ELSEVIER

Carbohydrate Research 294 (1996) 53–64

CARBOHYDRATE RESEARCH

First synthesis of cellooctaose by a convergent synthetic method [1]

Takeshi Nishimura *, Fumiaki Nakatsubo

Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

Received 26 February 1996; accepted 23 July 1996

Abstract

The first chemical synthesis of cellooctaose by a convergent synthetic method is described. A challenging glycosylation between cellotetraosyl donor **5** and acceptor **7** proceeded in a one-step reaction using a high-vacuum system for anhydrous glycosylation and minimizing imidate side reactions such as hydrolysis and glycosyl fluoride formation. Pivaloyl, allyl, and benzyl protecting groups of cellooctaose derivative **8** were completely removed with SeO_2–AcOH, NaOMe–MeOH, and $H_2/Pd(OH)_2$–C, respectively. The acetylation after each deprotection step finally led to cellooctaose hexacosaacetate (**20**), which is useful for purification and structural identification. Finally, the acetyl derivative **20** was deacetylated with 1,8-diazabicyclo[5.4.0.]undec-7-ene (DBU) in 20% MeOH–CH_2Cl_2 to give pure cellooctaose (**21**). The analogous synthetic route to the present convergent synthetic design of cellooctaose may be a most promising one that enables us to synthesize cellulose with a defined degree of polymerization (dp). © 1996 Elsevier Science Ltd.

Keywords: Cello-oligosaccharide; Convergent synthetic method; High-vacuum system; Glycosyl fluoride; Glycosyl imidate; Glycosylation

1. Introduction

Higher cello-oligosaccharides, and also celluloses with a definite dp are considered to be very useful for research into physicochemical properties and their dependence on dp [1–4]. Now, such cello-oligosaccharides up to celloheptaose are obtainable by partial

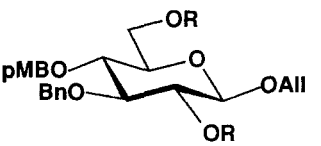
* Corresponding author. Present address: Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Norin Kenkyu Danchi-Nai, Ibaraki 305, Japan. Fax: +81-298-733797. E-mail: tnishi@ffpri.affrc.go.jp.

[1] Synthetic Studies of Cellulose, Part XIII; for Part XII: see ref. [8].

PII S0008-6215(96)00203-0

degradation of cellulose [4]. However, stepwise synthesis from glucose is considered to be one of the most suitable methods for preparing higher cello-oligosaccharides, which will afford much useful information on the unknown properties of these oligosaccharides.

As for synthetic studies for cello-oligosaccharides, cellotetraose was synthesized by Schmidt et al. [5] and Takeo et al. [6]. Recently, we succeeded in the first chemical synthesis of per-*O*-acetyl cellooctaose **20** from allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (**1**) by a linear synthetic method [7,8]. However, cellobiosyl or cellotetraosyl α-trichooroacetoimidate (glycosyl donor) with 2,3,6-tri-*O*-benzyl substituents derived from compound **1** was not obtained as a pure compound, as these imidoylation reactions always gave a mixture consisting of α- and β-anomers, and the separation of these highly reactive compounds was impossible because of their extreme instability on silica gel. For this reason, a convergent synthetic method starting from compound **1** was not successful.



1 R = Bn

2 R = Piv

Piv: $-COC(CH_3)_3$

Bn: $-CH_2C_6H_5$

pMB: $-CH_2C_6H_4OCH_3$

All: $-CH_2CHCH_2$

Then, allyl 3-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-2,6-di-*O*-pivaloyl-β-D-glucopyranoside (**2**) was selected as an alternative starting material for the convergent synthesis after considering substituent effects [9]. The suitable combination of acyl and benzyl groups brings the proper balance of the stability and reactivity of the imidate in glycosylation [10], and both 3-*O*-benzyl and 2-*O*-pivaloyl groups are known to be indispensable for highly stereoselective β-glycosylation [11].

In the previous paper, we described the following crucial points for the high-yield convergent synthesis of cellotetraose [12]: (1) The reactivity of the glycosyl donor and acceptor in glycosylation decreases with an increase of anhydroglucose repeating units. Actually, the cellobiose glycosyl donor and acceptor derived from compound **2** have lower reactivity than the monomeric compounds in glycosylation. (2) In the imidate method, the use of a larger amount of catalyst (BF_3 etherate), or application of higher temperature for getting higher reactivity of both glycosyl donor and acceptor gives rise to glycosyl fluoride formation or cleavage of the *p*-methoxybenzyl group of the glycosyl donor as a side reaction. (3) In order to prevent such side reactions on synthesizing tetraose derivative **3** in high yields, use of the acetyl group instead of the *p*-methoxybenzyl group as an O-4′ temporary protective group of the cellobiose derivative is effective.

In this paper, we report the high-yield β-glycosylation between cellotetraosyl glycosyl donor **5** and acceptor **7** to yield cellooctaose derivative **8**, and also the effectiveness

of the use of a high-vacuum system for more strictly controlled anhydrous reaction conditions.

2. Results and discussion

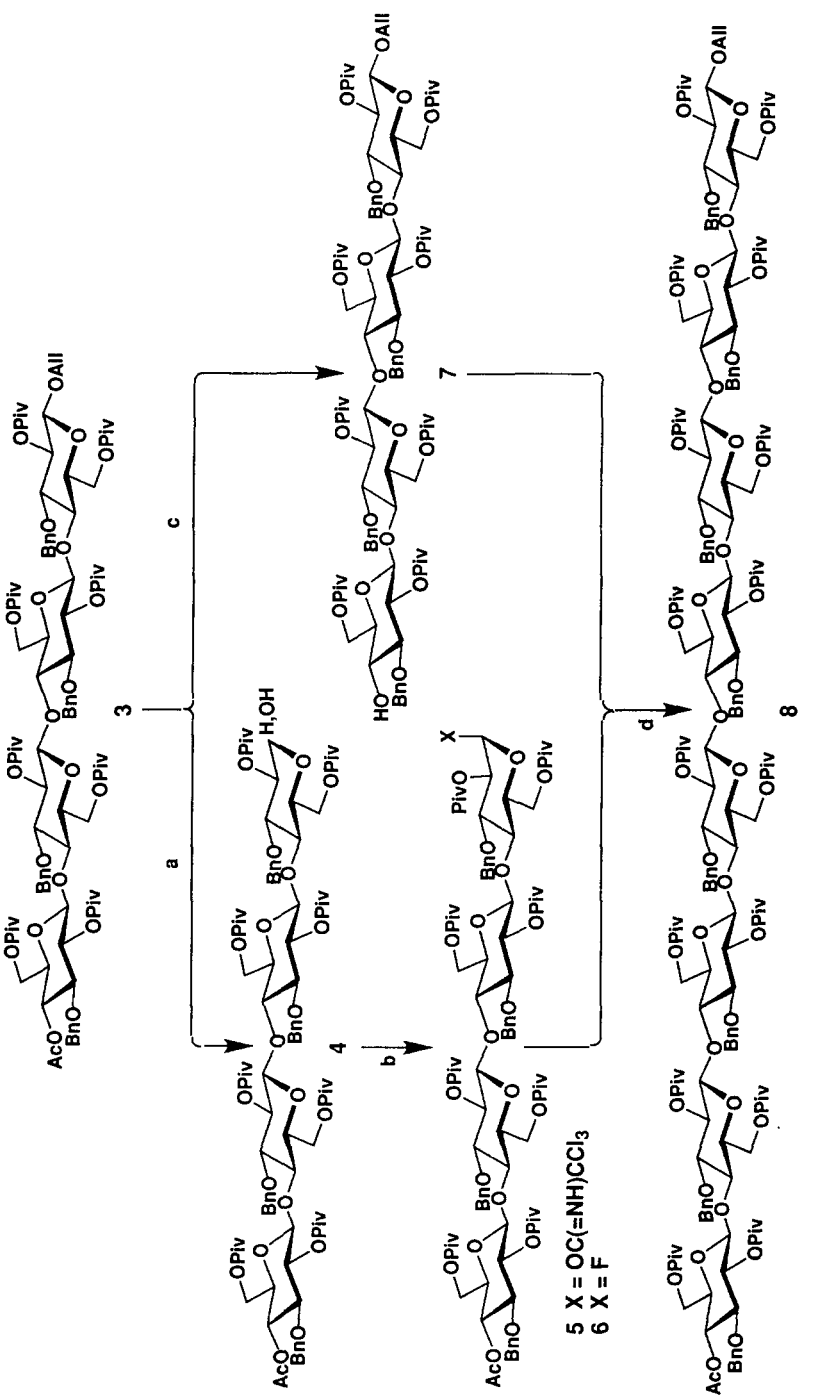
Deallylation of cellotetraose derivative **3** by selenium dioxide (SeO_2) oxidation gave **4** in 79% yield (see Scheme 1). The product was subsequently treated with trichloroacetonitrile (CCl_3CN) in the presence of DBU in dichloromethane (CH_2Cl_2) at room temperature to give the thermodynamically stable α-imidate **5** in a 96% yield. Under these reaction conditions using CH_2Cl_2 as solvent, the acetyl group does not cleave. Selective deacetylation of **3** in the presence of pivaloyl groups was carried out using DBU in MeOH at room temperature [13] to afford glycosyl acceptor **7** in a 65% yield.

Glycosylation of glycosyl acceptor **7** with glycosyl donor **5** did not proceed under the reaction conditions used for tetramerization [12], i.e., 0.05 mol equiv of BF_3–etherate (based on glycosyl donor) at -15 °C in anhydrous CH_2Cl_2. Then, the glycosylation under the more drastic reaction conditions (0.15 mol equiv of BF_3–etherate at 0 °C for 12 h) was tried, and the cellooctaose derivative **8** was obtained only in a 26% yield. Due to the fact that the glycosyl donor **5** was mainly converted into the hydrolysis product **4** in 30% yield, and into cellobiosyl α-fluoride **6** in about 20% yield, as judged by TLC. The latter product was identified by its characteristic anomeric peak appearing at δ 5.58 ppm (dd, 1 H, $J_{1,2}$ 2.5 Hz, $J_{1,F}$ 54.0 Hz, H-1) in the ^{1}H NMR spectrum. This product appeared even if a simple vacuum system previously reported was used [9]. Thus, it was evident that the yield must be increased by complete removal of water from the reaction system.

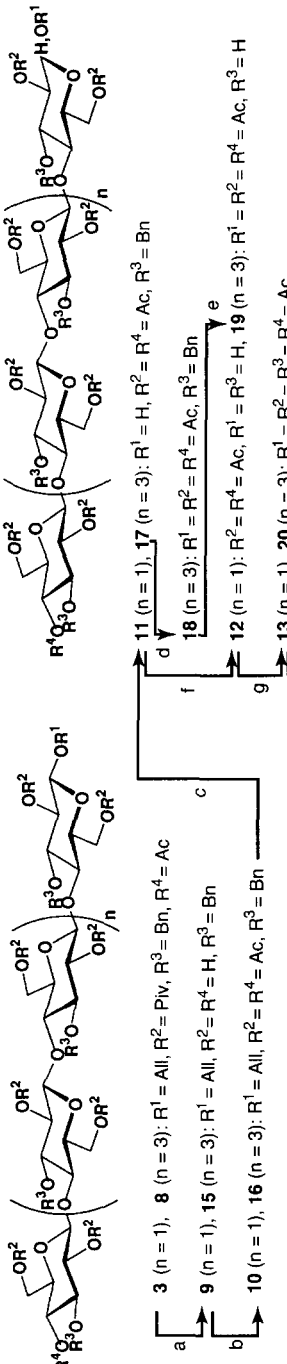
The use of 4 Å molecular sieves was not effective in this case, but the use of a high-vacuum system capable of maintaining vacuum below 1×10^{-3} Torr (< 0.13 Pa) shown in Fig. 1 was found to be useful. After removing water several times from reaction vessel A containing starting materials (as an azetropic mixture with toluene that had been distilled from NaH), the starting materials **5** (2.0 mol equiv) and **7** (1.0 mol equiv) were dissolved in CH_2Cl_2 that had been distilled from P_2O_5 or CaH_2. The reaction vessel was then removed from the system by melting off at the position marked 'B', and 0.10 mol equiv of freshly distilled BF_3–etherate was added at 0 °C. Under such reaction conditions, the formation of byproducts **4** and **6** was greatly depressed, and the expected cellooctaose derivative **8** was obtained in a 95% yield based on the glycosyl acceptor **7**. The excess amount of glycosyl donor was almost quantitatively recovered as the hydrolysis product **4**, which was recycled as the glycosyl donor after trichloroacetimidoylation.

The structure of **8** was confirmed by ^{1}H and ^{13}C NMR, and by FABMS spectrometric analyses. These data may be useful for the structural characterization of the higher oligosaccharide and polysaccharide derivatives of this series.

The deprotection of both cellotetraose derivative **3** and cellooctaose derivative **8**, resulting in cellotetraose and cellooctaose, respectively, were carried out as follows (see Scheme 2): Compound **3** was depivaloylated with NaOMe in MeOH under reflux to afford **9** in a quantitative yield, and the product was subsequently acetylated with



Scheme 1. Reagents and conditions: (a) SeO_2, AcOH, dioxane, 80 °C, 79%; (b) DBU, CCl_3CN, CH_2Cl_2, r.t., 96%; (c) DBU, CH_3OH, r.t., 65%; (d) BF_3–Et_2O, CH_2Cl_2, 0 °C, 95% (based on **7**).



Scheme 2. Reagents and conditions: (a) NaOMe–MeOH, reflux; (b) Ac_2O, Py, 50 °C; (c) SeO_2, AcOH, dioxane, 80 °C; (d) Ac_2O, Py, 50 °C; (e) $Pd(OH)_2$–C, H_2, THF, r.t.; (f) **11** ← **13**: Pd–C, H_2, 4:1 EtOH–AcOH, 50 °C; (g) Ac_2O, Py, 50 °C; (h) 2:8 MeOH–CH_2Cl_2, DBU, r.t.

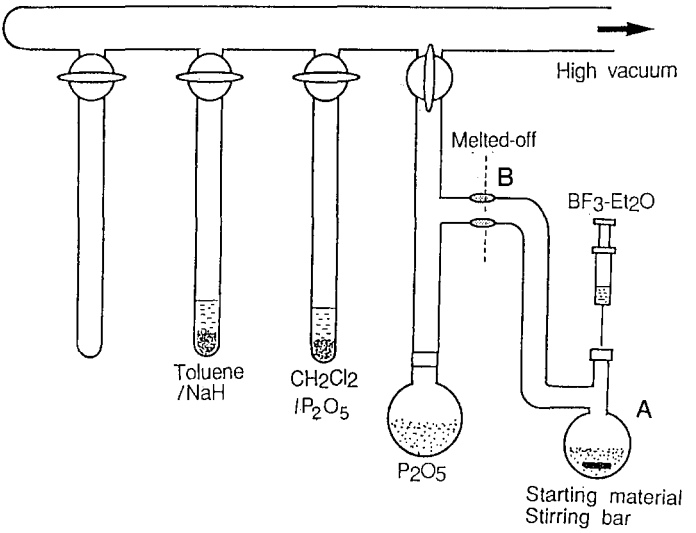


Fig. 1. High-vacuum system for conducting glycosylation reactions under anhydrous conditions.

Ac_2O–Py to give **10** in an 82% yield. An allyl group of 10 was then removed by SeO_2 oxidation to give **11** in a 54% yield. Catalytic hydorogenation of **11** using 10% Pd–C/H_2 in ~4:1 EtOH–AcOH (v/v) [14] afforded **12** in a 92% yield. Compound **12** was subsequently acetylated with Ac_2O–Py to afford cellotetraose hexacosaacetate **13** in a 72% yield. Both ^1H and ^{13}C NMR data of **13** were identical with those obtained on a product of the acetolysis of cellulose, except for the α/β-anomeric ratio [3], which was calculated to be 2:1 from their peak areas of the anomeric proton signals appearing at δ 6.23 (J 3.5 Hz) and 5.63 (J 8.0 Hz). The ~1:1 anomeric ratio of the compound **13** was also supported by the $[\alpha]_D$ value of +1.08°, as the values for the α- and β-anomers have been reported to be +13° and −18°, respectively [1]. Finally, deacetylation of **13** with DBU in 20:8 MeOH–CH_2Cl_2 afforded cellotetraose **14** as a colorless powder in a 75% yield, which was crystallized from 95% EtOH to yield colorless needles (mp 250–251 °C dec, lit mp 251–253 °C dec [6], 252–253 °C dec [15]). The structure of **14** was also confirmed by comparison of its ^{13}C NMR data in D_2O with those reported for cellotetraose [16].

The conversion of **8** into cellooctaose **21** was carried out by the procedure described above for those of **3**, except for the debenzylation. After **17** was acetylated to **18**, **18** was debenzylated by catalytic hydorogenation using $Pd(OH)_2$–C/H_2 in anhydrous tetrahydrofuran (THF) at room temperature. The product was subsequently acetylated to yield cellooctaose hexacosaacetate **20** consisting of a 2:1 α/β-anomeric mixture. ^1H and ^{13}C NMR data of the synthetic acetate **20** were identical in all respects to those obtained by the acetolysis product of cellulose [3] and by our linear synthesis [8]. Finally, deacetylation of **20** with DBU in 20:80 MeOH–CH_2Cl_2 afforded cellooctaose **21** as a colorless powder in a 87% yield. Interestingly, the ^{13}C NMR spectrum of **21** in Me_2SO-d_6 closely resembled those of low-dp cellulose with small dispersion [17] as shown in Fig. 2. The

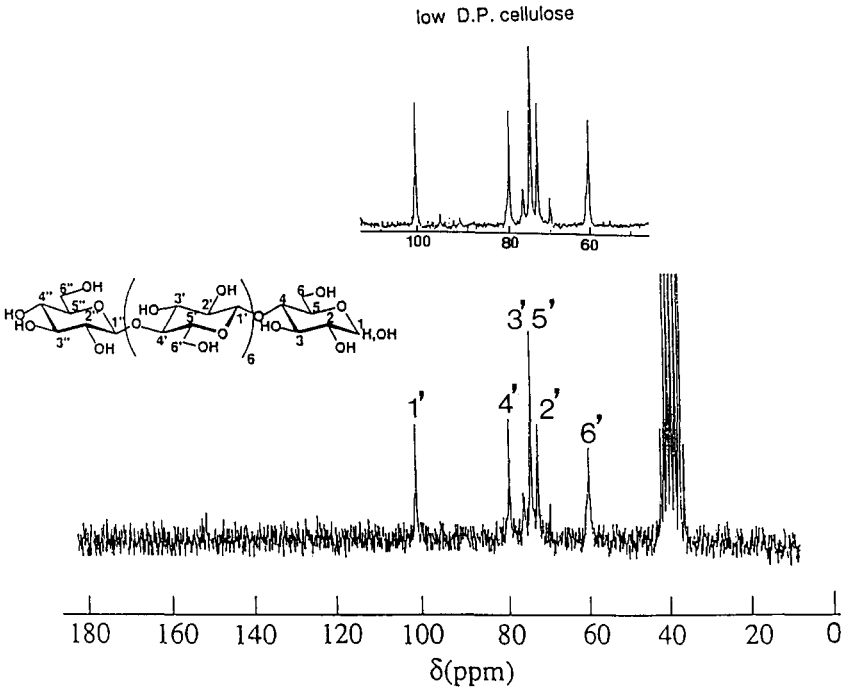


Fig. 2. ^{13}C NMR spectrum of synthesized cyclooctaose **21**.

cellooctaose thus obtained dissolved only in Me_2SO, and crystallization from Me_2SO–H_2O gave rise to gelation after cooling, without giving crystals.

Thus, we succeeded for the first time in synthesizing of cellooctaose by a convergent synthetic method. The synthetic route established here based on convergent synthetic design is a most promising one that may enable us to synthesize cellulose with defined dp [18]. Further elongation of the cello-oligosaccharide chain starting from **8** and **5** is thought to be possible, a topic that will be the subject of a subsequent paper.

3. Experimental

General.—Melting points are uncorrected. ^1H NMR and ^{13}C NMR spectra were taken with a Varian XL-200 (200 MHz), a JEOL FX-90Q (22.5 Hz), respectively, with Me_4Si as an internal standard. 2D COSY spectra of compound **8** were taken with a JEOL ALPHA-500 FTNMR (500 MHz) spectrometer, with Me_4Si as an internal standard. Chemical shifts and coupling constants are given in δ-values and Hz, respectively. Optical rotation was measured using a JASCO Dip-4 digital polarimeter in $CHCl_3$. Preparative TLC was done on silica gel plates (Kiesgel 60 F254, E. Merck). The mass spectrum was obtained on a JEOL JMS-DX303 HF mass spectrometer and JMA-DA500 mass data system equipped with an FAB-gun operated at 2 keV. Anhy-

drous CH_2Cl_2 was distilled from P_2O_5 or CaH_2. The standard workup included diluting with EtOAc, washing with aq $NaHCO_3$ and brine, drying over Na_2SO4, and evaporating in vacuo. Cerium(IV) ammonium nitrate (CAN), selenium dioxaide (SeO_2), 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU), trichloroacetonitrile (CCl_3CN), boron trifluoride–diethyl ether complex (BF_3–etherate) were purchased from Nacalai tesque Co., Ltd. Palladium(II) hydroxide-on-carbon [$Pd(OH)_2$] was purchased from Aldrich Chemical Company, Inc.

4-O-Acetyl-3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranosyl)-(1 → 4)-(3-O-benzyl-2,6-di-O-pivaloyl)$_2$-(1 → 4)-3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranose (**4**).—To a stirred solution of **3** (292 mg, 0.163 mmol) in dioxane (15 mL), were added SeO_2 (27.7 mg, 0.25 mmol) and AcOH (14.1 μL, 0.25 mmol). The solution was stirred for 26 h at 80 °C and worked up by the standard method. The product was purified by preparative TLC (4:1 hexane–EtOAc) to afford **4**, consisting of an epimeric mixture as a colorless syrup (225 mg, 79%); $[\alpha]_D^{28}$ +4.6° (*c* 6.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$): signals assigned to the allyl group disappeared, δ 1.01–1.17 (72 H, CH_3), 1.88 (s, 3 H, CH_3), 7.06–7.41 (m, 20 H, Ar H). Anal. Calcd for $C_{94}H_{132}O_{30}$: C, 64.81; H, 7.64. Found: C, 64.21; H, 7.58.

4-O-Acetyl-3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranosyl)-(1 → 4)-3-O-benzyl-2,6-di-O-pivaloyl-α-D-glucopyranosyl trichloroacetimidate (**5**).—To a stirred solution of **4** (104 mg, 60 μmol) in anhydrous CH_2Cl_2 (5 mL), were added DBU (9 μL, 0.060 mmol) and CCl_3CN (72 μL, 0.72 mmol). The solution was stirred for 7 h at room temperature, and then directly applied on alumina column (ICN Alumina B, activity I) eluted with CH_2Cl_2. The eluent was concentrated in vacuo below 25 °C. The residue was purified by column chromatography on silica gel (4:1 hexane–EtOAc) to afford **5** as a colorless syrup (108.5 mg, 96%); $[\alpha]_D^{35}$ +18° (*c* 6.5, $CHCl_3$); ^{1}H NMR ($CLCl_3$): δ 1.03, 1.05, 1.06, 1.07, 1.09, 1.11, 1.16, 1.20 [72 H, $C(CH_3)_3$], 1.89 (s, 3 H, CH_3), 6.40 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 7.16–7.44 (m, 20 H, Ar H), 8.59 (s, 1 H, NH). Anal. Calcd for $C_{94}H_{132}O_{30}$: C, 61.12; H, 7.05. Found: C, 60.84; H, 7.22.

Allyl 3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranosyl)-(1 → 4)-(3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranosyl)$_2$-(1 → 4)-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranoside (**7**).—To a stirred solution of **3** (351 mg, 0.197 mmol) in MeOH (50 mL), was added DBU (211 μL, 1.41 mmol). The solution was stirred for 2 h at room temperature and then diluted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The product was purified by preparative TLC (3:1 hexane–EtOAc) to afford **7** as a colorless syrup (224 mg, 65%); $[\alpha]_D^{35}$ −8.4° (*c* 4.83, $CHCl_3$); ^{1}H NMR ($CHCl_3$): Signals of methyl proton at 1.89 ppm disappeared, δ 1.05–1.20 [72 H, $C(CH_3)_3$], 7.06–7.45 (m, 20 H, Ar H). Anal. Calcd for $C_{95}H_{134}O_{29}$: C, 65.57; H, 7.76. Found: C, 65.00; H, 7.86.

Allyl 4-O-acetyl-3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranosyl)-(1 → 4)-(3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranosyl)$_6$-(1 → 4)-3-O-benzyl-2,6-di-O-pivaloyl-β-D-glucopyranoside (**8**).—The high-vacuum system for the anhydrous glycosylation reaction is shown in Fig. 1. A reaction vessel with glycosyl donor **5** (50 mg, 27 μmol), glycosyl acceptor **7** (23 mg, 13 μmol), and a magnetic stirring bar (A) was connected to the system, and the pressure was reduced below 10^{-3} Torr (< 0.13 Pa). Toluene (500 μL, distilled from NaH) was transferred to the reaction vessel, and the samples were

dissolved. By transferring the toluene to the initial reaction vessel repeatedly, water contained in these samples was removed as an azetropic mixture. The sample was further dried under high vacuum for 10 h. Dichloromethane (500 μL) dried over P_2O_5 or CaH_2 was degassed by freezing and thawing three times. The solvent was transferred to the reaction vessel, which was then sealed and melted off at constriction B and subsequently cooled to 0 °C. To a stirred solution was added the solution of BF_3–etherate (0.33 μL, 2.7 μmol) in anhydrous CH_2Cl_2 (10 μL) through a rubber septum by a syringe. After stirring for 12 h at the same temperature, the reaction mixture was worked up by the standard method. The product was purified by preparative TLC (4:1 hexane–EtOAc, developed three times) to give **8** (44 mg, 95% yield based on **7**; 47% yield based on **5**) as a colorless syrup; $[\alpha]_D^{28}$ −11.08° (*c* 1.90, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.00, 1.03, 1.04, 1.05, 1.06, 1.07, 1.09, 1.14, 1.16, 1.20, [144 H, $C(CH_3)_3$], 1.89 (s, 3 H, CH_3), 3.35 (m, H-5′), 3.47 (H-3′), 3.64 (H-4′), 3.83 (H-6′), 4.09 (H-6′), 4.28 (H-1′), 4.44 (PhCH_2), 4.84 (H-2′), 4.97 (PhCH_2), 5.15 [dd, 1 H, $J_{H,H}$ 16 and 1.5 Hz, –CH=CH_2 (trans)], 5.20 [dd, 1 H, $J_{H,H}$ 9.5 Hz and 1.5 Hz, –CH=CH_2 (cis)], 5.79 (m, 1 H, –C*H*=CH_2), 7.08–7.26 (m, 40 H, Ar H); ^{13}C NMR ($CDCl_3$): δ 20.7 (CH_3), 27.1, 27.2 [$C(CH_3)_3$], 38.7 [C–$C(CH_3)_3$], 62.5 (C-6′), 72.4 (C-2′), 73.4 (C-5′), 74.8 (Ph*C*H_2), 76.6 (C-4), 80.8 (C-3′), 100.3 (C-1′), 126.8, 127.1, 128.1, 138.7 (Ar C), 169.2 (C=O), 176.3 (C=O), 177.6 (C=O), 177.7 (C=O); FABMS: *m/z* 3504 $[M + K]^+$. Anal. Calcd for $C_{189}H_{264}O_{58}$: C, 65.53; H, 7.68. Found: C, 65.03; H, 7.66.

Allyl 3-O-benzyl-β-D-glucopyranosyl-(1 → 4)-(3-O-benzyl-β-D-glucopyranosyl)$_2$-(1 → 4)-3-O-benzyl-β-D-glucopyranoside (**9**).—To a stirred solution of **3** (276 mg, 0.15 mmol) in methanol (30 mL) was added 28% NaOMe–MeOH (354 μL, 6.2 mmol). The solution was stirred under reflux for two days, neutralized with N HCl, and worked up by the standard method to afford **9** (160 mg, 100%), which gave one spot on TLC (1:9 MeOH–CH_2Cl_2). The product was used for the subsequent step without further purification; $[\alpha]_D^{25}$ −9.38° (*c* 5.33, $CHCl_3$).

Allyl 2,4,6-tri-O-acetyl-3-O-benzyl-β-D-glucopyranosyl-(1 → 4)-(2,6-di-O-acetyl-3-O-acetyl-3-O-benzyl-β-D-glucopyranosyl)$_2$-(1 → 4)-2,6-di-O-acetyl-3-O-benzyl-β-D-glucopyranoside (**10**).—A solution of crude **9** (160 mg, 0.15 mmol) in acetic anhydride (5 mL) and pyridine (5 mL) was stirred for 2 h at r.t. and concentrated in vacuo. The product was purified by preparative TLC (1:1 hexane–EtOAc) to afford **10** as a colorless syrup (177 mg, 82%); $[\alpha]_D^{25}$ +10.02° (*c* 3.39, $CHCl_3$). The product was used directly in the next step.

2,4,6-Tri-O-acetyl-3-O-benzyl-β-D-glucopyranosyl-(1 → 4)-(2,6-di-O-acetyl-3-O-benzyl-β-D-glucopyranosyl)$_2$-(1 → 4)-2,6-di-O-acetyl-3-O-benzyl-β-D-glucopyranose (**11**).—To a stirred solution of **10** (67 mg, 46 μmol) in dioxane (2 mL), was added SeO_2 (7.8 mg, 0.070 mmol) and AcOH (4 μL, 0.070 mmol). The solution was stirred for 16 h at 80 °C and worked up by the standard method. The product was purified by preparative TLC (1:1 hexane–EtOAc) to afford **11** as colorless syrup (35 mg, 54%), $[\alpha]_D^{25}$ +29.9° (*c* 4.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.83–2.12 (9 s, 27 H, CH_3), 7.10–7.45 (m, 20 H, Ar H).

2,4,6-Tri-O-acetyl-β-D-glucopyranosyl-(1 → 4)-(2,6-di-O-acetyl-β-D-glucopyranosyl)$_2$-(1 → 4)-2,6-di-O-acetyl-β-D-glucopyranose (**12**).—A mixture of **11** (48 mg, 33 μmol) and 10% Pd–C (120 mg) in 10:1 EtOH–AcOH (v/v, 7.7 mL) was

stirred under hydrogen at 50 °C for 5 h, then filtered and concentrated in vacuo to afford crude **12** as a colorless syrup (31.8 mg, 92%). The product was used for the subsequent reaction without further purification; $[\alpha]_D^{25}$ −3.77° (*c* 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.05–2.20 (27 H, CH_3), 5.38 (d, 1 H, $J_{H,H}$ 3.5 Hz, H-1).

Tetradeca-O-acetylcellotetraose (**13**).—A solution of crude **12** (31.8 mg, 33 μmol) in pyridine (2 mL) and acetic anhydride (2 mL) was stirred at room temperature for 18 h and concentrated in vacuo. The residue was purified by column chromatography on silica gel (1:9 MeOH–CH_2Cl_2) to afford **13** (2:1 α- and β-anomeric mixture) as a colorless syrup (27.6 mg, 72%), $[\alpha]_D^{25}$ +1.08 (*c* 0.92, $CHCl_3$); lit. +12.8° for the α-anomer; −18° for the β-anomer [1], ^{1}H NMR ($CDCl_3$): δ 1.94–2.10 (42 H, CH_3), 3.48–3.70 (3 H, H-5′, H-5″), 3.72 (broad t, 3 H, $J_{3,4}$ 9.5 Hz, H-4, H-4′), 3.96–4.20 (5 H, H-5, H-6, H-6′, H-6″), 4.34–4.66 (7 H, H-1′, H-1″, H-6, H-6′, H-6″), 4.80–5.00 (4 H, H-2, H-2′, H-2″), 5.00–5.24 (4 H, H-3′, H-3″, H-4″), 5.41 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 5.63 [d, $J_{1,2}$ 8.0 Hz, H-1(β)], 6.23 [d, $J_{1,2}$ 3.5 Hz, H-1(α)]; ^{13}C NMR (22.5 MHz, $CDCl_3$): δ 20.5, 20.8 (CH_3), 61.6, 62.1 (C-6, C-6′, C-6″), 67.9 (C-4″), 69.4 (C-2, C-3), 70.8 (C-5), 71.7 (C-2″), 71.9 (C-2′), 72.1 (C-5″), 72.7 (C-3′, C-3″), 72.9 (C-5′), 76.2 (C-4, C-4′), 89.0 [C-1(α)], 91.7 [C-1(β)], 100.5 (C-1′), 100.8 (C-1′), 168.8, 169.0, 169.2, 169.7, 170.1, 170.4 (C=O).

Cellotetraose (**14**).—To a stirred solution of compound **13** (8.7 mg, 7 μmol) in 2:8 MeOH–CH_2Cl_2 (1 mL), was added DBU (43 μL, 0.29 mmol). The solution was stirred for two days at r.t., and cellotetraose **14** precipitated as a white powder. The precipitate was centrifuged at 3000 rpm for 5 min, and the supernatant solution was removed. The white powder thus obtained was further washed twice with hexane (1 mL) to remove completely DBU. Cellotetraose **14** was thus obtained (3.5 mg, 75% yield): mp 250–251 °C dec.; ^{13}C NMR (D_2O), chemical shifts in D_2O were expressed by setting C-1″ atom equal to the value observed in cellobiose (see ref. [16]): δ 60.9 (C-6, C-6′), 61.5 (C-6″), 70.4 (C-4″), 70.9 [C-5(α)], 72.2 [C-2(α), C-3(α)], 73.9 (C-2′), 74.1 (C-2″), 75.0 [C-2(β), C-3(β), C-3′], 75.7 [C-5(β), C-5], 76.4 (C-3″), 76.9 (C-5″), 79.4 (C-4, C-4′), 92.6 [C-1(α)], 96.7 [C-1(β)], 103.2 (C-1′), 103.4 (C-1″); (Me_2SO-d_6), 60.2 (C-6, C-6′, C-6″), 69.9 [C-4″, C-2, C-3(α)], 72.8 (C-2′), 73.1 (C-2′), 74.6 [C-3(β), C-3′, C-5′], 76.3 (C-3″), 76.6 (C-5″), 80.0 (C-4, C-4′), 91.8 [C-1(α)], 96.5 [C-1(β)], 102.6 (C-1′), 103.0 (C-1″). Anal. Calcd for $C_{24}H_{42}O_{21}$: C, 43.24; H, 6.35. Found: C, 42.53; H, 6.83.

Allyl 3-O-benzyl-β-D-glucopyranosyl-(1 → 4)-(3-O-benzyl-β-D-glucopyranosyl)$_6$-(1 → 4)-3-O-benzyl-β-D-glucopyranoside (**15**).—To a stirred solution of **8** (52 mg, 15 μmol) in methanol (3 mL) was added 28% NaOMe–MeOH (100 μL). The solution was stirred under reflex for 18 h. The mixture was treated with excess of Amberlyst-15 resin, filtered, and concentrated in vacuo. The product was purified by preparative TLC (5:95 MeOH–CH_2Cl_2) to afford **15** as a colorless syrup (24.2 mg, 78%); $[\alpha]_D^{31}$ +28.6° (*c* 1.47, $CHCl_3$).

Allyl 2,4,6-tri-O-acetyl-3-O-benzyl-β-D-glucopyranosyl-(1 → 4)-(2,6-di-O-acetyl-3-O-benzyl-β-D-glucopyranosyl)$_6$-(1 → 4)-2,6-di-O-acetyl-3-O-benzyl-β-D-glucopyranoside (**16**).—A solution of **15** (24.2 mg, 11.7 μmol) in acetic anhydride (1 mL) and pyridine (1 mL) was stirred at 50 °C for 15 min and diluted with EtOAc. The solution was successively washed with N HCl, aq $NaHCO_3$ and brine, and concentrated in vacuo. The product was purified by column chromatography on silica gel (5:95 MeOH–CH_2Cl_2) to

afford **16** as a colorless syrup (33.3 mg, 100%); ^{1}H NMR ($CDCl_3$): signals assigned to pivaloyl groups disappeared; δ 1.89–1.98 (48 H, CH_3), 2.10 (s, 3 H, CH_3), 5.15 [dd, 1 H, $J_{H,H}$ 1.5 and 8.5 Hz, –CH=CH_2 (cis)], 5.21 [dd,1 H, $J_{H,H}$ 1.5 and 15.5 Hz, –CH=CH_2 (trans)], 5.80 (m, 1 H, –CH=CH_2), 7.14–7.50 (m, 40 H, Ar H).

*2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-β-*D*-glucopyranosyl-(1 → 4)-(2,6-di-*O*-acetyl-3-*O*-benzyl-β-*D*-glucopyranosyl)$_6$-(1 → 4)-2,6-di-*O*-acetyl-3-*O*-benzyl-β-*D*-glucopyranose* (**17**).—To a stirred solution of **16** (33.3 mg, 12 μmol) in dioxane (3 mL) were added SeO_2 (2 mg, 0.018 μmol) and AcOH (1.03 μL, 0.018 mmol). The solution was stirred at 80 °C for two days and worked up by the standard method. The product was purified by preparative TLC (5:95 MeOH–CH_2Cl_2) to afford **17** as colorless syrup (25.4 mg, 77%); $[\alpha]_D^{27}$ +27.2° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): signals assigned to the allyl group disappeared; δ 1.76–1.95 (48 H, CH_3), 2.08 (s, 3 H, CH_3), 7.10–7.44 (m, 40 H, Ar H).

*2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-β-*D*-glucopyranosyl-(1 → 4)-(2,6-di-*O*-acetyl-3-*O*-benzyl-β-*D*-glucopyranosyl)$_6$-(1 → 4)-1,2,6-tri-*O*-acetyl-3-*O*-benzyl-β-*D*-glucopyranose* (**18**).—A solution of **17** (13 mg, 4.8 μmol) in acetic anhydride (1 mL) and pyridine (mL) was stirred at 50 °C for 45 min and diluted with EtOAc. The solution was successively washed with N HCl, aq $NaHCO_3$ and brine, and concentrated in vacuo. The water was removed as an ethanol azetrope. The product was purified by column chromatography on silica gel (5:95 MeOH–CH_2Cl_2) to afford **18** as a colorless syrup (13.7 mg, 100%); $[\alpha]_D^{21}$ +17.6° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.76–2.16 (54 H, CH_3), 5.52 [d, 0.3 H, $J_{H,H}$ 8.0 Hz, H-1 (β-anomer)], 6.18 [d, 0.7 H, $J_{H,H}$ 4.0 Hz, H-1 (α-anomer)], 7.10–7.44 (m, 40 H, Ar H).

*Hexacosa-*O*-acetylcellooctaose* (**20**).—A mixture of **18** (16 mg, 5.76 μmol) and $Pd(OH)_2$–C (10 mg) in anhydrous THF (1 mL) was stirred under hydrogen at room temperature for 8 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo to afford rude **19**, which was subsequently acetylated (Ac_2O–Py) and further purified by column chromatography on silica gel (CH_2Cl_2 to 2:8 MeOH–CH_2Cl_2) to afford pure product **20** as a colorless syrup (8.9 mg, 64%); $[\alpha]_D^{25}$ −2.25° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.80–2.10 (78 H, CH_3), 3.40–3.80 (14 H, H-4, H-4′, H-5′, H-5″), 3.85–4.10 (9 H, H-6, H-6′, H-6″, H-5), 4.26–4.50 (15 H, H-1′, H-1″, H-6, H-6′, H-6″), 4.68–4.92 (7 H, H-2′, H-2″), 4.94–5.20 (9 H, H-2, H-3′, H-3″, H-4″), 5.41 (1 H, H-3), 5.63 [d, 0.3 H, $J_{1,2}$ 8.0 Hz, H-1(β)], 6.23 [(d, 0.7, H, $J_{1,2}$ 4.0 Hz, H-1(α)]; ^{13}C NMR ($CDCl_3$): δ 20.2–20.7 (CH_3), 61.9, 62.3 (C-6, C-6′, C-6″), 67.9 (C-4″), 69.4 (C-2, C-3), 70.8 (C-5), 72.0 (C-2′, C-2″, C-5″), 72.7 (C-3′), 72.9 (C-5′, C-3″), 76.1 (C-4, C-4′), 89.0 [C-1(α)], 91.9 [C-1(β)], 100.5, 100.8 (C-1′, C-1″), 168.9–170.7 (C=O).

Cellooctaose (**21**).—To a stirred solution of compound **20** (10.5 mg, 4.36 μmol) in 2:8 MeOH–CH_2Cl_2 (1 mL), was added DBU (51 μL, 0.34 mmol). The solution was stirred for two days at r.t., and cellooctaose 21 precipitated as a white powder. The precipitate was centrifuged at 3000 rpm for 5 min, and the supernatant solution was removed. The resulting white powder was further washed twice with hexane (1 mL) to completely remove DBU. Cellooctaose **21** was thus obtained (5.0 mg, 87% yield); ^{13}C NMR (Me_2SO-d_6): δ 60.1 (C-6, C-6′, C-6″), 69.8 (C-4″), 72.8, 73.1 (C-2′, C-2″), 74.6 (C-2, C-3′, C-5, C-5′), 76.2 (C-3″), 76.6 (C-5″), 79.9 (C-4, C-4′), 96.6 [C-1(α)], 102.5

(C-1′, C-1″). Anal. Calcd for $C_{48}H_{82}O_{41}$: C, 43.84; H, 6.28. Found (after correction for a water content of 2.5%): C, 43.74; H, 6.29.

Acknowledgements

The authors thank Dr. James L. Davis of the University of Wisconsin Madison, for his critical reading of the manuscript. The authors thank Dr. T. Umezawa of Wood Research Institute, Kyoto University, for the FABMS measurement. This investigation was supported in part by the Scientific Research Fund of the Ministry of Education Science and Culture of Japan (No. 074566080).

References

[1] T. Mizuno, *Denpun-Tou-Gikenho*, 37 (1968) 66–79.
[2] B. Henrisaat, P. Serge, I. Ivaska, and W.T. Winter, *ACS Symp. Ser.*, 340 (1987) 38–67; K. Okuyama and K. Nogichi, *Kobunshi*, 43 (1994) 848–858.
[3] C.M. Buchanann, J.A. Hyatt, S.S. Kelley, and J.L. Little, *Macromolecules*, 23 (1990) 3747–3755.
[4] G.L. Miller, J. Dean, and R. Blum, *Arch. Biochem. Biophys.*, 91 (1960) 21–26.
[5] R.R. Schmidt and J. Michael, *Angew. Chem. Int. Ed. Engl.*, 21 (1982) 72–73.
[6] K. Takeo, K. Okushio, K. Fukuyama, and T. Kuge, *Carbohydr. Res.*, 21 (1983) 163–173.
[7] T. Kawada, F. Nakatsubo, and K. Murakami, *Cellulose Chem. Technol.*, 24 (1990) 343–350.
[8] T. Kawada, F. Nakatsubo, and K. Murakami, *Mokuzai Gakkaishi*, 41 (1994) 738–743.
[9] T. Nishimura, T. Takano, F. Nakatsubo, and K. Murakami, *Mokuzai Gakkaishi*, 39 (1993) 40–47.
[10] H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 21 (1982) 155–173; T. Takano, Y. Harada, F. Nakatsubo, and K. Murakami, *Cellulose Chem. Technol.*, 24 (1990) 333–342.
[11] P. Sinaÿ, *Pure Appl. Chem.*, 50 (1978) 1437–1452; H. Kunz and A. Harreus, *Liebigs Ann. Chem.*, (1982) 41–48; T. Takano, F. Nakatsubo, and K. Murakami, *Cellulose Chem. Technol.*, 22 (1988) 135–145; T. Takano, Y. Harada, F. Nakatsubo, and K. Murakami, *Mokuzai Gakkaishi.*, 36 (1990) 212–217.
[12] T. Nishimura, F. Nakatsubo, and K. Murakami, *Mokuzai Gakkaishi*, 40 (1994) 44–49.
[13] L.H.B. Baptistella, J.F. dos Santos, K.C. Ballabio, and A.J. Marsaiol, *Synthesis*, 21 (1989) 436.
[14] T. Kawada, F. Nakatsubo, K. Murakami, and T. Sakuno, *Mokuzai Gakkaishi*, 38 (1991) 930–934.
[15] M.L. Wolfrom and J.C. Dacons, *J. Am. Chem. Soc.*, 74 (1952) 5331–5333.
[16] J.C. Gast, R.H. Atalla, and R.D. Makolovey, *Carbohydr. Res.*, 84 (1980) 137–146.
[17] A. Isogai and M. Usuta, *Mokuzai Gakkaishi*, 37 (1991) 339–344.
[18] L. Velluz, J. Valls, and J. Mathieu, *Angew. Chem. Int. Ed. Engl.*, 6 (1967) 778–789; F. Nakatsubo, T.T. Akano, T. Kawada, H. Someya, T. Harada, H. Shiraki, and K. Murakami, *Mem. Coll. Agric. Kyoto. Univ.*, No. 127 (1985) 37–47.