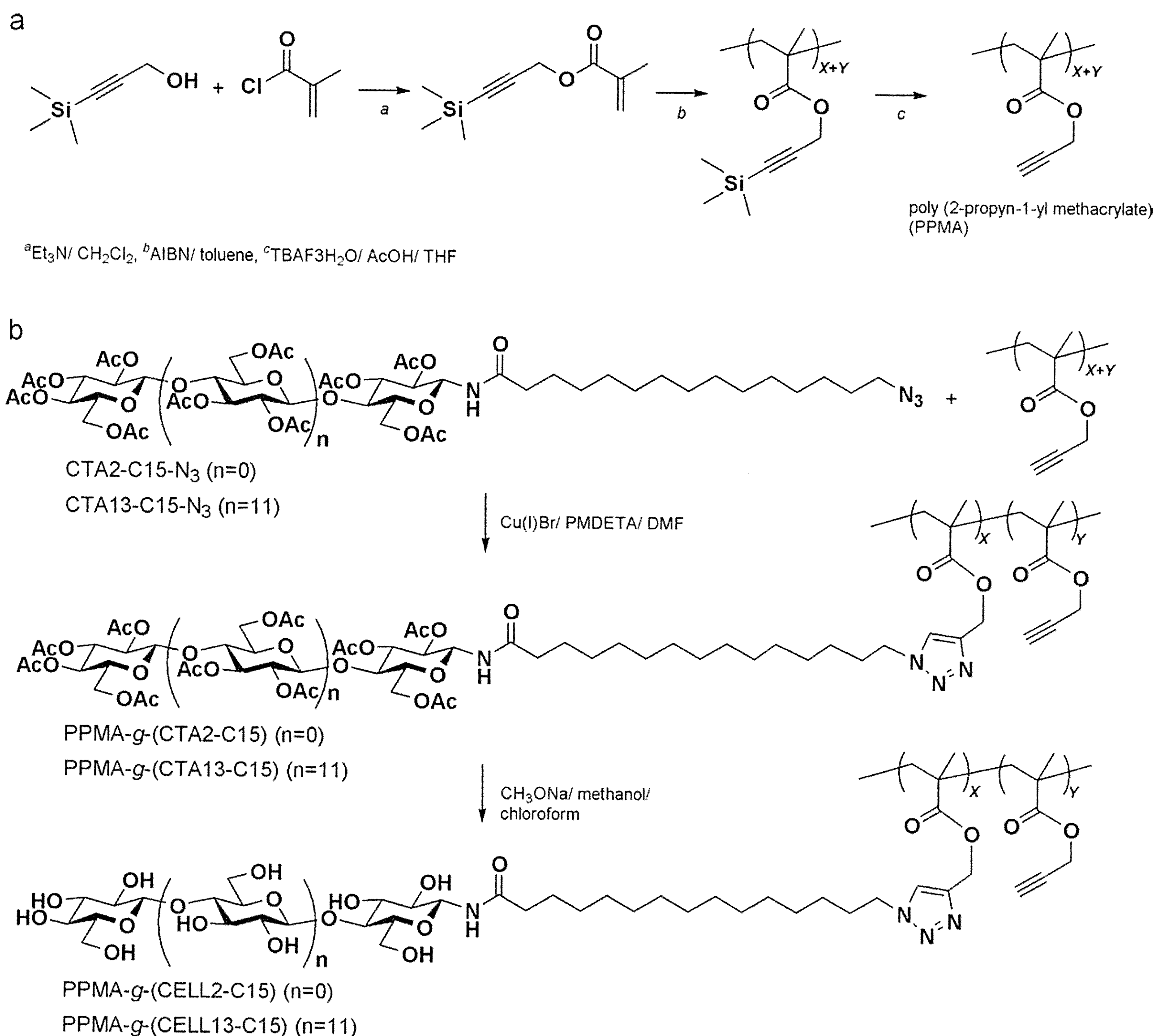


**Fig. 1.** Preparation of: (a) poly (2-propyn-1-yl methacrylate) (PPMA), (b) PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15).

## 2.6. PPMA-g-(CTA13-C15)

CTA13-C15-$N_3$ (42.2 mg, 1 eq. to PMA monomer) and PPMA (1.3 mg) were treated in the same manner with that for PPMA-*g*-(CTA2-C15) using PMDETA (18.2 μl, 10 eq. to PMA monomer) and Cu(I)Br (15.0 mg, 10 eq. to PMA monomer) in DMF (0.3 ml). The remaining CTA13-C15-$N_3$ in the crude product was removed by gel permeation chromatography with chloroform using a Shimadzu SEC system equipped with a fraction collector to give an amorphous solid PPMA-*g*-(CTA13-C15) (11.6 mg, 26.6% yield). The area ratio ($A(\%)$) of the high molecular fraction to the total area including the remaining CTA13-C15-$N_3$ in chromatogram (RI) of the crude product was $A(\%) = 43.8\%$. Number of graft chains ($X$) was calculated as $2.45 \times 10^2$ from $X = DP_w(\text{PPMA}) \times A(\%) = 5.59 \times 10^2 \times 43.8(\%)$, on the assumption that the initial molar ratio of CTA13-C15-$N_3$ to PMA monomer unit was 1.0. The number of PMA units ($Y$) in PPMA-*g*-(CTA13-C15) was calculated to be $3.14 \times 10^2$ from $Y = DP_w(\text{PPMA}) - X = 5.59 \times 10^2 - 2.45 \times 10^2$. The molecular weight of PPMA-*g*-(CTA13-C15) was estimated to be $1.38 \times 10^6$ from $M(\text{PPMA-}g\text{-(CTA13-C15)}) = M_w(\text{PPMA}) + X \times M_{w,PS}(\text{CTA13-C15-}N_3) = 6.94 \times 10^4 + 2.45 \times 10^2 \times 5.33 \times 10^3$. $^1$H NMR ($CDCl_3$): δ 1.25 (m, aliphatic-H), 1.9–2.1 ($CH_3$–CO–), 3.56 (C5-H), 3.72 (C4-H), 4.06 (C6-$H_b$), 4.37–4.43 (C6-$H_a$, C1-H), 4.81 (C2-H), 5.08 (C3-H). $^{13}$C NMR ($CDCl_3$): δ 20.5 ($CH_3$–CO–), 29.5 (aliphatic-C), 62.0 (C6), 71.7 (C2), 72.4 (C5, C3), 100.5 (C1), 169.3, 169.7, 170.2 ($CH_3$–CO– of C2, C3, C6, respectively).

## 2.7. PPMA-g-(CELL2-C15)

To a solution of PPMA-*g*-(CTA2-C15) (25 mg) in methanol/chloroform (1/4, v/v, 1 ml), sodium methoxide (50 μl, 0.03 mmol) was added at room temperature, and stirred for 4 h under nitrogen. The precipitated compound was filtered and washed by methanol to give an amorphous solid, PPMA-*g*-(CELL2-C15) (14.9 mg, quantitative). $^1$H NMR (DMSO-$d_6$): δ 1.21, 1.46, 1.79, 2.08 (br. s, aliphatic-H), 3.0–3.2, 3.7, 4.2, 4.6, 5.0 (ring-H), 4.74 (C1-H), 8.14 (triazole), 8.32 (NH). $^{13}$C NMR (DMSO-$d_6$): δ 25.9 (C1–NH–CO–$CH_2$–$CH_2$–), 29.1 (aliphatic-C), 35.4 (C1–NH–CO–$CH_2$–), 44.4 ($CH_2$–C–$CH_3$), 49.4 ($CH_2$–O), 57.7 ($CH_2$–C), 60.3 (C6′), 61.1 (C6), 70.0 (C4′), 72.1 (C2), 73.3 (C2′), 75.8 (C5′), 76.4 (C3, C3′), 76.8 (C5), 79.2 (C4), 80.4 (C1), 103.2 (C1′), 125.0 (CH (triazole)), 140.8 (C (triazole)), 172.9 (C1–NH–CO–), 176.0 (CO).

*2.8. PPMA-g-(CELL13-C15)*

To a solution of PPMA-g-(CTA13-C15) (4.8 mg) in methanol/chloroform (1/4, v/v, 0.3 ml), sodium methoxide (50 μl, 0.03 mmol) was added at room temperature, and stirred for 4 h under nitrogen. The precipitated compound was collected by centrifugation at 1000 rpm for 3 min to give an amorphous solid, PPMA-g-(CELL13-C15) (2.4 mg, 88.9% yield).

*2.9. General measurements*

$^{1}H$, $^{13}C$, and two-dimensional NMR spectra including H-H COSY (correlation spectroscopy), HSQC (heteronuclear single quantum coherence) were recorded with a Varian INOVA300 FT-NMR (300 MHz) or a JEOL JNM-A500 FT-NMR (500 MHz) spectrometer in $CDCl_3$ or DMSO-$d_6$ with tetramethylsilane (TMS) as internal standard. Chemical shifts ($\delta$) and coupling constants ($J$) are reported in (ppm) and (Hz), respectively. Fourier transform infrared (FT-IR) spectra were recorded on a FTIR-4000 spectrophotometer equipped with ATR attachment (Durasampl IR II).

*2.10. SEC–MALS measurement*

Size exclusion chromatography–multi-angle laser light scattering (SEC–MALS) measurements were carried out at 25 °C using a Shimadzu SEC system (CBM-10A, SPD-10A, SIL-10A, LC-10AT, FCV-10AL, CTO-10A, RID-10A, and FRC-10, Shimadzu, Japan) and MALS detector (DAWN EOS, Wyatt Technology Co. Ltd., U.S.A.) ($\lambda$ = 690 nm). Chloroform was used as eluent. The flow rate was 1.0 ml/min. The photometer was calibrated with pure toluene. Shodex columns (K802, K802.5, and K805) were used. Number and weight average molecular weights ($M_{n,PS}$, $M_{w,PS}$) and polydispersity index (PDI, $M_{w,PS}/M_{n,PS}$) were estimated using polystyrene standards (Shodex). An absolute molecular weight ($M_w$) was determined by MALS measurements using Zimm plots. Refractive index (RI) increments ($dn/dc$) were calculated assuming 100% recovery of injected mass. The $dn/dc$ values were 0.047 for PPMA-g-(CTA2-C15) and 0.064 for poly(2-propyn-1-yl methacrylate) (PPMA).

*2.11. X-ray diffraction measurements*

X-ray diffraction measurements were carried out with a Rigaku diffractometer Ultima IV. A Nickel-filtered CuKα radiation was used at 40 kV and 30 mA. Cellulose microcrystalline (Avicel, Merck) and low-molecular-weight cellulose (Isogai & Usuda, 1991) was used to obtain reflection pattern of cellulose I and cellulose II, respectively. The peak of X-ray diffraction curves are resolved to four peaks due to (1 −1 0), (1 1 0), (2 0 0) and amorphous reflections. Crystallinity index was calculated from the ratio of the sum of integrated intensities of (1 −1 0), (1 1 0), (2 0 0) reflections to the sum of integrated intensities of the four peaks (Ishikawa, Okano, & Sugiyama, 1997).

## 3. Results and discussion

*3.1. Preparation and characterization of PPMA-g-(CTA2-C15)*

Poly(2-propyn-1-yl methacrylate) (PPMA) was obtained *via* free radical polymerization of trimethylsilyl methacrylate monomer using AIBN and subsequent deprotection of trimethylsilyl group using TBAF·$3H_2O$ according to the previous article (Ladmiral et al., 2006), as described in Fig. 1a. We have prepared cellulose derivatives with a ω-azidopentadecanoyl group at the reducing-end in previous work (Enomoto, Kamitakahara, Takano, & Nakatsubo, 2006; Kamitakahara et al., 2005; Kamitakahara & Nakatsubo, 2005).

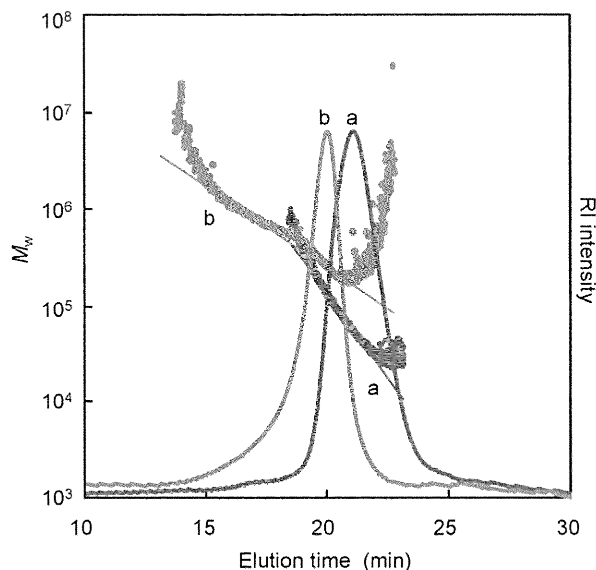


**Fig. 2.** The chromatograms (RI) and the molecular weight ($M_w$) plots of: (a) PPMA and (b) PPMA-g-(CTA2-C15).

*N*-(15-azidopentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosylamine (CTA2-C15-$N_3$), was grafted onto the PPMA main-chain at the molecular ratio [PMA monomer]/[CTA2-C15-$N_3$] = 1/1, *via* click chemistry using Cu(I)Br and PMDETA in DMF (Fournier & Du Prez, 2008), as described in Fig. 1b.

The chromatograms (RI) and plots of the molecular weight determined by MALS ($M_w$) of PPMA and PPMA-g-(CTA2-C15) are shown in Fig. 2. No peak corresponding to a free CTA2-C15-$N_3$ was observed in its chromatogram as shown in Fig. 2b. The characteristics of the copolymers are summarized in Table 1. In $^{1}H$ NMR spectrum of PPMA-g-(CTA2-C15) in Fig. 3a, the resonances assigned to the triazole appeared at 7.93 ppm and that assigned to the alkyne proton of the PPMA main-chain disappeared. In Fig. 3b, $^{13}C$ resonances assigned to the triazole were observed at 124.4 and 141.6 ppm. The heteronuclear connectivity (CH) of the triazole was confirmed by its HSQC spectrum (data not shown). The relative integral area of the triazole (1H) to the ring-H (14H) was 1.12–14, indicating a quantitative formation of the triazole. In the FT-IR spectrum of PPMA-g-(CTA2-C15) in Fig. 4c, disappearance of the $N_3$ absorbance at 2120 $cm^{-1}$ after click reaction supports a quantitative formation of the triazole.

*3.2. Structural characterization of PPMA-g-(CTA2-C15)*

Conformational structure of PPMA-g-(CTA2-C15) in chloroform was investigated by means of SEC–MALS measurements. The absolute molecular weight ($M_w$) of PPMA chain was determined to be $6.94 \times 10^4$ by MALS measurements. The weight average degree of polymerization of the PPMA ($DP_w$, $X+Y$) was calculated as $5.59 \times 10^2$ from $M_w$(PPMA)/$M$(PMA), where the molecular weight of PMA monomer is 124.21. The molecular weight ($M_{w,PS}$) ($7.58 \times 10^4$) of PPMA-g-(CTA2-C15) was underestimated by PS standards, compared to the absolute molecular weight ($M_w$) ($42.2 \times 10^4$) determined by MALS measurements. It is well known that SEC, when calibrated only with linear standard polymers such as polystyrene (PS), severely underestimates the molecular weight of a branched polymer which has a more compact molecular volume in solution than that of a corresponding linear polymer with the same molecular weight (Ito et al., 1992). This fact supports successful grafting of CTA2-C15-$N_3$ onto PPMA and the branched structures of the copolymers with CTA side-chains as

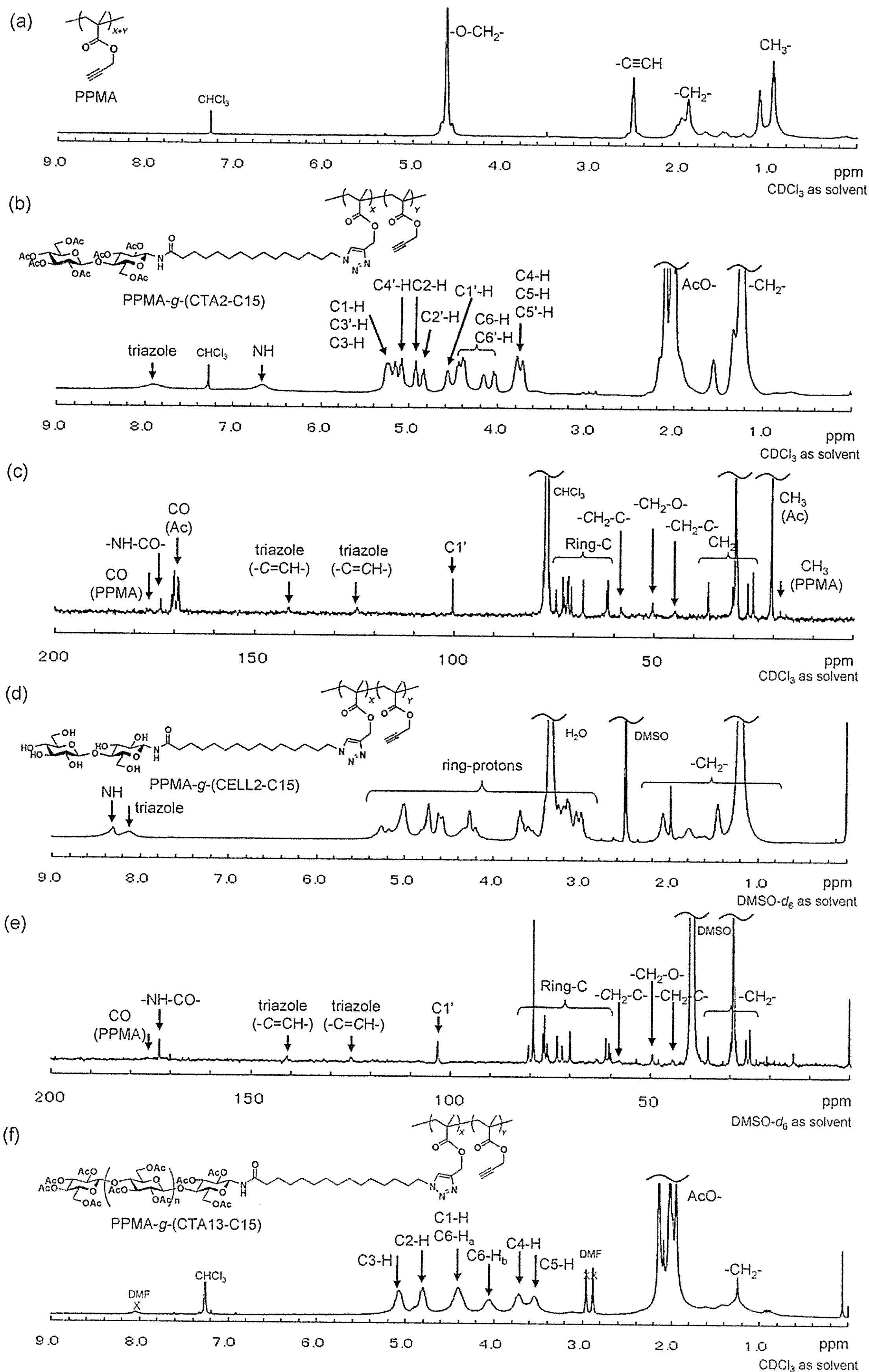


**Fig. 3.** (a) $^1$H NMR spectrum of PPMA, (b) $^1$H and (c) $^{13}$C NMR spectra of PPMA-g-(CTA2-C15), (d) $^1$H and (e) $^{13}$C NMR spectra of PPMA-g-(CELL2-C15) (f) $^1$H NMR spectrum of PPMA-g-(CTA13-C15).

**Table 1**
Characteristics of PPMA, PPMA-g-(CTA2-C15) and PPMA-g-(CTA13-C15).

| Polymers | $A$(%)[a] | Yield (%)[c] | $M_{n,PS}$ ($10^{-4}$)[d] | $M_{w,PS}$ ($10^{-4}$)[d] | $M_{w,PS}/M_{n,PS}$[d] | $M_w$ ($10^{-4}$) | $X+Y$ ($10^{-2}$)[f] | $X$ ($10^{-2}$) | $Y$ ($10^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|
| PPMA | | | 1.92 | 3.54 | 1.84 | 6.94[e] | 5.59 | | 5.59 |
| PPMA-g-(CTA2-C15) | [b] | 88.6 | 7.58 | 22.4 | 2.95 | 42.2[e] | 5.59 | 4.03[g] | 1.56[h] |
| PPMA-g-(CTA13-C15) | 43.8 | 26.6 | 22.9 | 87.5 | 3.81 | 137.57[j] | 5.59 | 2.45[i] | 3.14[h] |

[a] Peak area ratio (%) of the graft copolymer to total peak area of the graft copolymer and remaining CTA-C15-$N_3$ calculated from SEC(RI) elution curves.
[b] Quantitative.
[c] Yield of the graft copolymers.
[d] Estimated by polystyrene standards.
[e] Determined by MALS measurements.
[f] DPw of PPMA main-chain calculated as $X+Y=M_w$(PPMA)/$M$(PMA).
[g] Number of CTA2-C15 chains calculated from $X=(M_w$(PPMA-g-(CTA2-C15)) − $M_w$(PPMA))/$M_{w,PS}$(CTA2-C15-$N_3$). $M$(PMA) = 124.21. $M$(CTA2-C15-$N_3$) = 874.97.
[h] Number of PMA units calculated from $Y=(X+Y)-X$.
[i] Number of CTA13-C15 graft chains calculated from $X$ = DP$_w$(PPMA) × $A$(%).
[j] Molecular weight calculated from $M_w=M_w$(PPMA) + $X\times M_{w,PS}$ (CTA13-C15-$N_3$). $M_{n,PS}$ and $M_{w,PS}$ of CTA13-C15-$N_3$ = $4.12\times10^3$ and $5.33\times10^3$.

discussed in our previous articles (Enomoto-Rogers, Kamitakahara, Nakayama, et al., 2009; Enomoto-Rogers, Kamitakahara, Takano, et al., 2009). The PPMA-g-(CTA2-C15) has the same PPMA main-chain with the same DP$_w$ value. The number of CTA chains ($X$) of PPMA-g-(CTA2-C15) was calculated as $4.03\times10^2$ from $X=(M_w$ (PPMA-g-(CTA2-C15)) − $M_w$ (PPMA))/$M$(CTA2-C15-$N_3$). The number of PMA monomers ($Y$) was calculated as $1.56\times10^2$ from $Y=(X+Y)-X$.

### 3.3. Preparation and characterization of PPMA-g-(CTA13-C15)

Under conditions the same as those for CTA2-C15-$N_3$, $N$-(15-azidopentadecanoyl)-tri-$O$-acetyl-β-cellulosylamine (CTA13-C15-$N_3$, DP$_n$ = 13) was grafted onto the PPMA main-chain with the molecular ratio [PMA monomer]/[CTA13-C15-$N_3$] = 100% to obtain PPMA-g-(CTA13-C15), as described in Fig. 1b. In chromatogram of PPMA-g-(CTA13-C15) before purification, the two

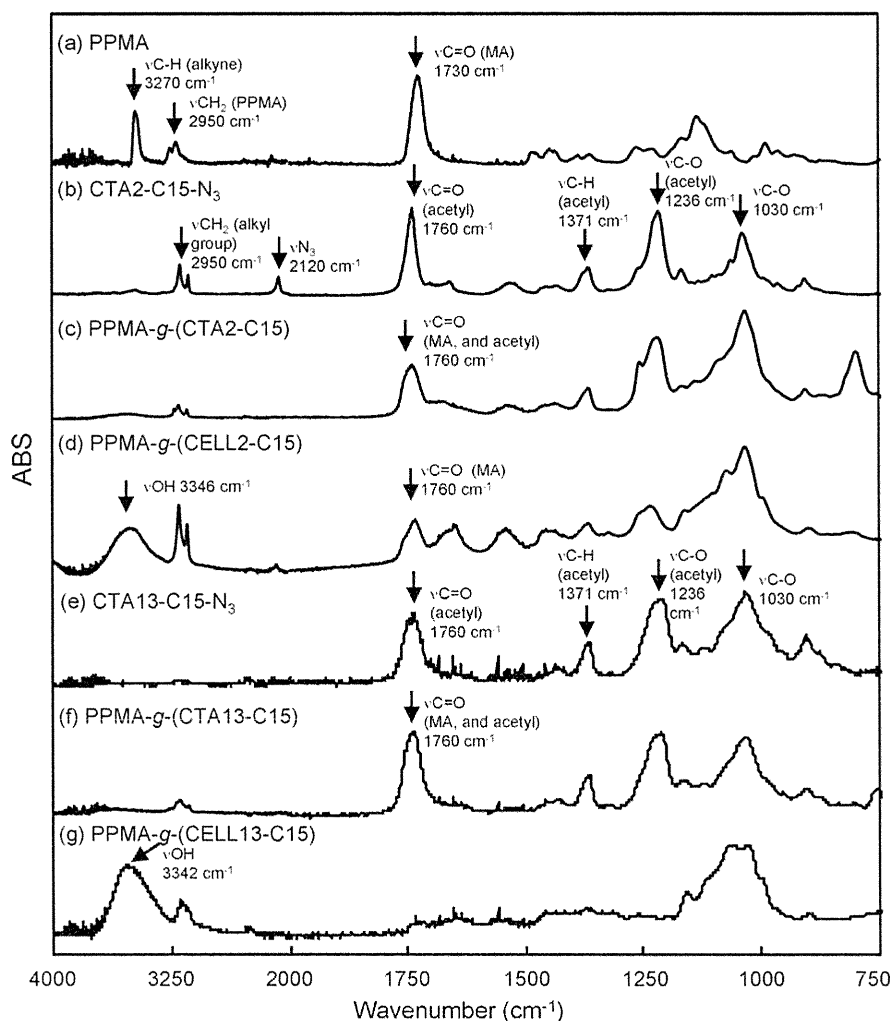


**Fig. 4.** FT-IR spectra of: (a) PPMA, (b) CTA2-C15-$N_3$, (c) PPMA-g-(CTA2-C15), (d) PPMA-g-(CELL2-C15), (e) CTA13-C15-$N_3$, (f) PPMA-g-(CTA13-C15) and (g) PPMA-g-(CELL13-C15).

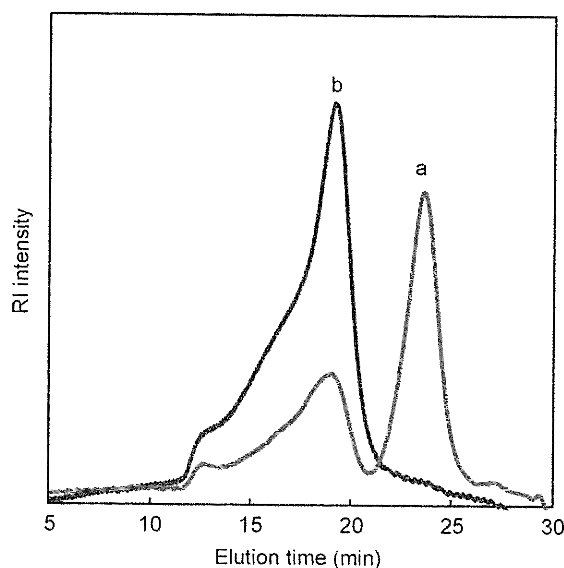


**Fig. 5.** Chromatograms (RI) of PPMA-*g*-(CTA13-C15) (a) before and (b) after purification.

peaks consisting of a remaining CTA13-C15-$N_3$ and the fraction with higher molecular weight were observed, as shown in Fig. 5a, indicating a formation of graft copolymer with CTA side-chains. The high-molecular-weight fraction was collected by SEC system equipped with a fraction collector, and PPMA-*g*-(CTA13-C15) was obtained in 26.6% yield as shown in Fig. 5b. In both FT-IR and $^1$H NMR spectra of purified PPMA-*g*-(CTA13-C15), peaks assigned to CTA chains were observed in Figs. 3f and 4f, indicating that the compounds are composed of CTA chains. The resonances of the triazole and PPMA of PPMA-*g*-(CTA13-C15) were however not identified in both NMR and FT-IR spectra, because of the low PPMA content of the copolymer.

The $M_{n,PS}$ and $M_{w,PS}$ of PPMA-*g*-(CTA13-C15) were estimated to be $2.29 \times 10^5$ and $8.75 \times 10^5$, respectively by SEC analysis using PS standards. The molecular weight of the branched copolymer should be underestimated by PS standards compared to the absolute molecular weight determined by MALS analysis, as discussed in the previous section. It was, however, impossible to measure an absolute molecular weight of PPMA-*g*-(CTA13-C15) by MALS analysis because of high scattering intensity of the compounds (see supplemental data). This high intensity is due to small amount of insoluble fraction with a large radius, which could not be removed and not dispersed at low concentration or after long-term storage in chloroform. The molecular weight was calculated as $1.2 \times 10^7$, and overestimated compared to an possible maximum value ($3.03 \times 10^6$), which was calculated by initial molar ratio [CTA13-C15-$N_3$]/[PMA] = 1.0 and 100% grafting rate.

According to the chromatogram of refractive index (RI), the area ratio of the high molecular fraction to the total area including the remaining CTA13-C15-$N_3$ was $A(\%) = 43.8\%$. Therefore, $X$ value of PPMA-*g*-(CTA13-C15) was calculated as $2.45 \times 10^2$ from $X = DP_w(\text{PPMA}) \times A(\%) = 5.59 \times 10^2 \times 43.8(\%)$, on the assumption that the initial molar ratio of CTA13-C15-$N_3$ to PMA monomer unit was 1.0. The number of PMA units ($Y$) in PPMA-*g*-(CTA13-C15) was calculated to be $3.14 \times 10^2$ from $Y = DP_w(\text{PPMA}) - X$. The molecular weight of PPMA-*g*-(CTA13-C15) was estimated to be $1.38 \times 10^6$ from $M(\text{PPMA-g-(CTA13-C15)}) = M_w(\text{PPMA}) + X \times M_{w,PS}(\text{CTA13-C15-N}_3) = 6.94 \times 10^4 + 2.45 \times 10^2 \times 5.33 \times 10^3$. The density of cellulose side-chains per PPMA main-chain increased compared to that of the copolymer PMMA-*g*-(CTA13-C15) (3.84 CTA side-chains per PMMA main-chain with $DP_w$ of $4.14 \times 10^2$) obtained *via* radical copolymerization of a cellulose macromonomer with methyl methacrylate studied previously (Enomoto-Rogers, Kamitakahara, Takano, et al., 2009). Thus, "graft onto" strategy and "click reaction" were efficient methods to obtain a copolymer with cellulosic side-chains.

### 3.4. Preparation and characterization of PPMA-g-(CELL2-C15) and PPMA-g-(CELL13-C15)

PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15) were treated with sodium methoxide to remove acetyl groups, as described in Fig. 1b, and the deprotected copolymers, PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15), were obtained. Completion of deacetylation was supported by a strong OH absorbance appeared at $\nu = 3446\,\text{cm}^{-1}$ in FT-IR spectrum as shown in Fig. 4d and g. The PPMA-*g*-(CELL2-C15) was soluble in DMSO, and insoluble in water. The PPMA-*g*-(CELL13-C15) was insoluble in water and other common solvents, such as DMSO and DMF. In $^1$H NMR spectrum of PPMA-*g*-(CELL2-C15) in DMSO-$d_6$ (Fig. 3d), the resonances of acetyl protons disappeared, and those of ring-protons of cellobiose moieties remained. In addition, the resonances at 8.14 and 8.32 ppm were assigned to the triazole and amide protons (NH), respectively. In the $^{13}$C NMR spectrum (Fig. 3e), the resonances assigned to two carbons of the triazole were observed at 124.4 and 141.6 ppm. The carbonyl carbons of the ester linkage (O–CO) of PPMA and the amide linkage (C1-NH-CO) remained at 176.0 and 172.9 ppm, respectively. These data indicate that the acetyl groups of the copolymers were selectively removed without cleavage of the triazole, ester and amide linkages. The comb-shaped copolymers having cellulose side-chains with "head-to-tail" orientation were successfully obtained.

### 3.5. X-ray diffraction measurements

In general, cellulose I, the native form, is believed to have parallel orientation (Gardner & Blackwel, 1974; Sugiyama, Vuong, & Chanzy, 1991). On the other hand, cellulose II, the regenerated or mercellized form, is believed to have anti-parallel orientation (Kolpak & Blackwell, 1976; Langan, Nishiyama, & Chanzy, 1999). X-ray diffraction measurements were carried out to analyze crystalline pattern of PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15). The cellobiose chains of PPMA-*g*-(CELL2-C15) showed no crystalline pattern as shown in Fig. 6c. The cellulose chains of PPMA-*g*-(CELL13-C15) had crystalline structure with crystallinity index = 42.2%, as shown in Fig. 6e, but exhibited crystalline pattern of cellulose II with three (1 −1 1), (1 1 0), and (2 0 0) reflections located at $d = 0.739$, 0.451, and 0.407 nm, respectively (Isogai, Usuda, Kato, Uryu, & Atalla, 1989). The reason for amorphous pattern of cellulose is likely that crystallization of cellulose chains *via* hydrogen bonding was inhibited by their covalent grafting on PPMA chain at the longer interchain distances than those suitable for the crystallization, as discussed in our previous work (Enomoto-Rogers, Kamitakahara, Yoshinaga, & Takano, 2011b). Regarding the crystalline structure, there are some possibilities for the structures of cellulose chains of PPMA-*g*-(CELL13-C15). Parallel cellulose chains might show diffraction pattern of cellulose II, or anti-parallel cellulose chains might be formed by interdigitation of cellulose chains on the PPMA chain and give diffraction pattern of cellulose II. In our previous study, nanoparticles consisting with cellulose chains having radial "head-to-tail" orientation showed a crystalline pattern of cellulose II (Enomoto-Rogers, Kamitakahara, Yoshinaga, & Takano, 2011a). Thus, the XRD data of PPMA-*g*-(CELL13-C15), which has cellulose side-chains with "head-to-tail" orientation, suggested the possibility that not only anti-parallel but also "parallel" orientations of cellulose chains might give a crystal structure of cellulose II.

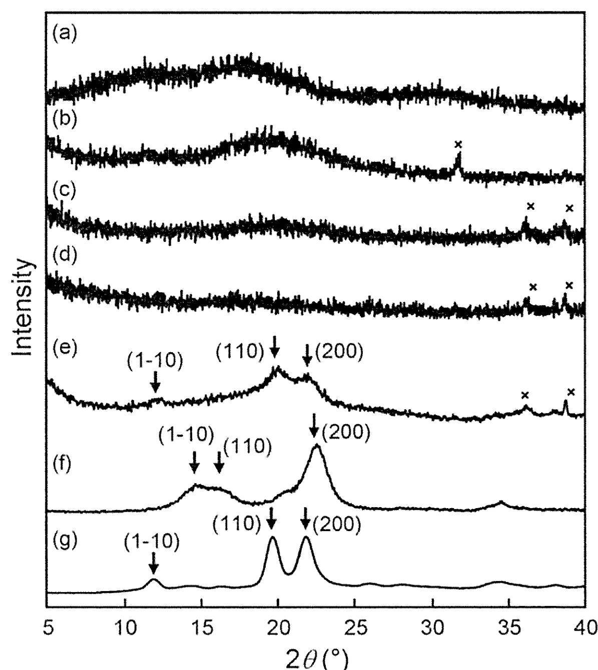


**Fig. 6.** Wide angle X-ray diffractgrams of: (a) PPMA, (b) PPMA-*g*-(CTA2-C15), (c) PPMA-*g*-(CELL2-C15), (d) PPMA-*g*-(CTA13-C15), (e) PPMA-*g*-(CELL13-C15), (f) microcrystalline cellulose (cellulose I) and (g) regenerated cellulose (cellulose II).

## 4. Conclusions

Comb-shaped graft copolymers with cellulose triacetate (CTA) side-chains, PPMA-g-(CTA2-C15) and PPMA-g-(CTA13-C15), were prepared by grafting CTA2-C15-$N_3$ and CTA13-C15-$N_3$ ($DP_n$ = 13) onto PPMA ($DP_w$ = 5.59 × $10^2$), respectively, *via* "click chemistry". The numbers of CTA side-chains (*X*) of PPMA-g-(CTA2-C15) and PPMA-g-(CTA13-C15) were calculated as 4.03 × $10^2$ and 2.45 × $10^2$, respectively. The density of cellulose side-chains per main-chain increased compared to that of the copolymers obtained *via* radical copolymerization of a cellulose macromonomer. PPMA-g-(CELL2-C15) and PPMA-g-(CELL13-C15) were successfully obtained by deacetylation of PPMA-g-(CTA2-C15) and PPMA-g-(CTA13-C15), respectively. PPMA-g-(CELL13-C15) exhibited reflection pattern of cellulose II, suggesting the possibility that not only anti-parallel but also parallel orientations of cellulose chains might give a crystal structure of cellulose II.


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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.carbpol.2011.10.055.

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# Synthesis of blockwise alkylated tetrasaccharide–organic quantum dot complexes and their utilization for live cell labeling with low cytotoxicity

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**Abstract** Bioimaging is a key to understanding immune responses, cell differentiation, and development. Quantum dots (QDs) conjugated with monoclonal antibodies and other biomolecules are currently utilized for flow cytometry and immunohistochemistry, but monoclonal antibody–QD complexes are of limited use when cell surface markers are not available. In this study, we synthesized novel amphiphilic blockwise alkylated tetrasaccharides and developed a simple method for labeling a wide variety of live cells with organic QDs encapsulated with these carbohydrates. The novel amphiphilic blockwise alkylated tetrasaccharides were as follows: 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 (**1**), methyl β-D-galactopyranosyl-(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 (**2**), ethyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside, (**3**), and ethyl β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside (**4**). The newly synthesized blockwise alkylated tetrasaccharides spontaneously assembled into micelle-like particles, in which the hydrophobic moiety of the blockwise alkylated tetrasaccharides played an important role. They were less toxic to human cells than octyl β-D-glucopyranoside, a commonly used amphiphilic glucoside. Flow cytometry and confocal laser scanning microscopy revealed that the blockwise alkylated tetrasaccharide–organic QD complexes were stably attached to live cells. The affinity of compounds 1 and 2 to the live cell surface was slightly higher than that of compounds 3 and 4. Because the preparation of these carbohydrate–QD complexes is simple and does not require sophisticated equipment, and because the complexes can be autonomously attached to a wide spectrum of cell lines, they can be used as cell labeling reagents in biomedical studies.

**Keywords** Cello-oligosaccharide · Carbohydrate · Organic quantum dots · Live cell labeling

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## Introduction

Quantum dots (QDs) are semiconductor nanoparticles that have been widely used for flow cytometry (Wu et al. 2010; DePasquale and Way 2009; Kapoor et al. 2009; Ibanez-Peral et al. 2008; Buller et al. 2007; Ferrari and Bergquist 2007; Kahn et al. 2006a; Kahn et al. 2006b; Roederer et al. 2004), immunohistochemistry (Xing et al. 2007; Shi et al. 2008; Chen et al. 2009), cell tracking (Derfus et al. 2004; Lei et al. 2008), and bioimaging (Zrazhevskiy et al. 2010; Kim et al. 2008). Because QDs are synthesized in organic solvents and are hydrophobic, their surfaces must be chemically modified with hydrophilic coatings for use in biomedical studies. An alternative means of dispersing QDs in aqueous solvents is through the use of liposomes (Chen et al. 2006; Dudu et al. 2008) and polymeric micelles composed of block copolymers (Cheyne and Moffitt 2007). Liposomes can be formed spontaneously when phosphatidylcholine derivatives assemble to form micelle-like structures; however an extruder equipped with a special filter is indispensable to prepare liposomes with monodisperse diameters. It is therefore difficult to prepare liposomes in standard biomedical laboratories. The preparation of polymeric micelles, which usually consist of amphiphilic polymers (Nida et al. 2008), requires sophisticated equipment and technique if the polymers are to have well-controlled monodisperse structures. Accordingly, the development of a new carrier consisting of a simple compound with low molecular weight to replace phospholipids, thereby simplifying the preparation of micelles, would be beneficial.

Interactions between live cells and bacteria, viruses, cancer cells, enzymes, and antibodies are essential for biological recognition. It is therefore important to identify the roles of the molecules expressed on the surfaces of live cells. The use of fluorescent probes to label live cells, and especially the surfaces of live cells, is a promising tool to analyze such biological recognitions. QDs have recently been utilized as fluorescent probes, being relatively bright and detectable by means of confocal laser microscopy as well as transmission electron microscopy. The physicochemical properties of QDs have allowed QD-antibody conjugates to be used for labeling of specific surface proteins of live cells (Sun et al. 2001; Jaiswal et al. 2003). This method, however, is applicable only for the limited subset of cells whose cell surface protein profiles have been identified. Because of this disadvantage associated with QD-antibody conjugates, the development of a novel strategy that will allow a wide spectrum of cells to be labeled is critical. Because live cells mostly express cell surface receptors that are specific to carbohydrates during migration, development, and homeostasis, it is possible to harness this carbohydrate-mediated recognition by live cells for the development of a versatile system for cell labeling.

We have recently reported a mixture of blockwise methylated cello-oligosaccharides (Kamitakahara et al. 2006), monodisperse diblock-trimer, -tetramer, -pentamer, -hexamer (Kamitakahara et al. 2007), and ABA and BAB tri-block-hexamers (Kamitakahara and Nakatsubo 2010). A mixture of blockwise methylated cello-oligosaccharides self-assembled to form nanoparticles (Kamitakahara et al. 2008). We thus attempted to use the novel amphiphilic tetrasaccharides derivatives for the efficient dispersion of organic QDs and for live cell imaging. As we have already developed diblock and triblock cello-oligomers, the synthetic strategy can be generally used for the combinatorial synthesis of a series of blockwise alkylated cello-oligosaccharides. In this study, we describe an improved synthetic method for blockwise alkylated cello-tetramers, which spontaneously assemble to form micelle-like structures and can disperse organic QDs in aqueous solvents. In addition, we show that the novel blockwise alkylated tetrasaccharides–QD complexes attach to a wide variety of human cell lines by simply mixing cells with the carbohydrate–QD complexes.

## Experimental procedures

### Physicochemical measurements

The $^{1}$H- and $^{13}$C-NMR spectra were recorded on a Varian INOVA 300 spectrometer in chloroform-*d* with tetramethylsilane as an internal standard, using pulse sequences for one- and two-dimensional spectra. Dynamic light scattering data were collected on a Particle Size Analyzer ELSZ-2 (Otsuka Electronics, Osaka, Japan). Hydrodynamic diameters were determined using a cumulant method.

Synthesis of blockwise ethylated tetrasaccharides

*Phenyl 4,6-O-p-methoxybenzylidene-2,3-di-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-1-thio-β-D-glucopyranoside (6)*

To a solution of phenyl 4,6-*O*-*p*-methoxybenzylidene-β-D-glucopyranosyl-(1 → 4)-1-thio-β-D-glucopyranoside (**5**) (180 mg, 0.326 mmol) in DMF (5 mL), sodium hydride (NaH, 60% in mineral oil, 98 mg, 2.445 mmol) and ethyl iodide (196 μL, 2.445 mmol) were added at room temperature and the reaction mixture was stirred at room temperature overnight. The reaction was monitored by means of analytical TLC. NaH (600 mg) and ethyl iodide (1.2 mL) were then added to the mixture, which was then stirred at 50 °C for an additional 23 h. The reaction mixture was extracted with ethyl acetate, washed with distilled water, and brine, dried over sodium sulfate, and concentrated to dryness to produce crude crystals. The products were washed with cold ethanol to produce purified crystals (143.4 mg, 68% yield).

$^1$H-NMR ($CDCl_3$): δ1.14–1.25 ($OCH_2CH_3$), 3.08 (t, 1H, *J* = 8.1, H2′), 3.20 (t, 1H, *J* = 9.0, H2), 3.32 (t, 1H, *J* = 8.7, H3), 3.46 (t, 1H, *J* = 7.2, H3′), 3.80 ($OCH_3$), 4.40 (dd, 1H, *J* = 4.8, *J* = 10.2, H6′), 4.49 (d, 1H, *J* = 7.5, H1′), 4.51 (d, 1H, *J* = 9.6, H1), 5.49 (s, 1H, =*C*HPh), 6.89 (d, 2H, *J* = 8.7, aromatic protons ($-CH-)_2$ C–$OCH_3$), 7.40 (d, 2H, *J* = 9.0, aromatic protons =CH–C($-CH-)_2$–), 7.2–7.3, 7.5–7.6 (aromatic protons, –SPh); $^{13}$C-NMR ($CDCl_3$): δ15.4, 15.6, 15.8, 55.2 (Ph–$OCH_3$), 65.9, 66.7, 68.4, 68.6, 68.8, 77.7, 79.3, 80.3, 81.3, 81.5, 82.4, 84.9, 87.3 (C1), 100.9 (=*C*HPh), 103.4 (C1′), 113.5 (aromatic, ($-CH-)_2$ C–$OCH_3$), 127.2, 128.7, 129.8, 131.7, 133.9 (aromatic, C1–S–C($-CH-)_2$), 159.9 (aromatic, =*C*–$OCH_3$).

*Phenyl 4-O-p-methoxybenzyl-2,3-di-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-1-thio-β-D-glucopyranoside (7)*

To a solution of phenyl 4,6-*O*-*p*-methoxybenzylidene-2,3-di-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**6**) (0.2513 g, 0.363 mmol) in anhydrous dichloromethane (0.41 mL), borane–tetrahydrofuran complex in tetrahydrofuran (2.6 mL, 14.5 mmol) was added at −20 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 38.8 μL, 0.214 μmol) was then added to the reaction mixture. The temperature of the reaction mixture was gradually brought up to 0 °C over a period of 6 h, then reduced to −15 °C. Triethylamine (0.72 mL) and a small amount of methanol were added to the solution. The mixture was diluted with ethyl acetate, washed with 4 *N* HCl, saturated aq. $NaHCO_3$ and brine, dried over $Na_2SO_4$, and concentrated to dryness to produce crude crystals. The products were washed with *n*-hexane to produce purified crystals (0.1577 g, 0.227 μmol, 63% yield).

$^1$H-NMR ($CDCl_3$): δ1.17, 1.18, 1.22, 1.26, (t, 3H, 6H, 3H, 3H, $OCH_2CH_3$), 3.04 (t, 1H, *J* = 8.1, H2′), 3.80 (Ph-$OCH_3$), 4.35 (d, 1H, *J* = 7.8, H1′), 4.52 (d, 1H, *J* = 9.9, H1), 4.50–4.54 (d, 1H, $CH_2$Ph–$OCH_3$), 4.77 (d, 1H, *J* = 10.5, $CH_2$Ph–$OCH_3$);$^{13}$C-NMR ($CDCl_3$): δ15.3, 15.6, 15.6, 15.7, 15.9, 55.2 (Ph–$OCH_3$), 62.5, 66.7, 68.3, 68.6, 68.9, 68.9, 69.0, 74.5 ($CH_2$Ph), 74.7, 77.2, 78.0, 79.3, 80.6, 82.5, 84.5, 85.0, 87.5 (C1), 102.5 (C1′), 113.9, 127.1, 128.7, 129.7, 130.1, 131.5, 134.2, 159.4 (aromatic, =*C*–$OCH_3$).

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

To a solution of phenyl 4-*O*-*p*-methoxybenzyl-2,3-di-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**7**) (120 mg, 0.173 mmol) in THF (5 mL), NaH (60% in mineral oil, 8.3 mg, 0.207 mmol) and ethyl iodide (16.6 μL, 0.207 mmol) were added at room temperature and the reaction mixture was stirred at room temperature for 3.7 h, then heated to 50 °C overnight. The reaction was monitored by means of analytical TLC. NaH (10.7 mg) and ethyl iodide (17 μL) were added to the mixture, which was then stirred at 50 °C for an additional 40 h. The reaction mixture was extracted with ethyl acetate, washed with distilled water and brine, dried over sodium sulfate, and concentrated to dryness to produce crude crystals (117.8 mg).

$^1$H-NMR ($CDCl_3$): δ1.14–1.26 ($CH_3$–$CH_2$–), 3.03 (t, 1H, *J* = 8.1, H2′), 3.80 (s, 3H, –$OCH_3$), 4.31 (d, 1H, *J* = 8.1, H1′), 4.52 (d, 1H, *J* = 9.6, H1), 4.55 (d, 1H, *J* = 10.8, –$CH_2$Ph), 4.76 (d, 1H, *J* = 10.5, –$CH_2$Ph), 6.88 (d, 2H, *J* = 8.7, aromatic), 7.22–7.26 (5H, aromatic), 7.54 (2H, *J* = 7.8, aromatic); $^{13}$C-NMR ($CDCl_3$): δ15.2, 15.3, 15.5, 15.6, 15.7, 16.0, 55.3, 66.6, 66.7, 68.5, 68.8, 68.8, 69.0, 74.6 (–$CH_2$Ph), 74.7 (C5′), 77.2 (C4′), 77.6 (C4), 79.4 (C5), 80.4 (C2), 82.4

(C2′), 84.9, 85.0, 87.4 (C1), 103.2 (C1′), 113.8, 127.1, 128.7, 129.7, 130.6, 131.6, 134.2, 159.2.

*Phenyl 2,3,6-tri-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-1-thio-β-D-glucopyranoside (**9**)*

To a solution of phenyl 4-*O*-*p*-methoxybenzyl-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**8**) (100 mg, 0.138 mmol) in acetonitrile/distilled water (5 mL, 9/1, v/v), ammonium cerium (IV) nitrate (151.7 mg, 0.277 mmol, 2 equiv) was added at 0 °C. After stirring for 4.7 h, the reaction mixture was extracted with ethyl acetate, washed with saturated aq. $NaHCO_3$, distilled water, and brine, dried over sodium sulfate, and concentrated to dryness to produce crude oil. The crude product was purified by preparative TLC (eluent: ethyl acetate/*n*-hexane = 1/2, v/v) to produce colorless crystals (68.4 mg, 82% yield).

$^1$H-NMR ($CDCl_3$): δ1.14–1.26 (18H, $CH_3$–$CH_2$–), 3.02 (t, 1H, *J* = 7.8, H2′), 3.19 (d, 2H, *J* = 9, H2, H3′), 3.31 (t, 1H, *J* = 8.7, H3), 3.30–3.38 (m, 2H, H5, H5′), 3.48–3.58 (H4′), 3.58–3.66 (H6′), 3.66–3.73, 3.73–3.79 (H6), 3.74–3.82 (H6′), 4.38 (d, 1H, *J* = 7.8, H1′), 4,51 (d, 1H, *J* = 9.6, H1), 7.2–7.3 (3H, aromatic H), 7.54 (d, 2H, *J* = 8.1, aromatic H), $^{13}$C-NMR ($CDCl_3$): δ15.1, 15.3, 15.6, 15.7, 15.8, 66.7, 67.2, 68.3, 68.5, 68.6, 68.7, 68.9, 71.7 (C6′), 72.7 (C5′), 73.1 (C4′), 77.5 (C4), 79.4 (C5), 80.4 (C2), 81.9 (C2′), 84.3 (C3′), 84.9 (C3), 87.4 (C1), 103.1 (C1′), 127.1, 128.7, 131.6, 134.2.

Synthesis of blockwise alkylated tetrasaccharides

*Phenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-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 (12)*

Phenyl 2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**10**) (59.1 mg, 0.1139 mmol) (Kamitakahara et al. 2006), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl 2,2,2-trichloroacetimidate (Urban et al. 1990) (**11**) (177.5 mg, 0.2278 mmol), and MS4Å (200 mg) were dried in a reaction ampule using a high-vacuum manifold overnight. Dichloromethane (2 mL) was added to the other ampule with $CaH_2$, and degassed by being frozen and thawed several times using the same high-vacuum manifold overnight. The solvent was transferred to the reaction ampule under high vacuum. The reaction ampule was sealed and placed at 0 °C. Trimethylsilyl trifluoromethanesulfonate (4.12 μL, 0.02278 mmol, 20 mol% to the glycosyl acceptor) was added into the reaction ampule through a rubber septum using a syringe. The reaction mixture was stirred at 0 °C for 22.3 h. MS4Å was removed by filtration and washed with ethyl acetate. The combined washings and filtrate were diluted with ethyl acetate, washed with aq. $NaHCO_3$, distilled water, and brine, dried over $Na_2SO_4$, and concentrated to dryness. The product was isolated by preparative TLC (eluent: ethyl acetate/*n*-hexane = 1:1, v/v, twice) to produce phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-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 (**12**) (98.6 mg, 76.1% yield).

$^1$H-NMR ($CDCl_3$): δ1.98, 2.01, 2.01, 2.04, 2.05, 2.09, 2.11 ($COCH_3$), 2.91 (t, 1H, *J* = 8.1, H2′), 3.07 (t, 1H, *J* = 8.7, H2), 3.17 (t, 1H, *J* = 8.7, H3′), 3.18–3.26 (m, 1H, H5′), 3.26 (t, 1H, *J* = 8.7, H3), 3.3–3.4 (m, 1H, H5), 3.37, 3.40, 3.52, 3.54, 3.59 ($OCH_3$), 3.80 (t, 1H, *J* = 9.3, H4″), 4.03 (dd, 1H, *J* = 2.1, *J* = 12.3, H6‴), 4.17 (dd, 1H, *J* = 4.8, *J* = 12.3, H6″), 4.28 (d, 1H, *J* = 7.8, H1′), 4.38 (dd, 1H, *J* = 4.2, *J* = 12.3, H6″), 4.46–4.54 (H6″), 4.50 (d, 1H, *J* = 9.9, H1), 4.51 (d, 1H, *J* = 7.8, H1‴), 4.70 (d, 1H, *J* = 8.1, H1″), 4.88 (dd, 1H, *J* = 8.4, *J* = 9.6, H2″), 4.92 (t, 1H, *J* = 8.4, H2‴), 5.07 (t, 1H, *J* = 9.3, H4‴), 5.14 (t, 1H, *J* = 9.0, H3‴), 5.15 (t, 1H, *J* = 9.6, H3″), 7.2–7.32, 7.5–7.54 (aromatic H); $^{13}$C-NMR: δ20.4, 20.5, 20.6, 20.7 (–$COCH_3$), 59.0, 59.3, 60.3, 60.5, 60.6, 60.7, 61.3 (C6‴), 61.6 (C6″), 67.5 (C4‴), 69.9 (C6 or C6′), 70.1 (C6 or C6′), 71.4 (C2‴), 71.8 (C2″), 71.9 (C5‴), 72.3 (C5″), 72.7 (C3″ or C3‴), 72.7 (C3″ or C3‴), 74.1 (C5′), 76.3 (C4″), 77.0 (C4′), 77.5 (C4), 78.8 (C5), 81.7 (C2), 83.6 (C2′), 84.7 (C3′), 86.5 (C3), 87.2 (C1), 100.2 (C1″), 100.6 (C1‴), 103.1 (C1′), 127.2, 128.7, 131.6, 133.7 (aromatic C), 168.9, 169.2, 169.4, 169.7, 170.1, 170.2, 170.4 (–$OCOCH_3$); MALDI-TOF MS calculated for $C_{50}H_{72}O_{27}S$ = 1136.4; found *m*/*z* $[M + Na]^+$ = 1159.57.

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

To a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**12**) (98.6 mg, 0.0867 mmol) in methanol/dichloromethane (10 mL, 1/4, v/v), *N*-iodosuccinimide (NIS) (27.3 mg, 0.121 mmol, 1.4 equiv) and a catalytic amount of AgOTf were added at room temperature. The reaction mixture was stirred for 1.3 h at room temperature. Solid $NaHCO_3$ was added to the reaction mixture. The mixture was concentrated to dryness. The crude compound was purified by silica gel column chromatography (eluent: dichloromethane, methanol/dichloromethane = 1:4, v/v) and by gel filtration on Sephadex LH-20 (eluent: methanol/dichloromethane, 1/4, v/v) to produce ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (**13**) (87.7 mg, 96% yield).

$^{1}$H-NMR ($CDCl_3$): $\delta$1.99, 2.01, 2.04, 2.05, 2.09, 2.12 (COC$H_3$), 2.94 (t, 1H, $J = 8.4$, H2′), 3.00 (t, $J = 8.1$, H2β), 3.18 (t, 1H, $J = 9.0$, H3′), 3.22 (dd, H2α), 3.38, 3.39, 3.40, 3.42, 3.50, 3.52, 3.53, 3.55, 3.56 (OC$H_3$), 4.03 (dd, $J = 2.4$, $J = 12.6$, H6‴), 4.15 (d, $J = 7.2$, H1β), 4.17 (dd, $J = 4.5$, $J = 11.4$, H6″), 4.25 (d, $J = 8.1$, H1′), 4.38 (dd, $J = 4.5$, $J = 12.6$, H6‴), 4.50 (dd, $J = 1.9$, $J = 12.6$, H6″), 4.51 (d, $J = 7.5$, H1‴), 4.71 (d, $J = 8.1$, H1″), 4.83 (d, $J = 3.6$, H1α), 4.89 (t, $J = 9.6$, H2″), 4.92 (t, $J = 8.1$, H2‴), 5.07 (t, $J = 9.3$, H4‴), 5.15 (t, $J = 9.6$, H3″); $^{13}$C-NMR: $\delta$20.4, 20.5, 20.6 (–CO$CH_3$), 20.7, 55.1, 56.8, 58.8, 59.0, 59.3, 59.4, 60.3, 60.4, 60.5, 60.6 (O$CH_3$), 61.3 (C6‴), 61.6 (C6″), 67.5 (C4‴), 69.6 (C5), 69.9 (C6 and C6′), 70.1, 71.4 (C2‴), 71.8 (C5‴), 71.9 (C2″), 72.3 (C5″), 72.7 (C3″ or C3‴), 72.7 (C3″ or C3‴), 74.2 (C5′), 74.4, 76.3 (C4″), 77.0 (C4′), 77.5 (small peak), 77.8 (C4), 80.9 (C2 (C1α)), 81.2 (C3 (C1α)), 82.8 (C2 (C1β)), 83.6 (C2′), 84.4, 84.8 (C3′), 97.3 (C1α), 100.2 (C1″), 100.6 (C1‴), 103.0, 103.2 (C1′), 104.0 (C1β), 168.9, 169.2, 169.5, 169.7, 170.1, 170.2, 170.4 (–OCOCH$_3$); MALDI-TOF MS calculated for $C_{45}H_{70}O_{28}$ = 1058.41; found *m/z* $[M + Na]^+$ = 1181.47.

*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 (1)*

To a solution of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (**13**) (87.7 mg, 0.0829 mmol) in methanol (10 mL), 28% sodium methoxide in methanol (66.3 μL, 1.16 mmol) was added at room temperature. The reaction mixture was kept stirring at room temperature for 2 h. The reaction mixture was neutralized with Dowex in a $H^+$ form to produce 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 (**1**) (61.3 mg, 97% yield).

$^{1}$H-NMR ($D_2O$): $\delta$3.10 (t, $J = 8.4$, H2′), 3.28 (t, $J = 9.0$, H2″), 3.31 (t, $J = 8.4$, H2‴), 3.3–3.4 (H2), 3.37, 3.45, 3.51, 3.57, 3.57 (OC$H_3$), 3.81 (dd, $J = 4.2$, $J = 12.6$, H6‴), 3.86 (dd, $J = 12.0$, H6″), 3.96 (dd, $J = 11.4$, H6‴), 4.39 (d, 1H, $J = 7.8$, H1′), 4.40 (d, $J = 7.5$, H1‴), 4.48 (d, $J = 7.8$, H1″), 4.97 (d, $J = 3.3$, H1α); $^{13}$C-NMR ($D_2O$): $\delta$57.6 (C1α-O$CH_3$), 59.9 (C1β-O$CH_3$), 60.5, 61.1, 61.1, 61.7, 61.8, 62.0, 62.6, 62.7 (C6), 63.1, 63.2 (C6), 71.9, 72.1, 72.5 (C6 or C6′), 72.6 (C6 or C6′), 75.8 (C2″ or C2‴), 75.9 (C2″ or C2‴), 76.2, 76.9, 77.6, 78.0, 78.1, 78.6, 79.2 (C4), 81.3 (C4′), 82.0 (C2 (C1α)), 82.8 (C3 (C1α)), 84.5, 85.1 (C2′), 85.5, 85.9 (C3′), 99.1 (C1α), 104.9 (C1″), 105.2 (C1′), 105.2 (C1‴), 105.6 (C1β); MALDI-TOF MS calculated for $C_{31}H_{56}O_{21}$ = 764.33; found *m/z* $[M + Na]^+$ = 787.462, $[M + K]^+$ = 803.433.

*Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-methyl-1-thio-D-glucopyranoside (15)*

Phenyl 2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**10**) (59.1 mg, 0.1139 mmol), 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl 2,2,2-trichloroacetimidate(Amvamzollo and Sinay 1986; Urban et al. 1990; Dean et al. 1993) (**14**) (177.5 mg, 0.2278 mmol), and MS4Å (200 mg)

were dried in a reaction ampule using a high-vacuum manifold overnight. Dichloromethane (2 mL) was added to the other ampule with $CaH_2$, and degassed by being frozen and thawed several times using the same high-vacuum manifold overnight. The solvent was transferred to the reaction ampule under high vacuum. The reaction ampule was sealed and placed at 0 °C. Trimethylsilyl trifluoromethanesulfonate (4.12 μL, 0.0227 mmol, 20 mol% to the glycosyl acceptor) was added into the reaction ampule through a rubber septum using a syringe. The reaction mixture was stirred at 0 °C for 18.3 h. MS4Å was removed by filtration and washed with ethyl acetate. The combined washings and filtrate were diluted with ethyl acetate, washed with aq. $NaHCO_3$, distilled water, and brine, dried over $Na_2SO_4$, and concentrated to dryness. The product was isolated by preparative TLC (eluent: ethyl acetate/*n*-hexane = 1:1, v/v) to produce phenyl 2,3,4,6-tetra-*O*-acetyl *β*-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-α-D-glucopyranoside (**15α**) (28.9 mg, 22.3%) and phenyl 2,3,4,6-tetra-*O*-acetyl *β*-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-*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 (**15*β***) (75.7 mg, 58.4% yield) (total yield: 80.7%).

**15α**: $^1$H-NMR ($CDCl_3$): δ1.97, 2.05, 2.11, 2.15 ($COCH_3$), 3.36, 3.41, 3.50, 3.54, 3.56, 3.59 ($-OCH_3$), 4.27 (d, 1H, $J = 8.1$, H1′), 4.48 (d, 1H, $J = 7.8$, H1‴), 4.72 (d, 1H, $J = 8.1$, H1″), 4.88 (dd, 1H, $J = 8.1, 9.3$, H2″), 4.95 (dd, 1H, $J = 3.6, 10.5$, H3‴), 4.88 (dd, 1H, $J = 8.1, 9.6$, H2‴), 5.17 (t, 1H, $J = 9.6$, H3″), 5.35 (H4‴), 5.74 (d, 1H, $J = 5.4$, H1), 7.2–7.6 (aromatic H); $^{13}$C-NMR: 20.5, 20.6, 20.8, 58.1, 58.9, 59.1, 59.4, 59.5, 60.4, 60.6, 60.7 (C6‴), 60.8, 60.8, 61.8 (C6″), 66.5 (C4‴), 69.0 (C2‴), 69.8 (C6 or C6′), 70.0 (C6 or C6′), 70.6 (C5‴), 70.9 (C3‴), 72.2 (C2″), 72.4 (C5″), 73.1 (C3″), 74.3 (C5′), 76.2 (C4″), 77.1 (C4′), 77.5 (C4), 80.8 (C5), 81.7 (C2), 83.7 (C2′), 84.9 (C3′), 86.3 (C3), 87.3 (C1), 100.1 (C1″), 101.0 (C1‴), 103.3 (C1′), 126.9, 128.8, 128.9, 130.9, 131.7, 169.1, 169.7, 169.8, 170.1, 170.1, 170.3.

**15*β***: $^1$H-NMR ($CDCl_3$): δ1.97, 2.05, 2.11, 2.16, 2.92 (t, 1H, $J = 8.4$, H2′), 3.07 (t, 1H, $J = 8.7$, H2), 3.18 (t, 1H, $J = 9.0$, H3′), 3.18–3.26 (m, 1H, H5′), 3.32–3.40 (m, 1H, H5), 3.37 (3H, $OCH_3$), 3.40 (3H, $OCH_3$), 3.52 (3H, $OCH_3$), 3.53 (3H, $OCH_3$), 3.59 (6H, $OCH_3$), 3.56–3.64 (H5″), 3.58–3.76 (m, H4, H4′, H6, H6′), 3.82 (t, 1H, $J = 9.0$, H4″), 3.87 (broad t, 1H, $J = 6.6$, H5‴), 4.04–4.14 (2H, H6‴), 4.17 (dd, 1H, $J = 4.8, 11.7$, H6″), 4.28 (d, 1H, $J = 7.5$, H1′), 4.44–4.50 (dd, 1H, $J = 1.7$, H6″), 4.48 (d, 1H, $J = 7.7$, H1‴), 4.50 (d, 1H, $J = 9.9$, H1), 4.71 (d, 1H, $J = 8.1$, H1″), 4.88 (t, 1H, $J = 9.6$, H2″), 4.95 (dd, 1H, $J = 3.3, 10.5$, H3‴), 5.11 (dd, 1H, $J = 10.4, 7.8$, H2‴), 5.17 (t, 1H, $J = 9.3$, H3″), 5.35 (broad d, 1H, $J = 3.4$, H4‴); $^{13}$C-NMR: δ 20.4, 20.6, 20.7, 20.7, 59.0, 59.4, 60.4, 60.5 (C6‴), 60.6, 60.7, 60.7, 61.7 (C6″), 66.4 (C4‴), 68.9 (C2‴), 70.0 (C6 or C6′), 70.2 (C6 or C6′), 70.5 (C5‴), 70.8 (C3‴), 72.1 (C2″), 72.3 (C5″), 73.1 (C3″), 74.2 (C5′), 76.1 (C4″), 77.0 (C4′), 77.6 (C4), 78.8 (C5), 81.8 (C2), 83.8 (C2′), 84.8 (C3′), 86.6 (C3), 87.2 (C1), 100.1 (C1″), 101.0 (C1‴), 103.2 (C1′), 127.2, 128.7, 131.7, 169.0, 169.6, 169.7, 170.0, 170.1, 170.3; MALDI-TOF MS calculated for $C_{50}H_{72}O_{27}S = 1136.4$; found *m*/*z* $[M + Na]^+ =$ 1159.45.

*Methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-methyl-D-glucopyranoside (**16**)*

To a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-*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 (**15*β***) (75.7 mg, 0.0666 mmol) in methanol/dichloromethane (10 mL, 1/4, v/v), NIS (21.0 mg, 0.0932 mmol, 1.4 equiv) and a catalytic amount of AgOTf were added at room temperature. The reaction mixture was stirred for 0.5 h at room temperature. Solid $NaHCO_3$ was then added to the reaction mixture, which was concentrated to dryness. The crude compound was purified by silica gel column chromatography (eluent: dichloromethane, methanol/dichloromethane = 1:4, v/v) and by gel filtration on Sephadex LH-20 (eluent: methanol/dichloromethane, 1/4, v/v) to produce methyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (**16**) (76.2 mg, quantitative yield).

$^{13}$C-NMR: δ 20.4, 20.5, 20.7, 55.1, 56.8, 58.9, 59.0, 59.4, 60.3, 60.5, 60.6, 61.8, 66.5, 67.9, 69.0, 69.6, 70.5, 70.8, 72.1, 72.3, 73.1, 74.2, 77.8, 80.9, 81.3, 83.6, 84.8, 97.4 (C1α), 100.1, 101.0, 103.2, 104.1 (C1β), 169.0, 169.6, 169.7, 170.0, 170.1, 170.3.

Compound **16** (28.9 mg, 0.0254 mmol) was separated by preparative TLC (eluent: ethyl acetate: *n*-hexane = 1:1, v/v) to give α- (**16α**, 5.4 mg, 0.0071 mmol) and β- (**16β**, 9.9 mg, 0.013 mmol) anomers (79% yield).

**16α**: $^{1}$H-NMR ($CDCl_3$): δ1.97, 2.04, 2.05, 2.07, 2.11, 2.16 (Ac), 2.94 (dd, 1H, *J* = 7.8, 9.0, H2′), 3.18 (t, 1H, *J* = 9.0, H3′), 3.22 (dd, 1H, *J* = 3.6, 9.9, H2), 3.18–3.26 (H5′), 3.19–3.25 (H3), 3.46–3.54 (H5), 3.39 (3H, $OCH_3$), 3.40 (3H, $OCH_3$), 3.42 (3H, $OCH_3$), 3.51 (3H, $OCH_3$), 3.54 (3H, $OCH_3$), 3.55 (3H, $OCH_3$), 3.57 (3H, $OCH_3$), 3.55–3.82 (H5″, H4, H4′, H6, H6′), 3.79 (t, 1H, *J* = 9.0, H4″), 3.86 (broad t, *J* = 7.2, H5‴), 4.04–4.14 (2H, H6‴), 4.18 (dd, 1H, *J* = 4.5, 11.7, H6″), 4.25 (d, 1H, *J* = 8.1, H1′), 4.45 (1H, H6″), 4.48 (d, 1H, *J* = 7.8, H1‴), 4.72 (d, 1H, *J* = 7.8, H1″), 4.83 (d, 1H, *J* = 3.6, H1), 4.88 (dd, 1H, *J* = 8.1, 9.6, H2″), 4.95 (dd, 1H, *J* = 3.6, 10.5, H3‴), 5.11 (dd, 1H, *J* = 8.1, 10.5, H2‴), 5.17 (t, 1H, *J* = 9.3, H3″), 5.35 (d, 1H, *J* = 3.0, H4‴)

**16β**: $^{1}$H-NMR ($CDCl_3$): δ1.97, 2.06, 2.07, 2.11, 2.17 (Ac), 2.94 (t, 1H, *J* = 9.3, H2′), 3.02 (t, 1H, *J* = 9.0, H2), 3.18–3.26 (m, 1H, H5′), 3.19 (t, 1H, *J* = 9.3, H3′), 3.24 (t, 1H, *J* = 9.0, H3), 3.35 (m, 1H, *J* = 9.9, H5), 3.39 (3H, $OCH_3$), 3.41 (3H, $OCH_3$), 3.54 (6H, $OCH_3$), 3.57 (6H, $OCH_3$), 3.60–3.78 (H5″, H4, H4′, H6, H6′), 3.83 (t, 1H, *J* = 9.3, H4″), 3.88 (broad t, *J* = 7.2, H5‴), 4.04–4.14 (2H, H6‴), 4.17 (d, 1H, *J* = 7.5, H1), 4.18 (dd, 1H, *J* = 4.8, 12.3, H6″), 4.29 (d, 1H, *J* = 7.8, H1′), 4.47 (dd, 1H, *J* = 1.7, 12.2, H6″), 4.49 (d, 1H, *J* = 8.1, H1‴), 4.72 (d, 1H, *J* = 8.1, H1″), 4.88 (dd, 1H, *J* = 7.8, 9.6, H2″), 4.97 (dd, 1H, *J* = 3.3, 10.5, H3‴), 5.11 (dd, 1H, *J* = 7.8, 10.2, H2‴), 5.18 (t, 1H, *J* = 9.6, H3″), 5.35 (broad d, 1H, *J* = 3.0, H4‴); $^{13}$C-NMR: δ 20.5, 20.6, 20.8, 56.9 (C1-$OCH_2CH_3$), 59.1, 59.4, 60.3, 60.4, 60.6, 60.7 (C6‴), 61.9 (C6″), 66.5 (C4‴), 69.0 (C2‴), 70.0 (C6 or C6′), 70.2 (C6 or C6′), 70.5 (C5‴), 70.9 (C3‴), 72.2 (C2″), 72.3 (C5″), 73.1 (C3″), 74.2 (C5′), 74.5 (C5), 76.2 (C4″), 77.2 (C4′), 77.6 (C4), 82.9 (C2), 83.7 (C2′), 84.4 (C3), 84.8 (C3′), 100.2 (C1″), 101.0 (C1‴), 103.1 (C1′), 104.1(C1), 169.2, 169.7, 169.9, 170.2, 170.2, 170.5.

*Methyl β-D-galactopyranosyl-(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 (**2**)*

To a solution of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-D-glucopyranoside (**16**) (76.2 mg, 0.0745 mmol) in methanol (10 mL), 28% sodium methoxide in methanol (53.3 μL, 0.933 mmol) was added at room temperature. The reaction mixture was continuously stirred at room temperature for 2 h. The reaction mixture was neutralized with Dowex in a $H^+$ form to produce methyl β-D-galactopyranosyl-(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 (**2**) (56.1 mg, quantitative yield).

$^{13}$C-NMR ($CDCl_3$): δ 53.4, 55.2, 56.8, 58.9, 59.5, 60.4, 60.7, 67.7, 69.7, 70.1, 72.0, 73.0, 73.2, 74.6, 77.2, 77.8, 80.9, 81.3, 83.6, 97.4, 103.3, 104.1; $^{1}$H-NMR ($D_2O$): δ3.11 (dd, 1H, *J* = 8.1, 9.3, H2′), 3.32 (dd, 1H, *J* = 8.4, 9.6, H2″), 3.38, 3.39, 3.45, 3.51, 3.57, 3.58 ($OCH_3$), 3.89 (d, 1H, *J* = 3.3, H4‴), 3.96 (dd, 1H, *J* = 1.8, 12.3, H6‴), 4.40 (d, 1H, *J* = 7.4, H1′), 4.42 (d, 1H, *J* = 7.8, H1″), 4.43 (d, 1H, *J* = 7.8, H1‴), 4.98 (d, 1H, *J* = 3.6, H1); $^{13}$C-NMR (D2O): δ 57.6 (C1(α)-$OCH_3$), 59.9 (C1(β)-$OCH_3$), 60.5, 61.0, 61.1 (small), 61.7 (s), 61.8, 62.1, 62.6, 62.8 (C6‴), 63.1, 63.7 (C6″), 69.2 (s), 71.2 (C4‴), 71.9 (C5), 72.5 (C6), 72.6 (C6), 73.6, 75.2, 75.8 (C2″), 76.2, 77.0 (C3″), 77.6, 78.0 (C4′), 79.2 (C4), 81.0 (C4″), 82.0 (C3), 82.8 (C2), 84.3 (s), 85.1 (C2′), 85.5 (s), 86.0 (C3′), 99.1 (C1α), 104.9 (C1″), 105.2 (C1′), 105.6 (C1‴); MALDI-TOF MS calculated for $C_{31}H_{56}O_{21}$ = 764.33; found *m/z* $[M + Na]^+$ = 787.437, $[M + K]^+$ = 803.433.

*Phenyl 2,3,4,6-tetra-O-acetyl β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-1-thio-β-D-glucopyranoside (**17**)*

Phenyl 2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**9**)

(25.0 mg, 0.0415 mmol), 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-*α*-D-glucopyranosyl 2,2,2-trichloroacetimidate (Urban et al. 1990) (**14**) (64.7 mg, 0.0830 mmol), and MS4Å (110 mg) were dried in a reaction ampule using a high-vacuum manifold overnight. Dichloromethane (1 mL) was added to the other ampule with $CaH_2$, and degassed by being frozen and thawed several times using the same high-vacuum manifold overnight. The solvent was transferred to the reaction ampule under high vacuum. The reaction ampule was sealed and placed at 0 °C. Trimethylsilyl trifluoromethanesulfonate (1.50 µL, 0.00830 mmol, 20 mol% to the glycosyl acceptor) was added into the reaction ampule through a rubber septum using a syringe. The reaction mixture was stirred at 0 °C for 7.5 h. MS4Å was removed by filtration and washed with ethyl acetate. The combined washings and filtrate were diluted with ethyl acetate, washed with aq. $NaHCO_3$, distilled water, and brine, dried over $Na_2SO_4$, and concentrated to dryness. The product was isolated through preparative TLC (eluent: ethyl acetate/*n*-hexane = 9:10, v/v) to produce compound **17** (35.8 mg, 70.6% yield).

$^1$H-NMR ($CDCl_3$): δ1.14–1.26 (18H, $CH_3$-$CH_2$-), 1.98–2.12 (–CO$CH_3$), 2.99 (t, 1H, *J* = 9.3, H2′), 3.10–3.24 (m, 3H, H2, H3′ H5′), 3.30 (t, 1H, *J* = 8.7, H3), 3.29–3.37 (m, 1H, H5), 3.9–4.0 (m, 1H, C3-O$CH_2$-$CH_3$), 4.02 (dd, 1H, *J* = 2.1, 12.6, H6‴), 4.17 (dd, 1H, H6″), 4.27 (d, 1H, *J* = 7.8, H1′), 4.39 (dd, 1H, *J* = 3.9, 12.6, H6‴), 4.43 (dd, 1H, *J* = 2.0, 12.0, H6″), 4.49 (d, 1H, H1‴), 4.51 (d, 1H, *J* = 9.6, H1), 4.70 (d, 1H, *J* = 8.1, H1″), 4.88 (dd, 1H, *J* = 8.9, 8.1, H2″), 4.92 (t, 1H, *J* = 8.1, H2‴), 5.07 (t, 1H, *J* = 9.3, H4‴), 5.12 (t, 1H, *J* = 9.3, H3‴), 5.13 (t, 1H, *J* = 9.0, H3″), 7.2–7.6 (aromatic H); $^{13}$C-NMR ($CDCl_3$): δ15.3, 15.5, 15.6, 15.7, 20.5, 20.5, 20.6, 20.7, 61.4 (C6‴), 61.9 (C6″), 66.6, 67.6 (C4‴), 67.9, 68.4, 68.3, 68.8, 71.5 (C2‴), 71.9 (C2″), 72.0 (C5‴), 72.3 (C5″), 72.9 (C3″, C3‴), 74.4 (C5′), 76.4 (C4″), 77.2 (C4′), 77.7 (C4), 79.4 (C5), 80.4 (C2), 81.9 (C2′), 82.9 (C3′), 84.8 (C3), 87.3 (C1), 100.0 (C1″), 100.9 (C1‴), 103.1 (C1′), 127.1, 128.7, 131.6, 169.1, 169.3, 169.5, 169.8, 170.2, 170.2, 170.5; MALDI-TOF MS: calculated for $C_{56}H_{84}O_{27}S$ 1220.49; found *m/z* $[M + Na]^+$ = 1243.79; $[M + K]^+$ = 1259.79

*Ethyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-D-glucopyranoside (**18**)*

To a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-*β*-D-glucopyranoside (**17**) (36.2 mg, 0.0296 mmol) in ethanol/dichloromethane (10 mL, 1/4, v/v), NIS (9.3 mg, 0.0415 mmol, 1.4 equiv) and a catalytic amount of AgOTf were added at room temperature. The reaction mixture was stirred for 3.5 h at room temperature. *N*-Bromosuccinimide (NBS) (7.4 mg, 0.042 mmol) was then added to the reaction mixture. After stirring for 0.5 h, solid $NaHCO_3$ was added to the reaction mixture, which was concentrated to dryness. The crude compound was purified by silica gel column chromatography (eluent: methanol/dichloromethane, 1/4, v/v) and gel filtration on Sephadex LH-20 (eluent: methanol/dichloromethane, 1/4, v/v) to produce ethyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-*β*-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside (**18**) (31.4 mg, 92% yield).

$^1$H-NMR ($CDCl_3$): δ1.0–1.3 (21H, –$CH_2$–$CH_3$), 1.9–2.2 (21H, CO$CH_3$), 2.96–3.06 (m, H2′), 3.07–3.27 (m, H5′, H3′), 3.29 (dd, 1H, H2α), 3.4–3.98 (H5″, H3a, H5‴, H5, H4, H4′, H4‴, –$CH_2CH_3$), 4.02 (dd, 1H, *J* = 1.9, 12.4, H6‴), 4.18 (dd, 1H, *J* = 4.5, 12.6, H6″), 4.25 (d, 1H, *J* = 8.1, H1′), 4.39 (dd, 1H, *J* = 12.0, 4.2, H6‴), 4.43 (dd, 1H, *J* = 1.4, 11.6, H6″), 4.49 (d, 1H, *J* = 7.8, H1‴), 4.72 (d, 1H, *J* = 8.1, H1″), 4.89 (t, 1H, *J* = 9.6, H2″), 4.89 (d, 1H, *J* = 3.6, H1α), 5.07 (t, 1H, *J* = 9.3, H4‴), 5.10 (t, 1H, *J* = 9.6, H3‴), 5.13 (t, 1H, *J* = 9.0, H3″); $^{13}$C-NMR ($CDCl_3$): δ14.9, 15.2, 15.3, 15.5, 20.5, 20.5, 20.6, 20.8, 61.4 (C6‴), 62.0 (C6″), 63.1 (C1–O–$CH_2CH_3$), 65.4 (small), 66.4, 66.6 (small), 66.7, 66.8, 67.6 (C4‴), 67.9, 68.3, 68.3, 68.4, 70.0 (C5′), 71.5 (C2‴), 71.9 (C2″), 72.1 (C5‴), 72.3 (C5″), 72.9 (C3″, C3‴), 74.5 (C5), 75.1 (small), 76.4 (C4″), 77.2 (C4′), 78.0 (C4), 79.5 (C2α), 79.7 (C3α), 81.6 (small), 81.8 (C2′), 82.9 (small), 83.0 (C3′), 96.5 (C1α), 100.1 (C1″), 100.9 (C1‴), 103.1 (C1′), 169.1, 169.3, 169.5 (small), 169.6, 169.8, 170.2, 170.3, 170.5.

*Ethyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-D-glucopyranoside (3)*

To a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside (**18**) (24.1 mg, 0.0208 mmol) in methanol (3 mL), 28% sodium methoxide in methanol (16.7 μL, 0.2915 mmol) was added at room temperature. The reaction mixture was continuously stirred at room temperature for 2 h. The reaction mixture was neutralized with Dowex in a $H^+$ form to produce ethyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside (**3**) (17.3 mg, 96% yield).

$^1$H-NMR ($CDCl_3$): δ1.0–1.4 (–$CH_2$-$CH_3$), 2.95–3.15 (m), 3.2–3.4 (m), 3.4–4.1 (m), 4.23 (d, $J = 6.9$), 4.29 (d, $J = 7.7$), 4.51 (m), 4.60 (m), 4.90 (d, $J = 3.0$, H1α); $^{13}$C-NMR ($CDCl_3$): δ14.9, 15.2, 15.3, 15.6, 15.7, 15.7, 15.7, 61.2, 63.2, 65.4, 66.5, 66.8, 67.3, 68.3, 68.9, 69.1, 69.2, 69.3, 69.4, 70.1, 72.7, 73.1, 73.4, 73.5, 74.2, 74.1, 74.2, 74.4, 74.6, 74.6, 74.7, 74.7, 74.9, 75.0, 75.1, 75.2, 76.0, 76.0, 79.5, 79.6, 79.7, 81.6, 82.2, 83.5, 96.5, 103.1; $^1$H-NMR ($D_2O$): δ3.11 (t, $J = 8.1$, H2 (H1β)), 3.16 (t, $J = 8.4$, H2′), 3.29 (t, 1H, $J = 9.3$, H2″ or H2‴), 3.29 (t, 1H, $J = 9.6$, H2″ or H2‴), 3.34–4.04 (m), 4.36 (d, $J = 8.1$, H1′), 4.42 (d, $J = 8.1$, H1″), 4.49 (d, $J = 7.8$, H1‴), 5.02 (d, $J = 2.1$, H1α); $^{13}$C-NMR ($D_2O$): δ16.7, 16.9, 16.9, 17.0, 17.3, 17.4, 63.0 (C6‴), 63.2 (C6″), 66.5 (C1-$OCH_2CH_3$), 69.4, 69.5, 69.6, 69.8, 70.7, 71.1, 71.4, 71.7, 72.0, 72.1 (CH), 72.3 (CH), 75.8 (C2″ or C2‴), 75.9 (C2″ or C2‴), 76.4, 76.7, 77.0, 77.8, 78.1, 78.3 (C4′), 78.6, 80.1 (C4), 81.0, 81.5 (C4″, C2), 83.4 (small), 83.7 (C2′), 84.5 (small), 84.9 (C3′), 98.4 (C1α), 104.6 (C1″), 105.3 (C1‴), 105.5 (C1′); MALDI-TOF MS: calculated for $C_{38}H_{70}O_{21}$ 862.44; found *m/z* $[M + Na]^+ = 885.65$, $[M + K]^+ = 901.60$.

*Phenyl 2,3,4,6-tetra-O-acetyl β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-1-thio-β-D-glucopyranoside (19)*

Phenyl 2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**9**) (17.2 mg, 0.0286 mmol), 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl 2,2,2,-trichloroacetimidate (**14**) (44.5 mg, 0.0571 mmol), and MS4Å (100 mg) were dried in a reaction ampule using a high-vacuum manifold overnight. Dichloromethane (1 mL) was added to the other ampule with $CaH_2$, and degassed by being frozen and thawed several times using the same high-vacuum manifold overnight. The solvent was transferred to the reaction ampule under high vacuum. The reaction ampule was sealed and placed at 0 °C. Trimethylsilyl trifluoromethanesulfonate (1.03 μL, 0.00571 mmol, 20 mol% to the glycosyl acceptor) was added into the reaction ampule through a rubber septum using a syringe. The reaction mixture was stirred at 0 °C for 17 h. MS4Å was removed by filtration and washed with ethyl acetate. The combined washings and filtrate were diluted with ethyl acetate, washed with aq. $NaHCO_3$, distilled water, and brine, dried over $Na_2SO_4$, and concentrated to dryness. The product was isolated by preparative TLC (eluent: ethyl acetate/*n*-hexane = 1:1, v/v) to produce phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**19**) (25.7 mg, 74% yield).

$^1$H-NMR ($CDCl_3$): δ1.0–1.3 (–$CH_2$-$CH_3$), 1.97, 2.04, 2.11, 2.16 ($COCH_3$), 2.99 (dd, 1H, $J = 8.1$, 9.0, H2′), 3.1–3.25 (H5′), 3.17 (t, 1H, $J = 9.6$, H2), 3.19 (t, 1H, $J = 9.0$, H2), 3.30 (t, 1H, $J = 8.7$, H3), 3.3–3.38 (H5), 4.02–4.2 (2H, H6‴), 4.17 (dd, 1H, $J = 4.2$, 12.3, H6″), 4.27 (d, 1H, $J = 7.8$, H1′), 4.41 (dd, 1H, $J = 2.1$, 12.3, H6″), 4.46 (d, 1H, $J = 7.8$, H1‴), 4.508 (d, 1H, $J = 9.9$, H1), 4.71 (d, 1H, $J = 7.8$, H1″), 4.87 (dd, 1H, $J = 8.1$, 9.6, H2″), 4.93 (dd, 1H, $J = 3.6$, 10.5, H3‴), 5.11 (dd, 1H, $J = 7.8$, 10.7, H2‴), 5.15 (t, 1H, $J = 9.3$, H3″), 5.34 (dd, 1H, $J = 0.7$, 3.3, H4‴); $^{13}$C-NMR ($CDCl_3$): δ15.3, 15.5, 15.6, 15.7, 20.5, 20.6, 20.8, 60.6 (C6‴), 62.1 (C6″), 66.5, 66.6, 67.9, 68.4, 68.8, 69.0 (C2‴), 70.5 (C5‴), 71.0 (C3‴), 72.2 (C2″), 72.3 (C5″), 73.2 (C3″), 74.4 (C5′), 76.2 (C4″), 77.2 (C4′), 77.7 (C4), 79.4 (C5), 80.4 (C2), 81.9 (C2′), 82.9 (C3′), 84.8 (C3), 87.4 (C1), 100.0 (C1″), 101.2 (C1‴), 103.1 (C1′), 127.1, 128.9, 130.6, 131.6, 134.2, 169.2, 169.6, 169.8, 170.1, 170.1, 170.3; MALDI-TOF MS: calculated for $C_{56}H_{84}O_{27}S$ 1220.49; found *m/z* $[M + Na]^+ = 1243.62$; $[M + K]^+ = 1259.62$.

*Ethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-D-glucopyranoside (**20**)*

To a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**19**) (35.6 mg, 0.0291 mmol) in ethanol/dichloromethane (5 mL, 1/4, v/v), NIS (9.2 mg, 0.0408 mmol, 1.4 equiv) and a catalytic amount of AgOTf were added at room temperature. The reaction mixture was stirred for 3 h at room temperature. Solid $NaHCO_3$ was then added to the reaction mixture, which was concentrated to dryness. The crude compound was purified by silica gel column chromatography (eluent: ethyl acetate/*n*-hexane = 1:1, v/v) to produce ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside (**20**) (16.3 mg, 67% yield).

$^1$H-NMR ($CDCl_3$): 1.09–1.20 (–$CH_2$–$CH_3$), 1.97, 2.04, 2.05, 2.06, 2.11, 2.16 (–CO$CH_3$), 3.01 (t, 1H, $J$ = 9.0, H2′), 3.1–3.2 (t, H3′), 3.30 (dd, 1H, $J$ = 3.9, 9.6, H2), 3.4–3.6 (–$CH_2$–$CH_3$), 4.18 (dd, 1H, $J$ = 6.0, 13.8, H6″), 4.25 (d, 1H, $J$ = 7.8, H1′), 4.41 (dd, 1H, $J$ = 1.8, 12.0, H6″), 4.46 (d, 1H, $J$ = 7.8, H1‴), 4.73 (d, 1H, $J$ = 7.8, H1″), 4.88 (dd, 1H, $J$ = 8.4, 9.6, H2″), 4.89 (d, 1H, $J$ = 3.9, H1), 4.93 (dd, 1H, $J$ = 3.3, 10.5, H3‴), 5.11 (dd, 1H, $J$ = 8.1, 10.5, H2‴), 5.15 (t, 1H, $J$ = 9.6, H3″), 5.35 (dd, 1H, $J$ = 0.9, 3.3, H4‴); $^{13}$C-NMR ($CDCl_3$): δ14.9, 15.3, 15.4, 15.5, 15.6 (–O$CH_2CH_3$), 20.5, 20.6, 20.8 (–CO$CH_3$), 60.6 (C6‴), 62.1 (C6″), 63.1, 66.5 (C4‴), 66.7, 66.9, 67.9, 68.3, 68.3, 68.5, 69.0 (C2‴), 70.1 (C5′), 70.6 (C5‴), 71.0 (C3‴), 72.2 (C2″), 72.3 (C5″), 73.3 (C3″), 74.5 (C5), 76.2 (C4″), 77.2 (C4′), 77.9 (C4), 79.5 (C2), 79.7 (C3), 81.1, 81.6, 81.9 (C2′), 82.9, 83.0 (C3′), 96.5 (C1α), 100.0 (C1″), 101.2 (C1‴), 103.1 (C1′), 169.2, 169.7, 169.7, 170.1, 170.4, 170.4 (–$COCH_3$); MALDI-TOF MS: calculated for $C_{52}H_{84}O_{28}$ 1156.51; found $m/z$ $[M + Na]^+$ = 1179.69; $[M + K]^+$ = 1195.68.

*Ethyl β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-O-ethyl-D-glucopyranoside (4)*

To a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside (**20**) (16.3 mg, 0.0141 mmol) in methanol (3 mL), 28% sodium methoxide in methanol (11.3 μL, 0.1973 mmol) was added at room temperature. The reaction mixture was continuously stirred at room temperature for 1.3 h. The reaction mixture was neutralized with Dowex $H^+$ to produce ethyl β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-D-glucopyranoside (**4**) (12.3 mg, quantitative yield).

$^{13}$C-NMR ($CDCl_3$): δ14.8, 15.2, 15.3, 15.6, 15.7, 15.7, 15.7, 60.7, 60.8, 61.1, 61.4, 63.2, 65.4, 66.5, 66.9, 68.4, 70.1, 73.0, 74.4, 74.9, 79.5, 79.7, 81.6, 82.2, 82.9, 83.5, 87.6, 96.6, 102.9, 103.1, 103.4, 103.6; $^1$H-NMR ($D_2O$): δ1.19 (t, O$CH_2CH_3$), 3.16 (t, $J$ = 9.0, H2), 3.90 (d, 1H, $J$ = 3.3, H4‴), 4.36 (d, $J$ = 7.8, H1′ or H1″ or H1‴), 4.37 (d, $J$ = 7.2, H1′ or H1″ or H1‴), 4.43 (d, $J$ = 7.8, H1′ or H1″ or H1‴), 5.02 (d, $J$ = 3.6, H1α); $^{13}$C-NMR ($D_2O$): δ16.7, 16.9, 16.9, 17.3, 17.4, 63.0, 63.7, 66.5 (C1-O$CH_2CH_3$), 69.4, 69.5, 69.6, 69.8, 70.7, 71.1, 71.2 (C4‴), 71.7, 72.0, 72.3 (C5), 73.6, 75.2, 75.8, 76.4,76.6, 77.1, 77.8, 78.0, 78.3 (C4′), 80.1 (C4), 81.0, 81.3 (C4″), 81.5 (C2), 83.7 (C2′), 84.9 (C3′), 98.4 (C1α), 104.6 (C1″), 105.5 (C1′), 105.7 (C1‴); MALDI-TOF MS: calculated for $C_{38}H_{70}O_{21}$ 862.44; found $m/z$ $[M + Na]^+$ = 885.45, $[M + K]^+$ = 901.38.

Preparation of blockwise alkylated tetrasaccharide–organic QD complexes

QDot 605 ITK Organic QDs (1 μM solution, 4 nmoles in decane 4 mL, Invitrogen Corp., Carlsbad, CA, USA) 200 μL (0.2 nmol) was placed in a micro tube. Methanol/2-propanol (75/25, v/v) 800 μL was then added to the micro tube, and the mixture was agitated

on a rotational shaker (MS3 Digital, IKA, Osaka, Japan) for 1 min, then dispersed using an ultrasonic cleaner (Yamato 2210, Yamato, Tokyo, Japan) for 1 min. The suspension was centrifuged in a micro-centrifuge (Centrifuge Micro 6 CFM-100, IWAKI Glass Co., Tokyo, Japan; 5 min) and the supernatant was removed by decantation.

Methanol 1 mL was added to the micro tube containing QDs. The mixture was agitated on a rotary shaker (IKA) for 1 min and was dispersed using an ultrasonic cleaner (Yamato 2210) for 1 min. The suspension was centrifuged in a micro-centrifuge (Iwaki; 5 min) and the supernatant was removed by decantation. The QDs were collected as the pellet. This procedure was then repeated. The collected QDs were dried under a vacuum for 2 h.

Blockwise alkylated tetrasaccharides (ca. 1 mg) were dissolved in dichloromethane (100 μL) in a second microtube. The dichloromethane solution was transferred into the microtube containing the dried QDs. The second microtube, now containing trace amounts of tetrasaccharides, was washed with dichloromethane and the washings were also added to the microtube containing the tetrasaccharides and QDs. The mixture of QDs and blockwise alkylated tetrasaccharides in dichloromethane was then agitated using an ultrasonic cleaner (Yamato 2210) for 30 s. The dichloromethane in the mixture was evaporated under a stream of nitrogen gas, and the mixture was heated to 50 °C for several minutes, then dried under a vacuum overnight to completely remove the dichloromethane. One hundred μL of PBS solution (Invitrogen, Cat. No. 12720-017), pH 7.4, was then added to the mixture of blockwise alkylated tetrasaccharide and QDs, which was dispersed using an ultrasonic cleaner for 1 min. Another 100 μL of PBS (pH 7.4) was added to the suspension, and the QD complexes were completely dispersed using an ultrasonic cleaner for 1 min.

### Cell lines

The human tumor cell lines U937 histocytoma, Daudi Burkitt's lymphoma, Raji Burkitt's lymphoma, RPMI8226 multiple myeloma, J.RT3-T3.5 $\alpha\beta$ T cell lymphoma, PEER $\gamma\delta$ T cell lymphoma, and MOLT3 T cell lymphoma were purchased from Human Science Research Resource Bank (Sen-nan, Osaka, Japan) and maintained in complete RPMI1640 medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% fetal calf serum (FCS, Sigma–Aldrich), $10^{-5}$ M 2-mercaptoethanol (Nacalai Tesque, Nakagyo-Ku, Kyoto, Japan), 100 U/mL penicillin (Meiji Seika Kaisha, Chuo-Ku, Tokyo, Japan), and 100 μg/mL streptomycin (Meiji Seika).

### Cytotoxicity assay

Compound **1** was dispersed in PBS to a concentration of 4 mg/mL and serially-diluted in two-fold steps in a 96-well flat bottom plate (50 μL/well, Corning Incorporated, Corning, NY, USA). U937 cells were suspended in complete RPMI1640 medium at $1 \times 10^4$ cells/mL and the cell suspension was added to the 96-well flat bottom plate ($5 \times 10^2$ cells/50 μL/well). The plate was incubated at 37 °C with 5% $CO_2$ for 4 days. The plate was allowed to stand at room temperature for 30 min and 100 μL of CellTiter-Glo Luminescent Cell Viability Assay reagent (Promega Corp., Madison, WI, USA) was added to each well. After being mixed thoroughly, the contents of each well were transferred into Optiplate™-96 (Perkin Elmer, Waltham, MA, USA). The cell viability was determined by measuring the luminescence through an ARVO™ SX Delfia 1420 Multilabel Counter (Perkin Elmer Life and Sciences, Shelton, CT, USA).

### Flow cytometric analysis

Human tumor cell lines were suspended in complete RPMI medium at $2 \times 10^5$ cells/45 μL and placed in 96-well round-bottom plates. The blockwise alkylated tetrasaccharide–QD complexes (5 μL) were then added to the cell suspension. The cells were incubated at 37 °C with 5% $CO_2$ for 16 h, and washed two times with 200 μL of complete RPMI1640 medium. For the assessment of cytotoxicity, the cells were resuspended in 200 μL of PBS/2% FCS and treated with propidium iodide (Sigma–Aldrich). For an assay of conjugation between human tumor cells and blockwise alkylated tetrasaccharide–QD complexes, the cells were washed once more with complete RPMI1640 medium. The cells were finally analyzed using a FACSCalibur flow cytometer (Becton–Dickinson, Franklin Lakes, NJ, USA).

### Confocal laser scanning microscopy

After $2.5 \times 10^6$ U937 cells had been labeled with the blockwise alkylated tetrasaccharide–QD complexes

as described above, the cells were purified using a FACSAria cell sorter (Becton–Dickinson). The $1.25 \times 10^6$ sorted cells were resuspended in 4 mL of complete RPMI1640 medium supplemented with 5 μM 5-chloromethylfluorescein diacetate (CellTracker™ Green CMFDA, Molecular Probes, Inc., Eugene, OR, USA), and incubated at room temperature for 15 min. After being washed once with complete RPMI1640 medium, the cells were incubated at 37 °C with 5% $CO_2$ for 30 min. The cells were washed three times with complete RPMI1640 medium, resuspended in 5 mL of the medium, and placed in a 35 mm glass-base dish (Asahi Glass Co., Ltd., Chiyoda-Ku, Tokyo, Japan) for observation of fluorescence using an LSM 710 confocal laser scanning microscope (Karl Zeiss AG, Oberkochen, Germany).

## Results and discussion

### Synthesis of blockwise alkylated tetrasaccharides

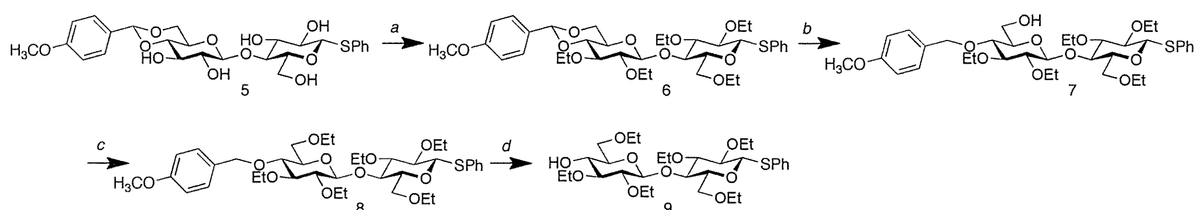
Using the same methodology as described for the synthesis of phenyl 2,3,6-tri-*O*-methyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methyl-1-thio-β-D-glucopyranoside (**10**) in our previous article (Kamitakahara et al. 2006), we prepared 2,3,6-tri-*O*-ethyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-ethyl-1-thio-β-D-glucopyranoside (**9**) as shown in Scheme 1.

Our previous reports demonstrated that a glycosyl donor could be converted into a glycosyl acceptor for further glycosylation (Kamitakahara et al. 2007, 2010). We therefore developed a novel synthetic strategy, through which glycosyl donors can be obtained with a good yield in a short period of time. The glycosyl donors, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl 2,2,2,-trichloroacetimidate (**11**) and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl 2,2,2,-trichloroacetimidate (**14**), were prepared from cellobiose and lactose, respectively, in three reaction steps.

Scheme 2 shows the synthetic routes for blockwise alkylated tetrasaccharides **1**–**4**. Glycosylation reactions were carried out in the presence of TMSOTf under high vacuum and anhydrous conditions. Different combinations of the two glycosyl donors and two acceptors afforded a set of four different glycosylation products containing only β-configuration, but not α-glycosyl moieties. The removal of a phenylthio group followed by the introduction of an alkyl group at the reducing-end and the deprotection of acetyl groups resulted in amphiphilic tetrasaccharides **1**–**4**.

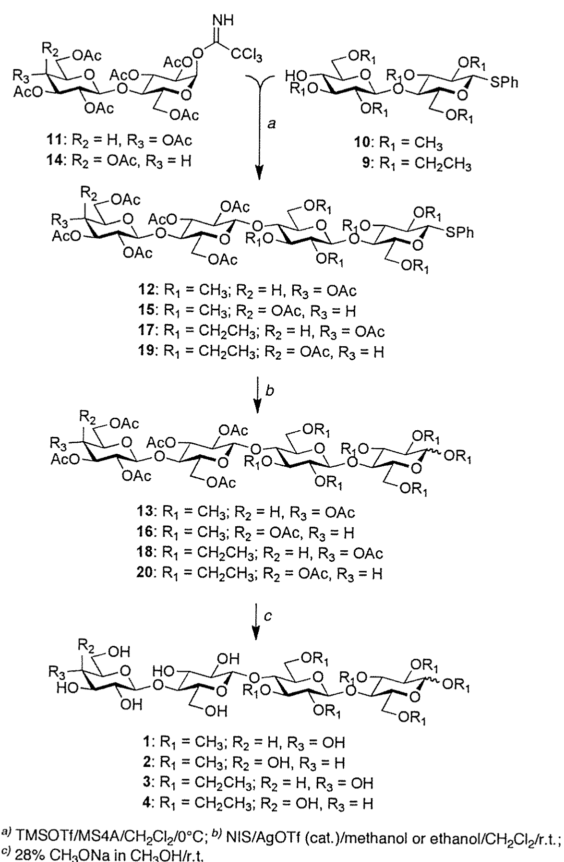
Chemical structures of compounds **1**, **2**, **3**, and **4** were studied by NMR spectra (See Supplementary Material). The C1α protons of blockwise methylated compounds **1** and **2** appeared at 4.97 and 4.98 ppm, respectively, and those of blockwise ethylated compounds **3** and **4** at 5.02 and 5.02 ppm, respectively. The C1α, C1′, C1″, and C1‴ protons of compounds **1**, **2**, **3**, and **4** appeared in the regions of 4.39–4.48, 4.37–4.43, 4.36–4.49, and 4.36–4.43 ppm, respectively. Especially, C1″ protons of compounds **1**, **2**, and **3** appeared at 4.48, 4.42, and 4.42 ppm with $J = 7.8$, 7.8, and 8.1 Hz, respectively, indicating that β-glycosylation proceeded. Coupling constants of H1′, H1″, and H1‴ of compound **4** showed the β-configurations, whereas those protons were not identified. The C4‴ protons of galactosyl residue of compounds **2** and **4** appeared at 3.89 ppm with coupling constant $J =$ 3.3 Hz. Furthermore, no aromatic and acetyl protons appeared in the spectra of compounds **1**–**4**, indicating that all reactions illustrated in Scheme 2 underwent successfully.

The C1α carbons of compounds **1**, **2**, **3**, and **4** appeared at 99.1, 99.1, 98.4, and 98.4 ppm, respectively.




[a), c)] Ethyl iodide/NaH/DMF/r.t.→50°C/a: 68%, c: quantitative yield; [b)] $BH_3$-THF complex in THF/TMSOTf/anhydrous $CH_2Cl_2$/-15°C→0°C/63%; [d)] CAN/$CH_3CN/H_2O$ (9/1, v/v)/0°C/82%

**Scheme 1** Synthesis of ethylated cellobiose derivatives




**Scheme 2** Synthetic routes for compounds **1**, **2**, **3**, and **4**

The C1″ of compounds **1**, **2**, **3**, and **4** appeared at 104.9, 104.9, 104.6, and 104.6 ppm, respectively, confirmed by HSQC and HMBC experiments. These facts also indicate that *β*-glycosylation proceeded successfully. Methyl carbon of the methyl group at C1 position of compounds **1** and **2** appeared at 57.6 and 57.6 ppm, respectively, indicating that phenylthio groups of compounds **12** and **15** were replaced with methoxyl group under reaction condition b in Scheme 2. The C4‴ carbon of galactosyl residue of compounds **2** and **4** appeared at 71.2 ppm. (Proton and Carbon NMR spectra are provided as electronic supplementary materials.)

Furthermore, MALDI-TOF MS measurement of compounds **1**, **2**, **3**, and **4** gave those theoretical molecular weights as described in the "Experimental" section. Consequently, all analytical data indicated that compounds **1**, **2**, **3**, and **4** were successfully synthesized.

## Self-assembled structure of amphiphilic tetrasaccharides in aqueous solution

Dynamic light scattering of aqueous blockwise alkylated tetrasaccharides revealed that the amphiphilic tetrasaccharide derivatives spontaneously assembled to form micelle-like nanoparticles at ambient temperature. As shown in Fig. 1, the average diameter of aggregates composed of compound **1** was approximately 180 nm.

## Preparation of blockwise alkylated tetrasaccharide–organic QD complexes

We next attempted to disperse organic QDs in aqueous solution using the newly synthesized amphiphilic tetrasaccharide derivatives. QDot 605 ITK Organic QDs (Invitrogen) have core (CdSe)/shell (ZnS) structure with the surface of trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO). Hydrophobic octyl groups exist on the surface of QDot 605 ITK organic QDs. QDot 605 ITK organic QDs were originally dispersed in decane. To replace the solvent with dichloromethane, a low boiling point solvent, the QDs were precipitated in methanol/2-propanol, and resuspended in a solution of blockwise alkylated

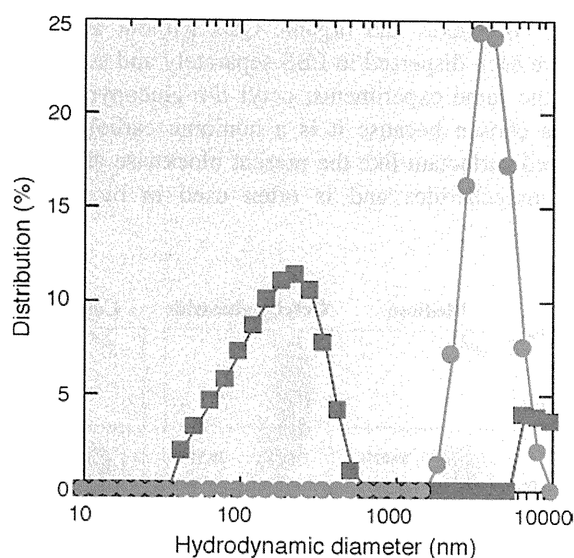



**Fig. 1** Hydrodynamic diameters of compound **1** and compound **1**–QD complex as measured by means of dynamic light scattering; *solid squares* compound **1**; *solid circles* compound **1**–QD complex