Synthesis of Thermosensitive Glycopolymers Containing D-Glucose Residue: Copolymers with *N*-isopropylacrylamide

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ABSTRACT: A new glycomonomer, 3-acrylamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, was synthesized from D-glucose. This monomer was homopolymerized and copolymerized with *N*-isopropylacrylamide in different compositions by free-radical polymerization. The composition of the copolymer was determined with ^{1}H-NMR spectroscopy. On acid hydrolysis, water-soluble deprotected copolymers were obtained. The protected and deprotected copolymers showed a sharp cloud-point temperature. A linear correlation was obtained between the lower critical solution temperatures and the concentration of glycomonomer in the copolymers © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 2607–2615, 2009

Key words: copolymerization; gel permeation chromatography (GPC); radical polymerization; stimuli-sensitive polymers; water-soluble polymers

INTRODUCTION

Sugar-containing synthetic macromolecules are generally called glycopolymers and can mimic naturally occurring glycoconjugates.[1] Glycopolymers with different architectures such as linear polymers, comb polymers, dendrimers, and crosslinked hydrogels have been reported.[2,3] These polymers are widely used as macromolecular drugs,[4,5] biocatalysts,[6] biosensitive hydrogels,[7] matrices for controlled cell culture,[8,9] stationary phases for separation problems,[10] surface modifiers,[11,12] artificial tissues and artificial organ substrates,[13,14] and in drug delivery systems.[15,16] Polymerizations of glycomonomers with different functionalities such as alkenyl,[17] alkynyl,[18] acryloyl,[19,20] methacryloyl,[21,22] acrylamide,[23,24] styryl,[25,26] and vinyl ether[27,28] have been successfully achieved. Glycopolymers have been prepared by different polymerization techniques, which include free-radical polymerization,[29] ionic polymerization,[27,30] coordination polymerization,[31] ring-opening polymerization,[32] ring-opening metathesis polymerization,[33] reversible addition–fragmentation chain transfer polymerization,[34] nitroxide-mediated polymerization,[25] cyanoxyl radical mediated polymerization,[35,36] and atom transfer radical polymerization.[19,37]

Stimuli-responsive polymers exhibit reversible phase changes in response to changes in environmental factors such as the temperature, pH, light, electric field, chemicals, and ionic strength.[38] In this respect, temperature- and pH-sensitive polymers have been extensively probed because they are more convenient and effective controlled-release systems.[39] Poly(*N*-isopropylacrylamide) (PNIPAm) is the most widely studied thermosensitive polymer and shows a coil-to-globule transition at a lower critical solution temperature (LCST) of 32–33°C.[40] The transition temperature can be controlled by the incorporation of a hydrophobic comonomer, which reduces the LCST, and a hydrophilic comonomer, which increase the LCST. Thus, one can tailor the hydrophilic and hydrophobic contents of a polymer to obtain a desired LCST, and this has wide applications in nanotechnology and biological systems.[41,42] In our previous work,[43] we demonstrated that the chemical structure of the hydrophobe and its concentration determine the LCST and heat of transition of hydrophobically modified PNIPAm copolymer gels.

Despite the increasing demand for thermosensitive glycopolymers, only a few reports are available on well-defined glycopolymers. Raku and Tokiwa[44] reported copolymers of 6-*O*-vinyladipoyl-D-glucose with *N*-isopropylacrylamide (NIPAm), which resulted in an increase in the LCST accompanied by a decrease in the heat of transition. Kim and Park[45]

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found that the copolymerization of hydrophilic acrylamido-2-deoxy-D-glucose with NIPAm produced an upward shift in the LCST. It was also noted that a copolymer of NIPAm with glucosyloxyethyl methacrylate shifted the LCST to higher temperatures.[46] Zhou et al.[47] found an increase in the LCST of a copolymer gel of NIPAm with acrylamidolactamine. Voit et al.[48] synthesized copolymers of NIPAm with 3′-(1′,2′:5′,6′-di-*O*-isopropylidene-α-D-glucofuranosyl)-6-methacrylamido hexanoate and with 3′-(1′,2′:5′,6′-di-*O*-isopropylidene-α-D-glucofuranosyl)-6-methacrylamido undecanoate. After deprotection, it was shown that the LCSTs of the copolymers were affected by the comonomer content, the spacer chain length of the glycomonomer, and the chain architecture of the copolymers. Stenzel et al.[49] reported the synthesis of thermosensitive diblock copolymers based on PNIPAm and poly (acryloyl glucosamine) by reversible addition–fragmentation chain transfer polymerization. Recently, Alexander et al.[50] described the reversible aggregation of a bacterial strain, *Escherichia coli*, controlled by a thermoresponsive glycopolymer through a combination of a cluster glycoside effect and polymer conformation.

This work reports the synthesis of a new glycomonomer, 3-acrylamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (AmIGlc or **8**), which was copolymerized with NIPAm to produce a hydrophobically modified copolymer by free-radical polymerization with 2,2′-azobisisobutyronitrile (AIBN) as an initiator. The isopropylidene groups of the sugar moiety of the copolymer were deprotected by aqueous formic acid to obtain a water-soluble polymer. The protected and deprotected copolymers showed downward and upward shifts in the LCST with respect to that of PNIPAm. A linear relation was obtained between the concentration of the glycomonomer and the LCSTs of the copolymers.

EXPERIMENTAL

Materials

NIPAm was obtained from Aldrich Chemicals (Milwaukee, WI), and was recrystallized from *n*-hexane. AIBN, purchased from Aldrich Chemicals, was purified by recrystallization in methanol (MeOH). All other chemicals were analytical-grade and were used as such. The solvents and reagents were dried before use, as reported elsewhere.[51]

Methods

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury NMR apparatus (Palo Alto, CA) in the solvents $CDCl_3$ and D_2O with tetramethylsilane as an internal standard. The chemical shifts are reported in δ (ppm) units with proton and carbon frequencies of 300 and 75 MHz, respectively. Fourier transform infrared (FTIR) spectra were recorded on a Shimadzu FTIR 8400 spectrometer. Optical rotations were measured with a Jasco P-1020 polarimeter at 25°C. Elemental analysis was carried out on a Thermo-Electron Corp. CHNS analyzer (Flash-EA 1112).

Synthesis of the monomer

A new glycomonomer (**8**) was synthesized from D-glucose according to Scheme 1. 3-Amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**7**) was prepared according to earlier reports.[52,53]

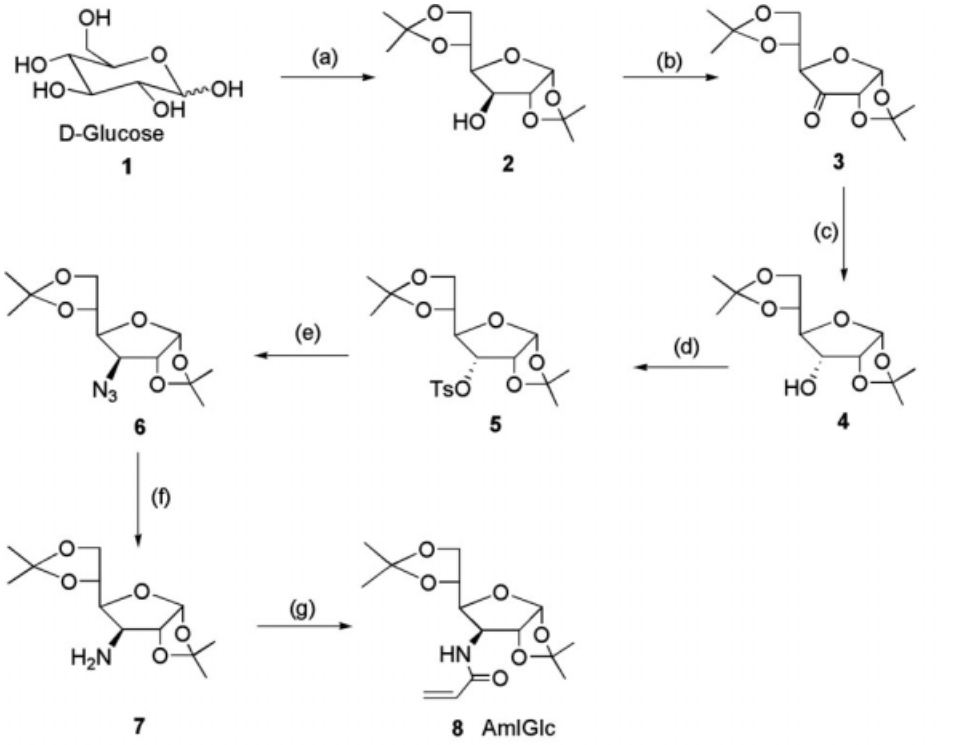
1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**2**)

D-Glucose (100 g, 555.6 mmol) was added to dry acetone (2 L) at room temperature and was followed by anhydrous $CuSO_4$ (100 g, 625 mmol). The reaction mixture was cooled to 0°C, and a catalytic amount of concentrated H_2SO_4 (4 mL, 16.8 mmol) was added dropwise over a period of 10 min. The reaction mixture was stirred at room temperature for 30 h. It was then neutralized with a saturated K_2CO_3 solution. The solution was filtered, and the filtrate was evaporated under reduced pressure. The residue thus obtained was extracted with chloroform (60 mL × 3). The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotavapor to afford a yellowish solid, which was recrystallized from chloroform–hexane (1 : 9) to give white crystals of **2** (85 g, yield = 59%).

mp: 108–110°C. Retention factor (R_f): 0.4 (ethyl acetate/hexane = 3/7). $[\alpha]^{25}_D$: −12.5 (c = 1, $CHCl_3$). ANAL. Calcd for $C_{12}H_{20}O_6$: C, 55.37%; H, 7.74%. Found: C, 55.10%; H, 7.04%. IR (KBr, disk, cm^{-1}): 1024.1 (C—O), 3354.2 (OH). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.31 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 2.97 (bs, 1H —OH, D_2O exchangeable), 3.98–4.03 (m, 2H), 4.14 (m, 1H, H-4), 4.29 (m, 2H), 4.59 (d, J = 4.0 Hz, 1H, H-2), 5.91 (d, J = 3.8 Hz, 1H, H-1). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 25.20, 26.20, 26.84, 26.98 (4 × CH_3), 67.56 (^{6}C), 73.12 (^{3}C), 74.84 (^{5}C), 81.15 (^{4}C), 85.07 (^{2}C), 105.12 (^{1}C), 109.45 (quaternary C), 111.63 (quaternary C).

1,2:5,6-Di-*O*-isopropylidine-α-D-glucofuran-3-one (**3**)

To a mixture of dry pyridinium chlorochromate (PCC; 150 g) and powdered 4-Å molecular sieves (150 g) in CH_2Cl_2 (300 mL) was added a solution of **2** (50 g) in dry CH_2Cl_2 (200 mL), and the reaction mixture was stirred at room temperature for 12 h. The product (**3**) was filtered through a silica gel column with ether as an eluent. This filtrate was evaporated



Scheme 1 Synthesis of glycomonomer AmIGlc.

under reduced pressure to obtain the keto compound. This crude, sticky, white solid (27.7 g, yield = 93%) was used directly for further reaction.

R_f: 0.5 (ethyl acetate/hexane = 3/7). $[\alpha]^{25}_D$: +44.0 (c = 1, $CHCl_3$). ANAL. Calcd for $C_{12}H_{18}O_6$: C, 55.82%, H, 7.02%. Found: C, 55.21%; H, 7.47%. IR (KBr, disk, cm^{-1}): 1081.1 (C—O), 1773.2 (C=O). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.31 (s, 6H, 2 × CH_3), 1.45 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 4.02 (m, 2H), 4.33–4.39 (m, 3H), 6.12 (d, J = 4.5 Hz, 1H, H-1). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 25.44, 26.12, 27.32, 27.71 (4 × CH_3), 64.43 (^{6}C), 76.52 (^{4}C), 77.40 (^{5}C), 79.10 (^{2}C), 103.75 (^{1}C), 109.84 (quaternary C), 112.63 (quaternary C), 209.05 (C=O).

1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**4**)

To a solution of ketone **3** (18.4 g, 71.4 mmol) in MeOH (100 mL) and water (10 mL), sodium borohydride ($NaBH_4$; 3.2 g, 85.6 mmol) was added in portions (0.2 g each with 7.5-min intervals over 2 h) at −10°C, and the mixture was stirred continuously. After completion of the reaction, 10% aqueous HCl was added until pH 7 to quench the excess of $NaBH_4$. It was then extracted with CH_2Cl_2 and washed with water. The organic layer was dried over anhydrous sodium sulfate and evaporated on a rotavapor to get a white solid **4**; (17.6 g, yield = 95%).

mp: 75–76°C. R_f: 0.3 (ethyl acetate/hexane = 3/7). $[\alpha]^{25}_D$: +37.6 (c = 1, $CHCl_3$). ANAL. Calcd for $C_{12}H_{20}O_6$: C, 55.37%, H, 7.74%. Found: C, 55.97%; H, 7.47%. IR (KBr, disk, cm^{-1}): 1026.1 (C—O), 3359.5 (OH). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.35 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 2.55 (d, J = 8.4 Hz 1H —OH, D_2O exchangeable), 3.80 (dd, J = 4.8, 8.5 Hz, 1H, H-6b), 3.97–4.08 (m, 3H, H-3, H-4, H-6a), 4.29 (ddd, J = 4.9, 6.6, 6.6 Hz, 1H, H-5), 4.59 (dd, J = 4.0, 5.1 Hz, 1H, H-2), 5.80 (d, J = 3.8 Hz, 1H, H-1). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 25.20, 26.24, 26.41, 26.59 (4 × CH_3), 65.72 (^{6}C), 72.44 (^{3}C), 75.51 (^{5}C), 78.81 (^{4}C), 79.69 (^{2}C), 103.75 (^{1}C), 109.84 (quaternary C), 112.63 (quaternary C).

1,2:5,6-Di-*O*-isopropylidene-3-*O*-tosyl-α-D-allofuranose (**5**)

To a solution of **4** (17.3 g, 66.5 mmol) in dry pyridine (125 mL), tosyl chloride (13.9 g, 73.2 mmol) followed by a catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP; 0.02 g, 0.16 mmol) was added at 0°C under a nitrogen atmosphere. Then, the reaction mixture

was stirred for 6 h at room temperature. After completion of the reaction, the mixture was neutralized (pH = 7) with 10% aqueous HCl. Then, it was extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulfate and evaporated under reduced pressure. Column purification of the product afforded a white, crystalline solid **5**; (27.2 g, yield = 98%).

mp: 107–109°C. R_f: 0.6 (ethyl acetate/hexane = 2/8). $[\alpha]^{25}_D$: +64.00 (c = 0.084, $CHCl_3$). ANAL. Calcd for $C_{19}H_{26}O_8S$: C, 55.06%; H, 6.32%. Found: C, 55.91%; H, 6.71%. IR (KBr, disk, cm^{-1}): 1026.1 (C—O), 1371.3 (S=O), 2987 (C—H). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.32 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 2.45 (s, CH_3—Ar), 3.78 (t, J = 8.4 Hz, 1H, H-4), 3.92 (dd, J = 6.6, 8.1 Hz, 1H, H-3), 4.13–4.22 (m, 2H, H-5 and H-6a), 4.67 (m, 2H, H-2 and H-6b), 5.75 (d, J = 3.0 Hz, 1H, H-1), 7.33 (d, J = 7.8 Hz, 2H, ArH), 7.85 (d, J = 7.8 Hz, 2H, ArH). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 21.75 (Ar-CH_3), 25.15, 26.14, 26.67, 26.73 (4 × CH_3), 65.20 (^{6}C), 74.66 (^{5}C), 76.61 (^{4}C), 77.01 (^{3}C), 77.96 (^{2}C), 103.75 (^{1}C), 109.84 (quaternary C), 113.52 (quaternary C), 128.24 (2 × Ar), 129.56 (2 × Ar), 133.04 (Ar—C—CH_3), 145.05 (Ar—C—SO_3).

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**)

To a stirred solution of **5** (11.0 g, 26.6 mmol) in anhydrous dimethylformamide (DMF; 120 mL), sodium azide (4.3 g, 66.5 mmol) and tetrabutylammonium iodide (TBAI; 4.9 g, 13.3 mmol) were added under a nitrogen atmosphere. The solution was heated at 110°C in an oil bath for 72 h. After completion of the reaction, the solvent was removed under reduced pressure. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated on a rotavapor. Silica gel column purification afforded a thick, yellow liquid **6**; (5.5 g, yield = 72%).

R_f: 0.7 (ethyl acetate/hexane = 1.5/8.5). $[\alpha]^{25}_D$: −24.37 (c = 0.087, $CHCl_3$). ANAL. Calcd for $C_{12}H_{19}N_3O_5$: C, 50.52%; H, 6.71%; N, 14.73%. Found: C, 51.01%; H, 6.81%; N, 14.12%. IR (thin film, cm^{-1}): 1072 (C—O), 2108 (—N=N=N), 2985 (C—H). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.33 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 3.98 (dd, 1H, J = 4.8 and 8.7 Hz, H-4), 4.07–4.16 (m, 3H, H-3, H-6a, and H-6b), 4.23 (m, 1H, H-5), 4.61 (d, 1H, J = 3.6 Hz, H-2), 5.9 (d, 1H, J = 3.6 Hz, H-1). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 24.92, 24.95, 26.41, 26.63 (4 × CH_3), 66.08 (^{3}C), 67.32 (^{6}C), 72.74 (^{5}C), 80.19 (^{4}C), 83.10 (^{2}C), 105.08 (^{1}C), 109.01 (quaternary C), 111.83 (quaternary C).

3-Amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**7**)

A solution of **6** (1.6 g, 5.6 mmol) in dry MeOH (20 mL) and 5% Pd/C (0.05 g) was hydrogenated under 2.76 × 10^6 Pa for 30 min. After completion of the reaction, it was filtered through a Celite bed, washed with MeOH, and concentrated on a rotavapor to give amine **7** as a sticky, white solid (1.4 g, yield = 98.5%).

R_f: 0.2 (ethyl acetate). $[\alpha]^{25}_D$: −19.82 (c = 0.032, $CHCl_3$). ANAL. Calcd for $C_{12}H_{21}NO_5$: C, 55.58%; H, 8.16%; N, 5.40%. Found: C, 55.31%; H, 8.99%; N, 5.84%. IR (KBr, disk, cm^{-1}): 3405 and 1590 (N—H), 1110 (C—N). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.31 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.92 (bs, 2H, —NH_2, D_2O exchangeable), 3.57 (d, 1H, J = 2.7 Hz, H-3), 3.98 (dd, 1H, J = 4.5 and 8.4 Hz, H-6a), 4.03 (dd, 1H, J = 2.7 and 8.7 Hz, H-4), 4.15 (dd, 1H, J = 6 and 8.4 Hz, H-6b), 4.21 (m, 1H, H-5), 4.43 (d, 1H, J = 3.3 Hz, H-2), 5.90 (d, 1H, J = 3.5 Hz, H-1). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 25.32, 26.25, 26.81, 26.93 (4 × CH_3), 57.41 (^{3}C), 68.12 (^{6}C), 72.84 (^{5}C), 81.19 (^{4}C), 86.10 (^{2}C), 104.88 (^{1}C), 109.41 (quaternary C), 111.63 (quaternary C).

3-Acrylamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**8**)

To a stirred solution of **7** (1.5 g, 5.8 mmol) in CH_2Cl_2 (20 mL) was added triethyl amine (NEt_3, 0.8 mL, 6.9 mmol), which was followed by the dropwise addition of acryloyl chloride (0.5 mL, 6.3 mmol) through a syringe at 0°C. The mixture was stirred at the same temperature (10 min) and quenched by the addition of water (10 mL). The mixture was extracted with CH_2Cl_2 (15 mL × 3) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford compound **8** as a white, crystalline solid (1.54 g, yield = 85%).

mp: 151–152°C. R_f: 0.7 (ethyl acetate). $[\alpha]^{25}_D$: −58.21 (c = 0.070, $CHCl_3$). ANAL. Calcd for $C_{15}H_{23}NO_6$: C, 57.50%; H, 7.40%; N, 4.47%. Found: C, 57.31%; H, 7.09%; N, 4.84%. IR (KBr, disk, cm^{-1}): 3319 (N—H), 1656 (C=O), 1626 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.31 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 3.82 (app. t, 1H, J = 7.2 Hz and 8.1 Hz, H-3), 4.13 (dd, 1H, J = 6.3 Hz and 8.1 Hz, H-4), 4.23 (m, 1H, H-5), 4.42 (m, 2H, H-6a and H-6b), 4.69 (d, 1H, J = 3.3 Hz, H-2), 5.68 (d, 1H, J = 10.2 Hz, H-9), 5.88 (d, 1H, J = 3.3 Hz, H-1), 6.06 (dd, 1H, J = 10.2 and 16.8 Hz, H-8), 6.32 (d, 1H, J = 17.1 Hz, H-10), 6.57 (bs, 1H, —NH, D_2O exchangeable). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 25.02, 26.05, 26.48, 30.96 (4 × CH_3),

TABLE I
Summary of the Copolymers of NIPAm and AmIGlc

Sample	Feed ratio (NIPAm:AmIGlc)	Composition (NIPAm:AmIGlc)[a]	M_n ($\times 10^{-3}$ g/mol)[b]	PDI[b]	T_c (°C)	
					Protected[c]	Deprotected[d]
PNIPAm	100 : 00	100 : 00	—	—	33.5	—
S-5	95 : 05	97 : 03	—	—	28.6	37.6
S-10	90 : 10	91 : 09	11.71	1.49	24.3	42.5
S-20	80 : 20	86 : 14	09.58	2.05	16.1	57.1
S-25	75 : 25	80 : 20	11.86	1.82	13.1	64.6
PAmIGlc	00 : 100	00 : 100	03.03	1.52	—	—

[a] Determined from ^{1}H-NMR integrations.
[b] Polydispersity index determined from GPC (polystyrene calibration).
[c] Cloud temperature of the copolymer containing a protected sugar moiety.
[d] Cloud temperature of the copolymer containing a deprotected sugar moiety.

56.43 (^{3}C), 69.35 (^{6}C), 73.16 (^{5}C), 79.06 (^{4}C), 83.87 (^{2}C), 104.22 (^{1}C), 109.69 ($^{1\circ}$C), 111.84 (^{13}C), 127.63 (^{8}C), 130.11 (^{9}C), 165.31 (^{7}C).

Polymerization

Homopolymers of AmIGlc and NIPAm and their copolymers (S-5, S-10, S-20, and S-25) were prepared by free-radical polymerization with AIBN at 65°C in 1,4-dioxane for 24 h according to the feed ratio given in Table I. In a typical procedure, a mixture of monomer **8** (0.064 g, 0.20 mmol), NIPAm (0.43 g, 3.85 mmol), and AIBN (7.0 mg) in 1,4-dioxane (5 mL) was placed in a glass tube, and nitrogen gas was bubbled through the tube for 20 min. The reaction was maintained at 65°C for 24 h. The content was precipitated in distilled *n*-hexane and again dissolved in acetone and reprecipitated in *n*-hexane. Finally, the product was dried under reduced pressure at 50°C for 2 days. All polymers were obtained as white powders in quantitative yields.

Deprotection

The 1,2- and 5,6-di-isopropylidene protection of the sugar moiety of the polymer was removed under mildly acidic conditions.[19] The protected polymer (200 mg) was dissolved in 23 mL of a formic acid solution (85%) and stirred for 48 h at room temperature. The resulting solution was dialyzed (Sigma–Aldrich; molecular weight cutoff = 1000) against double-distilled water for 2 days and freeze-dried. The deprotected polymer was obtained as a white powder (120 mg, yield = 60%).

Characterization

Each polymer was characterized with ^{1}H-NMR and ^{13}C-NMR spectroscopy. The average molecular weights of the protected homopolymer and copolymers were determined with a Thermofinigan gel permeation chromatography (GPC) apparatus (Waltham, MA) with a refractive-index detector and with tetrahydrofuran as an eluent at flow rate of 1.5 mL/min at room temperature. A column of 10-μ SDV gel was used. For calibration, narrow-polydispersity polystyrene standards (Polymer Standards Services) with a molecular weight range of 500–50,000 Da were used.

Cloud-point (LCST) measurements

The cloud points of 0.2% solutions of the copolymers in double-distilled water were determined by the measurement of the temperature-dependent optical density at 500 nm with a PerkinElmer Lambda 35 ultraviolet–visible spectrometer (Waltham, MA) equipped with a temperature-regulated bath. The temperature scanning rate was 1°C/min.

RESULTS AND DISCUSSION

Glycomonomer synthesis

As shown in Scheme 1, D-glucose was converted to **2** and oxidized to ketone **3** with PCC. The reduction of **3** with $NaBH_4$ gave **4**, which on tosylation gave the tosyl-protected product **5**. Compound **5** on treatment with sodium azide in DMF at 110°C afforded azido compound **6**, which on hydrogenation gave amine **7**. The amine on reaction with acryloyl chloride furnished glycomonomer AmIGlc in a 31% overall yield from D-glucose. The glycomonomer was characterized with ^{1}H-NMR and ^{13}C-NMR (Figs. 1 and 2, respectively) with full signal assignments.

Homopolymers and copolymers with NIPAm

As shown in Scheme 2, random copolymers of AmIGlc and NIPAm were prepared with an initiator, AIBN, in 1,4-dioxane at 65°C by free-radical polymerization. Figure 3 shows the ^{1}H-NMR spectra of copolymers S-5, S-10, S-20, and S-25 and the spectra of two homopolymers, PNIPAm and

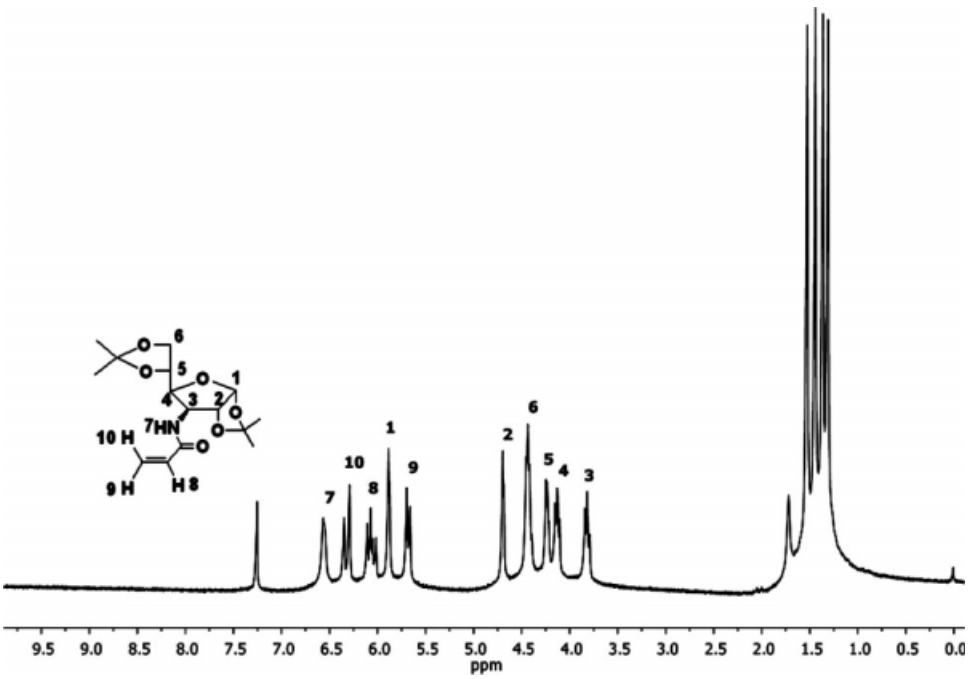


Figure 1 ^{1}H-NMR spectrum of AmIGlc.

poly(3-acrylamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose) (PAmIGlc). In the PAmIGlc homopolymer and copolymer spectra, the disappearance of olefinic proton peaks and appearance of signals for the backbone —CH and —CH_2 peaks at 1.5–2.5 ppm confirmed the formation of the polymers.

The incorporation of the glycomonomer into the copolymer was confirmed by a careful analysis of all the spectra. The ^{1}H-NMR spectra of the homopolymer of PAmIGlc showed a characteristic peak of the H-1 proton at 5.88 ppm for the glucofuranose ring, which was absent in the ^{1}H-NMR spectrum of the PNIPAm homopolymer. However, this peak grew distinctly in copolymers S-5, S-10, S-20, and S-25 as the glycomonomer content increased. This clearly indicated the incorporation of the glycomonomer into the copolymer. The composition of the copolymers was quantitatively estimated from the ratio of the integrations of the H-1 proton of the glucofuranose ring and the —CH proton of the isopropyl group of NIPAm. These values were compared with the feed composition. There was fairly

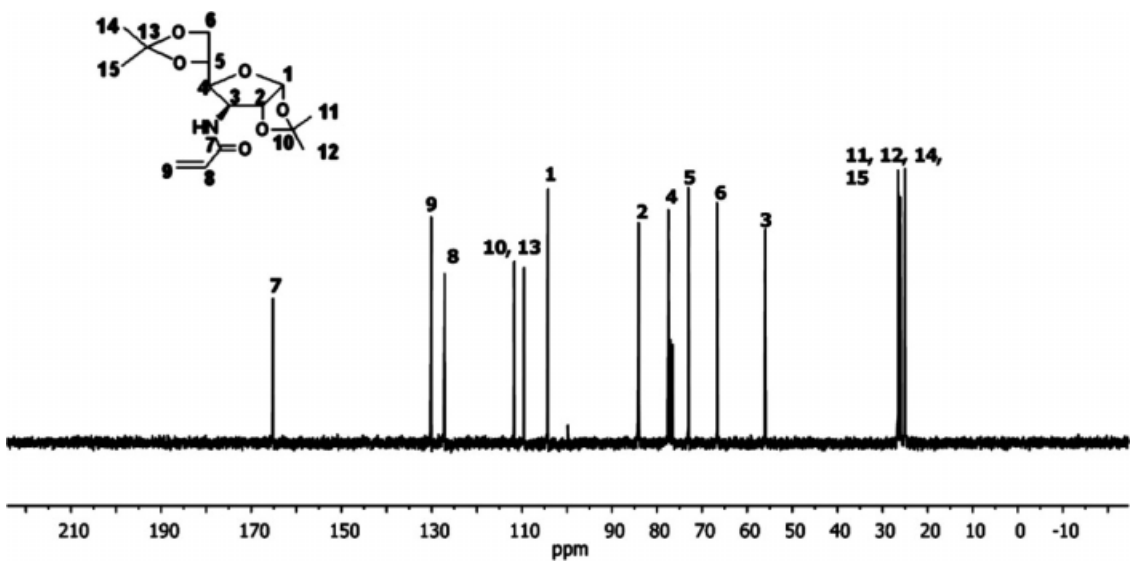
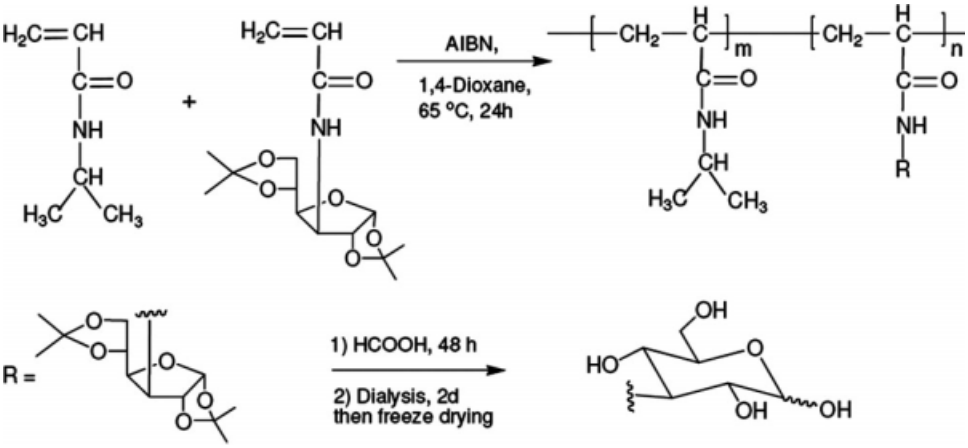


Figure 2 ^{13}C-NMR spectrum of AmIGlc.



Scheme 2 Reaction scheme for the synthesis of copolymers of PNIPAm and PAmIGlc.

good agreement with the values of the feed, and this indicated that the reaction proceeded to completion.

As shown in Table I, the molecular weights of the polymers were found to be 3000–11,800 g/mol with a polydispersity index of 1.49–2.05. However, the determination of the molecular weight of PNIPAm by GPC was difficult as it formed hydrogen bonds and showed a thermosensitive phase transition, which caused serious problems in the GPC analysis. Therefore, we did not determine the number-average molecular weight for the PNIPAm homopolymer and S-5 copolymer.

The PAmIGlc homopolymer and its copolymers were converted into water-soluble polymers by a treatment with 85% formic acid for 48 h followed by dialysis against water. The ^{1}H-NMR spectra of the protected homopolymer (PAmIGlc) and deprotected homopolymer Poly (3-acrylamido-3-deoxy-D-glucopyranose) (PAmGlc) are shown in Figure 4. The disappearance of the isopropylidene proton peaks at 1.3–1.5 ppm and the upfield shift of H-1 of the glucofuranose ring from 5.88 to 5.14 ppm confirmed the quantitative deprotection of isopropylidene groups and the formation of the glucopyranose ring system. Other evidence for quantitative deprotection was shown by FTIR spectroscopy. Figure 5 shows the FTIR spectra of both PAmIGlc and PAmGlc. PAmGlc showed a broad absorption around 3300 cm^{-1} due to free hydroxyl groups of sugar, which also confirmed the deprotection of the isopropylidene group.

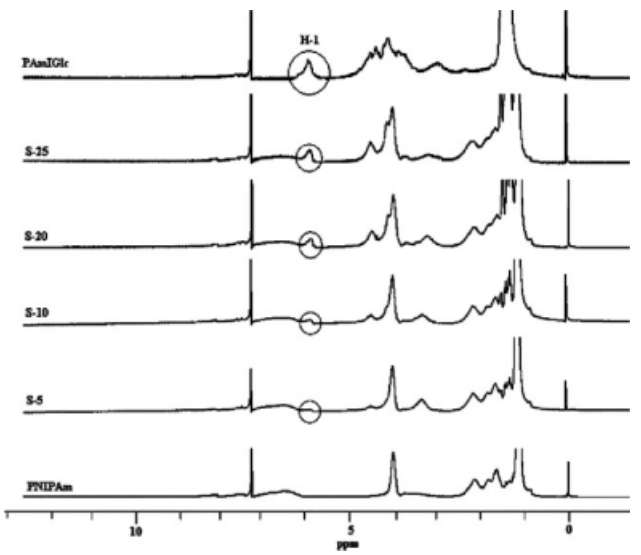


Figure 3 Comparison of ^{1}H-NMR spectra of the homopolymers and copolymers.

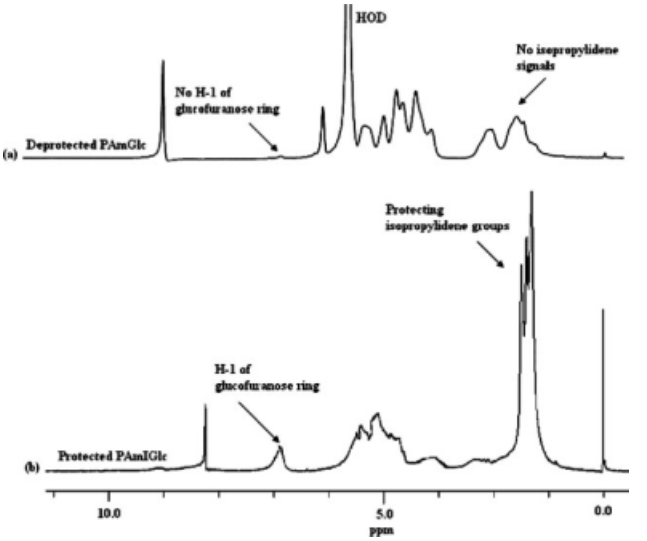


Figure 4 ^{1}H-NMR spectra of (a) the PAmIGlc homopolymer (in $CDCl_3$) and (b) the corresponding PAmGlc polymer (in D_2O) obtained after the removal of isopropylidene group protection.

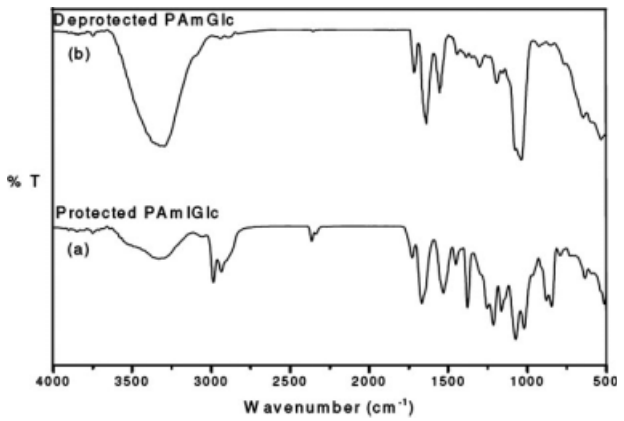


Figure 5 FTIR spectra of (a) protected PAmIGlc and (b) deprotected PAmGlc.

Cloud-point temperature

The cloud-point temperatures of both the protected and deprotected polymers were determined by temperature-dependent ultraviolet–visible absorption in water at 500 nm. As shown in Figure 6, the PNIPAm homopolymer clearly had an LCST at 33.5°C. The protected copolymers showed LCSTs lower than that of the PNIPAm homopolymer because of an increase in the overall hydrophobic content of the polymer. As the amount of the glycomonomer increased, the cloud-point temperature of the copolymer decreased.

It is interesting that the same copolymer after deprotection showed higher LCSTs than those of the protected copolymer and PNIPAm. As shown in Figure 7, an increase in the LCSTs of deprotected copolymers S-5, S-10, S-20, and S-25 could be attributed to the increase in hydrophilicity because, after deprotection, the hydroxyl groups of the sugar moiety became free and could easily form hydrogen bonds with water even after the LCST of PNIPAm.

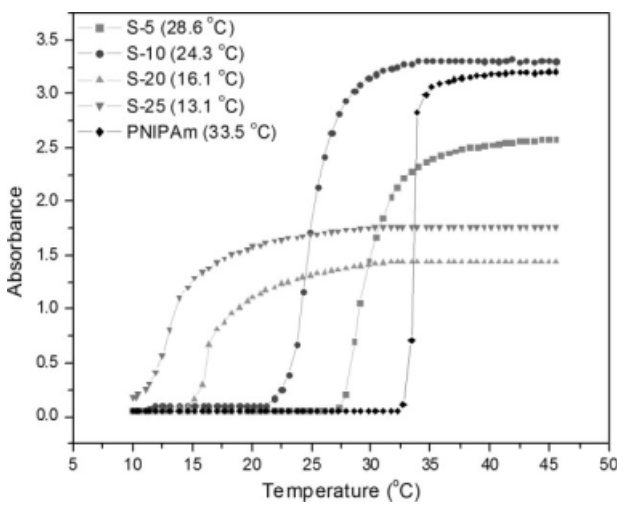


Figure 6 Cloud points of copolymers of PNIPAm with the protected sugar moiety. The values in parentheses indicate the LCSTs.

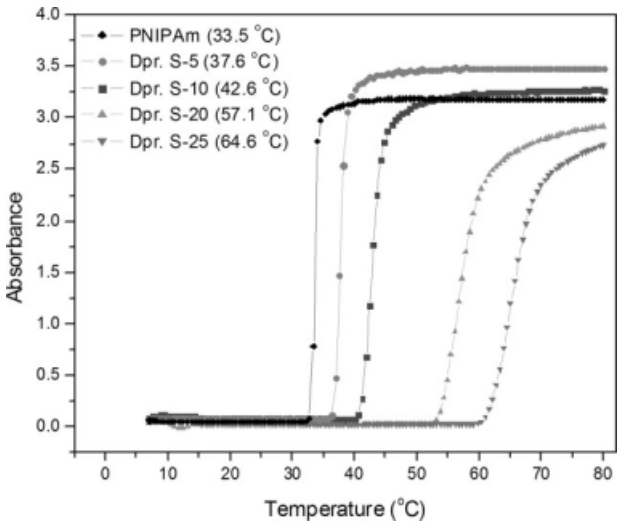


Figure 7 Cloud points of PNIPAm and deprotected (Dpr.) copolymers with PAmGlc. The values in parentheses indicate the LCSTs.

These observations also support the fact that copolymerizing NIPAm with a hydrophobic comonomer resulted in a lowering of LCST, whereas copolymerizing NIPAm with a hydrophilic comonomer resulted in a higher LCST. The effect of an increase in the concentration of the glycomonomer on the LCST is shown in Figure 8. A linear correlation between the LCST and concentration of the glycomonomer was obtained for both protected and deprotected copolymers. These correlations are useful for designing tailor-made thermosensitive

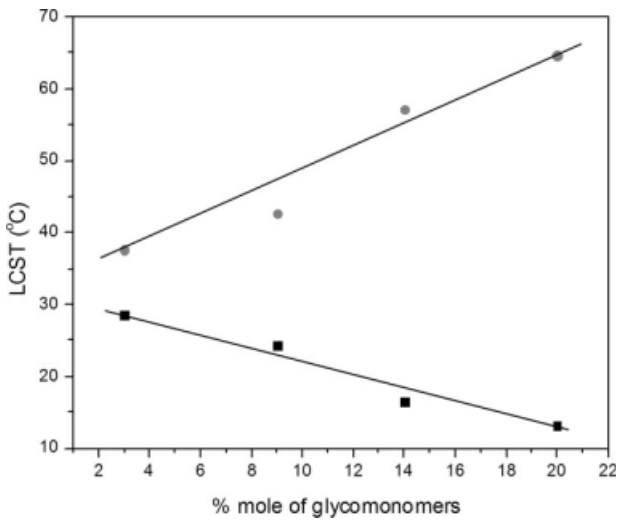


Figure 8 Correlation between the LCST and molar percentage of the sugar moiety (the actual molar composition of the glycomonomer determined by NMR) in the copolymers.

glycopolymers. These polymers can be used for the study of carbohydrate–protein interactions.

CONCLUSIONS

We synthesized a new glycomonomer (AmIGlc) from cheaply available D-glucose with a 31% overall yield. The homopolymerization and copolymerization of the glycomonomer with NIPAm in different compositions afforded thermosensitive glycopolymers. Acid hydrolysis of the protected glycopolymers produced water-soluble polymers. The protected copolymers showed lower LCSTs whereas the deprotected copolymers showed higher LCSTs than that of PNIPAm. The increase or decrease in the LCST was found to be proportional to the concentration of the glycomonomer. A linear correlation between the LCST and the concentration of the glycomonomer was found to exist in these copolymers. Such a correlation could be a useful tool in designing a thermosensitive glycopolymer with a desired LCST.

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