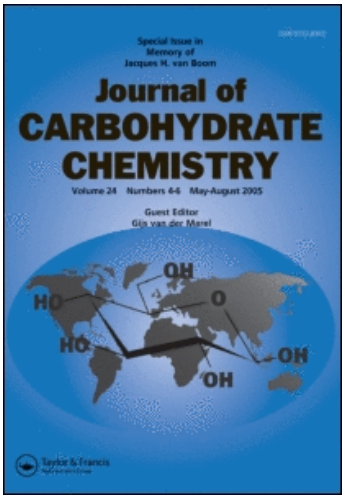

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EASY CHEMO-ENZYMATIC SYNTHESIS OF HUMAN MILK TRISACCHARIDES FROM A COMMON SELECTIVELY PROTECTED LACTOSE BUILDING BLOCK

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ABSTRACT

Three glycosyllactosides, contained in the neutral fraction of human milk oligosaccharides, were synthesised in a simple and straightforward manner through a sequence based on a chemo-enzymatic approach. Lipase catalysed regioselective 6'-*O*-acylation of benzyl β-lactoside, followed by the introduction of an isopropylidene group and acetylation afforded, depending on the reaction conditions, compounds **4a** and **4b**, which allow selective access to positions 3, 3' and 6'. Glycosylation with proper donors gave trisaccharides **6**, **9** and **12**.

INTRODUCTION

In recent years there has been a growing interest in the use of lipases and proteases both for hydrolysis and transesterification reactions as a tool for protection and deprotection of carbohydrates.[1] In many cases the enzymatic method showed noteworthy

regioselectivity, often offering much simpler experimental conditions with respect to the corresponding regioselective chemical acylation (if the latter is feasible at all).

Despite the large number of examples of such reactions on different mono- and disaccharides published so far,[2-6] the enzymatically protected sugars have rarely been utilised for the synthesis of more complex derivatives.

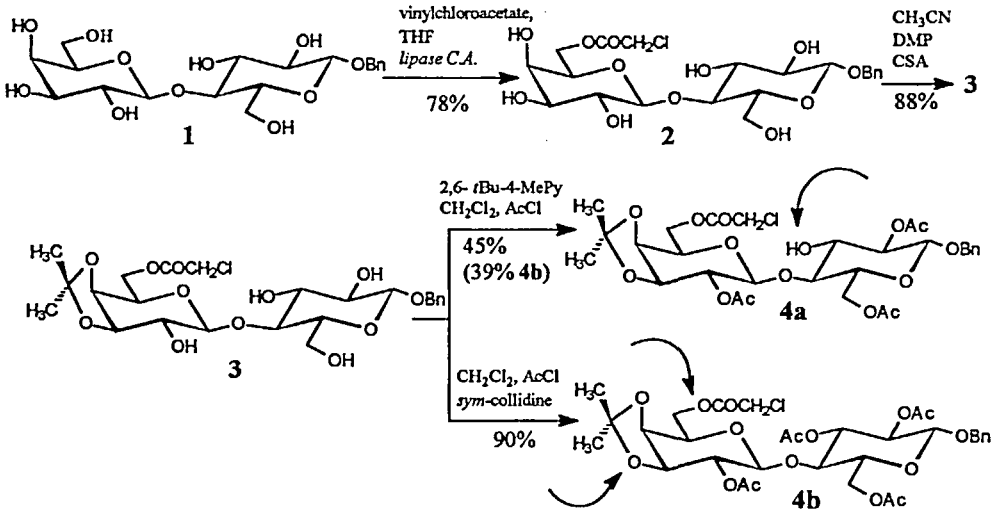
We recently gained interest in human milk oligosaccharides, when it has been demonstrated that these compounds, most of which are unique to the human species, possess antiadhesive properties and inhibitory activity towards many pathogens, representing a protection against infections for breast-fed infants during the lactation period.[7] It could then be of great importance to have access to such compounds to be used as additives in artificial milk for infants or aged people.

However, it is still unclear which of the above oligosaccharides exert this function. It will therefore be useful to have a flexible method for an easy access to different oligosaccharidic structures from common building blocks. We recently published a study on regioselective enzymatic acylation of common, commercially available disaccharides such as cellobiose, maltose and lactose.[8] As lactose is the most abundant component and a fundamental constituent of almost all the milk oligosaccharidic structures characterised so far, we focussed our attention on benzyl lactoside as a substrate for enzymatic acylation, showing that it is possible to selectively and sequentially acylate the hydroxy groups in position 6' and 2' with two different esters.

Exploiting some of the results previously described,[8] we planned a chemo-enzymatic approach aimed at obtaining versatile building blocks for the preparation of some human milk oligosaccharidic structures. The philosophy of such an approach consists in exploring a way to generate a family of oligosaccharides for biological testing in a reasonable amount of time instead of optimisation of a synthetic scheme for the preparation of specific target molecules. Using an appropriate pattern of protecting groups, introduced both with chemical and enzymatic methods, it is possible in principle to have selective access to each hydroxy group of lactose. As an example of such a strategy, we wish now to describe the synthesis of three lactose derived trisaccharides contained in the neutral fraction of human milk oligosaccharides. Antiadhesion studies on these compounds, after their complete deprotection, will be reported in due course.

RESULTS AND DISCUSSION

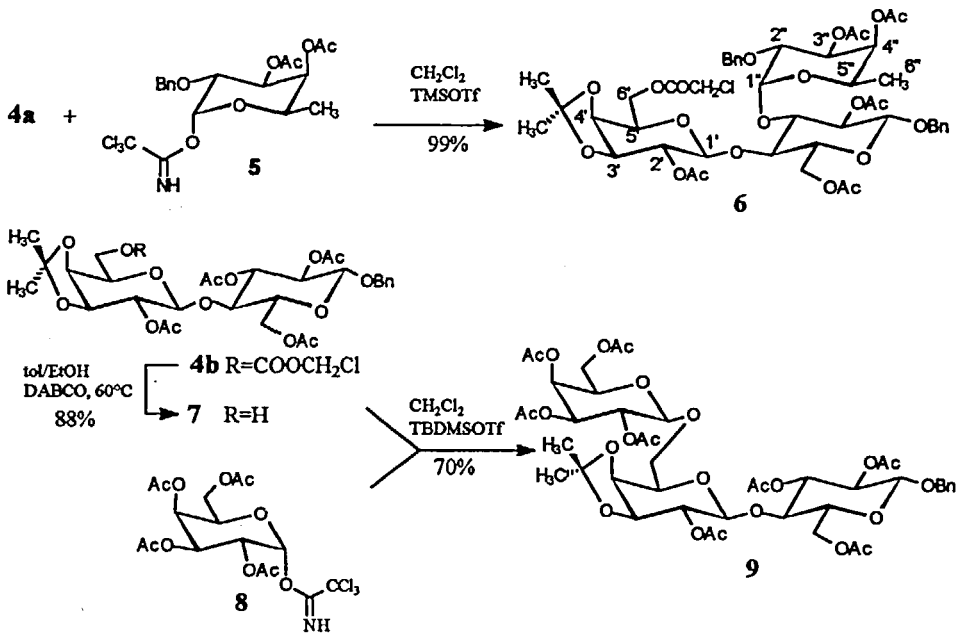
As mentioned above, we reported the highly regioselective 6'-*O*-acylation of benzyl β-lactoside **1** using lipase from *Candida antarctica*.[8] The reaction was carried out in *tert*-amyl alcohol with trifluoroethyl chloroacetate as acylating agent and afforded benzyl 6'-*O*-chloroacetyl-β-lactoside **2** in 81% yield. The choice of *tert*-amyl alcohol was determined by the necessity to solubilize the substrate, but the high boiling point of this solvent makes the work-up of the reaction and the purification of the product a laborious task, especially in light of a possible large scale synthesis. These difficulties and the observation of the activity of these lipases in other solvents,[9] suggested that we perform the reaction under different conditions. Using THF as solvent we obtained the desired selectively acylated benzyl 6'-*O*-chloroacetyl-β-lactoside **2** in comparable yield (78%) and with a much easier procedure for the recovery of the product (Scheme 1).



Scheme 1

Positions 3',4' of compound **2** were then protected by the introduction of an isopropylidene group; subsequent acetylation of compound **3** afforded the triacetylated compound **4a** and the tetraacetylated compound **4b**, depending on the reaction conditions. As a matter of fact, when acetyl chloride and 2,6-di-*tert*-butyl-4-methyl-

pyridine were used, an easily separable mixture of compounds **4a** (45%) and **4b** (39%) was obtained. In contrast, carrying out the reaction with acetyl chloride in the presence of *sym*-collidine gave the fully acetylated product, and compound **4b** was recovered in 90% yield. Compounds **4a** and **4b** give selective access to positions 3, 3' and 6' (Scheme 1).

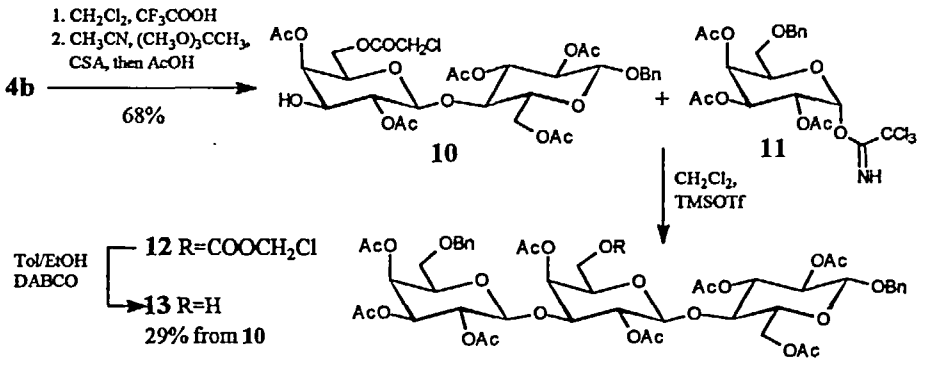


Scheme 2

Protected 3-*O*-fucosyl lactose **6** (Scheme 2) was obtained in excellent yield (99%) by fucosylation of compound **4a** with donor **5**,[10] using TMSOTf (0.01 eq) in CH_2Cl_2 at 0 °C. The newly synthesized glycosidic linkage was confirmed as having an α configuration by ^1H NMR spectroscopy; the signal corresponding to H-1" appeared as a doublet at 5.31 ppm ($J_{1,2}$ = 4.0 Hz), as expected for a 1,2-*cis* linkage.

Chemoselective removal of the 6'-*O*-chloroacetyl group from compound **4b** (DABCO, 87% yield) gave compound 7 which was then glycosylated with galactosyl trichloroacetimidate **8**[11] to afford the protected 6'-*O*-galactosyl lactose **9** (Scheme 2, 71% yield). The ^1H NMR spectrum of trisaccharide **9** showed a doublet at 4.64 ppm ($J_{1,2}$ = 7.9 Hz), confirming the β configuration of the newly formed glycosidic linkage. Finally, hydrolysis of the isopropylidene group on compound **4b**, followed by regioselective 4'-

O-acetylation, led to acceptor **10** (Scheme 3). Glycosylation of **10** with donor **8** gave unexpectedly poor yields of the trisaccharide, in spite of many attempts with different Lewis acids (TMSOTF or $BF_3{\cdot}OEt_2$, from –20 to 0 °C). Using the more reactive donor **11**,[12] trisaccharide **12** was obtained. However, the presence of an inseparable by-product prevented compound **12** from being isolated in pure enough form for full characterization. Removal of the chloroacetyl group from **12** afforded trisaccharide **13** (29% overall yield from compound **10**). The structure of trisaccharide **13** was ascertained by ^1H NMR spectroscopy; H-1" showed a doublet at 4.48 ppm ($J_{1,2}$ = 7.8 Hz), as expected for a 1,2-*trans* (β) linkage.



Scheme 3

The lipase catalyzed regioselective acylation will be further investigated on disaccharides others than lactose, in order to obtain building blocks suitable for the synthesis of higher human milk oligosaccharides.

EXPERIMENTAL

General methods. Melting points are uncorrected. Optical rotations were measured with a Perkin Elmer 241 digital polarimeter. NMR spectra were recorded on Varian XL200 (200 MHz for ^1H and 50.29 MHz for ^{13}C) and Bruker AC300 (300 MHz for ^1H and 75.44 MHz for ^{13}C) spectrometers. Chemical shifts are expressed in parts per million downfield from TMS. In ^{13}C NMR spectra description, the signals corresponding to aromatic carbons are omitted. Reactions were followed on TLC using silica gel $60F_{254}$

(E. Merck); flash column chromatography was performed on silica gel 60 (0.040-0.063 mm, E. Merck). Lipase from *Candida antarctica* was purchased from Boehringer Mannheim (Chirazyme® L-2, c.-f. C2 lyo).

Benzyl (6-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (2). Benzyl β-D-lactoside **1**[13] (1.57 g, 3.63 mmol) was suspended in dry THF (100 mL). Vinyl chloroacetate (2 mL, 19.75 mmol) and lipase from *Candida antarctica* (1 g) were added, the suspension was stirred mechanically for 48 h at rt and monitored by TLC (EtOAc/MeOH/H_2O 8:1.5:0.5 v/v). The enzyme was filtered, and the solvent was removed under reduced pressure; purification by flash chromatography (eluent ethyl acetate/methanol 10:1.5 v/v) afforded compound **2** as a white solid (1.44 g, 78% yield): mp 71-75 °C; $[\alpha]_D$ -10.1° (*c* 1.1, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 3.33 (m, 1H, H-5'), 3.43 (m, 1H, H-5), 3.46-3.59 (m, 4H, H-2, H-3, H-4, H-2'), 3.85 (dd, 1H, $J_{6a,5}$ = 4.2 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 3.86-3.94 (m, 2H, H-3', H-4'), 3.93 (dd, 1H, $J_{5,6b}$ = 2.3 Hz, H-6b), 4.24 (d, 1H, J = 15.5 Hz, CHCl), 4.30 (d, 1H, CHCl), 4.34 (dd, 1H, $J_{5,6'a}$ = 4.3 Hz, $J_{6'a,6'b}$ = 11.4 Hz, H-6'a), 4.37 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.39 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.41 (dd, 1H, $J_{5,6'b}$ = 8.5 Hz, H-6'b), 4.67 (d, 1H, J = 11.8 Hz, C*H*Ph), 4.91 (d, 1H, C*H*Ph), 7.26-7.48 (m, 5H, H_{Ar}); ^{13}C NMR (50.29 MHz, CD_3OD) δ 41.85 (t, CH_2Cl), 62.15, 66.24, 71.90 (3t, C-6, C-6', CH_2Ph), 70.10, 72.24, 74.10, 74.61, 74.85, 76.39, 76.39, 81.95 (8d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 103.2, 105.3 (2d, C-1, C-1'), 169.2 (s, *C*O).

Anal. Calcd for $C_{21}H_{29}O_{12}Cl$ (508.91): C, 49.56; H, 5.74; Cl, 6.97. Found: C, 49.50; H, 5.77; Cl, 6.92.

Benzyl (6-*O*-chloroacetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (3). Compound **2** (620 mg, 1.21 mmol) was dissolved in dry CH_3CN (7 mL) under an inert atmosphere, then dimethoxypropane (451 μL, 3 equiv) and a catalytic amount of CSA was added. The reaction was monitored by TLC (eluent ethyl acetate/methanol 10:1.5 v/v); after 1 h the solution was neutralized with $NaHCO_3$ and the solvent was removed under reduced pressure. Chromatographic purification (eluent ethyl acetate) afforded compound **3** as a white foam (584 mg, 88% yield); $[\alpha]_D$ +7.7° (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 1.34, 1.50 (2s, 6H, $C(CH_3)_2$), 3.32 (m, 1H, H-5'), 3.43 (m, 1H, H-5), 3.43-3.57 (m, 4H, H-2, H-3, H-4, H-2'), 3.82 (dd, 1H, $J_{6a,5}$ = 4.1 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.91 (dd, 1H, $J_{5,6b}$ = 2.1 Hz, H-6b), 4.08 (bt, 1H, $J_{2',3'} = J_{3',4'}$

= 6.1 Hz, H-3'), 4.21-4.25 (m, 2H, H-4', H-6'a), 4.29 (s, 2H, CH_2Cl), 4.37 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.39 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.41 (bd, 1H, $J_{6'a,6'b}$ = 6.3 Hz, H-6'b), 4.66 (d, 1H, J = 11.8 Hz, C*H*Ph), 4.91 (d, 1H, C*H*Ph), 7.25-7.46 (m, 5H, H_{Ar}); ^{13}C NMR (75.44 MHz, CD_3OD) δ 26.80, 28.65 (2q, *C*H_3C), 41.97 (t, *C*H_2Cl), 62.26, 66.13, 72.16 (3t, C-6, C-6', *C*H_2Ph), 72.48, 74.43, 75.05, 75.18, 76.56, 76.56, 81.18, 82.35 (8d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 103.42, 104.57 (2d, C-1, C-1'), 111.72 (s, *C*$(CH_3)_2$), 169.4 (s, *C*O).

Anal. Calcd for $C_{24}H_{33}O_{12}Cl$ (548.97): C, 52.51; H, 6.06; Cl, 6.46. Found: C, 52.49; H, 6.00; Cl, 6.41.

Benzyl (2-*O*-acetyl-6-*O*-chloroacetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-acetyl-β-D-glucopyranoside (4a). Compound **3** (272 mg, 0.49 mmol) was dissolved in dry CH_2Cl_2 (5 mL) under an inert atmosphere, then 2,6-di-*tert*-butyl-4-methyl pyridine (1.63 g, 7.92 mmol) and acetyl chloride (352 μL, 494 mmol) were added in two portions over 24 h. After 48 h the reaction was quenched with a saturated solution of $NaHCO_3$ (5 mL) and the organic layer was washed with HCl 5% and then with water. It was dried over Na_2SO_4 and the solvent removed under reduced pressure; purification of the crude by flash chromatography (eluent petroleum ether/ethyl acetate = 1:1 v/v) afforded compound **4a** as an amorphous white solid (152 mg, 45% yield) and compound **4b** also as an amorphous white solid (138 mg, 39% yield). **4a**: $[\alpha]_D$ –2.0° (*c* 1.5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30, 1.56 (2s, 6H, C(CH_3)$_2$), 2.12 (s, 3H, OAc), 2.14 (s, 6H, 2 OAc), 3.43-3.62 (m, 2H, H-4, H-5), 3.69 (bt, 1H, $J_{2,3}$ = $J_{3,4}$ = 8.6 Hz, H-3), 4.08 (dd, 1H, $J_{5,6a}$ = 4.5 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.16 (s, 2H, CH_2Cl), 4.11-4.26 (m, 3H, H-3', H-4', H-5'), 4.36 (dd, 1H, $J_{5,6b}$ = 1.9 Hz, H-6b), 4.39 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 4.46 (bd, 1H, $J_{6'a,6'b}$ = 12.3 Hz, H-6'a), 4.47 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.50 (bd, 1H, H-6'b), 4.61 (d, 1H, 12.2 Hz, C*H*Ph), 4.88 (d, 1H, C*H*Ph), 4.95 (dd, 1H, $J_{2',3'}$ = 9.2 Hz, H-2'), 4.99 (bt, 1H, H-2), 7.25-7.45 (m, 5H, H_{Ar}); ^{13}C NMR (75.44 MHz, $CDCl_3$) δ 21.39 (3q, *C*H_3CO), 26.84, 28.05 (2q, *C*H_3C), 41.19 (t, *C*H_2Cl), 63.24, 64.99, 71.21 (3t, C-6, C-6', *C*H_2Ph), 71.72, 72.46, 73.08, 73.08, 73.50, 73.87, 77.84, 83.24 (8d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 99.86, 102.0 (2d, C-1, C-1'), 111.9 (s, *C*$(CH_3)_2$), 167.9, 170.3, 170.3, 171.2 (4s, *C*O).

Anal. Calcd for $C_{30}H_{39}O_{15}Cl$ (675.08): C, 53.38; H, 5.82; Cl, 5.25. Found: C, 53.45; H, 5.80; Cl, 5.15.

For characterization of **4b**, see below.

Benzyl (2-*O*-acetyl-6-*O*-chloroacetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (4b). Compound **3** (540 mg, 0.98 mmol) was dissolved in dry CH_2Cl_2 (10 mL) under inert atmosphere, then *sym*-collidine (1.1 mL, 7.84 mmol) and acetyl chloride (490 μL, 6.86) were added. The reaction was monitored by TLC (eluent petroleum ether/ethyl acetate = 5:7 v/v). After 10 h *sym*-collidine (2.2 mL, 15.68 mmol) and acetyl chloride (980 μL, 13.72 mmol) were added and the solution was left stirring for 48 h. The reaction was quenched with a satured solution of $NaHCO_3$ (5 mL) and the organic layer was washed with 5% HCl, then with water, dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude by flash chromatography (eluent petroleum ether/ethyl acetate = 6:4, then 1:1 v/v) afforded compound **4b** as a glassy solid (630 mg, 90% yield); $[\alpha]_D$ -8.4° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31, 1.53 (2s, 6H, C(CH_3)$_2$), 2.00, 2.07, 2.08, 2.12 (4s, 12H, OAc), 3.59 (ddd, 1H, $J_{5,6b}$ = 2.0 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{5,4}$ = 9.5 Hz, H-5), 3.82 (bt, 1H, H-4), 3.78-3.95 (m, 4H, H-3', H-4', H-5', H-6'a), 4.10-4.16 (m, 4H, H-6a, H-6'b, CH_2Cl), 4.39 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 4.49 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.51 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.58 (d, 1H, J = 12.3 Hz, C*H*Ph), 4.83 (m, 1H, H-2'), 4.84 (d, 1H, C*H*Ph), 4.97 (bt, 1H, H-2), 5.12 (t, 1H, $J_{2,3}$ = 9.1 Hz, H-3), 7.22-7.38 (m, 5H, H_{Ar}); ^{13}C NMR (75.44 MHz, $CDCl_3$) δ 20.62, 20.80 (4q, CH_3CO), 26.04, 27.24 (2q, CH_3C), 40.61 (t, CH_2Cl), 62.23, 64.50, 70.70 (3t, C-6, C-6', CH_2Ph), 70.61, 71.71, 72.41, 72.65, 72.82, 72.82, 75.77, 76.77 (8d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 99.11, 100.2 (2d, C-1, C-1'), 110.9 (s, C(CH_3)$_2$), 167.1, 169.2, 169.5, 169.9, 170.4 (5s, CO).

Anal. Calcd for $C_{32}H_{41}O_{16}Cl$ (717.12): C, 53.60; H, 5.76; Cl, 4.94. Found: C, 53.63; H, 5.74; Cl, 4.90.

Benzyl (2-*O*-acetyl-6-*O*-chloroacetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2,6-di-*O*-acetyl-β-D-glucopyranoside (6). Compound **4a** (100 mg, 0.148 mmol) was dissolved under an inert atmosphere in dry CH_2Cl_2 (2 mL). At 0 °C TMSOTf (16 μL of a 0.1M solution in dry CH_2Cl_2, 0.0016 mmol) was added. Compound **5** dissolved in dry CH_2Cl_2 (1 mL) was added dropwise to the solution. After 30 min the reaction was neutralized with $NaHCO_3$ and chromatographic purification (eluent petroleum ether/ethyl acetate = 1:1 v/v) of the crude afforded pure compound **6** as a white foam (148 mg, 99% yield): $[\alpha]_D$ -50.1° (*c* 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (d, 3H, $J_{5'',6''}$ = 6.6 Hz, H-6''),

1.31, 1.50 (2s, 6H, C(CH_3)$_2$), 1.91, 1.96, 2.07, 2.10, 2.13 (5s, 15H, OAc), 3.47 (m, 1H, H-5), 3.82-3.96 (m, 2H, H-2'', H-4'), 3.89 (t, 1H, $J_{3,4} = J_{4,5} = 8.5$ Hz, H-4), 3.98 (t, 1H, $J_{2,3} = 8.5$ Hz, H-3), 3.89-4.08 (m, 1H, H-5'), 4.09-4.17 (m, 4H, H-6a, H-3', CH_2Cl), 4.28 (d, 1H, $J_{1',2'} = 8.6$ Hz, H-1'), 4.42 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1), 4.46 (d, 1H, J = 11.5 Hz, C*H*Ph), 4.53 (d, 1H, J = 12.5 Hz, C*H*Ph), 4.62 (d, 1H, C*H*Ph), 4.51-4.64 (m, 2H, H-6b, H-6'a), 4.72 (dd, 1H, $J_{5',6'b} = 8.0$ Hz, $J_{6'a,6'b} = 11.9$ Hz, H-6'b), 4.83 (d, 1H, C*H*Ph), 4.81-4.93 (m, 2H, H-2', H-5''), 5.15 (bt, 1H, H-2), 5.24 (bs, 1H, H-4''), 5.27 (m, 1H, H-3''), 5.31 (d, 1H, $J_{1'',2''} = 4.0$ Hz, H-1''), 7.18-7.40 (m, 10H, H_{Ar}); ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 15.84 (q, C-6''), 20.63, 20.77, 20.97 (3q, 5C, CH_3CO), 26.09, 27.56 (2q, CH_3C), 40.90 (t, CH_2Cl), 61.63, 64.01, 70.13, 72.61 (4t, C-6, C-6', CH_2Ph, CH_2Ph), 64.25, 70.00, 70.65, 71.85, 72.73, 73.23, 73.23, 73.35, 74.00, 74.32, 74.51, 77.18 (12 d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2'', C-3'', C-4'', C-5''), 96.34, 98.96, 100.2 (3d, C-1, C-1', C-1''), 110.9 (s, $C(CH_3)_2$), 167.7, 168.8, 169.1, 169.6, 170.5, 170.5 (6s, *C*O).

Anal. Calcd for $C_{47}H_{59}O_{21}Cl$ (995.43): C, 56.71; H, 5.97; Cl, 3.56. Found: C, 56.68; H, 6.01; Cl, 3.55.

Benzyl (2-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (7). Compound **4b** (88 mg, 0.12 mmol) was dissolved in toluene/ethanol = 1:1 v/v (8 mL), then DABCO (100 mg, 8 equiv) was added, and the solution was stirred at 60 °C. After 30 min the reaction mixture was concentrated and chromatographic purification (eluent petroleum ether/ethyl acetate = 4:6 v/v) afforded compound **7** as a white foam (68 mg, 88% yield): $[\alpha]_D$ -9.9° (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30, 1.52 (2s, 6H, C(CH_3)$_2$), 1.99, 2.06, 2.08, 2.11 (4s, 12H, OAc), 2.50 (bs, OH), 3.58 (ddd, 1H, $J_{5,6b} = 1.9$ Hz, $J_{5,6a} = 5.1$ Hz, $J_{5,4} = 9.4$ Hz, H-5), 3.82 (t, 1H, $J_{3,4} = 9.4$ Hz, H-4), 3.76-3.95 (m, 3H, H-5', H-6'a, H-6'b), 4.00-4.29 (m, 3H, H-3', H-4', H-6a), 4.38 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'), 4.49 (dd, 1H, $J_{6a,6b} = 12.2$ Hz, H-6b), 4.50 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 4.52 (d, 1H, J = 12.3 Hz, C*H*Ph), 4.85 (m, 1H, H-2'), 4.85 (d, 1H, C*H*Ph), 4.97 (dd, 1H, $J_{2,3} = 9.4$ Hz, H-2), 5.12 (t, 1H, H-3), 7.26-7.48 (m, 5H, H_{Ar}); ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 20.58, 20.78 (4q, CH_3CO), 26.12, 27.33 (2q, CH_3C), 62.10, 62.27, 70.65 (3t, C-6, C-6', CH_2Ph), 71.58, 72.68, 72.97, 72.97, 73.60, 73.60, 75.67, 77.06 (8d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 99.02, 100.3 (2d, C-1, C-1'), 110.8 (s, $=C(CH_3)_2$), 169.2, 169.6, 170.3, 170.4 (4s, *C*O).

Anal. Calcd for $C_{30}H_{40}O_{15}$ (640.64): C, 56.25; H, 6.29. Found: C, 56.28; H, 6.32.

Benzyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-(2-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (9). Compound **7** (50 mg, 0.078 mmol.) and compound **8** (77 mg, 0.156 mmol) were dissolved in dry CH_2Cl_2 (3 mL) under an inert atmosphere. TBDMSOTf (39 μL of a 0.1 M solution in dry CH_2Cl_2, 0.0039 mmol.) was then added. After 1 h the reaction was quenched with TEA and the solvent removed under reduced pressure. Chromatographic purification (eluent petroleum ether/ethyl acetate = 6:4, then 1:1 v/v) afforded compound **9** as a white foam (53 mg, 70% yield): $[\alpha]_D$ -13.3° (*c* 1.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29, 1.52 (2s, 6H, $C(CH_3)_2$), 1.92, 1.98, 2.02, 2.04, 2.07, 2.11, 2.15, 2.17 (8s, 24H, OAc), 3.57 (ddd, 1H, $J_{5,6b}$ = 1.4 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,4}$ = 9.5 Hz, H-5), 3.78 (t, 1H, $J_{3,4}$ = 9.5 Hz, H-4), 3.77-3.83 (m, 2H, H-6'a, H-6'b), 3.94 (bt, 1H, $J_{5'',6''}$ = 6.6 Hz, H-5''), 4.00-4.22 (m, 6H, H-6a, H-3', H-4', H-5', H-6''a, H-6''b), 4.36 (d, 1H, $J_{1',2'}$ = 7.1 Hz, H-1'), 4.44 (dd, 1H, $J_{6a,6b}$ = 11.9 Hz, H-6b), 4.51 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.59 (d, 1H, J = 12.3 Hz, C*H*Ph), 4.64 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1''), 4.81 (m, 1H, H-2'), 4.84 (d, 1H, C*H*Ph), 4.95 (bd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 5.07 (dd, 1H, $J_{3'',4''}$ = 3.4 Hz, $J_{2'',3''}$ = 10.4 Hz, H-3''), 5.13 (t, 1H, H-3), 5.21 (dd, 1H, H-2''), 5.40 (d, 1H, H-4''), 7.23-7.35 (m, 5H, H_{Ar}); ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 20.70 (1q, 8C, CH_3CO), 26.00, 27.21 (2q, CH_3C), 61.10, 62.22, 68.11, 70.70 (4t, C-6, C-6', C-6'', CH_2Ph), 67.02, 68.82, 70.71, 71.45, 72.61, 72.61, 72.61, 72.61, 72.93, 73.13, 75.32, 76.60 (12d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2'', C-3'', C-4'', C-5''), 99.13, 100.3, 100.8 (3d, C-1, C-1', C-1''), 110.7 (s, $C(CH_3)_2$), 169.1, 169.4, 169.4, 169.6, 169.9, 170.1, 170.2, 170.5 (8s, *C*O).

Anal. Calcd for $C_{44}H_{58}O_{24}$ (970.93): C, 54.43; H, 6.02. Found: C, 54.45; H, 5.99.

Benzyl (2,4-di-*O*-acetyl-6-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (10). Compound **4b** (100 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (3 mL) and 50% aq CF_3COOH was added. The reaction was left stirring at 50 °C and after 2 h was neutralized with a saturated solution of $NaHCO_3$. The organic layer was washed with water then dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was dissolved in dry CH_3CN under an inert atmosphere and $(CH_3O)_3CCH_3$ (48 μL, 0.42 mmol.) and a catalytic amount of CSA was added. After 10 min AcOH 80% was added, and the reaction was left stirring for 30 min. The reaction was diluted with CH_2Cl_2 and neutralized with a saturated solution of $NaHCO_3$, the organic layer was washed with water then dried over Na_2SO_4,

and the solvent was removed under reduced pressure. Chromatographic purification (eluent petroleum ether/ethyl acetate = 3:7 v/v) afforded 68 mg (68% yield) of compound **8** as a glassy solid: $[\alpha]_D$ -20.6° (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.03, 2.05, 2.11, 2.14, 2.17 (5s, 15H, OAc), 2.74 (bs, OH), 3.60 (ddd, 1H, $J_{5,6b}$ = 1.9 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{5,4}$ = 9.9 Hz, H-5), 3.78 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, $J_{2',3'}$ = 10.0 Hz, H-3'), 3.81 (bt, 1H, H-4), 3.85 (bt, 1H, $J_{5',6'}$ = 6.4 Hz, H-5'), 4.08 (s, 2H, C*H*$_2$Cl), 4.10 (dd, 1H, $J_{6a,6b}$ = 12.6 Hz, H-6a), 4.14-4.20 (m, 2H, H-6'a, H-6'b), 4.43 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.52 (dd, 1H, H-6b), 4.53 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.59 (d, 1H, J = 12.4 Hz, C*H*Ph), 4.85 (dd, 1H, H-2'), 4.86 (d, 1H, C*H*Ph), 4.95 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 5.14 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 9.2 Hz, H-3), 5.27 (d, 1H, H-4'), 7.24-7.37 (m, 5H, H_{Ar}); ^{13}C NMR (50.29 MHz, $CDCl_3$) δ 20.74 (5q, *C*H$_3$CO), 40.44 (t, *C*H$_2$Cl), 62.10, 62.97, 70.71 (3t, C-6, C-6', *C*H$_2$Ph), 69.09, 71.22, 71.22, 71.51, 72.61, 72.61, 72.77, 76.02 (8d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 93.99, 100.5 (2d, C-1, C-1'), 166.9, 169.5, 169.9, 170.5, 170.6, 170.9 (6s, *C*O).

Anal. Calcd for $C_{31}H_{39}O_{17}Cl$ (719.09): C, 51.78; H, 5.47; Cl, 4.93. Found: C, 51.80; H, 5.45; Cl, 4.98.

Benzyl (2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(2,4-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (13). Compound **10** (190 mg, 0.26 mmol) and compound **11** (226 mg, 0.42 mmol) were dissolved in dry CH_2Cl_2 (2 mL) under an inert atmosphere. Then TMSOTf (39 μL of a 0.1 M solution in dry CH_2Cl_2, 0.05 equiv) was added. After 1 h the reaction was neutralized with $NaHCO_3$ and the solvent removed under reduced pressure. The crude was partially purified by flash chromatography (eluent petroleum ether/ethyl acetate = 4:6 v/v). The fractions containing the product were dissolved in EtOH/toluene = 1:1 (4 mL) and a catalytic amount of DABCO was added. After 6 h the solvent was removed and chromatographic purification (eluent petroleum ether/ethyl acetate 1:1, then 4:6 v/v) afforded compound **13** as a glassy solid (77 mg, 29% yield): $[\alpha]_D$ -15.4° (*c* 1.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94, 1.99, 1.99, 2.01, 2.07, 2.08, 2.12, 2.16 (8s, 24H, OAc), 2.82 (bt, OH), 3.33-3.66 (m, 5H, H-4, H-5, H-5', H-6'a, H-6'b), 3.74-3.81 (m, 2H, H-3', H-5''), 4.08 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 4.11 (dd, 1H, $J_{5'',6a''}$ = 7.1 Hz, $J_{6''a,6''b}$ = 14.1 Hz, H-6''a), 4.36 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.42 (d, 1H, J = 12.0 Hz, C*H*Ph), 4.42-4.54 (m, 2H, H-6b, H-6''b), 4.48 (d, 1H, $J_{1'',2''}$ = 7.8 Hz, H-1''), 4.49

(d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.52 (d, 1H, C*H*Ph), 4.57 (d, 1H, J = 12.3 Hz, C*H*Ph), 4.83 (d, 1H, C*H*Ph), 4.91 (dd, 1H, $J_{3'',4''}$ = 3.7 Hz, $J_{2'',3''}$ = 10.4 Hz, H-3''), 4.93 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 5.04 (dd, 1H, H-2''), 5.10 (t, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.11 (t, 1H, $J_{2',3'}$ = 8.1 Hz, H-2'), 5.24 (d, 1H, $J_{3',4'}$ = 3.3 Hz, H-4'), 5.40 (d, 1H, H-4''), 7.22-7.39 (m, 10H, H_{Ar}); ^{13}C NMR (75.44 MHz, $CDCl_3$) δ 20.46, 20.56, 20.69, 20.78 (4q, 8C, CH_3CO), 60.18, 62.16, 67.47, 70.70, 73.59 (5t, C-6, C-6', C-6'', CH_2Ph, CH_2Ph), 67.35, 68.80, 70.01, 70.87, 71.05, 71.74, 72.43, 72.67, 73.36, 74.00, 76.03, 76.12 (12d, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2'', C-3'', C-4'', C-5''), 99.01, 101.0, 101.4 (3d, C-1, C-1', C-1''), 168.6, 169.1, 169.5, 169.8, 170.0, 170.1, 170.4, 172.2 (8s, *C*O).

Anal. Calcd for $C_{48}H_{60}O_{24}$ (1020.99): C, 56.47; H, 5.92. Found: C, 56.43; H, 5.90.

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