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Synthesis and thermal properties of poly(methyl methacrylate)-*graft*-(cellobiosylamine-C15)

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Abstract Model experiments for synthesis of a comb-shaped copolymer with cellulose side-chains were performed with cellobiose derivatives. A novel cellobiose monomer, *N*-(15-methacryloyloxypentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosylamine (**2**) was prepared from heptaacetylcellobiosyl- amine. Homopolymerization of cellobiose monomer **2** and copolymerization of monomer **2** with methyl methacrylate (MMA) were performed using 2,2′-azobis (isobutyronitrile) (AIBN) as an initiator to obtain homopolymers **3**-i (i = 1–4) and copolymers **3**-i (i = 5–7), poly(methyl methacrylate)-*graft*-(heptaacetylcellobiosylamine-C15). The size exclusion chromatography—multi-angle laser light scattering (SEC-MALS) measurements revealed that comb-shaped homopolymers **3**-i (i = 1–4) had more compact structures compared to copolymers **3**-i (i = 5–7) at the same elution volume. Selective deacetylation of polymers **3**-i (i = 1–7) gave novel cellobiose polymers **4**-i (i = 1–7), poly(methyl methacrylate)-*graft*-(cellobiosylamine-C15). The amide linkages between cellobiose moiety and long-chain alkyl group, and the ester linkages between PMMA main-chain and long-chain alkyl group remained after deprotection.

The differential scanning calorimetry (DSC) measurements revealed that the T_gs of the polymers **4**-i (i = 1, 5, 6, 7) increased with increasing cellobiose composition in the polymers. It was indicated that cellobiose moieties of polymers **4**-i (i = 1, 5, 6, 7) reduced the mobility of PMMA main-chain.

Keywords Graft copolymer · Cellulosic side-chain · Reducing-end · Free radical polymerization · Thermal properties

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Introduction

Cellulose is a linear (1 → 4)-*β*-glucopyranan having three hydroxyl groups at C2, C3, and C6 positions per anhydro glucose unit. In addition, the cellulose molecule has only one hemiacetal hydroxyl group at the reducing end, which can be substituted with other functional groups with high regioselectivity (Feger and Cantow 1980; Nakatsubo et al. 1987). Recently, we have succeeded to prepare a novel A(cellulose)-B(long-chain alkyl groups) type cellulose diblock copolymer by introducing long-chain alkyl groups into the reducing-end of cellulose chain step by step *via* amide linkages (Enomoto et al. 2006; Kamitakahara et al. 2005; Kamitakahara and

Nakatsubo 2005). This synthetic method made it possible to introduce other functional groups regioselectively into the reducing-end of cellulose, to give desired cellulose derivatives with well-defined structure.

Based on our synthetic method, we planned to prepare a novel cellulose macromonomer and its copolymer which has comb-shaped structure with cellulose side-chains. A macromonomer is a polymeric monomer with a polymerizable functional group such as methacryloyl or styryl groups at the one end that is capable of further polymerization (Ito et al. 1992; Shinoda et al. 2001). It is a common way to prepare a comb-shaped graft copolymer. The comb-shaped cellulose copolymer, which has cellulose side-chains grafted at the reducing end onto main-chain, can be a novel type of cellulose copolymer. The strategy could achieve parallel orientation of cellulose chains. Cellulose crystal with parallel orientation has not prepared from regenerated cellulose or by chemical synthesis pathway yet, although there are some attempts using cello-oligosaccharide analogues (Bernet et al. 2000; Murty et al. 2006).

There are many reports on cellulose graft copolymers, and a variety of polymers can be grafted onto the cellulose chain through its hydroxyl groups at C2, C3, and C6 positions to alter properties of cellulose or cellulosic materials (Nishio 2006). However, the cellulose chain is usually used only as main-chain of graft copolymers. In addition, there are also many reports on glycopolymers having carbohydrate as a pendent group prepared by polymerization of a carbohydrate monomer carrying a polymerizable group (Ohno et al. 1998a; Ohno et al. 1998b). However, carbohydrate residues are limited to mono- or di-saccharides such as glucose or lactose. Synthesis of a cellulose macromonomer or a copolymer with cellulose side-chains, have not been reported yet.

Therefore, as model experiments for the well-defined cellulose macromonomer and for its polymerization, we describe the design and synthesis of a novel cellobiose monomer carrying a methacryloyl group at the reducing-end, and its homopolymers and copolymers. The obtained polymers were analyzed by SEC-MALS measurements, Fourier transform infrared (FT-IR) measurements, and DSC measurements to investigate their structure or interactions between side-chains.

Experimental

General measurements

^{1}H-, ^{13}C-, and two-dimensional NMR spectra were recorded on Varian INOVA300 FT-NMR (300 MHz) spectrometer, in $CDCl_3$ or DMSO-d_6 with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) and coupling constants (J) are given in value (ppm) and (Hz), respectively. Open preparative column chromatography was performed using silica gel (Wakogel C-200, Wako pure chemical). Melting temperature (T_m) was determined on FP900 Thermosystem (Mettler Toledo) with FP82 Microscope Hot Stage. Optical rotations were measured on a JASCO Dip-1000 digital polarimeter.

SEC-MALS measurement

Size exclusion chromatography—multi-angle laser light scattering (SEC-MALS) measurements were carried out at 25 °C using SEC system (CBM-10A, SPD-10A, SIL-10A, LC-10AT, FCV-10AL, CTO-10A, RID-10A, and FRC-10, Shimadzu, Japan) and MALS detector (DAWN EOS, Wyatt Technology Co., Ltd., U.S.A.) ($\lambda = 690$ nm). Shodex column (K802, K802.5, and K805) was used. Number and weight averaged molecular weights ($M_{n,PS}$, $M_{w,PS}$) and polydispersity index ($M_{w,PS}/M_{n,PS}$) were estimated using polystyrene standards (Shodex). The photometer was calibrated with pure toluene. Chloroform was used as eluent. The flow rate was 1.0 mL/min. Refractive index increments (dn/dc) were measured at 25 °C by DRM1021 (Otsuka Electronics Co, Ltd, Japan) ($\lambda = 633$ nm). The dn/dc values were 0.063 for PMMA, 0.054 for homopolymers **3**-i (i = 1–4), and 0.057 for copolymers **3**-i (i = 5–7).

FT-IR measurement

Fourier transform infrared (FT-IR) spectra were recorded on FTIR-4000 spectrophotometer (Shimadzu, Japan). Samples were mixed with KBr and pressed into disks. The FT-IR spectra of PMMA, polymers **3**-i (i = 5, 6, 7), and **4**-i (i = 1, 5, 6, 7) were normalized to the band at 1387 cm^{-1} for the symmetric methyl CH_3 bending vibration mode of PMMA main-chain (νCH_3(PMMA)) (Willis et al. 1969). The overlap between νCH_3(PMMA) and the

band for CH bending vibration mode of acetyl groups at 1371 cm^{-1} (νCH(acetyl)) (Lee et al. 2003) was ignored for the spectra of copolymers **3**-i (i = 5, 6, 7). The spectrum of homopolymer **3**-1 was not normalized.

DSC measurement

Differential scanning calorimetry (DSC) thermograms were recorded on DSC823^e (Mettler Toledo) under nitrogen atmosphere. The samples were first cooled from 25 to −40 °C, heated to 210 °C (first heating scan) at a heating rate of 10 °C/min, and then immediately quenched to −40 °C. The second heating scans were run from −40 to 490 °C at heating rate of 10 °C/min, to record stable thermograms. The glass transition temperature was recorded as the midpoint temperature of the heat capacity transition of the second heating run.

Materials

2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosylamine was prepared, as described in our previous article (Kamitakahara and Nakatsubo 2005). Methyl methacrylate (MMA) monomer was distilled under reduced pressure. 2,2′-Azobis(isobutyronitrile) (AIBN) as an initiator for free radical polymerization was crystallized from ethanol before use. 15-Hydroxypentadecanoic acid, 1-ethylcarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), 1,8-diazabicyclo[5,4,0]-7-undecene (DBU), and all other reagents were commercially obtained and used without further purification.

N-(15-Hydroxypentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosylamine (**1**)

To a solution of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosylamine (4.5 g, 7.1 mmol) and 15-hydroxypentadecanoic acid (2.45 g, 9.4 mmol) in dichloromethane/ dimethylformamide (DMF) (9:1, v/v) (60 mL) was added EEDQ (2.44 g, 9.9 mmol). The mixture was stirred for 12 h at 30 °C under nitrogen. After completion of the reaction, the mixture was filtered and concentrated to dryness. Crude product was purified by open column chromatography (eluent: ethyl acetate/ *n*-hexane (1:1, v/v)) to give colorless solid (**1**) (6.00 g, 96.8% yield). T_m 147–150 °C $[\alpha]_D^{30}$ +0.97° (c 1.00, chloroform); ^{1}H-NMR ($CDCl_3$): δ 1.25 (m, 20H, aliphatic-H), 1.57 (m, 4H, C1–NH–CO–CH_2–CH_2–, –CH_2–CH_2–OH), 1.99, 2.01, 2.03, 2.04, 2.10, 2.12 (s, 21H, CH_3–CO–), 2.10–2.13 (2H, C1–NH–CO–CH_2–), 3.64 (t, 2H, J = 6.8, –CH_2–OH), 3.61–3.70 (overlapped, C5′–H, C5–H), 3.75 (d, 1H, $J_{3,4}$ = 6.0, C4–H), 4.04 (dd, 1H, $J_{5',6'}$ = 2.4, $J_{6'a,6'b}$ = 12.6, C6′–H_b), 4.13 (dd, 1H, $J_{5,6b}$ = 4.2, $J_{6a,6b}$ = 12.0, C6–H_b), 4.37 (dd, 1H, $J_{5',6'}$ = 4.5, $J_{6'a,6'b}$ = 12.3, C6′–H_a), 4.46 (d, 1H, $J_{6a,6b}$ = 12.6, C6–H_a), 4.50 (d, 1H, $J_{1',2'}$ = 7.8 C1′–H), 4.83 (t, 1H, $J_{2,3}$ = 9.6, C2–H), 4.92 (t, 1H, $J_{2',3'}$ = 8.6, C2′–H), 5.07 (t, 1H, $J_{4',5'}$ = 9.3, C4′–H), 5.14 (t, 1H, J = 9.2, C3′–H), 5.21 (t, 1H, $J_{1,2}$ = 9.5, C1–H), 5.28 (br t, 1H, $J_{3,4}$ = 9.3, C3–H), 6.22 (d, 1H, $J_{NH,1}$ = 9.3, C1–N*H*–CO–). ^{13}C-NMR ($CDCl_3$): δ 20.5, 20.6, 20.8 (CH_3–CO–), 25.1 (C1–NH–CO–CH_2–CH_2–), 25.7 (–CH_2–CH_2–CH_2–OH), 29.0, 29.2, 29.3, 29.5, 29.5 (aliphatic-C), 32.7 (–CH_2–CH_2–OH), 36.6 (C1–NH–CO–CH_2–), 61.6 (C6′), 61.8 (C6), 63.0 (–CH_2–OH), 67.8 (C4′), 70.8 (C2), 71.5 (C2′), 71.9 (C5′), 72.1 (C3), 72.8 (C3′), 74.4 (C5), 76.2 (C4), 77.9 (C1), 100.6 (C1′), 169.0, 169.3, 169.4, 170.2, 170.2, 170.5, 171.2 (CH_3CO), 173.3 (C1–NH–*C*O–). *Anal.* Calc. for $C_{41}H_{65}NO_{19}$: C, 56.22; H, 7.48; N, 1.60; O, 34.70. Found: C, 56.01; H, 7.44; N, 1.61; O, 34.94.

N-(15-Methacryloyloxypentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosylamine (**2**)

To a solution of *N*-(15-hydroxypentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosylamine (**1**) (2.1 g, 2.28 mmol) in dichloromethane (5 mL) were added methacryloyl chloride (0.7 mL, 6.3 mmol) and triethylamine (Et_3 N) (1.0 mL, 13.5 mmol). The mixture was stirred for 0.5 h at room temperature under nitrogen. After completion of the reaction, the mixture was extracted with ethyl acetate, washed with water and brine, dried with Na_2SO_4, and concentrated to dryness. Crude product was purified by open preparative column chromatography (eluent: ethyl acetate/ *n*-hexane (1:1, v/v)) to give colorless solid (**2**) (1.86 g, 82.0% yield). T_m 115–117 °C $[\alpha]_D^{30}$ +0.68° (c 1.00, chloroform); ^{1}H-NMR ($CDCl_3$): δ 1.25 (m, 20H, aliphatic-H), 1.57 (m, 2H, C1–NH–CO–CH_2–CH_2–), 1.67 (m, 2H, –CH_2–CH_2–O–CO–), 1.94

(m, 3H, CH_2 = C–CH_3), 1.99, 2.01, 2.03, 2.05, 2.10, 2.12 (s, 21H, CH_3–CO), 2.10–2.13 (2H, C1–NH–CO–CH_2–), 3.65 (m, 1H, C5′–H), 3.68 (m, 1H, C5–H), 3.78 (d, 1H, $J_{3,4}$ = 9.9, C4–H), 4.03 (dd, 1H, $J_{5',6'}$ = 1.8, $J_{6'a,6'b}$ = 12.6, C6′–H_b), 4.14 (t, 2H, J = 6.5, –CH_2–O–CO–), 4.11–4.16 (overlapped, 1H, C6–H_b), 4.38 (dd, 1H, $J_{5',6'}$ = 4.5, $J_{6'a,6'b}$ = 12.5, C6′–H_a), 4.46 (d, 1H, $J_{6a,6b}$ = 11.7, C6–H_a), 4.51 (d, 1H, $J_{1',2'}$ = 7.8 C1′–H), 4.83 (t, 1H, $J_{2,3}$ = 9.6, C2–H), 4.93 (t, 1H, $J_{2',3'}$ = 8.6, C2′–H), 5.07 (t, 1H, $J_{4',5'}$ = 9.3, C4′–H), 5.15 (t, 1H, J = 9.4, C3′–H), 5.21 (t, 1H, $J_{1,2}$ = 9.5, C1–H), 5.29 (br t, 1H, $J_{3,4}$ = 9.2, C3–H), 5.55, 6.10 (s, 2H, CH_2 = C–), 6.24 (d, 1H, $J_{NH,1}$ = 9.3, C1–N*H*–CO–). ^{13}C-NMR ($CDCl_3$): δ 18.2 (CH_2 = C–CH_3), 20.4, 20.6, 20.8 (CH_3–CO–), 25.1 (C1–NH–CO–CH_2–CH_2–), 25.8 (–CH_2–CH_2–CH_2–O–CO–), 28.5 (–CH_2–CH_2–O–CO–), 29.0, 29.1, 29.4, 29.4, 29.5 (aliphatic-C), 36.5 (C1–NH–CO–CH_2–), 61.5 (C6′), 61.8 (C6), 64.7 (–CH_2–O–CO), 67.7 (C4′), 70.7 (C2), 71.4 (C2′), 71.8 (C5′), 72.0 (C3), 72.8 (C3′), 74.3 (C5), 76.2 (C4), 77.9 (C1), 100.5 (C1′), 125.1 (CH_2 = C–CH_3), 136.4 (CH_2 = C–CH_3), 167.5 (–CH_2–O–CO–), 168.9, 169.2, 169.3, 170.2, 170.2, 170.4, 171.1 (CH_3–CO–), 173.3 (C1–NH–CO–). *Anal.* Calc. for $C_{45}H_{69}NO_{20}$: C, 57.25; H, 7.37; N, 1.48; O, 33.90. Found: C, 56.97; H, 7.29; N, 1.48; O, 34.26.

General procedures for free radical polymerization of cellobiose monomer **2**

Cellobiose monomer **2** (115 mg, 0.0278 mmol) and the amount of AIBN necessary to give the desired monomer-initiator ratio were weighed into a glass tube, and degassed on a vacuum line. Copolymerization with MMA was carried out by the same procedure with the appropriate amount of MMA. Benzene for 10 w/v% of monomer concentration was degassed by freeze-pump-thaw cycles typically three times until no oxygen remained, and loaded into the tube. The tube was sealed under vacuum, and placed in an oil bath at 60 °C for 3 days. The tube was cooled to room temperature, and opened. Conversion (%) of MMA was calculated from the weight of remaining MMA removed by extraction with methanol from the mixture obtained by drying *in vacuo* after the reaction. The reaction mixture was analyzed by ^{1}H-NMR. Conversion (%) of monomer **2** was calculated by comparing peak areas of olefinic protons of the methacryloyl group and sugar ring protons. The remaining monomers were removed by reprecipitation from chloroform into methanol. The compounds were filtered off, dried, to give polymers **3**-i (i = 1–7). Degree of polymerization of each monomer was defined as X for MMA, and Y for monomer **2**. Typical ^{1}H- and ^{13}C-NMR spectra of copolymer **3**-i (i = 7) were assigned as follows: ^{1}H-NMR ($CDCl_3$): δ 0.84, 1.02, 1.21 (–CH_2–C–CH_3), 1.25, 1.58 (br. s, aliphatic-H), 1.82, 1.90 (–CH_2– (MMA)), 1.99, 2.01, 2.03, 2.05, 2.10, 2.12 (s, CH_3–CO), 3.61 (CH_3–O–), 3.74 (C5′–H, C5–H), 3.79 (C4–H), 4.01–4.15 (C6′–H_b, C6–H_b), 4.38–4.48 (C6′–H_a, C6–H_a), 4.57–4.60 (C1′–H), 4.84 (br. t, C2–H), 4.93 (br. t, C2′–H), 5.07–5.27 (C4′–H, C3′–H, C1–H, C3–H), 6.76 (br. s, C1–N*H*–CO–). ^{13}C-NMR ($CDCl_3$): δ 16.2, 18.2 (–CH_2–C–CH_3), 20.4, 20.6, 20.8 (CH_3–CO–), 25.1 (C1–NH–CO–CH_2–CH_2–), 25.9 (–CH_2–CH_2–CH_2–O–CO–), 28.0 (–CH_2–CH_2–O–CO–), 29.4–29.7 (aliphatic-C), 36.4 (C1–NH–CO–CH_2–), 44.5 (–CH_2–C–CH_3), 51.8 (CH_3O–), 53.9 (–CH_2–C–CH_3), 61.4 (C6′), 61.8 (C6), 64.9 (–CH_2–O–CO–), 67.7 (C4′), 70.7 (C2), 71.4 (C2′), 71.7 (C5′), 72.3 (C3), 72.8 (C3′), 74.4 (C5), 76.1 (C4), 77.8 (C1), 100.5 (C1′), 168.9, 169.2, 169.4, 170.1, 170.2, 170.4, 170.9 (CH_3–CO–), 173.5 (C1–NH–CO–), 177.4 (–CH_2–O–CO–).

Deprotection of acetyl groups of polymers **3**-i (i = 1–7)

To a solution of the protected homopolymers **3**-1 (40.1 mg) in methanol/ dichloromethane (1:4, v/v) was added DBU (169.3 μL, 1.11 mmol) at room temperature. The solution was stirred for 3 h under nitrogen. The mixture was added to methanol and the precipitate was filtered off, washed with methanol, and dried *in vacuo* to give an amorphous solid, homopolymer **4**-1 (20.1 mg, 72.8% yield). The same procedure was applied to the deprotection of polymers **3**-i (i = 2–7) with the appropriate amount of DBU. Typical ^{1}H- and ^{13}C-NMR spectra of copolymer **4**-i (i = 1) were assigned as follows: ^{1}H-NMR (DMSO-d_6): δ 1.24 (br. s, aliphatic-H), 1.47–1.6 (–CH_2–CH_2–O–CO–, –CH_2–CH_2–CH_2–O–CO, –C1–NH–CO–CH_2–CH_2–), 2.10 (br. s, C1–NH–CO–CH_2–), 3.02–3.10 (C2′–H, C4′–H), 3.18 (C2–H), 3.2–3.4 (C4–H, C5–H, C5′–H), 3.7–3.8(C6–H, C6′–H), 4.27–4.30 (C1′–H), 4.74 (C1–H), 5.12 (C3–H), 3.61 (C3′–H), 8.41

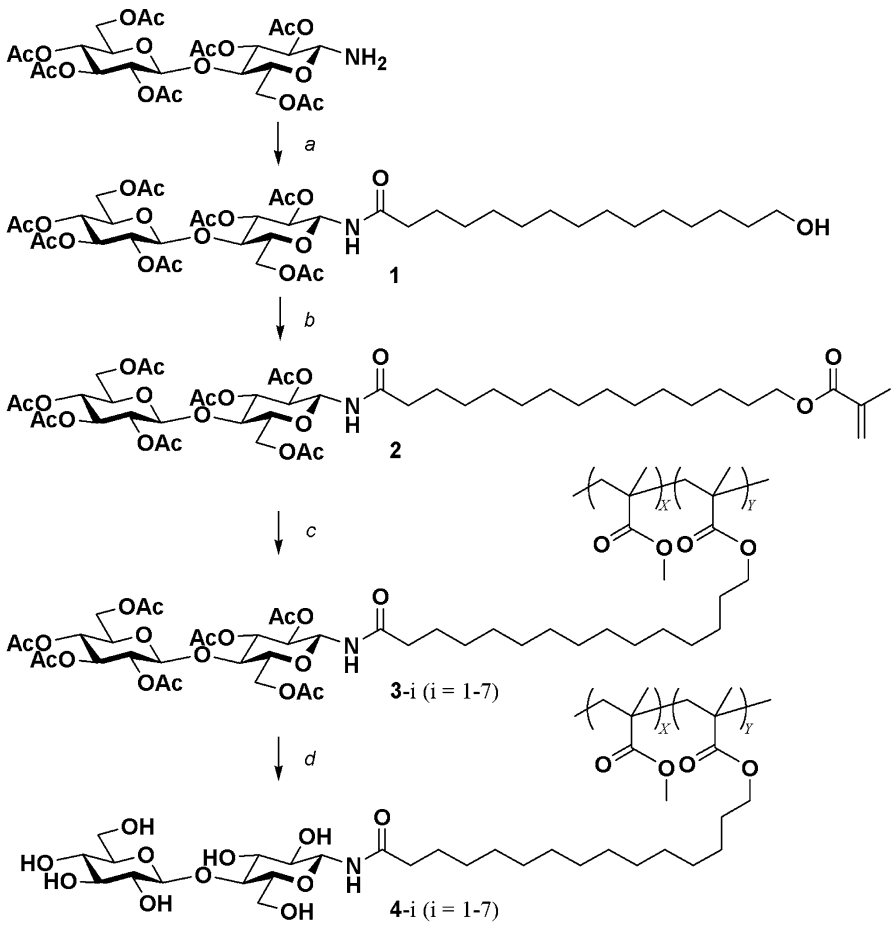
(br. s, C1–N*H*–CO–). ^{13}C-NMR (DMSO-d_6): δ 16.3, 18.6 (–CH_2–C–CH_3), 25.2 (C1–NH–CO–CH_2–CH_2–), 25.9 (–CH_2–CH_2–CH_2–O–CO–), 27.9 (–CH_2–CH_2–O–CO–), 29.5 (aliphatic-C), 35.6 (C1–NH–CO–CH_2–), 44.2 (–CH_2–C–CH_3), 53.5 (–CH_2–C–CH_3), 60.4 (C6′), 61.2 (C6), 64.6 (–CH_2–O–CO–), 70.2 (C4′), 72.1 (C2), 73.5 (C2′), 75.9 (C5′), 76.5 (C3, C3′), 76.9 (C5), 79.3 (C1), 80.4 (C4), 103.3 (C1′), 173.1 (C1–NH–CO–), 176.9 (–CH_2–O–CO–).

Results and discussion

Synthesis of cellobiose monomer **2**

We designed cellobiose monomer **2** as a model of a cellulose macromonomer, as described in Fig. 1. Methacryloyl group was selected as a polymerizable group, because ester bonds of polymethacrylate are known to be stable under the alkaline conditions of the subsequent deprotection procedure. 15-Hydroxypentadecanoic acid was introduced to the reducing-end as a spacer to avoid steric hindrance for radical polymerization.

Cellobiose derivative **1** was obtained in 96.8% yield by condensation of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosylamine and 15-hydroxypentadecanoic acid with 1-ethylcarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) which is a common condensation reagent in peptide synthesis. Next, cellobiose monomer **2** was obtained in 82.0% yield by further condensation of compound **1** and methacryloyl chloride with triethylamine (Et_3 N). Structure of monomer **2** was identified by ^{1}H-, ^{13}C-, and two-dimensional NMR measurements. All protons and carbons are assigned



[a]15-Hydroxypentadecanoic acid/ EEDQ/ DMF/ CH_2Cl_2 , [b]Methacryloyl chloride/ Et_3N/ CH_2Cl_2 , [c](MMA)/ AIBN/ benzene , [d]DBU/ methanol/ CH_2Cl_2.

Fig. 1 Synthetic route of cellobiose derivatives **1**, **2**, **3**-i (i = 1–7), and **4**-i (i = 1–7). [a]15-Hydroxypentadecanoic acid/EEDQ/DMF/CH_2Cl_2, [b]Methacryloyl chloride/ Et_3 N/CH_2Cl_2, [c](MMA)/ AIBN/benzene, [d]DBU/ methanol/CH_2Cl_2

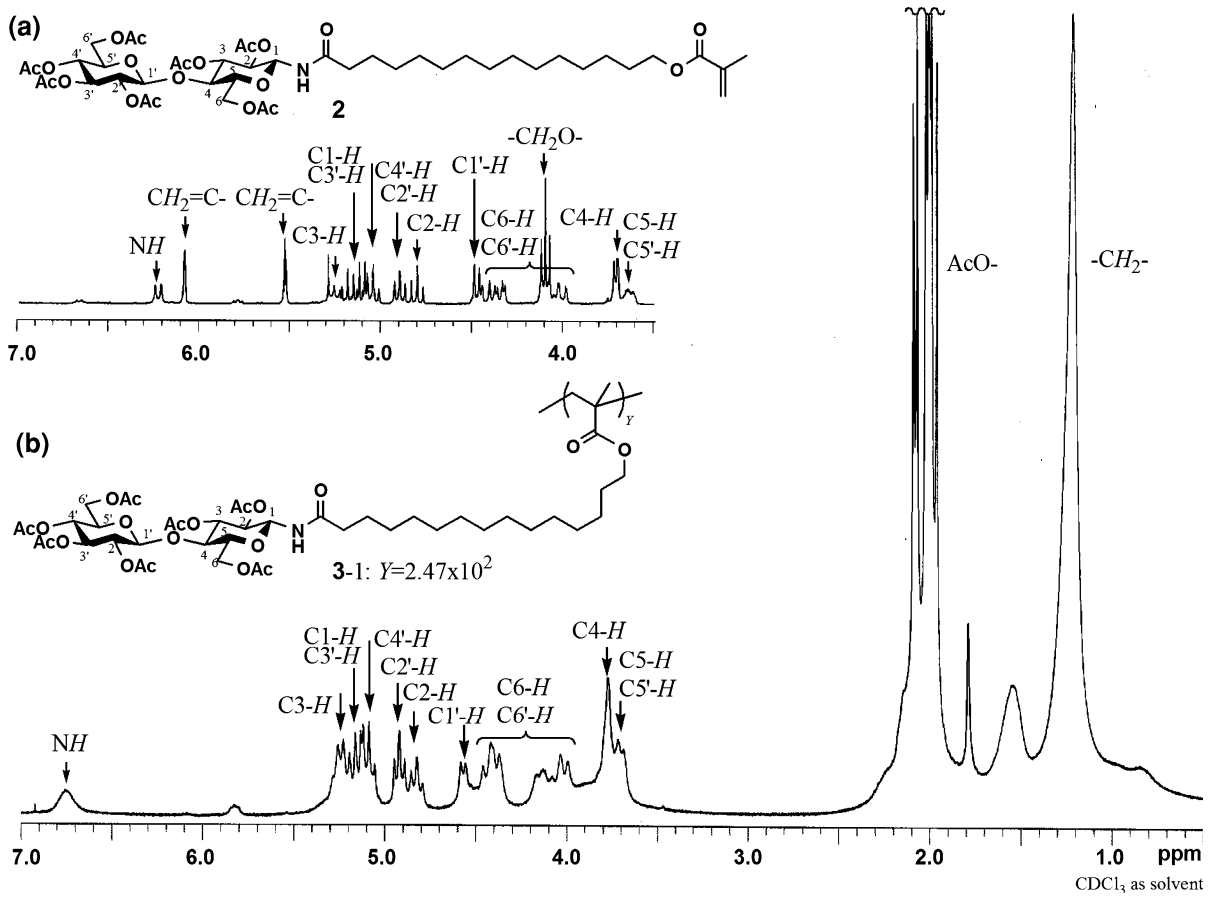


Fig. 2 ^{1}H-NMR spectra of (**a**) cellobiose monomer **2** and (**b**) homopolymer **3**-1

as shown in Figs. 2a and 5a. Olefinic protons (CH_2 = C–, δ 5.55 and 6.10 ppm) of the methacryloyl group and methylene protons (–CH_2–O–, δ 4.14 ppm) of the long-chain alkyl group were observed in its ^{1}H-NMR spectrum as shown in Fig. 2a.

It was proved that the novel cellobiose monomer **2** was successfully prepared from heptaacetylcellobiosylamine in high yield *via* condensation. The same synthetic route may be applicable to obtain the corresponding cellulose macromonomer having a methacryloyl group at the reducing-end.

Polymerization of cellobiose monomer **2**

Free radical polymerization of cellobiose monomer **2** was carried out, using 2,2′-azobis(isobutyronitrile) (AIBN) as an initiator, to give homopolymers **3**-i (i = 1–4). Monomer **2** was also copolymerized with MMA with different monomer ratio, to give copolymers **3**-i (i = 5–7). The results of polymerization are summarized in Table 1. The chemical structures of polymers **3**-i (i = 1–7) were identified by ^{1}H- and ^{13}C-NMR measurements. The molecular weights and the polydispersity index of polymers **3**-i (i = 1–7) were analyzed by SEC-MALS measurements.

Homopolymers **3**-i (i = 1–4) were obtained in high polymer yield and high monomer conversion. The polymer yield reached up to 89.7%, and the monomer conversion reached up to 96.4% in the case of homopolymer **3**-2. The $M_{w,LS}$s increased with increase in initial [monomer **2**]$_0$/[AIBN]$_0$ value. The polymer yield and the monomer conversion for homopolymer **3**-4 were 34.9% and 35.4%, respectively, and were lower than those of homopolymers **3**-i (i = 1–3). It is due to the too high value of initial monomer-initiator ratio ([monomer **2**]$_0$/[AIBN]$_0$ = 5000). However, degree of polymerization of monomer **2** (*Y*) reached

Table 1 Results of polymerization of cellobiose monomer **2** and MMA

Polymers **3**-i	$[MMA]_0/[monomer\ \mathbf{2}]_0/[AIBN]_0^a$	Polymer yield (%)	Conv. (MMA) (%)	Conv. (**2**)[b] (%)	$M_{w,PS}$ [c,d] (10^{-5})	$M_{w,PS}/M_{n,PS}$ [c,d]	$M_{w,LS}$ [d,e] (10^{-5})	$M_{w,PS}/M_{w,LS}$	X (MMA) [f,h] (10^{-3})	Y (**2**) [g,h] (10^{-2})
3-1	0/300/1	75.2	–	77.2	1.39	1.89	2.33	0.59	-	2.47
3-2	0/500/1	89.7	–	96.4	3.33	2.48	6.40	0.52	-	6.78
3-3	0/800/1	82.7	–	84.3	3.23	2.29	6.10	0.53	-	6.46
3-4	0/5000/1	34.9	–	35.4	6.35	3.43	15.5	0.41	-	16.4
3-5	300/3/1	Quantative	100	100	3.01	2.19	2.79	1.08	2.55	0.25
3-6	300/6/1	Quantative	100	100	1.69	2.29	1.45	1.17	1.22	0.24
3-7	300/30/1	Quantative	100	86.1	1.22	1.87	0.95	1.28	0.52	0.45

[a] Concentration of monomer **2**: 10 wt%, solvent: benzene, temperature: 60 °C, time: 3 days. [b] Determined by ^{1}H-NMR spectra from the ratio of peak areas of methacryloyl to C2 and C2′ protons. [c] Estimated by SEC measurement using polystyrene standards. [d] Chloroform as eluent. [e] Determined by MALS measurement. [f] Degree of polymerization of MMA. [g] Degree of polymerization of monomer **2**. [h] Calculated from $M_{w,LS}$ and the theoritical molecular weights of monomers (M(MMA) = 100.12, M(**2**) = 944.02)

up to 16.4×10^2. Fig. 2b shows the representative ^{1}H-NMR spectrum of homopolymer **3**-1. The proton signals assigned to PMMA chain were observed, in addition to ring-protons and acetyl groups of cellobiose heptaacetate moieties. The broad peaks of cellobiose heptaacetate groups were observed in its spectrum, indicating that polymerization of cellobiose monomer **2** successfully proceeded. The weak proton signals at 5.55 and 6.10 ppm in Fig. 2b and the carbon signals at 125.1 and 136.4 ppm in Fig. 3b, are due to traces of olefinic monomers.

In the case of copolymers **3**-i (i = 5–7), conversion of MMA was 100% for all samples, and conversion of monomer **2** was 100% for **3**-5 and **3**-6, and 86.1% for **3**-7. In the representative ^{1}H-NMR spectrum of copolymer **3**-7 (Fig. 4), the signals assigned to both PMMA and cellobiose heptaacetate groups are observed, indicating successful copolymerization of cellobiose monomer **2** with MMA.

It was confirmed that homopolymers **3**-i (i = 1–4) and copolymers **3**-i (i = 5–7) were prepared from cellobiose monomer **2** and MMA in high polymer yield and high monomer conversion. The same polymerization procedure may be applicable to obtain the corresponding copolymers with cellulose side-chains and PMMA main-chain.

Structural characterization of polymers **3**-i (i = 1–7) by SEC-MALS measurements

The molecular weights and conformation of polymers **3**-i (i = 1–7) in solution were analyzed by SEC-MALS measurements, using chloroform as eluent. The $M_{w,LS}$s of homopolymer **3**-i (i = 1–4) were higher than the $M_{w,PS}$s determined by polystyrene standards, as indicated by the $M_{w,PS}/M_{w,LS}$ values of 0.59, 0.52, 0.53, 0.41, respectively (Table 1). It is well known that SEC, when calibrated only with linear standard polymers such as polystyrene (PS), severely underestimates the molecular weight of a branched polymer which has a more compact molecular volume in solution than that of a corresponding linear polymer with the same molecular weight (Ito et al. 1992). The SEC-MALS measurements revealed that comb-shaped homopolymers **3**-i (i = 1–4) have more compact structures due to the pendent cellobiose heptaacetate moieties, compared to linear polystyrene (PS) standards at the same elution volume. On the other hand, the $M_{w,PS}/M_{w,LS}$ values of copolymers **3**-i (i = 5–7) were 1.08, 1.17, 1.28, respectively, indicating that structure of copolymers **3**-i (i = 5–7) are linear like PS standards.

The representative elution curves and plots of molecular weights ($M_{w,LS}$) vs elution volume for homopolymer **3**-1 and copolymer **3**-7 are shown in Fig. 5. Homopolymer **3**-1 has higher molecular weight than copolymer **3**-7 at the same elution volume, indicating that homopolymer **3**-1 had more compact structure compared to copolymer **3**-7. The short side-chains of copolymers **3**-i (i = 5–7) did not affect the elution volume of the copolymers because of their low composition of monomer **2** units. However, we expect that cellulose side-chains with higher degree of polymerization than cellobiose will

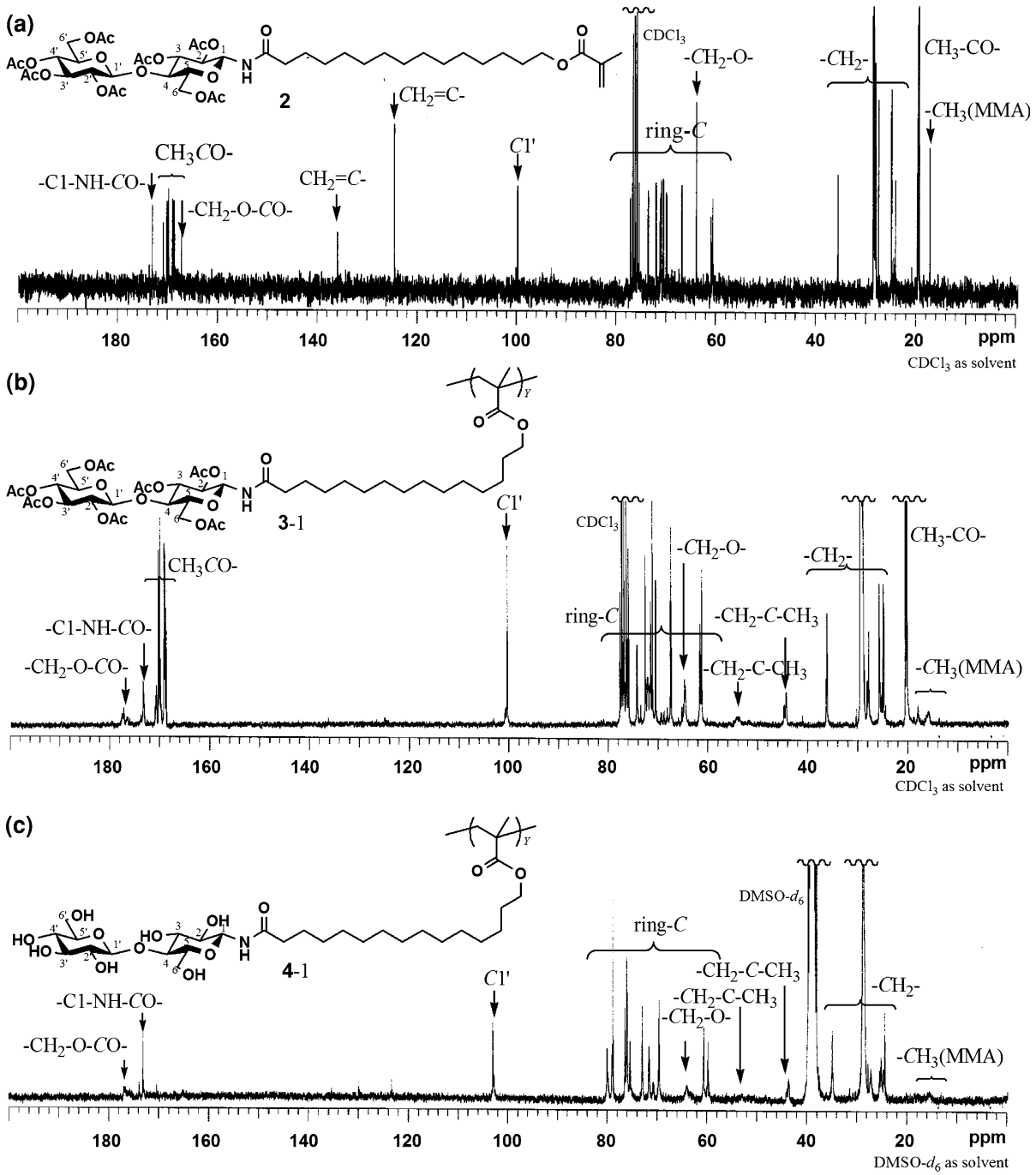


Fig. 3 ^{13}C-NMR spectra of (**a**) cellobiose monomer **2**, (**b**) homopolymer **3**-1 and (**c**) homopolymer **4**-1

have an effect on conformation of the corresponding cellulose copolymers even at low cellulose side-chain content.

From these data, it was revealed that homopolymer **3**-i (i = 1–4) had more compact structure compared to copolymers **3**-i (i = 5–7), and that it was due to

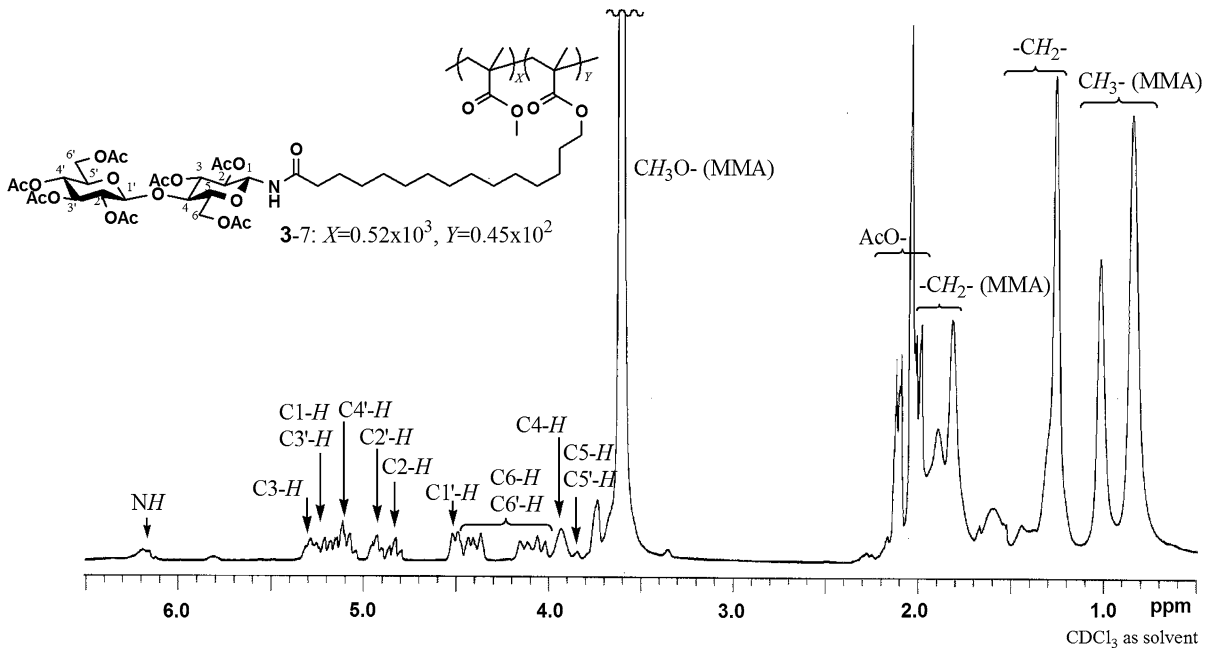


Fig. 4 ^{1}H-NMR spectrum of copolymer **3**-7

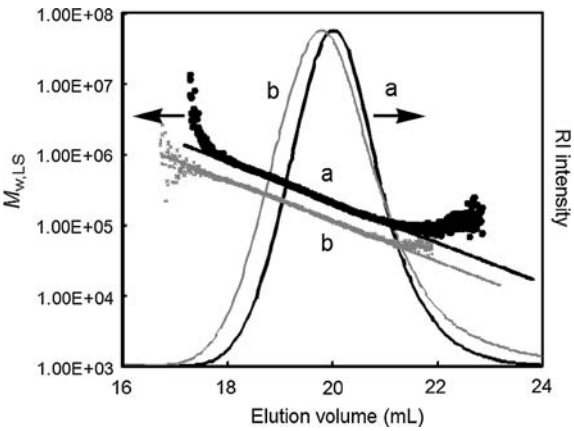


Fig. 5 SEC (RI) elution curves and molecular weight ($M_{w,\,LS}$) plots of (a) homopolymer **3**-1 and (b) copolymer **3**-7

higher density of cellobiose side-chains grafted on PMMA main-chain.

Selective deprotection of acetyl groups of polymers **3**-i (i = 1–7)

Deprotection of acetyl groups on polymers **3**-i (i = 1–7) was carried out with 1,8-diazabicyclo[5,4,0]-7-undecene (DBU) (Baptistella et al. 1989; Enomoto et al. 2006) to obtain the deprotected polymers **4**-i (i = 1–7). Polymers **4**-i (i = 1–7) were insoluble in water. Polymers **4**-i (i = 1–7) were identified by means of ^{1}H- and ^{13}C-NMR measurements in DMSO-d_6. The ^{13}C-NMR spectra of homopolymers **3**-1 and **4**-1, before and after the deprotection, are shown in Figs. 3b and 3c, respectively. Methyl carbons ($\underline{C}H_3$–CO–, ca. δ 20.6 ppm) and carbonyl carbons (CH_3–$\underline{C}O$–, ca. δ 170 ppm) of acetyl groups completely disappeared after the deprotection, and carbonyl carbon (C1–NH–$\underline{C}O$–, δ 173.1 ppm) of long-chain alkyl group at the C1 position was still observed in the ^{13}C-NMR spectrum of homopolymer **4**-1. The stability of the amide linkage (C1–NH–CO–) at C1 position has already been confirmed by two-dimensional NMR measurements in our previous study (Enomoto et al. 2006). In addition, the signals of carbonyl carbons (–CH_2–O–$\underline{C}O$–, δ 176.9 ppm) of methacryloyl groups and methylene carbons (–$\underline{C}H_2$–O–CO–, δ 64.6 ppm) were still observed at the same position before and after the deprotection. These assignments revealed that deprotection of acetyl groups proceeded quantitatively and that cellobiose moieties remained on the PMMA main-chain.

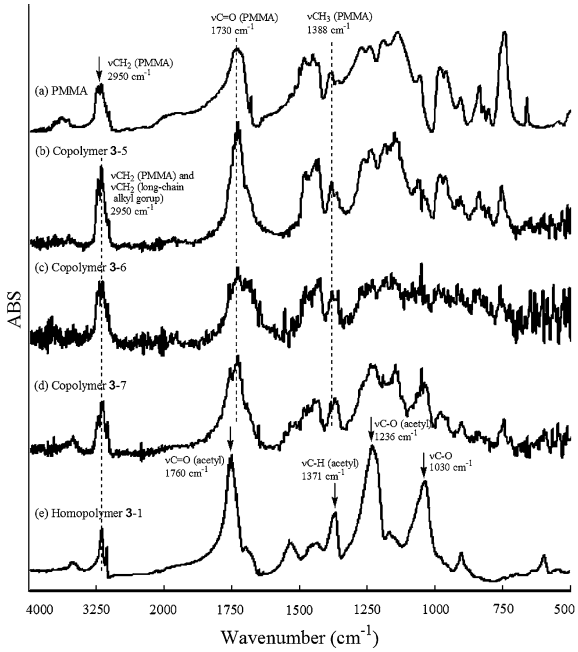


Fig. 6 FT-IR spectra of (a) PMMA, copolymers (b) **3**-5, (c) **3**-6, (d) **3**-7 with initial monomer ratio $[MMA]_0/[monomer\ \mathbf{2}]_0 = 300/3, 300/6, 300/30$, respectively, and (e) homopolymer **3**-1

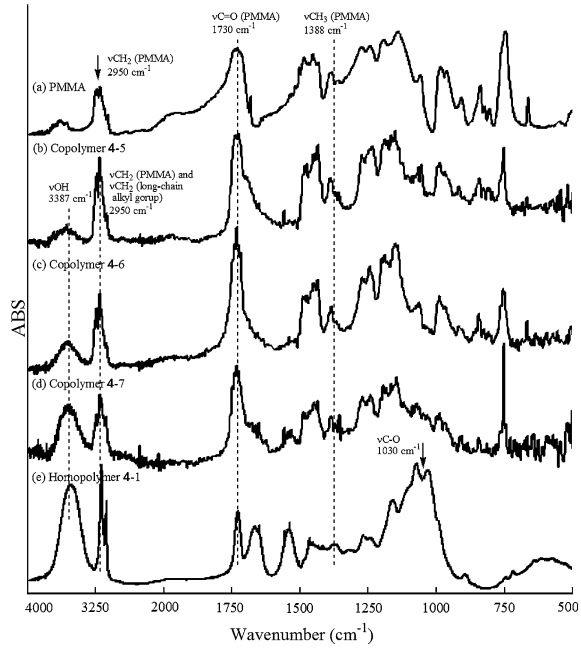


Fig. 7 FT-IR spectra of (a) PMMA, copolymers (b) **4**-5, (c) **4**-6, (d) **4**-7 with initial monomer ratio $[MMA]_0/[monomer\ \mathbf{2}]_0 = 300/3, 300/6, 300/30$, respectively, and (e) homopolymer **4**-1

The FT-IR spectra of the acetylated polymers **3**-i (i = 1, 5, 6, 7) and the deprotected polymers **4**-i (i = 1, 5, 6, 7) are shown in Figs. 6 and 7, respectively. The OH absorbance (*ν*OH ca. 3,400 cm^{-1}) of cellobiose groups appeared after the deprotection (Figs. 6b–e, 7b–e), indicating that acetyl groups were removed and that free hydroxyl groups of cellobiose appeared. The OH absorbance increased with the increase in composition of cellobiose moieties (Fig. 7b–e), indicating that cellobiose moieties remained on PMMA main-chain at the same molar composition as the original polymers **3**-i (i = 1, 5, 6, 7).

Furthermore, PMMA homopolymer was treated with DBU under the same conditions. The $M_{w,PS}$ value, ^1H- and ^{13}C-NMR spectra did not change after DBU-treatment (data not shown). These facts indicated the stability of methyl ester groups of PMMA main-chain.

Thus, it was revealed that selective deprotection of acetyl groups preceded by use of DBU, and that cellobiose groups still remained on PMMA main-chain *via* ester linkage, to give the desired branched structure of polymers **4**-i (i = 1–7).

Thermal properties of polymers **3**-i and **4**-i (i = 1, 5, 6, 7)

Thermal properties of the PMMA homopolymer and those of polymers **3**-i (i = 1, 5, 6, 7) and **4**-i (i = 1, 5, 6, 7) were examined by DSC, to investigate the composition dependence of transition behavior. All thermograms were recorded in second heating cycle to obtain stable data at a heating rate of 10 °C/min.

The acetylated polymers **3**-i (i = 1, 5, 6, 7) gave a single glass transition temperature T_g as shown in Fig. 8. The T_gs of PMMA and polymers **3**-i (i = 1, 5, 6, 7) decreased smoothly from 120.4 to 95.2 °C with increase in the number of cellobiose units on the PMMA main-chain (Figs. 8 and 10a). It was indicated that cellobiose heptaacetate moieties induced the mobility of PMMA main-chain, and behaved as a plasticizer.

On the other hand, the T_gs of PMMA and the deprotected polymers **4**-i (i = 1, 5, 6, 7) increased from 120.4 to 133.1 °C with increasing number of appended cellobiose monomers (Figs. 9 and 10b). PMMA is non-crystalline and hydrophobic, but

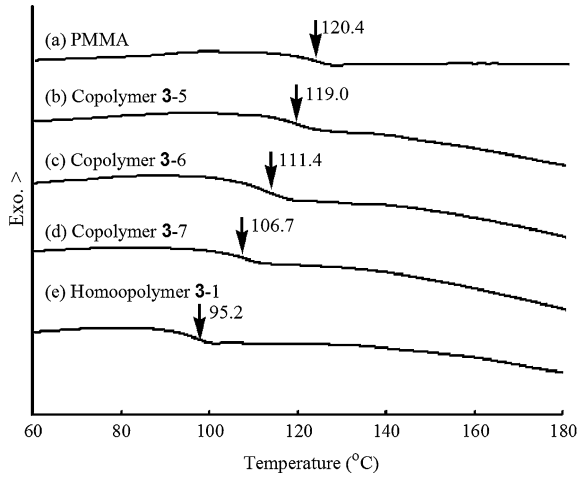


Fig. 8 DSC thermograms of (a) PMMA, copolymers (b) **3**-5, (c) **3**-6, (d) **3**-7 with initial monomer ratio $[MMA]_0/[monomer\ \mathbf{2}]_0 = 300/3, 300/6, 300/30$, respectively, and (e) homopolymer **3**-1

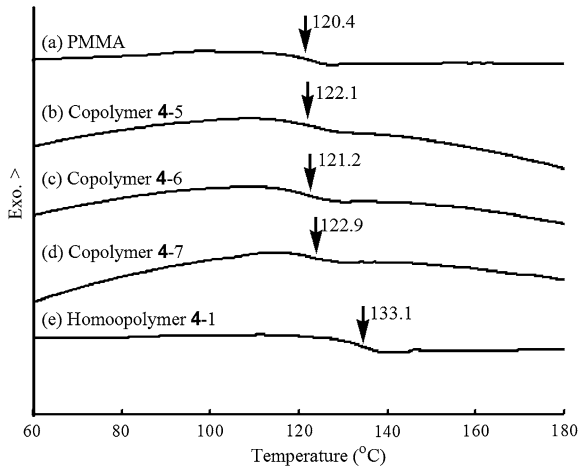


Fig. 9 DSC thermograms of (a) PMMA, copolymers (b) **4**-5, (c) **4**-6, (d) **4**-7 with initial monomer ratio $[MMA]_0/[monomer\ \mathbf{2}]_0 = 300/3, 300/6, 300/30$, respectively, and (e) homopolymer **4**-1

cellobiose is crystalline and hydrophilic. Cellobiose has decomposition temperature at 240.0 °C, and does not have T_g (data not shown). Thus, the T_gs observed for polymers **4**-i (i = 1, 5, 6, 7) were assigned to glass transitions of PMMA main-chains. It was indicated that cellobiose moieties of polymers **4**-i (i = 1, 5, 6, 7) reduced the mobility of PMMA main-chain with the increase in cellobiose composition, and that the T_g of PMMA main-chain could be controlled by cellobiose composition in the polymer.

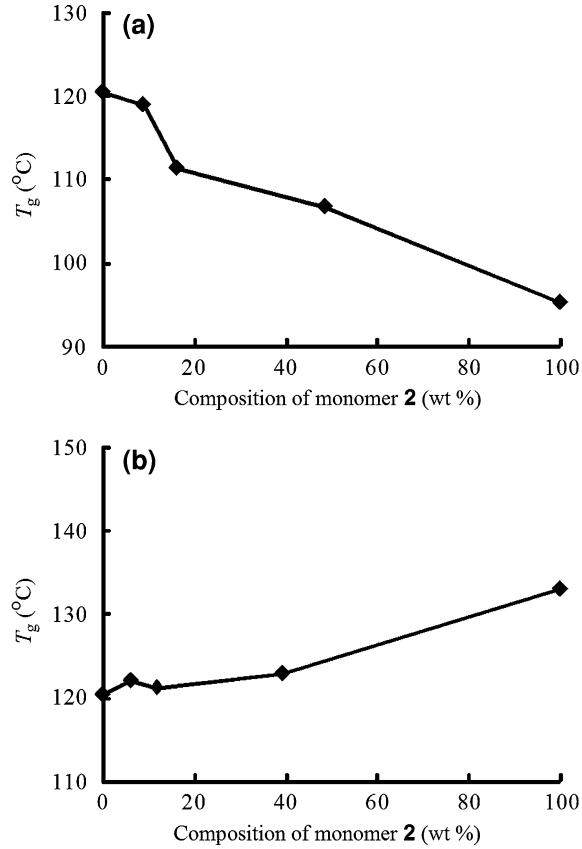


Fig. 10 Relationship between T_gs and composition of cellobiose monomer **2** (wt%) for (**a**) PMMA, polymers **3**-5, -6, -7 and -1, and (**b**) PMMA, polymers **4**-5, -6, -7 and -1

Conclusions

The novel cellobiose monomer **2** was prepared from heptaacetylcellobiosylamine. Homo- and co-polymerization of monomer **2** gave polymers **3**-i (i = 1–7), namely poly(methyl methacrylate)-*graft*-(heptaacetylcellobiosylamine-C15). The SEC-MALS measurements revealed that homopolymers **3**-i (i = 1–4) had more compact structure due to the increase in the density of cellobiose side-chains on PMMA main-chain, compared to copolymers **3**-i (i = 5–7).

Acetyl groups of polymers **3**-i (i = 1–7) were selectively removed by use of DBU, to yield the novel polymers **4**-i (i = 1–7), namely poly(methyl methacrylate)-*graft*-(cellobiosylamine–C15). Cellobiose groups remained attached to the PMMA main-chain after the deprotection *via* ester hydrolysis, to maintain the desired branched structure. The DSC

measurements indicated that cellobiose moieties of polymers **4**-i (i = 1, 5, 6, 7) reduced the mobility of PMMA main-chain.

The synthetic route described in this article will be applied to the corresponding cellulose derivatives. Furthermore, atom transfer radical polymerization (ATRP), which is known as an excellent method to obtain a copolymer with well-controlled structure and low polydispersity, can be applied to polymerization of monomer **2**. These studies will be reported elsewhere.

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