



# Synthesis of blockwise alkylated (1→4) linked trisaccharides as surfactants: influence of configuration of anomeric position on their surface activities

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## ABSTRACT

New carbohydrate-based surfactants consisting of hydrophilic cellobiosyl and hydrophobic glucosyl residues, methyl  $\beta$ -D-glucopyranosyl-(1→4)- $\alpha$ -D-glucopyranosyl-(1→4)-2,3,6-tri-O-methyl- $\alpha$ -D-glucopyranoside **1** ( $G_{\beta}G_{\alpha}M_{\alpha}$ , G: glucopyranosyl residue,  $\alpha$  and  $\beta$ :  $\alpha$ -(1→4)- and  $\beta$ -(1→4) glycosidic bonds, M: methyl group), **2** ( $G_{\beta}G_{\beta}M_{\alpha}$ ), **3** ( $G_{\beta}G_{\alpha}M_{\beta}$ ), **4** ( $G_{\beta}G_{\beta}M_{\beta}$ ), **5** ( $G_{\beta}G_{\alpha}E_{\alpha}$ , E: ethyl group), **6** ( $G_{\beta}G_{\beta}E_{\alpha}$ ), **7** ( $G_{\beta}G_{\alpha}E_{\beta}$ ), **8** ( $G_{\beta}G_{\beta}E_{\beta}$ ) and eight  $\alpha$ - and  $\beta$ -glycoside mixtures (a mixture of **1** and **2**: **1/2** = 62/38 (**9**), 32/68 (**10**); a mixture of **3** and **4**: **3/4** = 69/31 (**11**), 32/68 (**12**); a mixture of **5** and **6**: **5/6** = 62/38 (**13**), 33/67 (**14**); a mixture of **7** and **8**: **7/8** = 59/41 (**15**), 29/71 (**16**)) were synthesized via combined methods consisting of acid-catalyzed alcoholysis of cellulose ethers and glycosylation of phenyl thio-cellobioside derivatives. Their surface activities in aqueous solution depended on their chemical structures:  $\alpha$ - or  $\beta$ -(1→4) linkage between hydrophilic cellobiosyl and hydrophobic glucosyl blocks, methyl or ethyl groups of hydrophobic glucosyl block, and  $\alpha$ - or  $\beta$ -linked ether group at the C-1 of hydrophobic glucosyl block. The mixing effect of  $\alpha$ - and  $\beta$ -glycosides on surface activities was also investigated. As a result, ethyl  $\beta$ -D-glucopyranosyl-(1→4)- $\alpha$ -D-glucopyranosyl-(1→4)-2,3,6-tri-O-ethyl- $\beta$ -D-glucopyranoside **7** ( $G_{\beta}G_{\alpha}E_{\beta}$ ) had the highest surface activity, and its critical micellar concentration (CMC) and  $\gamma_{CMC}$  (surface tension at CMC) values of compound **7** were 0.5 mM (ca. 0.03 wt %) and 34.5 mN/m, respectively. The surface tensions of  $\alpha$ - and  $\beta$ -glycoside mixtures except for compounds **9** and **10** were almost equal to those of pure compounds. The syntheses of the mixtures of  $\alpha$ - and  $\beta$ -glycosides without purification process are easier than those of pure compounds. Thus, the mixtures should be more practical compounds for industrial use as a surfactant.

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## 1. Introduction

Nonionic carbohydrate-based surfactants such as alkylpolyglucosides and polysaccharide derivatives capable of being produced from renewable resources have recently been receiving attention because they show good surface activities.<sup>1</sup> It is well known that not only the kind of hydrophilic saccharide moiety but also the length of the hydrophobic chain influences the physicochemical properties, such as surface activities.<sup>2</sup> In addition, the properties of the aqueous solutions of nonionic carbohydrate-based surfactants are influenced by  $\alpha$ - or  $\beta$ -(1→4) linkage between hydrophilic and hydrophobic moieties.<sup>3</sup> Carbohydrate-based surfactants having different lengths of sugar chain and hydrocarbon chain have been synthesized to gain detailed information about the relationships between the chemical structure and their properties and to obtain surfactants having good surface activities.<sup>4–8</sup> The mixing effect of surfactants on their physicochemical properties has also been reported since surfactants are used in formulations containing a mixture of different compounds.<sup>9–12</sup> The practical utilization

of mixed surfactants is based on economical as well as synergetic reasons.<sup>13</sup>

The general carbohydrate-based surfactants synthesized so far consist of sugar chains as hydrophilic block and hydrocarbon chains as hydrophobic block. We have recently reported a novel class of carbohydrate-based surfactants consisting of only sugar chains in both hydrophilic and hydrophobic blocks. As a model of methylcellulose (MC), a polydisperse mixture of diblock co-oligomers of tri-O-methylated and unmodified cello-oligosaccharides were synthesized, and their properties in aqueous solution were investigated.<sup>14,15</sup> Moreover, monodisperse diblock-trimer, -pentamer, and -hexamer,<sup>16</sup> and ABA- and BAB-triblock hexamers of tri-O-methylated and unmodified cello-oligosaccharides were prepared to investigate relationships between their chemical structure and solubility.<sup>17</sup> These reports suggest that monodisperse model compounds with blocky structure along molecular chain have higher surface activity than MC. Namely, amphiphilic diblock trisaccharide derivatives should have a potential to act as high-performance detergents.

Thus, diblock co-trimers of tri-O-alkylated and unmodified oligosaccharides were designed to obtain surfactants with higher surface activity compared to MC via novel synthetic route. For the

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purpose, the mixing effects of glycosides having  $\alpha$ - and  $\beta$ -(1 $\rightarrow$ 4) linkages between hydrophilic and hydrophobic blocks on their surface activities were investigated with comparison to pure compounds to develop a facile preparation method for a carbohydrate-based surfactant with high surface activity. In addition, cellulose ethers converted from abundant natural resource, cellulose, should be used as raw materials for carbohydrate-based surfactants from economical aspects. Hydrophobic blocks of surfactants, methyl 2,3,6-tri-*O*-methyl- and ethyl 2,3,6-tri-*O*-ethyl-*D*-glucopyranosides having a free hydroxyl group at C-4, were therefore prepared in a large scale by sulfuric-acid-catalyzed alcoholysis of completely alkylated celluloses, tri-*O*-methyl- and tri-*O*-ethyl-celluloses, respectively. Commercially available MC (*DS* = 1.8) and ethylcellulose (EC, *DS* = 2.5) were selected to prepare completely alkylated celluloses. Furthermore, cellobiose was chosen as a hydrophilic block of surfactants to synthesize diblock trimers having new hydrophilic–lipophilic balance. Here we describe synthesis and structure–surface activity relationship of novel diblock trisaccharide derivatives.

## 2. Results and discussion

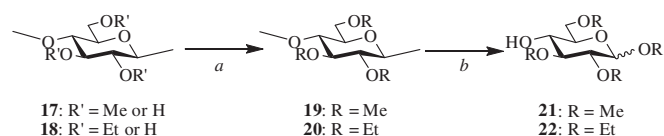
### 2.1. Syntheses of blockwise methylated or ethylated trisaccharide derivatives 1–8

The synthetic route for blockwise methylated or ethylated trisaccharide derivatives is illustrated in Figure 1. Phenyl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -*D*-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzyl-1-thio- $\beta$ -*D*-glucopyranoside (**30**) as a glycosyl donor derived from commercially available cellobiose was glycosylated with methyl 2,3,6-tri-*O*-methyl- (**21**) or ethyl 2,3,6-tri-*O*-ethyl-*D*-glucopyranoside (**22**) as glycosyl acceptors from commercially available MC or EC, respectively. Benzyl groups of glycosylated products were removed to give methylated or ethylated trisaccharide derivatives **1**–**8**.

#### 2.1.1. Syntheses of glycosyl acceptors as hydrophobic blocks from industrially produced MC and EC

Sulfuric-acid-catalyzed methanolysis of 2,3,6-tri-*O*-methyl-cellulose was reported by BeMiller et al. in 1967.<sup>18</sup> The production rate of methyl 2,3,6-tri-*O*-methyl-*D*-glucopyranoside and methyl 2,3,4,6-tetra-*O*-methyl-*D*-glucopyranoside was monitored as a function of depolymerization time in order to investigate the mechanism of acid-catalyzed hydrolysis of polysaccharide. Because the completely methylated derivatives are insoluble in water, methanolysis was applied instead of hydrolysis. As shown in Figure 2, industrially produced MC (*DS* = 1.8) was completely methylated to give 2,3,6-tri-*O*-methyl-cellulose (**19**) in 78.4% yield, and then compound **19** was depolymerized by sulfuric-acid-catalyzed methanolysis to give compound **21** in 95.6% yield on a gram scale.

Compound **28** was synthesized as an authentic compound from commercially available methyl  $\alpha$ -*D*-glucopyranoside (**23**) in 53.3%



**Figure 2.** Synthetic route for glycosyl acceptors **21** and **22**. (a) MeI or EtI/NaH/DMSO/50 °C/2 days; (b) H<sub>2</sub>SO<sub>4</sub>/MeOH or EtOH/CHCl<sub>3</sub>/60 °C.

overall yield in 5 reaction steps, as shown in Figure 3. Compound **23** was converted to methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl- $\alpha$ -*D*-glucopyranoside (**25**)<sup>19,20</sup> by 4,6-*O*-benzylideneation and subsequent methylation in 81.5% and 93.0% yield, respectively. Reductive cleavage of 4,6-*O*-benzylidene group by BH<sub>3</sub>–THF complex in THF and TMSOTf in CH<sub>2</sub>Cl<sub>2</sub> afforded methyl 4-*O*-benzyl-2,3-di-*O*-methyl- $\alpha$ -*D*-glucopyranoside (**26**) in 75.8% yield. Methylation of compound **26** with MeI and NaH in THF at 40 °C gave methyl 4-*O*-benzyl-2,3,6-tri-*O*-methyl- $\alpha$ -*D*-glucopyranoside (**27**) in 93.0% yield. Removal of benzyl groups of compound **27** using 10% Pd on C in EtOH and THF under hydrogen atmosphere at rt provided methyl 2,3,6-tri-*O*-methyl- $\alpha$ -*D*-glucopyranoside (**28**)<sup>21</sup> in quantitative yield.

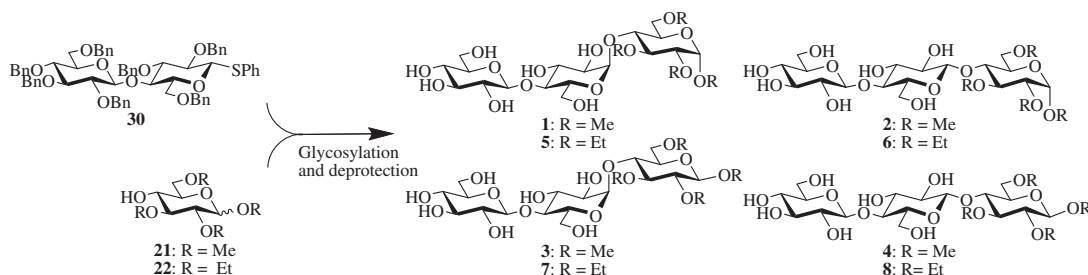
In <sup>13</sup>C NMR spectra of compounds **21** and **28**, chemical shifts of compound **21** completely correspond with those of compound **28**. Thus, the glucose derivative **21** having a free hydroxyl group at C-4 was successfully produced by sulfuric-acid-catalyzed methanolysis of 2,3,6-tri-*O*-methyl-cellulose.

This result prompted us to prepare ethyl 2,3,6-tri-*O*-ethyl-*D*-glucopyranoside from industrially produced EC. As shown in Figure 2 and 2,3,6-tri-*O*-ethyl-cellulose (**20**) was prepared from industrially produced EC in 61.9% yield, and ethyl 2,3,6-tri-*O*-ethyl-*D*-glucopyranoside (**22**) was successfully obtained by sulfuric-acid-catalyzed ethanolysis in 95.4% yield on a gram scale.

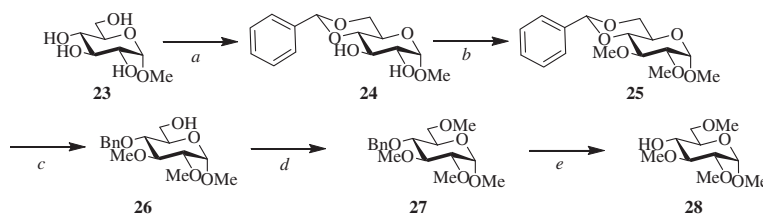
The syntheses of glucose derivatives having a free hydroxyl group at C-4 are required multiple reaction steps including 4,6-*O*-benzylideneation and reductive cleavage of 4,6-*O*-benzylidene group.<sup>22</sup> Whereas methyl 2,3,6-tri-*O*-methyl- $\alpha$ -*D*-glucopyranoside (**28**) was prepared from methyl  $\alpha$ -*D*-glucopyranoside (**23**) in 53.3% overall yield as shown in Figure 3, the synthesis of methyl 2,3,6-tri-*O*-methyl-*D*-glucopyranoside (**21**) from industrially produced MC (**17**) was achieved in 75.0% overall yield. Consequently, complete alkylation of cellulose followed by sulfuric-acid-catalyzed alcoholysis gave hydrophobic blocks of carbohydrate-based surfactants effectively.

#### 2.1.2. Synthesis of glycosyl donor as hydrophilic block

Fukase et al. reported in 1995 that glycosylation without neighboring group participation in ether using phenylthio glycoside combined by NBS and strong acid salts, LiClO<sub>4</sub> or LiNO<sub>3</sub> as a catalyst afforded  $\alpha$ -glycosides predominantly.<sup>23</sup> On the other hand, a combination of NBS with Ph<sub>2</sub>IOTf, Bu<sub>4</sub>NOTf, or Bu<sub>4</sub>NClO<sub>4</sub> was advantageous for  $\beta$ -selective glycosylation with 2-*O*-benzylated donors by the known solvent effect of nitrile.<sup>24</sup>



**Figure 1.** Syntheses of blockwise methylated or ethylated trisaccharide derivatives **1**–**8**.



**Figure 3.** Synthetic route for methyl 2,3,6-tri-O-methyl- $\alpha$ -D-glucopyranoside (**28**). <sup>(a)</sup>Benzaldehyde dimethylacetal/*p*-TsOH/DMF/40 °C→80 °C/5 h/81.5%; <sup>(b)</sup>Mel/NaH/THF/rt/6 days/93.0%; <sup>(c)</sup>BH<sub>3</sub>-THF/TMSOTf/CH<sub>2</sub>Cl<sub>2</sub>/−40→0 °C/5 h/75.8%; <sup>(d)</sup>Mel/NaH/THF/40 °C/overnight/93.0%; <sup>(e)</sup>10% Pd on C/THF/EtOH/under H<sub>2</sub>/rt/5 days/99.8%.

Thus, the phenylthio glycoside method without neighboring group participation was selected as a glycosylation method in this study to investigate the mixing effect of  $\alpha$ - and  $\beta$ -glycosides on surface activities. In addition, the benzyl protective groups have the advantages of good stability to a wide range of acidic and basic conditions and easy removal under mild hydrogenation conditions<sup>25</sup> and are suitable for purification by a preparative thin layer chromatography (P-TLC).

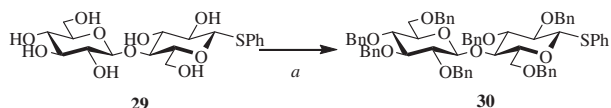
Phenyl 2,3,4,6-tetra-O-benzyl- $\beta$ -D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-1-thio- $\beta$ -D-glucopyranoside (**30**)<sup>26</sup> was converted from phenyl  $\beta$ -D-glucopyranosyl-(1→4)-1-thio- $\beta$ -D-glucopyranoside (**29**)<sup>27</sup> as a glycosyl donor in 78.3% yield, as shown in Figure 4.

### 2.1.3. Glycosylation and debenzoylation

Figure 5 shows synthetic route for blockwise alkylated trisaccharide derivatives **1–16**. The glycosylation, purification of glycosylated products by P-TLC, and removal of benzyl groups as temporary protective groups gave final products. Glycosylation of donor **30** with acceptors **21** or **22** using NBS and AgOTf under high vacuum condition<sup>28,29</sup> gave crude glycosylated products in 55–62% yields (see Table 1). In the case of glycosylation reactions in CH<sub>2</sub>Cl<sub>2</sub>, the ratio of  $\alpha$ -glycosides was higher than that of  $\beta$ -glycosides (Table 1, entry 1:  $\alpha/\beta$  = 35/20, entry 2:  $\alpha/\beta$  = 38/24), whereas glycosylation reaction in EtCN afforded  $\beta$ -glycosides with moderate stereoselectivity (Table 1, entry 3:  $\alpha/\beta$  = 18/39, entry 4:  $\alpha/\beta$  = 18/38). The  $\alpha$ -glycosides **31–34** having an axial substituent group at C-1 were easily isolated by P-TLC from the  $\beta$ -glycosides **35–38** having an equatorial substituent group, because *R<sub>f</sub>* values were different each other (see Section 4). In order to investigate a mixing effect of  $\alpha$ - and  $\beta$ -glycosides on surface activities, it is indispensable to know each surface activity of pure  $\alpha$ - and  $\beta$ -glycosides. Thus, compounds **39, 40, 43** and **44** were purified from compounds **31, 32, 33** and **34**, respectively. In the same way, compounds **41, 42, 45**, and **46** were obtained from compounds **35, 36, 37**, and **38**, respectively. Although *R<sub>f</sub>* values between  $\alpha$ -glycosides **39, 41, 43**, and **45** and  $\beta$ -glycosides, **40, 42, 44**, and **46** were close, respectively, six-times-purification-process on P-TLC plate (eluent; EtOAc/*n*-hexane 1:4) gave pure  $\alpha$ - and  $\beta$ -glycosides **39–46**. Benzyl groups of trisaccharide derivatives **31–46** were removed by 20% Pd(OH)<sub>2</sub> on C in EtOH and THF under hydrogen atmosphere at rt to give desired blockwise alkylated trisaccharide derivatives **1–16**.

### 2.1.4. Solubilities and chemical structures of compounds 1–16

Solubilities of compounds **1–16** in CHCl<sub>3</sub> and water were investigated. Methylated derivatives **1–4** and **9–12** were not completely soluble in CHCl<sub>3</sub> at concentration of 1.0 wt %, whereas ethylated



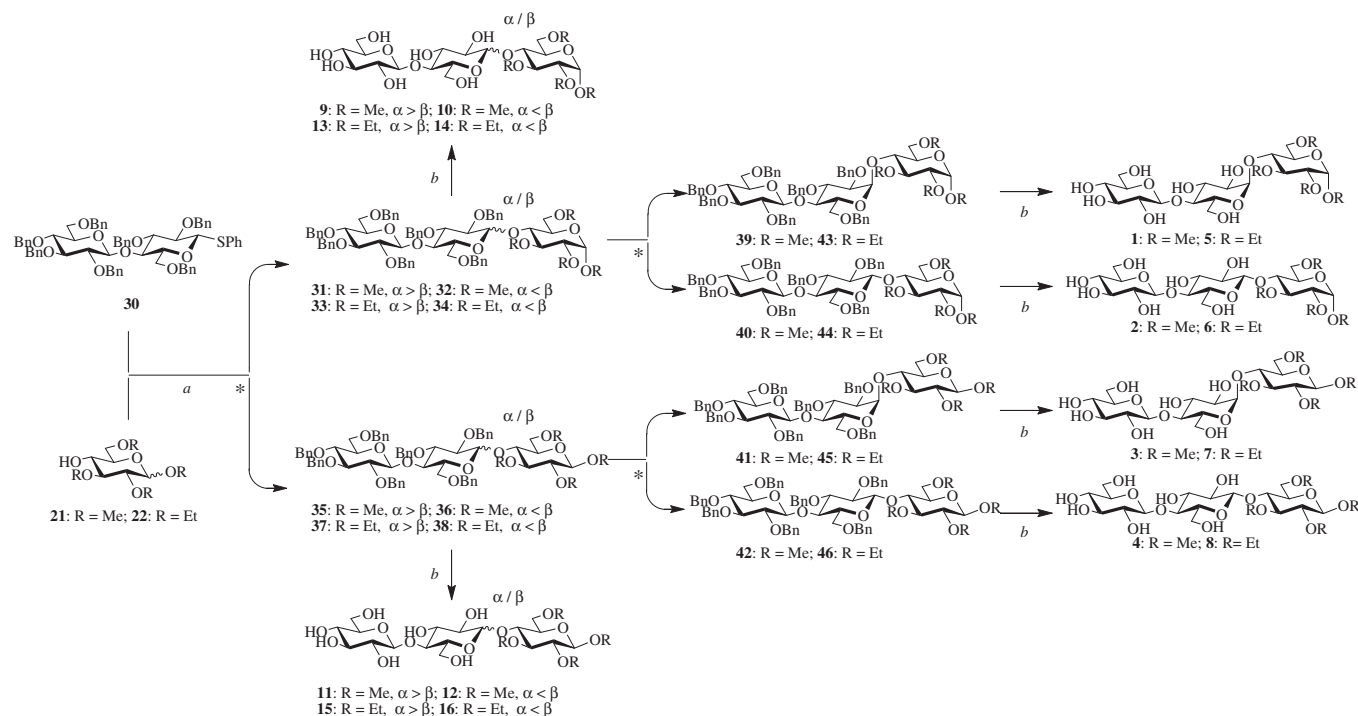
**Figure 4.** Synthesis of glycosyl donor **30**. <sup>(a)</sup>BnBr/NaH/TBNI/THF/DMF/80 °C/6 h.

derivatives **5–8** and **13–16** were dissolved in CHCl<sub>3</sub> to give clear solutions. On the other hand, compounds **1–16** were soluble in water. Chemical shifts and coupling constants of compounds **1–8** were summarized in Table 2. In the case of compounds **1, 3, 5**, and **7** having  $\alpha$ -(1→4) linkage between hydrophobic and hydrophilic blocks, C-1' protons appeared at 5.40, 5.38, 5.47, and 5.44 ppm as a doublet (*J* = 3.9, 3.9, 3.6, and 3.9 Hz, respectively). On the other hand, in the case of compounds **2, 4, 6**, and **8** having  $\beta$ -(1→4) linkage between hydrophobic and hydrophilic blocks, C-1' protons appeared at 4.40, 4.41, 4.41, and 4.41 ppm as a doublet (*J* = 8.1, 7.8, 7.8, and 6.3 Hz, respectively). The C-1' protons of compounds **1, 3, 5**, and **7** appeared at lower magnetic field than those of compounds **2, 4, 6**, and **8**. The C-1 anomeric protons with  $\alpha$  configurations were observed at 4.97, 4.98, 5.03, and 5.01 ppm as a doublet (*J* = 3.3, 3.6, 3.6, and 3.3 Hz, respectively), whereas those with  $\beta$  configurations appeared at 4.40, 4.40, 4.46, and 4.44 ppm as a doublet (*J* = 7.8, 7.5, 8.4, and 8.1 Hz, respectively). The C-1 protons of compounds **1, 3, 5**, and **7** appeared at lower magnetic field than those of compounds **2, 4, 6**, and **8**. The C-1 carbons of compounds **1, 2, 5**, and **6** appeared at 99.2, 99.2, 98.3, and 98.5 ppm, respectively, whereas the C-1 $\beta$  carbons of compounds **3, 4, 7**, and **8** appeared at 105.7, 105.6, 104.5, and 104.7 ppm, respectively. The C-1' $\alpha$  carbons of compounds **1, 3, 5**, and **7** appeared at 100.7, 100.5, 100.4, and 99.6 ppm, respectively, whereas the C-1' $\beta$  carbons of compounds **2, 4, 6**, and **8** appeared at 105.0, 104.9, 104.7, and 105.2 ppm, respectively. The C-1'' carbons derived from cellobiose blocks of compounds **1–8** appeared at ca. 105 ppm.

The optical rotations of compounds **1–16** measured in water were summarized in Table 3. In the case of pure compounds **1–8**, the optical rotations of compounds having  $\alpha$ -(1→4) linkage between hydrophilic cellobiosyl and hydrophobic glucosyl blocks were higher than those of compounds having  $\beta$ -(1→4) linkage: +59.1° (**1**: *G<sub>B</sub>G<sub>G</sub>M<sub>α</sub>*) > +37.9° (**2**: *G<sub>B</sub>G<sub>B</sub>M<sub>α</sub>*); +32.0° (**3**: *G<sub>B</sub>G<sub>G</sub>M<sub>β</sub>*) > −15.9° (**4**: *G<sub>B</sub>G<sub>B</sub>M<sub>β</sub>*); +56.0° (**5**: *G<sub>B</sub>G<sub>G</sub>E<sub>α</sub>*) > +51.0° (**6**: *G<sub>B</sub>G<sub>B</sub>E<sub>α</sub>*); +28.6° (**7**: *G<sub>B</sub>G<sub>G</sub>E<sub>β</sub>*) > −2.9° (**8**: *G<sub>B</sub>G<sub>B</sub>E<sub>β</sub>*). In addition, the optical rotations of compounds having  $\alpha$ -glycosidic bond at C-1 were higher than those of compounds having  $\beta$ -linkage: +59.1° (**1**: *G<sub>B</sub>G<sub>G</sub>M<sub>α</sub>*) > +32.0° (**3**: *G<sub>B</sub>G<sub>G</sub>M<sub>β</sub>*); +37.9° (**2**: *G<sub>B</sub>G<sub>B</sub>M<sub>α</sub>*) > −15.9° (**4**: *G<sub>B</sub>G<sub>B</sub>M<sub>β</sub>*); +56.0° (**5**: *G<sub>B</sub>G<sub>G</sub>E<sub>α</sub>*) > +28.6° (**7**: *G<sub>B</sub>G<sub>G</sub>E<sub>β</sub>*); +51.0° (**6**: *G<sub>B</sub>G<sub>B</sub>E<sub>α</sub>*) > −2.9° (**8**: *G<sub>B</sub>G<sub>B</sub>E<sub>β</sub>*). The optical rotations of mixtures **9, 10**, and **13** were higher than those of pure compounds, whereas the optical rotation values of compounds **11, 12, 14, 15**, and **16** were in between pure  $\alpha$ - and  $\beta$ -glycosides.

### 2.2. Surface activities of blockwise methylated or ethylated trisaccharide derivatives 1–16

The influence of chemical structure of blockwise alkylated trisaccharides **1–8** on the surface tension of their aqueous solutions was investigated. The differences of surface tension between **1** (*G<sub>B</sub>G<sub>G</sub>M<sub>α</sub>*) and **2** (*G<sub>B</sub>G<sub>B</sub>M<sub>α</sub>*), between **3** (*G<sub>B</sub>G<sub>G</sub>E<sub>α</sub>*) and **4** (*G<sub>B</sub>G<sub>B</sub>E<sub>α</sub>*), between **5** (*G<sub>B</sub>G<sub>G</sub>M<sub>β</sub>*) and **6** (*G<sub>B</sub>G<sub>B</sub>M<sub>β</sub>*), and between **7** (*G<sub>B</sub>G<sub>G</sub>E<sub>β</sub>*) and **8** (*G<sub>B</sub>G<sub>B</sub>E<sub>β</sub>*) would reveal the influence of  $\alpha$ - or  $\beta$ -(1→4) linkage between hydrophilic cellobiosyl and hydrophobic glucosyl blocks



**Figure 5.** Synthetic routes for blockwise methylated or ethylated trisaccharide derivatives **1–16**. <sup>(a)</sup>NBS/AgOTf/MS 3 Å or MS 4 Å/EtCN or CH<sub>2</sub>Cl<sub>2</sub>/rt/under vacuum, <sup>(b)</sup>Pd(OH)<sub>2</sub> on C/THF/EtOH/under H<sub>2</sub>/rt. \*Purification by a preparative thin layer chromatography (P-TLC).

**Table 1**  
Glycosylation of phenyl thio-cellobioside derivative **30** with acceptors **21** or **22**

Entry	Donor	Acceptor	Substituent group (R)	Solvent	Yield (%)				
					Total	G <sub>β</sub> G <sub>α</sub> R <sub>α</sub>	G <sub>β</sub> G <sub>α</sub> R <sub>β</sub>	G <sub>β</sub> G <sub>β</sub> R <sub>α</sub>	G <sub>β</sub> G <sub>β</sub> R <sub>β</sub>
1	<b>30</b>	<b>21</b>	Me	CH <sub>2</sub> Cl <sub>2</sub>	55	26	9	16	4
2	<b>30</b>	<b>22</b>	Et	CH <sub>2</sub> Cl <sub>2</sub>	62	28	10	17	7
3	<b>30</b>	<b>21</b>	Me	EtCN	57	12	6	26	13
4	<b>30</b>	<b>22</b>	Et	EtCN	56	14	4	28	10

Donor (1.0 equiv) and acceptor (1.5 equiv) were used for glycosylation. The glycosylation reaction was carried out at rt for 24 h. CH<sub>2</sub>Cl<sub>2</sub>: dichloromethane, EtCN: propionitrile.

on surface activities. The differences of surface tensions between **1** and **5**, between **2** and **6**, between **3** and **7**, and between **4** and **8** would reveal the influence of methyl or ethyl group as substituent group of hydrophobic block on their surface activities. The differences of surface tension between **1** and **3**, between **2** and **4**, between **5** and **7**, and between **6** and **8** would reveal the influence of α- and β-linkages at C-1 on their surface activities.

### 2.2.1. Surface activities of pure compounds **1–8**

Surface tensions were measured in the range of 0.01–10 wt % concentration. Surface tensions of compounds **1–8** and industrially produced MC in aqueous solution decreased with increasing concentration, as shown in Figure 6. Surface activities of pure compounds **1–8** depended upon their chemical structures and ranked as follows: **7** > (**3** ≅ **5** ≅ **8**) > (**1** ≅ **4** ≅ **6**) > **2** (Fig. 6). Among pure compounds, compounds **7** (G<sub>β</sub>G<sub>α</sub>E<sub>β</sub>) and **2** (G<sub>β</sub>G<sub>β</sub>M<sub>α</sub>) had highest and lowest surface activity, respectively. The surface tensions of blockwise methylated trisaccharide derivatives **1–4** decreased with increasing concentrations even over 0.1 wt % concentration which was CMC of MC (SM-4). The surface tensions of compounds **1–4** were lower than that of MC at 0.5 wt % concentration. These facts indicate that blockwise methylated trisaccharides have abilities to decrease surface tensions over CMC of MC. The surface tensions of compounds **1–8** showed three trends as follows:

1. The glycosides having α-(1→4) linkage between hydrophilic cellobiosyl and hydrophobic glucosyl blocks are more surface active than those having β-(1→4) linkage.
2. The glycosides having ethyl groups have higher surface activity than those having methyl groups.
3. β-Glycosides have higher surface activity than α-glycosides.

The above-mentioned 1, 2, and 3 are separately discussed in Figures 7–9 in the following sections.

#### 2.2.1.1. Influence of α- and β-linkages between hydrophilic and hydrophobic blocks on surface activities of compounds **1–8**

The surface tensions of the glycosides having α-(1→4) linkage between hydrophilic cellobiosyl and hydrophobic glucosyl blocks were compared with those of the glycosides having β-(1→4) linkage. The differences of surface tension between **1** (G<sub>β</sub>G<sub>α</sub>M<sub>α</sub>) and **2** (G<sub>β</sub>G<sub>β</sub>M<sub>α</sub>), between **3** (G<sub>β</sub>G<sub>α</sub>M<sub>β</sub>) and **4** (G<sub>β</sub>G<sub>β</sub>M<sub>β</sub>), between **5** (G<sub>β</sub>G<sub>α</sub>E<sub>α</sub>) and **6** (G<sub>β</sub>G<sub>β</sub>E<sub>α</sub>), and between **7** (G<sub>β</sub>G<sub>α</sub>E<sub>β</sub>) and **8** (G<sub>β</sub>G<sub>β</sub>E<sub>β</sub>) shown in Figure 7a–d indicate that α-glycosides have higher surface activities than β-glycosides. It is likely that α-glycosides are more hydrophobic than β-glycosides.<sup>30</sup> Dupuy et al. reported in 1997 that the glycosides having different configuration at anomeric center, α-1-*n*-dodecyl-*D*-maltoside and β-1-*n*-dodecyl-*D*-maltoside, formed different micellar aggregates in water.<sup>31</sup>



**Table 2**  
Chemical shifts of compounds 1–8

Compound	<sup>1</sup> H NMR					<sup>13</sup> C NMR				
	$\delta$ (ppm)					$\delta$ (ppm)				
	H-1''	H-1'	H-1	C1-OCH <sub>3</sub>	C2-OCH <sub>3</sub>	C3-OCH <sub>3</sub>	C6-OCH <sub>3</sub>	C-1''	C-1'	C-1
1 (G <sub>β</sub> G <sub>α</sub> M <sub>α</sub> )	4.52 (7.8)	5.40 (3.9)	4.97 (3.3)	3.39	3.46	3.56	3.33	105.1	100.7	99.2
2 (G <sub>β</sub> G <sub>β</sub> M <sub>α</sub> )	4.50 (7.8)	4.40 (8.1)	4.98 (3.6)	3.39	3.46	3.55	3.37	105.2	105.0	99.2
3 (G <sub>β</sub> G <sub>α</sub> M <sub>β</sub> )	4.50 (8.1)	5.38 (3.9)	4.40 (7.8)	3.54	3.56	3.59	3.33	105.1	100.5	105.7
4 (G <sub>β</sub> G <sub>β</sub> M <sub>β</sub> )	4.49 (7.5)	4.41 (7.8)	4.41 (7.5)	3.53	3.55	3.57	3.37	105.2	104.9	105.6
5 (G <sub>β</sub> G <sub>α</sub> E <sub>α</sub> )	4.51 (7.8)	5.47 (3.6)	5.03 (3.6)					105.1	100.4	98.3
6 (G <sub>β</sub> G <sub>β</sub> E <sub>α</sub> )	4.49 (7.8)	4.41 (8.1)	5.01 (3.3)					105.2	104.7	98.5
7 (G <sub>β</sub> G <sub>α</sub> E <sub>β</sub> )	4.49 (8.1)	5.44 (3.9)	4.46 (8.4)					104.0	99.6	104.5
8 (G <sub>β</sub> G <sub>β</sub> E <sub>β</sub> )	4.41 (7.5)	4.41 (6.3)	4.44 (8.1)					104.5	105.2	104.7

Values in brackets are coupling constants (Hz).

The difference suggests that the configuration of the head group affects the orientation of the polar residue and hence the packing of monomers during self-assembly. Consequently, in the case of blockwise alkylated trisaccharides 1–8,  $\alpha$ - and  $\beta$ -glycosides in this study should form different micellar aggregates in water, and as a result, had different surface activities.

### 2.2.1.2. Influence of methyl or ethyl group on surface activities of compounds 1–8.

In general, surface activity of a surfactant in an aqueous solution increases with increasing number of carbon atoms in the hydrophobic moiety.<sup>4</sup> The differences of surface tension between 1 (G<sub>β</sub>G<sub>α</sub>M<sub>α</sub>) and 5 (G<sub>β</sub>G<sub>α</sub>E<sub>α</sub>), between 2 (G<sub>β</sub>G<sub>β</sub>M<sub>α</sub>) and 6 (G<sub>β</sub>G<sub>β</sub>E<sub>α</sub>), between 3 (G<sub>β</sub>G<sub>α</sub>M<sub>β</sub>) and 7 (G<sub>β</sub>G<sub>α</sub>E<sub>β</sub>), and between 4 (G<sub>β</sub>G<sub>β</sub>M<sub>β</sub>) and 8 (G<sub>β</sub>G<sub>β</sub>E<sub>β</sub>) shown in Figure 8 indicate that the glycosides having ethyl groups had higher surface activities than those having methyl groups, as expected.

### 2.2.1.3. Influence of $\alpha$ - and $\beta$ -glycosides on surface activities of compounds 1–8.

Similar to the influence of  $\alpha$ - and  $\beta$ -linkage between hydrophilic and hydrophobic blocks on surface tension, compounds 1, 2, 5, and 6 having  $\alpha$ -glycosidic bond at C-1 were expected to have higher surface activities than those having  $\beta$ -linkage.<sup>31,32</sup> However, it was unexpectedly found that  $\beta$ -glycosides 3, 4, 7, and 8 had higher surface activities than  $\alpha$ -glycosides, resulting from the differences of surface tension between 1 (G<sub>β</sub>G<sub>α</sub>M<sub>α</sub>) and 3 (G<sub>β</sub>G<sub>α</sub>M<sub>β</sub>), between 2 (G<sub>β</sub>G<sub>β</sub>M<sub>α</sub>) and 4 (G<sub>β</sub>G<sub>β</sub>M<sub>β</sub>), between 5 (G<sub>β</sub>G<sub>α</sub>E<sub>α</sub>) and 7 (G<sub>β</sub>G<sub>α</sub>E<sub>β</sub>), and between 6 (G<sub>β</sub>G<sub>β</sub>E<sub>α</sub>) and 8 (G<sub>β</sub>G<sub>β</sub>E<sub>β</sub>), as shown in Figure 9.

### 2.2.2. Surface activities of $\alpha$ - and $\beta$ -glycoside mixtures 9–16

The surface tensions of mixtures 9–16 were measured to know mixing effects of glycosides having  $\alpha$ - and  $\beta$ -(1→4) linkages between hydrophilic cellobiosyl and hydrophobic glucosyl blocks on their surface activities. The  $\alpha/\beta$  ratios of compounds 9–16 are summarized in Table 3. Compounds 9, 11, 13, and 15 are  $\alpha$ -rich, and compounds 10, 12, 14, and 16 are  $\beta$ -rich. The surface tension curves of pure and mixed compounds were similar to each other, as shown in Figure 10. Interestingly, compounds 9 and 10 had higher surface activities than pure compounds 1 and 2 at all range of concentration tested. A special mixing effect was found in the case of mixtures 9 and 10. The surface tensions of mixtures 11–16 roughly followed the additivity rule. The surface tensions of compounds 11, 13, 14, 15 and 16 were lower than those of pure compounds below 0.2 mg/mL concentration, whereas the surface activities of compounds 11, 13, 14, 15 and 16 were approximately the average of those of the two pure compounds above 0.2 mg/mL concentration.

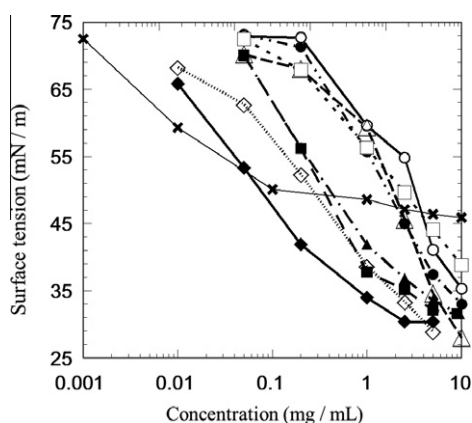
### 2.2.3. Comparisons of surface activity of blockwise alkylated trisaccharide derivatives with those of other commercially available surfactants

The surface activities of compounds 1–16 were compared with those of commonly-used surfactants, MC (Methocel Premium A15, mean molecular weight 14 kDa, methyl substitution between 27.5% and 31.5%),<sup>33</sup> *n*-octyl  $\beta$ -D-glucoside,<sup>34</sup> dodecyl octaethylene glycol (C<sub>12</sub>E<sub>8</sub>),<sup>35</sup> sodium dodecyl sulfate (SDS),<sup>36</sup> *n*-dodecyl  $\beta$ -D-maltoside,<sup>37</sup> cetyltrimethylammonium bromide (CTAB),<sup>36</sup> polyethylene glycol *p*-(1,1,3,3-tetramethylbutyl)-phenyl ether (Triton X-100),<sup>38</sup> as shown in Table 4. In general,  $\gamma_{\text{CMC}}$  (surface tension at CMC) is the minimum value of surface tension. The CMC value and  $\gamma_{\text{CMC}}$  of compound 7 (G<sub>β</sub>G<sub>α</sub>E<sub>β</sub>) which show the highest surface activity among pure compounds 1–8 were 0.5 mM (ca. 0.03 wt %) and 34.5 mN/m, respectively. These values were almost same or better compared with other surfactants in Table 4. The CMC values and  $\gamma_{\text{CMC}}$  of compound 7 were lower than those of MC, indicating that the surface activity of compound 7 is superior to that of MC.

**Table 3**  
Optical rotations of compounds 1–16

Mixture	$\alpha/\beta$ ratio calculated by		Optical rotations of mixtures	Optical rotations of pure compounds	
	$^1\text{H}$ NMR	Optical rotations		$\alpha^a$	$\beta^a$
<b>9</b>	1/2 = 62/38	—	+83.7°	+59.1° (1: $G_\beta G_\alpha M_\alpha$ )	+37.9° (2: $G_\beta G_\beta M_\alpha$ )
<b>10</b>	1/2 = 32/68	—	+67.7°		
<b>11</b>	3/4 = 62/38	3/4 = 62/38	+16.7°	+32.0° (3: $G_\beta G_\alpha M_\beta$ )	−15.9° (4: $G_\beta G_\beta M_\beta$ )
<b>12</b>	3/4 = 33/67	3/4 = 41/59	+3.9°		
<b>13</b>	5/6 = 69/31	—	+68.4°	+56.0° (5: $G_\beta G_\alpha E_\alpha$ )	+51.0° (6: $G_\beta G_\beta E_\alpha$ )
<b>14</b>	5/6 = 32/68	5/6 = 32/68	+51.7°		
<b>15</b>	7/8 = 59/41	7/8 = 82/18	+22.8°	+28.6° (7: $G_\beta G_\alpha E_\beta$ )	−2.9° (8: $G_\beta G_\alpha M_\beta$ )
<b>16</b>	7/8 = 29/71	7/8 = 17/83	+2.5°		

<sup>a</sup>  $\alpha$ - or  $\beta$ -glycosides having  $\alpha$ - or  $\beta$ -(1→4)-linkage between hydrophilic cellobiosyl and hydrophobic glucosyl block.



**Figure 6.** Surface tension–concentration curves of blockwise methylated or ethylated trisaccharide derivatives and SM-4. ●: compound 1; ○: compound 2; ▲: compound 3; △: compound 4; ■: compound 5; □: compound 6; ◆: compound 7; ◇: compound 8; ×: SM-4.

### 3. Conclusions

A synthetic method for blockwise alkylated trisaccharides from cellobiose and commercial cellulose derivatives, was established. Glucose derivatives having a free hydroxyl group at C-4 were prepared from MC and EC by sulfuric-acid-catalyzed alcoholysis on a gram scale. Glycosylation of benzylated thio-cellobioside with such glucose derivatives successfully afforded blockwise alkylated trisaccharide derivatives as a new class of carbohydrate-based surfactant. The blockwise ethylated trisaccharides were for the first time synthesized. The surface tension measurements of blockwise alkylated trisaccharide derivatives revealed the following three influences of their chemical structures on surface activity:

1. The type of linkage,  $\alpha$ - or  $\beta$ -(1→4) linkage between hydrophilic cellobiosyl and hydrophobic glucosyl blocks, influences surface activity:  $\alpha$ -glycosides are more surface active than  $\beta$ -glycosides.
2. The ethyl derivatives have higher surface activity than methyl derivatives.
3. The compounds having  $\beta$ -glycosidic bond at C-1 have higher surface activity than those having  $\alpha$ -glycosidic bond.

Consequently, among pure compounds, compounds 7 ( $G_\beta G_\alpha E_\beta$ ) and 2 ( $G_\beta G_\beta M_\alpha$ ) showed highest and lowest surface activities, respectively. Thus, important structural factors for a surfactant with high activity were found, resulting from the detailed investigation on the chemical structure–surface activity relationships of blockwise alkylated trisaccharides. The surface activities of mixtures of the glycosides having  $\alpha$ - and  $\beta$ -(1→4) linkages be-

tween hydrophilic cellobiosyl and hydrophobic glucosyl blocks were almost equal to those of pure compounds except for compounds 9 and 10. Taking the practical use into account, the syntheses and following utilization of  $\alpha$ - and  $\beta$ -anomer mixtures as a surfactant were easier than those of pure compounds. Carbohydrate-based surfactants consisting of only sugar chains in both hydrophilic and hydrophobic blocks had almost equal surface activities to other commercially available surfactants. In addition, these findings will contribute to novel utilization of MC and EC.

## 4. Experimental details

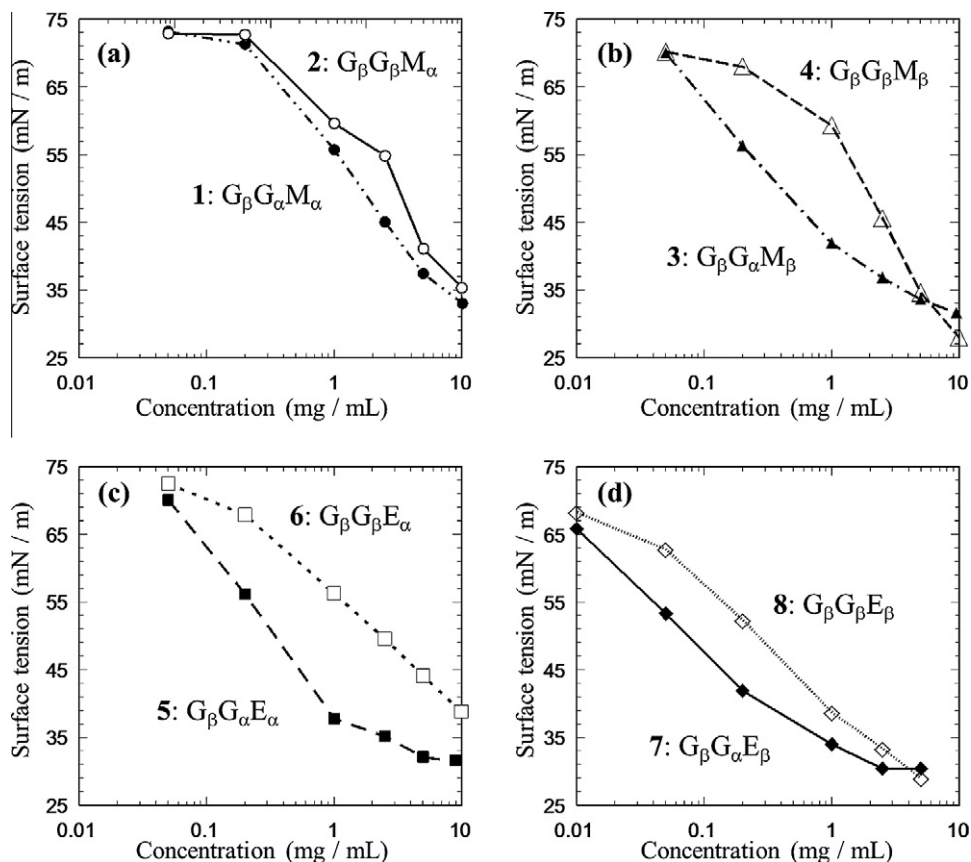
### 4.1. Materials and measurements

#### 4.1.1. Materials

SM-4 (methoxyl content: 29.2%; DS = 1.76; viscosity of 2% aqueous solution: 4.08 mPa s;  $M_w = 2.5 \times 10^4$ ) and SM-400 (methoxyl content: 27.5–31.5%; viscosity of 2% aqueous solution: 400 mPa s) were kindly provided by Shinetsu Chemical, Japan. Ethyl cellulose (abt. 49% ethoxy content) was purchased from Wako Pure Chemical Industries, Ltd. The products were purified on silica gel column chromatography (Wakogel C-200, Wako Pure Chemical Industries or Silica Gel 60N [spherical, neutral], 100–210  $\mu\text{m}$ , Kanto Chemical Co., Inc.) or thin layer chromatography (Silica Gel 60 F254, 0.5 mm or 2 mm thickness, Merck, Germany).

#### 4.1.2. General measurements

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with a Varian Inova 300 FT-NMR (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. Proton and carbon resonances were assigned by two-dimensional NMR experiments (gCOSY, TOCSY, gHSQC, and gHMBC). Chemical shifts ( $\delta$ ) and coupling constants (*J*) are given in  $\delta$ -values (ppm) and Hertz, respectively. Matrix assisted laser desorption/ionization time-of-flight mass (MALDI-TOF MS) spectra were recorded with a Bruker MALDI-TOF MS REFLEX III in the positive ion and reflector modes. For ionization, a nitrogen laser was used. All spectra were measured in the reflector mode using external calibration. MALDI-TOF MS spectra of compounds were measured with 2,5-dihydroxybenzoic acid (DHB) as a matrix. A Shimadzu SEC system (CBM-10A, SPD-10A, SIL-10A, LC-10AT, FIC-10AL, CTO-10A, RID-10A, and FRC-10, Shimadzu, Japan) and Shodex columns (K802, K802.5, and K805) were used. Number and weight averaged molecular weights ( $M_n$ ,  $M_w$ ) and polydispersity indices ( $M_w/M_n$ ) were estimated using polystyrene standards (Shodex). A flowrate of 1 mL/min at 40 °C was chosen. Optical rotations were measured using a JASCO Dip-1000 digital polarimeter. Melting points were determined on a FP900 Thermosystem (Mettler Toledo) with FP82 Microscope HotStage.



**Figure 7.** Surface tension–concentration curves of pure compounds **1–8**: the effect of  $\alpha$ - and  $\beta$ -(1 $\rightarrow$ 4) linkages between hydrophobic and hydrophilic moieties on surface activity;  $\bullet$ : compound **1**;  $\circ$ : compound **2**;  $\blacktriangle$ : compound **3**;  $\triangle$ : compound **4**;  $\blacksquare$ : compound **5**;  $\square$ : compound **6**;  $\blacklozenge$ : compound **7**;  $\diamond$ : compound **8**.

#### 4.1.3. Surface tension measurements

Compounds were dissolved in water at various concentrations and the values of their surface tension were measured by the Wilhelmy method using a CBVP-A3 surface tensiometer (Kyowa Interface Science, Co. Ltd, Tokyo) at 20 °C. The values were used when the values were stable after 30 min. The surface tension of distilled water is 72.8 mN/m at 20 °C.

### 4.2. Syntheses of blockwise methylated or ethylated trisaccharide derivatives

#### 4.2.1. 2,3,6-Tri-*O*-methyl-cellulose (**19**)

To a solution of MC (**17**) (SM-400,  $DS = 1.8$ , 3.03 g, 0.016 mol) in DMSO (230 mL), NaH (60% in mineral oil, 2.6 g, 0.064 mol, 4.0 equiv per anhydroglucopyranose) was added at 0 °C. The reaction mixture was stirred at 50 °C overnight. Then MeI (4.0 mL, 0.064 mol, 4.0 equiv per anhydroglucopyranose) was added into the solution. The reaction mixture was stirred at 50 °C for 24 h. MeOH (5.5 mL) was added to the reaction mixture, and then the mixture was poured slowly into water (3.0 L). The resulting precipitates were washed with water for three times and EtOH twice, centrifuged (12,000 rpm/10 min/2 °C), and concentrated to dryness to give compound **19** (2.587 g, 78.4% yield).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.96 (H-2), 3.22 (H-3), 3.31 (H-5), 3.39 (C6-OCH<sub>3</sub>), 3.62–3.66 (H-6a), 3.69 (H-4), 3.72–3.81 (H-6b), 4.33 (H-1);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  59.1 (C6-OCH<sub>3</sub>), 60.3 (C2-OCH<sub>3</sub>), 60.5 (C3-OCH<sub>3</sub>), 70.2 (C-6), 74.8 (C-5), 77.1 (C-4), 83.4 (C-2), 84.9 (C-3), 103.1 (C-1). Mp 208–209 °C.  $[\alpha]_{\text{D}}^{20.0} -7.8$  (c 0.937,  $\text{CHCl}_3$ ).  $M_n = 2.04 \times 10^4$ ,  $M_w = 4.82 \times 10^4$ ,  $M_w/M_n = 2.36$ ,  $DP_n = 100.0$ ,  $DP_w = 236.2$ .

#### 4.2.2. 2,3,6-Tri-*O*-ethyl-cellulose (**20**)

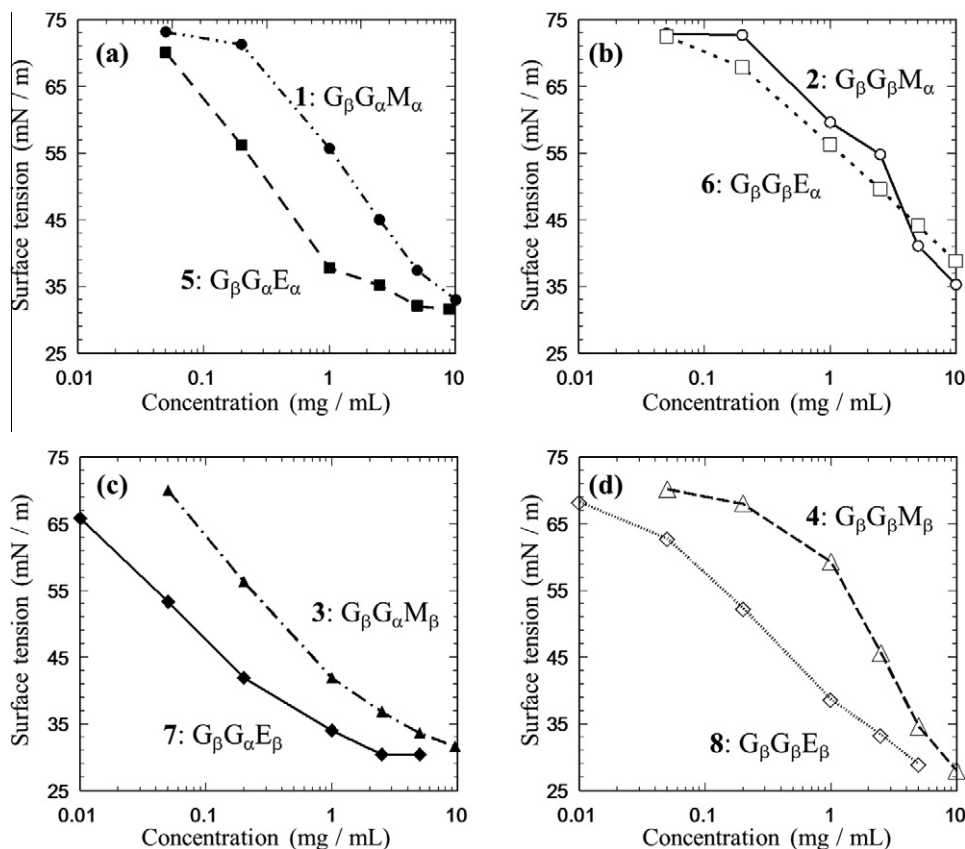
To a solution of EC (**18**) ( $DS = 2.5$ , 10.6095 g, 0.045 mol) in DMSO (140 mL), NaH (60% in mineral oil, 1.75 g, 0.043 mol, 1.0 equiv per anhydroglucopyranose) was added at 0 °C. The reaction mixture was stirred for 4 h at 50 °C. Then EtI (3.45 mL, 0.043 mmol, 1.0 equiv per anhydroglucopyranose unit) was added into the solution. The reaction mixture was stirred at 50 °C for 6 days. MeOH (3.0 mL) was added to the reaction mixture, and the mixture was poured slowly into water (3.0 L). The resulting precipitates were washed with water for three times and MeOH twice, centrifuged (12,000 rpm/10 min/2 °C), concentrated to dryness to give compound **20** (6.57 g, 61.9% yield).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.14, 1.14, 1.16 (–CH<sub>3</sub>), 3.02 (H-2), 3.16–3.25 (H-3, H-5), 3.39–4.07 (–CH<sub>2</sub>–), 3.52 (H-6), 3.73 (H-4), 4.33 (H-1);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  15.2, 15.5, 15.6 (–CH<sub>3</sub>), 66.3 (C-6), 68.2, 68.3 (–CH<sub>2</sub>–), 75.1 (C-5), 77.2 (C-4), 81.7 (C-2), 83.4 (C-3), 102.8 (C-1). Mp 185–186 °C.  $[\alpha]_{\text{D}}^{21.0} +7.7$  (c 0.913,  $\text{CHCl}_3$ ).  $M_n = 1.17 \times 10^4$ ,  $M_w = 2.45 \times 10^4$ ,  $M_w/M_n = 2.09$ ,  $DP_n = 47.5$ ,  $DP_w = 99.5$ .

#### 4.2.3. Methyl 2,3,6-tri-*O*-methyl- $\alpha$ -glucopyranoside (**21**)

To a solution of compound **19** (105.2 mg, 0.5156 mol) in  $\text{CHCl}_3$  and MeOH (5 mL, 4:1, v/v), concentrated sulfuric acid and MeOH (5 mL, 1:4, v/v) were added at 0 °C. The reaction mixture was stirred at 60 °C for 9 h. The solution was neutralized with  $\text{NaHCO}_3$ . The salts were filtered off and washed with EtOAc. The combined filtrate and washings were concentrated to dryness to give compound **21** (114.8 mg, 95.6% yield,  $\alpha/\beta = 75/25$ ).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.01 (dd, 1H,  $J = 7.5$ ,  $J_{2,3} = 7.8$ , H-2 $\beta$ ), 3.13 (t, 1H,  $J = 8.7$ ,  $J_{3,4} = 9.0$ , H-3 $\beta$ ), 3.25 (dd, 1H,  $J = 3.6$ ,  $J_{2,3} = 3.6$ , H-2 $\alpha$ ), 3.39–3.73 (10H, H-3 $\alpha$ , H-4 $\alpha$ , H-5 $\alpha$ , H-6 $\alpha$ , H-4 $\beta$ , H-5 $\beta$ , H-6 $\beta$ , –OCH<sub>3</sub>), 4.20 (d, 1H,  $J_{1,2} = 7.5$ , H-1 $\beta$ ), 4.86 (d, 1H,  $J_{1,2} = 3.6$ , H-1 $\alpha$ );



**Figure 8.** Surface tension–concentration curves of pure compounds **1–8**: the effect of methyl or ethyl group on surface activity; ●: compound **1**; ○: compound **2**; ▲: compound **3**; △: compound **4**; ■: compound **5**; □: compound **6**; ◆: compound **7**; ◇: compound **8**.

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  54.9, 56.7, 58.2, 59.1, 59.3, 59.9, 60.5, 60.8 (– $\text{OCH}_3$ ), 69.7 (C-4 $\alpha$ ), 69.8 (C-5 $\alpha$ ), 70.3, 71.5 (C-6 $\alpha$ ), 72.0, 73.9, 81.4 (C-2 $\alpha$ ), 82.7 (C-3 $\alpha$ ), 83.1, 85.6, 97.2 (C-1 $\alpha$ ), 104.1 (C-1 $\beta$ ).  $[\alpha]_{\text{D}}^{18.7} +102.8$  (c 0.997,  $\text{CHCl}_3$ ).

#### 4.2.4. Ethyl 2,3,6-tri-O-ethyl-D-glucopyranoside (**22**)

To a solution of compound **20** (504.7 mg, 2.051 mmol) in  $\text{CHCl}_3$  and EtOH (25 mL, 4:1, v/v), concentrated sulfuric acid and EtOH (25 mL, 1:4, v/v) were added at 0 °C. The reaction mixture was stirred at 60 °C for 21 h. The solution was neutralized with  $\text{NaHCO}_3$ . The salts were filtered off and washed with EtOAc. The combined filtrate and washings were concentrated to dryness to give compound **22** (481.5 mg, 95.4% yield,  $\alpha/\beta$  = 67/33).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.17–1.28 (– $\text{CH}_3$ ), 3.05 (dd, 1H,  $J$  = 7.2,  $J_{2,3}$  = 9.6, H-2 $\beta$ ), 3.21 (t, 1H,  $J$  = 9.0,  $J_{3,4}$  = 8.7, H-3 $\beta$ ), 3.32 (dd, 1H,  $J$  = 3.6,  $J_{2,3}$  = 3.6, H-2 $\alpha$ ), 3.39–4.01 (H-3 $\alpha$ , H-4 $\alpha$ , H-5 $\alpha$ , H-6 $\alpha$ , H-4 $\beta$ , H-5 $\beta$ , H-6 $\beta$ , – $\text{CH}_2$ –), 4.29 (d, 1H,  $J_{1,2}$  = 7.2, H-1 $\beta$ ), 4.93 (d, 1H,  $J_{1,2}$  = 3.6, H-1 $\alpha$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  14.8, 15.0, 15.1, 15.5, 15.6, 15.8 (– $\text{CH}_3$ ), 63.1, 65.5, 66.3, 67.0, 67.1, 68.1, 68.5, 68.6, 69.5, 70.0, 70.9, 71.1, 71.9, 72.6, 73.4, 80.1, 80.8, 81.7, 83.9, 96.4 (C-1 $\alpha$ ), 103.3 (C-1 $\beta$ ).  $[\alpha]_{\text{D}}^{19.7} +50.4$  (c 1.038,  $\text{CHCl}_3$ ).

#### 4.2.5. Methyl 4-O-benzyl-2,3-di-O-methyl- $\alpha$ -D-glucopyranoside (**26**)

To a solution of methyl 4,6-O-benzylidene- $\alpha$ -D-glucopyranoside (**24**) (10.0822 g, 0.035 mol) in THF (75 mL), NaH (60% in mineral oil, 2.2158 g, 0.055 mol, 1.5 equiv) and MeI (3.3 mL, 1.5 equiv) were added at 0 °C. The reaction mixture was stirred at rt overnight. Then NaH (1.4349 g, 0.035 mol, 1.0 equiv) and MeI (2.2 mL, 1.0 equiv) were added into the solution. The reaction mixture was stirred at rt for 5 days. The reaction was quenched by addition

of MeOH (2 mL). The reaction mixture was diluted with EtOAc, washed with water and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to dryness to give crude product **25** (11.715 g, 93.0% yield).

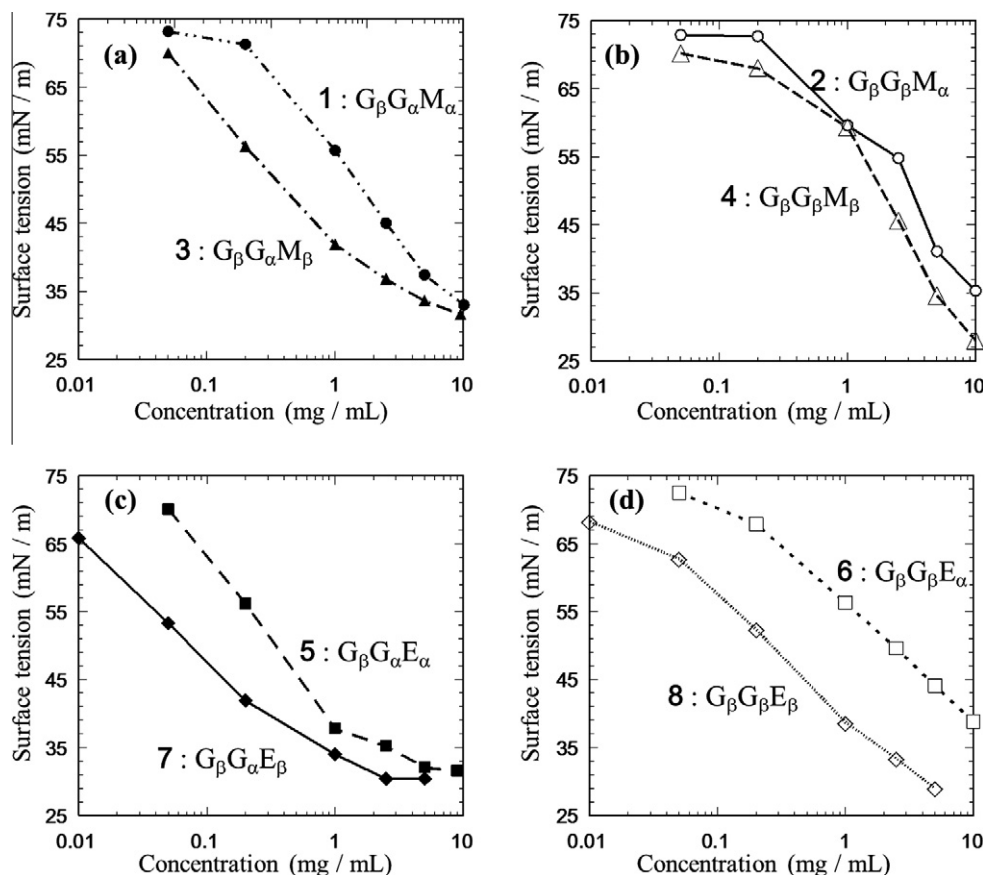
To a solution of compound **25** (5.0054 g, 16.128 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (6 mL),  $\text{BH}_3$ –THF complex in THF (59 mL, 0.6164 mol, 38.5 equiv) were added at –41 °C. After 1 h, TMSOTf (1.7 mL, 9.6718 mmol, 0.6 equiv) was added to the reaction mixture at –41 °C. The temperature of the reaction mixture was gradually raised up to 0 °C in 2 h and the mixture was kept at 0 °C for 4.5 h. The mixture was cooled under –40 °C and then,  $\text{Et}_3\text{N}$  (16.4 mL) was added to the solution. Next, MeOH was added to the solution until hydrogen was not produced. The mixture was diluted with EtOAc, washed with 4 M-HCl, saturated aq  $\text{NaHCO}_3$  and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to dryness to give crude crystals. The crude compound was purified by silica gel column chromatography eluted with  $\text{CH}_2\text{Cl}_2$  to give compound **26** (3.8225 g, 75.8% yield).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.21 (dd, 1H,  $J_{1,2}$  = 4.0, H-2), 3.37 (s, 3H, C1– $\text{OCH}_3$ ), 3.45 (t, 1H,  $J$  = 9.0,  $J$  = 10, H-4), 3.49 (s, 3H, C2– $\text{OCH}_3$ ), 3.58–3.63 (5H, H-3, H-5, C3– $\text{OCH}_3$ ), 3.69–3.80 (2H,  $J$  = 4.5,  $J$  = 4.0,  $J$  = 2.5,  $J$  = 2.5, H-6), 4.65 (1H,  $J$  = 11,  $\text{CH}_2\text{Ph}$ ), 4.80 (d,  $J_{1,2}$  = 4.0, H-1), 4.85 (1H,  $J$  = 11,  $\text{CH}_2\text{Ph}$ ), 7.25–7.35 (5H, aromatic H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  54.6 (C1– $\text{OCH}_3$ ), 58.4 (C2– $\text{OCH}_3$ ), 60.5 (C3– $\text{OCH}_3$ ), 61.0 (C-6), 70.4 (C-5), 74.4 (– $\text{CH}_2\text{Ph}$ ), 76.9 (C-4), 81.5 (C-2), 83.1 (C-3), 96.9 (C-1), 127.3–138 (aromatic C).

#### 4.2.6. Methyl 4-O-benzyl-2,3,6-tri-O-methyl- $\alpha$ -D-glucopyranoside (**27**)

To a solution of compound **26** (3.8225 g, 12.24 mmol) in THF (30 mL), NaH (60% in mineral oil, 0.5960 g, 14.9 mmol, 1.2 equiv) and MeI (0.91 mL, 14.6 mmol, 1.2 equiv) were added at 0 °C. After





**Figure 9.** Surface tension–concentration curves of pure compounds **1–8**: the effect of  $\alpha$ - and  $\beta$ -linkages at C-1 on surface activity;  $\bullet$ : compound **1**;  $\circ$ : compound **2**;  $\blacktriangle$ : compound **3**;  $\triangle$ : compound **4**;  $\blacksquare$ : compound **5**;  $\square$ : compound **6**;  $\blacklozenge$ : compound **7**;  $\diamond$ : compound **8**.

3 h, NaH (60% in mineral oil, 0.1590 g, 3.975 mmol, 0.3 equiv) and MeI (0.23 mL, 3.694 mmol, 0.3 equiv) were added into the solution. The reaction mixture was stirred at rt overnight. The reaction was quenched by addition of MeOH (0.75 mL). The reaction mixture was diluted with EtOAc, washed with water and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to dryness to give crude products. The compound was purified by silica gel column chromatography eluted with  $\text{CH}_2\text{Cl}_2$  to give compound **27** (3.7077 g, 93.0% yield).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.26 (dd, 1H,  $J = 3.6$ ,  $J_{2,3} = 3.6$ , H-2), 3.35 (s, 3H, C6-OCH<sub>3</sub>), 3.39 (s, 3H, C1-OCH<sub>3</sub>), 3.47–3.51 (4H, H-4, C2-OCH<sub>3</sub>), 3.52–3.60 (3H, H-3, H-6), 3.63 (s, 3H, C3-OCH<sub>3</sub>), 3.67 (m, 1H, H-5), 4.60 (d, 1H,  $J = 11$ ,  $\text{CH}_2\text{Ph}$ ), 4.86 (d, 1H,  $J = 11$ ,  $\text{CH}_2\text{Ph}$ ), 7.27–7.35 (5H, aromatic H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  54.7 (C1-OCH<sub>3</sub>), 58.5 (C2-OCH<sub>3</sub>), 58.8 (C6-OCH<sub>3</sub>), 60.6 (C3-OCH<sub>3</sub>), 69.4 (C-5), 70.5 (C-6), 74.5 ( $-\text{CH}_2\text{Ph}$ ), 77.0 (C-4), 81.5 (C-2), 83.3 (C-3), 97.1 (C-1), 127.3, 127.6, 128.0, 138.0 (aromatic C).

#### 4.2.7. Methyl 2,3,6-tri-O-methyl- $\alpha$ -D-glucopyranoside (**28**)

To a solution of compound **27** (1.7282 g, 5.2953 mmol) in THF (5 mL) and EtOH (5 mL), 10% Pd on C (0.9886 g) was added at rt. The reaction mixture was stirred at rt under hydrogen atmosphere overnight. Then 10% Pd on C (0.5099 g) was added into the solution. The 10% Pd on C was filtered off and washed 20% MeOH/ $\text{CH}_2\text{Cl}_2$  (v/v) and MeOH. The combined washings and filtrate were concentrated to give compound **28** (1.249 g, 99.8%).

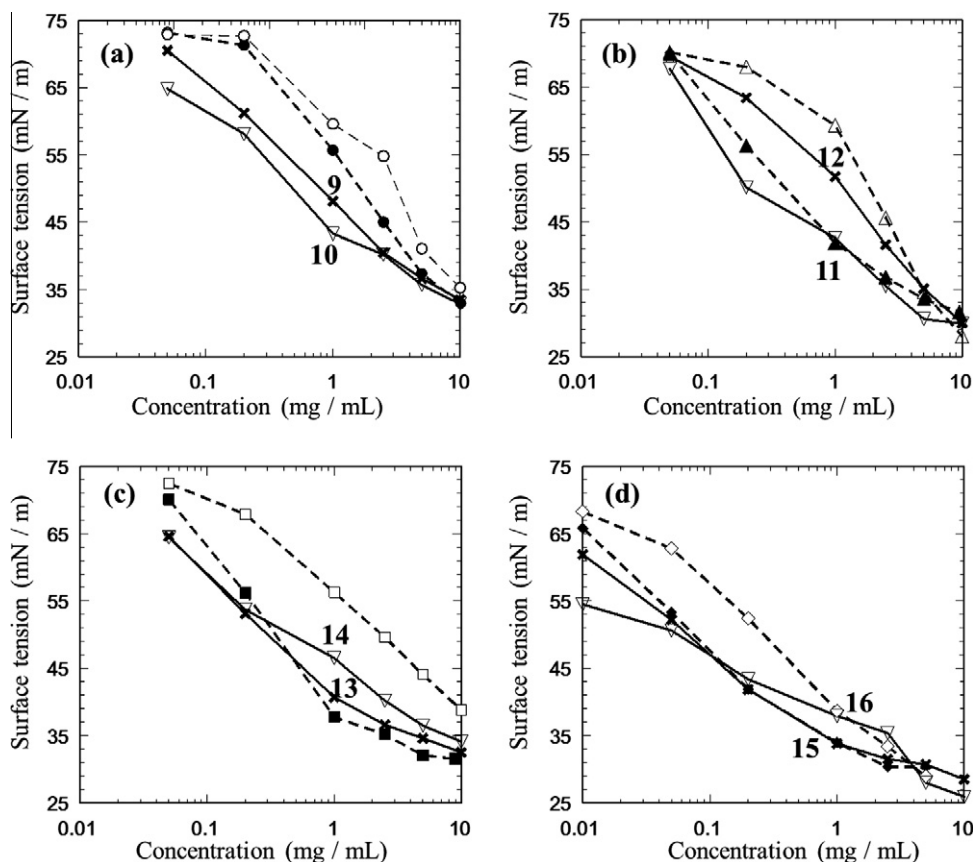
$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.18 (dd, 1H,  $J = 3.6$ ,  $J_{2,3} = 3.3$ , H-1), 3.34 (3H, C6-OCH<sub>3</sub>), 3.37–3.38 (4H, H-3, C1-OCH<sub>3</sub>), 3.41–3.45 (3H, C2-OCH<sub>3</sub>), 3.47–3.63 (6H, H-4, H-6, C3-OCH<sub>3</sub>), 4.79 (d, 1H,  $J_{1,2} = 3.6$ , H-1);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  55.0 (C1-OCH<sub>3</sub>), 58.2 (C2-OCH<sub>3</sub>), 59.2

(C6-OCH<sub>3</sub>), 60.9 (C3-OCH<sub>3</sub>), 69.6 (C-4), 69.9 (C-5), 71.5 (C-6), 81.4 (C-2), 82.7 (C-3), 97.2 (C-1).

#### 4.2.8. Phenyl 2,3,4,6-tetra-O-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl-1-thio- $\beta$ -D-glucopyranoside (**30**)

To a solution of phenyl  $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside **29** (0.9263 g, 2.1320 mmol) in THF (20 mL) and DMF (5 mL), NaH (1.1939 g, 29.848 mmol, 60% in mineral oil) and tetra-*n*-butyl ammonium iodide (114.5 mg, 0.309 mmol) were added and then BnBr (3.6 mL, 30.268 mmol) was added slowly dropwise at 0 °C. The reaction mixture was stirred for 6 h at 80 °C. MeOH (1.8 mL) was added to the reaction mixture for the decomposition of excess BnBr. The reaction mixture was diluted with EtOAc, washed with saturated aq  $\text{NaHCO}_3$  and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to dryness to give slightly yellow crystals. The crude crystals were recrystallized from EtOH to give colorless crystals **30** (1.778 g, 78.3% yield).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.30–3.47 (4H, H-2', H-4', H-5), 3.44 (t, 1H,  $J = 9.6$ ,  $J_{2,3} = 9.0$ , H-2), 3.53–3.76 (5H, H-3, H-3', H-5', H-6'), 3.84 (d,  $J = 4.2$ , H-6), 4.03 (t, 1H,  $J_{3,4} = 9.6$ ,  $J_{4,5} = 9.6$ , H-4), 4.49 (d, 1H,  $J_{1',2'} = 7.8$ , H-1'), 4.62 (d, 1H,  $J_{1,2} = 9.9$ , H-1), 4.38–4.82 (12H,  $-\text{CH}_2\text{Ph}$ ), 4.89 (d,  $J = 14.7$ ,  $-\text{CH}_2\text{Ph}$ ), 5.13 (d,  $J = 11.1$ ,  $-\text{CH}_2\text{Ph}$ ), 7.14–7.56 (40H, aromatic H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  68.1 (C-6), 68.8 (C-6'), 73.1, 73.2, 74.8, 74.9, 75.3, 75.4, 75.6, 76.3 (C-4), 77.9 (C-5'), 79.2 (C-5), 80.0 (C-2), 82.7 (C-2'), 84.8 (C-3or C-3'), 84.9 (C-3or C-3'), 87.3 (C-1), 102.5 (C-1'), 127.3–139 (aromatic C). Mp 147–148 °C (mp 152 °C).<sup>26</sup>  $[\alpha]_D^{14.7} +10.4$  (c 1.081,  $\text{CHCl}_3$ ) MALDI-TOF MS: calcd for  $\text{C}_{67}\text{H}_{68}\text{O}_{10}\text{S}$  1064.45 found  $[\text{M}+\text{Na}]^+ = 1087.39$ ,  $[\text{M}+\text{K}]^+ = 1103.39$ .



**Figure 10.** Surface tension–concentration curves of pure compounds **1–8** and mixtures **9–16**; broken lines are surface tension curves of pure compounds and solid lines are those of mixtures. ●: compound **1**; ○: compound **2**; ▲: compound **3**; △: compound **4**; ■: compound **5**; □: compound **6**; ◆: compound **7**; ◇: compound **8**; ×: mixtures  $\alpha > \beta$ ; ▽: mixtures  $\alpha < \beta$ .

### 4.3. General method for glycosylation

The glycosylation was carried out under a high vacuum system. Glycosyl donor (compound **30** (1.0 equiv)) and acceptor (compounds **27** or **28** (1.5 equiv)) were dissolved in anhydrous EtCN or  $\text{CH}_2\text{Cl}_2$  (2.0 mL) with activated molecular sieves 3 Å or 4 Å (ca. 250 mg), respectively. NBS (1.4 equiv) and AgOTf (a few mg) were added into the solution at rt. The reaction mixture was stirred at rt for 24 h. The reaction mixture was filtered with Celite and washed with EtOAc. The filtrate and washings were diluted with EtOAc, washed with saturated aq  $\text{NaHCO}_3$  and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to dryness to give products. After glycosylation, a part of the products was purified by P-TLC (eluent: EtOAc/*n*-hexane = 1/2, 1/4(v/v), several times) to give compounds **31–46**.

#### 4.3.1. Methyl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-methyl- $\alpha$ -D-glucopyranoside (**39**)

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.96 (C6- $\text{OCH}_3$ ), 3.26 (dd, 1H,  $J = 3.6$ ,  $J_{2,3} = 3.9$ , H-2), 3.31–3.37 (3H, H-2'', H-6), 3.39 (C1- $\text{OCH}_3$ ), 3.46 (C2- $\text{OCH}_3$ ), 3.47 (C3- $\text{OCH}_3$ ), 3.50–3.94 (12H, H-4, H-5, H-2', H-3', H-5', H-6', H-3'', H-4'', H-5'', H-6''), 3.70 (t, 1H,  $J = 9.3$ ,  $J_{3,4} = 9.3$ , H-3), 4.08 (t, 1H,  $J = 9.3$ , H-4'), 4.34 (d,  $J = 12.3$ ,  $-\text{CH}_2\text{Ph}$ ), 4.44 (d,  $J = 12.3$ ,  $-\text{CH}_2\text{Ph}$ ), 4.48 (d, 1H,  $J_{1'',2''} = 7.8$ , H-1''), 4.54 (d, 1H,  $J = 10.8$ ,  $-\text{CH}_2\text{Ph}$ ), 4.64 (d, 1H,  $J = 12.0$ ,  $-\text{CH}_2\text{Ph}$ ), 4.68–4.88 (9H,  $-\text{CH}_2\text{Ph}$ ), 4.80 (d, 1H,  $J = 3.6$ , H-1), 5.11 (d, 1H,  $J = 11.1$ ,  $-\text{CH}_2\text{Ph}$ ), 5.62 (d, 1H,  $J = 3.9$ , H-1'), 7.18–7.39 (35H, aromatic H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  55.0 (C1- $\text{OCH}_3$ ), 58.6 (C2- $\text{OCH}_3$ ), 58.7 (C6- $\text{OCH}_3$ ), 60.1 (C3- $\text{OCH}_3$ ), 67.6, 68.8, 68.9, 70.8 (C-6), 71.2 (C-5'), 73.2, 73.4, 73.7, 74.3, 74.7, 75.2, 75.2, 75.6, 76.2 (C-4'), 78.0, 78.7, 80.3 (C-4), 82.1 (C-2), 82.4 (C-2''), 83.3 (C-3), 84.9 (C-2'), 96.4 (C-1'), 97.0 (C-

1), 102.3 (C-1''), 126.9–139.4 (aromatic C).  $R_f$  (EtOAc/*n*-hexane 1:1, six times) 0.48.  $[\alpha]_D^{19.2} + 62.5$  (c 1.043,  $\text{CHCl}_3$ ). MALDI-TOF MS: calcd for  $\text{C}_{71}\text{H}_{82}\text{O}_{16}$  1190.56 found  $[\text{M}+\text{Na}]^+ = 1213.56$ ,  $[\text{M}+\text{K}]^+ = 1229.53$ .

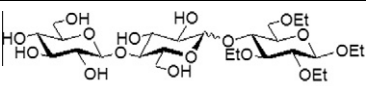
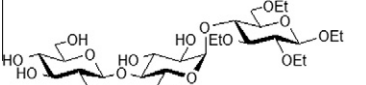
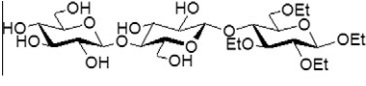
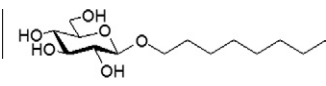
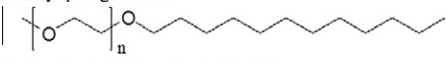
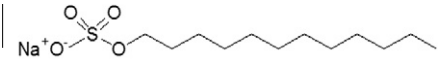
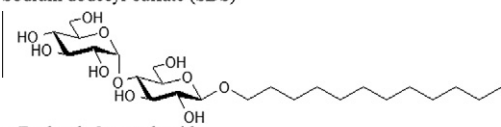
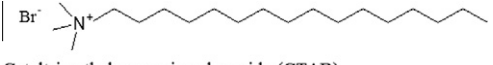
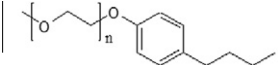
#### 4.3.2. Methyl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-methyl- $\alpha$ -D-glucopyranoside (**40**)

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.21 (dd, 1H,  $J = 3.6$ ,  $J_{2,3} = 3.6$ , H-2), 3.25 (C6- $\text{OCH}_3$ ), 3.30–3.74 (15H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4'', H-5, H-5', H-5'', H-6, H-6', H-6''), 3.40 (C1- $\text{OCH}_3$ ), 3.52 (C2- $\text{OCH}_3$ ), 3.55 (C3- $\text{OCH}_3$ ), 3.88 (dd, 2H,  $J = 3.3$ ,  $J = 3.0$ , H-6'), 4.08 (t, 1H,  $J = 9.3$ , H-4'), 4.36 (d, 1H,  $J_{1',2'} = 6.0$ , H-1'), 4.54 (d, 1H,  $J_{1'',2''} = 6.9$ , H-1''), 4.81 (d, 1H,  $J_{1,2} = 3.0$  Hz, H-1), 4.49–4.91 (12H,  $-\text{CH}_2\text{Ph}$ ), 5.13 (d, 1H,  $J = 11.4$ ,  $-\text{CH}_2\text{Ph}$ ), 7.15–7.35 (35H, aromatic H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  55.1 (C1- $\text{OCH}_3$ ), 58.8 (C2- $\text{OCH}_3$ ), 59.2 (C6- $\text{OCH}_3$ ), 60.9 (C3- $\text{OCH}_3$ ), 67.8 (C-6'), 68.8 (C-6''), 69.7 (C-5), 70.0 (C-6), 73.1 ( $-\text{CH}_2\text{Ph}$ ), 73.2 ( $-\text{CH}_2\text{Ph}$ ), 74.8 ( $-\text{CH}_2\text{Ph}$ ), 74.9 ( $-\text{CH}_2\text{Ph}$ ), 75.1 (C-5'), 75.6 (C-5''), 76.2 (C-4'), 77.4 (C-4), 77.9 (C-4''), 80.9 (C-2), 81.5 (C-3), 81.9 (C-2'), 82.7 (C-2''), 83.4 (C-3'), 84.8 (C-3''), 97.5 (C-1), 102.4 (C-1'), 102.9 (C-1''), 127.0–139.2 (aromatic C).  $R_f$  (EtOAc/*n*-hexane 1:1, six times) 0.48.  $[\alpha]_D^{18.6} + 42.7$  (c 0.994,  $\text{CHCl}_3$ ). MALDI-TOF MS: calcd for  $\text{C}_{71}\text{H}_{82}\text{O}_{16}$  1190.56 found  $[\text{M}+\text{Na}]^+ = 1214.51$ ,  $[\text{M}+\text{K}]^+ = 1229.48$ .

#### 4.3.3. Methyl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-methyl- $\beta$ -D-glucopyranoside (**41**)

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.01 (C6- $\text{OCH}_3$ ), 3.05 (dd, 1H,  $J = 7.8$ ,  $J_{2,3} = 9.0$ , H-2), 3.34–3.85 (24H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4'', H-5, H-5', H-5'', H-6, H-6', H-6''), 3.44 (C3- $\text{OCH}_3$ ), 3.50 (C1-

**Table 4**Comparisons of surface tensions of compounds **7**, **8** and **15** with those of commonly-used surfactants

Surfactants	CMC (mM)	$\gamma_{\text{CMC}}$ (mN/m)	Ref.
 <b>15</b>	0.48 (0.03 wt %)	32.2	—
 <b>7</b>	0.48 (0.03 wt %)	34.5	—
 <b>8</b>	1.6 (0.1 wt %)	36.5	—
Methylcellulose ( $DS \approx 1.8$ )	—	46.0 (0.5 wt %)	33
 <i>n</i> -Octyl $\beta$ -D-glucoside	22.0	30.8	34
 Dodecyl octaethyleneglycol ( $C_{12}E_8$ )	0.058	32.7	35
 Sodium dodecyl sulfate (SDS)	8.0–9.0	35.0	36
 <i>n</i> -Dodecyl $\beta$ -D-maltoside	0.17	35.3	37
 Cetyltrimethylammonium bromide (CTAB)	0.8	36.0	36
 Polyethylene glycol <i>p</i> -(1,1,3,3-tetramethylbutyl)-phenyl ether (Triton X-100)	0.27	40.8	38

Surface tensions of compounds **7**, **8**, and **15** at concentration of 0.5 wt % were 30.4, 29.0, and 28.0 mN/m, respectively.

OCH<sub>3</sub>), 3.54 (C2–OCH<sub>3</sub>), 3.92 (dd, 2H,  $J = 2.4$ ,  $J = 2.1$ , H-6'), 4.06 (t, 1H,  $J = 9.3$ , H-4'), 4.15 (d, 1H,  $J_{1,2} = 7.8$ , H-1), 4.31–4.88 (14H, –CH<sub>2</sub>Ph), 5.12 (d, 1H,  $J = 11.1$ , –CH<sub>2</sub>Ph), 4.43 (d, 1H,  $J_{1',2'} = 10.5$ , H-1'), 5.60 (d, 1H,  $J_{1',2'} = 3.9$ , H-1'), 7.17–7.30 (35H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  56.8 (C1–OCH<sub>3</sub>), 59.0 (C6–OCH<sub>3</sub>), 59.3 (C3–OCH<sub>3</sub>), 60.1 (C2–OCH<sub>3</sub>), 67.6 (C-6''), 68.9 (C-6'), 70.8 (C-6), 71.0, 73.3 (–CH<sub>2</sub>Ph), 73.4 (–CH<sub>2</sub>Ph), 73.6, 73.8, 74.4, 74.7, 75.2, 75.6, 76.4 (C-4'), 77.2, 78.0, 78.5 (C-2'), 80.2 (C-3'), 82.4 (C-2''), 83.7 (C-2), 84.8 (C-3''), 86.2 (C-3), 96.3 (C-1'), 102.4 (C-1''), 104.2 (C-1), 127.0–138.2 (aromatic C).  $R_f$  (EtOAc/*n*-hexane 1:1, six times) 0.76.  $[\alpha]_D^{20.0} +17.7$  (c 1.012, CHCl<sub>3</sub>). MALDI-TOF MS: calcd for C<sub>71</sub>H<sub>82</sub>O<sub>16</sub> 1190.56 found  $[M+Na]^+ = 1213.56$ ,  $[M+K]^+ = 1229.51$ .

#### 4.3.4. Methyl 2,3,4,6-tetra-O-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-methyl- $\beta$ -D-glucopyranoside (**42**)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  3.01 (dd, 1H,  $J = 7.8$ ,  $J_{2,3} = 9.0$ , H-2), 3.19–3.44 (9H, H-2'', H-2'', H-3, H-5, H-5', H-5'', –OCH<sub>3</sub>), 3.24 (C6–OCH<sub>3</sub>), 3.46–3.72 (19H, H-3'', H-3'', H-4, H-4'', H-6, H-6'', H-6'' –CH<sub>3</sub>), 3.51 (C1–OCH<sub>3</sub>), 3.55 (C3–OCH<sub>3</sub>), 3.57 (C2–OCH<sub>3</sub>), 3.88 (dd, 2H,  $J = 3.3$ ,  $J = 3.0$ , H-6'), 4.08 (t, 1H,  $J = 9.3$ , H-4'), 4.15 (d, 1H,  $J_{1,2} = 7.8$ , H-1), 4.40 (d, 1H,  $J_{1',2'} = 8.1$ , H-1''), 4.55 (d, 1H,  $J_{1',2'} = 7.8$ , H-1'), 4.37–

4.91 (15H, –CH<sub>2</sub>Ph), 5.13 (d, 1H,  $J = 11.1$ , –CH<sub>2</sub>Ph), 7.15–7.33 (35H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  56.9 (C1–OCH<sub>3</sub>), 59.0 (C6–OCH<sub>3</sub>), 60.5 (C3–OCH<sub>3</sub>), 60.6 (C2–OCH<sub>3</sub>), 67.8 (C-6'), 68.8 (C-6''), 70.2 (C-6), 73.1 (–CH<sub>2</sub>Ph), 73.2 (–CH<sub>2</sub>Ph), 74.6 (C-5 or C-5' or C-5''), 74.8 (C-5 or C-5' or C-5''), 74.9 (–CH<sub>2</sub>Ph), 75.0 (–CH<sub>2</sub>Ph), 75.6 (C-5 or C-5' or C-5''), 76.2 (C-4'), 77.2 (C-4 or C-4'), 77.9 (C-4 or C-4'), 81.8 (C-2''), 82.7 (C-2'), 82.9 (C-2), 83.3 (C-3' or C-3''), 84.5 (C-3), 84.8 (C-3' or C-3''), 102.4 (C-1'), 102.8 (C-1''), 104.1 (C-1), 127.0–138.5 (aromatic C).  $R_f$  (EtOAc/*n*-hexane 1:1, six times) 0.76.  $[\alpha]_D^{18.6} +6.17$  (c 1.004, CHCl<sub>3</sub>). MALDI-TOF MS: calcd for C<sub>71</sub>H<sub>82</sub>O<sub>16</sub> 1190.56 found  $[M+Na]^+ = 1213.51$ ,  $[M+K]^+ = 1229.45$ .

#### 4.3.5. Ethyl 2,3,4,6-tetra-O-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-ethyl- $\alpha$ -D-glucopyranoside (**43**)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.9–1.25 (12H, –CH<sub>2</sub>–CH<sub>3</sub>), 3.09–3.94 (25H, H-2, H-2'', H-2'', H-3, H-3', H-3'', H-4, H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'', –CH<sub>2</sub>–CH<sub>3</sub>), 4.11 (t, 1H,  $J = 9.6$ , H-4'), 4.32–4.87 (14H, H-1'', –CH<sub>2</sub>Ph), 4.89 (d, 1H,  $J_{1,2} = 3.6$ , H-1), 5.12 (d,  $J = 10.2$ , –CH<sub>2</sub>Ph), 5.73 (d, 1H,  $J_{1',2'} = 3.9$ , H-1'), 7.19–7.39 (35H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  14.8, 15.0, 15.6, 15.9 (–CH<sub>2</sub>–CH<sub>3</sub>), 63.1, 66.5, 66.7, 67.9,

68.9, 69.1, 69.2 (C-6, C-6', C-6'', -CH<sub>2</sub>-CH<sub>3</sub>), 70.8, 71.5, 73.2, 73.4, 74.0, 74.4, 74.7, 75.2 (-CH<sub>2</sub>Ph), 75.6, 76.2 (C-4'), 78.1, 78.8, 80.4 (C-4), 80.7 (C-2), 81.6 (C-3), 82.5 (C-2''), 84.8 (C-2'), 96.0 (C-1'), 96.2 (C-1), 102.4 (C-1''), 127.0–139.4 (aromatic C). *R*<sub>f</sub> (EtOAc/*n*-hexane 1:2, six times) 0.65. [ $\alpha$ ]<sub>D</sub><sup>17.0</sup> +58.6 (c 0.775, CHCl<sub>3</sub>). MALDI-TOF MS: calcd for C<sub>75</sub>H<sub>90</sub>O<sub>16</sub> 1246.62 found [M+Na]<sup>+</sup> = 1269.40, [M+K]<sup>+</sup> = 1285.34.

#### 4.3.6. Ethyl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-ethyl- $\alpha$ -D-glucopyranoside (44)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.09–1.27 (12H, -CH<sub>2</sub>-CH<sub>3</sub>), 3.29 (dd, 1H, *J* = 3.6, *J*<sub>2,3</sub> = 3.6, H-2), 3.37–3.95 (24H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'', -CH<sub>2</sub>-CH<sub>3</sub>), 4.09 (t, 1H, *J* = 9.0, *J* = 9.3, H-4'), 4.37 (d, 1H, *J*<sub>1',2'</sub> = 8.7, H-1''), 4.49 (d, 1H, *J*<sub>1',2'</sub> = 9.6, H-1'), 4.87 (d, 1H, *J*<sub>1,2</sub> = 3.9, H-1), 4.36–4.9 (13H, -CH<sub>2</sub>Ph), 5.12 (d, 1H, *J* = 11.1, -CH<sub>2</sub>Ph), 7.16–7.35 (35H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  14.9, 15.1, 15.6, 15.7 (-CH<sub>2</sub>-CH<sub>3</sub>), 63.1, 66.3, 66.9, 67.9, 68.0, 68.6, 68.8 (-CH<sub>2</sub>-CH<sub>3</sub>), 69.9, 73.1, 74.7, 74.9, 75.0, 75.1 (-CH<sub>2</sub>Ph), 75.6, 76.3 (C-4'), 77.5 (C-4), 77.9 (C-4''), 79.4 (C-3), 79.7 (C-2), 81.8 (C-2''), 82.6 (C-2'), 83.4 (C-3''), 84.8 (C-3'), 96.6 (C-1), 102.4 (C-1'), 102.9 (C-1''), 127.0–139.3 (aromatic C). *R*<sub>f</sub> (EtOAc/*n*-hexane 1:2, six times) 0.65. [ $\alpha$ ]<sub>D</sub><sup>17.5</sup> +44.7 (c 0.853, CHCl<sub>3</sub>). MALDI-TOF MS: calcd for C<sub>75</sub>H<sub>90</sub>O<sub>16</sub> 1246.62 found [M+Na]<sup>+</sup> = 1269.42, [M+K]<sup>+</sup> = 1285.39.

#### 4.3.7. Ethyl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-ethyl- $\beta$ -D-glucopyranoside (45)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.89–1.25 (12H, -CH<sub>2</sub>-CH<sub>3</sub>), 3.18–3.94 (24H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'', -CH<sub>2</sub>-CH<sub>3</sub>), 3.12 (dd, 1H, *J* = 8.1, *J*<sub>2,3</sub> = 9.3, H-2), 4.08 (t, 1H, *J* = 9.3, H-4'), 4.23 (d, 1H, *J*<sub>1,2</sub> = 7.5, H-1), 4.32–4.46 (13H, -CH<sub>2</sub>Ph), 4.45 (d, 1H, *J*<sub>1',2'</sub> = 6.0, H-1''), 5.12 (d, 1H, *J* = 11.4, -CH<sub>2</sub>Ph), 5.68 (d, 1H, *J* = 3.9, H-1'), 7.17–7.39 (35H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  15.1, 15.2, 15.6, 15.8 (-CH<sub>2</sub>-CH<sub>3</sub>), 65.4, 67.0, 67.6, 68.1 (-CH<sub>2</sub>-CH<sub>3</sub>), 68.9, 69.4, 70.8 (C-4), 71.4, 73.2, 73.4, 74.0, 74.3, 74.6, 74.7, 75.1, 75.2 (-CH<sub>2</sub>Ph), 75.6, 76.4 (C-4'), 77.2, 78.0, 78.5 (C-2'), 80.4 (C-3'), 82.4 (C-2), 82.5 (C-2''), 84.7 (C-3 or C-3''), 84.8 (C-3 or C-3''), 96.1 (C-1'), 102.5 (C-1''), 103.1 (C-1), 127.0–139.4 (aromatic C). *R*<sub>f</sub> (EtOAc/*n*-hexane 1:2, six times) 0.79. [ $\alpha$ ]<sub>D</sub><sup>16.7</sup> +22.2 (c 0.862, CHCl<sub>3</sub>). MALDI-TOF MS: calcd for C<sub>75</sub>H<sub>90</sub>O<sub>16</sub> 1246.62 found [M+Na]<sup>+</sup> = 1269.45, [M+K]<sup>+</sup> = 1285.39.

#### 4.3.8. Ethyl 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzyl- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-ethyl- $\beta$ -D-glucopyranoside (46)

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.08–1.24 (12H, -CH<sub>2</sub>-CH<sub>3</sub>), 3.09 (dd, 1H, *J* = 8.1, *J*<sub>2,3</sub> = 8.7, H-2), 3.26–3.92 (24H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'', -CH<sub>2</sub>-CH<sub>3</sub>), 4.09 (t, 1H, *J* = 9.0, *J* = 9.6, H-4'), 4.22 (d, 1H, *J*<sub>1,2</sub> = 8.1, H-1), 4.35–4.90 (16H, H-1', H-1'', -CH<sub>2</sub>Ph), 5.12 (d, 1H, *J* = 11.4, -CH<sub>2</sub>Ph), 7.17–7.35 (35H, aromatic H); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  15.2, 15.7, 15.8 (-CH<sub>2</sub>-CH<sub>3</sub>), 65.4, 66.5, 67.8, 68.3, 68.5, 68.7, 68.8 (C-6, C-6', C-6'', -CH<sub>2</sub>-CH<sub>3</sub>), 73.1, 74.7, 74.9 (-CH<sub>2</sub>Ph), 75.6, 76.3 (C-4'), 77.2, 77.7, 77.9 (-CH<sub>2</sub>Ph), 81.6 (C-2), 81.8 (C-2''), 82.6 (C-2' or C-3), 82.9 (C-2' or C-3), 83.3 (C-3' or C-3''), 84.8 (C-3' or C-3''), 102.4 (C-1'), 102.9 (C-1''), 103.2 (C-1), 127.0–139.3 (aromatic C). *R*<sub>f</sub> (EtOAc/*n*-hexane 1:2, six times) 0.79. [ $\alpha$ ]<sub>D</sub><sup>16.9</sup> +11.6 (c 0.684, CHCl<sub>3</sub>). MALDI-TOF MS: calcd for C<sub>75</sub>H<sub>90</sub>O<sub>16</sub> 1246.62 found [M+Na]<sup>+</sup> = 1269.42, [M+K]<sup>+</sup> = 1285.40.

#### 4.4. General method for debenzylation

To a solution of benzylated trisaccharide derivative in THF (0.1 mL) and EtOH (0.9 mL), Pd(OH)<sub>2</sub> on C was added. The reaction

mixture was stirred at rt under hydrogen atmosphere overnight. The Pd(OH)<sub>2</sub> on C was filtered off and washed with 20% MeOH/CH<sub>2</sub>Cl<sub>2</sub> (v/v) and MeOH. The combined filtrate and washings were concentrated to dryness to give products.

#### 4.4.1. Methyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-methyl- $\alpha$ -D-glucopyranoside (1)

Debenzylation of compound **1** gave compound **39** (17.9 mg) in ca. 100% yield (8.7 mg).

<sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  3.15–3.77 (18H, H-2, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4', H-4'', H-5, H-5', H-5'', H-6, H-6', H-6''), 3.33 (C6-OCH<sub>3</sub>), 3.39 (C1-OCH<sub>3</sub>), 3.46 (C2-OCH<sub>3</sub>), 3.56 (C3-OCH<sub>3</sub>), 4.52 (d, 1H, *J*<sub>1',2'</sub> = 7.8, H-1''), 4.97 (d, 1H, *J*<sub>1,2</sub> = 3.3, H-1), 5.40 (d, 1H, *J*<sub>1',2'</sub> = 3.9, H-1'); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  57.6 (C1-OCH<sub>3</sub>), 60.6 (C2-OCH<sub>3</sub>), 60.9 (C6-OCH<sub>3</sub>), 62.1 (C-6' or C-6''), 62.4 (C3-OCH<sub>3</sub>), 63.2 (C-6' or C-6''), 71.2, 72.1, 73.1 (C-6), 73.7, 73.9, 74.5, 75.8 (C-2''), 78.2 (C-2' or C-3''), 78.6 (C-2' or C-3'), 80.7 (C-3'), 83.4 (C-2), 85.7 (C-3), 99.2 (C-1), 100.7 (C-1'), 105.1 (C-1''). [ $\alpha$ ]<sub>D</sub><sup>19.2</sup> +59.1 (c 0.220, water). MALDI-TOF MS: calcd for C<sub>22</sub>H<sub>40</sub>O<sub>16</sub> 560.23 found [M+Na]<sup>+</sup> = 583.29.

#### 4.4.2. Methyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-methyl- $\alpha$ -D-glucopyranoside (2)

Debenzylation of compound **2** gave compound **40** (25.6 mg) in 95% yield (11.4 mg).

<sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  3.15–3.77 (18H, H-2, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4', H-4'', H-5, H-5', H-5'', H-6, H-6', H-6''), 3.37 (C6-OCH<sub>3</sub>), 3.39 (C1-OCH<sub>3</sub>), 3.46 (C3-OCH<sub>3</sub>), 3.55 (C2-OCH<sub>3</sub>), 4.40 (d, 1H, *J*<sub>1',2'</sub> = 8.1, H-1''), 4.50 (d, 1H, *J*<sub>1',2'</sub> = 7.8, H-1''), 4.98 (d, 1H, *J*<sub>1,2</sub> = 3.6, H-1); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  57.6 (C1-OCH<sub>3</sub>), 60.5 (C2-OCH<sub>3</sub>), 60.8 (C6-OCH<sub>3</sub>), 62.1 (C3-OCH<sub>3</sub>), 62.6 (C-6'), 63.2 (C-6''), 71.8, 72.1, 72.4 (C-6), 75.8 (C-2' or C-2''), 75.9 (C-2' or C-2''), 76.9, 77.5, 78.1, 78.3, 78.6 (C-4), 81.1 (C-4'), 82.1 (C-2), 83.0 (C-3), 99.2 (C-1), 105.0 (C-1'), 105.2 (C-1''). [ $\alpha$ ]<sub>D</sub><sup>20.0</sup> +37.9 (c 0.222, water). MALDI-TOF MS calcd for C<sub>22</sub>H<sub>40</sub>O<sub>16</sub> 560.23 found [M+Na]<sup>+</sup> = 583.18.

#### 4.4.3. Methyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-methyl- $\beta$ -D-glucopyranoside (3)

Debenzylation of compound **3** gave compound **41** (17.5 mg) in ca. 100% yield (8.6 mg).

<sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  3.25–3.91 (18H, H-2, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4', H-4'', H-5, H-5', H-5'', H-6, H-6', H-6''), 3.10 (t, 1H, *J* = 8.4, *J* = 8.7, H-2), 3.33 (C6-OCH<sub>3</sub>), 3.53 (C1-OCH<sub>3</sub>), 3.56 (C2-OCH<sub>3</sub>), 3.59 (C3-OCH<sub>3</sub>), 4.40 (d, 1H, *J*<sub>1,2</sub> = 7.8, H-1), 4.50 (d, 1H, *J*<sub>1',2'</sub> = 8.1, H-1''), 5.38 (d, 1H, *J*<sub>1',2'</sub> = 3.9, H-1'); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  59.9 (C1-OCH<sub>3</sub>), 61.0 (C6-OCH<sub>3</sub>), 62.1 (C-6' or C-6''), 62.2 (C2-OCH<sub>3</sub>), 62.6 (C3-OCH<sub>3</sub>), 63.2 (C-6' or C-6''), 72.1, 73.2 (C-6), 73.7, 73.8, 73.9 (C-4), 74.5, 75.5 (C-3'), 75.8 (C-2''), 78.3 (C-2' or C-3''), 78.6 (C-2' or C-3''), 80.7 (C-4'), 85.7 (C-2), 88.3 (C-3), 100.5 (C-1'), 105.1 (C-1''), 105.7 (C-1). [ $\alpha$ ]<sub>D</sub><sup>20.8</sup> +32.0 (c 0.220, water). MALDI-TOF MS: calcd for C<sub>22</sub>H<sub>40</sub>O<sub>16</sub> 560.23 found [M+Na]<sup>+</sup> = 583.4.

#### 4.4.4. Methyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-methyl- $\beta$ -D-glucopyranoside (4)

Debenzylation of compound **4** gave compound **42** (24.1 mg) in 98.2% yield (11.1 mg).

<sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  3.08 (t, 1H, *J* = 8.1, *J*<sub>2,3</sub> = 9.3, H-2), 3.32–3.62 (10H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4', H-5, H-5', H-5''), 3.37 (C6-OCH<sub>3</sub>), 3.39 (C1-OCH<sub>3</sub>), 3.46 (C3-OCH<sub>3</sub>), 3.55 (C2-OCH<sub>3</sub>), 3.67–3.98 (7H, H-4', H-6, H-6', H-6''), 4.40 (d, 1H, *J* = 7.8, H-1 or H-1'), 4.41 (d, 1H, *J* = 7.5, H-1 or H-1'), 4.49 (d, 1H, *J*<sub>1',2'</sub> = 7.8, H-1''); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  59.8 (C1-OCH<sub>3</sub>), 60.9 (C6-OCH<sub>3</sub>), 62.0 (C2-OCH<sub>3</sub> or C3-OCH<sub>3</sub>), 62.6 (C-6' or C-6' or C2-OCH<sub>3</sub> or C3-OCH<sub>3</sub>), 63.2 (C-6' or C-6''), 72.1, 72.6 (C-6), 75.8 (C-2' or C-2''), 75.9 (C-2' or C-2''), 76.1, 76.9, 77.5, 78.1, 78.4, 78.6 (C-4'), 81.1 (C-4), 84.4

(C-2), 85.7 (C-3), 104.9 (C-1'), 105.2 (C-1''), 105.6 (C-1).  $[\alpha]_D^{22.8} -15.9$  (c 0.220, water). MALDI-TOF MS: calcd for  $C_{22}H_{40}O_{16}$  560.23 found  $[M+Na]^+ = 583.51$ .

#### 4.4.5. Ethyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-ethyl- $\alpha$ -D-glucopyranoside (5)

Debenzylation of compound **5** gave compound **43** (18.0 mg) in 96% yield (8.6 mg).

$^1H$  NMR ( $D_2O$ ):  $\delta$  1.14–1.23 (12H,  $-CH_2-CH_3$ ), 3.28–3.96 (26H, H-2, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'',  $-CH_2-CH_3$ ), 4.51 (d,  $J_{1',2'} = 7.8$ , H1'), 5.03 (d,  $J_{1,2} = 3.6$ , H1), 5.47 (d,  $J_{1',2'} = 3.6$ , H1'');  $^{13}C$  NMR ( $D_2O$ ):  $\delta$  16.7, 17.3, 17.4 ( $-CH_2-CH_3$ ), 62.2, 63.2, 66.5, 69.5, 69.6, 71.3, 71.4, 71.6, 72.1, 73.7, 73.8, 74.1, 74.8, 75.8 (C-2''), 78.3, 78.6 (C-2'), 80.6, 82.1 (C-2), 84.1, 98.3 (C-1), 100.4 (C-1'), 105.1 (C-1'').  $[\alpha]_D^{21.0} +56.0$  (c 0.220, water). MALDI-TOF MS: calcd for  $C_{26}H_{48}O_{16}$  616.29 found  $[M+Na]^+ = 639.24$ .

#### 4.4.6. Ethyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-ethyl- $\alpha$ -D-glucopyranoside (6)

Debenzylation of compound **6** gave compound **44** (40.7 mg) in 96.9% yield (19.5 mg).

$^1H$  NMR ( $D_2O$ ):  $\delta$  1.15–1.22 (12H,  $-CH_2-CH_3$ ), 3.26–3.99 (26H, H-2, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4', H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'',  $-CH_2-CH_3$ ), 4.41 (d, 1H,  $J_{1',2'} = 7.8$ , H-1'), 4.49 (d, 1H,  $J_{1',2'} = 7.8$ , H-1''), 5.01 (d, 1H,  $J_{1,2} = 3.3$ , H-1);  $^{13}C$  NMR ( $D_2O$ ):  $\delta$  16.6, 16.8, 17.2, 17.3 ( $-CH_2-CH_3$ ), 62.9, 63.2, 66.4, 69.3, 69.8, 70.5, 71.5 ( $-CH_2-CH_3$ ), 72.0, 72.1, 75.8 (C-2' or C-2''), 75.9 (C-2' or C-2''), 76.9, 77.7, 78.1, 78.6, 78.8, 81.1 (C-2), 81.4, 81.8, 98.5 (C-1), 104.7 (C-1'), 105.2 (C-1'').  $[\alpha]_D^{22.8} +51.0$  (c 0.220, water). MALDI-TOF MS: calcd for  $C_{26}H_{48}O_{16}$  616.29 found  $[M+Na]^+ = 639.48$ .

#### 4.4.7. Ethyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-ethyl- $\beta$ -D-glucopyranoside (7)

Debenzylation of compound **7** gave compound **45** (7.1 mg) in ca. 100% yield (3.8 mg).

$^1H$  NMR ( $D_2O$ ):  $\delta$  1.13–1.23 (12H,  $-CH_2-CH_3$ ), 3.15 (t, 1H,  $J = 8.4$ ,  $J_{2,3} = 9.0$ , H-2), 3.27 (t, 1H,  $J = 7.8$ ,  $J_{2',3'} = 9.3$ , H-2''), 3.37–3.96 (24H, H-2', H-3, H-3', H-3'', H-4, H-4', H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'',  $-CH_2-CH_3$ ), 4.46 (d, 1H,  $J_{1,2} = 8.4$ , H-1), 4.49 (d, 1H,  $J_{1',2'} = 8.1$ , H-1'), 5.44 (d, 1H,  $J_{1',2'} = 3.9$ , H-1'');  $^{13}C$  NMR ( $D_2O$ ):  $\delta$  16.1, 16.4, 16.7, 16.8 ( $-CH_2-CH_3$ ), 61.6, 62.6, 68.7, 69.0, 70.9, 71.1, 71.2, 71.4, 73.1, 73.2, 73.4 (C-4), 74.2, 75.1 (C-3'), 75.2 (C-2''), 77.7 (C-3''), 78.0 (C-2'), 80.1 (C-4'), 83.8 (C-2), 86.2 (C-3), 99.6 (C-1'), 104.0 (C-1''), 104.5 (C-1).  $[\alpha]_D^{22.7} +28.6$  (c 0.220, water). MALDI-TOF MS: calcd for  $C_{26}H_{48}O_{16}$  616.29 found  $[M+Na]^+ = 639.50$ .

#### 4.4.8. Ethyl $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-ethyl- $\beta$ -D-glucopyranoside (8)

Debenzylation of compound **8** gave compound **46** (7.7 mg) in ca. 100% yield (3.8 mg).

$^1H$  NMR ( $D_2O$ ):  $\delta$  1.15–1.23 (12H,  $-CH_2-CH_3$ ), 3.25–3.98 (25H, H-2', H-2'', H-3, H-3', H-3'', H-4, H-4', H-4'', H-5, H-5', H-5'', H-6, H-6', H-6'',  $-CH_2-CH_3$ ), 3.09 (t, 1H,  $J_{1,2} = 8.1$ ,  $J_{2,3} = 9.0$ , H-2), 4.41 (d, 1H,  $J_{1',2'} = 6.3$ , H-1'), 4.44 (d, 1H,  $J_{1,2} = 8.1$ , H-1), 4.48 (d, 1H,  $J_{1',2'} = 7.8$ , H-1'');  $^{13}C$  NMR ( $D_2O$ ):  $\delta$  16.8, 16.9, 17.2, 17.3 ( $-CH_2-CH_3$ ), 62.8, 63.2, 69.3 (C-2' or C-2''), 69.5 (C-2' or C-2''), 70.7, 71.8, 72.0, 75.8, 75.9, 76.4, 76.9, 77.7, 78.1 (C-3''), 78.6, 78.8 (C-4), 81.4 (C-4'), 83.5 (C-2), 84.6 (C-3), 104.5 (C-1''), 104.7 (C-1), 105.2 (C-

1').  $[\alpha]_D^{22.9} -2.9$  (c 0.220, water). MALDI-TOF MS: calcd for  $C_{26}H_{48}O_{16}$  616.29 found  $[M+Na]^+ = 639.48$ .

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