One-Pot Synthesis of Hyperbranched Glycopolymers by RAFT Polymerization

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ABSTRACT: Soluble hyperbranched glycopolymers were prepared by copolymerization of glycan monomers with reversible addition-fragmentation chain transfer polymerization (RAFT) inimers in a simple one-pot reaction. Two novel RAFT inimers, 2-(methacryloyloxy)ethyl 4-cyano-4-(phenylcarbonothioylthio) pentanoate (MAE-CPP) and 2-(3-(benzylthiocarbonothioylthio) propanoyloxy)ethyl acrylate (BCP-EA) were synthesized and used to prepare hyperbranched glycopolymers. Two types of galactose-based saccharide monomers, 6-*O*-methacryloyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (proGal-M) and 6-*O*-(2′-acrylamido-2′-methylpropanoate)-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (proGal-A), containing a methacrylate and an acrylamide group, respectively, were also synthesized and polymerized under the mediation of the MAE-CPP and BCP-EA inimers, respectively. In addition, hyperbranched poly (proGal-M), linear poly(proGal-A), and hyperbranched poly(proGal-A) were generated and their polymerization kinetics were studied and compared. An unexpected difference was observed in the kinetics between the two monomers during polymerization: the relationship between polymerization rate and concentration of inimer was totally opposite in the two monomer–inimer systems. Branching analysis was conducted by using degree of branching (DB) as the measurement parameter. As expected, a higher DB occurred with increased inimer content. Furthermore, these polymers were readily deprotected by hydrolysis in trifluoroacetic acid solution resulting in water-soluble polymers. The resulting branched glycopolymers have potential as biomimetics of polysaccharides. © 2012 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 000: 000–000, 2012

KEYWORDS: biomimetic; glycopolymer; hyperbranched; inimer; reversible addition-fragmentation chain transfer polymerization (RAFT)

INTRODUCTION Polysaccharide mimetics, based on the free radical polymerization of glycans, which attempt to mimic the structure and biological properties of natural sugars, have attracted increasing interest because of their potential application in a wide range of fields including as biomaterials.[1,2] Since the first work by Horejsi et al.,[3] glycopolymers having a variety of architectures have been prepared, such as block copolymers,[4] star polymers,[5] hyperbranched polymers,[6] and dendrimers.[7]

As the macromolecular architecture of sugars influences their properties,[8,9] many researchers have prepared glycopolymers with precise molecular structures during the past decade.[10,11] Of particular interest is the application of living free radical polymerization to prepare polysaccharide mimetics. Narain and Armes[12] used atom transfer radical polymerization to synthesize poly(2-gluconamidoethyl methacrylate) in water. Lowe et al.[13] prepared poly(2-methacryloxyethyl glucoside) via reversible addition-fragmentation chain transfer polymerization (RAFT), after which a number of researchers have used the versatility and simplicity of RAFT polymerization to synthesize glycopolymers. For example, well-defined linear and star-like polymers of poly(6-*O*-vinyladipoyl-D-glucopyranose) and poly(acryloyl glucosamine) were synthesized from both *Z*- and *R*-type RAFT agents by Bernard et al.;[5,14] functional RAFT agents containing saccharide moieties were also used to generate graft or end-functional glycopolymers,[15,16] and Morinloto et al.[17] prepared stimuli-responsive nanogels formed by glycopolymers-*g*-poly(*N*-isopropylacrylamide).

Polysaccharides are an important class of biopolymer because of their role as structural components and their bioactivity (e.g., cell–cell signaling).[18] Many essential natural saccharides are hyperbranched, such as amylopectin and glycogen. Hyperbranched polymers, as less precise analogs of dendrimers, are capable of carrying significant numbers of functional groups and have a major advantage in that they are prepared using a much simpler synthetic procedure.[19–21] Since the first reports by Wang et al.,[22] Carter et al.,[23,24] and

Liu et al.,[25,26] RAFT polymerization has proven to be an efficient method to synthesize hyperbranched polymers.[26–37] Several hyperbranched polymers, such as polystyrene and poly(methyl methacrylate), were prepared via RAFT polymerization in the presence of divinyl crosslinkers[25–32] or polymerizable RAFT agents (inimers).[22–24,33–37] To the best of our knowledge, there is no reported synthesis of hyperbranched glycopolymers using RAFT *inimers* and glycan monomers.

Our aim was to prepare potentially bioactive hyperbranched glycopolymers through RAFT polymerization. The use of RAFT inimers provided a relatively simple methodology by which the hyperbranched polymers were prepared in a one-pot reaction without the disadvantages of multistep syntheses typically required to prepare polysaccharides. Our preliminary work on the synthesis of hyperbranched galactose glycopolymers, both protected and deprotected, via direct RAFT polymerization is reported here. It differs from the work of Semsarilar et al.,[38] in which click chemistry was used to conjugate saccharides onto the polymer backbone after RAFT polymerization. Polymerization and kinetic studies of two different types of galactose monomers using different polymerizable RAFT agents (inimers) are discussed.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA, inhibited with 20 ppm MEHQ; Ubichem), 2-hydroxyethyl acrylate (HEA; Sigma), methacrylic acid, vinyl azlactone, 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 99%; Merck), toluene (99.9%; Merck), and 4,4′-azobis(cyanopentanoic acid) (98%; Fluka) were used as received without purification. Other chemicals used in this work were purchased from Aldrich and used without purification. 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPAD) and 3-((benzylthio)carbonothioyl)thio)propanoic acid (BCPA) were synthesized as described in the literature.[39–41]

Characterization and Instrumentation

NMR Analysis

All the products were analyzed by ^{1}H and ^{13}C NMR on a 400 MHz Bruker Ultrashield spectrometer (Bruker, Germany).

Size Exclusion Chromatography Analysis

The dried polymer (~10 mg) was dissolved in tetrahydrofuran (THF, 4 mL) and filtered through a 0.22 μm pore-size disposable filter prior to analysis. Size exclusion chromatography (SEC) data were collected from a system consisting of a series of four "PLGel" columns (3 × 5 μm Mixed-C and 1 × 3 μm Mixed-E; Polymer Laboratories, Church Stretton, Shropshire, UK) and a Waters (Milford, MA) 2414 refractive index detector. THF was used as the mobile phase at a flow rate of 1.0 mL min^{-1}. Measurement was conducted at a temperature of 25 °C with an injection volume of 10 μL. The SEC instrument was calibrated with narrow polydispersity polystyrene standards with peak molecular weight (M_p) in the range of 256–264,000 g mol^{-1} (Polymer Laboratories), and the molecular weights were reported as polystyrene equivalents.

MS Analysis

Positive ion EI mass spectra were obtained on a ThermoQuest MAT95XL mass spectrometer using an ionization energy of 70 eV. Accurate mass measurements were conducted with a resolution of 5000–10,000 using perfluorokerosene as the reference compound.

Synthesis of Inimers 2-(Methacryloyloxy)ethyl 4-cyano-4-(phenylcarbonothioyl-thio)pentanoate (1) and 2-(3-(Benzylthiocarbonothioylthio)propanoyloxy) Ethyl Acrylate (2)

Preparation of 2-(methacryloyloxy)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate (MAE-CPP) (**1**) is described below. CPAD (1.0 g, 3.6 mmol) and HEMA (0.51 g, 4 mmol) were dissolved in dry toluene (10 mL), followed by the addition of DMAP (44 mg, 0.36 mmol). After the dissolution of DMAP, 1,3-dicyclohexyl carbodiimide (DCC; 0.82 g, 4 mmol) was added and the mixture was stirred for 12 h at room temperature. The reaction mixture was then filtered and the solution was dried on a rotary evaporator to afford the crude product. The crude product was further purified through a silica gel column (eluent: ethyl acetate:hexane, 1:3 v/v) to afford inimer MAE-CPP (**1**) as a pink liquid on drying (1.1 g, 75% yield).

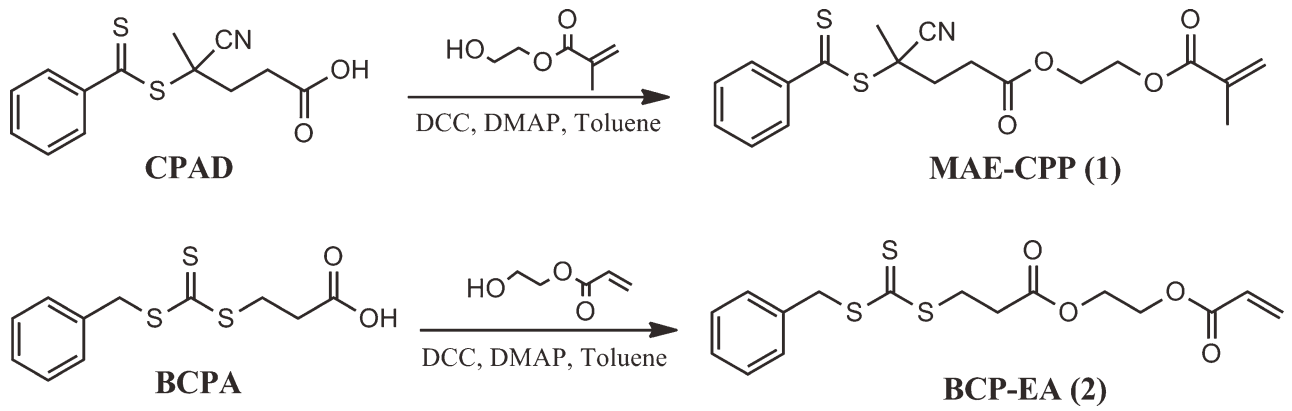
^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.61 (s, 3H, C(CH_3)(CN)), 1.95 (s, 3H, CH_3—C=C), 2.38 (s, 1H, C(CH$_3$)(CN)—CHH), 2.68 (t, 2H, CH_2(CO)O), 2.79 (s, 1H, C(CH$_3$)(CN)—CHH), 4.35 (m, 4H, (CO)OCH_2CH_2O(CO)), 5.61 (s, 1H, C=C-H_b), 6.13 (s, 1H, C=C-H_a), 7.28–7.95 (m, 5H, Φ). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 18.2, 24.1, 29.7, 33.3, 45.7, 62.2, 62.7, 76.4, 77.0, 77.6, 126.2, 126.6, 128.6, 133.0, 135.8, 144.5, 171.3. FTIR (NaCl, thin film, cm^{-1}): 2957, 1738, 1636, 1445, 1381, 1296, 1161, 1048, 945, 868, 763, 688, 650. HRMS (EI, m/z): calculated for $C_{19}H_{21}O_4NS_2$ [M]$^+$: 391.0907; found: 391.0903.

The procedure for preparing 2-(3-(benzylthiocarbonothioylthio)propanoyloxy)ethyl acrylate (BCP-EA) (**2**) was the same as that of MAE-CPP (**1**), except CPAD and HEMA were substituted with BCPA and HEA, respectively. The final product was a yellow liquid (82% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.80 (t, 2H, CH_2(CO)O), 3.63 (t, 2H, S(S=C)S—CH_2), 4.36 (m, 4H, (CO)OCH_2 and Φ-CH_2), 4.60 (s, 2H, CH_2O(CO)), 5.85–5.88 (d, 1H, C=C-H_c), 6.10–6.17 (tetra, 1H, H_b-C=C), 6.41–6.46 (d, 1H, C=C-H_a), 7.26–7.33 (m, 5H, Φ). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ, ppm): 31.2, 33.0, 41.5, 62.1, 62.6, 76.7, 77.0, 77.3, 127.8, 127.9, 128.7, 129.2, 131.5, 134.8, 138.2, 165.8, 171.2, 222.9. FTIR (NaCl, thin film, cm^{-1}): 3062, 3030, 2956, 1731, 1635, 1494, 1453, 1407, 1373, 1348, 1182, 1065, 982, 900, 888, 802, 730, 680, 638. HRMS (EI, m/z): calculated for $C_{16}H_{18}O_4S_3$ [M]$^+$: 370.0362; found: 370.0360.

Synthesis of 6-*O*-Methacryloyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (3)

1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (3.12 g, 12 mmol), methacrylic acid (1.12 mL, 13.2 mmol), and DMAP (0.16 g, 1.32 mmol) were dissolved in dichloromethane (30 mL). The solution was immersed in an ice bath and DCC (2.72 g, 13.2 mmol) was added. The reaction was then



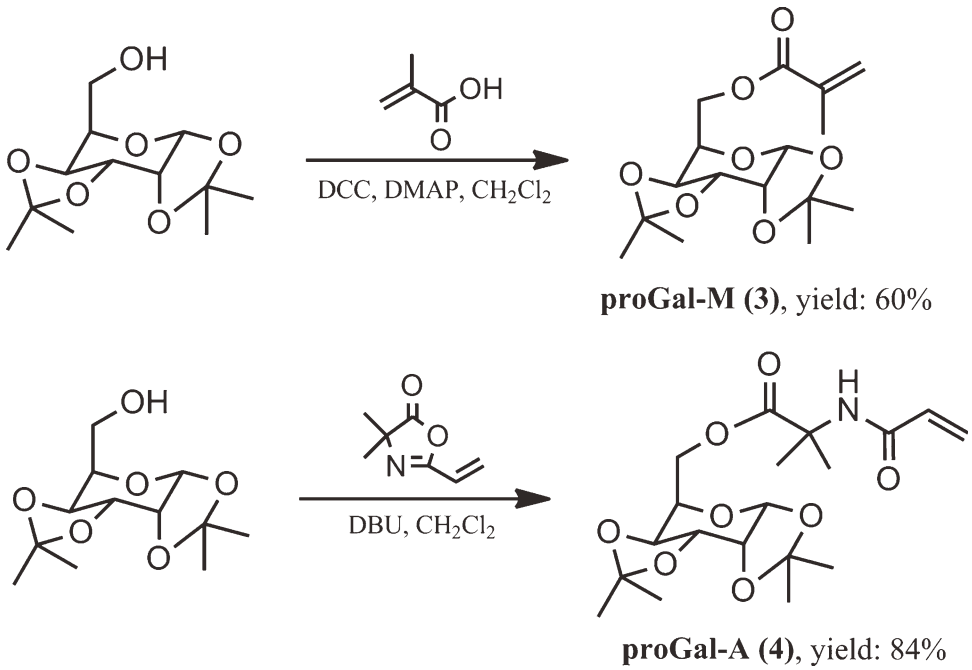
SCHEME 1 Synthetic routes of polymerizable RAFT agents (inimers).

stirred overnight at room temperature. The solvent was removed under vacuum to afford the crude product, which was further purified via a silica column (eluent: ethyl acetate:hexane, 1:3 v/v) to afford 6-*O*-methacryloyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (proGal-M) (**3**) as a white powder on drying (2.38 g, 60% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.24 (1s, 3H, CH_3), 1.25 (1s, 3H, CH_3), 1.36 (1s, 3H, CH_3), 1.41 (1s, 3H, CH_3), 1.85 (s, 3H, CH_2=CH(CH_3)), 4.00 (m, 1H, C*H*), 4.16–4.26 (m, 4H, 1C*H*H+3C*H*), 4.54 (dd, 1H, CH*H*), 5.43 (d, 1H, anomeric C*H*), 5.48 (m, 1H, CH_bH=C(CH_3)), 6.04 (s, 1H, CHH_a=C(CH_3)). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 18.4, 24.6, 25.2, 26.1, 63.9, 66.3, 70.7, 70.9, 71.4, 96.5, 108.9, 109.7, 125.9, 136.4, 167.2. HRMS (EI, *m/z*), calculated for $C_{16}H_{24}O_7$ $[M+1]^+$: 329.1600, found: 329.1608.

Synthesis of 6-*O*-(2′-Acrylamido-2′-methylpropanoate)-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (4)

1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (0.52 g, 2 mmol) and 1,8-diazabicycloundec-7-ene (30 μL, 0.2 mmol) were dissolved in dichloromethane (5 mL). The solution was immersed in an ice bath and vinyl azlactone (0.28 g, 2 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight and was then washed with 0.5 M HCl (5 mL × 2), 0.5 M Na_2CO_3 (5 mL × 2), and water (5 mL × 2) sequentially. The organic layer was dried with Na_2SO_4 and the solvent was removed under vacuum to afford the crude product, which was further purified via a silica column (eluent: ethyl acetate:hexane = 3:2 v/v) to afford 6-*O*-(2′-acrylamido-2′-methylpropanoate)-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (proGal-A) (**4**) as a white powder on drying (0.67 g, 84% yield).



SCHEME 2 Synthetic routes of glycan monomers proGal-M (**3**) and proGal-A (**4**).

TABLE 1 Polymerization Parameters and Results of proGal-M

Type	[M]/[RAFT]	Time (h)	Conversion (%)	M_n (theoretical)	M_n (NMR)	M_n (SEC)	PDI	DB
Hyperbranched	10	3	48	1,600	2,529	3,100	1.28	0.221
		3.5	54	1,800	2,704	3,400	1.30	0.214
		4	61	2,000	3,131	3,600	1.31	0.185
		5	70	2,300	3,388	4,300	1.36	0.188
		6	77	2,500	3,815	4,800	1.38	0.166
		8	81	2,600	4,026	5,400	1.42	0.181
		24	92	3,000	4,952	6,000	1.47	0.145
	20	3	36	2,400	4,431	4,000	1.25	0.115
		3.5	43	2,800	4,921	4,600	1.26	0.107
		4	51	3,300	5,366	4,900	1.29	0.098
		5	58	3,800	6,511	5,400	1.33	0.083
		6	62	4,100	6,678	6,000	1.35	0.084
		8	72	4,700	7,736	6,700	1.39	0.090
		24	91	6,000	9,730	7,700	1.48	0.071
	50	3	17	2,800	7,187	5,000	1.22	0.062
		3.5	23	3,800	7,515	5,100	1.22	0.062
		4	28	4,600	7,774	5,300	1.23	0.061
		5	42	6,900	11,025	6,500	1.27	0.038
		6	54	8,800	12,168	7,300	1.28	0.056
		8	63	10,300	14,065	8,800	1.32	0.048
		24	82	13,400	22,968	12,000	1.44	0.029
	100	2.5	12	3,900		36,900	1.63	
		3	22	7,200	64,713	46,200	1.81	0.010
		3.5	29	9,500	59,305	49,500	1.85	0.011
		4	33	10,800	56,376	51,900	1.90	0.012
		6	51	16,700	69,678	63,200	2.04	0.010
		8	60	19,700		60,900	2.34	
		24	96	31,500	69,219	50,500	3.04	0.010

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.24 (1s, 3H, CH_3), 1.25 (1s, 3H, CH_3), 1.36 (1s, 3H, CH_3), 1.41 (1s, 3H, CH_3), 1.60 (s, 6H, C(CH_3)$_2$), 4.00 (m, 1H, CH), 4.16–4.26 (m, 4H, 1CHH+3CH), 4.61 (dd, 1H, CHH), 5.51 (d, 1H, anomeric CH), 5.63 (m, 1H, CH_bH=CH), 6.10 (m, 1H, CH$_2$=CH), 6.23 (m, 1H, CHH_a=CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 26.7, 56.3, 60.9, 71.8, 72.8, 76.5, 96.9, 107.6, 111.7, 129.6, 130.1, 166.2, 172.3. HRMS (EI, m/z), calculated for $C_{19}H_{29}NO_8$ $[M]^+$: 399.1888, found: 399.1873.

Polymerization of ProGal-M and ProGal-A

ProGal-M (**3**) was polymerized with MAE-CPP (**1**) to obtain hyperbranched poly(proGal-M). ProGal-A (**4**) was polymerized separately with BCPA and BCP-EA (**2**) to produce both linear and hyperbranched poly(proGal-A). A typical procedure for the RAFT polymerization is described below. ProGal-M (0.7 g, 2.13 mmol), MAE-CPP (41.5 mg, 0.11 mmol), and AIBN (5.8 mg, 0.03 mmol) were dissolved in ethyl acetate (3.5 mL) in a Schlenk flask. The solution was subjected to three cycles of freeze-vacuum-thaw degassing process before immersing in an oil bath at 70 °C under N_2. Reaction samples were removed at regular intervals and precipitated three times in diethyl ether, after which precipitates were dried under vacuum at 40 °C and characterized by gravimetric, NMR, and SEC analysis.

Poly(proGal-M): ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.88–2.12 (m, 17H, —CH_2—C(CH_3)— and —C(CH_3)), 3.94–4.36 (b, 5H, 1CHH+4CH), 4.64 (b, 1H, CHH), 5.53 (b, 1H, anomeric CH). Poly(proGal-A): ^{1}H NMR (400 MHz, d_6-DMSO, δ, ppm): 0.95–1.74 (m, 21H, —CH_2—CH— and —C(CH_3)), 3.85–4.33 (b, 5H, 1CHH+4CH), 4.58 (b, 1H, CHH), 5.41 (b, 1H, anomeric CH).

Deprotection of Poly(proGal-M) and Poly(proGal-A)

The protected glycopolymer (0.1 g) was dissolved in 90% aqueous trifluoroacetic acid (0.3 mL) and stirred at room temperature for 5 h, after which the trifluoroacetic acid was removed by dialysis against water overnight. The deprotected glycopolymer was recovered by freeze drying giving a yield of 95%.

TABLE 2 Polymerization Parameters and Results of proGal-A

Type	[M]/[RAFT]	Time (h)	Conversion (%)	M_n (theoretical)	M_n (NMR)	M_n (SEC)	PDI	DB
Linear	20	3	10	800	1,400	1,800	1.10	–
		4	25	2,000	1,800	2,400	1.14	–
		5	60	4,800	3,400	3,900	1.14	–
		6	82	6,500	4,400	5,000	1.14	–
		8	91	7,300	5,200	5,600	1.13	–
		24	93	7,400	5,200	5,700	1.14	–
Hyperbranched	10	3	15	600	1,700	1,600	1.18	0.156
		4	33	1,300	2,300	2,100	1.22	0.176
		5	48	2,000	3,000	2,600	1.25	0.128
		6	69	2,700	6,000	3,200	1.30	0.107
		8	90	3,500	8,200	3,900	1.37	0.101
		24	100	4,000	9,800	4,500	1.42	0.083
	20	2	10	1,000	2,400	1,200	1.09	0.082
		2.5	16	1,400	3,000	1,500	1.16	0.101
		3	35	2,700	6,100	2,300	1.26	0.104
		5	76	5,900	14,200	5,800	1.39	0.053
		8	94	7,400	18,500	6,700	1.44	0.051
	50	2	16	3,200	5,700	2,200	1.30	0.061
		3	51	10,000	13,640	4,500	1.31	0.032
		4	86	17,000	23,700	8,300	1.45	0.013
		5	92	18,000	26,600	8,600	1.48	0.009
		6	96	19,000	28,500	8,900	1.48	0.008
		24	100	20,000	28,600	9,100	1.49	0.008

Poly(Gal-M): ^{1}H NMR (400 MHz, D_2O, δ, ppm): 0.63–1.90 (m, 5H, —CH_2—C(CH_3)—), 3.44–4.18 (b, 5H, 1C*H*H+4CH), 4.48 (b, 1H, CH*H*), 5.21 (b, 1H, anomeric C*H*). Poly(Gal-A): ^{1}H NMR (400 MHz, D_2O, δ, ppm): 0.90–1.69 (m, 9H, —CH_2—C*H*— and —C(CH_3)), 3.29–4.01 (br s, 5H, 1C*H*H+4CH), 4.31 (b, 1H, CH*H*), 5.08 (b, 1H, anomeric C*H*).

RESULTS AND DISCUSSION

Synthesis of Inimers MAE-CPP (1) and BCP-EA (2)

Two new RAFT inimers MAE-CPP (**1**) and BCP-EA (**2**) were synthesized as shown in Scheme 1. The structures of both inimers were confirmed by ^{1}H NMR, ^{13}C NMR, and accurate MS measurements as shown in the experimental section. The inimers were designed to incorporate two different types of RAFT agents, namely, a dithioester and a trithiocarbonate agent, to facilitate controlled polymerization of different types of monomers. Theoretically, MAE-CPP (**1**), a dithioester, was expected to be a suitable RAFT agent for most methacrylates, methacrylamides, styrenics, acrylates, and acrylamides; BCP-EA (**2**), a trithiocarbonate, was expected to be a suitable RAFT agent for most styrenics, acrylates, acrylamides, and vinyl esters.[42]

Synthesis of Saccharide Monomers ProGal-M (3) and ProGal-A (4)

The synthetic routes to the two saccharide monomers proGal-M (**3**) and proGal-A (**4**) are shown in Scheme 2. ProGal-M (**3**) is a methacrylate-type protected galactose monomer, and proGal-A (**4**) is an acrylamide-type protected galactose monomer. NMR and high-resolution MS analysis were consistent with the proposed structure of the two monomers. The protected monomers were used for polymerization because of the solubility of both monomers and polymers in organic solvents. The free hydroxyl groups could be readily recovered from the two protected galactose polymers through a simple hydrolysis process (see Hydrolysis of Protected Hyperbranched Glycopolymers section).

Polymerization of ProGal-M and ProGal-A

Both galactose monomers were polymerized using different ratios of monomer to inimer. The galactose methacrylate monomer [proGal-M (**3**)] was polymerized in the presence of inimer MAE-CPP (**1**) with monomer to inimer ratios of 10, 20, 50, and 100:1, whereas the galactose acrylamide monomer [proGal-A (**4**)] was polymerized in the presence of inimer BCP-EA (**2**) with monomer to inimer ratios of 10, 20, and 50:1. The hyperbranched polymers prepared in this study did not contain insoluble gels as was expected for RAFT inimer polymerizations,[23,24,32–35] unlike hyperbranched polymers prepared using divinyl crosslinkers[25,28] in which insoluble gels are formed. RAFT polymerizations of proGal-A (**4**) to yield linear polymers were conducted for comparison with the hyperbranched polymers; however, as the linear RAFT polymerization of proGal-M (**3**)

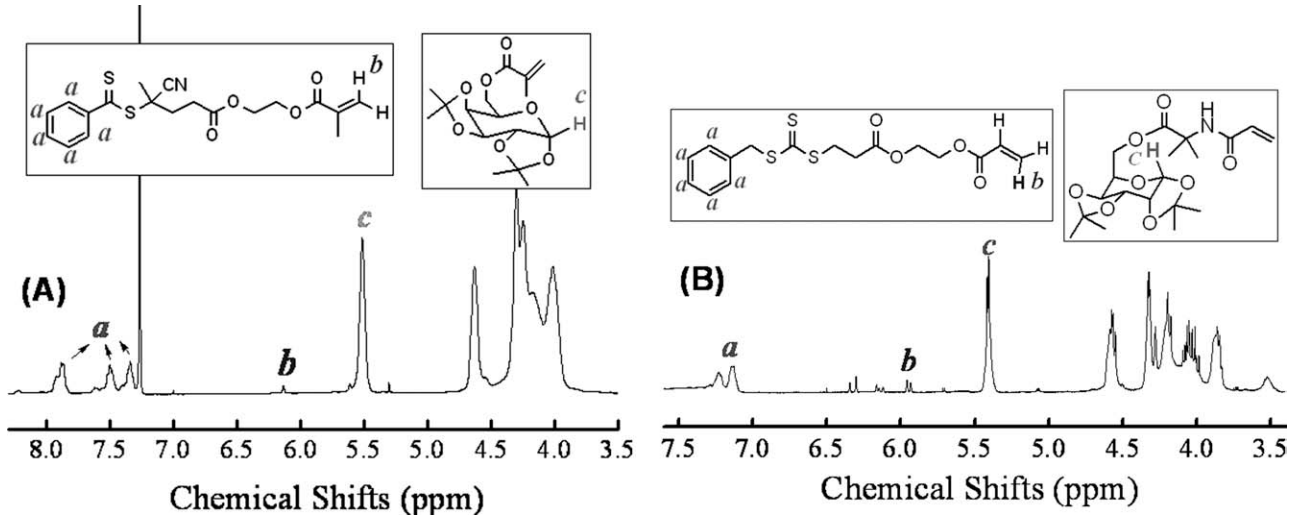


FIGURE 1 ^{1}H NMR spectra used to calculate DB: (A) proGal-M (**3**) polymerized with MAE-CPP (**1**) and (B) proGal-A (**4**) polymerized with BCP-EA (**2**).

has already been reported in the literature,[43–48] it was not repeated in this study. Polymerizations were all conducted using an initiator (AIBN) to inimer ratio of 1:3, and the conditions and results are summarized in Tables 1 and 2. The M_n (NMR) was estimated by end-group analysis from NMR spectroscopy based on an equation used for linear RAFT polymerization (eq 1), where A_a and A_c are the integrations of the RAFT phenyl end-group (5H) and monomer anomeric proton (1H) peaks, respectively, as shown in Figure 1.

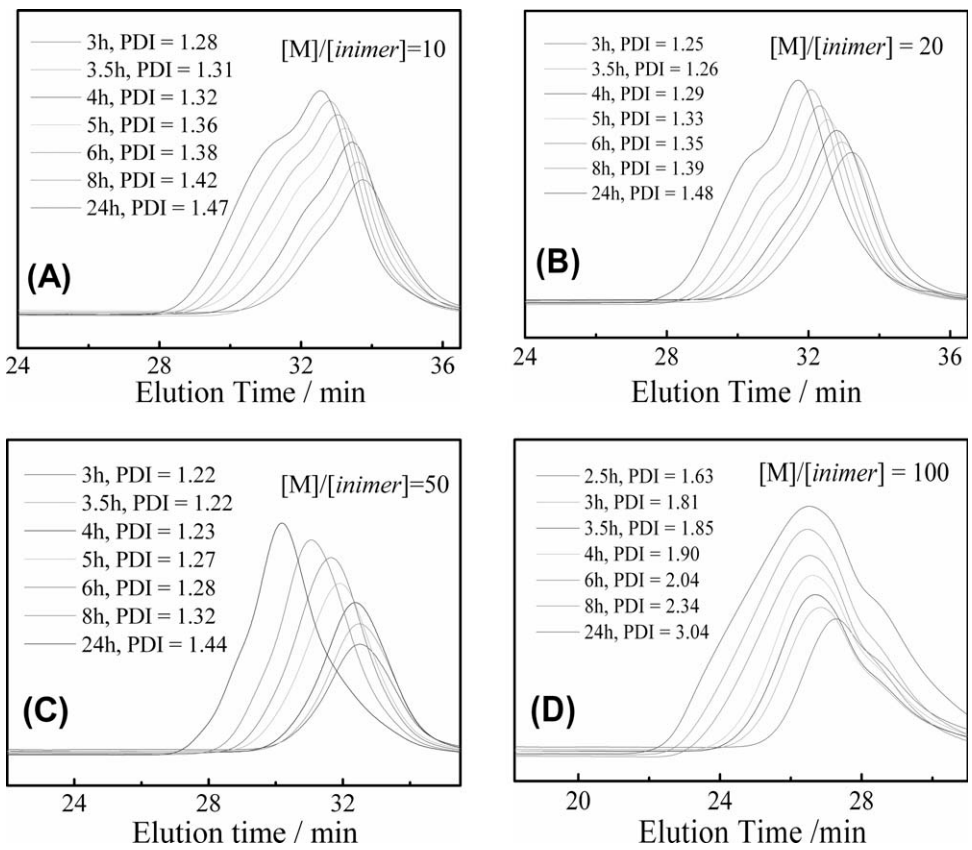


FIGURE 2 SEC curves of hyperbranched poly(proGal-M) prepared with a feed ratio of monomer to inimer of 10 (A); 20 (B); 50 (C); and 100 (D).

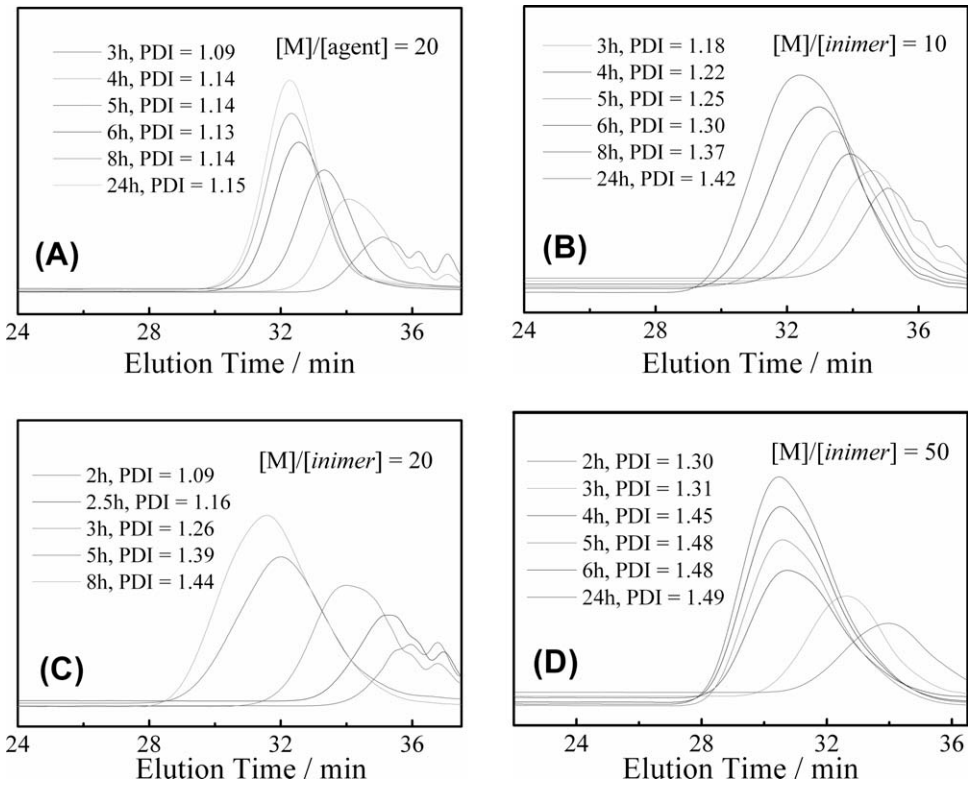


FIGURE 3 SEC curves of poly(proGal-A): linear RAFT polymers prepared using a feed ratio of monomer to RAFT agent of 20 (A); hyperbranched polymers prepared using a feed ratio of monomer to inimer of 10 (B); 20 (C); and 50 (D).

$$M_{\mathrm{n}}(\mathrm{NMR}) = \frac{5A_{\mathrm{c}}}{A_{\mathrm{a}}} \times M_{\mathrm{monomer}} + M_{\mathrm{inimer}} \quad (1)$$

The monomer conversion was calculated by gravimetric analysis, whereas the theoretical molecular weight was estimated based on conversion for a linear RAFT polymerization using eq 2.

$$M_{\mathrm{n}}(\mathrm{theo}) = M_{\mathrm{monomer}} \times \mathrm{DP}_{\mathrm{theo}} \times \mathrm{Conversion} + M_{\mathrm{inimer}} \quad (2)$$

where M_{monomer} is the molecular weight of glycan monomer; $\mathrm{DP}_{\mathrm{theo}}$ is the designated degree of polymerization, which is determined by the feed ratio of monomer to inimer; and M_{inimer} is the molecular weight of the inimer.

Figures 2 and 3 show the SEC results of hyperbranched poly(proGal-M), linear poly(proGal-A), and hyperbranched poly (proGal-A), respectively. By comparing the SEC results, it is clear that the glycopolymers have a hyperbranched structure [Figs. 2 and 3(B–D)] as they exhibited multiple and broad peaks, very different from that of linear RAFT glycopolymers reported in the literature[43–48] and also shown in Figure 3(A). This difference in SEC is consistent with that for hyperbranched polymers, as reported in the literature.[25,32,33]

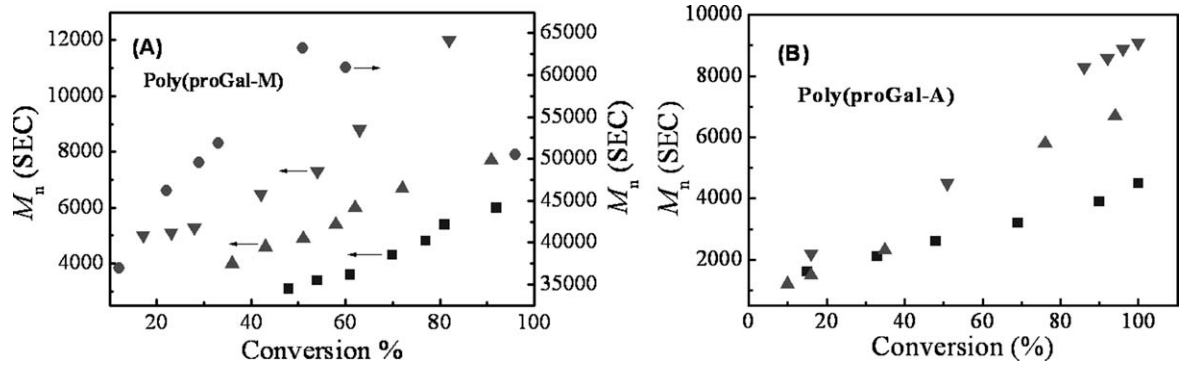


FIGURE 4 Molecular weight versus conversion for (A) hyperbranched poly(proGal-M) prepared using a ratio of monomer to inimer of 10 (■), 20 (▲), 50 (▼), and 100 (●) and (B) hyperbranched poly(proGal-A) prepared using a ratio of monomer to inimer of 10 (■), 20 (▲), and 50 (▼).

Moreover, it was also observed in Figure 2(D) that a low-molecular-weight shoulder appears in the copolymerization of proGal-M (**3**) with MAE-CCP (**1**) at a monomer to inimer ratio of 100:1, irrespective of reaction time, indicating that the polymers contained a portion of dead polymer chains, which do not grow with time. The linear poly(proGal-A) had a PDI of 1.14 at 93% conversion, consistent with other reports of linear RAFT glycopolymers.[43–48] However, in the presence of inimers, the resulting polymers had significantly higher PDIs in the range of 1.42–3.04 consistent with that of other RAFT inimer polymerizations.[33–35] The polydispersity of polymers prepared under the monomer to inimer ratio of 100:1 is significantly higher than that of polymers prepared under lower monomer to inimer ratios. This implies a partially uncontrolled polymerization under a higher ratio of monomer to inimer (100:1) and is discussed in later sections.

Polymerization Kinetics Study

The relationship of molecular weight versus conversion for the polymerization of proGal-M is shown in Figure 4(A) in which a fairly linear relationship was observed at lower ratios of [M]/[inimer] (10:1, 20:1), indicating the characteristics of living polymerization. However, at higher ratios of [M]/[inimer] (50:1, 100:1), the relationship was no longer linear. For example, in Figure 4(A), the polymerization at a ratio of 100:1 shows a linear trend in the molecular weight of polymers below 50% conversion, but decreases after reaching 50% conversion. Furthermore, the molecular weight, measured by SEC, of the polymers prepared at the ratio of 100:1 was much higher than predicted. For example, when the conversion was around 60%, the molecular weight of the polymer prepared at the ratio of [M]/[inimer] = 100 was about 60k compared with the predicted value of 19.7k. In contrast, the molecular weights of the polymers prepared at ratios of [M]/[inimer] = 10, 20, and 50 were only 3.6k, 6k, and 8.8k, respectively, which are reasonably close to calculated values. The observation of the unchanging SEC low MW shoulder in Figure 2(D) shows that the polymerization of proGal-M under [M]/[inimer] = 100 was not well controlled. Therefore, in our later studies on both poly (proGal-M) and poly(proGal-A), the ratio of monomer to RAFT agent was restricted to below 50:1. The polymerization of proGal-A was similar to that of proGal-M as shown by similar M_n versus conversion graphs [Fig. 4(B)], indicating that the polymerization of proGal-A proceeded in a controlled manner.

In RAFT polymerization, it is typically expected that the polymerization rate decreases with increasing concentration of the RAFT agent. This retardation effect has been more pronounced with the use of dithiobenzoates[49–51] than with the use of aliphatic dithioesters[52,53] or trithiocarbonates.[54] However, in the datasets arising from our experiments, this typical retardation was not observed. In the polymerization of proGal-M mediated by MAE-CPP, where a dithiobenzoate-type inimer was used, the polymerization rate surprisingly became faster as the inimer concentration was increased [Fig. 5(A)]. In contrast to proGal-M, the trithiocarbonate-type

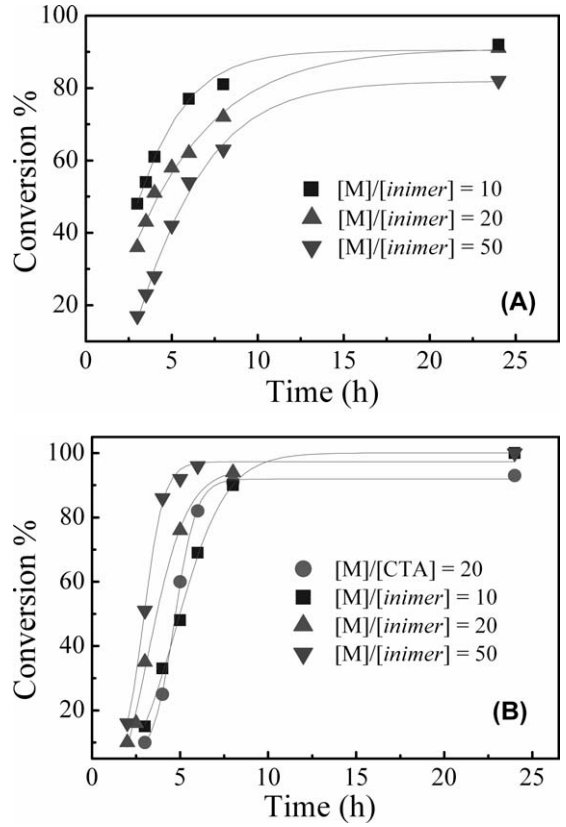


FIGURE 5 Monomer conversion versus reaction time for (A) hyperbranched poly(proGal-M) prepared using a ratio of monomer to inimer of 10 (■), 20 (▲), and 50 (▼); (B) hyperbranched poly(proGal-A) prepared using a ratio of monomer to inimer of 10 (■), 20 (▲), and 50 (▼), as well as linear poly(proGal-A) prepared using a ratio of monomer to RAFT agent of 20 (●).

inimer actually retarded the polymerization of proGal-A. A slower reaction rate was observed at a higher inimer concentration [Fig. 5(B)]. This effect may be related to the structure of the inimers, which are different from regular RAFT agents that contain no polymerizable groups. From the comparison of linear and hyperbranched polymerization of proGal-A shown in Figure 5(B), which were both conducted at a monomer/RAFT agent ratio of 20:1, the hyperbranched polymerization rate was faster than the linear one. As all the other parameters in the two polymerization systems were the same, we hypothesize that this difference in polymerization rate was caused by the existence of the polymerizable groups of the inimers. The polymerizable groups of the inimer may have been able to accelerate the polymerization by participating in the polymerization to form hyperbranching points. This hypothesis could explain the unusual phenomenon in kinetics shown in Figure 5(A,B). As the effects of both acceleration and retardation coexist in inimers, in the case of the polymerization of proGal-M [Fig. 5(A)], the acceleration effect was likely to have been stronger than the retardation effect; however, in the case of the polymerization of proGal-A [Fig. 5(B)], the retardation effect might be stronger than acceleration.

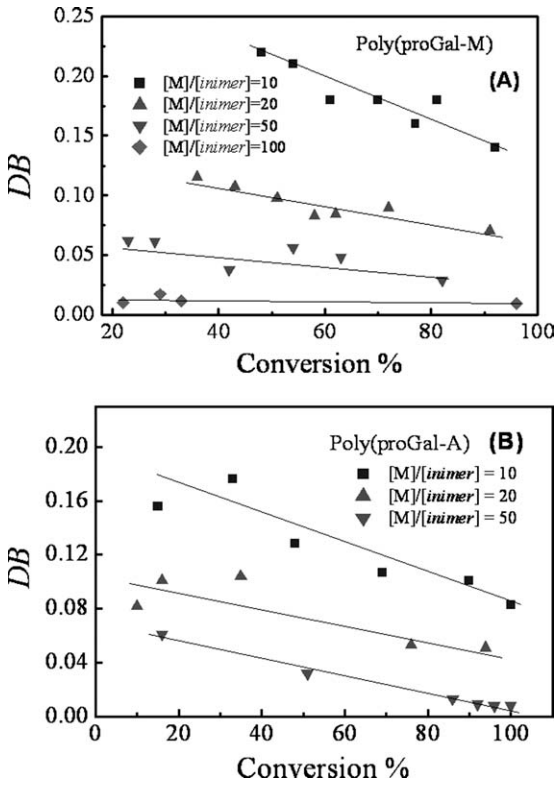


FIGURE 6 Degree of branching (DB) versus monomer conversion: (A) poly(proGal-M) and (B) poly(proGal-A), prepared using monomer to inimer ratios of 10 (■), 20 (▲), 50 (▼), and 100 (◆).

Hyperbranching Analysis

Degree of branching (DB) is an important parameter used to describe the fraction of branched units within the macromolecular structure of branched polymers. According to the definition of DB in the literature,[55] DB is calculated using the equation DB = (2 × number of dendritic units)/(total number of units − 1). A higher DB means more branching points for a branched polymer. The value of DB for a branched polymer should be between 0 and 1, with a linear polymer at 0 and a perfect dendrimer at 1. In our cases, DB can be calculated approximately by eq 3.

$$\mathrm{DB} = \frac{2 \times \left(\frac{A_a}{5} - A_b\right)}{\frac{A_a}{5} + A_c - 1} \tag{3}$$

where A_a, A_b, and A_c are the integrals of the protons on the phenyl and vinyl groups of the inimer and the anomeric proton of the monomer, respectively (Fig. 1). $\left(\frac{A_a}{5} - A_b\right)$ represents the dendritic units, and $\frac{A_a}{5} + A_c - 1$ represents the total number of units.

A graph of calculated DB versus monomer conversion (Fig. 6) was consistent with that observed for *N*-isopropylacrylamide hyperbranched polymers prepared using styryl dithioester as the RAFT inimer.[24] It was clearly observed that in the polymerization of both proGal-M and proGal-A, a higher inimer concentration resulted in a higher DB. As the participation of inimers in the polymerization resulted in hyperbranching points, this result was expected. DB decreased in a linear fashion with conversion for both the proGal-M/MAE-CPP and proGal-A/BCP-EA polymerizations. It should be noted that the DB values for the proGal-M/MAE-CPP polymerization at a [M]/[inimer] = 100 ratio have a significant error due to integration error of the very small vinyl peaks resulting from unpolymerized inimer and weak aromatic protons in the ^{1}H NMR spectrum. For all polymer series, the calculated DB values were comparable with the theoretical DB values of 0.2, 0.1, 0.04, and 0.02 for the [M]/[inimer] ratios of 10:1, 20:1, 50:1, and 100:1, respectively, assuming complete polymerization of the inimer and monomer. Although the inimer vinyl group could be observed in the ^{1}H NMR spectrum during the early stages of the polymerizations (up to about 70% conversion), complete consumption of the inimer vinyl groups was observed as the polymerization approached higher conversions (>70%). In addition, the DB of poly(proGal-M) was higher than that of

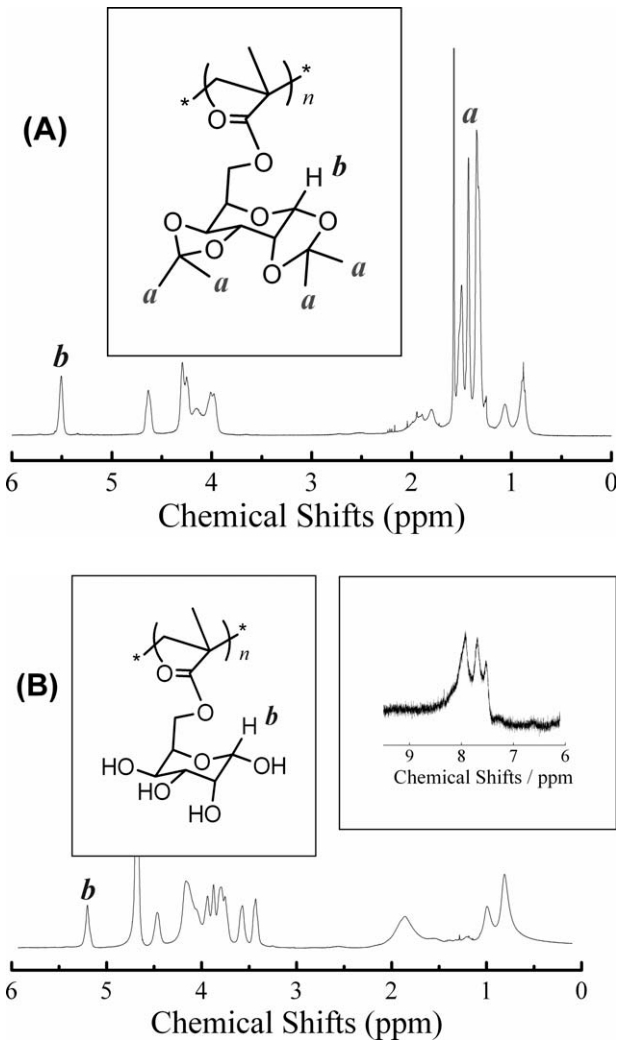


FIGURE 7 ^{1}H NMR spectra of poly(proGal-M): (A) before deprotection (in $CDCl_3$) and (B) after deprotection (in D_2O).

poly(proGal-A) when they were prepared at the same ratio of monomer to inimer.

Hydrolysis of Protected Hyperbranched Glycopolymers

To recover the water solubility of the hyperbranched glycopolymers, a deprotection procedure was conducted to cleave off the isopropylidene groups. Both poly(proGal-M) and poly (proGal-A) were converted to their corresponding deprotected glycopolymers bearing free hydroxyl groups. Trifluoroacetic acid was used in the deprotection reaction. Figure 7 shows the ^{1}H NMR spectra of poly(proGal-M) before and after deprotection. The disappearance of isopropylidene signals around 1.4 ppm and a slightly downfield shift of protons on sugar rings proved that the protecting groups were essentially quantitatively removed. Moreover, the RAFT agent endgroups could still be seen after deprotection [enlarged area in Fig. 7(B)], which provides the possibility to further modify these glycopolymers. The ^{1}H NMR data for the water-soluble deprotected poly(proGal-M) and poly(proGal-A) was recorded in the Experimental section.

CONCLUSIONS

This is the first report of a successful synthesis of hyperbranched glycopolymers via a one-pot RAFT polymerization without the formation of insoluble gels. Two types of hyperbranched glycopolymers (polygalactose analogs) were prepared using two novel RAFT inimers. The hyperbranched structures of the glycopolymers were confirmed by SEC and NMR analyses. The polymerization processes of the two systems showed living characteristics according to the kinetics study. The DB increased with decreasing [monomer]/[inimer] ratio during the polymerization of both monomers. The relationship between polymerization rate and [monomer]/[inimer] ratio was different in the polymerization of the two monomers. The glycopolymers were readily deprotected in TFA solution, resulting in water-soluble polymers. By controlling branching points (inimer concentration) and composition (saccharide monomers), the method described in this study has the potential to be used to synthesize a large array of hyperbranched glycopolymers, including bioactive glycopolymers which will be the subject of future work.

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