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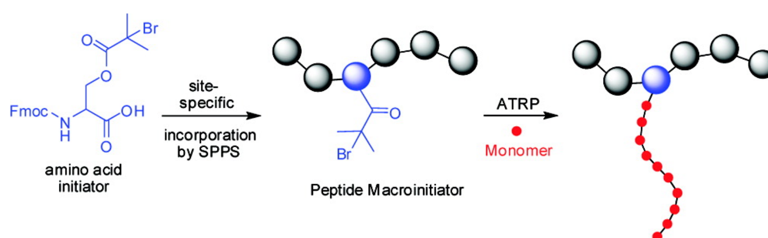

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Designed Amino Acid ATRP Initiators for the Synthesis of Biohybrid Materials

Rebecca M. Broyer, Grace M. Quaker, and Heather D. Maynard*

Department of Chemistry and Biochemistry and the California NanoSystems Institute, University of California, 607 Charles E. Young Drive East, Los Angeles, California 90096-1569

Received September 19, 2007; E-mail: maynard@chem.ucla.edu

Abstract: A synthetic strategy to prepare peptide−polymer conjugates with precise sites of attachment is described. Amino acids modified with atom transfer radical polymerization (ATRP) initiators for the polymerization of styrenes and methacrylates were prepared. Fmoc-4-(1-chloroethyl)-phenylalanine (**5**) was synthesized in four steps from Fmoc-tyrosine. HATU-mediated amidation with glycine-OMe resulted in dipeptide (**6**). The initiator was effective for Cu(I)/bipyridine mediated bulk polymerization of styrene. Kinetic studies indicated a controlled polymerization, with high conversion (97%), and a polydispersity index (PDI) of 1.25. Fmoc-*O*-(2-bromoisobutyryl)-serine *tert*-butyl ester (**10**) was synthesized from Fmoc-Ser(OTrt)-OH in three steps. This initiator was employed in the ATRP of 2-hydroxyethyl methacrylate (HEMA), and kinetic studies indicated a controlled polymerization. Different monomer to initiator ratios resulted in poly-(HEMA) of different molecular weights and narrow PDIs (1.14−1.25). Conversions were between 70 and 99%. HEMA modified with *N*-acetyl-D-glucosamine (GlcNAc) was also polymerized to 84% conversion and the resulting PDI was 1.19. The *t*-butyl ester protecting group of **10** was removed, and the resulting amino acid (**11**) was incorporated into VM(**11**)VVQTK by standard solid-phase peptide synthesis. Polymerization resulted in the glycopolymer−peptide conjugate in 93% conversion and a PDI of 1.14.

Introduction

Polymer conjugates with peptides and proteins have been widely used for applications in the areas of medicine, biotechnology, and materials science. These biohybrids make up an important class of polymer therapeutics.[1,2] In this context, covalent attachment of synthetic polymers to proteins or peptides offers a number of advantages. For example, the larger size facilitates longevity by reducing metabolic elimination, and the polymer reduces immunogenicity. Another important group of bioconjugates are "smart" polymer conjugates that respond to external stimuli such as changes in temperature or exposure to light.[3,4] Typically, upon stimulation, these "smart" polymers undergo a hydrophobic collapse and phase separation, transferring this property to the attached biomolecule. This technology has been used for enzyme switching, microfluidics, and affinity separations. Polymer conjugates can undergo self-assembly in aqueous solution driven by the hydrophobicity of the polymer chain; a property that is useful in the design of bioactive nanomaterials.[5] "Giant amphiphiles" consisting of a protein or enzyme polar head group and a nonpolar polystyrene tail have been shown to form bioactive nanostructures.[6,7] Further, conjugates can self-organize, driven by the interaction of the peptide or protein moieties.[5,8,9] These types of systems are especially useful to form hydrogels for drug delivery and tissue engineering.

The broad range of successful applications has increased the demand for efficient methods to prepare synthetic biological hybrids. Controlled radical polymerizations (CRPs), such as atom transfer radical polymerization (ATRP),[10,11] reversible addition-fragmentation chain transfer (RAFT) polymerization,[12−14] and nitroxide mediated radical polymerization (NMRP),[15] have emerged as powerful tools to make peptide and protein conjugates. Important for resultant conjugate properties is that (1) the binding site be defined on the protein and (2) the polymer chain exhibit a narrow polydispersity.[16] This has been achieved using CRPs by three main approaches.[17,18] First, polymers have been synthesized from functionalized initiators or chain-transfer agents with narrow molecular weight distributions and the direct

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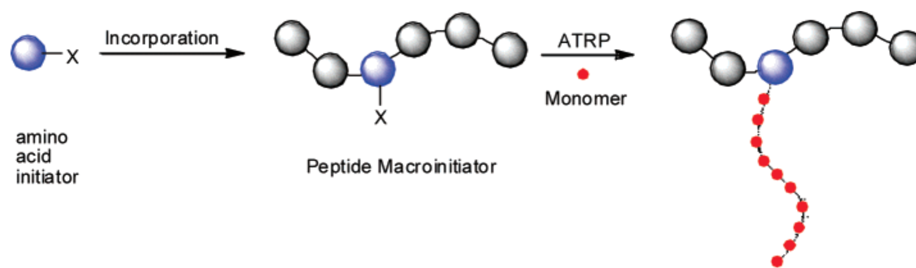
Scheme 1. Incorporation of Amino Acid Initiator for Precise Biohybrid Synthesis[a]



[a] Polymer modification occurs at the desired residue only, without having to rely on that amino acid being distinct within the sequence.

ability to bind to specific amino acid side chains.[19] Second, polymers have been prepared by polymerizing from modified proteins and peptides.[20,21−27] The third route involves side-chain polymers that have been produced by polymerizing peptide monomers or by post-polymerization conjugations.[28] Herein, we report a method to prepare peptide−polymer conjugates whereby the polymer is grown from a predetermined amino acid side chain of a peptide.

There are several examples of peptide−polymer conjugates produced by polymerizing from the biomolecule. Becker, Liu, and Wooley demonstrated that this method was amenable to resin-bound peptides.[21] The N-terminus of the protein transduction domain (PTD) of the HIV-TAT protein was modified with a NMRP initiator. This resin-bound peptide initiator was successful in generating block copolymers. The same group later utilized this technique to prepare poly(acrylic acid)-*block*-polystyrene from the antimicrobial peptide tritrpticin using both NMRP and ATRP.[24] The resulting conjugates formed well-defined micelles and were shown to have enhanced antimicrobial activity compared to the free peptide. ATRP has also been employed for the polymerization of 2-hydroxyethyl methacrylate (HEMA) from a resin-supported 2-bromopropionyl-functionalized sequence of the protein fibronectin, GRGDS (Gly-Arg-Gly-Asp-Ser).[25] The bioconjugate exhibited cell adhesion properties, indicating that the peptide was still active. Using solid-phase peptide synthesis (SPPS), Börner and co-workers prepared oligopeptide GDGFD functionalized at the N-terminus with a 2-bromopropionate or a dithioester group for ATRP[22] and RAFT,[23] respectively. Cleavage of the macroinitiators from the solid support followed by controlled polymerization of *n*-butyl acrylate resulted in well-defined bioconjugates. Biesalski and co-workers demonstrated that cyclic peptides modified with initiators at the lysine residues self-assembled into peptide nanotubes, orienting the initiating groups toward the outer surface of the nanotube.[26,29] The ensembles were utilized to polymerize *N*-isopropylacrylamide. Van Hest synthesized ABA triblock copolymers with a peptide as the center block.[30] This was accomplished by selective removal of serine protecting groups on the resin bound peptide, followed by modification with 2-bromoisobutyric acid and polymerization in solution from the cleaved peptide. More recently, a valine based haloamide initiator was used to polymerize *tert*-butyl acrylate and chain extended with styrene in order to create a diblock copolymer with amino acid functionality.[27] All of these methods, while effective to prepare biohybrids, involve modifying an existing peptide either at the N or C terminus or at reactive side-chains with the appropriate initiating group.

We were interested developing an approach that would allow us to modify precisely one amino acid in a peptide or protein without having to rely on that amino acid occurring infrequently. For example, we desired a strategy that would enable us to modify one serine in a peptide or protein that contains many serines, threonines, and tyrosines. Such a strategy would allow us to construct precise bioconjugates for finely tailored applications. One of the many possible uses of this approach would be to synthesize peptides or proteins modified at a controlled number of sites with glycopolymers to form conjugates that better mimic glycosylation patterns found in nature.[31] We envisioned that by designing an unnatural amino acid containing an ATRP initiator at the side chain, incorporating it into a peptide, and polymerizing from the biomolecule, we would be able to achieve such specific modification (Scheme 1). To our knowledge this tactic has not yet been undertaken. We targeted two classes of initiators, one modified with a 1-chloroethyl-

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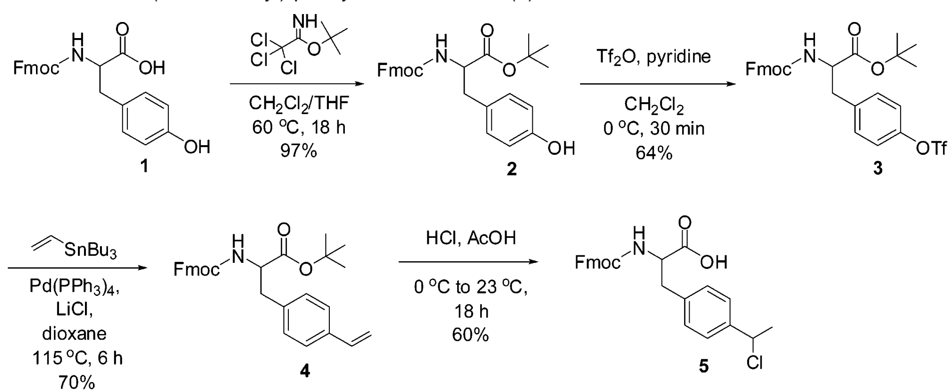
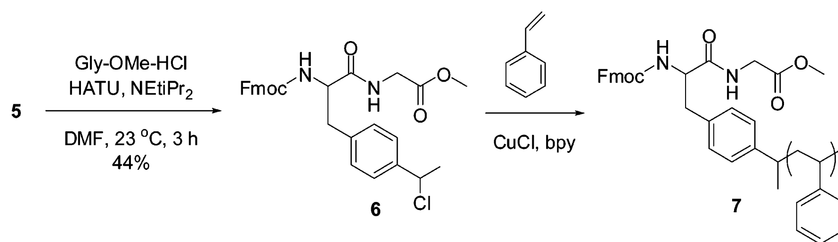
Scheme 2. Synthesis of *N*-Fmoc-4-(1-chloroethyl)-phenylalanine Initiator (**5**)



Scheme 3. Synthesis of Dipeptide and Polymerization of Styrene



phenyl group designed for the ATRP of styrenes and one modified with a 2-bromoisobutyrate group for the ATRP of methacrylates. In designing derivatives of phenylalanine and serine, respectively, we chose a 9-fluorenylmethoxycarbonyl (Fmoc) protection route that would conveniently allow for solid-phase peptide synthesis of the resulting amino acid initiators. Amino acid and peptide synthesis and polymerization of styrene, HEMA, and a β-linked *N*-acetyl-D-glucosamine (O-GlcNAc) modified monomer is reported. In addition, a single site modification of a peptide containing more than one amino acid with alcohol functionality is described.

Results and Discussion

Synthesis of Polystyrene-Peptide Conjugates. We started with a commercially available tyrosine to prepare an artificial amino acid containing the requisite phenylethyl halide moiety for the polymerization of styrene (Scheme 2). Chemical manipulation of Boc-protected tyrosine to 4-hydroxymethyl-L-phenylalanine via a key aryl triflate derivative had been reported previously by Morera et al.[32] We adopted a similar intermediate to prepare *N*-Fmoc-4-(1-chloroethyl)-phenylalanine (**5**). Commercially available Fmoc-L-tyrosine was protected as the *tert*-butyl ester.[33] Subsequent treatment with triflic anhydride[34] using 2.5 equiv of pyridine gave the triflate intermediate **3** in 64% yield. This compound was then subjected to Stille coupling conditions,[35] using tetrakis(triphenylphosphine) palladium(0) ($Pd(PPh_3)_4$) and dry lithium chloride to introduce a vinyl group on the aromatic ring (**4**) in 70% yield. Simultaneous chlorine installation and deprotection of the ester protecting group was achieved by treating **4** with HCl in acetic acid to give the 1-chloroethyl derivative **5** in 60% yield. The free acid end of **5** was coupled to glycine methyl ester hydrochloride to form dipeptide initiator **6** (Scheme 3). The coupling reaction was surveyed using a variety of conditions, and the most effective involved using excess *N*,*N*′-diisopropylethylamine (DIPEA) (3 equiv) along with 2-(1H-9 azobenzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate (HATU) as a coupling agent to give the amide product in 44% yield.[36] The origin of the relatively low yield of the coupling reaction was likely due to the reactivity of the chloroethyl-phenyl side chain. To increase the yields and to be able synthesize longer sequences, we envision direct incorporation of the free acid of vinyl precursor **4** during solid-phase synthesis followed by concurrent installation of the halogen and cleavage from the resin. Not only would such a strategy avoid side reactions, particularly during the Fmoc deprotection steps of the synthesis, it would produce the desired peptide macroinitiator in one fewer step.

We next investigated the ATRP of styrene utilizing the dipeptide as an initiator (Scheme 3). Styrene was polymerized in bulk at 110 °C using 100 equiv to initiator **6**, CuCl and 2,2′-bipyridine (bipy) as the catalyst (**6**:CuCl:bipy = 1:1:3). The resulting polymer was synthesized in 92% conversion, with a number-average molecular weight (M_n) of 10 100 Da and a polydispersity index (PDI) of 1.40 (Table 1). Maintaining a 110 °C reaction temperature, while reducing the equivalents of bipy from 3 to 2 resulted in a polymer with a narrower molecular

Table 1. Results of ATRP of Styrene

initiator	$[M]_o/[I]_o$	bipy (eq.)	T (°C)	conv (%)[a]	M_n (theory)	M_n[b]	PDI[b]
6	100:1	3	110	92	9600	10 100	1.40
6	100:1	2	110	54	5600	11 800	1.30
6	100:1	3	130	97	10 100	12 600[c]	1.25
5	100:1	3	130	95	9900	13 300	1.27

[a] Determined from the ^1H NMR spectra. [b] Determined by GPC in THF against polystyrene standards prior to precipitation. [c] After purification by precipitation, the M_n of this polymer was 13 500 and the PDI 1.23.

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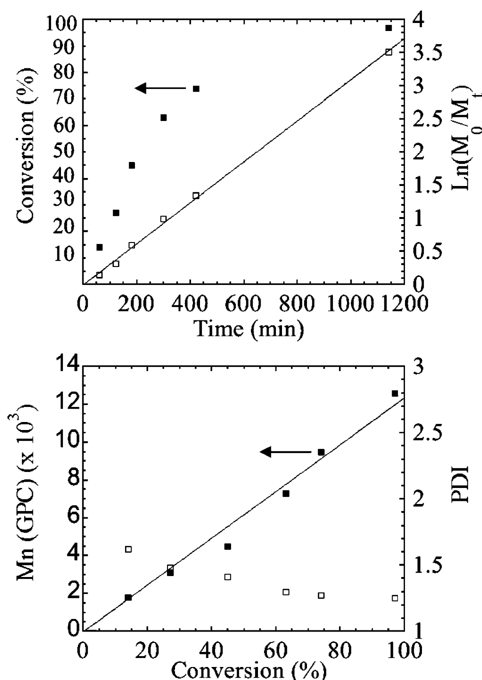
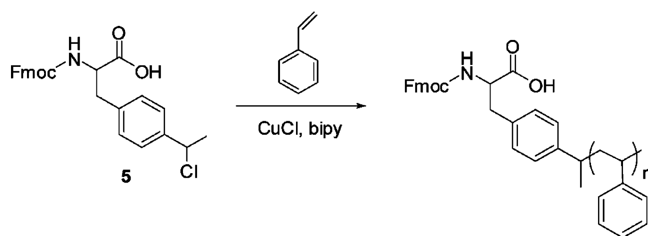


Figure 1. Kinetic plot (top) determined by ^{1}H NMR and plot of the evolution of M_n with conversion (bottom) determined by GPC in THF for the bulk polymerization of styrene **6**:[bipy]:[styrene] = 1:3:100 at 130 °C.

Scheme 4. Polymerization of Styrene from **5**



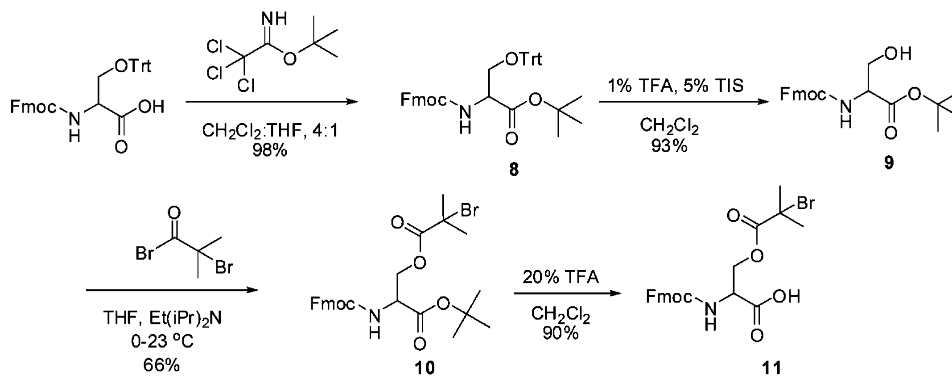
weight distribution (PDI = 1.30), although the conversion was only 54%. The dipeptide initiator **6** contained an amide group that may have competitively bound to the catalyst, requiring additional bipy to maintain enough copper-bound ligand to achieve high conversions. The reaction was then carried out at higher temperatures according to the original conditions used by Matyjaszewski.[37] Styrene was polymerized in bulk at 130 °C using 100 equiv to initiator **6** and CuCl and bipy as the catalyst (**6**:CuCl:bipy = 1:1:3). In this case, the reaction proceeded to high conversion (97%) and the polymer (**7**) had a M_n of 12 600 Da and a narrow PDI of 1.25 (Table 1). Aliquots removed periodically during the polymerization were analyzed by both ^{1}H NMR spectroscopy and GPC. The semilogarithmic kinetic plot was linear and the polymer M_n increased linearly with conversion (Figure 1). The PDI decreased throughout the reaction, similar to what had been observed for the ATRP of styrene utilizing unfunctionalized initiator, 1-phenylethyl chloride.[37] These results indicated a controlled polymerization from the dipeptide initiator.

To ensure that the biohybrid material had been synthesized, polymer **7** was purified by precipitation into methanol and subjected to analysis by ^{1}H NMR spectroscopy. The peaks corresponding to the peptide end group were clearly visible in the spectrum (see Supporting Information). In particular, peaks corresponding to the Fmoc hydrogens of the initiator (at 7.76, 7.55, and 7.38 ppm) were observed, providing good evidence that this moiety did not thermally deprotect during the polymerization. These peaks also enabled the estimation of molecular weight from the ^{1}H NMR spectrum. The result was 13 900 Da, which was in excellent agreement with the molecular weight observed by GPC after precipitation of the polymer (13 500 Da). This data additionally suggested that the peptide was conjugated to the chain.

In a study of various carboxylic acid-containing initiators, Matyjaszewski found that unprotected carboxylic acids had low efficiency in the ATRP of styrene.[38] Haddleton also found that addition of high concentrations of carboxylic acids to the ATRP of methyl methacrylate was deleterious to the polymerization.[39] However, Matyjaszewski also found that initiators with halide groups remote from the carboxylic acid moiety gave reasonable efficiencies for the polymerization of styrene.[38] Thus, although **5** contained a free acid, we decided to explore this initiator in the ATRP of styrene (Scheme 4) employing the optimized conditions found for initiator **6**. Styrene (100 equiv) was polymerized in bulk at 130 °C (**5**:CuCl:bipy = 1:1:3). The resulting polymer was synthesized with high conversion (95%), a M_n of 13 300 Da and a PDI of 1.27. This result indicated that **5** could effectively produce well-defined polystyrene, which suggested that the chloride was remote enough to avoid complications with the free acid moiety. Purity of the initiator was important as polymerizations that used impure samples showed remarkably increased polydispersities (data not shown).

These results demonstrated that dipeptide initiator **6** with a 1-chloroethyl group was highly effective for the polymerization of styrene. The ATRP amino acid was synthesized in a few steps. The peptide initiator was produced, not by the typical modification of the side chain, but rather by incorporation of the initiation moiety during synthesis. The polymerizations were

Scheme 5. Synthesis of the Fmoc-*O*-(2-bromoisobutyryl)-serine Initiator



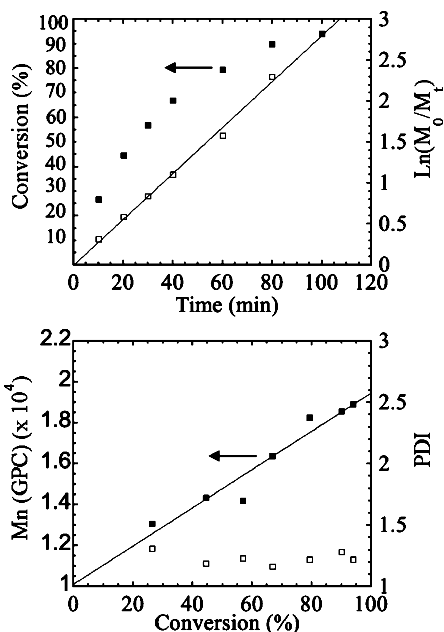


Figure 2. Kinetic plot (top) determined by ^{1}H NMR and plot of the evolution of M_n with conversion (bottom) determined by GPC in DMF (0.1 M LiBr) for the polymerization of HEMA in methanol-d_4 [**10**]:[bipy]:[HEMA] = 1:2:50 at 23 °C.

controlled and reached high conversions when three equivalents of bipy were utilized in the reaction at 130 °C. The resulting biohybrid materials were well-defined. Interestingly, amino acid **5** with a free acid could also be utilized as an initiator, suggesting that the acid was far enough removed from the initiating species. With these promising results, the scope of the types of polymers accessible with this strategy was next explored.

Synthesis of Polymethacrylate-Amino Acid Conjugates. We were also interested in synthesizing amino acid-based initiators that would be optimal to polymerize methacrylate monomers. Therefore, Fmoc protected serine derivative (**10**) was designed because the 2-bromoisobutyrate moiety is a well-known initiator for the ATRP of methacrylates.[10,11] The synthesis began by preparation of *N*-α-Fmoc-L-serine-*tert*-butyl ester **9** in two steps following a previously reported procedure (Scheme 5).[33] The protected serine **9** was subsequently treated with 2-bromoisobutyryl bromide in the presence of DIPEA to afford the Fmoc-*O*-(2-bromoisobutyryl)-serine *tert*-butyl ester initiator **10** in 66% yield.

HEMA was selected for polymerization from amino acid **10** because polyHEMA is a biocompatible material known to form hydrogels. It is widely used, for example, in the production of contact lenses.[40] Copper-mediated ATRP of HEMA (50 equiv) was explored using a 1:1:2 ratio of **10**:CuBr:bipy in methanol at 23 °C. Progression of the polymerization was monitored by ^{1}H NMR spectroscopy. The kinetic plot and M_n versus conversion were both linear to 94% conversion (Figure 2), indicating a controlled polymerization. The system was robust in that the initial monomer-to-initiator ratios could be changed while maintaining relatively high conversions. Conversions of 99% and 70% were achieved for monomer to initiator ratios of 20:1 and 100:1, respectively (Table 2). Increasing M_n values were observed as the monomer to initiator ratio was increased (4400, 5900, and 11 800 Da, for 20, 50, and 100 equiv of monomer, respectively). These molecular weights were estimated from the ^{1}H NMR spectra (see Supporting Information) after extensive purification of the samples. GPC was employed before and after to ensure that fractionation did not occur during this purification process. In all cases, the PDIs were narrow (1.14−1.25), indicating that well-defined polymers were formed.

Table 2. Results of ATRP of Methacrylate Monomers from Initiator **10**

polymer	$[M]_0/[I]_0$	conv[a] (%)	M_n (theory)	M_n[a]	PDI[b]
polyHEMA[c]	20	99	2500	4400	1.14
polyHEMA[c]	50	94	6300	5900	1.22
polyHEMA[c]	100	70	9100	11 800	1.25
12[d]	50	84	14 000	15 400	1.19

[a] Determined by ^{1}H NMR. [b] Determined by GPC in DMF (0.1 M LiBr) against polymethyl methacrylate standards. [c] Polymerization conditions: MeOH, 23 °C, $[\mathbf{10}]_0$:$[CuBr]_0$:$[bipy]_0$ = 1:1:2. [d] Polymerization conditions: MeOH:H_2O (85:15), 23 °C, $[\mathbf{10}]_0$:$[CuBr]_0$:$[bipy]_0$ = 1:1:2.

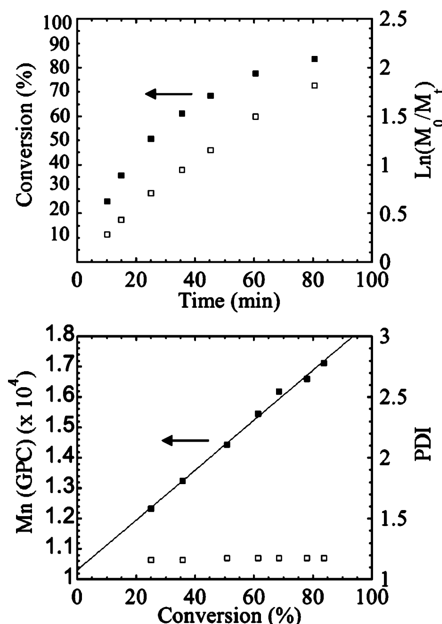


Figure 3. Kinetic plot (top) determined by ^{1}H NMR and plot of the evolution of M_n with conversion (bottom) determined by GPC in DMF (0.1 M LiBr) for the polymerization of the glycomonomer in methanol-d_4:D_2O (85:15), [**10**]:[bipy]:[glycomonomer] = 1:2:50 at 23 °C.

With this success, next we investigated the polymerization of a GlcNAc-modified HEMA. O-GlcNAc glycosylation is a specific protein post-translational modification that covalently attaches the sugar to a serine or threonine residue of proteins. This type of post-translational modification has been detected on transcription factors, protein kinases and cytoskeletal proteins and is important in a range of diseases.[41−43] In addition, glycopolymers are known to mimic the bioactivities of natural polysaccharides,[44] yet are significantly easier to synthesize than oligosaccharides. O-GlcNAc modified synthetic polymers at-

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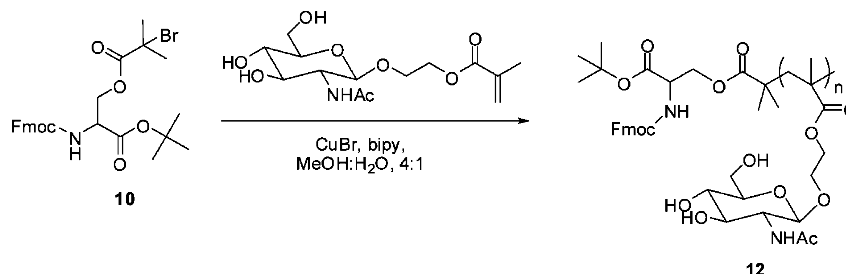
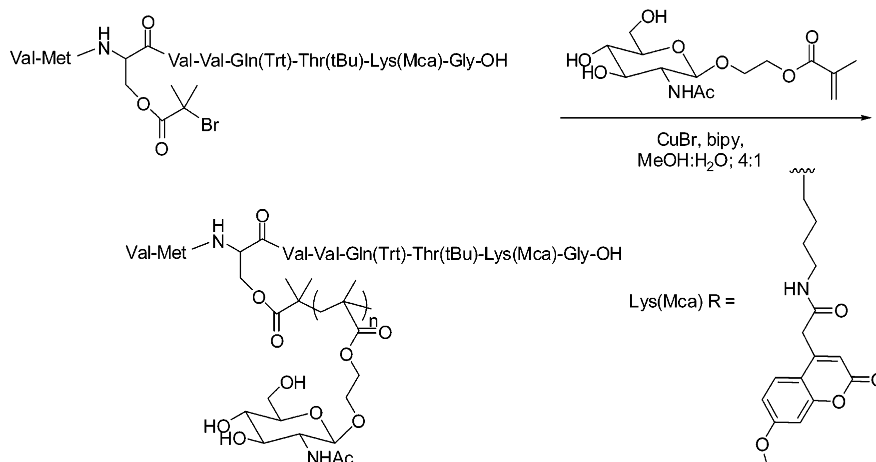
Scheme 6. ATRP of O-GlcNAc Glycomonomer from Serine Initiator **10**



Scheme 7. Polymerization from VM(**11**)VVQ(Trt)T(*t*Bu)K(Mca)G Peptide



tached to peptides or proteins may mimic biological glycosolation, particularly if the attachment can be made site specifically.[45] It was for these reasons that the polymerization of a GlcNAc-modified HEMA from initiator **10** was explored.

ATRP of the glycomonomer was carried out using initiator **10** with CuBr and bipy ($[\text{monomer}]_0$:$[\text{initiator}]_0$:$[\text{CuBr}]_0$:$[\text{bipy}]_0$ = 50:1:1:2). Initially, ATRP was explored using methanol as the solvent. However, high molecular weights of the glycopolymer were insoluble in methanol, making it difficult to obtain reproducible results. Therefore, methanol and water mixtures were investigated, with the idea that water would help solubilize the growing polymer. Indeed, it was found that the polymerization was homogeneous in MeOH:H_20 (85:15, $[M]_0 = 0.9$ M) and afforded polymer **12** in high conversion (84%) in 80 min. Kinetic analysis indicated slight curvature in the kinetic plot; yet the M_n versus conversion plot was linear and the PDIs were narrow throughout the polymerization (Figure 3). The M_n after extensive purification of the polymer was 15 400 Da by ^{1}H NMR spectroscopy. Importantly, the PDI was remarkably narrow at 1.19, indicating that well-defined glycopolymers were accessible.

With these promising results, **10** was deprotected and incorporated into a model peptide, VMSVVQTK, using solid-phase peptide synthesis. This particular peptide was chosen because the sequence is known to be O-GlcNAc modified in the human cellular factor (HCF) protein, an abundant chromatin-associated factor involved in cell proliferation and transcriptional regulation.[42] In addition, this peptide contains two different alcohol groups (the serine and threonine side chains) making it an interesting model system for site-specific modification. Deprotection of the *tert*-butyl ester initiator **10** with 20% trifluoroacetic acid (TFA) in dichlormethane (CH_2Cl_2) for 1 h gave the acid form (**11**) in 90% yield (Scheme 5). Glycine functionalized chlorotrityl resin was selected for the peptide synthesis, as the resulting peptide could be cleaved from the resin using relatively mild conditions (acetic acid:trifluoroethanol:CH_2Cl_2; 2:2:6) leaving the amino acid side chain protecting groups intact. At the C-terminal residue, the lysine was replaced with a lysine derivative modified at the epsilon amine with a (7-methoxycoumarin-4-yl)acetyl (Mca) group, so that the end group could be monitored by fluorescence measurements. The Mca group is known to fluoresce at 405 nm when stimulated at 340 nm.[46] Synthesis of VM(**11**)VVQ(Trt)T(*t*Bu)K(Mca) was achieved from glycine modified chlorotrityl resin, employing Fmoc-based solid-phase peptide synthesis. The peptide was purified by reverse phase preparatory HPLC prior to use.

ATRP from the protected peptide was conducted as for **10** using MeOH:H_2O (85:15), 23 °C, $[M]_0$:$[\text{peptide}]_0$:$[\text{CuBr}]_0$:$[\text{bipy}]_0$ = 20:1:1:2, and $[M]_0 = 0.9$ M (Scheme 7). Similarly, the polymerization reached 93% conversion to polymer in 80 min. Polymerization from the peptide resulted in a polymer with a PDI of 1.14, demonstrating that a well-defined conjugate was obtained. The M_n of the polymer was determined by GPC to be 12 200 Da. Conjugate formation was confirmed using a fluorometer after extensive purification to remove any unattached peptide. Upon stimulation at 340 nm, fluorescence was observed at 405 nm, as expected for the modified lysine Mca derivative. This provided good evidence that the peptide was indeed attached to the polymer and that the biohybrid was formed.

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These results demonstrated that a serine-derived initiator was highly effective for the polymerization of methacrylate-based monomers. The amino acid initiator was prepared in a few steps and was efficient in the synthesis of two important biopolymers: polyHEMA and an O-GlcNAc side chain glycopolymer. This amino acid was readily incorporated into a biologically relevant peptide, and the corresponding glycopolymer-biohybrid material was formed directly. Importantly, all polymers exhibited narrow molecular weight distributions, demonstrating that well-defined polymers were obtained utilizing this strategy.

Conclusions

Herein, a straightforward methodology to prepare peptide−polymer biohybrid materials was described. Artificial amino acids with side-chain ATRP initiators were designed and synthesized. Use of these amino acids to prepare well-defined polystyrene, polyHEMA, and glycopolymer biohybrid materials was demonstrated. Previous technologies were limited to modifying pre-existing peptides making it difficult to conjugate to one particular amino acid in a peptide that contained many of the residue. This methodology is unique in that it provides a means to polymerize from a peptide at any point along the sequence. Unlike use of selective deprotection group strategies and subsequent modification, this approach should result in quantitative incorporation of the initiation moiety. A variety of applications may be envisioned. For example, biohybrid materials composed of polystyrene are known to produce self-assembled nanostructures that are bioactive.[5] Until now, these have been synthesized by conjugation of a preformed polymer chain. The approach described here should allow the peptide to be modified at a precise site, potentially resulting in increased bioactivity or unique self-assembling properties of the conjugate. In addition, polyHEMA is a well-known hydrogel material, and formation of this polymer from peptides could be utilized to produce modified gels for a variety of biomaterial applications. Glycopolymers, including GlcNAc-modified materials, are useful as mimetics of natural polysaccharides, and are significantly more straightforward to synthesize compared to oligosaccharides.[44] We expect to be able to modify a wide variety of peptides at one particular amino acid with a glycopolymer. This should enable us to mimic glycosylation patterns found in nature, whereby a particular amino acid is modified with a saccharide or polysaccharide. In addition, this may result in glycopoolymer−peptide conjugates with unique bioactivities. We also anticipate that other monomers will be readily polymerized. In addition, we expect that polymerization from peptides supported on a solid resin should be possible. Thus far, we demonstrated utilizing solid-phase synthesis to incorporate an artificial amino acid into a short peptide. This same approach may then be extended to the incorporation of the amino acid initiator into longer peptides or proteins by known native chemical ligation technologies. Another potential use of these amino acids is incorporation of the deprotected versions into proteins by recombinant methods.[47−51] These and other applications of this method to produce peptide or protein conjugates with unprecedented control over the placement of the polymer chain are currently being pursued.

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Supporting Information Available: Experimental details, ^{1}H NMR spectra of polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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