Glycoconjugated Polymer. 3. Synthesis and Amphiphilic Property of Core-Glycoconjugated Star-Shaped Polystyrene

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ABSTRACT: Coupling reactions of 2,2,6,6-tetramethylpiperidinyloxy-terminated polystyrene (PS-TEMPO) using divinylbenzene (DVB) as a linking agent were performed in the presence of 4-vinylbenzyl glucoside peracetate (**1a**) and 4-vinylbenzyl maltohexaoside peracetate (**1b**). To prepare core-glycoconjugated star-shaped polymers with various amphiphilic properties, the molar ratio of **1** and DVB in the feed (F_1, [**1**]/[DVB]) was changed from 0.13 to 0.38 for the coupling reaction. Star-shaped polystyrenes having **1a** and **1b** units in their cores (**2a** and **2b**, respectively) were obtained by purification using preparative SEC. The weight-average molecular weights determined by static laser light scattering measurements ($M_{w,SLS}$), the polydispersity indices determined by size exclusion chromatography (M_w/M_n), the number of polystyrene arms (N_{arm}), and the core-composition ratio (f_1, [**1** unit]/[DVB unit]) were 2.89×10^5−3.31×10^5, 1.36−1.38, 18, and 0.09−0.23 for **2a** and 2.28×10^5−2.60×10^5, 1.29−1.35, 11−14, and 0.05−0.17 for **2b**, respectively. Amphiphilic star-shaped polystyrenes with cores containing glucose and maltohexaose as hydrophilic segments (**3a** and **3b**, respectively), which were obtained by the deacetylation of **2a** and **2b**, exhibited an encapsulation ability toward water-soluble molecules, such as methyl orange (MO), in chloroform. The amounts of encapsulated MO remarkably increased with the increasing hydrophilic property of **3**. A positive Cotton effect was observed in the circular dichroism spectrum for the MO/**3** system in the adsorption area of MO, indicating that MO existed in a chiral segment, i.e., the glycoconjugated core.

Introduction

Considerable interest has been drawn to hydrophobic polymers containing covalently bonded hydrophilic blocks or segments, which exhibit a unique amphiphilic nature in the solid as well as in the solution states. Thus, precise synthetic techniques involving living polymerization[1] were used in order to construct well-defined amphiphilic macromolecular architectures.[2] Kanaoka et al. reported, as an example, amphiphilic star-shaped poly(vinyl ether)s having hydrophilic segments in their arms and cores through living cationic polymerization.[3] Tsitsilianis et al. reported the amphiphilic heteroarm star copolymer of polystyrene and poly(ethylene oxide) through living anionic polymerization.[4]

Recently, controlled radical polymerizations[5] have been interestingly developed for the synthesis of well-defined macromolecular architectures, in particular, star-shaped polymers. There are two basic synthetic routes for star-shaped polymers using living radical polymerization systems: (1) polymerization using a multifunctional initiator, namely the “core-first” method, and (2) coupling reaction of preformed living polymer (arm) using a difunctional cross-linking reagent, namely the “arm-first” method. Atom transfer radical polymerization was used for the preparation of the star-shaped polymers in the “core-first” [6] and “arm-first”[7] methods. For the nitroxide-controlled free-radical polymerization, Hawker reported that a trifunctional initiator has been used to produce a three-arm polystyrene.[8] Solomon et al. and other research groups[9] reported that star-shaped polymers were formed by the coupling reaction of alkoxyamine-terminated polystyrenes using divinyl compounds. These radical polymerization processes have advantages as being applicable to a wide variety of functional groups. Thus, of great interest is to extend the controlled radical polymerization to the synthesis of amphiphilic star-shaped polymers.

We now report the synthesis of a novel amphiphilic star-shaped polystyrene having a glycoconjugated core (**3**) through the nitroxide-controlled free-radical polymerization followed by deacetylation. The coupling reactions of 2,2,6,6-tetramethylpiperidinyloxy-terminated polystyrene (PS-TEMPO) were carried out using divinylbenzene (DVB) as a linking agent in the presence of glycoconjugated vinyl comonomers, such as 4-vinylbenzyl glucoside peracetate (**1a**) and 4-vinylbenzyl maltohexaoside peracetate (**1b**) as shown in Scheme 1. Because saccharides possess multiple hydroxyl groups in the molecule, amphiphilic core-glycoconjugated star-shaped polystyrenes (**3**) are expected to have interesting hydrophilic properties. Thus, we report the encapsulation ability of **3** toward water-soluble molecules such as methyl orange (MO).

Experimental Section

Materials. Styrene (St) (Kanto Chemical Co., >99.0%), divinylbenzene (DVB) (Aldrich, tech., mixture of isomers, 80%), and *m*-xylene (Kanto Chemical Co., >99.0%) were distilled just before use. 1-Phenyl-1-(2′,2′,6′,6′-tetramethyl-1′-piperidinyloxy)ethane,[10] 4-vinylbenzyl alcohol,[11] and maltohexaose peracetate[12] were prepared according to literature procedures. Dry dichloromethane (CH_2Cl_2) (Kanto Chemical Co., >99.5%), dry tetrahydrofuran (THF) (Kanto Chemical Co., >99.5%), piperidine (Kanto Chemical Co., >98.0%), trichloroacetonitrile (Al-

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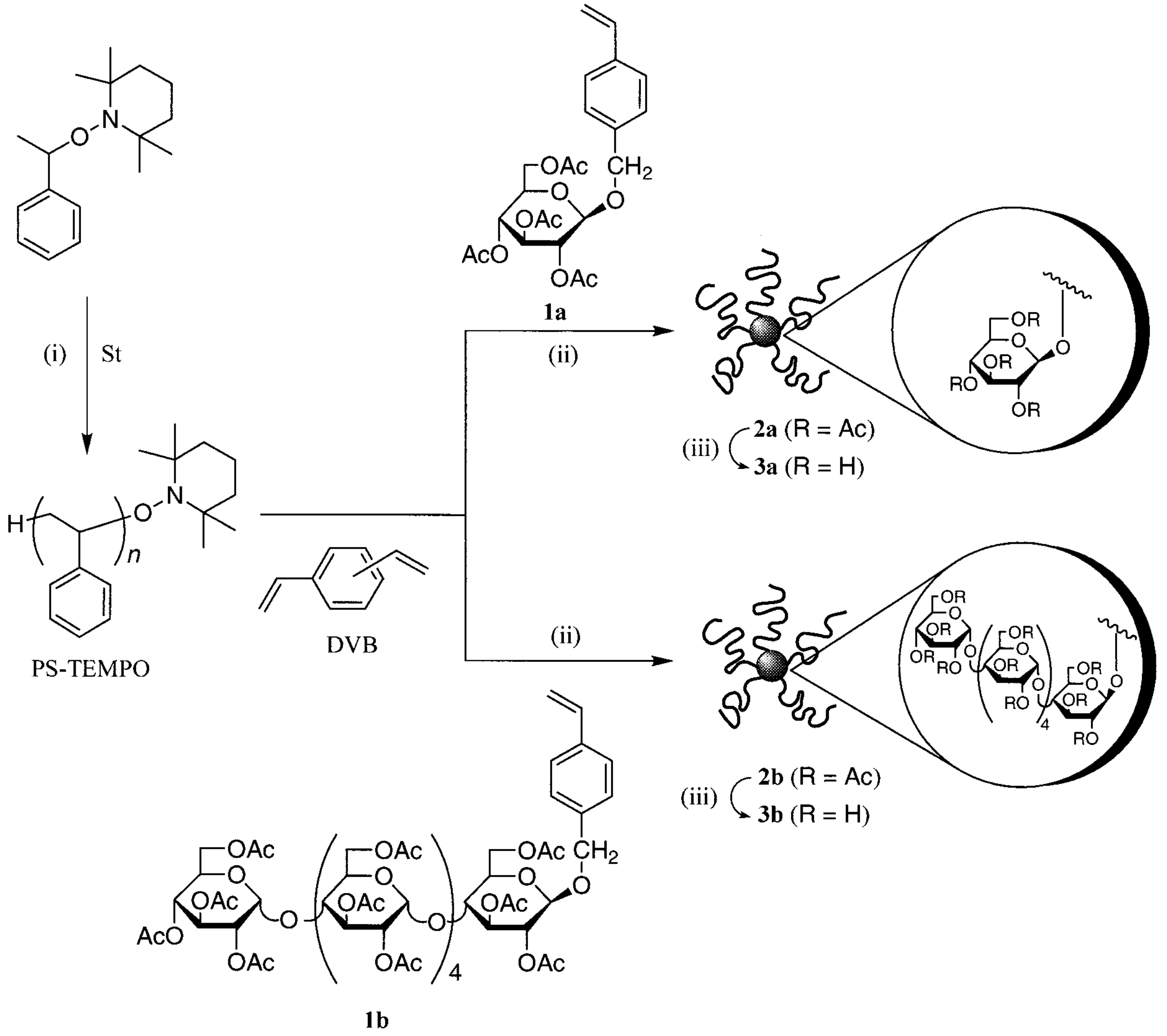
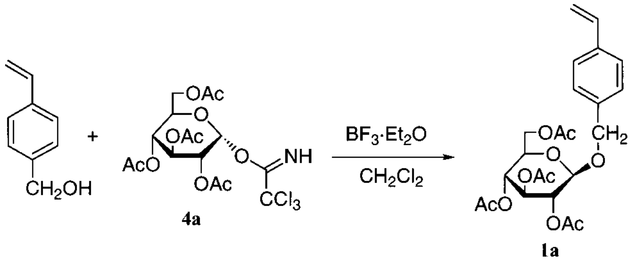
Scheme 1. Synthesis of Core-Glycoconjugated Star-Shaped Polystyrene: (i) Bulk Polymerization of Styrene; (ii) Coupling Reactions of PS-TEMPO Using Divinylbenzene (DVB) as a Linking Agent in the Presence of 1; (iii) Deacetylation of 2



drich, 98%), boron trifluoride diethyl ether complex (BF$_3$·OEt$_2$) (Kanto Chemical Co., >95%), sodium methoxide (Wako, 28% in methanol), and methyl orange (Wako) were used without further purification. A seamless cellulose tube UC24-32-100 was obtained from Viskase Sales Co.

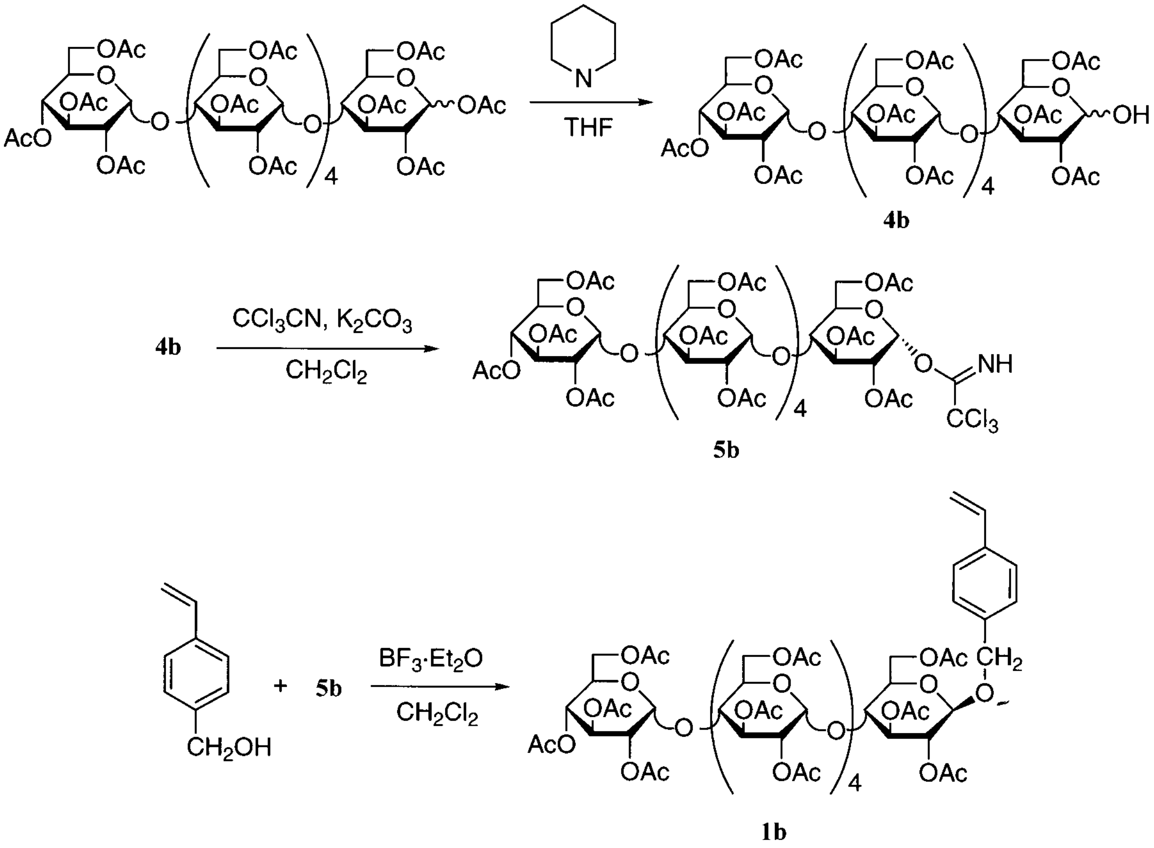
Measurements. The ^{1}H and ^{13}C NMR spectra were recorded using a Bruker ASX300 NMR and JEOL JNM-GX270 instruments. MS were recorded on JEOL JMS-SX102A and JEOL JMS-AX500 mass spectrometers (GC-MS & NMR Laboratory, Faculty of Agriculture, Hokkaido University). Optical rotations were measured using a Jasco DIP-1000 digital polarimeter. The size exclusion chromatography (SEC) was performed at 40 °C in chloroform (1.0 mL min^{-1}) using a Jasco GPC-900 system equipped with a Waters Ultrastyragel 7 mm column (linear, 7.8 mm × 300 mm) and two Shodex KF-804L columns (linear, 8 mm × 300 mm). The weight-average molecular weight ($M_{\mathrm{w,\,SEC}}$) and polydispersity (M_w/M_n) of the polymers were calculated on the basis of a polystyrene calibration. The preparative SEC was performed at 23 °C in THF (4.0 mL min^{-1}) using a JAI LC-908 equipped with two JAI JAIGEL-3H polystyrene columns (20 mm × 600 mm) and JAI UV-310 and JAI RI-5HC detectors. The static laser light scattering (SLS) measurement was performed in toluene at 25 °C on an Otsuka electronics DLS-7000 light scattering spectrophotometer ($\lambda = 632.8$ nm; four-point measurements; $c = 2 \sim 10$ mg/mL). The refractive index increment (dn/dc) was measured in toluene at 25 °C on an Otsuka electronics DRM-1021 double-beam differential refractometer ($\lambda = 632.8$ nm). Ultraviolet−visible (UV−vis) spectra were measured at 23 °C in CHCl$_3$ and DMSO/CHCl$_3$ (7/1, v/v) with 1 and 5 mm path lengths using a Jasco V-550 spectrophotometer. Circular dichroism (CD) spectra were measured at 23 °C in CHCl$_3$ with a 1 mm path length using a Jasco J-720 spectropolarimeter.

Scheme 2. Synthesis of 4-Vinylbenzyl Glucoside Peracetate (1a)



4-Vinylbenzyl 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranoside (1a). A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[13] (**4a**) (9.2 g, 19 mmol), 4-vinylbenzyl alcohol (16 g, 120 mmol), and powdered molecular sieves 4A in dry CH$_2$Cl$_2$ (250 mL) was stirred at −25 °C for 30 min under a nitrogen atmosphere (Scheme 2). To the mixture, BF$_3$·OEt$_2$ (2.6 mL, 21 mmol) was dropwise added for 10 min with stirring, and the reaction was immediately quenched with pyridine (5.0 mL). The reaction mixture was evaporated, and the residue was dissolved in CHCl$_3$ (300 mL) and washed with

Scheme 3. Synthesis of 4-Vinylbenzyl Maltohexaoside Peracetate (1b)



1 N HCl (300 mL). The aqueous layer was extracted with $CHCl_3$ (2 × 200 mL), and the combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine and dried with anhydrous $MgSO_4$. The $CHCl_3$ solution was evaporated to dryness, and the crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (2/1, v/v) to give **1a** as a white solid. Yield: 7.2 g (83%). ^1H NMR (270 MHz, $CDCl_3$): δ (ppm) = 7.39 (d, J = 7.9 Hz, 2H, Ar), 7.24 (d, J = 7.9 Hz, 2H, Ar), 6.71 (dd, J = 10.9, 17.5 Hz, 1H, $-CH{=}$), 5.76 (d, J = 17.5 Hz, 1H, $=CH_2$), 5.26 (d, J = 10.9 Hz, 1H, $=CH_2$), 5.17−5.06 (m, 3H, H-2 + H-3 + H-4), 4.88 (d, J = 12.4, 1H, $-CH_2O-$), 4.61 (d, J = 12.4 Hz, 1H, $-CH_2O-$), 4.54 (d, J = 7.6 Hz, 1H, H-1), 4.28 (dd, J = 4.3, 12.2 Hz, 1H, H-6), 4.17 (dd, J = 1.6, 12.2 Hz, 1H, H-6), 3.70−3.65 (m, 1H, H-5), 2.11 (s, 3H, $-CH_3$), 2.02 (s, 3H, $-CH_3$), 2.01 (s, 3H, $-CH_3$), 2.00 (s, 3H, $-CH_3$). ^{13}C NMR (67.7 MHz, $CDCl_3$): δ (ppm) = 170.6 (C=O), 170.1 (C=O), 169.3 (C=O), 169.2 (C=O), 137.3 (Ar), 136.3 ($-CH{=}$), 136.1 (Ar), 127.9 (Ar), 126.2 (Ar), 114.1 ($=CH_2$), 99.2 ($-CH-$), 72.8 ($-CH-$), 71.8 ($-CH-$), 71.2 ($-CH-$), 70.4 ($-CH_2O-$), 68.4 ($-CH-$), 61.9 ($-CH_2-$), 20.7 ($-CH_3$), 20.5 ($-CH_3$). $[\alpha]^{23}_D$ = −51.0° (c 1.0, $CHCl_3$). Anal. Calcd for $C_{23}H_{28}O_{10}$: C, 59.48; H, 6.08. Found: C, 59.34; H, 6.07.

4-Vinylbenzyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-[(*O*-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)]$_4$-*O*-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranoside (1b). 1b was synthesized via a three-step reaction from maltohexaose peracetate as the starting material as shown in Scheme 3.

To a solution of maltohexaose peracetate (32 g, 17 mmol) in dry THF (400 mL) was added piperidine (6 mL), and the mixture was stirred at room temperature until the starting material had been consumed as judged by the TLC analysis (18 h). The reaction mixture was evaporated, and the residue was dissolved in $CHCl_3$ (500 mL) and washed with 2 N HCl (ca. 300 mL). The aqueous layer was extracted with $CHCl_3$ (2 × 200 mL), and the combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried with anhydrous $MgSO_4$. The $CHCl_3$ solution was evaporated to dryness, and the crude product was purified by column chromatography on silica gel with toluene/acetone (4/1, v/v) to give *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-[(*O*-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)−(1→4)]$_4$-*O*-2,3,6-tri-*O*-acetyl-α,*β*-D-glucopyranose (**4b**) as a white solid. Yield: 25 g (80%). ^1H NMR (270 MHz, $CDCl_3$): δ (ppm) = 5.61−5.55, 5.42−5.29, 5.10−5.03, 4.88−3.67 (m, 42H, $-CH_2-$ + $-CH-$), 2.20−2.00 (m, 57H, $-CH_3$). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ (ppm) = 170.6−169.4 (C=O) 95.6 ($-CH-$), 94.8($-CH-$), 89.9($-CH-$), 74.8−67.6 ($-CH-$), 62.9−61.3 ($-CH_2-$) 20.8−20.5 ($-CH_3$). $[\alpha]^{23}_D$ = +140.4° (c 1.0, $CHCl_3$). Anal. Calcd for $C_{74}H_{100}O_{50}$: C, 49.67; H, 5.63. Found: C, 49.22; H, 5.54. MS (FD) (m/e) Calcd for (M^+): 1789. Found: 1789.

To a mixture of **4b** (47 g, 26 mmol) and anhydrous K_2CO_3 (36 g, 260 mmol) in dry CH_2Cl_2 (400 mL) was added trichloroacetonitrile (66 mL, 650 mol). The reaction mixture was stirred at room temperature under a nitrogen atmosphere until the starting material had been consumed as judged by the TLC analysis (20 h). K_2CO_3 was filtered off using Celite 535, and the filtrate was evaporated to dryness; then the crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (1/2, v/v) to give *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-[*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)]$_4$-*O*-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**5b**) as a white solid. Yield: 44 g (87%). ^1H NMR (270 MHz, $CDCl_3$): δ (ppm) = 8.67 (s, 1H, NH), 6.48 (d, J = 3.6 Hz, 1H, H-1), 5.63−5.56, 5.42−5.29, 5.10−3.75 (m, 41H, $-CH-$ and $-CH_2-$), 2.20−1.98 (m, 57H, $-CH_3$). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ (ppm) = 170.6−168.9 (C=O) 160.9 (C=NH) 95.7 ($-CH-$), 92.3 (CCl_3) 90.7($-CH-$), 73.5−67.6 ($-CH-$), 62.3−61.3 ($-CH_2-$) 20.8 ($-CH_3$), 20.6 ($-CH_3$), 20.5 ($-CH_3$). $[\alpha]^{23}_D$ = +147.7° (c 1.0, $CHCl_3$). Anal. Calcd for $C_{76}H_{100}Cl_3NO_{50}$: C, 47.20; H, 5.21; N, 0.72; Cl, 5.50. Found: C, 47.94; H, 5.24; N, 0.85; Cl, 5.85. MS (FAB) (m/e) Calcd for $(M + K)^+$: 1970. Found: 1970.

The same procedure as that for **1a** was applied to **5b** (4.4 g, 2.4 mmol), 4-vinylbenzyl alcohol (4.1 g, 30 mmol), and $BF_3 \cdot OEt_2$ (0.25 mL, 2.0 mmol) in dry CH_2Cl_2 (250 mL). The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (2:1 → 1:1 → 2:3, v/v) to give **1b** as a white solid. Yield: 2.5 g (54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.39 (d, J = 8.1 Hz, 2H, Ar), 7.25 (d, J = 8.0 Hz, 2H, Ar), 6.71 (dd, J = 17.6 Hz, J = 10.9 Hz, 1H, $-CH=$), 5.75 (d, J = 17.6 Hz, 1H, $=CH_2$), 5.42−3.67 (m, 45H, $=CH_2$ + $-CH-$ + $-CH_2-$ + $-OCH_2-$), 2.07−1.98 (m, 57H, $-CH_3$). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ (ppm) = 171.0−169.9 (C=O), 137.3 (Ar), 136.3 ($-CH=$), 136.1 (Ar), 127.9, (Ar), 126.2 (Ar), 114.1 ($=CH_2$), 99.0 ($-CH-$), 96.1 ($-CH-$), 75.8 70.8 ($-CH-$), 70.4 ($-CH_2O-$), 69.7−68.3 ($-CH-$), 62.8 61.7 ($-CH_2-$), 21.2−20.9 ($-CH_3$). $[\alpha]^{23}_D$ = +105.2° (c 1.0, $CHCl_3$). Anal. Calcd for $C_{83}H_{108}O_{50}$: C, 52.31; H, 5.71. Found: C, 52.35; H, 5.75.

TEMPO-Terminated Polystyrene (PS-TEMPO). 1-Phenyl-1-(2′,2′,6′,6′-tetramethyl-1′-piperidinyloxy)ethane (1.25 g, 4.80 mmol) was dissolved in styrene (125 g, 1.20 mol). Oxygen was removed from the solution by freezing in liquid nitrogen, evacuating the flask, warming to room temperature, and flushing the flask with argon gas. This procedure was repeated three times. The polymerization mixture was then stirred at 120 °C for 6 h. After cooling in liquid nitrogen, the mixture was diluted with chloroform (ca. 100 mL) and then poured into methanol (ca. 2 L). The precipitate was purified by reprecipitation with chloroform−methanol and dried in vacuo to give PSt-TEMPO as a white powder. Yield: 57.8 g (45.2%). $M_{w,\,SEC} = 1.00 \times 10^4$, $M_w/M_n = 1.09$. $M_{w,\,SLS} = 1.11 \times 10^4$, d$n$/d$c$ = 0.1051 mL g^{-1}.

Star-Shaped Polymer 2. An example of the procedure is described for the preparation of **2a-III**. A solution of PS-TEMPO (1.00 g, 0.10 mmol), DVB (457 mg, 3.51 mmol), and **1a** (489 mg, 1.05 mmol) in m-xylene (4.50 mL) was degassed by the same procedure as that for PS-TEMPO and then stirred at 138 °C for 20 h. After cooling in liquid nitrogen, the mixture was diluted with chloroform (ca. 30 mL) and then poured into methanol (ca. 900 mL). The precipitate was purified by reprecipitation with chloroform−methanol and dried in vacuo to give a white powder (original product). Yield: 1.54 g (79.0% based on the total feed of PS-TEMPO, DVB, and **1a**). $M_{w,SEC} = 7.57 \times 10^4$, $M_w/M_n = 2.01$. $[\alpha]^{23}_D$ = −8.0° (c 1.0, $CHCl_3$).

The original product (737 mg) was redissolved in THF (8 mL). The solution (2 mL) was applied to preparative SEC, and the eluents with the retention times of 33−47 min were fractionalized. This procedure was repeated four times. The combined fractions were evaporated, purified by reprecipitation with chloroform−methanol two times in order to remove the THF stabilizer, and then dried in vacuo to give **2a-III**. Yield: 604 mg (81.8% from the original product). $M_{w,SEC} = 7.61 \times 10^4$, $M_w/M_n = 1.38$. $M_{w,SLS} = 3.31 \times 10^5$, d$n$/d$c$ = 0.0929 mL g^{-1}. $[\alpha]^{23}_D$ = −9.3° (c 1.0, $CHCl_3$).

Arm-Number (N_{arm}) and Core-Composition Ratio ([1 unit]/[DVB unit], f_1) of 2. An example of the procedure for the estimation of **2a-III** is described. From the purification by preparative SEC, the original product (1.54 g) was found to be a mixture of **2a-III** (1.26 g) and a low-molecular-weight polymer (0.28 g). The weight fraction of the St unit in **2a-III** (W_{St}) was 0.57, which was determined from eq 2: (feed of PS-TEMPO: 1.00 g) = (yield of **2a-III**: 1.26 g) × W_{St} + (yield of low-molecular-weight polymer: 0.28 g), when low-molecular-weight polymer was regarded as unreacted PS-TEMPO. The arm-number (N_{arm}) of **2a-III** was 18, which was determined from eq 1: N_{arm} = ($M_{w,SLS}$ of **2a-III**: 3.31×10^5) × (W_{St}: 0.57)/ ($M_{w,SLS}$ of PS-TEMPO: 1.11×10^4).

The weight fraction of the **1a** units in **2a-III** (W_{1a}) was 0.18, which equaled $[\alpha]_D^{23}$ (c 1.0, chloroform) of **2a-III** (−9.3°) divided by that of poly-**1a** (−50.7°). The weight-fraction of the DVB units in **2a-III** (W_{DVB}) was 0.25, which was determined from eq 4: $W_{St} + W_1 + W_{DVB} = 1$. The core-composition ratio ([**1** unit]/[DVB unit], f_1) of **2a-III** is 0.20, which was determined from eq 3: f_1 = (W_1: 0.18/molecular weight of **1**: 464)/(W_{DVB}: 0.25/molecular weight of DVB: 130).

Star-Shaped Polymer 3. The typical procedure was as follows: A solution of **2a-III** (260 mg) in dry THF (10 mL) was added to a dry THF solution containing 2 wt % sodium methoxide (2 mL). The reaction mixture was stirred for 24 h at room temperature and then poured into water (ca. 80 mL). The mixture was transferred to a cellulose tube and dialyzed for 2 days against distilled water, followed by freeze-drying to yield **3a-III** as a white powder. Yield: 220 mg (95.7%).

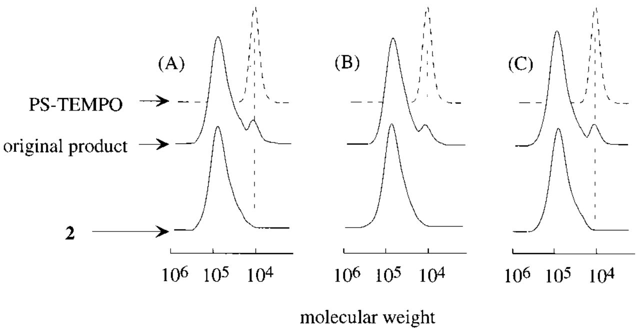


Figure 1. SEC traces of the original products (before fractionation, solid middle line) and **2** (after fractionation, solid bottom line) obtained from the reactions of PS-TEMPO (shown as upper broken line) and DVB in the presence of (A) **1a** ([**1a**]/[DVB] = 0.13), (B) **1b** ([**1b**]/[DVB] = 0.13), and (C) in the absence of **1**.

Encapsulation Ability of 3 Toward Water-Soluble Molecule. The typical procedure was as follows: To a solution of **3b-I** (40.0 mg, 1.86×10^{-7} mol) in $CHCl_3$ (2.00 mL) was added methyl orange (MO) (40.0 mg). The heterogeneous mixture was shaken at 40 °C for 18 h. After cooling, any undissolved MO was removed using 0.45 μm membrane filters to give a yellow solution. The yellow solution (MO/**3b-I** system) was characterized by UV−vis and CD spectra. UV−vis ($CHCl_3$, 1 mm cell): λ_{max}(abs) = 432 nm (1.04).

The MO/**3b-I** system (100 μL) was diluted with DMSO (700 μL) and characterized by its UV−vis spectrum for the quantitative analysis. Precipitation of **3b-I** was not observed in the solution even after adding DMSO. UV−vis (DMSO/$CHCl_3$ = 7/1, 5 mm cell): λ_{max}(abs) = 442 nm (0.38). Yield of MO in MO/**3b-I** system: 2.4 mg [based on UV−vis spectrum (DMSO/$CHCl_3$ = 7/1, v/v) of MO: λ_{max} (ϵ) = 442 nm (1.68×10^3 dm^3 mol^{-1} cm^{-1})].

Results and Discussion

Synthesis of PS-TEMPO. The synthetic procedure for the core-glycoconjugated star-shaped polystyrene is illustrated in Scheme 1. Styrene (St) was polymerized using 1-phenyl-1-(2′,2′,6′,6′-tetramethyl-1′-piperidinyloxy)ethane as an initiator (I) (bulk, [St]/[I] = 200/1) at 120 °C for 6 h to afford 2,2,6,6-tetramethylpiperidinyloxy-terminated polystyrene (PS-TEMPO) with a weight-average molecular weight ($M_{w,SEC}$) of 1.00×10^4 and a polydispersity (M_w/M_n) of 1.09, which were estimated from the linear polystyrene-calibrated size exclusion chromatography (SEC) using a differential refractometer (RI) detector. The weight-average molecular weight ($M_{w,SLS}$) of 1.11×10^4, which was determined by a static laser light scattering (SLS) measurement, was similar to the $M_{w,SEC}$ value.

Core-Glycoconjugated Star-Shaped Polystyrene (2). (I) Formation of Star-Shaped Polymer. Coupling reactions of PS-TEMPO using divinylbenzene (DVB) in the presence of 4-vinylbenzyl glucoside peracetate (**1a**) or 4-vinylbenzyl maltohexaoside peracetate (**1b**) were carried out in m-xylene at 138 °C for 20 h. Feeds of PS-TEMPO and DVB in m-xylene were constant for all the coupling reactions, while the molar ratio of **1** and DVB in the feed ([**1**]/[DVB], F_1) was changed from 0.13 to 0.38. The SEC chromatograms of the obtained polymer (original products) exhibited two peaks as shown in Figure 1. Similar observations were reported for the synthesis of the star polymers by other living polymerization techniques. A small amount of low-molecular-weight polymer was separated using

Table 1. Synthesis and Characterization of Star-Shaped Polymer[a]

sample	monomer	F_1[b]	yield[c] (%)	$M_{w,SEC} \times 10^{-4}$ [d]	M_w/M_n[d]	$M_{w,SLS} \times 10^{-5}$ [e]	$[\alpha]_D$ [f] (deg)	f_1[g]	N_{arm}[h]
2a-I	**1a**	0.13	68.5	7.54	1.36	2.89	−3.5	0.09	18
2a-II		0.25	66.7	7.77	1.37	3.01	−6.3	0.13	18
2a-III		0.38	64.7	7.61	1.38	3.31	−9.3	0.20	18
2b-I	**1b**	0.13	59.3	7.19	1.35	2.28	+15.8	0.04	13
2b-II		0.25	53.0	6.24	1.29	2.65	+31.0	0.09	12
2b-III		0.38	51.4	5.88	1.32	2.60	+44.1	0.14	10
2c	none		72.1	8.27	1.36	3.15			21

[a] PS-TEMPO, 1.0 g (9.0×10^{-5} mol); 80% DVB, 0.46 g (ca. 2.8×10^{-3} mol); solvent, *m*-xylene (4.5 mL); time, 20 h; temp, 138 °C. [b] The molar ratio of **1** and DVB ([**1**]/[DVB]) in the feed. [c] Yield of polymer **2** (weight, g) based on the total feed (weight, g). [d] Determined by SEC using polystyrene standards. [e] Determined by SLS measurement. [f] Measured in $CHCl_3$ at 23 °C (*c* 1.0). [g] The molar ratios of **1** units and DVB units ([**1** unit]/[DVB unit]) in the core of **2**. [h] The arm numbers of **2**.

preparative SEC from the original products obtained through the coupling reactions with **1a** and **1b** giving high-molecular-weight polymers (**2a** and **2b**, respectively) as white powders. In the absence of **1**, the same coupling reaction was also performed, followed by separation using preparative SEC to produce high-molecular-weight polymers **2c** as white powders. The yields of **2** from the original products were very similar in the all cases, i.e., 82.0−83.1% for **2a**, 83.4−85.0% for **2b**, and 82.2% for **2c**.

Table 1 lists the results for a series of coupling reactions. The weight-average molecular weights ($M_{w,SEC}$) and polydispersity indices (M_w/M_n), which were determined by SEC, ranged 7.54×10^4−7.77×10^4 and 1.36−1.38 for **2a**, 5.88×10^4−7.19×10^4 and 1.29−1.35 for **2b**, and 8.27×10^4 and 1.36 for **2c**, respectively. The $M_{w,SEC}$ values of **2** were 5−8 times greater than that of the starting arm PS-TEMPO ($M_{w,SEC} = 1.00 \times 10^4$). However, these values were apparent molecular weights when the hydrodynamic volumes of **2** were different from those of the linear polystyrenes. Thus, the weight-average molecular weights of **2** were determined by SLS measurements ($M_{w,SLS}$). The $M_{w,SLS}$'s ranged 2.89×10^5−3.31×10^5 for **2a**, 2.28×10^5−2.65×10^5 for **2b**, and 3.15×10^5 for **2c**. These values were 3−4 times greater than the $M_{w,SEC}$ values, indicating that the hydrodynamic volumes of **2** were considerably small compared to the linear polystyrene with the same molecular weight; i.e., a star-shaped polymer should be produced in the coupling reactions even in the presence of **1**.

(II) Formation of DVB Microgel Core Containing 1 Unit. The yields of **2** (weight, g) from the total feed of the starting materials (weight, g), including PS-TEMPO, DVB, and **1**, were estimated as shown in Table 1. The yields decreased in the order of **2c** (72.1%) > **2a** (64.7−68.5%) > **2b** (51.4−59.3%). The yields of **2** were also dependent on the reaction conditions such as [**1**]/[DVB] (F_1), i.e., decreased with increasing the F_1. Hence, we have to confirm the introduction of **1** in **2**.

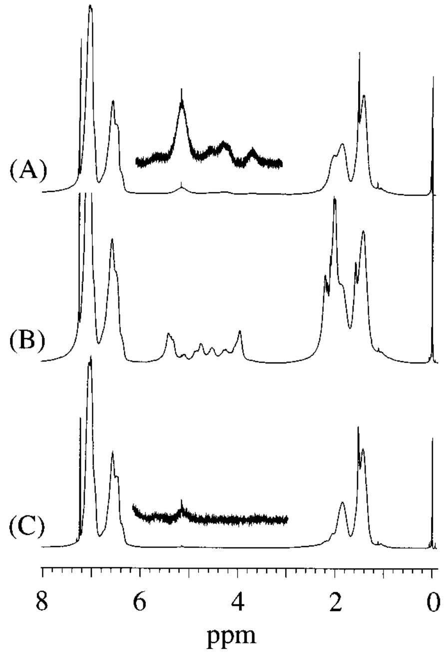


Figure 2. ^1H NMR spectra in $CDCl_3$ of (A) **2a-III**, (B) **2b-III**, and (C) **2c**.

Figure 2 shows the ^1H NMR spectra of **2**. For **2c**, which does not include any saccharide, the large signals due to the St and DVB units, i.e., aromatic protons (6.0−7.0 ppm) and the methine and methylene protons (0.5−3.5 ppm), appeared along with a very small characteristic signal due to the unreacted vinyl protons (4.8−5.8 ppm). The signal due to the unreacted vinyl pendant groups was broadened with respect to the loss of intramolecular mobility. In addition, in the ^1H NMR spectra of both **2a** and **2b**, the broadened signals derived from the **1** units appeared, i.e., 3.5−6.0 ppm due to the methine and methylene protons of the saccharides and 2.0−2.5 ppm due to the acetyl protons. This result indicated that the **1** units were introduced into the DVB microgel core in a star-shaped polymer. The introduction of the **1** units in **2** was also confirmed by their specific rotations ($[\alpha]^{23}_D$, *c* 1.0 $CHCl_3$,). As the F_1 value increased from 0.13 to 0.38, the absolute values of $[\alpha]^{23}_D$ increased from −3.5° to −9.3° for **2a** and from +15.8° to +44.1° for **2b**.

(III) Characterizations of Arm Number and Core Composition. The arm number of the star-shaped polymer **2** (N_{arm}) was calculated using the following equation:[14]

$$N_{arm} = (M_{w,SLS} \text{ of } \mathbf{2}) \times W_{St}/(M_{w,SLS} \text{ of PS-TEMPO}) \tag{1}$$

where W_{St} represents the weight fractions of the styrene (St) unit in **2**.

As described above, a small amount of low-molecular-weight polymers were separated using preparative SEC from the original products. Because the low-molecular-weight polymer was very similar to the molecular weight of PS-TEMPO, it was regarded as unreacted PS-TEMPO. Throughout the purification procedure of **2**, the St unit is completely recovered (little if any loss by the absorbent). Hence, there is the following relation according to the stoichiometric balance of the St unit, when the low-molecular weight polymer was regarded as unreacted PS-TEMPO.

$$(\text{feed of PS-TEMPO}) = (\text{yield of } \mathbf{2}) \times W_{\mathrm{St}} + (\text{yield of low-molecular-weight polymer}) \quad (2)$$

Equations 1 and 2 yield the N_{arm} of **2**, i.e., 18 for **2a**, 10−13 for **2b**, and 21 for **2c**. The coupling reaction in the present study made it possible to prepare **2** with many arms ($10 < N_{\mathrm{arm}} < 18$). This result is attributed to the nitroxide-controlled free-radical procedures, which are compatible with the functionalized vinyl monomer such as **1**.

The N_{arm}'s decreased in the order of **2c** > **2a** > **2b**. In addition, the N_{arm} of **2b** slightly decreased from 13 to 10 with increasing F_1. These results may be attributed to the steric hindrance due to the bulky **1** units. In the coupling reactions, a few DVB units add to PS-TEMPO along with **1** to form short block copolymers with pendant vinyl groups. The reactive chain ends then add to the pendant vinyl groups in another chain end to form a microgel core. Finally, the core−core coupling reaction occurs to form a star polymer, when it is sterically accessible. The core−core coupling reactions may be hindered when the bulky unit **1a** or much more bulky **1b** unit exists in the neighborhood of the pendant vinyl groups producing a decrease in the N_{arm}'s of **2a** and **2b** as above.

The core-composition ratio (f_1), which means the molar ratios of the **1** unit and the DVB units ([**1** unit]/[DVB unit]) in the core, was determined by the following relations:

$$f_1 = (W_1/\text{molecular weight of } \mathbf{1}) / (W_{\mathrm{DVB}}/\text{molecular weight of DVB}) \quad (3)$$

$$W_{\mathrm{St}} + W_1 + W_{\mathrm{DVB}} = 1 \quad (4)$$

where W_1 and W_{DVB} represent the weight fraction of the **1** unit and the DVB unit in **2**, respectively. W_1 equals the $[\alpha]^{23}_{\mathrm{D}}$ of **2** divided by that of poly-**1**. Equations 2 and 4 yield W_{DVB}. The f_1 values ranged 0.09−0.20 for **2a** and 0.04−0.14 for **2b** as listed in Table 1.

Core-Glycocojugated Star-Shaped Polystyrene (3): Synthesis and Amphiphilic Property. To obtain the amphiphilic star-shaped polymer, the deacetylation of **2a** and **2b** was carried out by alkaline hydrolysis using sodium methoxide in dry THF to afford **3a** and **3b**, respectively. Figure 3 shows the ^{1}H NMR spectra of **3** in pyridine-d_5. In the ^{1}H NMR spectra of **3**, the signals due to the St and DVB units (1.0−2.5 and 6.5−7.5 ppm) appeared along with the characteristic signals due to the saccharide residues (3.8−6.5 ppm), whereas the signals due to the acetyl groups disappeared. Thus, **3a** and **3b** were assigned to the star-shaped polystyrenes having a glucose- and maltohexaose-conjugated core, respectively.

We examined the solubility characteristics of **3a**−**b** (and **2c**, which does not include any saccharide moiety) in good solvents for polystyrene, such as toluene, $CHCl_3$, and dioxane, as summarized in Table 2. For **3a**, the hydrophilic property was found to be apparently increased with a small increase in the f_1 values, i.e., in the order **3a-I** < **3a-II** < **3a-III**. This result is attributable to the strong hydrophilic property of the saccharide molecules. For **3b**, the hydrophilic property also increased in the order **3b-I** < **3b-II** < **3b-III**. In general, **3b** was more hydrophilic than **3a**.

The star-shaped polymers **3** constitute a novel structure, i.e., saccharide molecules protected by many polystyrene arms; therefore, they are expected to show a characteristic amphiphilic property. To encapsulate a water-soluble molecule in good solvents for polystyrene is one of the desired abilities of **3**. To the respective solutions of **3a-I**, **3a-II**, and **3b-I** in $CHCl_3$ was added methyl orange (MO), and then the heterogeneous mixtures were shaken for 18 h at 40 °C. After removal of any undissolved MO using membrane filters, the $CHCl_3$ solution was apparently colorized. Figure 4 shows the ultraviolet−visible (UV−vis) spectra of MO encapsulated in the core-glycoconjugated star-shaped polystyrene (MO/**3** system). The characteristic absorption due to MO appeared in the visible area from 350 to 550 nm, except for the MO/**2c** systems. The absorbance increased in the order of the MO/**3a-I** < MO/**3a-II** < MO/**3b-I** systems. This fact indicates that encapsulation remarkably increased with the increasing hydrophilic property of the core in **3**.

The solution of the MO/**3** system diluted with dimethyl sulfoxide (DMSO) was characterized by UV−vis spectroscopy in order to determine the concentration of MO in the MO/**3** system. The molar ratios of MO and **3** ([MO]/[**3**]) were found to be 11, 34, and 39 in the MO/**3a-I**, **3a-II**, and **3b-I** systems, respectively, as shown in Table 2. Furthermore, the chiroptical property of the MO/**3b-I** system was examined using circular dichroism (CD) spectroscopy (Figure 5). In the CD spectrum, the positive Cotton effect was observed in the area from 400 to 550 nm corresponding to the absorption of MO,

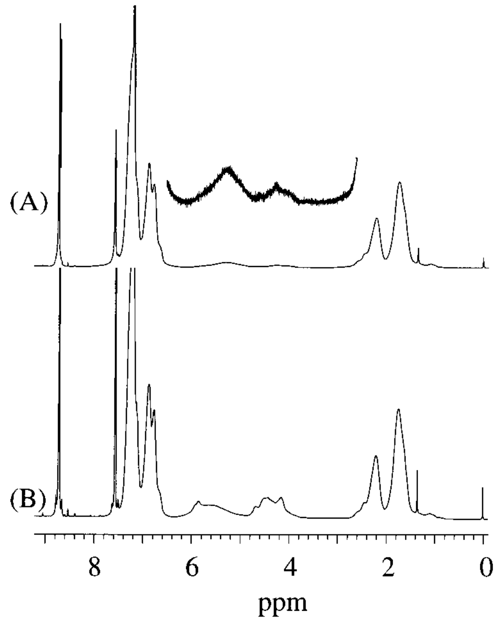


Figure 3. ^{1}H NMR spectra in pyridine-d_5 of (A) **3a-III** and (B) **3b-III**.

Table 2. Amphiphilic Property of Star-Shaped Polymer 3 (and 2c)

sample[a]	solubility[b] toluene	CHCl$_3$	dioxane	[MO]/[**3**] in CHCl$_3$
3a-I	○	○	○	11
3a-II	×	○	○	34
3a-III	×	×	○	
3b-I	×	○	○	39
3b-II	×	×	○	
3b-III	×	×	×	
2c	○	○	○	

[a] Prepared from deacetylation of **2** using NaOMe in dry THF. [b] ○, soluble; ×, swelled or insoluble.

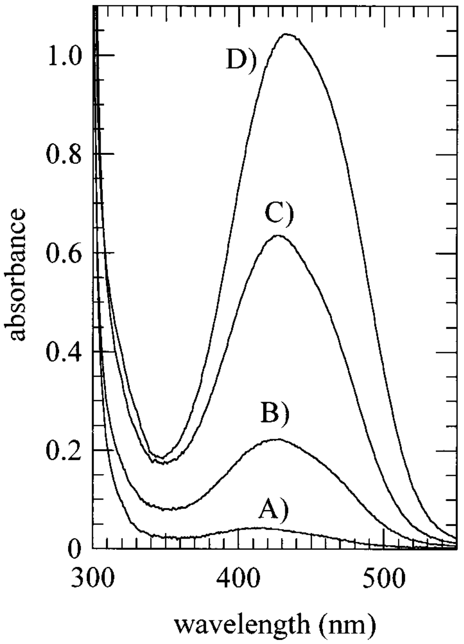


Figure 4. UV−vis spectra of (A) MO/**2c**, (B) MO/**3a-I**, (C) MO/**3a-II**, and (D) MO/**3b-I** systems, recorded in chloroform using a path length of 1 mm.

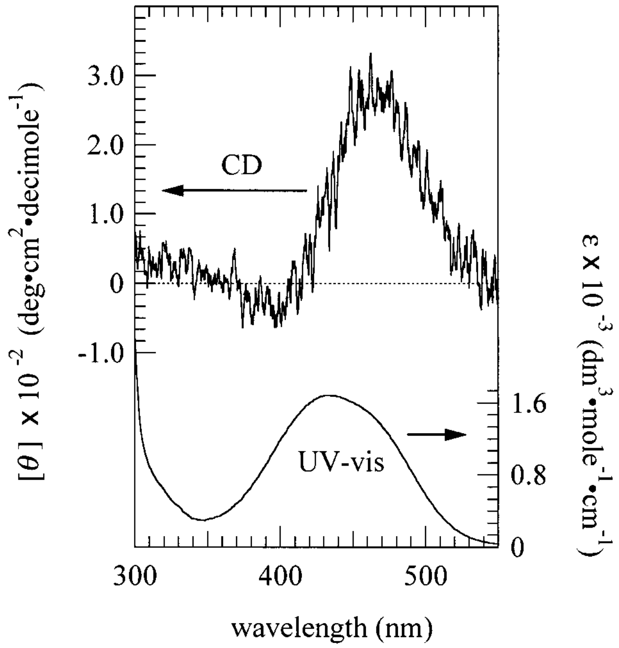


Figure 5. CD (upper line) and UV−vis (bottom line) spectra of MO/**3b-I** system, recorded in chloroform using a path length of 1 mm. The molar ellipticity ([θ]) value was estimated on the basis of the concentration of MO (3.6×10^{-3} mol L^{-1}, see Experimental Section).

indicating that MO existed in a chiral segment, i.e., the glycoconjugated core in **3**.

Conclusion

Coupling reactions of PS-TEMPO using DVB as a linking agent were performed in the presence of monovinyl monomers having saccharides (**1**). The reactions gave star-shaped polystyrenes with a core having **1** unit (**2**) along with a small amount of low-molecular-weight polymers. After purification using preparative HPLC, **2** were isolated, followed by deacetylation to give star-shaped polystyrenes with a core (**3**). The encapsulation ability of **3** toward water-soluble molecule, such as methyl orange (MO), in chloroform was examined. The UV spectra of MO/**3** systems indicated that MO was encapsulated into **3**, and also the encapsulation of MO dramatically increased with the increasing hydrophilic property of **3**. A positive Cotton effect was observed in the CD spectrum for the MO/**3** system, indicating that MO existed in a chiral segment, i.e., the glycoconjugated core in **3**.

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