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Synthesis of chitotetraose and chitohexaose based on dimethylmaleoyl protection

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Abstract

tert-Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside was readily transformed into the disaccharide glycosyl donor, 3,4,6-tri-*O*-acetyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-α/β-D-glucopyranosyl trichloroacetimidate, and the disaccharide glycosyl acceptor, *tert*-butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside. A TMSOTf-catalysed coupling of the acceptor with the donor afforded the respective tetrasaccharide derivative, which can be transformed to chitotetraose. *tert*-Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-4-*O*-phenoxyacetyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside was converted into donor 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-4-*O*-phenoxyacetyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl trichloroacetimidate. Its coupling with benzyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside, followed by dephenoxyacetylation, gave benzyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-D-glucopyranoside, whose glycosylation furnished, after replacement of the DMM-group by the acetyl moiety and subsequent deprotection, chitohexaose.

Keywords: Chitotetraose; Chitohexaose; Chitooligomers; Glucosamine; Protective groups; Dimethylmaleoyl

1. Introduction

The β-(1–4) linked *N*-acetylglucosamine moiety is a frequently occurring structural unit in various naturally and biologically important oligosaccharides and their conjugates. Chitin (**1d**) is a structural polysaccharide in crab shells, and chitobiose (**1a**) is usually found at the reducing end of *N*-glycan residues of glycoproteins. Several biological

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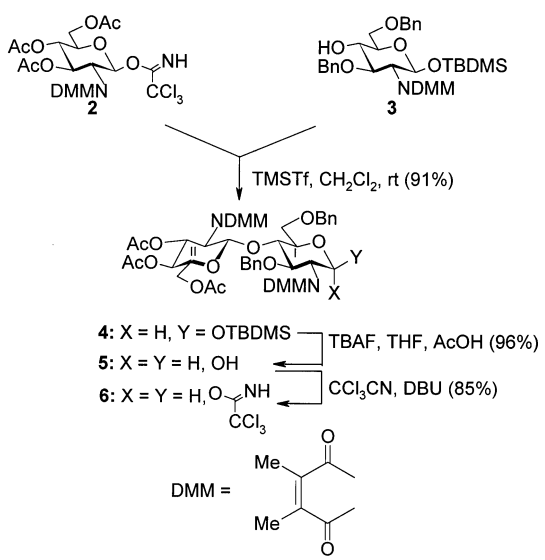
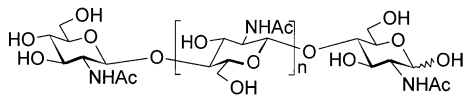
functions are related to chitin oligomers. Thus, they have been used as lysozyme substrates,[1–3] for biological studies on lectins,[4–7] as antitumor agents[8] and as immunopotentiating agents.[9,10] Of these biologically active chitooligosaccharides, chitotetraose (**1b**) has the highest affinity among chitooligosaccharides to bind to rat NKR-PL antigen, a carbohydrate-binding protein in rat natural killer (NK) cells.[11] On the other hand, chitohexaose (**1c**) showed a remarkable antitumor activity against *Sarcoma 180* solid tumors in *Bal b/c* mice; it is also an efficient elicitor of chitinase (EC 3.2.1.14) activity in melon plants.[12]

Chemical and enzymatic hydrolysis of chitin and chitosan are the commercial methods for obtaining chitooligosaccharides,[12–14] but these procedures give low yields. Therefore, we report herein the application of the dimethylmaleoyl (DMM) group as an amino protective group to the synthesis of chitotetraose (**1b**) and chitohexaose (**1c**). Its ease of attachment and subsequent cleavage, as well as its enhanced capability to form β linkages and its compatibility with different protective group manipulations made it a versatile amino protective group in glucosamine-containing oligosaccharide synthesis.[15–17] Our strategy is based on using DMM-protected glucosamine moieties as glycosyl donors and glycosyl acceptors for constructing these chitinoligomers.



Scheme 1.



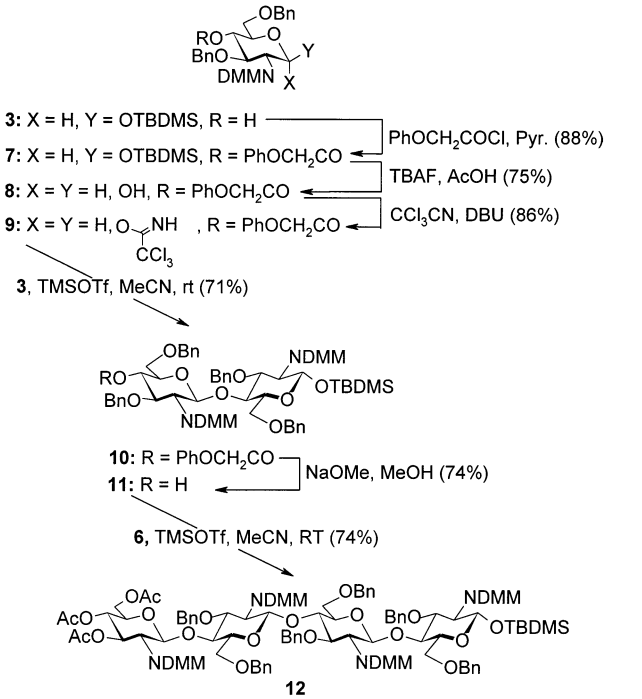
1a: n = 0; 1b: n = 2; 1c: n = 4; 1d: n = poly

2. Results and discussion

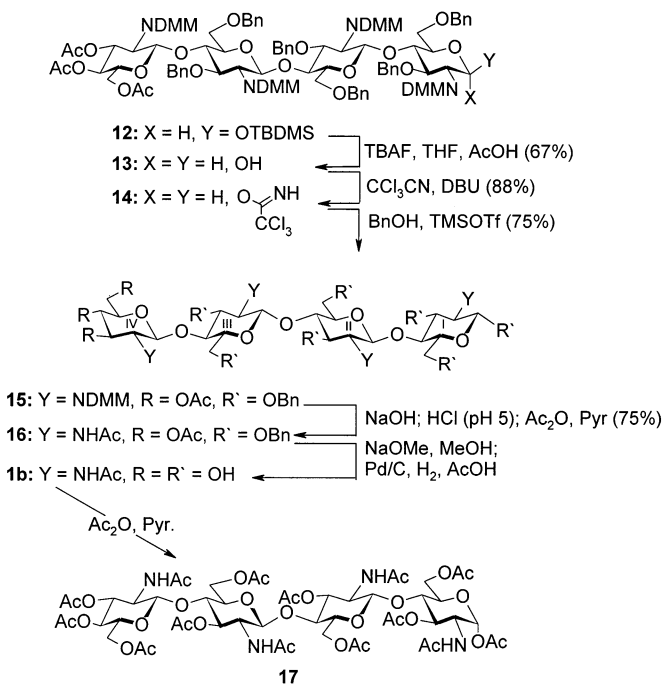
Synthesis of chitotetraose.—Glycosylation of known *tert*-butyldimethylsilyl 3,6-di-*O*-benzyl-2-dimethylmaleimido-β-D-glucopyranoside (**3**)[15] as a glycosyl acceptor with 4-*O*-acetyl-3,6-di-*O*-benzyl-2-dimethylmaleimido-α/β-D-glucopyranosyl trichloroactimidate (**2**)[15] as glycosyl donor using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst in dichloromethane at room temperature led to disaccharide derivative **4** in low yield.[15] However, coupling of **3** with glycosyl donor **2** using TMSOTf as catalyst in acetonitrile as solvent afforded the corresponding β-linked disaccharide **4** in excellent yield (91%). Its ^{1}H NMR specrum (δ 5.35, $J_{1,2}$ 8.4 Hz, H-1^{II}) supported the β anomeric linkage. Compound **4** was transformed into trichloroacetimidate **6** in high overall yield by 1-O-desilylation with tetrabutylammonium fluoride (TBAF) and acetic acid (AcOH) in dry tetrahydrofuran (THF) (**5**) and subsequent reaction with trichloroacetonitrile (TCA) in the presence of 1,8-diaza[5.4.0]bicycloundec-7-ene (DBU) as base (Scheme 1).

Treatment of **3** with phenoxyacetyl chloride in pyridine led to the 4-*O*-phenoxyacetyl derivative **7**. Ensuing 1-O-desilylation with TBAF gave **8** which upon trichloroacetimidation afforded glycosyl donor **9**. TMSOTf-catalysed glycosylation of acceptor **3** with **9** in acetonitrile as solvent afforded the β linked disaccharide **10** in good yield (71%). [^{1}H NMR: δ 5.07 ($J_{1,2}$ 8.4 Hz, H-1^{II})]. Deacylation of **10** with sodium methanolate in dry methanol (Zemplén conditions)[18] led to **11**, a disaccharide glycosyl acceptor. Glycosylation of **11** with the disaccharide donor **6** under catalysis by TMSOTf afforded the desired β linked tetrasaccharide **12** in 74% yield [^{1}H NMR: δ 4.93 ($J_{1,2}$ 8.6 Hz, H-1^{III})] (Scheme 2).

Anomeric O-desilylation of **12** with TBAF and acetic acid gave **13**, which was transformed into trichloroacetimidate **14**. Coupling of **14** as glycosyl donor with benzyl alcohol in



Scheme 2.



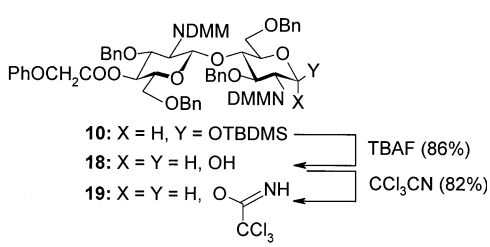
Scheme 3.

the presence of TMSOTf as catalyst led to benzyl glycoside **15**. Removal of the DMM groups in **15** and N-acetylation was performed in a one-pot reaction under standard conditions[15] to give **16** in 75% yield [HMQC: δ 4.71 ($J_{2,NH}$ 8.5 Hz, NH-2^{IV}), 4.92 ($J_{2,NH}$ 9.7 Hz, NH-2^{III}), 5.82 ($J_{2,NH}$ 9.8 Hz, NH-2^{II}), 6.29 ($J_{2,NH}$ 9.0 Hz, NH-2^{I})]. The small coupling constant for H-1^{I} [^{1}H NMR: [δ 4.67 ($J_{1,2}$ 4.7 Hz)]] may be attributed to a $^4C_1 \rightarrow {}^1C_4$ inversion of conformation at ring I. Deacetylation of **16** with sodium methanolate in methanol and subsequent hydrogenolytic O-debenzylation with palladium-on-carbon catalyst gave the desired chitotetraose **1b** in high overall yield. For complete structural assignment, **1b** was peracetylated with acetic anhydride in pyridine to afford the *O*-peracetyl derivative **17**, which showed the desired signals [HMQC: δ 4.38, 4.42, 4,45 (3d, J 8–10 Hz, H-1^{II}, H-1^{III}, H-1^{IV}) and 6.10 ($J_{1,2}$ 3.6 Hz, H-1^{I})] (Scheme 3).

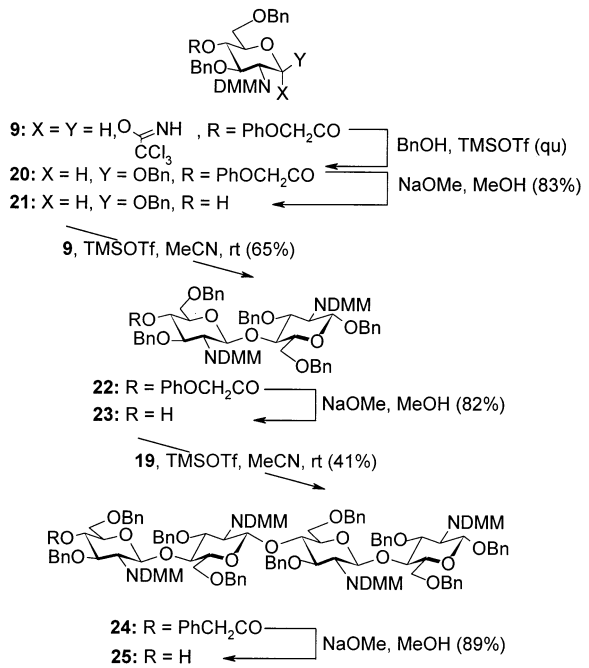
Synthesis of chitohexaose.—The synthesis of chitohexaose (**1c**) could be readily achieved from the available disaccharide precursors **6**, **9**, and **10**. Thus, anomeric O-desilylation of **10** gave **18**; ensuing transformation into trichloroacetimidate **19** furnished the desired disaccharide glycosyl donor **19** in high overall yield (Scheme 4).

Reaction of **9** with benzyl alcohol in the presence of TMSOTf as catalyst gave benzyl glycoside **20** which was O-deacylated to afford the glycosyl acceptor **21**. Glycosylation of acceptor **21** with **9** as donor afforded chitobioside derivative **22** in good yield. 4-O-Deacylation of **22** yielded glycosyl acceptor **23**. Similarly, coupling of **23** with glycosyl donor **19** furnished chitotetraose derivative **24**, which on 4-O-deacylation afforded the desired tetrasaccharide glycosyl acceptor **25** (Scheme 5).

Glycosylation of **25** with disaccharide donor **6** under standard conditions led to β-linked hexasaccharide derivative **26** in reasonable yield [^{1}H NMR: δ 4.96 ($J_{1,2}$ 8.2 Hz, H-1^{V})]. The six DMM-groups were removed in a one-pot procedure by treatment with a sodium hydroxide solution in a methanol–dioxane–



Scheme 4.



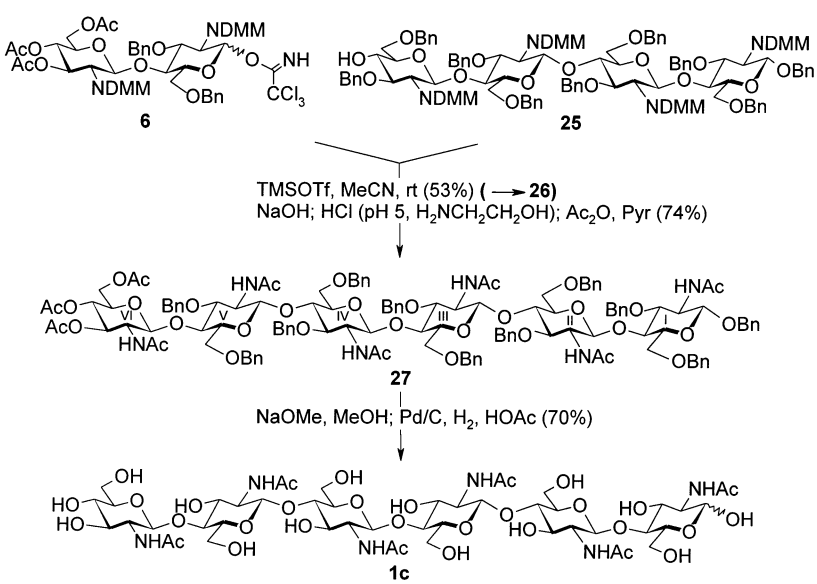
Scheme 5.

water mixture, followed by addition of HCl in the presence of ethanolamine to pH 5.[15] Then acetylation with acetic anhydride in pyridine afforded hexasaccharide **27** in 74% yield [HMQC: δ 4.11 (NH-2^{V}, NH-2^{VI}), 5.19 (NH-2^{III}), 5.42 (NH-2^{IV}), 5.82 (NH-2^{II}), 6.35 (NH-2^{I})]. The structure of **27** could be confirmed by the ^{1}H NMR data [δ 4.61 (d, $J_{1,2}$ 5.0 Hz, H-1^{I}), 4.27 (d, $J_{1,2}$ 8.8 Hz, H-1^{II}), 4.22 (d, $J_{1,2}$ 10.0 Hz, H-1^{V}), 4.21 (d, $J_{1,2}$ 8.8 Hz, H-1^{IV}), 4.20 (d, $J_{1,2}$ 10.0 Hz, H-1^{VI}), 4.12 (d, $J_{1,2}$ 8.8 Hz, H-1^{III})]; the small coupling constant for H-1^{I} was again due to conformational changes. O-Deacetylation of **27**, followed by catalytic O-debenzylation, afforded the desired chitohexaose (**1c**) (MALDIMS: m/z 1259.9 $[M + Na]^{+}$) (Scheme 6).

In conclusion, the use of DMM-protected glucosamine derived glycosyl donors and acceptors led to the efficient generation of chitobiose building blocks which readily provided chitotetraose, and chitohexaose intermediates, thus permitting the synthesis of the target molecules

3. Experimental

General methods.—Melting points are uncorrected. Thin-layer chromatography (TLC) was performed on plastic plates Silica Gel 60 F_{254} and on HPTLC plates NH_2 F_{254} S (E. Merck, layer thickness 0.2 mm). The detection was achieved by treatment with a solution of 20 g ammonium molybdate and 0.4 g cerium-(IV) sulfate in 400 mL of 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) and Lichroprep NH_2 (E. Merck, 40–63 μm). Medium-pressure liquid chromatography (MPLC): LiChroprep Si 60 (E. Merck, 15–25 μm), detection by differential refractometer. Optical rotations were de-



Scheme 6.

termined 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, using tetramethylsilane as an internal standard. The assignment of ^{1}H NMR spectra were based on chemical shift correlation (DQFCOSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY). The assignment of ^{13}C NMR spectra were based on carbon–proton shift-correlation heteronuclear multiple quantum coherence (HMQC). MS spectra were recorded with MALDI-Kompakt (Kratos) and FAB with Finningen MAT 312/AMD. Microanalyses were performed in the unit of Microanalysis at the Fakultät für Chemie, Universität Konstanz.

tert-*Butyldimethylsilyl 3,4,6-tri*-O-*acetyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**4**).—A solution of **2** (0.27 g, 0.484 mmol) and **3** (0.2 g, 0.343 mmol) in dry CH_2Cl_2 (1 mL) was stirred under N_2 at rt while TMSOTf (0.01 M in CH_2Cl_2, 0.52 mL) was added dropwise (10 min). After 4 h the reaction mixture was neutralized with Et_3N, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (2.5:1 petroleum ether–EtOAc) to yield **4** (0.305 g, 91%) as a white foam. TLC (2.5:1 petroleum ether–EtOAc): R_f 0.09; $[\alpha]_D$ +14.4° (*c* 0.53, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37–7.12 (m, 10 H, 2 Ph), 5.59 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 9.2 Hz, H-3^{II}), 5.35 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{II}), 5.05–5.02 (m, 2 H, H-1^{I}, H-4^{II}), 4.80, 4.41 (2 d, 2 H, J_{gem} 12.7 Hz, CH_2Ph), 4.60, 4.57 (2 d, 2 H, J_{gem} 12.2 Hz, CH_2Ph), 4.16 (dd,1 H, J_{gem} 12.2, $J_{5,6}$ 4.4 Hz, H-6′^{II}), 4.07–4.03 (m, 3 H, H-2^{II}, H-3^{I}, H-4^{I}), 3.89 (dd, 1 H, J_{gem} 12.2, $J_{5,6}$ 1.8 Hz, H-6^{II}), 3.81 (m, 1 H, H-2^{I}), 3.53–3.48 (m, 2 H, H-5^{II}, H-6′^{I}), 3.41 (dd, 1 H, J_{gem} 11.1, $J_{5,6}$ 3.7 Hz, H-6^{I}), 3.35 (m, 1 H, H-5^{I}), 1.97, 1.94, 1.88 (3 s, 9 H, 3 CH_3CO), 1.75 (br.s, 12 H, 4 CH_3), 0.67 [s, 9 H, $SiC(CH_3)_3$], −0.02 (s, 3 H, $SiCH_3$), −0.14 (s, 3 H, $SiCH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 97.20 (C-1^{II}), 93.60 (C-1^{I}), 77.10 (C-3^{I}, C-4^{I}), 74.90 (C-5^{I}), 74.20 (CH_2Ph), 73.40 (CH_2Ph), 71.70 (C-5^{II}), 71.10 (C-3^{II}), 69.10 (C-4^{II}), 68.50 (C-6^{I}), 62.00 (C-6^{II}), 57.90 (C-2^{I}), 55.40 (C-2^{II}). MALDIMS (positive-ion mode, DHB–THF matrix): *m*/*z*: 1001 $[M+Na]^+$. Anal. Calcd for $C_{50}H_{64}N_2O_{16}Si$ (977.11): C, 61.45; H, 6.60; N, 2.86. Found: C, 61.52; H, 6.67; N, 2.87.

3,4,6-Tri-O-*acetyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-α/β*-D-*glucopyranosyl trichloroacetimidate* (**6**).—A mixture of **4** (1.854 g, 1.89 mmol), AcOH (0.126 g, 2.1 mmol, 0.12 mL) in dry THF (5 mL) was stirred in an ice–salt bath while TBAF (0.549 g, 2.1 mmol, 2.1 mL) was added dropwise. After 30 min at −5 °C the cooling bath was removed, and the mixture was stirred at rt overnight. The reaction mixture was diluted with satd NaCl (25 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was separated and dried with anhyd $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc) to yield **5** (1.579 g, 96%) as a white foam. TLC (1:1 petroleum ether–EtOAc): R_f 0.16.

A mixture of **5** (1.579 g, 1.82 mmol), CCl_3CN (1.5 mL, 2.26 mmol) and DBU (0.05 mL, 0.33 mmol) in dry CH_2Cl_2 (6 mL) was stirred at rt. After 7 h the reaction mixture was concentrated in vacuo, and the residual syrup was purified by flash chromatography (1.5:1 EtOAc–petroleum ether, +1% Et_3N) to yield **6** (1.571 g, 85%) as a white foam in the α/β ratio 1:1.5. TLC (1.5:1 petroleum ether–EtOAc + 1% Et_3N): R_f 0.6; ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.49 (s, 1 H, NH), 7.44–7.12 (m, 10 H, 2 Ph), 6.14 (d, 0.6 H, $J_{1,2}$ 8.7 Hz, H-1$^{I}_{\beta}$), 6.13 (d, 0.4 H, $J_{1,2}$ 3.9 Hz, H-1$^{I}_{\alpha}$), 5.60 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.1 Hz, H-3^{II}), 5.36 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{II}), 5.05 (dd, 1 H, $J_{3,4}=J_{4,5}$ 9.1 Hz, H-4^{II}), 4.86, 4.43 (2 d, 2 H, J_{gem} 12.7 Hz, CH_2Ph), 4.62 (br.s, 2 H, CH_2Ph), 4.31–3.37 (m, 10 H), 1.99, 1.98, 1.96, 1.90, 1.74 (5 s, 21 H, 4 CH_3, 3 CH_3CO). FABMS (positive-ion mode, NBOH–NaI matrix): *m*/*z* 1030 $[M+Na]^+$. Anal. Calcd for $C_{46}H_{50}Cl_3N_3O_{16}$ (1007.25): C, 54.84; H, 5.00; N 4.17. Found: C, 54.97; H 5.00; N, 4.45.

tert-*Butyldimethylsilyl 3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-4-phenoxyacetyl-β*-D-*glucopyranoside* (**7**).—A solution of **3** (4.21 g, 7.23 mmol) in Py (12 mL) was cooled in an ice–salt bath, then phenoxyacetyl chloride (3.705 g, 21.7 mmol, 3.0 mL) was added drop-

wise. After 2 h the reaction mixture was co-evaporated with toluene in vacuo. The residue was dissolved in CH_2Cl_2, and the solution was then washed successively with HCl (3%, 50 mL), water (50 mL), and satd $NaHCO_3$ (50 mL), and then dried with $MgSO_4$. The solvent was evaporated, and traces were removed by coevaporation with toluene in vacuo. The residue was purified by flash chromatography (8:1, petroleum ether–EtOAc) to yield **7** (4.6 g, 88%) as a colorless oil. TLC (8:1 petroleum ether–EtOAc): R_f 0.13; $[\alpha]_D$ +17.3° (*c* 0.33, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.33–6.80 (m, 15 H, 3 Ph), 5.18 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.15 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 4.54–4.29 (2 d, 2 H, J_{gem} 12.1 Hz, CH_2Ph), 4.48–4.30 (m, 5 H, H-3, CH_2Ph, PhOCH_2CO), 3.98 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 10.9 Hz, H-2), 3.74–3.66, 3.57–3.55 (2 m, 3 H, H-5, H-6, H-6′), 1.82 (br.s, 6 H, 2 CH_3), 0.74 [s, 9 H, SiC(CH_3)$_3$], 0.05 (s, 3 H, SiCH_3), −0.05 (s, 3 H, SiCH_3). MALDIMS (positive-ion mode, DHB–THF matrix) *m*/*z*: 739 [M + Na]$^+$. Anal. Calcd for $C_{40}H_{49}NO_9Si$ (715.88): C, 67.10; H, 6.89; N, 1.95. Found: C, 66.82; H, 6.80; N, 1.71.

*3,6-Di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-4-phenoxyacetyl-α/β-*D*-glucopyranosyl trichloroacetimidate* (**9**).—A mixture of **7** (2.728 g, 3.81 mmol) and AcOH (0.26 g, 4.3 mmol) in dry THF (10 mL) was treated with TBAF (1.0 M solution in THF, 4.29 mL, 4.29 mmol) and then worked up as described for the synthesis of **5**. The residue was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) to yield **8** (1.824 g, 79%) as a white foam. TLC (1.5:1 petroleum ether–EtOAc): R_f 0.13.

A mixture of **8** (1.824 g, 3.02 mmol), CCl_3CN (2.0 mL, 1.2 mmol) and DBU (0.07 mL, 0.42 mmol) in dry CH_2Cl_2 (7 mL) was stirred at rt for 2 h, then concentrated in vacuo. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc + 1% Et_3N) to yield **9** (1.954 g, 86%) as a yellow foam in the α/β ratio 1:6. TLC (1:1 petroleum ether–EtOAc + 1% Et_3N): R_f 0.58 (β-form) and R_f 0.69 (α-form); ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.61 (s, 0.14 H, NH_α), 8.59 (s, 0.86 H, NH_β), 7.33–6.78 (m, 15 H, 3 Ph), 6.26–3.55 (m, 13 H), 1.78 (s, 6 H, 2 CH_3). FABMS (positive-ion mode, NBOH–NaI matrix): *m*/*z* 769 [M + Na]$^+$ and *m*/*z* 919 [M + NaI]Na$^+$. Anal. Calcd for $C_{36}H_{35}Cl_3N_2O_9$ (746.03): C, 57.95; H, 4.72; N, 3.75. Found: C, 58.04; H, 4.82; N, 3.74.

tert-*Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-4-*O*-phenoxyacetyl-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranoside* (**10**).—A mixture of **9** (1.519 g, 2.036 mmol) and **3** (1.0 g, 1.716 mmol) in dry MeCN (4 mL) was stirred under N_2 at rt for 10 min while TMSOTf (0.01 M in MeCN, 2.03 mL) was added dropwise. After stirring overnight the reaction mixture was neutralized with triethylamine, the solvent was evaporated, and the residue was dried in vacuo. The residue was purified by flash chromatography (6:1 petroleum ether–EtOAc) to yield **10** (1.425 g, 71%) as a white foam. TLC (3:1 petroleum ether–EtOAc): R_f 0.29; $[\alpha]_D$ +30.4° (*c* 0.46, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.26–6.72 (m, 25 H, 5 Ph), 5.14 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{II}), 5.07 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{II}), 4.97 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{I}), 4.74, 4.36 (2 d, 2 H, J_{gem} 12.6 Hz, CH_2Ph), 4.46, 4.18 (2 d, 2 H, J_{gem} 12.2 Hz, CH_2Ph), 4.34–4.24 (m, 5 H, H-3^{II}, 2 CH_2Ph), 4.27 (m, 2 H, PhOCH_2), 4.00–3.93 (m, 3 H, H-4^{I}, H-2^{II}, H-3^{I}), 3.76 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.2 Hz, H-2^{I}), 3.47–3.44 (m, 1 H, H-5^{II}), 3.43–3.40 (m, 2 H, H-6′^{I}, H-6′^{II}), 3.33 (dd, 1 H, J_{gem} 10.4, $J_{5,6}$ 4.4 Hz, H-6^{II}), 3.26–3.25 (m, 2 H, H-5^{I}, H-6^{I}), 1.72–1.69 (br.m, 12 H, 4 CH_3), 0.64 [s, 9 H, SiC(CH_3)$_3$], −0.09 (s, 3 H, SiCH_3), −0.20 (s, 3 H, SiCH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 97.30 (C-1^{II}), 93.30 (C-1^{I}), 77.00 (C-3^{II}), 76.90 (C-3^{I}), 76.30 (C-4^{I}), 74.60 (C-5^{I}), 74.20 (CH_2Ph), 73.80 (CH_2Ph), 73.60 (C-4^{II}, CH_2Ph), 72.80 (CH_2Ph), 72.50 (C-5^{II}), 69.60 (C-6^{II}), 67.90 (C-6^{I}), 65.20 (PhOCH_2), 57.60 (C-2^{I}), 55.90 (C-2^{II}). MALDIMS (positive-ion mode, DHB–THF matrix): *m*/*z* 1191 [M + Na]$^+$. Anal. Calcd for $C_{66}H_{76}N_2O_{15}Si$ (1165.37): C, 68.01; H, 6.57; N, 2.40. Found: C, 67.61; H, 6.57; N, 1.91.

tert-*Butyldimethylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranoside* (**11**).—A solution of **10** (1.425 g, 1.222 mmol) in MeOH

(11.7 mL) was stirred with NaOMe (0.195 M, 0.63 mL). After 75 min the reaction mixture was neutralized with Amberlite IR120 resin (H^+-form) and filtered, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (3.5:1 petroleum ether–EtOAc) to yield **11** (0.942 g, 74%) as a white foam. TLC (3.5:1 petroleum ether–EtOAc): R_f 0.12; $[\alpha]_D$ −0.94° (*c* 0.53, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.35–7.12 (m, 20 H, 4 Ph), 5.15 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{II}), 5.05 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{I}), 4.82 (d, 1 H, J_{gem} 12.5 Hz, CHHPh), 4.59, 4.53 (2 d, 2 H, J_{gem} 12.2 Hz, CH_2Ph), 4.52–4.47 (m, 3 H, 1.5 CH_2Ph), 4.79, 4.44 (2 d, 2 H, J_{gem} 12.5 Hz, CH_2Ph), 4.11 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 8.6 Hz, H-3^{II}), 4.05–4.03 (m, 2 H, H-3^{I}, H-4^{I}), 3.92 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.7 Hz, H-2^{II}), 3.83 (dd, 1 H, $J_{1,2}$ 8.2, $J_{2,3}$ 10.3 Hz, H-2^{I}), 3.75 (dd, 1 H, $J_{3,4}$ 8.6, $J_{4,5}$ 9.2 Hz, H-4^{II}), 3.67 (dd, 1 H, J_{gem} 10.0, $J_{5,6'}$ 4.4 Hz, H-$6'^{II}$), 3.54–3.50 (m, 2 H, H-$6'^{I}$, H-6^{II}), 3.42–3.33 (m, 3 H, H-5^{I}, H-6^{I}, H-5^{II}), 3.05 (s, 1 H, OH), 1.90–1.77 (br.m, 12 H, 4 CH_3), 0.72 [s, 9 H, $SiC(CH_3)_3$], 0.01 (s, 3 H, $SiCH_3$), −0.11 (s, 3 H, $SiCH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 97.60 (C-1^{II}), 93.70 (C-1^{I}), 79.30 (C-3^{II}), 77.20 (C-3^{I}), 76.50 (C-4^{I}), 75.60 (C-4^{II}), 75.10 (C-5^{I}), 74.70 (CH_2Ph), 74.40 (2 CH_2Ph), 73.20 (C-5^{II}, CH_2Ph), 71.40 (C-6^{II}), 68.50 (C-6^{I}), 58.10 (C-2^{I}), 56.20 (C-2^{II}). MALDIMS (positive-ion mode, DHB–THF matrix): *m/z* 1054 $[M + Na]^+$. Anal. Calcd for $C_{58}H_{70}N_2O_{13}Si$ (1031.24): C, 67.54; H, 6.84; N, 2.71. Found: C, 67.24; H, 7.00; N, 2.78.

tert-*Butyldimethylsilyl 3,4,6-tri*-O-*acetyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**12**).—A mixture of **6** (0.764 g, 0.758 mmol) and **11** (0.613 g, 0.594 mmol) in dry MeCN (2 mL) was stirred under N_2 at rt while TMSOTf (0.01 M in MeCN (0.76 mL) was added dropwise, and the mixture was stirred overnight. The reaction mixture was then neutralized with Et_3N, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (1.75:1 petroleum ether–EtOAc) to yield **12** (0.828 g, 74%) as a white foam. TLC (1.75:1 petroleum ether–EtOAc): R_f 0.13; $[\alpha]_D$ −1.8° (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.22–6.97 (m, 30 H, 6 Ph), 5.53 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.2 Hz, H-3^{IV}), 5.25 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{IV}), 5.00 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4^{IV}), 4.96 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{I}), 4.95 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1^{II}), 4.93 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1^{III}), 4.86–4.82 (m, 2 H, CH_2Ph), 4.73 (d, 1 H, J_{gem} 13.0 Hz, CHHPh), 4.50–4.30 (m, 9 H, 4.5 CH_2Ph), 4.11–4.10 (m, 2 H, H-$6'^{IV}$, H-4^{II}), 4.00–3.88 (m, 6 H, H-4^{I}, H-3^{I}, H-3^{III}, H-4^{III}, H-2^{IV}, H-3^{II}), 3.83–3.82 (m, 3 H, H-2^{III}, H-6^{IV}, H-2^{II}), 3.73 (dd, 1 H, $J_{1,2}$ 8.1 Hz, H-2^{I}), 3.49 (d, 1 H, J_{gem} 10.8 Hz, H-$6'^{II}$), 3.40–3.37 (m, 2 H, H-6^{I}, H-$6'^{III}$), 3.31 (m, 1 H, H-5^{IV}), 3.24–3.22 (m, 2 H, H-5^{I}, H-6^{I}), 3.20 (dd, 1 H, H-6^{II}), 3.08 (dd, 1 H, H-6^{III}), 3.04–3.03 (m, 2 H, H-5^{III}, H-5^{II}), 1.95–1.51 (several s, 21 H, 8 CH_3, 3 CH_3CO), 0.64 [s, 9 H, $SiC(CH_3)_3$], 0.64 [s, 9 H, $SiC(CH_3)_3$], −0.20 (s, 3 H, $SiCH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 97.40 (C-1^{II}, C-1^{III}), 97.10 (C-1^{IV}), 93.60 (C-1^{I}), 78.00 (C-3^{III}), 77.90 (C-3^{II}), 77.50 (C-3^{I}), 76.30 (C-4^{I}), 76.10 (C-4^{III}, C-4^{II}), 75.00 (C-5^{I}), 74.80 (C-5^{III}, CH_2Ph), 74.70, 74.50 (2 CH_2Ph), 74.50 (C-5^{II}), 73.20, 73.00, 72.80 (3 CH_2Ph), 71.60 (C-5^{IV}), 71.20 (C-3^{IV}), 69.00 (C-4^{IV}), 68.40 (C-6^{I}), 67.70 (C-6^{II}), 67.40 (C-6^{III}), 61.70 (C-6^{IV}), 58.00 (C-2^{I}), 56.70 (C-2^{III}, C-2^{II}), 55.50 (C-2^{IV}). MALDIMS (positive-ion mode, DHB–THF matrix): *m/z* 1899 $[M + Na]^+$, *m/z* 1915 $[M + K]^+$. Anal. Calcd for $C_{102}H_{118}N_4O_{28}Si$ (1876.07): C, 65.29; H, 6.33; N, 2.98. Found: C, 64.68; H, 6.34; N, 2.53.

3,4,6-Tri-O-*acetyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl trichloroacetimidate* (**14**).—A mixture of **12** (1.044 g, 0.556 mmol) and AcOH (0.042 g, 0.61 mmol, 0.035 mL) in dry THF (3 mL) was treated with TBAF (1.0 M in THF, 0.56 mL, 0.56 mmol) and then worked up as described for the synthesis of **5**. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc) to yield **13** (0.659 g, 67%) as a white foam. TLC (1:1 petroleum ether–EtOAc): R_f 0.17.

A mixture of **13** (0.659 g, 0.374 mmol), CCl_3CN (0.5 mL, 4.986 mmol) and DBU (0.05 mL, 0.33 mmol) in dry CH_2Cl_2 (6 mL) was stirred at rt. After 8 h the rection mixture was concentrated in vacuo, and the residual syrup was purified by flash chromatography (1:1 petroleum ether–EtOAc + 1% Et_3N) to yield **14** (0.634 g, 88%) as a yellow foam. TLC (1.5:1 petroleum ether–EtOAc + 1% Et_3N): R_f 0.67; $[\alpha]_D$ +16.7° (*c* 0.4, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 8.44 (s, 1 H, NH), 7.37–6.96 (m, 30 H, 6 Ph),. 6.10 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^I), 5.58 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.3 Hz, H-3^{IV}), 5.28 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{IV}), 5.08–2.65 (m, 37 H), 1.66–2.03 (several s, 33 H, 8 CH_3, 3 CH_3CO). Anal. Calcd for $C_{98}H_{104}Cl_3N_5O_{28}$ (1906.22): C, 61.74; H, 5.49; N 3.67. Found: C, 62.45; H, 5.74; N, 3.32.

Benzyl 3,4,6-tri-O-*acetyl-2-deoxy-2-dimethylmaleimido* - *β* - D - *glucopyranosyl* - *(1→4)-3,6-di* - O - *benzyl* - *2* - *deoxy* - *2* - *dimethylmaleimido* - *β* - D - *glucopyranosyl* - *(1→4)* - *3,6-di* - O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido* - *β* - D - *glucopyranoside* (**15**).—A mixture of **14** (0.151 g, 0.079 mmol) and benzyl alcohol (0.015 g, 0.138 mmol, 15.0 μL) in dry MeCN (1 mL) was stirred under N_2 at rt while TMSOTf (0.01 M in MeCN, 0.09 mL) was added dropwise. After 2 h the reaction mixture was neutralized with Et_3N, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) to yield **15** (0.109 g, 75%) as an amorphous mass. TLC (1.5:1 petroleum ether–EtOAc): R_f 0.14; $[\alpha]_D$ +2.6° (*c* 0.6, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.26–7.00 (m, 35 H, 7 Ph), 5.57 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.0 Hz, H-3^{IV}), 5.29 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{IV}), 5.04 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4^{IV}), 5.00 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{III}), 4.95 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{II}),. 4.89 (d, 1 H, J_{gem} 12.9 Hz, CHHPh), 4.87 (d, 1 H, J_{gem} 12.5 Hz, CHHPh), 4.80 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^I), 4.75 (d, 1 H, J_{gem} 13.0 Hz, CHHPh), 4.69 (d, 1 H, J_{gem} 12.4 Hz, CHHPh), 4.54–4.34 (m, 10 H, 5 CH_2Ph), 4.14–3.85 (m, 12 H, H-2^I, H-2^{II}, H-2^{III}, H-2^{IV}, H-3^I, H-3^{II}, H-3^{III}, H-4^I, H-4^{II}, H-4^{III}, H-6^{IV}, H-$6'^{IV}$), 3.52–3.51 (m, 2 H, H-$6'^I$, H-$6'^{III}$), 3.42 (dd, 1 H, J_{gem} 11.2, $J_{5,6'} < .1.0$ Hz, H-$6'^{II}$), 3.35–3.32 (m, 2 H, H-6^I, H-5^{IV}), 3.27 (m, 1 H, H-5^I), 3.22 (dd, 1 H, J_{gem} 11.1, $J_{5,6}$ 3.1 Hz, H-6^{III}), 3.11 (dd, 1 H, J_{gem} 11.2, $J_{5,6}$ 3.2 Hz, H-6^{II}), 3.07 (m, 1 H, H-5^{III}), 2.98 (m, 1 H, H-5^{II}), 1.99–1.60 (m, 33 H, 8 CH_3, 3 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 171.91–169.43 (8 CO, 3 CH_3CO), 138.49–136.47, 128.26–126.69 (7 Ph-C), 97.24 (C-1^I), 96.99 (C-1^{III}), 96.86 (C-1^{II}), 96.73 (C-1^{IV}), 77.50 (C-3^{III}), 77.60 (C-3^{II}), 77.10 (C-3^I), 75.70 (C-4^{III}), 75.60 (C-4^{II}), 75.50 (C-4^I), 74.71 (CH_2Ph), 74.50 (C-5^I), 74.30 (C-5^{II}, CH_2Ph), 74.20 (CH_2Ph), 74.00 (C-5^{III}), 72.74 (CH_2Ph), 72.49 (CH_2Ph), 72.39 (CH_2Ph), 71.22 (C-5^{IV}), 70.80 (C-3^{IV}), 70.37 (CH_2Ph), 68.63 (C-4^{IV}), 68.10 (C-6^I), 67.30 (C-6^{III}), 67.08 (C-6^{II}), 61.37 (C-6^{IV}), 56.39 (C-2^{II}), 56.31 (C-2^{III}), 55.42 (C-2^I), 55.12 (C-2^{IV}), 20.57, 20.51 (3 CH_3CO), 8.84, 8.43 (8 CH_3). MALDIMS (positive-ion mode, DHB–THF matrix): *m/z* 1872 $[M + Na]^+$, *m/z* 1887.3 $[M + K]^+$. $C_{103}H_{110}N_4O_{28}$ (1851.94).

Benzyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di* - O - *benzyl* - *2* - *deoxy* - *β* - D - *glucopyranoside* (**16**).—A mixture of **15** (0.109 g, 0.059 mmol) and NaOH (0.6 g, 15.0 mmol) in 5:2:1 MeOH–dioxane–water (8 mL) was stirred at rt overnight. The mixture was neutralized with 2 N HCl, and the pH was adjusted to pH 5 with N HCl in the presence of ethanolamine (0.02 g, 0.332 mmol, 0.02 mL). After 1 day the solution was neutralized with ethanolamine and evaporated, and the residue was thoroughly dried in vacuo. The residue was treated with Py 2:1 Ac_2O (15 mL) and stirred overnight. The reaction mixture was worked up as described for the synthesis of **7**. The residue was purified by flash chromatography (2:1 toluene–acetone), and the fractions of partially deprotected products were collected and treated again similarly as described before to yield **16** (0.078 g, 75%) as an amorphous mass. TLC (1:1 toluene–acetone): R_f 0.34; $[\alpha]_D$ −11.5° (*c* 0.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.40–7.25 (m, 35 H, 7 Ph), 6.29 (br.d, 1 H, $J_{2,NH}$ 9.0 Hz, NH-2^I), 5.82 (br.d, 1 H, $J_{2,NH}$ 9.8 Hz, NH-2^{II}), 5.04 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4^{IV}), 4.92 (br.d, NH-2^{III}), 4.88–4.70 (m, 9 H, 7 CHHPh, NH-2^{IV},

H-3^{IV}), 4.67 (d, 1 H, $J_{1,2}$ 4.7 Hz, H-1^{I}), 4.63–4.47 (m, 6 H, 6 CHHPh), 4.36 (d, 1 H, J_{gem} 11.8 Hz, CHHPh), 4.30 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1^{II}), 4.29 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{IV}), 4.26 (m, 1 H, H-2^{I}), 4.21 (dd, 1 H, J_{gem} 12.4, $J_{5,6'}$ 4.5. Hz, H-6′^{IV}), 4.12–3.92 (m, 8 H, H-1^{III} H-2^{II}, H-2^{III}, H-2^{IV}, H-4^{I}, H-4^{II}, H-4^{III},, H-6^{IV}), 3.83–3.74 (m, H-3^{I}, 4 H, H-5^{I}, H-6^{I}, H-6′^{I}), 3.64–3.56 (m, 3 H, H–6^{II}, H-6′^{II}, H-6′^{III}), 3.49–3.44 (m, 4 H, H-3^{II}, H-5^{IV}, H-5^{II}, H-6^{III}), 3.26 (dd, 1 H, $J_{2,3} = J_{3,4}$ 7.8 Hz, H-3^{III}), 3.22 (m, 1 H, H-5^{III}), 2.04, 2.03, 1.97, 1.80, 1.78, 1.76, 1.67 (7 s, 21 H, 7 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.89–169.27 (7 CH_3CO), 138.61–137.54, 128.95–127.50 (7 C–Ph), 100.47 (C-1^{II}), 100.00 (C-1^{IV}), 99.78 (C-1^{III}), 99.53 (C-1^{I}), 79.74 (C-3^{II}), 79.43 (C-3^{III}), 78.28 (C-3^{I}), 75.80 (C-4^{III}), 75.08–72.03 (6 CH_2Ph, C-3^{IV}, C-5^{I}, C-4^{II}, C-5^{III}, C-4^{I}, C-5^{II}), 71.46 (C-5^{IV}), 70.15(CH_2Ph), 69.94 (C-6^{I}), 69.94 (C-6^{II}), 68.26 (C-4^{IV}), 68.09 (C-6^{III}), 61.71 (C-6^{IV}), 53.97 (C-2^{III}, C-2^{IV}), 53.32 (C-2^{II}), 51.10 (C-2^{I}), 29.67, 23.28, 23.21, 20.60 (7 CH_3CO). MALDIMS (positive-ion mode, DHB–THF matrix): m/z 1612.3 $[M + Na]^+$; m/z 1628.5 $[M + K]^+$. Anal. Calcd for $C_{87}H_{102}N_4O_{24}$ (1587.71): C, 65.80; H, 6.47; N, 3.52. Found: C, 65.26; H, 6.43; N, 3.13.

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-*D*-glucopyranose* (**1b**).—A solution of **16** (36.0 mg, 22.0 μmol) in dry MeOH (6.85 mL) was treated with NaOMe (0.195 M, 0.45 mL) and then worked up as described for the synthesis of **11**. The residue was dissolved in 1:1:1 MeOH–AcOH–dioxane (3 mL) and then hydrogenated in the presence of Pd/C (10% Pd, 0.02 g). After 3 days the reaction mixture was filtered through Celite, washed with (1:1 MeOH–water) and evaporated in vacuo. The residue was purified by flash chromatography NH_2-phase (3:1 EtOH–water) to yield **1b** (0.018 g, quant). TLC (3:1 EtOH–water): R_f 0.65. MALDIMS (positive-ion mode, DHB–water matrix): m/z 851.7 $[M + Na]^+$. $C_{32}H_{54}N_4O_{21}$ (830.78).

*Acetyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-*D*-glucopyranoside* (**17**).—A mixture of **1b** (22.0 μg, 26.4 μmol), Py (8 mL) and Ac_2O (4 mL) was stirred at rt. After 3 days the reaction mixture was worked up as described for the synthesis of **7**. The residue was purified by flash chromatography (3:1 acetone–toluene) to yield **17** (0.018 g, 54%). TLC (3:1 acetone–toluene): R_f 0.13; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.24 (br.t, 1 H, NH), 6.10 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1$^{I}_{\alpha}$), 5.96 (br.d, 1 H, NH), 5.59 (br.d, 1 H, NH), 5.35–5.02, 4.45–3.25 (2 m, 27 H), 2.19–1.93 (several s, 42 H, 14 CH_3CO). $C_{52}H_{76}N_4O_{31}$ (1253.16).

*3,6-Di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-4-*O*-phenoxyacetyl-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranosyl trichloroacetimidate* (**19**).—A mixture of **10** (1.74 g, 1.493 mmol) and AcOH (0.105 g, 1.748 mmol, 0.1 mL) in dry THF (5 mL) was treated with TBAF (1.0 M solution in THF, 1.6 mL, 1.6 mmol) and then worked up as described for the synthesis of **5**. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc) to yield **18** (1.364 g, 86%) as a white foam. TLC (1:1 petroleum ether–EtOAc): R_f 0.24.

A mixture of **18** (1.688 g, 1.605 mmol), CCl_3CN (2.448 g, 16.9 mmol, 1.7 mL) and DBU (0.1 mL, 0.66 mmol) in dry CH_2Cl_2 (8 mL) was stirred at rt for 2 h then concentrated in vacuo. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc, + 1% Et_3N) to yield **19** (1.618 g, 82%) as a yellow foam in the α/β ratio 1:9. TLC (1:1 petroleum ether–EtOAc, + 1% Et_3N): R_f 0.66; ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.50 (s, 0.1 H, NH_α), 8.48 (s, 0.9 H, NH_β), 7.37–6.79 (m, 25 H, 5 Ph), 6.14 (d, 0.1 H, $J_{1,2}$ 2.3 Hz, H-1$^{I}_{\alpha}$), 6.12 (d, 0.9 H, $J_{1,2}$ 8.3 Hz, H-1$^{I}_{\beta}$), 5.22 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{II}), 5.14 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{II}), 4.84 (d, 1 H, J_{gem} 12.7 Hz, CHHPh), 4.57–3.35 (m, 20 H), 1.95–1.73 (m, 12 H, 4 CH_3). FABMS (positive-ion mode, NBOH–NaI matrix): 1216 $[M + Na]^+$; m/z 1218 $[M + Na + 2]^+$. Anal. Calcd for $C_{62}H_{62}Cl_3N_3O_{15}$ (1195.51): C, 62.28; H, 5.22; N, 3.51. Found: C, 62.17; H, 5.26; N, 3.16.

Benzyl 3,6-di-O-*benzyl-2-deoxy-2-dimethylmaleimido-4*-O-*phenoxyacetyl-β*-D-*glucopyranoside* (**20**).—A mixture of **9** (0.719 g, 0.963 mmol) and benzyl alcohol (0.125 g, 1.155 mmol, 0.12 mL) in dry MeCN (2 mL) was stirred at rt while TMSOTf (0.01 M in MeCN, 1.1 mL) was added dropwise. After 6 h the reaction mixture was neutralized with Et_3N, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to yield **20** (0.66 g, quant) as a colorless oil. TLC (4:1 petroleum ether–EtOAc): R_f 0.11; $[\alpha]_D$ −4.0° (*c* 0.6, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 7.35–6.79 (m, 20 H, 4 Ph), 5.21 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 4.98 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.79 (d, 1 H, J_{gem} 12.3 Hz, C*H*HPh), 4.08 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.7 Hz, H-2), 4.54–4.22, 3.72–3.58 (2 m, 13 H, H-5, H-6, H-6′, H-3, 3.5 CH_2Ph, CH_2OPh), 1.75 (br.s, 6 H, 2 CH_3). MALDIMS (positive-ion mode, DHB–THF matrix): *m*/*z* 716 $[M + Na]^+$; *m*/*z* 732 $[M + K]^+$. Anal. Calcd for $C_{41}H_{41}NO_9$ (691.74): C, 71.18; H, 5.97; N, 2.02. Found: C, 70.66; H, 6.07; N, 1.81.

Benzyl 3,6-di-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**21**).—A solution of **20** (0.674 g, 0.974 mmol) in MeOH (9.2 mL) was treated with NaOMe (0.195 M, 0.49 mL) and worked up as described for the synthesis of **11**. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to yield **21** (0.456 g, 83%) as a colorless oil. TLC (3:1 petroleum ether–EtOAc): R_f 0.13; $[\alpha]_D$ −7.0° (*c* 0.4, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 7.40–7.09 (m, 15 H, 3 Ph), 5.00 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1),. 4.79, 4.73 (2 d, 1 H, J_{gem} 12.4 Hz, CH_2Ph), 4.65, 4.58 (2 d, 2 H, J_{gem} 12.0 Hz, CH_2Ph), 4.48, 4.45 (2 d, 2 H, J_{gem} 12.3 Hz, CH_2Ph), 4.12–3.54 (m, 6 H, H-2, H-3, H-4, H-5, H-6, H-6′), 2.83 (d, 1 H, $J_{4,OH}$ 2.2 Hz, OH), 1.77 (br.s, 6 H, 2 CH_3). MALDIMS (positive-ion mode, DHB–THF matrix): *m*/*z* 582 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{35}NO_7$ (557.61): C, 71.07; H, 6.32; N, 2.51. Found: C, 70.81; H, 6.40; N, 2.33.

Benzyl 3,6-di-O-*benzyl-2-deoxy-2-dimethylmaleimido-4*-O-*phenoxyacetyl-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**22**)

Method A. A mixture of **9** (1.38 g, 1.849 mmol) and **21** (0.765 g, 1.371 mmol) in dry MeCN (5 mL) was stirred under N_2 at rt while TMSOTf (0.01 M in MeCN, 1.8 mL) was added dropwise. After 2 h the reaction mixture was neutralized with Et_3N, the solvent was evaporated, and the residue was dried in vacuo. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to yield **22** (1.025 g, 65%) as a white foam. TLC (3:1 petroleum ether–EtOAc): R_f 0.12; $[\alpha]_D$ −38.0° (*c* 0.4, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.28–6.79 (m, 30 H, 6 Ph). 5.21 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{II}), 5.12 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{II}), 4.86 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{I}), 4.72 (d, 1 H, J_{gem} 12.2 Hz, CHHPh), 4.59–4.52 (m, 3 H, 1.5 CH_2Ph), 4.41–4.31 (m, 7 H, PhOCH_2, 2.5 CH_2Ph), 4.31 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.4 Hz, H-3^{II}), 4.06 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4^{I}), 4.02 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.6 Hz, H-2^{II}), 4.00 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.4 Hz, H-3^{I}), 3.94 (dd, 1 H, $J_{1,2}$ 8.8, $J_{2,3}$ 10.6 Hz, H-2^{I}), 3.56 (dd, 1 H, J_{gem} 10.6, $J_{5,6'} < 1.0$ Hz, H-6′^{I}), 3.48–3.45 (m, 2 H, H-5^{II}, H-6′^{II}), 3.40–3.37 (m, 2 H, H-6^{I}, H-6^{II}), 3.33 (m, 1 H, H-5^{I}), 1.71–1.85 (br.m, 12 H, 4 CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 167.84 (4 CO), 157.62 (PhOCH_2CO), 139.00–136.60, 129.53–114.57 (6 Ph-C), 97.38 (C-1^{I}), 97.11 (C-1^{II}), 77.10 (C-3^{II}), 77.00 (C-3^{I}), 76.02 (C-4^{I}), 74.60 (C-5^{I}), 74.45–72.87 (C-4^{II}, 4 CH_2Ph), 72.54 (C-5^{II}), 70.41 (CH_2Ph), 69.62 (C-6^{II}), 68.10 (C-6^{I}), 65.22 (PhOCH_2), 55.90 (C-2^{II}), 55.40 (C-2^{I}), 8.53 (4 CH_3). MALDIMS (positive-ion mode, DHB–THF matrix): *m*/*z* 1164.7 $[M + Na]^+$. Anal. Calcd for $C_{67}H_{68}N_2O_{15}$ (1141.23): C, 70.50; H, 6.00; N, 2.45. Found: C, 70.45; H, 5.96; N, 2.16.

Method B. A mixture of **19** (0.36 g, 0.301 mmol) and benzyl alcohol (0.032 g, 0.301 mmol) in dry MeCN (2 mL) was stirred under N_2 at rt for 10 min while TMSOTf (0.01 M in MeCN, 0.3 mL) was added dropwise. After 2 h the reaction mixture was neutralized with Et_3N, and the solvent was evaporated in vacuo. The residue was purified as previously described to yield **22** (0.273 g, 81%).

Benzyl 3,6-di-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**23**).—A solution of **22** (1.025

g, 0.898 mmol) in dry 6:2.5 MeOH–THF (8.5 mL) was treated with NaOMe (0.195 M, 0.46 mL) and then worked up as described for the synthesis of **11**. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to yield **23** (0.742 g, 82%) as a white foam. TLC (3:1 petroleum ether–EtOAc): R_f 0.08; $[\alpha]_D$ +4.5° (*c* 0.4, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.29–7.07 (m, 25 H, 5 Ph), 5.12 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{II}), 4.85 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{I}), 4.80, 4.48 (2 d, 2 H, J_{gem} 12.3 Hz, CH_2Ph), 4.76, 3.38 (2 d, 2 H, J_{gem} 12.6 Hz, CH_2Ph), 4.72, 4.40 (2 d, 2 H, J_{gem} 12.4 Hz, CH_2Ph), 4.58–4.54 (q, 2 H, CH_2Ph), 4.47 (br.s, 2 H, CH_2Ph), 4.10–4.05 (m, 2 H, H-4^{I}, H-3^{II}), 3.99 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 8.4 Hz, H-3^{I}), 3.95–3.90 (m, 2 H, H-2^{II}, H-2^{I}), 3.73 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4^{II}), 3.66 (dd, 1 H, J_{gem} 10.0, $J_{5,6'}$ 4.3 Hz, H-$6'^{II}$), 3.58 (dd, 1 H, J_{gem} 11.0, $J_{5,6'}$ 1.5 Hz, H-$6'^{I}$), 3.51 (dd, 1 H, J_{gem} 10.0, $J_{5,6}$ 6.1 Hz, H-6^{II}), 3.42 (dd, 1 H, J_{gem} 11.0, $J_{5,6}$ 4.0 Hz, H-6^{I}), 3.34–3.30 (m, 2 H, H-5^{I}, H-5^{II}), 3.00 (br.s, 1 H, OH), 1.89–1.70 (m, 12 H, 4 CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 139.10–136.63, 128.51–126.88 (5 Ph), 97.34 (C-1^{I}), 97.02 (C-1^{II}), 78.92 (C-3^{II}), 77.00 (C-3^{I}), 75.62 (C-4^{I}), 75.21 (C-4^{II}), 74.72 (C-5^{I}), 74.32 (CH_2Ph), 74.12 (CH_2Ph), 73.70 (CH_2Ph), 72.87 (C-5^{II}), 72.79 (CH_2Ph), 71.01 (C-6^{II}), 70.42 (CH_2Ph), 68.26 (C-6^{I}), 55.86 (C-2^{II}), 55.44 (C-2^{I}), 8.52 (4 CH_3). MALDIMS (positive-ion mode, DHB–THF matrix): *m/z* 1033 $[M+Na]^+$. Anal. Calcd for $C_{59}H_{62}N_2O_{13}$ (1007.92): C, 70.30; H, 6.20; N, 2.77. Found: C, 69.94; H, 6.37; N, 2.18.

*Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-4-*O*-phenoxyacetyl-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranoside* (**24**).—A mixture of **19** (0.38 g, 0.317 mmol) and **23** (0.27 g, 0.267 mmol) in dry MeCN (2 mL) was stirred under N_2 at rt while TMSOTf (0.01 M in MeCN, 0.31 mL) was added dropwise. After 3 h the reaction mixture was neutralized with Et_3N, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (2.5:1 petroleum ether–EtOAc) to yield **24** (0.225 g, 41%) as a white foam. TLC (2.5:1 petroleum ether–EtOAc): R_f 0.09; $[\alpha]_D$ +33.8° (*c* 0.13, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.26–6.77 (m, 50 H, 10 Ph), 5.18 (m, 1 H, H-4^{IV}), 5.08 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{IV}), 4.97–2.98 (several signals, 46 H), 1.88–1.64 (m, 24 H, 8 CH_3). MALDIMS (positive-ion mode, DHB–THF matrix): *m/z* 2063.6 $[M+Na]^+$. Anal. Calcd for $C_{119}H_{112}N_4O_{27}$ (2040.19): C, 70.05; H, 6.02; N, 2.74. Found: C, 70.13; H, 6.39; N, 2.53.

*Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-dimethylmaleimido-β-*D*-glucopyranoside* (**25**).—A solution of **24** (1.003 g, 0.491 mmol) in dry 2:1 MeOH–THF (6.9 mL) was treated with NaOMe (0.195 M, 0.39 mL) and then worked up as described for the synthesis of **11.** The residue was purified by flash chromatography (1.75:1 petroleum ether–EtOAc) to yield **25** (0.834 g, 89%) as a white foam. TLC (1.75:1 petroleum ether–EtOAc): R_f 0.18; $[\alpha]_D$ +34.6° (*c* 0.26, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.26–6.97 (m, 45 H, 9 Ph), 5.06 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{IV}), 4.97 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{III}), 4.95 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^{II}), 4.87 (d, 1 H, J_{gem} 13.0 Hz, 1 H, CHHPh), 4.82–4.78 (m, 3 H, H-1^{I}, 2 CHHPh), 4.75 (d, 1 H, J_{gem} 13.0 Hz, CHHPh), 4.69 (d, 1 H, J_{gem} 12.4 Hz, CHHPh), 4.50–4.33 (m, 13 H, 6.5 CH_2Ph), 4.09–4.06 (m, 2 H, H-3^{IV}, H-4^{III}), 4.01–3.84 (m, 9 H, H-2^{I}, H-2^{II}, H-2^{III}, H-2^{IV}, H-3^{I}, H-3^{II}, H-3^{III}, H-4^{I}, H-4^{II}), 3.70 (br.t, 1 H, H-4^{IV}), 3.61 (dd, 1 H, $J_{5,6'}$ 3.9 Hz, H-$6'^{IV}$), 3.51–3.47 (m, 2 H, H-$6'^{I}$, H-$6'^{III}$), 3.44–3.41 (m, 2 H, H-$6'^{II}$, H-6^{IV}), 3.32 (dd, 1 H, J_{gem} 11.0, $J_{5,6}$ 3.5 Hz, H-6^{I}), 3.27–3.28 (m, 2 H, H-5^{I}, H-5^{IV}), 3.17 (br.d, 1 H, H-6^{III}), 3.10 (br.d, 1 H, H-6^{II}), 3.03 (m, 1 H, H-5^{III}), 2.99 (m, 1 H, H-5^{II}), 1.88–1.56 (m, 24 H, 8 CH_3). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 97.90 (C-1^{I}), 97.70 (C-1^{III}), 97.60 (C-1^{IV}), 97.50 (C-1^{II}), 79.50 (C-3^{IV}), 78.20 (C-3^{I}), 77.80 (C-3^{II}, C-3^{III}), 76.30 (C-4^{II}), 76.20 (C-4^{I}), 76.00 (C-4^{III}, C-4^{IV}), 75.20–73.30 (8 CH_2Ph, C-5^{III}, C-5^{II}, C-5^{I}), 73.00 (C-5^{IV}), 71.80 (C-6^{IV}), 71.20

(CH_2Ph), 68.70 (C-6^{I}), 68.00 (C-6^{III}), 67.70 (C-6^{II}), 56.90–56.00 (C-2^{I}, C-2^{III}, C-2^{II}, C-2^{IV}). MALDIMS (positive-ion mode, DHB–THF matrix): m/z 1928.9 $[M+Na]^+$. Anal. Calcd for $C_{111}H_{116}N_4O_{25}$ (1906.06): C, 69.94; H, 6.13; N, 2.93. Found: C, 69.81; H, 6.26; N, 2.74.

Benzyl 3,4,6-tri-O-*acetyl-2-deoxy-2-dimethylmaleimido - β - D - glucopyranosyl - (1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy - 2 - dimethylmaleimido - β - D - glucopyranosyl - (1→4) - 3,6 - di - O - benzyl - 2 - deoxy - 2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-dimethylmaleimido-β*-D-*glucopyranoside* (**26**).—A mixture of **6** (0.52 g, 0.516 mmol) and **25** (0.834 g, 0.437 mmol) in dry MeCN (3 mL) was stirred under N_2 at rt while TMSOTf (0.01 M in MeCN, 0.51 mL) was added dropwise. After 3 h the reaction mixture was neutralized with Et_3N, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (1.25:1 petroleum ether–EtOAc) to yield **26** (0.638 g, 53%) as a white foam. TLC (1.25:1 petroleum ether–EtOAc): R_f 0.12; $[\alpha]_D$ +6.9° (c 0.13, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.26–7.02 (m, 55 H, 11 Ph), 5.57 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.3 Hz, H-3^{VI}), 5.28(d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{VI}), 5.03 (dd, 1 H, $J_{3,4}=J_{4,5}$ 9.3 Hz, H-4^{VI}), 4.96 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{V}), 4.93–4.79 (m, 8 H, 2 CH_2Ph, H-1^{I}, H-1^{II}, H-1^{III}, H-1^{IV}), 4.72 (d, 1 H, J_{gem} 12.9 Hz, CHHPh), 4.67 (d, 1 H, J_{gem} 12.6 Hz, CHHPh), 4.51–4.29 (m, 16 H, 8 CH_2Ph), 4.14–4.12 (m, 2 H, H-6′^{VI}, H-4^{V}), 4.02–3.87 (m, 11 H, H-2^{I}, H-2^{VI}, H-3^{I}, H-3^{II}, H-3^{III}, H-3^{IV}, H-3^{V}, H-4^{I}, H-4^{II}, H-4^{III}, H-4^{IV}), 3.87-3.81 (m, 5 H, H-2^{II}, H-2^{III}, H-2^{IV}, H-2^{V}, H-6^{VI}), 3.51–3.49 (m, 2 H, H-6′^{I}, H-6′^{V}), 3.41–3.39 (m, 3 H, H-6′^{II}, H-6′^{III}, H-6′^{IV}), 3.38–3.31 (m, 2 H, H-6^{I}, H-5^{VI}), 3.26 (m, 1 H, H-5^{I}), 3.20 (m, 1 H, H-6^{V}), 3.08–3.03 (m, 4 H, H-5^{V}, H-6^{II}, H-6^{III}, H-6^{IV}), 2.97–2.95 (m, 3 H, H-5^{II}, H-5^{III}, H-5^{IV}), 1.97–1.63 (m, 45 H, 12 CH_3, 3 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 97.60 (C-1^{I}), 97.30 (C-1^{II}, C-1^{III}, C-1^{IV}, C-1^{V}), 97.10 (C-1^{VI}), 78.00 (C-3^{II}, C-3^{III}, C-3^{IV}), 77.90 (C-3^{V}), 77.50 (C-3^{I}), 76.10 (C-4^{V}), 76.00 (C-4^{I}), 75.90 (C-4^{II}, C-4^{III}, C-4^{IV}), 75.00–72.80 (C-5^{V}, C-5^{II}, C-5^{III}, C-5^{IV}, C-5^{I}, 10 CH_2Ph), 71.60 (C-5^{VI}), 71.20 (C-3^{VI}), 70.70 (CH_2Ph), 69.00 (C-4^{VI}), 68.50 (C-6^{I}), 67.70 (C-6^{V}), 67.50 (C-6^{II}, C-6^{III}, C-6^{IV}), 61.80 (C-6^{VI}), 56.70 (C-2^{II}, C-2^{III}, C-2^{IV}, C-2^{V}), 55.80 (C-2^{I}), 55.50 (C-2^{VI}). MALDIMS (positive-ion mode, DHB–THF matrix): m/z 2773.6 $[M+Na]^+$. Anal. Calcd for $C_{115}H_{164}N_6O_{40}$ (2750.91): C, 67.67; H, 6.00; N, 3.05. Found: C, 67.21; H, 5.95; N, 2.5

Benzyl 2-acetamido-3,4,6-tri-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl-(1→4)-2-acetamido-3,6-di - O - benzyl - 2 - deoxy - β - D - glucopyranoside* (**27**).—A mixture of **26** (0.535 g, 0.194 mmol) and NaOH (1.5 g, 37.5 mmol) in 5:2:1 MeOH–dioxane–water (8 mL) was stirred at rt overnight. The reaction mixture was neutralized with 2 N HCl, extracted with CH_2Cl_2 (2 × 20 mL), and the solvent was evaporated in vacuo. The crude product was redissolved in 5:2:1 MeOH–dioxane–water (8 mL), treated with ethanolamine (0.082 g, 1.35 mmol, 0.08 mL), and the pH was adjusted and kept at pH 5 by addition of N HCl. After 1 day the reaction mixture was neutralized with ethanolamine, the solvent was evaporated, and the residue was dried well in vacuo. The residue was treated with 2:1 pyr/Ac_2O (15 mL), stirred for 20 h and then worked up as described for **7**. The residue was purified by flash chromatography (1.5:1 toluene–acetone), and the fractions of partially deprotected products were collected and treated again as described before to yield **27** (0.328 g, 74%) as an amorphous mass. TLC (1.5:1 toluene–acetone): R_f 0.1; $[\alpha]_D$ −22.6° (c 0.53, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 7.34–7.19 (m, 35 H, 11 Ph), 6.35 (br s, 1 H, NH-2^{I}), 5.82 (br.s, 1 H, NH-2^{II}), 5.42 (br.s, 1 H, NH-2^{IV}), 5.19 (br.s, 1 H, NH-2^{III}), 5.11 (br.d, 2 H, NH-2^{V}, NH-2^{VI}), 4.98 (dd, 1 H, $J_{3,4}=J_{4,5}$ 9.9 Hz, H-4^{VI}), 4.84–4.65 (m, 12 H, H-3^{VI}, 5.5 CH_2Ph), 4.61 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1^{I}), 4.60–4.31 (m, 11 H, 5.5 CH_2Ph), 4.27 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1^{II}), 4.24 (m, 1 H,

H-2^{I}), 4.22 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1^{V}), 4.21 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1^{IV}), 4.20 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1^{VI}), 4.16 (dd, 1 H, J_{gem} 12.6, $J_{5,6'}$ 4.4 Hz, H-6′^{VI}), 4.12 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1^{III}), 4.05–3.91 (m, 10 H,, H-2^{II}, H-2^{III}, H-2^{IV,} H-2^{V}, H-2^{VI},H-4^{I}, H-4^{II}, H-4^{IV}, H-4^{V}, H-6^{VI}), 3.85–3.71 (m, 4 H, H-5^{I}, H-6^{I}, H-6′^{I}, H-3^{I}), 3.84 (dd, 1 H, $J_{3,4} = J_{4,5}$ 7.0 Hz, H-4^{III}), 3.59–3.44 (m, 10 H, H-3^{II}, H-5^{II}, H-6^{III}, H-6′^{III}, H-6^{V}, H-6′^{V}, H-6^{IV}, H-6′^{IV}, H-6^{II}, H-6′^{II}), 3.38–3.25 (m, 7 H, H-5^{III}, H-5^{V}, H-5^{IV}, H-3^{V}, H-3^{III}, H-3^{IV}, H-5^{VI}), 2.02, 2.00, 1.95, 1.94, 1.74, 1.73, 1.72, 1.69 (8 s, 27 H, 9 CH_3CO). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.86–170.06 (9 CH_3CO), 138.90–137.62, 128.62–127.45 (11 Ph-C), 100.50 (C-1^{II}), 100.30 (C-1^{IV}), 100.10 (C-1^{VI}), 99.77 (C-1^{III}, C-1^{V}), 99.50 (C-1^{I}), 80.20 (C-3^{IV}), 80.00 (C-3^{III}), 79.57 (C-3^{V}), 79.60 (C-3^{II}), 78.20 (C-3^{I}), 75.40 (C-4^{III}), 75.30 (C-4^{II}), 75.00 (C-5^{II}), 74.80 (C-5^{IV}, C-4^{V}, C-4^{IV}), 74.30 (C-5^{III}, C-5^{V}), 74.28 (C-4^{I}), 74.02 (C-5^{I}), 73.70–72.62 (9 CH_2Ph), 72.60 (C-3^{VI}), 71.43 (CH_2Ph), 71.30 (C-5^{VI}), 69.90 (C-6^{I}, CH_2Ph), 69.10 (C-6^{II}), 68.87 (C-6^{III}, C-6^{IV}), 68.40 (C-6^{V}), 68.25 (C-4^{VI}), 61.67 (C-6^{VI}), 54.05–53.95 (C-2^{III}, C-2^{IV}, C-2^{V}, C-2^{VI}), 53.38 (C-2^{II}),. 50.78 (C-2^{I}), 23.28, 23.17, 20.58 (9 CH_3CO). MALDIMS (positive-ion mode, DHB–THF matrix): m/z 2375.4 $[M + K]^+$. Anal. Calcd for $C_{131}H_{154}N_6O_{41}\cdot H_2O$ (2356.59): C, 66.76; H, 6.58; N, 3.56. Found: C, 66.49; H, 6.64; N, 2.85.

2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**1c**).—A solution of **27** (39.0 mg, 16.6 μmol) in dry MeOH (6.85 mL) was treated with NaOMe (0.195 M, 0.45 mL) and then worked up as described for the synthesis of **11**. The residue was dissolved in 2:1:1 MeOH–AcOH–water (4 mL) and then hydrogenated in the presence of Pd/C (10% Pd, 0.02 g). After 3 days the reaction mixture was filtered through Celite, the filtrate was washed with (1:1 EtOH–water), and the solvents were evaporated in vacuo. The residue was purified by flash chromatography NH_2-phase (2:1 EtOH–water) to yield **1c** (0.014 g, 70%). TLC (1.5:1 EtOH–water): R_f 0.72. MALDIMS (positive-ion mode, DHB–water matrix): m/z 1259.9 $[M + Na]^+$. $C_{48}H_{80}N_6O_{31}\cdot$(1237.17).

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References

1. Yang, Y.; Hamaguchi, K.; Kuramitsu, S. *J. Biochem. (Tokyo)* **1981**, *89*, 1357–1366.
2. Lumb, K. J.; Alpin, R. T.; Radford, S. E.; Archer, D. B.; Jeenes, D. J.; Lambert, N.; Mackenzie, D. A.; Dobson, C. M.; Lowe, G. *FEBS Letts*. **1992**, *296*, 153–157.
3. Sharon, N. *Proc. R. Soc. London Ser. B* **1967**, *167*, 402–415.
4. Bains, G.; Lee, R. T; Lee, Y.; Freire, E. *Biochemistry* **1992**, *31*, 12624–12628.
5. Kochibe, N.; Matta, K. L. *J. Biol. Chem*. **1989**, *264*, 173–177.
6. Anantharan, V.; Patanjali, S. R.; Swamy, J. M.; Sanadi, A. R.; Goldstein, I. J.; Surolia, A. *J. Biol. Chem*. **1986**, *261*, 14621–14627.
7. Cederberg, B. M.; Gray, G. R. *Anal Biochem*. **1979**, *99*, 221–230.
8. Mizuno, T.; Kawagishi, H.; Ito, H.; Shimura, K. *Shizuoka Daigaku Nogakubu Kenkyu Hokoku* **1988**, 29–35; *Chem. Abstr*. **1994**, *111*, 331886.
9. Suzuki, S.; Suzuki, K.; Tokuro, A.; Okawa, Y.; Suzuki, M. *Immunopotentiating Chitin Nature and Technology; Proceedings International Conference Chitin Chitosan*, Muzzarelli, R. A. A.; Jeuniaux, C.; Gooday, G. W. Eds.; third ed. Plenum Press: New York, 1986.
10. Suzuki, K.; Tokorno, A.; Okawa, Y.; Suzuki, S.; Suzuki, M. *Microbiol. Immunol*. **1986**, *30*, 777–787.
11. Sedmera, P.; Prikrylova, V.; Bezouska, K.; Rajnochova, E.; Thiem, J.; Kren, V. *J. Carbohydr. Chem*. **1998**, *17*, 1351–1357 and references therein.
12. Usui, T.; Matsui, H.; Isobe, K. *Carbohydr. Res*. **1990**, *203*, 65–77.
13. Singh, S.; Gallagher, R.; Derrick, P. J.; Crout, D. H. G. *Tetrahedron: Asymmetry* **1995**, *6*, 2803–2810.
14. Nanjo, F.; Kazuo, S.; Ishikawa, M.; Isobe, K.; Usui, T. *Agric. Biol. Chem*. **1989**, *53*, 2189–2195.

15. Aly, M. R. E.; Castro-Palomino, J. C.; Ibrahim, E. I.; El Ashry, E. S. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **1998**, 2305–2316
16. Aly, M. R. E.; Ibrahim, E. I.; El Ashry, E. S. H.; Schmidt, R. R. *Carbohydr. Res.* **1999**, *316*, 121–132.
17. Aly, M. R. E.; Ibrahim, E. I.; El Ashry, E. S. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 319–326.
18. Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1927**, *60*, 1555–1564.