Solid Phase Syntheses of Oligomannosides and of a Lactosamine Containing Milk Trisaccharide Using a Benzoate Linker

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Abstract: Galactose and mannose building blocks 9 and 12 were designed for the solid phase synthesis of oligosaccharides (SPOS). Both compounds were employed after condensation with benzoic acid function containing resin 10 in SPOS of human milk trisaccharide 1 and oligomannosides 2–4 (α-(1→2)-linked hexamer). Thus, in this approach a special linker development was not required and with the temporary protective groups phenoxyacetyl (PA) and 9-fluorenlymethoxy carbonyl (Fmoc) as part of compounds 7–12 the strategy offers the additional advantage of having the anomeric centre at the reducing end available for further manipulations.

Key words: solid-phase synthesis, Fmoc-protected hydroxy groups, glycosidations, trichloroacetimidates, oligosaccharides.

Oligosaccharides in their functions as cell surface carbohydrates and soluble glycoforms play an important role in nature.1–3 Although research in this field is still in its infancy, today there is already striking evidence that glycoconjugates are involved in several important processes, for instance, cellular differentiation and recognition, signal transduction pathways and certain disease states.4,5 In spite of the achievements in the chemical synthesis of oligosaccharides based on the development of highly reactive sugar donors,6,7 glycosylation strategies and advanced protective group chemistry in recent years,8–10 one of the future tasks is to combine this knowledge with the advantages of the solid phase synthesis and support glycobiology studies with a variety of well defined oligosaccharides and glycoconjugates. Therefore, we decided to focus our research on the synthesis of biologically important structures occurring as parts of N-glycans, GPI-anchors and human milk oligosaccharides, both in solution11–14 and on solid phase.15–22

Since in SPOS23 quite a different linker systems were developed,15,17–22,24–31,42 we use in our strategy a commercially available resin already bearing benzoic acid functions as the linker moiety.21 Furthermore, we describe herein the application of recently reported O-Fmoc protected20 O-glycosyl trichloroacetimides as glycosyl donors6,7 to the solid phase synthesis of target oligosaccharides 1–4 (Scheme 1); these compounds occur in nature as part of human milk oligosaccharides33, high mannose-type N-glycans and glycolipids,34 and within GPI-anchors.35 Therefore, in the retrosynthetic analysis polymer-bound target molecules 5 and 6–n were disconnected into the corresponding O-glycosyl trichloroacetimides 7,8,20 and 11 as glycosyl donors, and into primary alcohol residues 9 and 12 which were directly attached to the polymeric benzoic acid support via simple condensation reactions.

Two galactose building blocks 7 and 9 were prepared starting from known derivative 13,36 bearing two free hydroxy functions in the C2 and C3 position (Scheme 2). Regioselective introduction of the Fmoc group to the more reactive O3 position of building block 13 was achieved by using FmocCl in MeCN together with a catalytic amount of DMAP and pyridine (1 equiv). After conversion of 95% of the starting material, compound 14 was isolated in 67% yield. Unreacted 13 was separated (28%) and recycled. Installation of the benzoyl group to the remaining 2-hydroxy group of 14 was successful by using benzoyl chloride in a mixture of CH2Cl2/pyridine (→ 15). Under these conditions, no loss of the Fmoc group was observed. Subsequent 1-O-desilylation of derivative 15 using an excess of HF-pyridine complex37 in pyridine at 0 °C furnished compound 16. As described previously,20 the trichloroacetimidoyl moiety was introduced to compound 16 by treatment with CCl3CN in the presence of NaH at 0 °C leading to galactosyl donor 7 (1H NMR: δ = 6.85, J1,2 = 3.4 Hz, 1-H, containing only trace amounts of β anomers) in 86% yield.20 Fully protected compound 18 was obtained starting from known 1336 by a highly regioselective introduction of the phenoxyacetyl (PA) group (→ 17) at low temperature in 92% yield and subsequent treatment with benzoyl cyanide and Et3N in MeCN. Derivative 18 was converted into primary alcohol 9 via regioselective opening38 of the 4,6-O-benzylidene group under reductive reaction conditions (BH3:THF, 10 equiv and 1 M dibutyl boron triflate, 1.0 equiv) in high yield (90%).

3,6-Di-O-benzyl glucosamine derivative 1939 was prepared from D-glucosamine in five steps, using a strategy based on a dibutyltin oxide assisted regioselective benzylolation of the 3- and 6-O-positions (Scheme 3).39,40 Thus, the Fmoc group was introduced to the 4-O-position of compound 19 using FmocCl with a catalytic amount of DMAP in pyridine at r.t. 1-O-Desilylation of derivative 20 using an excess of HF-pyridine complex37 in THF furnished compound 21, which was subsequently trans-
formed into β-glycosyl trichloroacetimidate 8\textsuperscript{20} in the presence of NaH (0.1 equiv) in CCl\textsubscript{3}CN as solvent in 97% yield; only the β-isomer was obtained (\textsuperscript{1}H NMR: δ = 6.41, J\textsubscript{1,2} = 8.4 Hz, 1-H).

The first galactose residue, primary alcohol 9, was attached to the solid support 10 via a condensation reaction with N,N\textprime;-diisopropyl carbodiimide (DIC)\textsuperscript{41} and a catalytic amount of DMAP in CH\textsubscript{2}Cl\textsubscript{2} (Scheme 4). The remaining carboxylate functions were transformed into the methyl ester. Loading of resin 22 was determined to be 0.05 mmol/g after recycling and purification of starting material 9. Resin 22 was swollen in THF and PA-cleavage reaction with an 8 M solution of MeNH\textsubscript{2} in EtOH was repeated until the generated UV spot of the washing solutions completely disappeared, thus leading to polymer bound acceptor 23. An independent PA-cleavage experiment in solution with compound 18, applying the conditions described above proved the stability of Bz in 2-position of the galactose residue.\textsuperscript{43}

Glycosylation of resin 23 with donor 8 under standard solid phase glycosylation conditions (0.3 equiv TMSOTf; 3.0 equiv donor) at low temperature followed by removal of the Fmoc group\textsuperscript{22} afforded disaccharide acceptor resin 24. The 1-O-TDS group proved to be stable under these conditions.\textsuperscript{43} Glycosylation with donor 7 under the same conditions furnished fully protected trisaccharide resin 5. Completion of the reaction at each step was monitored by TLC and MALDI-TOF after analytical cleavage of a small resin sample (2–4 mg). The mass spectra indicated that the 2-O-benzoyl group was retained. Therefore, after preparative cleavage\textsuperscript{21} from the resin 5 we performed an additional O-acetylation step affording human milk trisaccharide 1 (\textsuperscript{1}H NMR: δ = 4.54, d, J\textsubscript{1,2} = 7.5 Hz, 1a-H; C-1a: δ = 96.80) in 61% overall yield from 22 (92% per step).

The reiterative solid phase cycle for the construction of the biologically important α-(1→2) linked mannose oligomers\textsuperscript{34,35} starts with the synthesis of appropriate mannose building blocks 11 and 12 (Scheme 5). Since it is known that O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl) trichloroacetimidate\textsuperscript{44} was already successfully applied to the synthesis of α-mannosides in solution\textsuperscript{14,45} and on solid phase,\textsuperscript{15,31} we decided to evaluate the usefulness of the corresponding 2-O-Fmoc protected donor 11 for SPOS.
Known precursor 25 was treated with FmocCl and a catalytic amount of DMAP in pyridine to furnish fully protected 26 in excellent yield. Deallylation 47 was performed with Wilkinson catalyst in a two-step sequence. Hydrolysis of the enol ether with iodine afforded 27, which was subsequently transformed into the trichloroacetimidate 11 at 0 °C in 93% yield. Primary alcohol 12 was synthesized in two steps from known 28. Introduction of the Fmoc group as described above furnished 29 in 95% yield. Subsequent removal of the 6-O-TBDPS group with excess HF/pyridine, THF afforded compound 12.

Primary alcohol 12 was subsequently attached to the carboxylic acid resin 10 as described above to furnish resin 30 (Scheme 6). In this approach loading of resin 30 was determined to be 0.20 mmol/g twice, via recycling of unconsumed starting material after treatment with Et3N and purification, as well as via preparative cleavage of allyl 3,4-di-O-benzyl-α-D-mannopyranoside from resin 30. Removal of the Fmoc group by treatment of resin 30 with Et3N furnished acceptor resin 31. The maximum
chain distance for a six membered ring of six bonds between acceptor hydroxyl function and position of linkage as pointed out in resin 31 proved to be very useful in the glycosylation reaction with donor 11 performed under standard conditions at r.t. as described above. Repetition of the glycosylation (→ 6-n) and deprotection (→ 32-n) sequence in a cyclic manner (n = 1 to 5) furnished the corresponding oligomannosides 2 to 4 up to the hexameric stage with high stereoselectivity and in excellent overall yields.

In summary, we present highly efficient synthetic routes for the SPOS via a simple ester-type linkage. The new Fmoc bearing building blocks were successfully applied to the solid phase synthesis of human milk trisaccharide 1 and oligomannosides 2 to 4. The target molecules were isolated as anomerically pure derivatives in high overall yields; they permit additional elongation at their reducing end. Investigations on further suitable building blocks for the synthesis of more complex, branched glycostructures are currently underway.

Solvents were purified and dried in the usual way. All reactions were performed with anhydrous solvents and under argon unless otherwise stated. TLC’s were performed on plastic plates Silica Gel 60 (Baker 30–60 μm). Petroleum ether (PE) was used with the boiling range 35–70 °C; toluene, CH2Cl2, MeOH and EtOAc were distilled. Optical rotations were determined at 21 °C with a Perkin-Elmer 241/ MC polarimeter (1 dm cell). NMR spectra were recorded with Bruker AC 250 and 600 DRX instruments, with tetramethylsilane as internal standard. MS spectra were recorded with a MALDI-kompakt (Kratos) instrument in the positive mode using 2,5-dihydroxybenzoic acid in dioxane as matrix. Microanalyses were performed in the unit of Microanalysis at the Fachbereich Chemie, Universität Konstan.

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\text{O-(2-O-Benzoyl-4,6-O-benzylidene-3-O-(9-fluorenyl-methoxy-carbonyl)-α-D-galactopyranosyl) trichloroacetiminate (7)}
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Compound 16 (78 mg, 131 μmol) was dissolved in CH2Cl2 (2 mL). The solution was stirred at 0 °C after the addition of NaH (0.5 mg) for 15 min. The crude reaction mixture was adsorbed onto silica gel and the residue was purified by flash chromatography through a short plug of silica (toluene–acetone, 10:1) to furnish compound 7 (83 mg, 112 μmol, 86%) as a white foam (containing only trace amounts of β-anomer).

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R_\text{f} \text{ (toluene–acetone, 5:1)} = 0.70.
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[\alpha]_D = +81.3 \text{ (c = 1.0, CHCl}_3\text{).}
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1^H \text{NMR (600 MHz, CDCl}_3\text{): } \delta = 4.06 \text{ (s, 1 H, 5-H), 4.10 \text{ (d, 1 H, J} \text{em} = 12.9 \text{ Hz, 6-H), 4.17 \text{ (t, 1 H, } J = 7.5 \text{ Hz, 9-H), 4.34–4.40 (m, 3 H, 6'-H, CH}_2\text{O (Fmoc)), 4.65 (d, 1 H, } J = 3.5 \text{ Hz, 4-H), 5.49 (dd, 1 H, } J_{1,2} = 10.6 \text{ Hz, } J_{1,3} = 3.5 \text{ Hz, 3-H), 5.58 (s, 1 H, CHPh), 5.89 \text{ (dd, 1 H, } J_{1,2} = 3.4 \text{ Hz, } J_{2,3} = 10.6 \text{ Hz, 2-H), 6.85 (d, 1 H, } J_{1,2} = 3.4 \text{ Hz, 1-H), 7.04–8.00 (m, 18 H, Ar), 8.55 (s, 1 H, NH).}
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1^C \text{NMR (150.9 MHz, CDCl}_3\text{): } \delta = 46.5 \text{ (1 C, C-9), 64.9 \text{ (1 C, C-5), 67.5 \text{ (1 C, C-2), 68.8 \text{ (1 C, C-6), 70.3 \text{ (1 C, CH}_2\text{O (Fmoc)), 72.2 \text{ (1 C, C-3), 73.4 \text{ (1 C, C-4), 94.6 \text{ (1 C, C-1), 101.0 \text{ (1 C, CHPh).}}}
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Anal. Calcd for C_{17}H_{27}Cl_{13}N_{9}O_{33}: C: 60.14; H: 4.09; N: 1.90. Found: C: 60.17; H: 4.13; N: 1.82.

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\text{O-(3,6-Di-O-benzyl-2-desoxy-4-O-(9-fluorenylmethoxy-carbonyl)-2-N-phthalimido-β-D-glucopyanosyl) trichloroacetiminate (8)}
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Compound 21 (115 mg, 0.161 mmol) was dissolved in CH2Cl2 (3 mL). The solution was stirred after the addition of NaH (0.5 mg) for 15 min. The crude reaction mixture was adsorbed onto silica gel and the residue was purified by flash chromatography through a short plug of silica (toluene–acetone, 10:1) to furnish compound 8 (133 mg, 0.155 mmol, 97%) as a white foam.
Rr (toluene-acetone, 5:1) = 0.73.

[α]D = +70.1 (c = 2.0, CHCl3).

1H NMR (250 MHz, CDCl3); δ = 3.69, 3.74 (m, 2 H, 6-H, 6’-H), 4.00 (dt, 1 H, J3a = 10.0 Hz, J2a = 3.7 Hz, 5-H), 4.12 (t, 1 H, J = 7.0 Hz, J = 2.9 Hz, 4.29–4.66 (m, 4 H, 2 CH2Ph), 4.32, 4.38 (m, 2 H, CH2O (Fmoc)), 4.51 (m, 1 H, 2-H), 4.59 (m, 1 H, 3-H), 5.10 (dd, 1 H, J1a = 8.6 Hz, J3a = 10.0 Hz, 4-H), 6.41 (d, 1 H, J = 8.4 Hz, 1-H), 6.82–7.77 (m, 22 H, Ar), 8.38 (s, 1 H, NH).

13C NMR (150.9 MHz, CDCl3); δ = 47.07 (1 C, C-9), 54.75 (1 C, C-2), 69.26 (1 C, C-6), 70.36 (1 C, CH2O (Fmoc)), 74.39 (1 C, C-5), 74.52 (2 C, 2 CH2Ph), 76.89 (1 C, C-3), 76.90 (1 C, C-4), 94.22 (1 C, C-1).

Calcd. Calc’d, H: 4.31; N: 3.01.

Thexyldimethylsilyl-2-O-benzyl-4-O-benzyl-3-O-phenoxycetoxyethyl-8-p-galactopyranoside (9)

To a cooled solution of compound 18 (2.00 g, 3.08 mmol) in CH2Cl2 (20 mL) at 0 °C were added dropwise 1M BH3·THF (31 mL, 10.0 equiv) and 1 M dibutyl boron triflate (3.1 mL, 1.0 equiv) under argon. After stirring for 0.5 h the reaction mixture was treated with Et3N (-10 mL) and neutralized with treatment with solid CaCO3 and H2O (0.1 mL). The mixture was filtered through MgSO4 and concentrated in vacuo. Flash chromatography (toluene–acetonitrile, 20:1) of the residue furnished 12 (261 mg, 0.42 mmol, 93%) as a white foam.

Rr (toluene-acetone, 20:1) = 0.01

[α]D = +13.2 (c = 2.0, CHCl3).

1H NMR (600 MHz, CDCl3); δ = 3.92 (t, 1 H, J = 3.7 Hz, 5-H, OH), J, = 2.9 Hz, 3-H), 4.36 (d, 1 H, J = 16.4 Hz, PhOC(O)CH2), 4.44 (d, 1 H, J, = 16.4 Hz, PhOC(O)CH2), 4.56 (d, 1 H, J, = 12.0 Hz, CH2Ph), 4.74 (d, 1 H, J, = 12.0 Hz, 1 H, CH2Ph), 4.84 (d, 1 H, J = 7.5 Hz, 1-H), 5.28 (dd, 1 H, J, = 10.5 Hz, J1a = 3.1 Hz, 3-H), 5.62 (dd, 1 H, J1a = 7.5 Hz, J = 10.5 Hz, 2-H), 6.66–8.03 (m, 15 H, Ar).

13C NMR (150.9 MHz, DMSO); δ = 58.80 (1 C, C-6), 63.94 (1 C, PhOCH3), 71.96 (1 C, C-2), 73.41 (1 C, C-3), 74.15 (2 C, C-4, C-5), 74.29 (1 C, CH2Ph), 94.97 (1 C, C-1).

Maldi-MS, m/z calc’d for: [MNa]+, 647.8; [MK]+, 869.9. Found: [MNa]+, 645.1; [MK]+, 689.4.

Anal. Calc’d for C45H40Cl3NO8 (817.15): C, 64.67; H, 4.93; N, 1.71.

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Thexyldimethylsilyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(9-fluorenylmethoxycarbonyl)-β-D-galactopyranoside (15)

To a cooled solution of compound 14 (0.33 g, 0.52 mmol) in CHCl₃- pyridine (2:1, 6 ml) under argon was added at 0 °C benzoyl chloride (100 μl, 1.5 equiv). After stirring for 12 h at r.t. the reaction mixture was diluted with EtOAc (10 ml), neutralized by sat. NH₄Cl and extracted with H₂O (20 ml) and sat. NaCl (20 ml). The combined organic layers were dried with MgSO₄ and concentrated in vacuo. The residue was purified by flash chromatography (toluene, pure) to furnish 15 (364 mg, 0.49 mmol, 95%) as a white foam. TLC

Rₖ (toluene–acetone, 20:1) = 0.52.

[α]D = +45.2 (c = 2.0, CHCl₃).

1H NMR (250 MHz, CDCl₃): δ = 0.20, 0.24 (2s, 6 H, 2 SiCH₃), 0.89–0.93 (m, 12 H, 4 CH₂), 1.61–1.73 (m, 1 H, CH(CH₃)₂), 2.16 (d, 1 H, J₂,₃ = 2.2 Hz, OH), 3.50 (s, 1 H, 5-H), 3.95 (dd, 1 H, J₃,₂ = 7.5 Hz, J₂,₃ = 10.2 Hz, 2-H), 4.04 (dd, 1 H, J₃,₂ = 1.7 Hz, J₂,₃ = 10.7 Hz, 6-H), 4.27 (d, 1 H, J₂,₃ = 12.4 Hz, 6-H’), 4.43 (d, 1 H, J₂,₃ = 3.6 Hz, 4-H), 4.63 (d, 1 H, J₂,₃ = 7.5 Hz, 1-H), 4.70 (bs, 2 H, PhOCH₂), 4.95 (dd, 1 H, J₂,₃ = 10.2 Hz, J₃,₂ = 3.7 Hz, 3-H), 5.48 (s, 1 H, CHPh), 6.86–7.50 (m, 10 H, Ar).

MALDI-MS, m/z calculated for: [MNa⁺], 567.7; [MK⁺], 583.8. Found: [MNa⁺], 568.3 ([MK⁺], 584.1.


Thexyldimethylsilyl 2-O-benzoyl-4,6-O-benzylidene-3-O-phenoxycetyl-β-D-galactopyranoside (18)

Et,N (1.7 ml, 1.1 equiv) and benzoyl cyanide (2.16 g, 1.5 equiv) were added under argon to a solution of compound 17 (6.00 g, 11.00 mmol) in MeCN (90 ml). After stirring for 2.5 h the reaction solution was treated with MeOH (40 ml) and concentrated in vacuo. The residue was purified by flash chromatography (toluene–acetone, 20:1) to give 18 (6.64 g, 10.23 mmol, 93%) as a beige, amorphous solid.

Rₖ (toluene–acetone, 5:1) = 0.66.

[α]D = +47.0 (c = 3.0, CHCl₃).

1H NMR (250 MHz, CDCl₃): δ = 0.10, 0.20 (2s, 6 H, 2 SiCH₃), 0.71–0.74 (m, 12 H, 4 CH₂), 1.52 (sp, 1 J = 6.8 Hz, 1 H, CH(CH₃)₂), 3.58 (s, 1 H, 5-H), 4.10 (dd, 1 H, J₂,₃ = 12.2 Hz, 6-H), 4.32 (dd, 1 H, J₂,₃ = 12.3 Hz, 6-H’), 4.47 (s, 1 H, 4-H’), 4.49 (d, 1 H, J₂,₃ = 16.5 Hz, PhOCH₂), 4.62 (d, 1 H, J₂,₃ = 16.5 Hz, PhOCH₂), 4.93 (d, 1 H, J₂,₃ = 7.6 Hz, 1-H), 5.25 (dd, 1 H, J₁,₂ = 10.4 Hz, J₃,₂ = 3.7 Hz, 3-H), 5.53 (s, 1 H, CHPh), 5.65 (dd, 1 H, J₁,₂ = 7.6 Hz, J₃,₂ = 10.5 Hz, 2-H), 6.69–8.03 (m, 15 H, Ar).

MALDI-MS, m/z calculated for: [MNa⁺], 671.8; [MK⁺], 687.9. Found: [MNa⁺], 671.2; [MK⁺], 687.3.

Anal. Calcd for: C₉₈H₇₉O₅Si (648.81): C, 66.64; H, 6.84. Found: C, 66.43; H, 6.50.

Thexyldimethylsilyl 3,6-di-O-benzyl-2-desoxy-4-O-(9-fluorenylmethoxycarbonyl)-2-N-phthalimidol-β-D-glucopyranoside (20)

DMAP (2 mg, 0.01 equiv) and FmocCl (0.38 g, 1.1 equiv) were added under argon to a solution of compound 19 (0.84 g, 1.33 mmol) in pyridine (6 ml). After stirring for 2 h the mixture was concentrated in vacuo and co-evaporated twice with toluene. Flash chromatography (toluene–EtOAc, 30:1) afforded compound 20 (0.93 g, 1.09 mmol, 82%) as a white foam.

Rₖ (toluene–acetone, 20:1) = 0.51.

[α]D = +38.6 (c = 2.0, CHCl₃).

1H NMR (250 MHz, CDCl₃): δ = −0.04, 0.12 (2 s, 6 H, 2 SiCH₃), 0.56–0.62 (m, 12 H, 4 CH₂), 1.31–1.42 (m, 1 H, CH(CH₃)₂), 3.67 (m, 2 H, 6-H, 6’-H), 3.82 (m, 1 H, 5-H), 4.14 (t, 1 H, J₂,₃ = 7.1 Hz, 9-H), 4.18 (dd, 1 H, J₁,₂ = 8.1 Hz, J₂,₃ = 10.9 Hz, 2-H), 4.28–4.63 (m, 4 H, 2 CH₂Ph), 4.30–4.38 (m, 2 H, CH₂O (Fmoc)), 4.86 (dd, 1 H, J₁,₂ = 10.9 Hz, J₃,₂ = 8.8 Hz, 3-H), 4.95 (dd, 1 H, J₁,₂ = 8.8 Hz, J₃,₂ = 9.9 Hz, 4-H), 5.35 (d, 1 H, J₁,₂ = 8.1 Hz, 1-H), 6.85–7.76 (m, 22 H, Ar).

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13C NMR (150.9 MHz, CDCl3): δ = 46.6 (1 C, C-9), 57.4 (1 C, C-2), 69.8 (1 C, C-6), 69.9 (1 C, CH2O (Fmoc)), 73.0 (1 C, C-5), 73.9 (2 C, 2 CH2Ph), 76.5 (1 C, C-3), 77.3 (1 C, C-4), 93.4 (1 C, C-1). MALDI-MS, m/z calculated for: [MNa+], 877.1; [MK+], 893.2. Found: [MNa+], 877.4; [MK+], 893.4.

Anal. Caled for C28H30NO8: 854.07; C, 71.72; H, 6.49; N, 1.64. Found: C, 71.55; H, 6.28; N, 1.35.

3.6-Di-O-benzyl-2-desoxy-4-O-(9-fluorenylmethoxycarbonyl)-2-N-phthalimido-α-D-glucopyranoside (21)

To a solution of compound 20 (296 mg, 0.35 mmol) in THF (3 mL) was added an excess of HF-pyridine complex (1 mL, 10 mol equiv). After stirring for 2.5 d the reaction solution was diluted with EtOAc (5 mL) and neutralized by treatment with solid CaCO3 and H2O (0.1 mL). The mixture was filtered through MgSO4 and concentrated in vacuo. Flash chromatography (toluene–EtOH–H2O (20:10:1, 15.5 mL) was added Wilkinson catalyst (tris(triphenyl phosphine) rhodium(I)-chloride, 96 mg, 0.2 eq) and the solution was refuxed for 1 h. The solid was filtered off, washed with toluene (2 x 20 mL), concentrated in vacuo and coevaporated twice with toluene–EtOH (1:1). The crude residue was dissolved in THF–H2O (4:1, 5 mL) and treated with iodine (0.25 g, 2.0 eq) for 30 min. The reaction mixture was diluted with EtOAc (30 mL) and extrated with Na2S2O3 (sat. solution, 3 x 20 mL) and H2O (3 x 20 mL). The combined organic layers were filtered through MgSO4, silica and activated carbon. Flash chromatography (toluene–acetone, 20:1) after removal of the solvents in vacuo afforded compound 27 (0.27 g, 0.40 mmol, 82%) as a white foam (αf, 61). Rf (toluene–acetone, 5:1) = 0.42.

Allyl 3,4,6-tri-O-benzyl-2-O-(9-fluorenylmethoxycarbonyl)-α-D-mannopyranoside (26)

DMAP (6 mg, 0.01 eq) and FmocCl (5.22 g, 4.0 eq) were added under argon to a solution of compound 25 (2.60 g, 5.05 mmol) in pyridine (30 mL). After stirring for 30 min the solid was filtered off, washed with toluene (2 x 30 mL) concentrated in vacuo and coevaporated twice with toluene. Flash chromatography (toluene–acetone) furnished 26 (3.38 g, 4.75 mmol, 94%) as a yellow oil. Rf (toluene–acetone, 10:1) = 0.16 (β).

[α]D = +462.3 (c = 2.0, CHCl3).

1H NMR (250 MHz, CDCl3): δ = 3.65, 3.67 (2s, 2 H, 6–H, 6’–H), 3.88 (dd, 1 H, J6,6’ = 10.1 Hz, J4,4’ = 4.4 Hz, 5–H), 4.14 (t, 1 H, J1,2 = 7.0 Hz, 9–H), 4.16 (dd, 1 H, J1,2 = 8.6 Hz, J3,4 = 10.6 Hz, 2–H), 4.28 (dd, 1 H, J1,2 = 12.3 Hz, CH2Ph), 4.32–4.54 (m, 5 H, 3–H, 6–H, 6’–H, CH2O (Fmoc)), 4.59 (4H, 4–H, CH2Ph), 5.00 (dd, 1 H, J1,2 = 9.0 Hz, J9,9’ = 9.9 Hz, 4–H), 5.37 (3d, 1 H, J1,2 = 8.5 Hz, 1–H), 6.84–7.77 (m, 22 H, Ar).

MALDI-MS, m/z calculated for: [MNa+], 734.8; [MK+], 750.9. Found: [MNa+], 734.5; [MK+], 750.5.

Anal. Caled for C28H30NO8: 717.16; C, 72.56; H, 5.24; N, 1.98. Found: C, 72.44; H, 5.43; N, 1.83.

Allyl 3,4,6-tri-O-benzyl-2-O-(9-fluorenylmethoxycarbonyl)-α-D-mannopyranoside (26)

DMAP (6 mg, 0.01 eq) and FmocCl (5.22 g, 4.0 eq) were added under argon to a solution of compound 25 (2.60 g, 5.05 mmol) in pyridine (30 mL). After stirring for 30 min the solid was filtered off, washed with toluene (2 x 30 mL) concentrated in vacuo and coevaporated twice with toluene. Flash chromatography (toluene–acetone) furnished 26 (3.38 g, 4.75 mmol, 94%) as a yellow oil. Rf (toluene–acetone, 10:1) = 0.76.

[α]D = +97.7 (c = 1.5, CHCl3).

1H NMR (600 MHz, CDCl3): δ = 3.77 (dd, 1 H, J1,2 = 1.2 Hz, J1,3 = 10.6 Hz, 6–H), 3.85 (dd, 1 H, J1,2 = 4.8 Hz, J6,6’ = 10.7 Hz, 6’–H), 3.89 (m, 1 H, 5–H), 3.99 (t, 1 H, J1,2 = 9.4 Hz, 4–H), 4.04 (dd, 1 H, J1,2 = 6.2 Hz, J1,3 = 12.8 Hz, OCH2CH=CH2), 4.07 (dd, 1 H, J1,2 = 9.3 Hz, J6,6’ = 9.1 Hz, 3–H), 4.22 (dd, 1 H, J1,2 = 5.2 Hz, J1,2 = 12.8 Hz, OCH2(CH2)3), 4.28 (t, 1 H, J1,2 = 7.5 Hz, 9–H), 4.34 (dd, 1 H, J1,2 = 10.3 Hz, J3,4 = 11.3 Hz, CH2O (Fmoc)), 4.46 (dd, 1 H, J1,2 = 7.3 Hz, J1,2 = 10.2 Hz, CH2O (Fmoc)), 4.57 (t, 2 H, J1,2 = 11.1 Hz, 12.5 Hz, CH2Ph), 4.62 (dd, 1 H, J1,2 = 11.3 Hz, CH2Ph), 4.73 (d, 1 H, J1,2 = 12.1 Hz, CH2Ph), 4.79 (d, 1 H, J1,2 = 11.3 Hz, CH2Ph), 4.92 (d, 1 H, J1,2 = 10.7 Hz, CH2Ph), 5.05 (s, 1 H, J1,2 = 1.0 Hz, 1–H), 5.21–5.32 (m, 3 H, 2–H, OCH2CH=CH2), 5.89–5.94 (m, 1 H, OCH2CH=CH2), 7.18–7.79 (m, 23 H, Ar).

13C NMR (150.9 MHz, CDCl3): δ = 46.60 (1 C, C-9), 68.15 (1 C, OCH2CH=CH2), 68.97 (1 C, C-6), 70.24 (1 C, CH2O (Fmoc)), 71.62 (1 C, C-5), 71.85 (1 C, CH2O (Fmoc)), 72.69 (1 C, C-2), 73.45 (1 C, CH2Ph), 74.40 (1 C, C-4), 75.31 (1 C, CH2Ph), 78.27 (1 C, C-3), 96.64 (1 C, C-1).

MALDI-MS, m/z calculated for: [MNa+], 735.8; [MK+], 751.9. Found: [MNa+], 735.4; [MK+].

751.5.
resin washed alternately with THF (3 x 15 mL/g resin) and CH₂Cl₂ (3 x 15 mL/g resin) and dried under high vacuum.

**General Procedure for Fmoc-Deprotection (Procedure B)**
Fmoc-Cleavage was performed on solid phase according to the procedure previously described.²²

**General Procedure for the Solid-Phase Glycosylation (Procedure C)**
Dry acceptor-loaded resin was directly swollen in a CH₂Cl₂ solution (15 mL/g resin) containing the appropriate donor (3.0 equiv) under argon. After 10 min under agitation, a freshly prepared 0.5 M TM-SOTT solution in CH₂Cl₂ (0.3 equiv) was added and shaking was continued for 30 min. The resin was filtered off, washed alternately with THF (3 x 15 mL/g resin) and CH₂Cl₂ (3 x 15 mL/g resin) and dried under high vacuum.

**General Procedure for Cleavage (Procedure D)**
Cleavage was performed according to the procedure previously described.²³

**Resin 5**
Resin 24 was glycosylated with 7 according to procedure C, at –20 °C.

**Resins 6-n**
Resins 32-n and resin 31 were glycosylated with 11 according to procedure C.

**Resin 22**
Resin 10 was loaded with the primary alcohol 9 (0.05 equiv) according to procedure A. Loading of resin 22 was determined to be 0.05 mmol/g by recovering of starting material 9 after purification (as described above).

**Resin 23**
To a suspension of resin 22 in THF (4 mL/g resin) was added MeNH₂ (8 M solution in EtOH, 4 mL/g resin). The resin was filtered off after 15 min and the procedure repeated, until the UV spot of the washing solution completely disappeared. Finally, the resin was washed alternately with THF (3 x 15 mL/g resin) and CH₂Cl₂ (3 x 15 mL/g resin) and dried under high vacuum.

**Resin 24**
Resin 23 was glycosylated with 8 according to procedure C, but at –20 °C. Subsequently it was treated according to Procedure B yielding resin 24.

**Resin 30**
Resin 10 was loaded with the primary alcohol 12 (0.22 equiv) according to procedure A. Loading of resin 30 was determined to be 0.20 mmol/g by treatment of the combined filtrates with Et₃N (1 mL per 4 mL of filtrate) for 4 h and was recovered by preparative cleavage (using procedure D) of allyl-3,4-di-O-benzyl-o-D-mannopyranoside⁴⁸ after purification.

**Resin 31**
Fmoc-cleavage of the resin bound monosaccharide (resin 30) was achieved subsequently according to procedure B yielding resin 31.

**Resins 32-n**
The Fmoc groups of resins 6-n were deprotected according to procedure B.

**Thexydylimethylsilyl (2,3-di-O-acetyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-desoxy-2-N-
\noctylamido-β-D-glucopyranosyl)-(1→3)-6-O-acetyl-2-O-
benzoyl-4-O-benzyl-β-D-galactopyranoside (1)**
Resin 5 (4.9 μmol) was treated as described in procedure D. After removal of the solvents in vacuo the crude cleavage residue was treated with Ac₂O (1 mL) and pyridine (1 mL) for 12 h. The resulting mixture was concentrated in vacuo and coevaporated twice with toluene. The residue was purified by flash chromatography (toluene-acetone, 20:1) to furnish 4 (4.1 mg, 61% overall yield based on resin 22) as an amorphous solid.

Rₜ (toluene-acetone, 5:1) = 0.40.

1H NMR (600 MHz, CDCl₃): δ = -0.01 (s, 3 H, SiCH₃), 0.07 (s, 3 H, SiCH₃), 0.52–0.56 (m, 12 H, 4 CH₃), 1.48–1.54 (m, 1 H, CH(CH₃)₂), 1.91 (s, 3 H, COCH₂), 2.04 (s, 3 H, COCH₂), 2.05 (s, 3 H, COCH₂), 3.21 (s, 1 H, 5c-H), 3.60 (m, 1 H, 5a-H), 3.61 (m, 1 H, 5b-H), 3.85 (m, 2 H, 6b-H, 6b'-H), 3.86 (m, 1 H, 3a-H), 3.88 (m, 1 H, 6c-H), 3.96 (d, 1 H, J = 3.7 Hz, 4a-H), 3.97 (m, 1 H, 6a-H), 4.11 (m, 1 H, 4b-H), 4.17 (m, 1 H, 6-H), 4.22 (m, 2 H, 2b-H, 6c-H), 4.25 (d, 1 H, J = 3.2 Hz, 4c'-H), 4.29 (m, 1 H, 3b-H), 4.41 (d, 1 H, J = 12.2 Hz, CH(Ph)), 4.54 (d, 1 H, J₃ = 7.5 Hz, 1a-H), 4.58 (d, 1 H, J = 11.8 Hz, 1H, ), 4.64 (d, 1 H, J = 11.6 Hz, CH(Ph)), 4.67 (d, 1 H, J = 7.8 Hz, 1c-H), 4.72–4.77 (m, 1 H, CH(Ph)), 4.82 (dd, 1 H, J₃ = 10.4 Hz, J₄ = 3.7 Hz, 3c-H), 4.93 (d, 1 H, J = 12.2 Hz, CH(Ph)), 5.06 (d, 1 H, J = 11.8 Hz, CH(Ph)), 5.25 (d, 1 H, J = 8.4 Hz, 1b-H), 5.26 (m, 1 H, 2a-H), 5.36 (m, 1 H, 2c-H), 5.41 (s, 1 H, CH(Ph)), 6.68–8.05 (m, 25 H, Ph).

13C NMR (150.9 MHz, CDCl₃): 56.40 (1 C, C-2c), 63.88 (1 C, C-6a), 66.61 (1 C, C-5c), 68.67 (1 C, C-6b), 68.97 (1 C, C-6c), 69.71 (1 C, C-2c), 72.50 (1 C, C-3e), 72.78 (1 C, C-5a), 73.66 (1 C, C-4c), 73.68 (1 C, C-2a), 75.1 (1 C, C-5b), 76.04 (1 C, C-4a), 77.16 (1 C, C-3b), 78.69 (1 C, C-4b), 80.67 (1 C, C-3a), 96.80 (1 C, C-1a), 100.13 (1 C, C-1b), 100.84 (1 C, C-1c), 101.44 (1 C, CH(Ph)).

MALDI-MS, m/z calc for: [MNa+]*, 1387.5. Found: [MNa+], 1387.7.

Anal. Calcd for C₃₆H₅₃NO₁₂Si (1364.56): C, 66.01; H, 6.28; N, 1.03. Found: C, 66.32; H, 6.29; N, 1.00.

**Allyl (3,4,6-tri-O-benzyl-o-D-mannopyranosyl)-(1→2)-3,4-di-O-
benzyl-o-D-mannopyranoside (2)**
Resin 6-1 (9.8 μmol) was treated as described in procedure D. The residue was purified by flash chromatography (toluene-EtOAc, 10:1) to afford 2 (7.0 mg, 86% overall yield based on resin 30) as a colorless oil.

Rₜ (toluene-acetone, 5:1) = 0.37.

1H NMR (600 MHz, CDCl₃): δ = +1.12 (s, 1 H, CH(OH)).
Solid Phase Syntheses of Oligomannosides and of a Lactosamine Containing Milk Trisaccharide

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C5(b), 72.30 (1 C, C-5a), 72.57 (1 C, C2H5Ph), 72.63 (1 C, CH2Ph), 73.85 (1 C, CH2Ph), 74.70 (1 C, C-6b), 74.94 (1 C, C-4a), 75.41 (1 C, C-2a), 75.69 (2 C, 2 CH2Ph), 79.97 (1 C, C-3a), 80.14 (1 C, C-3b), 98.51 (1 C, C-1a), 101.53 (1 C, C-1b).

MALDI-MS, m/z calc'd for: [MNa]+, 856.0. Found: [MNa]+, 856.1.


Allyl (3,4,6-tri-O-benzyl-D-mannopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-D-mannopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-D-mannopyranosyl)-(1→2)-3,4-di-O-benzyl-D-mannopyranoside (3)

Resin 6-5 (52 μmol) was treated as described in procedure D. The residue was purified by flash chromatography (toluene–acetonitrile, 40:1) to afford 4 (38 mg, 19% overall yield based on resin 30) as a white, amorphous solid.

Rf (toluene:acetonitrile, 5:1) = 0.67.

[α]D20 +62.3 (c = 1.0, CHCl3).

(H NMR (600 MHz, CDC13): δ = 1.95 (bs, 1 H, 6a-CH2), 2.32 (s, 1 H, 2d-CH2), 3.42 (d, 1 H, J6awebsite, 9.6 Hz, 6c-CH2), 3.49 (m, 1 H, 5a-CH), 3.53 (dd, 1 H, J5,6a = 4.0 Hz, J6,6a = 10.6 Hz, 6b-CH2), 3.53 (m, 1 H, 6c-CH2), 3.60 (m, 1 H, 4b-CH2), 3.61 (m, 1 H, 6a-CH2), 3.62 (m, 2 H, 6d-CH2), 3.63 (m, 1 H, 6b′-CH2), 3.65 (m, 1 H, 4a-CH2), 3.67 (m, 3 H, OCH2CH2CH3), 3.68 (m, 1 H, 6a′-CH2), 3.77 (m, 1 H, 3b-CH2), 3.78 (m, 3 H, 3a-CH2, 5b-CH2, 4d-CH2), 3.80 (m, 1 H, 4c-CH2), 3.81 (m, 3 H, 3d-CH2), 3.84 (m, 1 H, 5d-CH2), 3.85 (m, 2 H, 3c-CH2), 3.90 (m, 1 H, 2a-CH2), 3.91 (m, 1 H, OCH2CH2CH2OCH2CH3), 4.01 (t, 1 H, J1,2′ = 2.4 Hz, 2b′-CH2), 4.05 (m, 1 H, 2c-CH2), 4.06 (m, 1 H, 2d-CH2), 4.90 (d, 1 H, J2,3′ = 12.2 Hz, C2H2Ph), 4.72 (d, 1 H, J1,2′ = 12.2 Hz, 2H, CH2Ph), 4.73–4.76 (m, 4 H, 2 CH2Ph), 4.86 (d, 1 H, J1,2′ < 1.0 Hz, 1a-CH2), 5.01 (d, 1 H, J2,3′ = 10.4 Hz, 1 H, OCH2CH2CH2OCH2CH3), 5.06 (d, 1 H, J1,2′ < 1.0 Hz, 1 H, 1d-CH2), 5.05–5.09 (m, 1 H, OCH2CH2CH2OCH2CH3), 5.13 (d, 1 H, J1,2′ = 1.3 Hz, 1b-CH2), 5.15 (d, 1 H, J1,2′ = 1.3 Hz, 1c-CH2), 5.68–5.72 (m, 1 H, OCH2CH2CH2OCH2CH3), 6.98–7.24 (m, 55 H, Ar).

13C NMR (150.9 MHz, CDC13): δ = 62.21 (1 C, C-6a), 67.99 (1 C, OCH2CH2CH2OCH2CH3), 68.49 (1 C, C-2d), 68.79 (1 C, C-6c), 69.50 (1 C, C-6b), 69.65 (1 C, C-6d), 71.60 (1 C, CH2Ph), 71.74 (1 C, C-5d), 71.80 (1 C, C-5a), 72.07 (1 C, CH2Ph), 72.20 (1 C, CH2Ph), 72.30 (1 C, C-5b), 72.50 (1 C, C-3c), 72.55 (1 C, CH2Ph), 73.31 (1 C, CH2Ph), 74.30 (1 C, C-4c), 74.42 (1 C, CH2Ph), 74.84 (1 C, C-4a), 74.94 (1 C, C-4d), 75.08 (1 C, C-4b), 75.14 (1 C, C-2c), 75.38 (1 C, C-2b), 75.40 (1 C, C-4d), 75.48 (1 C, C-3b), 75.71 (2 C, CH2Ph), 77.09 (1 C, CH2Ph), 77.11 (2 C, CH2Ph), 77.82 (1 C, C-3b), 79.17 (1 C, C-3a), 79.41 (1 C, C-3c), 80.09 (1 C, C-3d), 98.05 (1 C, C-1a), 100.93 (1 C, C-1c), 100.98 (2 C, C-1b, C-1d).

MALDI-MS, m/z calc'd for: [MNa]+, 2586.0. Found: [MNa]+, 2586.7.


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