Fig. 7 $^1$H-NMR spectrum of compound 7 taken in CDCl$_3$
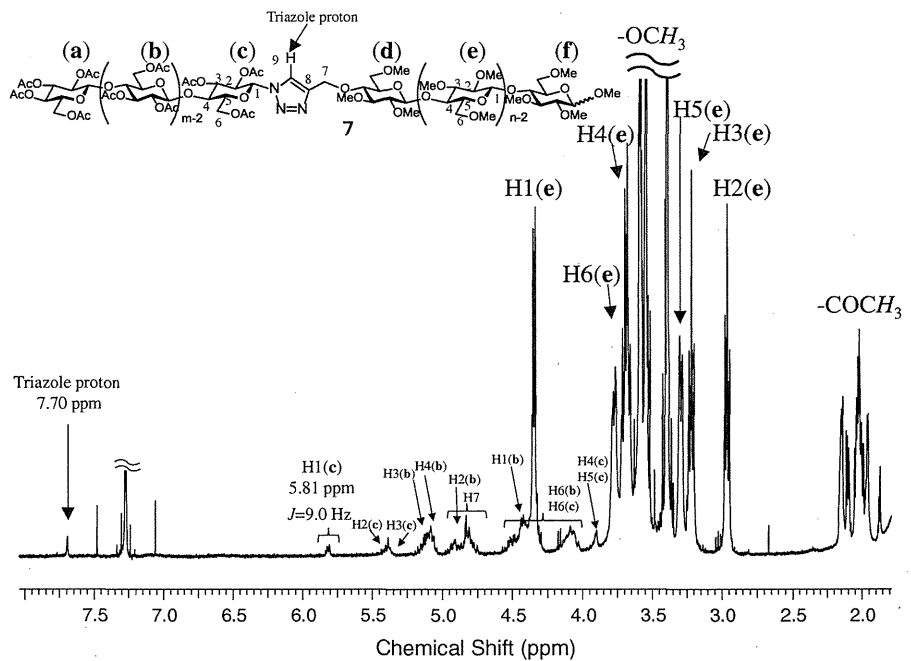


Fig. 8 $^1$H-NMR spectrum of compound 8 taken in D$_2$O
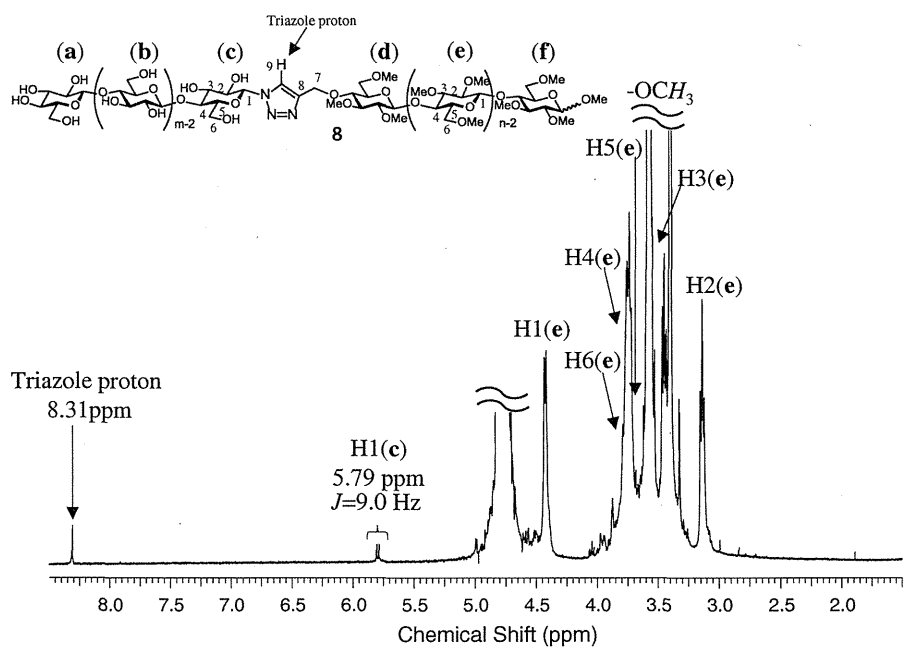
10 min after dissolving at $\sim 0$ °C. Compounds 5a and 8a showed the same macroscopic hydrogel formation as that of GG-236MC. Thus, we investigated thermal properties of 2.0 wt% aqueous solutions of compounds 5a, 5b, 8a, and 8b by means of DSC measurements to compare with that of GG-236MC.

DSC analysis of aqueous solution of GG-236MC showed endo- and exothermic peaks under heating and cooling, respectively, depending on the molecular weight and concentration (Nakagawa et al. 2011a). These endo- and exothermic peaks have suggested that the dehydration of hydrated compounds and

**Table 2** The $M_n$, $M_w/M_n$, $DP_n$ of hydrophobic block, and endothermic peak of compounds **5a**, **5b**, **8a**, **8b**, and **GG-236MC**

| Compound | $M_n$ ($\times 10^3$) | $M_w/M_n$ | $DP_n$ | | $DS$ | Endothermic peak (°C) |
|---|---|---|---|---|---|---|
| | | | Hydrophilic block[a] | Hydrophobic block[a] | | |
| **5a** | 7.4 | 1.39 | 2.0 | 33.0 | 2.80 | 33 |
| **5b** | 3.6 | 1.28 | 2.0 | 14.1 | 2.63 | 42 |
| **8a** | 8.2 | 1.35 | 9.0 | 26.7 | 2.23 | 35 |
| **8b** | 6.5 | -1.22 | 5.4 | 23.4 | 2.45 | 37 |
| GG-236MC | 6.4 | 1.27 | 2.0 | 26.2 | 2.76 | 33 |

[a] Calculated by $^1$H-NMR spectroscopy after acetylation
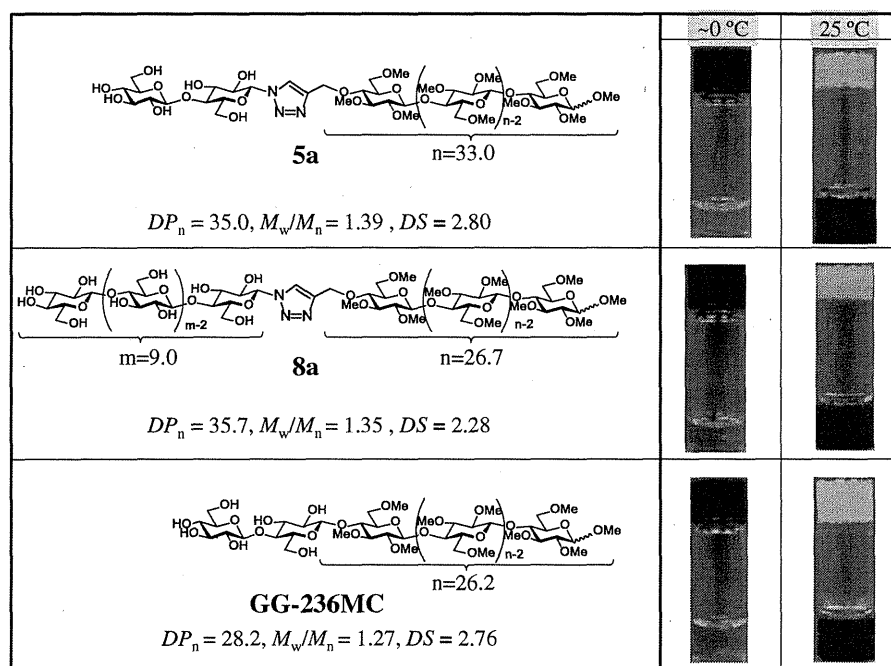


**Fig. 9** Photographs of 2.0 wt% aqueous solutions of compounds **5a**, **8a**, and GG-236MC

rehydration of dehydrated compounds occurred upon heating and cooling, respectively (Sarkar and Walker 1995).

DSC analysis of diblock MC analogues **5a**, **5b**, **8a**, **8b** and real diblock MC (GG-236MC) showed endo- and exothermic peaks under heating and cooling process, respectively. DSC curves at the second cycle of GG-236MC and the diblock MC analogues **5a**, **5b**, **8a**, **8b** with a heating and cooling rate 3.5 °C/min are shown in Fig. 10. In the case of GG-236MC having $M_n = 6.3 \times 10^3$, endothermic peaks appeared at 33 °C, whereas exothermic peaks appeared at 7 °C.

Compound **5a** having $M_n = 7.4 \times 10^3$ exhibited the same endo- and exothermic peaks as GG-236MC. In contrast, the endo- and exothermic peak temperatures of compound **5b** having $M_n = 3.6 \times 10^3$ were higher than those of compound **5a**. The temperature of endo- and exothermic peaks decreased with increasing the hydrophobic saccharide chain length. The endothermic peak temperature of compound **8** increased from 35 to 37 °C with decreasing $M_n$ from $8.2 \times 10^3$ to $6.5 \times 10^3$. Consequently, DSC measurement revealed that the peak temperatures of diblock MC analogues **5a** and **8a** were almost the same as those of GG-
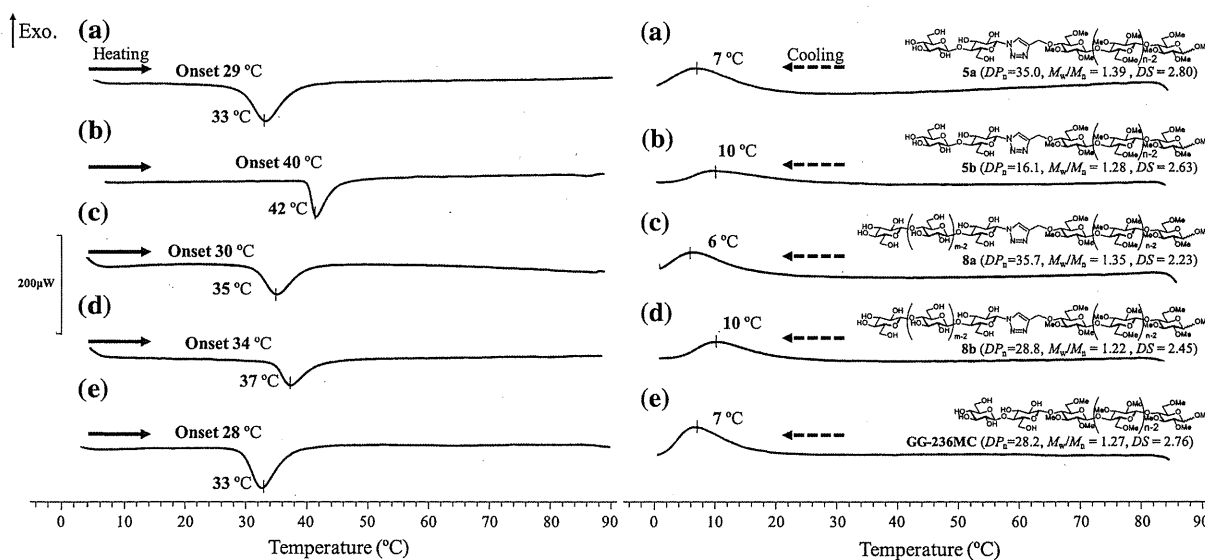
**Fig. 10** The second heating and cooling runs of 2.0 wt% aqueous solutions of compounds **5a**, **5b**, **8a**, **8b**, and GG-236MC at 3.5 °C/min. *a* **5a**; *b* **5b**; *c* **8a**; *d* **8b**; *e* GG-236MC

236MC, suggesting that the triazole between hydrophilic and hydrophobic blocks did not affect the hydration–dehydration phenomena obviously.

## Conclusions

This paper represents the new synthesis method based on 'click chemistry' for well-defined 1,2,3-triazole-linked diblock MC analogues to unravel a key structure for the thermoreversible gelation of diblock copolymers consisting of only sugar chains in both hydrophobic and hydrophilic blocks. Huisgen 1,3-dipolar cycloaddition of peracetyl glycosyl azides of cellobiose and low *DP* cellulose with terminal alkyne derivatives converted from 2,3,6-tri-*O*-methyl-cellulose afforded the desired 1,2,3-triazole-linked diblock copolymers successfully. 'Click chemistry' was capable of reacting a polymeric terminal alkyne with peracetylated cellulosyl azide having $DP_n$ = ca. 7.0. Our synthetic method via "click chemistry" made it possible not only to require less amount of a hydrophilic building block, peracetyl cellobiosyl azide, but also to link a hydrophilic cello-oligosaccharide block having $DP_n$ of ca. 7.0 with a hydrophobic 2,3,6-tri-*O*-methyl-cellulose block compared to glycosylation reaction. Consequently, 2.0 wt% aqueous solutions of the resultant diblock copolymers consisting of

hydrophilic cellobiose and low *DP* cellulose and hydrophobic sequence of 2,3,6-tri-*O*-methyl-cellulosyl blocks formed hydrogels at r.t. DSC measurements revealed that the triazole unit between hydrophilic and hydrophobic blocks of such 1,2,3-triazole-linked diblock copolymers had almost no influence on endo- and exothermic peak temperatures caused by dehydration and hydration, respectively. Our results demonstrated that the diblock MC structure of hydrophilic unmodified cello-oligosaccharides and a hydrophobic 2,3,6-tri-*O*-methyl-cellulose having $DP_n$ of 20–30 is sufficient condition for the thermoreversible gelation of aqueous MC solution. The synthetic strategy using click reaction reported in this paper should help not only in controlling the thermal properties of diblock MC analogues, but also in understanding the relationship between structure of commercial MC and thermoresponsive property.

## Experimental details

### Materials

SM-400 methylcellulose sample (methoxyl content: 27.5–31.5 %; viscosity of 2 % aqueous solution: 400 mPa s) was kindly provided by Shinetsu Chemical Co., Japan. The products were purified on silica

gel column chromatography (Wakogel C-200, Wako Pure Chemical Industries or Silica Gel 60 N [spherical, neutral], 100–210 μm, Kanto Chemical Co.).

General measurements

$^1$H- and $^{13}$C-NMR spectra were recorded with a Varian Inova 500 FT-NMR (500 MHz) spectrometer in chloroform-$d$ with tetramethylsilane as an internal standard or in deuterium oxide with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as an external standard. Chemical shifts ($\delta$) and coupling constants ($J$) are given in $\delta$-values (ppm) and Hz, respectively. Matrix assisted laser desorption/ionization time-of-flight mass (MALDI-TOF MS) spectra were recorded with a Bruker MALDI-TOF MS Autoflex III in the positive ion and linear modes. For ionization, a nitrogen laser was used. All spectra were measured in the linear mode using external calibration. MALDI-TOF MS spectra were measured with 2,5-dihydroxy-benzoic acid (DHB) as a matrix. A Shimadzu liquid chromatography injector (LC-10ATvp), a Shimadzu column oven (CTO-10Avp), a Shimadzu ultraviolet visible detector (SPD-10Avp), a Shimadzu refractive index detector (RID-10A), a Shimadzu communication bus module (CBM-10A), a Shimadzu LC workstation (CLASS-LC10), and Shodex columns (KF802, KF802.5, and KF805) were used. Number and weight averaged molecular weights ($M_n$, $M_w$) and polydispersity indices ($M_w/M_n$) were estimated using polystyrene standards (Shodex). A flow rate of 1 mL/min at 40 °C was chosen. Chloroform was used as eluent.

Methyl 2,3,6-tri-$O$-methyl celluloside having a propargyl group at C-4 of non-reducing end (2)

Methyl 2,3,6-tri-$O$-methyl celluloside (1) was synthesized from industrially produced MC (SM-400) according to the procedure in the literature (Nakagawa et al. 2011b). To a solution of compound (1) (1.0171 g, 4.98 mmol) in DMF (40 mL), NaH (2.4898 g, 62.2 mmol, 60 % in mineral oil) was added at r.t. Then 3-bromopropyne (4.5 mL, 60.1 mmol) was added into the solution at 0 °C. The reaction mixture was stirred at 70 °C for 48 h. MeOH (2.0 mL) was added to the reaction mixture, and then the mixture was poured slowly into MeOH (200 mL). The resulting precipitates were washed with MeOH (400 mL) and $n$-hexane (100 mL) and dried to give compound

2a (0.5502 g, 54 % yield), 2b (56 % yield). Compound 2a: $^1$H-NMR (CDCl$_3$): $\delta$ 2.46 (CH$_2$C≡C$H$), 2.95 (H-2), 3.22 (H-3), 3.30 (H-5), 3.39 (C6–OC$H_3$), 3.54 (C2–OC$H_3$), 3.58 (C3–OC$H_3$), 3.62–3.66 (H-6a), 3.69 (H-4), 3.72–3.81 (H-6b), 4.33 (H-1), 4.29–4.43 (m, C$H_2$C≡CH). MALDI-TOF MS (positive linear mode; DHB as matrix): the $m/z$ with starting mass: $DP = 7$, Calcd. = 1,498; Found [M + Na]$^+$ = 1,521, the increment between $DP_n$ and $DP_{n+1}$: 204, the range of detected compounds: $DP = 7$–34.

Peracetylated glycosyl azides

Peracetylated glycosyl azides of cellobiose and low-molecular-weight cellulose were synthesized from cellobiose and cellulose, respectively, according to the literature (Kamitakahara and Nakatsubo 2005; Kamitakahara et al. 2005).

General method for Huisgen 1,3-dipolar cycloaddition

To a solution of peracetylated glycosyl azide (2.0 equiv.) and methyl 2,3,6-tri-$O$-methyl celluloside having terminal alkyne at C-4 of reducing end (1.0 equiv.) in MeOH/CH$_2$Cl$_2$ (2 mL, 1/4, v/v), Cu (I) Br (10 equiv.), sodium ascorbate (20 equiv. 4 M in H$_2$O), and PMD-ETA (10 equiv.) were added at r.t. The reaction mixture was stirred at r.t. for 24 h. After 24 h, the mixture was passed through silica gel chromatography column eluted with 20 % MeOH/CH$_2$Cl$_2$ to give a crude product. The crude product was purified by silica gel column chromatography (eluent: EtOAc → 20 % MeOH/CH$_2$Cl$_2$) to give a reaction product.

Compound 4 (85 % yield): $^1$H-NMR (CDCl$_3$): $\delta$ 1.87–2.10 (OCOC$H_3$), 2.95 (H-2d), 3.21 (H-3d), 3.29 (H-5d), 3.38 (C6–OC$H_3$), 3.65–3.78 (H-6d), 3.69 (H-4d), 3.88–3.96 (H-4b, H-5b), 4.05–4.51 (H-6a, H-6b), 4.34 (H-1d), 4.56 (H-1a), 4.75–4.95 (H-7), 4.93 (H-2a), 5.09 (H-4a), 5.17 (H-3a), 5.38 (H-3b), 5.43 (H-2b), 5.82 (H-1b), 7.69 (H-9). MALDI-TOF MS (positive linear mode; DHB as matrix): the $m/z$ with starting mass: $DP = 11$, Calcd. = 2,568; Found [M + Na]$^+$ = 2,590, the increment between $DP_n$ and $DP_n + 1$: 204, the range of detected compounds: $DP = 11$–35.

Compound 7 (72 % yield): $^1$H-NMR (CDCl$_3$): $\delta$ 1.86–2.14 (OCOC$H_3$), 2.95 (H-2d), 3.21 (H-3d), 3.29 (H-5d), 3.38 (C6–OC$H_3$), 3.62–3.77 (H-6d), 3.69

(H-4d), 3.88–3.94 (H-4c, H-5c), 4.02–4.52 (H-6b, H-6c), 4.34 (H-1d), 4.42 (H-1b), 4.74–4.97 (H-7), 4.82 (H-2b), 5.03–5.17 (H-3b, H-4b), 5.34–5.43 (H-2c, H-3c), 5.81 (H-1c), 7.70 (H-9).

## General method for deacetylation

To a solution of acetylated MC analogue in MeOH (1 mL) and THF (1 mL), NaOMe (10 equiv. per AGU) were added at r.t. The mixture was stirred at r.t. overnight. The solution was neutralized with DOWEX $H^+$. DOWEX $H^+$ was filtered off and washed with 20 % MeOH/$CH_2Cl_2$. Combined filtrate and washings were concentrated to dryness to give diblock MC analogue.

Compound **5** (99 % yield): $^1$H-NMR ($D_2O$): $\delta$ 3.14 (H-2d), 3.40 (C6–OC$H_3$), 3.45 (H-3d), 3.56 (C2–OC$H_3$), 3.58 (C3–OC$H_3$), 3.62–3.96 (H-4d, H-5d, H-6d), 4.42 (H-1d), 8.31 (H-9). MALDI-TOF MS (positive linear mode; DHB as matrix): the $m/z$ with starting mass: $DP = 7$, Calcd. $= 1,457$; Found $[M + Na]^+ = 1,480$, the increment between $DP_n$ and $DP_n + 1$: 204, the range of detected compounds: $DP = 7–32$.

Compound **8** (96 % yield): $^1$H-NMR ($D_2O$): $\delta$ 3.14 (H-2d), 3.41 (C6–OC$H_3$), 3.45 (H-3d), 3.57 (C2–OC$H_3$), 3.59 (C3–OC$H_3$), 3.65–3.96 (H-4d, H-5d, H-6d), 4.43 (H-1d), 8.31 (H-9).

## Differential scanning calorimetry (DSC) measurement

DSC thermograms were recorded on a DSC823$^e$ (Mettler Toledo, Zurich, Switzerland) under nitrogen atmosphere during a heating/cooling cycle (0 → 90 → 0 °C) with a heating and cooling rate 3.5 °C/min. Each temperature cycle was sequentially repeated three times in order to ensure and check the reproducible response of the instrument.

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# 2-O-Methyl- and 3,6-Di-O-methyl-cellulose from Natural Cellulose: Synthesis and Structure Characterization

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**ABSTRACT:** For the first time, 2-O-methyl- (2MC) and 3,6-di-O-methyl-cellulose (36MC) were synthesized via 3-O-allyl- and 3-O-methyl-cellulose, respectively. Position 6 of 3-O-allyl- and 3-O-methyl-cellulose was protected with the 4-methoxytrityl groups. The reaction time and temperature were optimized to achieve a high regioselectivity at C-6 and to prevent the introduction of the 4-methoxytrityl group at C-2 of the polymer. It was found that the substituent at C-3 of 3-O-functionalized celluloses influenced the reactivity of the hydroxyl group at C-6. The structure was characterized by means of $^1$H and $^{13}$C NMR spectroscopy of the acetates of 2MC and 36MC. 2MC and 36MC were soluble in water and did not show thermoreversible gelation.
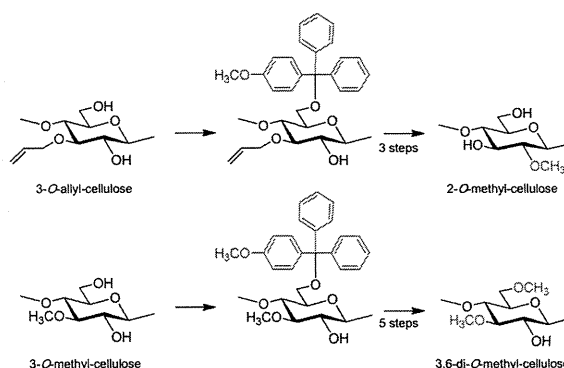
## INTRODUCTION

Regioselective functionalization of hydroxyl groups of polysaccharides still remains difficult and challenging. The regioselective synthesis of polysaccharide derivatives is of crucial importance to understand their structure–property relationships in detail.[1] To facilitate regioselective conversion, the use of the protecting groups is the most important approach today.[2] In cellulose chemistry, the substituent distribution of cellulose derivatives within the anhydroglucopyranose unit (AGU) and along the cellulose chain has a decisive influence on their properties.[3]

Methylcellulose (MC) is one of the most important cellulose ethers from the viewpoint of both application and basic research.[1] MCs are widely used as thickeners for foods and cosmetics, water binders for ceramic and cement, and film forming agents due to the water solubility, surface activity, and thermal gelation ability.[4] MCs are recognized as acceptable food additives by the U.S. Food and Drug Administration.[5] In addition, it has been reported that tetrasaccharide derivatives having methyl groups with a diblock structure were less toxic to human cells than octyl β-D-glucopyranoside, a commonly used surfactant.[6] Recently, MC has become even more interesting as a biomaterial for various applications in the biomedical and pharmaceutical areas due to the physiological inertness and the storage stability.[7,8] Usually, commercial MCs are synthesized starting from alkali cellulose in a slurry process using methyl chloride as an alkylating agent. Therefore, the commercial MCs are heterogeneous polymers consisting of highly substituted and less-substituted zones.[9] The structure–property relationship of MC with a heterogeneous functionalization pattern is complicated because MC can be regarded as a random copolymer consisting of one unsubstituted and seven substituted (2-O-, 3-O-, 6-O-, 2,3-di-O-, 2,6-di-O-, 3,6-di-O-, 2,3,6-tri-O-methyl) anhydroglucopyranose units and these eight anhydroglucopyranose units are sequenced at random. Thus, model compounds of commercial MC having a well-defined structure were required to investigate the substituent distribution–property relationships in detail.

There have been reports on the synthesis of regioselectively methylated cellulose derivatives from natural cellulose, 3-O-,[10] 6-O-,[11] 2,3-di-O-,[12–14] 2,6-di-O-,[15] 2,3,6-tri-O-methylcelluloses,[16] and their properties such as solubility in water and common organic solvents, thermal aggregation behavior, and thermoreversible gelation. Furthermore, they were used for understanding of relationships between inter- and intramolecular hydrogen bonds and the related properties like solubility and crystallinity. On the contrary, Karakawa et al. reported regiospecifically methylated cellulose derivatives that were synthesized via polymerization of glucose orthopivalate, and their solubilities in water as well as their crystallinities were investigated.[17] Regioselectively methylated cellulose derivatives from natural cellulose inevitably contain substructures to a low extent. However, the comparison of regioselectively methylated cellulose derivatives from natural cellulose with those from synthetic cellulose derivatives had provided the essential knowledge about the structure–property relationships of industrially produced MC.[15] It is therefore of crucial

importance to obtain all seven regioselectively methylated cellulose derivatives from natural cellulose.

Note that there have been no reports on the synthesis of 3,6-di-O-methyl-cellulose (36MC) from natural cellulose, while Sugihara et al. reported in 1949 that alkali cellulose after removal of water by coevaporation with butanol was reacted with MeI in a bomb tube at 100 °C to afford 2-O-methyl-cellulose (2MC) having 1.03 of DS and 88 of DP.[18] However, no NMR data were shown in the old Sugihara report. Furthermore, Hess et al.[19] reported that MC having a heterogeneous distribution of methyl groups within AGU was obtained from the alkali cellulose after the same pretreatment, as described by Sugihara et al.[18]

This paper describes the first synthesis of 36MC via 3-O-methyl-cellulose derived from natural cellulose and the new synthetic route for 2MC starting from 3-O-allyl-cellulose.

## ■ EXPERIMENTAL SECTION

**Materials.** 3-O-Allyl- (1) and 3-O-methyl-cellulose (6) were synthesized according to the method of Koschella et al. via 2,6-di-O-thexyldimethylsilyl-cellulose from Avicel and cotton liners.[10] ^{13}C NMR spectra of compounds 1 and 6 were measured in DMSO-$d_6$. The carbon resonances of compounds 1 and 6 were assigned according to the literature.[10] The DS values of allyl and methyl groups of compounds 1 and 6 were determined by ^{1}H NMR spectroscopy after peracetylation.

**Measurements.** Fourier transform infrared (FT-IR) spectra were recorded on a FTIR-4000 spectrophotometer (Shimadzu, Japan). Samples were mixed with KBr and pressed into disks. [1]H, ^{13}C, and two-dimensional NMR spectra were recorded on a Varian INOVA 500 FT-NMR (500 MHz) spectrometer in CDCl$_3$ with tetramethylsilane (TMS) as an internal standard. Chemical shifts ($\delta$) are reported in ppm. A Shimadzu SEC system (CBM-10A, SPD-10A, SIL-10A, LC-10AT, FCV-10AL, CTO-10A, RID-10A, and FRC-10, Shimadzu, Japan) and Shodex columns (K802, K802.5, and K805) were used. Number and weight averaged molecular weights ($M_n$, $M_w$) and polydispersity indices ($M_w/M_n$) were estimated using polystyrene standards (Shodex). A flow rate of 1 mL/min at 40 °C was chosen. Chloroform was used as eluent.

**Synthesis of 2-O-Methyl-cellulose.** *3-O-Allyl-cellulose (1).* ^{13}C NMR (DMSO-$d_6$, 296 K): $\delta$ 60.1 (C-6), 72.8 (-CH$_2$CH=CH$_2$), 74.5 (C-2), 74.9 (C-5), 76.5 (C-4), 83.1 (C-3), 102.8 (C-1), 115.2 (-CH$_2$CH=CH$_2$), 136.6 (-CH$_2$CH=CH$_2$). DS$_{allyl}$ = 1.04 (determined by ^{1}H NMR spectroscopy after peracetylation).

*6-O-(4-Methoxytrityl)-3-O-allyl-cellulose (2).* 3-O-Allyl-cellulose (1, 113.9 mg, 0.56 mmol) was suspended in 2.5 mL of DMAc and stirred at 130 °C for 2 h. After the slurry was allowed to cool to 100 °C, anhydrous LiCl (162.5 mg) was added. Compound 1 dissolved completely during cooling to room temperature under constant stirring. Pyridine (0.2 mL, 4.5 equiv) was added to this solution, followed by adding 4-methoxytrityl chloride (536 mg, 3.0 equiv). The reaction mixture was stirred at 70 °C. After 4 h reaction time, the mixture was poured into MeOH (50 mL). The precipitate was filtered off, washed with MeOH (500 mL), and dried to a constant weight in vacuo while increasing the temperature to 60 °C (0.2227 g, 83.3% yield).

^{1}H NMR (CDCl$_3$): $\delta$ 2.64–4.66 (AGU, CH$_2$CH=CH$_2$), 3.71 (OCH$_3$-trityl), 4.95 (CH$_2$CH=CH$_2$), 5.70 (CH$_2$CH=CH$_2$), 6.58–7.69 (aromatic H).

*3-O-Allyl-6-O-(4-methoxytrityl)-2-O-methyl-cellulose (3).* 6-O-(4-Methoxytrityl)-3-O-allyl-cellulose (2, 0.2216 g, 0.46 mmol) was suspended in DMF (10 mL) under anhydrous conditions. NaH (193.4 mg, 10.0 equiv) was added to the slurry at 0 °C, followed by MeI (0.3 mL, 10.0 equiv). The mixture was stirred for 1 day at room temperature and further 3 days at 50 °C. The reaction mixture was poured into distilled water (150 mL). The precipitate was collected, washed with distilled water (400 mL) and MeOH (150 mL), and dried

to a constant weight in vacuo while increasing the temperature to 60 °C (0.1983 g, 91% yield).

^{1}H NMR (CDCl$_3$): $\delta$ 2.50–4.45 (AGU-protons, -CH$_2$CH=CH$_2$), 3.28 (C-2-OCH$_3$), 3.77 (-OCH$_3$, 4-methoxytrityl), 5.02 (-CH$_2$CH=CH$_2$), 5.78 (-CH$_2$CH=CH$_2$), 6.49–7.72 (aromatic H).

FT-IR (KBr): 3058 (=C−H), 2931, 2835 (C−H), 1645 (C=C), 1606, 1510 (arom C−C), 1251 (PhOCH$_3$), 833 (arom C−H).

*3,6-Di-O-acetyl-2-O-methyl-cellulose (4).* To a solution of 3-O-allyl-6-O-(4-methoxytrityl)-2-O-methyl-cellulose (3, 0.1917 g, 0.39 mmol) in MeOH (1 mL) and CHCl$_3$ (4 mL), PdCl$_2$ (20 mg) was added. After 24 h, the reaction mixture was concentrated to dryness. The dried product was treated with acetic anhydride (1 mL), pyridine (1 mL), and DMAP (16.7 mg) at 80 °C for 24 h. The reaction mixture was poured into EtOH. The precipitate was collected, washed with EtOH (50 mL) and n-hexane (10 mL), and dried to a constant weight in vacuo while increasing the temperature to 60 °C (85.5 mg, 83.7% yield in two steps).

^{1}H NMR (CDCl$_3$): $\delta$ 2.02, 2.11 (OCOCH$_3$), 3.01 (H-2), 3.42 (C-2-OCH$_3$), 3.52 (H-5), 3.60 (H-4), 4.18 (H-6b), 4.24 (H-1), 4.52 (H-6a), 5.03 (H-3). ^{13}C NMR (CDCl$_3$): $\delta$ 20.8 (OCOCH$_3$), 60.8 (C-2-OCH$_3$), 62.3 (C-6), 72.9 (C-5), 73.5 (C-3), 76.3 (C-4), 81.3 (C-2), 102.6 (C-1), 170.1 (C=O).

*2-O-Methyl-cellulose (5).* To a solution of 3,6-di-O-acetyl-2-O-methyl-cellulose (4, 86.5 mg) in MeOH (1 mL) and CHCl$_3$ (4 mL), 28% NaOMe in MeOH (0.27 mL) was added. The reaction mixture was stirred at room temperature for 24 h and concentrated to dryness. The crude product was dialyzed against distilled water for a week, and then lyophilized to give purified product 5 (48.6 mg, 83% yield).

^{1}H NMR (D$_2$O): $\delta$ 3.07 (H-2), 3.56 (C-2-OCH$_3$), 3.60 (H-5), 3.64 (H-3, H-4), 3.78 (H-6b), 3.94 (H-6a), 4.53 (H-1). ^{13}C NMR (D$_2$O): $\delta$ 62.5 (C-6), 63.3 (C-2-OCH$_3$), 76.4 (C-3), 77.6 (C-5), 80.9 (C-4), 85.3 (C-2), 104.9 (C-1)

**Synthesis of 3,6-Di-O-methyl-cellulose.** *3-O-Methyl-cellulose (6).* ^{13}C NMR (DMSO-$d_6$/LiCl, 333K): $\delta$ 59.64 (C-3-OCH$_3$), 60.81 (C-6), 74.59 (C-2), 75.66 (C-5), 77.52 (C-4), 85.47 (C-3), 103.47 (C-1). DS$_{Me}$ = 1.06 (determined by ^{1}H NMR spectroscopy after peracetylation).

*6-O-(4-Methoxytrityl)-3-O-methyl-cellulose (7).* 3-O-Methyl-cellulose (6, 0.638 g, 3.62 mmol) was suspended in DMAc (16 mL) and stirred at 130 °C for 2 h. After the slurry was allowed to cool to 100 °C, anhydrous LiCl (1.05 g) was added. Compound 6 dissolved completely during cooling to room temperature under constant stirring. Pyridine (1.31 mL, 16.2 mmol, 4.5 equiv) was added to this solution, followed by adding 4-methoxytrityl chloride (3.354 g, 10.8 mmol, 3.0 equiv). The reaction mixture was stirred at 50 °C. After a 4 h reaction time the mixture was poured into MeOH (150 mL). The precipitate was filtered off, washed with MeOH (800 mL), and concentrated to dryness to give products (1.533 g, 94.4% yield).

^{1}H NMR (CDCl$_3$): $\delta$ 2.71–4.25 (AGU-protons), 3.59 (C-3-OCH$_3$), 3.71 (-OCH$_3$, 4-methoxytrityl), 6.59–7.84 (aromatic H).
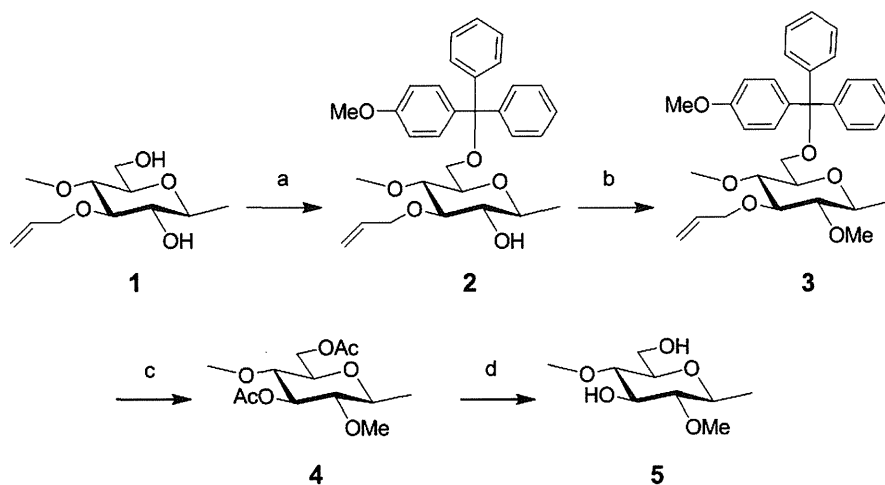
*2-O-Allyl-6-O-(4-methoxytrityl)-3-O-methyl-cellulose (8).* 6-O-(4-Methoxytrityl)-3-O-methyl-cellulose (7, 1.516 g, 3.38 mmol) was suspended in DMF (30 mL) under anhydrous conditions. NaH (1.409 g, 35.2 mmol, 10.0 equiv) was added to the slurry at 0 °C, followed by allyl bromide (3.0 mL, 35.4 mmol, 10.0 equiv). The mixture was stirred for 1 day at room temperature and an additional 3 days at 50 °C. The reaction mixture was quenched by addition of MeOH (1.0 mL) and water (2.0 mL) and poured into distilled water (100 mL). The precipitate was collected, washed with distilled water (700 mL) and MeOH (700 mL), and dried to a constant weight in vacuo while increasing the temperature to 60 °C (1.508 g, 91.2% yield).

^{1}H NMR (CDCl$_3$): $\delta$ 2.51–4.53 (AGU-protons, -CH$_2$CH=CH$_2$), 3.53 (C-3-OCH$_3$), 3.76 (-OCH$_3$, 4-methoxytrityl), 4.89 (-CH$_2$CH=CH$_2$), 5.57 (-CH$_2$CH=CH$_2$), 6.55–7.63 (aromatic H).

FT-IR (KBr, cm$^{-1}$): 3479.0 (OH), 3059, 3029.2, 2932 (arom, C−H), 2836.6 (OCH$_3$, C−H), 1607.9, 1509.5 (arom, C−C), 1251.3 (PhOCH$_3$), 1075.7, 1039.7 (C−O−C$_{AGU}$), 831.8 (arom, C−H).

*2-O-Allyl-3-O-methyl-cellulose (9).* To a solution of 2-O-allyl-6-O-(4-methoxytrityl)-3-O-methyl-cellulose (8, 1.484 g, 3.04 mmol) in CH$_2$Cl$_2$ (75 mL), FeCl$_3$ 6H$_2$O (2.494 g, 9.22 mmol, 3.0 equiv) was

**Figure 1.** Synthetic route for 2-O-methyl-cellulose (**5**): (a) 4-methoxytrityl chloride/pyridine/DMAc/LiCl/4 h/70 °C; (b) MeI/NaH/DMF/1 day/rt, 3 days/50 °C; (c) PdCl₂/MeOH/CHCl₃/rt/24 h; Ac₂O/pyridine/DMAP/80 °C/24 h; (d) NaOMe in MeOH/MeOH/THF/rt/24 h.

added. The mixture was stirred for 1 h at room temperature. The precipitates were washed with CH$_2$Cl$_2$ (250 mL) and distilled water (100 mL) to give product **9** (0.611 g, 92.9%).

*6-O-Acetyl-2-O-allyl-3-O-methyl-cellulose (10).* 2-O-Allyl-3-O-methyl-cellulose (**9**, 38.2 mg, 0.17 mmol) was treated with acetic anhydride (1.0 mL) and pyridine (1.0 mL) in the presence of DMAP (19.2 mg) at 90 °C for 24 h. The reaction mixture was poured into distilled water (20 mL). The precipitate was collected, washed with distilled water, n-hexane, and EtOH, and dried to a constant weight in vacuo while increasing the temperature to 60 °C (35.0 g, 76.7% yield).

$^1$H NMR (CDCl$_3$): δ 2.05 (OCOCH$_3$), 3.10 (H-2), 3.24 (H-3), 3.52 (C-3-OCH$_3$), 3.33–3.75 (H-4, H-5, H-6), 4.22 (–CH$_2$CH= CH$_2$), 4.35 (C-1), 4.52 (–CH$_2$CH=CH$_2$), 5.20 (–CH$_2$CH=CH$_2$), 5.89 (–CH$_2$CH=CH$_2$). $^{13}$C NMR (CDCl$_3$): δ 20.8 (OCOCH$_3$), 60.7 (C-3-OCH$_3$), 62.6 (C-6), 72.8 (C-5), 73.5 (-CH$_2$CH=CH$_2$), 78.4 (C-4), 81.2 (C-2), 84.6 (C-3), 102.7 (C-1), 116.5 (-CH$_2$CH=CH$_2$), 134.7 (-CH$_2$CH=CH$_2$), 170.4 (C=O).

*2-O-Allyl-3,6-di-O-methyl-cellulose (11).* 6-O-Acetyl-2-O-allyl-3-O-methyl-cellulose (**9**, 0.572 g, 2.64 mmol) were suspended in DMF (30 mL). NaH (1.098 g, 27.4 mmol, 10 equiv) was added to the slurry at 0 °C, followed by MeI (1.70 mL, 27.3 mmol, 10 equiv) after 0.5 h. The mixture was stirred for 2 h at room temperature and further 48 h at 50 °C. The mixture was poured into distilled water (200 mL). The precipitate was washed with water (400 mL) and n-hexane (100 mL) and dried in vacuo at 70 °C (0.452 g, 74.0%).

$^1$H NMR (CDCl$_3$): δ 3.11 (H-2), 3.23 (H-3), 3.38 (C-6-OCH$_3$), 3.57 (C-3-OCH$_3$), 3.50–3.90 (H-4, H-5, H-6), 4.22 (-CH$_2$CH= CH$_2$), 4.36 (H-1), 5.20 (-CH$_2$CH=CH$_2$), 5.91 (-CH$_2$CH=CH$_2$). $^{13}$C NMR (CDCl$_3$): δ 59.1 (C$_6$–OCH$_3$), 60.6 (C$_3$–OCH$_3$), 70.2 (C-6), 73.5 (-CH$_2$CH=CH$_2$), 74.9 (C-5), 77.7 (C-4), 81.5 (C-2), 85.0 (C-3), 102.9 (C-1), 116.2 (-CH$_2$CH=CH$_2$), 135.2 (-CH$_2$CH=CH$_2$).

*2-O-Acetyl-3,6-di-O-methyl-cellulose (12).* To a solution of 2-O-allyl-3,6-di-O-methyl-cellulose (**11**, 58.3 mg, 0.25 mmol) in MeOH (1 mL) and CHCl$_3$ (4 mL), PdCl$_2$ (11.5 mg) was added. After 24 h, the reaction mixture was concentrated to dryness. The dried product was treated with acetic anhydride (2 mL), pyridine (2 mL), and DMAP (20.1 mg) at 90 °C for 24 h to give a crude acetylated polymer. The polymer was dissolved in CHCl$_3$ and then poured into n-hexane (80 mL) to precipitate a polymer. The precipitated polymer was collected and purified by chromatography (Sephadex LH-20, eluted with MeOH/CH$_2$Cl$_2$ (1:4 v/v)) to afford compound **12** (52.4 mg, 89.1% yield in two steps).

$^1$H NMR (CDCl$_3$): δ 2.10 (OCOCH$_3$), 3.27 (H-5), 3.31 (H-3), 3.38 (C-6-OCH$_3$), 3.45 (C-3-OCH$_3$), 3.52–3.66 (H-6), 3.78 (H-4), 4.47 (H-1), 4.83 (H-2). $^{13}$C NMR (CDCl$_3$): δ 21.0 (OCOCH$_3$), 59.3 (C-6-OCH$_3$), 59.9 (C-3-OCH$_3$), 69.9 (C-6), 72.9 (C-2), 74.8 (C-5), 76.3 (C-4), 82.6 (C-3), 100.6 (C-1), 169.4 (C=O).

*3,6-Di-O-methyl-cellulose (13).* To a solution of 2-O-acetyl-3,6-di-O-methyl-cellulose (**12**, 52.4 mg, 0.22 mmol) in MeOH (1 mL) and CHCl$_3$ (4 mL), 28% NaOMe in MeOH (0.5 mL) was added. The reaction mixture was stirred at room temperature for 24 h and concentrated to dryness. The crude product was dialyzed against distilled water for three days and then lyophilized to give purified product **13** (40.8 mg, 95.1% yield).

$^1$H NMR (D$_2$O): δ 3.33 (H-2), 3.34 (C-6-OCH$_3$), 3.35 (H-3), 3.50 (C-3-OCH$_3$), 3.51 (H-5), 3.70 (H-4), 3.72 (H-6), 4.34 (H-1). $^{13}$C NMR (D$_2$O): δ 61.0 (C-6-OCH$_3$), 61.4 (C-3-OCH$_3$), 72.6 (C-6), 75.0 (C-2), 76.3 (C-5), 78.2 (C-4), 85.9 (C-3), 105.2 (C-1)

## ■ RESULTS AND DISCUSSION

The synthesis route via 4-methoxytritylation of the primary hydroxyl group of 3-O-allyl- (**1**)[10] and 3-O-methyl-cellulose (**6**)[10] and subsequent etherification of secondary hydroxyl group was considered to be achievable to synthesize 2MC and 36MC from cellulose via 2,6-di-O-thexyldimethylsilyl-cellulose. In addition, the etherification such as methylation or allylation of the cellulose derivatives having 4-methoxytrityl groups at C-6 was expected to proceed under the reaction conditions reported in the literature.[11,20]

Gomez et al. reported the 4-methoxytritylation of cellulose under homogeneous reaction conditions.[20] 4-Methoxytrityl chloride reacts 10 times faster with cellulose compared to the unsubstituted trityl chloride, and the regioselectivity at C-6 of cellulose is high in DMAc/LiCl within 4 h at 70 °C (DS = 1.03). The overtritylation at secondary hydroxyl groups has been reported in the literatures concerning 4-methoxytritylation of cellulose.[12,20] 4-Methoxytritylation of 3-O-functionalized celluloses (**1**) and (**6**) was therefore carried out under different reaction conditions to prevent 4-methoxytritylation at C-2. The overtritylation at C-2 of 3-O-functionalized celluloses resulted in the production of derivatives having acetyl groups at C-2 after detritylation and subsequent acetylation.

**Synthesis of 2-O-Methyl-cellulose (5).** According to the synthetic route in Figure 1, 2-O-methyl-cellulose (**5**) was synthesized starting from 3-O-allyl-cellulose (**1**).[10] The best condition for 4-methoxytritylation of compound **1** was found to be a conversion at 70 °C/4 h. Compound **1** was allowed to react with 3.0 equiv of 4-methoxytrityl chloride and 4.5 equiv of pyridine in DMAc/LiCl at 70 °C for 4 h to give compound **2a** in 83.3% yield. In the case of 50 °C/4 and 24 h, the isolated yield was low (50 °C/4 h, 28% yield; 50 °C/24 h, 30% yield).
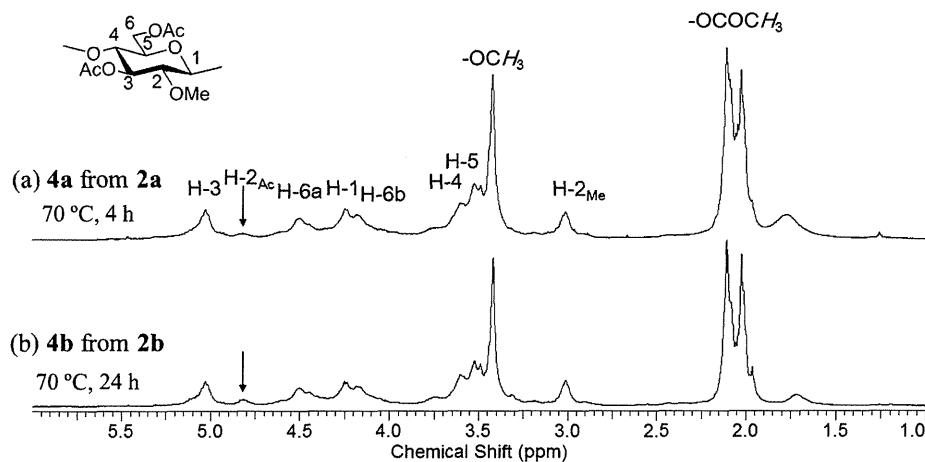
Figure 2. $^{1}$H NMR spectra of compounds **4a** (a) and **4b** (b) measured in CDCl$_3$.

Compound **2** was soluble in CHCl$_3$, but a 1.0 wt % CHCl$_3$ solution became a gel within 12 h. Nevertheless, a $^{1}$H NMR spectrum of compound **2** dissolved in CDCl$_3$ could be acquired. The methylation of the residual hydroxyl groups of compound **2** proceeded with MeI and NaH in DMF for 1 day at rt and for 3 days at 50 °C to afford 3-O-allyl-6-O-(4-methoxytrityl)-2-O-methyl-cellulose (**3**) in 91.0% yield. FT-IR spectroscopy of compound **3** showed no absorption of the OH vibration in the range around 3400 cm$^{-1}$, indicating the complete methylation of the residual hydroxyl groups of compound **2**.

We investigated the removal of 4-methoxytrityl groups of compound **3** using p-toluenesulfonic acid monohydrate[21] and concentrated HCl (35%).[12] Removal of 4-methoxytrityl groups of compound **3** using p-toluenesulfonic acid monohydrate in CHCl$_3$ at rt for 3 h gave a deprotected product, 2-O-allyl-3-O-methyl-cellulose in a good yield of 47.6%. Detritylation of compound **3** using concentrated HCl (35%) in THF at rt for 4 h provided a product that is insoluble in common organic solvents such as MeOH, EtOH, CH$_2$Cl$_2$, CHCl$_3$, DMSO, DMAc, DMF, and pyridine. Simultaneous detritylation and deallylation of compound **3** proceeded with PdCl$_2$ to give the deprotected product, 2MC, which could be acetylated with acetic anhydride, pyridine, and DMAP at 80 °C for 24 h to give 3,6-di-O-acetyl-2-O-methyl-cellulose (**4**) in 83.7% yield in two reaction steps.

$^{1}$H NMR spectra of compounds **4a** (from **2a**, 70 °C/4 h) and **4b** (from **2b**, 70 °C/24 h) showed small differences in substitution patterns, as shown in Figure 2. The H-2 resonances in the case of methylation at C-2 appeared at around 3.01 ppm, while those in the case of acetylation at C-2 were observed at around 4.82 ppm. The $^{1}$H NMR spectra of compounds **4a** and **4b** indicate the overtritylation at C-2. DS values of the methyl group at C-2 of compounds **4a** and **4b** were 0.82 and 0.77, respectively, as shown in Table 1. Compound **4a** shows an improved regioselectivity at C-2 and C-6 positions compared to compound **4b**.

The $^{13}$C NMR spectrum of compound **4** measured in CDCl$_3$ is shown in Figure 3 and the chemical shifts were summarized in Table 2. DS and DP$_n$ values of compound **4** were summarized in Table 3. The resonances of triphenylmethyl- and allyl groups disappeared, indicating complete removal of 4-methoxytrityl and allyl groups. Furthermore, no resonance in the case of acetylation at C-3 was found in the $^{13}$C NMR spectrum of compound **4**.

Table 1. DS Values of Compounds **4a** and **4b**[a]

| compound | reaction time for 4-methoxy tritylation (h) | total DS$_{Me}$ | DS$_2$ | DS$_3$ | DS$_6$ |
|---|---|---|---|---|---|
| | | | distribution of substitution | | |
| **4a** | 4 | 0.84 | 0.82 | 0.00 | 0.02 |
| **4b** | 24 | 0.87 | 0.77 | 0.00 | 0.10 |

[a]DS values were calculated by means of NMR spectroscopy.

Acetyl groups of compound **4** were removed using NaOMe to give a crude product that was dialyzed against distilled water for a week, and then lyophilized to afford 2MC (**5**) in 83% yield. 2MC (**5**) was soluble in water at ~25 °C and 2.0 wt % and 2.0 wt % aqueous solution did not exhibit thermoreversible gelation. Figure 4 shows $^{1}$H- and $^{13}$C NMR spectra of compound **5** measured in D$_2$O. The proton resonance of methyl groups at C-2 appeared at 3.56 ppm. The resonances of H-1, H-6a, H-6b and H-2 appeared at 4.53, 3.94, 3.78, and 3.07 ppm, respectively. Although the resonances of H-3, H-4, and H-5 overlapped around 3.60 ppm, these resonances were assigned by two-dimensional NMR experiments. The C-1 resonance appeared at 104.9 ppm as sharp signal, while the peaks of the other ring carbon atoms were detected at 62.5 (C-6), 76.4 (C-3), 77.6 (C-5), 80.9 (C-4), and 85.3 (C-2) ppm.

### Synthesis of 3,6-Di-O-methyl-cellulose (13).

Figure 5 shows a synthetic route for 36MC (**13**). 3-O-Methyl-cellulose (**6**) was allowed to react with 3.0 equiv of 4-methoxytrityl chloride and 4.5 equiv of pyridine in DMAc/LiCl at 30, 50, and 70 °C for 4 h. According to the synthetic route depicted in Figure 5, 2-O-allyl-3-O-methyl-cellulose (**9**) was synthesized starting from 6-O-(4-methoxytrityl)-3-O-methyl-cellulose (**7**). Because compound **9** was insoluble in CHCl$_3$, compound **9** was peracetylated to obtain chloroform soluble samples, 6-O-acetyl-2-O-allyl-3-O-methyl-celluloses (**10a−c**, see Table 4), that could be analyzed by NMR spectroscopy.

The H-2 resonances in the case of allylation at C-2 appeared at around 3.10 ppm, while those in the case of acetylation at C-2 were observed at around 4.82 ppm as shown in Figure 6. DS values of the allyl substituents at C-2 of compounds **10a−c** calculated from the signals of the $^{1}$H NMR spectra were 0.51, 0.84, and 0.75, respectively (Table 4). It should be noted that compound **10a** has the highest DS values of acetyl substituents at C-2, resulting from the low regioselectivity during the 4-methoxytritylation. After all, the 4-methoxytritylation of 3-O-
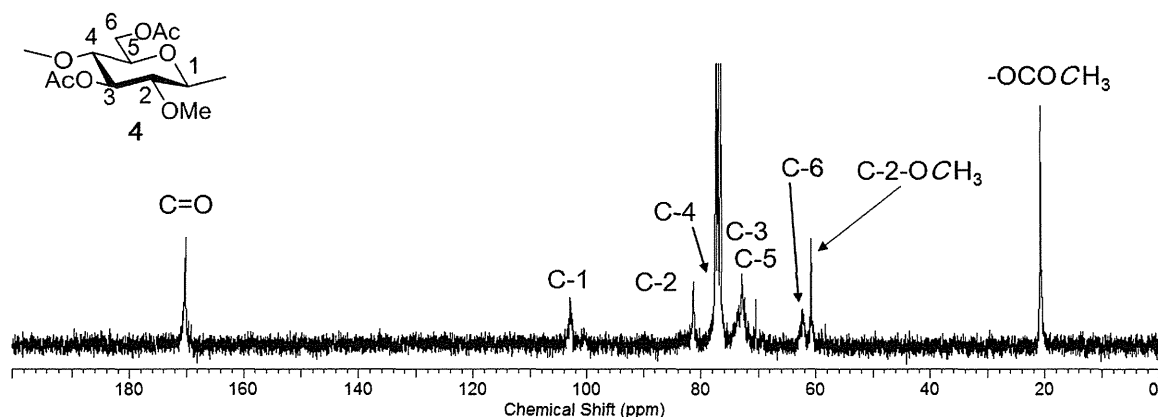
**Figure 3.** $^{13}$C NMR spectrum of compound 4 measured in CDCl$_3$.

**Table 2. Chemical Shifts of Compounds 4 and 12**

| | | | | | | $\delta$ (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $^1$H NMR | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | C-2-OCH$_3$ | C-3-OCH$_3$ | C-6-OCH$_3$ |
| 4 | 4.24 | 3.01 | 5.03 | 3.60 | 3.52 | 4.18, 4.52 | 3.42 | | |
| 12 | 4.47 | 4.83 | 3.31 | 3.78 | 3.27 | 3.52–3.66 | | 3.45 | 3.38 |
| | | | | | | $\delta$ (ppm) | | | | |
| $^{13}$C NMR | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | C-2-OCH$_3$ | C-3-OCH$_3$ | C-6-OCH$_3$ |
| 4 | 102.6 | 81.3 | 73.5 | 76.3 | 72.9 | 62.3 | 60.8 | | |
| 12 | 100.6 | 72.9 | 82.6 | 76.3 | 74.8 | 69.9 | | 59.9 | 59.3 |

**Table 3. DS and DP$_n$ of Compounds 4 and 12$^a$**

| | | distribution of substitution | | | | |
|---|---|---|---|---|---|---|
| compound | total DS$_{Me}$ | DS$_2$ | DS$_3$ | DS$_6$ | DP$_n$ | $M_w/M_n$ |
| 4 | 0.84 | 0.82 | 0.00 | 0.02 | 51.9 | 2.08 |
| 12 | 2.11 | 0.16 | 1.00 | 0.95 | 56.0 | 1.75 |

$^a$DS values were calculated by means of NMR spectroscopy.

methyl-cellulose (6) at 50 °C for 4 h gave the highest regioselectivity at C-6 among the reaction conditions studied.

While 4-methoxytritylation of 3-O-allyl-cellulose (1) at 70 °C for 4 h gave the best regioselectivity, the best reaction condition for 3-O-methyl-cellulose (6) was 50 °C/4 h. Increasing reaction temperature from 50 to 70 °C resulted in 4-methoxytritylation at C-2 of compound 6. These results suggested that steric hindrance of substituent group at C-3 might influence the reactivity of hydroxyl group at C-2. Consequently, it was found that the substituent groups at C-3 of 3-O-functionalized
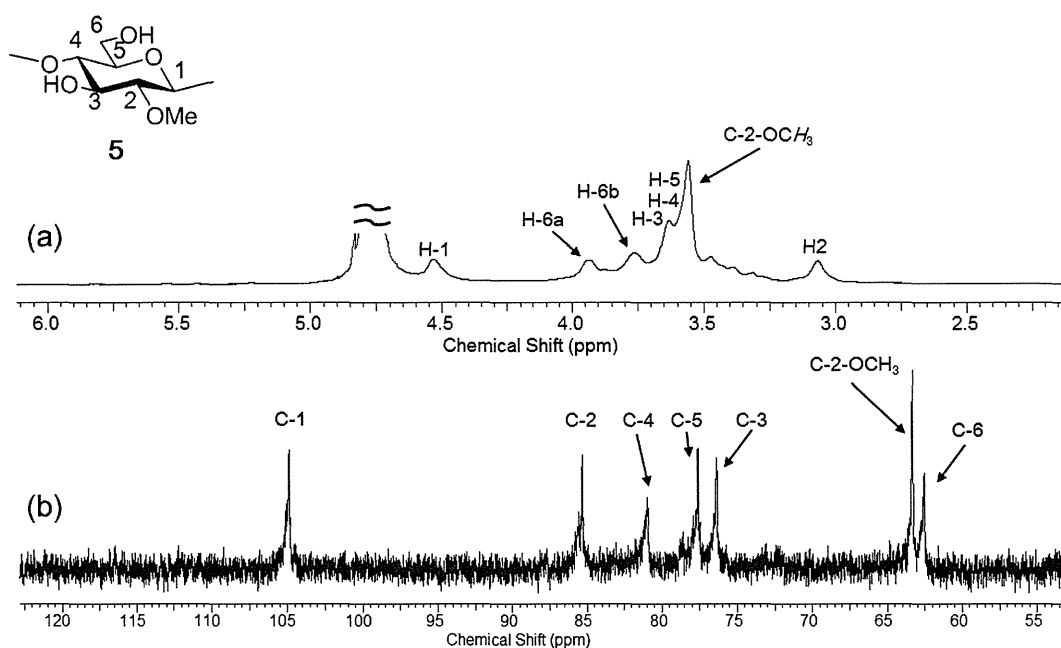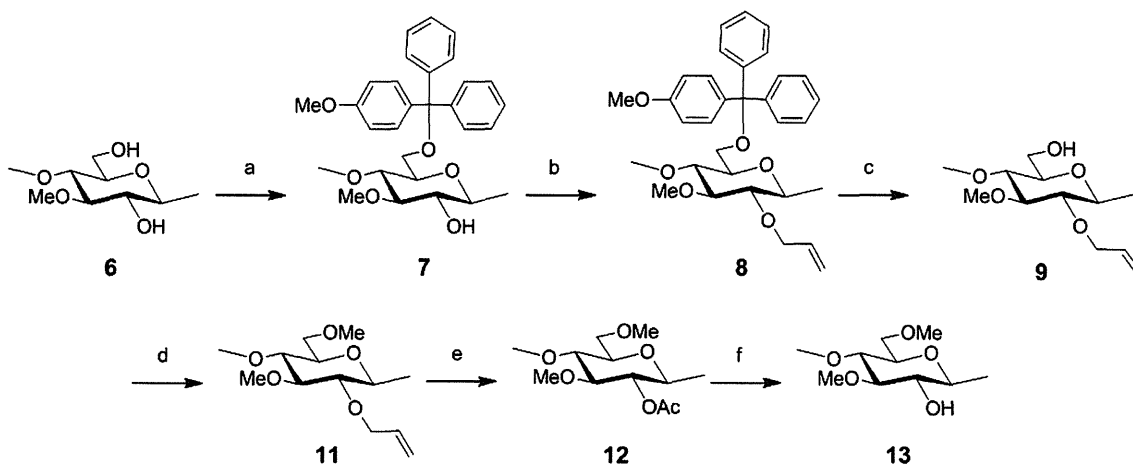


**Figure 4.** $^1$H (a) and $^{13}$C NMR (b) spectra of compound 5 measured in D$_2$O.

**Figure 5.** Synthetic route for 3,6-di-O-methyl-cellulose (**13**): (a) 4-methoxytrityl chloride/pyridine/DMAc/LiCl/4 h/50 °C; (b) allyl bromide/NaH/DMF/1 day/rt/3 days/50 °C; (c) FeCl₃ 6H₂O/CH₂Cl₂/1 h/rt; (d) MeI/NaH/DMF/1 day/rt/3 days/50 °C; (e) PdCl₂/MeOH/CHCl₃/rt/24 h; Ac₂O/pyridine/DMAP/80 °C/24 h; (f) NaOMe in MeOH/MeOH/THF/rt/6 h.

**Table 4. DS Values of Compounds 10a–c[a]**

| Chemical structure | Compound | Reaction condition for 4-methoxy tritylation | Distribution of substitution | | | | |
| | | | 2 | | 3 | 6 | |
| | | | $DS_{All}$ | $DS_{Ac}$ | $DS_{Me}$ | $DS_{All}$ | $DS_{Ac}$ |
|---|---|---|---|---|---|---|---|
| | **10a** | 30 °C, 4 h | 0.51 | 0.49 | 1.00 | 0.49 | 0.51 |
| | **10b** | 50 °C, 4 h | 0.84 | 0.16 | 1.00 | 0.00 | 1.00 |
| | **10c** | 70 °C, 4 h | 0.75 | 0.25 | 1.00 | 0.00 | 1.00 |

[a]DS values were calculated by means of NMR spectroscopy.
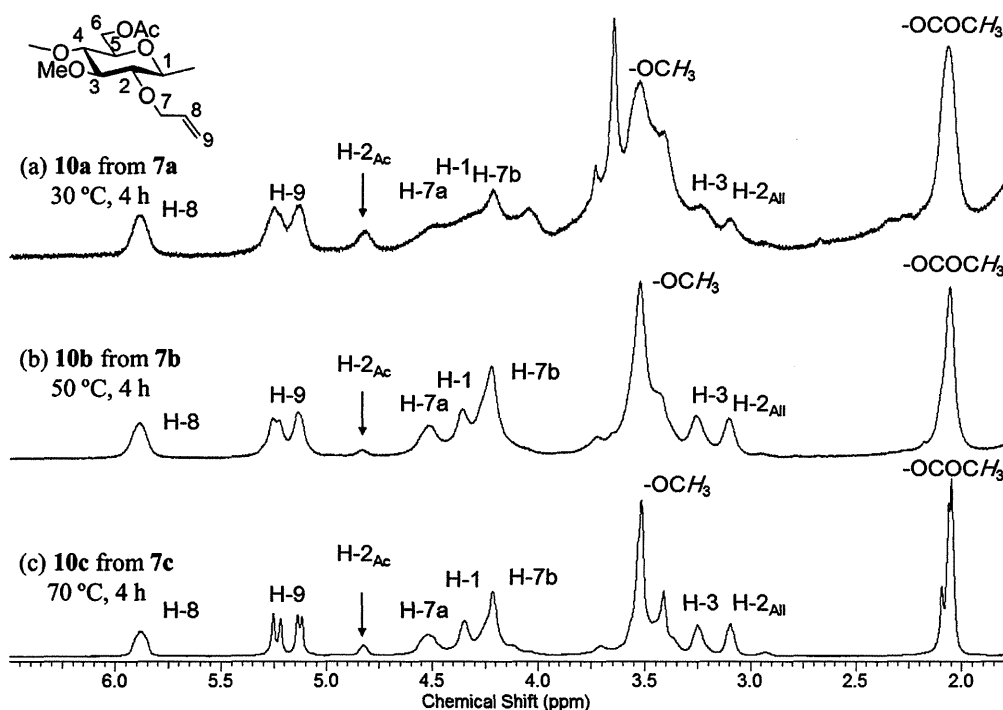


**Figure 6.** ¹H NMR spectra of compound **10a** (a), **10b** (b), and **10c** (c) measured in CDCl₃.

cellulose influenced 4-methoxytritylation not only at C-6 but also at C-2.

Compound **6**[10] was reacted with 4-methoxytrityl chloride in the presence of pyridine in DMAc/LiCl at 50 °C for 4 h to afford compound **7** in 94.4% yield. Compound **7** was soluble in

CHCl₃, but its 1.0 wt % solution in CHCl₃ became a gel within 12 h like compound **2**. However, a ¹H NMR spectrum of compound **7** in CDCl₃ could be acquired.

Subsequent allylation of compound **7** was carried out with allyl bromide in the presence of NaH in DMF for 1 day at rt
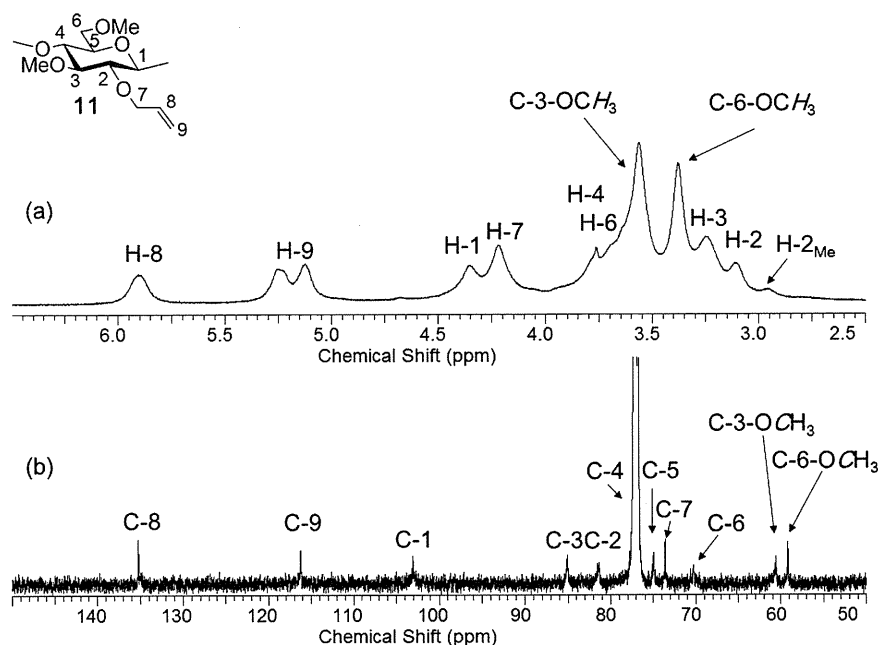
Figure 7. $^1$H (a) and $^{13}$C NMR (b) spectra of compound 11 measured in CDCl$_3$.



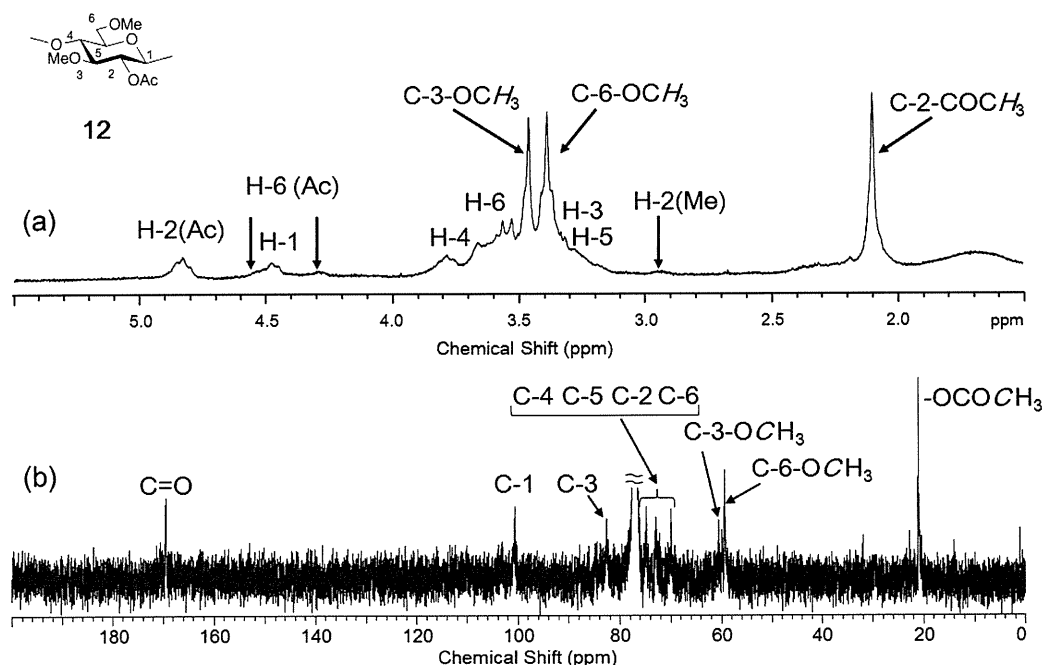Figure 8. $^1$H (a) and $^{13}$C NMR (b) spectra of compound 12 measured in CDCl$_3$.

and for 3 days at 50 °C to give 2-O-allyl-6-O-(4-methoxytrityl)-3-O-methyl-cellulose (8) in a yield of 91.2%. FT-IR spectroscopy of compound 8 showed no absorption of the OH vibration in the range around 3400 cm$^{-1}$, indicating the complete allylation of the residual hydroxyl groups of compound 7.

We also investigated the reaction conditions for the removal of 4-methoxytrityl groups of compound 8 using p-toluenesulfonic acid monohydrate,[21] concentrated HCl (35%),[12] and FeCl$_3$·6H$_2$O.[22,23] In the cases of p-toluenesulfonic acid monohydrate and concentrated HCl, the isolated yield of detritylated products was low (30−40%). In contrast, FeCl$_3$·6H$_2$O afforded the detritylated product, 2-O-allyl-3-O-

methyl-cellulose (9), in a high yield of 92.9%. Compound 8 was treated with FeCl$_3$·6H$_2$O in CH$_2$Cl$_2$ for 1 h at rt to give dichloromethane-insoluble precipitates. The precipitates were washed with CH$_2$Cl$_2$ and water (to remove the iron salt) to afford compound 9 (see Experimental Section).

Granström et al.[23] reported that in the case of detritylation of 2,6-di-O-(4-methoxytrityl)-cellulose, FeCl$_3$·6H$_2$O afforded the highest yield (100% yield) of the detritylated product, compared with other deprotection methods (concentrated HCl and 1.0% I$_2$/MeOH), presumably because FeCl$_3$·6H$_2$O may prevent the reinsertion of the released trityl carbocation, aiding the release of trityl groups (There are no descriptions regarding the reinsertion in the literatures[22,24] cited in
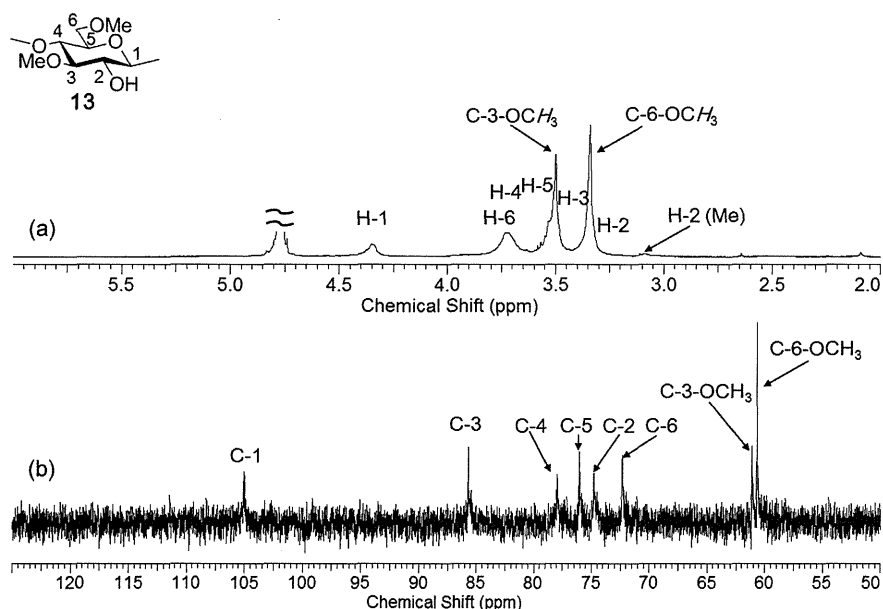
— 113 —

**Figure 9.** $^1$H (a) and $^{13}$C NMR (b) spectra of compound **13** measured in D$_2$O.

Granström et al.'s paper.). After all, FeCl$_3$·6H$_2$O had a good influence on the detritylation efficiency, resulting in the improvement of the isolated yield.

Methylation of compound **9** proceeded with MeI in the presence of NaH in DMF at rt for 12 h and at 50 °C for 48 h to give 2-O-allyl-3,6-di-O-methyl-cellulose (**11**) in 74.0% yield. Figure 7 shows the $^1$H and $^{13}$C NMR spectra of compound **11** measured in CDCl$_3$. The H-2 resonances in the case of allylation at C-2 appeared at 3.12 ppm, while those of methylation at C-2 were observed at 2.95 ppm. Consequently, we succeeded in the selective methylation at C-3 and C-6 of cellulose.

The deallylation and subsequent acetylation of compound **11** gave 2-O-acetyl-3,6-di-O-methyl-cellulose (**12**, 89.1% yield in two steps). Figure 8 shows $^1$H and $^{13}$C NMR spectra of compound **12** measured in CDCl$_3$, and the chemical shifts were summarized in Table 2. DS and DP$_n$ values of compound **12** were summarized in Table 3. The H-3 resonances of compound **12** having acetyl groups at C-3 was not detected in $^1$H NMR spectrum, while a small resonances at around 2.95, 4.30, and 4.53 ppm belonging to a substructure can be recognized, indicating the methylation at C-2 and the acetylation at C-6. These resonances may be due to the overtritylation at C-2 and the incomplete tritylation at C-6.

Acetyl groups of compound **12** were removed using NaOMe to give a crude product that was dialyzed against distilled water for three days and then lyophilized to afford 36MC (**13**) in 95.1% yield. 36MC (**13**) was soluble in water at ~25 °C and 2.0 wt %; the aqueous solution did not show thermoreversible gelation. Figure 9 shows $^1$H and $^{13}$C NMR spectra of 36MC measured in D$_2$O. The proton resonances of methyl groups at C-3 and C-6 appeared at 3.50 and 3.34 ppm, respectively. A small resonance at 3.09 ppm belonging to a substructure can be recognized, indicating the methylation at C-2. The C-1 resonance appeared at 105.2 ppm as sharp signal, while the peaks of the other ring carbon atoms were detected at 72.6 (C-6), 75.0 (C-2), 76.3 (C-5), 78.2 (C-4), and 85.9 (C-3) ppm.

## CONCLUSIONS

2-O-Methyl- and 3,6-di-O-methyl-cellulose were successfully synthesized from natural cellulose via 3-O-functionalized cellulose for the first time. These derivatives were the remaining challenges of regioselectively methylated celluloses synthesized from natural cellulose; thus, the "tool box" of all regioselectively functionalized methyl celluloses was filled. These derivatives are important for the characterization of properties such as solubility and crystallinity to compare with regioselectively methylated celluloses from natural and synthetic cellulose derivatives. Our synthetic strategy for 2-O- and 3,6-di-O-functionalized cellulose derivatives will be useful for the synthesis of 2-O- and 3,6-di-O-cellulose derivatives in general.

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**Notes**
The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

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# Thermoresponsive Hydrogel of Diblock Methylcellulose: Formation of Ribbonlike Supramolecular Nanostructures by Self-Assembly

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S Supporting Information

**ABSTRACT:** This article provides detailed insight into the thermoresponsive gelation mechanism of industrially produced methylcellulose (MC), highlighting the importance of diblock structure with a hydrophobic sequence of 2,3,6-tri-$O$-methyl-glucopyranosyl units for this physicochemical property. We show herein, for the first time, that well-defined diblock MC self-assembles thermoresponsively into ribbonlike nanostructures in water. A cryogenic transmission electron microscopy (cryo-TEM) technique was used to detect the ribbonlike nanostructures formed by the diblock copolymers consisting of hydrophilic glucosyl or cellobiosyl and hydrophobic 2,3,6-tri-$O$-methyl-cellulosyl blocks, methyl $\beta$-D-glucopyranosyl-(1→4)-2,3,6-tri-$O$-methyl-celluloside 1 (G-236MC, $DP_n$ = 10.7, DS = 2.65), and methyl $\beta$-D-glucopyranosyl-(1→4)-$\beta$-D-glucopyranosyl-(1→4)-2,3,6-tri-$O$-methyl-celluloside 2 (GG-236MC, $DP_n$ = 28.2, DS = 2.75). Rheological measurements revealed that the gel strength of a dispersion of GG-236MC (2, 2.0 wt %) in water at 70 °C was 3.0 times stronger than that of commercial MC SM-8000, although the molecular weight of GG-236MC (2) having $M_w$ = 8 × 10³ g/mol was 50 times smaller than that of SM-8000 having $M_w$ = 4 × 10⁵ g/mol. Cryo-TEM observation suggested that the hydrogel formation of the diblock copolymers could be attributed to the entanglement of ribbonlike nanostructures self-assembled by the diblock copolymers in water. The cryo-TEM micrograph of GG-236MC (2) at 5 °C showed rectangularly shaped nanostructures having a thickness from 11 to 24 nm, although G-236MC (1) at 20 °C showed no distinct self-assembled nanostructures. The ribbonlike nanostructures of GG-236MC (2) having a length ranging from 91 to 864 nm and a thickness from 8.5 to 27.1 nm were detected above 20 °C. Small-angle X-ray scattering measurements suggested that the ribbonlike nanostructures of GG-236MC (2) consisted of a bilayer structure with a width of ca. 40 nm. It was likely that GG-236MC (2) molecules were oriented perpendicularly to the long axis of the ribbonlike nanostructure. In addition, wide-angle X-ray scattering measurements revealed that GG-236MC (2) in its hydrogel formed the same crystalline regions as 2,3,6-tri-$O$-methylcellulose. The influence of the DP of diblock MC with a DS of around 2.7 on the gelation behavior will be discussed.
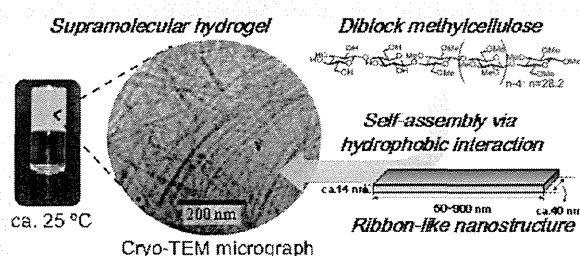
## ■ INTRODUCTION

Hydrogels are 3D networks of water-soluble polymers formed in water through either chemical or physical cross-linking[1] and are the most attractive soft materials used in many areas such as the biomedical, food, and pharmaceutical industries.[2] Many hydrogels undergo a sol–gel transition in response to external stimuli such as the temperature,[3,4] pH,[5] and coexisting solutes.[6]

Methylcellulose (MC) is one of the most extensively investigated thermoresponsive polymers[7–10] not only because MC is important for many practical applications but also because MC is the simplest cellulose ether derivative for the investigation of structure–property relationships. Industrially produced MCs with a degree of substitution (DS) of ca.1.8 are easy to apply to building materials, cosmetics, thickeners, coating materials, tissue engineering scaffolds, and ceramic materials[10,11] because they are soluble in water at room temperature. Aqueous solutions of industrially produced MCs with a DS of 1.4–2.0 undergo a sol–gel transition and typically form a physical hydrogel on heating to above approximately 60 °C.[12] In contrast, MCs with high DSs are soluble only in cold water.[13,14] There have been patent reports on MCs having a low molecular weight (3000–10 000 g/mol) and a high DS (1.8–2.7) as possible soil release agents in detergent compositions.[15,16]

The gelation is said to be due to the dehydration of hydrated methyl groups of MC in water, and the aggregation through hydrophobic interactions between densely methylated regions causes the formation of cross-linking points and subsequent 3D networks in which water molecules were entrapped, namely,

the formation of hydrogels.[17] In general, self-assembled fibers and their network, constructed through multiple weak interactions such as van der Waals interactions, hydrogen bonding, and dipole—dipole interactions, are indispensable to physical hydrogel formation.[18] The physical gelation process is complex because of the transient and reversible nature of the network formation and stabilization.[2,3] The cryo-TEM technique allows us to know the micro- and nanostructures in water most exactly because for cryo-TEM sample preparation a thin aqueous film of the sample on the TEM grid is rapidly frozen in liquid ethane in order to prevent the formation of ice crystals. In fact, Bodvik et al. have reported that in cryo-TEM micrographs of industrially produced MCs a network of fibrous structures was observed in MC solutions above 45 °C.[19] However, it was still unknown how the chemical structure of MCs played a critical role in the formation of fiber structures and subsequent 3D networks because the structure—property relationship of industrially produced MCs with heterogeneous functionalization patterns was complicated.

We have recently reported the syntheses and physical properties of diblock copolymers with regioselective functionalization patterns as model compounds for MC to determine a key structure for the thermoreversible gelation of MCs.[20,21] As a result, only diblock copolymers having 2,3,6-tri-O-methyl-glucopyranosyl units, methyl β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-methyl-celluloside 1 (G-236MC), and methyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-methyl-celluloside 2 (GG-236MC) aggregated with increasing temperature, accompanied by the formation of a hydrophobic environment. Interestingly, GG-236MC (2) formed a macroscopic hydrogel in water in the temperature range from ambient temperature to ca. 70 °C. The presence of more than ten 2,3,6-tri-O-methyl-glucopyranosyl units was crucially important to thermoreversible hydrogel formation of the diblock copolymers. We also investigated the mechanical properties of a dispersion of a diblock methylcellulose derivative, GG-236MC (2, 2.0 wt %), in water in order to compare the gel strength of a new hydrogel formed by the diblock copolymers with that of industrially produced MC (SM-8000) not only for the elucidation of the structure—property relationships of MC but also for end-use applications. Interestingly, the mechanical strength of a hydrogel of GG-236MC (2) was comparable to that of industrially produced MC at a 2.0 wt % concentration at 70 °C, although the molecular weight of GG-236MC (2) was 50 times lower than that of commercial MC. As a consequence, we successfully prepared a new cellulosic supramolecular hydrogelator having a low to moderate molecular weight, resulting from the elucidation of the chemical structure of cross-linking points in commercially produced MC gel by a chemical synthesis approach.

In contrast to the polymer hydrogel, there have recently been many reports on supramolecular self-assembled nanofibers and hydrogels consisting of low-molecular-weight compounds such as glycolipids,[18,22—24] bolaamphiphiles,[25] and peptide amphiphiles.[26,27] These amphiphiles are composed of a hydrophobic moiety, generally a single- or double-alkyl tail, linked to a hydrophilic saccharide or oligopeptide headgroup. These compounds are noncovalently self-assembled via well-defined molecular interactions such as hydrophobic interactions and hydrogen bonding, resulting in the formation of nanofibers and hydrogels. Such advanced supramolecular hydrogels, in which small molecules are self-assembled noncovalently, are expected

to be highly advantageous with respect to traditional hydrogels formed by the entanglement of water-soluble polymers because the properties of a supramolecular hydrogel such as the ability to respond to thermal stimuli and pH sensitivity can be easily tuned by adjusting the 1D chemical structures of a small molecule.[28] These supramolecular architectures are predominantly developed by the well-defined design of the chemical structure. The syntheses of numerous examples of amphiphiles having different building blocks are therefore required to understand how the 1D structure influences supramolecular morphologies such as fibers, tapes, nanotubes, vesicles, and spherical micelles. Whereas the supramolecular nanofibers and hydrogels of synthetic amphiphiles having a hydrophobic group such as a long alkyl chain have already been reported and extensively studied, to the best of our knowledge, there are no reports of supramolecularly self-assembled nanostructures formed by amphiphilic diblock copolymers consisting of only sugar chains in both hydrophilic and hydrophobic blocks. Self-assembled fibrous structures and their network should be formed in aqueous solution because a diblock MC derivative, GG-236MC (2), formed a hydrogel. It is of crucial importance to investigate not only how GG-236MC (2) forms a hydrogel but also what makes the hydrogel of GG-236MC (2) relatively tough. We report here for the first time hierarchical ribbonlike nanostructures formed by β-(1→4)-linked saccharide derivatives with diblock structure, as detected by a cryo-TEM technique in combination with small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) measurements.

## ■ EXPERIMENTAL SECTION

**Materials.** Syntheses of methyl β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-methyl-celluloside 1 (G-236MC) and methyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-methyl-celluloside 2 (GG-236MC) were described in our previous paper.[20] The chemical structures of compounds 1 and 2 are shown in Figure 1. Compounds 1 and 2 have almost the same DS, although the $DP_n$ of compound 2 is approximately three times larger than that of compound 1.



G-236MC (1), $DP_n$=10.7, $M_w/M_n$=1.49, DS=2.65

GG-236MC (2), $DP_n$=28.2, $M_w/M_n$=1.27, DS=2.75
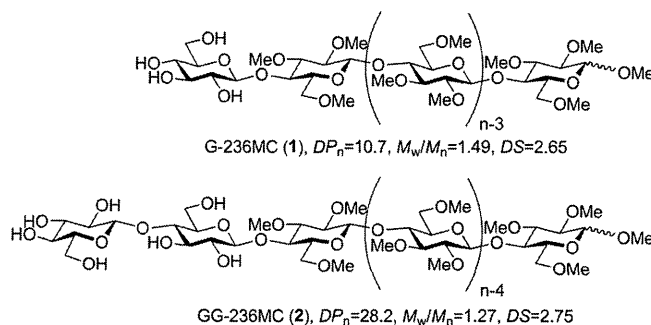
**Figure 1.** Chemical structures of diblock copolymers consisting of hydrophilic glucosyl or cellobiosyl and hydrophobic 2,3,6-tri-O-methyl-glucopyranosyl blocks.

**Rheological Measurements.** Rheological measurements were carried out using a Haake RS-6000 rheometer (Germany) with a cone—plate sensor system (cone plate C60/1) at 25 and 70 °C.

**Cryo-Transmission Electron Microscopy (Cryo-TEM).** For cryo-TEM of GG-236MC (2) at 5 °C (the representative example for sample preparation is described), the aqueous dispersion was stored for some months in the refrigerator at 9 °C. One hour before measurement, the dispersion was taken out of the refrigerator. During this time, the temperature of the sample increased to around 15—18 °C. Then the sample was precooled in a temperature-controlled water

bath at 3 °C for 20 min. After the sample was precooled, a volume of 5 $\mu$L of the dispersion was placed onto a grid covered by a perforated carbon support foil (Quantifoil Micro Tools Jena). The grid was blotted with a self-made autocontrolled blotting system equipped with a climatic chamber (5 °C) and then plunged rapidly into liquid ethane. The frozen sample was transferred with the Gatan-626 single tilt cryotransfer system to a Philips-CM120 cryo-electron microscope.

**Small-Angle X-ray Scatting (SAXS) Measurements.** SAXS experiments were carried out on a Bruker Nanostar with Cu K$\alpha$ radiation ($\lambda$ = 0.15405 nm) and a position-sensitive HiStar detector. The samples were prepared in a sealed quartz capillary with a diameter of 2 mm at 5, 30, 50, and 70 °C. SAXS data were fitted with a cylinder model using NANOFIT software.

**Wide-Angle X-ray Scattering (WAXS) Measurements.** WAXS experiments were carried out at approximately 15 °C on a Rigaku Ultima IV diffractometer. Nickel-filtered Cu K$\alpha$ radiation was used at 40 kV and 40 mA.

# RESULTS AND DISCUSSION

We have recently investigated the temperature-dependent behavior and thermoreversible gelation of diblock MC derivatives as model compounds for industrial MC to determine a key structure in the thermoreversible gelation of an aqueous solution of MC.[20,21] As a result, DSC, fluorescence, and DLS measurements revealed that only diblock copolymers having a sequence of 2,3,6-tri-$O$-methyl-glucopyranosyl units formed hydrophobic environments in water and aggregated with increasing temperature. In particular, the diblock copolymer consisting of hydrophilic cellobiosyl blocks and a hydrophobic 2,3,6-tri-$O$-methyl-cellulosyl block, GG-236MC (2, DP$_n$ = 28.2, DS = 2.75), exhibited thermoreversible gelation. We have also reported that the gelation temperature of diblock copolymers depended on the molecular weight when the DS values were almost the same.[21]

Figure 2 shows photographs of dispersions of GG-236MC (2, 1.0 and 2.0 wt %, DP$_n$ = 28.2, and DS = 2.75) in water at 25
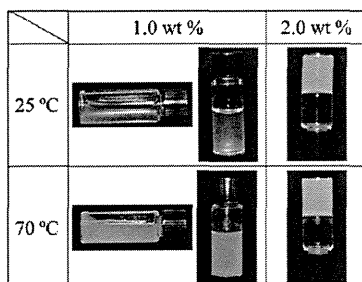
**Figure 2.** Photographs of dispersions of GG-236MC (2, 1.0 and 2.0 wt %, DP$_n$ = 28.2, and DS = 2.75) in water at 25 and 70 °C.

and 70 °C. Although a dispersion of GG-236MC (2, 1.0 wt %) in water forms a fluid gel even at 70 °C, a dispersion in water (2.0 wt %) forms stable hydrogels at 25 and 70 °C. Thus, we investigated the gel strength of a 2.0 wt % hydrogel of GG-236MC (2) at 25 and 70 °C.

**Mechanical Property of a Thermoresponsive Hydrogel Formed by Diblock Methylcellulose.** Figure 3 shows the dynamic storage modulus $G'$ and dynamic loss modulus $G''$ as a function of the angular frequency $\omega$ for a dispersion of GG-236MC (2.0 wt %, 2, DP$_n$ = 28.2, and DS = 2.75) in water at 25 and 70 °C. The $G'$ values at 25 °C (783 Pa at 0.9 rad s$^{-1}$) and 70 °C (7416 Pa at 0.9 rad s$^{-1}$) were around 15 and 10 times higher than the $G''$ values at comparable temperatures, and both moduli were almost independent of frequency $\omega$,
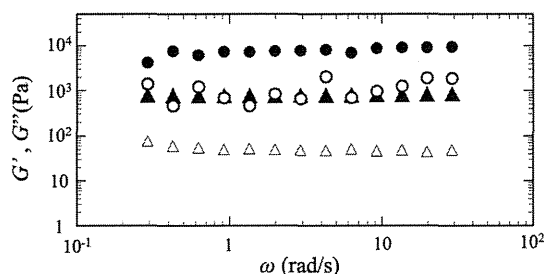
**Figure 3.** Dynamic storage modulus $G'$ and dynamic loss modulus $G''$ of a dispersion of GG-236MC (2, 2.0 wt %) in water as a function of angular frequency $\omega$ at 25 °C {$G'$ (▲) and $G''$ (△)} and 70 °C {$G'$ (●) and $G''$ (○)}.

indicating the formation of a well-developed gel network.[29] Between 25 and 70 °C, $G'$ increased by an order of magnitude. The $G'$ value of a dispersion of GG-236MC (2, 2.0 wt %) in water at 70 °C was approximately 7600 Pa, whereas the $G'$ value of a 2.0 wt % aqueous solution of industrially produced MC (SM-8000, weight-average molecular weight $M_w$ = 4 × 10$^5$ g/mol) at 70 °C was approximately 2500 Pa.[30,31] As a consequence, the rheological measurement revealed that a dispersion of diblock methylcellulose having $M_w$ = 8.1 × 10$^3$ g/mol, GG-236MC (2, 2.0 wt %), in water formed a thermoresponsive hydrogel above ambient temperature (25 °C) and the gel strength of GG-236MC (2) was 3.0 times stronger than that of commercial MC SM-8000 having $M_w$ = 4 × 10$^5$ g/mol. The interesting results concerning the rheological measurement of the GG-236MC (2) hydrogel prompted us to investigate the detailed structure of the diblock copolymers in water on the micro- and nanoscales using a cryo-TEM technique.

**Cryo-TEM Observation.** A cryo-TEM technique was employed to investigate the temperature-dependent nanostructures formed in a 0.2 wt % aqueous solution of G-236MC (1, DP$_n$ = 10.7 and DS = 2.65) and a dispersion of GG-236MC (2, 0.2 wt %, DP$_n$ = 28.2, and DS = 2.75). The cryo-TEM micrographs of the G-236MC (1) aqueous solution frozen from 5 to 20 °C showed no distinct structures such as fibrous structures (Figure 4a,b). At 50 °C, many ribbonlike supramolecular nanostructures were clearly seen. From the periodic change in the width of the elongated fibrous structures with minimum dimensions at crossover points, a twisted ribbon morphology can be concluded (as indicated by arrows in Figures 4c–f). A tilt series of micrographs in avi format is also available in the Supporting Information. In the micrographs at 70 °C, a solution structure consists of ribbonlike supramolecular nanostructures together with disklike and multilamellar nanostructures that were composed of stacked layers 3 to 4 nm thick (Figure 4l).

In contrast, the cryo-TEM micrograph of GG-236MC (2) at 5 °C showed rectanglar nanostructures having a thickness of 11 to 24 nm (tilt series of micrographs in avi format in the Supporting Information), although the cryo-TEM micrographs of G-236MC (1) at 20 °C showed no distinct self-assembled nanostructures. Ribbonlike supramolecular nanostructures were clearly observed in the solution held at 20 °C. In addition, GG-236MC (2) formed twisted ribbon structures at 20 °C (indicated by the arrows in Figure 5f,h,i,,l). With increasing temperature to 50 and 70 °C, the number of such ribbonlike supramolecular nanostructures increased, and the homogeneous solution structure consisting of predominantly the
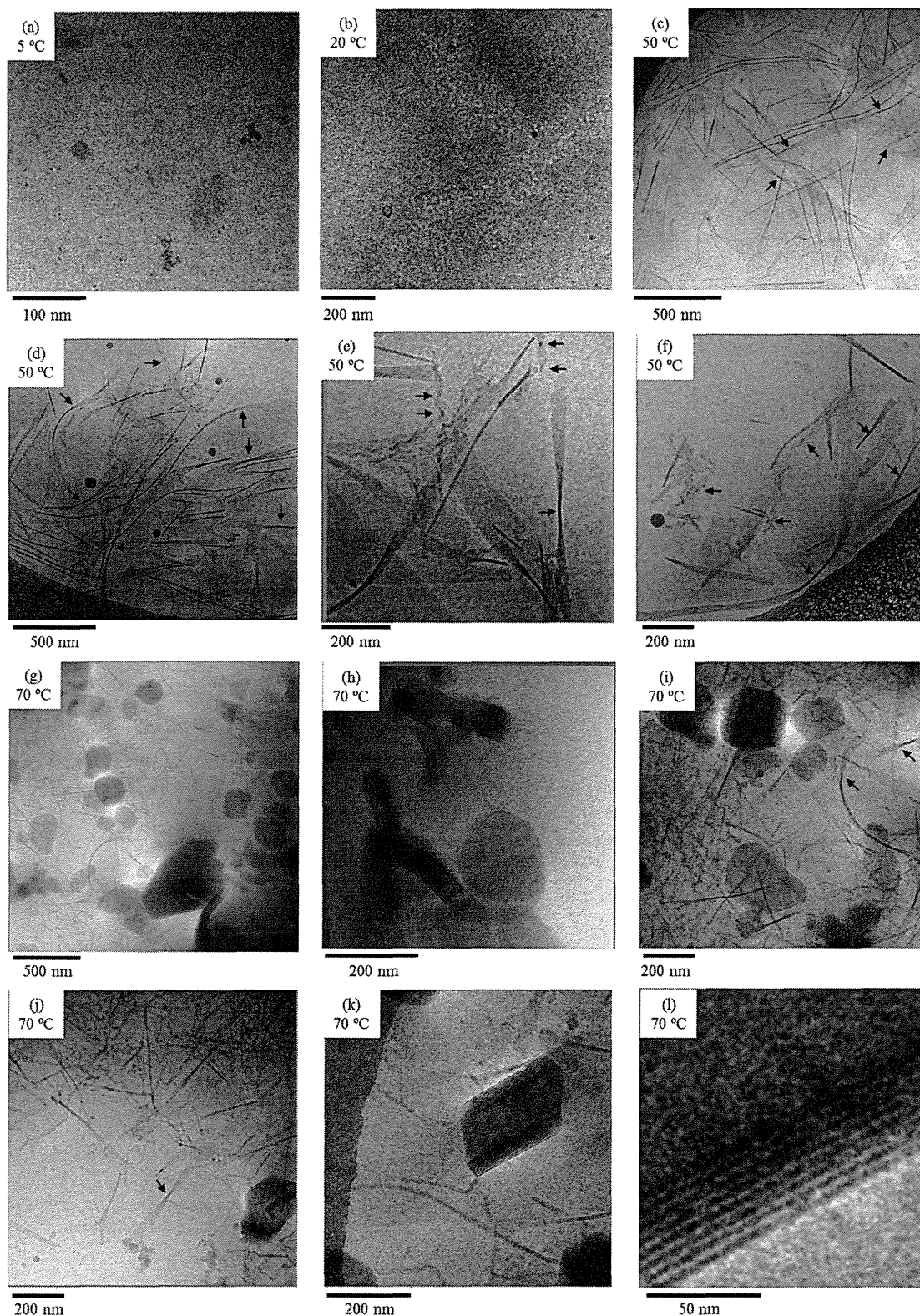
**Figure 4.** Cryo-TEM micrographs of G-236MC (1) at (a) 5, (b) 20, (c–f) 50, and (g–k) 70 °C. (l) Enlarged view of a multilamellar structure shown in k.

supramolecular nanostructures was visible. There were few multilamellar nanostructures in the case of a dispersion of GG-236MC (2) in water (not shown).

**Size Distributions of Self-Assembled Nanostructures.** The length and thickness of ca. 600 ribbonlike supramolecular nanostructures were measured directly from cryo-TEM micro-

graphs of GG-236MC (2) at 5, 20, 50, and 70 °C. Figure 6 shows the size distribution histograms, temperature-dependent average length and thickness, and schematic representation of the ribbonlike nanostructure.

GG-236MC (2) formed nanostructures at 5 °C, although the solution was apparently clear. At 5 °C, ribbonlike supra-
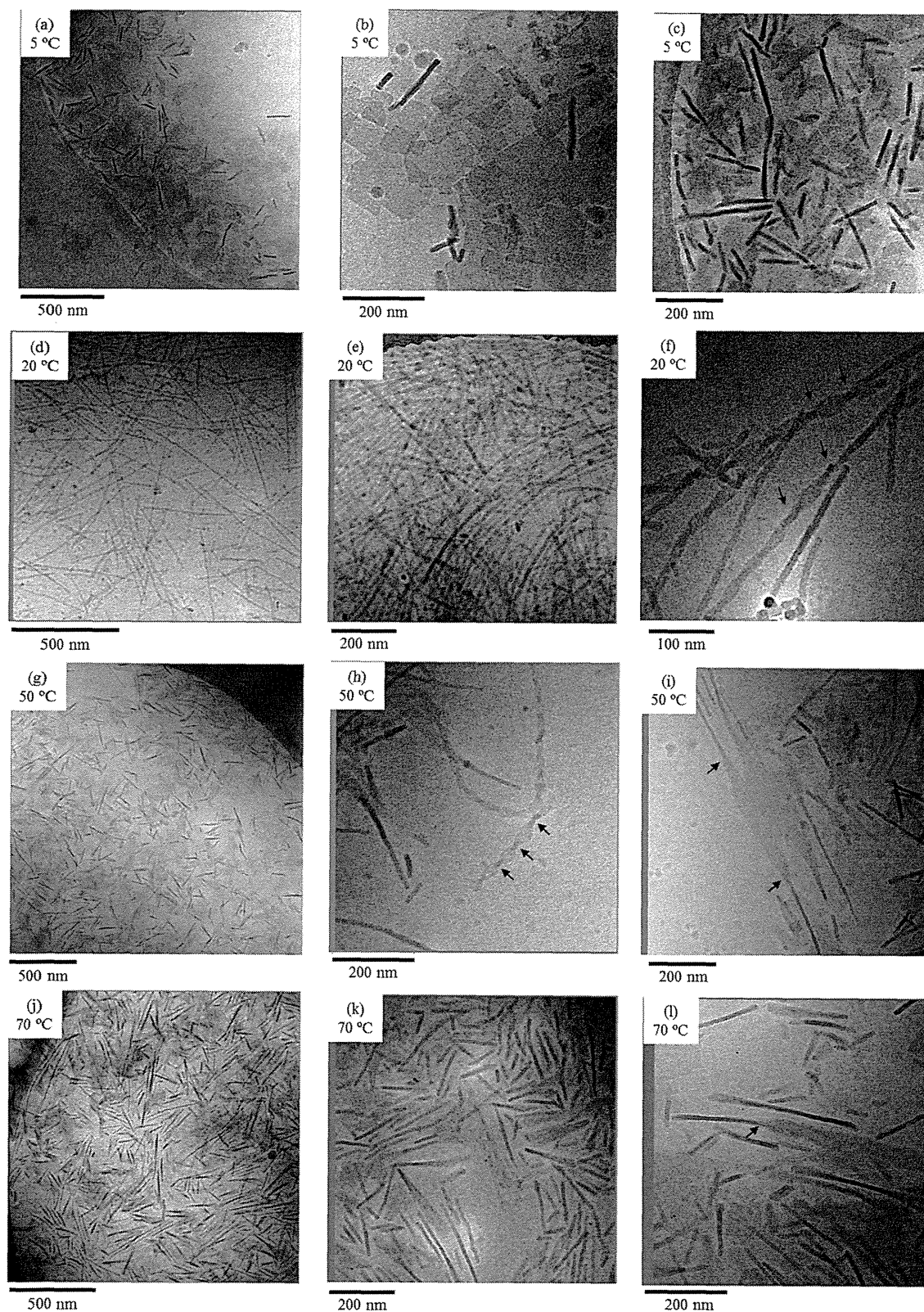
**Figure 5.** Cryo-TEM micrographs of GG-236MC (**2**) at (a–c) 5, (d–f) 20, (g–i) 50, and (j–l) 70 °C.

molecular nanostructures of GG-236MC (**2**) had a length ranging from 53 to 479 nm and a thickness ranging from 11.2 to 24.5 nm. At 20 °C, the length ranged from 91 to 864 nm and the thickness ranged from 8.5 to 27.1 nm. At 50 °C, the length ranged from 61.3 to 672 nm and the thickness ranged from 7.2 to 27.0 nm. At 70 °C, the length ranged from 51.6 to 559 nm

and the thickness ranged from 9.0 to 27.1 nm. The average lengths of supramolecular structures at 5, 20, 50, and 70 °C were 150, 310, 228, and 144 nm, respectively. Meanwhile, the average thicknesses at 5, 20, 50, and 70 °C were 16.4, 13.6, 13.4, and 13.7 nm, respectively. The self-assembled nanostructures of GG-236MC (**2**, $DP_n$ = 28.2 and DS = 2.75) became