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ORIGINAL PAPER

A novel polycondensation method for the synthesis of a two-faced β-1,4-glucan

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Abstract The stereospecific synthesis of a chitosan derivative repeating 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranose, which has two distinguishing faces, was achieved by polycondensation of the sole starting disaccharide, trichloroacetimidoyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranoside in a short and efficient way.

Keywords Carbohydrates · Protecting group · Glycosides · Polysaccharides

Introduction

The glucopyranose residues of a β-1,4-glucan present their surfaces in an alternating "obverse–reverse" mode. These two faces could be distinguished by introduction of a functional group at the same carbon of each alternative glucose unit. However, it seems to be difficult to attach a functional group at the same carbon of each alternative glucose unit directly into a polysaccharide chain [1].

In the previous papers [2, 3], we proposed a synthetic method for such a β-1,4-glucan having two faces using 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**), and reported the tetrasaccharide synthesis. During the synthesis of the tetrasaccharide, the phthalimido donor enables a stereospecific β-glycosidation (*trans*-glycosidation), which encourages us to use the repeating disaccharide unit **1** as a sole starting material for polycondensation. This way an one-step synthesis of β-1,4-glucan with a large degree of polymerization is achievable.

In this report, we present the conversion of the repeating disaccharide unit **1** into the sole starting disaccharide **4**, which in turn is used for a subsequent stereospecific polycondensation reaction.

Results and discussion

The essence of this polycondensation applied is a polyglycosylation reaction, which is based on a well-known glycosylation method. Thus, the imidate method [4], one of the most popular methods of glycosylation, was chosen, since the trichloroacetimidoyl group could be introduced and transformed under mild conditions [5]. Normally, the glycosyl imidate (glycosyl donor) reacts with a suitable glycosyl acceptor, having a free hydroxyl group. However, if the glycosyl imidate itself contains a free hydroxyl group, it should act as both glycosyl donor as well as glycosyl acceptor. Consequently, the chain reaction would be expected to afford a polysaccharide.

The repeating disaccharide unit **1** was converted into a sole starting disaccharide **4**, which possesses both characteristics, a glycosyl acceptor moiety (4′-OH) and a glycosyl donor residue (1-*O*-trichloroacetimidoyl group; Scheme 1). The 4′-*O*-acetyl group of the repeating disaccharide unit **1** and the *tert*-butyldimethylsilyl (TBDMS) group were cleaved subsequently. However, if the TBDMS group was cleaved prior to acetyl deprotection, the alkaline reaction

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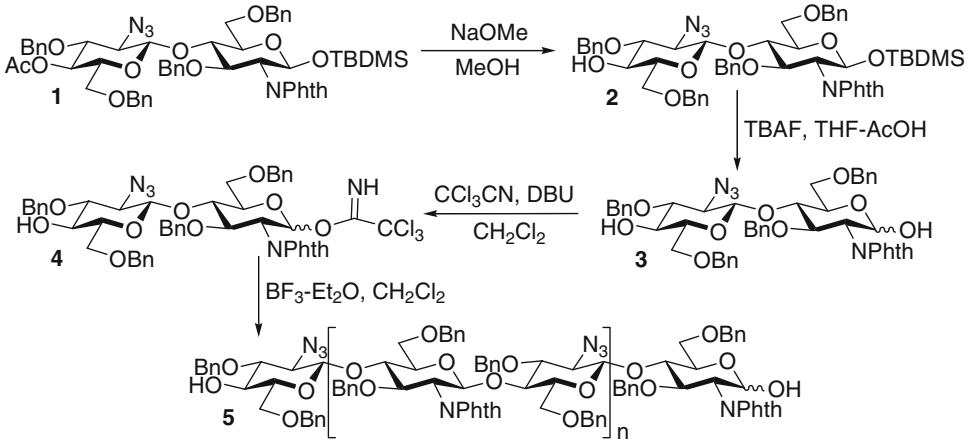
Scheme 1



conditions used for de-acetylation caused many side reactions leading to a dramatic decrease in yield.

It is indispensable to prepare a mono-imidate for conducting the polycondensation reaction. As shown in Scheme 1, disaccharide **3** was treated with trichloroacetonitrile (6.0 eq.) using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.2 eq.) [6] as a base for 10 min to give a mono-imidate **4** in 67% yield. An increase in reaction time led to the formation of significant amounts of 1,4′-di-imidate, albeit the yield of mono-imidate **4** was also slightly increased.

Mono-imidate **4** was polycondensed using boron trifluoride diethyl etherate (BF_3-Et_2O) as catalyst in anhydrous dichloromethane. After the reaction was finished within 5 min, the reaction mixture was neutralized by the addition of a saturated aqueous solution of sodium bicarbonate, washed with water and brine, and the solvent was evaporated to give polycondensed product **5**, which was used for chemical analysis without any purification.

The stereochemistry of the product **5** was determined by ^{13}C NMR. Signals derived from the newly generated glycosidic bonds appeared at $\delta = 97$ ppm, which indicates the expected β-configuration. The anomeric carbon at the reducing end gives a signal at $\delta = 93$ ppm, which suggests that even the free hydroxyl group is fixed in the β-configuration presumably affected by the neighboring phthaloyl group. The internal glycosidic bond neighboring with the azido group, which originally exists in the repeating disaccharide unit **1**, shows signals of around $\delta = 102$ ppm for the corresponding anomeric carbon atoms. The NMR studies show that the polycondensation reaction proceeded in a stereospecific manner, and the product **5** is a β-1,4-glucan.

The molecular weight was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS). A series of *chito*-oligosaccharide derivatives up to the dodecasaccharide derivative were detected. Each oligosaccharide gave major peaks corresponding to the molecular adduct with Na^+ $[M + Na]^+$, accompanied with minor peaks ionized with K^+ $[M + K]^+$. Other minor peaks correspond to products obtained by hydrogenation [M ($N_3 \rightarrow NH_2$)] of one or two azido group(s) to amino group(s) during MALDI-TOF-MS measurement.

GPC analysis shows that the numerical average of the degree of polymerization is 4.5, and the degree of polydispersion (Mw/Mn) is 1.96.

As mentioned above, the synthesis of chitosan derivatives containing two kinds of protecting groups at C-2 position in an alternate way was successfully achieved by polycondensation of the repeating disaccharide imidate **4**. Thus, "obverse and reverse" faces of the synthesized chitosan derivative could be distinguished due to phthalimido and azido groups at C-2; both could be easily converted into free amino and/or acetamido groups [3]. Benzyl groups also could be cleaved after suitable chemical processing at C-1, if appropriate.

Experimental

General methods

Nuclear magnetic resonance (NMR) spectra were measured with tetramethylsilane as an internal standard with a JEOL ARX-500. The assignments of the signals were determined using ^1H–^1H correlated spectroscopy and/or ^{13}C–^1H heteronuclear multiple-quantum correlation technique. Coupling constants (*J*) are given in Hz. MALDI-TOF MS spectra were recorded on Bruker Reflex III using 2,5-dihydroxy benzoic acid as a matrix. GPC analysis was done with an Asahipak GSM-700H. 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.

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 (**2**, $C_{54}H_{62}N_4O_{11}Si$)
To a solution of 426 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**, 0.25 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, and 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%) **2** as a colorless glass. $R_f = 0.43$ (EtOAc/*n*-hexane, 1:2, v/v); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = -0.11$ (s, 3H, Si*Me*), 0.04 (s, 3H, Si*Me*), 0.66 (s, 9H, *t*-Bu) 2.93 (d, 1H, $J_{4',OH} = 2.0$ Hz, 4′-O*H*), 3.19 (dd, 1H, $J_{2',3'} = 9.7$ Hz, $J_{3',4'} = 8.6$ Hz, H-3′), 3.24 (ddd, 1H, H-5′), 3.33 (dd, 1H, $J_{1',2'} = 8.1$ Hz, $J_{2',3'} = 9.7$ Hz, H-2′), 3.50 (dd, 1H, $J_{5',6'a} = 5.6$ Hz, $J_{6'a,6'b} = 10.1$ Hz, H-6′a), 3.61–3.68 (m, 3H, H-4′, H-6′b, H-5), 3.78 (dd, 1H, $J_{5,6a} = 1.5$ Hz, $J_{6a,6b} = 11.2$ Hz, H-6a), 3.98 (dd, 1H, $J_{5,6b} = 3.3$ Hz, $J_{6a,6b} = 11.2$ Hz, H-6b), 4.08–4.15 (m, 2H, H-2, H-4), 4.29 (dd, 1H, $J = 10.9$ Hz, $J = 8.4$ Hz, H-3), 4.43 (d, 1H, $J_{1',2'} = 8.1$ Hz, H-1′), 4.40–4.45 (m, 3H, CH_2Ph), 4.56 (d, 1H, CH_2Ph), 4.74 (2 × d, 2H, CH_2Ph), 4.85 (s, 2H, CH_2Ph), 5.32 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 6.81–6.86 (m, 3H, *Ph*), 6.95–6.98 (m, 2H, *Ph*), 7.18–7.43 (m, 15H, *Ph*), 7.58–7.79 (m, 4H, *Phth*) ppm; ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta = -5.4$ (Si*Me*), −4.1 (Si*Me*), 17.6 (Si*C*Me_3), 25.4 (*t*-Bu), 57.8 (C-2), 66.2 (C-2′), 68.1 (C-6), 70.8 (C-6′), 73.0 (C-5′), 73.37 (CH_2Ph), 73.44 (C-5 or C-4′), 73.7, 74.1 (2 × *C*H_2Ph), 74.8 (C-5 or C-4′), 75.1 (*C*H_2Ph), 76.5 (C-3), 78.0 (C-4), 82.6 (C-3′), 93.3 (C-1), 101.0 (C-1′), 123.0 (*Phth*), 126.8–128.4 (*Ph*), 131.5 (*Phth*), 133.5 (*Phth*), 137.3, 137.99, 138.03, 138.6 (3 × *Ph*) ppm.

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-glucopyranose (**3**, $C_{48}H_{48}N_4O_{11}$)
To a stirred solution of 1.7 g **2** (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 (TBAF, 1.0 M in THF, 3.2 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h, and then worked up. The residue was purified by flash column chromatography (EtOAc/toluene, 1:2, v/v) to yield 1.17 g (78%) **3** as colorless crystals. $R_f = 0.40$ (EtOAc/*n*-hexane, 1:1, v/v); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.95$ (d, 1H, $J_{4'OH} = 2.3$ Hz, 4′-O*H*), 3.12 (dd, 1H, $J_{2',3'} = 9.7$ Hz, $J_{3',4'} = 8.7$ Hz, H-3′), 3.17 (m, 1H, H-5′), 3.29 (dd, 1H, $J_{1',2'} = 8.1$ Hz, $J_{2',3'} = 9.7$ Hz, H-2′), 3.48 (dd, 1H, $J_{5',6'a} = 5.6$ Hz, $J_{6'a,6'b} = 10.1$ Hz, H-6′a), 3.53–3.66 (m, 3H, 1-O*H*, H-6′b, H-4′), 3.68 (ddd, 1H, $J_{4,5} = 9.9$ Hz, $J_{5,6a} = 1.5$ Hz, $J_{5,6b} = 3.3$ Hz, H-5), 3.81 (dd, 1H, $J_{5,6a} = 1.5$ Hz, $J_{6a,6b} = 10.9$ Hz, H-6a), 3.95 (dd, 1H, $J_{5,6b} = 3.3$ Hz, $J_{6a,6b} = 10.9$ Hz, H-6b), 4.04–4.11 (m, 2H, H-2, H-4), 4.29 (d, 1H, $J_{1',2'} = 8.1$ Hz, H-1′), 4.33 (dd, 1H, $J = 10.7$ Hz, $J = 8.4$ Hz, H-3), 4.36–4.49 (m, 4H, CH_2Ph), 4.71 (d, 1H, CH_2Ph), 4.75 (d, 1H, CH_2Ph), 4.83 (s, 2H, CH_2Ph), 5.32 (t, 1H, $J_{1,2} = 8.2$ Hz, $J_{1,OH} = 8.2$ Hz, H-1), 6.78–6.84 (m, 3H, *Ph*), 6.92–6.96 (m, 2H, *Ph*), 7.21–7.42 (m, 15H, *Ph*), 7.59–7.70 (m, 4H, *Phth*) ppm; ^{13}C NMR (67.8 MHz, $CDCl_3$): $\delta = 57.5$ (C-2), 66.1 (C-2′), 68.0 (C-6), 70.6 (C-6′), 73.0 (C-5′), 73.2 (C-4′), 73.5, 73.6, 74.4 (3 × *C*H_2Ph), 74.7 (C-5), 75.0 (*C*H_2Ph), 76.7 (C-3), 77.9 (C-4), 82.6 (C-3′), 92.8 (C-1), 100.9 (C-1′), 123.1 (*Phth*), 126.8–128.4 (*Ph*), 131.4 (*Phth*), 133.6 (*Phth*), 137.3, 137.6, 138.0, 138.4 (4 × *Ph*), 167.9 (*Phth*) ppm; MALDI-TOF-MS: $m/z = 895.32$ $[M + K]^+$, 879.35 $[M + Na]^+$, 869.35 $[M\ (N_3 \rightarrow NH_2) + K]^+$, 853.34 $[M\ (N_3 \rightarrow NH_2) + Na]^+$.

Trichloroacetimidoyl 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**, $C_{50}H_{48}Cl_3N_5O_{11}$)
To a solution of 366 mg **3** (0.43 mmol) in 18 cm^3 anhydrous CH_2Cl_2 13 mm^3 DBU (86 μmol) was added, and the solution was stirred for 30 min. Trichloroacetonitrile (357 mg, 2.6 mmol) was added and stirred for another 10 min at room temperature. The reaction mixture was directly applied on preparative TLC for purification (EtOAc/*n*-hexane, 1:2, v/v) to yield 289.6 mg (67%) **4** as colorless crystals. $R_f = 0.76$ (EtOAc/*n*-hexane, 1:2, v/v); ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 2.88$ (d, 1H, $J_{4'OH} = 2.1$ Hz, 4′-O*H*), 3.14 (dd, 1H, $J_{2',3'} = 9.7$ Hz, $J_{3',4'} = 8.7$ Hz, H-3′), 3.19 (m, 1H, H-5′), 3.32 (dd, 1H, $J_{1',2'} = 8.1$ Hz, $J_{2',3'} = 9.7$ Hz, H-2′), 3.51 (dd, 1H, $J_{5',6'a} = 5.6$ Hz, $J_{6'a,6'b} = 10.1$ Hz, H-6′a), 3.60–3.67 (m, 2H, H-4′, H-6′b), 3.84 (ddd, 1H, $J_{4,5} = 9.9$ Hz, $J_{5,6a} = 1.3$ Hz, $J_{5,6b} = 3.0$ Hz, H-5), 3.88 (dd, 1H, $J_{5,6a} = 1.3$ Hz, $J_{6a,6b} = 11.4$ Hz, H-6a), 4.03 (dd, 1H, $J_{5,6b} = 3.0$ Hz, $J_{6a,6b} = 11.4$ Hz, H-6b), 4.22 (dd, 1H, $J_{3,4} = 7.9$ Hz, $J_{4,5} = 9.9$ Hz, H-4), 4.34–4.54 (m, 7H, H-2, H-3, H-1′, CH_2Ph), 4.74–4.85 (m, 4H, CH_2Ph), 6.39 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 6.80–6.84 (m, 3H, *Ph*), 6.94–6.98 (m, 2H, *Ph*), 7.23–7.43 (m, 15H, *Ph*), 7.64–7.67 (m, 4H, *Phth*), 8.54 (s, 1H, N*H*) ppm.

[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-(1 →]$_n$ (**5**)

Carefully dried **4** (135 mg, 0.135 mmol) was dissolved in 10 cm^3 anhydrous CH_2Cl_2 and cooled to −78 °C. Then 4.9 mm^3 boron trifluoride diethyletherate (BF_3-Et_2O) (0.027 mmol) was added, and the reaction mixture was stirred for 5 min. Triethylamine (1 drop) was added for neutralizing the acidic catalyst and quenching the reaction. The reaction mixture was worked up, and the solvent was evaporated to obtain an oily syrup (107 mg). MALDI-TOF-MS: m/z = 5,070.92 $[M_{12} + Na]^+$, 4,248.57 $[M_{10} + K]^+$, 4,232.26 $[M_{10} + Na]^+$, 4,183.65 $[M_{10}$ $(2 \times N_3 \rightarrow 2 \times NH_2)]^+$, 3,410.25 $[M_8 + K]^+$, 3,394.28 $[M_8 + Na]^+$, 3,345.33 $[M_8$ $(N_3 \rightarrow NH_2)]^+$, 2,571.93 $[M_6 + K]^+$, 2,555.96 $[M_6 + Na]^+$, 2,507.01 $[M_6$ $(N_3 \rightarrow NH_2)]^+$, 853.34 $[M_2$ $(N_3 \rightarrow NH_2) + Na]^+$.

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