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PII: S0008-6215(19)30068-0

DOI: https://doi.org/10.1016/j.carres.2019.03.006

Reference: CAR 7688

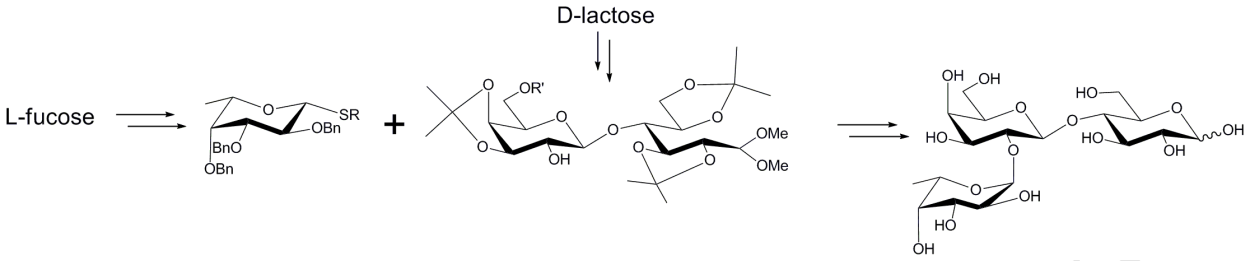
To appear in: *Carbohydrate Research*

Received Date: 29 January 2019

Revised Date: 12 March 2019

Accepted Date: 14 March 2019

Please cite this article as: K. Agoston, M.J. Hederos, I. Bajza, G. Dekany, Kilogram scale chemical synthesis of 2′-fucosyllactose, *Carbohydrate Research* (2019), doi: https://doi.org/10.1016/j.carres.2019.03.006.



Kilogram scale chemical synthesis of 2'-fucosyllactose

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KEYWORDS: oligosaccharide synthesis, carbohydrates, Human milk oligosaccharides, fucosylation, fucosyllactose

ABSTRACT: A scalable synthetic procedure to high quality 2`-fucosyllactose, the most abundant oligosaccharide in human breast milk, has been designed and validated in kilogram scale. The synthetic route has been developed to suit industrial environment and contains only a single chromatographic purification step.

1. INTRODUCTION

A correlation between infant health and breastfeeding has been undoubtful since the very first use of human milk substitutes developed by Justus von Liebig [1]. The health benefits of natural human milk over the synthetic substitutes are claimed to be the reduced number of bacterial and

viral infections in the breastfed infants. The specific protecting agents in human milk responsible for these effects are considered to be multiple such as secreted antibodies [2], enzymes such as lactoferrin or lysozyme [3] and/or oligosaccharides, so-called human milk oligosaccharides (HMOs) [4]. The mode of action of these oligosaccharides are both their prebiotic activity [5] and ability to bind to the host-cell receptors of the pathogens inhibiting the adhesion onto the host cells [6]. To date more than 140 different HMOs have been identified in human milk and this number is still increasing [7]. One of the most important HMOs is 2'-fucosyllactose (2'-FL), generally accepted as the HMO in highest concentration in mothers milk [8]. Several biological effects of 2'-FL have been suggested including but not limited to its prebiotic-, antibacterial- and antiviral effects [6] - making it an attractive target for nutritional applications. Since the publication [9] of the first chemical synthesis of 2'-FL several other chemical-, [10] chemo-enzymatic- [11] and enzymatic [12] approaches have been published, all together suffering from the limitation to offer a feasible route for large-scale synthesis of 2'-FL. In principle, all chemical and chemo-enzymatic synthesis contains multiple chromatographic purifications, which makes them unsuitable beyond laboratory scale. Sole enzymatic syntheses require the UDP sugars as glycosyl donors or genetically modified organisms as "catalyst" for the syntheses. Neither of these possibilities provides an unambiguous solution yet although, certain biotechnological methods have recently proven to be commercially attractive solutions [13]. However, chemical synthesis can provide suitable amount target compound within short time period if scale-up difficulties can be solved. Furthermore, chemical synthesis provides higher purity product for biological studies including well-defined impurity profile. In the chemical synthesis of 2'-FL the two most critical problems are purification of intermediates by column chromatography and the final isolation of the end-product.

Typically, in the literature wherein articles are claiming “large scale” synthesis of an oligosaccharide, the quantities of the target compound in the ends only milligrams (enzymatic methods [14]) or in max. ~10 g scale (chemical methods [15]). Within our earlier program and efforts to access high purity and large quantities (> 1 kg) of 2’-FL to drive pre-clinical / clinical programs and regulatory processes - we have extensively investigated the possibilities of the chemical synthesis of 2’-FL in kilogram scale. Similar to the results described herein has - to our best knowledge - never been published providing the targeted oligosaccharide in such a scale and purity.

2. RESULTS AND DISCUSSION

As starting point, our synthetic plan for the preparation of the glycosyl acceptor relied on an earlier published route [10b] where a lactose acceptor can be generated in two steps from commercially available lactose. In contrast to the 1-*O*-nitrophenyl fucose donor used for the synthesis [10b] we have selected 1-*S*-phenyl donor which can be readily prepared from L-fucose in four steps (Figure 1). One key focus area was to identify a 6’-*O* protecting group on the lactose acetonide acceptor with the ambition to obtain a crystalline acceptor with the option of purification of the crude glycosyl acceptor before the glycosylation step in large scale. Numerous different acyl- and alkyl groups (including but not limited to Ac, Bz, Piv, Lev, ClAc, Bn, Tr) were tried, but none of them provided a crystalline lactose acceptor. Finally and as a compromise, the choice fall on the 6’-O-pivaloate ester since it could be introduced with very high selectively (6’ >> 2’) and opened up the possibility of the use of the crude acceptor (**3, R= Piv)** directly in the glycosylation, even though purification by crystallization was not performed.

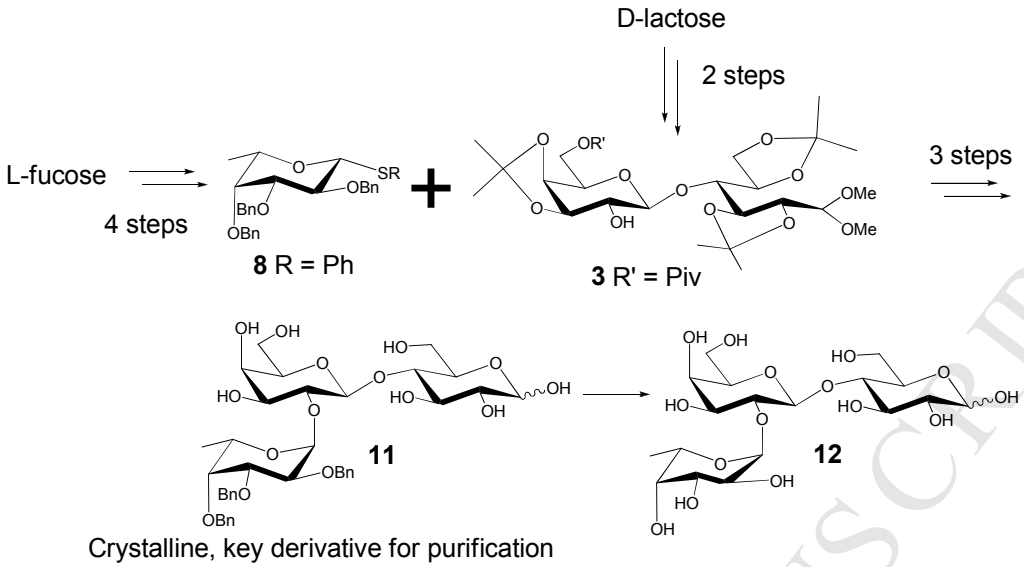


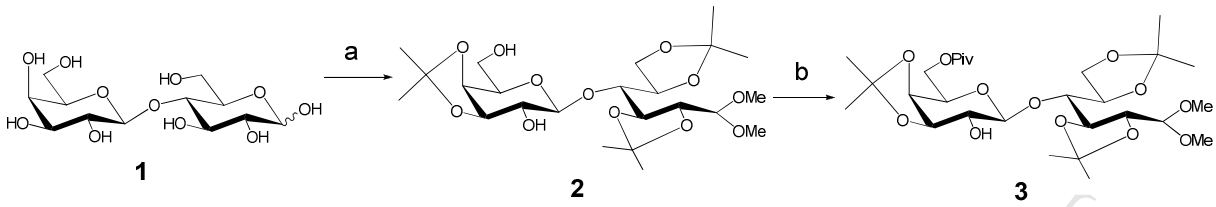
Figure 1. The synthetic route to 2'-FL

On the donor side several crystalline fucosyl donors have been published but in our hands - the thiophenyl derivative (**8, R= Ph**) was providing the best yields. For the activation of the glycosyl donor the traditional Lemieux`s method was selected because of the requirement of mild glycosylation reaction conditions due to the acid labile protecting groups. Direct activation of the donor using thiophyl promoters were considered, but not implemented at this stage. After the glycosylation three different type of protecting groups needed to be removed, i.e. acetals, esters and benzylic groups. From literature [9] it is known that one of the possible final intermediates, compound (**11**), is a crystalline compound. The glycosylation product compound (**9**) and partially deprotected compound (**10**) (See them below in Scheme 3) turned out not to be crystalline – even after purification and isolation by flash chromatography these two compounds failed to be crystallized. We anticipated that performing three consequently steps in a row (*i.e.* glycosylation, basic ester- and acidic acetal cleavages) and purifying afterwards would be very challenging, but not impossible due to the big difference in the polarity of the compounds in the

crude mixture. For example, all carbohydrate impurities derived from the fucosyl donor should still be very apolar due to the three benzyl groups, while the carbohydrate contaminants from the acceptor molecule would be very polar upon losing all the protecting groups. In that manner we assumed the polarity of the trisaccharide derivative (**11**) to be significantly different from above mentioned impurities which were targeted to be removed by means of extractive work-up. Another impurity to be eliminated is the β-2'-anomer of 2'-FL that can be formed during glycosylation and would render the β-2' stereoisomer of (**11**) after deprotection. The glycosylation method chosen is known to give about 10% beta isomer [16], which possibly could be removed at the stage of crystallization of final intermediate (**11)**. Final product (**12**) has been described as a solid when isolated from natural sources [17] and there is no reason to believe that the synthetic product would be different.

2.1. Synthesis of lactose acceptor

For the preparation of the glycosyl acceptor anhydrous lactose was used as a starting material in laboratory scale. In one step all hydroxyl functions but two were protected as acetonide ketal, or as dimethyl-acetal (**1**→**2**, Scheme 1). Compound **2** was isolated from the crude reaction mixture by crystallization with the yield of 65%. Upon execution in industrial scale a few parameters were revised; lactose monohydrate was used as starting material for the reaction due to the availability and price figure of anhydrous lactose. Furthermore, the concentration was increased by two-fold and the solvent used for the crystallization was changed from diethyl ether into MTBE as safety precaution. With these process adaptions the overall yield of the reaction dropped by 13 %, but still acceptable (52%).



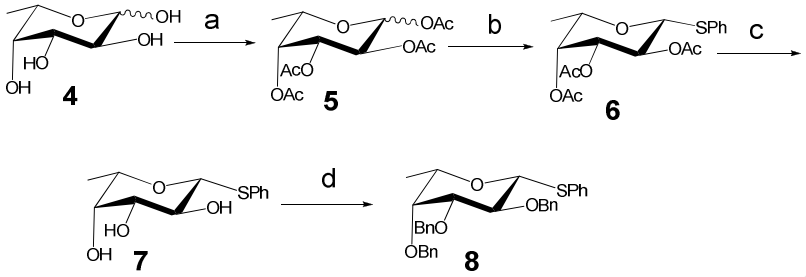
Scheme 1. Preparation of the glycosyl acceptor.

a) 2,2-Dimethoxypropane, pTsOH, reflux; b) Pivaloyl chloride, pyridine, DCM, -5 °C.

As discussed earlier, the pivaloyl ester was used to protect the 6' hydroxyl function of the lactose acetonide. The reaction was carried out in DCM using pyridine as base and pivaloyl chloride as acylating agent. Compound **3** was purified by column chromatography both in laboratory- and pilot scale (1 kg). In order to investigate the industrial feasibility of the whole process, a parallel experiment was done in pilot scale (~500 g) whereupon compound **3** was not purified by column chromatography using the crude reaction mixture directly in the glycosylation. This test batch was processed further to the final intermediate stage **11** to see the impact on eliminating the only chromatographic purification in the whole production, e.g. recovery yield, final intermediate product purity and process parameters such as extraction work-up performance.

2.2. Synthesis of fucosyl donor

The preparation of the glycosyl donor was done in four steps from commercially available L-fucose (Scheme 2). Both in laboratory and pilot scale the first three steps were done in "one pot". Conventional acetylation (**4**→**5**) followed by the introduction of the thiophenyl leaving group (**5**→**6**) and the catalytic removal of the acetate esters (**6**→**7**) were done in the same way both in laboratory and pilot scale. Only the solvent used for the crystallization of compound **7** was changed but had no significant influence on the product recovery yield.



Scheme 2. Preparation of the glycosyl donor.

a) Ac_2O, pyridine, 0 °C; b) HSPh, BF_3/Et_2O, DCM, 0 °C; c) NaOMe, MeOH; d) BnBr, NaH, DMF; or BnBr, KO*t*Bu, THF, 10 °C.

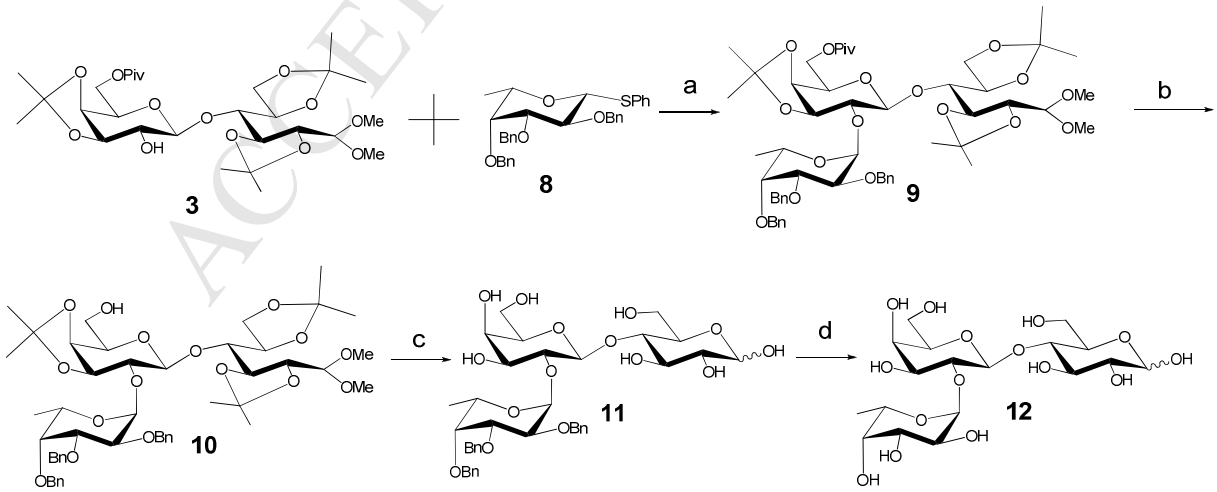
The benzylation of compound **7** was performed in DMF using NaH as base and benzyl bromide as alkylating agent in laboratory scale. The glycosyl donor (**8**) was isolated by crystallization from the mixture of Et_2O and hexane. In pilot scale the base has been changed to KO*t*Bu and the solvent to THF resulted in safer reaction conditions. The crystallization of the product was achieved from a mixture of EtOAc, MeOH and water allowing further simplification on the work-up procedure. The isolated yield of compound **8** in pilot scale was not significantly lower than in laboratory scale with the advantage of avoiding the use of sodium hydride [72% vs 67%].

2.3. Glycosylation and deprotection to final intermediate

For all the glycosylations conducted in pilot scale only crystalline donor compound **8** was used. In terms of the preparation of acceptor, two different qualities of acceptor were used. In all glycosylations conducted but one, the flash chromatographed and purified acceptor **3** was used. In one case and in parallel, the crude and non-purified (only extractive work-up) was used in the glycosylation with donor **8**. Typically, in a glycosylation sequence, compounds **9** and **10** – as being syrups – were only isolated in small scale for analytical purposes. At the stage of

compound **11** the preparative purification was conducted and the overall yields were determined. It was also at this stage the yields of using different quality of acceptors were compared. As a result of the low yield of using crude acceptor for the glycosylation only the experiment using the purified acceptor was scaled up to kg level.

In Scheme 3 is depicted the conditions for the glycosylation and deprotection to final end-product 2'-FL. The overall yield of the three steps procedure from glycosylation to compound **11** was 27.3% in the scale of 600 g of compound **3** or 19.8% in the scale of 5.0 kg of compound **3** In both cases the acceptor was purified by column chromatography before the glycosylation step. This equals to the yield of ~70% per step, which was acceptable considering the difficult glycosylation step. In case of using non-purified acceptor (**3c**) the isolated yield of compound **11** dropped to 8.5% over 4 steps (including the pivaloylation step). This means that the average yield is down to 54% per step, which was below our expectation. Clearly it would be a huge advantage to eliminate all chromatographic purifications, but the price paid for that is too high in this process. At this stage of the scale-up we still wanted to keep the last remaining chromatographic purification as a part of the overall synthetic procedure.



Scheme 3. Synthesis of 2`-FL.

a) i: Br_2, DCM, 5 °C, ii: TBABr, DMF, rt.; b) NaOMe, MeOH; c) AcOH, H_2O, 65 °C; d) Pd/C, H_2, IPA, MeOH, AcOH, water.

The reaction conditions used for all experiments (glycosylations and deprotections) were identical. First, compound **8** was dissolved in DCM and converted into bromosugar by adding bromine at 5 °C in DCM. The excess of Br_2 was neutralized by the addition of cyclohexene to the mixture. The solution of acceptor (**3**) and tetrabutylammonium bromide in DMF were added to the crude solution of the bromosugar. The mixture was stirred for 2 days and after work-up procedure the pivaloate ester was removed in MeOH using NaOMe, followed by the cleavage of the acetonide groups and the dimethyl-acetal with 60% acetic acid in water at elevated temperature. All the impurities derived from the acceptor were removed in this stage by extraction. Compound **11** was isolated by crystallization from EtOAc and purified further by re-crystallization from MeOH. The final intermediate was isolated with the yield of 67-73% per step by using the chromatographed acceptor whilst the use of “crude” acceptor resulted in 54% yield per step. No beta anomer (2’-β-fucosyl-lactose) was identified in either of the final intermediates by NMR or HPLC methods.

2.4. Final deprotection and isolation of targeted compound

The final deprotection step has been done in the mixture of isopropanol/methanol/water/acetic acid to provide solubility for both the starting compound and for the product. The benzyl groups were removed by catalytic hydrogenation using Pd/C and H_2. During the scale-up the concentration of the reaction mixture was increased, but that resulted in significantly slower reaction. Most probably the large amount of toluene being formed in the reaction afforded the aggregation of the catalyst and addition of fresh catalyst was necessary to drive the reaction to

completion. The final compound was isolated by precipitation using TBME in methanol. In Pilot scale a trituration in isopropanol was added to the procedure at the final stage to remove the solvent residues. 2`-Fucosyllactose was isolated in kilogram scale as a white amorphous solid and fully characterized by NMR and analyzed by HPLC and LCMS.

3. CONCLUSION

A kilogram scale preparation of 2`-fucosyllactose has been performed by synthesis of a fucosyl donor in ~10 kg scale and a lactose acceptor in ~1 kg scale. The developed route involves only one purification step using column chromatography contrary to the previously published routes which involve 4 to 8 chromatographic purification for the same target. At intermediate scale the synthesis has been conducted omitting chromatographic purification, but the overall yield turned out to be too low. For the glycosylation Lemieux`s method has been selected to provide high selectivity under mild reaction condition. The purification by crystallization of the final intermediate was the key for the success of synthesis. After the final hydrogenation step the product was isolated as an amorphous solid with high purity. The developed and presented procedure could provide the baseline for the synthesis of 2'-fucosyllactose in industrial scale.

4. EXPERIMENTAL

4.1. **General Methods**: Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. Pilot scale synthesis for compounds **2**, **3** and **9-12** were carried out in ERCOM Kft, Hungary and for compounds **5-8**

were carried out in NITRO Kft, Hungary. Both places the reactions were performed in 20-50 L double jacketed glass reactors except compound **2** which was prepared in 3000 L enameled reactor. NMR spectra were recorded on a Bruker AMX-400 (100.62 MHz for ^{13}C) or DRX-500 (125.83 MHz for ^{13}C) spectrometer using $CDCl_3$, DMSO-d_6, and D_2O as solvents. All chemical shifts are quoted in ppm downfield using solvent peaks as references ($CDCl_3$: ^{1}H: 7.26 ppm, ^{13}C: 77.1 ppm, DMSO-d_6: ^{1}H: 2.50 ppm, ^{13}C: 39.4 ppm, D_2O: ^{1}H: 4.79 ppm). Kieselgel 60 (E. Merck, Darmstadt, Germany) was used for column chromatography. Optical rotations were measured on a Perkin Elmer 241 polarimeter and the concentrations are given in units of g 100 mL^{-1}. $[\alpha]_D^{25}$ values are given in units of 10^{-1} deg cm^2 g^{-1}

4.2. *Compound **2***

4.2.1 *Laboratory scale*: *p*-Toluenesulphonic acid monohydrate (3.0 g, 16 mmol) was added to a suspension of anhydrous lactose (100 g, 0.29 mol) in 2,2-dimethoxypropane (700 mL). The mixture was heated up to reflux and stirred at reflux temperature for 1½ h. The mixture was cooled to RT and evaporated quickly at 40°C. The residue was dissolved in DCM (1 L) and washed thoroughly with water (4 × 500 mL) and with $NaHCO_3$ solution (saturated, 500 mL), dried ($MgSO_4$), filtered and evaporated in *vacuo* to give a yellow oil. The residue was dissolved in 230 ml Et_2O and a seeding crystal was added, left at 5°C overnight to crystallize. The crystals were filtered and washed with cold Et_2O (2 × 50 mL) to give 96.5 g white solid (yield: 65 %).

4.2.2 *Industrial scale*: Lactose monohydrate (328 kg, 0.91 kmol) was added to a solution of *p*TsOH (3.2 kg, 16.8 mol) in 2,2 dimethoxy-propane (975 kg). The suspension was warmed up to reflux temperature (85 ^{o}C mantle, 63.2 ^{o}C inner) and after this temperature was reached the mixture was stirred for 2 h. at reflux. Then the mixture was cooled down to 20-25 ^{o}C (inner

temperature) and at that temperature the volatile components were distilled off in *vacuo*. (614 kg solvents were collected). DCM (2160 kg) was added to the residue and extracted with water (3 × 1000 kg) then with a solution of $NaHCO_3$ (70 kg) in water (1000 kg) and finally with water (1000 kg). The organic phase was concentrated in *vacuo*. (1595 kg solvents were collected, at 40 °C mantle temperature). To the residue MTBE (370 kg) was added and the mixture was homogenized at 25 °C than cooled down to 0 °C (inner temperature) and stirred in this temperature for 8 h. The formed crystals were separated by centrifugation (each centrifuge batch was washed with 14 kg MTBE) affording 243 kg (yield: 52%) dry crystals. mp 131-133 °C, lit [18] mp 133-134 °C; $[\alpha]_D^{25}$ +38.8 (*c* 1, $CHCl_3$), lit [18] $[\alpha]_D^{25}$ +39.1 ($CHCl_3$); ^{1}H NMR. ($CDCl_3$, 400 MHz) δ: 4.56 (dd, 1 H, $J_{1,2}$ 6.7 Hz, $J_{2,3}$ 8.0 Hz, H-2), 4.39 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1´), 4.33 (d, 1 H, H-1), 4.28 (m, 1 H, H-5´), 4.16 and 3.98 (each m, 2 H, H-6), 4.02 (m, 1 H, H-5), 3.94 (m, 1 H, H-4´), 3.92 and 3.65 (each m, 2 H, H-6`), 3.86 (m, 1 H, H-3), 3.74 (m, 1 H, H-4), 3.48 (m, 1 H, H-3´), 3.47 (m, 1 H, H-2´), 3.45 and 3.45 (2 s, each 3 H, 2 × OMe), 1.46, 1.46, 1.35, 1.35, 1.28 and 1.27 (6 s, each 3 H, 6 × -CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 110.4, 109.8 and 108.2 (3 × -C(CH_3)$_2$), 107.0 (C-1), 103.3 (C-1´), 79.3 (C-5), 78.1 (C-3), 77.4 (C-5´), 75.7 (C-4´), 75.2 (C-2), 74.5, 74.0 and 73.4 (C-4, C-2´and C-3´), 64.4 and 62.3 (C-6 and C-6´), 57.4 and 54.3 (2 × OMe), 28.0, 27.0, 26.1, 26.1, 25.5 and 23.8 (6 × -CH_3). Anal. Calcd for $C_{23}H_{40}O_{12}$: C, 54.32; H, 7.93. Found: C, 54.37; H, 7.91.

4.3. *Compound **3***

4.3.1. *Laboratory scale*: Pyridine (16.4 mL, 202 mmol) was added to a solution of compound **2** (15 g, 29.5 mmol) in DCM (105 mL). The mixture was cooled down to -5 °C and pivaloyl chloride (5.7 mL, 46.3 mmol) in DCM (5 mL) was added and stirred for 15 h at -5 °C. MeOH (6

mL) was added to the mixture and stirred for 30 min. then extracted with water (75 mL), 1 N HCl solution (2 × 75 mL), water (75 mL) and with $NaHCO_3$ solution (75 mL). The organic phase was dried, filtered and concentrated. The crude mixture was purified by column chromatography (PE:EtOAc, 3:2) giving 13.8 g white foam (**3a**, 79%). $[\alpha]_D^{25}$ +35.4 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ: 4.44 (d, 1 H, $J_{1`,2`}$ 8.2 Hz, H-1`), 4.41 (dd, 1 H, $J_{2,3}$ 7.3 Hz, H-2), 4.36 (d, 1 H, $J_{1,2}$ 6.0 Hz, H-1), 4.31 (dd, 1H, $J_{5,6}$ 6.8 Hz, $J_{gem}$ 11.2 Hz, H-6`), 4.28 (m, 1 H), 4.24 (dd, 1 H, $J_{5`,6`}$ 6.3 Hz, J_{gem} 11.1 Hz, H-6`), 4.17 - 4.03 (m, 4 H), 3.99 (dd, 1 H, H-6), 3.95 (ddd, 1 H), 3.91 (dd, 1 H), 3.55 (dd, 1 H), 3.43, 3.42 (2 s, each 3 H, 2 × OMe), 1.50, 1.48, 1.37, 1.37, 1.32, 1.31 (6 s, each 3 H, 6 × -C(C<u>H</u>$_3$)$_2$), 1.20 (s, 9 H, OPiv). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 178.0 (C=O), 110.1, 110.1 and 108.2 (3 × -<u>C</u>$(CH_3)_2$), 104.9 and 103.4 (C1 and C-1'), 78.7, 77.8, 77.7, 77.6, 75.9, 74.9, 74.1 and 72.9 (C-2, 3, 4, 5, 2', 3', 4' and 5'), 64.5 and 62.7 (C-6 and 6') 56.2 and 53.1 (2 × OMe), 38.6, 28.0, 27.1, 27.0, 26.3, 26.1, 25.6, 24.4 and 24.3 (9 × -CH_3). Anal. Calcd for $C_{28}H_{48}O_{13}$: C, 56.74; H, 8.16. Found: C, 56.81; H, 8.19.

4.3.2. *Pilot scale with purification*: Pyridine (1.64 L, 20.2 mol) was added to a solution of compound **2** (1.5 kg, 2.95 mol) in DCM (10 L). The mixture was cooled down to -5 °C and pivaloyl chloride (570 mL, 4.63 mol) in DCM (500 mL) was added and stirred for 15 h between 0 and -5 °C. MeOH (600 mL) was added to the mixture and stirred for 30 min then extracted with water (10 L), 3 N HCl solution (10 L), water (10 L) and with $NaHCO_3$ solution (10 L). The organic phase was dried, filtered and concentrated. The crude mixture was purified by column chromatography (PE:EtOAc, 7:3, on 10 kg silicagel) giving 1.1 kg white foam (**3b**, 63%)

4.3.3. *Pilot scale without purification*: Compound **2** (515g, 1.01 mol) was dissolved in dry DCM (2.5 L) and cooled to -5^{o}C. Then pyridine (556 mL, 6.85 mol) was added at -5^{o}C. To this

solution pivaloyl chloride (200 mL, 1.62 mol) was added over a period of 2.5 h at -5^{o}C (exotermic). During the addition the clear solution turned to an off white suspension. The mixture was stirred at -5^{o}C for 6 h, then warmed up to 20 ^{o}C, and stirred for 12 h. Then the mixture was cooled to 0 ^{o}C, and methanol (200 mL) was added slowly while the suspension turned to clear solution. Then the mixture was allowed to warm up to 20 °C and stirred for 40 min then washed with water (2 × 1.3 L), 1 N HCl (3 × 1.3 L), water (2 × 1.2 L), sat. $NaHCO_3$ (1.5 L) and water (1.5 L). Then the mixture was concentrated to dryness and dry DMF (1.2 L), TBABr (23 g) and toluene (0.5 L) were added and again concentrated to remove the toluene. This mixture (**3c**) was used without any further purification for glycosylation later.

4.4. *Preparation of Compound **8** from **4**.*

4.4.1. *Laboratory Scale*: L-Fucose (**4**) (4.23 g, 25.7 mmol) was suspended in pyridine (20 mL). The mixture was cooled down to 0 °C and Ac_2O (20 mL) was added over a period of 30 min. then allowed to warm up to r.t. and stirred for 10 h. The mixture was concentrated and the residue was dissolved in DCM (150 mL) and washed with 1 N HCl (2 × 100 mL), water (75 mL) and with $NaHCO_3$ solution (75 mL). The organic phase was dried, filtered and concentrated. The crude residue (**5**, 8.45 g, colourless oil) was used for the next reaction.

Thiophenol (3.2 g, 29 mmol) was added to the solution of crude compound **5** (8.45 g, 25.4 mmol) in DCM (20 mL). The mixture was cooled down to 0 °C and BF_3/Et_2O (9.45 mL, 76.6 mmol) was added over a period of 10 min. The mixture was stirred for 2 h at 0 °C and 10 h at 5 °C. Then the mixture was diluted with DCM (200 mL) and washed with water (2 × 100 mL) and $NaHCO_3$ solution (100 mL). The organic phase was dried, filtered and concentrated affording 9.8 g crude product (**6**) as a pale yellow oil.

To the solution of the crude compound **6** (9.8 g) in MeOH (100 mL) NaOMe (150 mg) was added and the mixture was stirred for 6 h at 40 °C. Amberlite IR 120 (H^+) resin was added, stirred and the resin was filtered off. The methanol was removed in *vacuo* affording the crude product (6.25 g, yellow foam). The foam was dissolved in MeCN (30 mL) and washed with petrolether (3 × 60 mL) then the MeCN phase was concentrated and re-dissolved in warm EtOH (50 mL) followed by slow addition of petrolether (200 mL). The suspension was cooled to 0 °C and the formed crystals (**7**) were separated by filtration. (4.03 g, 61%, over 3 steps).

NaH (5.26 g, 109 mmol, 50% in mineral oil) was washed with hexane and suspended in dry DMF (10 mL) then the mixture was cooled down to 0 °C keeping the system all times under Ar. Slowly, over a period of 10 min, a solution of compound **7** (6.2 g, 24.5 mmol) in DMF (20 mL) was added followed by the addition of BnBr (13 mL, 109 mmol). The ice bath was removed and the mixture was stirred under Ar for 10 h. The mixture was cooled to 0 °C and MeOH (8 mL) was added over a period of 10 min. The mixture was stirred for 30 min, then concentrated. The residue was dissolved in DCM (150 mL) and washed with water (3 × 75 mL). The organic phase was dried, filtered and concentrated. The product was isolated by crystallization from the mixture of Et_2O and hexane (18 mL, each) affording a white solid (**8**, 9.1 g, 72%).

4.4.2. *Pilot Scale*: L-Fucose (**4**) (5 kg, 30.5 mol) was suspended in pyridine (12 L) in a 35 L glass reactor. The suspension was cooled down to 0 – 5 °C and Ac_2O (12 L) was added over a period of 3 h. The cooling was stopped and the mixture was allowed to warm up. (The temperature raised to 30 °C over a period of 2 h, then slowly cooled down to 21 °C. (ambient temperature)) The mixture was concentrated on a 20 L rota. The reaction was repeated with a same batch-size once more and the two residues after concentration were combined and

dissolved in DCM (50 L) and washed with 1 N HCl (2 × 20 L), water (20 L) and with $NaHCO_3$ solution (20 L). The organic phase was dried ($MgSO_4$) and filtered resulted in a 60 L paled yellow solution which was used for the next reaction without any further treatment.

Thiophenol (7.8 L, 75.9 mol) was added to the solution of crude compound **5** (60 L pale yellow solution in DCM, estimated 61 mol) in a 400 L enamelled reactor. The mixture was cooled down to 0 °C and BF_3/Et_2O (23 L, 186 mol) was added over a period of 3 h. The mixture was stirred for 8 h keeping the temperature between 0 – 5 °C. Then the mixture was washed with water (2 × 40 L), 1 N NaOH solution (2 × 40 L) and $NaHCO_3$ solution (1 × 20 L). The organic phase was dried, filtered and concentrated affording 25 kg crude product (**6**, yellow oil).

To the solution of the crude compound **6** (25 kg) in MeOH (40 L) NaOMe (300 mL, 30 % solution in MeOH) was added and the mixture was stirred for 6.5 h at 21 °C. Amberlite IR 120 (H^+) resin (5 kg) was added, stirred and the resin was filtered off. The methanol was removed in *vacuo* affording the crude product (14.3 kg, yellow foam). The foam was dissolved in warm EtOH (60 L) followed by slow addition of petrolether (70 L). The suspension was cooled to 0 °C and the formed crystals were separated by filtration (8.2 kg). The mother liquor was concentrated and crystallized from EtOH (18 L) and petrolether (14 L) affording 2.3 kg product (**7**). Total yield: 10.5 kg, 67.2%, over 3 steps.

KO*t*Bu (16 kg, 142.6 mol) was added to a solution of compound **7** (8.2 kg, 32 mol) in THF (60 L) over a period of 3 h in a 400 L enamelled reactor at 0 – 5 °C. After the addition was finished the mixture was stirred for 1 h then BnBr (23.04 kg, 134.8 mol) was added over a period of 4 h keeping the temperature below 5 °C. After the addition was finished the temperature was raised to 10 °C and stirred for 16 h at 10 °C. The mixture was cooled to 0 °C and MeOH (10 L) was

added and the mixture was stirred for 1 h. The mixture was diluted with EtOAc (60 L) and washed with water (2 × 60 L). To the organic phase MeOH (75 L) was added followed by the addition of water (48 L). The formed crystals were separated by filtration (11.2 kg, white solid, 67%) mp: 106-108 °C, $[\alpha]_D^{25}$ -13.8 (*c* 1, $CHCl_3$) mp: lit [19] 107-109 °C, $[\alpha]_D^{25}$ lit [19] -14.0 (*c* 0.7, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz): δ: 7.60-7.18 (m, 20 H, aromatics), 4.83, 4.76 and 4.73 (each ABq, each 2 H, 3 -CH_2Ph), 4.60 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.93 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 3.68 (dd, 1 H, $J_{4,5}$ <1 Hz, H-4), 3.59 (dd, 1 H, $J_{3,4}$ 2.8 Hz, H-3), 3.52 (m, 1H, H-5), 1.26 (d, 3 H, H-6). Anal. Calcd for $C_{33}H_{34}O_4S$: C, 75.83; H, 5.79. Found: C, 75.58; H, 5.76.

4.5. *Compound **9**, Glycosylations:*

4.5.1. *Experiment 1 using 600 g purified acceptor*: Compound **8** (600 g, 1.14 mol) was dissolved in dry DCM (2 L) and the solution was cooled down to 5 ^{o}C (Inner temperature, the cooling liquid was adjusted to 0 ^{o}C). To the solution Br_2 (73 mL, 1.42 mol) in dry DCM (200 mL) was added keeping the temperature between 5 and 10 ^{o}C over a period of 20 min. After the addition was finished the mixture was stirred for 10 min then cyclohexene (130 mL) was added to the mixture over a period of 20 min. keeping the temperature between 5 and 10 ^{o}C. The dark red solution turned to light yellow solution. To this crude solution of the bromosugar, compound **3** (600g, 1.01 mol) and TBABr (25 g, 77.5 mmol) in DMF (1.2 L) was added over a period of 30 min, then the cooling was removed and the solution was stirred for 48 h at 20 ^{o}C. The mixture was diluted with toluene (2 L) and washed with $NaHCO_3$ solution (saturated, 2 × 3 L) and with water (3 L). The organic phase was concentrated affording 2 L yellow oil. The crude mixture (**9a**) used for the next reaction without any further purification.

4.5.2. *Experiment 2 using 5 kg purified acceptor*: Compound **8** (5.0 kg, 9.49 mol) was dissolved in dry DCM (20 L) and the solution was cooled down to 5 ^{o}C (Inner temperature, the cooling liquid was adjusted to 0 ^{o}C). To the solution Br_2 (500 mL, 9.72 mol) in dry DCM (200 mL) was added keeping the temperature between 5 and 10 ^{o}C over a period of 1 h. After the addition was finished the mixture was stirred for 10 min then cyclohexene (820 mL) was added to the mixture over a period of 1 h. keeping the temperature between 5 and 10 ^{o}C. The dark red solution turned to light yellow solution. To the crude solution of the bromosugar, compound **3** (5.0 kg, 8.42 mol) and TBABr (250 g, 775 mmol) in DMF (12 L) was added over a period of 3 h, then the cooling was removed and the solution was stirred for 48 h at 22 ^{o}C. The mixture was diluted with toluene (20 L) and washed with $NaHCO_3$ solution (saturated, 2 × 30 L) and with water (30 L). The organic phase was concentrated affording 17 L yellow oil (**9b**). The crude mixture used for the next reaction without any further purification.

4.5.3. *Experiment 3 using 495 g crude acceptor*: Compound **8** (465 g, 0.88 mol) was dissolved in dry DCM (1.85 L) and the solution was cooled down to 5 ^{o}C (Inner temperature, the cooling liquid was adjusted to 0 ^{o}C). To the solution Br_2 (46 mL, 0.89 mol) in dry DCM (185 mL) was added keeping the temperature between 5 and 10 ^{o}C over a period of 20 min. After the addition was finished the mixture was stirred for 10 min then cyclohexene (76 mL) was added to the mixture over a period of 20 min. keeping the temperature between 5 and 10 ^{o}C. The dark red solution turned to light yellow solution. To this crude solution of the bromosugar, compound **3c** (crude from experiment *Pilot scale without purification* starting from 515 g of compound **2**) and TBABr (20 g, 62 mmol) in DMF (1.2 L) was added over a period of 30 min, then the cooling was removed and the solution was stirred for 48 h at 22 ^{o}C. The mixture was diluted with toluene (2 L) and washed with $NaHCO_3$ solution (saturated, 2 × 3 L) and with water (3 L). The organic

phase was concentrated affording 2 L yellow oil. The crude mixture (**9c**) used for the next reaction without any further purification.

200 mg of the sample (from experiment 2) was purified for analysis: $[\alpha]_D^{25}$ -42.0 (*c* 1, $CHCl_3$), ^{1}H NMR. ($CDCl_3$, 400 MHz) δ: 7.45-7.25 (m, 15 H, aromatic), 5.60 (d, 1 H, $J_{1'',2''}$ 3.2 Hz, H-1´´), 4.98 and 4.65 (ABq, 2 H, J_{gem} 11.6 Hz, -CH_2Ph), 4.88 and 4.74 (ABq, 2 H, J_{gem} 12.0 Hz, -CH_2Ph), 4.76 (s, 2 H, -CH_2Ph), 4.65 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1´), 4.48 (dd, 1 H, $J_{1,2}$ 6.0, $J_{2,3}$ 7.4 Hz, H-2), 4.38 (d, 1 H, H-1), 4.29 (m, 2 H, H-6), 4.24 (m, 1 H,), 4.19 (m, 1 H, H-3´), 4.08 (m, 2 H, H-4´ and H-5´), 4.08 and 3.88 (2 m, each 1 H, H-6´), 4.06 (m, 2 H, H-2´´ and H-3), 3.98 and 3.91 (2 m, each 1 H) 3.72 (dd, 1 H, H-2´), 3.66 (bs, 1 H,), 3.43 (2 s, each 3 H, 2 × OMe), 1.48, 1.42, 1.37, 1.36, 1.31 and 1.28 (6 s, each 3 H, 6 × -CH_3), 1.21 (s, 9 H, Piv), 1.10 (d, 3 H, $J_{5'',6''}$ 6.46 Hz, H-6´´). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 178.2 (CO), 110.4, 110.1 and 108.8 (3 × -$C(CH_3)_2$), 105.3 (C-1), 101.6 (C-1´), 95.3 (C-1´´), 80.4 (C-3´), 79.4, 78.2, 77.8, 77.8, 76.6, 75.6, 74.2, 72.8, 72.8, 70.6 and 66.6 (C-2, C-3, C-4, C-5, C-2´, C-4´, C-5´, C-2´´, C-3´´, C-4´´ and C-5´´), 75.0, 73.6 and 73.4 (3 CH_2Ph), 65.3 and 62.6 (C-6 and C-6´), 56.3 and 53.0 (2 × OMe), 39.0 (Piv), 28.1, 27.5, 27.4, 27.4, 27.3, 27.1, 27.0, 26.6 and 24.6 (9 × -CH_3), 17.1 (C-6´´).

4.6. *Compound **10***

4.6.1. *Experiment 1, using the crude product from 4.5.1.*: NaOMe (120 g, 2.22 mol) was added to a solution of compound **9** (**9a**, made from 1.01 mol compound **3**) in dry MeOH (1.8 L) and stirred for 18 h at 20 °C. (The yellow solution became turbid after 2 h.) Amberlite IR 120 resin (H^+ form, 600 g) was added to the mixture and stirred for 30 min then the resin was filtered off and the filtrate was concentrated affording 1 L dark yellow slurry (**10a**). The mixture was used for the next step without any purification.

4.6.2. *Experiment 2, using the crude product from 4.5.2.*: NaOMe (850 g, 15.73 mol) was added to a solution of compound **9** (**9b**, made from 8.42 mol compound **3**) in dry MeOH (5 L) and stirred for 18 h at 21 ^{o}C. (The yellow solution became turbid after 5 h.) Amberlite IR 120 resin (H^+ form, 5 kg) was added to the mixture and stirred for 30 min then the resin was filtered off and the filtrate was concentrated affording 10 L dark yellow slurry. The residue was taken up in EtOAc (30 L) and washed with brine (30 L). The organic phase was concentrated affording compound **10** (**10b**) and used for the next reaction.

4.6.3. *Experiment 3, using the crude product from 4.5.3.*: NaOMe (81 g, 1.59 mol) was added to a solution of compound **9** (**9c**, made from 1.01 mol compound **2**) in dry MeOH (2 L) and stirred for 18 h at 25 ^{o}C. Amberlite IR 120 resin (H^+ form, 500 g) was added to the mixture and stirred for 30 min then the resin was filtered off and the filtrate was concentrated affording 1 L dark yellow slurry. The residue was taken up in DCM (2 L) and washed with water (2 × 2 L). The organic phase was concentrated affording compound **10** (**10c**) and used for the next reaction.

200 mg of the sample (from experiment 2) was purified for analysis: $[\alpha]_D^{25}$ -39.0 (*c* 1, $CHCl_3$), ^{1}H NMR. ($CDCl_3$, 400 MHz) δ: 7.42-7.26 (m, 15 H, aromatic), 5.58 (d, 1 H, $J_{1'',2''}$ 3.2 Hz, H-1´´), 4.98 (d, 1 H, J_{gem} 11.6 Hz, -CH_2Ph), 4.88 (d, 1 H, J_{gem} 12.0 Hz, -CH_2Ph), 4.77-4.61 (m, 4 H, -CH_2Ph), 4.55 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1´), 4.48 (dd, 1 H, $J_{1,2}$ 6.0, $J_{2,3}$ 7.4 Hz, H-2), 4.36 (d, 1 H, $J_{1,2}$ 7.6 Hz H-1), 4.25-4.19 (m, 2 H), 4.09-3.90 (m, 8 H), 3.85-3.62 (m, 6 H), 3.43 (2 s, each 3 H, 2 × OMe), 1.48, 1.44, 1.39, 1.37, 1.31 and 1.29 (6 s, each 3 H, 6 × -CH_3), 1.10 (d, 3 H, $J_{5'',6''}$ 6.5 Hz, H-6´´). ^{13}C NMR ($CDCl_3$ 100 MHz) δ: 110.4, 109.7 and 108.7 (3 × -$C(CH_3)_2$), 107.6 (C-1), 101.4 (C-1´), 95.2 (C-1´´), 80.7 (C-3´), 79.0, 78.0, 77.9, 77.1, 76.3, 75.6, 74.7, 74.4, 74.3, 72.6 and 66.3 (C-2, C-3, C-4, C-5, C-2´, C-4´, C-5´, C-2´´, C-3´´, C-4´´ and C-5´´), 74.8, 73.9 and

73.0 (3 CH_2Ph), 65.0 and 62.4 (C-6 and C-6´), 57.7 and 54.0 (2 × OMe), 27.9, 27.0, 26.9, 26.5, 25.5 and 25.1 (6 × -CH_3), 16.8 (C-6´´).

4.7. *Compound **11***

4.7.1. *Experiment 1, using the crude product from 4.6.1.*: The crude mixture (**10a**, made from 1.01 mol compound **3**) from the depivaloylation reaction was suspended in the mixture of AcOH (1.5 L) and water (1 L). The suspension was stirred at 65 ^oC (inner temperature) for 3 d. The mixture was concentrated and taken up in EtOAc (3 L) and washed with water (2 × 4 L), the water phases were combined and washed with EtOAc (0.5 L). The organic phases were combined and stored in the freezer for 2 d. White crystals were formed which were isolated by filtration resulting 300 g crude crystals. The crude product was dissolved warm MeOH (750 mL) and cooled down. The clean product (**11**) was isolated after filtration (210 g, 0.276 mol, 27.3% over 3 steps, 73% per step)

4.7.2. *Experiment 2 using the crude product from 4.6.2.*: The crude mixture (**10b**, made from 8.42 mol compound **3**) from the depivaloylation reaction was suspended in the mixture of AcOH (15 L) and water (10 L). The suspension was stirred at 65 ^oC (inner temperature) for 1 d. The mixture was cooled down and extracted with 10 L hexane. The aqueous phase was concentrated and taken up in EtOAc (15 L) and washed with water (2 × 5 L), the water phases were combined and washed with EtOAc (2 L). The organic phases were combined and stored in the freezer for 2 d. White crystals were formed which were isolated by filtration resulting 1.95 kg crude crystals. The crude product was dissolved warm MeOH (5 L) and cooled down. The clean product (**11**) was isolated after filtration (1.27 kg, 1.67 mmol, 19.8% over 3 steps, 67% per step)

4.7.3. *Experiment 3, using the crude product from 4.6.3.*: The crude mixture (**10c**, made from 1.01 mol compound **2**) from the depivaloylation reaction was suspended in the mixture of AcOH (1.5 L) and water (1.0 L). The suspension was stirred at 65 ^{o}C (inner temperature) for 3 d. The mixture was cooled down and extracted with 1 L hexane. The aqueous phase was concentrated and taken up in EtOAc (1.5 L) and washed with water (2 × 0.5 L), the water phases were combined and washed with EtOAc (200 mL). The organic phases were combined and stored in the freezer for 2 d. White crystals were formed which were isolated by filtration resulting 70 g crude crystals. The crude product was dissolved warm MeOH (200 mL) and cooled down. The clean product (**11**) was isolated after filtration (65 g, 85.5 mmol, 8.5% over 4 steps, 54% per step)^{1}H NMR. (DMSO d6, 400 MHz) δ: 7.40-7.20 (m, 15 H, aromatic), 6.66 (d, 1 H, *J* 6.7 Hz 1-OH), 5.54 (br s, 1 H, H-1), 5.00-4.42 (m, 11 H, 6 × OBn and 5 × OH), 4.40-4.15 (m, 4 H, H-1', H-1'' and 2 × OH), 3.90-3.10 (m, 15 H), 2.98 (m, 1 H), 1.05 (d, 3 H, $J_{5'',6''}$ 6.5 Hz, H-6´´). ^{13}C NMR (DMSO d6, 100 MHz) δ: 139.6, 139.5 and 139.4 (aromatic quat), 101.4 (C-1´), 97.0 (C-1), 96.5 (C-1´´), 79.1, 78.6, 78.3, 75.8, 75.7, 75.3, 75.1, 75.0, 74.0, 69.1 and 65.9 (C-2, C-3, C-4, C-5, C-2´, C-3´, C-4´, C-5´, C-2´´, C-3´´, C-4´´ and C-5´´), 74.8, 72.4 and 70.7 (3 × CH_2Ph), 60.7 and 60.6 (C-6 and C-6´), 16.7 (C-6´´).

4.8. *Compound **12***

4.8.1. *Laboratory scale*: Compound **11** (10 g, 13.2 mmol) was suspended in the mixture of MeOH (100 mL), IPA (50 mL), and AcOH (5 mL). Then Pd/C (1 g, 10 %) was added in water (10 mL). The mixture was stirred under 6 bar H_2 pressure for 12 h. The H_2 atmosphere was changed into Ar and the catalyst was filtered off and the mixture was concentrated. The residue

was dissolved in MeOH (5 mL) and the product was precipitated by adding MTBE (25 mL) resulting a white powder (6.6 g, 13.1 mmol, quant.).

4.8.2. *Pilot scale*: Compound **11** (1.70 kg, 2.24 mol) was suspended in the mixture of MeOH (9 L), IPA (4.5 L), and AcOH (180 mL). Then Pd/C (180 g, 10 % in charcoal) was added in water (1 L). The mixture was stirred under 4.5 bar H_2 pressure for 24 h. TLC indicated that the reaction was still incomplete. Pd/C (90 g, 10 % in charcoal) was added in MeOH (1 mL) and the mixture was stirred under 4.5 bar H_2 pressure for 5 h, then again Pd/C (40 g, 10 % in charcoal) was added in MeOH (0.5 mL). The mixture was stirred for 12 h under H_2 pressure. The H_2 atmosphere was changed into Ar and the catalyst was filtered off and the mixture was concentrated. The residue was dissolved in MeOH (5 L) and the product was precipitated by adding MTBE (20 L) resulting a pale yellow powder (1.3 kg). The crude product was stirred in IPA (10 L) for 2 h, then filtered off, washed with IPA (0.5 L) and dried in *vacuo* affording a white solid (1.1 kg, 2.24 mmol, quant.). $[\alpha]_D^{25}$ -47.2 (initial) → -58.0 (72 h, *c* 2, H_2O), $[\alpha]_D^{25}$ lit. [16] -53.5 (initial) → -57.5 (72 h, *c* 2, H_2O). MS: ESI^+ 511.2 $[M+Na]^+$, 572.2 $[M+K]^+$; ESI^- 487.4 $[M-H]^-$, 523.2 $[M+Cl]^-$.

NMR data: See in Table 1.

Table 1, ^1H and ^{13}C NMR data of compound **12**.

Ring	1H	δ (ppm)	multiplicity	*J* (Hz)	13C	δ (ppm)
	H-1	5.22	d	4.0	C-1	94.5
	H-2	3.59	dd	9.2, 4.0	C-2	74.0

α-D glucose	H-3*	3.80	dd	9.6, 9.2	C-3*	72.3
	H-4	3.71	dd	9.6, 9.0	C-4	77.9
	H-5	3.91	m		C-5	73.1
	H-6a	3.90	m		C-6	62.7
	H-6b	3.80	m			
β-D glucose	H-1	4.63	d	8.1	C-1	98.6
	H-2	3.29	dd	9.3, 8.1	C-2	76.6
	H-3	3.58	dd	9.6, 9.3	C-3	77.0
	H-4	3.72	dd	9.8, 9.6	C-4	78.5
	H-5	3.47	ddd	9.8, 5.2, 1.8	C-5	78.0
	H-6a	3.94	dd	11.8, 1.8	C-6	62.9
	H-6b	3.76	dd	11.8, 5.2		
β-D galactose	H-1	4.52	d	7.8	C-1	102.9
	H-2	3.66	dd	9.0, 7.8	C-2	79.0
	H-3	3.88	m		C-3	76.3
	H-4	3.90	m		C-4	71.9
	H-5	3.81	m		C-5	74.0
	H-6a	3.81	m		C-6	63.8
	H-6b	3.74	m			
α-L-fucose	H-1	5.30	d	2.0	C-1	102.0
	H-2*	3.80	m		C-2*	72.4
	H-3*	3.80	m		C-3*	70.9
	H-4	3.82	d	1.0	C-4	74.4
	H-5	4.22, 4.25	qd	6.0 + 1.0	C-5	69.6
	CH_3	1.22	d	6.0	CH_3	18.0

* Interchangeable assignments due to non-first order, overlapping spin systems

ASSOCIATED CONTENT

Supporting Information. The following file is available free of charge; Analytical data (NMR, HPLC, and LCMS) of selected compounds (**3**, **8**, **11** and **12**).

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ACKNOWLEDGMENT

The presented work has been founded by Glycom A/S. Authors thank to Dr. Agnes Agoston for the HPLC analysis, Dr Gabor Tarkanyi for the NMR analysis of the final compound and to Dr. Laszlo Drahos for the LCMS analysis of the final compound.

REFERENCES

[1] L. Harvey, Revolution at the table: The transformation of the American diet: Oxford University Press, New York, 1988.

[2] L. Hanson, Comparative immunological studies of the immune globulins of human milk and of blood serum, Int. Arch. Allergy Appl. Immunol. 18 (1961) 241-267.

[3] D. S. Newburg, Innate Immunity and Human Milk, J. Nutr. (Suppl.) 135 (2005) 1308-1312.

[4] D. S. Newburg, S. H. Neubauer, Carbohydrates in milk. In *Handbook of Milk Composition,* ed. RG Jensen, pp. 273–349. Academic: San Diego, 1995.

[5] P. György, R. W. Jeanloz, H. von Nicolai, F. Zilliken, Undialyzable growth factors for *Lactobacillus bifidus* var. *pennsylvanicus*. Protective effect of sialic acid bound to glycoproteins and oligosaccharides against bacterial degradation, Eur. J. Biochem. 43 (1974) 29-33.

[6] D. S. Newburg, G. M. Ruiz-Palacios, A. L. Morrow, Human milk glycans protect infants against enteric pathogens, Annu. Rev. Nutr. 25 (2005) 37-58.

[7] B. Stahl, S. Thurl, J. Zeng, M. Karas, F. Hillenkamp, M. Steup, G. Sawatzki, Oligosaccharides from human milk as revealed by matrix–assisted laser desorption/ionization mass spectrometry, Anal. Biochem. 223 (1994) 218-226. X. Chen, Human Milk Oligosaccharides (HMOS): Structure, Function, and Enzyme-Catalyzed Synthesis, Adv. Carbohydr. Chem. Biochem. 72 (2015) 113-190. J. T. Smilowitz, C. B. Lebrilla, D. A. Mills, G. B. German, S. L. Freeman, Breast milk oligosaccharides: Structure-function relationships in the neonate, Annu. Rew. Nutr., 32 (2014) 142-169.

[8] C. Kunz, S. Rudloff, W. Baier, N. Klein, S. Strobel, Oligosaccharide from human milk: structural, functional and metabolic aspect, Annu. Rev. Nutr. 20 (2000) 699-722.

[9] S. A. Abbas, J. J. Barlow, K. L. Matta, Synthesis of O-α-L-fucopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (2′-O-α-L-fucopyranosyl-lactose), Carbohydr. Res. 88 (1981) 51-60.

[10] (a) A. Fernandez-Mayoralas, M. Martin-Lomas, Synthesis of 3- and 2′-fucosyl-lactose and 3,2′-difucosyl-lactose from partially benzylated lactose derivatives, Carbohydr. Res. 154 (1986) 93-101. (b) R. K. Jain, R. D. Locke, K. L. Matta, A convenient synthesis of O-α-L-fucopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (2'-O-α-fucosyllactose) Carbohydr. Res. 212 (1991) C1-C3. (c) M. Izumi, O. Tsuruta, S. Harayama, H. Hashimoto, Synthesis of 5-Thio-L-fucose-Containing Disaccharides, as Sequence-Specific Inhibitors, and 2'-Fucosyllactose, as a Substrate of α-L-Fucosidases, J. Org. Chem. 62 (1997) 992-998. (d) C. L. Pereira, F. E. McDonald, Synthesis of Human Milk Oligosaccharides: 2'- and 3'-Fucosyllactose Heterocycles 84 (2012) 637-655. (e) G-N. Wang, S. André, H.-J. Gabius, P. V. Murphy, Bi- to tetravalent glycoclusters: synthesis, structure-activity profiles as lectin inhibitors and impact of combining both valency and headgroup tailoring on selectivity, Org. Biomol. Chem., 10 (2012) 6893-6907.

[11] (a) A. Rencurosi, L. Poletti, L. Panza, L. Lay, Improvement on lipase catalyzed regioselective O-acylation of lactose: a convenient route to 2'fucosyllactose, J. Carbohydr. Chem. 20 (2001) 761-765. (b) A. M. Scheppokat, M. Morita, J. Thiem, H. Bretting, Enzymatic α(1-2)-L-fucosylation: investigation of the specificity of the α(1-2)-L-galactosyltransferase from *Helix pomatia*, Tetrahedron:*Asymmetry* 14 (2003) 2381-2386.

[12] (a) C. Albermann, W. Piepersberg, U. F. Wehmeier, Synthesis of the milk oligosaccharide 2'fucosyllactose using recombinant bacterial enzymes, Carbohydr. Res. 334 (2001) 97-103. (b)

E. Hüfner, J. Parkot, S. Jennewein, Synthesis of fucosylated compounds, PCT Int. Pat. Appl. WO/2010/070104A1, 2010.

[13] G. A. Sprenger, F. Baumgärtner, C. Albermann, Production of human milk oligosaccharides by enzymatic and whole-cell biotrasformations, J. Biotechnol. 258 (2017) 79-91.

[14] (a) D. Vasiliu, N. Razi, Y. Zhang, N. Jacobsen, K. Allin, X. Liu, J. Hoffmann, O. Bohorov, O. Blixt, Large-scale chemo enzymatic synthesis of blood group and tumor-associated poli-N-acetyllactosamine antigens, Carbohydr. Res. 341 (2006) 1447-1457. (b) S. Drouillard, H. Driguez, E. Samain, Large-scale synthesis of H-antigen oligosaccharides by expressing *Helicobacter pylori* alpha 1,2-fucosyltrasferase in metabolically engineered *E. coli* cells, Angew. Chem. Int. Ed. 45 (2006) 1778-1780. (c) G. Zhou, X. Liu, D. Su, L. Li, M. Xiao, P. G. Wang, Large scale enzymatic synthesis of oligosaccharides and a novel purification process, Bioorg. Med. Chem. Lett. 21 (2011) 311-314.

[15] G. Kretzschmar, W. Stahl, Large scale synthesis of linker-modified sialyl Lewis^x, Lewis^x and N-acetyllactosamine, Tetrahedron 54 (1998) 6341-6359.

[16] Fügedi P. Glycosylation methods. In Organic Chemistry of Sugars; Levy, D.E.; Fügedi, P. (Eds.) pp. 89-180; CRC Press LLC, Boca Raton, 2006.

[17] R. Kuhn, H. H. Baer, A. Gauhe, Fucosidolactose, das Trisaccharid der Frauenmilch, Chem. Ber. 88 (1955) 1135-1146.

[18] L. Hough, A. C. Richardson, L. A. W. Thelwall, Reaction of lactose with 2,2-dimethoxypropane. A tetraacetal of novel structure, Carbohydr. Res. 75 (1979) C11-C12.

[19] S. Komba, H. Ishida, M. Kiso, A. Hasegawa, Synthesis and biological activities of three sulfated sialyl Le^x ganglioside analogues for clarifying the real carbohydrate ligand structure of L-selectin, Bioorg. Med. Chem. 4 (1996) 1833-1847.

Kilogram scale chemical synthesis of 2'-fucosyllactose

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Highlights

A kilogram scale preparation of 2`-fucosyllactose has been performed.

The developed route involves only one purification step using column chromatography.

At intermediate scale, the synthesis has been conducted omitting column purification.

The product was isolated as an amorphous solid with high purity.