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ORIGINAL PAPER

Synthesis of a *chito*-tetrasaccharide β-1,4-Glc*N*Ac-β-1,4-Glc*N* repeating unit

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Abstract *tert*-Butyldimethylsilyl (4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (Kawada and Yoneda [MOCHEM-D-09-00120], 2009), designed as a repeating disaccharide unit in a β-glucan having two different faces, was converted into a glycosyl donor and an acceptor. The glycosyl acceptor was glycosylated with the donor to afford a *chito*-tetrasaccharide derivative in good yield. Phthalimido and azido groups in the tetrasaccharide were successively converted into acetamido and free amino groups, and all other protecting groups were cleaved to obtain the *chito*-tetrasaccharide (2-amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose.

Keywords Carbohydrates · Protecting groups · Glycosides · Oligosaccharides

Introduction

A β-1,4-glucan chain exhibiting two different faces can be synthesized by attaching the same functional group at the same carbon position in each sugar residue of two neighboring monosaccharides. In a previous paper [1], we proposed a stepwise method for synthesizing β-1,4-glucan and reported on the preparation of the repeating disaccharide unit, *tert*-butyldimethylsilyl (4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**1**).

In this report, a conversion of the disaccharide unit **1** into a disaccharide acceptor, *tert*-butyldimethylsilyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-*O*-benzyl-2-phthalimido-β-D-glucopyranoside (**4**) as well as into a disaccharide donor precursor (4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-*O*-benzyl-2-phthalimido-D-glucopyranose (**2**) is reported and the synthesis of *chito*-tetrasaccharide **9** is described in detail. The glycosylation procedure for synthesizing the tetrasaccharide presents a versatile reaction for future applications in glycosylation reactions directed towards oligosaccharides and/or polysaccharides.

Results and discussion

The repeating disaccharide unit **1** was converted into glycosyl acceptor **4** and donor **3** (Scheme 1). The glycosyl acceptor **4** was prepared by cleavage of the 4′-*O*-acetyl group and the corresponding glycosyl donor precursor **2** by cleavage of the *tert*-butyldimethylsilyl (TBDMS) group [2] in the disaccharide unit **1** using tetra-*n*-butylammonium fluoride (TBAF) [3, 4] in 78% yield. The glycosyl donor **3** was prepared by attaching a trichloroacetimidate moiety using trichloroacetonitrile [5] and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [6] in 88% yield.

The glycosylation of the disaccharide acceptor **4** with the disaccharide imidate donor **3** presents an efficient precedent for elongation of the glucan chain. Thus, in glucan chains extensions can be done either by applying linear or convergent synthetic methods [7] as well as by

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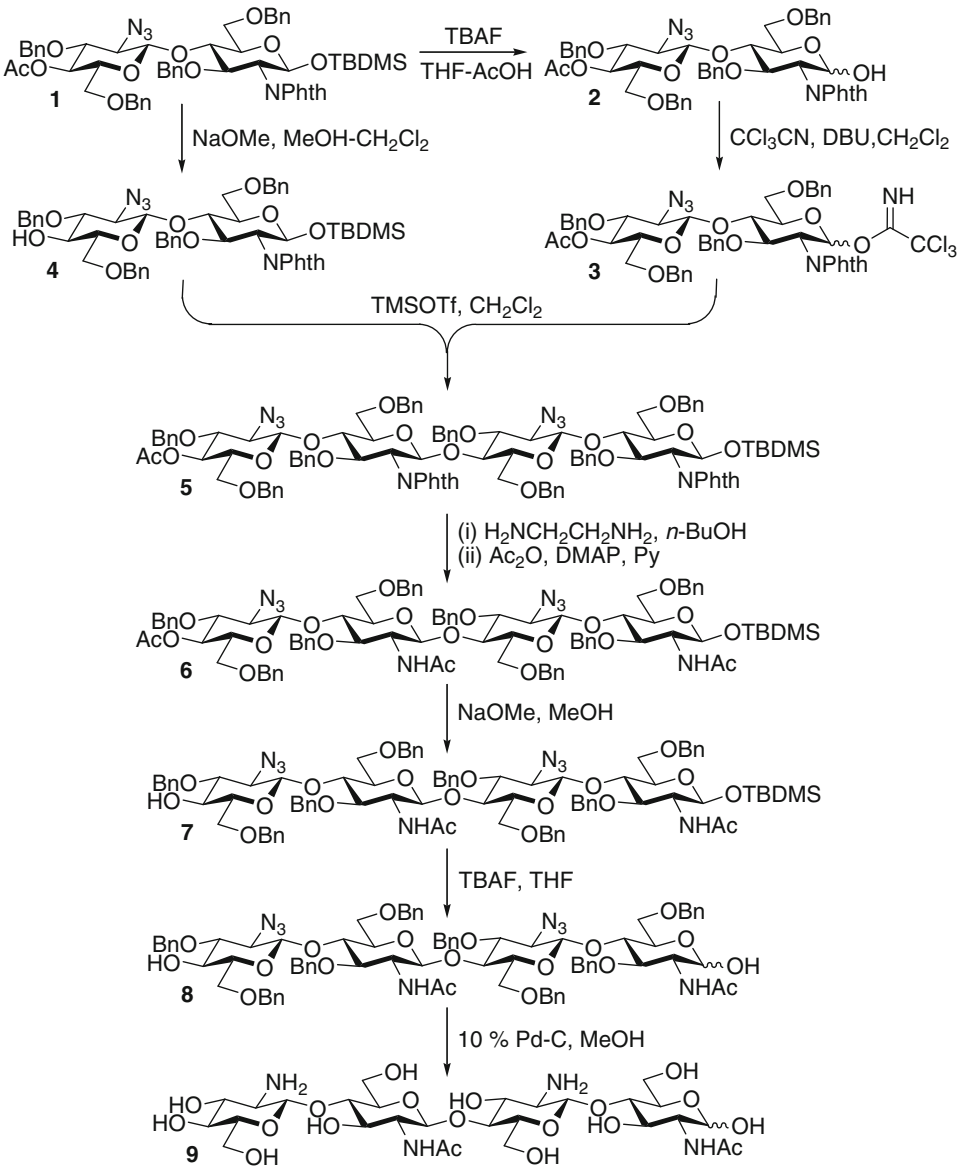
Scheme 1



using the polymerization method, wherein the disaccharide imidate **3** plays a central role as glycosyl donor. At the reducing end of donor **3** a phthalimido group at C-2 is attached and thus is expected to favor β-glycosidation due to neighboring group participation and steric hindrance [8].

A variety of glycosylation reaction conditions, conducted in dichloromethane, were investigated. The reaction temperature at -20 °C was found to be suitable as the starting disaccharide imidate **3** was consumed within 5 min, preventing complex side reactions. Higher reaction temperatures than -20 °C are leading to decomposition and consequently to a dramatic decrease in yield. Additionally, the use of boron trifluoride–diethyl etherate as glycosylation catalyst [5] generated a large number of byproducts including fluorides and α-glycoside. By applying a series of experiments the optimum reaction conditions were found using 0.01 equivalents (for the imidate **3**) of trimethylsilyl trifluoromethanesulfonate (TMSOTf) [9] as a catalyst to afford the stereochemically pure target β-glycoside. However, an increase of the amount of catalyst up to 0.05 equivalents caused unexpected side reactions and decreased the yield of the reaction. The produced tetrasaccharide **5** was purified by flash column chromatography. The ^{1}H NMR spectrum of **5** showed a doublet for the newly formed anomeric proton at $\delta = 5.20$ ppm, having a coupling constant of 8.3 Hz. This fact is consistent with a 1,2-*trans*-relationship between the newly formed anomeric proton and the C-2 proton typical for a β-linkage. In addition, the ^{13}C NMR spectrum of **5** showed a signal of C-1 at $\delta = 96.8$ ppm, which is empirically indicative for a β-glucoside [10, 11].

Tetrasaccharide **5** was transformed to *chito*-tetraose **9** by the conversion of the phthalimido groups into acetamido groups [12], cleavage of the 4′-*O*-acetyl group, cleavage of the anomeric TBDMS group [3, 4] and hydrogenolysis of benzyl and azide groups into free hydroxyl and free amino groups, respectively.

In conclusion, the disaccharide imidate **3** having phthalimido at the neighboring C-2 position stereospecifically afforded a β-glycosidic linkage using TMSOTf as catalyst and the disaccharide acceptor **4**. This result demonstrates that the disaccharide unit **1** is a suitable building block for constructing a β-glucan chain exhibiting two distinguished surfaces.

Experimental

General methods

Nuclear magnetic resonance (NMR) spectra were measured with tetramethylsilane as an internal standard. The assignments of the signals were determined using ^{1}H–^{1}H correlated spectroscopy and/or ^{13}C–^{1}H heteronuclear multiple-quantum correlation technique. Coupling constants (*J*) are given in Hz. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) spectra were recorded on Bruker Reflex III using 2,5-dihydroxybenzoic acid as a matrix. Anhydrous dichloromethane and tetrahydrofuran were prepared by distilling from phosphorus pentoxide and sodium/benzophenone, respectively. Flash column chromatography was performed on silica gel (Wakogel FC-40). Preparative thin-layer chromatography (TLC) was done on silica gel plates (Kieselgel 60 F_{254}, Merck). Results of elemental analyses agreed favorably with calculated values. Unless otherwise indicated, the usual workup for each reaction mixture consists of extraction with ethyl acetate, washing with brine, drying over sodium sulfate and evaporation in vacuo.

(4-O-Acetyl-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-O-benzyl-2-phthalimido-D-glucopyranose (**2**, $C_{50}H_{50}N_4O_{12}$)

To a stirred solution of 1.7 g *tert*-butyldimethylsilyl (4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-*O*-benzyl-2-phthalimido-β-D-glucopyranoside (**1**, 1.68 mmol) in 17 cm^3 anhydrous THF 46 mm^3 acetic acid (0.8 mmol) and 3.2 cm^3 tetra-*n*-butylammonium fluoride solution (1.0 M in THF, 3.2 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h and subsequently worked up. The residue was purified by flash column chromatography (EtOAc/toluene, 1:2, v/v) to give 1.17 g (78%) **2** as colorless crystals. $R_f = 0.45$ (EtOAc/toluene, 1:2, v/v); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.84$ (s, 3H, CO*Me*), 3.24 (t, 1H, $J_{2',3'} = 9.6$ Hz, $J_{3',4'} = 9.6$ Hz, H-3′), 3.30 (ddd, 1H, $J_{4',5'} = 9.6$ Hz, $J_{5',6'a} = 3.7$ Hz, $J_{5',6'b} = 5.3$ Hz, H-5′), 3.35 (dd, 1H, $J_{5',6'a} = 3.7$ Hz, $J_{6'a,6'b} = 10.6$ Hz, H-6′a), 3.40 (dd, 1H, $J_{1',2'} = 8.2$ Hz, $J_{2',3'} = 9.6$ Hz, H-2′), 3.44 (d, 1H, $J_{1,OH} = 8.5$ Hz, 1-O*H*), 3.47 (dd, 1H, $J_{5',6'b} = 5.3$ Hz, $J_{6'a,6'b} = 10.6$ Hz, H-6′b), 3.71 (ddd, 1H, $J_{4,5} = 10.1$ Hz, $J_{5,6a} = 1.6$ Hz, $J_{5,6b} = 3.4$ Hz, H-5), 3.83 (dd, 1H, $J_{5,6a} = 1.6$ Hz, $J_{6a,6b} = 11.0$ Hz, H-6a), 3.97 (dd, 1H, $J_{5,6b} = 3.4$ Hz, $J_{6a,ab} = 11.0$ Hz, H-6b), 4.09 (dd, 1H, $J_{1,2} = 8.5$ Hz, $J_{2,3} = 10.8$ Hz, H-2), 4.12 (dd, 1H, $J_{3,4} = 8.7$ Hz, $J_{4,5} = 10.1$ Hz, H-4), 4.32 (d, 1H, CH_2Ph), 4.35 (d, 1H, $J_{1',2'} = 8.2$ Hz, H-1′), 4.36 (dd, 1H, $J_{2,3} = 10.8$ Hz, $J_{3,4} = 8.7$ Hz, H-3), 4.40–4.43 (2 × d, 2H, CH_2Ph), 4.51 (d, 1H, CH_2Ph), 4.59 (d, 1H, CH_2Ph), 4.73–4.82 (3 × d, 3H, CH_2Ph), 4.96 (t, 1H, $J_{3',4'} = 9.6$ Hz, $J_{4',5'} = 9.6$ Hz, H-4′), 5.34 (t, 1H, $J_{1,2} = 8.5$ Hz, $J_{1,OH} = 8.5$ Hz, H-1), 6.81–6.84 (m, 3H, *Ph*), 6.94–6.96 (m, 2H, *Ph*), 7.22–7.39 (m, 15H, *Ph*), 7.61–7.79 (m, 4H, *Phth*) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 20.8$ (CO*Me*), 57.5 (C-2), 66.5 (C-2′), 68.0 (C-6), 69.4 (C-6′), 71.0 (C-4′), 73.3, 73.5, 73.6 (C-5′, 2 × *C*H_2Ph), 74.6, 74.7, 74.9 (C-5, 2 × *CH*$_2$Ph), 77.3 (C-3), 78.1 (C-4), 80.6 (C-3′), 92.9 (C-1), 100.9 (C-1′), 123.2 (*Phth*), 127.0–128.5 (*Ph*), 131.6, 133.7 (*Phth*), 137.6, 137.8, 137.9, 138.5 (*Ph*), 168.1 (*C*OMe) ppm.

Trichloroacetimidoyl (4-O-acetyl-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-O-benzyl-2-phthalimido-D-glucopyranoside
(**3**, $C_{52}H_{50}Cl_3N_5O_{12}$)

To a solution of 400 mg **2** (445 μmol) in 5 cm^3 anhydrous CH_2Cl_2 6.2 mm^3 1,8-diazabicyclo[5.4.0]undec-7-ene (45 μmol) and 318 mg trichloroacetonitrile (2.2 mmol) were added at 0 °C. After stirring at room temperature for 2 h, another portion of 3.0 mm^3 DBU (22 μmol) and 66.24 mm^3 trichloroacetonitrile (890 μmol) were added. The reaction mixture was stirred for 1 h, then purified by flash column chromatography (EtOAc/*n*-hexane, 1:3, v/v) to give 407 mg (88%) **3** as colorless crude crystals, which were directly used in the next step.

tert-Butyldimethylsilyl (2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-O-benzyl-2-phthalimido-β-D-glucopyranoside (**4**, $C_{54}H_{62}N_4O_{11}Si$)

To a solution of 874 mg *tert*-butyldimethylsilyl (4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-*O*-benzyl-2-phthalimido-β-D-glucopyranoside (**1**, 0.86 mmol) in 15 cm^3 MeOH and 10 cm^3 CH_2Cl_2 52 mm^3 sodium methoxide in MeOH (28%, 0.26 mmol) were added dropwise at 0 °C. The reaction mixture was stirred at room temperature overnight, then another portion of 52 mm^3 sodium methoxide in MeOH (28%, 0.26 mmol) was added. Stirring was continued

overnight. Subsequently the reaction mixture was neutralized with DOWEX 50W-X8 (H^+) resin. The resin was filtered off, and the filtrate was concentrated in vacuo and purified by flash column chromatography (EtOAc/toluene, 1:9, v/v) to yield 805 mg (96%) **4** as colorless glass. $R_f = 0.43$ (EtOAc/*n*-hexane, 1:2, v/v); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -0.11$ (s, 3H, Si*Me*), 0.03 (s, 3H, Si*Me*), 0.66 (s, 9H, SiC*Me*$_3$) 2.94 (d, 1H, $J_{4',OH} = 1.8$ Hz, 4′-O*H*), 3.20 (dd, 1H, $J_{2',3'} = 9.6$ Hz, $J_{3',4'} = 8.7$ Hz, H-3′), 3.24 (ddd, 1H, $J_{4',5'} = 9.9$ Hz, $J_{5',6'a} = 5.5$ Hz, $J_{5',6'b} = 4.4$ Hz, H-5′), 3.33 (dd, 1H, $J_{1',2'} = 8.0$ Hz, $J_{2',3'} = 9.6$ Hz, H-2′), 3.51 (dd, 1H, $J_{5',6'a} = 5.5$ Hz, $J_{6'a,6'b} = 9.9$ Hz, H-6′a), 3.62–3.67 (m, 3H, H-4′, H-6′b, H-5), 3.78 (dd, 1H, $J_{5,6a} = 1.4$ Hz, $J_{6a,6b} = 11.1$ Hz, H-6a), 3.97 (dd, 1H, $J_{5,6b} = 3.4$ Hz, $J_{6a,6b} = 11.1$ Hz, H-6b), 4.11–4.13 (m, 2H, H-2, H-4), 4.29 (dd, 1H, $J_{2,3} = 10.8$ Hz, $J_{3,4} = 8.6$ Hz, H-3), 4.41–4.47 (m, 4H, H-1′, C*H*$_2$Ph), 4.56 (d, 1H, C*H*$_2$Ph), 4.73–4.76 (2 × d, 2H, C*H*$_2$Ph), 4.86 (s, 2H, C*H*$_2$Ph), 5.32 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 6.81–6.85 (m, 3H, *Ph*), 6.96–6.98 (m, 2H, *Ph*), 7.24–7.42 (m, 15H, *Ph*), 7.60–7.78 (m, 4H, *Phth*) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -5.5$, −4.3 (Si*Me*), 17.5 (Si*C*Me$_3$), 25.3 (SiC*Me*$_3$), 57.8 (C-2), 66.2 (C-2′), 68.1 (C-6), 70.7 (C-6′), 73.0 (C-5′), 73.3, 73.3, 73.6 (*C*H$_2$Ph), 74.1, 74.8 (C-4′, C-5), 75.1 (*C*H$_2$Ph), 76.6 (C-3), 78.0 (C-4), 82.7 (C-3′), 93.3 (C-1), 101.0 (C-1′), 123.1 (*Phth*), 126.9–128.5 (*Ph*), 131.6, 133.6 (*Phth*), 137.5, 138.1, 138.1, 138.7 (*Ph*) ppm.

tert-Butyldimethylsilyl (4-O-acetyl-2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-(2-deoxy-3,6-di-O-benzyl-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-(2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-deoxy-3,6-di-O-benzyl-2-phthalimido-β-D-glucopyranoside (**5**, $C_{104}H_{110}N_8O_{22}Si$)

To a stirred solution of 886 mg **3** (0.85 mmol) and 882 mg **4** (0.91 mmol) in 20 cm^3 anhydrous CH_2Cl_2 1.5 mm^3 trimethylsilyl trifluoromethanesulfonate (8.5 μM) was added dropwise at −20 °C. After stirring for 10 min, the reaction mixture was neutralized with triethylamine and worked up. The crude material obtained was purified by flash column chromatography (EtOAc/toluene, 1:4, v/v) to yield 1.192 g (76%) **5** as colorless glass. $R_f = 0.67$ (EtOAc/toluene, 1:4, v/v); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -0.14$ (s, 3H, Si*Me*), 0.00 (s, 3H, Si*Me*), 0.64 (s, 9H, SiC*Me*$_3$), 1.81 (s, 3H, CO*Me*), 2.75 (br d, 1H, H-5′), 3.12 (t, 1H, $J = 8.7$ Hz, $J = 9.6$ Hz, H-3′), 3.20–3.28 (m, 5H, H-2′, H-6′a, H-5″, H-3‴, H-5‴), 3.35 (dd, 1H, $J = 4.6$ Hz, $J = 10.6$ Hz, H-6‴a), 3.38 (dd, 1H, $J = 8.0$ Hz, $J = 9.6$ Hz, H-2‴), 3.45–3.48 (m, 2H, H-6′b, H-6‴b), 3.55–3.58 (br d, 2H, H-5, H-6″a), 3.67 (dd, 1H, $J = 2.8$ Hz, $J = 11.0$ Hz, H-6″b), 3.70 (br d, 1H, H-6a), 3.89 (dd, 1H, $J = 2.8$ Hz, J = 11.0 Hz, H-6b), 4.00 (t, 1H, $J = 9.2$ Hz, H-4), 4.05 (dd, 1H, $J_{1,2} = 8.3$ Hz, $J_{2,3} = 10.5$ Hz, H-2), 4.10–4.16 (m, 3H, H-1′, H-4′, H-4″), 4.17–4.23 (m, 2H, H-3, H-2″), 4.26–4.41 (m, 10H, H-3″, H-1‴, C*H*$_2$Ph), 4.53 (d, 1H, C*H*$_2$Ph), 4.55 (d, 1H, C*H*$_2$Ph), 4.61 (d, 1H, C*H*$_2$Ph), 4.71–4.78 (3 × d, 3H, C*H*$_2$Ph), 4.81 (d, 1H, C*H*$_2$Ph), 4.97 (t, 1H, $J = 9.5$ Hz, H-4‴), 5.07 (d, 1H, C*H*$_2$Ph), 5.20 (d, 1H, $J_{1'',2''} = 8.3$ Hz, H-1″), 5.28 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 6.68–7.43 (m, 40H, *Ph*), 7.53–7.84 (m, 8H, *Phth*) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = -5.6$, −4.3 (Si*Me*), 17.5 (Si*C*Me$_3$), 20.7 (CO*Me*), 25.3 (SiC*Me*$_3$), 56.5 (C-2″), 57.8 (C-2), 66.4, 66.5 (C-2′, C-2‴), 67.5, 67.6, 68.0 (C-6, C-6′, C-6″), 69.4 (C-6‴), 71.0 (C-4‴), 72.4 (*C*H$_2$Ph), 73.0, 73.1, 73.5, 73.8, 74.2, 74.3, 74.69, 74.72, 74.77, 74.9 (C-5, C-4′, C-5′, C-5″, C-5‴, 7 × *C*H$_2$Ph), 76.6, 77.1 (C-3, C-3″), 77.7, 78.1 (C-4, C-4″), 80.6 (C-3‴), 80.9 (C-3′), 93.3 (C-1), 96.8 (C-1″), 100.7, 100.8 (C-1′, C-1‴), 123.0–123.2 (*Phth*), 126.8–129.0 (*Ph*), 131.4–131.7, 133.5–133.8 (*Phth*), 137.6, 137.9, 138.0, 138.1, 138.3, 138.4, 138.5, 138.7 (*Ph*), 167.6–168.2 (*Phth*), 169.6 (*C*OMe) ppm; MALDI-TOF-MS: $m/z =$ 1889.82 $[M + K]^+$, 1873.81 $[M + Na]^+$, 1863.76 $[M (N_3 \rightarrow NH_2) + K]^+$, 1847.81 $[M (N_3 \rightarrow NH_2) + Na]^+$.

tert-Butyldimethylsilyl (4-O-acetyl-2-azido-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-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**6**, $C_{92}H_{110}N_8O_{20}Si$)

To a solution of 515 mg **5** (0.28 mmol) in 5 cm^3 *n*-butanol 945 mm^3 ethylenediamine (14 mmol) was added at 90 °C. After stirring at 90 °C for 5 h, another portion of 189 mm^3 ethylenediamine (2.80 mmol) was added. The reaction mixture was stirred at 90 °C for 1 h and then co-evaporated with EtOH. The residue was re-dissolved in 7 cm^3 pyridine, and 3 cm^3 acetic anhydride and 6 mg 4-dimethylaminopyridine (49 μmol) were added at 0 °C. The reaction mixture was stirred overnight at room temperature and then worked up. The crude material was purified by flash column chromatography (EtOAc/toluene, 2:3, v/v) to yield 444 mg (95%) **6** as colorless glass. $R_f = 0.27$ (EtOAc/*n*-hexane, 1:1, v/v); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.06$ (s, 3H, Si*Me*), 0.11 (s, 3H, Si*Me*), 0.87 (s, 9H, SiC*Me*$_3$), 1.68 (s, 3H, CO*Me*), 1.78 (s, 3H, CO*Me*), 1.80 (s, 3H, CO*Me*), 3.07 (m, 1H, H-5′), 3.15–3.31 (m, 6H, H-2′, H-3′, H-3‴, H-5‴, H-6‴a, H-5$_{NHAc}$), 3.36–3.54 (m, 7H, H-2, H-3, H-6′a, H-2‴, H-6‴b, H-5$_{NHAc}$, H-6$_{NHAc}$), 3.61–3.68 (m, 3H, H-6′b, H-2″, H-6$_{NHAc}$), 3.74 (br d, 1H, H-6$_{NHAc}$), 3.87 (dd, 1H, $J = 3.2$ Hz, $J = 10.9$ Hz, H-6$_{NHAc}$), 3.91–3.95 (m, 2H, H-3, H-4′), 4.01–4.05 (m, 2H, H-4, H-4″), 4.21–4.37 (m, 6H, H-1′ ($\delta = 4.31$), H-1‴ ($\delta = 4.34$), C*H*$_2$Ph), 4.43 (d, 1H, C*H*$_2$Ph), 4.49–4.54

(m, 4H, H-1″, CH_2Ph), 4.56–4.60 (2 × d, 2H, CH_2Ph), 4.64–4.67 (2 × d, 2H, CH_2Ph), 4.71 (d, 1H, $J_{2'',NHAc}$ = 8.7 Hz, 2″-N*H*Ac), 4.76 (d, 1H, CH_2Ph), 4.86 (d, 1H, CH_2Ph), 4.92–4.95 (m, 2H, H-1, CH_2Ph), 4.97 (t, J = 9.7 Hz, H-4‴), 5.08 (d, 1H, CH_2Ph), 5.39 (d, 1H, J = 7.8 Hz, 2-N*H*Ac), 7.18–7.37 (m, 40H, Ph) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = −5.4, −4.3 (Si*Me*), 17.8 (Si*C*Me$_3$), 20.7, 23.3, 23.4 (CO*Me*), 25.5 (SiC*Me$_3$*), 57.8 (C-2″), 58.1 (C-2), 66.4, 66.5 (C-2′, C-2″), 67.8, 68.1, 68.3 (C-6, C-6′, C-6″), 69.4 (C-6″), 70.9 (C-4‴), 72.9, 73.0, 73.2, 73.5, 73.7, 73.9, 74.2, 74.5, 74.6, 74.8, 75.0 (C-5, C-5′, C-5″, C-5‴, 8 × *C*H$_2$Ph), 75.9 (C-3 or C-4′), 76.7, 77.3 (C-4, C-4″), 78.3 (C-3 or C-4′), 79.8 (C-3″), 80.3, 81.3 (C-3′, C-3‴), 95.1 (C-1), 100.0 (C-1″), 100.7, 100.9 (C-1′, C-1‴), 127.1–128.6 (*Ph*), 137.6, 137.8, 137.8, 138.0, 138.1, 138.6, 138.9, 139.2 (*Ph*), 169.6, 169.7, 169.8 (*C*OMe) ppm; MALDI-TOF-MS: *m*/*z* = 1713.77 $[M + K]^+$, 1697.78 $[M + Na]^+$, 1687.75 [M (N_3 → NH_2) + K]$^+$, 1671.76 [M (N_3 → NH_2) + Na]$^+$.

tert-Butyldimethylsilyl (2-azido-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-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoisde
(**7**, $C_{90}H_{108}N_8O_{19}Si$)

To a solution of 426 mg **6** (0.25 mmol) in 5 cm^3 MeOH 15 mm^3 sodium methoxide in MeOH (28%, 0.07 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature overnight, then neutralized with DOWEX 50W-X8 (H^+) resin. The resin was filtered off, and the filtrate was concentrated in vacuo and purified by flash column chromatography (EtOAc/toluene, 2:3, v/v) to yield 404 mg (97%) **7** as colorless glass. R_f = 0.29 (MeOH/CH_2Cl_2, 1:19, v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.06 (s, 3H, Si*Me*), 0.10 (s, 3H, Si*Me*), 0.87 (s, 9H, SiC*Me$_3$*), 1.67 (s, 3H, CO*Me*), 1.77 (s, 3H, CO*Me*), 2.93 (br s, 1H, 4‴-O*H*), 3.05–3.09 (m, 3H, H-3$_{azido}$, H-5′, H-5‴), 3.23 (t, 1H, J = 9.2 Hz, H-3$_{azido}$), 3.25–3.30 (m, 3H, H-2′, H-2‴, H-5$_{NHAc}$), 3.38–3.45 (m, 3H, H-2, H-3″, H-6), 3.48 (br s, 2H, 2 × H-6), 3.51–3.54 (m, 2H, H-5$_{NHAc}$, H-6), 3.59–3.68 (m, 4H, H-2″, H-4$_{azido}$, 2 × H-6), 3.74 (br d, 1H, H-6), 3.87 (dd, 1H, J = 3.2 Hz, J = 11.0 Hz, H-6), 3.90–3.95 (m, 2H, H-3, H-4$_{azido}$), 3.99–4.04 (m, 2H, H-4, H-4″), 4.23 (d, 1H, CH_2Ph), 4.28 (d, 1H, CH_2Ph), 4.30 (d, 1H, J = 8.2 Hz, H-1$_{azido}$), 4.31 (d, 1H, J = 7.8 Hz, H-1$_{azido}$), 4.37–4.43 (m, 3H, CH_2Ph), 4.47–4.53 (m, 4H, H-1″, CH_2Ph), 4.59 (d, 1H, CH_2Ph), 4.63–4.66 (2 × d, 2H, CH_2Ph), 4.71 (d, 1H, $J_{2'',NHAc}$ = 8.7 Hz, 2″-N*H*Ac), 4.81–4.83 (m, 3H, CH_2Ph), 4.91–4.95 (2 × d, 2H, H-1, CH_2Ph), 5.08 (d, 1H, CH_2Ph), 5.34 (d, 1H, $J_{2,NHAc}$ = 7.8 Hz, 2-N*H*Ac), 7.15–7.40 (m, 40H, *Ph*) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = −5.3, −4.2 (Si*Me*), 17.9 (Si*C*Me), 23.3, 23.4 (CO*Me*), 25.6 (SiC*Me$_3$*), 55.8 (C-2″), 58.2 (C-2), 66.2, 66.5 (C-2′, C-2‴), 67.9, 68.2, 68.4, 70.7 (C-6, C-6′, C-6″, C-6‴), 72.9, 73.0, 73.2, 73.3, 73.6, 73.8, 74.3, 74.6, 74.7, 75.0 (C-4$_{azido}$, C-5, C-5′, C-5″, C-5‴, 8 × *C*H$_2$Ph), 76.1, 76.5, 77.1, 78.4 (C-3, C-4, C-4″, C-4$_{azido}$), 79.8 (C-3″), 81.4, 82.3 (C-3′, C-3‴), 95.1 (C-1), 100.0 (C-1″), 100.7, 100.9 (C-1′, C-1‴), 127.1–128.9 (*Ph*), 137.4, 137.8, 138.1, 138.7, 139.0, 139.2 (*Ph*), 169.7, 169.8 (*C*OMe) ppm; MALDI-TOF-MS: *m*/*z* = 1671.65 $[M + K]^+$, 1655.66 $[M + Na]^+$, 1645.67 [M (N_3 → NH_2) + K]$^+$, 1629.67 [M (N_3 → NH_2) + Na]$^+$.

(2-Azido-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-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-D-glucopyranose (**8**, $C_{84}H_{94}N_8O_{19}$)

To a stirred solution of 458 mg **7** (0.28 mmol) in 5 cm^3 anhydrous THF 8 mm^3 acetic acid (0.14 mmol) and 0.56 cm^3 TBAF solution (0.56 mmol) were added at 0 °C. After stirring at room temperature for 24 h, another portion of 0.56 cm^3 TBAF (0.56 mmol) was added. The reaction mixture was stirred for 8 h and then worked up. The residue was purified by flash column chromatography (MeOH/CH_2Cl_2, 1:19, v/v) to yield 396 mg (93%) **8** as colorless glass. R_f = 0.24 (MeOH/CH_2Cl_2, 1:19, v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.68 (s, 3H, CO*Me*), 1.73 (s, 3H, CO*Me*), 2.97 (d, 1H, $J_{4''',OH}$ = 1.8 Hz, 4‴-O*H*), 2.98 (m, 1H, H-5′), 3.04–3.09 (m, 2H, H-3‴, H-5‴), 3.15 (t, 1H, J = 9.4 Hz, H-3′), 3.25–3.30 (m, 3H, H-2′, H-5″, H-2‴), 3.37 (t, 1H, J = 9.2 Hz, H-3″), 3.42 (dd, 1H, J = 6.0 Hz, J = 10.1 Hz, H-6), 3.48 (br s, 2H, 2 × H-6), 3.52 (dd, 1H, J = 4.6 Hz, J = 10.1 Hz, H-6), 3.60 (dt, 1H, $J_{4''',OH}$ = 1.8 Hz, J = 9.1 Hz, H-4‴), 3.64–3.69 (m, 5H, H-3, H-2″, 3 × H-6), 3.88 (dd, 1H, J = 2.8 Hz, J = 10.1 Hz, H-6), 3.90 (t, 1H, J = 9.2 Hz, H-4′), 3.95–4.08 (m, 4H, H-2, H-4, H-5, H-4″), 4.12 (d, 1H, J = 8.3 Hz, H-1$_{azido}$), 4.19 (d, 1H, CH_2Ph), 4.24 (br s, 1H, 1-O*H*), 4.28 (d, 1H, CH_2Ph), 4.30 (d, 1H, J = 8.3 Hz, H-1$_{azido}$), 4.37–4.49 (m, 7H, CH_2Ph), 4.45 (d, 1H, J = 8.3 Hz, H-1″), 4.62–4.65 (2 × d, 2H, CH_2Ph), 4.68 (d, 1H, $J_{2'',NHAc}$ = 8.8 Hz, 2″-N*H*Ac), 4.81–4.85 (m, 3H, CH_2Ph), 4.94 (d, 1H, CH_2Ph), 5.08 (d, 1H, CH_2Ph), 5.19 (br s, 1H, H-1″), 4.68 (d, 1H, $J_{2'',NHAc}$ = 8.2 Hz, 2″-N*H*Ac), 7.19–7.41 (m, 40H, *Ph*) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 23.2, 23.3 (CO*Me*), 52.8 (C-2), 55.5 (C-2″), 66.1, 66.4 (C-2′, C-2‴), 67.0, 67.8, 68.2 (3 × C-6), 70.2 (C-5), 70.6 (C-6), 72.9, 73.0, 73.2, 73.3, 73.6, 73.7, 74.0, 74.1, 74.6, 74.9, 75.0 (C-5′, C-5″, C-4‴, C-5‴, 8 × *C*H$_2$Ph), 76.1 (C-4′), 76.4, 77.1, 77.5 (C-3, C-4, C-4″), 80.0 (C-3″), 81.2 (C-3′), 82.3 (C-3‴), 91.5 (C-1), 100.1 (C-1″), 100.7, 101.0 (C-1′, C-1‴), 127.3–128.8 (*Ph*), 137.4, 137.7, 138.0, 138.6, 138.9, 139.1 (*Ph*), 169.8,

170.3 (*C*OMe) ppm; MALDI-TOF-MS: $m/z = 1557.53$ $[M + K]^+$, 1541.55 $[M + Na]^+$, 1515.54 $[M\ (N_3 \rightarrow NH_2) + Na]^+$.

(2-Amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose (**9**, $C_{28}H_{50}N_4O_{19}$)

Compound **8** (208 mg, 0.136 mmol) was dissolved in 5 cm^3 MeOH containing 400 mg 10% palladium on carbon, and the resulting mixture was stirred under hydrogen at normal pressure for 30 min at 50 °C. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was purified by gel filtration (Sephadex LH-20) using MeOH to yield 72.9 mg (72%) **9** as colorless crystals. $R_f = 0.20$ (AcOH/MeOH, 1:2, v/v); MALDI-TOF-MS: $m/z = 785.26$ $[M + K]^+$, 769.30 $[M + Na]^+$, 747.28 $[M + H]^+$.

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