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The chemical synthesis of human milk oligosaccharides: lacto-*N*-tetraose (Galβ1→3GlcNAcβ1→3Galβ1→4Glc)

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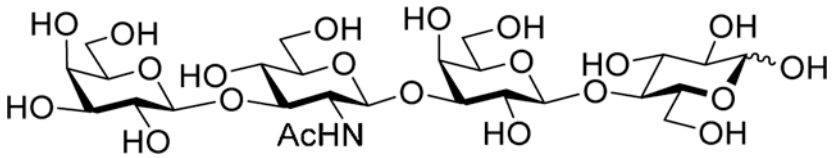
Abstract

The total chemical synthesis of lacto-*N*-tetraose (LNT) has been completed using both convergent and linear strategies. Similarly to that of our previous HMO syntheses, the donor-acceptor protecting-leaving group combinations were found to be of paramount significance to achieving successful glycosylations and minimizing side reactions.

Graphical Abstract



Carbohydrates are essential biomolecules that become our first food.[1] Oligosaccharides present in human milk (HMO) can supply building blocks for the development of the infants' cognition,[2] act as prebiotics[3] and antimicrobials.[4,5] Thanks to advances in glycol-sciences, chemical structures of 162 HMO have been elucidated to date,[6,7] but our understanding of how HMO function is incomplete.[8,9] All HMO are composed of five monosaccharides: including glucose (Glc), galactose (Gal), *N*-acetylglucosamine (Glc-NAc), fucose, and sialic acid.[10] Many efforts to prepare HMO enzymatically or chemically have been reported,[11–13] and importance of including HMO in infant formulas has been acknowledged.[14]

The total amount and composition of HMO varies between women and are dependent on maternal genetics, environment, and geographic location.[15] Lacto-N-tetraose (LNT) **1** represents one of the most common and abundant core structures and is classified as a type I HMO. It comprises a Galβ1→3GlcNAcβ1→3Galβ1→4Glc sequence shown in Scheme 1.

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Author Contributions

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Supporting Information

NMR spectra for all new compounds. This material is available free of charge via the Internet at

The authors declare no competing financial interests.

More specifically, LNT is a linear tetrasaccharide wherein the reducing end lactose disaccharide (Galβ1→4Glc) is elongated with lacto-N-biose disaccharide residue (Galβ1→3GlcNAc). Chemical[16–18] and enzymatic syntheses[19] of LNT have been reported, and several of its derivatives have been synthesized using chemical synthesis in solution and on solid phase.[20–24] Despite being one of the most abundant HMO core structures in human milk, LNT is not yet available in large quantities and at reasonable prices for research and application.

Previously, we reported the total synthesis of lacto-N-neotetraose that has been completed using both linear and convergent approaches.[25] Along the way, we developed the synthesis of key building blocks, accessed scalability, and refined coupling procedures to obtain different glycosidic linkages and sequences. Notably, the donor and acceptor protecting/leaving group combinations were found to be key parameters. In further synthetic studies of HMO in our lab reported herein is the synthesis of LNT **1**. First, we decided to investigate a convergent (2+2) synthetic strategy, according to which we chose to converge the protected lacto-*N*-biose donor **2** and lactose acceptor **3**.[25] Based on our previous synthetic endeavors and preliminary refinement of reaction conditions,[25] for the synthesis of disaccharide **2** we chose superarmed S-benzoxazolyl (SBox) galactosyl donor **4**[29,30] and glucosamine acceptor **5**. SBox galactosyl donor **4** was very instrumental in avoiding the unwanted aglycone transfer side reaction[12,26] that was taking place in other previously investigated building blocks.[25] As in our previous HMO synthesis, we chose benzyl ethers as semi-permanent protecting groups.

The synthesis of glucosamine thioglycoside acceptor **5** was achieved from known building block **6** as depicted in Scheme 2.[27] First, precursor **6** was reacted with tert-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in DMF at 90 °C to obtain compound **7** in 97% yield. The latter was then converted to intermediate **8** via the reductive regioselective opening of the benzylidene acetal by reaction with 1 M BH_3 in THF in the presence of catalytic TMSOTf in 82% yield. Subsequently, 6-OH in compound **8** was benzylated with BnBr in the presence of NaH in DMF to afford compound **9** in 77% yield. In order to minimize the formation of side products, temperature control is highly important in this reaction (see the experimental part for further details). Finally, the silyl group of compound **9** was removed with BF_3-Et_2O in CH_3CN at 0 °C to afford the desired glucosamine thioglycoside acceptor **5** in 87% yield.

We next turned our attention to the assembly of LNB disaccharide **2**. Selective activation of the SBox leaving group in glycosyl donor **4** over thioethyl anomeric moiety of acceptor **5** was achieved in the presence of silver trifluoromethanesulfonate (AgOTf) to afford β-linked disaccharide **2** in excellent yield of 99%. Direct coupling of disaccharide **2** with acceptor **3** was proven inefficient, perhaps due to a fairly unreactive donor and acceptor combination. We consistently saw incomplete reactions leading to very low yields of the tetrasaccharide product. To bypass this, we chose to employ the corresponding phosphate donor **10** that was obtained from thioglycoside **2** in 90% yield via an efficient one-step protocol developed by Seeberger and co-workers.[28] The coupling of phosphate donor **10** with lactose acceptor **3** in the presence of TMSOTf was more successful, and tetrasaccharide **11** was obtained in a moderate yield of 51% with complete β-stereoselectivity.

Despite numerous attempts involving modifying the reaction condition, and replacing the glycosyl donor with O- and S-imidoyl leaving groups, we failed to improve the outcome of this reaction. We were also unable to elucidate structures of byproducts forming alongside the desired tetrasaccharide **11**. Not being satisfied with the outcome of the convergent synthesis, we were curious to investigate whether the linear approach would be more successful in achieving a better outcome. Only a minimal strategic adjustment was required, and this involved conversion of building block **5** into its 4-O-Fmoc derivative **12** that was subsequently transformed into phosphate donor **13**. Glycosylation between donor **13** and lactose acceptor **3** in the presence of TMSOTf afforded trisaccharide **14** in 86% yield. The Fmoc protecting group was removed with 30% Et_3N in CH_2Cl_2 and glycosylation of the resulting trisaccharide acceptor **15** with SBox donor **4** in the presence of AgOTf afforded the desired β-linked tetrasaccharide **11** in 89% yield. Overall, the three-step linear assembly of **11** involving glycosylation of acceptor **3** with glycosyl donor **13**, interim Fmoc deprotection, followed by glycosylation with donor **4** proceeded with 68% overall yield for the synthesis of tetrasaccharide **11**. In contrast, the convergent approach was much less efficient, 45% over three steps, primarily due to the very low-yielding last coupling step between disaccharides **3** and **10**.

With the key tetrasaccharide intermediate **11** we endeavored to carry out its deprotection steps to obtain the target LNT tetrasaccharide **1**. Deprotection of the phthalimido and the ester groups was performed in the presence of NH_2NH_2-H_2O in refluxing MeOH. Subsequent N-acetylation with acetic anhydride in MeOH furnished tetrasaccharide intermediate **16** in 92% yield. Subsequently, benzyl ethers were hydrogenated the presence of 10% Pd/C in wet ethanol to afford the target trisaccharide **1** in 81% yield.

In summary, the total synthesis of lacto-N-tetraose has been completed using both linear and convergent synthesis approaches. The linear approach was significantly more effective in this application. Along the way, we have developed new synthetic protocols for different glycosidic linkages. Notably, the donor and acceptor protecting group and the leaving group combinations were found to be of paramount significance to successful glycosylations. The protecting groups in precursors used for the synthesis of the key building block **3** were chosen to provide access to variable glycosylation sites. In this application, 3’-OH acceptor **3** was achieved via the Fmoc group removal,[25] but the same precursors could also be used to achieve 6’-OH via the OPico group removal, or provide access to 3’,6’-diol for the synthesis of branched HMO. Further synthetic studies of HMO are underway in our laboratory. We expect that new methods for obtaining individual HMO will boost practical applications of these important biomolecules.

Experimental

General methods.

The reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70–230 mesh) and Sephadex G-25 size exclusion resin, reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by

examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 was distilled from CaH_2 directly prior to application. Molecular sieves (3Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2–3 h at 390 °C directly prior to application. AgOTf was co-evaporated with toluene (3 × 10 mL) and dried *in vacuo* for 2–3 h directly prior to application. Optical rotations were measured using a Jasco polarimeter. ^{1}H NMR spectra were recorded at 300 MHz or 600 MHz, and ^{13}C NMR spectra were recorded at 75 MHz or 151 MHz. The ^{1}H chemical shifts are referenced to the signal of the residual TMS (δ_H = 0.00 ppm) for solutions in $CDCl_3$ or the signal of the residual D_2O (δ_H = 4.79 ppm) for solutions in D_2O. The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.16 ppm) for solutions in $CDCl_3$ or the central signal of CD_3COCD_3 δ_C = 29.84 ppm) for solutions in D_2O. Accurate mass spectrometry determinations were performed using Agilent 6230 ESI TOF LCMS mass spectrometer.

Preparation of monosaccharide building blocks

Ethyl 4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (7).

TBDMSCl (0.37 g, 2.44 mmol) and imidazole (0.16 g, 2.44 mmol) were added to a solution of ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[27] (**6**, 0.54 g, 1.22 mmol) in DMF (7.0 mL) and the resulting mixture was heated at 90 °C for 3 h. After that, the reaction mixture was cooled to rt, diluted with CH_2Cl_2 (~250 mL) and washed with water (40 mL), sat. aq. $NaHCO_3$ (40 mL), and water (40 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white form in 97% yield (0.65 g, 1.18 mmol). Analytical data for **7**: R_f = 0.60 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{23}$ −2.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, −0.28, −0.12 (2 s, 6H, 2 x SiC*H*$_3$), 0.59 (s, 9H, Si^{t}Bu), 1.19 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.69 (m, 2H, CH_2CH_3), 3.59 (t, 1H, $J_{4,5}$ = 8.8 Hz, H-4), 3.71 (m, 1H, $J_{5,6a}$ = 10.0 Hz, *J*5,6b = 4.4 Hz, H-5), 3.81 (dd, 1H, $J_{6a,6b}$ = 10.0 Hz, H-6a), 4.32 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 4.39 (dd, 1H, H-6b), 4.67 (dd, 1H, $J_{3,4}$ = 8.8 Hz, H-3), 5.37 (d, 1H, $J_{1,2}$ = 10.7 Hz, H-1), 5.54 (s, 1H, *CH*Ph), 7.31–7.94 (m, 9H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, −5.1, −4.0, 15.0, 17.8, 24.2, 25.5 (×3), 56.7, 68.8, 70.6, 70.7, 81.9, 82.8, 102.1, 123.3, 123.8, 126.5 (×2), 128.3 (×2), 129.2, 131.7, 131.9, 134.3, 134.4, 137.2, 167.7, 168.4 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{29}H_{37}NNaO_6SSi$ 578.2009, found 578.1997.

Ethyl 4-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-*tert*butyldimethylsilyl-1-thio-β-D-glucopyranoside (8).

A 1 M solution of BH_3 in THF (43 mL, 43 mmol) was added to a solution of **7** (4.80 g, 8.66 mmol) in CH_2Cl_2 (45 mL). The resulting solution was cooled to 0 °C, TMSOTf (0.78 mL, 4.33 mmol) was added, and the resulting mixture was stirred for 5 h while the reaction temperature was allowed to gradually increase to rt. After that, the reaction was quenched with Et_3N (~2 mL) and MeOH (~5 mL), and the volatiles were removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~500 mL), washed with sat. aq. $NaHCO_3$ (50 mL) and water (2 × 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in*

vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a clear syrup in 82% yield (3.80 g, 7.06 mmol). Analytical data for **8**: R_f = 0.80 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{23}$ +27.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, −0.38, 0.00 (2 s, 6H, 2 x SiC*H*$_3$), 0.76 (s, 9H, Si^{t}Bu), 1.20 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.21 (m, 1H, OH), 2.68 (m, 2H, *CH*$_2$CH$_3$), 3.52–3.67 (m, 2H, H-4, 5), 3.73 (m, 1H, H-6a), 3.94 (m, 1H, H-6b), 4.27 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 4.56 (dd, 1H, $J_{3,4}$ = 8.0 Hz, H-3), 4.80 (dd, 2H, ^2J = 11.7 Hz, C*H*$_2$Ph), 5.40 (d, 1H, $J_{1,2}$ = 10.6 Hz, H-1), 7.27–7.95 (m, 9H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, −4.6, −4.0, 15.0, 17.7, 24.3, 25.7 (×3), 56.8, 62.0, 73.3, 74.7, 79.7 (×2), 81.2, 123.3, 123.7, 127.3 (×2), 127.6, 128.4 (×2), 131.7, 132.1, 134.3 (×2), 138.1, 167.6, 168.8 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{29}H_{39}NNaO_6SSi$ 580.2165, found 580.2163.

Ethyl 4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-*tert*butyldimethylsilyl-1-thio-β-D-glucopyranoside (9).

NaH (0.51 g, 0.021 mmol) was added portionwise to a cooled (−20 °C) solution of **8** (3.80 g, 7.06 mmol) in DMF (30 mL) and the resulting mixture was stirred under argon at −20 °C until gas evolution has ceased. After that, BnBr (1.06 mL, 9.18 mmol) was added and the resulting mixture was stirred for 6 h at −15 °C. The reaction mixture was cooled to −40 °C and glacial acetic acid (~2 mL) was added dropwise. The resulting mixture was allowed to attain rt, then diluted with EtOAc (~500 mL) and washed with water (50 mL), sat. aq. $NaHCO_3$ (50 mL) and water (2 × 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a colorless syrup in 77% yield (3.65 g, 5.42 mmol). Analytical data for **9**: R_f = 0.80 (ethyl acetate/hexane,3/7, v/v); $[\alpha]_D^{23}$ +39.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, −0.36, 0.00 (2 s, 6H, 2 x SiC*H*$_3$), 0.79 (s, 9H, Si^{t}Bu), 1.26 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.73 (m, 2H, C*H*$_2$CH$_3$), 3.63–3.71 (m, 2H, H-4, 5), 3.77–3.86 (br d, 2H, H-6a, 6b), 4.35 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.58 (dd, 1H, $J_{3,4}$ = 7.6 Hz, H-3), 4.64 (d, 2H, ^2J = 9.6 Hz, C*H*$_2$Ph), 4.79 (dd, 2H, ^2J = 12.0 Hz, C*H*$_2$Ph), 5.38 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 7.21–7.99 (m, 14H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, −4.6, −4.1, 15.1, 17.7, 23.9, 25.8 (×3), 56.8, 69.0, 73.4, 73.5, 74.6, 79.5, 80.0, 80.8, 123.2, 123.7, 127.1 (×2), 127.4, 127.6, 127.8 (×2), 128.4 (×4), 131.8, 132.2, 134.3 (×2), 138.3, 138.4, 167.7, 168.8 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{36}H_{45}NNaO_6SSi$ 670.2635, found 670.2633.

Ethyl 4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (5).

BF_3-Et_2O (1.05 mL, 8.29 mmol) was added to a solution of **9** (5.07 g, 7.54 mmol) in dry CH_3CN (90 mL) and the resulting mixture was stirred under argon for 20 min at 0 °C. After that, the reaction was quenched with sat. aq. $NaHCO_3$ (5 mL), and the volatiles were removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~500 mL) and washed with brine (2 × 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white amorphous solid in 87% yield (3.49 g, 6.54 mmol). Analytical data for **5**: R_f = 0.30 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{22}$ +7.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.20 (t, 3H, *J* = 7.4 Hz, CH_2CH_3). 2.36 (d, 1H, *J* = 4.5 Hz, OH), 2.67 (m, 2H, C*H*$_2$CH$_3$), 3.58–3.69 (m, 2H, H-4, 5),

3.75–3.85 (m, 2H, H-6a, 6b), 4.24 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2), 4.48 (m, 1H, H-3), 4.63 (dd, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.70 (m, 2H, ^2J = 11.5 Hz, CH_2Ph), 5.29 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 7.15–7.90 (m, 14H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.1, 24.1, 55.7, 69.0, 72.8, 73.6, 74.8, 79.3 (×2), 81.2, 123.4, 123.8, 127.8, 127.9 (×2), 128.0 (×2), 128.1, 128.5 (×2), 128.7 (×2), 131.7, 131.8, 134.2 (×2), 138.2 (×2), 168.1, 168.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{30}H_{31}NNaO_6S$ 556.1770, found 556.1767.

Ethyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (12).

FmocCl (3.88 g, 15.04 mmol) was added to a solution of **5** (2.08 g, 3.90 mmol) in CH_2Cl_2 (50 mL) and pyridine (1.72 mL) and the resulting mixture was stirred under argon for 2 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~500 mL) and washed with 1 M aq. HCl (50 mL) and water (2 × 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white amorphous solid in 92% yield (2.70 g, 3.58 mmol). Analytical data for **12**: R_f = 0.40 (ethyl acetate/hexane,2/3, v/v); $[\alpha]_D^{23}$ +55.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.22 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.70 (m, 2H, CH_2CH$_3$), 3.73–3.86 (m, 4H, H-5, 6a, 6b, OCOCH$_2$C*H*), 3.91–4.16 (m, 3H, H-4, OCOCH_2CH), 4.47 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2), 4.62 (dd, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.64 (dd, 2H, ^2J = 11.2 Hz, CH_2Ph), 5.46 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 5.75 (dd, 1H, $J_{3,4}$ = 8.9 Hz, H-3), 7.07–7.88 (m, 26H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.1, 24.2, 46.5, 54.1, 68.8, 70.3, 73.6, 75.0, 76.6, 78.5, 79.2, 81.0, 120.0 (×2), 123.7, 123.8, 125.1, 125.3, 127.3 (×2), 127.8 (×4), 127.9 (×4), 128.4 (×2), 128.5 (×2), 131.3, 131.8, 134.1, 134.4, 137.8, 138.2, 141.1, 141.2, 143.0, 143.3, 154.8, 167.5, 168.0 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{45}H_{41}NNaO_8S$ 778.2451, found 778.2451.

Di-*O*-butyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (13).

A mixture containing thioglycoside **12** (0.50 g, 0.66 mmol), dibutyl hydrogen phosphate (0.39 mL, 1.99 mmol), and molecular sieves (3 Å, 1.0 g) in CH_2Cl_2 (10 mL) was stirred under argon for 1h. The mixture was cooled to 0 °C, NIS (0.29 g, 1.32 mmol) and TfOH (10 μL, 0.13 mmol) were added, and the resulting mixture was stirred under argon for 20 min at 0 °C. After that, the solids were filtered off and washed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with 10% aq. $Na_2S_2O_3$ (15 mL), sat. aq. NaHCO3 (15 mL), and water (2 × 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white form in 94% yield (0.56 g, 0.62 mmol). Analytical data for **13**: R_f = 0.45 (ethyl acetate/hexane,1/4, v/v); $[\alpha]_D^{23}$ +43.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.71, 0.85 (2 t, 6H, 2 x O(CH$_2$)$_3$C*H*$_3$), 1.07 (m, 2H, O(CH$_2$)$_2$CH_2CH$_3$), 1.22–1.37 (m, 4H, OCH$_2$CH_2CH_2CH$_3$), 1.48–1.60 (m, 2H, OCH$_2$CH_2CH$_2$CH$_3$), 3.67–4.17 (m, 11H, 2 x OCH_2CH$_2$CH$_2$CH$_3$, H-4, 5, 6a, 6b, OCOCH_2CH, OCOCH$_2$C*H*), 4.49 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 4.60 (dd, 2H, ^2J = 11.9 Hz, CH_2Ph), 4.64 (dd, 2H, ^2J =11.2 Hz, CH_2Ph), 5.80 (dd, 1H, $J_{3,4}$ = 8.9 Hz, H-3), 6.02 (d, 1H, $J_{1,2}$ = 8.3, H-1), 6.97–7.86 (m, 22H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ,

13.5, 13.6, 18.4, 18.6, 31.9 (d, J = 7.0 Hz), 32.0 (d, J = 7.2 Hz), 46.4, 55.3 (d, J = 8.9 Hz). 67.9 (d, J 6.1 Hz), 68.1 (d, J = 6.0 Hz), 70.3, 73.6, 74.9, 75.2, 76.0, 76.8, 77.3, 93.9 (d, J = 4.6 Hz), 120.0 (×2), 123.6, 125.0, 125.2, 125.4, 127.2 (×2), 127.7 (×3), 127.8 (×3), 127.9 (×3), 128.3, 128.4 (×3), 128.5 (×3), 129.1, 134.2, 137.6, 137.9, 141.1, 141.2, 143.0, 143.2, 154.6 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{51}H_{54}NNaO_{12}P$ 926.3281, found 926.3285.

Synthesis of oligosaccharides

Ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2).

A mixture of benzoxazolyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[29,30] (**4**, 0.20 g, 0.29 mmol), acceptor **5** (0.12 g, 0.22 mmol), and freshly activated molecular sieves (3Å, 600 mg) in CH_2Cl_2 (7 mL) was stirred under argon for 2 h. The reaction mixture was cooled to −30 °C, and freshly conditioned AgOTf (0.15 g, 0.58 mmol) was added. The resulting mixture was stirred for 15 min while the temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off, and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 × 10 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 99% yield (0.23 g, 0.22 mmol). Analytical data for **2**: R_f = 0.55 (acetone/toluene, 1/9 v/v); $[\alpha]_D^{23}$ +34.1 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.09 (t, 3H, J = 7.4 Hz, CH_2CH_3). 2.56 (m, 2H, CH_2CH_3), 3.30–3.51 (m, 4H, H-3′, 5′, 6a′, 6b′), 3.57–3.72 (m, 2H, H-4, 5), 3.77 (br d, 2H, J = 2.1 Hz, H-6a, 6b), 3.94 (br d, 1H, $J_{3',4'}$ = 2.5 Hz, H-4′), 4.26 (dd, 2H, ^2J = 11.6 Hz, CH_2Ph), 4.29 (d, 1H, J = 12.0 Hz, CHPh), 4.30 (dd, 1H, $J_{2,3}$ = 10.3, H-2), 4.44 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.454.64 (m, 5H, 5 × CHPh), 4.83 (dd, 1H, $J_{3,4}$ = 8.0 Hz, H-3), 4.91 (d, 1H, J = 11.3 Hz, CHPh), 5.06 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.09 (d, 1H, J = 10.5 Hz, CHPh), 5.52 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, H-2′), 6.92–7.78 (m, 34H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 14.9, 23.7, 54.8, 67.8, 69.2, 71.7, 72.5, 72.8, 73.3, 73.4, 73.5, 74.8, 75.0, 77.4, 77.8, 79.5, 80.3, 81.0, 100.7, 127.2, 127.5 (×3), 127.6 (×2), 127.8 (×4), 128.0 (×3), 128.1 (×6), 128.2 (×3), 128.3 (×3), 128.4 (×3), 128.5 (×3), 130.0 (×2), 130.3, 131.5, 132.8, 134.0, 137.5, 138.0, 138.3, 138.7 (×2), 165.4 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{64}H_{63}NNaO_{12}S$ 1092.3969, found 1092.3981.

Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (10).

A mixture of compound **2** (0.236 g, 0.234 mmol), dibutyl hydrogen phosphate (0.14 mL, 0.802 mmol), and freshly activated molecular sieves (3 Å, 0.5 g) in CH_2Cl_2 (5.0 mL) was stirred under argon for 1 h at rt. The mixture was cooled to 0 °C, NIS (0.104 g, 0.468 mmol) and TfOH (4.15 μL, 0.047 mmol) were added, and the resulting mixture was stirred for 20 min at 0 °C. After that, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with 10% aq. $Na_2S_2O_3$ (15 mL) and sat. aq. $NaHCO_3$ (15 mL), and water (2 × 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on

silica gel (acetone - toluene gradient elution) to afford the title compound as an oily syrup in 94% yield (0.267 g, 0.219 mmol). Analytical data for **10**: R_f = 0.35 (acetone/toluene,1/9, v/v); $[\alpha]_D^{23}$ +26.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.67 (t, 3H, *J* = 7.2 Hz, $O(CH_2)_3CH_3$), 0.81 (t, 3H, *J* = 7.3 Hz, $O(CH_2)_3CH_3$), 0.90–1.06 (m, 2H, $O(CH_2)_2CH_2CH_3$), 1.11–1.31 (m, 4H, $O(CH_2)_2CH_2CH_3$, $OCH_2CH_2CH_2CH_3$), 1.38–1.53 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.31–3.96 (m, 13H, H-3′, 4, 4′, 5, 5′, 6a, 6b, 6a′, 6b′, 2 x $OCOCH_2(CH)_2CH_3$), 4.20 (d, 1H, ^2J = 11.7 Hz, CHPh), 4.24–4.32 (m, 3H, H-2, 2 x CHPh), 4.43–4.54 (m, 5H, H-1, 4 x CHPh), 4.59 (d, 1H, ^2J = 12.0 Hz, CHPh), 4.91 (m, 2H, H-3, CHPh), 5.09 (d, 1H, ^2J = 10.5 Hz, CHPh), 5.51 (dd, 1H, $J_{2',3'}$ = 8.8 Hz, H-2′), 5.69 (dd, 1H, $J_{1,2}$ = 7.4 Hz, H-1) 6.94–7.74 (m, 36H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.5, 13.6, 18.3, 18.6, 31.8 (d, *J* = 7.1 Hz). 32.0 (d, *J* = 7.2 Hz), 56.1, 56.2, 67.6, 67.8 (d, *J* = 4.5 Hz), 68.0 (d, *J* = 6.4 Hz), 68.6, 71.7, 72.5, 72.8, 73.3, 73.5 (×2), 74.8, 74.9, 75.6, 76.2, 80.2, 94.1 (d, *J* = 4.6 Hz), 100.8, 123.5, 127.3, 127.5 (×2), 127.6 (×3), 127.7, 127.8 (×3), 128.0 (×7), 128.1 (×4), 128.2 (×4), 128.3 (×2), 128.4 (×2), 128.5 (×2), 130.0, 130.2, 131.5, 132.7, 134.0, 137.5, 138.0, 138.1, 138.6, 138.7, 165.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{70}H_{76}NNaO_{16}P$ 1240.4799, found 1240.4829.

Benzyl *O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (14).

A mixture of donor **13** (0.14 g, 0.15 mmol), acceptor **3**[25] (0.12 g, 0.12 mmol) (reference from LNnT paper), and freshly activated molecular sieves (3Å, 450 mg) in CH_2Cl_2 (7.0 mL) was stirred under argon for 2 h. The mixture was cooled to −30 °C, TMSOTf (56 μL, 0.31 mmol) was added, and the resulting mixture was stirred for 15 min while the temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 × 10 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 86% yield (0.17 g, 0.10 mmol). Analytical data for **14**: R_f = 0.45 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{23}$ +11.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.91 (ddd, 1H, *J* = 1.8, 3.0, 9.9 Hz, H-5), 3.27 (dd, 1H, *J* = 9.5 Hz, H-6a), 3.33 (dd, 1H, $J_{2,3}$ = 8.4 Hz, H-2), 3.38–3.42 (m, 2H, H-3, 6b), 3.69–3.75 (m, 3H, H-3′, 5′, $OCOCH_2CH$), 3.79–3.85 (m, 4H, H-4, 5′′, 6a′′, 6b′′), 3.87–3.93 (m, 2H, H-4′′, $OCOCH_2CH$), 4.02 (dd, 1H, *J* = 10.5, 7.2 Hz, $OCOCH_2CH$), 4.11 (br d, 1H, *J* = 2.4 Hz, H-4′), 4.18–4.24 (m, 2H, H-6a′, CHPh), 4.27 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.34–4.40 (m, 2H, H-2′′, 6b′), 4.42–4.49 (m, 3H, $J_{1',2'}$ = 8.1 Hz, H-1′, 2 x CHPh), 4.5–4.69 (m, 7H, 7 x CHPh), 4.80 (d, 1H, ^2J = 12.0 Hz, CHPh), 4.81 (d, 1H, ^2J = 12.0 Hz, CHPh), 4.92 (d, 1H, ^2J = 10.4 Hz, CHPh), 5.12 (d, 1H, ^2J = 11.5 Hz, CHPh), 5.36 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, H-2′), 5.44 (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1′′), 5.66 (dd, 1H, *J* = 8.9, 10.7 Hz, H-3′′), 6.81–8.73 (m, 56H, aromatic) ppm; ^{13}C NMR (151 MHz, cdcl$_3$): δ, 46.4, 55.2, 63.8, 67.6, 68.9, 70.3, 71.0, 71.9, 72.0, 73.5 (×2), 73.6 (×2), 74.4, 74.7, 75.0 (×2), 75.2, 75.6, 76.2, 76.6, 80.6, 81.7, 82.6, 99.4, 100.4, 102.6, 120.0, 125.0, 125.3, 125.5, 126.9, 127.2 (×2), 127.3, 127.6 (×2), 127.7 (×3), 127.8 (×3), 127.9 (×5), 128.0, 128.1 (×9), 128.3 (×6), 128.4 (×3), 128.5 (×3), 128.6 (×9), 128.7 (×3), 129.4, 129.8, 132.9, 137.0, 137.6 (×2), 137.9, 138.3, 138.7 (×2), 139.0, 141.1, 141.2, 142.9,

143.3, 147.8, 150.0, 154.6, 164.5, 164.5 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{103}H_{94}N_2NaO_{21}$ 1718.6280, found 1718.6222.

Benzyl *O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (15).

Compound **14** (135 mg, 0.0796 mmol) was dissolved in a mixture of Et_3N in CH_2Cl_2 (5.0 mL, 3/7, v/v) and the resulting solution was stirred for 2 h at rt. After that, the reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 89% yield (104.8 mg, 0.0711 mmol). Analytical data for **15**: R_f = 0.55 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{22}$ −11.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.45 (d, 1H, *J* = 4.6 Hz, OH), 2.94 (ddd, 1H, *J* = 8.9 Hz, H-5), 3.29 (dd, 1H, *J* = 10.3 Hz, H-6a), 3.34 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.39–3.44 (m, 2H, H-3, 6b), 3.61 (dd, 1H, *J*3",4" = 9.1 Hz, *J*4",5" = 9.1 Hz, H-4′′), 3.67–3.76 (m, 3H, H-3′, 5′′, 6a′′), 3.78–3.88 (m, 3H, H-4, 5′, 6b′′), 4.11 (br s, 1H, H-4′), 4.13–4.26 (m, 3H, H-2′′, 6a′, C*H*Ph), 4.29 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.36 (dd, 1H, *J* = 6.1, 11.0 Hz, H-6b′), 4.44–4.52 (m, 4H, $J_{1',2'}$ = 8.0 Hz, H-1′, 3′′, 2 x C*H*Ph), 4.55–4.70 (m, 6H, 6 x C*H*Ph), 4.74 (d, 1H, ^2J = 11.4 Hz, C*H*Ph), 4.82 (d, 2H, $_2J$ = 12.0 Hz, C*H*Ph × 2), 4.94 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.14 (d, 1H, ^2J = 11.5 Hz, C*H*Ph), 5.22 (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1′′), 5.34–5.39 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, H-2′), 7.00–8.75 (m, 48H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 56.8, 63.9, 67.5, 69.1, 71.0 (×2), 72.0 (×2), 73.4, 73.6 (×2), 74.3, 74.9 (×3), 75.1, 75.6, 76.1, 79.2, 80.3, 81.6, 82.6, 99.7, 100.3, 102.5, 125.4, 126.9, 127.2, 127.5, 127.7 (×3), 127.8 (×3), 127.9, 128.0 (×8), 128.1 (×4), 128.2 (×3), 128.3 (×6), 128.4 (×3), 128.5 (×3), 128.6 (×6), 128.7 (×3), 129.4, 129.6, 132.9, 133.6, 137.0, 137.5, 137.9, 138.1, 138.2, 138.6, 138.8, 139.0, 147.7, 149.9, 164.4 (×2) ppm; ESI TOF LCMS $[M+H]^+$ calcd for $C_{88}H_{85}N_2O_{19}$ 1473.5747, found 1473.5757.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthlimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*- benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11).

Convergent method.—A mixture of donor **10** (80.0 mg, 0.065 mmol), acceptor **3** (49.8 mg, 0.050 mmol),[25] and freshly activated molecular sieves (3Å, 400 mg) in CH_2Cl_2 (7.0 mL) was stirred under argon for 2 h. The mixture was cooled to −60 °C, TMSOTf (24 μL, 0.131 mmol) was added, and the resulting mixture was stirred for 30 min while the temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 × 10 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 51% yield (66.6 mg, 0.033 mmol). *Linear method*. A mixture of benzoxazolyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[29,30] (**4**, 13.3 mg, 0.0194 mmol), acceptor **15** (22 mg, 0.0149 mmol), and freshly activated molecular sieves (3Å, 100 mg) in CH_2Cl_2 (2.0 mL) was stirred under argon for 2 h. The reaction mixture was cooled to −30 °C, freshly conditioned AgOTf (10.0 mg, 0.0387 mmol) was added, and the resulting mixture was stirred for 15 min while the

temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off and was rinsed successively with CH_2Cl_2. The combined filtrate (~30 mL) was washed with sat. aq. $NaHCO_3$ (7 mL) and water (2 × 7 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone -toluene gradient elution) to afford the title compound as a white foam in 89% yield (26.7 mg, 0.0132 mmol). Analytical data for **11**: R_f = 0.45 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ +2.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.85 (ddd, 1H, *J* = 9.6 Hz, H-5), 3.19 (dd, 1H, *J* = 10.2 Hz, H-6a), 3.25–3.37 (m, 6H, H-2, 3, 3′′′, 6a′′′, 6b, 6b′′′), 3.43 (m, 1H, H-5′′′), 3.58–3.66 (m, 3H, H-3′, 4′′, 5′), 3.70 (m, 1H, H-5′′), 3.84–3.75 (m, 3H, H-4, 6a′′, 6b′′), 3.88 (br d, 1 H, *J* = 1.9 Hz, H-4′′′), 3.98 (s, 1H, H-4′), 4.14–4.30 (m, 9H, H-1, 1′′′, 2′′, 6a′, 6b′, 4 x C*H*Ph), 4.33 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.41–4.60 (m, 9H, 9 x C*H*Ph), 4.66 (d, 1H, ^2J = 11.0 Hz, C*H*Ph), 4.78–4.82 (m, 3H, H-3′′, 2 x C*H*Ph), 4.89 (d, 2H, ^2J = 11.0 Hz, 2 x C*H*Ph), 4.95 (d, 1H, ^2J = 11.7 Hz, C*H*Ph), 5.00 (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1′′), 5.06 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.17 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2′), 5.45 (dd, 1H, $J_{2'',3''}$ = 8.9 Hz, H-2′′′), 6.75–8.69 (m, 68H, aromatic) ppm: ^{13}C NMR (151 MHz, $CDCl_3$): δ, 29.8, 31.1, 56.0, 63.8, 67.4, 67.8, 69.5, 71.0, 71.8, 72.0 (× 2), 72.6, 73.2, 73.5 (× 2), 73.6, 74.4, 74.8, 74.9, 75.0, 75.1, 75.2, 75.6, 75.8 (× 2), 76.1, 79.4, 80.2, 81.6, 82.6, 99.3, 100.4, 100.5, 102.6, 122.7, 123.7, 125.4, 126.8, 127.3, 127.4 (×2), 127.5 (×4), 127.6 (×2), 127.7, 127.8 (×7), 127.9, 128.0 (×3), 128.1 (×7), 128.2 (×8), 128.3 (×4), 128.4 (×7), 128.5 (×6), 128.6 (×2), 128.8 (×2), 129.6, 129.8, 130.0, 130.2, 130.9, 131.3, 132.7, 133.0, 133.4, 134.6, 136.9, 137.5, 137.6, 138.0, 138.1, 138.2, 138.5, 138.7, 138.8, 139.0, 147.8, 150.0, 164.3, 164.4, 165.5, 166.3, 168.4 ppm; ESI TOF LCMS $[M+H]^+$calcd for $C_{122}H_{117}N_2O_{25}$ 2010.7979, found 2010.7963.

Deprotection of tetrasaccharide 11

Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(3,6-di-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16).

Compound **11** (59.0 mg, 0.029 mmol) was dissolved in MeOH (3.0 mL), NH_2NH_2-H_2O (130 μL, 2.64 mmol) was added, and the resulting mixture was heated at 90 °C for 24 h. After that, the volatiles were removed under reduced pressure, and the residue was dried *in vacuo* for 3 h. The crude residue was dissolved in a mixture of Ac_2O and MeOH (2.0 mL, 1/1, v/v) and the resulting mixture was stirred for 12 h at rt. The volatiles were removed under reduced pressure, the residue was diluted with CH_2Cl_2 (50 mL), and washed with sat. aq. $NaHCO_3$ (10 mL) and 1 M HCl (10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 92% yield (42.9 mg, 0.026 mmol). Analytical data for **16**: R_f = 0.50 (acetone/toluene, 3/7 v/v); $[\alpha]_D^{23}$ +70.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 1.81 (s, 3H, CH_3CO), 2.95–4.03 (m, 24H, H-2, 2′, 2′′, 2′′′, 3, 3′, 3′′, 3′′′, 4, 4′, 4′′, 4′′′, 5, 5′, 5′′, 5′′′, 6a, 6a′, 6a′′, 6a′′′, 6b, 6b′, 6b′′,6b′′′), 4.23 (d, 1H, ^2J = 11.8 Hz, C*H*Ph), 4.30–4.58 (m, 10H, H-1, 1′, 1′′′, 7 x C*H*Ph), 4.60–4.96 (m, 11H, 11 x C*H*Ph), 5.01–5.04 (m, 2H, H-1′′, C*H*Ph), 6.48 (d, 1H, *J* = 6.3 Hz, N*H*COCH$_3$), 7.12–7.37 (m, 50H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 23.7, 58.4, 61.9, 68.2, 68.8, 69.4, 71.3, 71.6, 71.9,

72.1, 72.8, 73.5, 73.7 (×3), 73.8, 74.3, 74.7, 74.8, 74.9, 75.1 (×2), 75.2, 75.4, 76.6, 77.1, 81.9, 82.1, 83.0, 83.1, 83.7, 101.8, 102.9, 103.1, 104.5, 127.4, 127.5, 127.6 (×3), 127.7 (×2), 127.8 (×3), 127.9 (×4), 128.0 (×7), 128.2 (×8), 128.3 (×6), 128.4 (×4), 128.5 (×6), 128.7 (×3), 128.8 (×2), 137.6, 138.0 (×2), 138.1, 138.2, 138.4, 138.6, 138.7, 138.9, 139.0, 172.3 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{96}H_{105}NNaO_{21}$ 1631.7110, found 1631.7121.

O-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (1, LNT).

10% Pd on carbon (125 mg) was added to a solution of tetrasaccharide **16** (40 mg, 0.025 mml) in 80% aq. EtOH (5.0 mL), and the resulting mixture was stirred under hydrogen atmosphere for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol and water. The combined filtrate (~40 mL) was concentrated in *vacuo*. The residue was purified by size exclusion column chromatography on Sephadex G-25 using water as the eluent to afford the title compound as a white amorphous solid in 81% yield (14.3 mg, 0.020 mmol). Analytical data for **1**: R_f = 0.30 (chloroform/methanol/water, 2/1/0.4, v/v/v); ^{1}H NMR (600 MHz, D_2O): δ, 2.01 (s, 3H, C*H*$_3$CO), 3.24–3.51 (m, 2H), 3.49–3.96 (m, 33H), 4.14 (d, 1H, *J* = 3.3 Hz), 4.43 (d, 2H, *J* = 7.8 Hz), 4.65 (d, 1H, *J* = 8.0 Hz), 4.72 (dd, 1H, *J* = 2.5, 8.4 Hz), 5.21 (d, 1H, *J* = 3.8 Hz) ppm; ^{13}C NMR (151 MHz, D_2O): δ, 22.6, 55.0, 60.3, 60.4, 60.8, 61.3, 61.4, 68.7, 68.8, 68.9, 70.4, 70.5, 71.0, 71.5, 71.8, 72.8, 74.1, 74.7, 75.1, 75.2, 75.5, 75.6, 78.6, 78.7, 82.3, 82.4, 92.2, 96.0, 96.1, 102.9, 103.2, 103.3, 103.8, 175.3 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{26}H_{45}NNaO_{21}$ 730.2382, found 730.2361.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

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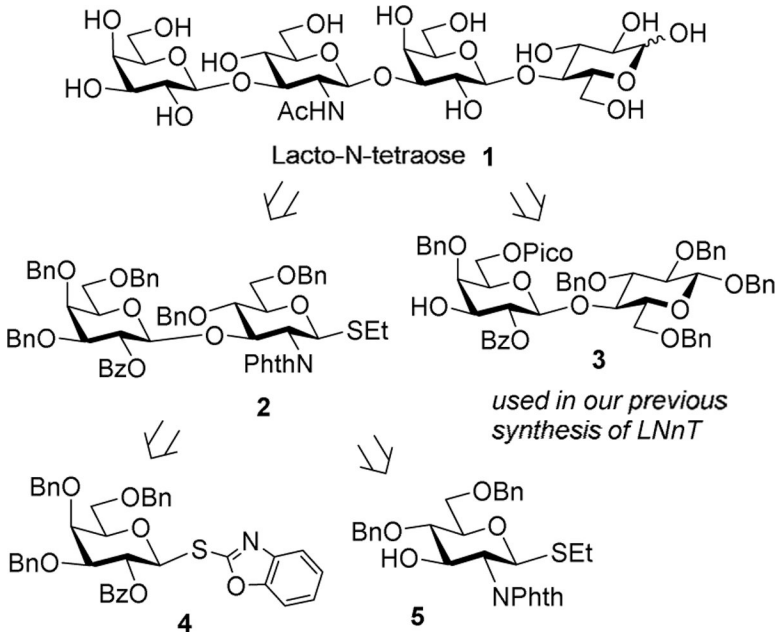
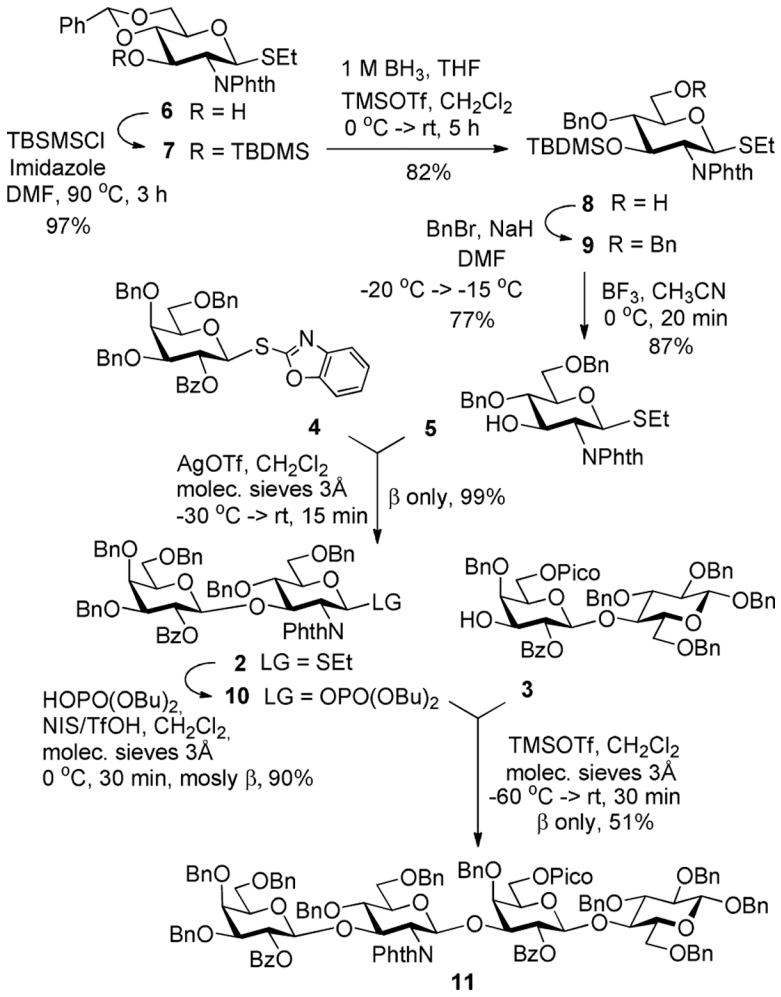
- The total synthesis of lacto-N-tetraose has been completed using both linear and convergent synthesis approaches;
- The linear approach was proven to be more efficient in this application;
- New synthetic protocols for different glycosidic linkages have been developed and refined;
- New methods for obtaining individual HMO help to improve understanding their roles and boost practical applications

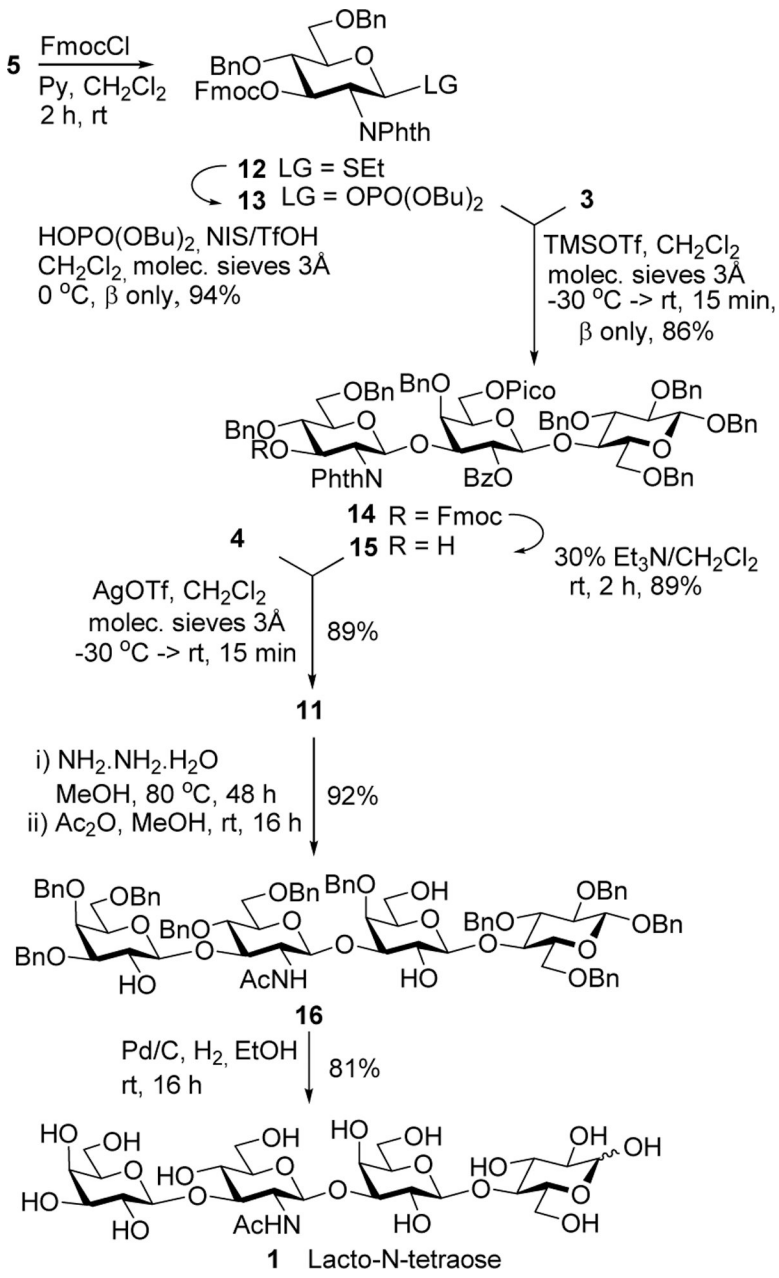


Scheme 1.
Retrosynthesis analysis of LNT 1.



Scheme 2.

Convergent synthesis of protected LNT 11.



Scheme 3.

The linear synthesis of tetrasaccharide 11 and its deprotection to obtain LNT 1.