Synthesis of Well-Defined Glycoconjugate Polyacrylamides via Preactivated Polymers Prepared by ATRP

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Received 21 January 2004; accepted 28 January 2005
DOI 10.1002/app.22041
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Poly(*N*-acryloxysuccinimide) (polyNAS) with narrow molecular weight distributions (MWD) applicable for the preparation of well-defined glycoconjugate polyacrylamides were successfully prepared by atom transfer radical polymerization (ATRP). The structures of polyNAS were characterized by ^{1}H-NMR and GPC. GPC results showed that the molecular weight polydispersity indices (PDI) range from 1.17 to 1.29. The molecular weights could be calculated based on ^{1}H-NMR results but GPC results of polyNAS by using 0.01*M* LiBr/DMF did not give accurate molecular weights, probably because of the complex interaction in the system. The effects of free *N*-hydroxysuccimide produced in the polymerization processes on the free-radical concentrations and apparent initiation efficiencies of ATRP were discussed. Well-defined glycoconjugate polyacrylamides (i.e., with narrow molecular weight distributions and designed glycoconjugate degrees) were prepared by substituting *N*-oxysuccimide units with galactosamine followed by reaction of ethanolamine. The galactose conjugate degrees were determined by ^{1}H-NMR and the total substitutions of *N*-oxysuccimides were verified by ^{1}H-NMR and FTIR. J Appl Polym Sci 98: 189–194, 2005

Key words: water-soluble polymers; glycopolymers; polyacrylamides; atom transfer radical polymerization (ATRP); functionalization of polymers

INTRODUCTION

Cell-surface carbohydrates from glycoproteins, glycolipids, and proteoglycans play important roles in many biological phenomena such as cell growth, cancer, fertilization, immunological protection, virus infection, and viral recognition.[1,2] However, it is difficult to obtain natural glycoproteins, glycolipids, and proteoglycan usually having complicated structures. Diverse synthetic glycopolymers mimicking cell-surface carbohydrates have been synthesized for different applications as models for investigation of biological interactions, potent inhibitors, and targeted drug delivery reagents.[2–5]

Linear glycopolymers, distinguished from dendritic glycopolymers (glycodendrimers) and glycoclusters, comprised linear polymer backbones and oligosaccharide side groups. Various oligosaccharides were grafted to various polymer backbones such as polyacrylamide, polystyrene, polylysine, polyvinylene, and polynorbonene.[2,3] Among the linear glycopolymers, glycoconjugate polyacrylamides have been demonstrated to be promising inhibitors of pathogen–host interactions and are applicable in many other fields.[5–10] There are two approaches to prepare glycoconjugate polyacrylamides (i.e., by attaching oligosaccharides to preactivated polymers[5,9,10] or by polymerizing oligosaccharide conjugate acrylamide monomers).[6–9] The advantages of the first approach lie in the ease of controlling oligosaccharide conjugate distribution and degrees along the polymer backbones rendering glycopolymers the optimal properties.[3,5] In comparison, the different reactivity of monomers used in the second approach to adjust the conjugate degrees probably leads to an uneven distribution of oligosaccharide side groups or even homopolymers of different monomers.[5,10] Two kinds of polymers [i.e., poly(*p*-nitrophenyl acrylate)[5] and poly(*N*-acryloxysuccinimide)[9,10]], prepared by normal radical polymerization, have been used as preactivated polymers for the preparation of glycopolymers, but these polymers have wide molecular weight distributions with molecular weight dispersion indices higher than 2, and the glycopolymers obtained from these polymers also have wide molecular weight distributions.[5,9,10] However, glycopolymers with narrow molecular weight distributions are expected for different applications.

Recently, controlled radical polymerization has been demonstrated to be a robust tool to produce well-defined polymers from many kinds of monomers.[11–13] Therefore, we were inspired to apply controlled radical polymerization to syntheses of preacti-

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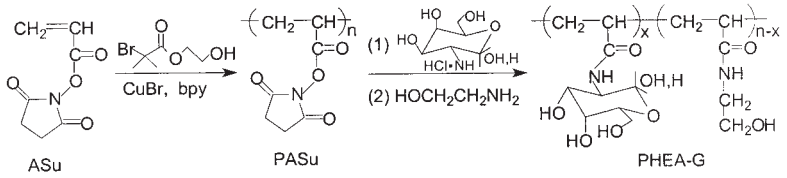
Contract grant sponsor: Program of the Chinese Academy of Science for Attracting Overseas Professionals.



Scheme 1

vated polymers for preparation of well-defined glycopolymers. First, atom transfer radical polymerization (ATRP) of *p*-nitrophenyl acrylate was investigated but the polymerization did not occur; then, ATRP of *p*-nitrophenyl methacrylate was found to give monomer conversions around 50% and produce homopolymers with polydispersity indices (PDI) around 2.0, although well-defined block copolymers, poly(styrene-*b*-*p*-nitrophenyl methacrylate), were obtained by starting from polystyrene macroinitiators.[14] The substitution of *p*-nitrophenyl in poly(*p*-nitrophenyl methacrylate) by galactosamine did not proceed in DMSO solution even at 90°C. In this article, we reported that ATRP of *N*-acryloxysuccinimide (NAS) provides preactivated polymers, poly(*N*-acryloxysuccimide) (polyNAS), with narrow molecular weight distribution, and well-defined glycoconjugated polyacrylamide with narrow molecular weight distributions and controllable glycoconjugate degrees were obtained by substituting *N*-oxysuccimide units using amino oligosaccharides as described in Scheme 1.

EXPERIMENTAL

Materials

Acryloyl chloride (Fluka, Steinheim, Switzerland) and *N*-hydroxysuccinimide (Acros, Geel, Belgium) were used as received. CuBr was purified by stirring it in acetic acid, washing with methanol, and drying *in vacuo* at 70°C. 2-Hydroxyethyl-2-bromoisobutyrate (HEBriB) was prepared according to the reported procedure.[15] 2,2′-Bipyridine (bpy) (Shanghai Chemical Reagent No. 1 Plant, Shanghai, China) and D-galactosamine hydrochloride (Fluka) were used as received. DMF was distilled from CaH_2 (calcium hydride) under reduced pressure and was subsequently stored over molecular sieves (4 Å). Triethylamine and *n*-butylamine were distilled over KOH after refluxing for 12 h.

General characterization

^{1}H-NMR studies were performed on a Bruker DRX-300 spectrometer. Gel permeation chromatography (GPC) was implemented on a Waters 2690 apparatus with a Waters 410 refractive index detector by using two columns in series (Waters Styragel HR 4E and 5E), 0.01*M* LiBr/DMF as eluant, and polystyrene standards. FTIR was obtained on a Bruker Equinox 55 spectrometer.

Preparation of NAS

Acryloyl chloride (4.9 mL, 0.06 mol) was added dropwise to a stirred solution of *N*-hydroxysuccinimide (5.75 g, 0.05 mol) and triethylamine (8.4 mL, 0.06 mol) in chloroform (80 mL) at 0°C. After stirring for 4 h at 0°C, the reaction mixture was washed with ice-cold saturated sodium bicarbonate solution four times and dried on $MgSO_4$ overnight. Then, the solution was filtered and chloroform was removed by evaporation in the presence of hydroquinone (6 mg). The obtained crude product was recrystallized twice from a solution of ethyl ether/hexane (1 : 1, v/v) to give white crystals in 73% yield.

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 6.15–6.74 (m, 3H, CH_2═CH), 2.87 (s, 4H, CH_2CH_2) and melting point (mp), 69–69.5°C.

ATRP of NAS

In a typical process, 2-hydroxyethyl-2-bromoisobutyrate, CuBr, and 2,2′-bipyridine (1 : 1 : 2 in mole) were mixed for 5 min in a glass tube. After an equimolar amount of NAS to HEBriB was added, the tube was degassed three times, sealed under vacuum, and finally placed in an oil bath thermostated at 80°C for a designed time. About 30 mg of the reaction mixture was dissolved in deuterium dimethyl sulfoxide (DMSO-d_6) for ^{1}H-NMR to determine monomer conversions based on:

$$\text{Monomer conversion}(\%) = 1.5 \times I_{2.07}/(1.5 \times I_{2.07} + I_{6.3\text{–}6.7}) \times 100\% \quad (1)$$

where $I_{2.07}$ and $I_{6.3\text{–}6.7}$ are the integral intensities of peaks corresponding to protons of methylene units in the main chains of polyNAS and in vinyl units of NAS, respectively.

The remained reaction mixture was dissolved in DMSO. The solution was precipitated in methanol three times followed by drying at 40°C under vacuum.

TABLE I
Results of ATRP Polymerization of *N*-acryloxysuccinimide in bulk at 80°C[a]

Entry	$[M]_0 : [I]_0$[b]	Time (h)	Conversion (%)	$M_{n(\text{theo})}$[c]	$M_{n(\text{NMR})}$	$M_{n(\text{GPC})}$[d]	M_w/M_n	f_{app}[e]
1	25 : 1	6	54	2490	5130	51,770	1.17	0.48
2	50 : 1	0.5	24	2240	8800	36,660	1.17	0.25
3	50 : 1	1.5	37	3340	7060	40,660	1.18	0.47
4	50 : 1	4.5	53	4690	10150	46,920	1.18	0.46
5	50 : 1	6	66	5790	9430	46,930	1.21	0.61
6	50 : 1	10	75	6550	7450	43,060	1.23	0.88
7	75 : 1	24	75	9720	15,120	42,740	1.29	0.64
8	100 : 1	24	77	13,220	14,290	46,910	1.22	0.92

[a] $[I]_0 : [\text{CuBr}]_0 : [\text{bpy}]_0 = 1 : 1 : 2$.
[b] Molar ratio of the initial monomer and initiator concentration.
[c] $M_{n(\text{theo})} = [M]_0/[I]_0 \times \text{conversion} \times 169 + 211$.
[d] $M_{n(\text{GPC})}$ was obtained by GPC by using 0.01*M* LiBr/DMF as eluant and polystyrene standards.
[e] Apparent initiator efficiency calculated by $f_{\text{app}} = M_{n(\text{theo})}/M_{n(\text{NMR})}$.

Substitution of polyNAS by *n*-butyl amine

Typically 0.15 g of polyNAS was dissolved in 2.0 mL of anhydrous DMF. Then, *n*-butylamine (BA) (BA : *N*-oxysuccinimide = 10 : 1 in mole) was added and the reaction mixture was stirred for 24 h under nitrogen atmosphere. Poly(*n*-butyl acrylamide) (polyBAD) was obtained by precipitating the reaction mixture in acetone/ethyl ether (1 : 6, v/v), washing thoroughly with ethyl ether, and drying under vacuum at 40°C for 24 h.

Preparation of glycoconjugate polyacrylamide

In a typical process, 160 mg of polyNAS (containing 0.91 mmol *N*-oxysuccinimide) and 40 mg of galactosamine hydrochloride (galactosamine/*N*-oxysuccinimide = 0.2 in mole) were mixed in 2.4 mL anhydrous DMF with 52 μL triethylamine (triethylamine/galactosamine = 2 : 1 in mole). The solution was stirred at 60°C for 6 h. Then, 90 μL of ethanolamine (ethanolamine/*N*-oxysuccinimide = 1.6 : 1 in mole) was added. After 6 h, the solution was precipitated in a mixture solution of diethyl ether/acetone (1 : 1, v/v), followed by drying in vacuum at 60°C.

RESULTS AND DISCUSSION

Preparation of polyNAS by ATRP

ATRP of NAS was performed in bulk. The reaction temperature was set at 80°C higher than the melting point of NAS (69°C). After performing the polymerization for a designed time, polymer was dissolved in DMSO followed by precipitation in methanol, producing a white powder. The results are listed in Table I. Table I shows that the monomer conversion rate is mainly determined by the reaction time and not by the initiator amount, as reflected by comparing entries 1 to 5 and 7 to 8. When the reaction time was long enough, the conversion rate reached 77% (entry 8).

The relationship between $\ln([M]_0/[M]_t)$ and reaction time (t) is shown in Figure 1. An apparent upward curvature appeared at the initial stage of polymerization, indicating that the polymerization system has a high and inconstant free-radical concentration. In addition, the color of reaction mixture changed gradually from brown (Cu^I species) to green (Cu^{II} species) during the polymerization process, indicating the accumulation of Cu^{II} species. So, it can be expected that the reversible equilibrium between growing free radicals and dormant species shifts to growing free radicals, therefore, leading to a high free radical concentration. Similar phenomena have been observed in the ATRP performed in high polar media presumably caused by the formation of coordinate complexes between copper-based catalyst, especially with Cu(II), and the polar medium [e.g., water, and/or the change from Cu(I) to Cu and Cu(II)].[16,17] In our work, NMR showed that some amount of *N*-hydroxysuccimide coexisted with the formed polymers even though NAS monomer purity was verified to be high. Probably

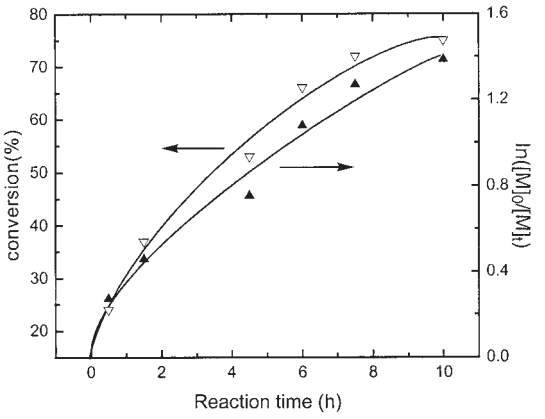


Figure 1 Relationship between $\ln([M]_0/[M]_t)$ and reaction time (t) for ATRP of NAS performed in bulk at 80°C.

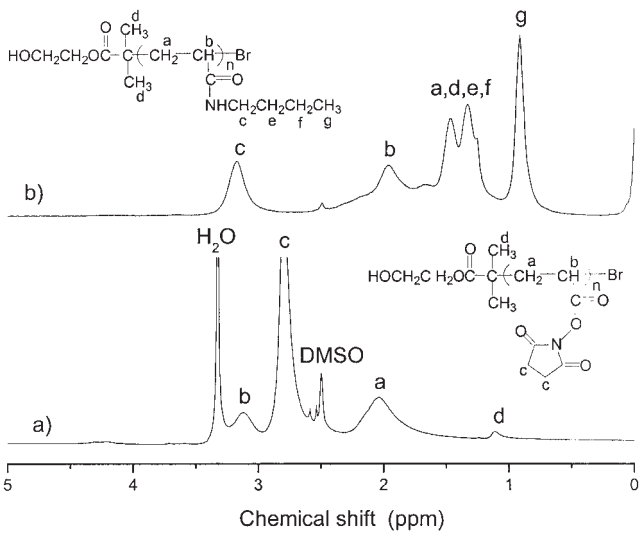


Figure 2 Typical ^{1}H-NMR spectrum of (a) polyNAS (entry 7 in Table I) in $CDCl_3$ and (b) polyBAD (obtained from entry 7 in Table I) in DMSO-d_6.

some *N*-hydroxysuccimide groups were unavoidably formed in the polymerization process. *N*-hydroxysuccimide should form a coordination complex with copper-based catalyst, especially Cu(II), leading to green color and higher free-radical concentrations.

PolyNAS dissolves in DMSO and DMF but is insoluble in THF, $CHCl_3$, and acetone. So, the molecular weights of the obtained polyNAS were measured using DMF as eluant for gel permeation chromatography (GPC) based on polystyrene standards. In pure DMF, significant aggregation of polyNAS occurred as indicated by an early appearance of the elution peaks, so LiBr was added. For 0.01 and 0.05*M* LiBr/DMF solutions, the elution peaks of the same polyNAS samples appeared at the same positions later than those using pure DMF when other conditions were kept constant; therefore, it can be assumed that the aggregations were prevented. However, the elution peaks of polystyrene standards were retarded significantly and the peak consequence showed no correspondence to the standard molecular weights when 0.05 LiBr/DMF eluant was used. However, the elution behavior was reasonable when 0.01*M* LiBr/DMF was adopted. Therefore, 0.01*M* LiBr/DMF was chosen as eluant. The phenomena probably reflect the existence of complex aggregation of polymers and interaction of polymers and columns. The totally different hydrodynamic volumes between polyNAS and polystyrene standards should be responsible for the unreasonable molecular weights of polyNAS obtained by GPC based on polystyrene standards. However, ^{1}H-NMR was a more reliable tool to give more accurate molecular weights of polyNAS. Figure 2(a) is a typical ^{1}H-NMR spectrum of polyNAS (entry 7). The molecular weights of polyNAS can be calculated based on

$$M_{n(\mathrm{NMR})} = 3 \times I_{2.07}/I_{1.13} \times 169 + 211 \quad (2)$$

where $I_{2.07}$ and $I_{1.13}$ stand for the integral intensity of peaks of protons attached to methylene units in the polymer backbones and methyl units in the end 2-hydroxyethyl-2-isobutyrate groups. The obtained values are listed in Table I. Further, polyBAD having good solubility in THF was produced by substituting *N*-oxysuccimide groups by *n*-butylamine as verified by ^{1}H-NMR. Figure 2(b) is a typical ^{1}H-NMR spectrum of polyBAD (obtained from entry 7 in Table I). Then, the molecular weight of polyBAD was evaluated by using THF as eluant. A typical GPC profile of polyBAD is shown in Figure 3. The molecular weight of polyBAD is around 16,810. Probably less polymer aggregation and interaction with columns exist in GPC systems using THF as eluant.

Furthermore, Table I shows that $M_{n(\mathrm{NMR})}$ of polyNAS is higher than $M_{n(\mathrm{theo})}$ calculated based on the assumption that all the initiator participated in the initiation of polymerization. The apparent initiation efficiencies of initiators (f_{app}) defined by eq. (3) are low

$$f_{\mathrm{app}} = M_{n(\mathrm{theo})}/M_{n(\mathrm{NMR})} \quad (3)$$

where $M_{n(\mathrm{NMR})}$ is calculated by eq. (2) and $M_{n(\mathrm{theo})}$ by

$$M_{n(\mathrm{theo})} = [M]_0/[I]_0 \times \mathrm{conversion} \times 169 + 211 \quad (4)$$

where $[M]_0/[I]_0$ presents the molar ratio of the feed monomer and initiator, and the conversion is calculated by eq. (1). Also, it indicated that f_{app} increased when the reaction time was extended.

In ATRP of styrene and methacrylate, usually the apparent initiation efficiencies are close to one.[18,19] However, apparent initiation efficiencies have been reported to be lower in ATRP performed in highly polar media such as aqueous solutions,[16,17] and ATRP of methyacryloxysuccimide (MNAS) with a structure similar to NAS performed in DMSO also had low apparent initiation efficiencies.[20] Probably the com-

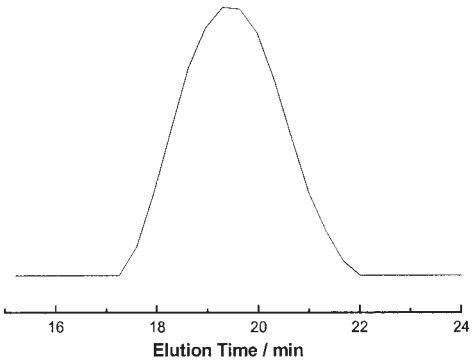


Figure 3 Typical GPC profile of polyBAD (obtained from entry 7 in Table I) in THF.

plex formation of copper-based catalyst with free *N*-hydroxysuccimide as discussed above retarded the polymerization; therefore, the apparent initiation efficiencies are low for these short-time polymerizations and increased when the reaction time was long enough.

However, as shown in Table I, bulk ATPR polymerizations of NAS produced polyNAS with narrow molecular weight distributions reflected by PDI ranging from 1.17 to 1.29, taking in account the peak broadening effects in GPC by using DMF as eluant. A longer reaction time led to a higher PDI, probably due to the retardation on polymerization for the formation of complexes of *N*-hydroxysuccimide with copper-based catalyst. The PDI of polyBAD obtained from entry 7 in Table I is around 1.26 obtained by GPC performed using THF as eluant, which also verifies the narrow molecular weight distribution of polyNAS. Therefore, ATRP of NAS in bulk can provide preactivated polyNAS with narrow molecular weight distributions for the preparation of well-defined glycoconjugate polyacrylamides.

Preparation of well-defined glycoconjugate polyacrylamides

The ease of substituting *N*-oxysucccimide units by amino-containing compounds has been well demonstrated.[9,10] We used commercially available galactosamine as a model compound to prepare well-defined glycoconjugate polyacrylamides (i.e., of controllable glycoconjugate degrees and narrow molecular weight distributions). Glycoconjugation was performed by substituting *N*-oxysuccimide units with a predetermined amount of galactosamine in DMF at

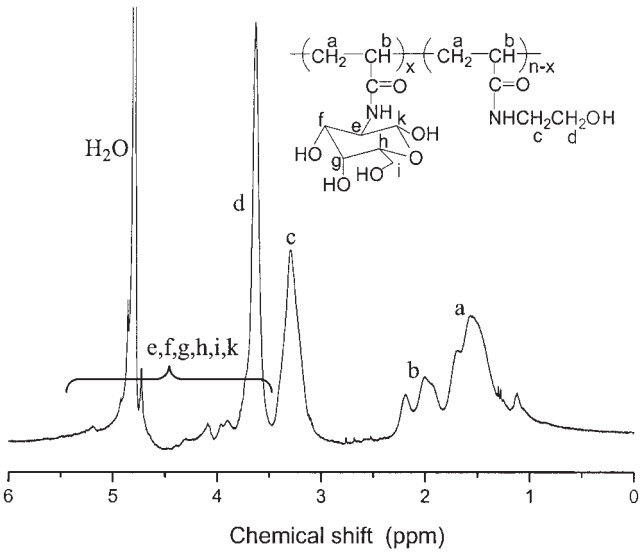


Figure 4 ^{1}H-NMR spectrum of galactose conjugate polyacrylamide in D_2O.

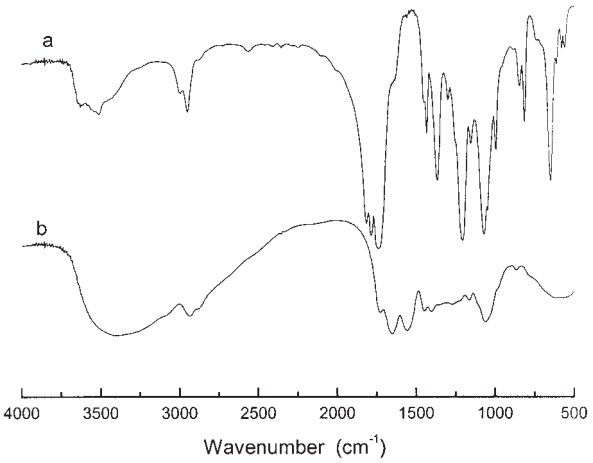


Figure 5 Comparison of FTIR spectrum of (a) polyNAS and (b) galactose conjugate polyacrylamide in KBr crystal piece.

60°C. Then, the remaining *N*-oxysuccimide units were substituted by ethanolamine. Two glycoconjugate polyacrylamides with the designed galactose conjugate degrees of 20 and 60% were prepared, respectively. Figure 4 is the ^{1}H-NMR spectrum of glycoconjugate polyacrylamide with 20% galactose conjugate degree. The total disappearance of the characteristic peak of *N*-oxysuccimide groups at around 2.80 ppm verifies the complete substitution by galactosamine and ethanolamine, which is further confirmed by FTIR results. As shown in Figure 5, the characteristic bands of ester carbonyl and two cyclic carbonyl groups at 1730–1822 cm^{-1} disappeared, and the absorption peaks of amide groups at around 1558–1651 cm^{-1} appeared. The glycoconjugate degrees, galactose %, could be calculated on the basis of ^{1}H-NMR results by

$$\text{Galactose \%} = (1 - 1.5 \times I_{3.3}/I_{1.3\text{–}2.3}) \times 100\% \quad (5)$$

where $I_{3.3}$ and $I_{1.3\text{–}2.3}$ are the integral intensities of peaks of protons attached to methylene in ethanolamine and polymer backbone, respectively. For the two batches, the galactose % determined is around 22 and 59%, respectively, agreeing well with the target values confirming that galactosamine can be quantitatively attached to preactivated polyNAS.

CONCLUSIONS

Preactivated polyNAS with narrow molecular weight distributions have been prepared by ATRP. However, ATRP initiators showed low apparent initiation efficiency; therefore, the molecular weights of polyacryloxysuccimides obtained are higher than the designed values, especially when the reaction times were not long. The low apparent initiation efficiencies of initiators probably result from the retardation effects of

free *N*-hydroxysuccimides formed unavoidably in the processes. The retardation effects assumably are caused by the formation of complexes between *N*-hydroxysuccimide and copper-based catalyst, which also lead to higher and changing free-radical concentrations in ATRP as indicated by the nonlinear relationship between $\ln([M]_0/[M]_t) \sim t$ (reaction time). Well-defined glycoconjugate polyacrylamides (i.e., with narrow molecular weight distributions and designed glycoconjugate degrees) were prepared by substituting *N*-oxysuccimide units by predetermined amounts of galactosamine followed by using ethanolamine to remove remained *N*-oxysuccimide units.

In addition, the obtained preactivated polyNAS also can be used for the preparation of other useful polymers with well-defined structures such as thermal sensitive poly(*N*-isopropyl acrylamide) widely investigated with many potential applications.

The financial support from the Program of the Chinese Academy of Science for Attracting Overseas Professionals is gratefully acknowledged.

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