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Controlled synthesis of amphiphilic block copolymers based on polyester and poly(amino methacrylate): Comprehensive study of reaction mechanisms

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Abstract

The synthesis of amphiphilic and adaptative block copolymers has been envisioned following a commutative two-step strategy involving atom transfer radical polymerization (ATRP) and the Huisgen-1,3-dipolar cycloaddition techniques. The reliability of this strategy is based on the use of an azido-containing ATRP initiator, the 2-(2-azidoethoxy)ethylbromoisobutyrate (N_3E^iBBr), able to be "clicked" to an alkyne-terminated derivative and to promote the ATRP polymerization from the active site. In the context of this work, an alkyne-terminated poly(ε-caprolactone) produced by ring-opening polymerization (ROP) of CL was employed as hydrophobic "clickable" segment. The N_3E^iBBr initiator was obtained by nucleophilic substitution of the chloride atom from 2-(2-chloroethoxy)ethanol by an azide function and followed by the esterification of the hydroxy function by bromoisobutyryl bromide. This initiator was employed in polymerization of *N*,*N*-dimethylamino-2-ethyl methacrylate (DMAEMA) monomer by ATRP in THF at 60 °C using CuBr complexed by 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) as catalytic complex. Low initiation efficiencies were obtained and they were ascribed to intramolecular cyclization during the polymerization as evidenced by ESI-MS and 2D NMR spectroscopy. The "Click" coupling reaction was performed in THF at r.t. and was found to be efficient when using CuBr complexed by 2,2′-bipyridine ligand. To circumvent the low initiation efficiency, the N_3E^iBBr could be "clicked" in a first step to PCL precursors before initiating the polymerization of DMAEMA monomer by ATRP. In this context, various catalytic complexes in different composition ratio were employed to optimize the "click" coupling step. Moreover, this strategy was found to be suitable to produce well-defined PCL-b-PDMAEMA block copolymers, characterized by narrow polydispersity indices. Since ATRP and the Huisgen-1,3-dipolar cycloaddition both require the use of a copper(I)-based catalyst, the two first strategies were merged in a "one-pot" process in order to obtain in one step a well-defined block copolymer characterized by a narrow polydispersity index and predictable composition and block lengths.

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1. Introduction

Well-defined synthetic amphiphilic and biocompatible block copolymers, in their diversities (selective solubility, molar mass balance, charge repartition, etc.), are extensively applied in various biotechnological areas such as prodrug systems, protein delivery, gene therapy, bioassays, or bioseparation [1–5]. Some of these block copolymers are called "smart" because they include at least one stimuli-responsive sequence, which brings further modulation potentialities to physico-chemical and self-assembling properties of the end-product. Among numerous examples, poly(*N*,*N*-dimethylamino-2-ethyl methacrylate) (PDMAEMA) is widely used as thermo- and pH-sensitive polymer [6–9]. Well-defined PDMAEMA can be obtained by controlled radical polymerization (CRP), and more particularly by atom transfer radical polymerization (ATRP), as first reported by Zhang et al. using either CuBr · 1,1,4,7,10,10-hexamethyltriethylenetetramine (CuBr · HMTETA) or CuBr · 2,2′-bipyridine (CuBr · Bpy) and halogenoesters as catalyst and initiator, respectively [10]. Generally, several routes may be considered for the synthesis of block copolymers among which the successive polymerization of two or more monomers with similar chemical reactivity (e.g., similar radical reactivity), the use of heterofunctional initiators allowing controlled polymerization of monomers with different reactivity (e.g., anionic and radical reactivity) and the so-called macroinitiator technique in which an adequately functionalized macromolecular precursor triggers the polymerization of a second monomer [11]. More recently, a novel method has been successfully applied for producing block copolymers, combining pericyclic [2 + 3] "Click" reaction and CRP such as reversible addition fragmentation chain transfer (RAFT) [12] and ATRP [13–15]. "Click" reactions, as termed by Sharpless et al. [16], have gained a great deal of attention due to their high specificity, quantitative yields and near-perfect fidelity in the presence of most functional groups. The most popular "Click chemistry" reaction is the copper(I)-catalyzed Huisgen-1,3-dipolar cycloaddition reaction between an azide and an alkyne leading to a 1,4-disubstituted 1,2,3-triazole structure [17,18]. Interestingly, both "Click chemistry" and ATRP mechanisms may require the use of a copper(I)-based catalyst making possible to combine them in a one-step strategy. To the best of our knowledge, the one-pot ATRP- "Click coupling" process has only been tempted for the synthesis of homo- or heterotelechelic polystyrene and poly(methyl methacrylate) [19,20].

Some of us have recently reported in a short communication the ATRP-"Click coupling" process for the preparation of amphiphilic, biocompatible and adaptative poly(ε-caprolactone)-b-poly(*N*,*N*-dimethylamino-2-ethyl methacrylate) (PCL-b-PDMAEMA) block copolymers [21]. The key strategy of this process relied on the preparation of an azido-containing initiator, the 2-(2-azidoethoxy)ethyl bromoisobutyrate (N_3E^iBBr) able to react by "Click chemistry" from the azido group and to initiate the ATRP of DMAEMA monomer from the bromoisobutyryl function. In a first strategy, the ATRP of DMAEMA was initiated by N_3E^iBBr yielding to α-azido poly(*N*,*N*-dimethylamino-2-ethyl methacrylate) (N_3–PDMAEMA) followed by the "Click" coupling process with alkyne-terminated PCL. The PCL block was synthesized by a perfectly controlled ring-opening polymerization operating via "living" coordination-insertion mechanism yielding to α-isopropoxy, ω-4-pentynoate poly(ε-caprolactone) (PCL–C≡CH). In the second strategy, the PCL–C≡CH block reacts by "Click" chemistry with N_3E^iBBr in order to generate α-isopropoxy, ω-2-(2-triazole ethoxy)ethyl bromoisobutyrate poly(ε-caprolactone) (PCL–Br) macroinitiator, followed by the ATRP of DMAEMA. Last but not least, both mechanisms could be combined in a one-pot process to generate in a one-step the expected amphiphilic block copolymer. In our short communication [21], optimized conditions for each step of the aforementioned strategies have been reported. However, before reaching these optimisations, many difficulties have been encountered, particularly in terms of mechanisms and choice of catalytic complexes, leading respectively to the formation of unexpected side-products and sharp decrease of the reactivity/reaction of control. Herewith, a comprehensive investigation of the reaction mechanisms versus experimental conditions, e.g., type of catalyst ligands, is proposed. Accordingly, characterization techniques like mass spectrometry

and multinuclear NMR spectroscopy have been approached.

2. Experimental section

2.1. Materials

ε-Caprolactone (CL, from Acros, 99%) was dried over calcium hydride for 48 h at room temperature and distilled under reduced pressure just before use. Aluminum triisopropoxide ($Al(O^iPr)_3$, from Acros, 98%) was purified by distillation under reduced pressure, quenched in liquid nitrogen, rapidly dissolved in dry toluene and stored under nitrogen. Accurate concentration was determined by back complexiometric titration of Al^{3+} using ethylenediaminetetraacetic acid disodium salt and $ZnSO_4$ at pH 4.8. *N*,*N*-dimethylamino-2-ethyl methacrylate (DMAEMA, from Aldrich, 98%) was passed through a column of basic alumina to remove the stabilizing agent and distilled over CaH_2 prior use. Copper bromide (CuBr, from Fluka, 98%) was purified by solubilization of oxidized copper(II) derivatives in glacial acetic acid (from Acros), washed by ethanol (from Acros) under nitrogen and dried under reduced pressure. Pentynoic acid (from Aldrich, 95%), *N*,*N*-dicyclohexylcarbodiimide (DCCI, from Aldrich, 99%), *N*,*N*-dimethylamino-4-pyridine (DMAP, from Acros, 99%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, from Aldrich, 97%), 2,2′-bipyridine (Bpy, from Acros, 99%), sodium azide (NaN_3, from Acros, 99.5%), 2-(2-chloroethoxy)ethanol (from Aldrich, 99%) and 2-bromoisobutyryl bromide (from Aldrich, 98%), ethyl acetate (EtOAc, from Aldrich, 99.5%) were used as received. Triethylamine (from Fluka, 99%) was dried over barium oxide for 48 h at r.t. and distilled under reduced pressure. Toluene (Labscan, 99%) was dried by refluxing over CaH_2 and distilled just before use. Tetrahydrofuran (THF, Labscan, 99%) was previously dried over molecular sieves (4 Å) and distilled over polystyryl lithium complex. Dichloromethane (CH_2Cl_2, from Aldrich, 99%) was dried over calcium hydride for 48 h at r.t. and distilled under reduced pressure.

2.2. Synthesis of α-isopropoxy, ω-hydroxy poly(ε-caprolactone) (PCL–OH)

Briefly, 30 ml (0.27 mol) of ε-caprolactone was added to 223 ml toluene in a round bottom flask under inert atmosphere. Polymerization was initiated at 0 °C by adding 17.5 ml (14 mmol) of aluminum triisopropoxide in toluene solution (0.77 mol L^{-1}). After 10 min, the reaction was stopped by adding 10 ml aqueous HCl solution (1 mol L^{-1}) and the polyester was selectively recovered by precipitation in cold heptane, filtration and drying under reduced pressure (yield > 99%). Al residues were removed by liquid/liquid extraction and α-isopropoxy, ω-hydroxy poly(ε-caprolactone) (PCL–OH) was recovered by precipitation. ^1H NMR (500 MHz, $CDCl_3$, δ (ppm), not shown here): 1.1 (d, $(CH_3)_2CH$–O–), 1.3–1.8 (m, –$(CH_2)_3$–), 2.3 (t, –CH_2–COO–), 3.65 (t, –CH_2–OH), 4.05 (t, –CH_2–OCO–), 5.1 $(CH_3)_2CH$–O–), M_n NMR = 2400 g mol^{-1}, M_n theor = 2300 g mol^{-1}, M_n ESI-MS = 2400 g mol^{-1}, M_n SEC = 2100 g mol^{-1} and $M_w/M_n = 1.28$.

2.3. Synthesis of α-isopropoxy, ω-4-pentynoate poly(ε-caprolactone) (PCL–C≡CH)

In a previously dried and nitrogen purged round bottom flask were dissolved 2.7 g of 4-pentynoic acid (28 mmol) and 2.6 g of *N*,*N*′-dicyclohexylcarbodiimide (DCCI, 13 mmol) in CH_2Cl_2 (25 ml). The mixture was stirred for 6 h at 0 °C to form dipentynoic anhydride. In a second dried round bottom flask, 14.5 g of PCL–OH (6 mmol, M_n = 2400 g mol^{-1}) and 0.249 g of DMAP (2 mmol) were dried by three successive toluene azeotropic distillations before adding 25 ml CH_2Cl_2. These solutions were mixed for 48 h at r.t. and α-isopropoxy, ω-4-pentynoate-poly(ε-caprolactone) (PCL–C≡CH) was recovered by selective precipitation in cold methanol, filtration and drying at 40 °C under reduced pressure. Yield: 87%. ^1H NMR (500 MHz, $CDCl_3$, δ (ppm), Fig. 1B): 1.1 (d, $6H_g$), 1.3–1.8 (m, $6H_{b,c}$), 1.9 (s, $1H_i$), 2.3 (t, $2H_a$), 2.45 (t, $2H_h$), 2.55 (t, $2H_e$), 4.05 (t, $2H_d$), 5.1 (sept, $1H_f$), M_n NMR = 2700 g mol^{-1}, M_n ESI-MS = 2665 g mol^{-1}, M_n SEC = 2600 g mol^{-1} and $M_w/M_n = 1.20$.

2.4. Synthesis of 2-(2-azidoethoxy)ethanol

In a round bottom flask surmounted by a reflux column were introduced 20 g of 2-(2-chloroethoxy)ethanol (161 mmol), 52.2 g of NaN_3 (803 mmol) and 80 ml of water. The mixture was refluxed for 24 h, then cooled down and treated with aqueous HCl solution (1 mol L^{-1}). The aqueous solution was extracted by EtOAc and the organic

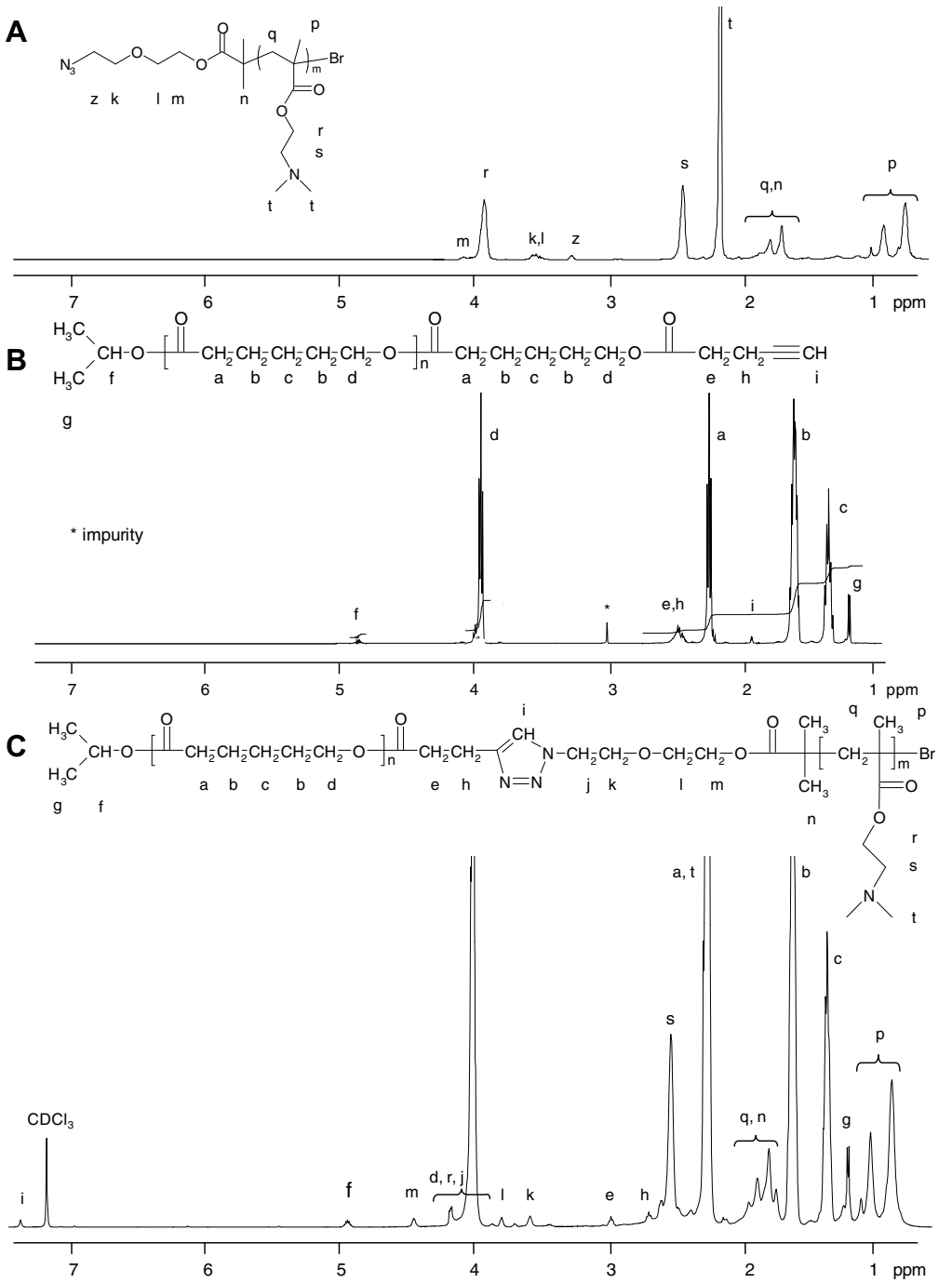


Fig. 1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) (A) of N_3-PDMAEMA as obtained by ATRP of DMAEMA initiated by N_3E^iBBr, (B) PCL–C≡CH as obtained by ROP and (C) PCL-b-DMAEMA as obtained by "Click chemistry".

solvent was evaporated under reduced pressure. Yield: 92%. ^{13}C NMR (300 MHz, D_2O, δ (ppm), not shown here): 50.96 (N_3–CH_2–), 61.22 (–CH_2–OH), 69.75 (N_3–CH_2–CH_2–O), 72.33 (O–CH_2–CH_2–OH).

2.5. Synthesis of 2-(2-azidoethoxy)ethyl bromoisobutyrate (N_3E^iBBr)

In a round bottom flask were introduced 15 g of 2-(2-azidoethoxy)ethanol (114 mmol), 32 ml of

NEt_3 (228 mmol) and 60 ml of THF. A solution of 15.6 ml of 2-bromoisobutyryl bromide (126 mmol) in 60 ml of THF was added dropwise at 0 °C. After 48 h at r.t., insoluble ammonium salts were filtered off and the filtrate was passed over a basic alumina column. The final product was recovered by evaporation of THF. Yield: 70%. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm), not shown here): 1.9 (s, $6H_a$), 3.35 (t, $2H_e$), 3.65 (t, $2H_d$), 3.75 (t, $2H_c$), 4.3 (t, $2H_b$). ^{13}C NMR (300 MHz, $CDCl_3$, δ (ppm), not shown here): 31 ($C(CH_3)_2$), 51 (N_3–CH_2–), 56 (–CH_2–O–C(O)–), 65.3 ($C(CH_3)_2$–), 69.1 (N_3–CH_2–CH_2–O), 70.5 (O–CH_2–CH_2–), 171.7 (O–C(O)–).

2.6. Synthesis of α-isopropyloxy, ω-2-(triazolethoxy)-ethyl bromoisobutyrate poly (ε-caprolactone) (PCL–Br) (see first step in Scheme 2)

Under inert atmosphere were introduced 0.014 g of CuBr (0.098 mmol), 0.048 g of 2,2′-dipyridyl (0.31 mmol), 0.251 g of PCL–C≡CH (0.094 mmol), 0.035 g of N_3E^iBBr (0.13 mmol) and 2.6 ml of THF. After 24 h at r.t., the catalytic complex was removed by filtration of the polymer solution through a basic alumina column. The final product (PCL–Br) was recovered by solvent evaporation. Yield: 95%. ^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm), Fig. 5): 1.1 (d, $6H_g$), 1.3–1.8 (m, $6H_{b,c}$), 1.95 (s, $6H_n$), 2.3 (m, $2H_a$), 2.7 (t, $2H_h$), 3.0 (t, $2H_e$), 3.7 (t, $2H_k$), 3.9 (t, $2H_l$), 4.1 (t, $2H_d$), 4.3 (t, $2H_j$), 4.5 (t, $2H_m$), 5.0 (m, $1H_f$), 7.4 (s, $1H_i$).

2.7. Synthesis of poly(ε-caprolactone-block-N,N-dimethylamino-2-ethyl methacrylate) (PCL-b-PDMAEMA) (see Scheme 2)

Under inert atmosphere were introduced 0.013 g of CuBr (0.093 mmol), 0.039 g of 2,2′-bipyridyl (0.25 mmol), 0.229 g of PCL–C≡CH (0.086 mmol), 0.026 g of N_3E^iBBr (0.091 mmol) and 2.6 ml of THF. After 24 h at r.t., the temperature was raised up to 60 °C and 0.7 ml of freshly distilled DMAEMA (4.55 mmol) was added. After 22 min of polymerisation, the copolymer was recovered by precipitation in cold heptane, filtration and drying. Yield: 68%. Copper catalyst was removed out by passing the copolymer solution in THF through a basic alumina column and the organic solvent was evaporated. ^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm), not shown here): 0.7–1.05 (m, $3H_p$), 1.1 (d, $6H_g$), 1.35 (m, $2H_c$), 1.61 (m, $4H_b$), 1.8–1.95 (m, $2H_q + 6H_n$), 2.3 (t, $2H_a + 6\ H_t$), 2.6 (t, $2H_s$), 2.75 (t, $2H_h$), 3.05 (t, $2H_e$), 3.65 (t, $2H_k$), 3.9 (t, $2H_l$), 4.05 (t, $2H_d + 2H_r + 2H_j$), 4.50 (t, $2H_m$), 5.0 (m, $1H_f$), 7.5 (s, $1H_i$). M_n theor = 10,520 g mol^{-1}, M_n NMR = 11,700 g mol^{-1} and $M_w/M_n = 1.34$.

2.8. Synthesis of α-azido poly(N,N-dimethylamino-2-ethyl methacrylate) (N_3-PDMAEMA) (see first step in Scheme 1)

In a round bottom flask were introduced 0.263 g of CuBr (1.8 mmol), 0.893 g of HMTETA (3.6 mmol) and 15 ml of DMAEMA (89 mmol). Three freezing/thawing cycles were performed under vacuum to get rid of trapped O_2. In a second round bottom flask were introduced 0.5 g of 2-(2-azidoethoxy)ethyl bromoisobutyrate (N_3E^iBBr, 1.8 mmol) and 2.5 ml of THF previously deprived of its stabilizer by filtration through a basic alumina column. N_2 was bubbled through the solution before transferring it into the first flask. After 22 min at 60 °C, the polymer was recovered by precipitation in cold heptane, filtration and drying. Yield: 19%. Copper catalyst was removed out by passing the polymer solution in THF through a basic aluminum oxide column and the organic solvent was evaporated. ^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm), Fig. 1A): 0.7–1.1 (m, $3H_p$), 1.6–1.9 (m, $2H_q + 6H_n$), 2.15 (s, $6H_t$), 2.45 (t, $2H_s$), 3.25 (t, $2H_z$), 3.5 (t, $2H_l$), 3.55 (t, $2H_k$), 3.9 (t, $2H_s$), 4.1 (t, $2H_m$). M_n NMR = 3600 g mol^{-1} and M_w/M_n = 1.24.

2.9. Synthesis of poly(ε-caprolactone-b-N,N-dimethylamino-2-ethyl methacrylate) block copolymer (PCL-b-PDMAEMA) (see second step in Scheme 1)

Under inert atmosphere were introduced 0.014 g of CuBr (0.098 mmol), 0.048 g of 2,2′-bipyridyl (0.31 mmol), 0.338 g of N_3-PDMAEMA (0.094 mmol, Table 1, entry 2), 0.251 g PCL–C≡CH (0.094 mmol) and 2.6 ml of THF. After 72 h at r.t., the reaction medium was diluted with THF, the catalytic complex was removed by passing through a basic alumina column and the organic solvent was evaporated under reduced pressure. Yield: 99%. ^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm), Fig. 1C): 0.7–1.05 (m, $3H_p$), 1.1 (d, $6H_g$), 1.35 (m, $2H_c$), 1.61 (m, $4H_b$), 1.8–1.95 (m, $2H_q + 6H_n$), 2.3 (t, $2H_a + 6H_t$), 2.6 (t, $2H_s$), 2.75 (t, $2H_h$), 3.05 (t, $2H_e$), 3.65 (t, $2H_k$), 3.90 (t, $2H_l$), 4.05 (t,

Table 1
Effect of the catalytic system on the "click reaction" conversion between 2-(2-azidoethoxy)ethyl bromoisobutyrate (N_3E^iBBr) and α-isopropoxy, ω-4-pentynoate poly(ε-caprolactone) (PCL–C≡CH) after 24 h reaction at r.t. in THF

Entry	CuBr · 3L	$[N_3E^iBBr]_0/[CuBr]_0/[L]_0$	Conv.[a] (%)
1	CuBr · 3HMTETA	1/1/3	55
2	CuBr · 3HMTETA	1/2/6	75
3	CuBr · 3HMTETA	1/3/9	75
4	CuBr · 3Bpy	1/1/3	>99

[a] As determined by ^{1}H NMR spectroscopy (500 MHz) from the relative intensities of triazole methine (H_i), isopropoxy methine (H_f) and residual azido methylene protons (H_z): Conv. (%) = $(I_i/I_f - (I_z/2))) \times 100$ (see Fig. 5).

$2H_d + 2H_r + 2H_j$), 4.50 (t, $2H_m$), 5.0 (m, $1H_f$), 7.5 (s, $1H_i$). $M_w/M_n = 1.50$.

2.10. One-pot synthesis of poly(ε-caprolactone-b-N,N-dimethylamino-2-ethyl methacrylate) (PCL-b-PDMAEMA) (see Scheme 3)

Under inert atmosphere were introduced 0.013 g of CuBr (0.090 mmol), 0.039 g of 2,2′-bipyridyne (0.246 mmol), 0.229 g of PCL–C≡CH (0.086 mmol), 0.026 g of 2-(2-azidoethoxy)ethyl bromoisobutyrate (N_3E^iBBr, 0.091 mmol), 0.7 ml of *N*,*N*-dimethylamino-2-ethyl methacrylate (4.15 mmol) and 1.8 ml of THF. After 24 h at r.t., temperature was raised up to 60 °C for 6 h to promote DMAEMA polymerization. The reaction medium was diluted with THF, the catalytic complex was removed by passing through a basic alumina column and the organic solvent was evaporated under reduced pressure. Yield: 95%. ^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm), not shown here): 0.7–1.05 (m, $3H_p$), 1.1 (d, $6H_g$), 1.35 (m, $2H_c$), 1.61 (m, $4H_b$), 1.8–1.95 (m, $2H_q + 6H_n$), 2.3 (t, $2H_a + 6H_t + 6H_{t'}$), 2.6 (t,$2H_s + 2H_{s'}$), 2.75 (t, $2H_h$), 3.05 (t, $2H_e$), 3.65 (t, $2H_k$), 3.90 (t, $2H_l$), 4.05 (t, $2H_d + 2H_r + 2H_j$), 4,1 (t, $2H_{r'}$), 4.50 (t, $2H_m$), 5.0 (m, $1H_f$), 5.6–6.15 (d, $2H_{u,v}$), 7.5 (s, $1H_i$). M_n theor = 11,000 g mol^{-1}, M_n NMR = 13,800 g mol^{-1} and $M_w/M_n = 1.31$.

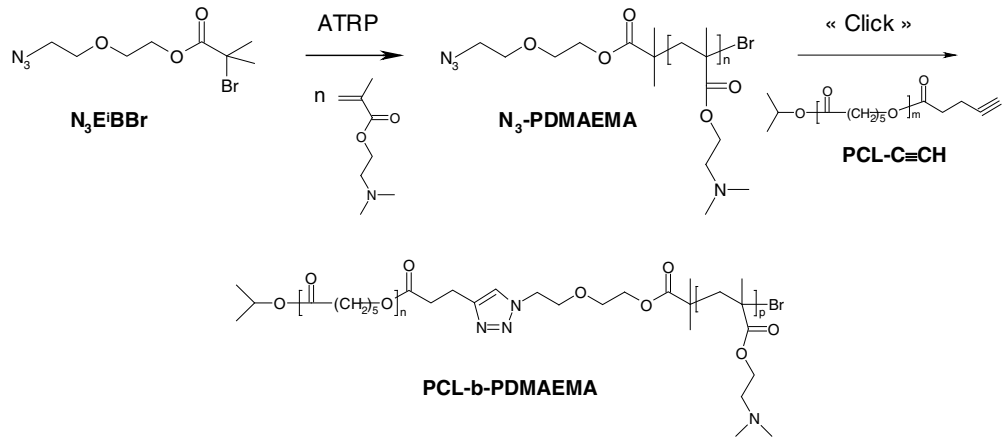
2.11. Characterization

^{1}H NMR spectra were recorded using a Bruker AMX-300 or AMX-500 apparatus at r.t. in $CDCl_3$ and D_2O (30 mg/0.6 ml). Size exclusion chromatography (SEC) was performed in THF (for polyesters) or THF + 2 wt% NEt_3 (for (co)polymers containing amino methacrylate) at 35 °C using a Polymer Laboratories liquid chromatograph equipped with a PL-DG802 degasser, an isocratic HPLC pump LC 1120 (flow rate = 1 ml/min), a Marathon autosampler (loop volume = 200 μl, solution conc. = 1 mg/ml), a PL-DRI refractive index detector and three columns: a PL gel 10 μm guard column and two PL gel Mixed-B 10 μm columns (linear columns for separation of MW_{PS} ranging from 500 to 10^6 daltons). Polystyrene and poly(methyl methacrylate) standards were used for calibration. Mass spectrometry measurements were performed on a Waters QToF2 apparatus equipped with an orthogonal electrospray ionisation (ESI) source (Z-spray) operating in positive ion mode. Samples were dissolved in acetonitrile ($\sim 10^{-5}$ mol L^{-1}) and infused into the ESI source at 5 μL min^{-1} rate with a Harvard syringe pump. Typical ESI conditions were capillary voltage 3.1 kV, cone voltage 80 V, source temperature 80 °C and desolvation temperature 120 °C. Dry nitrogen was used as the ESI gas. The quadrupole was set to pass ions from 100 to 3000 Th and all ions were transmitted into the pusher region of the time-of-flight analyser for mass-analysis with 1 s integration time. Data were acquired in continuum mode until acceptable average data were obtained. Gas chromatography analysis was performed with a GCQ type from Finnigan (Interscience) equipped with Rtx-5Sil MS column (30 m, 0.25 mm, 0.25 μm) in 5/95 polydiphenylsiloxane/polydimethylsiloxane. The heating program started at 60 °C for 1 min, followed by a ramp of 10 °C/min until 250 °C and an isotherm at 250 °C for 2 min. Injector temperature was fixed at 250 °C. Helium was used as mobile phase with a 30 cm/s rate. Two microliters of the sample were introduced in the column and the experiment was operating in positive mode EI^+ (electronic ionization) and CI^+ (chemical ionization) with an electronic energy of 70 eV. In CI^+ mode, methane was used as the reactive gas and the temperature of the source was hold at 200 °C. The transfer line was maintained at 260 °C.

3. Results and discussion

3.1. Synthesis of PCL-b-PDMAEMA block copolymers via the "ATRP first" strategy

According to the two-step strategy illustrated in Scheme 1, the controlled radical polymerization of DMAEMA has first been initiated by N_3E^iBBr



Scheme 1. Synthesis of PCL-b-PDMAEMA block copolymer following the "ATRP first" strategy.

using CuBr · 2HMTETA as catalytic complex in THF at 60 °C, i.e. under conventional conditions [10].

Initial monomer concentration was set up at 5 mol L^{-1} for initial $[DMAEMA]_0/[N_3E^iBBr]_0/[CuBr]_0/[HMTETA]_0$ molar ratios of 50/1/1/2. After a polymerization time reduced at 22 min to limit the degree of polymerization, a monomer conversion as low as 19% has been reached. Fig. 1A shows the ^1H NMR spectrum of the isolated α-azido poly(*N*,*N*-dimethylamino-2-ethyl methacrylate) (N_3–PDMAEMA). The number average molar

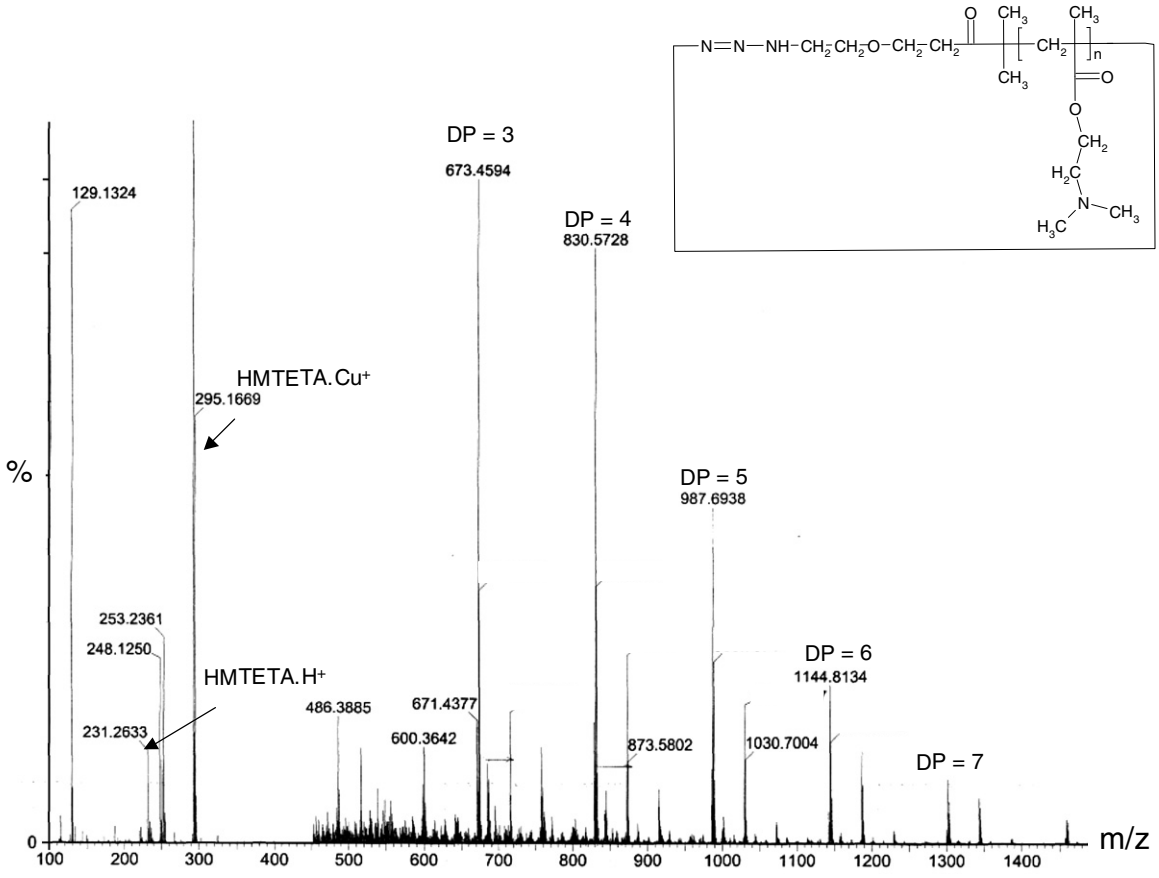


Fig. 2. ESI-MS spectrum of the presumed PDMAEMA cyclic derivative in $CDCl_3$.

mass of N_3–PDMAEMA has been determined from the relative intensity of terminal azido methylene protons at 3.3 ppm (H_z) and amino methyl protons of the repetitive units at 2.15 ppm (H_t) assuming that each polymer chain is end-capped by a 2-(2-azidoethoxy)ethyl isobutyrate group (M_n NMR $= (I_t/3I_z) \times M_W$ DMAEMA $+ M_W$ $N_3E^iBBr = 3600$ g mol^{-1}). Compared to the theoretical molar mass assuming a controlled process (M_n theor $=$ [DMAEMA]$_0$/[N_3E^iBBr]$_0 \times$ conv./100 $\times M_W$ DMAEMA $+ M_W$ $N_3E^iBBr = 1800$ g mol^{-1}), a very low initiation efficiency ($f = M_n$ theor/M_n NMR $=$ 0.41) is calculated. Such a behavior has been tentatively taken into account by the occurrence of intramolecular cyclization involving the azide moiety and the propagating center [20]. Indeed, it could be excluded that azido groups act themselves as initiators in ATRP since the relative intensities of protons from the 2-(2-azidoethoxy)ethyl isobutyrate group are kept unchanged, e.g., $I_z/(I_k + I_l)/I_m/I_n = 1/2/1/3$ (Fig. 1A). Such an active initiating role of azido groups would have also contributed to broaden the molar mass distribution, which contrasts with the narrow polydispersity observed by SEC ($M_w/M_n = 1.24$). In order to shed some light on the assumed intramolecular substitution of the bromide extremity by the azido species and therefore the resulting intramolecular cyclization, the polymerization of DMAEMA was initiated by N_3E^iBBr for a targeted DP of 2. The polymerization was carried out in the presence of the CuBr · 2HMTETA catalytic complex for an initial [DMAEMA]$_0$/[N_3E^iBBr]$_0$/[CuBr]$_0$/[HMTETA]$_0$ molar ratio of 2/1/1/2 and an initial monomer concentration of 5 mol L^{-1}. After a 5-min polymerization time in THF at 60 °C, the crude product was recovered by precipitation in cold heptane, purified by filtration through basic alumina column and characterized both by ESI-MS and NMR spectroscopy. As can be assumed from the isotopic distribution of the mono-charged family shown in the ESI spectrum (Fig. 2), the final structure does not contain any bromine extremity and is composed of few DMAEMA subunits. From the mass values of the predominant family, it can be assumed the presence of a cyclic structure incorporating a triazene moiety or an acyclic structure where the bromine atom is replaced by an hydrogen atom. Interestingly ^{1}H coupled to ^{13}C HSQCET 2D NMR studies tend to indicate the selective formation of cycles by the apparition of new protons q and f, corresponding to the protonated azide and the α-methylene azide protons, respectively, and new signals in the ^{13}C NMR spectrum at 31, 45 and 51 ppm corresponding to carbon atoms in the vicinity of the triazole link $C_{12'}$, $C_{13'}$ and C_8, respectively (Fig. 3). The selective formation of cycles rather than linear structures was further attested by DEPT ^{13}C NMR analysis attesting for the absence of terminal C–H type carbon signal. It is worth pointing out that N_3E^iBBr initiator did not prove to be able to self-react without incorporating DMAEMA subunits and therefore to yield cyclic derivatives when added with the Cu(I) catalyst (data not shown here).

In a next step, the 1,3-dipolar cycloaddition coupling reaction between N_3-PDMAEMA ($M_n = 3850$ g mol^{-1}) and PCL–C≡CH ($M_n = 2700$ g mol^{-1}) has been carried out by using CuBr · 3Bpy catalyst in THF at r.t. Initial concentrations in both azide and alkyne functions were maintained at 0.3 mol L^{-1} while it was taken great care to respect the exact stoichiometry ([N_3–PDMAEMA]$_0$/[PCL–C≡CH]$_0 = 1$). From ^{1}H NMR spectroscopy, it comes out that complete "Click" reaction conversion is reached, at least within the accuracy of ^{1}H NMR spectroscopy (Fig. 1C). No more azido end-group could be detected at 3.3 ppm while the relative intensities of polyester and polymethacrylate protons close to the triazole junction fit well with the theoretical ratios ($I_i/I_f/I_h/I_k/I_l/I_m = 1/1/2/2/2/2$). Fig. 4 shows that the SEC trace of the as-formed PCL-b-PDMAEMA diblock copolymer is shifted to lower elution volumes with respect to the starting PCL–C≡CH and N_3–PDMAEMA precursors ($M_w/M_n = 1.50$). This broad polydispersity might be ascribed to some trace of "unclicked" polymer precursors, not detected in the ^{1}H NMR spectrum. Indeed, a tiny shoulder can be observed in the SEC trace at higher retention volume.

3.2. Synthesis of PCL-b-PDMAEMA block copolymers via the "Click first" strategy

According to the two-step strategy depicted in Scheme 2 the Huisgen-1,3-dipolar cycloaddition was carried out between PCL–C≡CH and N_3E^iBBr in the presence of the copper(I)-based catalyst in THF. In order to reach quantitative conversion of both azide and alkyne functions into triazole, optimization of the "Click coupling" reaction was studied by varying the CuBr-based catalyst. For this purpose, 2,2′-bipyridine (Bpy) and 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) were

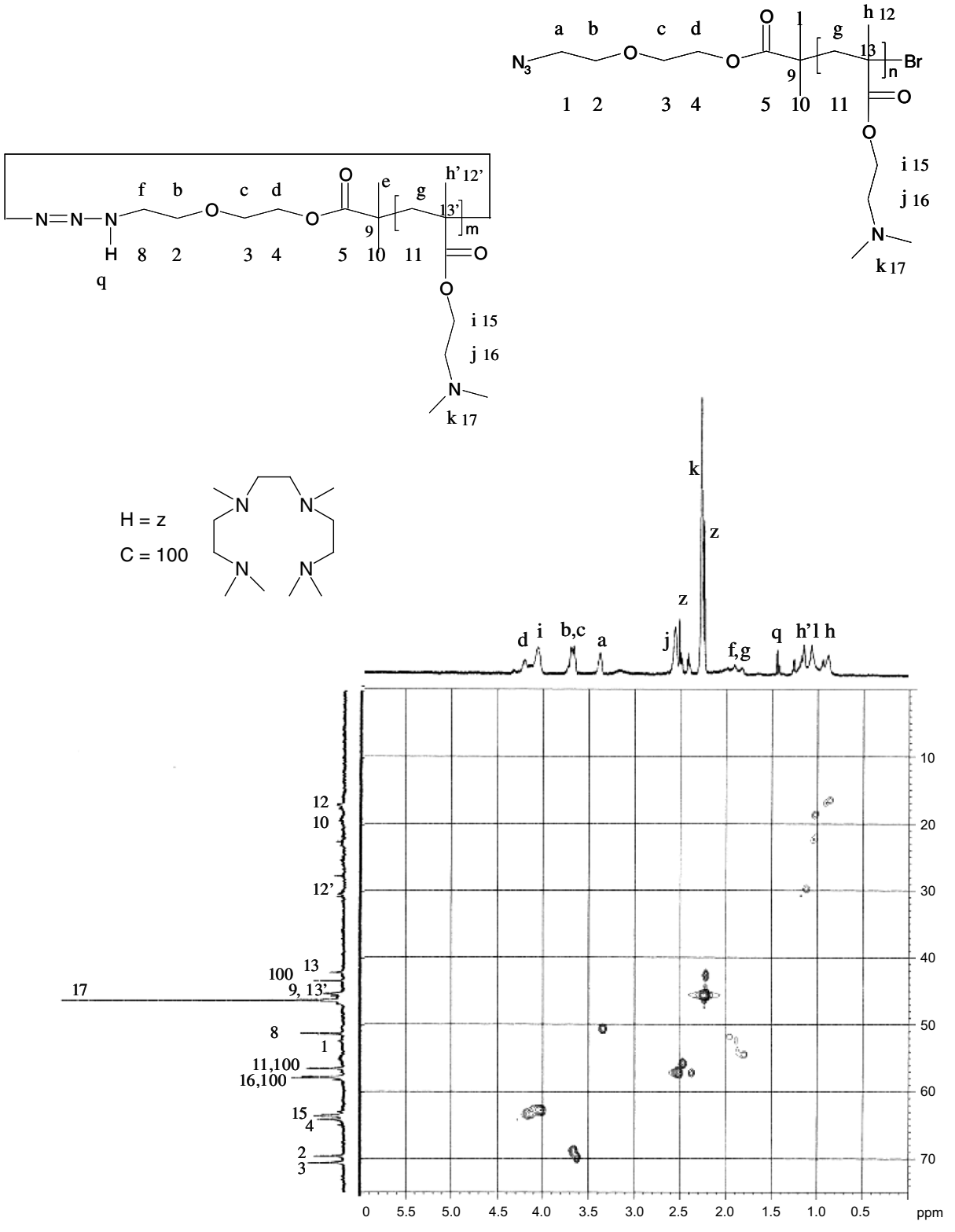


Fig. 3. 2D ^{1}H–^{13}C NMR spectrum of the presumed PDMAEMA cyclic derivative in $CDCl_3$.

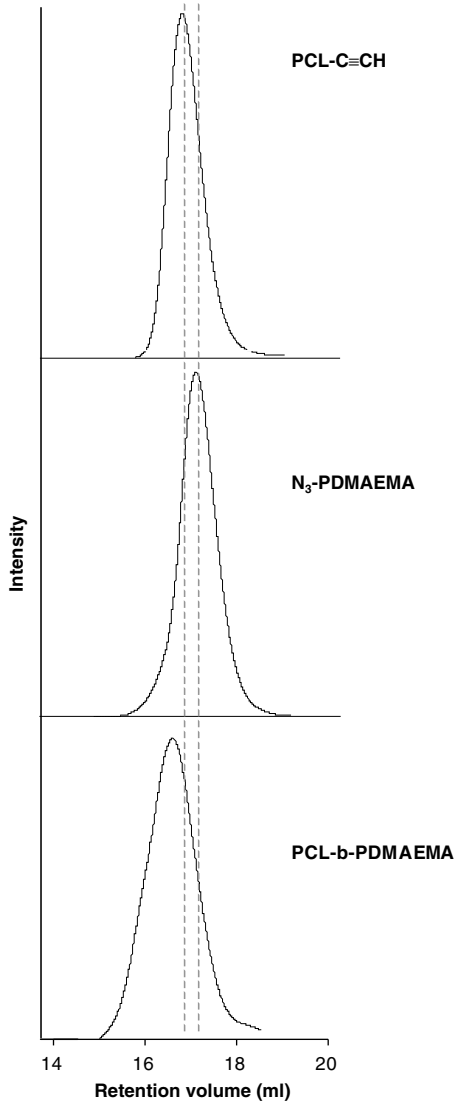
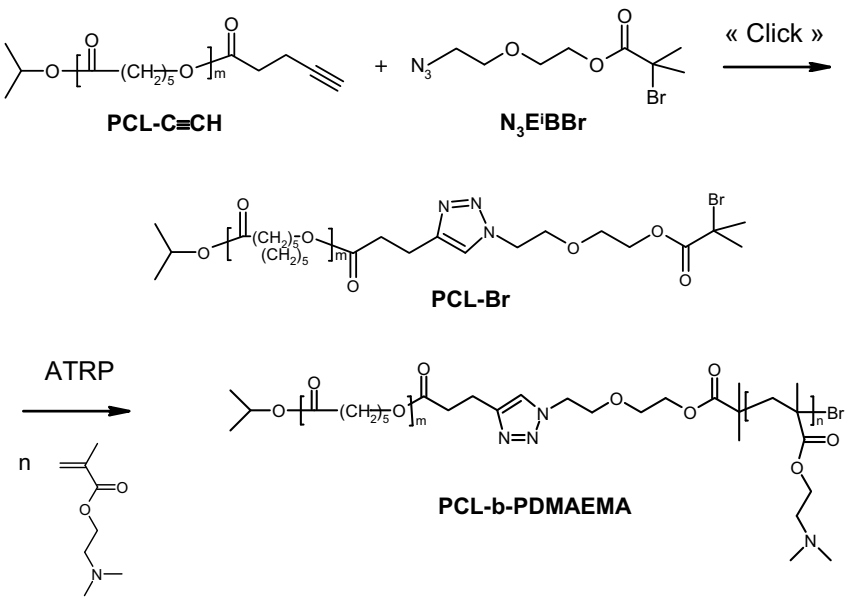


Fig. 4. SEC traces of PCL-b-PDMAEMA block copolymer compared to the first step formed homopolymers using CuBr.3Bpy as catalyst (strategy 1: "ATRP first").

investigated as ligands of CuBr. Those two ligands were selected not only because of their capability to favor the solubility of Cu(I) compound in organic solvent but also because of their high efficiency to modulate the redox potential of Cu(I) in atom transfer radical polymerization (ATRP) [22]. Practically, initial concentration in both azide and alkyne functions were maintained at 0.03 mol L^{-1} and great attention was taken to carry out this reaction in equimolar condition. The initial CuBr-to-ligand (L) molar ratio was fixed to 1/3 (CuBr.3L). From Table 1 (entries 1–3) the use of HMTETA as ligand results in uncomplete conversions of azide and alkyne groups into triazole even at higher content in catalytic complex. A maximum of 75% was reached after 24 h under the aforementioned conditions as determined by ^{1}H NMR spectroscopy. The non quantitativeness of the "Click" reaction can be ascribed to the high basicity of HMTETA ligand, which limits the transfer of proton to the triazole ring at the proteolyse step. In contrast, substituting CuBr · 3HMTETA for CuBr · 3Bpy (Bpy being less basic than HMTETA) allowed reaching quantitative "Click reaction" conversion within 24 h, at least within the accuracy limits of ^{1}H NMR spectroscopy (Fig. 5). No more azido end-group could be detected at 3.3 ppm while the relative intensities of protons close to the triazole link fit well with the theoretical ratios ($I_i/I_f/I_h/I_j/I_k/I_l/I_m = 1/1/2/2/2/2/2$). A further confirmation of "Click reaction" completion comes from ESI-MS, which selectively shows the formation of PCL-ω-(2-triazole ethoxy) 2-ethyl bromoisobutyrate as a twofold and threefold charged family centered at a m/z values of 1228.3 and 1690.5, respectively. Indeed, the comparison of ESI-MS spectra of PCL–C≡CH and PCL–Br for a range of m/z values comprised between 1680 and 1700 clearly demonstrates the absence of starting material at m/z value of 1690 ($DP_{CL} = 28$) (Fig. 6A). It is worth mentioning that PCL–C≡CH centered at a m/z value of 1680 finds its correspondent as a threefold charged isotopic distribution at a m/z value of 1228.3 as illustrated in Fig. 6B. In a next step, the ATRP of DMAEMA has been initiated by the as-formed PCL–Br. Practically, PCL–C≡CH was reacted with N_3E^iBBr in THF at 25 °C using CuBr · 3Bpy as catalytic complex for an initial molar concentration in N_3E^iBBr of 0.03 mol L^{-1} and an azide-to-alkyne molar ratio of 1. After 24 h of reaction, the temperature was increased to 60 °C and freshly distilled DMAEMA was added to the reaction medium for an initial $[DMAEMA]_0/[N_3E^iBBr]_0/[CuBr]_0/[Bpy]_0$ molar ratio fixed to 50/1/1/3. The crude sample was recovered after 22 min polymerization time and flash evaporation of the solvent while the rest of the solution was poured into a large excess of cold heptane. It comes out that monomer conversion was almost quantitative (~99%) as evidenced by the very low intensity of remaining methacrylic protons at 5.6 and 6.1 ppm (not shown here). Knowing the number average molar mass of the PCL block ($M_n = 2700$ g mol^{-1}), the number average molar mass of PDMAEMA segment has been calculated



Scheme 2. Synthesis of PCL-b-PDMAEMA block copolymer following the "Click first" strategy.

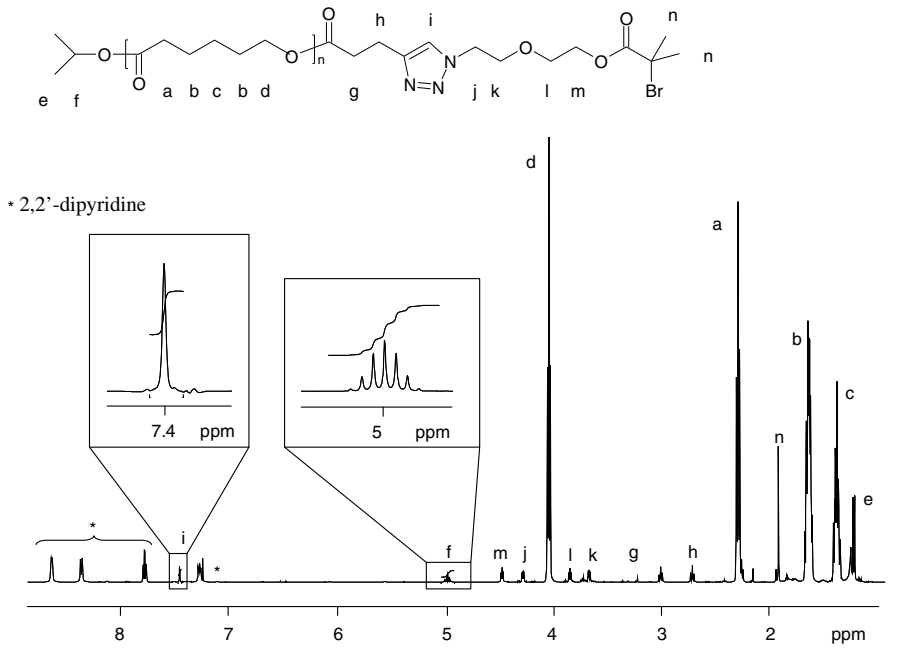


Fig. 5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of PCL–Br as obtained by "Click reaction" between N_3E^iBBr and PCL–C≡CH using CuBr.3Bpy as catalyst (entry 4, Table 1).

from the relative intensity of amino methylene protons (I_s) at 2.6 ppm (M_n NMR PDMAEMA = 9600 g mol^{-1}) and compared to the theoretical value assuming a controlled radical polymerization (M_n theor = ([DMAEMA]$_0$/[N_3E^iBBr]$_0$ × conv./100 × M_w DMAEMA) + M_w N_3E^iBBr = 8100 g mol^{-1}). A reasonable initiation efficiency (f) of 0.85 was found. Interestingly, the actual initiation efficiency f is thus highly improved compared to the one obtained after DMAEMA homopolymerization from N_3E^iBBr (f = 0.41), giving credit to our previous observations about intra-

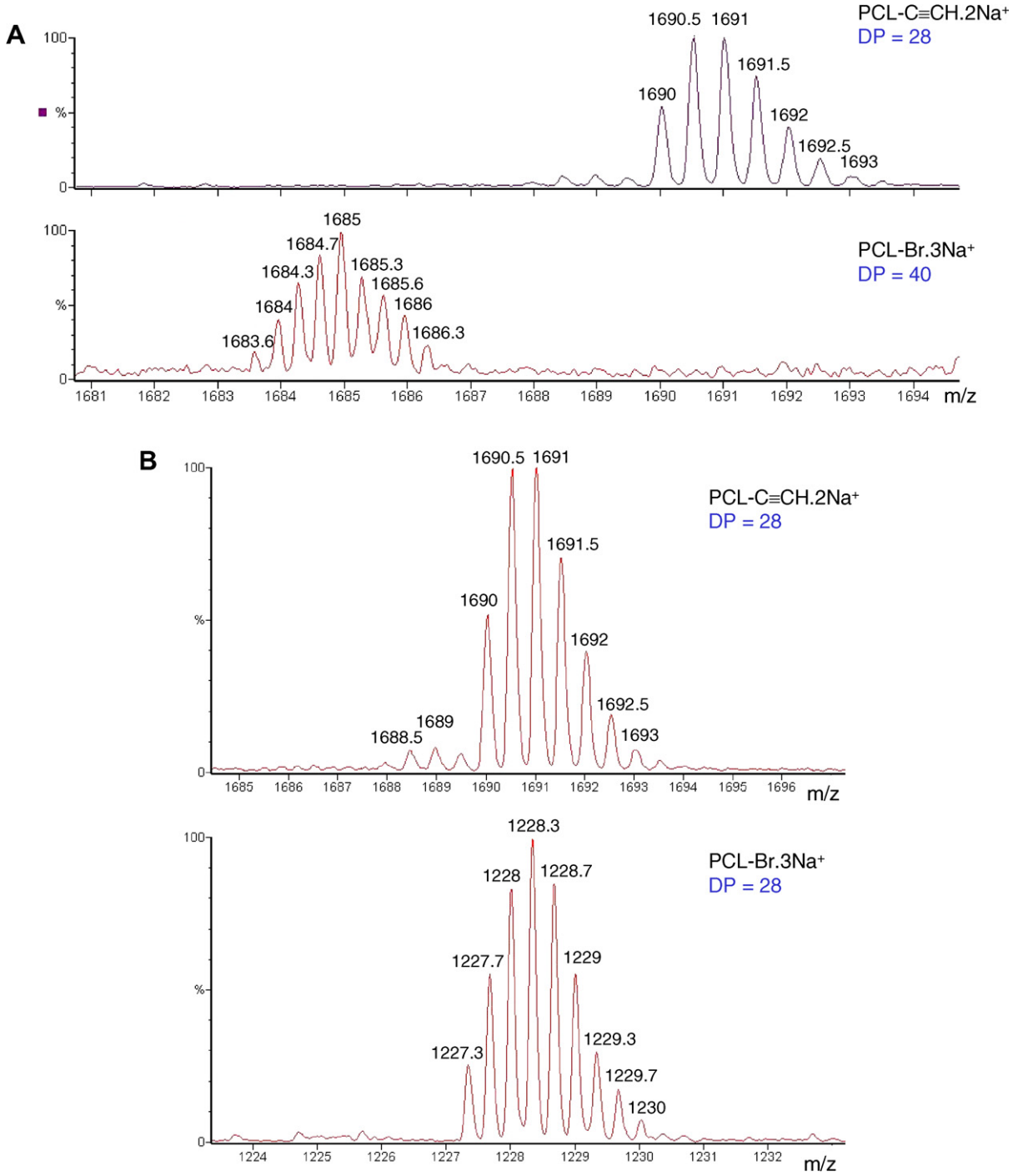
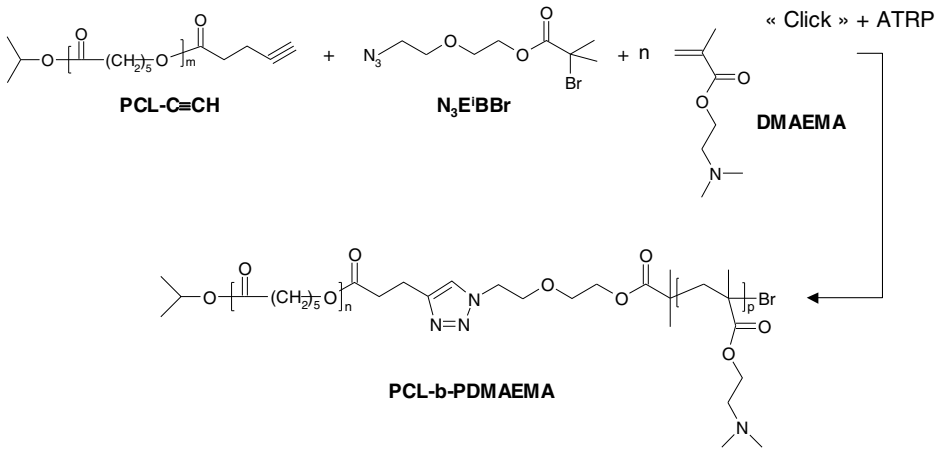


Fig. 6. (A) ESI-MS spectra of PCL–C≡CH and PCL–Br obtained after "Click reaction" using CuBr.3Bpy as catalyst for a reduced m/z range values and (B) ESI-MS spectra of PCL–C≡CH and PCL–Br obtained after "Click reaction" using CuBr.3Bpy as catalyst for a reduced m/z range values.

molecular cyclization involving the free azide moiety. The molecular weight distribution of the PCL-b-PDMAEMA diblock copolymer is unimodal and symmetrical ($M_w/M_n = 1.34$) and shifted to lower retention volume compared to the PCL–C≡CH precursor.



Scheme 3. Synthesis of PCL-b-PDMAEMA block copolymer following the "One-pot" strategy.

3.3. One-step synthesis of PCL-b-PDMAEMA block copolymers

Direct synthesis of PCL-b-PDMAEMA block copolymer has been performed starting from a mixture of PCL–C≡CH, N_3E^iBBr, DMAEMA in THF using CuBr · 3Bpy as the unique catalyst and applying a temperature gradient from 25 °C to 60 °C (Scheme 3). The initial $[DMAEMA]_0/[N_3E^iBBr]_0/[PCL–C{\equiv}CH]_0/[CuBr \cdot 3Bpy]_0$ molar ratios were fixed to 50/1/1/1 ($[N_3E^iBBr]_0 = 0.03$ mol L^{-1}). After 24 h at 25 °C, the temperature was raised up to 60 °C for 6 h in order to promote ATRP of DMAEMA. Then, the reaction medium was diluted by THF, the catalytic complex was removed out by passing through a basic alumina column and the organic solvent was evaporated under mild conditions.

The ^{1}H NMR spectrum is similar to the one shown in Fig. 1C attesting for the completion of both the "click" reaction and the ATRP of DMAEMA. Only traces of residual monomer can be detected at 5.5 and 6.1 ppm (conv. = 95%) while no azido methylene protons could be observed at 3.3 ppm. Knowing the number average molar mass of PCL–C≡CH ($M_n = 2700$ g mol^{-1}, $DP_n = 22$), M_n of the polymethacrylate block has been calculated from the relative intensity of amino methylene protons (I_s) at 2.6 ppm (M_n NMR PDMAEMA = 10,000 g mol^{-1}) and compared to the theoretical value assuming a controlled radical polymerization (M_n theor = $([DMAEMA]_0/[N_3E^iBBr]_0 \times M_w$ DMAEMA) + M_w N_3E^iBBr = 7900 g mol^{-1}). It comes out a higher initiation efficiency ($f = 0.79$) compared to the value obtained for the homopolymerization of DMAEMA using CuBr · 2HMTETA as catalyst attesting that CuBr · 3Bpy is not only an efficient catalyst for the "click" reaction but also for the ATRP of DMAEMA initiated by N_3E^iBBr.

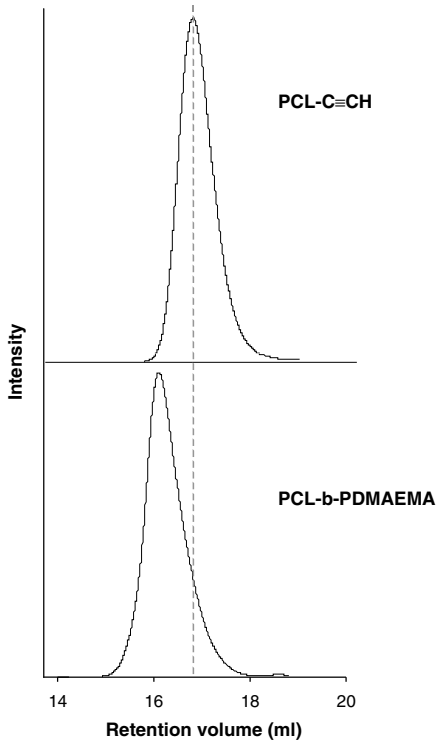


Fig. 7. SEC traces of PCL-b-PDMAEMA block copolymer compared to PCL–C≡CH precursor as obtained by a one-pot process and using CuBr.3Bpy as catalyst (strategy 3: "One-pot").

Fig. 7 shows the SEC trace of the diblock copolymer compared to the polyester block and the clear shift of the molar mass distribution to lower elution volumes. It is worth mentioning that the quite narrow molar mass distribution ($M_w/M_n = 1.31$) indicates a good control over the polymerization process.

4. Conclusions

Amphiphilic and adaptative PCL-b-PDMAEMA diblock copolymers have been successfully synthesized by combining ROP, ATRP and "Click" coupling reaction either by a two-step or a "one-pot" procedure. The use of CuBr.3Bpy catalytic complex was found to be the most efficient to achieve quantitative "Click reaction" between azide and alkyne functions, but also to enhance the initiation efficiency of DMAEMA monomer by ATRP. The physico-chemical properties of the so-produced amphiphilic block copolymers will be reported elsewhere, especially their behavior in aqueous solution with respect to pH and temperature variations.

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