

Research Article

New 1,2,3-Triazole Containing Polyesters *via* Click Step-Growth Polymerization and Nanoparticles Made of Them

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High-molecular-weight AA-BB-type aliphatic polyesters were synthesized *via* Cu(I)-catalyzed click step-growth polymerization (SGP) following a new synthetic strategy. The synthesis was performed between diyne and diazide monomers in an organic solvent as one pot process using three components and two stages. The dipropargyl esters of dicarboxylic acids (component 1) were used as diyne monomers, di-(bromoacetic acid)-alkylene diesters (component 2) were used as precursors of diazide monomers, and sodium azide (component 3) was used for generating diazide monomers. The SGP was carried out in two steps: at Step 1 dibromoacetates interacted with two moles of sodium azide resulting in diazide monomers which interacted in situ with diyne monomers at Step 2 in the presence of Cu(I) catalyst. A systematic study was done for optimizing the multiparameter click SGP in terms of the solvent, duration of both Step 1 and Step 2, solution concentration, catalyst concentration, catalyst and catalyst activator (ligand) nature, catalyst/ligand mole ratio, and temperature of both steps of the click SGP. As a result, high-molecular-weight (M_w up to 74 kDa) elastic film-forming click polyesters were obtained. The new polymers were found suitable for fabricating biodegradable nanoparticles, which are promising as drug delivery containers in nanotherapy.

1. Introduction

Copper(I) catalyzed and copper-free azide-alkyne cycloadditions (CuAAC and CuFAAC) are one of the most widely used click reactions. CuAAC click reaction has been extensively used in biochemistry and biotechnology [1], in polymer chemistry for dendrimer synthesis/functionalization, surface immobilization/modification, orthogonally functionalizing polymers, and its integration with ATRP (atom transfer radical polymerization) and ROP (ring-opening polymerization) for synthesizing block-copolymers, cyclic polymers, hyperbranched macromolecules, star-shaped polymers, and cross-linked polymeric networks [2–11]. CuAAC reaction was also widely employed in orthogonal multiple click reactions for constructing various well-defined complex macromolecular structures [12, 13].

The azide-alkyne click polymerization appears as a powerful tool for the synthesis of polymers with versatile architectures by step-growth polymerization (SGP). Due to the

high reaction rates and quantitative yields, high-molecular-weights can be achieved. It is important to note that CuAAC click reaction results in the formation of 1,2,3-triazole ring which is known as low toxic moiety [14, 15]. Furthermore, triazole rings-containing compounds display a broad spectrum of biological activities, including antimicrobial, antiviral, antifungal, anticancer, anti-inflammatory, antitubercular, antioxidant, analgesic, and antimalarial activities [16, 17]. 1,2,3-Triazoles are highly promising for the designing of various functional coatings such as anticorrosive, antimicrobial, self-healing, and hybrid nanocomposite [18].

There are rather limited publications on the application of CuAAC in SGP for constructing either AB or AA-BB type polymeric backbones: Díaz et al. reported about the synthesis of new adhesive polymers with N-arylsulfonamide moieties in the backbones [19]; Van Steenis et al. obtained novel conjugated aromatic polymers with molecular weights (MWs) of 60–396 kDa (M_w) by click SGP indicating that this reaction is suitable for constructing long-chain

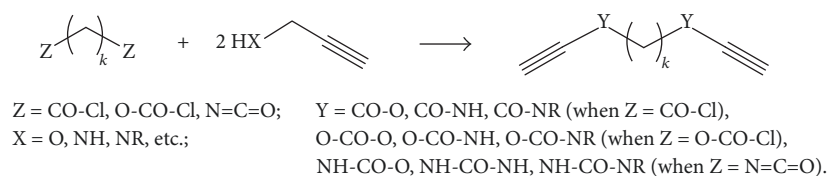


FIGURE 1: The synthesis of diyne monomers.

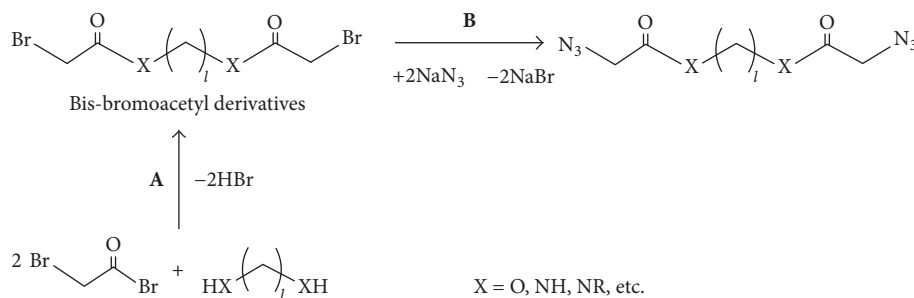


FIGURE 2: Synthesis of bis-bromoacetyl derivatives (A) and in situ generation of diazide monomers (B).

macromolecules with proper material properties [20]; AA-BB type aromatic polyethers having M_w of 27.0–32.8 kDa obtained by click chemistry were also reported [21]; liquid crystalline aromatic polyesters (PEs) [22], aliphatic polyethers constituting triethylene glycol [23], multifunctional poly(ethylene glycol)s (PEGs) [24], poly(alkyl aryl ether)s [25], poly(glycoamidoamine)s [26], linear polytriazoles composed of various 1,4:3,6-dianhydrohexitols [27], polytriazoles [28–30], and poly(aroyltriazole)s [31] were synthesized *via* CuAAC SGP as well. Triazole polymers (mostly polyethers on the basis of commercial polyols) were also synthesized under neat conditions, by azide-alkyne cycloaddition SGP at r.t. during 144 h without using metal catalyst [32].

There are limited publications on the synthesis of aliphatic triazole-linked AA-BB-type ester polymers by CuAAC click SGP. Nagao and Takasu [33] reported on the first synthesis of linear, biodegradable polyesters (PEs). Their synthetic strategy was based on the use of diester diyne monomers which were obtained by thermal (60°C) condensation of diacids with 3-butyl-1-ol and 2-propyn-1-ol (propargyl alcohol) under reduced pressure using Scandium(III) triflate as a catalyst $\text{Sc}(\text{OTf})_3$. Diazide monomers were either presynthesized or formed in consequence of in situ azidation, by interaction of α,ω -dibromoalkanes with sodium azide. The click PEs were obtained using CuBr as a catalyst *via* SGP by interaction of diester diyne monomers either with presynthesized dangerous diazide monomers or using more safe one-pot synthesis comprising in situ azidation. The click polyesters with the highest molecular weights (up to 66 kDa) were obtained using presynthesized diazide monomers; one-pot synthesis led to rather low-molecular-weight polymers (within 1–25 kDa) presumably due to the insufficient reactivity of dibromoalkanes. Bueno et al. [34] also reported on the synthesis of AA-BB-type biodegradable “click” PEs from erythritol having free hydroxyl groups. The authors synthesized

diester diyne monomers by thermal condensation (in refluxed toluene) of diols (1 mol) with propiolic acid (2 mol) using *p*-toluenesulfonic acid as a condensation catalyst. 1,4-Diazide-1,4-dideoxyerythritol was used as a counter-partner of the diester diyne monomers in the click SGP. The molecular weights of the obtained polyesters were in the range of 13–37 kDa.

For synthesizing high-molecular-weight click polymers we have elaborated different synthetic strategy. According to our approach the synthesis of click polymers is based on the use of diyne monomers obtained by interaction of acetylenic nucleophiles (both regular, such as propargyl alcohol, 3-butyl-1-ol, and propargylamines, and strained, such as hydroxy-dibenzocyclooctyne, dibenzocyclooctyne-amines, and related compounds) with activated bis-electrophiles such as diacid chlorides, bis-chloroformates, or diisocyanates. This approach broadens the range of applicable monomers, as diyne monomers containing various hetero-bonds (ester, amide, carbonate, urethane, or urea) can be obtained according to general scheme shown for propargyl derivatives in Figure 1.

According to our strategy, the click SGP is carried out as one-pot/two-step/three-component procedure. Diazide monomers are obtained by in situ azidation of bis-bromoacetyl derivatives (Figure 2,B).

Bis-bromoacetyl derivatives could be synthesized by interaction of bromoacetyl bromide with diols, diamines, or other bis-nucleophiles (Figure 2,A). The bis-bromoacetyl derivatives due to the influence of carbonyl group are more active in nucleophilic substitution reaction with nucleophiles including sodium azide as compared with α,ω -dibromoalkanes. This provides more effective formation of diazide monomers in one-pot procedure resulting in high-molecular-weight polymers (≤ 73 kDa, to be discussed below). The application of bis-bromoacetyl derivatives as precursors of diazide monomers allows introducing additional

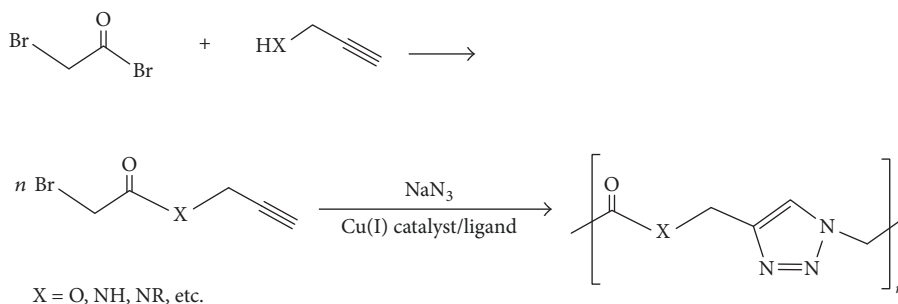


FIGURE 3: Scheme of the synthesis of AB-type click polymers.

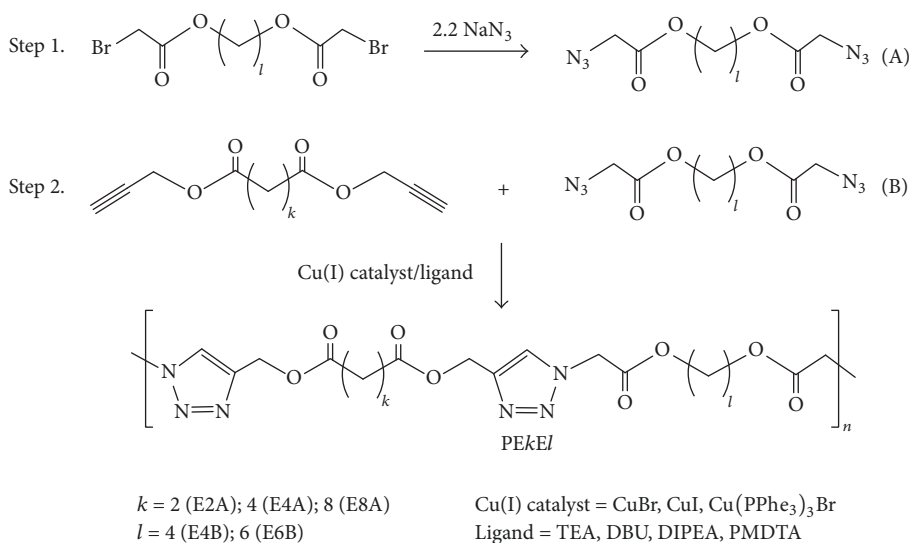


FIGURE 4: One-pot/two-step/three-component synthesis of click PEs.

hetero-bonds in the repeating units of the target polymers. As a result, the repeating units of the click polymers contain four hetero-bonds two of which come from the diyne monomers and an additional two, from the diazide monomers. Besides, the new synthetic strategy opens a way to AB-type click polymers such as polyesters, polyamides, and related polymers *via* click SGP using only two compounds, bromoacetyl bromide and functionalized acetylenic derivatives, according to a general scheme shown for propargyl derivatives in Figure 3.

Thus, the new click SGP approach is extremely versatile and allows synthesizing different types (AB and AA-BB) and classes (containing various hetero-bonds: ester, amide, carbonate, urethane, or urea) of heterochain click polymers with molecular architecture in which hetero-bonds are linked in 1,4-positions of 1,2,3-triazole rings through two methylene bridges. The new synthetic strategy looks promising for both CuAAC and CuFAAC click SGP.

As a part of our systematic study, in the present paper we report on the first synthesis of high-molecular-weight click polymers *via* new synthetic strategy. Aliphatic PEs were obtained in the first study *via* one-pot/three-component/two-stage process following the synthetic scheme represented in Figure 4.

This class of heterochain polymers was selected for the following reasons: (i) the new PEs are structurally closer to the traditional poly(alkylene dicarboxylate)s [35]; they can be considered as derivatives of alkylene dicarboxylates (AA-BB type PEs) that are obtained by the insertion of 1,2,3-triazole-containing fragments (which, in turn, coincides with the repeating unit of the AB-type click PE, depicted in Figure 3) between carbonyl groups and ether oxygens in the backbones of the poly(alkylene dicarboxylate)s (Figure 5); (ii) the genetic affinity of the new polymers to the poly(alkylene dicarboxylate)s allows better tracking of the influence of the triazole-containing fragments on physical-chemical properties of the PEs; (iii) PEs with triazole-containing fragments could be of interest as biodegradable polymers for fabricating various biomedical materials including nanoparticles, which could be modified by transformations of the 1,2,3-triazole rings, and finally (iv) it is expected that the new click PEs will possess an increased biodegradation rate having four hydrolyzable ester bonds per repeating unit versus two ester bonds in click PEs according to [33, 34].

The synthesis of the new click PEs was performed by one-pot/two-step/three-component SGP of diester-diyne ($Y = O-CO$ in Figure 4) with bis-bromoacetates of diols (X

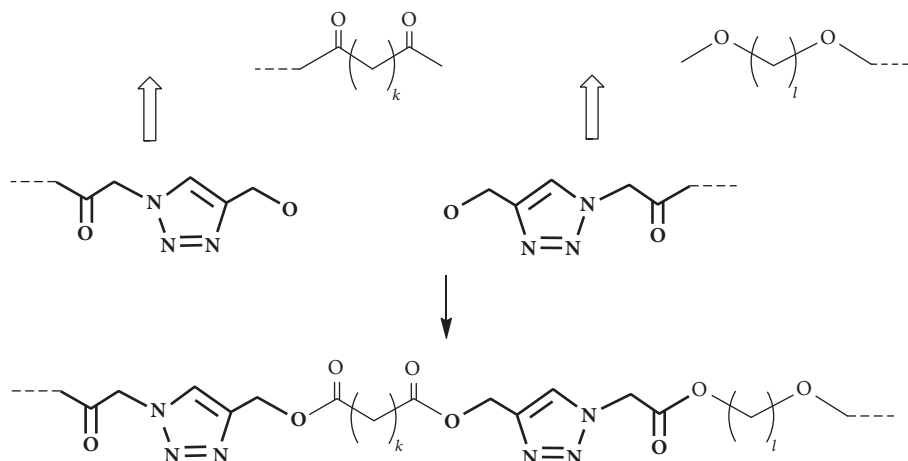


FIGURE 5: Schematic representation of PEs as products of the insertion (empty arrows) of 1,2,3-triazole-containing fragments (given in bold) between ester bonds of poly(alkylene dicarboxylates).

= O in Figure 2) in the presence of sodium azide using copper(I) catalysts. The CuAAC click SGP was optimized in terms of reaction conditions/parameters. As a result, a series of new high-molecular-weight 1,2,3-triazole ring-containing aliphatic “click” PEs on the basis of nontoxic building blocks, fatty diols and dicarboxylic acids, were obtained. The new biodegradable PEs having valuable material properties are promising for numerous practical applications in biomedicine including nanoparticles (NPs) with the focus on drug delivery nanocontainers. This was demonstrated in the present work by fabricating NPs using cost-effective polymer deposition/solvent displacement (nanoprecipitation) method.

2. Materials and Methods

2.1. Materials. Bromoacetyl bromide, sodium azide, copper(I) bromide, copper(I) iodide, Bromotris(triphenylphosphine)copper(I), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), N,N-diisopropylethylamine (DIPEA), N,N,N',N'',N'''-pentamethyldiethylenetriamine (PMDETA), propargyl alcohol, 1,4-butanediol, 1,6-hexanediol, sebacoyl chloride, adipoyl chloride, succinyl chloride, lithium bromide, acetone, ethanol, acetonitrile, hexane, ethyl acetate, dichloromethane (DCM), 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), trifluoroethanol (TFE), 1,3-dimethyl-2-imidazolidinone (DMI), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA), and Tween 20 were purchased from Sigma-Aldrich, MO, USA, and used without further purification. Dimethyl sulfoxide (DMSO) and N-methylpyrrolidone (NMP) were purchased from Carl Roth, Karlsruhe, Germany. Triethylamine (TEA) was purchased from Lancaster, UK. All the chemicals were used as received. The dialysis bag (MWCO 25 kDa) was purchased from Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA.

2.2. Characterization. The materials synthesized were characterized by FT-IR and ^1H and ^{13}C NMR spectroscopy, GPC, DSC, and viscometry. Thermo Nicolet Avatar 370 FT-IR

spectrophotometer (coupled with EZ OMNIC software) was used for IR analysis. The FT-IR spectra of crystalline products were recorded in fine powders using Avatar Multi-Bounce Flat Plate 45 degree Ge. To obtain the polymers spectra the thin films were cast from HFIP solution on KBr plates, solvent was evaporated at r.t., and films were dried in a vacuum at 40°C for 24 h. The ^1H and ^{13}C NMR spectra were recorded using JEOL ECA-400 MHz NMR spectrometer at r.t. in $\text{DMSO}-d_6$ as a solvent and internal standard.

The weight-average (M_w), number-average (M_n) MWs, and molecular weight distribution (Dispersity, \mathcal{D}) of the polymers were determined on a GPC instrument (Waters Associates, Inc., Milford, United States. Styragel columns in DMF: HR4, HR3, HR0.5 all $7.8\text{ mm} \times 300\text{ mm}$) equipped with a high-pressure liquid chromatography pump (Waters 1525 Binary HPLC) and a Waters refractive index detector 2414 and UV-detector (Waters 2487 dual absorbance detector, $\lambda = 240\text{ nm}$). A solution of LiBr (0.1 M) in DMF was used as an eluent. Injected volume of $100\ \mu\text{L}$, sample concentration 5.0 mg/mL , flow rate 1.0 mL/min , and temperature 35°C . The columns were calibrated with PMMA standards. Reduced viscosity (η_{red}) of the new polymers was determined in DMF solutions at a concentration of 0.5 g/dL and 25°C using automatic viscometer “Lauda” (Germany). The thermal properties of the polymers were measured by NETZSCH DSC 200 PC Phox within the temperature range of $20\text{--}200^\circ\text{C}$ under a dry nitrogen gas flow at a heating rate of 10°C/min .

The NPs were characterized by size (Mean Particle Diameter, MPD) and size distribution (Polydispersity Index, PDI) and zeta-potential (ZP) which were assessed by dynamic light scattering (DLS) using a particle size analyzer (Zetasizer Nano ZS, Malvern Instrument, Malvern, UK) at 25°C . The MPD and PDI are presented as an average of five measurements \pm standard deviation (SD). The $\text{PDI} < 0.04$ corresponds to a narrow distribution, $0.04 \leq \text{PDI} \leq 0.16$ to a mean distribution and $\text{PDI} > 0.16$ to a wide distribution.

2.3. Synthesis of Dipropargyl Esters of Dicarboxylic Acids (Bis-alkyne Monomers). Bis-alkyne monomers on the basis

of diacid chlorides were synthesized according to scheme depicted in Figure 1 ($Z = \text{COCl}$, $X = \text{O}$). The synthesis was carried out in DMA solution without using tertiary amine as HCl acceptor. In a typical procedure propargyl alcohol (17.71 g, 0.316 mol, an excess) was dissolved in 500 mL of DMA, the solution was chilled to -5°C , and 0.144 mol of a diacid chloride was added dropwise upon stirring using a magnetic stirrer. The reaction solution was stirred for 30 min at -5°C and then was continued at r.t. for 24 h. Afterwards the solution was poured into water and the vessel with precipitated white powder was placed overnight in a refrigerator. The obtained solid product was filtered off, dried under reduced pressure at r.t., and recrystallized from ethanol/hexane mixture. For labelling the obtained bis-alkyne monomers, see Figure 4.

E2A. Yield: 51.6%. m.p. $35\text{--}36^\circ\text{C}$. FTIR (cm^{-1}): 3256 ($\equiv\text{C-H}$), 2132 ($\text{C}\equiv\text{C}$), 1728 ($-\text{CO}-$ ester), 1211 (C-O-C). ^1H NMR (400 MHz, $\text{DMSO-}d_6$, δ): 2.64 (4H, s, CH_2CO); 3.00 (2H, t, J 2.5, sp-CH); 4.65 (4H, d, J 2.5, OCH_2). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$, δ) 28.0 (CH_2); 51.4 (OCH_2); 76.2 (sp-CH); 77.2 (sp-C); 170.1 (CO).

E4A. Yield: 56.0%. m.p. $24\text{--}25^\circ\text{C}$. FTIR (cm^{-1}): 3227 ($\equiv\text{C-H}$), 2116 ($\text{C}\equiv\text{C}$), 1736 ($-\text{CO}-$ ester), 1229 (C-O-C). ^1H NMR (400 MHz, $\text{DMSO-}d_6$, δ): 1.51–1.71 (4H, m, CH_2); 2.29–2.42 (4H, m, CH_2CO); 2.96 (2H, t, J 2.5, sp-CH); 4.63 (4H, d, J 2.5, OCH_2). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$, δ): 23.4 (CH_2); 32.6 (COCH_2); 51.0 (OCH_2); 75.9 (sp-CH); 77.4 (sp-C); 170.9 (CO).

E8A. Yield: 66.0%. m.p. $21\text{--}22^\circ\text{C}$. FTIR (cm^{-1}): 3238 ($\equiv\text{C-H}$), 2128 ($\text{C}\equiv\text{C}$), 1732 ($-\text{CO}-$ ester), 1217 (C-O-C). ^1H NMR: (400 MHz, $\text{DMSO-}d_6$, δ): 1.29–1.35 (8H, m, CH_2); 1.59 (4H, m, $\text{CH}_2\text{CH}_2\text{CO}$); 2.30 (4H, t, J 7.4, CH_2CO); 2.93 (2H, t, J 2.4, sp-CH); 4.61 (4H, d, J 2.4, OCH_2). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$, δ): 24.0 (CH_2); 28.2 (CH_2); 28.3 (CH_2); 33.0 (CH_2); 50.8 (OCH_2); 75.8 (sp-CH); 77.5 (sp-C); 155.4 (CO).

2.4. Synthesis of Di-(bromoacetic acid)-alkylene Diesters (Precursors of Diazide Monomers). The di-(bromoacetic acid)-alkylene diesters, precursors of diazide monomers, were synthesized as reported in [36] (according to scheme given in Figure 2,A): to a solution of 0.075 mol of a diol in 450 mL of DCM, 33.30 g (0.165 mol) of bromoacetyl bromide was added. The resulting yellow solution was refluxed for 20 h and the solvent was removed in a vacuum on a rotary evaporator yielding an orange crystalline solid. The obtained crystals were dried under reduced pressure at r.t. and recrystallized from acetone/water mixture. For labelling the obtained di-(bromoacetic acid)-alkylene diesters see Figure 4.

E4B. Yield: 90.7%. m.p. $66\text{--}68^\circ\text{C}$. FTIR (cm^{-1}): 1744 ($-\text{CO}-$ ester), 1294 (C-O-C). ^1H NMR (400 MHz, $\text{DMSO-}d_6$, δ): 1.64–1.69 (4H, m, CH_2), 4.13 (4H, s, CH_2Br), 4.14 (4H, t, CH_2O). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$, δ): 24.9 (CH_2), 25.0 (CH_2Br), 59.7 (CH_2O), 172.7 (CO).

E6B. Yield: 64.4%. m.p. $42\text{--}44^\circ\text{C}$. FTIR (cm^{-1}): 1748 ($-\text{CO}-$ ester), 1335 (C-O-C). ^1H NMR (400 MHz, $\text{DMSO-}d_6$, δ): 1.32–1.37 (4H, m, CH_2), 1.55–1.63 (4H, m, $\text{CH}_2\text{CH}_2\text{O}$), 4.10 (4H, t, J 6.58, CH_2O), 4.13 (4H, s, CH_2Br). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$, δ): 24.9 (CH_2), 25.0 (CH_2Br), 28.1 (OCH_2CH_2), 59.6 (CH_2O), 172.7 (CO).

2.5. General Procedure for Optimizing the Click SGP. A systematic study for optimizing the one-pot/three-component/two-stage CuAAC click SGP was performed on the example of click PE PE8E4 (Figure 4, $k = 8$, $l = 4$): 620.8 mg (1.870 mmol) of di-(bromoacetic acid)-tetramethylene diester (E4B) and 267.4 mg (4.114 mmol, 10 mol.% excess) of sodium azide were dissolved in a predetermined volume (a variable parameter) of a predetermined organic solvent (a variable parameter) and stirred at a predetermined temperature (a variable parameter) for a predetermined period of time (a variable parameter). Then 520.5 mg (1.870 mmol) of dipropargyl sebacate (E8A), a predetermined amount (a variable parameter) of a predetermined catalyst (a variable parameter), and a predetermined ligand (a variable parameter) were added to the reaction solution. The catalyst/ligand mole ratio was also varied. The reaction was carried out at a predetermined temperature (a variable parameter) for a predetermined period of time (a variable parameter). After completing the reaction, the resulting solution was diluted (2–3 times depending on a viscosity of the resulting solution) with the same organic solvent in which the reaction was carried out and the diluted solution was filtered off and poured into distilled water to separate the polymer. The precipitated polymer was filtered off, thoroughly washed with water, and dried in a vacuum at r.t. The obtained polymer was reprecipitated from the same organic solvent (at a concentration 10% w/v) in distilled water. The precipitated polymer was filtered off, thoroughly washed with water and dried in a vacuum at r.t. The yields of the click PEs depending on the reaction parameters/conditions are given in Tables 3–11.

The structure of the obtained click PE PE8E4 (selected as an object when optimizing the SGP; see below) was confirmed by FTIR and NMR spectroscopy. FTIR (cm^{-1}): 1732 ($-\text{CO}-$ ester), 1228 (C-O-C), 3086 (C=C-H of the 1,2,3-triazole moiety). ^1H NMR (400 MHz, $\text{DMSO-}d_6$, δ): 1.21 (8H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_4\text{CH}_2\text{CH}_2\text{O}$), 1.49 (4H, $\text{OCH}_2(\text{CH}_2)_2\text{CH}_2\text{O}$), 1.65 (4H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_4\text{CH}_2\text{CH}_2\text{O}$), 2.29 (4H, $\text{C(O)CH}_2(\text{CH}_2)_6\text{CH}_2\text{CO}$), 4.15 (4H, $\text{OCH}_2(\text{CH}_2)_2\text{CH}_2\text{O}$), 5.14 (4H, N- $\text{CH}_2\text{-CO}$), 5.40 (4H, C- $\text{CH}_2\text{-O}$), 8.15 (2H, C=CH). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$, δ): 24.1 (CH_2), 24.3 (CH_2), 28.1 (CH_2), 28.3 (CH_2), 33.1 (CH_2), 50.1 (CO- $\text{CH}_2\text{-N}$), 56.7 (OCH_2CH_2), 64.6 (C- $\text{CH}_2\text{-O}$), 126.0 (N-CH-C), 167.0 (CO- $\text{CH}_2\text{-N}$), 172.5 (CO- $\text{CH}_2\text{-CH}_2$).

2.6. Synthesis of Click Polyesters. Several click PEs were synthesized by one-pot/three-component/two-stage process according to scheme depicted in Figure 4 under the reaction parameters/conditions which were found as an optimal (given in bold and underlined): 6.270 mmol

of di-(bromoacetic acid)-alkylene diester and 896.7 mg (13.794 mmol) of sodium azide were dissolved in 9.576 mL of NMP and stirred at 20°C for 3 h. Then the reaction solution was chilled to 0°C in the ice bath and 6.270 mmol of a di-propargyl ester of dicarboxylic acid, 238.8 mg (1.254 mmol) of catalyst CuI (20 mol%), and 0.874 mL (6.270 mmol) of TEA as a ligand were added to the reaction solution. The catalyst/ligand mole ratio was 1:5. The monomer molar concentration in reaction solution was 0.6 mol/L. The reaction solution was stirred at 0°C until the solution became too viscous to stir (4–6 h) and then the reaction was carried out at 20°C for 9–11 h (after adding catalyst/ligand, the total reaction duration was 15 h). After completing the reaction, the resulting solution was diluted with NMP (2–3 times depending on a viscosity of the resulting solution), and the polymers were separated and purified according to the general procedure above.

2.7. Preparation of the NPs. The NPs were prepared according to the polymer deposition/solvent displacement (nanoprecipitation) method under the optimal conditions which were established previously for amino acid based biodegradable ester polymers [37]. All the experiments of the NPs fabrication were performed at r.t.: 6.0 mg of polymer was dissolved in 1.0 mL of the organic solvent miscible with water, DMSO. This organic phase was then added dropwise (dropping rate 12 drops/min) to 10.0 mL of the water phase containing 50.0 mg of a surfactant Tween 20 (the organic/water phases (O/W) ratio 1:10 v/v) at stirring rate of 700 rpm using a magnetic stirrer. In all cases, after adding the organic phase, the aqueous phase became turbid that indicated the formation of the NPs. The suspensions of the NPs, obtained after the complete addition of the organic phase, were stirred for 2–3 min and, finally, were dialyzed against distilled water for 72 h to remove the organic solvent and residual surfactant using the dialysis bag with MWCO 25 kDa. The obtained suspensions of the NPs were refrigerated. The NPs were characterized as both those freshly prepared and those after one and two months' storage.

3. Results and Discussion

3.1. Synthesis of Di-Propargyl Esters of Dicarboxylic Acids (Bis-alkyne Monomers). The synthesis of bis-alkyne monomers on the basis of dicarboxylic acids chlorides was discussed above (see Materials and Methods). Three bis-alkyne monomers, di-propargyl ester of succinic acid (labeled as E2A), di-propargyl ester of adipic acid (labeled as E4A), and di-propargyl ester of sebacic acid (labeled as E8A) were synthesized according to scheme depicted in Figure 1 (Z = CO-Cl, X = O, Y = CO-O). The characteristics of the obtained bis-alkyne monomers are given in Table 1. The yields of the obtained bis-alkyne monomers were in the range of 51.6–66.0%. Their structure was confirmed by FTIR and NMR spectroscopies.

3.2. Synthesis of Di-(bromoacetic acid)-alkylene Diesters (Precursors of Diazide Monomers). The synthesis of di-(bromoacetic acid)-alkylene diesters, precursors of diazide

TABLE 1: The characteristics of bis-alkyne monomers.

Bis-alkyne monomer	Empirical formula (FW)	Yield* (%)	m.p. (°C)
E2A	C ₁₀ H ₁₀ O ₄ (194.18)	51.6	35-36
E4A	C ₁₂ H ₁₄ O ₄ (222.24)	56.0	24-25
E8A	C ₁₆ H ₂₂ O ₄ (278.34)	66.0	21-22

* After recrystallization from ethanol/hexane mixture.

monomers, was described above (see Materials and Methods). Two precursors of diazide monomers (di-(bromoacetic acid)-tetramethylene diester (labeled as E4B) and di-(bromoacetic acid)-hexamethylene diester (labeled as E6B)) were synthesized according to the scheme depicted in Figure 2,A. It should be noted that low molecular weight organic azides may be unstable and should be handled with caution. The equation $(N_C + N_O)/N_N$ (so-called C/N ratio), where N_C signifies the number of carbon atoms, N_O signifies the number of oxygen atoms, and N_N signifies the number of nitrogen atoms, is used to evaluate the stability of the organic azides. Azides with a C/N ratio greater than one and no more than three can be synthesized and isolated but should be stored below r.t. at no more than 1 M concentration. Organic azides with $C/N < 1$ should never be isolated and practically they were used without further purification. The C/N ratios of diazide monomers, corresponding to their precursors E4B and E6B, are greater than one and no more than three (see Table 2). Therefore, to avoid the use of potentially dangerous diazide monomers, their dibromide precursors (di-(bromoacetic acid)-alkylene diesters) were synthesized instead, and the goal click PEs containing 1,2,3-triazole unites were obtained *via* one-pot/two-step/three-component SGP (Figure 4). The characteristics of the obtained di-(bromoacetic acid)-alkylene diesters are given in Table 2. The yields of the obtained compounds were in the range of 64.4–90.7%. Their structures were confirmed by FTIR and NMR spectroscopies.

3.3. The Optimization of the CuAAC Click SGP. We have carried out a systematic study of the click SGP for optimizing this multiparameter process in terms of the solvent, duration of diazide monomer formation (Step 1), solution concentration, catalyst concentration, catalyst and catalyst activator (ligand) nature, catalyst/ligand mole ratio, temperature of both steps of the one-pot/two-step/three-component reaction, diazide monomer formation (Step 1, Figure 4,A) and click SGP between diazide and bis-alkyne monomers (Step 2, Figure 4,B), and the duration of click SGP between diazide and bis-alkyne monomers (Step 2). The main goal of this optimization is to increase the yields and molecular weights (MWs) of the click polymers as the material characteristics depend on polymers MWs.

The PE PE8E4 based on the monomers E8A and E4B was selected as an object for a systematic study for optimizing the CuAAC click SGP since the mentioned monomers were

TABLE 2: The characteristics of di-(bromoacetic acid) alkylene diesters.

Dibromoacetate	Empirical formula (FW)	Yield* (%)	m.p. (°C)	$(N_C + N_O)/N_N^{**}$
E4B	$C_8H_{12}Br_2O_4$ (331.98)	90.7	66–68	2.00
E6B	$C_{10}H_{16}Br_2O_4$ (360.04)	64.4	42–44	2.30

* After recrystallization from acetone/water mixture. ** The ratio for the corresponding diazide monomers.

TABLE 3: The influence of the organic solvent on the characteristics of PE8E4.

Organic solvent	Yield (%)	M_w (g/mol)*	M_n (g/mol)*	\bar{D}^*	η_{red} (dL/g)**
DMSO	75.9	9,100	5,100	1.77	0.07
HMPA	70.4	9,300	5,600	1.65	0.09
DMA	64.7	16,900	8,600	1.97	0.14
DMI	75.6	18,500	9,500	1.93	0.15
NMP	90.8	26,600	13,300	1.99	0.17

* GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. ** Determined in DMF at 25°C.

obtained in the highest yields (see Tables 1 and 2). As it was mentioned above, to avoid the use of potentially dangerous diazide monomers, their dibromide precursors such as di-(bromoacetic acid)-alkylene diesters were obtained. The click PE PE8E4 was synthesized *via* one-pot/two-step/three-component procedure (Figure 4, $k = 8$, $l = 4$) by interaction of E4B with 10 mol.% excess (invariable parameter) of sodium azide resulting in the diazide monomer (Step 1), and subsequent click SGP reaction between diazide and bis-alkyne monomers (Step-2).

3.3.1. The Influence of the Organic Solvent. Five different organic solvents, DMSO, HMPA, DMA, DMI, and NMP (*variable* parameter), were used and their influence on the molecular weight of target PE PE8E4 was studied. Herein, the invariable parameters and conditions of the SGP were as follows: the duration of Step 1, 1 h; solution concentration, 0.6 mol/L (per a monomer); catalyst (CuI) concentration, 20 mol% (per a monomer); ligand, TEA; catalyst/ligand mole ratio, 1:10; temperature of both Step 1 and Step 2, 20°C; duration of the reaction between diazide and bis-alkyne monomers (Step 2), 24 h.

As one can see from the obtained data, listed in Table 3, the click polymer PE8E4 with the lowest MWs ($M_w = 9,100$ g/mol) and η_{red} (0.07 dL/g) was synthesized when DMSO was used as a solvent. Practically the same results were obtained in case of HMPA. The PEs with higher MWs and η_{red} were obtained in DMA and DMI ($M_w = 16,900$ and 18,500 g/mol, resp.), and PE8E4 with the highest MWs ($M_w = 26,600$ g/mol) and η_{red} (0.17 dL/g) was synthesized when NMP was used as a solvent. Dispersity of the PEs, \bar{D} , varied from 1.65 to 1.99. The highest yields of the target PEs were achieved when NMP was used as a solvent (90.8%, Table 3). Therefore, NMP was selected as a solvent in the subsequent optimization experiments.

3.3.2. The Influence of the Duration of Step 1. For obtaining high-molecular-weight PEs, the outcome of Step 1 should be close to 100%; otherwise stoichiometric imbalance in AA-BB type step-growth processes causes decrease of molecular weights of polymers. The completeness of Step 1 should depend on its duration.

To study the influence of the duration of the reaction between E4B and sodium azide on the one-pot/two-step/three-component click SGP, the time of Step 1 was varied from 1 to 5 h. The invariable parameters and conditions of the SGP were as follows: the solvent, NMP; monomer concentration, 0.6 mol/L; catalyst (CuI) concentration, 20 mol% (per a monomer); ligand, TEA; catalyst/ligand mole ratio, 1:10; temperature of both Step 1 and Step 2, 20°C; duration of Step 2, 24 h.

The completeness of Step 1 was assessed by the final results of the one-pot/two-step/three-component process by the PE yield and MWs. The results, given in Table 4, show that increasing the duration of Step 1 from 1 to 3 h drastically increased MWs (M_w from 26,600 to 50,500 g/mol, $M_n =$ from 13,300 to 21,500 g/mol) and η_{red} (from 0.17 to 0.25 dL/g) of the resulting PE. In this case slight increasing of \bar{D} was observed as well (from 1.99 to 2.34). Further increasing of the duration of Step 1 up to 5 h had negligible influence on the MWs, η_{red} , and \bar{D} of the resulting PE, indicating that for 3 h E4B completely transformed into corresponding diazide monomer. Variation of the duration of Step 1 from 1 to 5 h virtually had no influence on the yield of the PE. Based on the obtained results, 3 h was considered as an optimal duration of Step 1 and used in the subsequent experiments.

3.3.3. The Influence of the Solution Concentration. The solution concentration was varied within the range 0.15–1.20 mol/L (per a monomer) in order to find an

TABLE 4: The influence of the duration of Step 1 of the one-pot/two-step/three-component process on the characteristics of PE8E4.

Duration of reaction between E4B and NaN ₃ (h)	Yield (%)	M_W (g/mol)*	M_n (g/mol)*	\bar{D} *	η_{red} (dL/g)**
1	90.8	26,600	13,300	1.99	0.17
3	88.9	50,500	21,500	2.34	0.25
5	87.6	43,700	19,200	2.27	0.25

*GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. **Determined in DMF at 25°C.

TABLE 5: The influence of the solution concentration (per a monomer) on the characteristics of PE8E4.

A monomer concentration (mole/L)	Yield (%)	M_W (g/mol)*	M_n (g/mol)*	\bar{D} *	η_{red} (dL/g)**
0.15	81.3	20,400	9,600	2.11	0.16
0.30	87.1	44,800	18,200	2.46	0.21
0.60	88.9	50,500	21,500	2.34	0.25
1.20	85.8	21,000	10,700	1.96	0.14

*GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. **Determined in DMF at 25°C.

TABLE 6: The influence of the catalyst concentration on the characteristics of PE8E4.

Catalyst concentration (mol% per a monomer)	Yield (%)	M_W (g/mol)*	M_n (g/mol)*	\bar{D} *	η_{red} (dL/g)**
5	86.5	34,200	16,200	2.12	0.21
20	88.9	50,500	21,500	2.34	0.25
50	81.7	22,100	10,800	2.04	0.14
80	78.4	21,200	10,700	1.98	0.13

*GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. **Determined in DMF at 25°C.

optimal concentration for the one-pot/two-step/three-component synthesis of click SGP. The invariable parameters and conditions of the SGP were as follows: the solvent, NMP (found as the best); duration of Step 1, 3 h (found as an optimal); catalyst (CuI) concentration, 20 mol% (per a monomer); ligand, TEA; catalyst/ligand mole ratio, 1:10; temperature of both Step 1 and Step 2, 20°C; duration of Step 2, 24 h.

The results of the experiments, listed in Table 5, show that increasing the solution concentration from 0.15 mol/L to 0.60 mol/L causes significant increasing of MWs (M_W from 20,400 to 50,500 g/mol and M_n from 9,600 to 21,500 g/mol) and η_{red} (from 0.16 to 0.25 dL/g) of the resulting PE. A further increasing of the concentration up to 1.20 mol/L resulted in too viscous solution, difficult to stir, that led to drastic decreasing in the MWs and η_{red} of the PE. Based on the obtained data, solution concentration 0.60 mol/L was considered as an optimal and applied in subsequent experiments.

3.3.4. The Influence of the Catalyst Concentration. The effect of the catalyst concentration was studied for CuI. The concentration of the catalyst was varied within 5–80 mol% (per a monomer). The invariable parameters and conditions of the PE8E4's synthesis were as follows: the solvent, NMP; duration of Step 1, 3 h; solution concentration, 0.6 mol/L; ligand, TEA;

catalyst/ligand mole ratio, 1:10; temperature of both Step 1 and Step 2, 20°C; duration of Step 2, 24 h.

The data listed in Table 6 show that increasing the catalyst concentration from 5 to 20 mol% increased the MWs (M_W from 34,200 to 50,500 g/mol, M_n from 16,200 to 21,500 g/mol) and η_{red} (from 0.21 to 0.25 dL/g) of the resulting PE. Further increasing of the catalyst concentration up to 50 and 80 mol% significantly decreased the MWs and η_{red} of the PE; slight decreasing of the yield of the PE8E4 from 88.9 to 78.4% was observed as well. Based on the obtained data, the catalyst (CuI) concentration 20 mol% was considered as an optimal and applied in subsequent experiments.

3.3.5. The Influence of the Catalyst and Ligand Nature. Three catalysts (CuI, CuBr, and CuBr(PPhe₃)₃) and four ligands (TEA, DBU, DIPEA, and PMDTA) were examined in one-pot/two-step/three-component synthesis of click SGP. The invariable parameters and conditions of the SGP were as follows: the solvent, NMP; duration of Step 1, 3 h; solution concentration, 0.6 mol/L; catalyst concentration, 20 mol%; catalyst/ligand mole ratio, 1:10; temperature of both Step 1 and Step 2, 20°C; duration of Step 2, 24 h.

The obtained results are listed in Table 7. The least effective ligand was DBU leading to the PEs with the lowest MWs (M_W varied within 5,100–6,900 g/mol), η_{red} (0.03–0.04 dL/g), and yields (15.8–26.3%) regardless of the nature of the catalyst

TABLE 7: The influence of the catalyst and ligand's nature on the characteristics of PE8E4.

Catalyst/ligand	Yield (%)	M_W (g/mol)*	M_n (g/mol)*	\mathcal{D} *	η_{red} (dL/g)**
CuI/TEA	88.9	50,500	21,500	2.34	0.25
CuI/DBU	26.3	6,900	4,100	1.67	0.04
CuI/DIPEA	86.5	50,400	16,300	3.08	0.19
CuI/PMDTA	79.6	31,400	14,800	2.11	0.16
CuBr/TEA	87.1	39,200	18,500	2.12	0.18
CuBr/DBU	18.3	5,300	3,100	1.70	0.03
CuBr/DIPEA	87.2	50,300	16,900	2.98	0.16
CuBr/PMDTA	81.9	32,600	16,700	1.94	0.18
CuBr(PPhe ₃) ₃ /TEA	77.4	11,700	6,900	1.69	0.09
CuBr(PPhe ₃) ₃ /DBU	15.8	5,100	3,000	1.73	0.03
CuBr(PPhe ₃) ₃ /DIPEA	73.6	12,500	7,100	1.74	0.09
CuBr(PPhe ₃) ₃ /PMDTA	71.2	15,100	8,600	1.75	0.09

*GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. **Determined in DMF at 25°C.

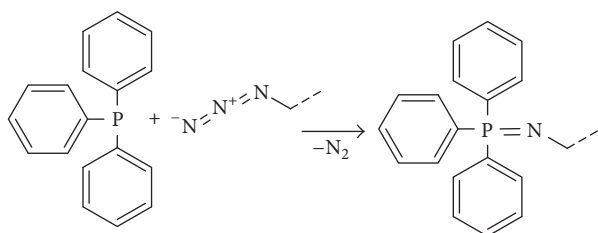


FIGURE 6: Scheme of the Staudinger reaction.

used. The PE8E4 with the highest MWs ($M_W = 50,500$ and $M_n = 21,500$ g/mol) and η_{red} (0.25 dL/g) together with a high yield (88.9%) was synthesized by using the pair of CuI/TEA. It should be noted that the polymers with a comparable weight-average MWs were obtained when using the DIPEA with CuI and CuBr ($M_W = 50,400$ and $50,300$ g/mol, resp.), but in this case molecular weight distribution of the PE was increased ($\mathcal{D} = 3.08$ and 2.98 , resp.) and reduced viscosity was lower ($\eta_{red} = 0.19$ and 0.16 , resp.); less effective ligand was PMDTA with both catalysts, CuI and CuBr ($M_W = 31,400$ and $32,600$ g/mol, $\eta_{red} = 0.16$ and 0.18 dL/g, resp.). The worst catalyst was Cu(PPhe₃)₃Br which resulted in the PE with the lowest characteristics regardless of the used ligand (M_W varies in the range of 5,100–15,100 g/mol, η_{red} within 0.03–0.09 dL/g, and the yield within 15.8–77.4%). Presumably, obtained low molecular weights are due to the Staudinger reaction (Figure 6) [38, 39]; a side reaction between azide group and triphenylphosphine of Cu(PPhe₃)₃Br may lead to break of the 1:1 stoichiometric balance of the functional groups, which in turn is decisive for obtaining high-molecular-weight polymers in SGP. Based on the obtained data, the selection of the proper catalyst/ligand system significantly determines the success of CuAAC click SGP. The pair CuI/TEA was considered as an optimal catalytic system and employed in subsequent experiments.

3.3.6. The Influence of the Catalyst/Ligand Mole Ratio. The mole ratio between the catalyst (CuI) and ligand (TEA) was

varied as 1:1, 1:2, 1:15, and 1:10. The invariable parameters and conditions of the SGP were as follows: the solvent, NMP; duration of Step 1, 3 h; solution concentration, 0.6 mol/L; catalyst (CuI) concentration, 20 mol%; temperature of both Step 1 and Step 2, 20°C; duration of Step 2, 24 h.

The results of the experiments, listed in Table 8, display that increasing the mole ratio CuI/TEA from 1:1 to 1:5 led to the significant increasing of MWs (M_W from 24,800 to 50,700 g/mol and M_n from 10,900 to 22,200 g/mol) and η_{red} (from 0.19 to 0.25 dL/g) of the resulting PEs, whereas further increasing of the quantity of ligand (CuI/TEA = 1:10) did not influence the results. The variation of the catalyst/ligand mole ratio has a negligible influence on the molecular weight distributions and yields of the PE. The CuI/TEA mole ratio 1:5 was considered as an optimal and used in the subsequent experiments.

3.3.7. The Influence of the Reaction Temperature. The temperature was varied at both steps of the one-pot/two-step/three-component/CuAAC click SGP. The invariable parameters and conditions of the SGP were as follows: the solvent, NMP; duration of Step 1, 3 h; catalyst concentration, 20 mol%; solution concentration, 0.6 mol/L; CuI/TEA molar ratio, 1:5; total duration of Step 2, 24 h.

The obtained results, given in Table 9 show that the PE8E4 with the highest characteristics (Sample 1: $M_W = 73,500$, $M_n = 32,300$ g/mol, $\eta_{red} = 0.45$ dL/g, yield 90.1%) was obtained when Step 1 was carried out at 20°C and Step 2 at 0°C for about 4 h (until the solution became too viscous to stir) and then proceeded at 20°C for an additional 20 h. Further decreasing of the temperature of Step 2 to -10°C (Sample 2) virtually did not influence characteristics of the PE8E4. Performing both Step 1 and Step 2 at 20°C (Sample 3) resulted in the PE8E4 with medium characteristics ($M_W = 50,700$, $M_n = 22,200$ g/mol, $\eta_{red} = 0.25$ dL/g, and yield 89.1%). Using 60°C for Step 1 and 20°C for Step 2 (Sample 4) also led to the satisfactory results ($M_W = 41,100$, $M_n = 18,000$ g/mol, $\eta_{red} = 0.22$ dL/g, and yield 86.3%). Increasing the temperature of both Step 1 and Step 2 up to 40 and 60°C gave the worst results (Samples 5 and 6) including the yields of the PE8E4. These

TABLE 8: The influence of the catalyst/ligand mole ratio on the characteristics of PE8E4.

Catalyst/ligand mole ratio	Yield (%)	M_w (g/mol)*	M_n (g/mol)*	\bar{D} *	η_{red} (dL/g)**
1:1	87.0	24,800	10,900	2.27	0.19
1:2	87.3	24,300	10,700	2.26	0.19
1:5	89.1	50,700	22,200	2.29	0.25
1:10	88.9	50,500	21,500	2.34	0.25

*GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. **Determined in DMF at 25°C.

TABLE 9: The influence of reaction temperature on the characteristics of PE8E4.

#	Temperature, °C		Yield (%)	M_w (g/mol)*	M_n (g/mol)*	\bar{D} *	η_{red} (dL/g)**
	Step 1	Step 2					
(1)	20	0 [†]	90.1	73,500	32,300	2.27	0.45
(2)	20	-10 [†]	88.6	70,900	30,600	2.31	0.44
(3)	20	20	89.1	50,700	22,200	2.29	0.25
(4)	60	20	86.3	41,100	18,000	2.28	0.22
(5)	40	40	79.8	22,000	11,100	1.97	0.18
(6)	60	60	77.2	16,800	9,100	1.83	0.14

*GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. **Determined in DMF at 25°C. [†]Step 2 was carried out at 0° or -10°C until the solution became too viscous to stir, and then the reaction was completed at 20°C.

TABLE 10: The influence of the reaction duration on the characteristics of PE8E4.

Duration of the Step 2 (h) [†]	Yield (%)	M_w (g/mol)*	M_n (g/mol)*	\bar{D} *	η_{red} (dL/g)**
5	65.5	46,600	20,000	2.33	0.24
10	80.2	60,200	26,000	2.31	0.36
15	89.7	73,700	32,200	2.28	0.45
24	90.1	73,500	32,300	2.27	0.45
48	89.5	73,800	32,400	2.28	0.45

*GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. **Determined in DMF at 25°C. [†]For the first 4 h Step 2 was performed at 0°C.

data could speak that the temperature of Step 2 is decisive for achieving high MWs in one-pot/two-step/three-component CuAAC click SGP.

3.3.8. The Influence of the Duration of Step 2 under the New Temperature Regime. It was found that an optimal duration of Step 1 is 3 h (see Section 3.3.2). As the final step of the optimization of the one-pot/two-step/three-component CuAAC click SGP we investigated an optimal duration of Step 2. The duration of Step 2 was varied from 5 to 48 h. In this case invariable was parameters and conditions found as optimal (see Sections 3.3.1–3.3.7): solvent, NMP; duration of Step 1, 3 h; catalyst concentration, 20 mol%; solution concentration, 0.6 mol/L; catalyst/ligand pair, CuI/TEA, and its molar ratio, 1:5; temperature of Step 1, 20°C, and of Step 2, 0°C (as an initial temperature). At 0°C Step 2 was performed for the first 4 h, and then the reaction was proceeded at 20°C till predetermined time 5–48 h, which is listed in Table 10 along with the obtained data. After the predetermined time the polymer was separated from the reaction solution as described in Section 2.5. According to the obtained data, increasing the total time of Step 2 from 5 to 10 h increased the characteristics of the PE8E4 (M_w from 46,600 to 60,200 g/mol, M_n from 20,000 to 26,000 g/mol, η_{red} from 0.24 to 0.36 dL/g, and

yield from 65.5 to 80.2%). Maximal characteristics (M_w 73,700 g/mol, M_n 32,200 g/mol, η_{red} 0.45 dL/g, and yield 89.7%) were achieved by increasing the total reaction time up to 15 h. Further increasing the total time from 24 to 48 h had no influence on the obtained data (minor differences lie within experimental errors).

3.4. Synthesis of Various Click PEs under the Established Optimal Parameters and Conditions. A series of new click PEs were synthesized via one-pot/two-step/three-component CuAAC click SGP under the established optimal parameters and conditions: the solvent, NMP; duration of Step 1, 3 h; catalyst concentration, 20 mol%; solution concentration, 0.6 mol/L; catalyst/ligand pair, CuI/TEA, and its mole ratio, 1:5; temperature of Step 1, 20°C, and of Step 2, 0°C for 4–6 h (until the solution became too viscous to stir) and 20°C for 9–11 h (i.e., for total 15 h).

According to the obtained data given in Table 11, the PEs PE8E4 and PE4E6 have the highest MWs (M_w 73,700 and 72,600 g/mol, M_n 32,200 and 21,900 g/mol, η_{red} 0.45 and 0.24 dL/g, and yields 89.7 and 91.3%, resp.). The PEs PE2E4, PE2E6, and PE8E6 have medium MWs and η_{red} , whereas the PE4E4 show the lowest MWs (M_w 26,000 and M_n 12,100 g/mol, and η_{red} 0.13 dL/g). All the synthesized PEs

TABLE 11: Characteristics of the obtained click PEs.

PE	Yield (%)	M_w (g/mol)*	M_n (g/mol)*	\bar{D} *	η_{red} (dL/g)**
PE2E4	83.1	44,900	19,800	2.26	0.23
PE4E4	82.6	26,000	12,100	2.13	0.13
PE8E4	89.7	73,700	32,200	2.28	0.45
PE2E6	80.2	42,300	17,300	2.43	0.16
PE4E6	91.3	72,600	21,900	3.31	0.24
PE8E6	82.8	54,500	16,600	3.28	0.23

* GPC in 0.1 M LiBr/DMF versus PMMA standards at a flow rate of 1.0 mL/min and 35°C. ** Determined in DMF at 25°C.

TABLE 12: Solubility of the click PEs in organic solvents (10 mg of polymer in 1 mL solvent).

Polymer	DMSO	NMP	DMF	DMA	DMI	TFE	HFIP	DCM	EtOH	Acetone	CH ₃ CN	EtAc
PE2E4	+	+	+t	+t	+t	+	-	-	-	-	-	-
PE4E4	+	+	+t	+t	+	+	+	-	-	-	-	-
PE8E4	+	+	+	+	+	+	+	-	-	-	-	-
PE2E6	+	+	+t	+t	+	+	+	-	-	-	-	-
PE4E6	+	+	+t	+t	+	+	+	-	-	-	-	-
PE8E6	+	+	+	+	+	+	+	+	-	-	-	-

“+”: soluble, “-”: insoluble, and “+t”: soluble upon heating.

reveal rather wide molecular weight distribution, and the polymers PE4E6 and PE8E6 show the widest dispersity (\bar{D} = 3.31 and 3.28, resp.). The obtained PEs, except for PE2E4 and PE4E4, possess elastic film-forming properties (cast from TFE solution). The films from PE2E4 and PE4E4 were brittle and grindable into fine powders.

3.5. The Structure of the Click PEs. The FTIR study well confirmed the presumed structure of PEs in which all the expected absorption bands were observed: 1740–1728 cm^{-1} (ester C=O) and 1233–1209 cm^{-1} (C-O-C), as well as 3092–3080 cm^{-1} that corresponds to stretching vibration of C=C-H of the 1,2,3-triazole moiety. The detailed ^1H and ^{13}C NMR study was performed with the selected sample PE8E4. A signal of 1,2,3-triazole proton (C=C-H) at 8.15 ppm in the ^1H NMR spectrum and a signal of C₅ carbon at 126 ppm in the ^{13}C NMR spectrum of the PE8E4 indicate exclusive formation of 1,4-disubstituted triazoles and speak on a high regioselectivity of Cu(I) catalyzed click SGP.

3.6. Solubility of the Click PEs. The solubility of the obtained click polymers in organic solvents (10.0 mg in 1.0 mL) is given in Table 12. The click PEs were insoluble in common solvents such as EtOH, acetone, acetonitrile, and ethyl acetate even upon heating. At the same time the polymers showed a good solubility in polar solvents such as DMSO, NMP, DMF, DMA, DMI, TFE, and HFIP at r.t. and some of them at elevated temperatures, except for the PE2E4 which was insoluble in HFIP. Among the PEs, only PE8E6 was well-soluble in DCM, which contains the longest polymethylene chains in the backbones. In whole, the new click PEs showed poor solubility in organic solvents as compared with poly(alkylene dicarboxylate)s presumably owing to the increased rigidity of the polymer backbone and more strong intermolecular forces stipulated by 1,2,3-triazole rings.

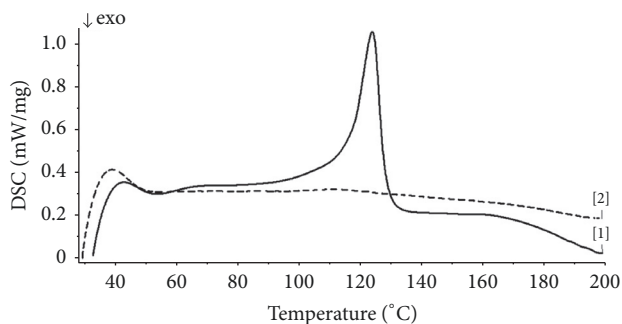


FIGURE 7: The representative DSC profile of the click PE PE8E4: [1], the first heating scan; [2], the second heating scan after a heating-cooling cycle.

3.7. Thermal Properties of the Click PEs. A high rigidity of the polymeric chains and more strong intermolecular forces stipulated by 1,2,3-triazole rings determine the thermal properties of the new click PEs. For a comparison, the melting temperatures (T_m) of the click PEs and some poly(alkylene dicarboxylate)s are given in Table 13. It is evident that T_m of the click PEs are much higher (within 123–192°C) than T_m of poly(alkylene dicarboxylate)s with an equal number of methylene groups in a repeating unit (within 63–178°C). Thus, the insertion of the rigid 1,2,3-triazole rings in the PEs backbone can substantially improve the thermal properties of the polymers. All synthesized click PEs are difficult or impossible to recrystallize; on the second heating curve of DSC measurement endothermic peak disappears (a representative thermogram of PE8E4 is given in Figure 7).

3.8. MPD, PDI, and ZP of the Fabricated NPs. The NPs on the basis of obtained click PEs were prepared according to

TABLE 13: Melting temperatures (T_m) of the click PEs versus T_m of the regular PEs.

Click PEs, (N)	T_m ($^{\circ}\text{C}$)	Poly(alkylene dicarboxylate)s, (N)	T_m ($^{\circ}\text{C}$) [Ref.]
PE2E4 (10)	138	Polyethylene sebacate (10)	78 [40]
PE4E4 (12)	131	Polybutylene sebacate (12)	63 [41]
PE8E4 (16)	123	-	-
PE2E6 (12)	160	Polybutylene sebacate (12)	63 [41]
PE4E6 (14)	168	Polyhexamethylene sebacate (14)	65 [42]
PE8E6 (18)	192	-	-

N : A number of methylene groups in a repeating unit.

TABLE 14: The MPD, PDI, and ZP of prepared NPs*.

Click PE	MPD (nm) \pm SD	PDI \pm SD	ZP (mV) \pm SD
PE2E4	171.5 \pm 3.6	0.150 \pm 0.009	-12.5 \pm 0.5
PE4E4	142.0 \pm 4.2	0.360 \pm 0.034	-13.6 \pm 0.3
PE8E4	128.5 \pm 1.0	0.203 \pm 0.010	-12.1 \pm 0.5
PE2E6	113.6 \pm 1.8	0.181 \pm 0.004	-12.9 \pm 0.9
PE4E6	130.8 \pm 5.3	0.412 \pm 0.029	-10.2 \pm 0.6
PE8E6	66.8 \pm 2.2	0.245 \pm 0.019	-11.6 \pm 0.3

*MPD: Mean Particle Diameter, PDI: Polydispersity Index, and ZP: zeta-potential.

TABLE 15: The stability of the prepared NPs upon storage at 4-5 $^{\circ}$ C.

Time	Click PE					
	PE2E4	PE4E4	PE8E4	PE2E6	PE4E6	PE8E6
	MPD (nm) \pm SD			[PDI \pm SD]		
Freshly prepared	171.5 \pm 3.6 [0.150 \pm 0.009]	142.0 \pm 4.2 [0.360 \pm 0.034]	128.5 \pm 1.0 [0.203 \pm 0.010]	113.6 \pm 1.8 [0.181 \pm 0.004]	130.8 \pm 5.3 [0.412 \pm 0.029]	66.8 \pm 2.2 [0.245 \pm 0.019]
A month later	198.3 \pm 5.3 [0.273 \pm 0.018]	152.4 \pm 3.1 [0.483 \pm 0.031]	136.2 \pm 2.3 [0.248 \pm 0.012]	128.1 \pm 2.4 [0.312 \pm 0.017]	Aggregation	Aggregation
Two months later	223.4 \pm 3.9 [0.315 \pm 0.012]	201.2 \pm 4.3 [0.492 \pm 0.026]	180.1 \pm 3.7 [0.443 \pm 0.016]	Aggregation	—	—

the polymer deposition/solvent displacement (nanoprecipitation) method using Tween 20 as a surfactant (see Materials and Methods). All the fabricated NPs were characterized by MPD, PDI, and ZP (Table 14) using DLS method. According to the obtained results, all the obtained click PEs are suitable polymers for preparing nanoscale particles with the MPD within 66.8–171.5 nm. As to particle size distribution, the PE2E4 formed NPs of mean distribution ($0.04 \leq \text{PDI} \leq 0.16$); all other click PEs resulted in the NPs of wide distribution ($\text{PDI} > 0.16$). All the fabricated NPs are negatively charged; the ZP varied within $-10.2 \div -13.6$ mV. Nevertheless, this weak negative charge is sufficient to ensure the stability of NPs upon a storage. Presumably, the negative ZP of the NPs can be caused by a partial hydrolysis of the ester links of the click PEs that generates free carboxyl groups (carboxylate anions $-\text{COO}^-$).

3.9. Stability of the NPs. The stability of the negatively charged NPs was studied up to 2 months at 4-5 $^{\circ}$ C (the samples were refrigerated). The data listed in Table 15 show that the NPs made of the PE4E6 and PE8E6 have the worse stability; the samples were virtually fully aggregated (micronized big

particles were formed) after a month of storage. The NPs made of the PE2E6 showed medium stability; they were aggregated after two months. The most stable were the NPs made of the PE2E4, PE4E4, and PE8E4, though they also showed a tendency of increasing MPD and PDI after two months' storage. We suppose that the stability of the NPs made of click PEs can be improved by imparting a high positive charge through the quaternization of the 1,2,3-triazole rings using quaternizing agents such as alkyl iodides or methoxy PEG bromides. Using the latter as quaternizing agent can at the same time enhance the biocompatibility of the materials. This study is in progress now.

4. Conclusions

In this article we reported a new strategy of the synthesis of 1,2,3-triazole rings-containing heterochain click polymers we have designed. The new strategy is based on the application of diyne derivatives of dicarboxylic acids as bis-alkyne monomers and bifunctional derivatives of di-bromoacetic acid as precursors of diazide monomers which are formed by substitution of bromine atom by

azide group in one pot procedure without separating. The new approach could be applied to both Cu(I)-catalyzed and copper-free click SGP. We used the new strategy in the synthesis of AA-BB-type aliphatic polyesters *via* Cu(I)-catalyzed click SGP. Six click polyesters were synthesized *via* the new one-pot/two-stage/three-component SGP using three bis-propargyl dicarboxylates as diyne monomers and two di-(bromoacetic acid)-alkylene diesters. The polyesters with high MWs (M_w 26.0–73.7 kDa) in good yields were obtained. These polyesters contain 1,4-disubstituted 1,2,3-triazoles in the backbones. The presence of the main-chain triazoles significantly improved the thermal properties of the polyesters. Elastic films and nanoparticles made of the new PEs are suitable as biodegradable biomaterials for biomedical applications. Our work should encourage the synthesis of novel heterochain polymers of various types and classes with 1,4-disubstituted 1,2,3-triazoles by applying the new synthetic strategy to both Cu(I)-catalyzed and copper-free click step-growth polymerizations.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

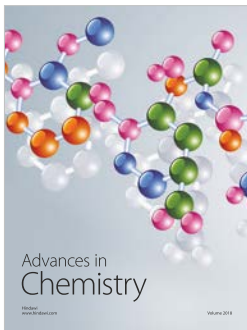
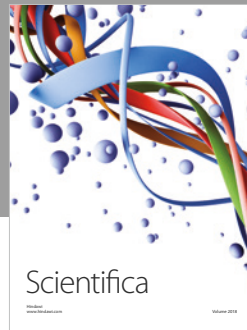
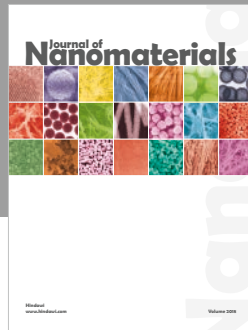
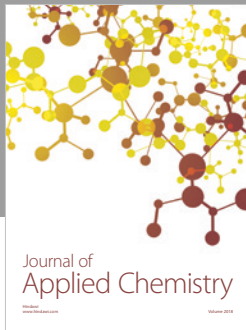
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