Complex Structures of Antennary Human Milk Oligosaccharides – Synthesis of a Branched Octasaccharide

Petra Knuhr,[a] Julio Castro-Palomino,[b] Matthias Grathwohl,[a] and Richard R. Schmidt*[a]

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We have developed a highly convergent synthetic route for the synthesis of the branched structure of human milk octasaccharide β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→4)-α,β-D-glucopyranose (**1**). In the retrosynthetic analysis, target structure **1** was disconnected into building blocks **2**–**6**. Starting from only four known building blocks – **4**, **7**, **8**, and **12** – the required three disaccharide units were obtained, resulting after further protecting group manipulation and glycoside bond formation in the desired tetrasaccharides **13** and **16**. Cleavage of the TBDMS group of **13** afforded tetrasaccharide **14**, which was transformed into isolactosamine-β-(1→3)-lactosamine trichloroacetimidate **15**. Removal of the 4b,6b-*O*-benzylidene group of tetrasaccharide **16** gave the lacto-*N*-tetraose acceptor **17**, to afford the protected octasaccharide **18** on glycosylation with donor **15**. Complete deprotection of the octasaccharide by way of **19** afforded target human milk oligosaccharide **1** in a short and efficient route.

Introduction

Cell surface carbohydrates and soluble glycoforms are major components of mammalian organisms. The corresponding oligosaccharide structures change dramatically during development. The different sets of glycostructures at each stage of differentiation are correlated to specific functions within different organisms.[1–3] Investigation of correlations between oligosaccharide structure and biological function is therefore one of the main interests of carbohydrate chemists. In particular, research on human milk oligosaccharides (HMOs), which belong mainly to the *lacto*- and the *lactoneo*- series, has received much attention in recent years. It began, however, about a century ago, with the assignment of the corresponding structural core elements of HMOs and the first determination of biological functions.[4–7] Today, there is striking evidence that HMOs are potent inhibitors of bacterial adhesion,[8–10] occurring with a high degree of variation,[11,12] and that their biological functions are closely related to their conformation.[13] The progress in this field concerning structural, functional, and metabolic aspects of HMOs has recently been reviewed.[14]

Until now, only a few well defined HMO structures have been synthesized, by solution synthesis,[15,16] chemoenzymatic,[17,18] and solid-phase approaches.[19] We decided to focus on different methods for the synthesis of more complex HMOs in solution[20,21] and on solid-phase synthesis.[22–24] Here we describe a pathway for the synthesis of HMOs, affording the branched antennary octasaccharide **1** (Scheme 1) incorporating *N*-acetylisolactosamine-β-(1→3)-*N*-acetyllactosamine-β-(1→6)-[*N*-acetylisolactosamine-β-(1→3)]lactose; full details of the synthesis are reported. As well as the lactose moiety, another central structural unit of the HMOs and also of most other glycoconjugates is D-glucosamine; it is mainly found as the *N*-acetyl derivative in β-glycosidic linkages,[25,26] for which good linkage methods exist.[25–28] In retrosynthetic analysis, target structure **1** was therefore disconnected into building blocks **2**–**6**, which are isolactosamine (**2, 3**), lactosamine (**5, 6**), and lactose (**4**) derivatives. In order to reach the target octasaccharide **1**, we decided to use the powerful trichloroacetimidate method[25,27] to connect the suitably protected building blocks.

Results and Discussion

Isolactosamine derivative **2** was obtained in high yield from the known acceptor **7**[28] and the known *O*-(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-α-D-galactopyranosyl)trichloroacetimidate (**8**)[29] acting as glycosyl donor, at 0 °C in the presence of TMSOTf as catalyst (Scheme 2). Galactosyl donor **8** has already been successfully applied to the synthesis of Lewis antigens[29] and of *N*-glycans.[30] In this synthesis, **8** offers opportunities to install the galactose residues both of the required isolactosamine trichloroacetimidate **3**, as glycosyl donor, and of the corresponding lactosamine **5** as acceptor. The 4a,6a-*O*-benzylidene group of **2** was removed by treatment with *p*TsOH in the presence of ethanethiol as nucleophile,[31] thus affording 4a,6a-*O*-unprotected disaccharide **9**. Treatment of **9** with acetic anhydride/pyridine af-

[a] Fachbereich Chemie, Universität Konstanz
Fach M 725, D-78457 Konstanz, Germany
Fax: (internat.) + 49-(0)7531/88-3135
E-mail: richard.schmidt@uni-konstanz.de
[b] Bayer AG, Business Group Pharma VI,
42096 Wuppertal, Germany
Fax: (internat.) + 49-(0)202/36-4883
E-mail: julio.castro-palomino.jc@bayer-ag.de

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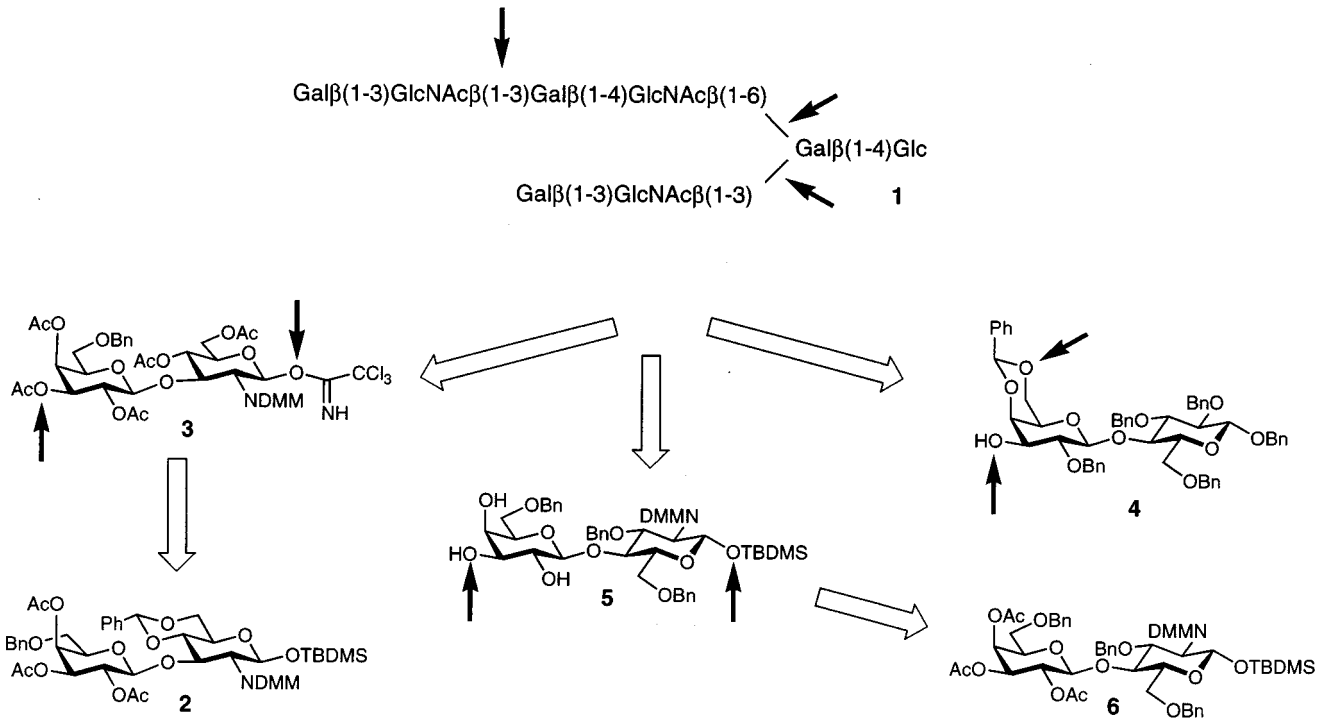
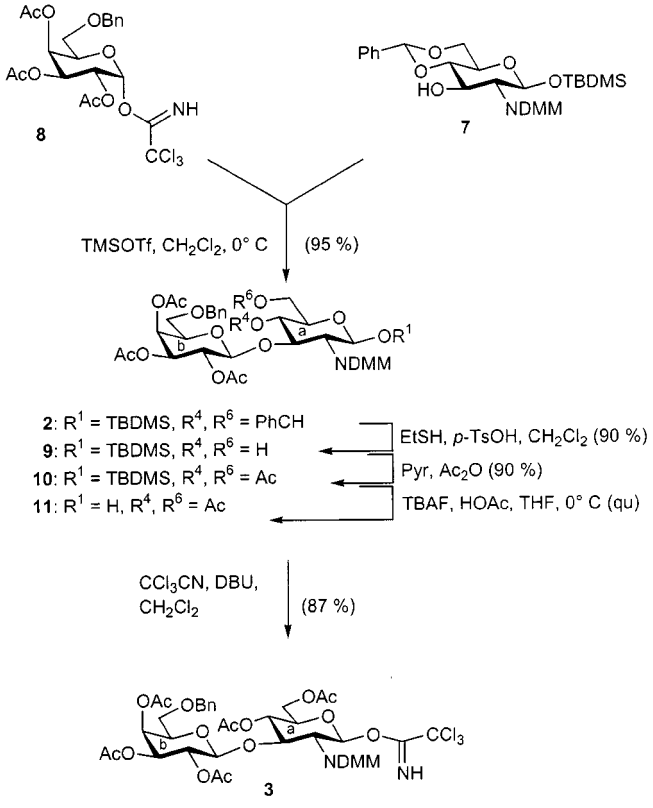


Scheme 1. Structure of the complex octasaccharide of human milk **1** and its retrosynthesis resulting in building blocks **2**–**6**

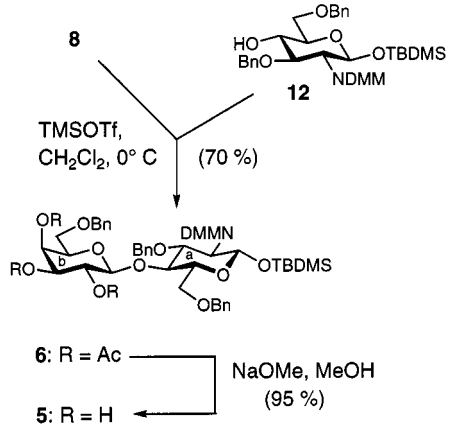


Scheme 2. Synthesis of the isolactosamine building block **3**

forded 4a,6a-di-*O*-acetyl derivative **10**, and cleavage of the TBDMS group with TBAF at 0 °C in the presence of acetic acid gave 1-*O*-unprotected derivative **11**. This in turn afforded the desired trichloroacetimidate **3** on treatment with CCl_3CN and a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Only the β anomer was obtained (^{1}H NMR: $J_{1,2}$ = 8.9 Hz, 1a-*H*).

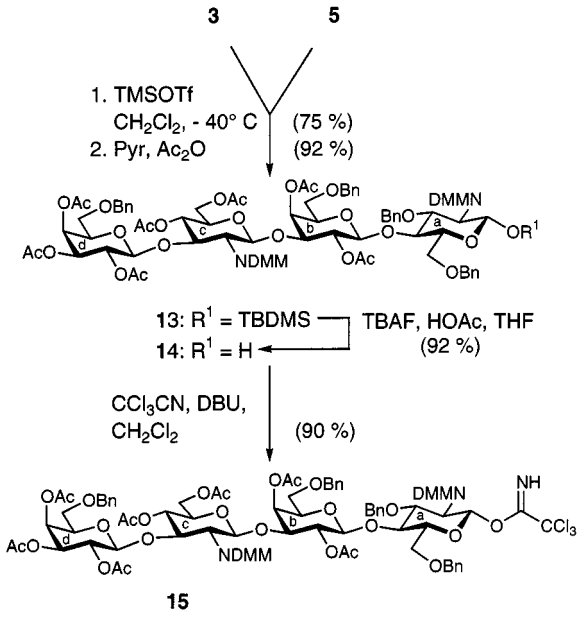
The lactosamine building block **5** was readily obtained from known glucosamine derivative **12**[28] as acceptor and galactosyl donor **8**[29] (Scheme 3); glycosylation at 0 °C in the presence of TMSOTf as catalyst furnished the desired β-linked disaccharide **6** (^{1}H NMR: $J_{1,2}$ = 7.8 Hz, 1b-*H*). This afforded 2b,3b,4b-*O*-unprotected acceptor **5** on de-*O*-acetylation under Zemplén conditions.[32]

The next convergent part of the synthesis design is the connection of isolactosamine trichloroacetimidate **3** both with known acceptor **4**[33] and also with **5**, thus affording the desired isolacto-*N*-tetraose donor **15** and the lacto-*N*-tetraose acceptor **17**, respectively. Glycosylation of 2b,3b,4b-*O*-unprotected acceptor **5** with donor **3** proceeded

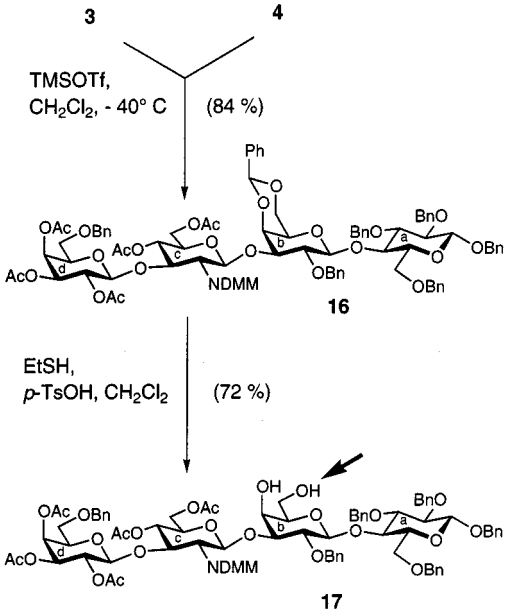


Scheme 3. Synthesis of the lactosamine building block **5**

regioselectively at −40 °C under TMSOTf catalysis conditions to furnish the desired tetrasaccharide in 75% yield. This gave hepta-*O*-acetyl derivative **13** on *O*-acetylation (Scheme 4). Subsequent treatment of **13** with TBAF and acetic acid in THF resulted in desilylation to give **14**, which can easily be transformed into trichloroacetimidate **15** by employing standard conditions. The lacto-*N*-tetraose building block **16** was again prepared by using the trichloroacetimidate **3** as the donor in the glycosylation of known lactose acceptor **4**,[33] using the same conditions as described above (Scheme 5). The β linkage of residue c was ascertained from NMR spectroscopic data ($J_{1,2}$ = 8.4 Hz, 1c-*H*; 99.7, *C*-1c).



Scheme 4. Synthesis of the tetrasaccharide donor **15**

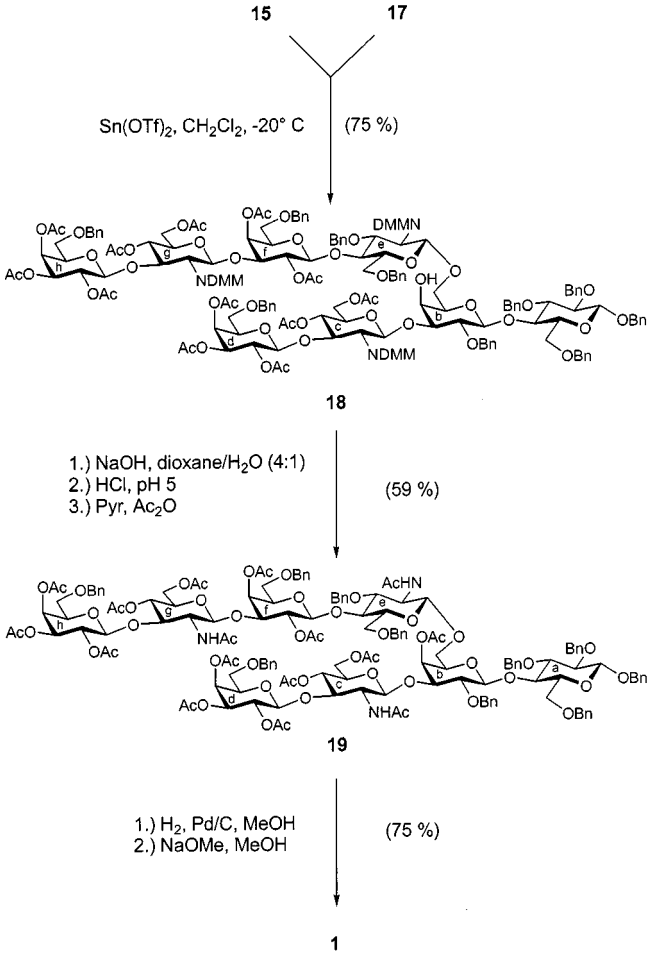


Scheme 5. Synthesis of lacto-*N*-tetraose acceptor **17**

Removal of the 4b,6b-*O*-benzylidene group of **16** by treatment with *p*TsOH in the presence of ethanethiol afforded the desired 4b,6b-*O*-unprotected lacto-*N*-tetraose acceptor **17**.

Glycosylation of acceptor **17** with donor **15** to furnish the protected target octasaccharide **18** (Scheme 5) proceeded smoothly and regioselectively by use of catalytic amounts of $Sn(OTf)_2$ as Lewis acid and by performing the glycosylation reaction at low temperature (−20 °C). The isolactosamine-β-(1→3)-lactosamine residue of donor **15** was regioselectively attached at the 6b-*O*-position of the tetrasaccharide acceptor **17** and the β anomer **18** was generated exclusively, as indicated by the NMR spectroscopic data ($J_{1,2}$ = 8.2 Hz, 1e-H; *C*-6b: δ = 67.1).

The three DMM groups in **18** were removed by treatment with sodium hydroxide followed by mild acidification (pH 5) as previously described,[20,21] thus affording the fully protected octasaccharide **19** after *N*,*O*-acetylation with acetic anhydride in pyridine. Complete deprotection of **19** was achieved by hydrogenolytic debenzylation (Pd/C, H_2) and complete de-*O*-acetylation using sodium methoxide in methanol, resulting in the target human milk octasaccharide **1** (Scheme 6).



Scheme 6. Synthesis of the target octasaccharide **1**

Conclusion

A highly convergent synthetic route to complex antennary structures of human milk oligosaccharides (HMOs) has been developed and the synthesis of branched octasaccharide **1** has been achieved by this approach. The efficiency is due to the convergency as well as to several regioselective and stereoselective glycosylation steps. Work based on this strategy and aimed at the synthesis of even more complex sialylated structures and higher oligomers of HMOs and the available versatile building blocks is currently underway.

Experimental Section

General Remarks: Solvents were purified and dried in the usual way. All reactions were performed with dry solvents and under argon unless otherwise stated. − TLCs were performed on 60 F_{254} silica gel plastic plates. − Detection was achieved by treatment with a solution of 20 g ammonium molybdate and 0.4 g cerium(IV) sulfate in 400 mL 10% H_2SO_4 or with 15% H_2SO_4, and heating at 150 °C. − Flash chromatography was carried out on silica gel (Baker 30−60 μm). Adsorption of crude reaction products was performed using silica gel (Baker 60−200 μm). Petroleum ether used was of the boiling range 35−70 °C; toluene, CH_2Cl_2, MeOH and EtOAc were distilled. − Optical rotations were determined at 21 °C with a Perkin−Elmer 241/MC polarimeter (1dm 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 Microanalysis Unit at the Fachbereich Chemie, Universität Konstanz.

***tert*-Butyldimethylsilyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (2):** TMSOTf (0.01 M in dichloromethane, 0.3 mL) was added dropwise under nitrogen at 0 °C to a stirred mixture of **7** (5.24 g, 10.70 mmol)[28] and **8** (6.94 g, 12.83 mmol)[29] in dry dichloromethane (10 mL). After 10 min the solution was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 3:1) to afford **2** (8.82 g, 95%) as a white foam. − TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.33. − $[\alpha]_D$ = + 4.3 (c = 2.0, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = −0.05 (s, 3 H, SiCH_3), 0.04 (s, 3 H, SiCH_3), 0.73 (s, 9 H, SiC(CH_3)$_3$], 1.86 (s, 3 H, CH_3), 1.90 (s, 3 H, CH_3), 1.97 (2s, 6 H, 2 COCH_3), 2.00 (s, 3 H, COCH_3), 3.31 (dd, $J_{5,6}$ = 5.8 Hz, $J_{6,6'}$ = 9.3 Hz, 1 H, 6b-*H*), 3.36 (dd, ^3J = 7.5, 9.2 Hz, 1 H, 6′b-*H*), 3.51 (m, 1 H, 5a-*H*), 3.53 (m, 1 H, 5b-*H*), 3.73 (m, 1 H, 4a-*H*), 3.76 (m, 1 H, 6a-*H*), 3.99 (dd, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 10.5 Hz, 1 H, 2a-*H*), 4.22 (d, ^2J = 11.9 Hz, 1 H, 1/2 CH_2Ph), 4.26 (dd, $J_{5,6'}$ = 5.0 Hz, $J_{6,6'}$ = 10.5 Hz, 1 H, 6′a-*H*), 4.42 (d, ^2J = 11.9 Hz, 1 H, 1/2 CH_2Ph), 4.53 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1b-*H*), 4.56 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 8.8 Hz, 1 H, 3a-*H*), 4.83 (dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.5 Hz, 1 H, 3b-*H*), 5.00 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.2 Hz, 1 H, 2b-*H*), 5.18 (d, $J_{1,2}$ = 8.2 Hz, 1 H, 1a-*H*), 5.31 (t, ^3J = 3.5 Hz, 1 H, 4b-*H*), 5.47 (s, 1 H, C*H*Ph), 7.20−7.44 (m, 10 H, *Ph*). − ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 57.6 (*C*-2a), 66.8 (*C*-5b), 67.5 (*C*-4b), 67.5 (*C*-6b), 69.0 (*C*-6a), 70.1 (*C*-2b), 71.5 (*C*-3b), 71.7 (*C*-5a), 75.5 (*C*-3a), 81.3 (*C*-4a), 94.2 (*C*-1a), 100.5 (*C*-1b), 101.4 (*C*HPh). − $C_{44}H_{57}NO_{15}Si$ (868.01): calcd. C 60.88 H, 6.62, N 1.61; found C 60.78, H 6.65, N 1.49.

***O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl Trichloroacetimidate (3):** Glacial acetic acid (0.19 mL, 3.29 mmol) and TBAF (3.3 mL of a 1 M solution in THF, 3.29 mmol) were added whilst stirring to a solution of **10** (2.58 g, 2.99 mmol) in dry THF (30 mL) in an ice-salt bath. After 30 min the solution was treated with a saturated sodium chloride solution (50 mL) and extracted with dichloromethane (3 × 30 mL). The organic layer was separated and dried with anhydrous magnesium sulfate, and the solvents were evaporated in vacuo. The residue was purified by flash chromatography (toluene/acetone, 7:2) to yield **11** (2.24 g, quant.) as a white foam. − TLC (petroleum ether/ethyl acetate, 1:1): R_f = 0.5. − A mixture of this foam (2.24 g, 2.99 mmol), trichloroacetonitrile (2.58 mL, 25.74 mmol), and DBU (8 μL, 0.05 mmol) in dry dichloromethane (40 mL) was stirred at room temp. for 1 h and then concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone, 6:1 + 1% NEt_3) to give **3** (2.32 g, 87%) as a white foam. − TLC (petroleum ether/ethyl acetate, 1:2): R_f = 0.6. − $[\alpha]_D$ = +6.5 (c = 1.0, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.91 (2s, 6 H, 2 CH_3), 1.95 (2s, 6 H, 2 COCH_3), 2.00 (s, 3 H, COCH_3), 2.03 (s, 3 H, COCH_3), 2.08 (s, 3 H, COCH_3), 3.37 (t, ^3J = 8.0 Hz, 1 H, 5a-*H*), 3.52 (dd, $J_{5,6}$ = 5.6 Hz, $J_{5,6'}$ = 8.9 Hz, 1 H, 5b-*H*), 3.68 (t, ^3J = 5.6 Hz, 1 H, 6b-*H*), 3.84−3.88 (m, 1 H, 6′b-*H*), 4.11−4.16 (m, 1 H, 6a-*H*), 4.17 (d, $J_{1,2}$ = 7.4 Hz, 1 H, 1b-*H*), 4.24−4.33 (m, 1 H, 2a-*H*), 4.38 (d, ^2J = 12.5 Hz, 1 H, 1/2 CH_2Ph), 4.48−4.55 (m, 2 H, 6′a-H, 1/2 CH_2Ph), 4.63 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, 3a-*H*), 4.83 (dd, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.2 Hz, 1 H, 3b-*H*), 4.91 (dd, $J_{1,2}$ = 7.4 Hz, $J_{2,3}$ = 10.4 Hz, 1 H, 2b-*H*), 5.08 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 10.1 Hz, 1 H, 4a-*H*), 5.36 (d, $J_{3,4}$ = 3.2 Hz, 1 H, 4b-*H*), 6.13 (d, $J_{1,2}$ = 8.9 Hz, 1 H, 1a-*H*), 7.24−7.46 (m, 5 H, *Ph*), 8.59 (s, 1 H, N*H*). − $C_{37}H_{43}Cl_3N_2O_{17}$ (894.10): calcd. C 49.70, H 4.85, N 3.13; found C 49.97, H 5.19, N 2.58.

***tert*-Butyldimethylsilyl *O*-(6-*O*-Benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (5):** A solution of **6** (2.8 g, 2.92 mmol) in dry methanol (50 mL) was treated with a catalytic amount of sodium methoxide (0.1 M, 800 μL). After 1 h the solution was neutralized with ion-exchange resin (Amberlite IR-120 H^+), the resin was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone, 2:1) to give **5** (2.31 g, 95%) as a white foam. − TLC (toluene/acetone, 1:1): R_f = 0.29. − $[\alpha]_D$ = +38.0 (c = 1.0, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = −0.10 (s, 3 H, SiCH_3), 0.01 (s, 3 H, SiCH_3), 0.72 (s, 9 H, SiC(CH_3)$_3$], 1.75 (br. s, 6 H, 2 CH_3), 3.42−3.73 (m, 8 H, O*H*, 2b-*H*, 3b-*H*, 5a-*H*, 6a-*H*, 6b-*H*, 6′b-*H*), 3.85 (dd, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.7 Hz, 1 H, 2a-*H*), 3.92−4.08 (m, 3 H, 4a-*H*, 4b-*H*, 6′a-*H*), 4.25 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 8.7 Hz, 1 H, 3a-*H*), 4.41 (d, ^2J = 12.7 Hz, 1 H, 1/2 CH_2Ph), 4.42 (s, 2 H, CH_2Ph), 4.59 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.60 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1b-*H*), 4.72 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.86 (d, ^2J = 12.7 Hz, 1 H, 1/2 CH_2Ph), 5.09 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-*H*), 7.06−7.34 (m, 15 H, *Ph*). − $C_{45}H_{59}NO_{12}Si$ (834.04): calcd. C 64.80, H 7.13, N 1.68; found C 64.68, H 7.05, N 1.61.

***tert*-Butyldimethylsilyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (6):** TMSOTf (0.1 M in dichloromethane, 1.08 mL) was added dropwise under nitrogen at 0 °C to a stirred mixture of **12** (6.31 g, 10.84 mmol)[28] and **8** (7.03 g, 13.00 mmol)[29] in dry dichloromethane (15 mL). After 20 min the solution was

neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 4:1) to afford **6** (7.29 g, 70%) as a colorless oil. − TLC (petroleum ether/ethyl acetate, 1:1): R_f = 0.71. − $[\alpha]_D$ = +12.5 (c = 2.0, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = −0.09 (s, 3 H, SiCH_3), 0.02 (s, 3 H, SiCH_3), 0.73 [s, 9 H, SiC(CH_3)$_3$], 1.80 (br. s, 6 H, 2 CH_3), 1.94 (s, 3 H, COCH_3), 1.96 (s, 3 H, COCH_3), 1.97 (s, 3 H, COCH_3), 3.23 (dd, $J_{5,6}$ = 7.8 Hz, $J_{6,6'}$ = 9.5 Hz, 1 H, 6b-*H*), 3.34 (dd, $J_{5,6'}$ = 5.5 Hz, $J_{6,6'}$ = 9.5 Hz, 1 H, 6′b-*H*), 3.43 (m, $J_{4,5}$ = 9.6 Hz, 1 H, 5a-*H*), 3.60−3.74 (m, 3 H, 6a-*H*, 6′a-*H*, 5b-*H*), 3.86 (dd, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.8 Hz, 1 H, 2a-*H*), 3.96 (dd, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.6 Hz, 1 H, 4a-*H*), 4.12 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.5 Hz, 1 H, 3a-*H*), 4.23 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.39 (d, ^2J = 9.3 Hz, 1 H, 1/2 CH_2Ph), 4.44 (d, ^2J = 9.3 Hz, 1 H, 1/2 CH_2Ph), 4.49 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.60 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1b-*H*), 4.72 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.80 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.87 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.0 Hz, 1 H, 3b-*H*), 5.11 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.4 Hz, 1 H, 2b-*H*), 5.12 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-*H*), 5.37 (d, $J_{3,4}$ = 3.0 Hz, 1 H, 4b-*H*), 7.10−7.36 (m, 15 H, *Ph*). − $C_{51}H_{65}NO_{15}Si$ (960.15): calcd. C 63.80, H 6.82, N 1.46; found C 63.67, H 6.81, N 1.42.

***tert*-Butyldimethylsilyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (9):** A solution of **2** (3.21 g, 3.70 mmol) in dry dichloromethane (30 mL) was treated with ethanethiol (1.65 mL, 22.2 mmol) and *p*TsOH (0.14 g, 0.74 mmol) and stirred at room temp. After 1 h, the solution was neutralized with triethylamine and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:2) to give **9** (2.60 g, 90%) as a white foam. − TLC (petroleum ether/ethyl acetate, 1:1): R_f = 0.37. − $[\alpha]_D$ = + 9.4 (c = 1.5, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = −0.07 (s, 3 H, SiCH_3), 0.02 (s, 3 H, SiCH_3), 0.73 [s, 9 H, SiC(CH_3)$_3$], 1.80 (s, 3 H, CH_3), 1.91 (s, 3 H, CH_3), 1.95 (2s, 6 H, 2 COCH_3), 2.04 (s, 3 H, COCH_3), 3.40−3.59 (m, 4 H, 4a-*H*, 5a-*H*, 6b-*H*, 6′b-*H*), 3.71 (dd, $J_{5,6'}$ = 5.6 Hz, $J_{6,6'}$ = 11.5 Hz, 1 H, 6a-*H*), 3.82−3.92 (m, 3 H, 2a-*H*, 5b-*H*, 6′a-*H*), 4.31 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.4 Hz, 1 H, 3a-*H*), 4.36 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1b-*H*), 4.43−4.50 (m, 1 H, 1/2 CH_2Ph), 4.52 (d, ^2J = 11.8 Hz, 1 H, 1/2 CH_2Ph), 4.90 (dd, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.4 Hz, 1 H, 3b-*H*), 5.08 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-*H*), 5.08−5.14 (m, 1 H, 2b-*H*), 5.32 (d, ^3J = 2.7 Hz, 1 H, 4b-*H*), 7.24−7.34 (m, 5 H, *Ph*). − $C_{37}H_{53}NO_{15}Si$ (779.91): calcd. C 56.98, H 6.85, N 1.80; found C 56.87, H 6.73, N 1.58.

***tert*-Butyldimethylsilyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (10):** Compound **9** (2.50 g, 3.21 mmol) was treated with pyridine (15 mL) and acetic anhydride (15 mL) and the mixture was stirred overnight. It was concentrated in vacuo by co-distillation with toluene/ethanol and the residue was purified by flash chromatography (toluene/acetone, 9:1) to give **10** (2.63 g, 95%) as a white foam. − TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.32. − $[\alpha]_D$ = +16.5 (c = 0.5, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = −0.05 (s, 3 H, SiCH_3), 0.03 (s, 3 H, SiCH_3), 0.74 [s, 9 H, SiC(CH_3)$_3$], 1.90 (s, 3 H, CH_3), 1.91 (s, 3 H, CH_3), 1.95 (s, 3 H, COCH_3), 1.98 (2s, 6 H, 2 COCH_3), 2.00 (s, 3 H, COCH_3), 2.06 (s, 3 H, COCH_3), 3.36 (t, ^3J = 8.0 Hz, 1 H, 5a-*H*), 3.52 (dd, $J_{5,6}$ = 5.4 Hz, $J_{5,6'}$ = 9.1 Hz, 1 H, 5b-*H*), 3.65−3.71 (m, 2 H, 6b-*H*, 6′b-*H*), 3.94 (dd, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 10.9 Hz, 1 H, 2a-*H*), 4.13 (d, J = 4.2 Hz, 2 H, 6a-*H*, 6′a-*H*), 4.17 (d, $J_{1,2}$ = 7.4 Hz, 1 H, 1b-*H*), 4.39 (d, ^2J = 11.8 Hz, 1 H, 1/2 CH_2Ph), 4.53 (dd, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 8.9 Hz, 1 H, 3a-*H*), 4.53 (d, ^2J = 11.8 Hz, 1 H, 1/2 CH_2Ph), 4.80 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.1 Hz, 1 H, 3b-*H*), 4.88−4.96 (m, 2 H, 2b-*H*, 4a-*H*), 5.06 (d, $J_{1,2}$ = 8.2 Hz, 1 H, 1a-*H*), 5.35 (dd, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = 0.9 Hz, 1 H, 4b-*H*), 7.22−7.36 (m, 5 H, *Ph*). − $C_{41}H_{57}NO_{17}Si$ (863.98): calcd. C 57.00, H 6.65, N 1.62; found C 57.21, H 6.68, N 1.61.

***tert*-Butyldimethylsilyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2,4−di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside (13):** A solution of **5** (1.55 g, 1.86 mmol) in dry dichloromethane (3 mL) was cooled to −40 °C. TMSOTf (0.1 M in dichloromethane, 0.93 mL) was slowly added dropwise under nitrogen and the reaction mixture was stirred for 10 min. A solution of the trichloroacetimidate **3** (2.15 g, 2.40 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise at −40 °C and the mixture was stirred for 20 min, neutralized with triethylamine, and allowed to warm to room temp. The solvent was evaporated in vacuo, and flash chromatography (toluene/acetone, 9:2) of the residue afforded the tetrasaccharide (2.18 g, 75%) as a white foam (TLC (toluene/acetone, 1:1): R_f = 0.78). The tetrasaccharide (2.18 g, 1.40 mmol) was treated with acetic anhydride (10 mL) and pyridine (10 mL) and the reaction mixture was stirred overnight at room temp. The mixture was concentrated in vacuo by co-distillation with toluene and the residue was purified by flash chromatography (toluene/acetone, 7:1) to afford **13** (2.12 g, 92%) as a white foam. − TLC (toluene/acetone, 2:1): R_f = 0.60. − $[\alpha]_D$ = +7.0 (c = 1.0, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = −0.09 (s, 3 H, SiCH_3), 0.02 (s, 3 H, SiCH_3), 0.72 [s, 9 H, SiC(CH_3)$_3$], 1.77 (br. s, 6 H, 2 CH_3), 1.88 (s, 3 H, COCH_3), 1.90 (s, 6 H, 2 CH_3), 1.92 (s, 3 H, COCH_3), 1.95 (s, 3 H, COCH_3), 1.97 (s, 3 H, COCH_3), 1.99 (s, 3 H, COCH_3), 2.00 (s, 3 H, COCH_3), 2.03 (s, 3 H, COCH_3), 3.28 (m, 2 H, 6b-*H*, 6′b-*H*), 3.37 (m, 1 H, 6d-*H*), 3.39 (m, 1 H, 5a-*H*), 3.51 (dd, $J_{5,6'}$ = 5.4 Hz, $J_{6,6'}$ = 9.2 Hz, 1 H, 6′d-*H*), 3.56 (t, ^3J = 6.4 Hz, 1 H, 5b-*H*), 3.63 (m, 1 H, 6a-*H*), 3.65 (m, 2 H, 3b-H, 5c-*H*), 3.67 (m, 2 H, 6′a-H, 5d-*H*), 3.84 (m, 1 H, 2a-*H*), 3.85 (m, 1 H, 2c-*H*), 3.88 (m, 1 H, 4a-*H*), 4.08 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1d-*H*), 4.08 (m, 1 H, 6c-*H*), 4.09 (m, 1 H, 3a-*H*), 4.24 (d, ^2J = 11.7 Hz, 1 H, 1/2 CH_2Ph), 4.32 (dd, $J_{5,6'}$ = 2.5 Hz, $J_{6,6'}$ = 12.3 Hz, 1 H, 6′c-*H*), 4.33 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.37−4.40 (m, 2 H, CH_2Ph), 4.43 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1b-*H*), 4.46−4.49 (m, 1 H, 1/2 CH_2Ph), 4.47 (m, 1 H, 3c-*H*), 4.51 (d, ^2J = 11.8 Hz, 1 H, 1/2 CH_2Ph), 4.74 (d, ^2J = 12.1 Hz, 1 H, 1/2 CH_2Ph), 4.80 (m, 2 H, 3d-*H*, 1/2 CH_2Ph), 4.87 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1c-*H*), 4.87 (m, 1 H, 2d-*H*), 4.88 (m, 1 H, 2b-*H*), 4.95 (t, ^3J = 9.6 Hz, 1 H, 4c-*H*), 5.09 (d, $J_{1,2}$ = 8.2 Hz, 1 H, 1a-*H*), 5.31 (t, ^3J = 4.1 Hz, 1 H, 4b-*H*), 5.35 (t, ^3J = 3.9 Hz, 1 H, 4d-*H*), 7.07−7.33 (m, 20 H, *Ph*). − ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 55.7 (*C*-2c), 57.9 (*C*-2a), 62.2 (*C*-6c), 67.2 (*C*-6d), 67.5 (*C*-4d), 68.2 (*C*-6a), 68.6 (*C*-6b), 69.5 (*C*-2d), 69.7 (*C*-4c), 69.9 (*C*-4b), 71.4 (*C*-3d), 72.2 (*C*-3b, *C*-5c), 72.3 (*C*-2b), 73.2 (*C*-5b), 74.1 (*C*-3c), 74.8 (*C*-5d), 75.5 (*C*-5a), 77.6 (*C*-3a), 78.1 (*C*-4a), 93.9 (*C*-1a), 98.0 (*C*-1c), 100.6 (*C*-1b), 100.7 (*C*-1d). − $C_{84}H_{104}N_2O_{30}Si$ (1649.81): calcd. C 61.15, H 6.35, N 1.70; found C 61.42, H 6.49, N 1.65.

***O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2,4−di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranose (14):** Glacial acetic acid (52 μL, 0.91 mmol) and TBAF (0.91 mL of a 1 M solution in THF, 0.91 mmol) were added to a solution of **13** (1.36 g, 0.82 mmol) in dry THF (15 mL) in an ice-salt bath. After stirring overnight, the solution was treated with a saturated sodium chloride solution (25 mL) and extracted with dichlorome-

thane (3 × 15 mL). The organic layer was separated and dried with anhydrous magnesium sulfate, and the solvents were evaporated in vacuo. The residue was purified by flash chromatography (toluene/acetone, 4:1) to yield **14** (1.16 g, 92%) as a white foam. − TLC (toluene/acetone, 2:1): R_f = 0.49. − $[\alpha]_D$ = +5.2 (c = 0.5, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.90 (br. s, 6 H, 2 CH_3), 1.92 (s, 3 H, COCH_3), 1.98 (s, 3 H, COCH_3), 1.99 (s, 3 H, COCH_3), 2.00 (2s, 6 H, 2 COCH_3), 2.01 (s, 3 H, COCH_3), 2.06 (s, 3 H, COCH_3), 2.95 (d, $J_{H,OH}$ = 8.6 Hz, 1 H, OH), 3.28 (d, J = 6.2 Hz, 2 H, 6b-H, 6b′-H), 3.39−3.47 (m, 2 H, 5a-H, 6d-H), 3.51−3.72 (m, 7 H, 3b-H, 5b-H, 5c-H, 5d-H, 6a-H, 6′a-H, 6′d-H), 3.78−3.96 (m, 2 H, 2c-H, 4a-H), 3.87 (dd, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 11.0 Hz, 1 H, 2a-H), 4.09 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1d-H), 4.10−4.26 (m, 2 H, 3a-H, 6c-H), 4.24 (d, ^2J = 11.8 Hz, 1 H, 1/2 CH_2Ph), 4.31−4.45 (m, 6 H, 1b-H, 6′c-H, 2 CH_2Ph), 4.49−4.53 (m, 1 H, 3c-H), 4.54 (d, ^2J = 11.8 Hz, 1 H, 1/2 CH_2Ph), 4.77−4.91 (m, 6 H, 1c-H, 2b-H, 2d-H, 3d-H, CH_2Ph), 4.98 (t, ^3J = 9.4 Hz, 1 H, 4c-H), 5.09 (dd, $J_{H,OH}$ = $J_{1,2}$ = 8.3 Hz, 1 H, 1a-H), 5.31 (d, ^3J = 3.6 Hz, 1 H, 4b-H), 5.37 (d, ^3J = 2.8 Hz, 1 H, 4d-H), 7.05−7.39 (m, 20 H, Ph). − $C_{78}H_{90}N_2O_{30}$ (1535.56): calcd. C 61.01, H 5.91, N 1.82; found C 61.26, H 6.11, N 1.96.

***O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2,4−di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl Trichloroacetimidate (15):** A mixture of **14** (0.37 g, 0.24 mmol), trichloroacetonitrile (0.24 mL, 2.41 mmol), and DBU (6 μL, 0.04 mmol) in dry dichloromethane (8 mL) was stirred at room temp. for 1 h and then concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone, 6:1 + 1% NEt_3) to give **15** (0.36 g, 90%) as a white foam. − TLC (toluene/acetone, 2:1): R_f = 0.57. − $[\alpha]_D$ = +3.5 (c = 0.5, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.85 (br. s, 6 H, 2 CH_3), 1.91 (s, 3 H, COCH_3), 1.97 (s, 3 H, COCH_3), 1.99 (2s, 6 H, 2 COCH_3), 2.01 (s, 3 H, COCH_3), 2.03 (s, 3 H, COCH_3), 2.06 (s, 3 H, COCH_3), 3.28 (d, J = 6.3 Hz, 2 H, 6b-H, 6b′-H), 3.34−3.42 (m, 2 H, 5a-H, 6d-H), 3.50−3.71 (m, 7 H, 3b-H, 5b-H, 5c-H, 5d-H, 6a-H, 6′a-H, 6′d-H), 3.85 (dd, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 10.8 Hz, 1 H, 2a-H), 4.08 (d, $J_{1,2}$ = 7.4 Hz, 1 H, 1d-H), 3.99−4.51 (m, 12 H, 1b-H, 2c-H, 3a-H, 3c-H, 4a-H, 6c-H, 6′c-H, 41/2 CH_2Ph), 4.52 (d, ^2J = 11.8 Hz, 1 H, 1/2 CH_2Ph), 4.72−4.90 (m, 6 H, 1c-H, 2b-H, 2d-H, 3d-H, CH_2Ph), 4.96 (dd, ^3J = 9.2 Hz, 1 H, 4c-H), 5.30 (d, ^3J = 3.6 Hz, 1 H, 4b-H), 5.36 (d, ^3J = 2.9 Hz, 1 H, 4d-H), 6.14 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1a-H), 7.04−7.38 (m, 20 H, Ph). − $C_{80}H_{90}Cl_3N_3O_{30}$ (1679.93): calcd. C 57.20, H 5.40, N 2.50; found C 57.41, H 5.35, N 2.38.

Benzyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2-O-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (16): A mixture of **4** (0.91 g, 1.03 mmol)[33] and **3** (1.20 g, 1.34 mmol) in dry dichloromethane (3 mL) was cooled to −40 °C. TMSOTf (0.01 M in dichloromethane, 1.06 mL) was added dropwise under nitrogen. After 40 min the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (toluene/acetone, 6:1) to afford **16** (1.40 g, 84%) as a white foam. − TLC (toluene/acetone, 3:1) R_f = 0.52. − $[\alpha]_D$ = −20.0 (c = 0.3, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.83 (s, 3 H, COCH_3), 1.84 (s, 3 H, CH_3), 1.86 (s, 3 H, CH_3), 1.90 (s, 3 H, COCH_3), 1.93 (s, 3 H, COCH_3), 1.94 (s, 3 H, COCH_3), 2.10 (s, 3 H, COCH_3), 2.91 (d, J = 9.8 Hz, 1 H, 5a-H), 2.99 (s, 1 H, 5b-H), 3.31 (m, 1 H, 6d-H), 3.32 (m, 1 H, 6a-H), 3.37 (m, 2 H, 2a-H, 3b-H), 3.41 (m, 1 H, 3a-H), 3.47 (m, 1 H, 6′d-H), 3.49 (m, 1 H, 2b-H), 3.56 (m, 1 H, 5d-H), 3.59 (m, 1 H, 6′a-H), 3.62 (m, 1 H, 5c-H), 3.81 (d, $J_{5,6}$ < 1 Hz, $J_{6,6'}$ = 10.5 Hz, 1 H, 6b-H), 3.87 (dd, $J_{3,4}$ = 9.8 Hz, $J_{4,5}$ = 8.5 Hz, 1 H, 4a-H), 3.97 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1d-H), 4.05 (m, 2 H, 2c-H, 6c-H), 4.12 (d, ^3J = 3.6 Hz, 1 H, 4b-H), 4.17 (d, $J_{5,6'}$ < 1 Hz, $J_{6,6'}$ = 10.5 Hz, 1 H, 6′b-H), 4.21 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.24(d, $J_{5,6'}$ = 2.3 Hz, $J_{6,6'}$ = 12.1 Hz, 1 H, 6′c-H), 4.28 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1a-H), 4.31−4.35 (m, 3 H, 1b-H, CH_2Ph), 4.42−4.52 (m, 4 H, 2 CH_2Ph), 4.58 (d, ^2J = 10.7 Hz, 1 H, 1/2 CH_2Ph), 4.64 (d, ^2J = 10.9 Hz, 1 H, 1/2 CH_2Ph), 4.71 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.5 Hz, 1 H, 3d-H), 4.79−4.82 (m, 3 H, 2d-H, CH_2Ph), 4.92 (t, ^3J.= 9.2, 10.0 Hz, 1 H, 4c-H), 5.01 (d, ^2J = 10.6 Hz, 1 H, 1/2 CH_2Ph), 5.12 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1c-H), 5.29 (d, ^3J = 4.1 Hz, 1 H, 4d-H), 5.39 (s, 1 H, CHPh), 7.03−7.38 (m, 35 H, Ph). − ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 55.7 (C-2c), 62.6 (C-6c), 66.7 (C-5c), 67.1 (C-6d), 67.5 (C-4d), 68.2 (C-6a), 69.1 (C-6b), 69.4 (C-2d), 71.3 (C-3d), 72.0 (C-5d), 72.1 (C-5c), 74.8 (C-3c), 75.1 (C-5a), 76.2 (C-4b), 77.2 (C-4a), 77.9 (C-2b), 80.8 (C-3b), 82.0 (C-2a), 83.4 (C-3a), 99.7 (C-1c), 100.5 (C-1d), 100.9 (CHPh), 102.7 (C-1a), 102.8 (C-1b). − $C_{89}H_{97}NO_{27}$ (1612.71): calcd. C 66.28, H 6.06, N 0.87; found C 66.01, H 6.33, N 0.82.

Benzyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (17): A solution of **16** (1.18 g, 0.73 mmol) in dry dichloromethane (15 mL) was treated with ethanethiol (0.33 mL, 4.39 mmol) and *p*TsOH (28 mg, 0.15 mmol) and then stirred at room temp. After 2 h the solution was neutralized with triethylamine and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (toluene/acetone, 5:1) to give **17** (0.80 g, 72%) as a white foam. − TLC (toluene/acetone, 3:1): R_f = 0.27. − $[\alpha]_D$ = −8.0 (c = 0.5, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.82, 1.83 (m, 6 H, 2 CH_3), 1.97 (s, 6 H, 2 COCH_3), 1.99 (s, 6 H, 2 COCH_3), 2.02 (s, 3 H, COCH_3), 3.14 (m, 1 H, 5a-H), 3.18 (m, 1 H, 2a-H), 3.22 (m, 1 H, 5b-H), 3.29 (m, 1 H, 2b-H), 3.34 (m, 1 H, 6d-H), 3.35 (m, 1 H, 6a-H), 3.38 (m, 1 H, 3a-H), 3.39 (m, 1 H, 6b-H), 3.41 (m, 1 H, 3b-H), 3.43 (m, 1 H, 6′d-H), 3.55 (m, 2 H, 6′a-H, 6′b-H), 3.74 (t, ^3J = 9.3 Hz, 1 H, 4a-H), 3.85 (m, 2 H, 2c-H, 5c-H), 3.98 (d, ^3J = 3.4 Hz, 1 H, 4b-H), 4.04 (m, 1 H, 6c-H), 4.06 (m, 1 H, 5d-H), 4.09 (m, 1 H, 6′c-H), 4.27 (d, ^2J = 12.1 Hz, 1 H, 1/2 CH_2Ph), 4.29 (d, $J_{1,2}$ = 7.7 Hz, 1 H, 1d-H), 4.30 (d, $J_{1,2}$ = 7.7 Hz, 1 H, 1b-H), 4.30 (m, 1 H, 1/2 CH_2Ph), 4.37 (dd, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 8.8 Hz, 1 H, 3c-H), 4. (m, 1 H, 1a-H), 4.40−4.51 (m, 6 H, 3 CH_2Ph), 4.55 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.58 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.65 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.3 Hz, 1 H, 2d-H), 4.70 (t, ^3J = 9.1, 10.0 Hz, 1 H, 4c-H), 4.72 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.75 (d, ^2J = 12.2 Hz, 1 H, 1/2 CH_2Ph), 4.87 (d, ^2J = 10.6 Hz, 1 H, 1/2 CH_2Ph), 4.98 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.6 Hz, 1 H, 3d-H), 5.16 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1c-H), 5.19 (t, ^3J = 4.0 Hz, 1 H, 4d-H), 7.11−7.36 (m, 30 H, Ph). − ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 54.9 (C-2c), 59.7 (C-6b), 62.3 (C-6c), 66.5 (C-6d), 66.9 (C-4b, C-4d), 67.5 (C-6a), 68.9 (C-2d), 69.4 (C-4c), 70.1 (CH$_2$Ph), 70.3 (C-5d), 70.5 (C-3d), 70.6 (C-5c), 72.1 (CH$_2$Ph), 72.3 (CH$_2$Ph), 73.0 (CH$_2$Ph), 73.8 (C-5a), 74.1 (CH$_2$Ph), 74.5 (C-3a), 75.1 (C-4a), 77.4 (C-2b), 82.0 (C-2a), 82.1 (C-3a), 83.1 (C-3b), 98.8 (C-1c), 99.1 (C-1d), 101.4 (C-1b), 101.6 (C-1a). − $C_{82}H_{93}NO_{27}$ (1524.61): calcd. C 64.60, H 6.15, N 0.92; found C 63.31, H 6.14, N 0.69.

Benzyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)-(1→3)-(2,4−di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-dimethyl-

maleimido-β-D-glucopyranosyl)-(1→6)-[(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl)]-(1→3)-(2-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (18): A mixture of **17** (0.59 g, 0.39 mmol) and **15** (0.84 g, 0.50 mmol) in dry dichloromethane (4 mL) was cooled to −20 °C. $Sn(OTf)_2$ (16.3 mg, 0.04 mmol) was added and the resulting mixture was stirred under nitrogen. After 10 min the mixture was neutralized with triethylamine and concentrated in vacuo. The residue was purified by flash chromatography (toluene/ethyl acetate, 2:1) to afford **18** (0.89 g, 75%) as a white foam. − TLC (toluene/acetone, 3:1) R_f = 0.40. − $[\alpha]_D$ = −19.0 (c = 0.5, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.65−1.77 (m, 18 H, 6 CH_3), 1.87 (s, 3 H, COCH_3), 1.90 (s, 3 H, COCH_3), 1.91 (s, 3 H, COCH_3), 1.92 (s, 3 H, COCH_3), 1.96 (s, 3 H, COCH_3), 1.97 (s, 3 H, COCH_3), 1.98 (s, 3 H, COCH_3), 2.00 (2 s, 6 H, 2 COCH_3), 2.01 (s, 3 H, COCH_3), 2.04 (s, 3 H, COCH_3), 2.05 (s, 3 H, COCH_3), 2.89 (d, $^3J_{H,OH}$ = 3.7 Hz, 1 H, 4b-OH), 3.01 (d, ^2J = 11.8 Hz, 1 H, 5a-H), 3.17 (t, ^3J = 6.5 Hz, 1 H, 5b-H), 3.26 (m, 1 H, 3b-H), 3.28 (m, 2 H, 6f-H, 6′f-H), 3.31 (m, 1 H, 5e-H), 3.38 (m, 1 H, 3a-H), 3.39 (m, 3 H, 6a-H, 6d-H, 6h-H), 3.42 (m, 1 H, 2b-H), 3.44 (m, 2 H, 2a-H, 6b-H), 3.54 (m, 2 H, 6′d-H, 6′h-H), 3.56 (m, 1 H, 6′a-H), 3.57 (m, 1 H, 5f-H), 3.60 (s, 2 H, 6e-H, 6′e-H), 3.64 (m, 1 H, 5h-H), 3.66 (m, 1 H, 3f-H), 3.68 (m, 3 H, 5c-H, 5d-H, 5 g-H), 3.83 (m, 2 H, 4a-H, 4b-H), 3.85 (m, 1 H, 2g-H), 3.86 (m, 1 H, 4e-H), 3.87 (m, 1 H, 2e-H), 3.88 (m, 1 H, 6′b-H), 4.05 (m, 2 H, 2c-H, 1d-H), 4.06 (m, 1 H, 3e-H), 4.09 (m, 1 H, 1h-H), 4.10 (m, 1 H, 6d-H), 4.11 (m, 1 H, 6g-H), 4.14 (m, 1 H, 6′c-H), 4.24−4.88 (m, 17 H, 81/2 CH_2Ph), 4.26 (m, 1 H, 1b-H), 4.34 (d, $J_{1,2}$ = 7.7 Hz, 1 H, 1a-H), 4.35 (m, 1 H, 6′g-H), 4.38 (m, 1 H, 1f-H), 4.46 (m, 1 H, 3c-H), 4.49 (m, 1 H, 3g-H), 4.64 (d, ^2J = 10.6 Hz, 1 H, 1/2 CH_2Ph), 4.72 (m, 2 H, CH_2Ph), 4.78 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.6 Hz, 1 H, 3d-H), 4.82 (d, $J_{1,2}$ = 8.2 Hz, 1 H, 1e-H), 4.82 (m, 1 H, 1e-H, 3h-H), 4.87 (m, 1 H, 2d-H), 4.88 (m, 4 H, 4c-H, 2f-H, 1g-H, 2h-H), 4.97 (t, ^3J = 9.6 Hz, 1 H, 4g-H), 5.04 (d, $J_{1,2}$ = 8.5 Hz, 1 H, 1c-H), 5.33 (d, ^3J = 3.9 Hz, 1 H, 4f-H), 5.36 (d, ^3J = 3.6 Hz, 1 H, 4d-H), 5.37 (d, ^3J = 3.6 Hz, 1 H, 4h-H), 7.06−7.37 (m, 50 H, Ph). − ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 55.5 (*C*-2c), 55.7 (*C*-2e), 55.8 (*C*-2g), 62.2 (*C*-6g), 62.8 (*C*-6c), 66.9 (*C*-4b), 67.1 (*C*-6b), 67.2 (*C*-6d, *C*-6h), 67.4 (*C*-4d), 67.5 (*C*-4h), 67.9 (*C*-4c, *C*-4g), 69.7 (*C*-2d), 69.9 (*C*-4f), 71.4 (*C*-3h), 71.7 (*C*-3d), 72.2 (*C*-5c, *C*-5d, *C*-5g, *C*-5h), 72.3 (*C*-5b), 73.0 (*C*-5f), 74.4 (*C*-3g), 74.7 (*C*-3c), 74.8 (*C*-3f), 75.0 (*C*-5e), 75.1 (*C*-5a), 76.2 (*C*-4a), 77.6 (*C*-3e), 77.9 (*C*-4e), 78.3 (*C*-2b), 82.1 (*C*-2a), 83.2 (*C*-3a), 84.0 (*C*-3b), 97.9 (*C*-1g), 98.9 (*C*-1e), 99.0 (*C*-1c), 100.5 (*C*-1d), 100.6 (*C*-1h), 102.3 (*C*-1b), 102.8 (*C*-1a). − $C_{160}H_{181}N_3O_{56}$ (3042.14): calcd. C 63.17, H 6.00, N 1.38; found C 63.11, H 6.06, N 1.30.

Benzyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4−di-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-[(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)]-(1→3)-(4-*O*-acetyl-2-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (19): A mixture of **18** (122 mg, 0.04 mmol) and sodium hydroxide (0.01 g, 0.25 mmol) in a dioxane/water mixture (4:1, 3 mL) was stirred at room temperature. After 24 h the pH was adjusted to 5 with HCl (1 M) and the solution was stirred for 18 h at room temp. The solution was neutralized with potassium carbonate and dried in vacuo. The residue was treated with pyridine (5 mL) and acetic anhydride (2.5 mL) and stirred for 12 h at room temp. The solution was then evaporated in vacuo. The residue was purified by column chromatography on silica gel (toluene/acetone, 2:1) to afford **19** (68 mg, 59%) as a white solid. − TLC (toluene/ethyl acetate, 5:1): R_f = 0.41. − $[\alpha]_D$ = +12.5 (c = 0.5, $CHCl_3$). − ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.86−2.20 (m, 48 H, 16 COCH_3), 3.32 (m, 4 H, 6a-H, 6c-H, 6g-H, 6h-H), 3.53−3.60 (m, 8 H, 5a-H, 5h-H, 6′a-H, 6′b-H, 6′d-H, 6′c-H, 6′g-H, 6′h-H), 3.62−3.70 (m, 5 H, 5e-H, 5b-H, 5c-H, 5d-H, 5g-H), 3.80−4.20 (m, 9 H, 4a-H, 2b-H, 4b-H, 5f-H, 2a-H, 2c-H, 6e-H, 6f-H, 2g-H), 4.23 (m, 2 H, 3b-H, 3g-H), 4.31−4.53 (m, 25 H, 1b-H, 3a-H, 3c-H, 1e-H, 1g-H, 10 CH_2Ph), 4.65−5.12 (m, 11 H, 2d-H, 2e-H, 2 NH, 4f-H, 4g-H, 4d-H, 4c-H, 4h-H, 1a-H, 1c-H), 5.15 (m, 2 H, 1d-H, 1h-H), 5.21 (m, 3 H, 2d-H, 2f-H, 2h-H), 5.28−5.45 (m, 3 H, 3d-H, 3h-H, NH), 7.25−7.30 (m, 50 H, 10 Ph). − $C_{150}H_{177}N_3O_{54}$ (2886.00): calcd. C 62.43, H 6.18, N 1.46; found C 62.12, H 6.23, N 1.39.

β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→4)-α,β-D-glucopyranose (1): A solution of **19** (35 mg, 12 μmol) in methanol (5 mL) was hydrogenolyzed in the presence of Pd/C for 12 h. The mixture was then filtered through Celite and the resulted clear solution was treated with sodium methoxide (8 mg) and stirred for 5 h at room temp. The solution was then concentrated in vacuo. The residue was purified by RP-18 column chromatography (MeOH/H_2O, from 1:1 to 15:1) to afford **1** (13 mg, 75%) as a white solid. − TLC (methanol) R_f = 0.17. − $[\alpha]_D$ = +18.0 (c = 0.5, MeOH/$CHCl_3$, 1:1). − ^{1}H NMR (600 MHz, DMSO): δ = 1.98 (m, 6 H, 3 COCH_3), 4.22 (m, 3 H, 1d-H, 1h-H, 1b-H), 4.45 (m, 2 H, 1c-H, 1g-H), 4.52 (m, 3 H, 1e-H, 1f-H, 1a-H), 5.05 (m, 2 H, NH), 5.20 (m, 1 H, NH). − $C_{54}H_{91}N_3O_{41}$ (1438.30): MALDI-MS (positive mode, DHB/THF matrix): m/z = calcd. 1461.3 $[MNa]^+$; found 1460.9 $[MNa]^+$.

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