

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 $\rightarrow$ 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 $\rightarrow$ 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 $\rightarrow$ 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.

31 1. Introduction

32 Methylcellulose (MC) is one of the more common cellulose ethers and has been of particular
33 interest for the investigation of its structure–property relationships, such as the surface activity of its
34 aqueous solution and its thermo-reversible gelation properties at elevated temperature. These
35 properties of industrial and academic interest are attributed to the chemical structure of the
36 methylcellulose skeleton. Therefore, many researchers have previously investigated MC.
37 Commercial MC prepared under heterogeneous conditions is an alternating block copolymer
38 composed of densely substituted hydrophobic and less densely substituted hydrophilic block
39 sequences (Savage, 1957). The highly methylated region—a sequence of
40 2,3,6-tri-*O*-methyl-glucosyl residues—of the cellulose skeleton is said to cause micelles, that is,
41 liquid–liquid phase separations in aqueous solution (Rees, 1972). These micelles are known as
42 “crosslinking loci” (Kato, Yokoyama, & Takahashi, 1978). In addition, it is well known that
43 reversible crosslinks must exist in any reversible gel (Kato et al., 1978).

44 We have reported diblock methylcellulose derivatives with regioselective functionalization patterns
45 (Nakagawa, Fenn, Koschella, Heinze, & Kamitakahara, 2011b). We found direct evidence that a
46 sequence of 2,3,6-tri-*O*-methyl-glucopyranosyl units causes thermo-reversible gelation of aqueous
47 MC solution and that an idealized diblock structure consisting of 2,3,6-tri-*O*-methyl-glucopyranosyl
48 and unmodified cello-oligosaccharides caused gelation (Nakagawa, Fenn, Koschella, Heinze, &
49 Kamitakahara, 2011a). However, we had to simplify a synthetic route for new methylcellulose
50 derivatives possessing lower critical solution temperature (LCST) behaviors in aqueous solution.
51 Glycosylation of a cellobiose derivative with a polymeric methyl tri-*O*-methylcelluloside having
52 one hydroxy group at the C-4 position of the glucosyl residue at the non-reducing end consumed a
53 large amount of cellobiosyl trichloroacetimidate derivative to afford only the diblock
54 methylcellulose. To improve the efficiency of the coupling reaction between the hydrophobic and
55 hydrophilic segments, we synthesized a diblock methylcellulose analogue via Huisgen 1,3- dipolar
56 cycloaddition (Nakagawa, Kamitakahara, & Takano, 2012). A 2-propynyl group was introduced to
57 the C-4 hydroxy group at the non-reducing end of the methyl tri-*O*-methylcelluloside. Huisgen
58 1,3-dipolar cycloaddition was more efficient than glycosylation for connecting the hydrophobic and
59 hydrophilic segments.

60 Recently, we have reported a versatile pathway to heterobifunctional/telechelic cellulose ethers,
61 such as tri-*O*-methylcellulosyl azide and propargyl tri-*O*-methylcelluloside, with one free C-4
62 hydroxy group attached to the glucosyl residue at the non-reducing end for the use in the Huisgen
63 1,3-dipolar cycloaddition (Hiroshi Kamitakahara et al., 2016). This new method enables us to
64 prepare a hydrophobic segment for the Huisgen 1,3-dipolar cycloaddition from

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

66 If the chemical structure of trehalose-type diblock polysaccharide analogues exhibited the same
67 physical properties as those of the original diblock polysaccharides, the Huisgen 1,3-dipolar
68 cycloaddition of azido and alkyne derivatives could produce a variety of diblock polysaccharide
69 analogues more easily than a glycosylation method, to afford, for instance,
70 cellobiosyl-(1→4)-methylcelluloses. As an example, cellobiosyl-(1↔1)-methylcelluloside, a
71 trehalose-type diblock copolymer, possesses an analogous structure to
72 cellobiosyl-(1→4)-methylcellulose. Moreover,
73 1-methylcellulosyl-4-cellobiosyloxymethyl-1*H*-1,2,3-triazole and
74 4-methylcellulosyloxymethyl-1-cellobiosyl-1*H*-1,2,3-triazole have analogous structures to
75 cellobiosyl-(1↔1)-methylcelluloside. Therefore,
76 1-methylcellulosyl-4-cellobiosyloxymethyl-1*H*-1,2,3-triazole and
77 4-methylcellulosyloxymethyl-1-cellobiosyl-1*H*-1,2,3-triazole exhibit analogous structures to
78 cellobiosyl-(1→4)-methylcellulose, a diblock methylcellulose. These triazole-linked diblock
79 methylcellulose analogues would allow us to gain deep insights into not only fundamental but also
80 potential properties of methylcelluloses.

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

94 To gain deep insights into the influence of the hydrophilic segments of the diblock methylcellulose
95 analogues on the general properties of the original methylcellulose, we chose three hydrophilic
96 segments: β-D-glucopyranosyl-(1→4)-β-D-glucopyranose,
97 (6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranose, and
98 (β-D-glucopyranuronosyl)-(1→4)-β-D-glucopyranuronic acid.

99 Methylcellulose-based diblock copolymers bearing cationic or anionic hydrophilic segments would
100 enhance the physical performance of commercially available methylcellulose. Thus, we describe, in
101 this paper, the synthesis and structure–property relationships of
102 1-(tri-*O*-methyl-cellulosyl)-4-(β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxymethyl)-1*H*-1,2,3
103 -triazole (**1**),
104 1-(tri-*O*-methyl-cellulosyl)-4-((6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-
105 D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**2**), and
106 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-((β-D-glucopyranuronosyl)-(1→4)-β-D-glucopyranuronosyl
107)-1*H*-1,2,3-triazole (**3**). In particular, their surface activities, thermal properties, and
108 thermoresponsive gelation properties will be discussed.

109 **2. Experimental**

110 **2.1. General measurements**

111 ¹H- and ¹³C-NMR spectra were recorded with Varian 500 NMR (500 MHz) or Varian INOVA300
112 (300 MHz) spectrometer in chloroform-*d* with tetramethylsilane as an internal standard or in
113 deuterium oxide with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as an external standard.
114 Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively.
115 Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)
116 analysis was performed with a Bruker MALDI-TOF MS Autoflex III in the positive ion and linear
117 modes. For ionization, a smartbeam laser was used. All spectra were measured in the linear mode
118 by using external calibration. MALDI-TOF MS used 2,5-dihydroxybenzoic acid as a matrix. A
119 Shimadzu liquid chromatography injector (LC-10ATvp), Shimadzu column oven (CTO-10Avp),
120 Shimadzu ultraviolet visible detector (SPD-10Avp), Shimadzu refractive index detector (RID-10A),
121 Shimadzu communication bus module (CBM-10A), Shimadzu LC workstation (CLASS-LC10),
122 and Shodex columns (KF802, KF802.5, and KF805) were used. Number- and weight-averaged
123 molecular weights (M_n , M_w) and polydispersity indices (M_w/M_n) were estimated by using
124 polystyrene standards (Shodex). A flow rate of 1 mL/min at 40 °C was chosen. Chloroform was
125 used as the eluent.

126 **2.2. Differential scanning calorimetry (DSC) measurements**

127 DSC thermograms were recorded on a DSC823[°] instrument (Mettler Toledo, Zurich, Switzerland)
128 with an HSS7 sensor under a nitrogen atmosphere during a heating/cooling cycle (0→90→0 °C)
129 with a heating and cooling rate of 3.5 °C/min. Each temperature cycle was sequentially repeated
130 three times in order to ensure and check the reproducible response of the instrument. The sample
131 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.

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 NaHCO₃ was then added to the reaction mixture. The reaction mixture was extracted with dichloromethane, washed with water, sat. aq. NaHCO₃ solution, and brine, dried with Na₂SO₄, 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).

¹H NMR (Moni et al., 2013) (400 MHz): δ 5.21 (dd, 1H, *J*_{2,3} = *J*_{3,4} = 9.2 Hz, H-3), 5.14 (dd, 1H, *J*_{2',3'} = *J*_{3',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, *J*_{1,2} = 8.0 Hz, H-1), 4.54 (dd, 1H, *J*_{5,6a} = 2.0 Hz, *J*_{6a,6b} = 12.0 Hz, H6a), 4.51 (d, 1H, *J*_{1',2'} = 8.0 Hz, H-1'), 4.37 (dd, 1H, *J*_{5',6'a} = 4.5 Hz, *J*_{6'a,6'b} = 12.5 Hz, H6'a), 4.33 (d, 2H, *J* = 2.5 Hz, OCH₂CCH), 4.10 (dd, 1H, *J*_{5,6b} = 4.7 Hz, H-6b), 4.14 (dd, 1H, *J*_{5',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, OCH₂CCH), 2.19–2.01 (7 s, 21H, 7 Ac).
¹³C-NMR (125 MHz, CDCl₃): δ 20.5, 20.6, 20.7, 20.8, 55.9 (–CH₂CCH), 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 (CH₂CCH), 76.3 (C4), 78.0 (CH₂CCH), 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).

¹H NMR (400 MHz, D₂O) (Moni et al., 2013): δ 4.50 (d, 1H, *J*_{1,2} = 8.0 Hz, H-1), 4.34 (d, 1H, *J*_{1',2'} = 7.5 Hz, H-1'), 4.31 (dd, 2H, *J* = 16.0, 2.5 Hz, OCH₂CCH), 3.82 (dd, 1H, *J*_{5,6a} = 2.0 Hz, *J*_{6a,6b} = 12.5 Hz, H-6a), 3.75 (dd, 1H, *J*_{5',6'a} = 2.0 Hz, *J*_{6'a,6'b} = 12.2 Hz, H6'a), 3.65 (dd, 1H, *J*_{5,6b} = 4.5 Hz, H-6b), 3.56 (dd, 1H, *J*_{5',6'b} = 5.5 Hz, H-6'b), 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, OCH₂CCH).

¹³C-NMR (125 MHz, D₂O): 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*-toluenesulfonyl-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

200 (**13**, 2.17 g, 5.69 mmol) in pyridine (8 mL). The reaction mixture was stirred at 8 °C for 22.5 h.
 201 Brine was then added to the reaction mixture. The organic phase was extracted with ethyl acetate
 202 three times, and pyridine was azeotropically removed with ethanol to produce crude compound **14**.
 203 The crude product was purified by silica gel column chromatography (methanol/chloroform=1/5,
 204 v/v) to give 2-propynyl
 205 (6-*O-p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-6-*O-p*-toluenesulfonyl-β-D-glucopyranoside
 206 (**14**, 1.97 g, 50.5% yield).
 207 ¹H-NMR (500 MHz, CDCl₃): δ 2.40 (s, 3H, PhCH₃), 2.41 (s, 3H, PhCH₃), 2.49 (t, 1H, —CH₂CCH),
 208 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,
 209 CH₂CCH), 4.24-4.43 (H6', H6', H6, H6, CH₂CCH), 4.44 (d, 1H, *J*=8.0 Hz, H1), 4.50 (d, 1H, *J*=7.0
 210 Hz, H1'), 7.3-7.8 (aromatic H)
 211 ¹³C-NMR (125 MHz, CDCl₃): δ 21.7, 55.9 (—CH₂CCH), 68.9 (C6'), 69.1 (C6), 69.4 (C3'), 72.1,
 212 72.4, 73.0 (C2), 73.5, 73.7, 75.7, 75.7 (CH₂CCH), 77.0, 78.8 (CH₂CCH), 100.0, 101.3 (C1'), 128.0,
 213 129.8, 130.0, 132.2, 132.6, 144.9, 145.2 (aromatic C)
 214
 215 2-Propynyl
 216 (2,3,4-tri-*O*-acetyl-6-*O-p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O-p*-tolue
 217 nesulfonyl-β-D-glucopyranoside (**15**):
 218 Acetic anhydride (1 mL) was added to a solution of 2-propynyl
 219 (6-*O-p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-6-*O-p*-toluenesulfonyl-β-D-glucopyranoside
 220 (**14**, 0.763 g) in pyridine (5 mL). The reaction mixture was stirred at room temperature overnight.
 221 The reaction mixture was extracted with ethyl acetate, washed with 1 N HCl, sat. aq. NaHCO₃, and
 222 brine, dried over Na₂SO₄, and concentrated to dryness to give 2-propynyl
 223 (2,3,4-tri-*O*-acetyl-6-*O-p*-toluenesulfonyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-*O-p*-tolue
 224 nesulfonyl-β-D-glucopyranoside (**15**, 0.9409 g, 94.6% yield).
 225 ¹H-NMR (300 MHz, CDCl₃): δ 1.91, 1.98, 1.99, 2.00, 2.03 (COCH₃), 2.45 (t, 1H, —CH₂CCH),
 226 2.47 (s, 3H, PhCH₃), 2.48 (s, 3H, PhCH₃), 3.55 (m, 1H, *J*=2.0, *J*=3.5 Hz, *J*=9.5 Hz, H5), 3.65 (m,
 227 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,
 228 *J*=11.5 Hz, H6'), 4.14-4.28 (H6', H6, CH₂CCH), 4.33 (dd, 1H, *J*=2.0 Hz, *J*=11.0 Hz, H6), 4.38 (d,
 229 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,
 230 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),
 231 7.39-7.84 (aromatic H)
 232 ¹³C-NMR (75 MHz, CDCl₃): δ 20.5, 20.5, 20.6, 20.7, 21.6, 21.7, 55.7 (—CH₂CCH), 66.3 (C6'), 66.8
 233 (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
 234 (CH₂CCH), 77.9 (CH₂CCH), 97.7 (C1), 100.0 (C1'), 128.0, 128.1, 130.1, 130.1, 132.3, 132.6,
 235 145.4, 145.5 (aromatic C), 168.8, 169.3, 169.5, 169.9, 170.1 (COCH₃)

236

237 2-Propynyl

238 (2,3,4-tri-*O*-acetyl-6-azido-6-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl-6-azido-6-deoxy- β -
239 -D-glucopyranoside (**16**):

240 Sodium azide (0.3731 g) was added to a solution of 2-propynyl

241 (2,3,4-tri-*O*-acetyl-6-*O*-*p*-toluenesulfonyl- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl-6-*O*-*p*-tolue
242 nesulfonyl- β -D-glucopyranoside (**15**, 1.2888 g) in *N,N*-dimethylformamide (DMF; 5 mL). The

243 reaction mixture was stirred overnight at 50 °C. The mixture was then poured into distilled water

244 with ice. The organic phase was extracted with dichloromethane four times, dried over sodium

245 sulfate, and concentrated to dryness. The crude product was purified by silica gel column

246 chromatography (eluent: ethyl acetate/*n*-hexane=2/1, v/v) to afford 2-propynyl

247 (2,3,4-tri-*O*-acetyl-6-*O*-azido-6-*O*-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl-6-*O*-azido-6-
248 *O*-deoxy- β -D-glucopyranoside (**16**, 0.8904 g, 97% yield).

249 ¹H-NMR (500 MHz, CDCl₃): δ 1.99 (s, 3H, (CO)CH₃), 2.04 (s, 3H, (CO)CH₃), 2.05 (s, 3H,

250 (CO)CH₃), 2.05 (s, 3H, (CO)CH₃), 2.08 (s, 3H, (CO)CH₃), 2.47 (s, 1H, *J*=2.5 Hz, CH₂CCH), 3.37

251 (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

252 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,

253 1H, *J*=9.5 Hz, H4), 4.36 (d, 2H, *J*=2.5 Hz, CH₂CCH), 4.57 (d, 1H, *J*_{1,2}=8.0 Hz, H1'), 4.79 (d, 1H,

254 *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),

255 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)

256 ¹³C-NMR (125 MHz, CDCl₃): δ 20.5, 20.6, 20.6, 20.7, 20.8 ((CO)CH₃), 50.2 (C6), 50.9 (C6'), 55.8

257 (CH₂CCH), 69.1 (C4'), 71.3 (C2), 71.7 (C2'), 72.4 (C3), 72.6 (C3'), 72.7 (C5), 74.5 (C5'), 75.6

258 (CH₂CCH), 75.9 (C4), 78.0 (CH₂CCH), 97.8 (C1), 100.1 (C1'), 168.9, 169.4, 169.6, 169.8, 170.2

259 ((CO)CH₃)

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

261

262 2-Propynyl (6-azido-6-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-6-azido-6-deoxy- β -D-glucopyranoside
263 (**17**):

264 Sodium methoxide (28%) in methanol (28 μ L) was added to 2-propynyl

265 (2,3,4-tri-*O*-acetyl-6-azido-6-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl-6-azido-6-deoxy- β -
266 -D-glucopyranoside (**16**, 0.3028 g) in methanol (1.5 mL) and THF (1.5 mL). The reaction mixture

267 was stirred at room temperature for overnight. The mixture was neutralized with Amberlyst H⁺. The

268 Amberlyst H⁺ was removed by filtration and washed with methanol. The filtrate and washings were

269 concentrated to dryness to produce 2-propynyl

270 (6-*O*-azido-6-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-6-*O*-azido-6-deoxy- β -D-glucopyranoside (**17**,

271 0.1805 g, 87% yield).

¹H-NMR (500 MHz, D₂O): δ 2.92 (t, 1H, *J*=2.5 Hz, CH₂CCH), 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, (CH₂CCH)), 4.49 (d, 1H, *J*=8 Hz, H1'), 4.68 (d, 1H, *J*=7.5 Hz, H1)

¹³C-NMR (D₂O): δ 50.2 (C6 (b)), 50.8 (C6 (a)), 56.7 (CH₂CCH), 70.0 (C4'), 72.6 (C2), 73.0 (C2'), 73.6 (C5), 73.9 (C3), 74.2 (C5'), 75.2 (C3'), 76.4 (CH₂CCH), 78.5 (CH₂CCH), 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

282

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).

¹H-NMR (D₂O): 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, CH₂CCH), 4.47 (d, 1H, *J*=8.0 Hz, H1'), 4.64 (d, 1H, *J*=8.0 Hz, H1)

¹³C-NMR (D₂O): δ 41.1 (C6), 41.2 (C6'), 56.8 (CH₂CCH), 70.9 (C4'), 72.8 (C2), 73.3 (C2'), 74.2 (C3), 75.2 (C5), 75.4 (C3'), 75.6 (C5'), 75.9 (CH₂CCH), 78.4 (CH₂CCH), 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**.

306

307 2-Propynyl

2,3,4-tri-*O*-acetyl-6-deoxy-6-acetylamino- β -D-glucopyranosyl-(1 $\rightarrow$ 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 $\rightarrow$ 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 $\rightarrow$ 4)-2,3-di-*O*-acetyl-6-deoxy-6-acetylamino- β -D-glucopyranoside (**19**, 233.4 mg, 96% yield).

¹H-NMR (CDCl₃): δ 1.98, 2.03, 2.04, 2.05, 2.07, 20.8 (21H, O(CO)CH₃, NH(CO)CH₃), 2.50 (t, 1H, J =2.0, CH₂CCH), 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, CH₂CCH), 4.38 (dd, 1H, J =2.5 Hz, J =16 Hz, CH₂CCH), 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, C6NH(CO)CH₃), 6.63 (t, 1H, J =6.0 Hz, C6'NH(CO)CH₃)

¹³C-NMR (CDCl₃): δ 20.6, 20.7, 20.7, 20.9 (CH₃ (OAc)), 23.0, 23.3 (CH₃ (NHAc)), 39.1 (C6'), 39.8 (C6), 56.4 (CH₂CCH), 68.7 (C4'), 71.4 (C2), 71.7 (C2'), 72.3 (C5'), 72.7 (C3), 72.8 (C3'), 73.5 (C5), 75.5 (CH₂CCH), 76.2 (C4), 78.3 (CH₂CCH), 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 $\rightarrow$ 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 (Boc₂O; 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 $\rightarrow$ 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 $\rightarrow$ 4)-2,3-di-*O*-acetyl-6-[acetyl(*tert*-butoxycarbonyl)amino]-6-deoxy- β -D-glucopyranoside (**20**, 127.5 mg;

344 MW = 872.9, MALDI-TOF MS: m/z $[M+Na]^+ = 895.4$.
 345 Sodium methoxide (28%) in methanol (13 μ L) was added to a solution of crude compound **20** (97.3
 346 mg) in methanol (2 mL) and dichloromethane (1 mL). The reaction mixture was stirred at room
 347 temperature for 6 h. The mixture was neutralized with Amberlyst H⁺. After filtration of the
 348 Amberlyst H⁺ and washing with methanol, the combined filtrate and washings were concentrated to
 349 dryness to produce crude product. The crude product was purified by preparative thin-layer
 350 chromatography (PTLC; eluent: methanol/dichloromethane=1/9, v/v) to afford 2-propynyl
 351 (6-(*tert*-butoxycarbonyl)amino-6-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-6-(*tert*-butoxycarbonyl)amino
 352 -6-deoxy- β -D-glucopyranoside (**21**, 49.1 mg, MW = 578.6; MALDI-TOF MS: m/z $[M+Na]^+ =$
 353 601.4).
 354 Compound **21** (49.1 mg) was then dissolved in acetic anhydride (0.3 mL) and pyridine (2 mL). The
 355 reaction mixture was stirred at 60 °C for 2 h and concentrated azeotropically with toluene to give
 356 2-propynyl
 357 (2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-*O*-ac
 358 etyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy- β -D-glucopyranoside (**10**, 67.9 mg, 77% yield from
 359 compound **19**).
 360 ¹H-NMR (CDCl₃): δ 1.44, 1.46 (18H, COOC(CH₃)₃), 1.98, 2.04, 2.04, 2.06, 2.08 (15H, m, COCH₃),
 361 2.49 (t, 1H, $J=2.5$ Hz, CH₂CCH), 3.31-3.36 (m, 3H, H₆, H_{6'}, H_{6''}), 3.50-3.54 (m, 2H, H₅, H_{5'}),
 362 3.63-3.65 (m, 1H, H_{6'}), 3.69 (t, 1H, $J=9.5$ Hz, H₄), 4.31-4.39 (2H, m, (CH₂CCH)), 4.71 (d, 1H,
 363 $J=7.0$ Hz, H₁), 4.76 (broad d, 1H, $J=8.0$ Hz, H_{1'}), 4.90-4.96 (m, 4H, H₂, H_{2'}, H_{4'}, NH), 5.17 (t, 1H,
 364 $J=9.5$ Hz, H_{3'}), 5.20 (t, 1H, $J=9.5$ Hz, H₃), 5.15-5.22 (1H, NH)
 365 ¹³C-NMR (CDCl₃): δ 20.6, 20.7, 20.7, 20.8, 20.8 (COCH₃), 28.3, 28.4 (COOC(CH₃)₃), 40.6, 40.7
 366 (C₆, C_{6'}), 56.4 (CH₂CCH), 68.7 (broad, C_{4'}), 71.4 (C₂), 71.6 (C_{2'}), 72.1 (broad, C₃), 72.9 (C_{3'}),
 367 73.0 (C₅ or C_{5'}), 73.9 (broad, C₅ or C_{5'}), 75.4 (CH₂CCH), 75.9 (broad, C₄), 78.3 (CH₂CCH), 79.8,
 368 79.8 (broad, COOC(CH₃)₃), 98.5 (C₁), 99.2 (broad, C_{1'}), 155.7 (COOC(CH₃)₃), 169.4, 169.6,
 369 169.7, 170.2 (COCH₃)
 370 Mw=788.8 MALDI-TOF MS: m/z $[M+Na]^+ = 811.5$, m/z $[M+K]^+ = 827.4$
 371
 372 2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl azide
 373 (**22**):
 374 Compound **22** was prepared according to the method in our previous report (H. Kamitakahara &
 375 Nakatsubo, 2005).
 376
 377 β -D-Glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyl azide (**23**):
 378 Compound **23** was prepared according to the method in previous reports (Schamann & Schafer,
 379 2003; Ying & Gervay-Hague, 2003).

380

381 β -D-Glucopyranosiduronosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosiduronosyl azide (**24**):

382 Potassium bromide (18.8 mg) and TEMPO (16.7 mg) were added to a solution of

383 β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyl azide (**23**, 290 mg) in sat. aq. sodium

384 hydrogencarbonate (3 mL). Sodium hypochlorite (NaOCl, 3.9 mL) was then added to the reaction

385 mixture. The mixture was stirred at 0 °C for 1 h. TEMPO (8 mg) and NaOCl (3.9 mL) were further

386 added to the reaction mixture. After being stirred at 4 °C for one day, the reaction mixture was

387 extracted with distilled water and washed with dichloromethane four times. The aqueous layer was

388 adjusted to pH 2 with 2 N HCl, concentrated, and diluted with distilled water. This concentration

389 and dilution cycle was repeated several times until the color of the solution turned from yellow to

390 colorless. The aqueous layer was finally concentrated to dryness. The insoluble part was filtered off

391 and washed with methanol. The combined filtrate and washings were concentrated to dryness. This

392 procedure was repeated three times to give

393 β -D-glucopyranosiduronosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosiduronosyl azide (**24**, 278.7 mg) (Schamann

394 & Schafer, 2003; Ying & Gervay-Hague, 2003). The carboxylic acid moieties of crude compound

395 **24** were esterified without further purification.

396

397 Methyl [(methyl β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)- β -D-glucopyranosyl azide]uronate (**25**):

398 2,2-Dimethoxypropane (1.5 mL) and one drop of conc. HCl were added at room temperature to a

399 solution of β -D-glucopyranosiduronosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosiduronosyl azide (**24**, 278.7 mg)

400 in methanol (15 mL). The reaction mixture was stirred for one day and concentrated to dryness to

401 give methyl [(methyl β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)- β -D-glucopyranosyl azide]uronate (**25**,

402 273.3 mg) (Schamann & Schafer, 2003). Crude compound **25** was acetylated without further

403 purification.

404

405 Methyl [(methyl

406 2,3,4-tri-*O*-acetyl- β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl- β -D-glucopyranosyl

407 azide]uronate (**11**):

408 Methyl [(methyl β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)- β -D-glucopyranosyl azide]uronate (**25**, 92.5

409 mg) was dispersed in acetic anhydride (4 mL) with sodium acetate (70.8 mg). The reaction mixture

410 was stirred at 60 °C overnight. The organic layer was extracted with ethyl acetate, washed with

411 distilled water twice, aq. sodium hydrogen carbonate three times, and brine, and concentrated to

412 dryness. The crude product was purified by silica gel column chromatography (eluents: ethyl

413 acetate/*n*-hexane=1/1, v/v; methanol/dichloromethane=1/49, v/v) and by PTLC (eluent: ethyl

414 acetate/*n*-hexane=1/1, v/v) to give methyl [(methyl

415 2,3,4-tri-*O*-acetyl- β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl- β -D-glucopyranosyl

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

¹H-NMR (CDCl₃): δ 1.99, 2.01, 2.01, 2.06, 2.07 (15H, COCH₃), 3.73 (s, 3H, C6'OOCH₃), 3.87 (s, 3H, C6OOCH₃), 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)

¹³C-NMR (CDCl₃): δ 20.4, 20.5, 20.5 (COCH₃), 52.8 (C6OOCH₃), 53.2 (C6'OOCH₃), 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'OOCH₃), 167.1 (C6OOCH₃), 169.0, 169.3, 169.3, 170.0, 170.1 (COCH₃)

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

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

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

Propargyl tri-*O*-methyl cellulose (**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 H₂O), 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 cellulose azide (**7**, 98.5 mg, *M_n* = 5.37×10³, *DP_n* = 26.3, 18.4 mmol, 1.0 equiv.) in MeOH/CH₂Cl₂ (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/CH₂Cl₂ to give the crude product. The crude product was purified by silica gel column chromatography (eluent: EtOAc→20% MeOH/CH₂Cl₂) 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).

¹H-NMR (500 MHz, CDCl₃): δ 1.97, 1.99, 2.02, 2.07, 2.13 (COCH₃), 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, OCH₃), 3.52 (s, OCH₃), 3.56 (s, OCH₃), 3.6-3.9 (H5_{Ac}, H4_{Ac}, H5'_{Ac}), 3.65 (H6_{Me} (internal)), 3.68 (t,

452 $J=9.0$, H_{4Me} (internal)), 3.75 (m, H_{6Me} (internal)), 3.99 (m, $J=10.0$ Hz, $H_{5\alpha Me}$, α -anomer), 4.04
 453 (dd, 1H, $J=2.0$ Hz, $J=12.5$ Hz, $H_{6'Ac}$), 4.11 (dd, 1H, $J=5.0$ Hz, $J=12.0$ Hz, H_{6Ac}), 4.17 (t, $J=7.5$ Hz,
 454 $H_{3\alpha Me}$, α -anomer), 4.33 (d, $J=8.0$ Hz, H_{1Me} (internal)), 4.37 ($H_{6'Ac}$), 4.50 (d, $J=8.0$ Hz, $H_{1'Ac}$),
 455 4.54 (dd, $J=1.5$ Hz, $J=11.5$, H_{6Ac} , β -anomer), 4.55 (dd, $J=2.0$ Hz, $J=11.5$ Hz, H_{6Ac} , α -anomer),
 456 4.60 (d, $J=8.0$ Hz, H_{1Ac} , β -anomer of methylcellulose), 4.61 (d, $J=8.0$ Hz, H_{1Ac} , β -anomer of
 457 methylcellulose), 4.81 (d, 1H, $J=12.5$ Hz, OCH_2 -triazole), 4.87-4.94 (d, OCH_2 -triazole), 4.87-4.94
 458 (H_{2Ac} , $H_{2'Ac}$), 5.05 (t, $J=9.5$, $H_{4'Ac}$), 5.13 (t, $J=9.5$ Hz, H_{3Ac} , $H_{3'Ac}$), 5.44 (d, 1H, $J=9.0$ Hz,
 459 $H_{1\beta Me}$), 6.15 (d, $J=5.5$ Hz, $H_{1\alpha Me}$), 7.69 (s, triazole, β -anomer), 7.70 (s, triazole, α -anomer) (α/β
 460 ratio = 2/1)
 461 ^{13}C -NMR (125 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6, 20.8 ($COCH_3$), 59.1 (OCH_3), 60.2 (OCH_3), 60.5
 462 (OCH_3), 61.6, 61.7 ($C_{6'Ac}$), 61.8 (C_{6Ac}), 62.8 (OCH_2 -triazole), 67.7 ($C_{4'Ac}$), 70.2 (C_{6Me} (internal)),
 463 71.3 (C_{2Ac} or $C_{2'Ac}$), 71.5 (C_{2Ac} or $C_{2'Ac}$), 71.9, 72.0 (C_{5Ac}), 72.3 ($C_{5'Ac}$), 72.7 (C_{3Ac} or $C_{3'Ac}$),
 464 72.8 (C_{3Ac} or $C_{3'Ac}$), 73.0, 73.3, 73.7, 74.8 (C_{5Me} (internal)), 76.2 (C_{4Ac} (reducing end)), 77.4
 465 (C_{4Me} (internal)), 77.8 (C_{4Me} (reducing end)), 79.3, 79.5, 81.0 ($C_{3\alpha Me}$), 82.1 (C_{2Me} (reducing end)),
 466 83.3 ($C_{1\alpha Me}$), 83.4 (C_{2Me} (internal)), 83.7, 83.7, 84.0, 84.8, 85.0 (C_{3Me} (internal)), 85.3 (C_{4Me}
 467 (reducing end)), 86.1, 86.9, 87.3 ($C_{1\beta Me}$ (reducing end)), 99.6 (C_{1Ac}), 100.7 ($C_{1'Ac}$), 103.1 (C_{1Me}
 468 (internal)), 103.7 (C_{1Me}), 124.5 (triazole CH), 143.2 ($O-CH_2-C=$), 168.9, 169.2, 169.6, 169.7, 170.2,
 469 170.3, 170.4 ($COCH_3$)
 470

471 1-(2,3,6-Tri-*O*-methyl-cellulosyl)-4-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyloxymethyl]-1
 472 *H*-1,2,3-triazole (**1**):

473 Sodium methoxide (28%) in methanol (0.02 mL, 10 equiv. per AGU) was added at room
 474 temperature to a solution of

475 1-(2,3,6-tri-*O*-methyl-cellulosyl)-4-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-a
 476 cetyl- β -D-glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**4**, 76.3 mg) in MeOH (3 mL) and THF (3.5
 477 mL). The mixture was stirred overnight at room temperature. The solution was neutralized with
 478 Amberlyst H^+ . The Amberlyst H^+ was filtered off and washed with MeOH. The combined filtrate
 479 and washings were concentrated to dryness to give
 480 1-(2,3,6-tri-*O*-methyl-cellulosyl)-4-[β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyloxymethyl]-1*H*
 481 -1,2,3-triazole (**1**, 79.3 mg, quantitative yield).

482 1H -NMR (500 MHz, D_2O): δ 3.03 (t, $J=8.5$ Hz, H_{2Me} (internal)), 3.29 (s, OCH_3), 3.34 (t, 1H, $J=8.0$
 483 Hz, H_{3Me} (internal)), 3.46 (s, OCH_3), 3.47 (s, OCH_3), 4.32 (d, $J=7.0$ Hz, H_{1Me} (internal)), 4.38 (d,
 484 $J=8.0$ Hz), 4.46 (d, $J=7.5$ Hz), 4.46 (d, $J=8.0$ Hz), 4.67, 4.80 (d, $J=13.0$ Hz, OCH_2 -triazole), 4.90 (d,
 485 $J=12.5$ Hz, OCH_2 -triazole), 5.68 (d, 1H, $J=9.5$ Hz, $H_{1\beta Me}$), 6.42 (d, $J=5.5$ Hz, $H_{1\alpha Me}$), 8.16, 8.24,
 486 8.32 (s, triazole CH)

487 ^{13}C -NMR (125 MHz, D_2O): δ 58.3 (OCH_3 (internal)), 58.5, 58.8, 58.9 (OCH_3 (internal)), 59.8,

60.3 (OCH₃ (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**: $M_n=4.9\times10^3$, $M_w/M_n=1.7$, $DP_n=23$ (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-glucopyranosyloxy methyl)-1*H*-1,2,3-triazole (**5**):

CuSO₄·H₂O (34.0 mg, MW = 249.69, 136 mmol, 10 equiv.), sodium ascorbate (54.0 mg/68 μL, 20 equiv., 4 M in H₂O), 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, $M_n = 7.34\times10^3$, $DP_n = 35.8$, 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/CH₂Cl₂ to give the crude product. The crude product was purified by silica gel column chromatography (eluent: EtOAc→10% MeOH/CH₂Cl₂) 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).

¹H-NMR (500 MHz, CDCl₃): δ 1.44, 1.47 (CH₃ (NHBoc)), 1.97, 1.98, 2.00, 2.02, 2.02, 2.04, 2.05, 2.07 (CH₃ (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 (OCH₃), 3.54 (OCH₃), 3.58 (OCH₃), 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 (CH₂, triazole-alkene), 4.90-4.96 (H2_{Ac}, H2'_{Ac}, H4'_{Ac}, NH), 5.1-5.2 (H3_{Ac}, H3'_{Ac}), 5.46 (d, $J=9.0$ Hz, H1β_{Me}), 6.15 (d, $J=5.0$ Hz, H1α_{Me}), 7.71, 7.76 (H, triazole)

¹³C-NMR (125 MHz, CDCl₃): δ 20.6, 20.63, 20.65, 20.7, 20.8 (COCH₃), 28.4(COOC(CH₃)₃), 40.7 (C6, C6'), 59.0, 59.2 (OCH₃), 59.6, 60.1, 60.3 (OCH₃), 60.4, 60.5, 60.5 (OCH₃), 60.8, 62.8

524 (OCH₂-triazole), 68.5 (broad, C4' _{Ac}), 70.3 (C6_{Me} (internal)), 71.6 (C2_{Ac} and C2' _{Ac}), 72.1 (C3_{Ac}),
 525 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}
 526 (internal)), 75.5 (C4_{Ac}), 77.5 (C4_{Me} (internal)), 79.8 COOC(CH₃)₃, 83.3, 83.5 (C2_{Me} (internal)),
 527 83.7 (C1_α_{Me}), 84.9, 85.0 (C3_{Me} (internal)), 86.1, 99.1 (C1_{Ac}), 99.8 (C1' _{Ac}), 103.2 (C1_{Me} (internal)),
 528 122.2, 124.4 (triazole CH), 143.2, 144.2 (O-CH₂-C=), 155.8 (COOC(CH₃)₃), 169.3, 169.6, 169.7,
 529 170.2 (COCH₃)
 530
 531 1-(Tri-*O*-methyl-cellulosyl)-4-[(6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-
 532 D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**2**):
 533 Sodium methoxide (28%) in methanol (5.5 μL, 10 equiv. per AGU) were added at room
 534 temperature to a solution of
 535 1-(tri-*O*-methyl-cellulosyl)-4-[2,3,4-tri-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucop
 536 yranosyl-(1→4)-2,3-di-*O*-acetyl-6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymet
 537 hyl]-1*H*-1,2,3-triazole (**5**, 76.3 mg) in MeOH (2 mL), THF (2 mL), and CH₂Cl₂ (1 mL). The
 538 mixture was stirred overnight at room temperature. The solution was neutralized with Amberlyst H⁺.
 539 The Amberlyst H⁺ was filtered off and washed with MeOH. The combined filtrate and washings
 540 were concentrated to dryness to give
 541 1-(tri-*O*-methyl-cellulosyl)-4-[6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl-(1→4)-6
 542 -(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (78.9 mg).
 543 Trifluoroacetic acid (0.5 mL) was added at -20 °C to a solution of
 544 1-(tri-*O*-methyl-cellulosyl)-4-[6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyl-(1→4)-
 545 6-(*tert*-butoxycarbonyl)amino-6-deoxy-β-D-glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (84 mg)
 546 in CH₂Cl₂ (1 mL). The reaction mixture was stirred for 1.2 h at -20 °C. The mixture was
 547 concentrated to dryness at 4 °C to give the crude product. The crude product was purified by gel
 548 filtration chromatography (Sephadex LH-20) to give
 549 1-(tri-*O*-methyl-cellulosyl)-4-[6-amino-6-deoxy-β-D-glucopyranosyl-(1→4)-6-amino-6-deoxy-β-D-
 550 glucopyranosyloxymethyl]-1*H*-1,2,3-triazole (**2**, 82.7 mg, quantitative yield).
 551 ¹H-NMR (500 MHz, D₂O): δ 3.03 (t, *J*=8.5 Hz, H2_{Me} (internal)), 3.34 (t, 1H, *J*=8.5 Hz, H3_{Me}
 552 (internal)), 3.42-3.49 (H5_{Me} (internal)), 3.29 (OCH₃), 3.46 (OCH₃), 3.47 (OCH₃), 3.57-3.68 (H4_{Me}
 553 (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
 554 Hz), 4.79-4.95 (CH₂, triazole-alkene), 5.69 (H1_β_{Me} (reducing end), *J*=9 Hz), 6.42 (H1_α_{Me} (reducing
 555 end), *J*=5.5 Hz), 8.17, 8.25 (s, triazole CH)
 556 ¹³C-NMR (D₂O): δ 40.2 (C6-NH₂ or C6'-NH₂), 40.5 (C6-NH₂ or C6'-NH₂), 58.3 (OCH₃), 58.5, 58.9
 557 (OCH₃), 59.8, 60.3 (OCH₃), 69.8 (C6_{Me} (internal)), 70.8, 73.0, 63.6 (C5_{Me} (internal)), 74.2, 74.5,
 558 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,
 559 101.2, 101.4, 102.4 (C1_{Me} (internal)), 102.8

560 GPC analysis of acetylated compound **2**: $M_n=7.1\times 10^3$, $M_w/M_n=1.6$, $DP_n=33$ (including DP of
 561 hydrophilic segment)
 562
 563 4-(Tri-*O*-methyl-cellulosyloxymethyl)-1-[methyl {(methyl
 564 (2,3,4-tri-*O*-acetyl- β -D-glucopyranosyl)uronate)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl- β -D-glucopyranosyl}uronat
 565 e]-1*H*-1,2,3-triazole (**6**):
 566 Cu (I) Br (5.6 mg, MW = 143.45, 39 mmol, 10 equiv.), sodium ascorbate (15.5 mg/19 μ L, 20 equiv.,
 567 4 M in H₂O), and PMDETA (MW = 173.3, d = 0.83 g/mL, 8.2 μ L, 6.8 mg, 39 mmol, 10 equiv.)
 568 were added at room temperature to a solution of methyl [(methyl
 569 2,3,4-tri-*O*-acetyl- β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl- β -D-glucopyranosyl
 570 azide]uronate (**11**, 7.4 mg, MW = 633.5, 11.7 mmol, 3.0 equiv.) and propargyl tri-*O*-methyl
 571 cellulose (**8**, 30 mg, $M_n = 7.72\times 10^3$, $DP_n = 37.5$, 3.9 mmol, 1.0 equiv.) in MeOH/CH₂Cl₂ (2 mL,
 572 1/4, v/v). The reaction mixture was stirred overnight at room temperature under a nitrogen
 573 atmosphere. The mixture was concentrated and passed through a silica gel chromatography column
 574 eluted with 10% MeOH/CH₂Cl₂ to give the crude product. The crude product was purified by silica
 575 gel column chromatography (eluent: EtOAc $\rightarrow$ 10% MeOH/CH₂Cl₂) to give
 576 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-[methyl {(methyl (2,3,4-tri-*O*-acetyl- β -D-
 577 glucopyranosyl)uronate)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl- β -D-glucopyranosyl}uronate]-1*H*-1,2,3-triazole (**6**,
 578 29.9 mg, 92% yield).
 579 ¹H-NMR (500 MHz, CDCl₃): δ 1.86, 1.86, 2.00, 2.02, 2.02, 2.09, 2.10 (COCH₃), 2.96 (t, $J=8.5$ Hz,
 580 H2_{Me} (internal)), 3.22 (t, 1H, $J=9.0$ Hz, H3_{Me} (internal)), 3.29 (m, $J=9.0$ Hz, H5_{Me} (internal)),
 581 3.39 (OCH₃), 3.54 (OCH₃), 3.58 (OCH₃), 3.66 (H6_{Me} (internal)), 3.70 (t, $J=9.0$ Hz, H4_{Me}
 582 (internal)), 3.77 (broad dd, $J=3,5$ Hz, $J=11.0$ Hz, H6_{Me} (internal)), 3.84 (s, 3H, C6'OOCH₃), 3.85
 583 (s, 3H, C6OOCH₃), 4.03 (dd, $J=3.5$, $J=10.0$, H5'_{Ac} (non-reducing end)), 4.18 (dd, $J=2.0$ Hz, $J=10.0$
 584 Hz, H5_{Ac} (reducing end)), 4.29 (H4_{Ac} (reducing end)), 4.35 (d, $J=8.0$ Hz, H1_{Me} (internal)), 4.40 (d,
 585 $J=8.0$ Hz, H1_{Me}), 4.67 (d, $J=13.0$ Hz, OCH₂-triazole), 4.67-4.68 (d, $J=8$, H1'_{Ac} (non-reducing end)),
 586 4.82 (d, $J=13.5$ Hz, OCH₂-triazole), 4.85 (d, $J=13.0$ Hz, OCH₂-triazole), 4.90 (t, $J=8.5$ Hz, H2'_{Ac}
 587 (non-reducing end)), 4.96 (d, $J=13.0$ Hz, OCH₂-triazole), 5.03 (d, $J=3.5$ Hz, H1 α _{Me} (reducing end)),
 588 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,
 589 $J=9.0$ Hz, H2_{Ac} (reducing end)), 5.41 (t, $J=9.0$ Hz, H3_{Ac} (reducing end)), 5.84-5.87 (d, H1_{Ac}
 590 (reducing end)), 7.81 (s, triazole CH)
 591 ¹³C-NMR (125 MHz, CDCl₃): δ 20.1, 20.2, 20.4, 20.5 (COCH₃), 52.8, 53.3 (COOCH₃), 58.8, 59.0,
 592 59.1 (OCH₃), 59.3, 59.6, 60.1, 60.1, 60.3 (OCH₃), 60.4, 50.4, 60.5 (OCH₃), 60.6 (OCH₂-triazole,
 593 overlapped), 60.7, 60.8, 62.3 (OCH₂-triazole), 69.3 (C4'_{Ac} (non-reducing end)), 69.9, 70.0 (C3_{Ac}
 594 (reducing end)), 70.3 (C6_{Me} (internal)), 70.6, 71.0 (C2'_{Ac} (non-reducing end)), 71.6 (C2_{Ac} (reducing
 595 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_{αMe} (reducing end)), 100.3 (C1'_{Ac} (non-reducing end)), 103.2 (C1_{Me} (internal)), 103.4 (C1_{Me}), 121.4 (triazole CH), 145.1 (O-CH₂-C=), 166.6 (C6'_{Ac} OCH₃), 166.8 (C6_{Ac} OCH₃), 169.0, 169.3, 169.7, 169.8, 170.1 (COCH₃)

4-(Tri-*O*-methyl-cellulosityloxymethyl)-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-cellulosityloxymethyl)-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-cellulosityloxymethyl)-1-[(β-D-glucopyranuronosyl)-(1→4)-β-D-glucopyranuronosyl]-1*H*-1,2,3-triazole (**3**, 13.8 mg, 99% yield).

¹H-NMR (500 MHz, D₂O): 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 (OCH₃), 3.46 (OCH₃), 3.47 (OCH₃), 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 (OCH₂-triazole), 4.80 (d, *J*=14.0 Hz, OCH₂-triazole), 4.88 (d, *J*=13.5 Hz, OCH₂-triazole), 5.71 (d, *J*=8.5 Hz, H1_{OH}), 8.23 (s, triazole CH)
¹³C-NMR (125 MHz, D₂O): δ 58.3 (OCH₃), 58.5, 58.9 (OCH₃), 59.8, 60.3 (OCH₃), 60.4 (OCH₂-triazole), 62.2 (OCH₂-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 CH), 144.0 (O-CH₂-C=)

GPC analysis of acetylated compound **3**: *M*_n=9.1×10³, *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 $\rightarrow$ 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 $\rightarrow$ 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 $\rightarrow$ 4)-2,3-di-*O*-acetyl- β -D-glucopyranosyl
639 azide]uronate (**11**) were synthesized according to the synthetic routes shown in Scheme 1.

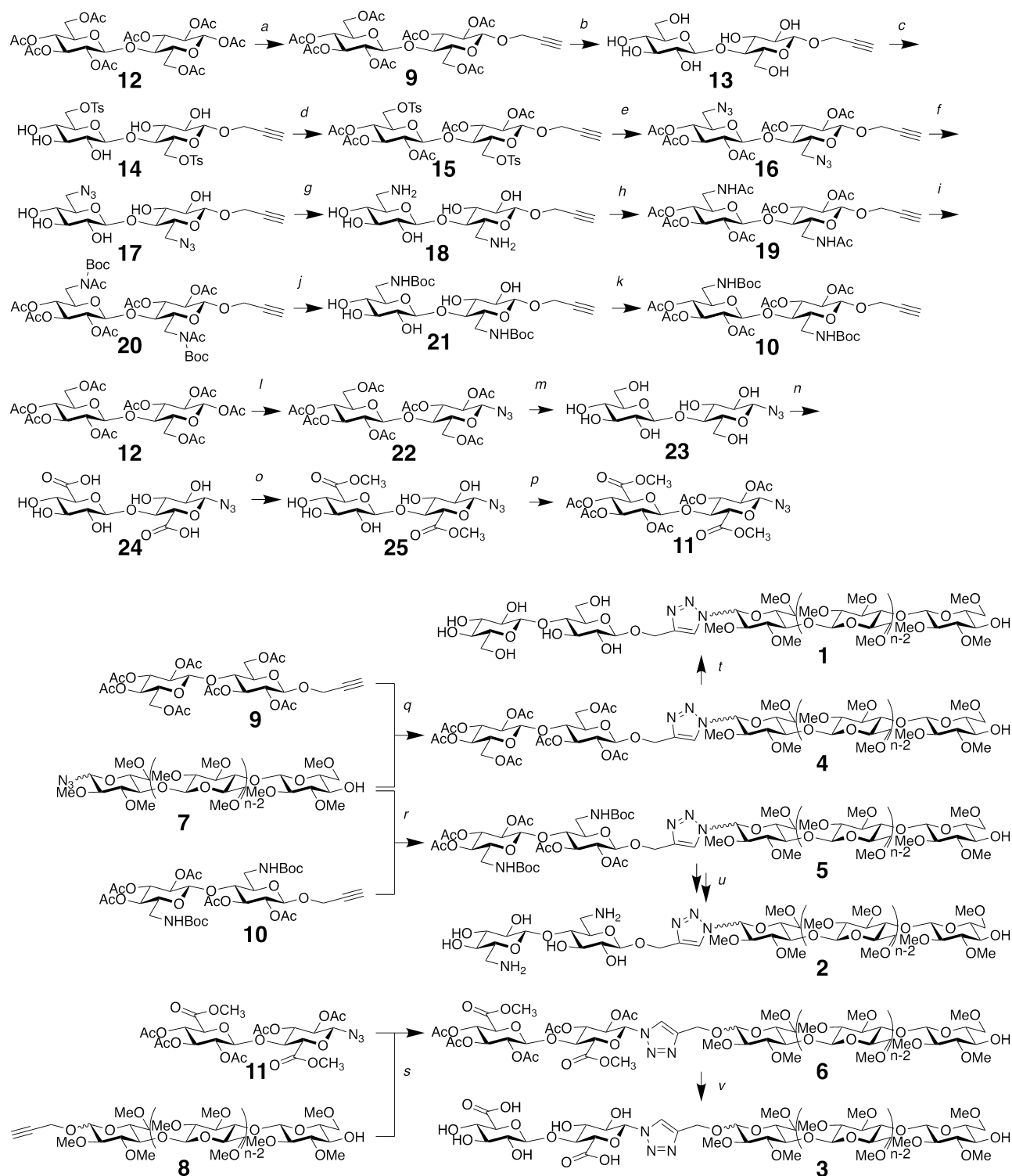
640 Compound **9** was prepared from cellobiose via cellobiose octaacetate (**12**) (H. Kamitakahara &
641 Nakatsubo, 2005) and is a precursor of the nonionic hydrophilic segment that gives compound **1**.

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 $\rightarrow$ 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



649

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

651

652 a) 2-propyn-1-ol/ $\text{BF}_3\text{Et}_2\text{O}$ / anhydrous CH_2Cl_2 /r.t./ 23h/99%; b) 28% NaOCH_3 in MeOH/ THF/MeOH/r.t./3h/96%; c)

653 TsCl /Pyridine/ 8°C / 2.5h/51%; d) Ac_2O /Pyridine/ r.t./overnight/ 95%; e) NaN_3 /DMF/ 50°C /overnight/97%; f)

654 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 /

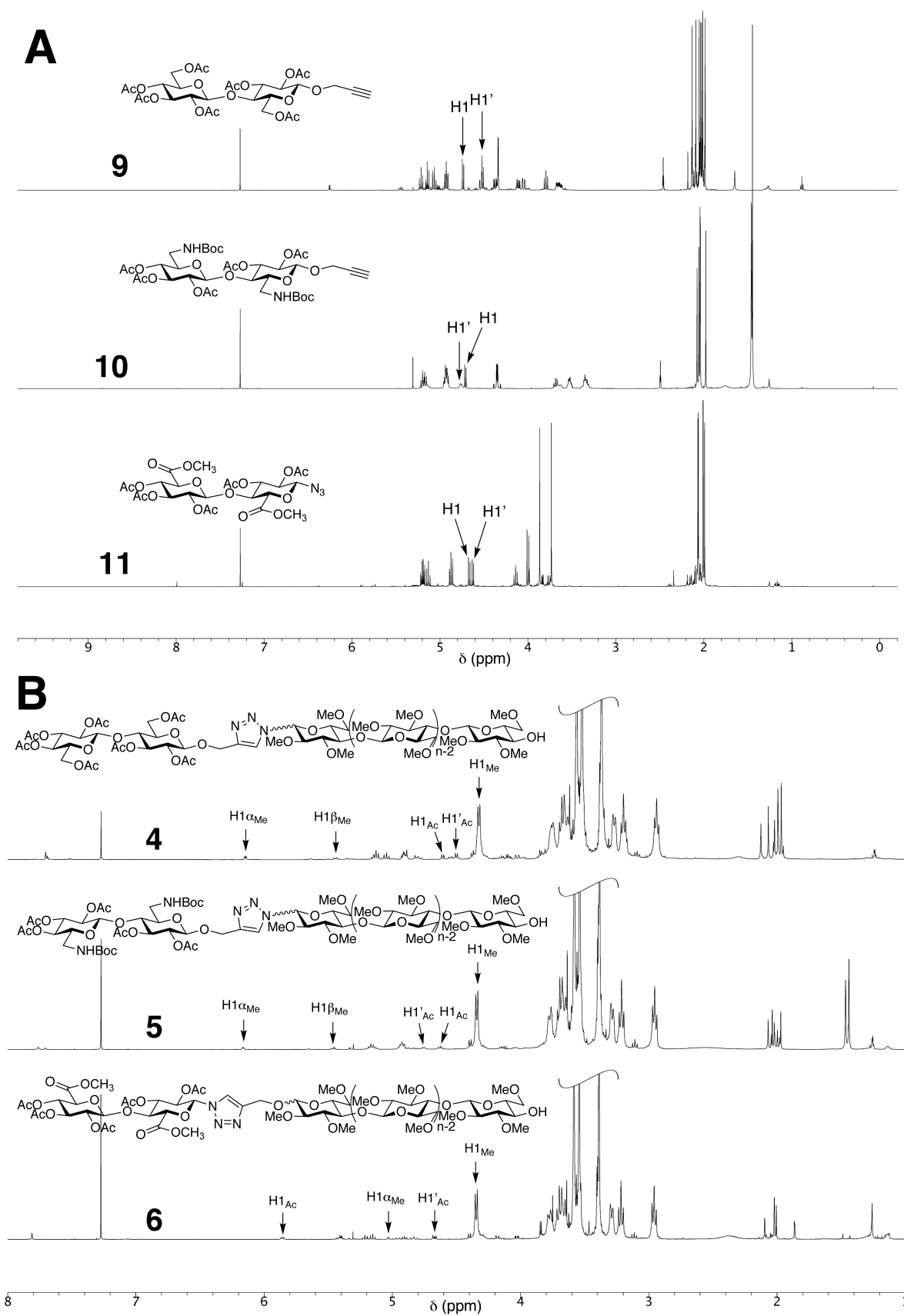
655 80°C /3h; Ac_2O /Pyridine/ 80°C /1h/ 96%; i) Boc_2O /DMAP/ THF/reflux/22.5h; j) 28% NaOCH_3 in MeOH/

656 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

657 in MeOH/ CH_2Cl_2 /MeOH/r.t./ quantitative yield; n) TEMPO / KBr / NaOCl /sat. aq. NaHCO_3 / 4°C /1d; o)

658 2,2-dimethoxypropane/conc. HCl/ MeOH/r.t./ 1d; p) Ac₂O/AcONa/60°C/overnight/ total yield 11% from compound **23**;
 659 q) Cu(I)Br/ sodium ascorbate in water/ PMDETA/MeOH:CH₂Cl₂ (1:4, v/v), distilled water/r.t./4 days; r) CuSO₄·H₂O/
 660 sodium ascorbate in water/ PMDETA/THF/50°C/overnight; s) CuSO₄·H₂O/ sodium ascorbate in water/
 661 PMDETA/THF/50°C/overnight; t) 28%NaOCH₃ in MeOH/ THF/MeOH/r.t./overnight; u) 28%NaOCH₃ in MeOH/
 662 THF/MeOH/CH₂Cl₂ /r.t./overnight; trifluoroacetic acid/-20°C/1.2 h; v) 0.0125 M NaOH/THF/r.t./30 min/99% yield
 663
 664 The synthesis of amino-functionalized cellobiose compound **10** from 2-propynyl
 665 (6-amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-glucopyranoside (**18**) was
 666 analogous to a method for a glucosamine derivative (Chen et al., 2010). Compound **10** was
 667 converted from compound **9** in 10 reaction steps. The *N*-acetyl groups at the C-6 positions of
 668 compound **19** would be relatively stable under alkali conditions. The amino groups of compound **18**
 669 are reactive and labile and are therefore protected by *tert*-butoxycarbonyl (Boc) groups. The
 670 primary hydroxy groups at the C-6 positions of compound **13** were tosylated with *p*-toluenesulfonyl
 671 chloride to give compound **14** in 51% yield. The four secondary hydroxy groups of compound **14**
 672 were acetylated with acetic anhydride in pyridine to give compound **15** in 95% yield. The tosylate
 673 **15** was treated with sodium azide to give azide derivative **16** in 97% yield via a nucleophilic
 674 substitution at the C-6 positions. Removal of the acetyl groups of compound **16** produced
 675 compound **17** in 87% yield. A Staudinger reaction afforded amino derivative **18** from azido
 676 compound **17** in 98% yield. Compound **18** is an analogous derivative of a chitosan dimer. Because
 677 the reactivity and stability of compound **18**, without protective groups for the amino and hydroxy
 678 groups, are unknown, the protected compound **10** was selected as the reactant for Huisgen
 679 1,3-dipolar cycloaddition. Compound **18** was acetylated to give 6-(acetyl)amino derivative **19**.
 680 Butoxycarbonylation of *N*-(acetyl)amino compound **19** followed by removal of the acetyl groups
 681 afforded 6-(Boc)amino derivative **21** via 6-acetyl(Boc)amino derivative **20**. Compound **21** was
 682 acetylated to give 2-propynyl
 683 (2,3,4-tri-*O*-acetyl-6-(Boc)amino-6-deoxy-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-(Boc)ami
 684 no-6-deoxy-β-D-glucopyranoside (**10**). Compound **10** is a precursor of the cationic hydrophilic
 685 segment that gives compound **2**.
 686 Compound **11** was prepared from cellobiose octaacetate (**12**) (H. Kamitakahara & Nakatsubo,
 687 2005) in five reaction steps and is a precursor of the anionic hydrophilic segment that gives
 688 compound **3**. Compound **11** is a glycosyl azide derivative, although compounds **9** and **10** are
 689 2-propenyl glycosides. The alkyne group was unstable under the reaction conditions for TEMPO
 690 oxidation of the primary alcohol. Methyl [(methyl
 691 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-2,3-di-*O*-acetyl-β-D-glucopyranosyl
 692 azide]uronate (**11**) was therefore chosen as the hydrophilic segment and treated with 2-propynyl
 693 tri-*O*-methyl-celluloside to produce compound **6**. Cellobiose octaacetate (**12**) was converted into
 694 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (**22**)

695 (H. Kamitakahara & Nakatsubo, 2005). Removal of the acetyl groups of compound **22** gave
696 β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyl azide (**23**). TEMPO oxidation of cellobiosyl azide
697 **23** gave (β -D-glucopyranuronosyl)-(1 $\rightarrow$ 4)- β -D-glucopyranuronosyl azide (**24**). The uronic acids of
698 compound **24** were esterified to give methyl [(methyl
699 β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)- β -D-glucopyranosyl azide]uronate (**25**). The remaining hydroxy
700 groups of compound **25** were acetylated to give methyl [(methyl
701 2,3,4-tri-*O*-acetyl- β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-2,3-di-*O*-acetyl- β -D-glucopyranosyl
702 azide]uronate (**11**).
703 Figure 1A shows ^1H -NMR spectra of cellobiose derivatives **9**, **10**, and **11**. Figure S1 in the
704 Supporting Information show ^{13}C -NMR spectra of cellobiose derivatives **9**, **10**, and **11**.



708 3.1.2. Synthesis of trehalose-type diblock methylcellulose analogues **1**, **2**, and **3**

709 The Huisgen 1,3-dipolar cycloaddition followed by removal of the protective groups afforded the
710 nonionic, cationic, and anionic diblock methylcellulose analogues
711 1-(tri-*O*-methyl-cellulosyl)-4-(β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranosyloxymethyl)-1*H*-1,2,3-
712 -triazole (**1**),
713 1-(tri-*O*-methyl-cellulosyl)-4-(6-amino-6-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-6-amino-6-deoxy- β -D-
714 glucopyranosyloxymethyl)-1*H*-1,2,3-triazole (**2**), and
715 4-(tri-*O*-methyl-cellulosyloxymethyl)-1-(β -D-glucopyranuronosyl-(1 $\rightarrow$ 4)-D-glucopyranuronosyl)-1-
716 *H*-1,2,3-triazole (**3**), as shown in Scheme 1.

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

724 Figure 1B shows the ^1H -NMR spectra of compounds **4**, **5**, and **6** obtained as a result of the Huisgen
725 1,3-dipolar cycloadditions. The proton resonances of the cellobiose segments were small relative to
726 those of the polymeric methylcelluloses. The triazole ring proton, however, appeared at
727 approximately 7.7–7.8 ppm, which enabled us to confirm the successful formation of the
728 trehalose-type diblock methylcellulose analogues. The ^1H -NMR spectrum of compound **4** revealed
729 that the C-1 proton of the α - and β -anomers of the methylcellulosyl residue appeared at 6.15 and
730 5.44 ppm, respectively. The α/β ratio was approx. 2/1. Two triazole protons appeared in the
731 ^1H -NMR spectra of compounds **4** and **5**, which means that the α - and β -anomers of the
732 methylcellulosyl residue affected the chemical shift of those 1,2,3-triazole protons. In contrast, one
733 triazole proton appeared in the ^1H -NMR spectra of compound **6**. The proton resonance
734 corresponding to the anomeric center of the methyl glucopyranosiduronate residue attached to the
735 triazole unit appeared at approx. 5.8–5.9 ppm as a doublet. The anomeric center at the reducing end
736 of the methylcelluloside appeared at 5.03 ppm as a doublet ($J=3.5$ Hz).
737 After the removal of the acyl groups of compounds **4**, **5**, and **6**, the proton resonances of the
738 unmodified cellobiosyl residues of compounds **1**, **2**, and **3** overlapped with those of methylcellulose
739 (data not shown). Therefore, we were unable to conclude from the results of the NMR analysis that
740 compounds **1**, **2**, and **3** were obtained. However, the MALDI-TOF mass spectra of compounds **1**, **2**,
741 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**

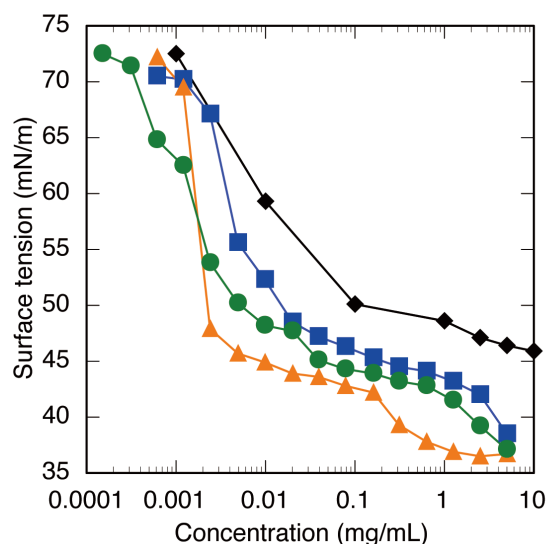
Compound no.	Zeta potential (mV)	Interfacial property		Aggregation temperature (°C) judged by DLS (0.2 wt%)	Thermal property detected by DSC (4.0 wt%)		Gelation property	
	0.2 wt%, 35°C	Critical Micelle Concentration (CMC) (mg/mL)	Surface tension at CMC (mN/m)		Endothermic peak (°C)	Exothermic peak (°C)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

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

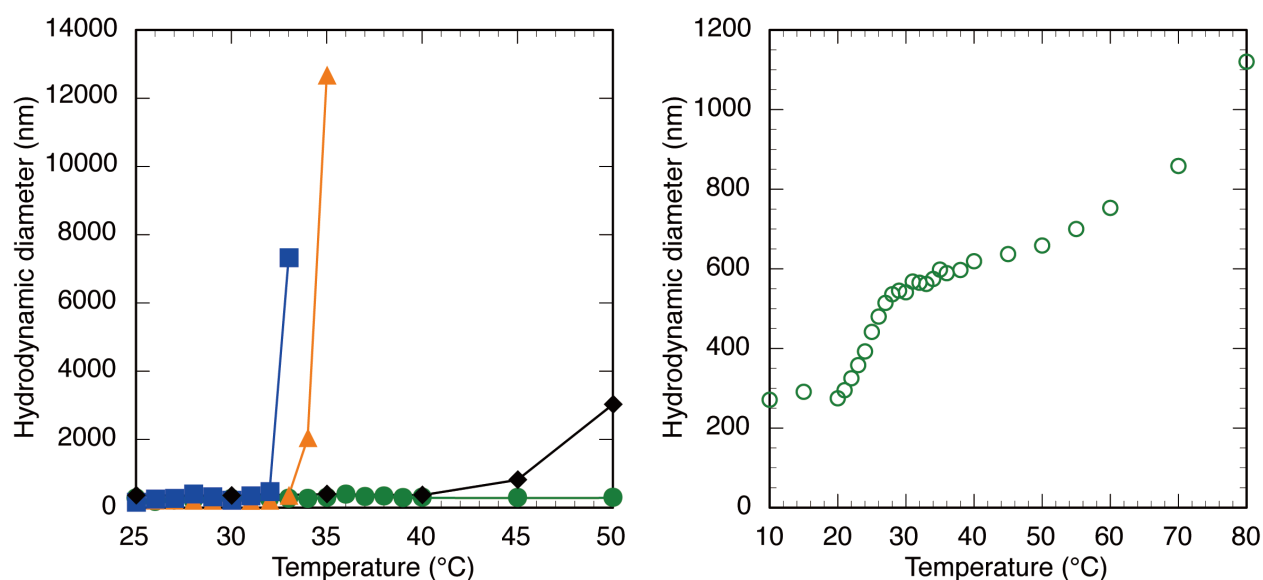
775 cationic segment with amino groups and a slightly anionic methylcellulose segment, affected its
 776 self-assembly behavior. Moreover, the surface-tension–concentration curve of compound **2** was
 777 atypical. Namely, any phase transition of compound **2** might occur over 0.16 mg/mL. The
 778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

780



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

784



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

789

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

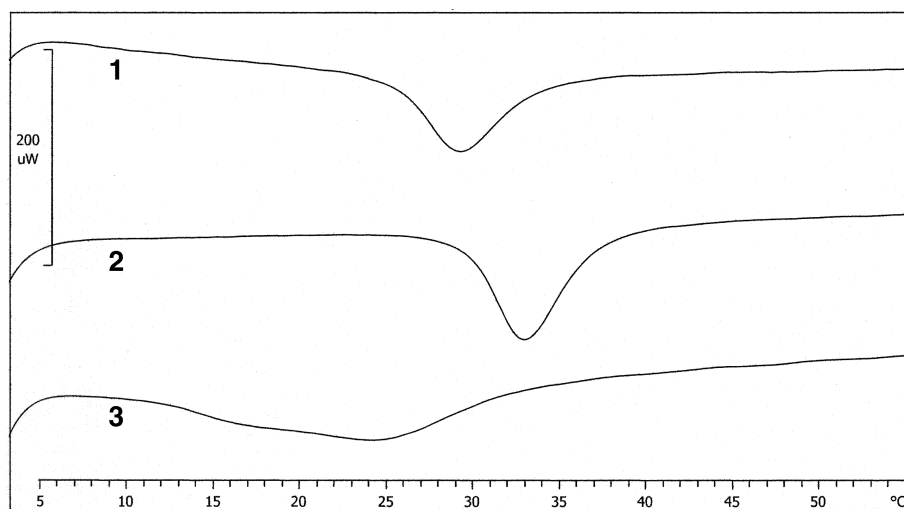
791 The aggregation properties of compounds **1**, **2**, and **3** in 0.2 wt. % aqueous media were different, as
792 shown in Figure 3. Compounds **1** and **2** suddenly aggregated at 33 °C and 34 °C, respectively, but
793 compound **3** did not show obvious aggregation properties. In contrast, the 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.

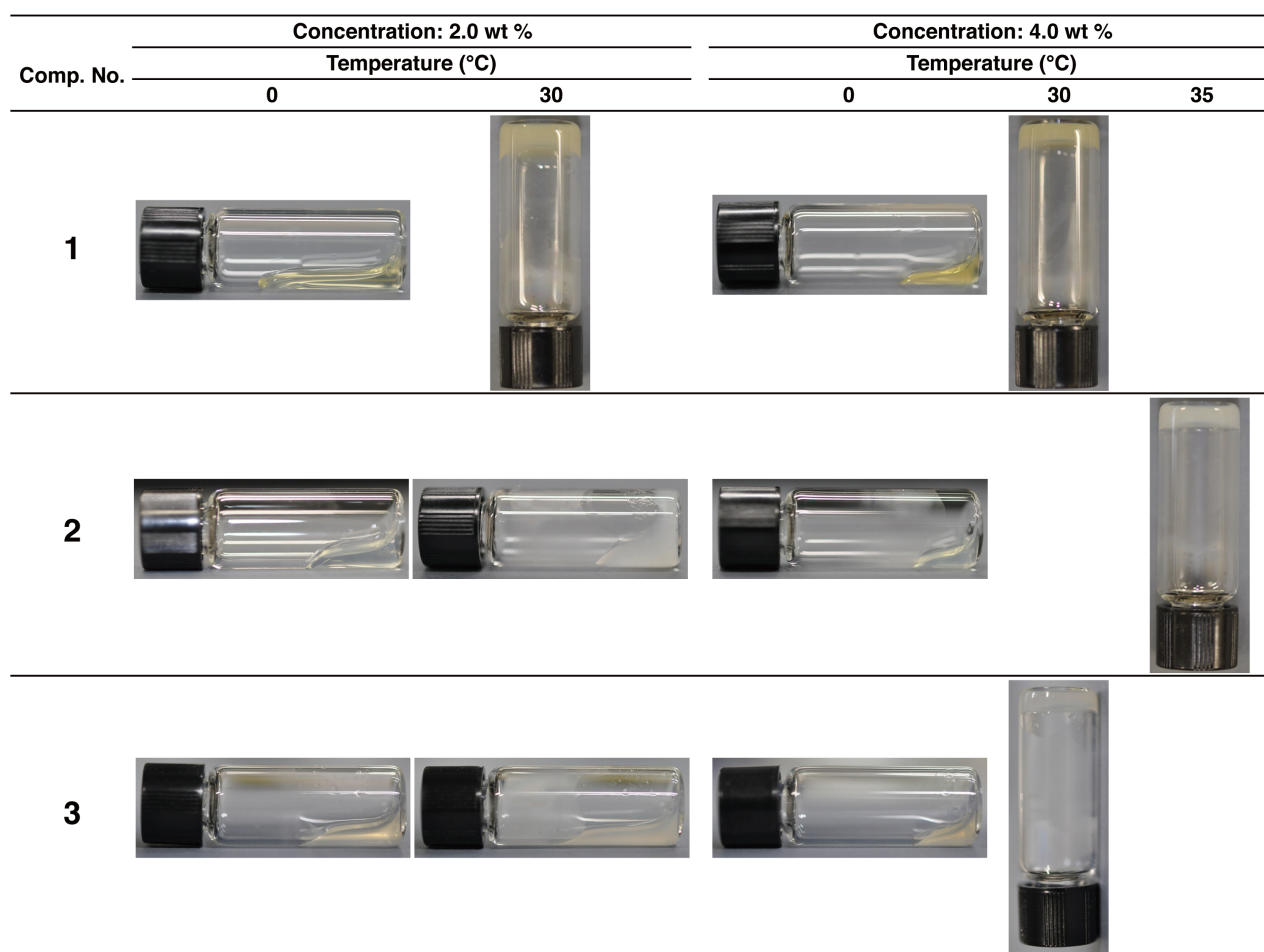
816

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.

828



829

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

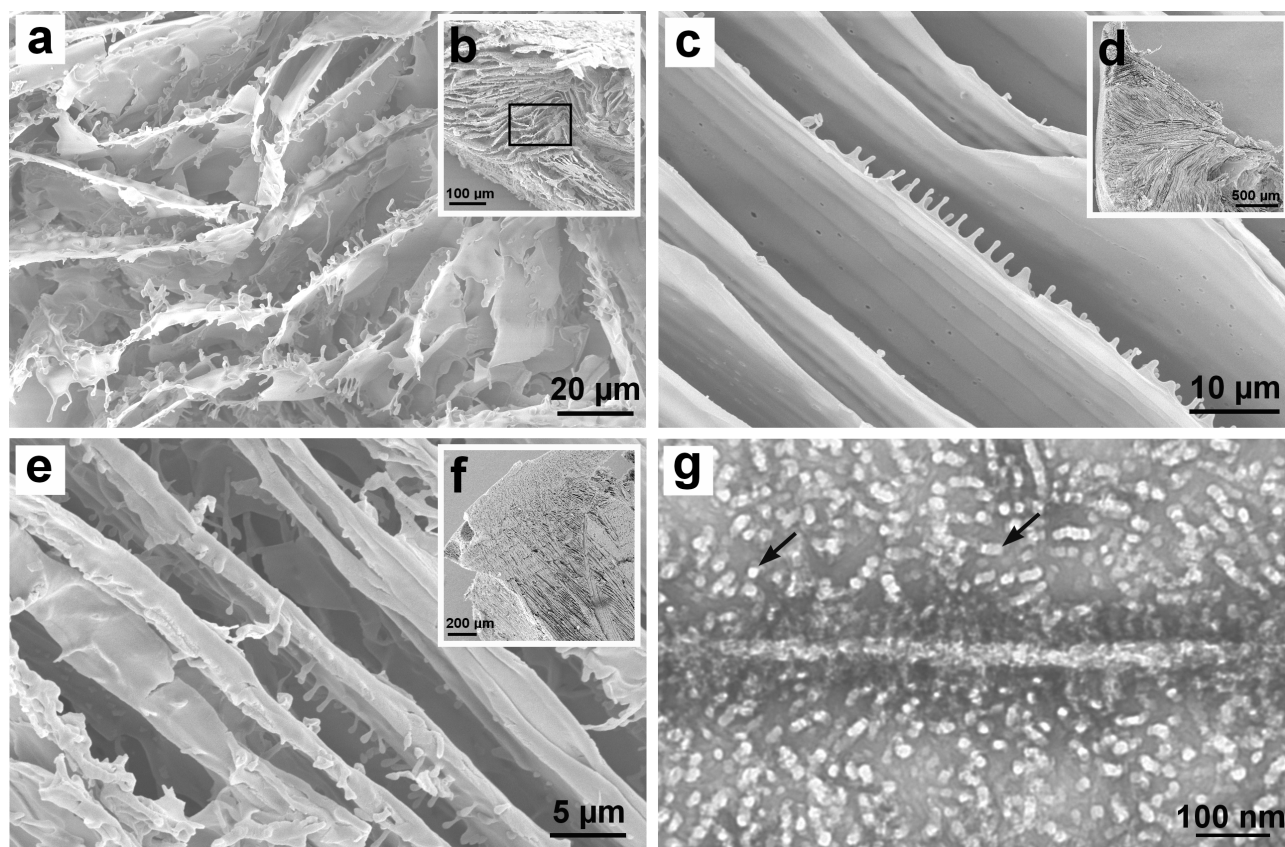
831

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

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

839



840 Figure 6. Scanning electron microscopic images of hydrogels from compounds **1** (a, b), **2** (c, d), and
 841 **3** (e, f); a transmission electron microscopic image of compound **1** (g). a, c, e were enlarged images
 842 from low magnified images (b, d and f). Arrows in g indicate square and rectangular aggregated
 843 particles.

844

845 3.3.3. Ultrastructures of thermoresponsive supramolecular hydrogel of compound **1**

846 A transmission electron microscopy image is shown in Figure 6 g. Square or rectangular structures
 847 of approximately 20 nm × 20–55 nm (Figure 6 g, arrows) can be seen. In addition, a long linear
 848 structure of approximately 20 nm width with short fine protuberances on both sides was observed.
 849 The molecular length of compound **1** is approx. 10 nm. Two molecules of compound **1** exist in the
 850 20 nm width for a short side of a rectangular structure. We propose a hypothesis for how the
 851 molecules of compound **1** self-assembled to form square or rectangular structures in Figure S3 in
 852 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.

860

861 **4. Conclusion**

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

880

881 **Acknowledgements**

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883 15H04531.

884

885 **Figure Legends**

886 Scheme 1. Synthetic routes for cellobiose derivatives

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

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

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

896 Figure 4. DSC thermograms of 2.0 wt. % aqueous solutions of compounds **1**, **2**, and **3**. Heating rate:
897 3.5°C/min.

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

899 Figure 6. Scanning electron microscopic images of hydrogels from compounds **1** (a, b), **2** (c, d), and
900 **3** (e, f); a transmission electron microscopic image of compound **1** (g). a, c, e were enlarged images
901 from low magnified images (b, d and f). Arrows in g indicate square and rectangular aggregated
902 particles.

903

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

905

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

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

909 Figure S3. Schematic figure of self-assembly process of compound **1** upon heating
910 Red hexagon: 2,3,6-tri-*O*-methyl glucose residue; Blue hexagon: unmodified glucose residue

911

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