ing effects were not evaluated because of its very low gene expression level in ScN2a cells.

3.2. Rescue of gene silencing effect

To confirm the specificity of gabrb1 gene silencing, a rescue experiment was performed using an expression vector for the mutated gabrb1 gene, which contained silent mutations at the targeting region of the double-stranded siRNA. Introduction of this mutated gabrb1 gene into ScN2a cells did not modify the PrPres level (Fig. 2; lanes 1 and 2). Co-transfection of the mutated gabrb1 expression vector and the double-stranded siRNA into ScN2a cells caused no reduction of the PrPres level, although co-transfection of the mock expression vector and the double-stranded siRNA caused substantial reduction in the PrPres level (Fig. 2; lanes 3 and 4). Consequently, the gabrb1 gene silencing effect on the PrPres formation was rescued by the expression of the silent mutation-containing gabrb1 gene.

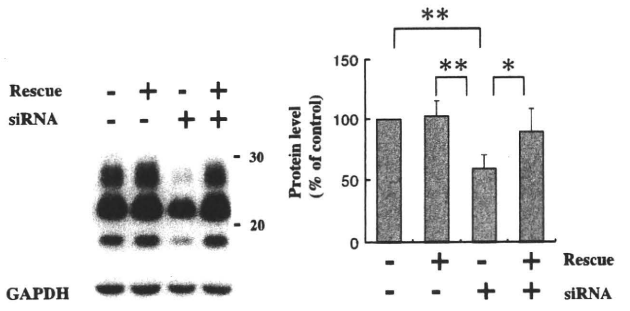

Fig. 2. Rescue of gabrb1 gene silencing. Immunoblot and protein level of PrPres from ScN2a cells transfected with silent mutation-containing gabrb1 expression vector (rescue +) or mock vector (rescue −) in the presence of (siRNA +) or the absence of (siRNA −) the siRNA used in the experiment shown in Fig. 1. Molecular size markers in the right side of the immunoblots are shown in kilodaltons. Immunoblot data shown here are representative examples; the graphic data shown here are the average and standard deviation from results of independent triplicate experiments (*$P < 0.05$, **$P < 0.01$).

3.3. Effects of GABAA receptor-related compounds

Salicylidene salicylhydrazide is a selective inhibitor of β1 subunit-containing GABAA receptors [19]. Treatment of ScN2a cells with this compound inhibited PrPres formation dose-dependently with a 50% inhibition dose of 450 nM (Fig. 3a). However, it did not modify the PrP mRNA level and the PrPc level, as demonstrated in the experiment using N2a cells (Supplementary Fig. 3). Another GABAA receptor antagonist, bicuculline methiodide [8], was also effective in inhibiting the PrPres formation dose-dependently but at almost a thousand times higher dose than that of salicylidene salicylhydrazide (Fig. 3b). No other GABAA receptor-related compound modified the PrPres level in ScN2a cells (Supplementary Fig. 4). The compounds tested in this study included an antagonist, picrotoxin [8], and agonists such as GABA [8], muscimol [8], pentobarbital [8], ethanol [20], and isoguvacine hydrochloride [21].

4. Discussion

Results show that gabrb1 is involved in the PrPres formation in ScN2a cells. The PrPres level was reduced significantly by gabrb1 gene silencing using either shRNA or siRNA, designed to target each different region of the gene. This gabrb1 gene silencing effect was rescued by co-transfection of silent mutation-containing gabrb1 gene, which was designed not to be targeted by the siRNA. Moreover, the results of the experiment with gabrb1-specific inhibitor salicylidene salicylhydrazide were coincident with those of gabrb1 gene silencing experiments. These results indicate that gabrb1 gene silencing effects are not artifacts such as off-targeting. We confirmed the gabrb1 gene silencing effects in the mRNA level but not in the protein level. We attempted unsuccessfully to detect gabrb1 in protein level using several gabrb1-specific antibodies ob-

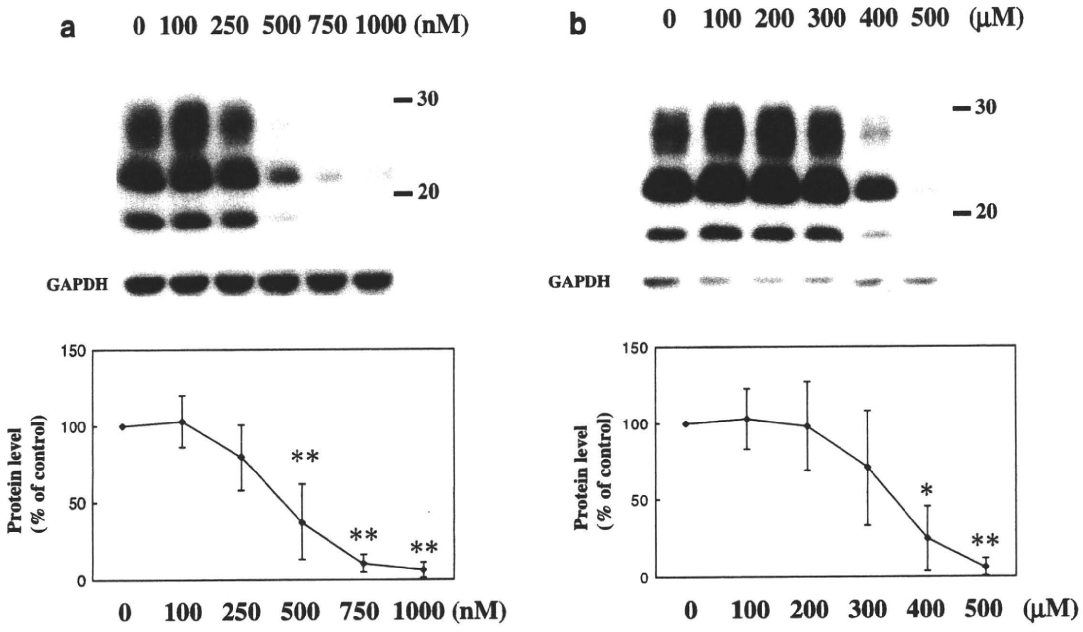

Fig. 3. Effects of gabrb1-specific inhibitor and GABAA receptor antagonist. Immunoblot and protein level of PrPres from ScN2a cells treated with gabrb1-specific inhibitor salicylidene salicylhydrazide (a) or GABAA receptor antagonist bicuculline methiodide (b) are shown. Molecular size markers in the right side of the immunoblots are shown in kilodaltons. Immunoblot data shown here are representative examples; the graphic data shown here are the average and standard deviation from results of independent triplicate experiments (*$P < 0.05$, **$P < 0.01$).

tained from different sources. Failure was mainly attributed to the very low expression level of gabrb1 protein in the cells and to technical difficulties in distinguishing gabrb1 signals from immunoglobulin heavy chain signals on the blot. Successful detection of endogenous gabrb1 protein in immunoblotting from cultured cells has not been reported.

Gene silencing of other GABAA receptor subunits tested in this study did not affect the PrPres formation in ScN2a cells, suggesting that gabrb1 involvement in the PrPres formation is in a manner irrespective of GABAA receptor. Results from treatments of ScN2a cells with an antagonist picrotoxin and agonists such as GABA, muscimol, pentobarbital, ethanol, and isoguvacine hydrochloride were consistent with this hypothesis. No new function of gabrb1 unrelated to GABAA receptors, except gabrb1 homomeric chloride ion channels, has been reported in the relevant literature. These homomeric channels, expressed in Xenopus oocytes or A293 cells, are sensitive to picrotoxin [22,23], but picrotoxin did not affect the PrPres formation in ScN2a cells. Consequently, gabrb1 function in ScN2a cells might differ from the homomeric channels, which remains to be evaluated.

The gabrb1 gene silencing increased the PrP mRNA level and PrPc protein level. It remains unclear how this happened and whether this resulted from upregulation of PrP gene transcription or from increased stability of PrP mRNA. However, it is noteworthy that the formation of PrPres was reduced despite the increased PrPc expression level. Furthermore, it is noteworthy that treatment with gabrb1 inhibitor, salicylidene salicylhydrazide, decreased the PrPres level but did not modify the PrP expression in either the mRNA level or protein level. The discrepancy between the results of gabrb1 gene silencing and those of gabrb1 inhibitor might reflect the difference in the mRNA/protein expression level of gabrb1: a decreased gabrb1 expression level in the gene silencing versus an unmodified gabrb1 expression level but functional inhibition in the inhibitor. However, further study is necessary to elucidate this speculation.

Compared to ScN2a cells (RML prion-infected N2a cells), N167 cells (22L prion-infected N2a cells) showed less remarkable reduction of PrPres formation in gabrb1 gene silencing. This gap of gabrb1 involvement between ScN2a cells and N167 cells might reflect prion strain difference. Gabrb1 might be more influential in the PrPres formation of RML prion strain, than 22L prion strain. However, we could not exclude possibilities that other factors than prion strain might be responsible for the gap observed between the two cells.

Involvement of GABAergic system in the pathogenesis of prion diseases has been reported [9–15]. The GABAergic neurons are degenerated in an early stage of the disease [13,14]. On the other hand, Trifilo and colleagues [17] report upregulation of GABAA receptor subunits in the prion-inoculated mouse brains expressing anchorless PrP. According to their speculation, inhibitory synaptic transmission is over-stimulated while PrPres formation is promoted; then the inhibitory synaptic transmission system collapses from overwork. Taken together with the findings in this study, suppression of gabrb1 function by inhibitors or other means might be useful for both calming overwhelmed GABAergic systems and inhibiting PrPres formation when conducted at an appropriate stage of the disease.

In conclusion, we identified gabrb1 as a new host factor involved in the PrPres formation in ScN2a cells. Although gabrb1 is a subunit of GABAA receptors, our results suggest that gabrb1 acts on the PrPres formation in a GABAA receptor-independent manner. Because previous literature has not revealed any association of gabrb1 with PrPc [24–30], gabrb1 might function through other cellular factors or directly interact with PrPres as observed in the prion-inoculated mouse brains expressing anchorless PrP [17], where direct association of GABAA receptors with PrPres was demonstrated. The mechanism of gabrb1 involvement in the PrPres formation remains to be elucidated.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.02.029.

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ABA- and BAB-triblock cooligomers of tri-*O*-methylated and unmodified cello-oligosaccharides: syntheses and structure-solubility relationship

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Abstract Triblock cooligomers consisting of tri-*O*-methyl-glucopyranosyl and unmodified glucopyranosyl residues, methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranoside (**1**: ABA triblock cooligomer; DS = 2.1) and β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (**2**: BAB triblock cooligomer; DS = 1.8) were prepared. Compound **1** dissolved both in distilled water and chloroform but compound **2** dissolved in distilled water not in chloroform, though compounds **1** and **2** consist of 4 tri-*O*-methyl-glucopyranosyl and 2 unmodified anhydro glucopyranosyl units.

Keywords Triblock cooligomer · Cello-oligosaccharides · Methylcellulose · Solubility

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Introduction

Water-soluble methylcellulose (MC) with degree of substitution (DS) ~ 1.8 is important industrial product since 1905 (Klemm et al. 2005). Its aqueous solution is put to various uses such as additive for building technologies and for pharmaceutics (Hartmann et al. 2007). The aqueous solution of MC shows thermoreversible gelation behavior. There have been many attempts to understand the physical basis of the peculiar behavior but with unsatisfactory success (Nishinari and Takahashi 2003). One possibility of the explanation is believed in amphiphilic segments of partially methylated cellulose chain which may associate via self-assembly (Savage 1957). Therefore, we have developed new approach to unravel structure-property relationships of methylcellulose (MC; Kamitakahara et al. 2006; 2007). A polydisperse mixture of block co-oligomers of tri-*O*-methylated and unmodified cello-oligosaccharides (Kamitakahara et al. 2006), monodisperse diblock co-oligomers (Kamitakahara et al. 2007), methyl β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (pentamer), methyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (hexamer), and methyl

β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (trimer) were synthesized. Furthermore, the polydisperse mixture of the block-like methylated cello-oligosaccharides self-assembled to form ellipsoidal particles with dimensions of about 50 nm for the semi-major axis and of circa 25 nm for the semi-minor axis in aqueous media (Kamitakahara et al. 2008).

Triblock co-oligomers of tri-*O*-methyl-glucopyranosyl and unmodified glucopyranosyl residues remain important synthetic targets, promising further information on structure-property relationship of MC. Thus, triblock cello-oligomers containing an unmodified block as the center part and methylated ones at the two ends (ABA triblock co-oligomer), and also to the inverted composition (BAB triblock co-oligomer) were for the first time synthesized. In this paper, we describe their synthetic methods and preliminary data on their difference of their solubilities.

Results and discussion

Synthesis of ABA triblock cooligomer: methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranoside (**1**)

The ABA triblock cooligomer **1** was synthesized according to the synthetic route as shown in Fig. 1. The compound **1** was prepared from three cellobiose building blocks **3**, **4**, and **8**. Namely, two methylated and one benzylated cellobiose building blocks were used for the ABA triblock cell-hexaose derivative **1**. Glycosylation experiments revealed that suitable combination between glycosyl acceptors and donors exists. After many trials, benzylated glycosyl trichloroacetoimidate **3** gave good result for glycosylation with methylated cellobiose acceptor **4**. 4-*O*-Acetyl-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,2,2-trichloro acetoimidate (**3**) was glycosylated with phenyl 2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**4**) to give a tetramer derivative, phenyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**5**) in 84% yield. The tetramer **5** is a precursor of an amphiphilic diblock cello-tetramer derivative. Details of preparation method for diblock cello-tetramer will be reported elsewhere. Phenylthio glycoside **5** was converted to methyl glycoside **6** with *N*-iodosuccinimide (NIS) and catalytic amount of AgOTf in methanol / dichloromethane. Acetyl group at C4 position of the non-reducing end of compound **6** was removed to give a cello-tetraosyl acceptor, methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (**7**).

On the other hand, methylated phenylthio glycoside was found to be the best glycosyl donor for benzylated glycosyl acceptor. Methylated cellobiosyl trichloroacetoimidate derivative had poor reactivity with benzylated glycosyl acceptor. Thus, phenyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**8**) was chosen and glycosylated with benzylated cello-tetraosyl acceptor **7** to give ABA triblock cello-hexamer derivative, methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranoside (**9**) in 25% yield with 22% α-glycoside formation. Removal of six benzyl groups in B block, two internal anhydro glucose units, generated a target compound, methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranoside (**1**) (DS = 2.1) in a quantitative yield.

The compound **1** was soluble in chloroform, methanol, and water. Figures 2a and 3a show ^{1}H- and ^{13}C-NMR spectra measured in D_2O of compound **1**, respectively. Anomeric proton of α configuration at the reducing-end appeared at 4.99 ppm with coupling constant $J = 3.0$ Hz. The C1α carbon appeared at 99.1 ppm. The C1β may overlap in the C1 region at ~ 105 ppm. The methyl carbon of the methoxyl group at the C1 position appeared in lower magnetic field at 57.6 ppm, compared to other methyl

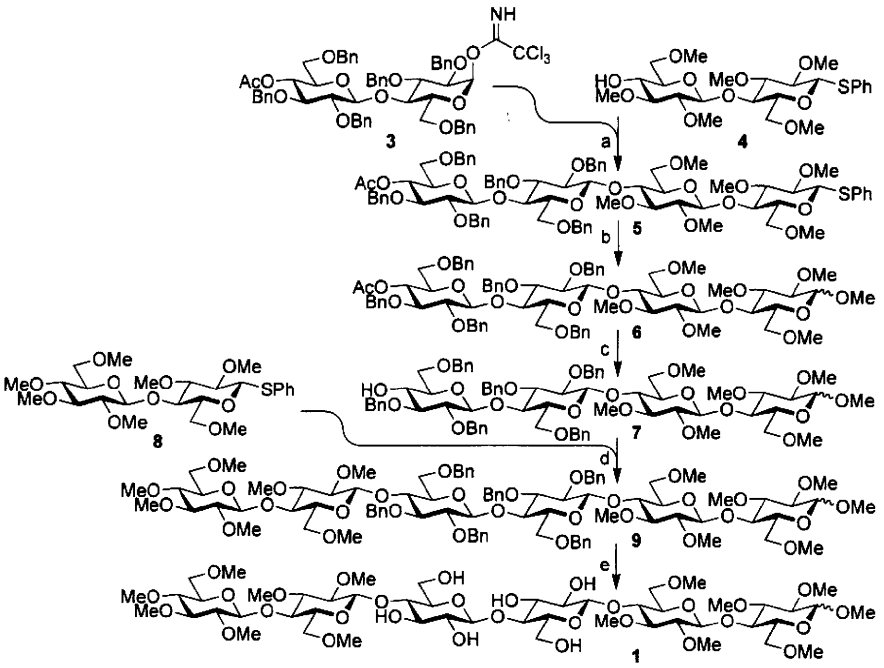

Fig. 1 Synthetic route for ABA triblock cello-hexamer **1**. **a** BF_3Et_2O/anhydrous CH_2Cl_2/−78 °C for 44 h → (3 h) −60 °C/84%; **b** NIS/AgOTf/MeOH/CH_2Cl_2/1 h; **c** 28% $NaOCH_3$ in MeOH/THF/MeOH/r.t./12 h/quantitative from compound **5**; **d** NIS/AgOTf/C_2H_5CN/MS 3 Å/r.t./24 h/25%; **e** 20% $Pd(OH)_2$ on carbon/H_2/EtOH/r.t./12 h/quantitative

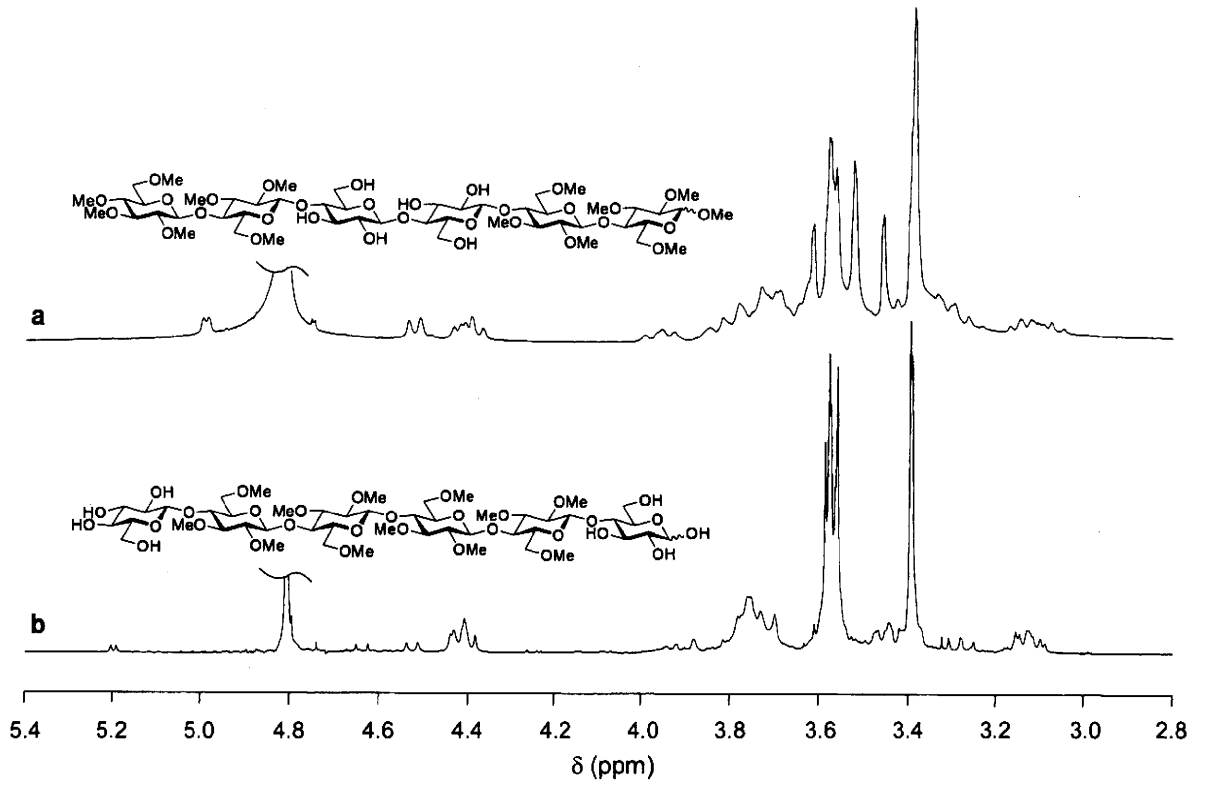

Fig. 2 ^{1}H-NMR spectra of ABA and BAB triblock cello-hexamers **1** (**a**) and **2** (**b**) measured in D_2O (3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as external standard)

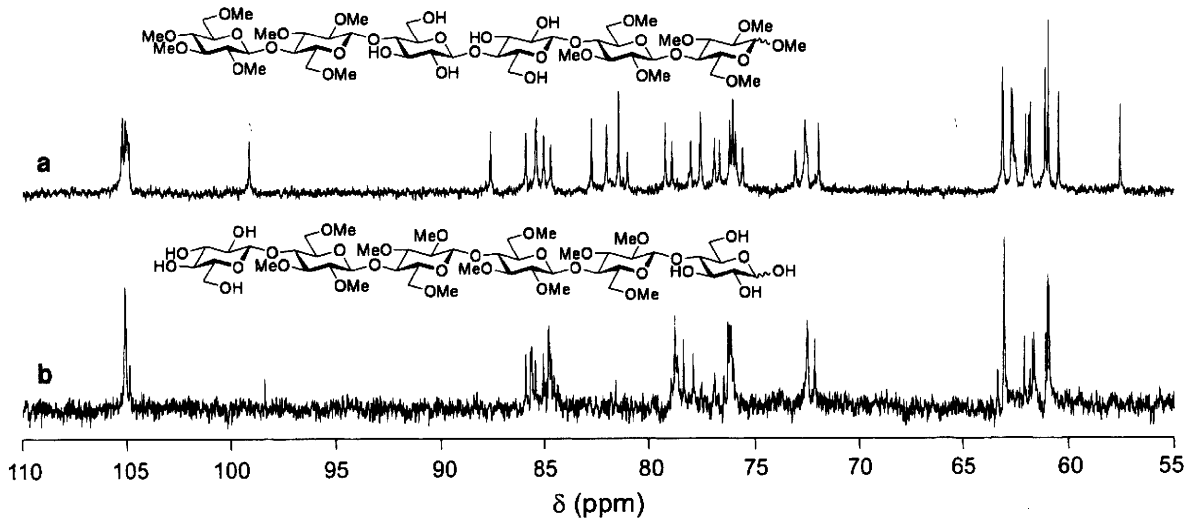

Fig. 3 ^{13}C-NMR spectra of ABA and BAB triblock cello-hexamers **1** (**a**) and **2** (**b**) measured in D_2O (3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as external standard)

carbons. These data indicate that compound **1** having α anomeric configuration is predominant.

Synthesis of BAB triblock cooligomer: β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (**2**)

The BAB triblock cooligomer **2** was synthesized from two unmodified glucose and two tri-*O*-methylated cellobiose building blocks as shown in Fig. 4. The compound **2** has hemiacetalic hydroxyl group at the reducing-end. First, phenyl 3-*O*-benzyl-2,6-di-*O*-pivaloyl-1-thio-β-D-glucopyranoside was used as a building block at the reducing-end instead of benzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**12**). A "peeling off" reaction, however, occurred during deprotection procedure even under relatively mild basic condition with sodium methoxide at 50 °C. Therefore, the removal of benzyl groups was chosen as the last reaction to give compound **2**. Namely, compound **12** was selected as a glucosyl residue at the reducing-end. The compound **12** was converted from glucose in three reaction steps. Glucose was 4,6-*O*-benzylidenated to give 4,6-*O*-benzylidene-D-glucopyranose (**10**) (Kami takahara et al. 1994). The compound **10** was benzylated to give benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**11**). The 4,6-benzylidene group of compound **11** was reductively cleaved by use of trimethylsilyl chloride and sodium cyanoborohydride to give benzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**12**).

Best glycosylation method between benzylated glycosyl acceptor and methylated donor was thio-glycoside method as mentioned before. Therefore, phenyl 4-*O*-acetyl-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**13**) (Kamitakahara et al. 2006) was glycosylated with compound **12** to give benzyl 4-*O*-acetyl-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**14**) in 30% yield. Acetyl group of compound **14** was removed under basic condition to give cello-trimeric glycosyl acceptor, benzyl 2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**15**).

On the other hand, glycosylation of 4-*O*-acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-D-glucopyranosyl fluoride with phenyl 2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside gave a cello-trimer derivative, phenyl 4-*O*-acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**16**) as reported previously (Kamitakahara et al. 2007).

Best glycosylation method between methylated glycosyl acceptor and methylated donor was the

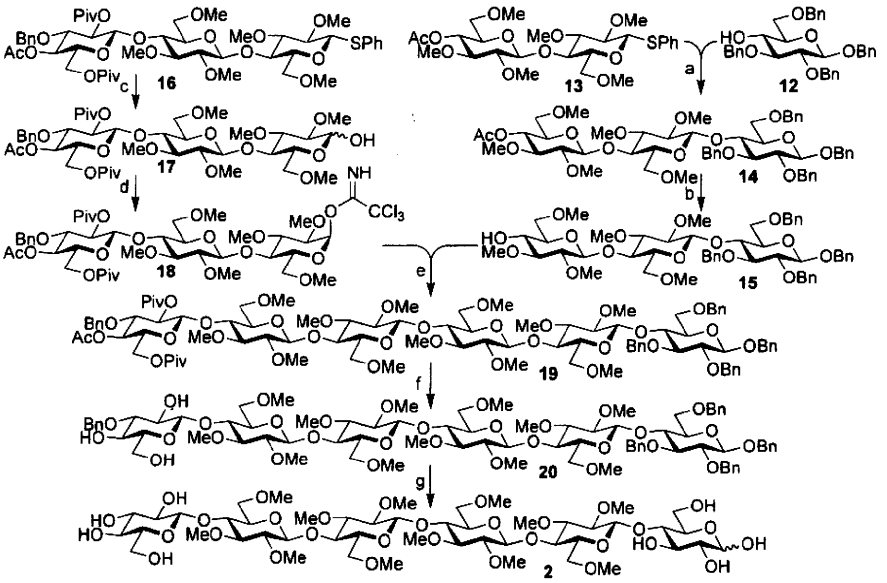

Fig. 4 Synthetic route for BAB triblock cello-hexamer **2**. **a** NIS/AgOTf/ C_2H_5CN/MS 3 Å/ 0 °C → r.t./20 h/30%; **b** 28% $NaOCH_3$ in MeOH/ MeOH/r.t. for 14.5 h; 50 °C for 21.5 h/89% **c** NBS/ AgOTf/acetone/distilled water/r.t./27 h; **d** DBU/ CCl_3CN/anhydrous CH_2Cl_2/r.t./98%; **e** BF_3Et_2O/anhydrous CH_2Cl_2/−30 °C/ 62.5 h/38%; **f** 28% $NaOCH_3$ in MeOH/r.t. for 3.5 h; 50 °C for 12 h; reflux temperature for 40 h/73%; **g** 10% Pd on carbon/20% $Pd(OH)_2$ on carbon/H_2/ EtOH/r.t./64.5 h/67%

glycosyl imidate method among other trials. Phenylthio group of compound **16** was removed to afford 4-*O*-acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranose (**17**). And then, hemiacetalic hydroxyl group of compound **17** attacked to trichloroacetonitrile under basic condition of DBU to give 4-*O*-acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranosyl trichloroacetoimidate (**18**) in 98% yield.

Cello-trimeric glycosyl acceptor **15** was glycosylated with cello-trimeric glycosyl donor **18** to give BAB triblock cello-hexamer derivative, benzyl 4-*O*-acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**19**) in 38% yield without production of α-glycoside. Removal of ester groups of compound **19** gave benzyl 3-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**20**) only having benzyl protective groups in 73% yield. After debenzylation of compound **20**, a target compound, β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (**2**) was obtained in 67% yield.

Figures 2b and 3b show ^{1}H- and ^{13}C-NMR spectra of compound **2**, respectively. Anomeric protons of α and β configurations appeared at 5.19 ppm (J = 3.9 Hz) and at 4.65 ppm (J = 8.4 Hz), respectively. The C1α and C1β carbons appeared at 94.5 ppm and at 98.4 ppm, respectively. Compared to the peaks of the methyl protons of compound **1**, those of compound **2** appeared as simple peaks.

The BAB triblock cello-hexamer **2** (DS = 1.80) having hydrophilic glucosyl residues at two ends did not dissolve in chloroform, though it was soluble in water and methanol. Only the compound **2** does not dissolve in chloroform, although monodisperse AB diblock cello-trimer (DS = 1.91), pentamer (DS = 2.29), hexamer (DS = 1.95), and polydisperse mixture of diblock cello-oligomers (DS = ca. 2.21) were soluble in chloroform, methanol, and water (Kamitakahara et al. 2006; 2007). Furthermore, an AB diblock tetramer, methyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (DS = 1.50) is soluble in chloroform, methanol, and water (unpublished data), although DS value of the AB diblock tetramer (DS = 1.50) is lower

than that of compound **2** (DS = 1.80). Thus, a sequence of tri-*O*-methyl-glucosyl residues along cellulose chain, a blocky structure, is more important compared to overall average DS value of cello-oligosaccharide derivatives. Such peculiar solubility of compound **2** (DS = 1.80) is of importance to unravel structure-property relationship of industrial MC.

Other properties of the ABA and BAB triblock cello-hexamers such as thermo-responsive property and aggregation property in aqueous media will be discussed elsewhere.

Experimental section

General. All melting points (m.p.) are uncorrected. Optical rotation values were obtained with a JASCO DIP-1000 digital polarimeter. ^{1}H-NMR spectra and ^{13}C-NMR spectra were recorded with a Varian INO-VA300 FT-NMR (300 MHz) spectrometer or with a Bruker AVANCE400 (400 MHz), in chloroform-*d* or methanol-d_4 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 (δ) and coupling constants (J) are given in δ-values (ppm) and Hz, respectively. Matrix assisted laser desorption/ionization time-of-flight mass (MALDI-TOF MS) spectra were recorded with a Bruker MALDI-TOF MS REFLEX III. For ionization, a nitrogen laser was used. All spectra were measured in the reflector mode using external calibration. Compounds were measured with 2,5-dihydroxybenzoic acid (DHB) as a matrix. Anhydrous dichloromethane was distilled from P_2O_5. Preparative thin layer chromatography (PTLC) was performed on silica-gel plates (Kieselgel 60 F_{254}, Merck). The standard work-up procedure included diluting with an ethyl acetate, washing with aq. $NaHCO_3$, and a brine, drying over Na_2SO_4, and evaporating *in vacuo*.

4-*O*-Acetyl-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,2,2-trichloroacetoimidate (**3**)

Compound **3** was prepared according to our previous report (Kamitakahara et al. 2007).

Phenyl 2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**4**)

Compound **4** was prepared according to our previous report (Kamitakahara et al. 2006).

Phenyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**5**)

Compounds **3** (84 mg, 0.0787 mmol) and **4** (34 mg, 0.0656 mmol) were dried in a reaction ampule using high-vacuum system overnight. Dichloromethane was distilled from CaH_2, and degassed by freezing and thawing a few times. The solvent was transferred under high vacuum. The reaction ampule was separated by melting off and placed at −78 °C. Boron trifluoride diethylether (2.5 μL, 0.0197 mmol: 25 mol% to compound **3**) was added into the reaction ampule through a rubber septum by a syringe. The reaction mixture was stirred at −78 °C for 44 h and reaction temperature was gradually raised up to −60 °C for 3 h. The reaction mixture was treated with the standard work-up procedure. The product was isolated on preparative TLC (eluent: ethyl acetate:*n*-hexane = 1:2, v/v) to give compound **5** (78.6 mg, 84% yield): ^{1}H-NMR ($CDCl_3$): δ 1.81 ($COCH_3$), 2.95 (t, 1H, J = 9.0, H2′), 3.09 (t, 1H, J = 8.7, H2), 3.18 (t, 1H, J = 9.3, H3′), 3.28, 3.40, 3.55, 3.56, 3.59, 3.60 (OCH_3), 4.04 (t, 1H, J = 9.3, H4″), 4.23 (d, 1H, J = 11.7, CH_2Ph), 4.31 (d, 1H, J = 7.8, H1′), 4.37 (d, 1H, J = 12.0, CH_2Ph), 4.43 (d, 1H, J = 7.5, H1″), 4.51 (d, 1H, J = 10.2, H1), 4.56 (d, 1H, J = 7.2, H1‴), 4.1–4.9 (CH_2Ph), 4.96 (t, 1H, J = 8.7, H4‴), 5.02 (d, 1H, J = 10.8, CH_2Ph), 7.2–7.6 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 20.8 ($COCH_3$), 59.0, 59.2, 60.7, 60.7, 60.8, 67.7, 69.9, 70.4, 71.5 (C4‴), 73.3, 73.5, 74.7, 75.1, 75.2, 76.4 (C4″), 77.2 (C4′), 77.9 (C4), 78.9, 82.0, 82.5, 83.2, 83.4 (C2′), 85.0 (C3′), 86.7 (C3), 87.2 (C1), 102.2 (C1‴), 102.8 (C1″), 103.4 (C1′), 127.3,127.4, 127.5, 127.5, 127.7. 127.7, 127.9, 127.9, 128.0, 128.2, 128.2, 128.3, 128.8, 131.7, 133.8, 136.5, 138.1, 138.3, 138.3, 139.0, 169.9 ($COCH_3$); MALDI-TOF MS: calculated for $C_{80}H_{95}O_{21}S$ = 1,424.62; found m/z $[M + Na]^+$ = 1,447.84.

Methyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (**6**)

To a solution of compound **5** (78.6 mg. 0.0558 mmol) in methanol/dichloromethane (5 mL, 1/4, v/v), *N*-iodosuccinimide (NIS; 17.6 mg, 0.0781 mmol) and catalytic amount of silver trifluoromethanesulfonate (AgOTf) was added at room temperature. The reaction mixture was kept stirring for 5 h. NIS (5.4 mg) was added to the reaction mixture. The reaction mixture was stirred for 1 h. Solid $NaHCO_3$ was added to the reaction mixture. The reaction mixture was concentrated, extracted with ethyl acetate, washed with aq. $NaHCO_3$, distilled water, and brine, dried over Na_2SO_4, and concentrated to dryness to give crude compound **6** (82.7 mg). The crude compound was used for the next reaction without further purification: ^{1}H-NMR ($CDCl_3$): δ 1.84 ($COCH_3$), 2.98 (t, 1H, J = 9.0, H2′), 3.19 (t, 1H, J = 9.0, H3′), 3.29, 3.42, 3.51, 3.57, 3.58 (OCH_3), 3.88 (dd, 1H, H6″), 4.05 (t, 1H, J = 9.5, H4″), 4.23 (d, 1H, J = 4.2, CH_2Ph), 4.30 (d, 1H, J = 7.9, H1′), 4.38 (d, 1H, J = 11.8, CH_2Ph), 4.48 (d, 1H, J = 7.8, H1″), 4.51 (d, 1H, J = 12.1, CH_2Ph), 4.56 (d, 1H, J = 7.7, H1‴), 4.61 (d, 1H, J = 11,4, CH_2Ph), 4.66 (d, 1H, J = 12.0, CH_2Ph), 4.71 (d, 1H, J = 10.9, CH_2Ph), 4.72 (d, 1H, J = 10.9, CH_2Ph), 4.75–4.81 (4H, CH_2Ph), 4.84 (d, 1H, J = 3.6, H1α), 4.96 (t, 1H, J = 9.4, H4‴), 5.02 (d, 1H, J = 10.9, CH_2Ph), 7.1–7.5 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 20.8, 55.2, 56.9, 58.9, 59.0, 59.1, 60.3, 60.3, 60.5, 60.6, 67.7, 69.7, 69.9, 70.0, 70.2, 71.4, 73.2, 73.4, 74.6, 74.7, 75.0, 75.0, 75.1, 75.2, 75.3, 76.3, 77.8, 78.1, 81.1, 81.4, 82.0, 82.4, 83.0, 83.2, 83.3, 84.5, 85.1, 97.4 (C1α), 102.1, 102,8, 103.4, 104.1, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.7, 127.8, 127.9, 127.9, 128.0, 128.1, 128.2, 128.3, 138.1, 138.2, 138.3, 138.3, 138.9, 169.8; MALDI-TOF MS: calculated for $C_{75}H_{94}O_{22}$ = 1,346.62; found m/z $[M + Na]^+$ = 1,346.62.

Methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (**7**)

To a solution of crude compound **6** (82.7 mg) in tetrahydrofuran/methanol (5 mL, 4:1, v/v), 28% sodium methoxide in methanol (3.5 μL, 1 equiv.) was added at room temperature. After 1 h, 28% sodium methoxide in methanol (20 μL) was added at room temperature. The reaction mixture was kept stirring for 12 h, and then extracted with ethyl acetate, washed with distilled water and brine, dried over Na_2SO_4, and concentrated to dryness to give compound **7** (71.9 mg, 99.9% yield from compound **5**): $[\alpha]_D^{23.5}$ = +54.2 (c 0.055, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 2.98 (t, 1H, J = 8.9, H2′), 3.19 (t, 1H, J = 9.0, H3′), 3,29, 3.42, 3.51, 3.57, 3.58 (OCH_3), 4.04 (t, 1H, J = 9.4, H4″), 3.87 (dd, 1H, J = 3.2, J = 11.4, H6″), 4.30 (d, 1H, J = 7.9, H1′), 4.37 (d, 1H, J = 12.0, CH_2Ph), 4.43 (d, 1H, J = 12.1, CH_2Ph), 4.47 (d, 1H, J = 7.7, H1″), 4.50 (d, 1H, J = 12.0, CH_2Ph), 4.55 (d, 1H, J = 7.6, H1‴), 4.64 (d, 1H, J = 12.1, CH_2Ph), 4.72 (d, 1H, J = 11.0, CH_2Ph), 4.74 (d, 1H, J = 11.4, CH_2Ph), 4.74 (d, 1H, J = 11.5, CH_2Ph), 4.77 (d, 1H, J = 12.3, CH_2Ph), 4.80 (d, 1H, J = 11.2, CH_2Ph), 4.81 (d, 1H, J = 11.8, CH_2Ph), 4.85 (d, 1H, J = 4.1, H1α), 4.87 (d, 1H, J = 11.3, CH_2Ph), 5.10 (d, 1H, J = 11.0, CH_2Ph), 7.18–7.35 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 55.1, 56.8, 58.9, 59.0, 60.4, 60.6, 60.6, 60.6, 60.7, 67.7, 69.7, 70.0, 70.2, 73.1, 73.3, 73.5, 74.8, 74.9, 75.0, 75.1, 75.2, 76,2, 77.2, 77.9, 78.2, 81.1, 81.5, 82.0, 82.0, 83.1, 83.2, 83.3, 84.3, 84.6, 85.1, 97.4 (C1α), 102.3, 102.7, 103.3, 103.4, 104.1, 127.1, 127.4, 127.5, 127.6, 127.6, 127.6, 127.7, 127.8, 127.8, 127.9, 128.1, 128.2, 128.3, 128.3, 128.3, 137.8, 138.3, 138.4, 138.6, 139.1; MALDI-TOF MS: calculated for $C_{73}H_{92}O_{21}$ = 1,304.61; found m/z $[M + Na]^+$ = 1,327.67.

Phenyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**8**)

To a solution of phenyl β-D-glucopyranosyl-(1 → 4)-1-thio-β-D-glucopyranoside (500 mg (1.15 mmol) in *N*,*N*-dimethylformamide (DMF; 5 mL), sodium hydride (367 mg, 60% in mineral oil, 8.0 equiv) and methyl iodide (0.55 mL, 7.7 equiv) were added at room temperature. The reaction mixture was kept stirring for 22.5 h at room temperature. After addition of methanol to inactivate sodium hydride, the reaction mixture was treated with the standard work-up procedure to give crude crystals **8** (619.5 mg). The

crude crystals **8** were recrystalized from *n*-hexane to give pure crystals **8** (309.1 mg, 51% yield): m.p. = 72.4–74.5 °C; $[\alpha]_D^{24.6} = -22.6$ (*c* 1.04, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 2.97 (t, 1H, $J = 8.6$, H2′), 3.14 (dd, 1H, $J = 8.6$, $J = 9.8$, H2), 3.18 (t, 1H, $J = 9.0$, H3′), 3.34 (t, 1H, $J = 8.7$, H3), 3,42, 3,45, 3.58, 3.59, 3.64, 3.66, 3.67 (OCH_3), 4.35 (d, 1H, $J = 7.8$, H1′), 4.56 (d, 1H, $J = 9.9$, H1), 7.2–7.7 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 59.1, 59.2, 60.3, 60.5, 60.6, 60.6, 60.7, 70.5, 71.1, 74.6, 77.7, 78.9, 79.2, 81.8, 84.0, 86.6, 86.8, 87.2, 103.3 (C1′), 127.2, 128.7, 131.7, 133.8; MALDI-TOF MS: calculated for $C_{25}H_{40}O_{10}S$ = 532.23; found *m/z* $[M + Na]^+ = 555.29$, $[M + K]^+ = 571.28$.

Methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranoside (**9**)

To a solution of compound **8** (21.5 mg, 0.0404 mmol, 2 equiv) and **9** (26.4 mg, 0.0202 mmol) with molecular sieves 3 Å (100 mg) in anhydrous propionitrile (1 mL), *N*-iodosuccinimide (NIS; 12.7 mg, 0.0566 mmol, 2.8 equiv) and catalytic amount of AgOTf was added at room temperature. The reaction mixture was kept stirring for ~24 h. Solid $NaHCO_3$ was added to the reaction mixture. The reaction mixture was filtered off and washed with ethyl acetate. The combined filtrate and washings were concentrated, extracted with ethyl acetate, washed with distilled water, and brine, dried over Na_2SO_4, and concentrated to dryness. Crude compound was purified on preparative TLC (eluent: ethyl acetate: *n*-hexane = 1:4, v/v) to give a target compound **9** (8.7 mg, 25% yield) with an α-glycoside (7.6 mg, 22% yield): $[\alpha]_D^{23.5} = +61.5$ (*c* 0.063, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 2.88 (t, 1H, $J = 8.4$, H2′′′′′), 2.94 (t, 1H, $J = 8.4$ H2′′′′), 2.9–3.0 (m, H5′′′′), 2.97 (t, 1H, $J = 8.7$, H2′), 3.04 (t, 1H, $J = 9.0$, H3′′′′), 3.20–3.26 (dd, 1H, $J = 3.9$, H2), 3.19, 3.28, 3.39, 3.42, 3.42, 3.46, 3.47, 3.51, 3.52, 3.54, 3.56, 3.58, 3.61 (OCH_3), 3.87 (dd, $J = 2.6$, $J = 11.4$, H6′′), 3.99 (t, 1H, $J = 9.6$, H4′′′), 4.06 (t, 1H, $J = 9.3$, H4′′), 4.23 (d, 1H, $J = 7.8$, H1′′′′′), 4.29 (d, 1H, $J = 7.5$, H1′), 4.35 (d, 1H, $J = 7.5$, H1′′′′), 4.43 (d, 1H, $J = 12.6$, CH_2Ph), 4.43 (d, 1H, $J = 7.8$, H1′′), 4.47 (d, 1H, $J = 12.3$, CH_2Ph), 4.53 (d, 1H, $J = 7.5$, H1′′′), 4.62 (d, 1H, $J = 12.6$, CH_2Ph), 4.64–4.76 (6H, CH_2Ph), 4.83 (d, 1H, $J = 3.6$, H1), 5.11 (d, 1H, $J = 11.1$, CH_2Ph), 5.13 (d, 1H, $J = 11.1$, CH_2Ph), 7.1–7.4 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 55.2, 58.7, 59.0, 59.1, 59.4, 60.0, 60.3, 60.5, 60.7, 60.8 (OCH_3), 67.9 (C6′′ or C6′′′), 68.2 (C6′′ or C6′′′), 69.8 (CH (C5) and CH_2 are overlapped), 70.0, 70.2, 71.1, 72.7 (CH_2Ph), 73.1 (CH_2Ph), 74.6, 74.7, 74.8, 75.1 (CH_2Ph), 75.2 (CH_2Ph, overlapped), 76.4, 76.6 (overlapped), 77.0 (overlapped), 77.2, 78.4 (C4), 79.2 (C4′′′′′), 81.2 (C2), 81.5, 82.0, 82.1, 83.3, 83.3, 83.5, 83.6, 84.0, 84.6 (C3′′′′), 85.2 (C3′), 86.8 (C3′′′′′), 97.5 (C1), 102.3, 102.6, 103.1, 103.5, 127.0, 127.3, 127.4, 127.6, 127.7, 127.8, 128.0, 128.2, 128.2, 128.5, 138.5, 138.5, 138.6, 139.3, 139.3 (aromatic C); MALDI-TOF MS: calculated for $C_{92}H_{126}O_{31}$ = 1,726.83; found *m/z* $[M + Na]^+ = 1{,}749.73$.

Methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranoside (**1**)

To a solution of compound **9** (8.7 mg, 0.00503 mmol) in ethanol (1 mL), 20% $Pd(OH)_2$ on carbon (24.3 mg) was added. The reaction mixture was stirred under hydrogen atmosphere at room temperature for 12 h. The reaction mixture was filtered off and washed with methanol. The combined filtrate and washings were concentrated to dryness to give compound **1** (6 mg, quantitative): $[\alpha]_D^{27.0}$=+45.5 (*c* 0.21, H_2O); ^{1}H-NMR ($CDCl_3$): δ 2.92 (t, 1H, $J = 8.4$, H2′′′′′), 2.99 (t, 1H, $J = 9.0$, H2), 3.03 (t, 1H, $J = 8.4$, H2′′′′), 3.12 (t, 1H, $J = 8.7$, H3′′′′′), 3.18 (t, 1H, $J = 7.8$, H4′′′′′), 3.38, 3.40, 3.42, 3.46, 3.51, 3.53, 3.54, 3.56, 3.57, 3.59, 3.62, 3.63 (OCH_3), 4.26 (d, 1H, $J = 7.8$, H1′′′′′), 4.28 (d, 1H, $J = 7.5$, H1′), 4.32 (d, 1H, $J = 7.8$, H1′′′′), 4.57 (d, 1H, $J = 7.5$, H1′′), 4.61 (d, 1H, $J = 7.8$, H1′′′), 4.83 (d, 1H, $J = 3.6$, H1α); ^{13}C-NMR ($CDCl_3$): δ 55.3, 58.9, 59.0, 59.1, 59.3, 60.1, 60.4, 60.5, 60.6, 60.8 (C6′′ or C6′′′) (OCH_3 overlapped), 60.9, 61.4 (C6′′ or C6′′′), 69.7 (C5), 70.0 (C6 or C6′ or C6′′ or C6′′′), 70.7 (C6 or C6′ or C6′′ or C6′′′), 70.9 (C6 or C6′ or C6′′ or C6′′′), 71.1 (C6 or C6′ or C6′′ or C6′′′), 71.9 (C2′′), 72.9 (C2′′′), 74.3, 74.5, 74.7, 74.9, 75.0, 75.2, 77.7 (C4), 78.0, 79.2 (C4′′′′′), 80.7, 81.1 (C2), 81.4 (C3),

82.8 (C2''''), 84.0 (C2'''''), 84.2 (C2'), 84.7 (C3''''), 85.5 (C3'), 86.9 (C3'''''), 97.5 (C1α), 102.0 (C1''), 102.7 (C1'''), 103.3, 103.4; ^{1}H-NMR (D_2O): δ 3.08 (t, 1H, J = 8.1), 3.15 (t, 1H, J = 7.5), 3.0–3.2 (1H), 3.39, 3.46, 3.52, 3.56, 3.58, 3.58, 3.61 (OCH_3), 3.9–4.0 (H6'', H6'''), 4.38 (d, 1H, J = 7.8), 4.40 (d, 1H, J = 7.2), 4.42 (d, 1H, J = 7.5), 4.52 (d, 2H, J = 8.1), 4.99 (d, 1H, J = 3.0, H1α); ^{13}C-NMR (D_2O): δ 57.6 (C1-OCH_3), 60.5, 61.0, 61.1, 61.8, 61.9, 62.0, 62.5 (C6'' or C6'''), 62.6 (C6'' or C6''') (OCH_3, overlapped), 62.7, 63.1 (OCH_3), 71.9 (C5), 72.5 (C6, C6', C6'''', C6'''''), 72.6 (C6, C6', C6'''', C6''''') (OCH_3 overlapped), 73.1 (C6, C6', C6'''', C6'''''), 75.6, 75.9, 76.0, 76.2, 76.7, 76.9, 77.6, 78.0 (C4), 78.9, 79.2, 81.0, 81.5, 82.0 (C2), 82.8 (C3), 84.7, 85.1, 85.4, 85.9, 87.6, 99.1 (C1), 104.9, 105.0, 105.0, 105.1, 105.2 (C1', C1'', C1''', C1'''', C1'''''); MALDI-TOF MS: calculated for $C_{50}H_{90}O_{31}$ = 1,186.55; found m/z $[M + Na]^+$ = 1,209.73, $[M + K]^+$ = 1,225.55.

4,6-*O*-Benzylidene-D-glucopyranose (**10**)

The compound **10** was prepared according to our previous paper (Kamitakahara and Nakatsubo 1996).

Benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**11**)

To a solution of compound **10** (3.00 g, 0.0112 mmol) in THF (20 mL), sodium hydride (1.34 g, 60% in mineral oil, 0.0336 mmol), tetrabutylammonium iodide (0.62 g, 0.15 equiv.), and benzyl bromide (3.00 mL, 0.0336 mmol) were added at 0 °C. The reaction temperature was raised gradually up to room temperature. Additional THF (10 mL) was added after 16 h. After 4.5 h, the reaction mixture was kept stirring at 45 °C for 3.5 h. After addition of sodium hydride (0.7 g) and benzyl bromide (2.0 mL), the reaction mixture was kept stirring for 17 h at 50 °C. The mixture was diluted with ethyl acetate, washed with distilled water and brine, dried over Na_2SO_4 to give crude crystals **11**. Crude crystals were recrystallized from ethanol to give pure crystals **11** (1.139 g, 18.9% yield): m.p. = 135.3–137.1 °C; $[\alpha]_D^{24.6}$ = −53.4 (c 1.07, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 3.43 (m, 1H, H5), 3.54 (t, 1H, J = 7.2, H2), 3.71 (t, 1H, J = 9.6, H4), 3.76 (t, 1H, J = 7.2, H3), 3.82 (t, 1H, J = 10.2, H6), 4.39 (dd, 1H, J = 5.1, J = 10.5, H6), 4.63 (d, 1H, J = 7.8, H1), 4.67 (d, 1H, J = 12.0, CH_2Ph), 4.77 (d, 1H, J = 12.0, CH_2Ph), 4.79 (d, 1H, J = 11.4), 4.90 (d, 1H, J = 11.1, CH_2Ph), 4.91 (d, 1H, J = 11.4), 4.95 (d, 1H, J = 11.7, CH_2Ph), 5.59 (s, 1H, –CHPh), 7.2–7.6 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 66.0 (C5), 68.7 (C6), 71.5 (CH_2Ph), 75.1 (CH_2Ph), 75.4 (CH_2Ph), 80.9 (C3), 81.4 (C4), 82.1 (C2), 101.1 (CHPh), 103.0 (C1), 126.0, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.3, 128.4, 128.9, 137.0, 137.2, 138.2, 138.4 (aromatic C); MALDI-TOF MS: calculated for $C_{34}H_{34}O_6$ = 538.24; found m/z $[M + Na]^+$ = 561.23.

Benzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**12**)

To a solution of compound **11** (1.077 g, 2.0 mmol) in acetonitrile (20 mL), powdered Molecular Sieves 4 Å (1.0 g) and sodium cyanoborohydride (0.53 g, 8 mmol) were added. Trimethylchlorosilane (2.0 mL, 15.8 mmol) was added dropwise over a period of 2 h to the reaction mixture. The reaction mixture was kept at room temperature overnight, filtered by use of Celite 535 and the residue was washed with ethyl acetate. The combined filtrate and washings were diluted with ethyl acetate, washed with distilled water and brine, dried over Na_2SO_4 to afford yellow syrup. The crude syrup was purified on preparative TLC (eluent: ethyl acetate: n-hexane = 1:4, v/v) to give colorless crystals **12** (0.833 g, 77% yield): m.p. = 60.4–64.4 °C; $[\alpha]_D^{24.6}$ = −39.6 (c 1.19, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 3.42–3.54 (H5, H3, H2), 3.62 (t, 1H, J = 9.0, H4), 3.73 (dd, 1H, J = 10.2, J = 5.1, H6), 3.80 (dd, 1H, J = 10.5, J = 3.9, H6), 4.53 (d, 1H, J = 7.2, H1), 4.58 (d, 1H, J = 12.0, CH_2Ph), 4.63 (d, 1H, J = 12.0, CH_2Ph), 4.66 (d, 1H, J = 12.0, CH_2Ph), 4.72 (d, 2H, J = 11.4, CH_2Ph, CH_2Ph), 4.93 (d, 1H, J = 11.4, CH_2Ph), 4.96 (d, 1H, J = 10.8, CH_2Ph), 4.96 (d, 1H, J = 12.3, CH_2Ph), 7.25–7.40 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 70.2 (C6), 71.2 (CH_2Ph), 71.5 (C4), 73.6 (CH_2Ph), 74.0 (C5), 74.8 (CH_2Ph), 75.3 (CH_2Ph), 81.8 (C2), 84.0 (C3), 102.6 (C1), 127.7, 127.8, 127.8, 128.0, 128.2, 128.3, 128.4, 128.5, 137.3, 137.9, 138.3, 138.5 (aromatic C); MALDI-TOF MS: calculated for $C_{34}H_{36}O_6$ = 540.25; found m/z $[M + Na]^+$ = 563.27, $[M + K]^+$ = 579.25.

Phenyl 4-*O*-acetyl-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-*β*-D-glucopyranoside (**13**)

Compound **13** was prepared according to our previous paper (Kamitakahara et al. 2006).

Benzyl 4-*O*-acetyl-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (**14**)

To a solution of compound **12** (100.8 mg. 0.186 mmol) and **13** (104.5 mg, 0.186 mmol) with molecular sieves 3 Å (300 mg) in anhydrous propionitrile (3 mL), *N*-iodosuccinimide (NIS; 58.7 mg, 0.264 mmol, 1.4 equiv) and catalytic amount of AgOTf was added at 0 °C. Temperature of the reaction mixture was raised gradually up to room temperature. The reaction mixture was kept stirring for 20 h. The reaction mixture was filtered off and washed with ethyl acetate. The combined filtrate and washings were diluted with ethyl acetate, washed with aq. $NaHCO_3$, distilled water, and brine, dried over Na_2SO_4, and concentrated to dryness. The crude compound was purified on preparative TLC (eluent: ethyl acetate:*n*-hexane = 1:4, v/v) to give a target compound **14** (55.8 mg, 30% yield) with α-glycoside (47.5 mg, 26% yield): $[\alpha]_D^{23.5} = +0.1$ (c 0.35, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 2.07 ($COCH_3$), 2.93 (t, 1H, J = 8,1, H2′), 2.99 (t, 1H, J = 8.4, H2″), 2.98–3.06 (m, 1H, H5′), 3.08 (t, 1H, J = 9.0, H3′), 3.21 (t, 1H, H = 9.2, H″), 3.22 (C6′-OCH_3), 3.34, 3.49, 3.50, 3.54 (OCH_3), 4.71 (t, 1H, J = 9.0, H4′), 3.95 (t, 1H, J = 9.0, H4), 4.33 (d, 1H, J = 7.8, H1″), 4.40 (d, 1H, J = 7.8, H1′), 4.50 (d, 1H, J = 7.5, H1), 4.58 (d, 1H, J = 12.0, CH_2Ph), 4.64–4.76 (4H, CH_2Ph), 4.87 (t, 1H, J = 9.6, H4″), 4.89 (d, 1H, J = 10.8, CH_2Ph), 4.96 (d, 1H, J = 12.0, CH_2Ph), 5.06 (d, 1H, J = 11.4, CH_2Ph), 7.2–7.4 (aromatic H); ^{13}C-NMR: δ 20.9 (–$COCH_3$), 58.7. 59.4, 60.0, 60.3, 60.5, 60.5, 68.4 (C6), 69.7 (C6′), 70.9 (C4″), 70.9 (CH_2Ph), 72.1 (C6″), 72.9 (C5″), 73.1 (CH_2Ph), 74.6 (C5′), 74.8 (CH_2Ph), 74.9 (CH_2Ph), 75.0 (C5), 77.0 (C4′), 77.4 (C4), 81.7 (C2), 83.2 (C3), 83.2 (C2′), 83.5 (C2″), 83.8 (C3″), 84.6 (C3′), 102.3 (C1″), 102.7 (C1′), 102.9 (C1), 127.0, 127.4, 127.5, 127.5, 127.6, 127.8, 128.0, 128.1, 128.2, 128.3, 137.3, 138.3, 138.4, 139.3, 169.8 (-$COCH_3$); MALDI-TOF MS: calculated for $C_{54}H_{70}O_{17}$ = 990.46; found *m/z* $[M+Na]^+$ = 1,013.594.

Benzyl 2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (**15**)

To a solution of compound **14** (55.8 mg, 0.0563 mmol) in methanol (11 mL), 28% sodium methoxide in methanol (3.5 μL, 0.0619 mmol, 1.1 equiv) was added at room temperature. The reaction mixture was kept stirring at room temperature for 14.5 h, and at 50 °C for 21.5 h. According to a monitoring of the reaction by means of analytical TLC, 28% sodium methoxide in methanol (10 μL) was added twice to the reaction mixture. The reaction mixture was neutralized with Dowex H^+, filtrated, and washed with methanol, and concentrated to dryness to give compound **15** (47.6 mg, 0.0486 mmol, 89% yield): $[\alpha]_D^{23.5} = -2.1$ (c 0.35, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 2.88–2.98 (t, t, 2H, H2′,H2″), 2.98–3.07 (m, 1H, H5′), 3.04–3.13 (t, t, 2H, H3′, H3″), 3.28–3.38 (m, 1H, H5″), 3.22, 3.40, 3.48. 3.49, 3.54, 3.63 (OCH_3), 3.64–3.75 (t, t, 2H, H4′ H4″), 3.87–3.91 (H6), 3.95 (t, 1H, J = 9.0, H4), 4.31 (d, 1H, J = 7.5, H1″), 4.40 (d, 1H, J = 7.8, H1′), 4.50 (d, 1H, J = 7.8, H1), 4.58 (d, 1H, J = 12.3, CH_2Ph), 4.64–4.77 (4H, CH_2Ph), 4.89 (d, 1H, J = 10.5, CH_2Ph), 4.97 (d, 1H, J = 12.0, CH_2Ph), 5.06 (d, 1H, J = 11.4, CH_2Ph), 7.2–7.4 (aromatic H); ^{13}C-NMR: δ 58.7. 59.5, 59.8, 60.3, 60.5, 60.8 (OCH_3), 68.4 (C6), 69.7 (C6′), 70.9 (C1-OCH_2Ph), 71.8(C4″), 72.9 (C6″), 73.1 (CH_2Ph), 73.3 (C5″), 74.7 (C5′), 74.9 (CH_2Ph), 74.9 (CH_2Ph), 75.0 (C5), 76.8 (C4′), 77.4 (C4), 81.7 (C2), 83.1 (C3), 83.6 (C2′, C2″, two carbons), 84.6 (C3′), 86.0 (C3″), 102.3 (C1″), 103.1 (C1′), 102.9 (C1), 127.0, 127.4, 127.5, 127.5, 127.6, 127.8, 128.0, 128.0, 128.2, 128.2, 137.4, 138.3, 138.4, 139.3; MALDI-TOF MS: calculated for $C_{52}H_{68}O_{16}$ = 948.45; found *m/z* $[M + Na]^+$ = 971.472.

Phenyl 4-*O*-acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-*β*-D-glucopyranoside (**16**)

Compound **16** was prepared according to our previous paper (Kamitakahara et al. 2007).

4-*O*-Acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranose (**17**)

To a solution of compound **16** (61.5 mg, 0.0627 mmol) in acetone/distilled water (10 mL, 9/1, v/v), *N*-bromosuccinimide (NBS; 15.4 mg, 0.0865 mmol, 1.4 equiv) and catalytic amount of AgOTf were added at room temperature. The reaction mixture was stirred for 4 h at room temperature. A catalytic amount of AgOTf and *N*-bromosuccinimide (NBS; 18.7 mg, 0.105 mmol; 18.0 mg, 0.101 mmol; 16.3 mg, 0.0916 mmol) were added to the reaction mixture, monitoring a reaction by use of analytical TLC. The reaction mixture was kept stirring for 23 h. Solid $NaHCO_3$ was added to the reaction mixture. The mixture was concentrated to dryness. The crude compound was extracted with diethyl ether. Diethyl ether-insoluble part was removed from the suspension by filtration. The combined filtrate and washings were concentrated to dryness to give crude compound **17**. The crude compound was purified by silica gel column chromatography (Wakogel C-300, eluent: dichloromethane, 20% methanol/dichloromethane, v/v) to give a compound **17** (59.8 mg) with a small amount of impurity. The compound was used for the next reaction without further purification: ^{1}H-NMR ($CDCl_3$): δ 1.19, 1.22 (s, s, 9H, 9H, $COC(CH_3)_3$), 1.94 ($COCH_3$), 2.90, 2.91 (t, t, J = 9.0, J = 8.1, H2′), 2.99 (dd, J = 7.8, J = 9.0, H2 (H1β)), 3.19 (t, J = 9.0, H3′), 3.15–3.25 (H2 (H1α), H5′, H5 (H1β)), 3.25 (t, J = 8.7, H3 (H1β)), 3.39, 3.43, 3.52, 3.54, 3.58, 3.62 (OCH_3), 4.0 (H5α), 4.12–4.25 (dd, dd, 2H, H6″), 4.25 (d, J = 7.8, H1′), 4.54 (d, J = 11.1, CH_2Ph), 4.58–4.63 (H1β), 4.62 (d, 1H, J = 7.5, H1″), 4.63 (d, J = 11.1, CH_2Ph), 5.09 (dd, J = 9.3, J = 7.8, H2″), 5.19 (t, J = 9.6, H4″), 5.33 (d, J = 3.6, H1α), 7.1–7.5 (aromatic H); ^{13}C-NMR ($CDCl_3$): δ 20.7 ($COCH_3$), 27.0 ($COC(CH_3)_3$), 27.2 ($COC(CH_3)_3$), 38.8 ($COC(CH_3)_3$), 58.7, 58.9, 59.3, 59.4, 60.4, 60.5, 60.6, 60.7 (OCH_3), 62.0 (C6″), 69.2 (C4″), 69.7 (C5α), 70.2 (C6 or C6′), 70.6 (C6 or C6′), 72.0 (C5″), 72.5 (C2″), 73.2 (CH_2Ph), 74.3 (C5β), 74.5 (C5′), 75.7 (C4′), 78.2 (C4β), 78.3 (C4α), 80.7 (C3″), 81.0 (C3α), 81.2 (C2α), 83.3 (C2′), 83.3 (C2′), 84.3 (C2β or C3β), 84.4 (C2β or C3β), 84.6 (C3′), 90.4 (C1α), 97.0 (C1β), 99.8 (C1″), 103.3 (C1′), 103.4 (C1′), 127.5, 127.7, 128.3, 137.5 (aromatic C), 169.2 ($COCH_3$), 176.5 ($COC(CH_3)_3$), 178.2 ($COC(CH_3)_3$); MALDI-TOF MS: calculated for $C_{43}H_{68}O_{19}$ = 888.44; found m/z $[M + Na]^+$ = 911.45.

4-*O*-Acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-α-D-glucopyranosyl trichloroacetoimidate (**18**)

To a solution of compound **17** (59.8 mg) in anhydrous dichloromethane (10 mL), *1,8*-diazabicyclo [5,4,0]-*7*-undecene (DBU; 10.1 μL, 0.0675 mmol, 1.0 equiv.) and trichloroacetonitrile (80.9 μL, 0.807 mmol, 12 equiv.) were added at room temperature. The reaction mixture was stirred for 5.5 h. The mixture was purified by alumina column chromatography to give compound **18** with predominant α-anomer (68 mg, 0.0658 mmol, 98% yield): ^{1}H-NMR ($CDCl_3$): δ 1.20, 1.22 (s, s, 9H, 9H, $COC(CH_3)_3$), 1.94 ($COCH_3$), 2.91 (t, 1H, J = 8.7, H2′), 3.18 (t, J = 8.7, H3′), 3.15–3.22 (m, 1H, H5′), 3.37, 3.43, 3.48, 3.53, 3.58, 3.60 (OCH_3), 3.35–3.45 (H2), 3.50–3.85 (m, H3, H5″, H6′, H3″, H4, H4′, H6), 3.85–3.93 (m, 1H, H5), 4.05–4.35 (dd, dd, 2H, H6″), 4.28 (d, 1H, J = 8.1, H1′), 4.54 (d, 1H, J = 10.8, CH_2Ph), 4.62 (d, 1H, J = 7.8, H1″), 4.64 (d, 1H, J = 10.8, CH_2Ph), 5.09 (dd, 1H, J = 9.3, J = 7.8, H2″), 5.19 (t, 1H, J = 9.3, H4″), 6.50 (d, 1H, J = 3.3, H1), 7.18–7.38 (aromatic H), 8.61 (s, 1H, (OC(=N*H*)CCl_3); ^{13}C-NMR ($CDCl_3$): δ 20.7 ($COCH_3$), 27.1 ($COC(CH_3)_3$), 27.2 ($COC(CH_3)_3$), 38.8 ($COC(CH_3)_3$), 58.7, 58.9, 59.5, 60.7, 60.7, 60.8 (OCH_3), 62.1 (C6″), 69.2 (C4″), 69.5 (C6), 70.2 (C6′), 72.0 (C5″), 72.5 (C2″), 72.7 (C5), 73.2 (CH_2Ph), 74.6 (C5′), 75.8 (C4′), 77.3 (C4), 80.4 (C2″), 80.8 (C3 and C3″), 83.3 (C2′), 84.8 (C3′), 91.2 (OC(=NH)CCl_3), 93.7 (C1), 99.9 (C1″), 103.4 (C1′), 127.5, 127.7, 128.4, 137.6 (aromatic C), 161.2 (OC(=NH)CCl_3), 169.2 ($COCH_3$), 176.5 ($COC(CH_3)_3$), 178.2 ($COC(CH_3)_3$).

Benzyl 4-*O*-acetyl-3-*O*-benzyl-2,6-di-*O*-pivaloyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (**19**)

Compounds **15** (47.6 mg, 0.0486 mmol) and **18** (68.0 mg, 0.0658 mmol) were dried in a reaction

ampule using high-vacuum system overnight. Dichloromethane (1 mL) was distilled from CaH_2, and degassed by freezing and thawing a few times. The solvent was transferred under high vacuum. The reaction ampule was separated by melting off and placed at −30 °C. Boron trifluoride diethylether (0.83 μL, 0.00658 mmol: 10 mol% to compound **18**) was added into the reaction ampule through a rubber septum by a syringe. The reaction mixture was stirred at −30 °C for 20 h. Boron trifluoride diethylether (0.85 μL) was added again at −30 °C. Reaction temperature was kept at −30 °C for 42.5 h, and gradually raised up to 0 °C for 3 h, and kept at 0 °C for 3.5 h. The reaction mixture was treated with the standard work-up procedure. The product was isolated on preparative TLC (eluent: ethyl acetate:*n*-hexane = 2:1, v/v) to give compound **19** (34.9 mg, 38% yield): $[\alpha]_D^{23.5} = +11.2$ (*c* 0.14, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 1.19, 1.22 (s, s, 9H, 9H, $COC(CH_3)_3$), 1.93 ($COCH_3$), 2.88 (t, 1H, $J = 9.3$, H2′′′′), 2.92 (t, 1H, $J = 8.7$, H2′ or H2′′ or H2′′′), 2.94 (t, 1H, $J = 7.8$, H2′ or H2′′ or H2′′′), 2.98–3.06 (m, 1H, H2′′ or H2′′′), 2.98–3.06 (m, 1H, H5′) 3.08 (t, 1H, $J = 9.0$, H3′), 3.13–3.24 (H3′′, H3′′′,H3′′′′), 3.27 (m, 2H, H5′′ and H5′′′), 3.21, 3.38, 3.42, 3.49, 3.53, 3.54, 3.56, 3.56, 3.57, 3.58, 3.57 (OCH_3), 3.4–3.5 (H5), 3.4–3.5 (H2), 3.5–3.6 (H3, H5′′′′′), 3.6–3.7 (H4′, H4′′, H4′′′, H6′, H6′′, H6′′′), 3.65–3.75 (H3′′′′′), 3.6–3.8 (H6′′′′), 3.7–3.8 (H4′′′′), 3.89 (d, 1H, $J = 2.7$, H6), 3.95 (t, 1H, $J = 9.0$, H4), 4.15 (dd, 1H, $J = 2.4$, $J = 12.0$, H6′′′′′), 4.21 (dd, 1H, $J = 12.3$, $J = 4.2$, H6′′′′′), 4.26 (d, 1H, $J = 8.7$, H1′′ or H1′′′), 4.29 (d, 1H, $J = 8.7$, H1′′′′), 4.34 (d, 1H, $J = 7.5$, H1′′ or H1′′′), 4.39 (d, 1H, $J = 7.2$, H1′), 4.50 (d, 1H, $J = 7.8$, H1), 4.54 (d, 1H, $J = 11.1$, CH_2Ph), 4.58 (d, 1H, $J = 12.0$, CH_2Ph), 4.62 (d, 1H, $J = 7.8$, H1′′′′′), 4.63 (d, 1H, $J = 11.7$, CH_2Ph), 4.67 (d, 1H, $J = 12.3$, CH_2Ph), 4.68 (d, 1H, $J = 12.3$, CH_2Ph), 4.73 (d, 1H, $J = 11.7$, CH_2Ph), 4.88 (d, 1H, $J = 10.5$, CH_2Ph), 4.96 (d, 1H, $J = 12.0$, CH_2Ph), 5.06 (d, 1H, $J = 11.1$, CH_2Ph), 5.09 (dd, 1H, $J = 8.1$, $J = 9.3$, H2′′′′′), 5.19 (t, 1H, $J = 9.3$, H4′′′′′), 7.2–7.4 (aromatic H); ^{13}C-NMR: δ 20.7 (–$COCH_3$), 27.0 (C2′′′′′-$COC(CH_3)_3$), 27.2 (C6′′′′′-$COC(CH_3)_3$), 38.8, 58.7, 59.1, 59.1, 59.4, 60.2, 60.4, 60.5, 60.5, 60.7, 60.8, 62.0 (C6′′′′′), 68.4 (C6), 69.2 (C4′′′′′), 69.7, 70.1, 70.2 (C6′, C6′′, C6′′′, C6′′′′), 70.9 (C1-OCH_2Ph), 72.0 (C5′′′′′), 72.5 (C2′′′′′), 73.1 (CH_2Ph), 73.2 (CH_2Ph), 74.5, 74.7, 74.8 (C5′, C5′′, C5′′′, C5′′′′), 74.9 (CH_2Ph), 74.9 (CH_2Ph), 75.0 (C5), 75.8 (C4′′′′), 77.2, 77.5 (C4, C4′, C4′′, C4′′′), 80.8 (C3′′′′′), 81.7 (C2), 83.2 (C3), 83.4, 83.5 (C2′, C2′′, C2′′′, C2′′′′), 84.7, 84.8, 84.9, 85.0 (C3′, C3′′, C3′′′, C3′′′′), 99.9 (C1′′′′′), 102.3 (C1), 102.7 (C1′), 103.1 (C1′′, C1′′′, C1′′′′) 127.0, 127.5, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.3, 137.4, 137.6, 138.3, 138.4, 139.3, 169.2 (C4′′′′′-$OCOCH_3$), 176.5 (C2′′′′′-$COC(CH_3)_3$), 178.2 (C6′′′′′-$COC(CH_3)_3$); MALDI-TOF MS: calculated for $C_{95}H_{134}O_{34} = 1{,}818.88$; found *m/z* $[M + Na]^+ = 1{,}841.817$.

Benzyl 3-*O*-benzyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**20**)

To a solution of compound **19** (33.9 mg, 0.0186 mmol) in methanol (10 mL), 28% sodium methoxide in methanol (26 μL) was added at room temperature. The reaction mixture was kept stirring at room temperature for 3.5 h, and at 50 °C for 12 h. The reaction mixture was neutralized with Dowex H^+ to give a reaction product having one pivaloyl group at C2′′′′′ position: benzyl 3-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside: ^{1}H-NMR ($CDCl_3$): δ 1.20 (s, 9H, $COC(CH_3)_3$), 2.91 (t, $J = 9.3$, H2′ or H2′′ or H2′′′ or H2′′′′), 2.92 (t, $J = 8.7$, H2′ or H2′′ or H2′′′ or H2′′′′), 2.94 (t, $J = 7.8$, H2′ or H2′′ or H2′′′ or H2′′′′), 2.88–2.98 (H2′ or H2′′ or H2′′′ or H2′′′′), 2.99–3.06 (m, H5′), 3.08 (t, 1H, $J = 9.0$, H3′), 3.13–3.3 (H3′′, H3′′′, H3′′′′), 3.26 (m, 2H, $J = 9.9$, H5′′ and H5′′′), 3.21, 3.39, 3.41, 3.49, 3.52, 3.53, 3.56, 3.56, 3.57, 3.57 (OCH_3), 3.35–3.45 (H5′′′′′), 3.46–3.6 (H3′′′′′), 3.64–3.74 (H4′′′′′), 3.84–3.94 (H6′′′′′), 3.90 (dd, 1H, $J = 11.7$, H6), 3.95 (t, 1H, $J = 9.3$, H4), 4.26 (d, 1H, $J = 8.1$, H1′′ or H1′′′ or H1′′′′), 4.29 (d, 1H, $J = 8.1$, H1′′ or H1′′′ or H1′′′′), 4.34 (d, 1H, $J = 7.8$, H1′′ or H1′′′ or H1′′′′), 4.39 (d, 1H, $J = 7.8$, H1′), 4.49 (d, 1H, $J = 7.8$, H1), 4.58 (d, 1H, $J = 7.8$, H1′′′′′), 4.58 (d, 1H, $J = 12.0$, CH_2Ph), 4.66 (d, 1H,

J = 11.4, CH_2Ph), 4.64–4.70 (d, d, 2H, CH_2Ph), 4.68 (d, 1H, J = 12.3, CH_2Ph), 4.73 (d, 1H, J = 11.7, CH_2Ph), 4.74 (d, 1H, J = 11.4, CH_2Ph), 4.88 (d, 1H, J = 11.1, CH_2Ph), 4.96 (d, 1H, J = 12.0, CH_2Ph), 5.01 (dd,, 1H, H2$''''$), 5.06 (d, 1H, J = 12.0, CH_2Ph), 7.2–7.4 (aromatic H); ^{13}C-NMR: δ 27.2 (C2$''''$-COC(CH_3)$_3$), 38.8 (C2$''''$-$COC(CH_3)_3$), 58.7, 59.1, 59.1, 59.4, 60.2, 60.4, 60.4, 60.5, 60.6, 60.6, 61.0 (OCH_3), 62.5 (C6$''''$), 68.4 (C6), 69.7, 70.1, 70.2, 70.3 (C6′, C6″, C6‴, C6$''''$), 70.7 (C3$''''$), 70.9 (C1-OCH_2Ph), 73.0 (C2$''''$), 73.1 (CH_2Ph), 74.2 (CH_2Ph), 74.5, 74.7, 74.7, 74.8, 74.9 (CH_2Ph), 74.9 (CH_2Ph), 75.0 (C5$''''$), 75.0 (C5), 75.5 (C4$''''$), 77.1, 77.4 (C4, C4′, C4″, C4‴, C4$''''$), 81.7 (C2), 83.2 (C3), 83.4, 83.4, 84.6 (C2′, C2″, C2‴, C2$''''$), 84.7, 84.9 (C3′, C3″, C3‴, C3$''''$), 99.6 (C1$''''$), 102.3 (C1), 102.7 (C1′), 103.1 (C1″, C1‴, C1$''''$) 127.0, 127.4, 127.5, 127.5, 127.7, 127.9, 127.9, 128.0, 128.1, 128.2, 128.3, 128.3, 128.6, 137.4, 137.9, 138.3, 138.4, 139.3 (aromatic C), 176.8 (C2$''''$-$COC(CH_3)_3$).

To a solution of the product having one pivaloyl group in methanol (10 mL), 28% sodium methoxide in methanol (24 μL) was added. The reaction mixture was kept stirring at reflux temperature for 22 h. After adding 28% sodium methoxide in methanol (0.2 mL), the reaction mixture was kept stirring at reflux temperature for 2 h. Furthermore, after adding 28% sodium methoxide in methanol (1.0 mL), the reaction mixture was kept stirring at reflux temperature for 16 h. The reaction mixture was neutralized with Dowex H^+ to give a crude compound. The crude compound was purified by silica gel column chromatography (eluent: dichloromethane and 20% methanol/ dichloromethane), and preparative TLC (5% methanol/dichloromethane, v/v) to give compound **20** (21.8 mg, 0.0135 mmol, 73% yield): ^{1}H-NMR ($CDCl_3$): δ 2.88–2.99 (4H, H2′, H2″, H2‴, H2$''''$), 2.98–3.07 (m, 1H, H5′), 3.09 (t, 1H, J = 9.0, H3′), 3.14–3.3 (H3″, H3‴, H3$''''$), 3.2–3.36 (H5″, H5‴, H5$''''$), 3.34–3.44 (H5$''''$, H3$''''$), 3.4–3.5 (H5), 3.42–3.54 (H2, H2$''''$), 3.52–3.62 (H3), 3.21 (C6′-OCH_3), 3.38, 3.39, 3.47, 3.47, 3.49, 3.54, 3.56, 3.58, 3.63 (OCH_3), 3.8–3.9 (H6), 3.88–3.98 (H6″ or H6‴ or H6$''''$), 3.94 (t, 1H, J = 9.3, H4), 4.26 (d, 1H, J = 7.5, H1″ or H1‴ or H1$''''$), 4.34 (d, 1 J, J = 8.1, H1″ or H1‴ or H1$''''$), 4.34 (d, 1H, J = 8.1, H1″ or H1‴ or H1$''''$), 4.39 (d, 1H, J = 7.8, H1′), 4.50 (d, 1H, J = 7.8, H1), 4.58 (d, 1H, J = 11.7, CH_2Ph), 4.61 (d,, 1H, J = 7.2, H1$''''$), 4.67 (d, 1H, J = 12.6, CH_2Ph), 4.67 (d, 1H, J = 10.5, CH_2Ph), 4.68 (d, 1H, J = 12.3, CH_2Ph), 4.73 (d, 2H, J = 11.4, CH_2Ph), 4.88 (d, 1H, J = 10.5, CH_2Ph), 4.96 (d, 1H, J = 12.3, CH_2Ph), 5.06 (d, 1H, J = 11.4, CH_2Ph), 5.06 (d, 1H, J = 11.4, CH_2Ph), 7.2–7.4 (aromatic H); ^{13}C-NMR: δ 58.8, 59.1, 59.2, 59.9, 60.2, 60.5, 60.5, 60.5, 60.6, 60.6, 60.9 (OCH_3), 62.4 (C6$''''$), 68.4 (C6), 69.7 (C4$''''$, C6′), 70.0 (C6″ or C6‴ or C6$''''$), 70.2 (C6″ or C6‴ or C6$''''$), 70.9 (C1-OCH_2Ph), 71.3 (C6″ or C6‴ or C6$''''$), 73.1 (CH_2Ph), 73.9, 74.6 (CH_2Ph), 74.7, 74.9 (CH_2Ph), 74.9 (CH_2Ph), 75.0, 75.3 (C5$''''$), 76.6, 77.1, 77.2, 77.5 (C4′, C4″, C4‴, C4$''''$), 77.5 (C4), 81.7 (C2), 83.2 (C3), 83.4, 83.5, 84.4 (C2′, C2″, C2‴, C2$''''$), 83.7 (C3$''''$), 84.8, 85.0, 85.6 (C3′, C3″, C3‴, C3$''''$), 102.4 (C1), 102.7 (C1′), 103.1, 103.1, 103.2 (C1″, C1‴, C1$''''$), 103.4 (C1$''''$), 127.0, 127.5, 127.5, 127.6, 127.7, 127.9, 128.0, 128.0, 128.1, 128.2, 128.3, 128.3, 128.6, 137.4, 138.3, 138.4, 138.6, 139.3 (aromatic C); MALDI-TOF MS: calculated for $C_{83}H_{116}O_{31}$ = 1,608.75; found m/z $[M + Na]^+$ = 1,631.591.

β-D-Glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (**2**)

To a solution of compound **20** (21.8 mg, 0.0135 mmol) in ethanol (2 mL), 10% palladium on carbon (50 mg) was added. The reaction mixture was kept stirring under hydrogen atmosphere at room temperature for 18 h. Twenty % palladium hydroxide on carbon (50 mg) was added to the reaction mixture. The mixture was kept stirring for 22.5 h at room temperature. Furthermore, after adding palladium hydroxide (50 mg) again, the mixture was kept stirring for 24 h at room temperature. Palladium hydroxide on carbon and palladium on carbon were filtered off and washed with 20% methanol/ dichloromethane and then with methanol. The combined filtrate and washings were concentrated to dryness to give compound **2** (10.5 mg, 67% yield): $[\alpha]_D^{28.0}$ = +0.7 (c 0.744, H_2O); ^{1}H-NMR (D_2O): δ 3.18–3.20 (H2′, H2″, H2‴, H2$''''$), 3.28 (dd, J = 8.1, J = 9.0, H2(H1β), H2$''''$), 3.36–3.50 (H3′, H3″, H3‴, H3$''''$), 3.5–3.6 (H2(H1α)), 3.50–3.64 (H5′, H5″, H5‴, H5$''''$), 3.40, 3.57, 3.58 (OCH_3), 3.64–3.84 (H4′, H4″, H4‴, H4$''''$, H6′, H6″, H6‴, H6$''''$), 4.38–4.45,

4.52 (H1′, H1″, H1‴, H1⁗, H1⁗′), 4.65 (d, J = 8.4, H1β), 5.19 (d, J = 3.9, H1α); ^{13}C-NMR (D_2O): δ 60.9, 61.0, 61.1, 61.6, 61.7, 61.8, 62.1 (OCH_3), 63.1 (C6⁗′, OCH_3), 63.4 (C6 or C6⁗′), 72.2 (C4⁗′), 72.5 (C6′, C6″, C6‴, C6⁗), 76.0, 76.1, 76.2, 76.3, 76.5 (C2β), 76.9 (C3β), 77.5 (C5β), 77.9, 78.4 (C3⁗′), 78.8 (C5⁗′), 81.6, 84.4, 84.6, 84.7, 84.8, 85.1, 85.5, 85.7, 86.0, 94.5 (C1α), 98.4 (C1β), 104.9, 105.1 (C1′, C1″, C1‴, C1⁗, C1⁗′); MALDI-TOF MS: calculated for $C_{48}H_{86}O_{34}$ = 1,158.52; found m/z $[M + Na]^+$ = 1,181.560.

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Synthesis and Structure/Property Relationships of Regioselective 2-*O*-, 3-*O*- and 6-*O*-Ethyl Celluloses

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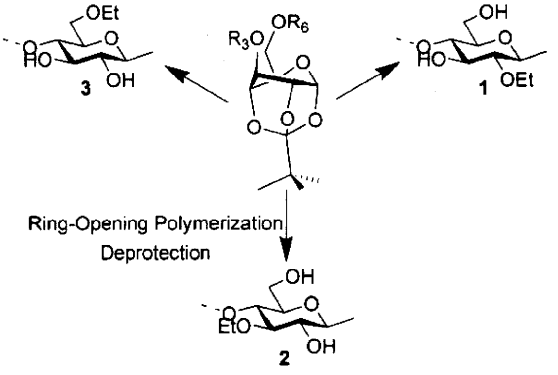
Regioselectively ethylated celluloses, 2-*O*- (**1**), 3-*O*- (**2**), and 6-*O*-ethyl- (**3**) celluloses were synthesized via ring-opening polymerization of glucopyranose orthopivalate derivatives. The number-average degrees of polymerization (DP_ns) of compounds **1** and **2** were calculated to be 10.6 and 49.4, respectively. Three kinds of compound **3** with different DP_ns were prepared: DP_ns = 12.9 (**3-1**), 60.3 (**3-2**), and 36.1 (**3-3**). The 2-*O*-, 3-*O*-, and 6-*O*-ethylcelluloses were soluble in water, confirmed by NMR analysis. Furthermore, the 3-*O*- (**2**), and 6-*O*-ethyl- (**3-2**) celluloses showed thermo-responsive aggregation behavior and had a lower critical solution temperature (LCST) at about 40 °C and 70 °C, respectively, based on the results from turbidity tests and DSC measurements. The 6-*O*-ethyl-cellulose (**3-3**) with $DP_n = 36.1$ and $DP_w = 54.6$ showed gelation behavior over approx 70 °C, whereas the 6-*O*-ethyl-celluloses **3-1** and **3-2** with lower and higher molecular weight, such as DP_ns 12.9 and 60.3, did not show gelation behavior at this temperature. It was revealed that the position of ethyl group affected the phase transition temperature. According to our experiments, the 3-*O*-ethyl and 6-*O*-ethyl groups along the cellulose chains caused the thermo-responsive property of their aqueous solutions. The appropriate DP of the regioselective 6-*O*-ethyl-cellulose existed for gelation of the aqueous solution.



Introduction

Ethyl cellulose (EC) (ethoxyl content: 43–50%; DS = ca. 2.1–2.6) is commercially used as a coating film for drug tablets to control the rate of release of active ingredients. The Ethocel polymers are used as organic binders in high-tech paste inks for printing circuit patterns that are then furnace-fired to a final form. Highly substituted EC (ethoxyl content: 50–52.5%; DS = ca. 2.6–2.8) is soluble in hydrocarbon solvents, but loses solubility in oxygenated solvents.[1]

On the other hand, low DS EC (ethoxyl content: 19–35%; DS = approx. 0.8–1.7) is water soluble,[1–3] although water soluble EC is not commercially available now. Investigation into the structure-property relationship of EC, especially that of water soluble EC, is of importance not only to develop new utilization of EC, but also to compare the properties of EC with commercially available water soluble methylcellulose (MC) (DS = ca. 1.8).

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Commercial MC, having a DS of approximately 1.6–2.0 is water soluble. The substituent distribution of methyl groups on the cellulose skeleton affects the solution properties of MC. The regioselectively substituted MCs with DS = 1 and 2, 2-*O*-methyl-, 3-*O*-methyl-, 6-*O*-methyl-, 2,3-di-*O*-methyl-, 2,6-di-*O*-methyl-, and 3,6-di-*O*-methyl-celluloses, prepared according to the ring-opening polymerization of glucose orthoester derivatives[4] were water insoluble. Furthermore, it was also found that 3-*O*-methyl-cellulose[5] and 2,6-di-*O*-methyl cellulose[6] from natural cellulose are water insoluble. It was revealed in general that regioselective derivatized MCs have poor solubility in water.

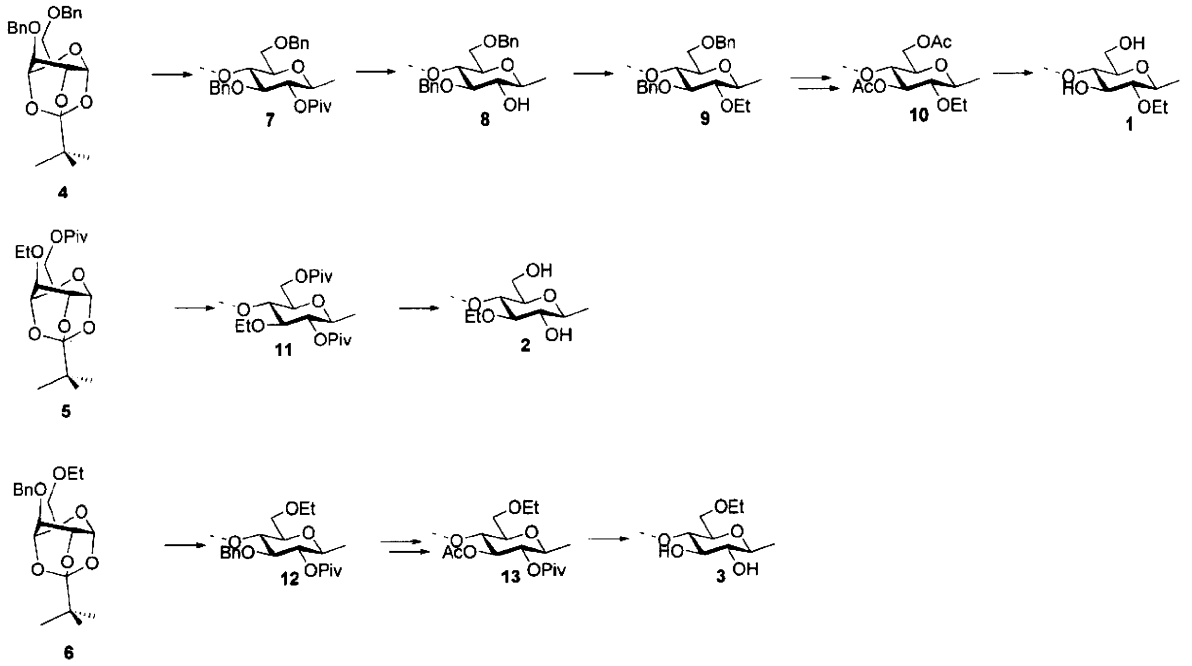
Furthermore, we have recently reported that replacement of one or both methyl groups on 2,6-*O*-methyl cellulose (2,6-di-*O*-ethyl-cellulose, 2-*O*-ethyl-6-*O*-methyl-cellulose, and 6-*O*-ethyl-2-*O*-methyl-cellulose) with an ethyl group increased solubility in organic media such as chloroform.[7] Regioselective 6-*O*-ethyl/methyl-cellulose with ethyl/methyl ratios of 10:0, 9:1, 5:5 1:9 have also been prepared by ring-opening polymerization of glucose orthoester derivatives.[8] The 6-*O*-ethyl group enhanced the solubility of regioselectively 6-*O*-alkylated celluloses in water.

The structure-property relationships of the above-mentioned regioselectively etherified celluloses with relatively low DP (3–150) are of industrial significance to improve the properties of cellulose ethers produced. Especially, cellulosic diblock and triblock cooligomers, trimer to octamer, were found to be amphiphilic, to show higher surface activities than industrially produced methylcellulose, and to form nano particles.[9–12] Thus, such a cellulosic material having a lower molecular weight can be one of the fields of application.

In this paper, we report synthesis of the next synthetic targets, regioselectively mono-*O*-ethylated celluloses via the ring-opening polymerization of glucose orthoester derivatives and their structure-property relationships. In particular, the influence of the introduced positions of ethyl groups on the water solubilities of regioselectively mono-*O*-ethylated celluloses was investigated. It is of significance from both fundamental and commercial aspects to investigate the water solubilities of regioselective ECs with DS = 1.

Regioselective 6-*O*-ethyl (6EC) and 3-*O*-ethyl (3EC) celluloses from natural cellulose have been prepared by Kondo[13] and Koschella et al.,[14] respectively. There was no analytical data about 6EC in the literature. 6EC was water soluble, as reported in our recent paper.[8] Moreover, the influence of the DP of 6EC on the physical properties, such as gelation behavior, is described in the present paper. 3EC was reported to be water soluble and to show thermoreversible gelation.[14] A 2-*O*-ethyl cellulose (2EC) has not been synthesized up to now. The influence of regioselective substitution of the ECs on their physical properties is of interest in connection with the commercial production of EC and fundamental cellulose chemistry.

In this paper, we describe the synthesis and properties of 2EC, 3EC and 6EC via the ring-opening polymerization of



■ *Figure 1.* Synthesis routes of compounds **1**, **2** and **3**.

Table 1. Results of ring-opening polymerizations of α-D-glucopyranose 1,2,4-orthopivalates **4**, **5**, and **6**. Monomer concentration: 100 g/100 mL. Initiator: BF_3Et_2O; 5 mol-%. Solvent: dichloromethane. Yield: no monomer remained after polymerization.

Run	Monomer	Temp.	Time	Comp. No.	$\overline{M}_w/\overline{M}_n$	DP_w	DP_n
		°C	h				
1	**4**	−10	12.25	**7**	1.56	27.8	17.8
2	**5**	−40 → r.t.	72 → 0.1	**11**	1.95	83.1	42.6
3	**6**	−30	48	**12-1**	2.31	26.8	11.6
4	**6**	−10	24	**12-2**	1.87	115.2	61.6
5	**6**	0	24	**12-3**	2.34	99.2	42.4

glucopyranose 1,2,4-orthopivalate derivatives. Solubilities, crystallinities, surface activities and thermo-responsive aggregation and/or gelation behaviors of three kinds of mono-*O*-ethyl-celluloses will be discussed here.

Results and Discussion

Synthesis of 2-*O*-, 3-*O*- and 6-*O*-Ethyl Celluloses

Synthesis routes for 2EC, 3EC, and 6EC are shown in Figure 1. In the case of 2EC (**1**), the ethyl group was introduced after polymerization of compound **4** during the deprotection/substitution procedure. 3EC (**2**) and 6EC (**3**) were prepared via ring-opening polymerization of mono-*O*-ethylated glucose 1,2,4-orthopivalate derivatives **5** and **6**, respectively. Therefore, 3EC (**2**) and 6EC (**3**) have ethyl substituent groups with DS 1.0 definitely. Benzyl and pivaloyl groups are usually used as temporary protective groups for the syntheses of regioselective functionalization of cellulose.[15,16]

The results of the ring-opening polymerizations of compounds **4**, **5**, and **6** are summarized in Table 1. The

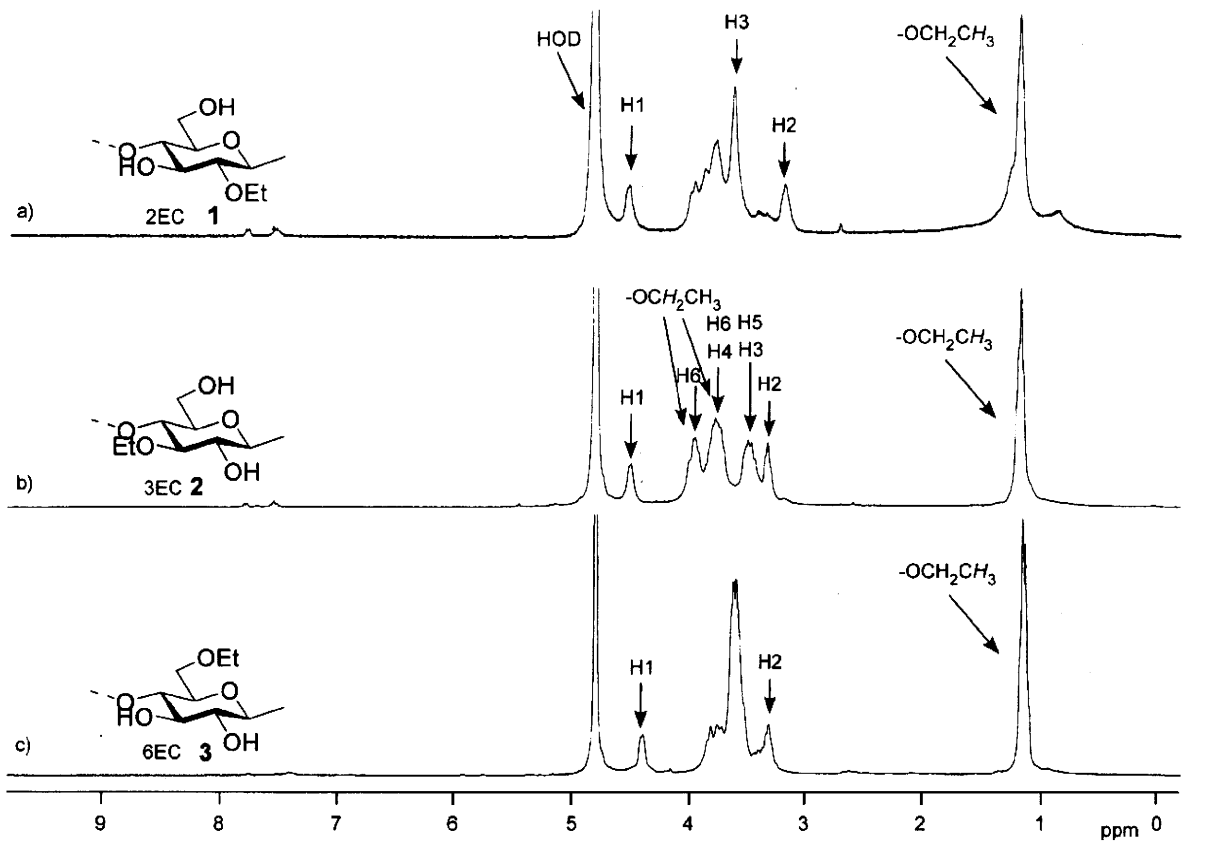


Figure 2. ^1H NMR spectra of compound **1**, **2** and **3**, measured in D_2O.

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