*C*H_2COO lev.), 26.2 (2 CH_3 isoprop.). Anal. Calcd for $C_{27}H_{38}O_{13}$ (570.583): C, 56.83, H, 6.71. Found: C, 56.95 H, 6.67.

Benzyl 2-O-benzoyl-3,4-O-isopropylidene-6-O-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (**4**).—Compound **3** (272 mg, 0.48 mmol) was dissolved in dry CH_2Cl_2 (5 mL) under N_2 atmosphere, then pyridine (410 μL, 5.1 mmol), DMAP (cat) and benzoyl chloride (360 μL, 3.1 mmol) were added at 0 °C and the reaction mixture was stirred 48 h at rt. The reaction was quenched with MeOH and the solution was concentrated under diminished pressure. Purification by flash chromatography (6.5:3.5 petroleum ether–EtOAc) afforded **4** as white foam (378 mg, 80%): $[\alpha]_D^{20}$ +24.4° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.10–7.08 (m, 25 H, H_{Ar}), 5.63 (t, 1 H, $J_{3,2} = J_{3,4}$ 9.3 Hz, H-3), 5.44 (t, 1 H, H-2), 5.09 (t, 1 H, $J_{1',2'} = J_{2',3'}$ 7.4 Hz, H-2′), 4.80 (d, 1 H, *J* 12.6 Hz, C*H*HPh), 4.68 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.64 (dd, 1 H, $J_{6a,5}$ 2.8 Hz, H-6a), 4.62 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.57 (d, 1 H, C*H*HPh), 4.50 (dd, 1 H, $J_{6a,6b}$ 11.8, $J_{6b,5}$ 4.8 Hz, H-6b), 4.24–4.18 (m, 2 H, H-4, H-3′), 4.05 (dd, 1 H, $J_{4',5'}$ 1.7, $J_{4',3'}$ 5.8 Hz, H-4′), 3.95 (dd, 1 H, $J_{6'a,6'b}$ 11.5, $J_{6'a,5'}$ 4.9 Hz, H-6′a), 3.82–3.73 (m, 2 H, H-5, H-5′), 3.64 (dd, 1 H, $J_{6'b,5'}$ 7.0 Hz, H-6′b), 2.77–2.73 (m, 2 H, CH_2 lev.), 2.56–2.51 (m, 2 H, CH_2 lev.), 2.20 (s, 3 H, CH_3 lev.), 1.48 (s, 3 H, CH_3 isoprop.), 1.22 (s, 3 H, CH_3 isoprop.); ^{13}C NMR ($CDCl_3$, 50.29 MHz): δ 206.3 (CO lev.), 172.2 (COO lev.), 165.9, 165.4, 165.1, 164.8 (COOBz), 110.7 (Cq isoprop.), 100.2, 99.1 (C-1, C-1′), 76.9, 75.5, 73.6, 73.2, 73.0, 72.7, 72.0, 70.9 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.3 (*C*H_2Ph), 62.7 (C-6, C-6′), 37.9 (*C*H_2COCH$_3$ lev.), 29.7 (*C*H_3CO lev.), 27.9 (*C*H_2COO lev.), 27.3, 26.0 (2 CH_3 isoprop.); Anal. Calcd for $C_{55}H_{54}O_{17}$ (987.007): C, 66.93; H, 5.51. Found: C, 66.99; H, 5.55.

Benzyl 2-O-benzoyl-6-O-levulinoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (**5**).—Compound **4** (400 mg, 0.41 mmol), was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. A 60% aq CF_3COOH (4 mL) was added under vigorous stirring (TLC 1:1 petroleum ether–EtOAc). After 30 min at 0 °C and 30 min at rt, the reaction mixture was diluted

with CH_2Cl_2, neutralised with $NaHCO_3$ and partitioned between water and CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under diminished pressure. Purification by flash chromatography (1:4 petroleum ether–EtOAc), afforded **6** (325 mg, 85%) as an amorphous white solid: $[\alpha]_D^{20}$ +19.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.09–7.08 (m, 25 H, H_{Ar}), 5.56 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 5.46 (dd, 1 H, $J_{1,2}$ 7.4 Hz, H-2), 5.31 (t, 1 H, $J_{1',2'} = J_{2',3'}$ 8.5 Hz, H-2′), 4.80 (d, 1 H, *J* 12.5 Hz, C*H*HPh), 4.65 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.60–4.47 (m, 4 H, H-6a, H-6b, H-1′, C*H*HPh), 4.13 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4), 3.82–3.35 (2 m, 8 H, H-5, H-3′, H-4′, H-5′, H-6′a, H-6′b, 2 OH), 2.74–2.67 (m, 2 H, CH_2 lev.), 2.51–2.43 (m, 2 H, CH_2 lev.), 2.16 (s, 3 H, CH_3 lev.); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 206.7 (CO lev.), 172.4 (COO lev.), 166.1, 166.0, 165.3 (COOBz), 101.1, 98.8 (C-1, C-1′), 76.4, 75.5, 73.5, 73.1, 72.4, 72.3, 71.7, 68.6 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.3 (*C*H_2Ph), 62.8, 61.8 (C-6, C-6′), 37.9 (*C*H_2COCH$_3$ lev.), 29.8 (*C*H_3CO lev.), 27.7 (*C*H_2COO lev.). Anal. Calcd for $C_{52}H_{50}O_{17}$ (946.943): C, 65.96; H, 5.32. Found: C, 65.92; H, 5.34.

*Benzyl 2-*O*-benzoyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**6**).—Compound **4** (356 mg, 0.36 mmol) was dissolved in 1:1 EtOH–Et_2O (8 mL), then a 1 M solution of $AcONH_3NH_2$ in EtOH (0.4 mL) was dropped at rt. After 20 min, the solvents were removed under diminished pressure and purification by flash chromatography (3:1 toluene–EtOAc) afforded compound **5** as a white solid (309 mg, 97%): mp 202–203 °C; $[\alpha]_D^{20}$ +26.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.05–7.15 (m, 25 H, H_{Ar}), 5.62 (t, 1 H, $J_{3,4} = J_{2,3}$ 8.8 Hz, H-3), 5.49 (dd, 1 H, $J_{1,2}$ 7.3 Hz, H-2), 5.14 (t, 1 H, $J_{2',3'}$ 7.3 Hz, H-2′), 4.81 (d, 1 H, *J* 12.5 Hz, C*H*HPh), 4.71 (d, 1 H, H-1), 4.64 (dd, 1 H, $J_{6a,6b}$ 12.2, $J_{6a,5}$ 2.2 Hz, H-6a), 4.61 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.58 (d, 1 H, C*H*HPh), 4.47 (dd, 1 H, $J_{6b,5}$ 4.7 Hz, H-6b), 4.25–4.16 (m, 2 H, H-4, H-3′), 4.00 (dd, 1 H, $J_{4'3'}$ 5.65, $J_{4',5'}$ 2.01 Hz, H-4′), 3.8 (ddd, 1 H, H-5), 3.56 (m, 1 H, H-5′), 3.43–3.17 (m, 2 H, H-6′a, H-6′b), 1.51 (s, 3 H, CH_3 isoprop.), 1.28 (s, 3 H, CH_3 isoprop.); ^{13}C NMR ($CDCl_3$, 50.29 MHz): δ 165.9, 165.5, 165.2, 164.9 (COOBz), 110.7 (Cq isoprop.), 100.5, 99.0 (C-1, C-1′), 77.0, 75.8, 73.9, 73.4, 73.3, 73.0, 71.8 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.3 (*C*H_2Ph), 62.8, 61.8 (C-6, C-6′), 27.4, 26.1 (2 CH_3 isoprop.). Anal. Calcd for $C_{50}H_{48}O_{15}$ (888.907): C, 67.56; H, 5.44. Found: C, 67.62; H, 5.51.

*Benzyl 3,4-*O*-isopropylidene-6-*O*-levulinoyl-β-*D*-galactopyranosyl-(1→4)-2,6-di-*O*-benzoyl-β-*D*-glucopyranoside* (**7**).—To a solution of **3** (300 mg, 0.53 mmol) in dry THF (7.5 mL), TEA (0.37 mL, 2.65 mmol) was added under Ar atmosphere. The reaction mixture was cooled to −25 °C, and a solution of BzCN (204 mg, 1.56 mmol) in THF (2.5 mL) was added dropwise. The reaction mixture was stirred for 17 h (TLC 1:9 MeOH–EtOAc), diluted with EtOAc, quenched with MeOH (0.3 mL) and allowed to warm at rt. After concentration under diminished pressure, sequential purification by flash and MP chromatography (8:1.5 toluene–acetone) afforded **7** (203 mg, 50%), as a white foam. $[\alpha]_D^{20}$ −15.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.11–7.15 (m, 15 H, H_{Ar}), 5.21 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-2), 4.87 (2d overlapped, 2 H, H-6a, C*H*HPh), 4.70 (d, 1 H, *J* 12.6 Hz, C*H*HPh), 4.60 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.55 (dd, 1 H, $J_{6a,6b}$ 12.0, $J_{6b,5}$ 5.2 Hz, H-6b), 4.39 (dd, 1 H, $J_{6'a,6'b}$ 12.1, $J_{6'a,5'}$ 2.8 Hz, H-6′a), 4.30 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.25 (dd, 1 H, $J_{6'b,5'}$ 9.4 Hz, H-6′b), 4.10–4.06 (m, 3 H, H-3′, H-4′, H-5′), 3.87 (t, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 3.69 (br dd, 1 H, $J_{4,5}$ 10.0 Hz, H-5), 3.63 (br dd, 1 H, $J_{2,3}$ 6.3 Hz, H-2′), 3.61 (t, 1 H, H-4), 2.54–2.35 (m, 4 H, 2 CH_2 lev.), 2.02 (s, 3 H, CH_3 lev.), 1.51 (s, 3 H, CH_3 isoprop.), 1.32 (s, 3 H, CH_3 isoprop.); ^{13}C NMR ($CDCl_3$, 150.86 MHz): δ 206.8 (CO lev.), 171.6, 166.8, 165.3 (CO lev., Bz), 110.7 (Cq isoprop.), 103.4 (C-1′), 98.9 (C-1), 82.2 (C-4), 79.1 (C-3′ or C-4′), 73.7 (C-3), 73.6 (C-5), 73.4 (C-2′), 73.1 (C-2), 73.0 (C-3′ or C-4′), 71.5 (C-5′), 70.3 (*C*H_2Ph), 63.9 (C-6), 63.2 (C-6′), 37.6 (CH_3 lev.), 29.6, 28.0 (2 CH_2 lev.), 27.8, 26.2 (2 CH_3 isoprop.). Anal. Calcd for $C_{41}H_{46}O_{15}$ (778.795): C, 63.23; H, 5.95. Found: C, 63.20; H, 5.89.

*Benzyl 6-*O*-levulinoyl-β-*D*-galactopyranosyl-(1→4)-2,6-di-*O*-benzoyl-β-*D*-glucopyranoside* (**8**).—Compound **7** (564 mg, 0.72 mmol) was submitted to the same conditions described for the preparation of **5**. Purification by flash chromatography (EtOAc) afforded **8** (490 mg, 92%) as an amorphous glassy solid: $[\alpha]_D^{20}$ −37.0° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.15–7.11 (m, 15 H, H_{Ar}), 5.19 (t, 1 H, $J_{2,3}$ 8.7 Hz, H-2), 4.85 (d, 1 H, H-6a), 4.81 (d, 1 H, C*H*HPh), 4.62 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 4.59 (d, 1 H, *J* 11.3 Hz, C*H*HPh), 4.54 (dd, 1 H, $J_{6b,5}$ 5.6, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.44 (br s, 1 H, OH), 4.34 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1′), 4.28 (dd, 1 H, $J_{6'a,5'}$ 3.3 Hz, H-6′a), 4.24 (dd, 1 H, $J_{6'b,5'}$ 4.4, $J_{6'a,6'b}$ 11.8 Hz, H-6′b), 4.01 (br s, 1 H, OH), 3.90–3.63 (m, 7 H, H-3, H-4, H-2′, H-3′, H-4′, H-5′, OH), 3.54 (m, 1 H, H-5), 3.31 (br s, 1 H, OH), 2.57–2.40 (m, 4 H, 2 CH_2 lev.), 2.03 (s, 3 H, CH_3 lev.); ^{13}C NMR ($CDCl_3$, 50.29 MHz, 50 °C): δ 207.2 (CO lev.), 173.0, 167.1, 165.7 (COO lev., Bz), 104.3 (C-1′), 99.5 (C-1), 81.8, 73.8, 73.3, 71.3, 68.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.6 (*C*H_2Ph), 64.2, 63.6 (C-6, C-6′), 37.9 (*C*H_2COCH$_3$ lev.), 29.2 (CH_3 lev.), 28.1 (*C*H_2COO lev.). Anal. Calcd for $C_{38}H_{42}O_{15}$ (738.731): C, 61.68; H, 5.73. Found: C, 61.63; H, 5.77.

*Benzyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-non-2-ulopyranosyl-onate)-(2→6)-2-*O*-benzoyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-*D*-gluco-*

pyranoside (**10a**) *and its isomer* (**10b**).—Compound **5** (390 mg, 0.44 mmol) and phosphite donor **9**[13] (468 mg, 0.76 mmol) were dissolved in dry CH_3CN (4 mL), and cooled to −40 °C. A 0.5 M solution of trimethylsilyltrifluoromethansulfonate (TMSOTf) in CH_3CN (150 μL, 0.075 mmol), was dropped under vigorous stirring. After 1 h, the reaction mixture was neutralised with TEA and concentrated. Flash chromatography purification (95:5 toluene–EtOH) of the crude afforded a mixture of **10a**, **10b** and glycal[26] which was further purified by MP chromatography to give **10a** (236 mg, 39%) and **10b** (115 mg, 19%) as white amorphous solids: **10a**, $[\alpha]_D^{20}$ +6.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (C_6D_6, 500 MHz): δ 8.30–6.91 (2 m, 25 H, H_{Ar}), 5.92 (ddd, 1 H, $J_{8'',9''b}$ 7.7 Hz, H-8″), 5.87 (m, 2 H, H-2, H-3), 5.60 (t, 1 H, $J_{2',3'}$ 7.2 Hz, H-2′), 5.44 (dd, 1 H, $J_{6'',7''}$ 2.3, $J_{7'',8''}$ 7.9 Hz, H-7″), 4.89 (dd, 1 H, $J_{6a,6b}$ 11.8, $J_{6a,5}$ 6.2 Hz, H-6a), 4.80 (m, 3 H, H 6b, H-4″, H 1′), 4.70 (dd, 1 H, $J_{8'',9''a}$ 2.9, $J_{9''a,9''b}$ 12.2 Hz, H-9″a), 4.63 (d, 1 H, *J* 12.7 Hz, C*H*HPh), 4.52 (m, 1 H, H-4), 4.44 (d, 1 H, C*H*HPh), 4.41 (m, 2 H, H-1, H-5″), 4.29 (dd, 1 H, $J_{8'',9''b}$ 7.7 Hz, H-9″b), 4.10 (dd, 1 H, $J_{6'',7''}$ 2.3, $J_{5'',6''}$ 10.8 Hz, H-6″), 4.01 (dd, 1 H, $J_{4',5'}$ 2.0, $J_{3',4'}$ 5.4 Hz, H-4′), 3.96–3.87 (m, 3 H, H-3′, H-6′a, NH), 3.86 (br t, 1 H, H-5′), 3.59 (t, 1 H, $J_{6'a,6'b}$ 9.4, $J_{6'b,5'}$ 8.3 Hz, H-6′b), 3.42 (s, 3 H, $COOCH_3$), 3.34 (br t, 1 H, H-5), 2.73, (dd, 1 H, $J_{3''eq,3''ax}$ 12.8, $J_{3''eq,4''}$ 4.7 Hz, H-3″eq), 2.20 (s, 3 H, CH_3Ac), 2.07 (s, 3 H, CH_3Ac), 1.96 (t, 1 H, $J_{3''ax,4''}$ 12.0 Hz, H-3″ax), 1.92 (s, 3 H, CH_3Ac), 1.58 (s, 3 H, CH_3Ac), 1.56 (s, 3 H, $NHCOCH_3$), 1.54 (s, 3 H, CH_3 isoprop.), 1.40 (s, 3 H, CH_3 isoprop.); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 171.3, 171.1, 170.7, 170.4, 170.3, 168.3, 166.4, 165.8, 165.6, 165.4 (COOAc, NHAc, Bz, $COOCH_3$), 110.7 (Cq isoprop.), 100.2, 99.4, 99.4 (C-1, C-1′, C-2″), 77.4, 77.6, 74.3, 73.7, 73.2, 72.6, 71.7, 69.3, 69.0, 68.0 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-4″, C-6″, C-7″, C-8″), 70.7 (CH_2Ph), 63.5, 63.1, 62.9 (C-6, C-6′, C-9″), 53.2 ($COOCH_3$), 49.9 (C-5″), 38.1 (C-3″), 27.9, 26.6 (2 CH_3, isoprop.), 23.6 ($NHCOCH_3$), 21.4–21.2 (4 CH_3Ac). Anal. Calcd for $C_{70}H_{75}NO_{27}$ (1362.335): C, 61.71; H, 5.55; N, 1.03. Found: C, 61.66; H, 5.52; N, 0.97. **10b**, $[\alpha]_D^{20}$ +23.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (C_6D_6, 500 MHz): δ 8.19–6.98 (2 m, 25 H, H), 5.98 (t, 1 H, H-3), 5.88 (dd, 1 H, $J_{6'',7''}$ 2.0, $J_{7'',8''}$ 3.1 Hz, H-7″), 5.83 (t, 1 H, $J_{2,3}$ 8.8 Hz, H-2), 5.63 (t, 1 H, H-2′), 5.60 (ddd, 1 H, H-8″), 5.49 (ddd, 1 H, $J_{4'',5''}$ 10.1 Hz, H-4″), 5.26 (dd, 1 H, $J_{9''a,9''b}$ 12.4, $J_{9''a,8''}$ 2.5 Hz, H-9″a), 4.96 (d, 1 H, $J_{5'',NH}$ 9.5 Hz, NH), 4.79 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.77 (dd, 1 H, $J_{6a,5}$ 2.5, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.74 (dd, 1 H, $J_{6b,5}$ 5.1 Hz, H-6b), 4.69 (d, 1 H, *J* 12.7 Hz, C*H*HPh), 4.63 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 4.51 (dd, 1 H, $J_{9''b,8''}$ 8.1 Hz, H-9″b), 4.47–4.43 (m, 3 H, C*H*HPh, H-6″, H-5″), 4.28 (dd, 1 H, $J_{3,4}$ 8.4, $J_{4,5}$ 9.4 Hz, H-4), 4.17 (dd, 1 H, $J_{3',4'}$ 5.5, $J_{4',5'}$ 1.7 Hz, H-4′), 4.07 (m, 1 H, H-6′a), 3.97 (dd, 1 H, $J_{2,3}$ 7.3 Hz, H-3′), 3.88 (m, 2 H, H-5′, H-6′b), 3.61 (ddd, 1 H, H-5), 3.40 (s, 3 H, $COOCH_3$), 2.61 (dd, 1 H, $J_{3''eq,3''ax}$ 12.9, $J_{3''eq,4''}$ 4.9 Hz, H-3″eq), 1.92 (s, 3 H, CH_3Ac), 1.85 (s, 3 H, CH_3Ac), 1.82 (t, 1 H, $J_{3''ax,4''}$ 11.5 Hz, H-3″ax), 1.71 (s, 3 H, CH_3Ac), 1.65 (s, 3 H, CH_3 isoprop.), 1.59 (s, 3 H, CH_3Ac), 1.55 (s, 3 H, $HNCOCH_3$), 1.38 (s, 3 H, CH_3 isoprop.); ^{13}C NMR (C_6D_6, 125.72 MHz): δ 170.8, 170.4, 170.3, 169.6, 167.3, 166.1, 165.5, 165.2 (COOAc, NHAc, Bz, $COOCH_3$), 110.9 (Cq isoprop.), 100.7 (C-1′), 99.8 (C-1), 99.19 (C-2″), 77.1 (C-3′), 76.5 (C-4), 73.9 (C-2′), 73.6 (C-3), 73.5 (C-8″), 73.4 (C-4″), 73.3 (C-2) 73.1 (C-5), 72.9 (C-6″), 71.9 (C-5′), 70.4 (CH_2Ph), 69.0 (C-7″), 69.0 (C-4″), 63.6 (C-6), 63.2 (C-9″), 62.5 (C-6″), 52.4 ($COOCH_3$), 49.9 (C-5″), 38.0 (C-3″), 27.7 (CH_3 isoprop.), 26.7 (CH_3 isoprop.), 22.9 ($HNCOCH_3$), 20.8, 20.6, 20.48, 20.4 (4 CH_3Ac), Anal. Calcd for $C_{70}H_{75}NO_{27}$ (1362.335): C, 61.71; H, 5.55; N, 1.03. Found: C, 61.78; H, 5.54; N, 0.96.

Sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*non-2-ulopyranosylonate-(2→6)-β-D-galacto-pyranosyl-(1→4)-D-glucopyranose* (**11**).—To a solution of **10a** (235 mg, 0.17 mmol) in CH_2Cl_2, a 60% aq soln of CF_3COOH was added at 0 °C, and the reaction mixture was stirred at this temperature for 3 h (TLC 9:1 toluene–EtOH). After dilution with water, neutralisation with Na_2CO_3 and extraction with CH_2Cl_2, the organic phase was separated, dried over Na_2SO_4 and concentrated to dryness. The obtained residue was then dissolved in MeOH (4 mL) and treated with a 0.5 M solution of MeONa in MeOH (110 μL) for 48 h at rt (TLC 3:1:0.25 $CHCl_3$–MeOH–water). The reaction mixture was neutralised with Amberlite IR-120 resin (H^+ form), concentrated under diminished pressure, dissolved in water (3 mL) and freeze-dried. The obtained compound was dissolved in water (3 mL), treated with 1 M aq NaOH (180 μL) for 24 h and freeze-dried. The residue was dissolved in 1:4 MeOH–water (6 mL) and submitted to hydrogenolysis in the presence of 10% Pd/C for 24 h (TLC 6:3:1 acetone–BuOH–water); the mixture was filtered through Celite and freeze-dried. The residue was loaded onto a Sephadex G 10 column (V_0 = 150 mL, V_t = 300 mL) and eluted with 1:9 EtOH–water. The fractions containing the product were collected and freeze-dried giving **11** (82 mg, 74%) as a white foam. ^{1}H and ^{13}C NMR data for this compound are in agreement with those reported in the literature.[25]

Benzyl (methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulopyranosyl-onate)-(2→3)-6-levulinoyl-β*-D-*galactopyranosyl-(1→4)-2,6-di*-O-*benzoyl-β*-D-*glucopyranoside* (**12**).—Compound **8** (100 mg, 0.13 mmol) and phosphite donor **9**[13] (160 mg, 0.26 mmol) were dissolved in 2:1 CH_3CN–THF (1.5 mL), and cooled to −44 °C. A 0.5 M TMSOTf solution in CH_3CN (50 μL, 0.025 mmol) was dropped under vigorous stirring. After 2 h, the reaction mixture was neutralised with TEA and concentrated.

Flash chromatography purification (95:5 $CHCl_3$–MeOH) of the crude mixture afforded unreacted **8** (22 mg, 22%) and a mixture of **12,** glycal[26] and other unidentified compounds (presumably isomers of **12**), which was further purified by MP chromatography (3:2 toluene–acetone) to give **12** (81 mg, 51%) as a white amorphous solid: $[\alpha]_D^{20}$ −14.6° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): δ 8.09–7.10 (m, 15 H, H_{Ar}), 5.31 (td, 1 H, $J_{7'',8''} = J_{8'',9''a}$ 8.4, $J_{8'',9''b}$ 2.5 Hz, H-8″), 5.27 (br d, 1 H, H-7″), 5.25 (t, 1 H, $J_{1,2} = J_{2,3}$ 9.5 Hz, H-2), 5.22 (d, 1 H, $J_{5'',NH}$ 9.6 Hz, NH), 4.97 (td, 1 H, $J_{3''ax,4''} = J_{4'',5''}$ 10.5, $J_{3''eq,4''}$ 4.6 Hz, H-4″), 4.93 (br d, 1 H, $J_{6a,6b}$ 10.7 Hz, H-6a), 4.82 (d, 1 H, *J* 12.6 Hz, C*H*HPh), 4.61 (d, 1 H, C*H*HPh), 4.60 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.53 (dd, 1 H, $J_{6b,5}$ 5.6 Hz, H-6b), 4.52 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.42 (s, 1 H, OH), 4.30 (dd, 1 H, $J_{9''a,9''b}$ 12.4 Hz, H-9″a), 4.27 (m, 2 H, H-6′a, H-6′b), 4.10 (br d, 1 H, $J_{6'',5''}$ 9.2 Hz, H-6″), 4.08 (1 H, dd, $J_{2',3'}$ 9.1, $J_{3',4'}$ 3.4 Hz, H-3′), 3.93 (m, 2 H, H-5″, H-9″b), 3.84 (br t, 1 H, $J_{2,3} = J_{3,4}$ 8.8 Hz, H-3), 3.82–3.68 (m, 4 H, H-4, H-5, H-2′, H-5′), 3.80 (s, 3 H, $COOCH_3$), 3.63 (br s, 1 H, H-4′), 3.17 (d, 1 H, *J* 2.0 Hz, OH), 2.66 (dd, 1 H, $J_{3''eq,3''ax}$ 13.0 Hz, H-3″eq), 2.63–2.43 (m, 4 H, 2 CH_2 lev.), 2.38 (d, 1 H, $J_{4',OH}$ 3.0 Hz, OH), 2.13 (s, 3 H, CH_3 lev.), 2.05 (s, 3 H, CH_3Ac), 2.02 (s, 3 H, CH_3Ac), 2.01 (s, 3 H, CH_3Ac), 2.00 (t, 1 H, H-3″ax), 1.96 (s, 3 H, CH_3Ac), 1.88 (s, 3 H, $HNCOCH_3$); ^{13}C NMR ($CDCl_3$, 150.86 MHz): δ 206.9 (CO lev.), 172.6, 170.8, 170.5, 170.2, 170.1, 168.1, 166.2, 165.4 (9 COOAc, NHAc, Bz, $COOCH_3$, lev.), 104.2 (C-1′), 99.0 (C-1), 97.6 (C-2″), 82.3 (C-5), 76.5 (C-3′), 73.6 (C-3), 73.2 (C-7″), 73.0 (C-6″), 72.9 (C-4), 72.4 (C-5′), 70.1 (CH_2Ph), 69.0 (C-2′), 68.8 (C-8″), 68.2 (C-4′), 67.9 (C-4″), 67.0 (C-2), 63.6 (C-6), 63.2 (C-6′), 62.4 (C-9″), 53.3 ($COOCH_3$), 49.8 (C-5″), 37.7, 37.6 (C-3″, CH_2COCH_3 lev.), 30.9 (CH_3 lev.), 27.9 (CH_2COO lev.), 23.1 ($HNCOCH_3$), 21.1–20.6 (4 CH_3Ac). Anal. Calcd for $C_{58}H_{69}NO_{27}$ (1212.159): C, 57.47; H, 5.74; N, 1.16. Found: C, 57.54; H, 6.64; N, 1.19.

Sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*non*-2-*ulopyranosylonate*-(2→3)-β-D-*galacto*-*pyranosyl*-(1→4)-D-*glucopyranose* (**13**).—To a solution of **12** (35 mg, 0.029 mmol) in MeOH (2 mL), a 0.5 M solution of MeONa in MeOH (12 μL) was added and the reaction mixture was stirred for 48 h at rt (TLC 3:1:0.35 $CHCl_3$–MeOH–water). The solution was neutralised by Amberlite IR 120 resin (H^+ form), filtered, concentrated under diminished pressure and, after dilution in water, freeze-dried. The resulting product was dissolved in water (1 mL), treated with 1 M aq NaOH (80 μL) for 24 h and freeze-dried. The residue was dissolved in 1:4 MeOH–water (3 mL) and hydrogenolysed in the presence of 10% Pd/C for 24 h (TLC 6:3:1 acetone–BuOH–water). The mixture was filtered through Celite and freeze-dried. Finally the residue was loaded onto a Sephadex G 10 column ($V_0 = 17$ mL, $V_t = 23$ mL) and eluted with 1:9 EtOH–water. The fractions containing the product were collected and freeze-dried giving **13** (16 mg, 84%) as a white powder. ^1H and ^{13}C NMR data for this compound are in agreement with those reported in the literature.[20,25]

Benzyl 2,3,4-tri-O-*acetyl*-6-O-*levulinoyl*-β-D-*galacto*-*pyranosyl*-(1→4)-2,3,6-*tri*-O-*acetyl*-β-D-*glucopyranoside* (**14**).—To compound **2** (110 mg, 0.21 mmol) in pyridine (4 mL), Ac_2O (2 mL) was added and the mixture was stirred at rt for 24 h. The reaction mixture was quenched with MeOH and concentrated. The residue was diluted with EtOAc and washed with 5% HCl, then with water. The organic layer was dried over Na_2SO_4, concentrated and purified by flash chromatography (4:1 EtOAc–petroleum ether) affording **14** (130 mg, 80%) as a white foam: $[\alpha]_D^{20}$ −28.7° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 7.42–7.23 (m, 5 H, H_{Ar}), 5.33 (br d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4′), 5.17 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 5.10 (dd, 1 H, $J_{1,2}$ 7.6 Hz, H-2), 4.95 (m, 2 H, H-3′, H-4′), 4.85 (d, 1 H, *J* 12.3 Hz, C*H*HPh), 4.60 (d, 1 H, C*H*HPh), 4.49 (m, 3 H, H-1, H-1′, H-6a), 4.20–4.03 (m, 3 H, H-6b, H-6′a, H-6′b), 3.84 (m, 2 H, H-4, H-5′), 3.60 (ddd, 1 H, $J_{5,6a}$ 2.0, $J_{5,6b}$ 4.9, $J_{4,5}$ 9.8 Hz, H-5), 2.74 (m, 2 H, CH_2 lev.), 2.53 (m, 2 H, CH_2 lev.), 2.21, 2.16, 2.05, 2.00, 1.94 (5 s, 21 H, CH_3Ac, lev.); ^{13}C NMR ($CDCl_3$, 50.29 MHz): δ 206.1 (CO lev.), 172.1, 170.2, 170.0, 169.7, 169.4, 168.9 (6 COOAc), 100.9, 99.0 (C-1, C-1′), 76.1, 72.7, 71.8, 70.9, 70.5, 69.6, 66.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.6 (CH_2Ph), 62.0, 60.9 (C-6, C-6′), 37.7 (CH_2COCH_3 lev.), 29.5 (CH_3 lev.), 27.7 (CH_2COO lev.), 20.7–20.5 (6 CH_3Ac). Anal. Calcd for $C_{36}H_{46}O_{19}$ (782.739): C, 55.24; H, 5.92. Found: C, 55.29; H, 5.96.

Benzyl 2,3,4-tri-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-2,3,6-*tri*-O-*acetyl*-β-D-*glucopyranoside* (**15**).—Compound **14** (95 mg, 0.12 mmol) was dissolved in 1:1 EtOH–Et_2O (2 mL) and $AcONH_3NH_2$ (13 mg, 0.14 mmol) was added at rt. After 2 h, the mixture was concentrated and purified by flash chromatography (9:1 toluene–acetone) affording **15** (81 mg, 98%) as a white foam: $[\alpha]_D^{20}$ −13.3° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 7.35–7.13 (m, 5 H, H_{Ar}), 5.34 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 5.17 (t, 1 H, $J_{3,4} = J_{2,3}$ 9.2 Hz, H-3), 5.14 (dd, 1 H, $J_{1',2'}$ 7.6, $J_{2',3'}$ 10.4 Hz, H-2′), 4.98 (dd, 1 H, H-3′), 4.96 (dd, 1 H, $J_{1,2}$ 7.4 Hz, H-2), 4.86 (d, 1 H, *J* 12.2 Hz, C*H*HPh), 4.62–4.51 (m, 4 H, H-1, H-1′, H-6a, C*H*HPh), 4.09 (dd, 1 H, $J_{6a,6b}$ 11.9, $J_{6b,5}$ 5.2 Hz, H-6b), 3.86 (br t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.70–3.48 (m, 4 H, H-5, H-5′, H-6′a, H-6′b), 2.17–1.95 (6 s, 18 H, CH_3Ac); ^{13}C NMR ($CDCl_3$, 75.44 MHz): δ 170.9, 170.4, 170.1, 169.9, 169.6, 169.1 (6 COOAc), 101.1, 99.0 (C-1, C-1′), 76.2, 74.1, 73.5, 72.6, 71.7, 71.0, 69.5, 67.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.0 (CH_2Ph), 62.1, 60.8 (C-6, C-6′), 20.9–20.7 (6 CH_3Ac). Anal. Calcd for $C_{31}H_{40}O_{17}$ (684.639): C, 54.38; H, 5.89. Found: C, 54.36; H, 5.82.

Benzyl 6-O-sulfo-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside sodium salt (**16**).—Compound **15** (186 mg, 0.27 mmol), was dissolved in dry DMF (3 mL) in Ar atmosphere, the SO_3NMe_3 complex (56 mg, 0.40 mmol) was added and the reaction mixture was stirred at 60 °C (TLC 4:1 CH_2Cl_2–MeOH). After 48 h, the reaction was quenched with a few drops of MeOH and concentrated. Flash chromatography purification ($CHCl_3$, then 10:1 $CHCl_3$–MeOH, and 5:1 $CHCl_3$–MeOH) afforded a white amorphous solid (203 mg). The resulting product was dissolved in dry MeOH (8 mL) under nitrogen; after cooling to 0 °C a 1 M solution of MeONa in MeOH (400 μL) was added and the reaction mixture was stirred at rt for 7 h. The solution was percolated on a column (1 × 10 cm) filled with Dowex 50 WX8 (H^+ form) resin and the eluate was concentrated and purified by flash chromatography (5:8:0.7 MeOH–$CHCl_3$–water). The fractions containing the product were concentrated to a volume of 3 mL and percolated on a Dowex 50 WX8 (Na^+ form) column (1 × 10 cm). The resulting eluate was finally concentrated to dryness affording **16** (117 mg, 81%), as a white amorphous solid: $[\alpha]_D^{20}$ −11.5° (*c* 0.6, water); ^{1}H NMR (D_2O, 500 MHz): δ 7.51–7.42 (m, 5 H, H_{Ar}), 4.94 (d, 1 H, *J* 11.7 Hz, C*H*HPh), 4.77 (d, 1 H, C*H*HPh), 4.57 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.48 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1′), 4.21 (appearing as a d, 2 H, *J* 6.2 Hz, H-6′a, H-6′b), 4.00 (dd, 1 H, $J_{4',5'} < 2$ Hz, H-4′), 3.99–3.98 (m, 2 H, H-6a, H-5′), 3.81 (dd, 1 H, $J_{5,6b}$ 5.1, $J_{6a,6b}$ 12.4 Hz, H-6b), 3.69 (dd, 1 H, $J_{2',3'}$ 10.0, $J_{3',4'}$ 3.5 Hz, H-3′), 3.64 (m, 3 H, H-3, H-4, H-5), 3.56 (dd, 1 H, H-2′), 3.38 (t, 1 H, $J_{2,3}$ 9.4 Hz, H-2); ^{13}C NMR (D_2O, 125.72 MHz): δ 105.9 (C-1′), 103.9 (C-1), 82.3 (C-4), 77.5 (C-5), 77.3 (C-3), 75.6 (C-2), 75.7 (C-5′), 75.2 (C-3′), 74.3 (*C*H_2Ph), 73.5 (C-2′), 71.1 (C-4′), 70.0 (C-6′), 63.1 (C-6). Anal. Calcd for $C_{19}H_{27}NaO_{14}S$ (534.465): C, 42.70; H, 5.09. Found: C, 42.75; H, 5.17.

6-O-Sulfo-β-D-galactopyranosyl-(1→4)-D-glucopyranose sodium salt (**17**).—A solution of **16** (25 mg, 0.046 mmol) in 3:1 MeOH–water (4 mL) was hydrogenolysed in the presence of 10% Pd/C for 16 h, (TLC 6:3:1 acetone–BuOH–water). The mixture was filtered through Celite and freeze-dried. The residue was loaded onto a Sephadex G 10 column ($V_0 = 17$ mL, $V_t = 23$ mL) and eluted with 1:9 EtOH–water. The fraction containing the product were collected and freeze-dried affording **17** (18 mg, 88%) as a white powder: $[\alpha]_D^{20}$ +29.4° (*c* 1.0, water); ^{1}H NMR (D_2O, 200 MHz): δ 5.28 (d, 0.28 H, $J_{1d,2d}$ 3.4 Hz, H-1α), 4.72 (d, 0.7 H, $J_{1e,2e}$ 7.9 Hz, H-1β), 4.57 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.27 (appearing as a d, 2 H, H-6′a, H-6′b), 4.06–3.56 (m, 9.3 H, H-2α, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′), 3.36 (br t, 0.7 H, $J_{2,3}$ 9.1 Hz, H-2β); ^{13}C NMR ($CDCl_3$, 75.44 MHz): 103.9 (C-1′), 96.5 (C-1β), 92.6 (C-1α), 80.4, 80.2, 75.5, 75.2, 74.6, 73.7, 73.2, 72.3, 72.0, 71.6, 70.9, 69.1, 68.2 (C-6′), 61.1 (C-6α), 61.0 (C-6β). Anal. Calcd for $C_{12}H_{21}NaO_{14}S$ (444.342): C, 32.44; H, 4.76. Found: C, 32.49; H, 4.72.

Benzyl (3-O-sulfo-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside sodium salt (**18**).—Compound **1** (218 mg, 0.50 mmol) and Bu_2SnO (135 mg, 0.54 mmol) were stirred in refluxing dry MeOH (4 mL) for 2 h under nitrogen. The reaction mixture was concentrated to dryness and the dry dibutylstannylene intermediate was treated with SO_3NMe_3 complex (144 mg, 1.0 mmol) in dry dioxane (4 mL) at rt. After 48 h, the reaction was diluted with MeOH (3 mL), filtered and concentrated. The residue was then purified by flash chromatography (5:8:1 MeOH–$CHCl_3$–water); the fractions containing the product were concentrated, dissolved in MeOH and percolated into a cation exchange resin column (Dowex 50 WX8, H^+ form, 1 × 10 cm). The eluate was concentrated and percolated on a Dowex 50 column (Na^+ form, 1 × 10 cm) affording, after removal of the solvents, **18** (200 mg, 75%) as a white amorphous solid: $[\alpha]_D^{20}$ −17.2° (*c* 1.0, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 4.95 (d, 1 H, *J* 11.4 Hz, C*H*HPh), 4.78 (d, 1 H, C*H*HPh), 4.58 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.57 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.34 (dd, 1 H, $J_{2',3'}$ 9.8, $J_{3',4'}$ 3.3 Hz, H-3′), 4.30 (d, 1 H, $J_{4',5'} < 1$ Hz, H-4′), 4.01 (dd, 1 H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 12.4 Hz, H-6a), 3.84 (dd, 1 H, $J_{5,6b}$ 5.2 Hz, H-6b), 3.78 (m, 1 H, H-5′), 3.75 (m, 2 H, H-6′a, H-6′b), 3.70 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.70 (t, 1 H, H-2′), 3.64 (t, 1 H, $J_{3,4}$ 8.9 Hz, H-3), 3.59 (ddd, 1 H, H-5), 3.37 (t, 1 H, $J_{2,3}$ 9.2 Hz, H-2); ^{13}C NMR (D_2O, 125.72 MHz): δ 105.4 (C-1′), 103.9 (C-1), 82.9 (C-3′), 81.3 (C-4), 77.7 (C-5′), 77.6 (C-5), 77.2 (C-3), 75.8 (C-2), 74.3 (*C*H_2Ph), 71.9 (C-2′), 69.6 (C-4′), 63.7 (C-6′), 62.9 (C-6). Anal. Calcd for $C_{19}H_{27}NaO_{14}S$ (534.465): C, 42.70; H, 5.09. Found: C, 42.77; H, 5.12.

3-O-Sulfo-β-D-galactopyranosyl-(1→4)-D-glucopyranose sodium salt (**19**).—Compound **18** (40 mg, 0.075 mmol) was submitted to the same procedure described for **17**, affording **19** (32 mg, 96%) as a white powder: $[\alpha]_D^{20}$ +40.7° (*c* 1.1, water). ^{1}H and ^{13}C NMR data are in agreement with those reported in the literature for the natural compound.[6]

Benzyl 2,4,6-O-tri-O-acetyl-3-O-methoxycarbonylmethyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**23**).—Compound **1** (200 mg, 0.46 mmol) and Bu_2SnO (125 mg, 0.50 mmol) were stirred in refluxing dry MeOH (4 mL) under N_2 for 16 h. The reaction mixture was concentrated to dryness and the dibutylstannylene intermediate, after coevaporation with toluene, was dissolved in dry DMF (4 mL) under Ar and treated with ethyl bromoacetate (255 μL, 2.3 mmol) and TBAI (cat.) at 40 °C for 48 h (TLC 8:1.5 CH_2Cl_2–MeOH). The reaction mixture was concentrated under diminished pressure and chromatographed to give a pale yellow syrup (181 mg) containing two compounds, namely the expected product **20** and the corresponding lactone derivative **21**. The mixture of

these compounds was dissolved in dry MeOH (4 mL) and treated with a 1 M solution of MeONa (30 μL) in MeOH. After the complete conversion of the faster moving compound into the second (TLC 4:1 CH_2Cl_2–MeOH), the mixture was neutralised with Amberlite IR 120 (H^+ form) and concentrated leading to crude compound **22**. The residue was dissolved in pyridine (4 mL) and treated with Ac_2O (2 mL). After one night, the reaction mixture was concentrated, diluted with CH_2Cl_2 and washed with water. The organic layer was separated, dried over Na_2SO_4 and concentrated. Purification by flash chromatography (1:1 EtOAc–petroleum ether) afforded **23** as a white foam (193 mg, 56% from **1**): $[\alpha]_D^{20}$ −9.6° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 7.40–7.21 (m, 5 H, H_{Ar}), 5.39 (br d, 1 H, $J_{3',4'}$ 3.1 Hz, H-4′), 5.15 (t, 1 H, $J_{3,4} = J_{2,3}$ 9.0 Hz, H-3), 5.00 (t, 1 H, $J_{2',3'}$ 7.9 Hz, H-2′), 4.95 (t, 1 H, $J_{2,3}$ 7.8 Hz, H-2), 4.86 (d, 1 H, *J* 12.3 Hz, C*H*HPh), 4.59 (d, 1 H, C*H*HPh), 4.54 (dd overlapping signal, 1 H, H-6a), 4.52 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.46 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.19–4.08 (m, 5 H, H-6b, H-6a′, H-6b′, OCH_2COO), 3.80 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 3.75 (br t, 1 H, $J_{4',5'} = J_{5',6'}$ 7.0 Hz, H-5′), 3.72 (s, 3 H, $COOCH_3$), 3.63–3.58 (m, 2 H, H-5, H-3′), 2.13 (s, 9 H, 3 CH_3Ac), 2.08, 2.04, 1.98 (3 s, 9 H, 3 CH_3Ac); ^{13}C NMR ($CDCl_3$, 50.29 MHz): δ 170.3, 170.2, 170.0, 169.6 (6 COOAc, 2 overlapping signals), 101.0, 98.9 (C-1, C-1′), 78.3, 76.2, 72.9, 72.7, 71.7, 70.6, 70.5, 65.2 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 70.6 (*C*H_2Ph), 66.0, 62.1, 61.2 (C-6, C-6′, O*C*H_2COO), 51.7 (COO*C*H_3), 20.70–20.59 (CH_3Ac). Anal. Calcd for $C_{34}H_{44}O_{19}$ (756.702): C, 53.97; H, 5.86, Found: C, 54.01; H, 5.81.

3-O-*Carboxymethyl-β*-D-*galactopyranosyl-(1→4)*-D-*glucopyranose sodium salt* (**24**).—To a solution of compound **23** (20 mg, 0.026 mmol) in MeOH (3 mL), a 0.5 M solution of MeONa in MeOH (30 μL) was added and the reaction mixture was stirred at rt for 16 h. Then water (2 mL) and 0.5 M solution of MeONa in MeOH (30 μL) were added, the mixture was stirred for additional 20 h at rt, then concentrated under diminished pressure. The residue was dissolved in 1:1 MeOH–water and hydrogenolysed in the presence of 10% Pd/C for 16 h, (TLC 6:3:1 acetone–BuOH–water). The mixture was filtered through Celite and freeze-dried. The residue was dissolved in 0.1 M NaOH (0.5 mL), loaded onto a Sephadex G 10 column ($V_0 = 17$ mL, $V_t = 23$ mL) and eluted with 1:9 EtOH–water. The fraction containing the product were collected and freeze-dried giving **24** (9 mg, 91%) as a white powder: $[\alpha]_D^{20}$ +59.1° (*c* 0.5, water); ^1H NMR (D_2O, 300 MHz): δ 5.26 (d, H 0.3, $J_{1\alpha,2}$ 3.8 Hz, H-1α), 4.70 (d, H 0.7, $J_{1\beta,2}$ 7.9 Hz, H-1β), 4.52 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.11 (br s, 3 H), 4.06–3.56 (m, 10.3 H), 3.32 (bt, 0.70 H, *J* 8.3 Hz, H-2β); ^{13}C NMR (D_2O, 75.44 MHz): 179.2 (COO^-), 103.6 (C-1′), 96.6 (C-1β), 92.6 (C-1α), 82.7, 79.1, 79.0, 75.9, 75.6, 75.1, 74.6, 73.5, 72.2, 72.0, 70.9, 70.7, 66.2 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′,C-5′ α and β), 69.3 (O*C*H_2COO^-), 61.1 (C-6′), 60.9 (C-6β), 60.8 (C-6α). Anal. Calcd for $C_{14}H_{23}NaO_{13}$ (422.314): C, 39.82; H, 5.49. Found: C, 39.80; H, 5.57.

Benzyl 6-O-*thexyldimethylsilyl-β*-D-*galactopyranosyl-(1→4)-β*-D-*glucopyranoside* (**25**).—Compound **1** (200 mg, 0.46 mmol) and Bu_2SnO (125 mg, 0.50 mmol) were stirred in refluxing dry MeOH (4 mL) for 16 h. The reaction mixture was concentrated to dryness and the dibutylstannylene intermediate was dissolved in dry THF (3 mL) and treated with TDSCl (102 μL, 0.52 mmol) at rt for 18 h. After adding some drops of TEA, the solution was concentrated under diminished pressure. Purification by flash chromatography (95:5 then 9:1 CH_2Cl_2–MeOH) afforded **25** (201 mg, 76%). In order to allow an unambiguous identification of the product, a portion of **25** (50 mg) was submitted to standard acetylation (Ac_2O, Py) and characterised. $[\alpha]_D^{20}$ −29.4° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz): δ 7.38–7.22 (m, 5 H, H_{Ar}), 5.39 (br d, 1 H, $J_{3',4'}$ 3.1 Hz, H-4′), 5.14 (t, 1 H, $J_{3,4} = J_{2,3}$ 9.3 Hz, H-3), 5.08–4.91 (m, 3 H, H-2, H-2′, H-3′), 4.85 (d, 1 H, *J* 12.3 Hz, C*H*HPh), 4.59 (d, 1 H, C*H*HPh), 4.53–4.45 (m, 3 H, H-1, H-1′, H-6a), 4.10 (dd, 1 H, $J_{6a,6b}$ 11.8, $J_{5,6b}$ 4.8 Hz, H-6b), 3.82 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.70–3.50 (m, 4 H, H-5, H-5′, H-6′a, H-6′b), 2.12, 2.11, 2.03, 2.02, 1.99, 1.95 (6 s, 18 H, 6 CH_3Ac), 1.60 (dq, 1 H, H TDS), 0.91–0.79 (m, 12 H, 4 CH_3, TDS), 0.07, 0.04 (2 s, 6 H, 2 CH_3Si TDS).

Benzyl 2,3,4-tri-O-*benzyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**26**).—To a solution of **25** (165 mg, 0.29 mmol) in dry DMF (2 mL), BnBr (415 μL, 3.49 mmol) and NaH (60% suspension in mineral oil 104 mg, 2.60 mmol) were added, and the reaction mixture was stirred at rt overnight (TLC 1:9 EtOAc–petroleum ether). After quenching the excess of NaH with MeOH, the reaction mixture was concentrated, the residue was diluted with CH_2Cl_2 and the organic phase was washed with water. The organic layer was separated, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0.6:10 EtOAc–petroleum ether) affording a colourless syrup (300 mg). The resulting compound was dissolved in THF (10 mL) and, after cooling the solution to −12 °C, treated with a 1 M TBAF solution (540 μL, 0.54 mmol). The reaction mixture was stirred at rt for 6 h (TLC 2:3 EtOAc–petroleum ether), then the solution was diluted with EtOAc, and washed with satd NH_4Cl. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The crude compound was purified by flash chromatography (3:7 then 2:3 EtOAc–petroleum ether) affording **26** (185 mg, 66%) as a white foam: $[\alpha]_D^{20}$ −12.6° (*c* 1, $CHCl_3$), lit.[22] −14° (*c* 1.29, $CHCl_3$). NMR data for this compound are in agreement with those reported in the literature.[22]

Benzyl 2,3,4-tri-O-benzyl-6-O-tert-butoxycarboxymethyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**27**).—To a solution of compound **26** (120 mg, 0.12 mmol) in CH_2Cl_2 (0.2 mL), *tert*-butyl bromoacetate (377 μL, 1.47 mmol), Bu_4NHSO_4 (51 mg, 0.16 mmol) and 33% aq NaOH (0.8 mL) were added and the reaction mixture was stirred at rt for 4 h (TLC 2:3 EtOAc–petroleum ether). The reaction mixture was diluted with water and extracted with CH_2Cl_2, the organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The crude compound was purified by flash chromatography (1:9 then 1:4 EtOAc–petroleum ether) affording **27** (129 mg, 96%) as a colourless syrup: $[\alpha]_D^{20}$ + 3.2° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.00 (d, 1 H, *J* 10.7 Hz, C*H*HPh), 4.98 (d, 1 H, *J* 11.8 Hz, C*H*HPh), 4.94 (d, 1 H, *J* 12.1 Hz, C*H*HPh), 4.90 (d, 1 H, *J* 10.9 Hz, C*H*HPh), 4.80 (d, 1 H, *J* 11.2 Hz, C*H*HPh), 4.75 (d, 1 H, C*H*HPh), 4.74 (d, 1 H, C*H*HPh), 4.73 (d, 1 H, *J* 11.8 Hz, C*H*HPh), 4.69 (d, 1 H, C*H*HPh), 4.65 (d, 1 H, C*H*HPh), 4.63 (d, 1 H, C*H*HPh), 4.55 (d, 1 H, *J* 12.1 Hz, C*H*HPh), 4.48 (d, *J* 7.3 Hz, H-1 or H-1′), 4.42 (d, *J* 6.9 Hz, H-1 or H-1′), 4.41 (d, 1 H, C*H*HPh), 4.15–3.90 (m, 2 H), 3.83–3.70 (m, 4 H), 3.65 (d, 1 H, *J* 16.4 Hz, OC*H*HCOO), 3.57–3.29 (m, 8 H), 1.44 (s, 9 H, 3 CH_3*t*-Bu); ^{13}C NMR ($CDCl_3$, 75.44 MHz): δ 169.4 (COO*t*-Bu), 102.8, 102.5 (C-1, C-1′), 83.0, 82.5, 81.9, 80.0, 76.7, 75.2, 73.5, 73.0 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 75.3, 75.2, 75.0, 64.7, 73.2, 72.6, 70.9, 69.3, 69.0, 68.3 (7 *C*H_2Ph, C-6, C-6′, O*C*H_2COO*t*-Bu), 60.3 (Cq *t*-Bu), 28.1 (CH_3*t*-Bu). Anal. Calcd for $C_{67}H_{74}O_{13}$ (1087.297): C, 74.01; H, 6.86. Found: C, 74.09; H, 6.89.

6-O-Carboxymethyl-β-D-galactopyranosyl-(1→4)-D-glucopyranose sodium salt (**28**).—To a solution of **27** (49 mg, 0.045 mmol) in CH_2Cl_2, 10% CF_3COOH in CH_2Cl_2 (mL) was added. The reaction mixture was stirred at rt for 30 min (TLC 3:7 EtOAc–petroleum ether) and concentrated to dryness. The obtained compound was dissolved in 1:1 EtOAc–MeOH and submitted to hydrogenolysis in the presence of 10% $Pd(OH)_2$/C for 72 h (TLC 6:3:1 acetone–BuOH–water). The mixture was filtered through Celite and freeze-dried. The residue was dissolved in 0.1 M NaOH (0.5 mL), loaded onto a Sephadex G 10 column ($V_0 = 17$ mL, $V_t = 23$ mL) and eluted with 1:9 EtOH–water. The fraction containing the product were collected and freeze-dried giving **28** (18 mg, 95%) as a white powder: $[\alpha]_D^{20}$ + 24.2° (*c* 0.6, water); ^{1}H NMR (D_2O, 300 MHz): δ 5.35 (d, 0.3 H, $J_{1\alpha,2}$ 3.8 Hz, H-1α), 4.81 (H-1β), 4.58 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.17–3.65 (m, 13.3 H), 3.42 (t, 0.7 H, *J* 8.4 Hz, H-2β);^{13}C NMR (D_2O, 75.44 MHz): δ 178.7 (COO^-), 103.8 (C-1′), 96.6 (C-1β), 92.7 (C-1α), 79.8, 79.7, 75.7, 75.3, 74.7, 74.2, 72.3, 72.1, 71.8, 69.6 (C-2, C-3, C-4, C-5, C-1′, C-2′, C-3′, C-4′, C-5′ α and β), 70.9, 70.6 ($C$$H_2COO^-$, C-6′), 61.1 (C-6β), 61.0 (C-6α). Anal. Calcd for $C_{14}H_{23}NaO_{13}$ (422.314): C, 39.82; H, 5.49. Found: C, 39.88; H, 5.56.

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