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Synthesis of glycoconjugate polymer carrying globotriaose as artificial multivalent ligand for Shiga toxin-producing *Escherichia coli* O157: H7

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Abstract

As an artificial ligand, a glycoconjugate polymer carrying carbohydrate moiety of lactosyl ceramide or globotriaosyl ceramide (Gb_3) was synthesized. Gb_3 is known as the receptor of Shiga toxin-producing *Escherichia coli* O157: H7. The preparation of the glycoconjugate polymer initially involves the construction of the carbohydrate moiety of Gb_3 derivative which has *n*-pentenyl group as polymerizable group. In addition, the *n*-pentenyl group of the Gb_3 derivative was modified and different polymerizable groups such as acrylamide group were introduced at ω-position of the aglycon. Radical polymerization of the synthesized glycosyl monomers with or without acrylamide proceeded smoothly in water using ammonium persulfate and *N*, *N*, *N′*, *N′*-tetramethylethylenediamine as usual initiator system and gave water-soluble glycoconjugate polymers having various polymer compositions. These polymers have the potential to neutralize Shiga toxin by reason of cluster effect and multivalency.
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Keywords: *Escherichia coli*; Shiga toxins; Glycoconjugate polymer; Radical polymerization; Carbohydrates; Globotriaose

1. Introduction

The importance of cell-surface carbohydrates in initiating a wide variety of biological and pathological processes is now well recognized (Arya et al., 1999). Glycoconjugate polymers carrying biologically active carbohydrates as pendant groups constitute a new class of biomimetic and biomedical materials. They have provided access to many new methodologies in cell cultivation, tumor detection and diagnosis, and trapping of viruses and toxins. Their wide range of utility can be ascribed primarily to the widely occurring carbohydrate-binding proteins on the surfaces of cells, bacteria, and viruses (Debenham, Cossrow, & Toone, 1999; Dohi et al., 1999; Mylvaganam & Lingwood, 1999). Moreover, multivalency or cluster effects of carbohydrate integrate the binding affinity of glycoconjugate polymers to carbohydrate-binding proteins and contribute much to extend their potential utility (Gestwicki, Cairo, Strong, Oetjen, & Kiessling, 2002; Lee & Lee, 1995; Roy, 1996; Turnbull & Stoddart, 2002).

Shiga toxins (Stxs; Stx1 and Stx2) produced by pathogenic *Escherichia coli* O157: H7 have been associated with diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. The Stxs are a family of AB_5 subunit toxins. The enzymatic A subunit (32 kDa) is non-covalently associated with the pentamer of receptor-binding B subunits (7.5 kDa). The B-pentamer specifically binds to the globotriaosyl ceramide [Gb_3, Galα(1-4)Galβ(1-4)Glcβ-ceramide], a cell surface glycolipid (Kitov et al., 2000; Ling et al., 1998; Nishikawa et al., 2002; Soltyk et al., 2002). The importance of the B-pentamer–Gb_3 interaction is clearly illustrated by the fact that all cells susceptible to Stxs express Gb_3 on their cell surface, whereas cells that do not express Gb_3 are resistant to the toxins. Therefore, binding to the cell surface is a crucial initial step in cytotoxicity of Stxs (Bast, Banerjee, Clark, Read, & Brunton, 1999).

We describe herein the syntheses of a couple of new glycoconjugate polymers carrying the trisaccharide (globotriaose) moieties of Gb_3 as an artificial receptor for Stxs. These polymerizable saccharide derivatives having *n*-pentenyl group or acrylamide group at the ω-position of

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the aglycon are polymerized or co-polymerized with acrylamide. These artificial glycoconjugate polymers have the therapeutic potential for neutralization of Stxs because the polymers are water-soluble and the binding affinity is enhanced by cluster effects.

2. Results and discussion

2.1. Synthesis of polymerizable globotriose derivative

Our strategy for preparing the polymerizable globotriaose derivative involves the introduction of an olefin, *n*-pentenyl group, at the aglycon unit of the glycosyl acceptor and subsequent glycosidation with glycosyl donor (Matsuoka, Terabatake, Esumi, Terunuma, & Kuzuhara, 1999). After assembling the globotriaose structure, *n*-pentenyl group was modified, and to the ω-position of the aglycon was introduced an acrylamide group (Fig. 1). Consequently, glycosyl monomers having different polymerizable groups (*n*-pentenyl and acrylamide group) were prepared, respectively.

Scheme 1 describes the synthesis of glycosyl acceptor **3**. *n*-Pentenyl β-lactoside **1** which was prepared according to Matsuoka and Nishimura (1995), and Takano, Nakatsubo, and Murakami (1990) was selectively protected by formation of a benzylidene acetal intermediate. Subsequent benzylation of remaining OH groups afforded **2**. Selective reductive cleavage by treatment of **2** with $AlCl_3$ in the presence of $BH_3{\cdot}NMe_3$ in THF gave glycosyl acceptor **3** with 4′-OH in 76.3% yield.

Compound **4** (Koto, Morishima, Miyata, & Zen, 1976) gave the glycosyl donor **5** (Austin, Hardy, Buchanan, & Baddiley, 1965) quantitatively by treatment with $SOCl_2$ in

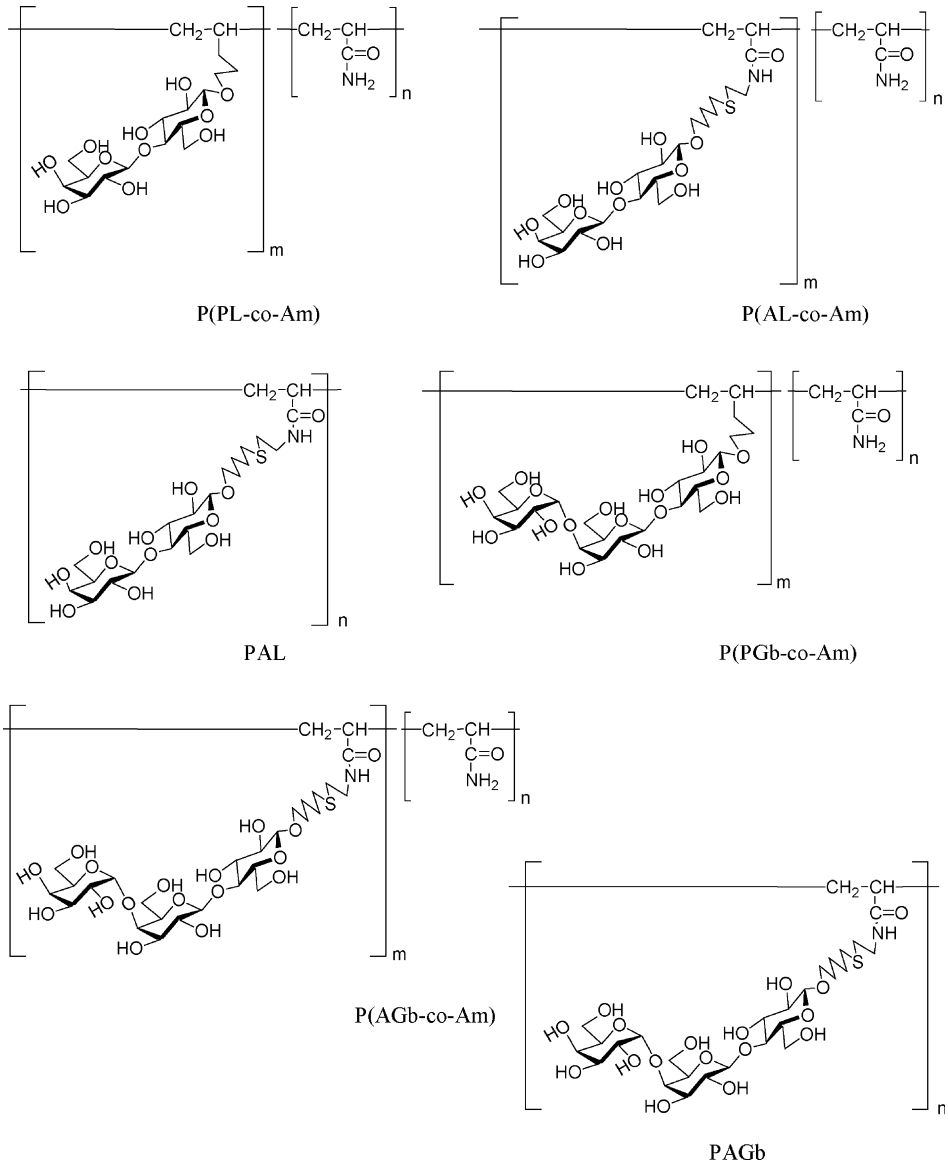
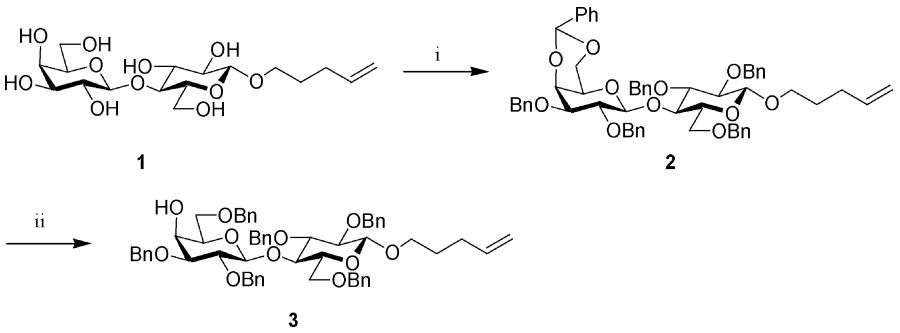
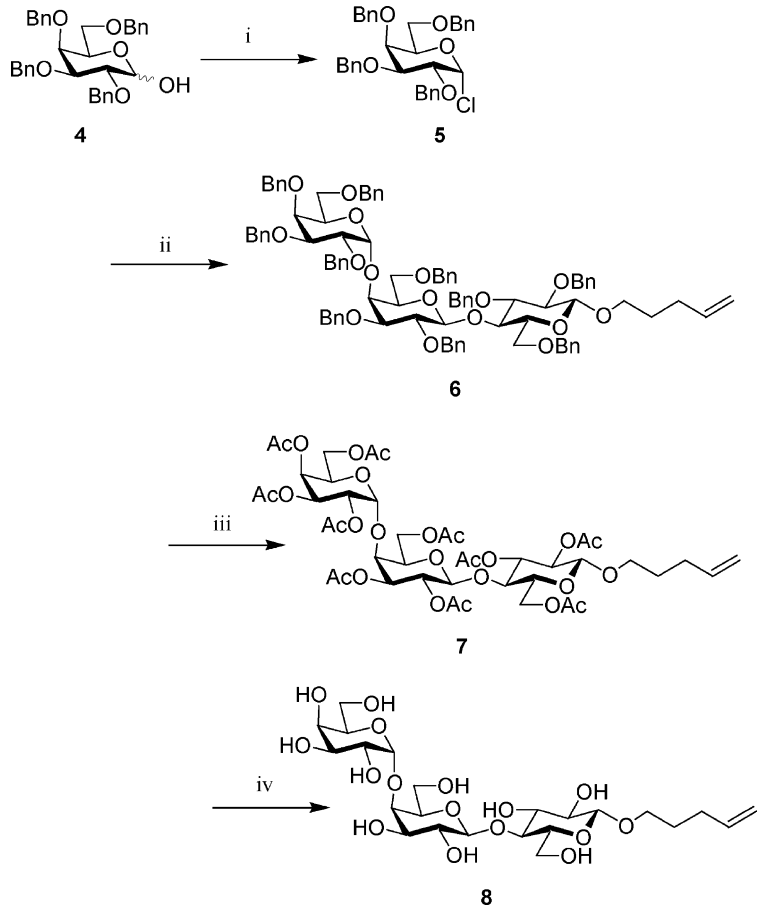


Fig. 1. Chemical structures of synthesized glycoconjugate polymers.

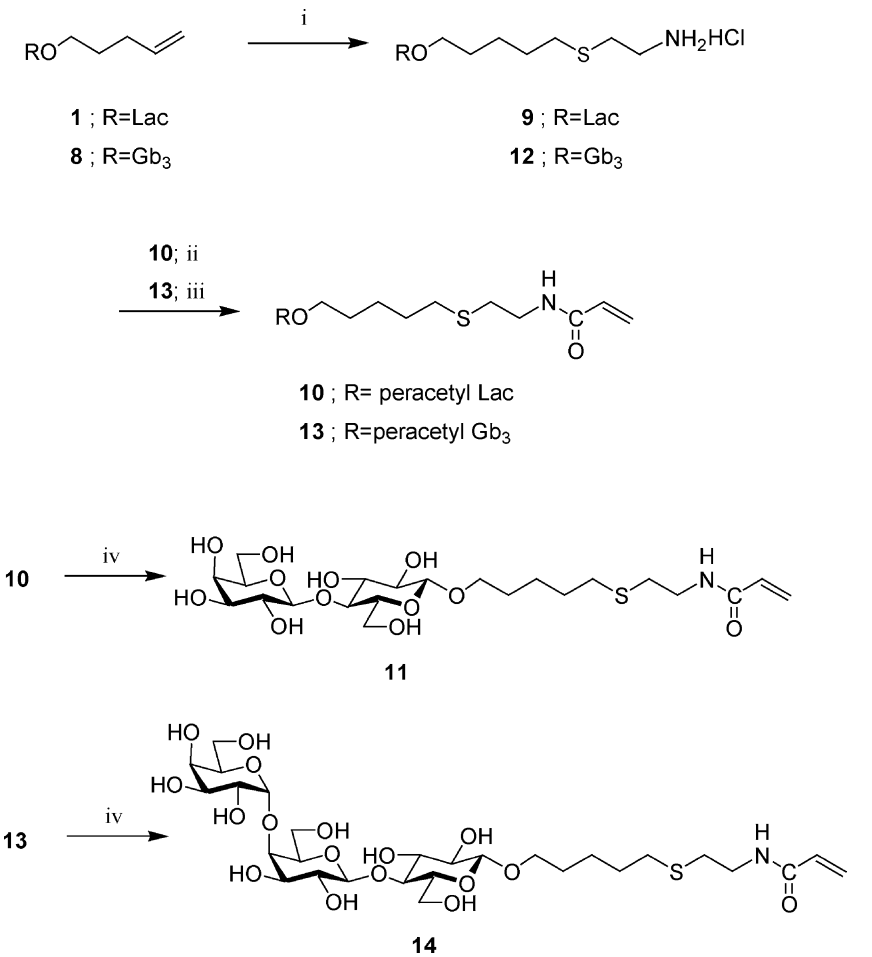


Scheme 1. Reagents and conditions: (i) $C_6H_5CH(OCH_3)_2$, CSA, 60°C, 2.5 h, under reduced pressure, then NaH, BnBr, DMF, rt, 1.5 h; (ii) $Me_3N{\cdot}BH_3$, $AlCl_3$, MS 4Å, THF, rt, 1 h.

the presence of DMF (Ogawa, Nakabayashi, & Kitajima, 1983). Stereoselective glycosidation (shown in Scheme 2) of glycosyl acceptor **3** with glycosyl donor **5** promoted by silver trifluoromethansulfonate in ether at −20 °C gave globotriaose derivative **6** in 71.3% yield. The NMR spectrum of the product confirms the α-linkage of the newly formed glycosidic bond [^{13}C NMR ($CDCl_3$) δ: 103.51 (β; C-1′), 102.80 (β; C-1), 100.63 (α; C-1″)]. Debenzylation of **6** without affecting the terminal double bond of the *n*-pentenyl aglycon was accomplished by Birch reduction. However, in the case of globotriaose derivatives having butenyl or allyl aglycon unit, these aglycons were slightly cleaved by Birch reduction. Compound **6** was treated with Na in liquid NH_3 at −78 °C, followed by acetylation to afford fully acetylated *n*-pentenyl globotriaose derivative **7**. After purification of **7**, subsequent deacetylation gave water-soluble *n*-pentenyl β-globotriaoside **8**, a glycosyl monomer with free hydroxyl groups and the *n*-pentenyl (olefin moiety) as polymerizable group.



Scheme 2. Reagents and conditions: (i) $SOCl_2$, DMF, $ClCH_2CH_2Cl$, 0 °C → rt, 20 h. (ii) AgOTf, MS 4Å, Et_2O, −20 °C, 3.5 h; (iii) Na, liq. NH_3, −78 °C, 20 min, then Ac_2O, pyridine, rt, 21 h; (iv) NaOMe, MeOH, rt, 16 h.



Scheme 3. Reagents and conditions: (i) $HSCH_2CH_2NH_2{\cdot}HCl$, MeOH, $h\nu$ (254 nm), 0 °C, 2.5 h; (ii) $CH_2{=}CHCOCl$, Et_3N, MeOH, 0 °C, then Ac_2O, pyridine, rt, 15 h; (iii) $CH_2{=}CHCOCl$, $NaHCO_3$, MeOH, 0 °C, then Ac_2O, pyridine, rt, 13 h; (iv) NaOMe, MeOH, rt, 3.0 h.

Scheme 3 describes the syntheses of glycosyl monomers having acrylamide group at the terminal. Initially, to *n*-pentenyl β-lactoside **1** was introduced an amino group at ω-position of the aglycon. *n*-Pentenyl β-lactoside **1** and cysteamine hydrochloride were irradiated (254 nm), yielding the amino terminated thioether **9** (Lee & Lee, 1974; Roy & Tropper, 1988; van Seeventer, van Dorst, Siemerink, Kamerling, & Vliegenthart, 1997). Then the amino group of **9** was *N*-acryloylated and then acetylated to give fully protected derivative. After usual purification, deacetylated **10** gave lactose monomer **11** having an acrylamide group at ω-position of the aglycon. By the same procedure, globotriaose monomer **14** was obtained. Radical addition of *n*-pentenyl β-globotriaoside **8** proceeded, followed by *N*-acryloylation and acetylation to afford **13** which was deacetylated to give globotriaose monomer **14** having an acrylamide group at ω-position of the aglycon.

2.2. Radical polymerization of glycoconjugate polymers

Glycosyl monomers were polymerized or copolymerized with acrylamide in distilled water at room temperature using *N*, *N*, *N′*, *N′*-tetramethylenediamine (TEMED) and ammonium persulfate (APS) as initiators (Matsuoka & Nishimura, 1995; Nishimura et al., 1994), and the products were purified by gel filtration.

The results of polymerization and copolymerization are summarized in Table 1. The unit ratio of the polymers abbreviated as 'polymer comps' was determined from the ^1H NMR results by comparing the intensity of the integration of the protons for 1, 1′-positions of lactose or globotriaose (at 4.4 ppm) due to glycosyl residue, and methine group (at 2.2 ppm) due to the main chain of the polymer (Figs. 2 and 3).

The sugar content of the polymer was determined as percent by weight of the glycosyl monomer in the polymer. As shown in Table 1, polymer composition was affected by the glycosyl monomer. The factors affecting polymerization involves the difference of the polymerizability of *n*-pentenyl and acrylamide groups that glycosyl monomers have and steric hindrance of bulky glycosides. Gb_3 was bulkier than lactose due to the additional α-galactose residue. However, the polymer molecular weight and sugar content seemed enough to inhibit

Table 1
Results of polymerization of glycosyl monomers with acrylamide

Polymer	Glycosyl monomer	Monomer ratio[a]	Total yield (%)	Polymer compsa	Sugar content (wt %)	Mw[b] (kDa)
P(PL-co-Am)	**1**	1:10	86.5	2:27	30	81.2
P(AL-co-Am)	**11**	1:10	92.2	1:6	55.9	<10
PAL	**11**	1:0	92.0	1:0	100	46.5
P(PGb-co-Am)	**13**	1:10	83.9	1:25	24.4	147
P(AGb-co-Am)	**14**	1:10	48.0	1:12	45.2	73.1
PAGb	**14**	1:0	80.0	1:0	100	36

[a] Ratio of glycosyl monomer to acrylamide.
[b] Mws were estimated by SEC method with Asahipack G-510 column (pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards).

cytotoxicity of Shiga toxins. These glycoconjugate polymers were assayed in vitro and glycoconjugate polymers carrying Gb_3 were found effective for neutralization of Shiga toxins, not only for Stx1 but also the clinically more relevant Stx2. Moreover, glycoconjugate polymers carrying Gb_3 were also found effective in vivo. Details of these results are discussed elsewhere (Watanabe et al., 2004).

In conclusion, we synthesized glycoconjugate polymers having lactose and globotriaose residues as biologically active pendants. These monomers of glyco-polymers were systematically synthesized. The construction of the trisaccharide moiety was accomplished from D-galactose and D-lactose by several chemical steps. The carbohydrate derivatives having *n*-pentenyl group at the aglycon were efficiently synthesized, and the elongation of the aglycon was performed to afforded corresponding glycosyl monomers having an acrylamide group. Polymerization of those monomers was accomplished and the results suggested that the acrylamide-type aglycon was found to be a better polymerizable group. This phenomenon gave us the glycosyl monomers having acrylamide group had appropriate length of flexible spacer arm and showed grater polymerizability. The glycoconjugate polymers having acrylamide-type aglycon had stronger neutralization potency against both Stxs.

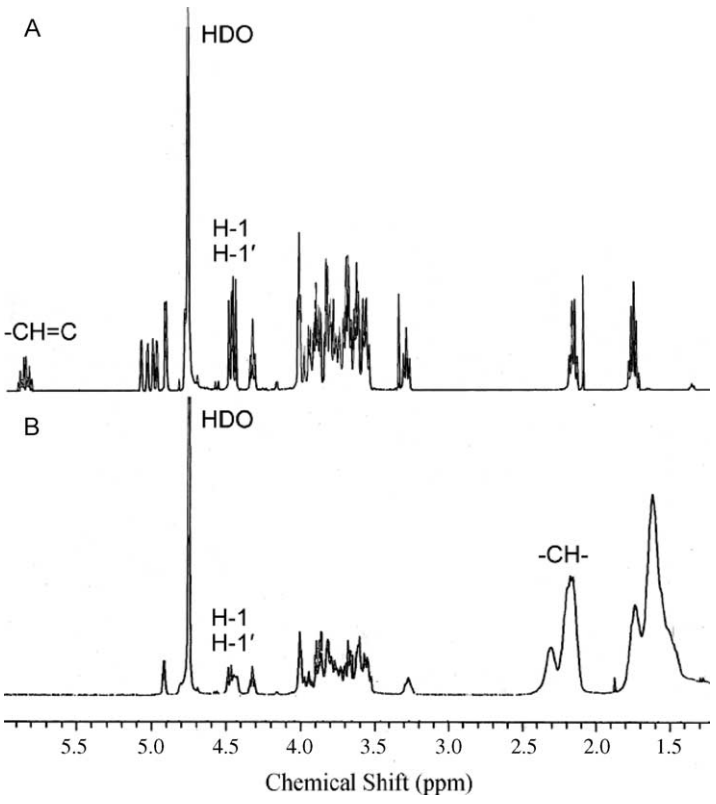


Fig. 2. ^{1}H NMR spectra of (A) glycosyl monomer **8**, (B) P(PGb-co-Am) in D_2O.

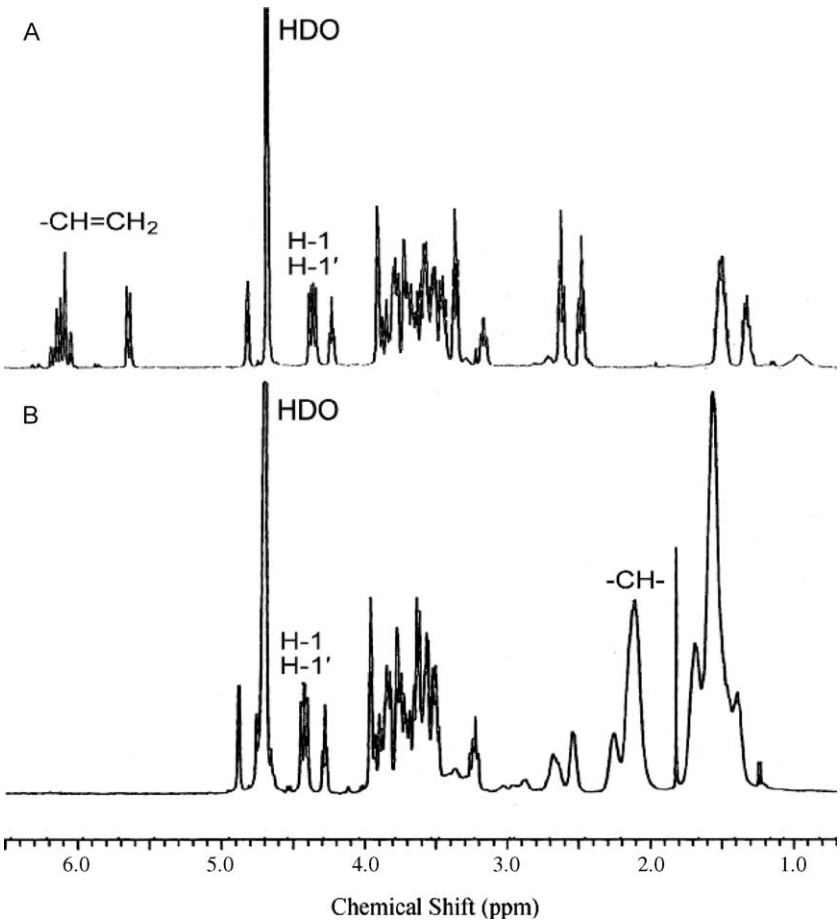


Fig. 3. ^{1}H NMR spectra of (A) glycosyl monomer **14**, (B) P(AGb-co-Am) in D_2O.

3. Experimental section

3.1. General procedures

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. *N,N*-Dimethylformamide (DMF), tetrahydrofuran (THF), 1,2-dichloroethane, dichloromethane, and pyridine were stored over molecular sieves 4 Å. Methanol was stored over molecular sieves 3 Å. Powdered molecular sieves were dried in vacuo at ca. 180 °C in 2 h. Acrylamide was recrystallized from benzene. The optical rotations were determined with a JASCO DIP-1000 digital polarimeter. IR spectra were measured in KBr disc for solid samples, or film on KBr for liquid samples with JASCO FT/IR-300E. ^{1}H NMR spectra were recorded at 200 or 400 MHz with Varian Gemini-200 or Bruker AM-400 spectrometer in chloroform-*d* or deuterium oxide. ^{13}C NMR spectra were recorded at 50.3 or 100.6 MHz with the same instruments. Tetramethylsilane (TMS), HDO (4.78 ppm) were used as internal standards. Proton assignments in NMR were made by first-order analysis of spectra, and supported by homonuclear decoupling experiments. Elemental analyses were performed with a Fisons EA1108 on samples extensively dried ca. 24 h in vacuo over phosphorus pentoxide. Average molecular weights of the polymers were estimated by size exclusion chromatography (SEC) method with a Shodex Asahipak GS-510 7E column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, 300 kDa, Shodex Standard P-82) were used as standards. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (layer thickness, 0.25 mm; E. Merk, Darmstadt, Germany). For detection of intermediates, TLC sheets were dipped with (a) a solution of 85:10:5 (v/v/v) methanol–*p*-anisaldehyde–concentrated sulfuric acid and heated for a few minutes (for carbohydrates); (b) an aqueous solution of 5 wt % potassium permanganate and heated similarly (for double bond). Column chromatography was performed on silica gel (Silica Gel 60; 40–63 μm, E. Merck), or (Silica Gel 60, spherical neutral; 40–100 μm, E. Merck).

3.2. n-Pentenyl 4-O-(2,3-di-O-benzyl-4,6-O-benzilidene-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (2)

To a solution of **1** (500 mg, 1.22 mmol) in DMF (2.5 ml) was added benzaldehyde dimethylacetal (275 μl, 1.83 mmol)

and (±)-camphor-10-sulfonic acid (28.3 mg, 122 μmol), and the mixture was stirred over evaporation at 60 °C for 2.5 h. The solution was cooled to room temperature, and triethylamine (34 μl, 244 μmol) added to neutralize. The solution was evaporated to give an intermediate mixture. A part of mixture was crystallized from 2-propanol to give a white crystals having m.p. 179–180 °C. The whole mixture was dissolved in DMF (15 ml), and the solution was added dropwise to NaH (420 mg, 17.6 mmol) in DMF (15 ml). Then benzyl bromide (1.39 ml, 11.7 mmol) was added dropwise to the reaction mixture, and the mixture was stirred at room temperature. After 40 min, the reaction was quenched with methanol and the mixture was evaporated. The residue was extracted with diethyl ether and washed with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 10:1 (v/v) toluene–ethyl acetate to give **2** (359 mg, 61.8%) as a syrup: $[\alpha]_D^{27.5} = +12.3°$ ($c = 1.60$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.20 (m, 30 H, Ph × 6), 5.81 (m, 1 H, –CH=C), 5.45 (s, 1 H, Ph–CH–O_2–), 4.46 (d, 1 H, $J_{1',2'} = 7.5$ Hz, H-1′), 4.37 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.02 (br-d, 1 H, $J_{3',4'} = 3.2$ Hz, H-4′), 3.93 (t, 1 H, $J_{4,5} = 6.6$ Hz, H-4), 3.86 (dd, 1 H, $J_{5,6b} = 4.6$ Hz, H-6b), 3.85 (dd, 1 H, $J_{6a,6b} = 10.7$ Hz, H-6a), 3.74 (m, 2 H, –OCH_2–), 3.62 (t, 1 H, $J_{2,3} = 8.8$ Hz, H-3), 3.53 (ddd, 1 H, $J_{5,6a} = 2.9$ Hz, H-5), 2.15 (m, 2 H, –CH_2–C=C), 1.75 (m, 2 H, –OC–CH_2–); Anal. $C_{59}H_{64}O_{11}$. Calcd: C, 74.66; H, 6.80. Found: C, 74.52; H, 6.80.

3.3. n-Pentenyl 4-O-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (3)

To a solution of **2** (119 mg, 125 μmol) in THF (1.95 ml) was added molecular seives 4 Å powder (119 mg) and stirred at 0 °C for 30 min. To subsequent solution was added trimethylamine–borane (63.8 mg, 875 μmol) and then aluminum chloride (117 mg, 875 μmol) added in numbers. The solution was stirred at room temperature for 1 h. The solution was filtered through Celite pad and the filtrate was extracted with chloroform and washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 3:1 (v/v) hexane–ethyl acetate to give **3** (91 mg, 76.3%) as an amorphous powder: $[\alpha]_D^{25.9} = +18.9°$ (c 1.91, $CHCl_3$); IR (KBr) ν 3506 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (m, 30 H, Ph × 6), 5.81 (m, 1 H, –CH=C), 4.53 (br-s, 1 H, H-1′), 4.44 (br-s, 1 H, H-1), 4.01 (m, 1 H, H-4′), 3.94 (br-d, 1 H, $J = 8.6$ Hz, H-4), 2.12 (m, 2 H, –CH_2–C=C), 1.65 (m, 2 H, –OC–CH_2–); Anal. $C_{59}H_{66}O_{11}$. Calcd: C, 74.50; H, 6.99. Found: C, 74.48; H, 7.05.

3.4. 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl chloride (5)

To a solution of **4** (4.00 g, 7.40 mmol) in 1,2-dichloroethane (30 ml) was added DMF (290 μl, 3.70 mmol) and cooled at 0 °C. To the solution was added thionyl chloride (3.22 ml, 444 mmol) and stirred at 0 °C for 20 h. The solution was filtered through silica gel and concentrated to give **5** (4.14 g, 100%): ^1H NMR ($CDCl_3$) δ 7.28 (m, 20 H, Ph × 4), 6.14 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-1), 4.45 (dd, 1 H, $J_{3,4} = 18.8$ Hz, H-3), 4.22 (dd, 1 H, $J_{2,3} = 11.9$ Hz, H-2).

3.5. n-Pentenyl 4-O-[4-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-β-D-glucopyranoside (6)

To a solution of **3** (4.35 g, 4.57 mmol) and **5** (6.10 g, 10.9 mmol) in distilled diethyl ether (200 ml) was added molecular seives 4 Å powder (4.14 g) and stirred for 30 min. To the mixture was added silver trifluoromethansulfonate (3.52 g, 13.7 mmol) and stirred at −20 °C for 3.5 h. The solution was diluted with chloroform and filtered through a pad of Celite, and the filtrate was extracted with chloroform and washed successively with aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 8:1 (v/v) hexane–ethyl acetate to give **6** (4.80 mg, 71.3%) as a syrup: $[\alpha]_D^{25.4} = +33.5°$ (c 0.51, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.22 (m, 50 H, Ph × 10), 5.80 (m, 1 H, –CH=C), 2.13 (m, 2 H, –CH_2–C=C), 1.73 (m, 2 H, –OC–CH_2–); ^{13}C NMR ($CDCl_3$) δ 103.51 (C-1′), 102.80 (C-1), 100.63 (C-1″), 114.79 (–C=CH_2); Anal. $C_{93}H_{100}O_{16}$. Calcd: C, 75.79; H, 6.84. Found: C, 75.83; H, 6.86.

3.6. n-Pentenyl 4-O-[4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-galactopyranosyl]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (7)

Na (1.97 g, 85.8 mmol) was added to liquid NH_3 (90 ml) at −78 °C and a solution of **6** (3.16 g, 2.15 mmol) in 1,2-dimethoxyethane (20 ml) was added dropwise to the mixture. After the mixture was stirred at −78 °C for 20 min, ammonium chloride (4.59 g, 85.8 mmol) was added to the reaction mixture and the mixture was stirred for 3 h. The mixture was evaporated and the residue was stirred with pyridine (45 ml) and acetic anhydride (30 ml) at room temperature for 21 h. The mixture was poured into ice–water. The extract with chloroform was washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 1:1 (v/v) hexane–ethyl acetate to give syrupy **7**

(1.53 g, 71.7%): $[\alpha]_D^{24.0} = +40.5°$ (*c* 1.11, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.78 (m, 1 H, –CH=C), 5.39 (dd, 1 H, $J_{3'',4''} = 3.3$ Hz, $J_{2''3''} = 11.0$ Hz, H-3″), 5.20 (t, 1 H, $J_{2,3} = 9.2$ Hz, $J_{3,4} = 9.4$ Hz, H-3), 5.18 (dd, 1 H, $J_{1'',2''} = 3.8$ Hz, H-2″), 5.10 (dd, 1 H, $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 10.8$ Hz, H-2′), 5.00 (m, 1 H, –C=CH_2), 4.99 (d, 1 H, $J_{1'',2''} = 2.9$ Hz, H-1″), 4.89 (br-t, 1 H, H-2), 4.73 (dd, 1 H, $J_{2',3'} = 10.9$ Hz, $J_{3',4'} = 2.4$ Hz, H-3′), 4.52 (d, 1 H, $J_{1',2'} = 7.6$ Hz, H-1′), 4.46 (d, 1 H, $J_{1,2} = 7.7$ Hz, H-1), 4.45 (m, 3 H, $J_{6a,6b} = 11.1$ Hz, $J_{5,6b} = 6.3$ Hz, H-6b, 6a″ and 6b″), 4.13 (m, 4 H, H-6a, 6a′, 6b'and 5″), 4.01 (br-d, 1 H, H-4′), 3.84 (t, 1 H, $J_{4,5} = 9.0$ Hz, H-4), 3.81 (m, 2 H, –OCH_2–), 3.79 (t, 1H, $J_{5',6b'} = 9.4$ Hz, H-5′), 3.48 (ddd, 1 H, $J_{5,6a} = 3.5$ Hz, $J_{5,6b} = 4.8$ Hz, $J_{4,5} = 9.6$ Hz, H-5), 2.08 (m, 32 H, –OAc × 10, –CH_2–C=C), 1.75 (m, 2 H, –OC–CH_2–); ^{13}C NMR ($CDCl_3$) δ 101.02 (C-1′), 100.49 (C-1), 99.54 (C-1″), 114.99 (–C=CH_2); Anal. $C_{43}H_{60}O_{26}$. Calcd: C, 52.01; H, 6.09. Found: C, 52.38; H, 6.15.

3.7. n-Pentenyl 4-O-[4-O-(α-D-galactopyranosyl)- β-D-galactopyranosyl]-β-D-glucopyranoside (8)

To a solution of **7** (1.10 g, 11.0 mmol) in methanol (11 ml) was added sodium methoxide (59.9 mg, 1.10 mmol), and the mixture was stirred for 16 h at room temperature. IR-120B (H^+) resin (875 μl) was added to neutralize the solution, and the suspension was filtered and evaporated to give **8** (632 mg, 99.9%): ^1H NMR (D_2O) δ 5.87 (m, 1 H, –CH=C), 5.05 (dd, 1 H, $J_{trans} = 17.1$ Hz, $J_{gem} = 1.35$ Hz, –C–C=CH), 4.98 (dd, 1 H, $J_{cis} = 10.2$ Hz, –C–C=CH), 4.90 (d, 1 H, $J_{1'',2''} = 3.8$ Hz, H-1″), 4.46 (d, 1 H, $J_{1',2'} = 8.0$ Hz, H-1′), 4.43 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1), 3.25 (t, 1 H, H-2), 2.10 (m, 2 H, –CH_2–C=C), 1.68 (m, 2 H, –OC–CH_2–).

3.8. 5-(2-aminoethylthio) pentyl 4-O-(β-D-galactopyranosyl)- β-D-glucopyranoside hydrochloride (9)

To a solution of **1** (200 mg, 487 μmol) in MeOH (3.0 ml) was added 2-aminoethanthiol hydrochloride (277 mg, 2.44 mmol) and irradiated with ultraviolet light (254 nm) at 0 °C for 3 h. The mixture was concentrated and then the residue was purified by gel filtration with aqueous 5% acetic acid to give crude **9** (294 mg) containing acetic acid as an impurity, which was used for next step without further purification: ^1H NMR (D_2O) δ 3.09 (dd, 2 H, –CH_2–N–C–), 2.74 (dd, 2 H, –S–CH_2–), 2.49 (dd, 2 H, –CH_2–S–).

3.9. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyrosyl)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (10)

Compound **9** (100 mg, 191 μmol) was dissolved in methanol (1.0 ml) and cooled at 0 °C. To the solution was simultaneously added triethylamine (41.4 μl, 573 μmol) and acryloyl chloride (19.5 μl, 229 μmol) dropwise five times. After removal of the solvent, acetic anhydride (3.0 ml) and pyridine (3.0 ml) was added to the mixture at room temperature and the mixture was stirring for 15 h, and concentrated. The residue was extracted with chloroform and washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 1:3 (v/v) toluene–ethyl acetate to give corresponding **10** (132 mg, 82.6%): $[\alpha]_D^{14.9} = -14.4°$ (*c* 1.36, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.30 (dd, 1H, $J_{trans} = 17.0$ Hz, $J_{gem} = 1.42$ Hz, –C–C=CH), 6.13 (m, 1 H, –NH–), 6.13 (dd, 1 H, $J_{cis} = 10.2$ Hz, –C–CH=C), 5.66 (dd, 1 H, –C–C=CH), 5.35 (br-d, 1 H, H-4″), 3.52 (m, 2 H, –CH_2–N–), 2.69 (t, 2 H, $J = 6.4$, –S–CH_2–C–N–), 2.52 (t, 2 H, $J = 7.2$, –C–CH_2–S–), 2.00 (m, 30 H, –OAc × 10), 1.58 (m, 4 H, –CH_2– × 2), 1.43 (m, 2 H, –CH_2–); Anal. $C_{36}H_{53}N_1O_{19}S_1$. Calcd: C, 51.73; H, 6.39; N, 1.68. Found: C, 51.79; H, 6.41; N, 1.51.

3.10. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-(β-D-galactopyranosyl)- β-D-glucopyranoside (11)

To a solution of **10** (950 mg, 1.14 mmol) in methanol (12 ml) was added sodium methoxide (43.0 mg, 795 μmol), and the mixture was stirred for 2.5 h at room temperature. IR-120B (H^+) resin (628 μl) was added to neutralize the solution, and the suspension was filtered and evaporated to give **11** (612 mg, 99.4%): m.p.: 159 °C; IR (KBr) ν 3420 (N–H), 2920 (O–H), 1653 (C=O), 1627 (N–H) cm^{-1}; ^1H NMR (D_2O) δ 6.10 (m, 1 H, –CH=C), 5.66 (br-d, 1 H, –C=CH), 4.35 (m, 2 H, H-1′ and 1), 3.82 (m, 1 H, H-4′), 3.68 (m, 1 H, H-3′), 3.56 (m, 2 H, H-3, 5), 3.45 (m, 2 H, H-2, 4), 3.38 (m, 2 H, –CH_2–N–), 3.20 (m, 3 H, H-2, –O–CH–), 2.64 (m, 2 H, –CH_2–S–), 2.49 (m, 2 H, –S–CH_2–), 1.51 (m, 4 H, –CH_2– × 2), 1.28 (m, 2 H, –CH_2–); Anal. $C_{36}H_{53}N_1O_{19}S_1 \cdot 0.25\ H_2O$. Calcd: C, 48.38; H, 7.29; N, 2.56. Found: C, 48.35; H, 7.05; N, 2.44.

3.11. 5-(2-aminoethylthio) pentyl 4-O-[4-O-(α-D-galactopyranosyl)- β-D-galactopyranosyl]-β-D-glucopyranoside hydrochloride (12)

To a solution of **8** (200 mg, 349 μmol) in MeOH (2.0 ml) was added 2-aminoethanthiol hydrochloride (198 mg, 1.75 mmol) and irradiated with ultraviolet light (254 nm) at 0 °C for 3 h. The mixture was concentrated and then the residue was purified by gel filtration with aqueous 5% acetic acid to give **12** (241 mg, 100%): ^1H NMR (D_2O) δ 3.09 (dd, 2 H, –CH_2–N–C–), 2.73 (dd, 2 H, –S–CH_2–), 2.47 (dd, 2 H, –CH_2–S–).

3.12. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-[4-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-2,3,6-tri-O-acetyl-β-D-galactopyranosyl]-2,3,6-tri-O-acetyl-β-D-glucopyranoside (13)

Compound **12** (225 mg, 328 μmol) was dissolved in methanol (3.0 ml) and cooled at 0 °C. Then to the solution was added sodium hydrogen carbonate (165 mg, 1.97 mmol) and acryloyl chloride (80.0 μl, 984 μmol) dropwise four times. To the mixture was added acetic anhydride (7.0 ml) and pyridine (10.0 ml) at room temperature for 13 h and concentrated. The residue was diluted with chloroform and washed successively with aqueous 1 M hydrochloric acid, aqueous sodium hydrogen carbonate and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by silica gel chromatography with 1:3 (v/v) toluene–ethyl acetate to give **13** (163 mg, 44.2%): $[\alpha]_D^{14.6} = +34.3°$ (*c* 1.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.24 (dd, 1 H, $J_{trans} = 17.1$ Hz, $J_{gem} = 1.1$ Hz, –C–C=CH), 6.22 (m, 1 H, –NH–), 6.09 (dd, 1 H, $J_{cis} = 10.2$ Hz, –C–C=CH), 5.60 (dd, 1 H, –C–C=CH), 5.53 (br-d, 1 H, H-4″), 5.33 (dd, 1 H, $J_{2'',3''} = 11.2$ Hz, $J_{3'',4''} = 3.2$ Hz H-3″), 5.14 (t, 1 H, $J_{2,3} = 9.1$ Hz, H-3), 5.13 (dd, 1 H, $J_{1'',2''} = 3.5$ Hz, H-2″), 5.05 (dd, 1 H, $J_{1',2'} = 7.5$ Hz, $J_{2',3'} = 10.7$ Hz, H-2′), 4.94 (d, 1 H, H-1″), 4.83 (dd, 1 H, $J_{1,2} = 8.0$ Hz, H-2), 4.69 (dd, 1 H, $J_{3',4'} = 2.7$ Hz, H-3′), 4.47 (d, 1 H, H-1′), 4.44 (m, 2 H, H-6a″, 6b″), 4.42 (d, 1 H, H-1), 4.38 (dd, 1 H, $J_{5,6b} = 6.7$ Hz, $J_{6a,6b} = 11.5$ Hz, H-6b), 4.07 (m, 4 H, H-6a, 6a′, 6b′ and 5″), 3.97 (br-d, 1 H, H-4′), 3.77 (m, 3 H, H-4, $-OCH_2$), 3.74 (t, 1 H, $J_{5',6a'} = J_{5',6b'} = 3.7$ Hz, H-5′), 3.43 (m, 2 H, $-CH_2-N-$), 2.63 (t, 2 H, $J = 2.6$ Hz, $-S-CH_2-C-N-$), 2.47 (t, 2 H, $J = 2.5$, $-C-CH_2-S-$), 1.99 (m, 30 H, –OAc × 10), 1.52 (m, 4 H, $-CH_2-$ × 2), 1.37 (m, 2 H, $-CH_2-$); Anal. $C_{48}H_{69}N_1O_{27}S_1$. Calcd: C, 51.29; H, 6.19; N, 1.25. Found: C, 51.27; H, 6.18; N, 1.22.

3.13. 5-(2-N-acryloylaminoethylthio) pentyl 4-O-[4-O-(α-D-galactopyranosyl)- β-D-galactopyranosyl]-β-D-glucopyranoside (14)

To a solution of **13** (225 mg, 200 μmol) in methanol (2.85 ml) was added sodium methoxide (10.8 mg, 200 μmol), and the mixture was stirred for 8 h at room temperature. IR-120B (H^+) resin (158 μl) was added to neutralize the solution, and the suspension was filtered and evaporated to give **14** (141 mg, 100%): IR (KBr) ν 3445 (N–H), 2922 (O–H), 1654 (C=O), 1623 (N–H) cm^{-1}; ^{1}H NMR (D_2O) δ 6.10 (m, 2 H, –CH=C, –C=CH), 5.66 (d, 1 H, –C=CH), 4.83 (d, 1 H, H-1″), 4.39 (d, 1 H, H-1′), 4.34 (d, 1 H, H-1), 4.23 (br-t, 1 H, H-5″), 3.92 (br-d, 1 H, H-4′), 3.80 (m, 1 H, H-5′), 3.73 (m, 1 H, H-2″), 3.62 (m, 2 H, H-3′ and 4″), 3.52 (m, 2 H, H-3 and 5), 3.46 (m, 2 H, H-3″, 2′ and 4), 3.36 (m, 2 H, $-CH_2-N-$), 3.24 (s, 2 H, $-OCH_2-$), 3.18 (t, 1 H, H-2), 2.65 (m, 2 H, $-S-CH_2-$), 2.51 (m, 2 H, $-CH_2-S-$), 1.52 (m, 4 H, $-CH_2-$ × 2), 1.30 (m, 2 H, $-CH_2-$); ^{13}C NMR (D_2O) δ 168 (–C=O), 130 (–*C*=C), 128 (–C=*C*), 103 (C-1′), 102 (C-1), 100 (C-1″).

3.14. Copolymerization of glycosyl monomer with acrylamide

A solution of the glycosyl monomer **1** (100 mg, 185 μmol) and 10 molar equiv of acrylamide (131 mg, 1.85 mmol) in distilled water (2.0 ml) was degassed using a diaphragm pump, and TEMED (2.74 μl, 18.5 μmol) and APS (1.67 mg, 7.3 μmol) were added. The reaction mixture was continuously stirred for 2 h at room temperature, diluted with 0.1 M acetic acid–pyridine buffer (pH 5.00), purified by using gel filtration (Sephadex G-50), and lyophilized to give the water-soluble copolymer as a white powder. The same procedure was carried out for each of the monomers **8**, **11** and **14**.

3.15. Polymerization of glycosyl monomer

A solution of a glycosyl monomer **11** or **14** (100 mg, 185 μmol) in distilled water (2.0 ml) was degassed using a diaphragm pump, and TEMED (2.74 μl, 18.5 μmol) and APS (1.67 mg, 7.3 μmol) were added. The reaction mixture was continuously stirred for 2 h at room temperature, diluted with 0.1 M acetic acid–pyridine buffer (pH 5.00), purified using gel filtration (Sephadex G-50), and lyophilized to give the water-soluble copolymer as a white powder.

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