Thermo-reversible supramolecular hydrogels of trehalose-type diblock methylcellulose analogues

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ABSTRACT:
This paper describes the design and synthesis of new trehalose-type diblock methylcellulose analogues with nonionic, cationic, and anionic cellobiosyl segments, namely 1-(tri-*O*-methyl-cellulosyl)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**1**), 1-(tri-*O*-methyl-cellulosyl)-4-[(6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**2**), and 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-[β-D-glucopyranuronosyl-(1→4)-β-D-glucopyranuronosyl]-1*H*-1,2,3-triazole (**3**), respectively. Aqueous solutions of all of the 1,2,3-triazole-linked diblock methylcellulose analogues possessed higher surface activities than that of industrially produced methylcellulose and exhibited lower critical solution temperatures, that allowed the formation of thermoresponsive supramolecular hydrogels at close to human body temperature. Supramolecular structures of thermo-reversible hydrogels based on compounds **1**, **2**, and **3** were investigated by means of scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Detailed structure-property-function relationships of compounds **1**, **2**, and **3** were discussed. Not only nonionic hydrophilic segment but also ionic hydrophilic segments of diblock methylcellulose analogues were valid for the formation of thermo-reversible supramolecular hydrogels based on end-functionalized methylcellulose.

Keywords: methylcellulose; polysaccharides; diblock copolymer; end-functionalization; surface activity; thermo-reversible supramolecular hydrogels.

1. Introduction

Methylcellulose (MC) is one of the more common cellulose ethers and has been of particular interest for the investigation of its structure–property relationships, such as the surface activity of its aqueous solution and its thermo-reversible gelation properties at elevated temperature. These properties of industrial and academic interest are attributed to the chemical structure of the methylcellulose skeleton. Therefore, many researchers have previously investigated MC. Commercial MC prepared under heterogeneous conditions is an alternating block copolymer composed of densely substituted hydrophobic and less densely substituted hydrophilic block sequences (Savage, 1957). The highly methylated region—a sequence of 2,3,6-tri-*O*-methyl-glucosyl residues—of the cellulose skeleton is said to cause micelles, that is, liquid–liquid phase separations in aqueous solution (Rees, 1972). These micelles are known as "crosslinking loci" (Kato, Yokoyama, & Takahashi, 1978). In addition, it is well known that reversible crosslinks must exist in any reversible gel (Kato et al., 1978).

We have reported diblock methylcellulose derivatives with regioselective functionalization patterns (Nakagawa, Fenn, Koschella, Heinze, & Kamitakahara, 2011b). We found direct evidence that a sequence of 2,3,6-tri-*O*-methyl-glucopyranosyl units causes thermo-reversible gelation of aqueous MC solution and that an idealized diblock structure consisting of 2,3,6-tri-*O*-methyl-glucopyranosyl and unmodified cello-oligosaccharides caused gelation (Nakagawa, Fenn, Koschella, Heinze, & Kamitakahara, 2011a). However, we had to simplify a synthetic route for new methylcellulose derivatives possessing lower critical solution temperature (LCST) behaviors in aqueous solution. Glycosylation of a cellobiose derivative with a polymeric methyl tri-*O*-methylcelluloside having one hydroxy group at the C-4 position of the glucosyl residue at the non-reducing end consumed a large amount of cellobiosyl trichloroacetimidate derivative to afford only the diblock methylcellulose. To improve the efficiency of the coupling reaction between the hydrophobic and hydrophilic segments, we synthesized a diblock methylcellulose analogue via Huisgen 1,3- dipolar cycloaddition (Nakagawa, Kamitakahara, & Takano, 2012). A 2-propynyl group was introduced to the C-4 hydroxy group at the non-reducing end of the methyl tri-*O*-methylcelluloside. Huisgen 1,3-dipolar cycloaddition was more efficient than glycosylation for connecting the hydrophobic and hydrophilic segments.

Recently, we have reported a versatile pathway to heterobifunctional/telechelic cellulose ethers, such as tri-*O*-methylcellulosyl azide and propargyl tri-*O*-methylcelluloside, with one free C-4 hydroxy group attached to the glucosyl residue at the non-reducing end for the use in the Huisgen 1,3-dipolar cycloaddition (Hiroshi Kamitakahara et al., 2016). This new method enables us to prepare a hydrophobic segment for the Huisgen 1,3-dipolar cycloaddition from

tri-*O*-methylcellulose in a one-step reaction.

If the chemical structure of trehalose-type diblock polysaccharide analogues exhibited the same physical properties as those of the original diblock polysaccharides, the Huisgen 1,3-dipolar cycloaddition of azido and alkyne derivatives could produce a variety of diblock polysaccharide analogues more easily than a glycosylation method, to afford, for instance, cellobiosyl-(1→4)-methylcelluloses. As an example, cellobiosyl-(1↔1)-methylcelluloside, a trehalose-type diblock copolymer, possesses an analogous structure to cellobiosyl-(1→4)-methylcellulose. Moreover, 1-methylcellulosyl-4-cellobiosyloxymethyl-1*H*-1,2,3-triazole and 4-methylcellulosyloxymethyl-1-cellobiosyl-1*H*-1,2,3-triazole have analogous structures to cellobiosyl-(1↔1)-methylcelluloside. Therefore, 1-methylcellulosyl-4-cellobiosyloxymethyl-1*H*-1,2,3-triazole and 4-methylcellulosyloxymethyl-1-cellobiosyl-1*H*-1,2,3-triazole exhibit analogous structures to cellobiosyl-(1→4)-methylcellulose, a diblock methylcellulose. These triazole-linked diblock methylcellulose analogues would allow us to gain deep insights into not only fundamental but also potential properties of methylcelluloses.

A hydrophilic segment would be chosen to tune the properties of the methylcellulose, thereby producing new functional methylcellulose derivatives. Methylcellulose is nonionic. Cationic and anionic cellulose ethers are also of industrial importance. Commercial cationic hydroxyethyl cellulose (QC-10), *O*-[2-hydroxy-3-(trimethylammonio)]propyl hydroxyethyl cellulose chloride, is well known as a conditioning polymer for hair-care products (Hossel, Dieing, Norenberg, Pfau, & Sander, 2000). Chitosan, poly(2-amino-2-deoxy-glucopyranose), an analogous structure to cellulose, is the second most abundant natural polymer (Rinaudo, 2006). 6-Amino-6-deoxycellulose (Teshirogi, Yamamoto, Sakamoto, & Tonami, 1979) is an analogous polymer to chitosan. Carboxymethyl cellulose (Heinze, Erler, Nehls, & Klemm, 1994) is an anionic cellulose ether, and its application fields are widely spread. Recently, cellouronic acid (Isogai & Kato, 1998) and cellulose nanofibers prepared by TEMPO (2,2,6,6-tetramethylpiperidinyloxy) oxidation (Saito, Kimura, Nishiyama, & Isogai, 2007; Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006) have gained increasing attention as anionic cellulosic materials.

To gain deep insights into the influence of the hydrophilic segments of the diblock methylcellulose analogues on the general properties of the original methylcellulose, we chose three hydrophilic segments: β-D-glucopyranosyl-(1→4)-β-D-glucopyranose, (6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranose, and (β-D-glucopyranuronosyl)-(1→4)-β-D-glucopyranuronic acid.

Methylcellulose-based diblock copolymers bearing cationic or anionic hydrophilic segments would enhance the physical performance of commercially available methylcellulose. Thus, we describe, in this paper, the synthesis and structure–property relationships of 1-(tri-*O*-methyl-cellulosyl)-4-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**1**), 1-(tri-*O*-methyl-cellulosyl)-4-((6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**2**), and 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-((β-D-glucopyranuronosyl)-(1→4)-β-D-glucopyranuronosyl)-1*H*-1,2,3-triazole (**3**). In particular, their surface activities, thermal properties, and thermoresponsive gelation properties will be discussed.

2. Experimental

2.1. General measurements

^{1}H- and ^{13}C-NMR spectra were recorded with Varian 500 NMR (500 MHz) or Varian INOVA300 (300 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 (δ) and coupling constants (J) are given in ppm and Hz, respectively. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis was performed with a Bruker MALDI-TOF MS Autoflex III in the positive ion and linear modes. For ionization, a smartbeam laser was used. All spectra were measured in the linear mode by using external calibration. MALDI-TOF MS used 2,5-dihydroxybenzoic acid as a matrix. A Shimadzu liquid chromatography injector (LC-10ATvp), Shimadzu column oven (CTO-10Avp), Shimadzu ultraviolet visible detector (SPD-10Avp), Shimadzu refractive index detector (RID-10A), Shimadzu communication bus module (CBM-10A), 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 by using polystyrene standards (Shodex). A flow rate of 1 mL/min at 40 °C was chosen. Chloroform was used as the eluent.

2.2. Differential scanning calorimetry (DSC) measurements

DSC thermograms were recorded on a DSC823^{e} instrument (Mettler Toledo, Zurich, Switzerland) with an HSS7 sensor under a nitrogen atmosphere during a heating/cooling cycle (0→90→0 °C) with a heating and cooling rate of 3.5 °C/min. Each temperature cycle was sequentially repeated three times in order to ensure and check the reproducible response of the instrument. The sample concentration for DSC measurements was 2.0 wt. %.

132 **2.3. Dynamic light scattering (DLS) measurements**

133 DLS measurements were performed with an ELS-Z zeta-potential and particle-size analyzer
134 (Otsuka Electronics Co., Ltd, Osaka, Japan) and observed in the temperature range from 10 to
135 90 °C. The sample solutions were kept for 5 min at the required temperature before each
136 measurement. The sample concentration for DLS measurements was 0.2 or 2.0 wt. %. The
137 hydrodynamic diameters were obtained by Cumulant method. Intensity and number size
138 distributions were obtained by Marquardt method.
139
140 **2.4. Surface tension measurements**

141 Surface tension was measured by the Wilhelmy method by using a CBVP-A3 surface tensiometer
142 (Kyowa Interface Science, Co. Ltd., Tokyo, Japan) at 25 °C. A Teflon cell containing 700 μL of
143 solution was used for the measurement. The surface tension gradually decreased during the
144 measurements. The values were stable after 30 min and were recorded.

145 **2.5. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)**

146 The three kinds of hydrogels from aqueous solutions of compounds **1**, **2** and **3** were frozen with
147 liquid nitrogen, lyophilized, and cut with a razor blade. The cut surfaces of the hydrogels were
148 sputter-coated with gold with an ion-coater (JFC-1100E, JEOL, Tokyo, Japan) and observed under
149 a scanning electron microscope (JSM-6060, JEOL) at an accelerating voltage of 5 kV.

150 A drop of aqueous dispersion of compound **1** was mounted on a copper grid with an elastic carbon
151 supporting film (Oken Shoji, Tokyo, Japan) and observed under a transmission electron microscope
152 (JEM1400, JEOL) at an accelerating voltage of 100 kV after negative staining with uranyl acetate.

153 **2.6. Syntheses**

154 Cellobiose octaacetate (**12**):
155 Cellobiose was acetylated to give cellobiose octaacetate (**12**) according to the method in our
156 previous paper (H. Kamitakahara, Nakatsubo, & Klemm, 2006). Cellobiose (12.04 g, 35.17 mmol)
157 and sodium acetate were added in acetic anhydride (60 mL). The reaction mixture was stirred at
158 55 °C over night and 100 °C for 3 h. The reaction mixture was poured into water with ice (600 mL).
159 Crude crystals were filtered and washed with distilled water and recrystallized with EtOH to give
160 colorless crystals. (20.8 g, 30.65 mmol, 87% yield). CAS Registry No. 5346-90-7
161
162 2-Propynyl (2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside
163 (**9**) (Moni et al., 2013):

2-Propyne-1-ol (1.05 mL, 18.2 mmol, 1.2 equiv.) was added to a solution of compound **12** (10.3 g, 15.2 mmol) in anhydrous dichloromethane (40 mL). The reaction mixture was cooled to 0 °C. Boron trifluoride diethyl ether complex (2.86 mL, 22.8 mmol, 1.5 equiv.) was added to the reaction mixture at 0 °C. The mixture was stirred for about one day. Solid $NaHCO_3$ was then added to the reaction mixture. The reaction mixture was extracted with dichloromethane, washed with water, sat. aq. $NaHCO_3$ solution, and brine, dried with Na_2SO_4, and concentrated to dryness. The obtained crude crystals were recrystallized with dichloromethane/*n*-hexane to produce 2-propynyl (2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**9**, 10.1223 g, 99% yield).

^{1}H NMR (Moni et al., 2013) (400 MHz): δ 5.21 (dd, 1H, J2,3 = J3,4 = 9.2 Hz, H-3), 5.14 (dd, 1H, J2',3' = J3',4' = 9.1 Hz, H-3'), 5.06 (dd, 1H, H-4'), 4.93 (2 dd, 2H, H-2, H-2') 4.73 (d, 1H, J1,2 = 8.0 Hz, H-1), 4.54 (dd, 1H, J5,6a = 2.0 Hz, J6a,6b = 12.0 Hz, H6a), 4.51 (d, 1H, J1',2' = 8.0 Hz, H-1'), 4.37 (dd, 1H, J5',6'a = 4.5 Hz, J6'a,6'b = 12.5 Hz, H6'a), 4.33 (d, 2H, J = 2.5 Hz, OCH_2CCH), 4.10 (dd, 1H, J5,6b = 4.7 Hz, H-6b), 4.14 (dd, 1H, J5',6'b = 2.0 Hz, H-6'b), 3.79 (dd, 1H, H-4), 3.68–3.60 (m, 2H, H-5, H-5'), 2.45 (t, 1H, OCH_2CCH), 2.19–2.01 (7 s, 21H, 7 Ac).

^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.5, 20.6, 20.7, 20.8, 55.9 (-$\underline{C}H_2CCH$), 61.5 (C6'), 61.7 (C6), 67.8 (C4'), 71.2 (C2), 71.6 (C2'), 71.9 (C5'), 72.4 (C3), 72.8 (C5 or C3'), 72.9 (C5 or C3'), 75.4 ($CH_2C\underline{C}H$), 76.3 (C4), 78.0 ($CH_2\underline{C}CH$), 97.9 (C1), 100.7 (C1'), 169.0, 169.3, 169.7, 169.7, 170.2, 170.3, 170.5

2-Propynyl β-D-cellobioside (**13**) (Moni et al., 2013):

Sodium methoxide (28%) in methanol (0.48 mL, 8.37 mmol, 1.4 equiv.) was added to a solution of compound **9** (4.0010 g, 5.93 mmol) in tetrahydrofuran (THF; 100 mL) and methanol (50 mL). The reaction mixture was stirred for 3.3 h at room temperature. Amberlyst H^+ was added to neutralize the mixture and was then filtered off. The combined filtrate and washings were then concentrated to dryness to give 2-propynyl β-D-cellobioside (**13**, 2.17 g, 96% yield).

^{1}H NMR (400 MHz, D_2O) (Moni et al., 2013): δ 4.50 (d, 1H, J1,2 = 8.0 Hz, H-1), 4.34 (d, 1H, J1' ,2' = 7.5 Hz, H-1'), 4.31 (dd, 2H, J = 16.0, 2.5 Hz, OCH_2CCH), 3.82 (dd, 1H, J5,6a = 2.0 Hz, J6a,6b = 12.5 Hz, H-6a), 3.75 (dd, 1H, J5',6'a = 2.0 Hz, J6'a,6'b = 12.2 Hz, H6'a), 3.65 (dd, 1H, J5,6b = 4.5 Hz, H-6b), 3.56 (dd, 1H, J5',6'b = 5.5 Hz, H-6b'), 3.51–3.42 (m, 3H, H-3, H-4, H-5), 3.36–3.12 (m, 5H, H-2, H-2' , H-3' , H-4' , H-5'), 2.75 (t, 1H, OCH_2CCH).

^{13}C-NMR (125 MHz, D_2O): δ 51.5, 59.3, 62.6, 63.2, 72.1, 75.4, 75.8, 76.9, 77.5, 78.2, 78.7, 79.0, 81.2, 81.4, 103.0, 105.2

2-Propynyl (6-*O*-*p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-6-*O*-*p*-toluenesulfony-β-D-glucopyranoside (**14**):

Tosyl chloride (3.48 g, 18.3 mmol) was added at 0 °C to a solution of 2-propynyl β-D-cellobioside

(**13**, 2.17 g, 5.69 mmol) in pyridine (8 mL). The reaction mixture was stirred at 8 °C for 22.5 h. Brine was then added to the reaction mixture. The organic phase was extracted with ethyl acetate three times, and pyridine was azeotropically removed with ethanol to produce crude compound **14**. The crude product was purified by silica gel column chromatography (methanol/chloroform=1/5, v/v) to give 2-propynyl (6-*O*-*p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (**14**, 1.97 g, 50.5% yield).

^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.40 (s, 3H, PhC*H*$_3$), 2.41 (s, 3H, PhC*H*$_3$), 2.49 (t, 1H, —CH_2CCH), 3.36 (t, 1H, *J*=8.5 Hz), 3.45 (t, 1H, *J*=8.5 Hz), 3.49-3.70 (m), 4.17 (dd, 1H, *J*=2 Hz, *J*=16 Hz, C*H*$_2$CCH), 4.24-4.43 (H6', H6', H6, H6, C*H*$_2$CCH), 4.44 (d, 1H, *J*=8.0 Hz, H1), 4.50 (d, 1H, *J*=7.0 Hz, H1'), 7.3-7.8 (aromatic H)

^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.7, 55.9 (-$\underline{C}H_2CCH$), 68.9 (C6'), 69.1 (C6), 69.4 (C3'), 72.1, 72.4, 73.0 (C2), 73.5, 73.7, 75.7, 75.7 ($CH_2C\underline{C}H$), 77.0, 78.8 ($CH_2\underline{C}CH$), 100.0, 101.3 (C1'), 128.0, 129.8, 130.0, 132.2, 132.6, 144.9, 145.2 (aromatic C)

2-Propynyl (2,3,4-tri-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (**15**):

Acetic anhydride (1 mL) was added to a solution of 2-propynyl (6-*O*-*p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (**14**, 0.763 g) in pyridine (5 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was extracted with ethyl acetate, washed with 1 N HCl, sat. aq. $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated to dryness to give 2-propynyl (2,3,4-tri-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (**15**, 0.9409 g, 94.6% yield).

^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.91, 1.98, 1.99, 2.00, 2.03 (COC*H*$_3$), 2.45 (t, 1H, —CH_2CCH), 2.47 (s, 3H, PhC*H*$_3$), 2.48 (s, 3H, PhC*H*$_3$), 3.55 (m, 1H, *J*=2.0, *J*=3.5 Hz, *J*=9.5 Hz, H5), 3.65 (m, 1H, *J*=2.5 Hz, *J*=4.5 Hz, *J*=10.0 Hz, H5'), 3.71 (t, 1H, *J*=10.0 Hz, H4), 4.10 (dd, 1H, *J*=5.0 Hz, *J*=11.5 Hz, H6'), 4.14-4.28 (H6', H6, C*H*$_2$CCH), 4.33 (dd, 1H, *J*=2.0 Hz, *J*=11.0 Hz, H6), 4.38 (d, 1H, *J*=7.5 Hz, H1'), 4.65 (d, 1H, *J*=8.5 Hz, H1), 4.80 (t, 1H, *J*=9.5 Hz, H2), 4.81 (t, 1H, *J*=8.5 Hz, H2'), 4.93 (t, 1H, *J*=10.0 Hz, H4'), 5.03 (t, 1H, *J*=9.0 Hz, H3'), 5.11 (t, 1H, *J*=9.0 Hz, H3), 7.39-7.84 (aromatic H)

^{13}C-NMR (75 MHz, $CDCl_3$): δ 20.5, 20.5, 20.6, 20.7, 21.6, 21.7, 55.7 (-$\underline{C}H_2CCH$), 66.3 (C6'), 66.8 (C6), 68.0 (C4'), 70.8 (C2), 71.4 (C2'), 71.5 (C5'), 71.8 (C3), 72.4 (C5), 72.8 (C3'), 74.9 (C4), 75.6 ($CH_2C\underline{C}H$), 77.9 ($CH_2\underline{C}CH$), 97.7 (C1), 100.0 (C1'), 128.0, 128.1, 130.1, 130.1, 132.3, 132.6, 145.4, 145.5 (aromatic C), 168.8, 169.3, 169.5, 169.9, 170.1 (*C*OCH_3)

2-Propynyl (2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside (**16**):

Sodium azide (0.3731 g) was added to a solution of 2-propynyl (2,3,4-tri-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (**15**, 1.2888 g) in *N*,*N*-dimethylformamide (DMF; 5 mL). The reaction mixture was stirred overnight at 50 °C. The mixture was then poured into distilled water with ice. The organic phase was extracted with dichloromethane four times, dried over sodium sulfate, and concentrated to dryness. The crude product was purified by silica gel column chromatography (eluent: ethyl acetate/*n*-hexane=2/1, v/v) to afford 2-propynyl (2,3,4-tri-*O*-acetyl-6-*O*-azido-6-*O*-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O*-azido-6-*O*-deoxy-β-D-glucopyranoside (**16**, 0.8904 g, 97% yield).

^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.99 (s, 3H, (CO)CH_3), 2.04 (s, 3H, (CO)CH_3), 2.05 (s, 3H, (CO)CH_3), 2.05 (s, 3H, (CO)CH_3), 2.08 (s, 3H, (CO)CH_3), 2.47 (s, 1H, *J*=2.5 Hz, CH_2CCH), 3.37 (dd, 1H, *J*=5.0 Hz, *J*=13.0 Hz, H6'), 3.40 (dd, 1H, *J*=4.5 Hz, *J*=13.0 Hz, H6), 3.43 (dd, 1H, *J*=3.0 Hz, *J*=13.5 Hz, H6'), 3.58 (dd, 1H, *J*=2.0 Hz, *J*=13.0 Hz, H6), 3.60-3.64 (2H, m, H5, H5'), 3.85 (t, 1H, *J* =9.5 Hz, H4), 4.36 (d, 2H, *J*=2.5 Hz, CH_2CCH), 4.57 (d, 1H, $J_{1',2'}$=8.0 Hz, H1'), 4.79 (d, 1H, $J_{1,2}$=8.0 Hz, H1), 4.89 (dd, 1H, *J*=8.0 Hz, *J*=9.5 Hz, H2'), 4.94 (dd, 1H, *J*=8.0 Hz, *J*=9.5 Hz, H2,), 4.99 (t, 1H, *J*=9.5 Hz, H4'), 5.16 (t, 1H, *J*=9.5 Hz, H3'), 5.22 (t, 1H, *J*=9.5 Hz, H3)

^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.5, 20.6, 20.6, 20.7, 20.8 ((CO)CH_3), 50.2 (C6), 50.9 (C6'), 55.8 (CH_2CCH), 69.1 (C4'), 71.3 (C2), 71.7 (C2'), 72.4 (C3), 72.6 (C3'), 72.7 (C5), 74.5 (C5'), 75.6 (CH_2C$\underline{C}$H), 75.9 (C4), 78.0 ($CH_2\underline{C}$CH), 97.8 (C1), 100.1 (C1'), 168.9, 169.4, 169.6, 169.8, 170.2 ((C)CH_3)

Mw=640.5, MALDI-TOF MS: *m/z* $[M+Na]^+$=663.6, *m/z* $[M+K]^+$=679.6

2-Propynyl (6-azido-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-azido-6-deoxy-β-D-glucopyranoside (**17**)**:**

Sodium methoxide (28%) in methanol (28 μL) was added to 2-propynyl (2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranoside (**16**, 0.3028 g) in methanol (1.5 mL) and THF (1.5 mL). The reaction mixture was stirred at room temperature for overnight. The mixture was neutralized with Amberlyst H^+. The Amberlyst H^+ was removed by filtration and washed with methanol. The filtrate and washings were concentrated to dryness to produce 2-propynyl (6-*O*-azido-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-*O*-azido-6-deoxy-β-D-glucopyranoside (**17**, 0.1805 g, 87% yield).

^{1}H-NMR (500 MHz, D_2O): δ 2.92 (t, 1H, *J*=2.5 Hz, $CH_2CC\underline{H}$), 3.30 (dd, 1H, *J*=8.0 Hz, *J*=9.0 Hz, H2'), 3.35 (dd, 1H, *J*=8.0 Hz, *J*=9.5 Hz, H2), 3.43 (t, 1H, *J*=9.0 Hz, H4'), 3.48 (t, 1H, *J*=9.0 Hz, H3'), 3.52 (dd, 1H, *J*=5.5 Hz, *J*=13.0 Hz, H6'), 3.58 (m, 1H, *J*=9.25 Hz, *J*=2.3 Hz, 5.5 Hz, H5'), 3.61-3.66 (3H, m, H3, H4, H6), 3.75 (dd, 1H, *J*=2.5 Hz, *J*=14.0 Hz, H6), 3.75-3.79 (1H, m, H5), 3.78 (dd, 1H, *J*=2.5 Hz, *J*=13.5 Hz, H6'), 4.46 (2H, ($C\underline{H_2}CCH$)), 4.49 (d, 1H, *J*=8 Hz, H1'), 4.68 (d, 1H, *J*=7.5 Hz, H1)

^{13}C-NMR (D_2O): δ 50.2 (C6 (b)), 50.8 (C6 (a)), 56.7 ($\underline{C}H_2CCH$), 70.0 (C4'), 72.6 (C2), 73.0 (C2'), 73.6 (C5), 73.9 (C3), 74.2 (C5'), 75.2 (C3'), 76.4 ($CH_2C\underline{C}H$), 78.5 ($CH_2\underline{C}CH$), 79.1 (C4), 100.4 (C1), 102.5 (C1')

Mw=430.4 MALDI-TOF MS: *m/z* $[M+Na]^+$=453.1, *m/z* $[M+K]^+$=469.1

2-Propynyl (6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranoside (**18**):

Triphenylphosphine (132.1 mg) was added to a solution of 2-propynyl (6-azido-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-azido-6-deoxy-β-D-glucopyranoside (**17**, 54.2 mg) in methanol (3 mL), THF (3 mL), and distilled water (0.7 mL). The reaction mixture was stirred at room temperature for 14 days. The reaction product was extracted with distilled water and washed with dichloromethane three times. The water layer was concentrated to dryness to afford 2-propynyl (6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranoside (**18**, 46.8 mg, 98% yield).

^{1}H-NMR (D_2O): δ 2.79 (dd, 1H, *J*=7.5 Hz, *J*=13.5 Hz, H6), 2.80 (dd, 1H, *J*=7.5 Hz, *J*=14.0 Hz, H6'), 3.07 (dd, 1H, *J*=3.0 Hz, *J*=14.0 Hz, H6'), 3.19 (dd, 1H, *J*=2.0v, *J*=13.0 Hz, H6), 3.29 (dd, 1H, *J*=7.5 Hz, *J*=9.0 Hz, H2'), 3.31 (t, 1H, *J*=8.0 Hz, H4') 3.33 (t, 1H, *J*=8.0 Hz, H2), 3.39 (m, 1H, *J*=9.5 Hz, *J*=2.5 Hz, *J*=7.0 Hz, H5'), 3.47 (t, 1H, *J*=9.5 Hz, H3'), 3.51-3.561 (m, 2H, H4, H5), 3.60 (t, 1H, *J*=8.5 Hz, H3), 4.46 (d, 2H, *J=1.5* Hz, $C\underline{H_2}CCH$), 4.47 (d, 1H, *J*=8.0 Hz, H1'), 4.64 (d, 1H, *J*=8.0 Hz, H1)

^{13}C-NMR (D_2O): δ 41.1 (C6), 41.2 (C6'), 56.8 ($\underline{C}H_2CCH$), 70.9 (C4'), 72.8 (C2), 73.3 (C2'), 74.2 (C3), 75.2 (C5), 75.4 (C3'), 75.6 (C5'), 75.9 ($CH_2C\underline{C}H$), 78.4 ($CH_2\underline{C}CH$), 80.2 (C4), 100.8 (C1), 102.7 (C1')

Mw=378.4 MALDI-TOF MS: *m/z* $[M+H]^+$=379.3, *m/z* $[M+Na]^+$=401.3, *m/z* $[M+K]^+$=417.2

Note that the carbon and proton resonances of the alkyne group did not appear with a good signal-to-noise ratio, although the molecular ion peak was properly detected by MALDI-TOF MS analysis. Moreover, the NMR spectra of compounds **19** and **10** indicate that the propargyl group is not affected by the Staudinger reaction of compound **17** to produce compound **18**.

2-Propynyl

2,3,4-tri-*O*-acetyl-6-deoxy-6-acetylamino-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-deoxy-6-acetylamino-β-D-glucopyranoside (**19**):

Sodium acetate (34.0 mg) was added to a dispersion of 2-propynyl (6-*O*-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-*O*-amino-6-*O*-deoxy-β-D-glucopyranoside (**18**, 157.1 mg) in acetic anhydride (3 mL). The reaction mixture was stirred at 80 °C for 3 h. The mixture was extracted with ethyl acetate, washed with distilled water and brine, dried over anhydrous sodium sulfate, and concentrated to dryness. The crude product was again acetylated with acetic anhydride (0.5 mL) in pyridine (2 mL) at 80 °C for 1 h. The reagents were azeotropically removed with toluene to afford 2-propynyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-acetylamino-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-deoxy-6-acetylamino-β-D-glucopyranoside (**19**, 233.4 mg, 96% yield).

^{1}H-NMR ($CDCl_3$): δ 1.98, 2.03, 2.04, 2.05, 2.07, 20.8 (21H, O(CO)C*H*$_3$, NH(CO)C*H*$_3$), 2.50 (t, 1H, *J*=2.0, $CH_2CC\underline{H}$), 3.40 (dd, 1H, *J*=6.0 Hz, 14.0 Hz, H6), 3.43 (dd, 1H, *J*=6.5 Hz, 14.5 Hz, H6'), 3.49 (ddd, 1H, *J*=3.0 Hz, *J*=6.0 Hz, *J*=14.5 Hz, H6'), 3.58-3.62 (m, 2H, H5, H5'), 3.78 (t, 1H, *J=9.5*, H4), 3.81 (ddd, 1H, *J*=3.5 Hz, *J*=6.0 Hz, *J*=14.5 Hz, H6), 4.33 (dd, 1H, *J*=2.5 Hz, *J*=16 Hz, $C\underline{H_2}CCH$), 4.38 (dd, 1H, *J*=2.5 Hz, *J*=16 Hz, $C\underline{H_2}CCH$), 4.71 (d, 1H, *J*=8.0 Hz, H1), 4.78 (d, 1H, *J*=7.5 Hz, H1'), 4.91 (t, 1H, *J* =9.5 Hz, H4'), 4.94 (dd, 1H, *J*=7.5 Hz, *J*=9.0 Hz, H2'), 4.98 (dd, 1H, *J*=8.0 Hz, *J*=9.0 Hz, H2), 5.13 (t, 1H, *J*=9.5 Hz, H3), 5.18 (t, 1H, *J* =9.5 Hz, H3'), 5.97 (t, 1H, *J*=6.0 Hz, C6N*H*(CO)CH_3), 6.63 (t, 1H, *J*=6.0 Hz, C6'N*H*(CO)CH_3)

^{13}C-NMR ($CDCl_3$): δ 20.6, 20.7, 20.7, 20.9 (CH_3 (OAc)), 23.0, 23.3 (CH_3 (NHAc)), 39.1 (C6'), 39.8 (C6), 56.4 ($\underline{C}H_2CCH$), 68.7 (C4'), 71.4 (C2), 71.7 (C2'), 72.3 (C5'), 72.7 (C3), 72.8 (C3'), 73.5 (C5), 75.5 ($CH_2\underline{C}CH$), 76.2 (C4), 78.3 ($CH_2C\underline{C}H$), 98.5 (C1), 98.7 (C1'), 169.4, 169.7, 169.8, 170.1, 170.2, 170.3, 170.6 (C=O (OAc, NHAc))

Mw= 672.6 MALDI-TOF MS: *m/z* $[M+Na]^+$ =695.2, *m/z* $[M+K]^+$ =711.2

2-Propynyl [2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl]-(1→4)-2,3-di-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranoside (**10**):

4-Dimethylaminopyridine (DMAP; 2.7 mg) and di-*tert*-butyl dicarbonate (Boc_2O; 0.1 mL) were added to a solution of 2-propynyl [2,3,4-tri-*O*-acetyl-6-*O*-(acetylamino)-6-deoxy-β-D-glucopyranosyl]-(1→4)-2,3-di-*O*-acetyl-6-*O*-(acetylamino)-6-deoxy-β-D-glucopyranoside (**19**, 75 mg) in THF (4 mL). The reaction mixture was stirred at reflux temperature for 5.5 h. The mixture was concentrated in vacuo to afford crude 2-propynyl (2,3,4-tri-*O*-acetyl-6-[acetyl(*tert*-butoxycarbonyl)amino]-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-[acetyl(*tert*-butoxycarbonyl)amino]-6-deoxy-β-D-glucopyranoside (**20**, 127.5 mg;

MW = 872.9, MALDI-TOF MS: *m/z* $[M+Na]^+$ = 895.4).

Sodium methoxide (28%) in methanol (13 μL) was added to a solution of crude compound **20** (97.3 mg) in methanol (2 mL) and dichloromethane (1 mL). The reaction mixture was stirred at room temperature for 6 h. The mixture was neutralized with Amberlyst H^+. After filtration of the Amberlyst H^+ and washing with methanol, the combined filtrate and washings were concentrated to dryness to produce crude product. The crude product was purified by preparative thin-layer chromatography (PTLC; eluent: methanol/dichloromethane=1/9, v/v) to afford 2-propynyl (6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-(*tert*-butoxycarbonyl)amino -6-deoxy-β-D-glucopyranoside (**21**, 49.1 mg, MW = 578.6; MALDI-TOF MS: *m/z* $[M+Na]^+$ = 601.4).

Compound **21** (49.1 mg) was then dissolved in acetic anhydride (0.3 mL) and pyridine (2 mL). The reaction mixture was stirred at 60 °C for 2 h and concentrated azeotropically with toluene to give 2-propynyl (2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-ac etyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranoside (**10**, 67.9 mg, 77% yield from compound **19**).

^{1}H-NMR ($CDCl_3$): δ 1.44, 1.46 (18H, COOC(CH_3)$_3$), 1.98, 2.04, 2.04, 2.06, 2.08 (15H, m, COCH_3), 2.49 (t, 1H, *J*=2.5 Hz, CH_2CCH), 3.31-3.36 (m, 3H, H6, H6', H6'), 3.50-3.54 (m, 2H, H5, H5'), 3.63-3.65 (m, 1H, H6'), 3.69 (t, 1H, *J*=9.5 Hz, H4), 4.31-4.39 (2H, m, (CH_2CCH)), 4.71 (d, 1H, *J*=7.0 Hz, H1), 4.76 (broad d, 1H, *J*=8.0 Hz, H1'), 4.90-4.96 (m, 4H, H2, H2', H4', N*H*), 5.17 (t, 1H, *J=9.5* Hz, H3'), 5.20 (t, 1H, *J=9.5* Hz, H3), 5.15-5.22 (1H, N*H*)

^{13}C-NMR ($CDCl_3$): δ 20.6, 20.7, 20.7, 20.8, 20.8 (COCH_3), 28.3, 28.4 (COOC(CH_3)$_3$), 40.6, 40.7 (C6, C6'), 56.4 (CH_2CCH), 68.7 (broad, C4'), 71.4 (C2), 71.6 (C2'), 72.1 (broad, C3), 72.9 (C3'), 73.0 (C5 or C5'), 73.9 (broad, C5 or C5'), 75.4 (CH_2CCH), 75.9 (broad, C4), 78.3 (CH_2CCH), 79.8, 79.8 (broad, COOC(CH_3)$_3$), 98.5 (C1), 99.2 (broad, C1'), 155.7 (COOC(CH_3)$_3$), 169.4, 169.6, 169.7, 170.2 (COCH_3)

Mw=788.8 MALDI-TOF MS: *m/z* $[M+Na]^+$ =811.5, *m/z* $[M+K]^+$ =827.4

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (**22**):

Compound **22** was prepared according to the method in our previous report (H. Kamitakahara & Nakatsubo, 2005).

β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyl azide (**23**):

Compound **23** was prepared according to the method in previous reports (Schamann & Schafer, 2003; Ying & Gervay-Hague, 2003).

β-D-Glucopyranosiduronosyl-(1→4)-β-D-glucopyranosiduronosyl azide (**24**):
Potassium bromide (18.8 mg) and TEMPO (16.7 mg) were added to a solution of β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl azide (**23**, 290 mg) in sat. aq. sodium hydrogencarbonate (3 mL). Sodium hypochlorite (NaOCl, 3.9 mL) was then added to the reaction mixture. The mixture was stirred at 0 °C for 1 h. TEMPO (8 mg) and NaOCl (3.9 mL) were further added to the reaction mixture. After being stirred at 4 °C for one day, the reaction mixture was extracted with distilled water and washed with dichloromethane four times. The aqueous layer was adjusted to pH 2 with 2 N HCl, concentrated, and diluted with distilled water. This concentration and dilution cycle was repeated several times until the color of the solution turned from yellow to colorless. The aqueous layer was finally concentrated to dryness. The insoluble part was filtered off and washed with methanol. The combined filtrate and washings were concentrated to dryness. This procedure was repeated three times to give β-D-glucopyranosiduronosyl-(1→4)-β-D-glucopyranosiduronosyl azide (**24**, 278.7 mg) (Schamann & Schafer, 2003; Ying & Gervay-Hague, 2003). The carboxylic acid moieties of crude compound **24** were esterified without further purification.

Methyl [(methyl β-D-glucopyranosyluronate)-(1→4)-β-D-glucopyranosyl azide]uronate (**25**):
2,2-Dimethoxypropane (1.5 mL) and one drop of conc. HCl were added at room temperature to a solution of β-D-glucopyranosiduronosyl-(1→4)-β-D-glucopyranosiduronosyl azide (**24**, 278.7 mg) in methanol (15 mL). The reaction mixture was stirred for one day and concentrated to dryness to give methyl [(methyl β-D-glucopyranosyluronate)-(1→4)-β-D-glucopyranosyl azide]uronate (**25**, 273.3 mg) (Schamann & Schafer, 2003). Crude compound **25** was acetylated without further purification.

Methyl [(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl azide]uronate (**11**):
Methyl [(methyl β-D-glucopyranosyluronate)-(1→4)-β-D-glucopyranosyl azide]uronate (**25**, 92.5 mg) was dispersed in acetic anhydride (4 mL) with sodium acetate (70.8 mg). The reaction mixture was stirred at 60 °C overnight. The organic layer was extracted with ethyl acetate, washed with distilled water twice, aq. sodium hydrogen carbonate three times, and brine, and concentrated to dryness. The crude product was purified by silica gel column chromatography (eluents: ethyl acetate/*n*-hexane=1/1, v/v; methanol/dichloromethane=1/49, v/v) and by PTLC (eluent: ethyl acetate/*n*-hexane=1/1, v/v) to give methyl [(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl

azide]uronate (**11**, 70 mg, total yield 11% from compound **23**).

^{1}H-NMR ($CDCl_3$): δ 1.99, 2.01, 2.01, 2.06, 2.07 (15H, COC*H*$_3$), 3.73 (s, 3H, C6'OOC*H*$_3$), 3.87 (s, 3H, C6OOC*H*$_3$), 4.0 (d, 2H, *J* =10 Hz, H5 and H5'), 4.14 (t, 1H, *J*=9.5 Hz, H4), 4.63 (d, 1H, *J*=8.0 Hz, H1'), 4.67 (d, 1H, *J*=8.5 Hz, H1), 4.88 (t, 2H, *J*=9.5 Hz, H2 and H2'), 5.13 (t, 1H, *J*=10.0 Hz, H4'), 5.19 (t, 1H, *J* =9.5 Hz, H3'), 5.20 (t, 1H, *J*=9.5 Hz, H3)

^{13}C-NMR ($CDCl_3$): δ 20.4, 20.5, 20.5 (CO*C*H$_3$), 52.8 (C6OO*C*H$_3$), 53.2 (C6'OO*C*H$_3$), 69.4 (C4'), 70.5 (C2'), 71.0 (C2), 71.5 (C3), 72.0 (C3'), 72.6 (C5'), 75.7 (C5), 76.4 (C4), 88.4 (C1), 100.4 (C1'), 166.7 (*C6'*OOCH$_3$), 167.1 (*C6*OOCH$_3$), 169.0, 169.3, 169.3, 170.0, 170.1 (*C*OCH$_3$)

Mw=633.5 MALDI-TOF MS: *m/z* $[M+Na]^+$ =656.4, *m/z* $[M+K]^+$ =672.3

Tri-*O*-methyl cellulosyl azide (**7**):

Compound **7** was prepared according to the method in our previous paper (Hiroshi Kamitakahara et al., 2016).

Propargyl tri-*O*-methyl celluloside (**8**):

Compound **8** was prepared according to the method in our previous paper (Hiroshi Kamitakahara et al., 2016).

1-(2,3,6-Tri-*O*-methyl-cellulosyl)-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**4**):

Cu (I) Br (26.7 mg, MW = 143.45, 186 mmol, 10 equiv.), sodium ascorbate (73.8 mg/0.09 mL, 20 equiv., 4 M in H_2O), and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, MW = 173.3, *d* = 0.83 g/mL, 0.04 mL, 0.0332 g, 192 mmol, 10 equiv.) were added at room temperature to a solution of 2-propynyl (2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**9**) (25.1 mg, MW = 674.6, 37.2 mmol, 2.0 equiv.) and tri-*O*-methyl cellulosyl azide (**7**, 98.5 mg, M_n = 5.37×10^3, DP_n = 26.3, 18.4 mmol, 1.0 equiv.) in MeOH/CH_2Cl_2 (4 mL, 1/4, v/v). Distilled water (0.2 mL) was added. The reaction mixture was stirred at room temperature for four days under a nitrogen atmosphere. The mixture was concentrated and passed through a silica gel chromatography column eluted with 20% MeOH/CH_2Cl_2 to give the crude product. The crude product was purified by silica gel column chromatography (eluent: EtOAc→20% MeOH/CH_2Cl_2) to give 1-(2,3,6-tri-*O*-methyl-cellulosyl)-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**4**, 86.8 mg, 78.5% yield).

^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.97, 1.99, 2.02, 2.07, 2.13 (COC*H*$_3$), 2.94 (t, *J*=8.5 Hz, $H2_{Me}$ (internal)), 3.20 (t, 1H, *J*=9.0 Hz, $H3_{Me}$ (internal)), 3.27 (m, *J*=9.5 Hz, $H5_{Me}$ (internal)), 3.37 (s, OCH_3), 3.52 (s, OCH_3), 3.56 (s, OCH_3), 3.6-3.9 ($H5_{Ac}$, $H4_{Ac}$, $H5'_{Ac}$), 3.65 ($H6_{Me}$ (internal)), 3.68 (t,

J=9.0, $H4_{Me}$ (internal)), 3.75 (m, $H6_{Me}$ (internal)), 3.99 (m, J=10.0 Hz, $H5\alpha_{Me}$, α-anomer), 4.04 (dd, 1H, J=2.0 Hz, J=12.5 Hz, $H6'_{Ac}$), 4.11 (dd, 1H, J=5.0 Hz, J=12.0 Hz, $H6_{Ac}$), 4.17 (t, J=7.5 Hz, $H3\alpha_{Me}$, α-anomer), 4.33 (d, J=8.0 Hz, $H1_{Me}$ (internal)), 4.37 ($H6'_{Ac}$), 4.50 (d, J=8.0 Hz, $H1'_{Ac}$), 4.54 (dd, J=1.5 Hz, J=11.5, $H6_{Ac}$, β-anomer), 4.55 (dd, J=2.0 Hz, J=11.5 Hz, $H6_{Ac}$, α-anomer), 4.60 (d, J=8.0 Hz, $H1_{Ac}$, β-anomer of methylcellulose), 4.61 (d, J=8.0 Hz, $H1_{Ac}$, β-anomer of methylcellulose), 4.81 (d, 1H, J=12.5 Hz, OC$\underline{H}_2$-triazole), 4.87-4.94 (d, OC$\underline{H}_2$-triazole), 4.87-4.94 ($H2_{Ac}$, $H2'_{Ac}$), 5.05 (t, J=9.5, $H4'_{Ac}$), 5.13 (t, J=9.5 Hz, $H3_{Ac}$, $H3'_{Ac}$), 5.44 (d, 1H, J=9.0 Hz, $H1\beta_{Me}$), 6.15 (d, J=5.5 Hz, $H1\alpha_{Me}$), 7.69 (s, triazole, β-anomer), 7.70 (s, triazole, α-anomer) (α/β ratio = 2/1)

^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6, 20.8 (CO*C*H_3), 59.1 (O$\underline{C}H_3$), 60.2 (O$\underline{C}H_3$), 60.5 (O$\underline{C}H_3$), 61.6, 61.7 ($C6'_{Ac}$), 61.8 ($C6_{Ac}$), 62.8 (OC$\underline{H}_2$-triazole), 67.7 ($C4'_{Ac}$), 70.2 ($C6_{Me}$ (internal)), 71.3 ($C2_{Ac}$ or $C2'_{Ac}$), 71.5 ($C2_{Ac}$ or $C2'_{Ac}$), 71.9, 72.0 ($C5_{Ac}$), 72.3 ($C5'_{Ac}$), 72.7 ($C3_{Ac}$ or $C3'_{Ac}$), 72.8 ($C3_{Ac}$ or $C3'_{Ac}$), 73.0, 73.3, 73.7, 74.8 ($C5_{Me}$ (internal)), 76.2 ($C4_{Ac}$ (reducing end)), 77.4 ($C4_{Me}$ (internal)), 77.8 ($C4_{Me}$ (reducing end)), 79.3, 79.5, 81.0 ($C3\alpha_{Me}$), 82.1 ($C2_{Me}$ (reducing end)), 83.3 ($C1\alpha_{Me}$), 83.4 ($C2_{Me}$ (internal)), 83.7, 83.7, 84.0, 84.8, 85.0 ($C3_{Me}$ (internal)), 85.3 ($C4_{Me}$ (reducing end)), 86.1, 86.9, 87.3 ($C1\beta_{Me}$(reducing end)), 99.6 ($C1_{Ac}$), 100.7 ($C1'_{Ac}$), 103.1 ($C1_{Me}$ (internal)), 103.7 ($C1_{Me}$), 124.5 (triazole *C*H), 143.2 (O-CH_2-*C*=), 168.9, 169.2, 169.6, 169.7, 170.2, 170.3, 170.4 (CO*C*H_3)

1-(2,3,6-Tri-*O*-methyl-cellulosyl)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**1**):

Sodium methoxide (28%) in methanol (0.02 mL, 10 equiv. per AGU) was added at room temperature to a solution of 1-(2,3,6-tri-*O*-methyl-cellulosyl)-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**4**, 76.3 mg) in MeOH (3 mL) and THF (3.5 mL). The mixture was stirred overnight at room temperature. The solution was neutralized with Amberlyst H^+. The Amberlyst H^+ was filtered off and washed with MeOH. The combined filtrate and washings were concentrated to dryness to give 1-(2,3,6-tri-*O*-methyl-cellulosyl)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**1**, 79.3 mg, quantitative yield).

^{1}H-NMR (500 MHz, D_2O): δ 3.03 (t, J=8.5 Hz, $H2_{Me}$ (internal)), 3.29 (s, OCH_3), 3.34 (t, 1H, J=8.0 Hz, $H3_{Me}$ (internal)), 3.46 (s, OCH_3), 3.47 (s, OCH_3),4.32 (d, J=7.0 Hz, $H1_{Me}$ (internal)), 4.38 (d, J=8.0 Hz), 4.46 (d, J=7.5 Hz), 4.46 (d, J=8.0 Hz), 4.67, 4.80 (d, J=13.0 Hz, OC$\underline{H}_2$-triazole), 4.90 (d, J=12.5 Hz, OC$\underline{H}_2$-triazole), 5.68 (d, 1H, J=9.5 Hz, $H1\beta_{Me}$), 6.42 (d, J=5.5 Hz, $H1\alpha_{Me}$), 8.16, 8.24, 8.32 (s, triazole C*H*)

^{13}C-NMR (125 MHz, D_2O): δ 58.3 (O$\underline{C}H_3$ (internal)), 58.5, 58.8, 58.9 (O$\underline{C}H_3$ (internal)), 59.8,

60.3 (O<u>C</u>H_3 (internal)), 60.5, 61.1, 64.0, 68.8, 69.4, 69.8 ($C6_{Me}$ (internal)), 70.4, 70.7, 72.7, 73.1, 73.6 ($C5_{Me}$ (internal)), 74.2, 74.8, 75.4, 75.7, 76.0 ($C4_{Me}$ (internal)), 76.9, 78.4, 82.1 ($C2_{Me}$ (internal)), 82.5, 83.0 ($C3_{Me}$ (internal)), 85.0, 101.3 ($C1_{OH}$), 102.4 ($C1_{Me}$ (internal)), 102.5 ($C1'_{OH}$)
GPC analysis of acetylated compound **1**: M_n=4.9×10^3, M_w/M_n=1.7, DP_n=23 (including DP of hydrophilic segment)

1-(Tri-*O*-methyl-cellulosyl)-4-(2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**5**)**:**
$CuSO_4·H_2O$ (34.0 mg, MW = 249.69, 136 mmol, 10 equiv.), sodium ascorbate (54.0 mg/68 μL, 20 equiv., 4 M in H_2O), and PMDETA (MW = 173.3, *d* = 0.83 g/mL, 28 μL, 23.2 mg, 134 mmol, 10 equiv.) were added to a solution of 2-propynyl [2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl]-(1→4)-2,3-di-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranoside (**10**, 32.2 mg, MW = 788.8, 40.8 mmol, 3.0 equiv.) and tri-*O*-methyl cellulosyl azide (**7**, 100 mg, M_n = 7.34×10^3, DP_n = 35.8, 13.6 mmol, 1.0 equiv.) in tetrahydrofuran (3 mL). The reaction mixture was stirred at 50 °C overnight in a nitrogen atmosphere. The mixture was concentrated and passed through a silica gel chromatography column eluted with 10% MeOH/CH_2Cl_2 to give the crude product. The crude product was purified by silica gel column chromatography (eluent: EtOAc→10% MeOH/CH_2Cl_2) to give 1-(tri-*O*-methyl-cellulosyl)-4-(2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**5**, 103.2 mg, 93% yield).
^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.44, 1.47 (CH_3 (NHBoc)), 1.97, 1.98, 2.00, 2.02, 2.02, 2.04, 2.05, 2.07 (CH_3 (OAc)), 2.95 (t, *J*=8.0 Hz, $H2_{Me}$ (internal)), 3.11 (t, *J*=9.0 Hz, $H3_{Me}$), 3.21 (t, 1H, *J*=9.0 Hz, $H3_{Me}$ (internal)), 3.29 (m, *J*=9.0 Hz, $H5_{Me}$ (internal)), 3.3-3.4 (H6, H6', H6'), 3.6-3.65 (H6'), 3.38 (OC<u>H</u>$_3$), 3.54 (OC<u>H</u>$_3$), 3.58 (OC<u>H</u>$_3$), 3.62-3.72 ($H6_{Me}$ (internal)), 3.69 (t, *J*=9.5 Hz, $H4_{Me}$ (internal)), 3.73-3.81 (m, $H6_{Me}$ (internal)), 3.40 ($H2_{Me}$, reducing-end, α-anomer), 3.77 ($H2_{Me}$, reducing-end, α-anomer), 3.77 ($H2_{Me}$, reducing-end, α-anomer), 3.83 ($H2_{Me}$, reducing-end, α-anomer), 3.89 ($H6_{Me}$, reducing-end, α-anomer), 4.04 (m, *J*=9.5 Hz, $H5_{Me}$, reducing-end, α-anomer), 4.15 (t, *J*=7.5 Hz, $H3_{Me}$, reducing-end, α-anomer), 4.34 (d, *J*=7.5 Hz, $H1_{Me}$ (internal)), 4.39 (d, *J*=8.0 Hz, $H1_{Me}$,), 4.6-4.64 (d, $H1_{Ac}$), 4.76 (broad d, 1H, *J*=8.0 Hz, $H1'_{Ac}$), 4.75-4.94 (CH_2, triazole-alkene), 4.90-4.96 ($H2_{Ac}$, $H2'_{Ac}$, $H4'_{Ac}$, N*H*), 5.1-5.2 ($H3_{Ac}$, $H3'_{Ac}$), 5.46 (d, *J*=9.0 Hz, $H1\beta_{Me}$), 6.15 (d, *J*=5.0 Hz, $H1\alpha_{Me}$), 7.71, 7.76 (H, triazole)
^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.6, 20.63, 20.65, 20.7, 20.8 (CO*C*H_3), 28.4(COOC(<u>C</u>H_3)$_3$), 40.7 (C6, C6'), 59.0, 59.2 (O<u>C</u>H_3), 59.6, 60.1, 60.3 (O<u>C</u>H_3), 60.4, 60.5, 60.5 (O<u>C</u>H_3), 60.8, 62.8

(OCH_2-triazole), 68.5 (broad, C4'$_{Ac}$), 70.3 (C6$_{Me}$ (internal)), 71.6 (C2$_{Ac}$ and C2'$_{Ac}$), 72.1 (C3$_{Ac}$), 72.9 (C3'$_{Ac}$), 73.0 (C5$_{Ac}$ or C5'$_{Ac}$), 73.1, 73.2, 73.9 (broad, C5$_{Ac}$ or C5'$_{Ac}$), 74.9 (C5$_{Me}$ (internal)),75.5 (C4$_{Ac}$), 77.5 (C4$_{Me}$ (internal)), 79.8 COO$\underline{C}$(CH$_3$)$_3$), 83.3, 83.5 (C2$_{Me}$ (internal)), 83.7 (C1α_{Me}), 84.9, 85.0 (C3$_{Me}$ (internal)), 86.1, 99.1(C1$_{Ac}$), 99.8 (C1'$_{Ac}$), 103.2 (C1$_{Me}$ (internal)), 122.2, 124.4 (triazole *C*H), 143.2, 144.2 (O-CH$_2$-*C*=), 155.8 (*C*OOC(CH$_3$)$_3$), 169.3, 169.6, 169.7, 170.2 (*C*OCH$_3$)

1-(Tri-*O*-methyl-cellulosyl)-4-[(6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**2**):

Sodium methoxide (28%) in methanol (5.5 µL, 10 equiv. per AGU) were added at room temperature to a solution of 1-(tri-*O*-methyl-cellulosyl)-4-[2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**5**, 76.3 mg) in MeOH (2 mL), THF (2 mL), and CH_2Cl_2 (1 mL). The mixture was stirred overnight at room temperature. The solution was neutralized with Amberlyst H^+. The Amberlyst H^+ was filtered off and washed with MeOH. The combined filtrate and washings were concentrated to dryness to give 1-(tri-*O*-methyl-cellulosyl)-4-[6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl-(1→4)-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (78.9 mg). Trifluoroacetic acid (0.5 mL) was added at −20 °C to a solution of 1-(tri-*O*-methyl-cellulosyl)-4-[6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (84 mg) in CH_2Cl_2 (1 mL). The reaction mixture was stirred for 1.2 h at −20 °C. The mixture was concentrated to dryness at 4 °C to give the crude product. The crude product was purified by gel filtration chromatography (Sephadex LH-20) to give 1-(tri-*O*-methyl-cellulosyl)-4-[6-amino-6-deoxy-β-D-glucopyranosyl-(1→4)-6-amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**2**, 82.7 mg, quantitative yield).

^{1}H-NMR (500 MHz, D_2O): δ 3.03 (t, *J*=8.5 Hz, H2$_{Me}$ (internal)), 3.34 (t, 1H, *J*=8.5 Hz, H3$_{Me}$ (internal)), 3.42-3.49 (H5$_{Me}$ (internal)), 3.29 (OC$\underline{H}_3$), 3.46 (OC$\underline{H}_3$), 3.47 (OC$\underline{H}_3$), 3.57-3.68 (H4$_{Me}$ (internal), H6$_{Me}$ (internal)), 4.32 (d, *J*=7.0 Hz, H1$_{Me}$ (internal)), 4.40 (d, J=8.0 Hz), 4.44 (d, *J*=6.0 Hz), 4.79-4.95 (CH_2, triazole-alkene), 5.69 (H1β$_{Me}$ (reducing end), *J=9* Hz), 6.42 (H1α$_{Me}$ (reducing end), *J=5.5* Hz), 8.17, 8.25 (s, triazole C*H*)

^{13}C-NMR (D_2O): δ 40.2 ($\underline{C}$6-NH_2 or $\underline{C}$6'-NH_2), 40.5 ($\underline{C}$6-NH_2 or $\underline{C}$6'-NH_2), 58.3 (O$\underline{C}$H$_3$), 58.5, 58.9 (O$\underline{C}$H$_3$), 59.8, 60.3 (O$\underline{C}$H$_3$), 69.8 (C6$_{Me}$ (internal)), 70.8, 73.0, 63.6 (C5$_{Me}$ (internal)), 74.2, 74.5, 74.7, 75.1, 75.7, 76.0 (C4$_{Me}$ (internal)), 82.0 (C2$_{Me}$ (internal)), 82.5, 83.0 (C3$_{Me}$ (internal)) 85.0, 101.2, 101.4, 102.4 (C1$_{Me}$ (internal)), 102.8

GPC analysis of acetylated compound **2**: M_n=7.1×10^3, M_w/M_n=1.6, DP_n=33 (including DP of hydrophilic segment)

4-(Tri-*O*-methyl-cellulosyloxymethyl)-1-[methyl {(methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl}uronate]-1*H*-1,2,3-triazole (**6**):

Cu (I) Br (5.6 mg, MW = 143.45, 39 mmol, 10 equiv.), sodium ascorbate (15.5 mg/19 μL, 20 equiv., 4 M in H_2O), and PMDETA (MW = 173.3, d = 0.83 g/mL, 8.2 μL, 6.8 mg, 39 mmol, 10 equiv.) were added at room temperature to a solution of methyl [(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl azide]uronate (**11**, 7.4 mg, MW = 633.5, 11.7 mmol, 3.0 equiv.) and propargyl tri-*O*-methyl celluloside (**8**, 30 mg, M_n = 7.72×10^3, DP_n = 37.5, 3.9 mmol, 1.0 equiv.) in MeOH/CH_2Cl_2 (2 mL, 1/4, v/v). The reaction mixture was stirred overnight at room temperature under a nitrogen atmosphere. The mixture was concentrated and passed through a silica gel chromatography column eluted with 10% MeOH/CH_2Cl_2 to give the crude product. The crude product was purified by silica gel column chromatography (eluent: EtOAc→10% MeOH/CH_2Cl_2) to give 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-[methyl {(methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl}uronate]-1*H*-1,2,3-triazole (**6**, 29.9 mg, 92% yield).

^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.86, 1.86, 2.00, 2.02, 2.02, 2.09, 2.10 (COC$\underline{H}_3$), 2.96 (t, J=8.5 Hz, $H2_{Me}$ (internal)), 3.22 (t, 1H, J=9.0 Hz, $H3_{Me}$ (internal)), 3.29 (m, J=9.0 Hz, $H5_{Me}$ (internal)), 3.39 (OC$\underline{H}_3$), 3.54 (OC$\underline{H}_3$), 3.58 (OC$\underline{H}_3$), 3.66 ($H6_{Me}$ (internal)), 3.70 (t, J=9.0 Hz, $H4_{Me}$ (internal)), 3.77 (broad dd, J=3,5 Hz, J=11.0 Hz, $H6_{Me}$ (internal)), 3.84 (s, 3H, C6'OOC$\underline{H}_3$), 3.85 (s, 3H, C6OOC$\underline{H}_3$), 4.03 (dd, J=3.5, J=10.0, $H5'_{Ac}$ (non-reducing end)), 4.18 (dd, J=2.0 Hz, J=10.0 Hz, $H5_{Ac}$ (reducing end)), 4.29 ($H4_{Ac}$ (reducing end)), 4.35 (d, J=8.0 Hz, $H1_{Me}$ (internal)), 4.40 (d, J=8.0 Hz, $H1_{Me}$), 4.67 (d, J=13.0 Hz, OC$\underline{H}_2$-triazole), 4.67-4.68 (d, J=8, $H1'_{Ac}$ (non-reducing end)), 4.82 (d, J=13.5 Hz, OC$\underline{H}_2$-triazole), 4.85 (d, J=13.0 Hz, OC$\underline{H}_2$-triazole), 4.90 (t, J=8.5 Hz, $H2'_{Ac}$ (non-reducing end)), 4.96 (d, J=13.0 Hz, OC$\underline{H}_2$-triazole), 5.03 (d, J=3.5 Hz, $H1\alpha_{Me}$ (reducing end)), 5.15 (t, J=9.5 Hz, $H4'_{Ac}$ (non-reducing end)), 5.21 (t, J=9.5 Hz, $H3'_{Ac}$ (non-reducing end)), 5.40 (t, J=9.0 Hz, $H2_{Ac}$ (reducing end)), 5.41 (t, J=9.0 Hz, $H3_{Ac}$ (reducing end)), 5.84-5.87 (d, $H1_{Ac}$ (reducing end)), 7.81 (s, triazole CH)

^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.1, 20.2, 20.4, 20.5 (COCH$_3$), 52.8, 53.3 (COO$\underline{C}$H$_3$), 58.8, 59.0, 59.1 (O$\underline{C}$H$_3$), 59.3, 59.6, 60.1, 60.1, 60.3 (O$\underline{C}$H$_3$), 60.4, 50.4, 60.5 (O$\underline{C}$H$_3$), 60.6 (O$\underline{C}$H$_2$-triazole, overlapped), 60.7, 60.8, 62.3 (O$\underline{C}$H$_2$-triazole), 69.3 ($C4'_{Ac}$ (non-reducing end)), 69.9, 70.0 ($C3_{Ac}$ (reducing end)), 70.3 ($C6_{Me}$ (internal)), 70.6, 71.0 ($C2'_{Ac}$ (non-reducing end)), 71.6 ($C2_{Ac}$ (reducing end)), 72.0 ($C3'_{Ac}$ (non-reducing end)), 72.2, 72.7 ($C5'_{Ac}$ (non-reducing end)), 73.2, 73.2, 74.6, 74.7,

74.9 ($C5_{Me}$ (internal)), 76.2 ($C4_{Ac}$ (reducing end)), 76.3 ($C5_{Ac}$ (reducing end)), 77.5 ($C4_{Me}$ (internal)), 83.5 ($C2_{Me}$ (internal)), 85.0 ($C3_{Me}$ (internal)), 86.1 ($C1_{Ac}$ (reducing end)), 95.7 ($C1\alpha_{Me}$ (reducing end)), 100.3 ($C1'_{Ac}$ (non-reducing end)), 103.2 ($C1_{Me}$ (internal)), 103.4 ($C1_{Me}$), 121.4 (triazole *C*H), 145.1 (O-CH_2-*C*=), 166.6 ($\underline{C6}'_{Ac}$ $OOCH_3$), 166.8 ($\underline{C6}_{Ac}$ $OOCH_3$), 169.0, 169.3, 169.7, 169.8, 170.1 ($COCH_3$)

4-(Tri-*O*-methyl-cellulosyloxymethyl)-1-[β-D-glucopyranuronosyl-(1→4)-β-D-glucopyranuronosyl] -1*H*-1,2,3-triazole (**3**):

An aqueous solution of sodium hydroxide (0.0125 M, 1.5 mL) was added at room temperature to a solution of 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-[methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranuronosyl]-1*H*-1,2 ,3-triazole (**6**, 14.4 mg) in THF (1 mL). The reaction mixture was stirred at room temperature for 30 min. The solution was neutralized with Amberlyst H^+. The Amberlyst H^+ was filtered off and washed with tetrahydrofuran. The combined filtrate and washings were concentrated to dryness to give the crude product. The crude product was purified by gel filtration chromatography (Sephadex LH-20) to give 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-[(β-D-glucopyranuronosyl)-(1→4)-β-D-glucopyranuronosyl]-1*H*-1,2,3-triaz ole (**3**, 13.8 mg, 99% yield).

^{1}H-NMR (500 MHz, D_2O): δ 3.03 (t, 1H, *J*=8.5 Hz, $H2_{Me}$ (internal)), 3.27 ($H2_{Me}$), 3.34 (t, 1H, *J*=9.0 Hz, $H3_{Me}$ (internal)), 3.47 ($H5_{Me}$ (internal)), 3.29 ($OC\underline{H}_3$), 3.46 ($OC\underline{H}_3$), 3.47 ($OC\underline{H}_3$), 3.64 ($H4_{Me}$ (internal)), 3.6-3.7 ($H6_{Me}$ (internal)), 3.77 ($H4_{OH}$), 3.96 ($H3_{OH}$), 4.02 ($H2_{OH}$), 4.31 (d, *J*=5.5 Hz, $C1_{Me}$ (internal)), 4.43 (d, *J*=7.0 Hz, $H1_{Me}$), 4.72 ($OC\underline{H}_2$-triazole), 4.80 (d, *J*=14.0 Hz, $OC\underline{H}_2$-triazole), 4.88 (d, *J*=13.5 Hz, $OC\underline{H}_2$-triazole), 5.71(d, *J*=8.5 Hz, $H1_{OH}$), 8.23 (s, triazole C*H*)

^{13}C-NMR (125 MHz, D_2O): δ 58.3 ($O\underline{C}H_3$), 58.5, 58.9 ($O\underline{C}H_3$), 59.8, 60.3 ($O\underline{C}H_3$), 60.4 ($O\underline{C}H_2$-triazole), 62.2 ($O\underline{C}H_2$-triazole), 68.8, 69.8 ($C6_{Me}$ (internal)), 71.8 ($C3_{OH}$), 73.0, 73.6 ($C5_{Me}$ (internal)), 74.2, 74.6 ($C4_{OH}$), 75.9 ($C4_{Me}$ (internal)), 78.0 ($C2_{OH}$), 80.3 ($C2_{Me}$), 82.1 ($C2_{Me}$ (internal)), 82.5, 83.0 ($C3_{Me}$ (internal)), 85.0 ($C3_{Me}$), 87.0 ($C1_{OH}$), 101.1, 101.3, 102.3 ($C1_{Me}$ (internal)), 124.5 (triazole *C*H), 144.0 (O-CH_2-*C*=)

GPC analysis of acetylated compound **3**: M_n=9.1×10^3, M_w/M_n=1.9, DP_n=43 (including DP of hydrophilic segment)

3. Results and Discussion

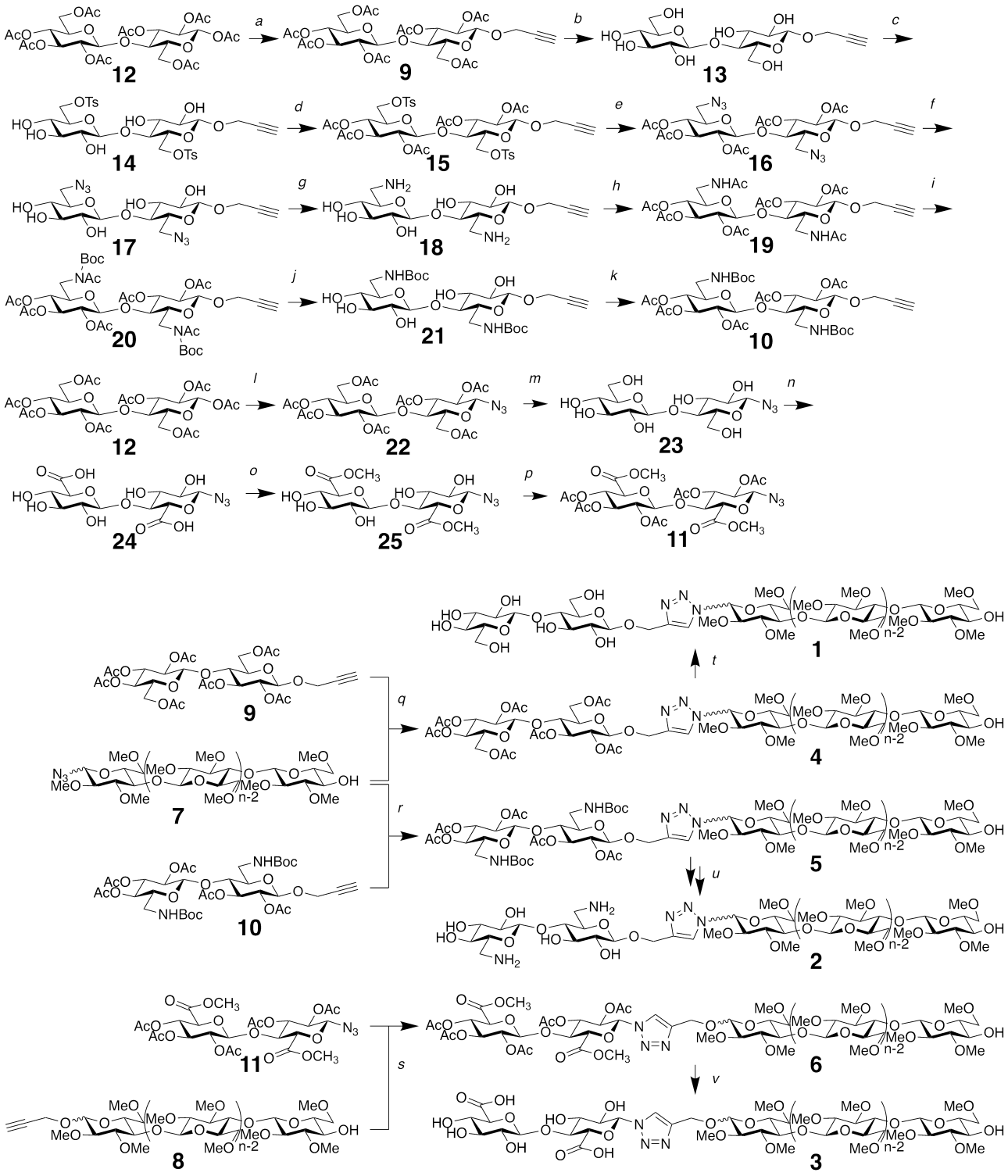
3.1. Synthesis of trehalose-type diblock methylcellulose analogues

Three hydrophilic segments, nonionic, cationic, and anionic cellobiosyl residues, were coupled with hydrophobic permethylated methylcellulose segments via the Huisgen 1,3-dipolar cycloaddition to produce trehalose-type diblock methylcellulose analogues.

632

633 3.1.1. Synthesis of hydrophilic segments

634 2-Propynyl 2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside
635 (**9**), 2-propynyl
636 2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acet
637 yl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranoside (**10**), and methyl [(methyl
638 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl
639 azide]uronate (**11**) were synthesized according to the synthetic routes shown in Scheme 1.
640 Compound **9** was prepared from cellobiose via cellobiose octaacetate (**12**) (H. Kamitakahara &
641 Nakatsubo, 2005) and is a precursor of the nonionic hydrophilic segment that gives compound **1**.
642 Huisgen 1,3-dipolar cycloaddition of compounds **7** and **9** produced compound **4**. The removal of
643 the acetyl groups of compound **4** afforded compound **1**. Compound **1** is analogous to
644 cellobiosyl-(1→4)-methylcellulose, diblock methylcellulose (Nakagawa et al., 2011b), which
645 possesses thermoreversible gelation properties. Nonionic compound **1** is also a standard for cationic
646 compound **2** and anionic compound **3**. In other words, nonionic compound **9** is also a standard for
647 cationic compound **10** and anionic compound **11**.
648



Scheme 1. Synthetic routes for trehalose-type diblock methylcellulose analogues **1**, **2**, and **3**

a) 2-propyn-1-ol/BF_3Et_2O/ anhydrous CH_2Cl_2/r.t./ 23h/99%; b) 28%$NaOCH_3$ in MeOH/ THF/MeOH/r.t./3h/96%; c) TsCl/Pyridine/ 8°C/ 2.5h/51%; d) Ac_2O/Pyridine/ r.t./overnight/ 95%; e) NaN_3/DMF/ 50°C/overnight/97%; f) 28%$NaOCH_3$ in MeOH/ THF/MeOH/r.t./overnight/ 87%; g) Ph_3P/ H_2O/ THF/MeOH/r.t./14d/98%; h) Ac_2O/AcONa/ 80°C/3h; Ac_2O/Pyridine/80°C/1h/ 96%; i) Boc_2O/DMAP/ THF/reflux/22.5h; j) 28%$NaOCH_3$ in MeOH/ CH_2Cl_2/MeOH/r.t./6h; k) Ac_2O/Pyridine/ 60°C/2h/77%; l) $TMSN_3/SnCl_4$/ $CHCl_3$/r.t./overnight/99%; m) 28%$NaOCH_3$ in MeOH/ CH_2Cl_2/MeOH/r.t./ quantitative yield; n) TEMPO/KBr/NaOCl/sat. aq. $NaHCO_3$/4°C/1d; o)

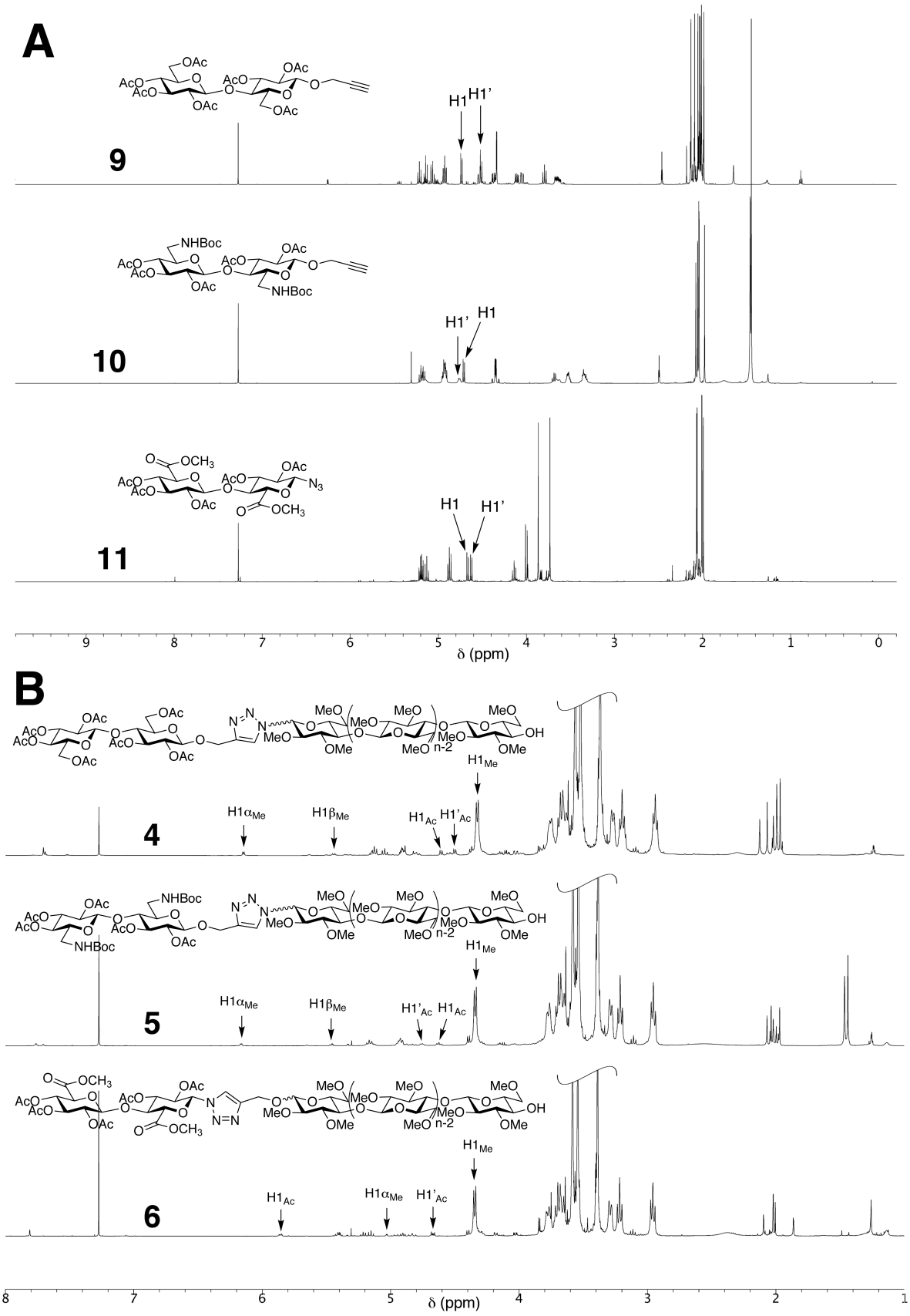
2,2-dimethoxypropane/conc. HCl/ MeOH/r.t./ 1d; p) Ac_2O/AcONa/60°C/overnight/ total yield 11% from compound **23**; q) Cu(I)Br/ sodium ascorbate in water/ PMDETA/MeOH:CH_2Cl_2 (1:4, v/v), distilled water/r.t./4 days; r) $CuSO_4 \cdot H_2O$/ sodium ascorbate in water/ PMDETA/THF/50°C/overnight; s) $CuSO_4 \cdot H_2O$/ sodium ascorbate in water/ PMDETA/THF/50°C/overnight; t) 28%$NaOCH_3$ in MeOH/ THF/MeOH/r.t./overnight; u) 28%$NaOCH_3$ in MeOH/ THF/MeOH/CH_2Cl_2 /r.t./overnight; trifluoroacetic acid/-20°C/1.2 h; v) 0.0125 M NaOH/THF/r.t./30 min/99% yield

The synthesis of amino-functionalized cellobiose compound **10** from 2-propynyl (6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranoside (**18**) was analogous to a method for a glucosamine derivative (Chen et al., 2010). Compound **10** was converted from compound **9** in 10 reaction steps. The *N*-acetyl groups at the C-6 positions of compound **19** would be relatively stable under alkali conditions. The amino groups of compound **18** are reactive and labile and are therefore protected by *tert*-butoxycarbonyl (Boc) groups. The primary hydroxy groups at the C-6 positions of compound **13** were tosylated with *p*-toluenesulfonyl chloride to give compound **14** in 51% yield. The four secondary hydroxy groups of compound **14** were acetylated with acetic anhydride in pyridine to give compound **15** in 95% yield. The tosylate **15** was treated with sodium azide to give azide derivative **16** in 97% yield via a nucleophilic substitution at the C-6 positions. Removal of the acetyl groups of compound **16** produced compound **17** in 87% yield. A Staudinger reaction afforded amino derivative **18** from azido compound **17** in 98% yield. Compound **18** is an analogous derivative of a chitosan dimer. Because the reactivity and stability of compound **18**, without protective groups for the amino and hydroxy groups, are unknown, the protected compound **10** was selected as the reactant for Huisgen 1,3-dipolar cycloaddition. Compound **18** was acetylated to give 6-(acetyl)amino derivative **19**. Butoxycarbonylation of *N*-(acetyl)amino compound **19** followed by removal of the acetyl groups afforded 6-(Boc)amino derivative **21** via 6-acetyl(Boc)amino derivative **20**. Compound **21** was acetylated to give 2-propynyl (2,3,4-tri-*O*-acetyl-6-(Boc)amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-(Boc)amino-6-deoxy-β-D-glucopyranoside (**10**). Compound **10** is a precursor of the cationic hydrophilic segment that gives compound **2**.

Compound **11** was prepared from cellobiose octaacetate (**12**) (H. Kamitakahara & Nakatsubo, 2005) in five reaction steps and is a precursor of the anionic hydrophilic segment that gives compound **3**. Compound **11** is a glycosyl azide derivative, although compounds **9** and **10** are 2-propenyl glycosides. The alkyne group was unstable under the reaction conditions for TEMPO oxidation of the primary alcohol. Methyl [(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl azide]uronate (**11**) was therefore chosen as the hydrophilic segment and treated with 2-propynyl tri-*O*-methyl-celluloside to produce compound **6**. Cellobiose octaacetate (**12**) was converted into 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (**22**)

(H. Kamitakahara & Nakatsubo, 2005). Removal of the acetyl groups of compound **22** gave β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl azide (**23**). TEMPO oxidation of cellobiosyl azide **23** gave (β-D-glucopyranuronosyl)-(1→4)-β-D-glucopyranuronosyl azide (**24**). The uronic acids of compound **24** were esterified to give methyl [(methyl β-D-glucopyranosyluronate)-(1→4)-β-D-glucopyranosyl azide]uronate (**25**). The remaining hydroxy groups of compound **25** were acetylated to give methyl [(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl azide]uronate (**11**).

Figure 1A shows ^{1}H-NMR spectra of cellobiose derivatives **9**, **10**, and **11**. Figure S1 in the Supporting Information show ^{13}C-NMR spectra of cellobiose derivatives **9**, **10**, and **11**.



705 Figure 1. ^{1}H-NMR spectra of (A) cellobiose derivatives **9**, **10**, and **11**, and (B) compounds **4**, **5**,
706 and **6** after CuAAC reaction
707

3.1.2. Synthesis of trehalose-type diblock methylcellulose analogues **1**, **2**, and **3**

The Huisgen 1,3-dipolar cycloaddition followed by removal of the protective groups afforded the nonionic, cationic, and anionic diblock methylcellulose analogues 1-(tri-*O*-methyl-cellulosyl)-4-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**1**), 1-(tri-*O*-methyl-cellulosyl)-4-(6-amino-6-deoxy-β-D-glucopyranosyl-(1→4)-6-amino-6-deoxy-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**2**), and 4-(tri-*O*-methyl-cellulosyloxylmethyl)-1-(β-D-glucopyranuronosyl-(1→4)-D-glucopyranuronosyl)-1*H*-1,2,3-triazole (**3**), as shown in Scheme 1.

The Huisgen 1,3-dipolar cycloaddition between the alkyne and azido derivatives was successfully carried out. An excess amount, three equivalents, of cellobiose derivatives **9**, **10**, and **11** relative to polymeric methylcellulose derivatives **7** and **8** produced trehalose-type diblock methylcellulose derivatives **4**, **5**, and **6** with no remaining **7** and **8**. In the MALDI-TOF mass spectra shown in Figure S2, we observed pseudo-molecular-ion peaks for compounds **4**, **5**, and **6** (see the Supporting Information), which means that complete end-functionalization of the methylcellulose derivatives was carried out via the Huisgen 1,3-dipolar cycloaddition.

Figure 1B shows the ^{1}H-NMR spectra of compounds **4**, **5**, and **6** obtained as a result of the Huisgen 1,3-dipolar cycloadditions. The proton resonances of the cellobiose segments were small relative to those of the polymeric methylcelluloses. The triazole ring proton, however, appeared at approximately 7.7–7.8 ppm, which enabled us to confirm the successful formation of the trehalose-type diblock methylcellulose analogues. The ^{1}H-NMR spectrum of compound **4** revealed that the C-1 proton of the α- and β-anomers of the methylcellulosyl residue appeared at 6.15 and 5.44 ppm, respectively. The α/β ratio was approx. 2/1. Two triazole protons appeared in the ^{1}H-NMR spectra of compounds **4** and **5**, which means that the α- and β-anomers of the methylcellulosyl residue affected the chemical shift of those 1,2,3-triazole protons. In contrast, one triazole proton appeared in the ^{1}H-NMR spectra of compound **6**. The proton resonance corresponding to the anomeric center of the methyl glucopyranosiduronate residue attached to the triazole unit appeared at approx. 5.8–5.9 ppm as a doublet. The anomeric center at the reducing end of the methylcelluloside appeared at 5.03 ppm as a doublet (J=3.5 Hz).
After the removal of the acyl groups of compounds **4**, **5**, and **6**, the proton resonances of the unmodified cellobiosyl residues of compounds **1**, **2**, and **3** overlapped with those of methylcellulose (data not shown). Therefore, we were unable to conclude from the results of the NMR analysis that compounds **1**, **2**, and **3** were obtained. However, the MALDI-TOF mass spectra of compounds **1**, **2**, and **3** proved that we succeeded in establishing synthetic routes for these target compounds (Figure

S2). The MALDI-TOF mass spectrum of compound **1** shows that the pseudo-molecular-ion peaks of compound **1** appeared as sodium adducts. In contrast, the MALDI-TOF mass spectrum of compound **2** revealed that amino derivative **2** was observed as a proton adduct.

The synthesis of compound **3** from compound **6** under alkali conditions did not remove the a proton (H-5) of the C-6 carbonyl carbon atom of the hydrophilic segment to promote β-elimination; therefore, there was no depolymerization of a glucuronic acid at the non-reducing end of the hydrophilic segment of compound **3**. We did not observe any evidence of the β-elimination, as shown in Figure 1, although there are unassigned peaks. Purity of compounds **1**, **2**, and **3** was confirmed by means of MALDI-TOF MS, GPC after acetylation, and analytical thin layer chromatography (TLC).

In addition, the zeta potential of compounds **1**, **2**, and **3** revealed that compounds **2** and **3** involve cationic and anionic functional groups, respectively, as summarized later in Table 1. The zeta potential data also proved that compounds **1**, **2**, and **3** were produced.

3.2. Physical properties of trehalose-type diblock methylcellulose analogues **1**, **2**, and **3**

Some physical properties of compounds **1**, **2**, and **3** are summarized in Table 1. We investigated the properties of the aqueous solutions. Nonionic compound **1** shows a negative zeta potential (-6.8 mV), likely because oxygen atoms along the methylcellulose residue affect the negative charge for the whole molecule. The zeta potential of cationic compound **2** was slightly higher than that of nonionic compound **1**. Two amino groups at the end of molecule **2** affected the total zeta potential of compound **2** in water. The zeta potential of compound **3** was the lowest among compounds **1**, **2**, and **3**, which means that the carboxylic acid at the end of the molecule affected the overall zeta potential of compound **3**. Table 1 also summarizes the interfacial properties, DLS data of the aqueous solutions, DSC data, and gelation properties.

Table 1. Physicochemical properties of compounds **1**, **2**, and **3**

Compound no.	Zeta potential (mV)	Interfacial property		Aggregation temperature (˚C) judged by DLS	Thermal property detected by DSC (4.0 wt%)		Gelation property	
	0.2 wt%, 35˚C	Critical Micelle Concentration (CMC) (mg/mL)	Surface tention at CMC (mN/m)	(0.2 wt%)	Endothermic peak (˚C)	Exothermic peak (˚C)	2.0 wt%	4.0 wt%
1	-6.8	6.5×10^{-3}	48.2	33	29	5	+ (30˚C)	+ (30˚C)
2	-3.9	2.6×10^{-3}	44.0	34	33	8	-	+ (35˚C)
3	-28.2	3.5×10^{-3}	44.3	20-29	24	3	-	+ (30˚C)

3.2.1. Surface activity of aqueous solutions of compounds **1**, **2**, and **3**

The trehalose-type diblock methylcellulose analogues **1**, **2**, and **3** exhibited amphiphilic properties and better surface activities than commercially available methylcellulose SM-4, as shown in Figure 2. The surface activity was in the order **2** > **3** > **1**. In particular, cationic compound **2** exhibited the best surface activity among the compounds tested, likely because its diblock structure, comprising a

cationic segment with amino groups and a slightly anionic methylcellulose segment, affected its self-assembly behavior. Moreover, the surface-tension–concentration curve of compound **2** was atypical. Namely, any phase transition of compound **2** might occur over 0.16 mg/mL. The mechanism of concentration-dependent aggregation behavior for trehalose-type diblock methylcellulose analogues is now under investigation.

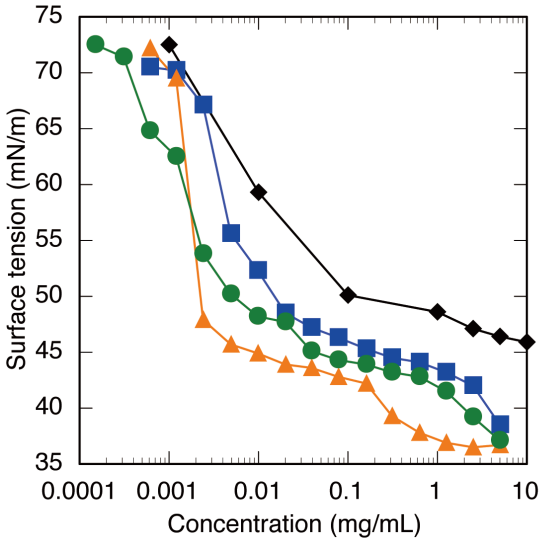


Figure 2. Surface tension-concentration curves of compounds **1**, **2**, and **3**; blue solid square: **1**; orange solid triangle: **2**; green solid circle: **3**; black solid diamond: commercially available methylcellulose SM-4.

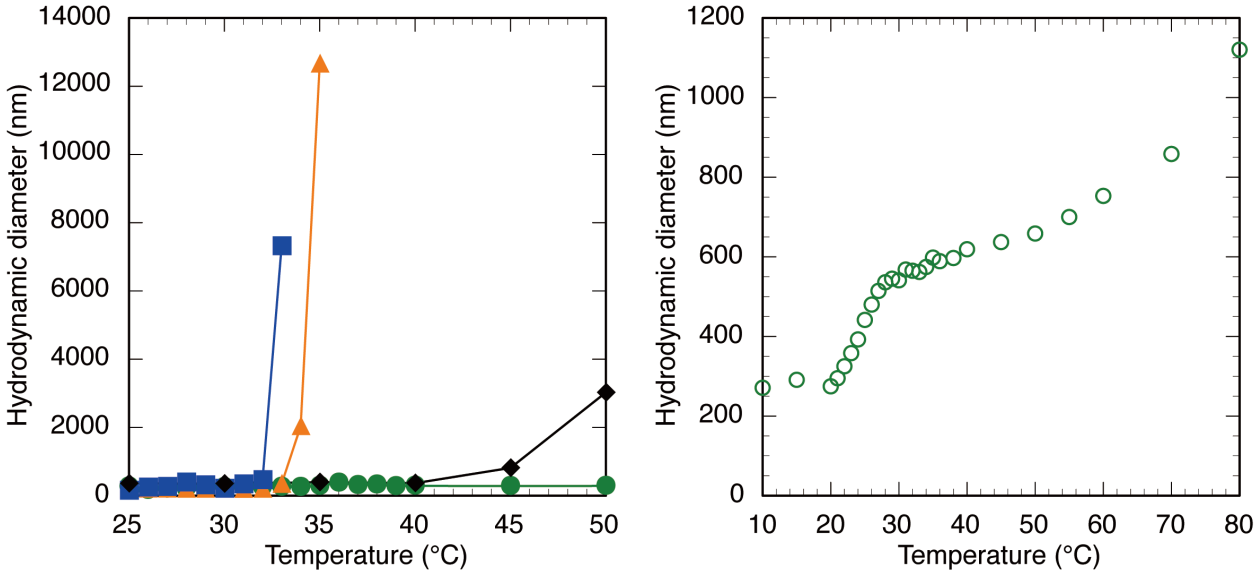


Figure 3. Hydrodynamic diameter of 0.2 wt.% aq. solution of compounds **1**, **2**, and **3** (a) and expanded graph of 2.0 wt.% aq. solution of compound **3** (b) as a function of temperature; blue solid square: **1**; orange solid triangle: **2**; green solid circle: **3**; black solid diamond: commercially available methylcellulose SM-4.

789

790 3.2.2. Thermoresponsive aggregation behavior of compounds **1**, **2**, and **3** in water

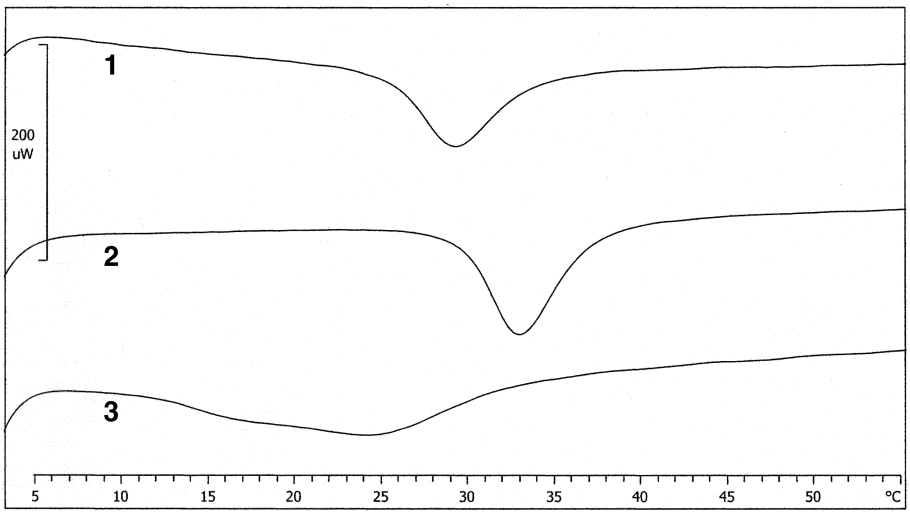
791 The aggregation properties of compounds **1**, **2**, and **3** in 0.2 wt. % aqueous media were different, as
792 shown in Figure 3. Compounds **1** and **2** suddenly aggregated at 33 °C and 34 °C, respectively, but
793 compound **3** did not show obvious aggregation properties. In contrast, the 2.0 wt. % aqueous
794 solution of compound **3** gradually aggregated in the range from 20 to 29 °C, likely because its
795 relatively large negative zeta potential would inhibit its self-aggregation behavior. The hydrophilic
796 segments affected molecular aggregation, with the result that the nonionic, cationic, and anionic
797 compounds exhibited different thermoresponsive temperatures.

798

799 3.2.2. Thermal properties of aqueous solutions of compounds **1**, **2**, and **3**

800 Figure 4 shows DSC data for aqueous solutions of compounds **1**, **2**, and **3**. Nonionic compound **1**
801 and cationic compound **2** exhibited endothermic peaks at 29 °C and 33 °C, respectively. In contrast,
802 anionic compound **3** exhibited a broad endothermic peak in the range from 13 to 30 °C. The
803 endothermic peak indicated dehydration surrounding the methylcellulose analogues. The results of
804 DLS experiments are consistent with those of DSC analysis, indicating that a dehydration process
805 followed by self-aggregation occurred. For instance, the endothermic peak of the aqueous solution
806 of compound **1** appeared at 29 °C upon heating (heating rate: 3.5 °C/min). After the endothermic
807 temperature of compound **1** at 29 °C was detected by DSC measurements, the aggregation of
808 compound **1** was apparently observed by DLS measurements at 33 °C, as summarized in Table 1.
809 The same tendency was also observed for compound **2**. The dehydration of compound **3** occurred
810 slowly, likely because the large negative zeta potential of compound **3** would disturb the
811 intermolecular aggregation process.

812



813

814 Figure 4. DSC thermograms of 2.0 wt. % aqueous solutions of compounds **1**, **2**, and **3**. Heating rate:
815 3.5°C/min.

816

817 **3.3. Observation of thermoresponsive supramolecular hydrogels comprising compounds 1**, **2**,
818 and **3**

819 3.3.1. Visual observation of thermoresponsive supramolecular hydrogels

820 Figure 5 shows photographs of dispersions of compounds **1**, **2**, and **3** in water at 0, 30, and 35 °C.
821 With increasing temperature, the trehalose-type methylcellulose analogues self-aggregated,
822 triggered by dehydration around the molecules. The multi-molecular assembly of the trehalose-type
823 methylcellulose analogues caused a macroscopic change from sol to hydrogel. A 2 wt. % aqueous
824 solution of nonionic compound **1** became a hydrogel at 30 °C. In contrast, 2 wt. % aqueous
825 solutions of cationic compound **2** and anionic compound **3** stayed in the sol state. However, 4 wt. %
826 aqueous solutions of ionic compounds **2** and **3** became hydrogels at 35 and 30 °C, respectively. A 4
827 wt. % aqueous solution of nonionic compound **1** also became a hydrogel at 30 °C.

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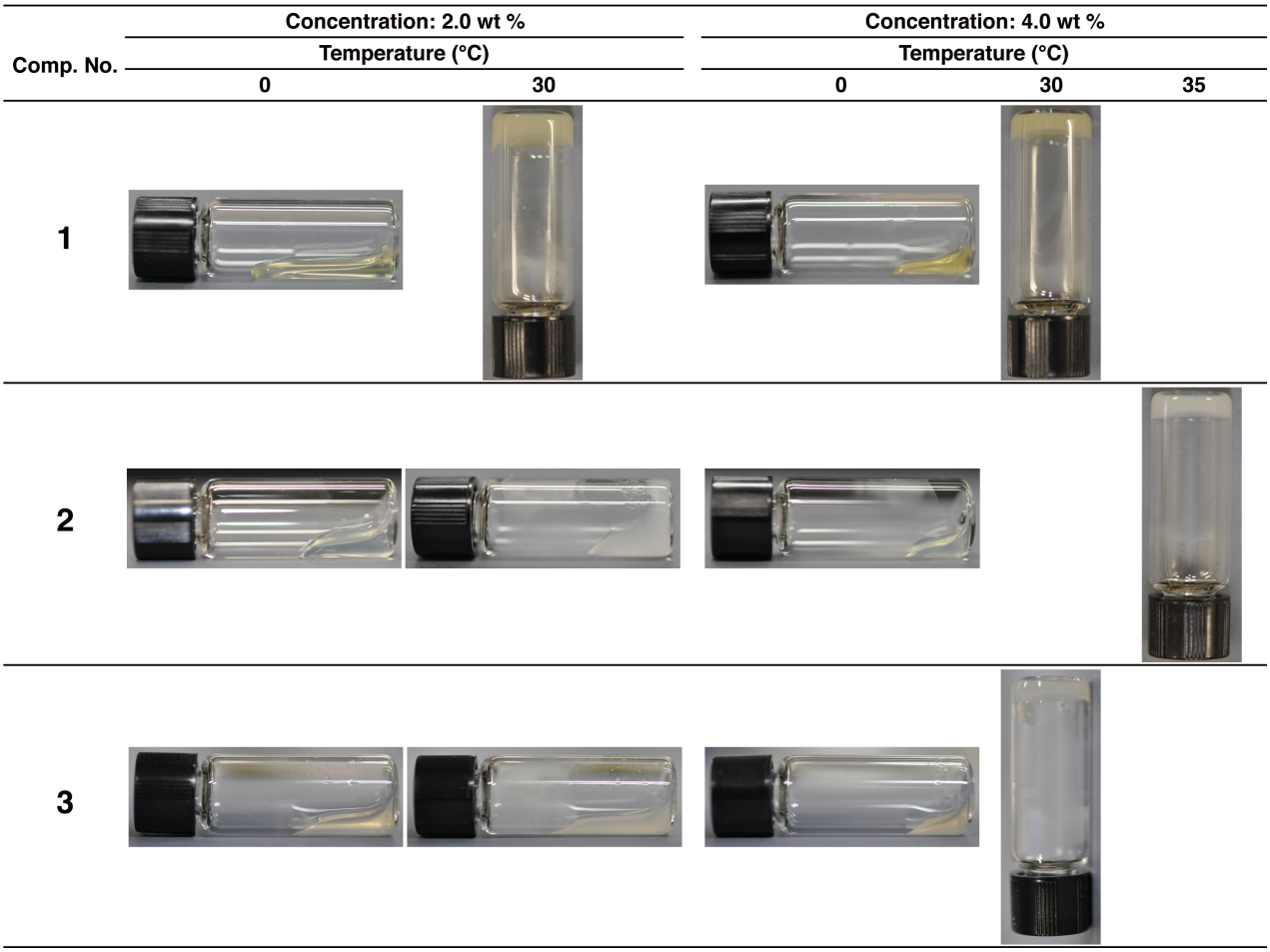

829

830 Figure 5. Photographs of dispersion of compounds **1**, **2**, and **3** in water at 0, 30, and 35°C

831

832 3.3.2. Layered structure of lyophilized hydrogels from compounds **1**, **2** and **3**.

833 Scanning electron microscopy images are shown in Figure 6 a–f. The sections of the three kinds of

hydrogels show layered structures (Figure 6, insets b, d, and f). Short, regularly arranged protuberances can be seen on one side of the layers (Figure 6 a, c and e). The surfaces of the layers in the hydrogel from cationic compound **2** were very smooth relative to those in the hydrogels from compounds **1** and **3**, which suggests that the chemical structure of the hydrophilic segments affects the structure of the hydrogels.

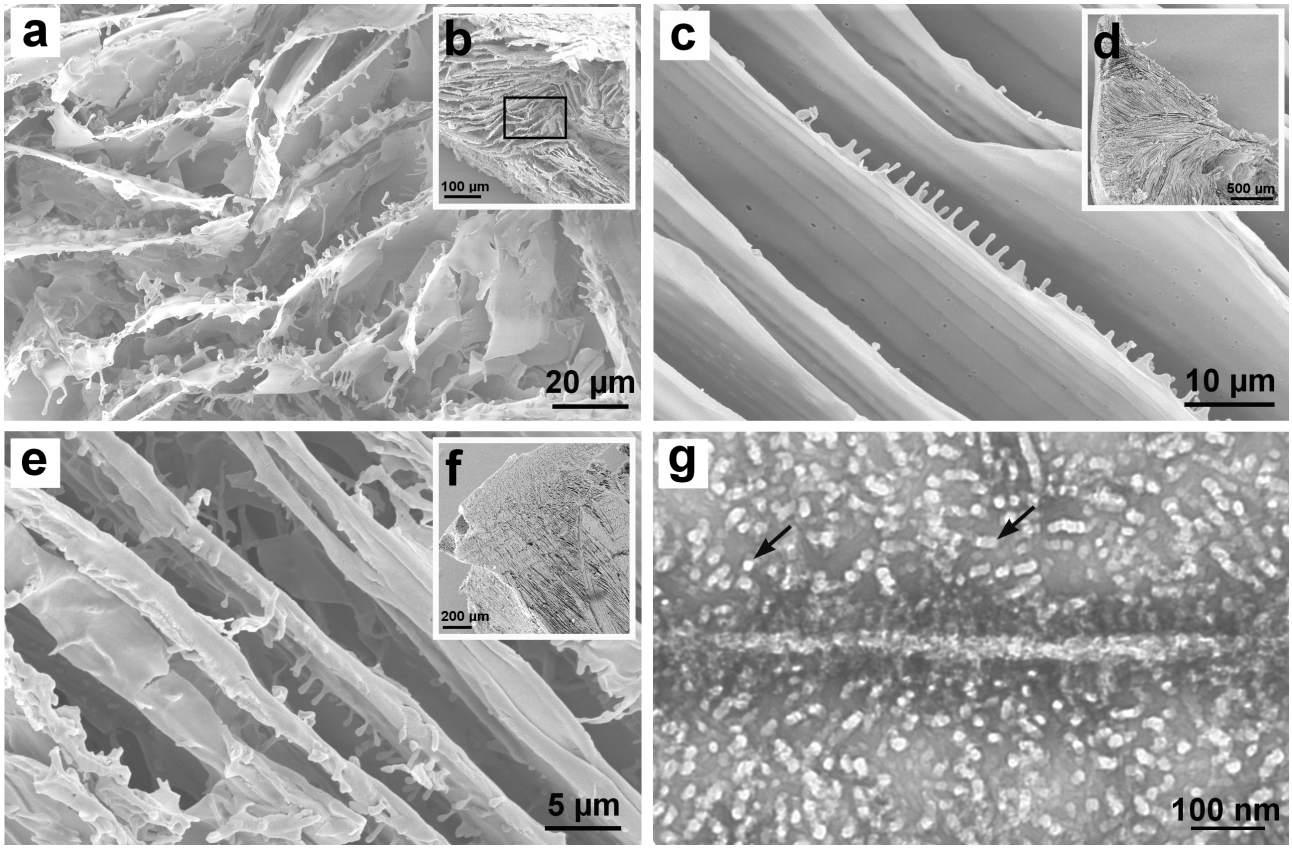

Figure 6. Scanning electron microscopic images of hydrogels from compounds **1** (a, b), **2** (c, d), and **3** (e, f)**;** a transmission electron microscopic image of compound **1** (g). a, c, e were enlarged images from low magnified images (b, d and f). Arrows in g indicate square and rectangular aggregated particles.

3.3.3. Ultrastructures of thermoresponsive supramolecular hydrogel of compound **1**

A transmission electron microscopy image is shown in Figure 6 g. Square or rectangular structures of approximately 20 nm × 20–55 nm (Figure 6 g, arrows) can be seen. In addition, a long linear structure of approximately 20 nm width with short fine protuberances on both sides was observed. The molecular length of compound **1** is approx. 10 nm. Two molecules of compound **1** exist in the 20 nm width for a short side of a rectangular structure. We propose a hypothesis for how the molecules of compound **1** self-assembled to form square or rectangular structures in Figure S3 in the Supporting Information. The thickness of those structures was approximately 1.2 nm, as

confirmed by atomic force microscopy (data not shown). The long side of the rectangular structure alters depending on the particles. Hydrophobic interactions between tri-*O*-methylcellulose segments would elongate the long side of the rectangular structure upon heating. Hydrogen bonding between hydrophilic segments would drive self-assembly between square and/or rectangular structures to form a linear structure over 1 μm. This combined structure would grow into a shish-kebab-like supramolecular structure. Finally those shish-kebab-like supramolecular structures would grow into a two-dimensional sheet structure, as shown in Figure 6 a.

4. Conclusion

We succeeded in the methycellulose analogues end-functionalized with nonionic and ionic cellobiose derivatives via Huisgen 1,3-dipolar cycloaddition. New trehalose-type diblock methylcellulose analogues, nonionic **1**, cationic **2**, and anionic **3**, provide understanding of the detailed structure–property relationships of cellulose ether derivatives. The synthetic routes for them were shortened, relative to those we have already reported (Nakagawa et al., 2012). The methodology described in this paper allows us to synthesize a variety of diblock methylcellulose analogues with a series of hydrophilic segments, thereby developing new applications of cellulose derivatives. Cationic compound **2** exhibited higher surface activity than anionic compound **3** and nonionic compound **1**. The two amino groups at the end of the trehalose-type diblock methylcellulose analogue affected its self-assembly behavior at the interface between water and air. Not only nonionic **1** but also cationic **2** and anionic **3** formed thermoresponsive supramolecular hydrogels in water at under 37 °C, close to human body temperature. This fact means that the methylcellulose-based hydrogels including a nonionic or ionic cellobiosyl segment would respond to human body temperature and are comparable with those based on poly(*N*-isopropyl acrylamide) (Ashraf, Park, Park, & Lee, 2016). These methylcellulose-based new materials will be applicable for the similar uses as poly(*N*-isopropyl acrylamide). Trehalose-type methylcellulose analogues from natural resource would produce eco-friendly surfactant, and safe thermoresponsive hydrogel matrices for drug release.

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Figure Legends

Scheme 1. Synthetic routes for cellobiose derivatives

Figure 1. ^{1}H-NMR spectra of (A) cellobiose derivatives **9**, **10**, and **11**, and (B) compounds **4**, **5**, and **6** after CuAAC reaction

Figure 2. Surface tension-concentration curves of compounds **1**, **2**, and **3**; blue solid circle: **1**; orange solid circle: **2**; green solid circle: **3**; black solid circle: commercially available methylcellulose SM-4.

Figure 3. Hydrodynamic diameter of 0.2 wt.% aq. solution of compounds **1**, **2**, and **3** (a) and expanded graph of 2.0 wt.% aq. solution of compound **3** (b) as a function of temperature; blue solid circle: **1**; orange solid circle: **2**; green solid circle: **3**; black solid circle: commercially available methylcellulose SM-4.

Figure 4. DSC thermograms of 2.0 wt. % aqueous solutions of compounds **1**, **2**, and **3**. Heating rate: 3.5°C/min.

Figure 5. Photographs of dispersion of compounds **1**, **2**, and **3** in water at 0, 30, and 35°C

Figure 6. Scanning electron microscopic images of hydrogels from compounds **1** (a, b), **2** (c, d), and **3** (e, f)**;** a transmission electron microscopic image of compound **1** (g). a, c, e were enlarged images from low magnified images (b, d and f). Arrows in g indicate square and rectangular aggregated particles.

Table 1. Physicochemical properties of compounds **1**, **2**, and **3**

Figure S1. ^{13}C-NMR spectra of cellobiose derivatives **9**, **10**, and **11**

Figure S2. MALDI-TOF MS spectra of compounds after CuAAC reaction and of compounds **1**, **2**, and **3** after removal of protective groups

Figure S3. Schematic figure of self-assembly process of compound **1** upon heating

Red hexagon: 2,3,6-tri-*O*-methyl glucose residue; Blue hexagon: unmodified glucose residue

References

Ashraf, S., Park, H.-K., Park, H., & Lee, S.-H. (2016). Snapshot of phase transition in thermoresponsive hydrogel PNIPAM: Role in drug delivery and tissue engineering. *Macromol. Res.,* 24(4), 297-304.

Chen, Q., Bian, N., Cao, C., Qiu, X. L., Qi, A. D., & Han, B. H. (2010). Glucosamine hydrochloride functionalized tetraphenylethylene: A novel fluorescent probe for alkaline phosphatase based on the aggregation-induced emission. *Chemical Communications,* 46(23), 4067-4069.

Heinze, T., Erler, U., Nehls, I., & Klemm, D. (1994). Determination of the substituent pattern of heterogeneously and homogeneously synthesized carboxymethyl cellulose by using high-performance liquid chromatography. *Angew. Makromol. Chem.,* 215, 93-106.

Hossel, P., Dieing, R., Norenberg, R., Pfau, A., & Sander, R. (2000). Conditioning

polymers in today's shampoo formulations - efficacy, mechanism and test methods. *Int. J. Cosmet. Sci.,* 22(1), 1-10.

Isogai, A., & Kato, Y. (1998). Preparation of polyuronic acid from cellulose by TEMPO-mediated oxidation. *Cellulose (London),* 5(3), 153-164.

Kamitakahara, H., & Nakatsubo, F. (2005). Synthesis of diblock copolymers with cellulose derivatives. 1. Model study with azidoalkyl carboxylic acid and cellobiosylamine derivative. *Cellulose,* 12(2), 209-219.

Kamitakahara, H., Nakatsubo, F., & Klemm, D. (2006). Block co-oligomers of tri-O-methylated and unmodified cello-oligosaccharides as model compounds for methylcellulose and its dissolution/gelation behavior. *Cellulose,* 13(4), 375-392.

Kamitakahara, H., Suhara, R., Yamagami, M., Kawano, H., Okanishi, R., Asahi, T., & Takano, T. (2016). A versatile pathway to end-functionalized cellulose ethers for click chemistry applications. *Carbohydrate Polymers,* 151, 88-95.

Kato, T., Yokoyama, M., & Takahashi, A. (1978). Melting temperatures of thermally reversible gels. *Colloid and Polymer Science,* 256, 15-21.

Moni, L., Marra, A., Skotnicki, J. S., Koehn, F. E., Abou-Gharbia, M., & Dondoni, A. (2013). Synthesis of rapamycin glycoconjugates via a CuAAC-based approach. *Tetrahedron Lett.,* 54(51), 6999-7003.

Nakagawa, A., Fenn, D., Koschella, A., Heinze, T., & Kamitakahara, H. (2011a). Physical Properties of Diblock Methylcellulose Derivatives with Regioselective Functionalization Patterns: First Direct Evidence that a Sequence of 2,3,6-Tri-O-methyl-glucopyranosyl Units Causes Thermoreversible Gelation of Methylcellulose. *Journal of Polymer Science Part B-Polymer Physics,* 49(21), 1539-1546.

Nakagawa, A., Fenn, D., Koschella, A., Heinze, T., & Kamitakahara, H. (2011b). Synthesis of Diblock Methylcellulose Derivatives with Regioselective Functionalization Patterns. *Journal of Polymer Science Part A-Polymer Chemistry,* 49(23), 4964-4976.

Nakagawa, A., Kamitakahara, H., & Takano, T. (2012). Synthesis and thermoreversible gelation of diblock methylcellulose analogues via Huisgen 1,3-dipolar cycloaddition. *Cellulose,* 19(4), 1315-1326.

Rees, D. A. (1972). Polysaccharide gels, A molecular view. *Chem. & Ind. (London),* 19, 630-636.

Rinaudo, M. (2006). Chitin and chitosan: properties and applications. *Prog. Polym. Sci.,* 31(7), 603-632.

Saito, T., Kimura, S., Nishiyama, Y., & Isogai, A. (2007). Cellulose Nanofibers

961 Prepared by TEMPO-Mediated Oxidation of Native Cellulose.
962 *Biomacromolecules,* 8(8), 2485-2491.
963 Saito, T., Nishiyama, Y., Putaux, J.-L., Vignon, M., & Isogai, A. (2006). Homogeneous
964 Suspensions of Individualized Microfibrils from TEMPO-Catalyzed Oxidation of
965 Native Cellulose. *Biomacromolecules,* 7(6), 1687-1691.
966 Savage, A. B. (1957). Temperature-Viscosity Relationships for Water-Soluble Cellulose
967 Ethers. *Industrial and Engineering Chemistry,* 49, 99.
968 Schamann, M., & Schafer, H. J. (2003). TEMPO-mediated anodic oxidation of methyl
969 glycosides and 1-methyl and 1-azido disaccharides. *Eur. J. Org. Chem.*(2),
970 351-358.
971 Teshirogi, T., Yamamoto, H., Sakamoto, M., & Tonami, H. (1979). Synthesis of
972 6-amino-6-deoxycellulose. *Sen'i Gakkaishi,* 35(12), T525-T529.
973 Ying, L. Q., & Gervay-Hague, J. (2003). General methods for the synthesis of
974 glycopyranosyluronic acid azides. *Carbohydrate Research,* 338(9), 835-841.
975