A Versatile Synthetic Approach to Polypeptide Based Rod−Coil Block Copolymers by Click Chemistry

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ABSTRACT: Well-defined block copolymers composed of a rigid poly(γ-benzyl-L-glutamate) (PBLG) sequence and a poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) block were synthesized by Huisgen's 1,3-dipolar cycloaddition (click chemistry) from homopolymers containing azide and alkyne functionalities. These functional groups were introduced in the α-position of both PBLG and PDMAEMA precursors using appropriate α−ω-functionalized initiators to trigger the living/controlled polymerization of the corresponding monomers. Both α-alkyne- and α-azido-PBLGs were synthesized by ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride at room temperature from amino-containing α-alkyne and α-azide difunctional initiators, using dimethylformamide as solvent. As for α-alkyne-PDMAEMA and α-azido-PDMAEMA, they were obtained by copper-mediated atom transfer radical polymerization of 2-(dimethylamino)ethyl methacrylate at 60 °C in tetrahydrofuran as solvent. The copper(I)-catalyzed 1,3-dipolar cycloaddition coupling reactions of the α-azido-PBLG with the α-alkyne-PDMAEMA, in the one hand, and of the α-alkyne-PBLG with the α-azido-PDMAEMA, on the other hand were conveniently performed in DMF, affording the targeted PBLG-*b*-PDMAEMA diblock copolymers. Removal of the residual PDMAEMA used in slight excess was facilitated by the retention of this homopolymer onto the stationary phase of the column chromatography. On the basis of size exclusion chromatography, IR and NMR analyses, click chemistry was found to be quantitative, yielding for the first time hybrid diblock copolymers based on a polypeptide and a vinylic polymer.

Introduction

Block copolymers enter into widespread applications as the result of their self-assembly properties, either in the solid state or in a selective solvent of one block, which provides a great variety of morphologies in the nanometer size range.[1−6] The multifaceted role played by these materials make them useful as compatibilizers, viscosity modifiers, dispersants to stabilize colloidal suspensions, nanocarriers for the encapsulation and controlled release of drugs, templates for mineralization, and supports for catalysis.[1−6] From a synthetic viewpoint, block copolymers are generally designed from "controlled/living" polymerization (CLP) techniques.[7−9] Strategies include (i) sequential addition of monomers, (ii) combination of two different modes of polymerization, (iii) one-pot initiation from dual ("double-head") bifunctional initiators, and (iv) coupling of preformed polymer segments possessing antagonist end groups.[10] In method i, it is usually essential to sequentially polymerize the two monomers in a certain order to access the targeted block. Whatever the CLP followed, the golden rule is to comply with the scale of reactivity of propagating species, starting by the polymerization of the monomer that will form the higher reactive propagating center and then polymerize the second monomer. The sequential anionic polymerization of styrene and dienes is the prototypical example of the synthesis of block copolymers from this route.[11] Using a preformed polymer that can be used as a macroinitiator for the growth of the second block by another mechanism is an alternative synthetic access to block copolymers, method ii.[12] One resorts to this "switch of mechanism" strategy when monomers to pair in a diblock structure do not polymerize by the same mechanism. "Dual" or "double-head" initiators contain two distinct sites for initiating the polymerization of monomers by different mechanisms with no interference between the two modes in the ideal case, method iii.[13] Finally, the covalent coupling of two polymeric chains at their respective ends also results in a diblock copolymer. Such a method, method iv, however, implies selective, fast and quantitative coupling reactions, criteria that are sometimes difficult to fulfill both because of the incompatibility and the steric hindrance of the blocks which decrease the reactivity between antagonist functions. One can combine this "coupling strategy" with method i, relying on sequential CLP; in this case, "living" blocks are linked onto difunctional coupling agents and produce ABA triblock copolymers. Because an excess of "living" chains is generally employed, a fractionation step is required to separate ABA-type triblocks from AB-type diblocks. Typical example is the synthesis of polystyrene-*b*-polyisoprene-*b*-polystyrene triblock copolymers, diblock precursors being obtained by sequential anionic copolymerization and dichlorodimethylsilane being the coupling agent.[14,15] To synthesize metallo-supramolecular block copolymers, Schubert and colleagues recently developed an elegant coupling strategy based on the formation of noncovalent metal−ligand interactions of polymers.[16] More recently, a synthetic coupling strategy to block copolymers based on "click chemistry" was successfully developed by a few groups.[17−20] The terms "click chemistry" were coined by Sharpless and colleagues,[21] who revisited the Huisgen's 1,3-dipolar cycloadditions between azides and alkynes (or nitriles) using copper salts as catalysts.[22] Generally speaking, click chemistry is a versatile method of C−C bond (or C−N) formation, combining mild experimental conditions, tolerance of functional groups, and high yields. Very recently, Binder and Sachsenhofer[23] reviewed the application of click chemistry in macromolecular engineering and in material science, including the synthesis of polymers, dendrimers, gels, etc. It turns out that, in the context of block copolymer

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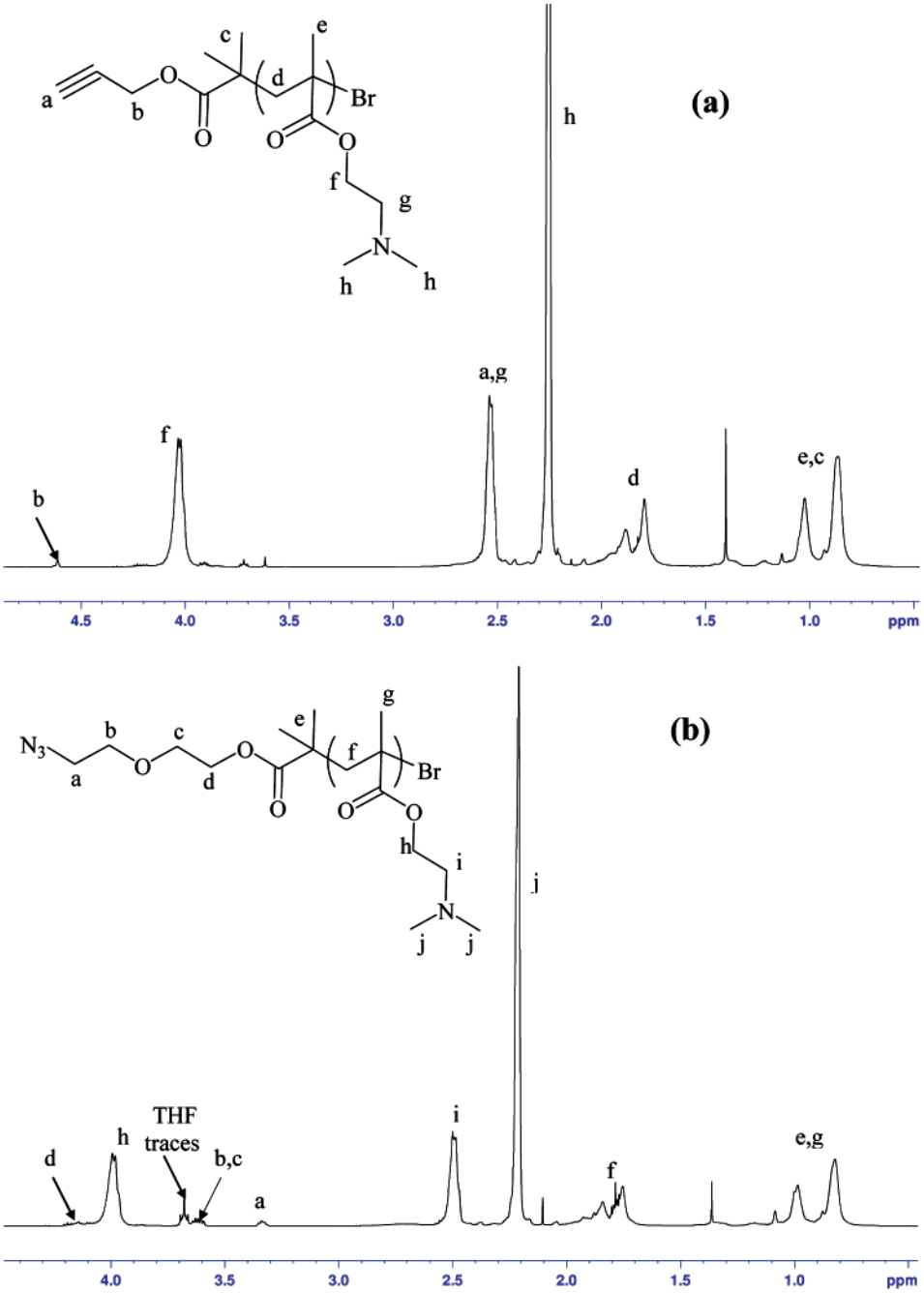


Figure 1. ^{1}H NMR ($CDCl_3$, 400 MHz) spectra of α-alkyne-PDMAEMA **7** (a) and of α-azido-PDMAEMA **4** (b).

synthesis, click chemistry was exploited to form vinylic-based polymers only, e.g., polystyrene-*b*-poly(ethylene oxide)[17−19] and polystyrene-*b*-poly(vinyl acetate).[20] In this contribution, we describe the synthesis of hybrid rod−coil block copolymers by combining ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride, atom transfer radical polymerization of 2-(dimethylamino)ethyl methacrylate, and subsequent click chemistry. Interest of synthetic polypeptide blocks originates from their peculiar change of structural conformations: they are optically active in nature since they are obtained from optically active *N*-carboxyanhydride, and are also capable of undergoing a reversible transition from a rod-like α-helix conformation to a coil conformation that is sensitive to a variation of temperature.[5,24−26] In addition, poly(γ-benzyl-L-glutamate) (PBLG) is the precursor for poly(glutamic acid) (PGA), which is a pH-sensitive and biocompatible synthetic polypeptide.[24−26] As for poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA), it is a water-soluble, temperature-sensitive, and weak polybase possessing inherent amine protonation in physiological media, which proved efficient as polycationic condensing agent for non-viral DNA.[27] PDMAEMA is therefore an interesting component in drug delivery systems. Block copolymers based on PDMAEMA and PBLG described here thus combine pH and temperature-sensitivity, in addition to the possibility of reversible transition from the coil to the α-helix rod conformation of the polypeptide block. To the best of our knowledge, this is the first example of the use of click chemistry as an easy and efficient way to prepare hybrid block copolymers based on a rod-like polypeptide and a coil-type vinylic polymer.

Experimental Section

Materials. Dichloromethane was dried over CaH_2 and subsequently purified by distillation. Tetrahydrofuran was first distilled

over CaH_2 and distilled over sodium. Dimethylformamide was dried over molecular sieves (3 and 4 Å) and cryodistilled. Propargyl alcohol (99%), 2-bromoisobutyric acid (+98%), dicyclohexylcarbodiimide (DCC) (99%), 4-dimethylaminopyridine (DMAP) (99%), 3-chloropropylamine hydrochloride (98%), sodium azide (99%), 2-(2-chloroethoxy)ethanol (98%), tetrabutylammonium iodide (98%), cis-dicyclohexano-18-crown-6 (98%), propargylamine (+98%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (97%), 2-butanone (+99%), and γ-benzyl-L-glutamate *N*-carboxyanhydride (Bz-L-GluNCA) (+96%) were used as received. 2-(dimethylamino)ethyl methacrylate (DMAEMA) (98%) was purified by passing two times over a basic alumina column and stored at −18 °C under dry nitrogen. CuBr was purified following a standard procedure.[28] All reagents were purchased from Aldrich except for tetrahydrofuran (J. T. Baker), dichloromethane (Xilab) dimethylformamide (Scharlau) and γ-benzyl-L-glutamate *N*-carboxyanhydride (Isochem).

Synthesis of α-Functionalized Initiators. Synthesis of 2-(2-Azidoethoxy)ethanol (2). To a solution of 2-(2-chloroethoxy)-ethanol (**1**) (5 mL, 47 mmol) in 2-butanone (25 mL) were added NaN_3 (4.5 g, 69 mmol), Bu_4NI (2.5 g, 6 mmol), and dicyclohexano-18-crown-6 (10 mg), and the mixture was stirred under reflux for 24 h. ^{13}C NMR spectroscopy of a supernatant showed the absence of a signal at δ 42.7 ppm and a strong signal at δ 50.0 ppm. The mixture was filtered, and the solids were thoroughly rinsed with acetone. After concentration of the combined solutions, the crude compound was distilled at 90 °C. Yield: 4.74 g (77%). ^1H NMR ($CDCl_3$): δ 3.70 (t, 2 H, CH_2OH), 3.65 (t, 2H, HOCH$_2$CH_2O), 3.56 (t, 2H, $N_3CH_2CH_2O$), 3.37 (t, 2H, CH_2N_3), and 2.56 (s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 72.47 (HOCH$_2$CH_2O), 69.97 (N_3-CH$_2$CH_2O), 61.67 (CH_2OH), 50.71 (CH_2N_3).

Synthesis of 2-(2-Azidoethoxy)bromoisobutyrate (3). 2-(2-azidoethoxy)ethanol (***2***) (2 g, 15.2 mmol) and 2-bromoisobutyric acid (5.09 g, 30.5 mmol) were dissolved in methylene chloride (30 mL). The reaction mixture was cooled in an ice−water bath, and a solution of dicyclohexyl carbodiimide (6.28 g, 30.5 mmol) in methylene chloride (15 mL) was slowly added while stirring. A solution of 4-dimethylaminopyridine (0.74 g) in methylene chloride (10 mL) was added over a period of 10 min. The mixture was stirred at 0 °C for 1 h and then at room temperature for 24 h. The precipitated dicyclohexylurea was filtered on cotton two times and washed with methylene chloride. The solution was extracted three times with a solution of $NaHCO_3$ (5%) and dried over $MgSO_4$. The volatiles were removed by reduced pressure and the crude product purified by column chromatography on silica gel (heptane: ethyl acetate 18:2). A colorless product was obtained and dried under vacuum overnight. Yield: 3.03 g (83%). ^1H NMR ($CDCl_3$): δ 4.30 (t, 2H, CH_2OCO), 3.73 (t, 2H, COOCH$_2$CH_2O), 3.67 (t, 2H, $N_3CH_2CH_2O$), 3.35 (t, 2H, CH_2N_3), and 1.92 (s, 6H, $(CH_3)_2C$). ^{13}C NMR ($CDCl_3$): δ 171.60 (C═O), 70.15 (COOCH$_2$CH_2O), 68.78 ($N_3CH_2CH_2O$), 65.02 (CH_2CH_2O), 55.79 (CBr), 50.73 (CH_2N_3), and 30.73 ((CH_3)$_2$C).

Synthesis of Propargyl 2-Bromoisobutyrate (6). This compound was synthesized following the procedure reported by Matyjaszewski and colleagues.[29] Propargyl alcohol (**5**) (1.5 g, 26.8 mmol) and 2-bromoisobutyric acid (4.47 g, 26.8 mmol) were dissolved in methylene chloride (20 mL). The reaction mixture was cooled in an ice−water bath and a solution of DCC (5.53 g, 26.8 mmol) in methylene chloride (10 mL) was slowly added while stirring. A solution of DMAP (0.18 g) in methylene chloride (10 mL) was then added over a period of 10 min. The mixture was stirred at 0 °C for 1 h and then at room temperature for 24 h. The precipitated dicyclohexylurea was filtered and washed with methylene chloride (50 mL). The solvent was removed on a rotary evaporator and the product was distilled under vacuum. Yield: 4.56 g (83%). ^1H NMR ($CDCl_3$): δ 4.73 (d, 2H, CH_2O), 2.50 (t, 1H, C≡CH), and 1.91 (s, 6H, $(CH_3)_2C$). ^{13}C NMR ($CDCl_3$): δ 170.90 (C═O), 76.94 (*C*≡CH), 75.55 (C≡*C*H), 54.94 (CBr), 53.43 (CH_2O), and 30.65 ((CH_3)$_2$C).

Synthesis of 1-Azido-3-aminopropane (9). This compound was synthesized following an already published procedure.[30] A solution of 3-chloropropylamine hydrochloride (4 g, 30.8 mmol) and sodium azide (6 g, 92.3 mmol, 3 equiv) in water (30 mL) was heated at 80 °C for 15 h. After most of the water was removed by distillation under vacuum, the reaction mixture was cooled in an ice bath. Diethyl ether (50 mL) and then KOH pellets (4 g) were added keeping the temperature below 10 °C. After separation of the organic phase, the aqueous layer was further extracted with diethyl ether (2 × 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give an oil which was purified by distillation. Yield: 2.3 g (75%).^1H NMR ($CDCl_3$): δ 3.4 (t, 2H, CH_2N_3), 2.65 (t, 2H, CH_2NH$_2$), 1.99 (s, 2H, NH_2), and 1.64 (q, 2H, $CH_2CH_2CH_2$). ^{13}C NMR ($CDCl_3$): δ 48.58 (CH_2N_3), 38.79 (CH_2NH_2), and 32.23 ($CH_2CH_2CH_2$).

Polymerization Reactions. All polymerizations were carried out in Schlenk flasks equipped with a magnetic bar.

Synthesis of α-Azido PDMAEMA (4) and α-Alkyne PDMAEMA (7). The two polymers were prepared according to the same procedure. In a typical experiment for synthesizing **4**, 2-(dimethylamino)ethyl methacrylate (10 mL, 58.2 mmol), 2-(2-azidoethoxy)bromoisobutyrate (**3**) (814 mg, 2.9 mmol), and HMTETA (1.63 mL, 5.81 mmol, 2 equiv) were added to an oven-dried Schlenk flask in 10 mL of anhydrous THF. The mixture was stirred for 5 min and degassed by freeze−thaw cycles and added into another Schlenk flask containing CuBr (417 mg, 2.9 mmol, 2eq) via a cannula. The Schlenk flask was placed in a constant temperature oil bath at 60 °C for 15 min. Conversion was evaluated by ^1H NMR comparing the signals h of the monomer and the polymer (see Figure 1). The reaction was stopped by adding more THF and the solution was passed through a basic alumina column to remove the catalyst. After being concentrated, the solution was precipitated into pentane and the polymer was dried under vacuum overnight and analyzed by SEC in DMF (LiBr, 60 °C) and ^1H NMR. Molar masses were determined by ^1H NMR (Figure 1 and Table 1) using the following equations

$$M_{n,PDMAEMA} = \frac{I_a M_{DMAEMA}}{I_i} + M_{init}(\alpha\text{-azido PDMAEMA } \mathbf{4})$$

$$M_{n,PDMAEMA} = \frac{I_b M_{DMAEMA}}{I_f} + M_{init}(\alpha\text{-alkyne PDMAEMA } \mathbf{7})$$

where I_b, I_a, I_f, I_i, M_{DMAEMA}, and M_{init} are, respectively, the intensity of methylene protons b (initiator **6**) and a (initiator **3**) from the side groups, the intensity of methylene protons h (α-alkyne PDMAEMA) and g (α-azido PDMAEMA) from the PDMAEMA main chain, the molar mass of the DMAEMA monomer unit, and the molar mass of initiator **6** and **3**.

Synthesis of α-Azido PBLG (10) and α-Alkyne PBLG (12). Also in these cases, the two polymers were synthesized following the same protocole. In a typical experiment for synthesizing **12**, Bz-L-GluNCA (4 g, 15.2 mmol) was weighed in a glovebox under pure argon, introduced in a flame-dried Schlenk, and dissolved with 40 mL of anhydrous DMF. The solution was stirred for 10 min, and propargylamine (12 μL, 175.2 μmol) was added with a nitrogen-purged syringe. The solution was stirred for 40 h at room temperature. The polymer was recovered by precipitation in diethylether and dried under high vacuum, analyzed by SEC in DMF (LiBr, 60 °C) and by ^1H NMR. Molar masses were determined by ^1H NMR (Figure 2 and Table 1) using the following equations

$$M_{n,PBLG} = \frac{2I_h M_{BLG}}{5I_a} + M_{init}(\alpha\text{-azido PBLG } \mathbf{10})$$

$$M_{n,PBLG} = \frac{2I_g M_{BLG}}{5I_b} + M_{init}(\alpha\text{-alkyne PBLG } \mathbf{12})$$

where I_a, I_b, I_h, I_g, M_{BLG}, and M_{init} are, respectively, the intensity of methylene protons a (initiator **9**) and b (initiator **11**) from the side groups, the intensity of methylene protons h (α-azido PBLG)

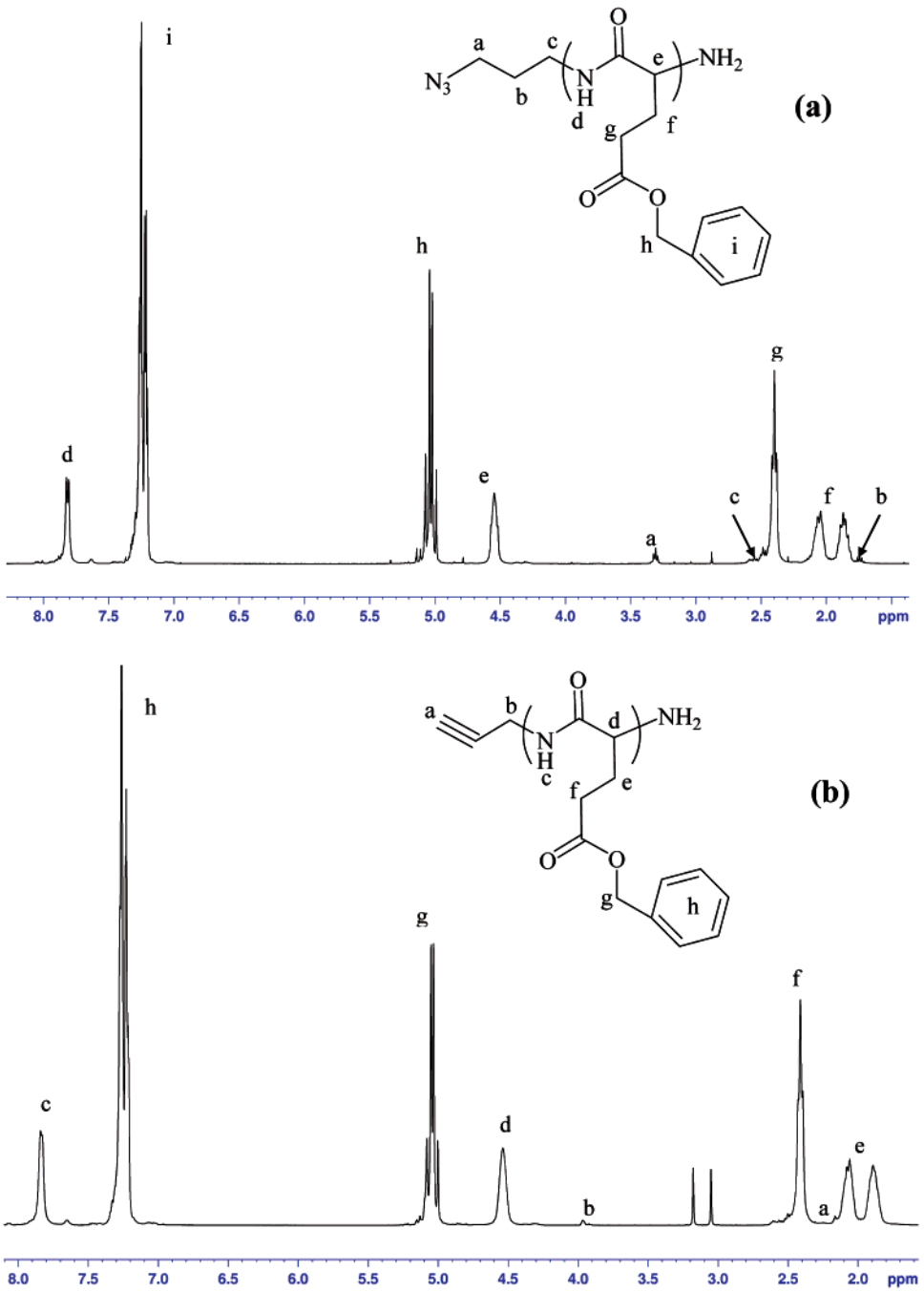


Figure 2. ^{1}H NMR ($CDCl_3$+15%TFA, 400 MHz) spectra of α-azido-PBLG **10** (a) and of α-alkyne-PBLG **12** (b).

Table 1. Molecular Characteristic of α-Functionalized PDMA and PBLG Homopolymers and of the Corresponding PDMAEMA-*b*-PBLG Block Copolymers Obtained by Click Chemistry

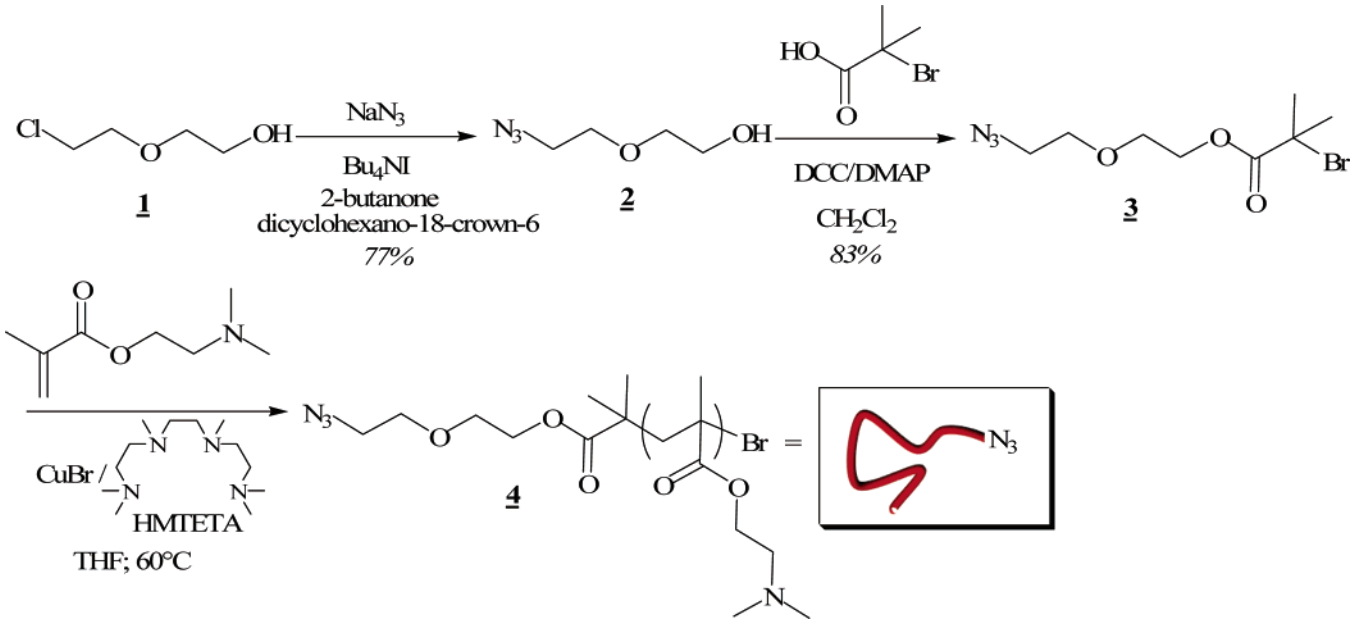
α-alkyne homopolymers				α-azido homopolymers				PDMAEMA-*b*-PBLG		
sample	$DP_{n,NMR}$[a]	$M_{n,SEC}$[b] (g mol^{-1})	PDI[b]	sample	$DP_{n,NMR}$[a]	$M_{n,SEC}$[b] (g mol^{-1})	PDI[b]	sample	$M_{n,SEC}$[b] (g mol^{-1})	PDI[b]
PBLG **12**	47	12 600	1.17	PDMAEMA **4**	27	8800	1.12	**13**	20 800	1.18
PDMAEMA **7**	35	10 400	1.17	PBLG **10**	40	11 100	1.17	**14**	21 100	1.15

[a] Experimental degree of polymerization obtained from the relative integration of characteristic protons of chain ends to protons of polymer main chain. [b] Determined by SEC in DMF at 60 °C in the presence of LiBr; calibration with polystyrene standards.

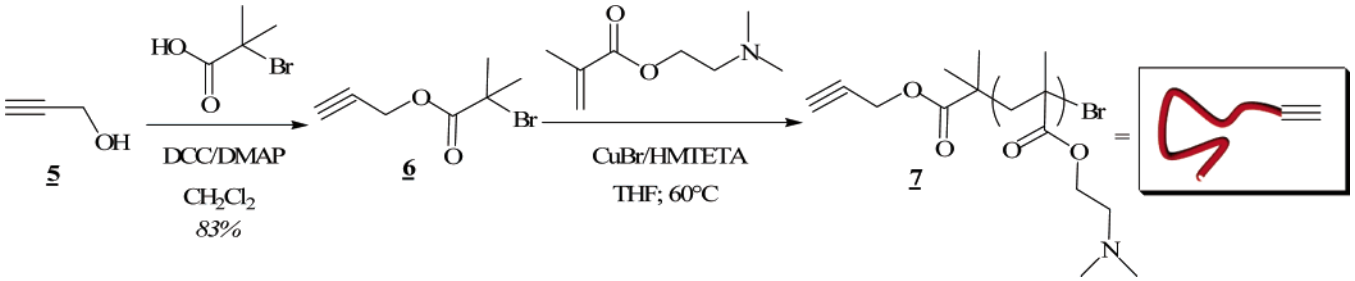
and g (α-alkyne PBLG) from the PBLG main chain, the molar mass of the BLG monomer unit, and the molar mass of initiators **9** and **11**.

Synthesis of PDMAEMA-*b*-PBLG Block Copolymers by Huisgen's 1,3-Dipolar Cycloadditions (Click Reactions). Click experiments between PDMAEMA and PBLG blocks were performed in DMF using either **4** and **12** or **7** and **10**, according to Scheme 5. In a typical experiment (entry 1, Table 1), **12** (0.19 g, 18.3 μmol), **4** (0.1 g, 22.1 μmol, 1.2 equiv), and PMDETA (7.6 μL, 36.6 μmol, 2 equiv) were added to a flask in 5 mL of anhydrous DMF. The mixture was stirred for 10 min and degassed by three freeze−thaw cycles and added in another Schlenk containing CuBr (5.2 mg, 36.2 μmol, 2 equiv) via N_2-purged syringe. The Schlenk flask was placed in a constant temperature oil bath at 25 °C for 24

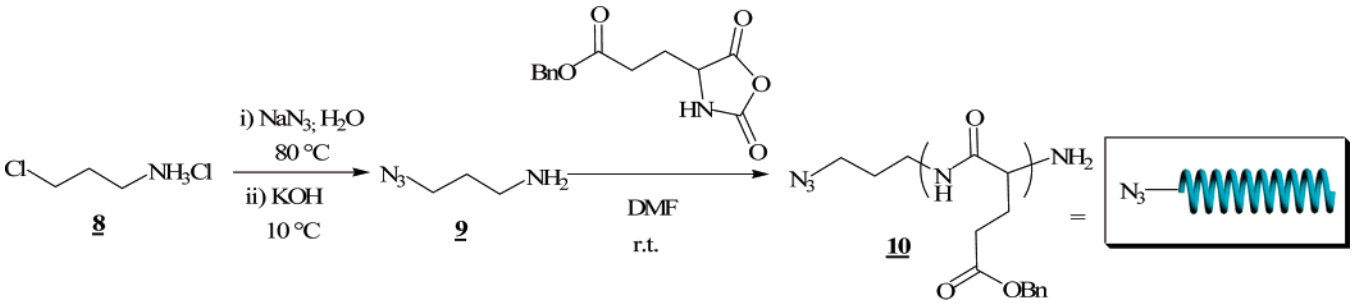
Scheme 1. Synthesis of α-Azido-PDMAEMA by Copper-Mediated ATRP of DMAEMA



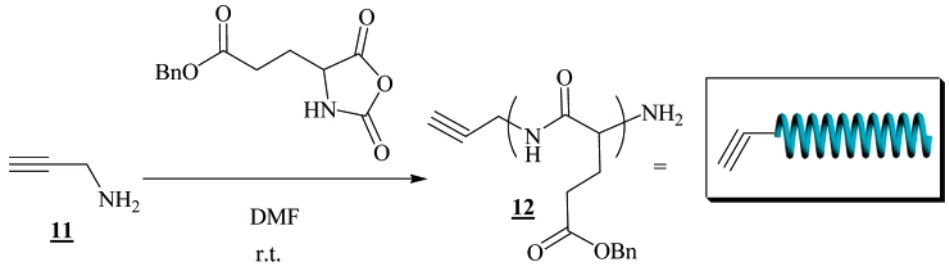
Scheme 2. Synthesis of α-Alkyne-PDMAEMA by Copper-Mediated ATRP of DMAEMA



Scheme 3. Synthesis of α-azido-PBLG by ROP of Bz-L-GluNCA



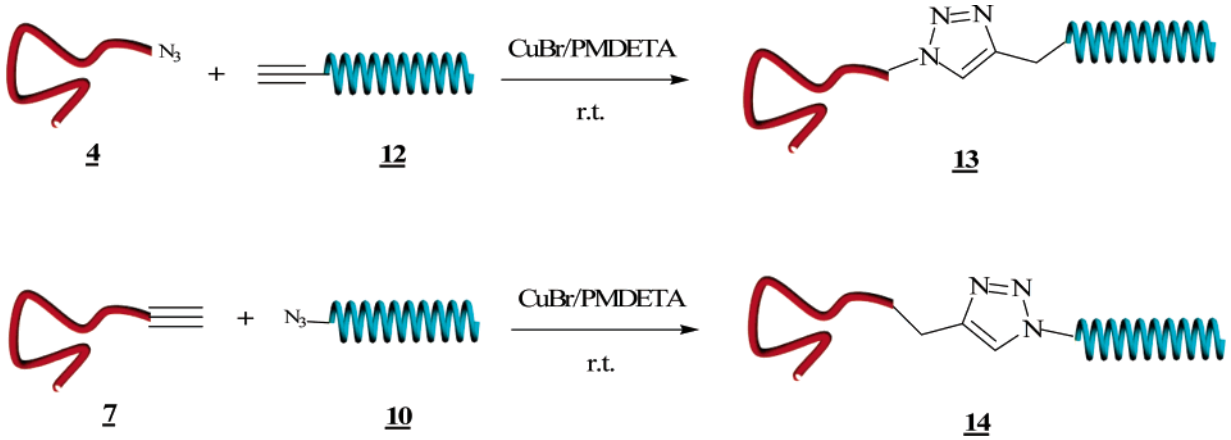
Scheme 4. Synthesis of α-alkyne-PBLG by ROP of Bz-L-GluNCA



h. The solution was passed through a neutral alumina column in order to remove copper salt and the excess of **4**. After concentration, the solution was poured into a large excess of diethylether and the suspension centrifuged. The final product was dried under vacuum overnight and analyzed by SEC in DMF containing LiBr at 60 °C (Figure 3), by ^{1}H NMR (Figure 4) and by infrared (Figure 5).

Characterization. ^{1}H NMR spectra were recorded on a Bruker AC 400 spectrometer. The molar masses were determined by size exclusion chromatography (SEC) equipped with two PLgel Mixed-C columns (7.5 × 300 mm) and one PLgel 5 μm guard column (7.5 × 50 mm), a refractive index detector (Jasco 1530-RI), a UV detector (Jasco 875-UV) and with dimethylformamide (DMF) as eluent (0.8 mL/min) at 60 °C in the presence of LiBr (1 g/L), and was calibrated using linear polystyrene samples. Infrared measurements were performed on a Bruker Tensor 27 spectrometer using the attenuated total reflection (ATR) method.

Scheme 5. Synthesis of PDMAEMA-*b*-PBLG Diblock Copolymers by Click Chemistry



Results and Discussion

Our synthetic approach to block copolymers based on PBLG and PDMAEMA is based on a three-step sequence combining (1) the synthesis of α-azide (**10**) or α-alkyne terminated PBLG (**12**) by ring-opening polymerization (ROP) of γ-benzyl-L-glutamate *N*-carboxyanhydride (Bz-L-GluNCA)[24−26] and (2) the synthesis of α-azide (**4**) or α-alkyne terminated PDMAEMA (**7**) by copper-mediated atom transfer radical polymerization (ATRP)[31] of 2-(dimethylamino)ethyl methacrylate (DMAEMA), followed by (3) the Huisgen's 1,3-dipolar cycloaddition (click chemistry) of precursors containing antagonist functionalities. The four α-functionalized homopolymers were synthesized using appropriate initiators possessing in α-position either the azide or the alkyne function, as depicted in Schemes 1−4. Three out of the four functionalized initiators were specifically designed to entail the functionality required for the subsequent coupling of the homopolymers by click chemistry (Scheme 5). Both azido- and alkyno-bromoester derivatives, **3** and **6,** aimed at triggering ATRP of DMAEMA were readily prepared by conventional reactions, from commercially available reagents, 2-(2-chloroethoxy)ethanol and propargyl alcohol, respectively (Schemes 1 and 2). Compound **6** was prepared following a similar procedure to that reported by Matyjaszewski and colleagues.[29] As for the aminoazide derivative **9** serving for the synthesis of the α-azido PBLG, it was obtained as described in Scheme 3, following an already published procedure.[30] Compounds **3**, **6**, and **9** were obtained in good yields and their purity was checked by ^{1}H and ^{13}C NMR spectroscopy (see Experimental Section). Finally the commercially available propargyl amine **11** was used to prepare the α-alkyne-PBLG (Scheme 4). As discussed below, we found that the alkyne hydrogen did not interfere either with ATRP of DMAEMA using **6** or with ROP of γ-benzyl-L-glutamate *N*-carboxyanhydride from **9**. Therefore, there was no need to protect the acetylene function by a trimethylsilyl group, as sometimes has been reported.[17−20]

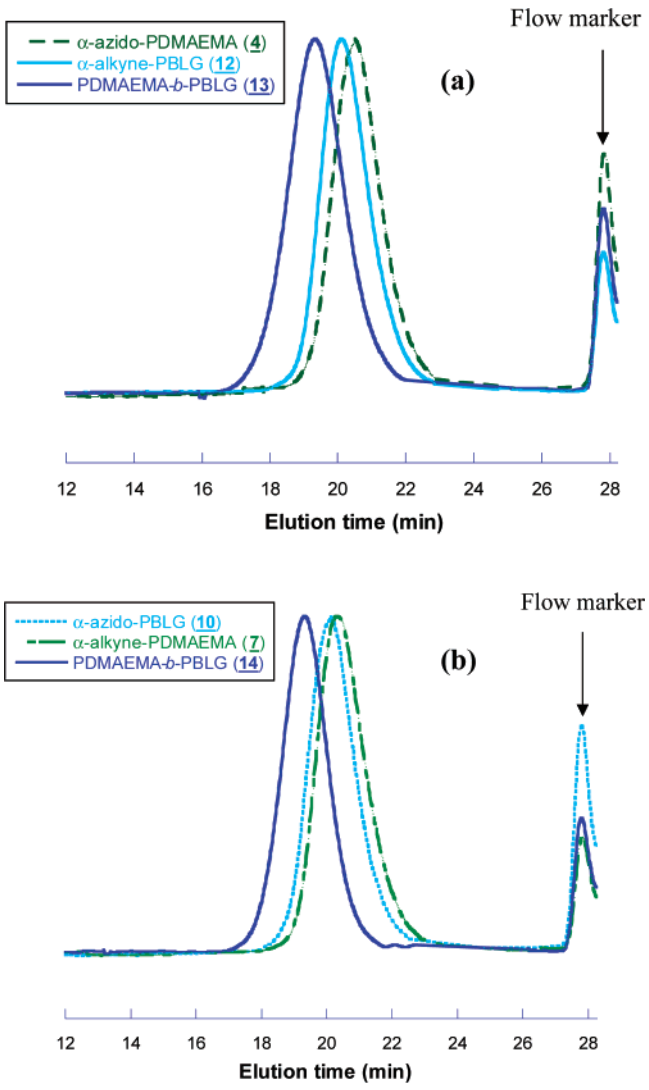


Figure 3. SEC trace in DMF in the presence of LiBr at 60 °C of PBLG-*b*-PDMAEMA diblock copolymers **13** (a) and **14** (b) also showing the starting homopolymeric precursors: α-azido-PDMAEMA **4** and α-alkyne-PBLG **12** (a); α-alkyne-PDMAEMA **7** and α-azido-PBLG **10** (b).

There have been several reports on ATRP of DMAEMA using different initiators than **3** and **6** with the purpose of obtaining well-defined pH-sensitive PDMAEMA's chains and related copolymers which exhibit self-assembly properties in water.[32−36] ATRP of DMAEMA can be performed under controlled conditions in a variety of organic solvents, including tetrahydrofuran (THF), dichlorobenzene but also in aqueous or methanolic solutions or even in bulk at room temperature. Well-defined PDMAEMAs can thus be synthesized by ATRP under relatively mild conditions, using copper bromide (CuBr) generally complexed by 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) as ligand, and 2-ethylbromoisobutyrate or 2-[monomethoxy-capped-poly(ethylene oxide)]bromoisobutyrate as the (macro)initiator.[32−36] In our case, the α-azido and α-alkyne PDMAEMAs, **4** and **7**, were prepared by ATRP of DMAEMA at 60 °C in THF, in the presence of CuBr/HMTETA as the catalytic system, from **3** and

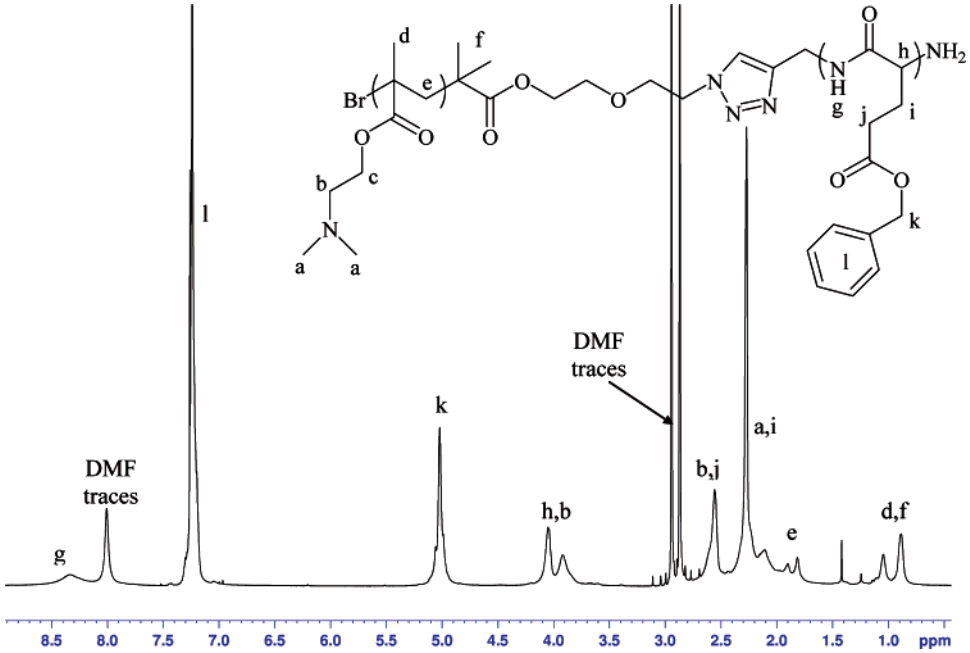


Figure 4. ^{1}H NMR ($CDCl_3$, 400 MHz) of PBLG-*b*-PDMAEMA diblock copolymer **13**.

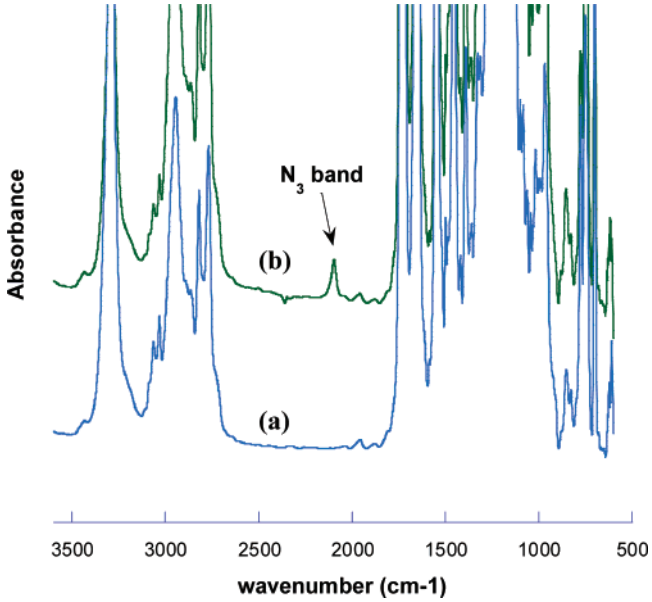


Figure 5. FTIR of PBLG-*b*-PDMAEMA diblock copolymer **13** (a) and of the corresponding homopolymers **4** and **12** (b).

6 as the initiator, respectively. Under such conditions, both the molar masses and the polydispersities of the samples could be controlled, as attested by ^{1}H NMR calculation of molar masses and by size exclusion chromatography (SEC) in hot DMF which showed monomodal traces and polydispersity indices less than 1.17 (Figure 1). In both cases, protons of PDMAEMA chain ends introduced by the functionalized initiators were clearly identified by ^{1}H NMR, which provided full evidence for a very high fidelity of both the azido and the alkyne functions of these PDMAEMA precursors. A typical ^{1}H NMR spectrum recorded in $CDCl_3$ of each α-functionalized PDMAEMA is shown in Figure 1. In the case of compound **7**, the signal of the terminal methylene protons (−C*H*$_2$−C≡CH) clearly appears at 4.6 ppm and the methylene protons in the α-position of the ester group of the PDMAEMA main chains are detected around 4 ppm. Values of molar masses delivered by SEC were relative to polystyrene standards used to calibrate the columns, so more accurate values were provided by ^{1}H NMR using the following equation

$$M_{n,PDMAEMA} = \frac{I_b M_{DMAEMA}}{I_f} + M_{init}$$

where I_b, I_f, M_{DMAEMA}, and M_{init} are, respectively, the intensity of methylene protons b from the side groups, the intensity of methylene protons f from the PDMAEMA main chain, the molar mass of the DMAEMA monomer unit and the molar mass of initiator **3** or **6**. Good agreement between experimental and theoretical molar masses confirmed the formation of well-defined PDMAEMAs and attested to the controlled character of ATRP of DMAEMA in THF at 60 °C using **3** and **6** as initiators (Table 1).

ROP of Bz-L-GluNCA was subsequently carried out in DMF at room temperature in the presence of **9** and **11** (Schemes 3 and 4). Masuda and colleagues.[37] previously reported the use of propargylamine **11** as initiator for ROP of the same monomer with the aim of synthesizing macromonomers. When subjected to a rhodium-catalyzed polymerization and copolymerization with an alanine-derived *N*-propargylamide, these macromonomers formed graft copolymers with a polyacetylene main chain. The authors performed the ROP of Bz-L-GluNCA in THF as solvent and noted that partial precipitation of the α-alkyne PBLG occurred in this solvent, giving rise to heterogeneous polymerization mixtures for degrees of polymerization (DPs) higher than 20. It was assumed that the heterogeneity of the polymerization was responsible for the obtainment of high polydispersity index (PDI) values.[37] In our case, when DMF was used as solvent, we did not observe any aggregation phenomenon/precipitation during ROP of Bz-L-GluNCA at room temperature. Moreover, as reported by Deming,[24] the conditions the best suited for SEC analysis of synthetic polypeptides such as PBLG consisted of using DMF as solvent of elution at 60 °C in the presence of LiBr in order to minimize the aggregation phenomena of PBLG blocks. In contrast to THF used as solvent at room temperature for SEC characterization, we observed monomodal and narrow molar masses distribution in hot DMF using LiBr (Figure 3). For the same reason mentioned above

for PDMAEMAs, molar masses were accurately determined from ^{1}H NMR spectroscopy (Experimental Section). Indeed, characteristic protons of chain ends arising from α-functionalized initiators **9** and **11** were clearly identified in the ^{1}H NMR spectra of PBLG's **10** and **12** run in $CDCl_3$ + 15%TFA. For instance, the methylenic protons adjacent to the azido function in **10** ($-CH_2CH_2N_3$) were observed at 3.3 ppm. Relative integration of this peak compared to that characteristic of protons of the PBLG main chain, e.g., methylenic protons due to the benzylic groups at 5.0 ppm (Figure 2), allowed us to determine the molar mass of polymer **10** in $CDCl_3$ in the presence of 15% of trifluoroacetic acid. In the case of polymer **12**, its molar mass was estimated from the relative intensity of peak at 3.95 ppm due to the methylenic protons in α-position of the acetylene function ($-CH_2-C\equiv CH$) with a peak characteristic of methylenic protons of the PBLG chain centered at 5.0 ppm. In both cases, the molar masses of the α-functionalized homopolymers **10** and **12** were close to the expected values and PDIs remained below 1.17, attesting to a quantitative introduction in α-position of the azido or the acetylene function in PBLG chains. Importantly, no interference of the azido or the alkyne α-functional group was noted during ROP of Bz-L-GluNCA.

Next was thus the "click chemistry" between the α-azide-PBLG **10** with the α-alkyne-PDMAEMA **7**, in the one hand, and of the α-alkyne-PBLG **12** with the α-azide-PDMAEMA **4**, on the other hand (Scheme 5). These copper(I)-catalyzed 1,3-dipolar cycloaddition coupling reactions were conveniently performed in solution in DMF at room temperature using CuBr complexed by pentamethyldiethylenetriamine (PMDETA).

After 24 h of reaction, click chemistry afforded the targeted PBLG-*b*-PDMAEMA block copolymers **13** and **14**, as illustrated in Scheme 5 and summarized in Table 1. In both cases, a slight excess of PDMAEMA was used (1.2 equiv) in order to drive the coupling reactions to completion. This excess of PDMAEMA was easily removed from the block copolymers afterward because it was retained onto neutral alumina used as the stationary phase of the column chromatography when eluted from the crude DMF solution. We indeed verified that PDMAEMAs **4** and **7** were not eluted on neutral alumina. The pure diblock copolymers **13** and **14** were thus obtained by precipitation in excess diethylether. Scheme 5 shows the slight difference between the two diblock compounds **13** and **14** due to the different position of the triazole ring separating the two types of blocks. Both SEC traces showed a clear shift toward the higher molar mass region after click chemistry in comparison with the starting homopolymers (Figure 3). Noteworthy, both SEC traces of diblock copolymers **13** and **14** were unimodal and symmetrical while remaining quite narrow, which attested to an efficient diblock formation.

The structure of both block copolymers was confirmed by ^{1}H NMR spectroscopy: Figure 4 shows a typical ^{1}H NMR in $CDCl_3$ spectrum of a purified diblock compound with all expected peaks. Efficiency of 1,3-dipolar cycloaddition couplings was also supported by FTIR analysis before and after coupling reaction. Figure 5 shows the FTIR spectrum of a mixture of PDMAEMA **4** and PBLG **12** homopolymers before coupling as well as the FTIR spectrum of the resulting copolymer after click chemistry. The complete disappearance of the azide signal at 2100 cm^{-1} in FTIR perfectly confirms completion of the coupling reactions.

A final treatment of these PBLG-*b*-PDMAEMA block copolymers with KOH in THF led to the expected PGA-*b*-PDMAEMA DHBC. Interestingly, such DHBC could be readily dissolved in water without the need of an organic cosolvent. We are in the process of investigating the self-assembly properties in water of these DHBC in details. Preliminary investigations by dynamic light scattering revealed the formation of well-defined micellar aggregates above the LCST of the PDMAEMA block, in the 100 nm range. Very recent measurements by small-angle neutron scattering have also been performed, and we are currently fitting the corresponding data to have an insight into the morphology of the nano-objects formed. Complete description of the self-assembling properties of such DHBC will be the topic of forthcoming publications.

Conclusion

Azido and akyne functionalities can be introduced as end groups in both PBLG and PDMAEMA by utilizing appropriate α-functionalized initiators for ROP of γ-benzyl-L-glutamate *N*-carboxyanhydride and copper-mediated ATRP of 2-(dimethylamino)ethyl methacrylate. Both types of polymerization prove "controlled/living" processes, which ensure control over molar masses and polydispersities of the corresponding homopolymers as well as a quantitative functionalization of polymer chain ends. Block copolymers composed of a PBLG sequence and a PDMAEMA block can be subsequently derived by the Huisgen's 1,3-dipolar cycloaddition ("click chemistry") from homopolymers possessing the azide and the alkyne functionalities, using a slight excess of the PDMAEMA precursor. Removal of the excess PDMAEMA is made easy by the retention of this polymer onto neutral alumina used as stationary phase of the column chromatography during purification. On the basis of size exclusion chromatography and NMR and IR spectroscopy, coupling reactions prove quantitative, yielding pure diblock copolymers composed of (i) a PBLG block capable of adopting either a rod-like α-helix conformation or a coil conformation as a function of the temperature and (ii) a PDMAEMA that is a temperature and pH-sensitive water-soluble and polymeric building block. On the other hand, PBLG is the convenient precursor of poly(glutamic acid) (PGA) which is an interesting pH-sensitive water-soluble and biocompatible polymer, whereas PDMAEMA can be readily protonated in physiological media affording polycationic condensing agent for non-viral-DNA. Association of PGA and cationized PDMAEMA therefore leads to hybrid double hydrophilic block copolymers combining biocompatibility, water solubility, and both pH and temperature sensitiveness. Investigations on the self-assembly properties in bulk and in solution of such rod−coil block copolymers are in progress and will be the topics of forthcoming publications.

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