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 RAFT inimers in a simple one-pot reaction. Two novel RAFT inimers, 2-(methacryloyloxy)ethyl 4-cyano-4-(phenylcarboxothioylthio)pentanoate (MAE-CPP) and 2-(3-(benzylthiocarboxothioylthio)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 unusual phenomenon was observed in the kinetics between the two monomers during polymerization. The relationship between polymerization rate and concentration of inimer was totally opposite in different monomer-inimer systems. Branching analysis was conducted by using degree of branching (DB) as the measurement parameter. A higher degree of branching occurred with increased inimer content. Furthermore, these polymers were readily
deprotected by hydrolysis in trifluoroacetic acid solution resulting in water soluble polymers.

**Keywords:** one-pot synthesis, hyperbranched glycopolymers, RAFT, *inimer*, polymerization

**Introduction**

Free radically polymerized glycans, which attempt to mimic the structure and biological properties of natural sugars, have attracted increasing interest as polysaccharide mimics due to their potential applications in a wide range of fields including 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 is significant to the properties of sugars [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 *et al.* used atom transfer radical polymerization (ATRP) to synthesize poly(2-gluconamidoethyl methacrylate) in water [12]. Lowe *et al.* prepared poly(2-methacycloxyethyl glucoside) via reversible addition-fragmentation chain transfer polymerization (RAFT) [13].

Since Lowe *et al.* reported their first work, a number of researchers have used RAFT polymerization to synthesize glycopolymers. This approach has proven to be a versatile and simple method. For example, well-defined linear and star-like multiple arms 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 used to generate graft or end-functional glycopolymers [15,16]; Morimoto *et al.* prepared stimuli-responsive nanogels formed by glycopolymers-g-poly(N-isopropylacrylamide) using RAFT polymerization [17].
To the best of our knowledge, there is as yet no report on hyperbranched glycopolymers prepared using RAFT *inimers* and glycan monomers. Polysaccharides are an important class of biopolymer due to their role as structural components and their bioactivity (e.g. cell–cell signalling) [18]. Many essential natural saccharides are hyperbranched, such as amylopectin and glycogen. As less precise analogues of dendrimers, hyperbranched polymers are capable of carrying significant numbers of functional groups and have a significant advantage in that they are prepared using a much simpler synthetic procedure [19-21]. Since the first reports by Rimmer et al. [22,23] and Liu et al. [24], RAFT polymerization has proven to be an efficient method to synthesize hyperbranched polymers [25-34]. Several hyperbranched polymers, such as polystyrene and poly(methyl methacrylate), were prepared via RAFT polymerization in the presence of cross-linkers [24-30] or polymerizable RAFT agents (*inimers*) [31-34].

Our aim was to prepare 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 disadvantage of typical multi-step syntheses required to prepare polysaccharides. Our preliminary work on the synthesis of hyperbranched glycopolymers via direct RAFT polymerization is reported here for the first time. It differs from Perrier’s work [16], in which click chemistry was used to conjugate saccharides on polymer backbone after RAFT polymerization. The polymerization and kinetic studies of two different types of galactose monomers using two different polymerizable RAFT agents (*inimers*) were discussed. Both protected and deprotected galactose polymers were obtained by RAFT process.

**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) (V501, 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)carbonothioylthio)propanoic acid (BCPA) were synthesized as described in the literature [35-37].

Characterization and Instrumentation

NMR analysis

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

Size exclusion chromatography (SEC) analysis

10 mg of the dried polymer was dissolved in 4 ml of THF and filtered through 0.22 µm pore-size disposable filter prior to analysis. The 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, USA) 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 25ºC with injection volume of 10 µl. The GPC instrument was calibrated with narrow polydispersity polystyrene standards with peak molecular weight (Mp) in the range of 264–256000 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 ionization energy of 70eV. Accurate mass measurements were conducted with a resolution of 5000-10000 using perfluorokerosene as the reference compound.
Synthesis of inimers 2-(methacryloyloxy)ethyl 4-cyano-4-(phenylcarbonothioylthio)pentanoate (MAE-CPP) (1) and 2-(3-(benzylthiocarbonothioylthio)propanoyloxy)ethyl acrylate (BCP-EA) (2)

Preparation of MAE-CPP (1) is described below. CPAD 1.0 g (3.6 mmol) and HEMA 0.51 g (4 mmol) were dissolved in 10 ml dry toluene, followed by 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 hours 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 upon drying (1.1 g, 75% yield).

\[ ^1 \text{H NMR, 400MHz (CDCl}_3, \delta \text{ ppm): 7.95-7.28 (m, 5H, } \Phi \text{), 6.13 (s, 1H, C=C- } H_6 \text{), 5.61 (s, 1H, C=C- } H_6 \text{), 4.61 (m, 4H, (CO)OC}_2H_2CH_2O(CO)), 2.79 (s, 1H, C(CH}_3(CN)-CHH), 2.68 (t, 2H, } CH_2(CO)O\text{), 2.38 (s, 1H, C(CH}_3(CN)-CHH), 1.95 (s, 3H, CH}_3-C=C\text{), 1.61 (s, 3H, C(CH}_3)(CN)). \]

\[ ^13 \text{C NMR, 75.4MHz (CDCl}_3, \delta \text{ ppm): 171.3, 144.5, 135.8, 133.0, 128.6, 126.6, 126.2, 77.6, 77.0, 76.4, 62.7, 62.2, 45.7, 33.3, 29.7, 24.1, 18.2. IR (liquid between NaCl discs, cm}^{-1}\text{): 2957, 1738, 1636, 1445, 1381, 1296, 1161, 1048, 945, 868, 763, 688, 650. HRMS (EI): calculated for C}_{19}H_{21}O_4NS_2 [M]^+: 391.0907; found: 391.0903. \]

The procedure of preparing BCP-EA (2) was the same as MAE-CPP (1), except for substituting CPAD and HEMA by BCPA and HEA, respectively. The final product was a yellow liquid (82% yield). \[ ^1 \text{H NMR, 400MHz (CDCl}_3, \delta \text{ ppm): 7.33-7.26 (m, 5H, } \Phi \text{), 6.46-6.41 (d, 1H, C=C- } H_6 \text{), 6.17-6.10 (tetra, 1H, } H_6-C=C- } H_6 \text{), 5.88-5.85 (d, 1H, C=C- } H_6 \text{), 4.60 (s, 2H, } CH_2O(CO)\text{), 4.36 (m, 4H, (CO)OC}_2H_2 \text{ and } \Phi-CH_2\text{), 3.63 (t, 2H, S(S=C)S-CH}_2\text{), 2.80 (t, 2H, } CH_2(CH}_2O)\text{). } ^13 \text{C NMR, 75.4MHz (CDCl}_3, \delta \text{ ppm): 222.9, 171.2, 165.8, 138.2, 134.8, 131.5, 129.2, 128.7, 127.9, 127.8, 77.3, 77.0, 76.7, 62.6, 62.1, 41.5, 33.0, 31.2. IR (liquid between NaCl discs, cm}^{-1}\text{): 3062, 3030, 2956, 1731, 1635, 1494, 1453, 1407, 1373, 1348, 1182, 1065, 982, 900, 888, 802, 730, 680, 638. HRMS (EI): calculated for C}_{16}H_{18}O_4S_3 [M]^+: 370.0362; found: 370.0360. \]
Synthesis of 6-O-methacryloyl-1,2:3,4-di-O-isopropylidene-D-galactopyranose (proGal-M) (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 stirred overnight at room temperature. The solvent was removed under vacuum to afford the crude product which was purified via a silica column (eluent: ethyl acetate:hexane = 1:3 v/v) to afford proGal-M (3) as a white powder upon drying (2.38 g, 60% yield). 1H NMR, 400MHz (CDCl$_3$, δ ppm): 6.04 (s, 1H, CH$_{a}=C$(CH$_3$)), 5.48 (m, 1H, CH$_{b}=C$(CH$_3$)), 5.43 (d, 1H, anomeric CH), 4.54 (dd, 1H, CHH), 4.16–4.26 (m, 4H, 1CHH+3CH), 4.00 (m, 1H, CH), 1.85 (s, 3H, CH$_2$=CH(CH$_3$)), 1.41 (1s, 3H, CH$_3$), 1.36 (1s, 3H, CH$_3$), 1.25 (1s, 3H, CH$_3$), 1.24 (1s, 3H, CH$_3$). 13C NMR, 75.4MHz (CDCl$_3$, δ ppm): 167.2, 136.4, 125.9, 109.7, 108.9, 96.5, 71.4, 70.9, 70.7, 66.3, 63.9, 26.1, 25.2, 24.6, 18.4. HRMS (EI), 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 (proGal-A) (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 drop wise. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was then washed with 0.5M HCl (5 ml × 2), 0.5M Na$_2$CO$_3$ (5 ml × 2) and water (5 ml × 2) sequentially. The organic layer was dried with Na$_2$SO$_4$ and the solvent was removed under vacuum to afford the crude product, which was purified via a silica column (eluent: ethyl acetate:hexane = 3:2 v/v) to afford proGal-A (4) as a white powder upon drying (0.67 g, 84% yield). 1H NMR, 400MHz (CDCl$_3$, δ ppm): 6.23 (m, 1H, CHH$_{a}$=CH), 6.10 (m, 1H, CH$_2$=CH), 5.63 (m, 1H, CHH$_{a}$=CH), 5.51 (d, 1H, anomeric CH), 4.61 (dd, 1H, CHH), 4.16–4.26 (m, 4H, 1CHH+3CH), 4.00 (m, 1H, CH), 1.60 (s, 6H, C(CH$_3$)$_2$), 1.41 (1s, 3H, CH$_3$), 1.36 (1s, 3H, CH$_3$), 1.25 (1s, 3H, CH$_3$), 1.24 (1s, 3H, CH$_3$). 13C NMR, 75.4MHz (CDCl$_3$, δ
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 of 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 subject to three cycles of freeze-vacuum-thaw degassing process before immersing in an oil bath at 70°C under N₂ atmosphere. Reaction samples were removed at regular time points and precipitated in diethyl ether. The precipitates were dried under vacuum at 40°C and characterized by gravimetric, NMR and SEC analysis. Poly(proGal-M): ¹H NMR, 400MHz (CDCl₃, δ ppm): 5.53 (b, 1H, anomeric CH), 4.64 (b, 1H, CHH), 4.36–3.94 (b, 5H, 1CHH+4CH), 2.12-0.88 (m, 17H, -C₆H₂-C(CH₃)- and -C(CH₃)₂). Poly(proGal-A): ¹H NMR, 400MHz (CDCl₃, δ ppm): 5.41 (b, 1H, anomeric CH), 4.58 (b, 1H, CHH), 4.33–3.85 (b, 5H, 1CHH+4CH), 1.74-0.95 (m, 21H, -CH₂-CH- and -C(CH₃)₂).

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

The protected glycopolymer (0.1 g) was dissolved in 0.3 ml of 90% aqueous trifluoroacetic acid and stirred at room temperature for 5 hours. The trifluoroacetic acid was then removed by dialysis against water overnight. The deprotected glycopolymer was recovered by freeze drying at a yield of 95%. Poly(Gal-M): ¹H NMR, 400MHz (D₂O, δ ppm): 5.21 (b, 1H, anomeric CH), 4.48 (b, 1H, CHH), 4.18–3.44 (b, 5H, 1CHH+4CH), 1.90-0.63 (m, 5H, -CH₂-C(CH₃)-). Poly(Gal-A): ¹H NMR, 400MHz (D₂O, δ ppm): 5.08 (b, 1H, anomeric CH), 4.31 (b, 1H, CHH), 4.01–3.29 (br s, 5H, 1CHH+4CH), 1.69-0.90 (m, 9H, -CH₂-CH- and -C(CH₃)₂).

Results and Discussion
1. 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, in order to facilitate controlled polymerization of different types of monomers. Theoretically, MAE-CPP (1), a dithioester, was expected to be suitable for most methacrylates, methacrylamides, styrenics, acrylates and acrylamides; BCP-EA (2), a trithiocarbonate, was expected to be suitable for most styrenics, acrylates, acrylamides and vinyl esters [38].

![Scheme 1. Synthetic routes of polymerizable RAFT agents (inimers).](image)

2. 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 is an acrylamide-type protected galactose monomer. NMR and accurate MS analysis were consistent with the proposed structures of the two monomers. The protected monomers were used for polymerization due to 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 Section 6).
3. Polymerization of proGal-M and proGal-A

Both monomers were polymerized under 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, while 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. Furthermore, a series of linear RAFT polymerization of proGal-A (4) were also conducted for comparison with hyperbranched polymers. As the linear RAFT polymerization of proGal-M (3) has already been reported in the literature [39-44] it was not repeated in this study. Polymerizations were all conducted using an initiator (AIBN) to inimer ratio of 1:3. Polymerization conditions and results are summarized in Tables 1 and 2. The monomer conversion was calculated by gravimetric analysis while theoretical molecular weight for a RAFT polymerization was calculated using Equation 1.

\[ Mn(\text{Theo}) = M_{\text{monomer}} \times DP_{\text{Theo}} \times \text{Conversion} + M_{\text{inimer}} \quad (\text{Equation 1}) \]

Where \( M_{\text{monomer}} \) is the molecular weight of saccharide monomer; \( DP_{\text{Theo}} \) is the designated degree of polymerization, which is determined by the feed ratio of monomer to inimer; \( M_{\text{inimer}} \) is the molecular weight of inimer.
Figures 1 and 2 show the SEC results for 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 (Figure 1, Figure 2(B, C, and D)) since they exhibited multiple and broad peaks, which are very different from linear RAFT glycopolymers as shown in both literature [39-44] and Figure 2(A). This difference in SEC is consistent with that of hyperbranched polymers reported in the literature [24,31,32]. Moreover, it is also observed in Figure 1(D) that a low molecular weight shoulder appears in all polymers prepared at different reaction times, indicating that the polymers contain a portion of dead polymer chains, which did not grow with time. This implies the partially uncontrolled polymerization under a higher ratio of monomer to inimer (100). This will be further discussed in later sections.
Figure 1. 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 2. 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).

4. Polymerization kinetics study

The relationship of molecular weight versus conversion for the polymerization of proGal-M is shown in Figure 3(A) which shows a close to linear relationship at lower ratios of [M]/[inimer] (10, 20), indicating the characteristics of living polymerization. However, at higher ratios of [M]/[inimer] (50, 100), the relationship was no longer linear. Particularly for the polymerization under the ratio of 100, the molecular weight of polymers firstly shows a linear trend below 50% conversion, but decreases after reaching 50% conversion. It is also observed that the molecular weight of the polymers prepared at the ratio of 100 was much higher than expected. For
example, when the conversion was around 60%, the molecular weight of the polymer prepared at the ratio of \([M]/[\text{inimer}] = 100\) was about 60k. In contrast, the molecular weight of the polymers prepared at ratios of \([M]/[\text{inimer}] = 10, 20, \) and 50 were only 3.6k, 6k and 8.8k, respectively, which are reasonably close to calculated values. The extremely high molecular weight together with the observation of the unchanging SEC low MW shoulder in Figure 1(D), shows that the polymerization of proGal-M under \([M]/[\text{inimer}] = 100\) 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. The polymerization of proGal-A was similar to that of proGal-M as shown by similar \(M_n\) vs conversion graphs (Figure 3B), 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 [45-47] than the use of aliphatic dithioesters [48-49] or trithiocarbonates [50]. However, in datasets arising from our experiments, this typical retardation was not observed. In the copolymerization of proGal-M and MAE-CPP, where a dithiobenzoate-type \(\text{inimer}\) was used, the polymerization rate surprisingly became faster as the \(\text{inimer}\) concentration was increased (Figure 4(A)). In contrast to proGal-M, the trithiocarbonate-type \(\text{inimer}\) actually retarded the polymerization of proGal-A. A slower reaction rate was observed at a higher \(\text{inimer}\) concentration (Figure 4(B)). This effect may be related to the structure of the \(\text{inimers}\), which are different from regular RAFT agents. From the comparison of linear and hyperbranched polymerization of proGal-A shown in Figure 4(B), which were both conducted at a monomer/RAFT agent ratio of 20, the hyperbranched polymerization rate was faster than the linear one. As all the other parameters in the two polymerization systems were the same, we propose that this difference in polymerization rate was caused by the existence of the polymerizable groups in \(\text{inimers}\). The polymerizable groups in \(\text{inimer}\) may have been able to accelerate the polymerization by participating in the polymerization to form hyperbranching points. This hypothesis could also be used to explain the unusual phenomenon in kinetics shown in Figures 4(A) and 4(B) referred above. Since the
effects of both acceleration and retardation co-exist in inimers, in the case of the polymerization of proGal-M (Figure 4(A)), the acceleration effect was likely to have been stronger than the retardation effect; whereas, in the case of polymerization of proGal-A (Figure 4(B)), the retardation effect might be stronger than acceleration.

**Figure 3.** Molecular weight versus conversion for (A) hyperbranched poly(proGal-M) prepared using a ratio of monomer to inimer of 10 (■), 20 (▲), 50 (▼), and 100 (●); (B) hyperbranched poly(proGal-A) prepared using a ratio of monomer to inimer of 10 (■), 20 (▲), and 50 (▼).
Figure 4. Monomer conversion versus reaction time for (A) hyperbranched poly(proGal-M) prepared using a ratio of monomer to \textit{inimer} of 10 (■), 20 (▲), and 50 (▼); (B) hyperbranched poly(proGal-A) prepared using a ratio of monomer to \textit{inimer} of 10 (■), 20 (▲), and 50 (▼), as well as linear poly(proGal-A) prepared using a ratio of monomer to RAFT agent of 20 (●).

5. Hyperbranching analysis

Degree of branching (\textit{DB}) is an important parameter to describe macromolecular structure of hyperbranched polymers. According to the definition of \textit{DB} in literature [51], \textit{DB} is calculated according to \textit{DB} = (2 \times \text{number of dendritic units})/(\text{total number of units - 1}). A higher \textit{DB} means more branching points for a branched polymer. The value of \textit{DB} should be between 0 and 1, with a linear polymer...
at 0 and a perfect dendrimer at 1. In our cases, $DB$ can be approximately calculated by Equation 2.

$$DB = \frac{2 \times (\frac{A_a}{S} - A_b)}{A_c - 1} \quad \text{(Equation 2)}$$

Where $A_a$, $A_b$, and $A_c$ are the integration of different peaks shown in Figure 5. 

$\left(\frac{A_a}{S} - A_b\right)$ represents the hyperbranching points and $A_c - 1$ represents the total number of repeating saccharide units.

![Chemical shifts of proGal-M and proGal-A](image)

**Figure 5.** $^1$H NMR spectra used to calculate $DB$. (A) proGal-M (3) polymerized with MAE-CPP (1); (B) proGal-A (4) polymerized with BCP-EA (2).
Calculated DB was plotted against monomer conversion in Figure 6. Firstly, it was clearly observed that in the polymerization of both proGal-M and proGal-A a higher inimer concentration resulted in a higher degree of branching. Since the participation of inimers in the polymerization resulted in hyperbranching points, this result was expected. Secondly, a similar changing trend of DB was observed in both poly(proGal-M) and poly(proGal-A): there seemed to be three stages during the polymerization of proGal-M (Figure 6(A)). DB decreased in the first stage, then increased or remained constant in the second stage, and then decreased again in the third stage. We hypothesize that this trend was caused by the propagation competition between monomer and inimer in the polymerization. At the early stage of polymerization, the concentration of sugar monomer was relatively high and thus the polymerization rate of the sugar monomer relative to that of the inimer double bond was high, resulting in decrease of DB. With the consumption of the sugar monomer (second stage), the reaction rate of double bonds in inimer started to become more significant, as a result the DB was able to keep constant or even slightly increased. At third stage, the double bonds in inimer ran out before the end of polymerization whilst the sugar monomer continued to polymerize, during which period the DB decreased again. In the case of the polymerization of proGal-A (Figure 6(B)), the acrylamide groups were more active than methacrylate groups as shown in Figure 4, which showed the polymerization rate of acrylamide monomer was much faster than methacrylate monomer. It is likely that this was the reason why the first stage could not be seen in the polymerization of proGal-A and only the last two (in the case of [M]/[inimer] = 10 and 20) or one (in the case of [M]/[inimer] = 50) stages could be observed. In addition, DB of poly(proGal-A) was higher than poly(proGal-M) when they were prepared at same ratio of monomer to inimer.
Figure 6. Degree of branching (DB) versus monomer conversion: (A) poly(proGal-M) and (B) poly(proGal-A), prepared using ratios of monomer to inimer of 10 (■), 20 (▲), and 50 (▼).

6. Hydrolysis of protected hyperbranched glycopolymers

In order to recover the water solubility of hyperbranched glycopolymers, a deprotection procedure (Scheme 3) 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 slightly downfield shift of protons on sugar rings proved that the protecting groups were quantitatively removed. Moreover,
the RAFT agent end groups could still be seen after deprotection (enlarged area in Figure 7(B)), which provides the possibility to further modify these glycopolymers. The $^1$H NMR data of the water soluble deprotected poly(proGal-M) and poly(proGal-A) was recorded in experimental section.

**Figure 7.** $^1$H NMR spectra of poly(proGal-M): (A) before deprotection (in CDCl$_3$); (B) after deprotection (in D$_2$O).
Table 1. Polymerization parameters and results of proGal-M

<table>
<thead>
<tr>
<th>Type</th>
<th>[M]/[RAFT]</th>
<th>Time</th>
<th>Conver.</th>
<th>Mn (theo.)</th>
<th>Mn (NMR)</th>
<th>Mn (SEC)</th>
<th>PDI</th>
<th>DB</th>
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<td></td>
<td>10</td>
<td>3h</td>
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Conclusions

This is the first report on successful preparation of hyperbranched glycopolymers by one-pot RAFT polymerization. Two types of hyperbranched glycopolymers (polygalactose analogues) were prepared using two novel inimers. The hyperbranched structures of the glycopolymers were confirmed by SEC and NMR analyses. The polymerization processes of two systems showed living characteristics according to the kinetics study. The degree of branching increased with the decreasing [monomer]/[inimer] ratio in polymerization of both monomers. The relationships of polymerization rate and [monomer]/[inimer] ratio were different in the polymerization of two monomers. The glycopolymers were also able to be readily deprotected in TFA resulting in water soluble polymers. The method described in this study has the potential to synthesize a large array of hyperbranched glycopolymers, including bioactive glycopolymers which will be reported in our later work, by controlling branching points (inimer concentration) and composition using different saccharide monomers.

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References


