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Synthesis of (meth)acrylamide-based glycomonomers using renewable resources and their polymerization in aqueous systems†

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In this work, we present the kinetically-controlled enzymatic synthesis of novel glycosyl-(meth)acrylamide monomers using β-glucosidase. Cellobiose served as the glycosyl donor in the enzyme catalyzed transglycosylation reaction and hydroxyl-alkyl (meth)acrylamides as the glycosyl acceptor. After optimization, we were able to increase the glycomonomer yield up to 68% by changing the glycosyl donor to *p*-nitrophenyl β-D-glucopyranoside and adding $BMIMPF_6$ as cosolvent. The structure of the glycomonomers was confirmed by ^1H NMR, ^{13}C NMR, and mass spectrometry experiments. Aqueous RAFT polymerization of the glycomonomers was successfully performed resulting in glycopolymers with molecular weights up to 30 kg mol^{-1} and relatively low polydispersity indices (PDI's < 1.30). Free radical polymerization of the glycomonomers was executed as well with the obtained glycopolymers resulting in higher molecular weights and PDI's than the glycopolymers prepared by RAFT polymerization. Thermal properties of the synthesized glycopolymers were investigated *via* differential scanning calorimetry.

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Introduction

Carbohydrates are the most abundant renewable biomass produced annually but only a small fraction of them is used by the chemical industry.[1] In principle, carbohydrates can be further utilized as an alternative resource for fossil-based chemicals in the field of polymers. For example, researchers used carbohydrates as starting materials to design polymers having pendant sugar moieties called glycopolymers.[2] Glycopolymers have gained much interest in the last decades especially because of their properties that can mimic glycolipids and glycoproteins.[3–5] The application of glycopolymers has been reported mainly for drug delivery,[6–8] tumor targeting,[9–11] immune stimulants,[12] and therapeutics.[13]

Glycomonomers, the precursor of glycopolymers, can be synthesized either by chemical or enzymatic methods.[14–23] Enzymatic reactions offer some advantages compared to chemical reactions; for instance, reaction specificity provided by the enzyme can avoid necessary protection steps of the saccharide-hydroxyl groups in the conventional synthetic procedures. In addition, enzymes are non-toxic catalysts, derived from renewable resources, and enzymatic reactions are generally performed under relatively mild conditions.[24,25]

Lipases[17,20,21] and glycosidases[14,18,22,23] are the most used biocatalysts for the synthesis of glycomonomers. Lipases catalyze the transesterification between a primary alcohol of mono- and disaccharides that function as the glycosyl donor and an activated ester serves as the glycosyl acceptor. Such reactions are usually carried out for at least 4 days in order to obtain a good yield. In contrast to lipases that require an activated molecule, glycosidases only need a natural primary or secondary alcohol to functionalize monosaccharides at the C-1 position with reasonable shorter reaction times than lipase. For example, we previously used commercially available unmodified hydroxy-alkyl (meth)acrylates to synthesize glycomonomers catalyzed by β-glucosidase under thermodynamically-controlled reaction conditions.[14] The maximum yield of the desired glycomonomers was obtained after one day of reaction and a lower glycomonomer yield was obtained when the kinetically-controlled reaction conditions were applied.

Most of reported glycopolymers, that can potentially be applied as biomimetic materials[6–13] employ an amide bond to attach a saccharide unit to the polymer backbone. This is due to the fact that amide groups provide better stability towards hydrolysis of the saccharide units than ester groups in aqueous media. In addition, we can hypothesize, that the amide bond mimics the peptide bond in glycoproteins.

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† Electronic supplementary information (ESI) available: Theoretical and observed ESI-MS spectra of Glc-β-EAAm, Glc-β-EMAAm, and Glc-β-BMAAm; NMR and DSC results of the polymers prepared by free radical polymerization. See DOI: 10.1039/c7gc03023a

Using the previously reported thermodynamically-driven approach and the same enzymes, we failed to obtain the desired glycomonomers with (meth)acrylamide-based alcohols serving as the glycosyl acceptor, but we were able to synthesize three glycosyl-(meth)acrylamide monomers under kinetically-controlled reaction conditions. Herewith, we present enzymatically-synthesized *N*-(β-glycosyloxy)-ethyl acrylamide (Glc-β-EAAm), *N*-(β-glycosyloxy)-ethyl methacrylamide (Glc-β-EMAAm), and *N*-(β-glycosyloxy)-butyl methacrylamide (Glc-β-BMAAm) monomers, and the study of improving monomer yield. This is the first report on the enzymatic synthesis of these glycomonomers; Glc-β-EAAm has been synthesized before but using a chemical approach involving 5 reaction steps[26,27] whereas Glc-β-EMAAm and Glc-β-BMAAm have never been reported in the literature. Furthermore, these novel glycomonomers were successfully polymerized by aqueous reversible addition–fragmentation chain-transfer (RAFT) polymerization and free radical polymerization (FRP).

Experimental section

Materials

β-Glucosidase from almonds with activity ≥2 units per mg solid, *N*-hydroxyethyl acrylamide (HEAAm) 97%, 4,4′-azobis(4-cyanovaleric acid) (ACVA) ≥98.0%, and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB) >97% were purchased from Sigma-Aldrich. Cellobiose 98% was obtained from Acros Organics. *p*-Nitrophenyl β-D-glucopyranoside (*p*-NPG) 98+% and 1-*N*-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$) 98+% were acquired from Alfa Aesar. Chloroform ($CHCl_3$), methanol (MeOH), and ethanol (EtOH) were obtained from Avantor. Silica gel was purchased from Silicycle. All chemicals were used as received. *N*-Hydroxyethyl methacrylamide (HEMAAm) and *N*-hydroxybutyl methacrylamide (HBMAAm) were synthesized according to literature.[28] RAFT agent, 3-benzylsulfanylthiocarbonylsufanyl-propionic acid (BSPA), for the acrylamide-based monomer was prepared according to literature.[29]

Characterization

^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz and 300 MHz Varian VXR Spectrometer, respectively, using deuterium oxide (99.9 atom % D, Aldrich) as the solvent. The acquired spectra were processed by MestReNova Software from Mestrelab Research S.L. Thin layer chromatography (TLC) was performed on aluminum sheet silica gel 60/kieselguhr (Merck) with $CHCl_3$/MeOH (4/1) mixture as the eluent. Spot visualization of the glycomonomers was done by spraying the TLC plate with 5% H_2SO_4 in EtOH followed by heating. Electrospray ionization mass spectrometry (ESI-MS) was executed on a Thermo Scientific LTQ Orbitrap Mass Spectrometer in positive ion mode and Milli-Q water was used as the solvent. Size exclusion chromatography (SEC) was carried out on a Viscotek GPCmax equipped with model 302 TDA detectors. Three columns were used: a guard column (PSS-GRAM, 10 μm, 5 cm) and two analytical columns (PSS-GRAM-1000/30 Å, 10 μm, 30 cm). The temperature for the columns and detectors were at 50 °C. DMF containing 0.01 M LiBr was used as the eluent at a flow rate of 1.0 ml min^{-1}. The samples were filtered through a 0.45 μm PTFE filter prior to injection. Narrow PMMA standards were used for calibration and molecular weights were calculated by universal calibration method with refractive index increment of PMMA (0.063 ml g^{-1}) was applied. The calculation was done using Viscotec Omnisec Software. Differential scanning calorimetry (DSC) was performed on a DSC Q1000 from TA Instruments by heating the samples to 200 °C with the heating and cooling rate of 10 °C min^{-1}.

Preparative synthesis Glc-β-EAAm, Glc-β-EMAAm, and Glc-β-BMAAm

In a 25 ml round-bottom flask, cellobiose (0.50 gram, 1.46 mmol) was dissolved in Milli-Q water (2.25 ml) at 50 °C. Subsequently, HEAAm (0.65 gram, 5.65 mmol), HEMAAm (0.75 gram, 5.80 mmol), or HBMAAm (0.9 gram, 5.72 mmol) was added into the cellobiose solution. The reaction was started by adding enzyme solution (50 mg in 0.25 ml H_2O). After 1 hour of reaction, the flask was put on boiled water to deactivate the enzyme and stop the reaction. TLC of the reaction mixture was performed and the reaction product was detected at retardation factor of 0.30, 0.35, and 0.37 for Glc-β-EAAm, Glc-β-EMAAm, and Glc-β-BMAAm, respectively. The water in the reaction mixture was evaporated and MeOH (10 ml) was added to precipitate the glucose and unreacted cellobiose. After centrifugation (4500 rpm, 10 min, 4 °C), the supernatant was concentrated by evaporation followed by purification through column chromatography with silica gel served as the stationary phase and $CHCl_3$/MeOH (4/1) mixture as the eluent. Eluent from the collected fractions containing the product was evaporated by rotary evaporation (<40 °C at reduced pressure) and the resulted product was stored in the fridge.

Glc-β-EAAm. Yellowish viscous liquid, 67 mg, yield: 16%, purity: 98%. ESI-MS: calculated for $C_{11}H_{19}NO_7$ + Na: 300.1054, observed: 300.1052. ^{1}H NMR (D_2O) δ in ppm: 6.15–6.30 (H11-*cis* and H9), 5.74 (H11-*trans*, J = 11.6 Hz), 4.45 (H1-axial, J = 8.0 Hz), 3.23–4.01 (H2, H3, H4, H5, H6, H7, H8). ^{13}C NMR (D_2O) δ in ppm: 169 (C12), 130 (C9), 127 (C11), 102 (C1β), 75.8 (C5), 75.6 (C3), 73 (C2), 70 (C4), 68 (C7), 61 (C6), 39 (C8).

Glc-β-EMAAm. Yellowish viscous liquid, 51 mg, yield: 12%, purity: 97%. ESI-MS: calculated for $C_{12}H_{21}NO_7$ + Na: 314.1210, observed: 314.1213. ^{1}H NMR (D_2O) δ in ppm: 5.69 (H11-*cis*), 5.44 (H11-*trans*), 4.45 (H1-axial, J = 8.0 Hz), 3.23–4.02 (H2, H3, H4, H5, H6, H7, H8), 1.91 (H10). ^{13}C NMR (D_2O) δ in ppm: 172 (C12), 139 (C9), 121 (C11), 102 (C1β), 75.8 (C5), 75.6 (C3), 73 (C2), 70 (C4), 68 (C7), 61 (C6), 39 (C8), 18 (C10).

Glc-β-BMAAm. Yellowish viscous liquid, 90 mg, yield: 18%, purity: 96%. ESI-MS: calculated for $C_{14}H_{25}NO_7$ + Na: 342.1523, observed: 342.1518. ^{1}H NMR (D_2O) δ in ppm: 5.65 (H11-*cis*), 5.41(H11-*trans*), 4.43 (H1-axial, J = 8.0 Hz), 3.21–3.95 (H2, H3, H4, H5, H6, H7, H8), 1.90 (H10), 1.62 (H7′ and H8′). ^{13}C NMR

(D_2O) δ in ppm: 172 (C12), 139 (C9), 121 (C11), 102 (C1β), 75.9 (C5), 75.8 (C3), 73 (C2), 70 (C4), 69.6 (C7), 61 (C6), 39 (C8), 26 (C7′), 25 (C8′), 18 (C10).

Time course of the reaction

The reaction mixture was prepared as described in the procedure above with HEAAm used as glycosyl acceptor. After 1, 2, and 3 hours of reaction time, an aliquot (~50 mg) was taken and directly put on boiled water to deactivate the enzyme. The aliquots were dissolved in D_2O (0.7 ml) and subsequently measured by ^{1}H NMR spectrometry.

Improvement of yield

In two 25 ml round-bottom flasks, *p*-NPG (0.50 gram, 1.62 mmol) was dissolved in Milli-Q water (4.50 ml) at 50 °C. Subsequently, HEAAm (0.65 gram, 5.65 mmol) was added into the *p*-NPG solution. Ionic liquid of $BMIMPF_6$ (2.0 ml, 30 v%) was added into one of the flasks and the reaction was started by adding the enzyme solution (50 mg in 0.25 ml H_2O). After 1 hour of reaction time, an aliquot (~50 mg) from each flask was taken and directly put on boiled water to deactivate the enzyme. The aliquots were dissolved in D_2O (0.7 ml) and subsequently measured by ^{1}H NMR Spectrometry. The rest was then put on boiled water to deactivate the enzyme and stop the reaction. The reaction mixture was then centrifuged (4500 rpm, 30 min, 4 °C) and two layers were observed. The water phase was collected and evaporated, then MeOH (10 ml) was added to precipitate the glucose and unreacted *p*-NPG. After another centrifugation (4500 rpm, 10 min, 4 °C), the supernatant was concentrated by evaporation followed by purification through column chromatography with silica gel served as the stationary phase and $CHCl_3$/MeOH (4/1) mixture as the eluent. Eluent from the collected fractions containing the product was evaporated and the obtained product was stored in the fridge.

RAFT polymerization of Glc-β-EAAm, Glc-β-EMAAm, and Glc-β-BMAAm

In a 10 ml round-bottom flask, Glc-β-EAAm (0.50 gram, 1.81 mmol), Glc-β-EMAAm (0.53 gram, 1.81 mmol), or Glc-β-BMAAm (0.58 gram, 1.81 mmol) was dissolved in Milli-Q water (1.60 ml). BSPA and CPADB were used as the RAFT agent for acrylamide- and methacylamide-based monomers, respectively. ACVA was used as the water-based initiator. The RAFT agent (25 mg in 0.5 ml DMF) and ACVA (25.3 mg in 1.0 ml DMF) solutions were prepared and 100 μl of each solution (18 μmol of RAFT agent or 9 μmol of initiator) was added to the monomer solution. The flask was then put into an ice bath and N_2 bubbling was performed for at least 1 hour. Subsequently, the flask was placed in an oil bath at 70 °C to start the reaction. An aliquot solution (100 μl) was drawn at a specified time to determine the monomer conversion by ^{1}H NMR. The polymer was isolated by precipitation of the reaction mixture into MeOH (10× volume) and reprecipitated two times. The gel-like precipitates were dried in vacuum oven (40 °C) overnight.

Free radical polymerization of Glc-β-EAAm, Glc-β-EMAAm, and Glc-β-BMAAm

In a 10 ml round-bottom flask, Glc-β-EAAm (0.20 gram, 0.72 mmol), Glc-β-EMAAm (0.21 gram, 0.72 mmol), or Glc-β-BMAAm (0.23 gram, 0.72 mmol) was dissolved in Milli-Q water (2.4 ml). An initiator solution (10 mg in 1.0 ml DMF) was prepared and 100 μl of it (3.57 μmol) was added into the monomer solution. The next steps use the same procedure as in the RAFT polymerization.

Poly(*N*-(β-glycosyloxy)-ethyl acrylamide) (P(Glc-β-EAAm)). RAFT product = pale yellowish powder, monomer conversion: 95%, yield: 45%. Free radical product = white powder, monomer conversion: 78%, yield: 54%. ^{1}H NMR (D_2O) δ in ppm: 7.86–8.30 (*N*H), 7.25–7.45 (H-aromatic from the RAFT agent), 4.52 (H1-axial, J = 6.8 Hz), 3.26–4.08 (H2, H3, H4, H5, H6, H7, H8), 1.94–2.33 (H9), 1.37–1.90 (H11). ^{13}C NMR (D_2O) δ in ppm: 176 (C12), 102 (C1β), 75.9 (C5), 75.7 (C3), 73 (C2), 70 (C4), 68 (C7), 61 (C6), 49 (C9), 42 (C11), 39 (C8).

Poly(*N*-(β-glycosyloxy)-ethyl methacrylamide) (P(Glc-β-EMAAm)). RAFT product = pale pinkish powder, monomer conversion: 88%, yield: 39%. Free radical product = white powder, monomer conversion: 82%, yield: 40%. ^{1}H NMR (D_2O) δ in ppm: 7.40–8.00 (*N*H and H-aromatic from the RAFT agent), 4.45 (H1-axial), 3.15–4.00 (H2, H3, H4, H5, H6, H7, H8), 1.23–2.05 (H11), 0.39–1.14 (H10). ^{13}C NMR (D_2O) δ in ppm: 179 (C12), 102 (C1β), 75.9 (C5), 75.7 (C3), 73 (C2), 70 (C4), 68 (C7), 61 (C6), 49 (C9), 45 (C11), 40 (C8), 17 (C10).

Poly(*N*-(β-glycosyloxy)-butyl methacrylamide) (P(Glc-β-BMAAm)). RAFT product = pale pinkish powder, monomer conversion: 94%, yield: 41%. Free radical product = white powder, monomer conversion: 90%, yield: 27%. ^{1}H NMR (D_2O) δ in ppm: 7.34–7.92 (*N*H and H-aromatic from the RAFT agent), 4.41 (H1-axial), 2.90–4.00 (H2, H3, H4, H5, H6, H7, H8), 1.40–2.08 (H11, H7′, H8′), 0.70–1.30 (H10). ^{13}C NMR (D_2O) δ in ppm: 179 (C12), 102 (C1β), 75.9 (C5), 75.7 (C3), 73 (C2), 70 (C4), 61 (C6), 45 (C11), 40 (C8), 26 (C7′), 24 (C8′), 17 (C10).

Results and discussion

The enzymatic synthesis of glycosyl-alkyl (meth)acrylamides was successfully performed using cellobiose as the glycosyl donor and HEAAm, HEMAAm, or HBMAAm as the glycosyl acceptors. Cellobiose, a disaccharide molecule consists of two β-glucose units, is preferred as starting materials since it is largely derived from renewable cellulose and a good substrate for the enzyme β-glucosidase. The glycomonomers synthesis was successfully carried out under the kinetically-controlled reaction conditions and we obtained three types of glycomonomers namely *N*-(β-glycosyloxy)-ethyl acrylamide (Glc-β-EAAm), *N*-(β-glycosyloxy)-ethyl methacrylamide (Glc-β-EMAAm), and *N*-(β-glycosyloxy)-butyl methacrylamide (Glc-β-BMAAm). The monomer Glc-β-EAAm has been synthesized previously by a chemical approach involving five reaction steps[26,27] while to the best of our knowledge, the monomers Glc-β-EMAAm and

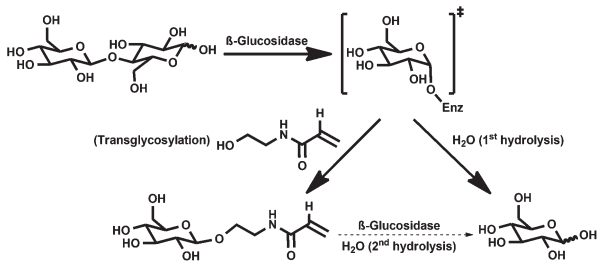
Glc-β-BMAAm have never been prepared before either by chemical or enzymatic methods. As shown in Scheme 1, the advantage of biocatalytic synthesis of glycomonomers is, that it is performed in only one reaction step generating purer products and lower environmental waste than the conventional reactions.

Fig. 1 shows ^{1}H NMR and ^{13}C NMR spectra of the purified glycosyl-alkyl (meth)acrylamide monomers. From the ^{1}H NMR spectra, only one anomeric proton signal was observed at 4.45 ppm corresponding to the axial position. Additionally, the ^{13}C NMR spectra demonstrating the anomeric carbon (C1) has only one chemical shift at 102 ppm that relates to a glycoside in β-configuration. These findings concluded that we obtained anomerically pure products with the linkage of (meth)acrylamide unit toward glucose at β-position. Besides, by comparing the peak integration of the anomeric proton with the vinyl protons (H9 & H11) it suggested that each glucose unit contains only one vinyl group. Consequently, the prepared glycomonomers were not only anomerically pure but also monofunctional; a unique feature provided by the enzymatic reaction offering high selectivity. In addition, molecular weights of the glycosyl-alkyl (meth)acrylamide monomers obtained from mass spectrometry experiments were almost identical to the calculated ones (see Fig. S1–S3 in ESI†). Combination of ^{1}H NMR, ^{13}C NMR, and mass spectrometry measurements confirmed the structure of the aimed glycomonomers as shown in Scheme 1.

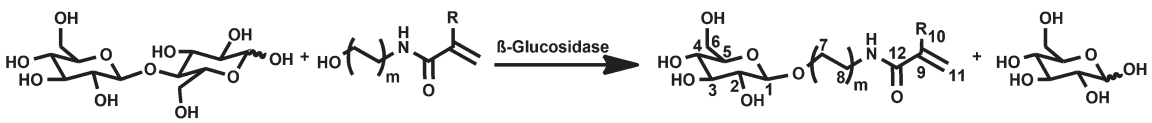
Time course of the reaction

The reaction mechanism of the kinetically-driven enzymatic synthesis of Glc-β-EAAm is shown in Scheme 2. According to this mechanism, a competition between transglycosylation and hydrolysis occurs during the enzymatic reaction. Therefore, it is crucial to determine the time needed for the reaction as short reaction times will lead to a low reactant conversion and low amounts of transglycosylation products while long reaction times risk the glycomonomers to be further hydrolyzed. In order to find the optimum time, ^{1}H NMR spectra of Glc-β-EAAm reaction mixtures were measured at certain time intervals and the results are displayed in Fig. 2.

According to Fig. 2(a), the anomeric proton peak of Glc-β-EAAm at 4.45 ppm was observed after a one-hour reaction and its peak area was further reduced with longer reaction time. In addition, the anomeric proton peak of cellobiose at 4.50 ppm almost completely disappeared at a reaction time of two hours. These observations indicated that the enzyme starts to hydrolyze the glycomonomer following 2nd hydrolysis pathway when almost all cellobiose is consumed. Hence, a one-hour reaction time is the optimum condition for this bio-



Scheme 2 Reaction mechanism of the synthesis of Glc-β-EAAm catalyzed by β-glucosidase.



Scheme 1 Kinetically-controlled synthesis of Glc-β-BMAAm (m = 2, R = CH_3), Glc-β-EMAAm (m = 1, R = CH_3), and Glc-β-EAAm (m = 1, R = H) catalyzed by β-glucosidase.

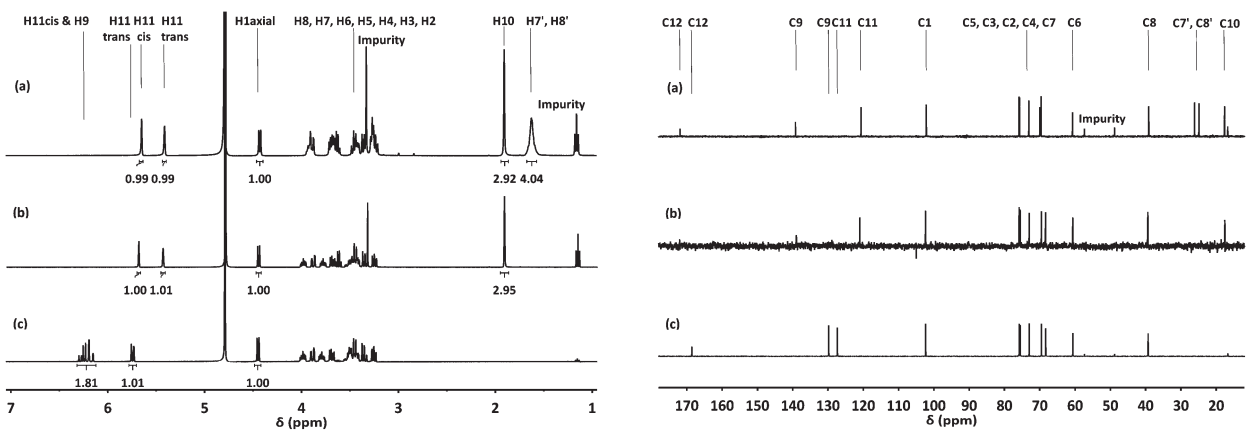


Fig. 1 ^{1}H NMR and ^{13}C NMR spectra of the enzymatically synthesized (a) Glc-β-BMAAm, (b) Glc-β-EMAAm, and (c) Glc-β-EAAm in D_2O.

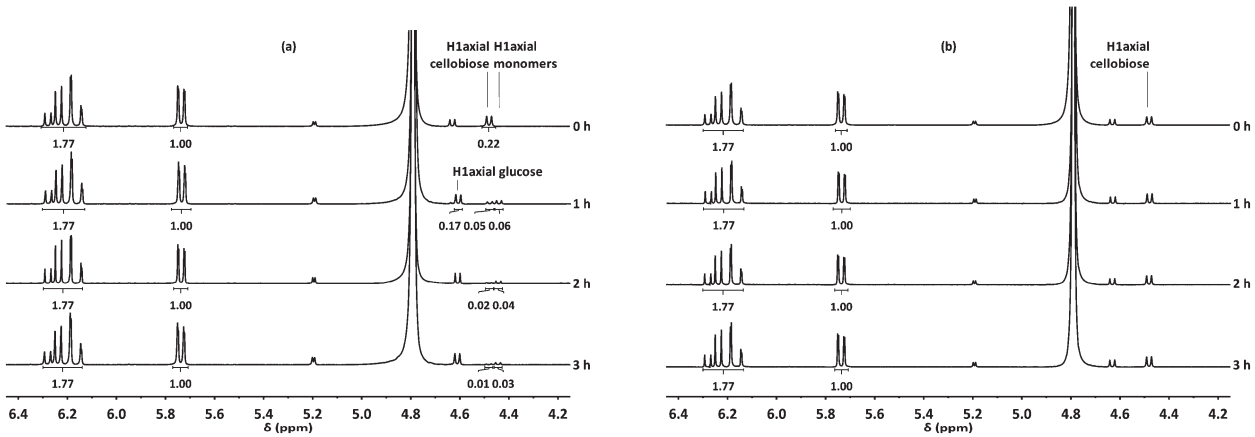


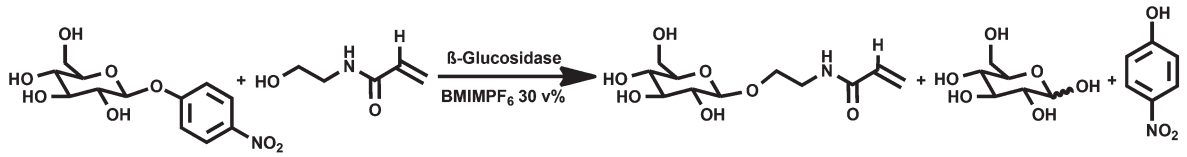
Fig. 2 ^{1}H NMR spectra of the solution mixtures from Glc-β-EAAm synthesis at different reaction times (a) with enzyme and (b) without enzyme in D_2O.

catalytic reaction. This result supports the advantage of a kinetically-controlled enzymatic reaction having short reaction times. Moreover, at a one-hour reaction, the peak area of the anomeric proton of glucose at 4.60 ppm is higher than the anomeric proton of Glc-β-EAAm indicating the 1st hydrolysis reaction in Scheme 2 is more favorable than transglycosylation. As a result, Glc-β-EAAm is produced less than glucose with the yield of about 16%. Furthermore, neither glycomonomers nor glucose proton peaks were observed in the control reaction (Fig. 2b) confirming the role of the enzyme in catalyzing the reaction.

Improvement of yield

The yield of the synthesized Glc-β-EAAm, Glc-β-EMAAm, and Glc-β-BMAAm was quite low of around 16%, 12%, and 18%, respectively, although the maximum concentration of cellobiose (0.60 M at 50 °C) was used together with the 2–4 times[30] concentration of glycosyl acceptor. It has been reported that aryl or vinyl units on an activated sugar are much better leaving-groups than glycosyl units on native sugars in transglycosylation reactions.[31,32] Hence, we might replace cellobiose with an activated sugar like *p*-NPG to make the transglycosylation route more favorable than hydrolysis. Another way to improve the glycomonomers yield is by adding cosolvents like ionic liquids[33–35] or organic solvents.[36–38] For example, Bayón *et al.*[33] used the ionic liquid $BMIMPF_6$ a a cosolvent and the transglycosylation yields improved up to 97%. Their molecular dynamic simulations revealed that the electrostatic interaction between enzyme and substrate was higher in water-$BMIMPF_6$ mixtures than in pure water, which leads to an increase of the reaction speed and improves the conversion rate. Additionally, our experiments showed that the immiscibility of $BMIMPF_6$ with water facilitates the separation from the reaction mixture just by centrifugation. As a result, $BMIMPF_6$ may potentially be reused for the next experiments. We, therefore, studied the yield improvement of Glc-β-EAAm synthesis by using *p*-NPG as an alternative glycosyl donor in transglycosylation reaction (see Scheme 3) and the synthesis was performed with and without $BMIMPF_6$.

Fig. 3 compares ^{1}H NMR spectra of the solution mixtures of the Glc-β-EAAm synthesis with different substrate compositions after a one-hour reaction time. The peak area at 4.45 ppm increased significantly when the combination of *p*-NPG and $BMIMPF_6$ was used. It indicates that higher glycomonomer yields were achieved by adjusting the reaction medium from the initial formulation that consists of only cellobiose without cosolvent. The obtained yield of Glc-β-EAAm was about 68%. In the event of using only *p*-NPG without cosolvent, the peak area of the anomeric proton was still higher than using cellobiose with a glycomonomer yield of about 49%. The latter case is remarkable considering the fact that the concentration of *p*-NPG was 40% less than cellobiose but it was able to produce higher Glc-β-EAAm yield. This outcome further confirms that using an activated monosaccharide as the glycosyl donor can facilitate the reaction to be more preferred towards transglycosylation than hydrolysis. Unfortunately, *p*-nitrophenol is generated as the side product when *p*-NPG was used and this compound is recognized to be



Scheme 3 Transglycosylation reaction of *p*-NPG and HEAAm catalyzed by β-glucosidase with $BMIMPF_6$ as cosolvent.

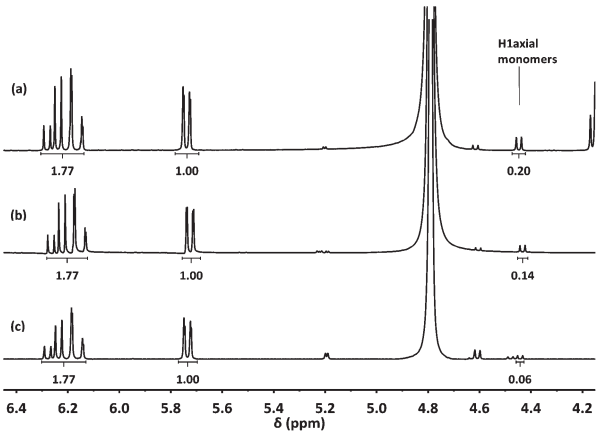
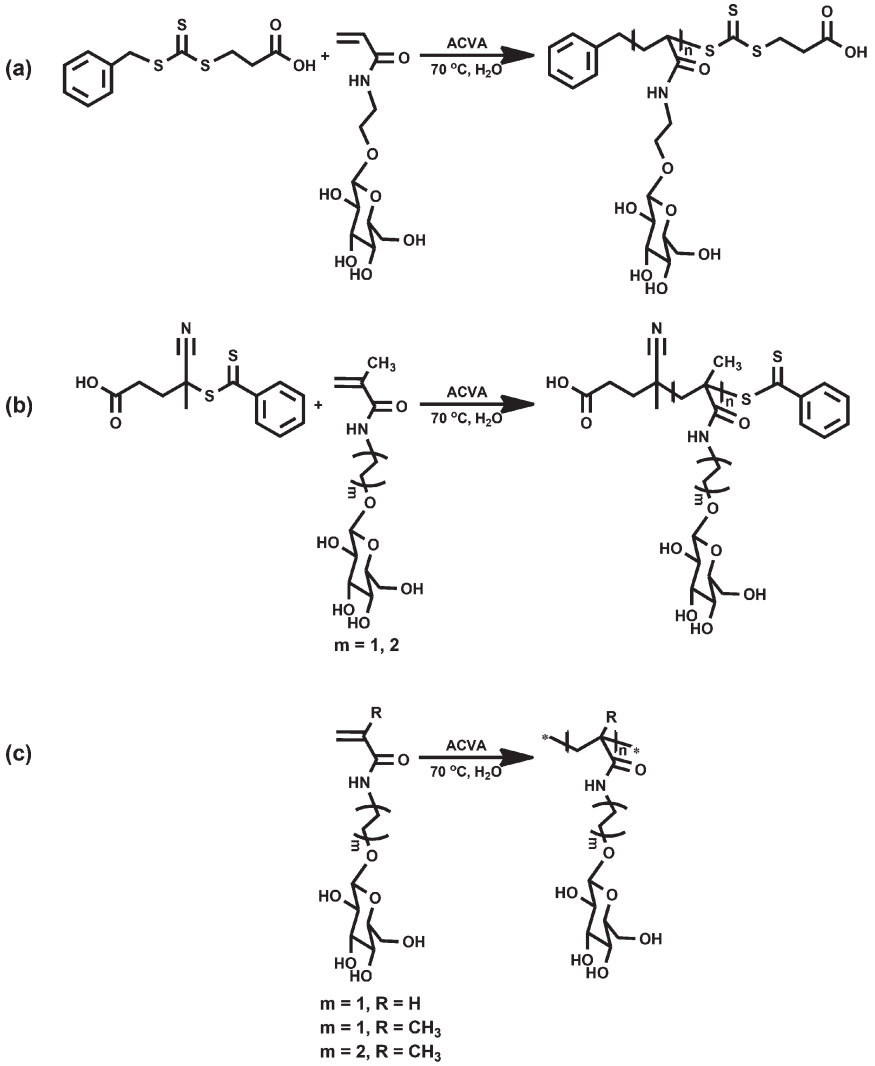


Fig. 3 ^{1}H NMR spectra of the solution mixtures of the Glc-β-EAAm synthesis after 1-hour reaction with different substrate compositions containing (a) 0.34 M *p*-NPG and $BMIMPF_6$, (b) 0.34 M *p*-NPG without cosolvent, and (c) 0.60 M cellobiose without cosolvent in D_2O.

highly toxic to living organisms. Therefore, another activated glucose such as vinyl-β-D-glucopyranoside may be exploited as a promising glycosyl donor and we will further study this in the future.

Polymerization of the prepared glycomonomers

The glycosyl-(meth)acrylamide monomers were successfully polymerized by aqueous RAFT polymerization and free radical polymerization (FRP). RAFT polymerization is one of the most versatile methods for the synthesis of well-defined polymers; on the other hand, FRP is a very robust technique for the production of high-molar-weight polymers in industry. The polymerization reactions are displayed in Scheme 4. Water was chosen in order to create the glycopolymers using a green solvent as we did with the synthesis of glycomonomers although a minor fraction of DMF (6 v%) was still needed to solubilize the RAFT agent. CPADB is a commercially available RAFT agent that is known to be well-suited for methacryla-



Scheme 4 Synthesis of (a) P(Glc-β-EAAm) and (b) P(Glc-β-EMAAm) or P(Glc-β-BMAAm) by aqueous RAFT polymerization. (c) Synthesis of the prepared glycomonomers by aqueous FRP.

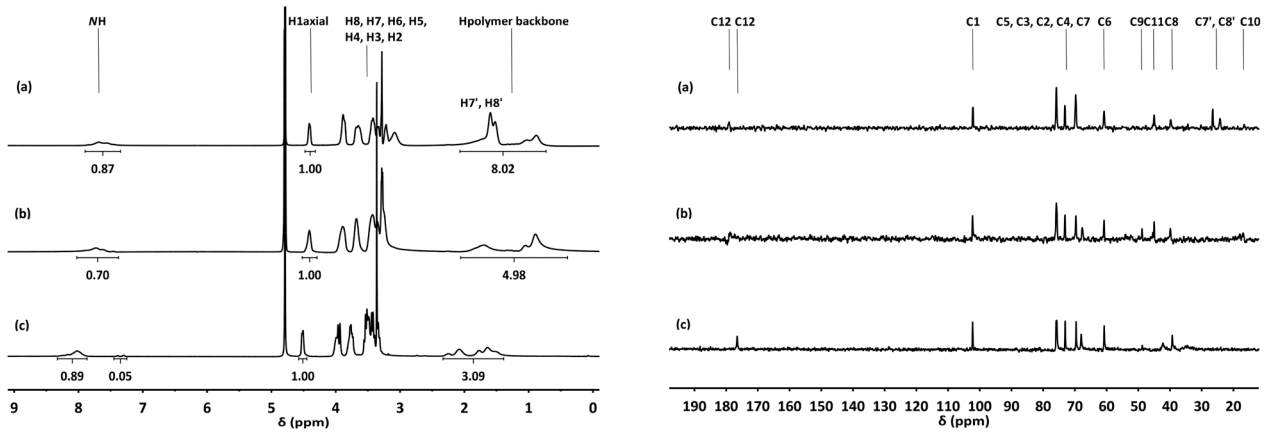


Fig. 4 ^{1}H NMR and ^{13}C NMR spectra of (a) P(Glc-β-BMAAm), (b) P(Glc-β-EMAAm), and (c) P(Glc-β-EAAm) synthesized *via* aqueous RAFT polymerization in D_2O.

mides[39,40] and BSPA, a trithiocarbonate-based RAFT agent, was relatively easy to prepare and has been successfully utilized in the preparation of polyacrylamides.[41,42] The presence of carboxylate unit on both RAFT agents maintains the solubility in aqueous medium.

Based on ^{1}H NMR spectra of the synthesized glycopolymers by RAFT polymerization in Fig. 4 (or Fig. S4† for the glycopolymers prepared by free radical polymerization), vinyl proton peaks of the monomers (H9 & H11 in Fig. 1) were no longer observed. In addition, broad peaks appeared around 0.5–2.3 ppm to be related to the protons at the polymer backbone. In the case of P(Glc-β-EMAAm) and P(Glc-β-BMAAm), aromatic proton peaks from the RAFT agent were mixed with a broad N–H peak around 7.25–8.0 ppm. The latter peak was also observed in the literature.[43] In agreement with the ^{1}H NMR spectra, ^{13}C NMR spectra of the prepared glycopolymers showed that the vinyl carbon peaks of the monomers (C9 & C11 in Fig. 1) were absent. Also, new carbon peaks (C9 & C11 in Fig. 4) assigned to the polymer backbone were detected.

Fig. 5 shows SEC chromatograms of the prepared glycopolymers by RAFT polymerization and FRP. Elugrams with relatively narrow peak and unimodal distribution were observed for the synthesized glycopolymers by RAFT polymerization. Also, these glycopolymers had low polydispersity indices (PDI's) as presented in Table 1. These results suggested that the controlled behavior has been achieved during RAFT polymerization of the glycomonomers. Moreover, molecular weights of these glycopolymers were calculated theoretically based on the monomer conversion obtained from ^{1}H NMR spectra of the reaction mixtures and the resulted molecular weights were in the range of 26–30 kg mol^{-1}. Nevertheless, the theoretical molecular weights of these glycopolymers were lower than the molecular weights gained from the SEC measurements because of the differences in hydrodynamic volumes of the glycopolymers and the standard PMMA. In comparison with the prepared glycopolymers by RAFT polymerization, the prepared glycopolymers by FRP have lower elution volumes and broader peaks in their elugrams. It

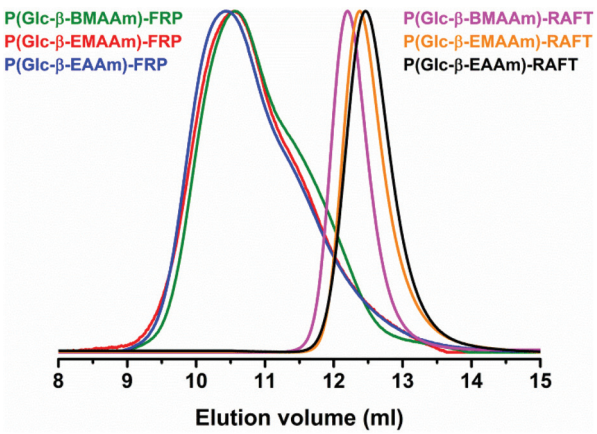


Fig. 5 SEC measurements (RI signals) of the synthesized glycopolymers by aqueous RAFT polymerization and FRP.

means that the latter polymers have higher molecular weights and PDI's than the former polymers. The absence of chain transfer agents during FRP allowing the polymer chains to growth uncontrolled lead to high molecular weights polymers and the termination reaction of FRP typically occurred *via* combination and disproportionation reactions causing broad polydispersities.

The glass transition temperature (T_g) of the prepared glycopolymers by RAFT polymerization was measured by differential scanning calorimetry and the thermograms are presented in Fig. 6 (or Fig. S5† for the glycopolymers prepared by free radical polymerization). The T_g's of respective glycopolymers prepared by RAFT polymerization and FRP were similar. Even though the glycopolymers synthesized by FRP had 7–8 times higher molecular weight than the glycopolymers synthesized by RAFT, both polymers have already reached the level of moderate to high molecular weights. Then according to the Fluory–Fox equation,[44,45] the difference between the T_g's for the same polymers are no longer significant. Besides, the measured T_g

Table 1 Overview of the synthesized glycopolymers by aqueous RAFT polymerization and FRP at 70 °C

Polymer[a]	t_R[b]	Conv. (%)	$M_{n,\ theory}$[c]	$M_{n,\ SEC}$[d]	PDI	T_g (°C)
P(Glc-β-BMAAm)-RAFT	4	94	30.4	66.4	1.22	130
P(Glc-β-EMAAm)-RAFT	4	88	26.0	49.4	1.29	165
P(Glc-β-EAAm)-RAFT	4	95	26.7	43.2	1.25	142
P(Glc-β-BMAAm)-FRP	4	90	—	214.3	2.46	131
P(Glc-β-EMAAm)-FRP	6	85	—	225.2	3.29	171
P(Glc-β-EAAm)-FRP	6	78	—	223.2	2.79	147

[a] [Monomer] for RAFT = 1.0 M and FRP = 0.29 M, [Monomer]:[RAFT agent]:[Initiator] = 100:1.0:0.5. [b] Reaction times in hours. [c] $M_{n,theory} = \frac{[Monomer]}{[RAFT\ Agent]} \times Conv. \times MW_{monomer} + MW_{RAFT\ agent}$. [d] Calculated molecular weights (in kg mol^{-1}).

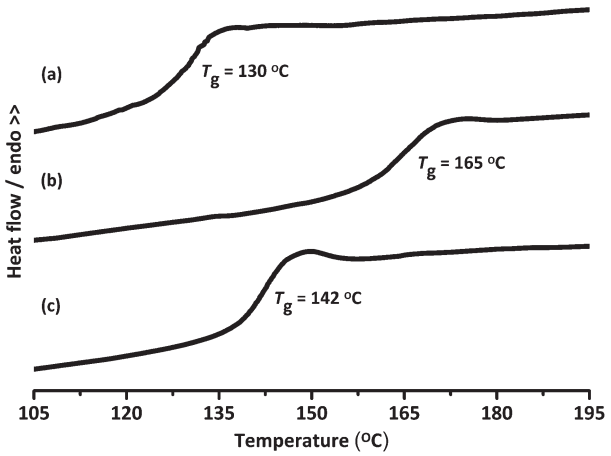


Fig. 6 DSC thermograms of (a) P(Glc-β-BMAAm), (b) P(Glc-β-EMAAm), and (c) P(Glc-β-EAAm) prepared *via* RAFT polymerization (2nd heating cycle).

of P(Glc-β-EAAm) was 142 °C, which is lower than the T_g of polyacrylamide[46] at 165 °C. This observation is reasonable because the glycosyl units increase the free volume of P(Glc-β-EAAm) while the polyacrylamide has a more rigid structure. The same holds for the P(Glc-β-BMAAm) with longer alkyl side chains resulting in bigger free volume thus lower T_g than P(Glc-β-EMAAm). Furthermore, the T_g of P(Glc-β-EMAAm) was higher compared to that of P(Glc-β-EAAm) as methyl group at the P(Glc-β-EMAAm) backbone hinders polymer chain mobility. Consequently, P(Glc-β-EMAAm) requires higher energy and higher temperature than P(Glc-β-EAAm) for the transition from the glassy-state to the rubbery-like material.

Conclusions

We have successfully synthesized three different types of glycosyl-(meth)acrylamide monomers using β-glucosidase from almond as the biocatalyst. Due to the enzyme selectivity, the prepared glycomonomers were found to be monofunctional and anomerically pure. The linkage of (meth)acrylamide units was observed at the anomeric β-position of glucose. Furthermore, the reaction condition for the kinetically-controlled enzymatic synthesis of the glycomonomers has been optimized to improve the glycomonomer yield. The structural characterization of the glycomonomers was conducted by ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

The synthesized glycomonomers were successfully polymerized by aqueous RAFT and free radical polymerization. The SEC measurements demonstrated that the glycopolymers synthesized by RAFT polymerization have lower molecular weights and PDI's than the glycopolymers prepared by FRP. The T_g's of both glycopolymers were around 142–171 °C as characterized by differential scanning calorimetry.

The synthesis of glycomonomers and glycopolymers have been performed in a green way, *i.e.* using renewable resources as starting materials, applying enzyme as the biocatalyst, and performing the reaction in water/water-ionic liquid mixture. In spite of the simple synthesis route in creating the glycomonomers as presented in this report, producing the monomer on a large scale remain challenging considering the price of the enzyme, the substrates (cellobiose and *p*-NPG), and cosolvent. Even though $BMIMPF_6$ can be reused, further experiments need to be performed to determine the number of cycles of used $BMIMPF_6$ that still resulting good amounts of glycomonomers. Moreover, the future investigation will be carried out in the direction of using these glycomonomers and glycopolymers as bio-related application materials.

Conflicts of interest

There are no conflicts to declare.

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