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One-pot synthesis of hyperbranched poly(amido amine) clicked with a sugar shell via Michael addition polymerization and thiol click reaction

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This paper reports the production of glycopolymers via a simple and flexible method. A novel glycopolymer with a hyperbranched poly(amido amine) core and a sugar shell (HPAA-GLc) was synthesized by using thiol-ene click reaction via facile one-pot method. Hyperbranched poly(amido amine) with vinyl terminals was first synthesized by Michael addition polymerization of *N,N′*-methylene bisacrylamide (MBA) with 1-(2-aminoethyl) piperazine (AEPZ). Subsequently, thiol-ene click reaction between vinyl units of hyperbranched poly(amido amine) and thio-glucose was performed *in situ*. Based on the NMR result, all the vinyl groups reacted with thiol-glucose in 120 min. Strong photoluminescence emission was observed from the aqueous solution of HPAA-GLc.

thiol-ene click reaction, glycopolymers, hyperbranched poly(amido amine), photoluminescence

1 Introduction

Thiol-ene click chemistry has attracted significant attention in the material field [1–4]. The reaction of thiols with enes is performed under various conditions including acid/base catalyzed thiol-Michael reaction and radical mediated radical thiol-ene reaction. The reaction displays many characteristics of click reaction [5], such as high efficiency, simple orthogonal reactions and biologically friendly nature (metal free). It has been very attractive in the synthesis of functional polymers, especially bio-related polymers [6, 7].

Glycopolymers are synthetic polymers with pendent sugar moieties [8, 9]. The pendent sugar moieties play a significant role in a number of significant biological processes including inflammation, cell-cell contacts, signal transmission and fertilization [10–13]. Therefore, polymers with sugar moieties have been of interest due to their biomimetic properties and significance in biological applications. Two different approaches have been developed to synthesize polymers with pendent sugar moieties: polymerization of a sugar-containing monomer and post-functionalization of preformed polymers using sugar-containing reagents. The latter approach is relatively simple and convenient because the synthesis of sugar-containing monomers often requires tedious multi-step reactions. However, the method frequently results in polymers having less regular structures because of incomplete reactions due to steric hindrance [14, 15]. In recent years, click reaction has been used to produce polymers with pendent sugar moieties. The most representative example is the construction of glycopolymers from alkyne backbone-functional polymers via Cu-catalyzed azide-alkyne click chemistry [16, 17] and thiol-ene click reaction [6, 7] under mild reaction conditions. Although great progress has been achieved in the synthesis of polymers with pendent sugar moieties, simple and flexible methods are still desirable for their preparation, especially one-pot method.

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Herein, we report a facile one-pot approach to synthesize hyperbranched poly(amido amine) (HPAA) with a sugar shell by using click chemistry. Hyperbranched poly(amido amine) with vinyl terminals (HPAA-vinyl) was synthesized using Michael addition polymerization of *N,N′*-methylene bisacrylamide (MBA) with 1-(2-aminoethyl) piperazine (AEPZ). The vinyl terminals subsequently reacted with thio-glucose *in situ* via thiol-ene click reaction under base catalysis with 100% conversion in a short time. HPAA clicked with a sugar shell shows strong photoluminescence emission.

2 Experimental

2.1 Materials

Pentaacetyl-β-D-glucopyranose (99%), 1-(2-aminoethyl) piperazine (AEPZ, 99%) and *N,N′*-methylene bisacrylamide (MBA, 99%) were purchased from Aldrich. Hydrogen bromide (HBr, 33% in acetic acid) was obtained from Xiangfan Wan Weiyang Chemical Co., Ltd. Thiourea, triethylamine (TEA, 99%), sodium metabisulphite ($Na_2S_2O_5$, 98%), and sodium methoxide (50% in methanol) were purchased from Sinopharm Chemical Reagent Co., Ltd. Cation exchange resin 7120H was purchased from Tianyuan Group Shanghai Resin Factory Co., Ltd. The resin was washed with methanol and water, and finally acidified with 10% hydrochloric acid. Other chemicals and solvents were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. and used as received without further purification.

2.2 Characterization

^{1}H NMR spectra (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on a Bruker AV 300 spectrometer. The number-average molecular weight (M_n) and polydispersity index of polymers were determined by size exclusion chromatography (SEC) using a Waters 2690 apparatus with two columns in series (Waters Styragel HR 4E and 5E) and polystyrene as standards. The SEC system was equipped with a mini-DAWN multiangle light scattering detector. DMF was used as the eluent at a flow rate of 1.0 mL/min and a temperature of 35 °C. SEC data were analyzed using Astra 4.50 software from Wyatt Technology. Excitation spectra and emission spectra were obtained on a Perkin-Elmer LS 55 luminescence spectrometer with a slit width of 5 nm using a 10-mm-path quartz cell under xenon discharge lamp excitation.

2.3 Synthesis of 1-thio-β-D-glucose (β-GlcSH)

*2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (**2**)*

Pentaacetyl-β-D-glucopyranose **1** (10.0 g, 25.6 mmol, 1 equiv) was added in to a 250 mL flask and dissolved in dichloromethane (75 mL). The solution was bubbled with argon for 5 min and sealed. Then, hydrogen bromide (40% in acetic acid, 60 mL) was injected, and the reaction mixture was stirred at room temperature for 2.5 h. After the reaction finished, the reaction mixture was partitioned between dichloromethane (200 mL) and water (200 mL), and the aqueous layer was re-extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with saturated sodium hydrogen carbonate aqueous solution, washed with brine (400 mL), dried over magnesium sulfate, filtered and evaporated in vacuum. Crystallization from ethyl acetate/petroleum ether afforded 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (8.80 g, 84%) as a white crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04, 2.06, 2.10, 2.11 (4s, 12H, COCH_3), 4.10–4.19 (m, 1H, H-6'), 4.26–4.36 (m, 2H, H-5/H-6), 4.81–4.87 (dd, 1H, H-2), 5.13–5.20 (t, 1H, H-4), 5.53–5.60 (t, 1H, H-3), 6.58–6.64 (d, 1H, H-1) ppm.

*2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-1-isothiouroniu m) bromide (**3**)*

Thiourea (2.4 g, 32.1 mmol, 1.5 equiv) and compound **2** (8.80 g, 21.4 mmol, 1 equiv) were dissolved in acetone (100 mL) under argon. The reaction was preformed at 60 °C under stirring. After 30 min, a white solid appeared, and was collected by filtration and the filtrate returned to reflux. This process was repeated until the solid ceased to precipitate. The combined precipitates were re-crystallized from acetone/petroleum ether to afford 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-1-isothiouronium bromide (4.91 g, 47 %) as a white crystalline solid. ^{1}H NMR (300 MHz, d_6-DMSO): δ 1.98, 2.00, 2.02, 2.06 (4s, 12H, COCH_3), 4.06–4.12 (m, 1H, H-6'), 4.17–4.24 (m, 2H, H-5/H-6), 5.08–5.14 (m, 2H, H-2/H-4), 5.25–5.38 (m, 1H, H-3), 5.66–5.71 (m, 1H, H-1), 9.17 (s, 4H, NH_2) ppm.

*2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranoside (**4**)*

Sodium metabisulphite (2.85 g, 15.0 mmol, 1.5 equiv) and compound 3 (4.90 g, 10.0 mmol, 1 equiv) were dissolved in dichloromethane (45 mL) and water (25 mL). The reaction mixture was heated to reflux under argon for 4 h. Then it was cooled to room temperature, and the phases separated. The aqueous layer was re-extracted with dichloromethane (3 × 60 mL), dried over magnesium sulfate, filtered and concentrated in vacuum to afford 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (3.73 g, 100%) as a white crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01, 2.03, 2.08, 2.10 (4s, 12H, COCH_3), 2.20–2.40 (d, 1H, SH), 3.70–3.76 (ddd, 1H, H-5), 4.09–4.20 (dd, 1H, H-6'), 4.24–4.4.28 (dd, 1H, H-6), 4.52–4.59 (t, 1H, H-1), 4.94–5.01 (t, 1H, H-2), 5.07–5.14 (t, 1H, H-4), 5.16–5.23 (t, 1H, H-3) ppm.

*1-Thio-β-D-glucose (β-GlcSH) (**5**)*

Sodium methoxide (50% in methanol, 880 mg, 8.2 mmol, 1.5 equiv) was added into a solution of 2,3,4,6-tetra-*O*-acetyl-

1-thio-β-D-glucopyranoside (2.0 g, 5.4 mmol, 1 equiv) in methanol (25 mL). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was acidified with 7120H cation exchange resin, filtered and concentrated in vacuum to afford 1-thio-β-D-glucose (β-GlcSH) (1.10 g, 96 %) as a pale yellow oil. ^{1}H NMR (300 MHz, D_2O): δ 3.20 (t, 1H, H-4), 3.40 (m, 3H, H-2/H-3/H-5) 3.64 (dd, 1H, H-6'), 3.82 (d, 1H, H-6), 4.50 (d, 1H, H-1) ppm; MS: calcd. for $C_6H_{12}O_9S$ ($M-H^+$) 195.03, found 195.04. The final product contains ~10% 1-thio-α-D-glucose (α-GlcSH) based on ^{1}H NMR results.

2.4 One-pot synthesis of HPAA with a sugar shell

MBA (2.467 g, 16.0 mmol) was added into a solution of AEPZ (1.038 g, 8.0 mmol) in a 24 mL solvent mixture (methanol/water = 70/30, *v*/*v*). After fully mixing, the reaction mixture was stirred at 50 °C until 25% of the total vinyl groups were left (monitored by ^{1}H NMR spectroscopy, a portion of the reaction solution was taken out and concentrated under a reduced pressure, followed by precipitating into cold acetone and drying in vacuum for measure). Then, predetermined β-GlcSH (The molar ratio to double bonds is 2.0) and TEA were added to the reaction mixture, and some of the solution was transferred to the NMR tube. The spectra were taken at different time intervals for the kinetic study. After 100% conversion of the double bonds (120 min), the reaction solution was concentrated under a reduced pressure, followed by precipitating into cold acetone and drying in vacuum.

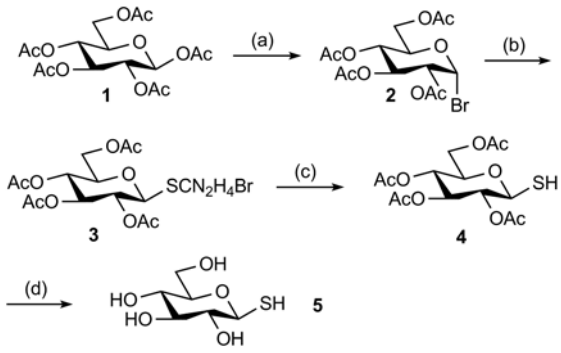
2.5 Effect of feed ratios on thiol click reactions

To further investigate the effect of β-GlcSH concentration, the thiol click reactions of HPAA-vinyl with different amounts of β-GlcSH (The molar ratios to double bonds are 1.0 and 1.4) were performed at room temperature for different reaction time. Typically, a predetermined amount of AEPZ and MBA reaction solution (0.25 mmol double bonds, 1.0 equiv) was added into a vial. β-GlcSH (49.0 mg, 0.25 mmol, 1.0 equiv) and 50 μL TEA were added under argon, and the reaction was performed at room temperature for 5 or 24 h. The final polymer was obtained via precipitating in cool acetone and drying under vacuum at room temperature.

3 Results and discussion

3.1 Synthesis of 1-thio-β-D-glucose

β-GlcSH could be easily synthesized following standard coupling methods [18, 19] at the anomeric center (Scheme 1). First, compound **1** was treated with HBr to afford the glycosyl halides (compound **2**). Then **2** was converted into **5** through treatment with thiourea to afford the isothiouronium



Scheme 1 Synthesis of 1-thio-β-D-glucose. Reaction conditions: (a) HBr (40% in acetic acid), dichloromethane (84%); (b) thiourea, acetone, 60 °C (47%); (c) $Na_2S_2O_5$, dichloromethane/water (100%); (d) MeONa, MeOH (96%).

salt, followed by mild hydrolysis with sodium metabisulphite and Zémplen deacetylation. All the compounds were confirmed by ^{1}H NMR.

3.2 Synthesis of HPAA with vinyl terminals (HPAA-vinyl)

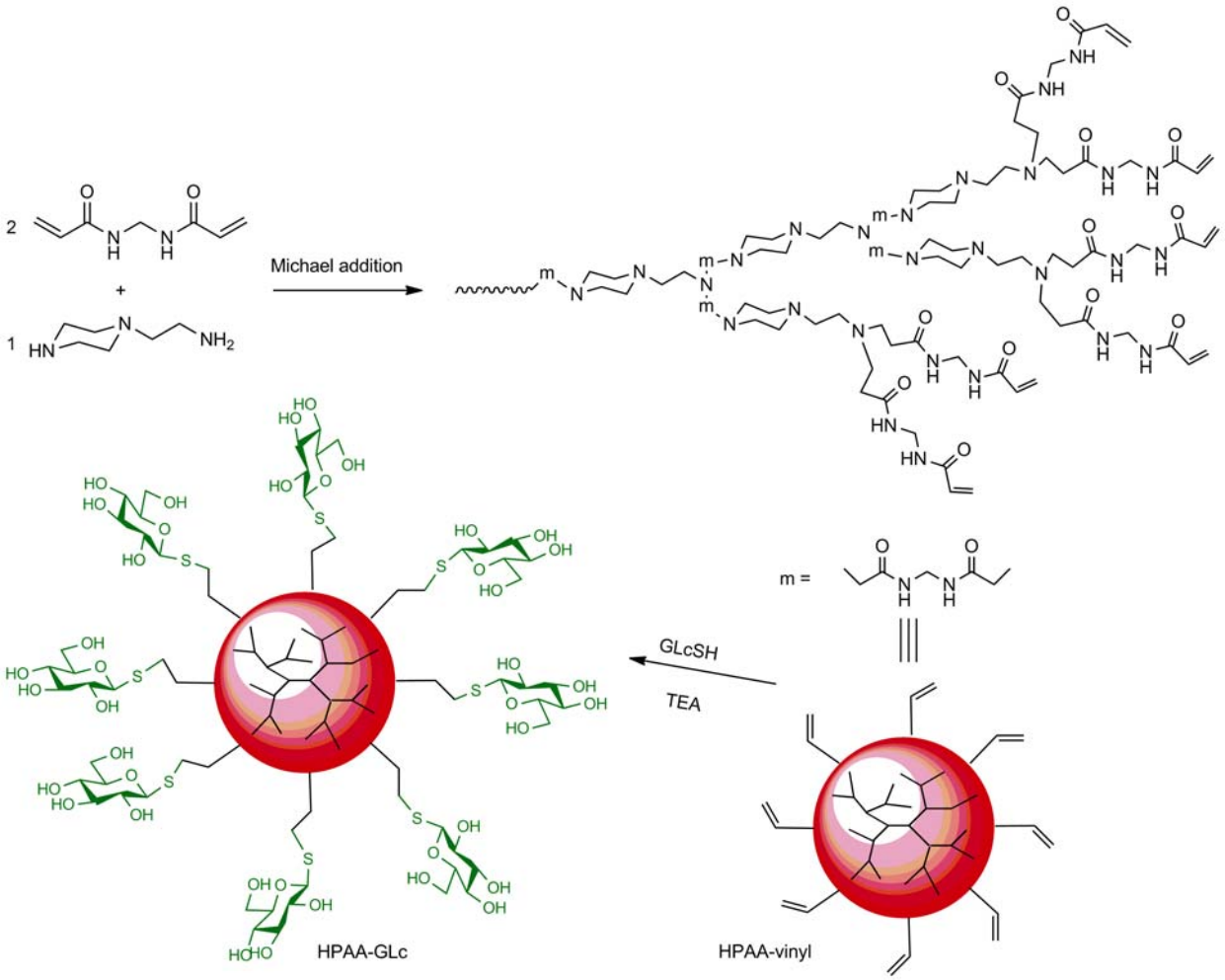
HPAA-vinyl was synthesized using Michael addition polymerization of MBA with AEPZ at a molar ratio of 2/1 (Scheme 2). Based on the Michael addition reaction mechanism, the resulting HPAA contains the residual 25 mol% vinyl groups after polymerization, which was confirmed by ^{1}H NMR spectroscopy of the reaction solution after 96 h reaction as shown in Figure 1(a). This residual vinyl groups value is 24% calculated from eq. (1) according to the ^{1}H NMR spectrum.

$$\text{Residual vinyl groups value}\ (\%) = I_{5.48-6.49}/3I_{4.26-4.49} \times 100\% \quad (1)$$

where $I_{5.48-6.49}$ and $I_{4.26-4.49}$ are integral values of the signals at 5.48–6.49 ppm attributed to vinyl protons and those at 4.26–4.49 ppm attributed to methylene protons from MBA in Figure 1(a), respectively. The resulting poly(amido amine) with a hyperbranched structure was confirmed by the appearance of peaks at 50–51 ppm in its ^{13}C NMR as shown in Figure 1(b). However, it is difficult to obtain the degree of branching from ^{1}H NMR and ^{13}C NMR, which is similar to the previous findings [20–22].

3.3 Synthesis of HPAA with a sugar shell via thiol-ene click reactions

The vinyl ends of HPAA are activated species which could easily react with β-GlcSH via thiol-ene click reaction under base-catalysis. Considering that thiol-ene click reactions could be performed in a wide range of solvents, here we synthesized HPAA with a sugar shell by hydrothiolation of vinyl groups *in situ* using one-pot method as shown in



Scheme 2 Synthetic strategy for preparation of HPAA with a sugar shell.

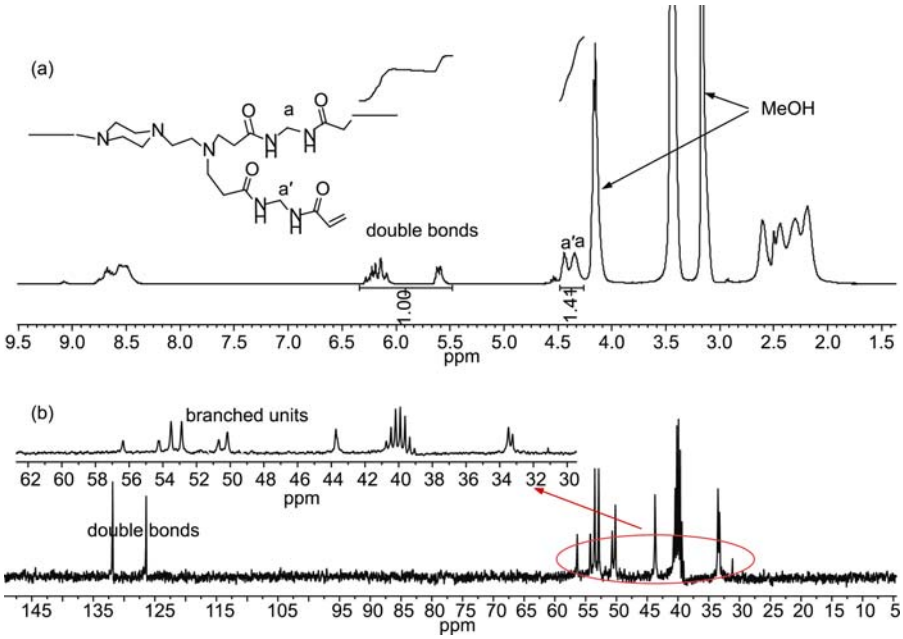


Figure 1 (a) ^{1}H NMR spectrum of 1-(2-aminoethyl) piperazine (AEPZ) and *N,N′*-methylene bisacrylamide (MBA) reaction solution in d_6-DMSO. (b) ^{13}C NMR spectrum of hyperbranched poly(amido amine) with vinyl terminals in d_6-DMSO.

Scheme 2. For better understanding of the kinetics of thiol-ene click reaction at room temperature, HPAA reacting with β-GlcSH was monitored by ^{1}H NMR. In its ^{1}H NMR, the integral value of vinyl double bond peaks decreases with the increase of reaction time as shown in Figure 2. The conversion of the vinyl was calculated from eq. (2) according to the ^{1}H NMR spectrum.

$$\text{Conversion}(\%) = \left(0.25 - I_{5.48-6.49}/3I_{4.30-4.50}\right)/0.25 \times 100\% \quad (2)$$

where $I_{5.48-6.49}$ and $I_{4.30-4.50}$ are integral values of the signals at 5.48–6.49 ppm attributed to vinyl protons and those at 4.30–4.50 ppm attributed to methylene protons from MBA. A conversion of 50% was achieved in 30 min and a complete conversion was achieved within 120 min.

HPAA with a sugar shell was obtained via precipitating from cool acetone. The M_n of HPAA with vinyl terminals is 19600, and the M_n of HPAA with a sugar shell is 28800, further confirming the success of the thiol-click reaction.

3.4 Effect of feed ratios on thiol click reactions

Post-functionalization of preformed polymers using sugar-containing reagents often results in incomplete conversion of the post-functional groups due to steric hindrance. Our research results show that quantitative conversion of the vinyl could be achieved within 2 h with the molar ratio of β-GlcSH to vinyl being 2.0. To further investigate the effect of β-GlcSH concentration on thiol click reactions, several control reactions were performed. The reaction conditions and results are shown in Table 1. A 78% conversion was obtained in 5 h at the feed molar ratio of 1.0, which indicates that the β-GlcSH concentration has a significant effect on thiol click reactions. Only 85% conversion was achieved even when the reaction was performed for 24 h. The low conversion may be attributed to three factors: the high steric hindrance of β-GlcSH, insufficient reaction time and some of the β-GlcSH oxidized to dimers. However, by increasing the feed ratio of β-GlcSH to vinyl to 1.4, complete conversion was achieved in 5 h.

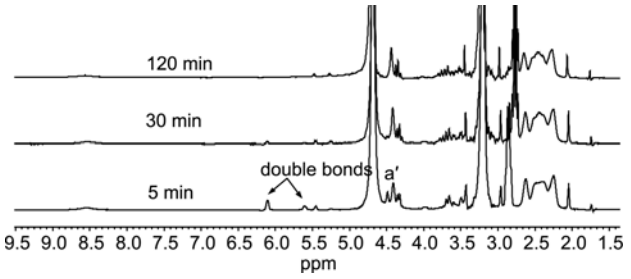


Figure 2 ^{1}H NMR spectra of thiol-ene click reaction at different time.

Table 1 Reaction conditions and results of thiol click reaction between HPAA-vinyl and β-GlcSH

Sample	Feed molar ratio β-GlcSH: vinyl	Reaction time (h)	Conversion of vinyl (%) [a]
HPAA-GLc1	1.0	5	78
HPAA-GLc2	1.0	24	85
HPAA-GLc3	1.4	5	100

a) Calculated according to eq. (2).

3.5 Photoluminescence properties of HPAA-GLc

Figure 3 shows the excitation and emission fluorescence spectra of HPAA-vinyl, HPAA-GLc1, HPAA-GLc2 and HPAA-GLc3 recorded at room temperature. The preformed polymer HPAA-vinyl exhibited very weak photoluminescence, but after clicked with β-GlcSH, HPAA-GLc1, HPAA-GLc2 and HPAA-GLc3 showed enhanced fluorescence emission with an emission band at 380 nm and an excitation band at 320 nm. This indicates that the β-GlcSH groups hydrothiolation to HPAA plays a significant role in the fluorescent properties. Those crowed terminal β-GlcSH groups may make the overall structure of HPAA much tighter and change the structure and the microenvironment of the fluorescence species, therefore enhancing the fluorescence emission. The fluorescence intensity was stronger with increasing conversion of vinyl, which further confirmed the above mechanism.

4 Conclusions

In summary, hyperbranched poly(amido amine) clicked with a sugar shell was synthesized via the thiol-ene click reaction between HPAA-vinyl and β-GlcSH in one pot. The highly efficient and simple reaction process is very suitable for large-scale synthesis of glycopolymers. The investigation of their potential applications in gene delivery and bioimaging is underway.

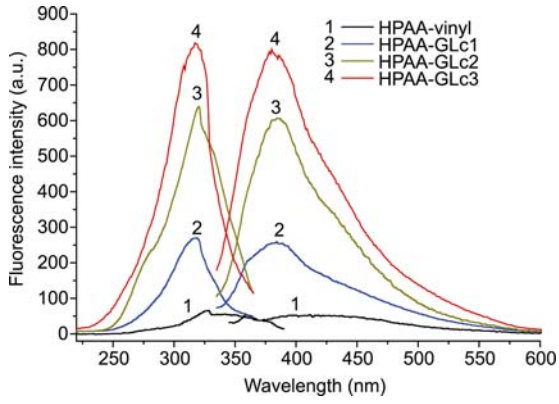


Figure 3 Fluorescence excitation and emission spectra of HPAA-vinyl, HPAA-GLc1, HPAA-GLc2 and HPAA-GLc3.

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