Combining ATRP and "Click" Chemistry: a Promising Platform toward Functional Biocompatible Polymers and Polymer Bioconjugates

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ABSTRACT: The bromine chain-ends of well-defined poly(oligo(ethylene glycol) acrylate) (POEGA) (M_n = 6850 g·mol^{-1}, M_w/M_n = 1.21) prepared using ATRP were successfully transformed into various functional end groups (ω-hydroxy, ω-amino, and ω-Fmoc-amino acid) via a two step pathway: (1) substitution of the bromine terminal atom by an azide function, (2) 1,3-dipolar cycloaddition of the terminal azide and functional alkynes (propargyl alcohol, propargylamine, and *N*-α-(9-fluorenylmethyloxycarbonyl)-L-propargylglycine). The efficient "click" cycloaddition was confirmed in all cases by ^{1}H NMR or SEC−UV analysis. Moreover, this two-step synthetic strategy was also studied for preparing polymer-*b*-oligopeptide bioconjugates. Well-defined POEGA-*b*-GGRGDG was obtained in high yields via the "click" ligation of the azido functional POEGA and the alkyne functional oligopeptide GGRGDG.

Introduction

Synthetic biocompatible polymers are crucial materials in modern biomedical applications.[1,2] For instance, water-soluble polymers, stimuli-responsive polymers, or polymer bioconjugates are extensively applied in various biotechnological areas such as pro-drug systems, protein delivery, gene therapy, bioassays, or bioseparation.[1−5] In this context, the development of novel synthetic methods at the interface of standard polymer chemistry and biochemistry is particularly important.

Recently, Sharpless et al. popularized in organic synthesis the 1,3-dipolar cycloaddition of azides and terminal alkynes, catalyzed by copper(I).[6] Such reactions were proven to be very versatile, since they can be performed in high yield, in multiple solvents (including water) and in the presence of numerous other functional groups. Hence, these cycloadditions were evidenced to be efficient tools for the ligation of synthetic organic molecules and biological structures such as peptides, proteins, viruses or cells.[7−14] Due to their efficiency and simplicity, azide/alkyne cycloadditions were ranked by Sharpless in the category of "click" reactions.[15]

During the last 2 years, "click" cycloadditions became also very popular in polymer chemistry, as a tool for functionalizing synthetic macromolecules.[16−41] The transfer of "click" chemistry in polymer synthesis first started with the very influential works of Fréchet and Hawker. Their early publications in the field illustrated that the 1,3-dipolar cycloaddition of azides and alkynes is a promising reaction for preparing either dendrimers[20,24,27,35] or functional linear polymers.[18,28] Shortly after, we and others reported the combination of "click" chemistry and atom transfer radical polymerization (ATRP).[26,30,34] Such a step was important since ATRP is probably one of the most employed polymerization method in modern material science.[42−44] Indeed, ATRP is a facile technique, which allows the preparation of well-defined polymers with narrow molecular weight distribution, predictable chain length, controlled microstructure, defined chain-ends and controlled architecture.[44−50] Nevertheless, the range of possibilities of ATRP can be further broadened by "click" chemistry. For example, the ω-bromine chain-ends of polymers prepared by ATRP can be transformed into azides by nucleophilic substitution and subsequently reacted with functional alkynes.[51,52] Such approach was used for preparing either well-defined telechelic polymers or block copolymers.[21,22,26,30,34] Additionally, functional initiators or monomers (i.e., azide or alkyne functional molecules) can be used in ATRP for preparing well-defined "clickable" polymers.[29,30,33,34,38]

Such combination of "click" chemistry and ATRP could be further developed as an interesting platform for building tailor-made biomaterials. However, so far, only a few steps were made in this direction,[21,38] even though, as mentioned above, "click" chemistry appears to be an interesting link between the synthetic and biological worlds.

Poly(ethylene glycol) (PEG) is undoubtedly the most studied and applied synthetic polymer in the biomedical field.[1] Indeed, PEG is an uncharged, water-soluble, nontoxic, nonimmunogenic polymer and therefore an ideal material for bioapplications. Classic examples of application include peptide/protein PEGylation, stealth drug carriers, and protein repellent surfaces.[5,53,54] In general, PEGylation chemistry relies on short end-functional linear PEG macromolecules. However, it was recently demonstrated than nonlinear PEG analogues, possessing a vinyl backbone and multiple PEG side chains, can also be used for building advanced biomaterials.[55−65] Such structures can be obtained via either radical (i.e., free radical polymerization or controlled radical polymerization techniques such as ATRP) or anionic polymerization of oligo(ethylene glycol) macromonomers.[66−70] Recent studies indicated that these brushlike PEG macromolecules are as biocompatible (e.g., nontoxic, nonimmunogenic) as their linear counterpart.[55,57,58,60−65] Moreover, nonlinear PEG analogues can exhibit new properties, which are typically not attainable with linear PEG. For example, we recently reported that the copolymerization of two oligo-

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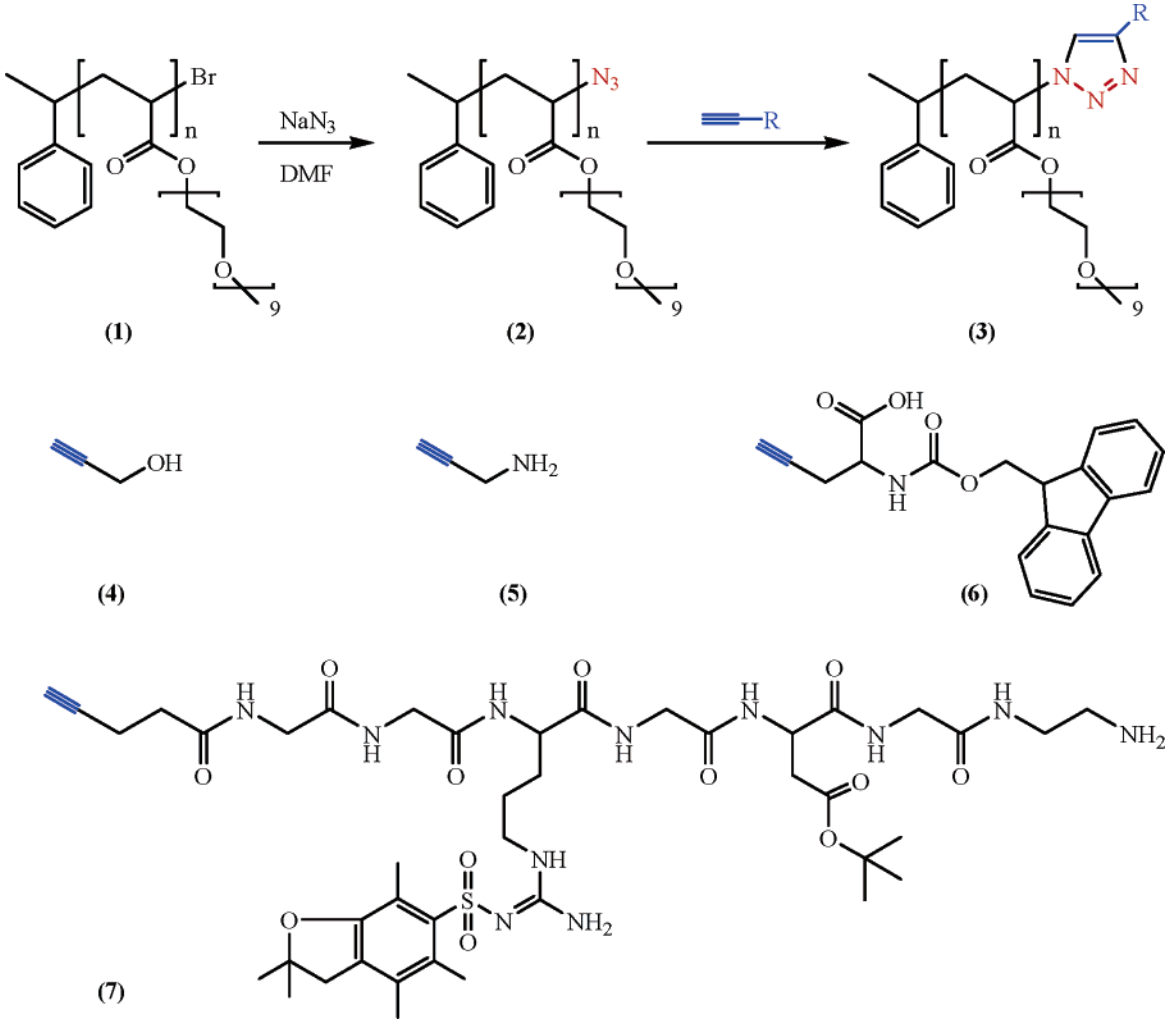
Scheme 1. Preparation of ω-Azide Functional Poly(oligo(ethylene glycol) acrylate) (POEGA) and Molecular Structures of the Various Alkynes Studied Herein for "Click" Functionalization of POEGA



(ethylene glycol) macromonomers of different chain-lengths lead to the formation of thermosensitive copolymers with a precisely tunable lower critical solution temperature (LCST).[71,72]

In the present article, we studied the 1,3-dipolar cycloaddition of azides and terminal alkynes as a general methods for functionalizing nonlinear PEG macromolecules prepared using ATRP (Scheme 1). The ω-chain-end of well-defined azide-terminal poly(oligo(ethylene glycol) acrylate) (POEGA) was studied as a reactive site for "click" coupling. This approach was preferred to the use of α-functional initiators (e.g., alkyne or azide functional initiators) since the ω-functionality is the only one available when polymers are grown from a surface (e.g., from inorganic nanoparticles or from flat substrates).[50] Our chemistry is therefore adapted for the post-functionalization of biocompatible surfaces. As a proof of concept, the "click" ligation of ω-azide functional POEGA to either low molecular weight functional alkynes or sequence-defined oligopeptides is described herein.

Experimental Part

Chemicals. Propargyl alcohol (Aldrich, 99%), propargylamine (Aldrich, 99%), oligo(ethylene glycol) methyl ether acrylate (Aldrich, M_n = 454 g·mol^{-1}), 2,2′-bipyridyl (Bipy) (Fluka, 98%), *N*,*N*,*N*′,*N*′,*N*″ pentamethyldiethylenetriamine (PMDETA) (Aldrich, 99%), 4,4′-Di-(5-nonyl)-2,2′-bipyridine (dNbipy) (Aldrich, 99%), (1-bromoethyl) benzene (Acros, 97%), and sodium azide (Aldrich, 99%) were used as received. Copper(I) bromide (Acros, 95%) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried. *N*-α-(9-Fluorenylmethyloxycarbonyl)-L-propargylglycine (Fmoc-L-pra-OH), *N*-α-(9-fluorenylmethyloxycarbonyl)-*N*′-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-L-arginine (Fmoc-L-Arg(Pbf)-OH), *N*-α-(9-fluorenyl- methyloxycarbonyl)glycine (Fmoc-Gly-OH), *N*-α-(9-Fluorenylmethyloxycarbonyl)-L-aspartic acid β-*tert*-butyl ester (Fmoc-L-Asp(*t*Bu)-OH), and the polystyrene-(2-chlorotrityl chloride) resin (loading: 0.25 mmol/g) were received from Iris Biotech GmbH (peptide grade).

Preparation of Bromine End-Functional Poly(oligo(ethylene glycol) acrylate) 1 via Atom Transfer Radical Polymerization. Copper bromide (CuBr) (172 mg, 1.20 mmol) and 2,2′-bipyridyl (Bipy) (375 mg, 2.40 mmol) were added into a dry flask. The flask was sealed with a septum and subsequently purged with dry argon for several minutes. Then, 20 mL of thoroughly degassed oligo(ethylene glycol) methyl ether acrylate (Aldrich, M_n = 454 g·mol^{-1}) was added with a degassed syringe. The mixture turned dark brown, indicating complexation of Cu^{I}Br and Bipy. Last, (1-bromoethyl) benzene (222 mg, 1.20 mmol) was added with a precision syringe. The mixture was heated at 90 °C in an oil bath. After 3 h, the experiment was stopped by opening the flask and exposing the catalyst to air. The final mixture was diluted with deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation. The purified polymer appeared as a clear yellowish oil.

Preparation of Azide End-Functional Poly(oligo(ethylene glycol) acrylate) 2. The procedure for transforming bromide

ω-functional POEGA into azide ω-functional POEGA was adapted from the literature.[52] Typically, the bromine end-functional poly(oligo(ethylene glycol) acrylate) **1** (6850 g·mol^{-1}) (5.5 g, 0.80 mmol), sodium azide (57 mg, 0.88 mmol) and dimethylformamide (6 mL) were added in a dry flask. The clear homogeneous solution was stirred at room temperature for 3 h. The final mixture was diluted with deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation.

General Procedure for the "Click" Coupling of Azide End-Functional Poly(oligo(ethylene glycol) acrylate) 2 and a Low Molecular Weight Functional Alkyne (either 4, 5, or 6). In a flask, polymer **2** (200 mg, 0.03 mmol), copper bromide (21 mg, 0.14 mmol) and 2 mL of tetrahydrofuran (THF) were added. The flask was capped with a septum and the solution was purged with dry argon for 5 min. Then, 4,4′-di(5-nonyl)-2,2′-bipyridine (dNbipy) (119 mg, 0.29 mmol) dissolved in 3 mL of degassed THF was added with a degassed syringe through the septum and the mixture turned to a homogeneous brown/dark red solution. Last, the functional alkyne (0.29 mmol) was added via a microliter syringe (only alkynes **4** and **5** are liquid—the solid alkyne **6** was added at the beginning of the reaction, together with CuBr). In all cases, the reaction mixture was stirred for 24 h at room temperature. After reaction, the final mixture was diluted in tetrahydrofuran (THF) and passed through a short silica column in order to remove copper catalyst. Then, the THF solution was further diluted with an excess deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation.

Synthesis of the Alkyne Functional Oligopeptide GGRGDG 7. The solid-phase supported peptide synthesis of **7** was performed on an Applied Biosystems ABI 433a peptide synthesizer using *N*-methyl-2-pyrrolidone (NMP) as solvent. The coupling of the amino acids was facilitated by HBTU/DIPEA and Fmoc protocols were followed as described previously.[73,74] A polystyrene-(2-chlorotrityl chloride) resin was preloaded with 1,2-ethanediamine via standard procedures[75] and subsequently used for attaching the Fmoc-amino acid derivatives to give the peptide GGRGDG. After removal of the final Fmoc group, the N-terminal amine group was amidated with 20 equiv excess of 4-pentynoic acid using standard DCC coupling protocols. **7** was liberated from the support by twice treatment with a cleavage mixture (2 vol % of trifluoroacetic acid (TFA) in dichloromethane) for 2 min, followed by washing cycles. After diethyl ether precipitation, centrifugation, and lyophilization from 50 vol % water/acetonitrile **7** was isolated in about 78% yield. ESI−MS: $C_{42}H_{65}N_{11}O_{12}S$, M_{th} = 947.45 Da; 948 *m*/*z* ($[M + H]^+$); 475 *m*/*z* ($[M + 2H]^{2+}$); 447 *m*/*z* ($[M\text{-}t\text{Bu} + 2H]^{2+}$.

General Procedure for the "Click" Coupling of Azide End-Functional Poly(oligo(ethylene glycol) acrylate) 2 and the Alkyne Functional Oligopeptide GGRGDG 7. Polymer **2** (100 mg, 0.015 mmol), the oligopeptide **7** (55 mg, 0.06 mmol), and copper bromide (10 mg, 0.07 mmol) were added together into a dry flask. The glassware was capped with a septum and purged with dry argon for several minutes. Then, Bipy (23 mg, 0.14 mmol) dissolved in 2 mL of degassed *N*-methyl pyrrolidone (NMP) was added via a degassed syringe. The mixture turned dark brown, indicating complexation of Cu(I)Br and Bipy. The reaction mixture was stirred for 24 h at room temperature. The final mixture was diluted in deionized water and subsequently purified by dialysis against pure water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by rotary evaporation.

Measurements and Analysis. Size Exclusion Chromatography, SEC. (1) Molecular weights and molecular weight distributions were determined by SEC performed in THF as eluent and using three 5 μ-MZ-SDV columns with pore sizes of 10^3, 10^5, and 10^6 Å (flow rate 1 mL·min^{-1}). The detection was performed with a RI− (Shodex RI-71). For calibration, linear polystyrene standards (PSS, Germany) were used. (2) Sanger tests SEC monitoring were performed at 20 °C using a Waters 515 HPLC isocratic pump equipped with a Waters 2414 Refractive Index detector, a Waters 2487 UV detector (set at a wavelength of 380 nm) and a set of Styragel columns (HR5, HR 45, HR 3, 500−100 000 Da) from Waters. Eluent: THF (HPLC, from Roth). Flow rate: 1 mL·min^{-1}. The degree of functionalization of ω-amino functional polymers was roughly estimated by comparing the intensities of the polymer signal and the signal of the unreacted Sanger reagent.

Table 1. Molecular Description of the Well-Defined POEGA Prepared by ATRP[a]

	monomer	t (h)	conv	M_n GPC	M_w/M_n	M_n NMR	$M_{n\ th}$[b]
1	OEGA	3	0.33	6850	1.21	7200	6000

[a] Experimental conditions: bulk, 60 °C, $[OEGA]_0/[PEBr]_0/[CuBr]_0/[Bipy]_0$ = 40/1/1/2. [b] $M_{n\ th}$ = conv. (454 $[OEGA]_0/[PEBr]_0$).

^{1}H NMR. ^{1}H NMR spectra were recorded at room temperature in acetone-d_6 or dimethyl sulfoxide-d_6 (DMSO-d_6) on a Bruker DPX-400 operating at 400.1 MHz. Monomer conversions were calculated from ^{1}H NMR spectra by comparing the integrations of the vinyl protons of the remaining monomer (5.90, 6.18, and 6.35 ppm) to the overall integration of the region 3.90−4.70 ppm where 2 protons of the remaining monomer and 2 protons of the formed polymer resonate.

Results and Discussion

A combination of ATRP and "click" chemistry was studied as a new strategy for preparing functional PEG analogues and PEGylated oligopeptides. Poly(oligo(ethylene glycol) acrylate) (POEGA) was chosen as model polymer in this study. The acrylate macromonomer (oligo(ethylene glycol) acrylate) (OEGA) was preferred to its methacrylate counterpart since the nucleophilic substitution of halogen end groups into azides was reported to be generally faster for poly(acrylates) than for poly(methacrylates).[45,52]

A well-defined POEGA sample **1** was prepared by bulk ATRP in the presence of the catalyst system Cu(I)Br/Bipy (Table 1). For this reaction, the initiator (1-bromoethyl) benzene (PEBr) was selected, since the phenyl protons of this moiety can be clearly detected and quantified in the ^{1}H NMR spectrum of POEGA.[76] As described in the literature, the polymerization was stopped at rather low monomer conversion in order to obtain a polymer with a high degree of terminal halogen functionality.[77,78] Previous reports indicated that at this stage of the polymerization (i.e., ∼30% monomer conversion), the bromine end-functionality of poly(acrylates) prepared by ATRP is above 90%.[76,78] However, after polymerization, the reaction mixture still contained a high proportion of unreacted OEGA. This excess of macromonomer was selectively separated form the polymer via dialysis in pure water as shown in Figure 1. Indeed, the molecular weight distribution of POEGA was slightly narrowed in this process since a fraction of the low molecular weight chains is lost during dialysis. Nevertheless, after purification, a well-defined POEGA was obtained (M_n = 6850 g·mol^{-1}, M_w/M_n = 1.21). The number-average molecular weight determined by SEC in THF for this sample was also confirmed by ^{1}H NMR (i.e., by comparing the integration of the protons of the initiator and the protons of the backbone). Nevertheless, number-average molecular weight and molecular weight distribution of the polymer were not affected by further dialysis steps using the same type of membranes (Figure 1).

To perform a 1,3-dipolar "click" cycloaddition azide/alkyne at the ω-chain-end, the bromo functional POEGA **1** was transformed in an azide ω-functional polymer via nucleophilic substitution with sodium azide, as described previously in the literature.[52] The resulting POEGA **2** was subsequently involved in "click" reactions with various low molecular weight functional alkynes (propargyl alcohol, the amino acid *N*-α-(9-

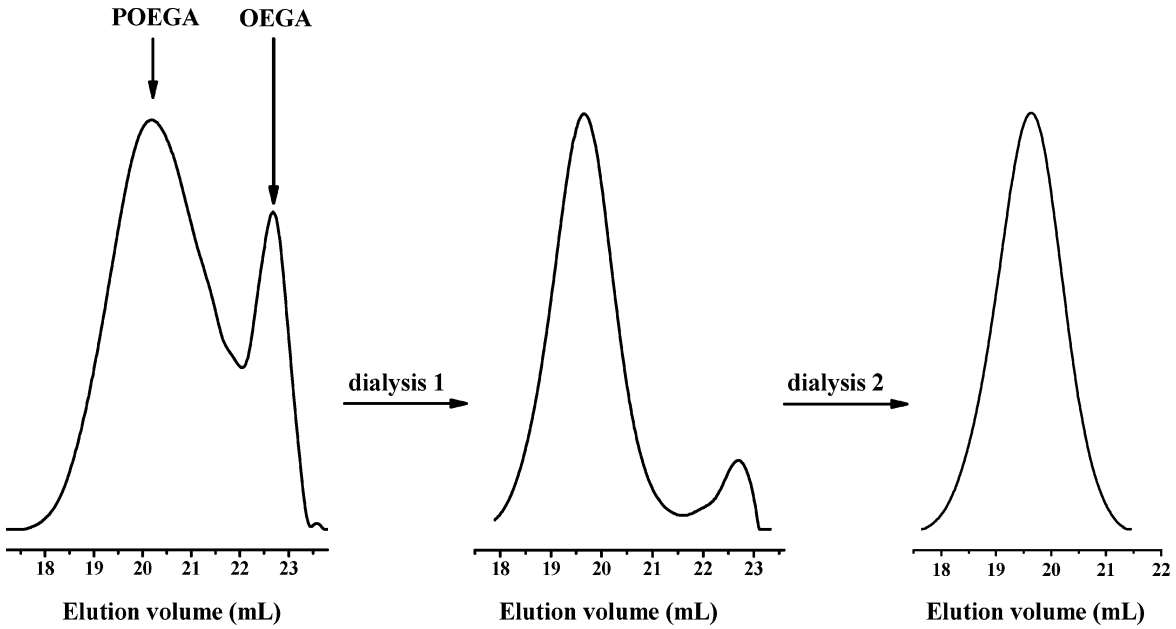


Figure 1. SEC chromatograms recorded in THF for a well-defined poly(oligo(ethylene glycol) acrylate) (POEGA) prepared by ATRP. Left: raw POEGA **1** at the end of the polymerization. Middle: purified POEGA **1** after dialysis in water. Right: POEGA **2** after reaction with NaN_3 and purification by dialysis in water.

fluorenylmethyloxycarbonyl)-L-propargylglycine (Fmoc-L-pra-OH) and propargylamine) in order to prepare functional PEG analogues. Typically, in the absence of an appropriate catalyst, the reaction between azides and terminal alkynes is quite slow since these alkynes are poor 1,3-dipole acceptors. However, in the presence of copper(I), which can bind to terminal alkynes, the cycloaddition reactions are dramatically accelerated, regioselective and highly efficient (yields are often above 95%). In the present work, copper(I) bromide and either the bidentate ligand 4,4′-di(5-nonyl)-2,2′-bipyridine (dNbipy) or the tridentate ligand PMDETA were used as catalyst for the azide/alkyne cycloaddition. Both combinations were selected since they lead to homogeneous reaction mixtures in THF.[22,26]

Figure 2 compares the ^{1}H NMR spectra measured for the azide ω-functional POEGA **2** before and after reaction with propargyl alcohol. The chain-ends of this polymer can be detected and quantified using NMR. From 7.05 to 7.40 ppm, a broad signal due to the aromatic protons of the initiator moiety (H_a) can be observed. After cycloaddition with propargyl alcohol, two new chain-ends signal appeared in the NMR spectrum. The signal at 4.50−4.80 ppm corresponds to the two methylene protons neighboring the terminal alcohol function (H_e) whereas the signal at 7.90−8.10 ppm was assigned to the proton of the triazole ring.[26] The integration of the signals due to α- and ω- chain-ends allows calculation of the fraction of chains initiated by (1-bromoethyl) benzene and terminated by an hydroxy methylene triazole. Degree of functionalization higher as 90% could be obtained (Figure 2B). It is important to remind that this number cannot reach 100% since atom transfer radical polymerizations are, by essence, subject to termination reactions.[77,78] The latter can be minimized but never completely suppressed. Nevertheless, these results indicate that the two chain-end modification steps (i.e., transformation into azide and "click" cycloaddition) are most likely quantitative as previously evidenced for polystyrene and poly(acrylate) models.[26,37]

The functionalization of synthetic polymers with Fmoc-protected amino acid moieties is an important issue since it would open the possibility of using short macromonomers in solid-phase peptide synthesis. Figure 2C shows the ^{1}H NMR spectra measured for a purified POEGA after reaction with Fmoc-L-pra-OH. The apparition of several broad signals between 7 and 8 ppm confirmed the efficient formation of an ω-amino acid POEGA. This particular region contains the aromatic protons of the initiator moiety, the proton of the triazole and the protons of the fluorene ring in Fmoc. In particular, the region 7.6−8.0 ppm contains the four most deshielded protons of the Fmoc moiety. Nevertheless, the proton of the triazole ring could not be separately observed in this spectrum. However, due to the Fmoc-amino acid neighboring group, this proton is theoretically expected to be more shielded than its counterpart in Figure 2B.

The functionalization of POEGA **2** with propargylamine was a less challenging target since terminal azides can also be directly reduced to primary amine via iminophosphorane intermediates.[79] Nevertheless, this reaction was investigated in order to illustrate further the versatility of "click" cycloadditions in polymer synthesis. However, the formed ω-primary amino functional POEGA could not be studied by ^{1}H NMR since the characteristic protons of the ω-chain-ends overlap with other signals. Thus, the existence of ω-amino end groups was confirmed by a standard colorimetric test. 1-Fluoro-2,4-dinitrobenzene (also known as the Sanger reagent) reacts selectively with primary amines via nucleophilic aromatic substitution, resulting in an intense yellow chromophore (Figure 3). The latter was effectively observed when ω-amino POEGA was put in the presence of the Sanger reagent in pure THF (control THF solutions are colorless). However, such visual test should not be overinterpreted since the yellowing could also result from the presence of low molecular weight impurities (e.g., traces of unreacted propargylamine). To rule out this possibility, the POEGA sample reacted with the Sanger reagent was investigated by SEC−UV (Figure 3). This measurement indicated the presence of primary amines functions at the ω-chain-ends of POEGA (the differences in elution volumes between Figures 1 and 3 are due to the use of two different SEC setups). Moreover, the signal intensity confirmed a high degree of chain-end functionalization.

The bioconjugation of nontoxic, nonimmunogenic polymers such as PEG with peptides, enzymes or proteins is an important aspect of modern biotechnology.[5] However, Haddleton and co-

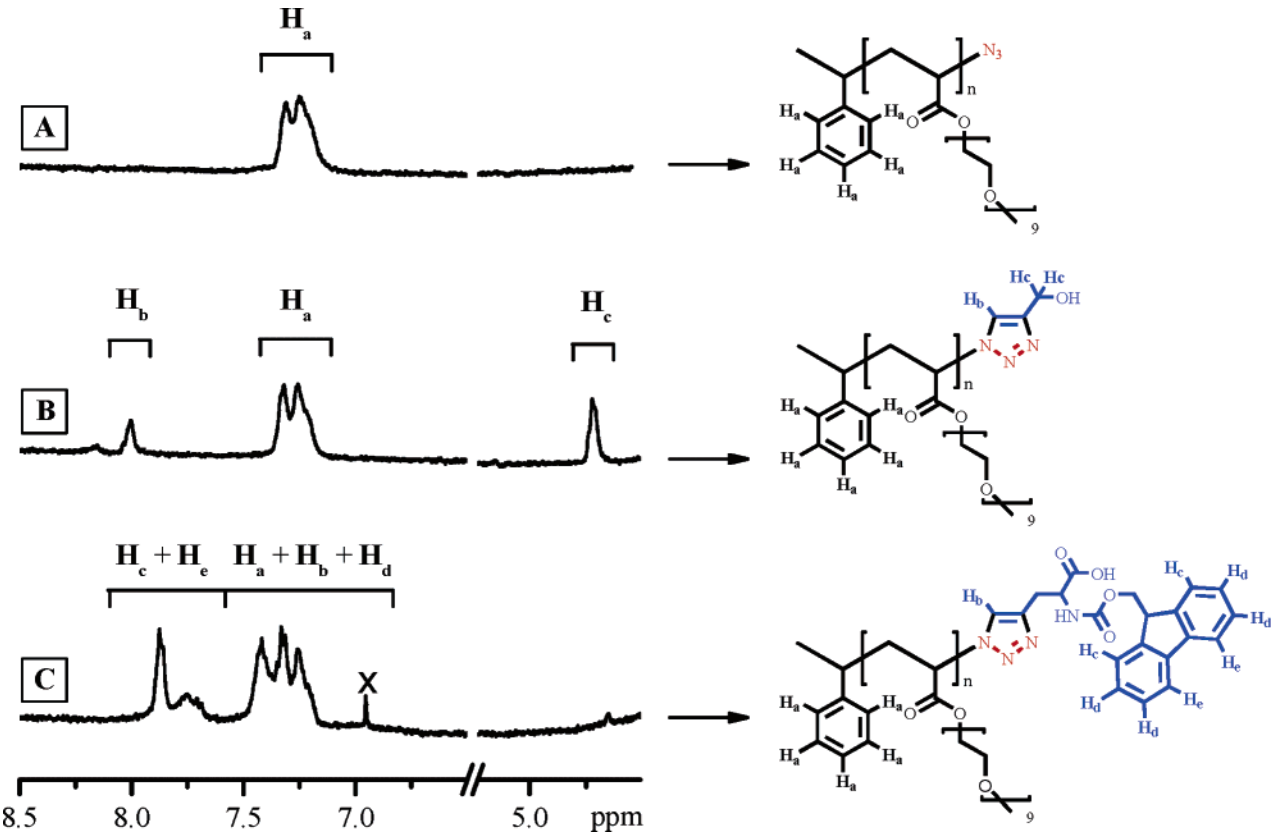


Figure 2. ^{1}H NMR spectrum (zooms of the regions 8.5−6.5 ppm and 5.2−4.5 ppm) of purified poly(oligo(ethylene glycol) acrylate) (POEGA): (A) azide ω-functional before "click" cycloaddition, (B) after "click" cycloaddition with propargyl alcohol, (C) after "click" cycloaddition with *N*-α-(9-fluorenylmethyloxycarbonyl)-L-propargylglycine. All spectra were recorded at room temperature in acetone-d_6.

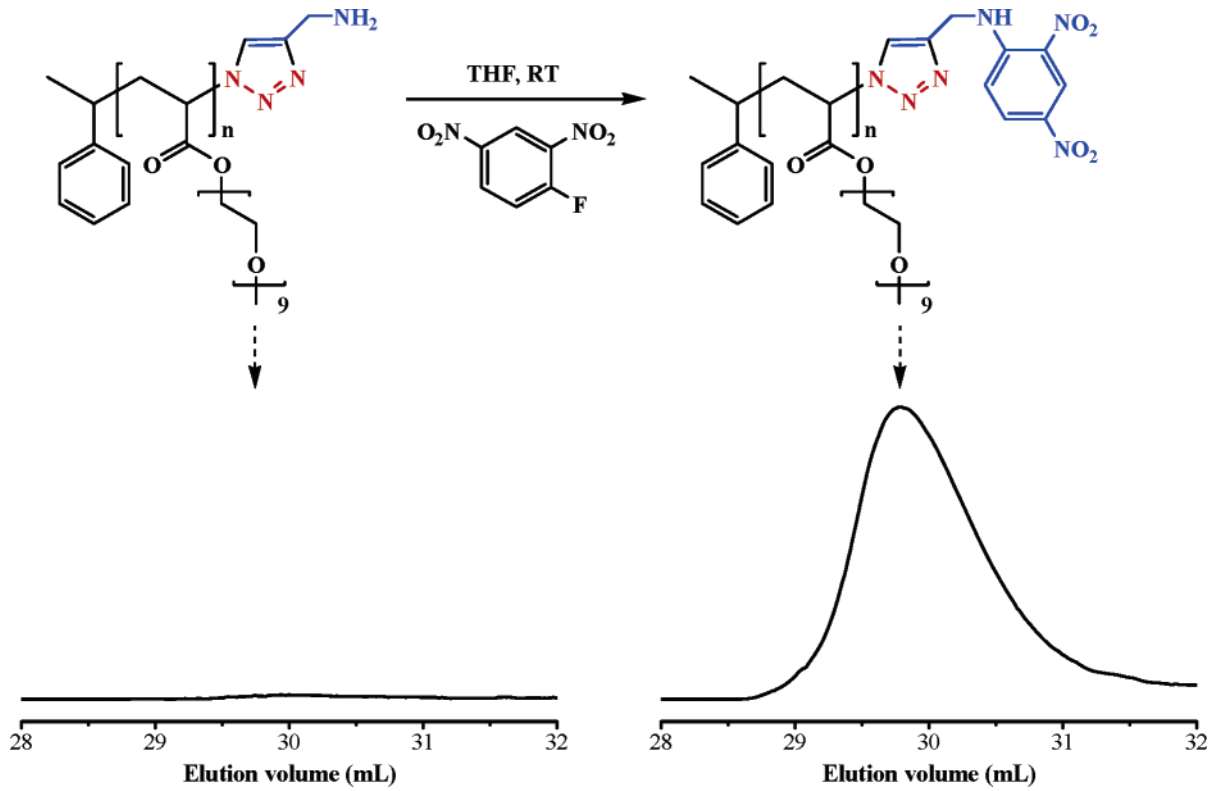


Figure 3. Schematic representation of a primary amine test using 1-fluoro-2,4-dinitrobenzene (Sanger reagent) and SEC chromatograms measured for ω-amino functional poly(oligo(ethylene glycol) acrylate) (POEGA) before and after reaction with the Sanger reagent. SEC chromatograms were recorded in THF with the help of UV detector set at a wavelength of 380 nm.

workers already demonstrated that macromolecules constructed with PEG macromonomers are promising alternative to standard linear PEG for the bioconjugation of proteins.[29,59] It was therefore important to expand our synthetic strategy for the preparation of well-defined bioconjugates of POEGA and oligopeptides.

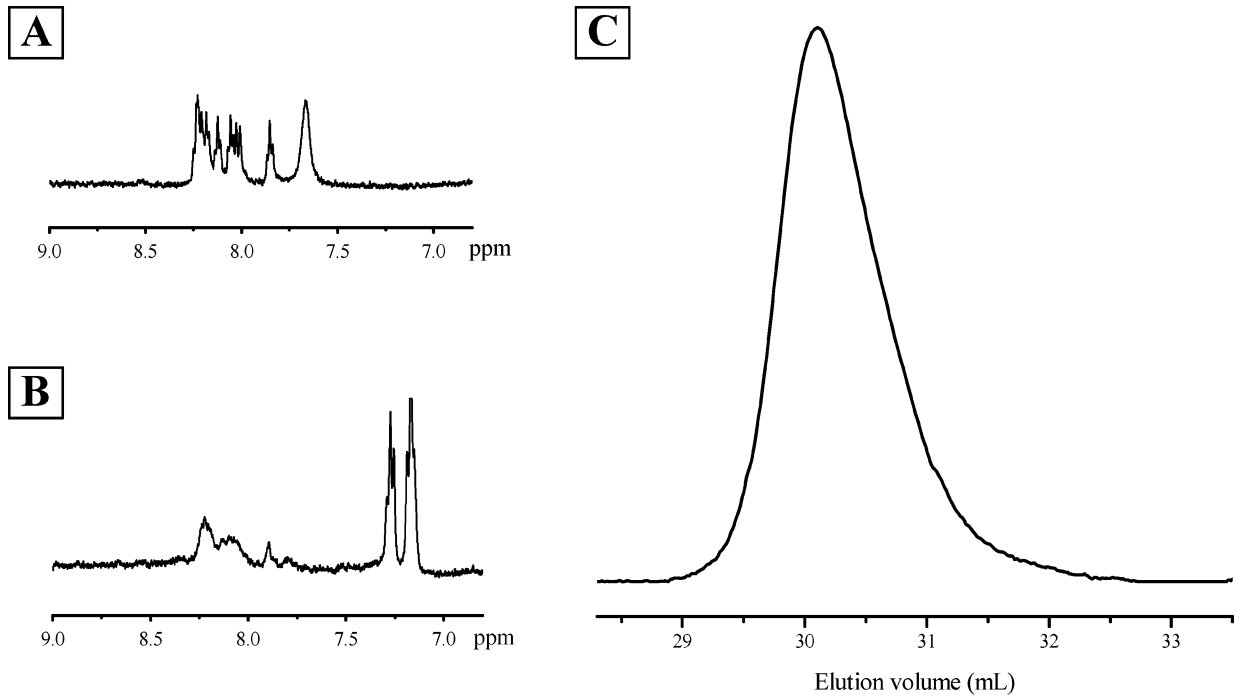


Figure 4. ^{1}H NMR spectrum (zooms of the region 9−6.8 ppm) recorded at room temperature in DMSO-d_6 for (A) the oligopeptide GGRGDG **7** and (B) poly(oligo(ethylene glycol) acrylate) (POEGA) after "click" cycloaddition with the oligopeptide **7**. (C) SEC chromatogram measured for ω-GGRGDG functional poly(oligo(ethylene glycol) acrylate) (POEGA) after reaction with the 1-fluoro-2,4-dinitrobenzene. The chromatogram was recorded in THF with the help of UV detector set at a wavelength of 380 nm.

Many cell receptors of the integrin family recognize the arginine-glycine-aspartic acid (Arg-Gly-Asp, RGD) segment within their ligands.[80] These ligands include fibronectin, fibrinogen, and other large glycoproteins.[81] Recently, it was demonstrated that the RGD-labels can be applied in a versatile manner to enhance the bioacceptability and the biocompatibility of surfaces[82,83] and to induce targeting of drugs or colloidal drug carriers,[84] as well as to control cell adhesion on synthetic materials.[85] Hence, the RGD motif was selected in the present study as a model oligopeptide for the "click" ligation with POEGA.

The oligopeptide GGRGDG **7** was prepared by solid-phase supported synthesis using standard Fmoc protection chemistry. While still on the solid support, the terminal amino-functionality of the polypeptide was transformed in an alkyne moiety via a coupling reaction with 4-pentynoic acid in the presence of *N,N′*-dicyclohexylcarbodiimide. The protecting groups of the arginine and aspartic acid amino acids were kept in the molecular structure after synthesis to allow sufficient THF solubility and therefore a reliable SEC analysis of the polymer bioconjugate. Additionally, the oligopeptide was functionalized with a primary amine end group (obtained when the peptide was cleaved from the solid support) to allow further coupling with the Sanger reagent and SEC−UV analysis.

The "click" reaction of POEGA **2** and the oligopeptide **7** was investigated in *N*-methyl pyrrolidone in the presence of the catalyst system Cu(I)Br/Bipy. As expected, the coupling between the azido functional POEGA and the alkyne functional GGRGDG occurred, as evidenced by ^{1}H NMR and SEC−UV. Parts A and B of Figure 4 compare the region 9−6.8 ppm of the ^{1}H NMR spectra measured respectively for the pristine oligopeptide **7** and the formed bioconjugate POEGA-*b*-GGRGDG after purification. The broad signals in Figure 4A are solely due to the secondary amine backbone protons of the oligopeptide. These protons still appear in Figure 4B, thus confirming the presence of the oligopeptide moiety in the bioconjugate POEGA-*b*-GGRGDG.

The integration of the secondary amine protons and the initiator protons in Figure 4B indicated that approximately 75% of the POEGA chains were functionalized by the oligopeptide. This degree of functionalization is lower than the one observed for low molecular weight alkynes and could be explained by steric hindrance of the functional macromolecules. However, it is noteworthy that secondary amine protons are usually leading to broader and weaker ^{1}H NMR signals in comparison to C−H protons. Hence, the number calculated above might also be slightly underestimated.

Nevertheless, the formation of a well-defined bioconjugate POEGA-*b*-GGRGDG was further confirmed by the Sanger test and subsequent SEC−UV−RI analysis. Figure 4C shows the chromatogram measured for POEGA-*b*-GGRGDG after treatment with 1-fluoro-2,4-dinitrobenzene. A monomodal well-defined polymer could be observed with both UV and RI detectors. In comparison, the pristine bioconjugate POEGA-*b*-GGRGDG (i.e., not reacted with the Sanger reagent) gave no UV signal at the studied wavelength. Moreover, SEC confirmed the complete removal of the excess of the oligopeptide **7** during the purification process.

Conclusion

The 1,3-dipolar cycloaddition of azides and terminal alkynes was investigated as a general tool for functionalizing the ω-chain-ends of well-defined poly(oligo(ethylene glycol) acrylate) prepared using ATRP. This synthetic approach permitted to functionalize "on demand" the polymer with either low molecular weight motifs (e.g., ω-hydroxy, ω-amino or ω-amino acid terminal functions) or sequence-defined oligopeptides. These new results confirm our previous study on well-defined polystyrene models and highlight that the combination of ATRP and click chemistry is a facile and versatile method for preparing

functional macromolecules.[26] Moreover, the synthetic strategy reported in this article can be potentially considered as an "universal" route toward the design of novel biomaterials such as tailor-made polymer bioconjugates and functional biosurfaces.

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