Journal of Balkan Science and Technology
journal homepage: www.jbst.nku.edu.tr/

Preparation and Dual-Functionalization of Pendant Aldehyde and Azide-Bearing Hydrophilic Polymer

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Research Article

Keywords:
Dual-reactive polymer,
RAFT,
Reductive amination,
Huisgen Click

Received: 04.07.2024
Accepted: 06.11.2024
Published: 30.12.2024

DOI: 10.55848/jbst.2024.45

ABSTRACT

Polymers with more than one functionality hold significant value due to their diverse applications across various biological fields. This study describes the synthesis of dual-reactive and hydrophilic polymer via Reversible Addition-Fragmentation Chain Transfer Polymerization (RAFT), suitable for drug delivery systems. Pendant hydrophilic polyethylene glycol units enhance the polymer's water solubility, while aldehyde and azide side chains create opportunities for further functionalization. Side chain functionalization studies were conducted using amine and alkyne-containing model molecules. Azide side chains were functionalized with ((prop-2-yn-1-yloxy)methyl)benzene through the Huisgen-Click reaction while the aldehyde side chains were linked to amyl amine via reductive amination reaction.

1. Introduction

The design and synthesis of polymers with pendant or end reactive groups have been gaining significant attention in material science [1-4]. Moreover, polymeric scaffolds with different reactive groups can be modified into multi-functional structures for desired applications through selective conjugation chemistries [5-8]. For example, Kilbinger and coworkers reported the synthesis of pendant pentafluorophenol ester, maleimide, and protected alkyne-carrying terpolymer via living ring-opening metathesis polymerization (ROMP). The polymer was stable and useful for the orthogonal triple-functionalization [9]. Tonga et al. synthesized anthracene and azide appended polymers and functionalized them via grafting with reactive polyester dendrons to obtain dendronized complex polymer architectures [10, 11]. However biocompatible, hydrophilic multi-reactive polymer designs are also required since they find utility in polymer-conjugated drug delivery systems, as well as in the development of biosensors and biochips for bioconjugations in biotechnology [12]. Water-soluble polymers having various reactive side groups that can be modified with molecules of interest such as therapeutics, biorecognition units, or imaging agents enable them useful in medicine. The first example of a drug-conjugated polymer goes back to the 1950s [13]. Jatzkewitz conjugated mescaline which is a natural drug known for its hallucinogenic effects, to a copolymer of N-vinylpyrrolidone and acrylic acid. The urinary excretion of mescaline was monitored in mice. The mescaline in the polymer-drug conjugate exhibited an extended circulation time of up to 21 days, while the free drug was rapidly cleared within approximately 16 hours. In the 1970s Ringsdorf proposed a targeted polymer drug carrier concept [14] that would enter clinical trials later [15]. The concept, called the Ringsdorf's model, comprised a polymeric backbone, a linker for the conjugation of the drug to the backbone, a drug, and a targeting group. Nowadays, pendant reactive polymeric systems based on Ringsdorf's model are widely investigated for drug delivery applications [16]. For example, Sanyal and coworkers reported a study regarding the synthesis of novel reactive carbonate monomer which enables the direct synthesis of copolymers with the combination of other reactive monomers such as azide or N-hydroxysuccinimide methacrylate. Successful orthogonal functionalization of pendant azide chains was carried out with azide-reactive alkyne-containing molecules and carbonate/N-hydroxysuccinimide side chains with amine-containing model molecules. It was demonstrated that even though both carbonate/N-hydroxysuccinimide groups are amine-reactive they can be selectively functionalized with different amine-containing molecules [17]. In another study, the carbonate monomer was used to synthesize orthogonally functionalizable soluble copolymers incorporating pendant carbonate groups with a thiol-reactive maleimide end group. The facile functionalization of carbonate side chains with amine-containing anticancer drug doxorubicin and maleimide end group modification with a thiol-containing targeting ligand derived from folic acid was demonstrated and cellular internalization, cytotoxicity, and drug release studies showed promising results [18].

The synthesis of reactive polymers is typically accomplished using various reactive monomers, often

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employing living controlled radical polymerization techniques. Using these polymerization techniques, one can easily vary the ratio of initiator, monomer, and solvent or the reaction time to adjust both the length of the polymer chain and the number of reactive groups in the polymer chain. Atomic Transfer Radical Polymerization (ATRP) [19], Reversible Addition-Fragmentation Chain Transfer Polymerization (RAFT) [20], Ring Opening Metathesis Polymerization (ROMP) [21], Nitroxide Mediated Polymerization (NMP) [22], are well-known living polymerization techniques [23]. Among these techniques, RAFT, first reported in 1998 is one of the most versatile and important for the synthesis of polymeric structures [24]. This is because it allows the design of polymeric architectures with foreseeable molecular weight, narrow molecular weight distribution, and high end-group fidelity. In addition, the use of thiocarbonylthio chain transfer agents in RAFT controls the distribution and end-groups without the need for any metal catalyst.

Although there are studies regarding the direct synthesis of reactive hydrophilic multi-reactive polymers there is still a need for more research in this area. For example, even various monomers were used together with living polymerization techniques through the direct polymerization of reactive monomers, as far as we know aldehyde and azide groups were not used together before to obtain a dual-reactive water-soluble polymer. In our previous study, we used aldehyde and azide monomers to obtain a dual-reactive hydrogel via ATRP [25]. In this work, a dual-reactive polymer was synthesized using azide- and aldehyde-bearing methacrylate monomers and a hydrophilic polyethylene glycol methacrylate. The capability of dual-functionalization of the polymer was evidenced with two model molecules one carrying an aldehyde-reactive amine group and the other one an azide-reactive alkyne unit (Figure 1). Consequently, the hydrophilic dual-reactive polymer system can find applications in polymer-conjugated drug delivery systems.

2. Material And Method

2.1. Materials and Characterizations

6-Azidohexyl methacrylate (AHMA) [25], 6-oxohexyl methacrylate (OHMA) [26], and ((prop-2-yn-1-yloxy)methyl)benzene [27] were synthesized as described in literature procedures.

Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, Mn 300), azobisisobutyronitrile (AIBN), 2-cyano-2-propyl benzodithioate, sodium acetate (NaOAc), sodium borohydride ($NaBH_4$), N", N"pentamethyldiethylenetriamine (PMDTA), sodium azide (NaN_3), methacryloyl chloride (MAC), amylamine, and 1,6-hexanediol were obtained from Sigma-Aldrich. 6-chloro-1-hexanol was purchased from TCI. Tetrahydrofuran (THF), methanol (MeOH), dimethyl formamide (DMF), dichloromethane (DCM), triethylamine (Et_3N), and pyridinium chlorochromate (PCC) were obtained from Merck. Column chromatography was performed using silica gel 60 (43-60 nm, Merck). Thin layer chromatography was performed using silica gel plates (Kieselgel 60 F254, 0.2 mm, Merck). Before use, PEGMEMA was filtered through a short plug of basic alumina to remove the inhibitor. H-NMR spectroscopy characterization of the monomers, ((prop-2-yn-1-yloxy)methyl)benzene and polymers was conducted by using a Varian 400 MHz instrument. The number average molecular weights (Mn) as well as the dispersity index (PDI) were determined by gel permeation chromatography (GPC) using a Shimadzu GPC system furnished with a PSS-SDV (length/ID 8×300 mm, 10µm particle size) linear M column. Polystyrene standards (1-150 kDa) from Viscotek were used for calibration. Tetrahydrofuran (THF) was used as eluent at a flow rate of 1 $mLmin^{-1}$ at 30 ºC.

2.2. Synthesis of Polymer, P1 via RAFT

6-azidohexyl methacrylate (11.50 mg, 0.054 mmol), 6-oxohexyl acrylate (50.00 mg, 0.271 mmol), polyethylene glycol methacrylate (Mn 300) (651.3 mg, 2.168 mmol), 2-cyano-2-propyl benzodithioate, (11. 95 mg, 0.054 mmol) and AIBN (0.89 mg, 0.005 mmol) were dissolved in previously degassed DMF in a flask (2M monomer concentration in solution) and degassed for another15 minutes. The flask in the oil bath was set to 70 ºC. After 5 hours of polymerization time, the reaction was cooled in an ice bath and precipitated in diethyl ether for purification. (yield 90%). Mn: 20 kDa. PDI: 1.3

2.3. Functionalized Polymer, P2 via Huisgen-Click

P1 (30.6 mg, 0.0017 mmol) was dissolved in anhydrous THF (0.8 mL) under a nitrogen atmosphere. PMDTA (1.3 mg, 0.007 mmol) was added to the solution. Then a solution of ((prop-2-yn-1-yloxy)methyl)benzene (10 mg, 0.07 mmol) and Cu(I)Br (1 mg, 0.007 mmol) in 0.2 mL THF was added to the mixture under nitrogen medium and stirred at 40 ºC for 24 h. The mixture was filtered through aluminum oxide and purified by precipitation in diethyl ether (yield 89%).

2.4. Functionalized Polymer, P3 via Aldehyde-Amine Reductive Amination

To a solution of P2 (20 mg, 0.001 mmol) in MeOH (1 mL) amyl amine (6 mg, 0.069 mmol) and, NaOAc (14 mg, 0.17 mmol) were added and stirred at room temperature overnight. Then the mixture was cooled down to 0 ºC and $NaBH_4$ (1.3 mg, 0.035 mmol) was added and stirred for 1 hour. After the reaction, excess $NaBH_4$ was hydrolyzed with $NaHCO_3$ solution (1 mL), evaporated to remove the MeOH, and extraction was carried out with dichloromethane. The dichloromethane phase was dried over Na_2SO_4 and evaporated to remove the solvent (yield 98%).

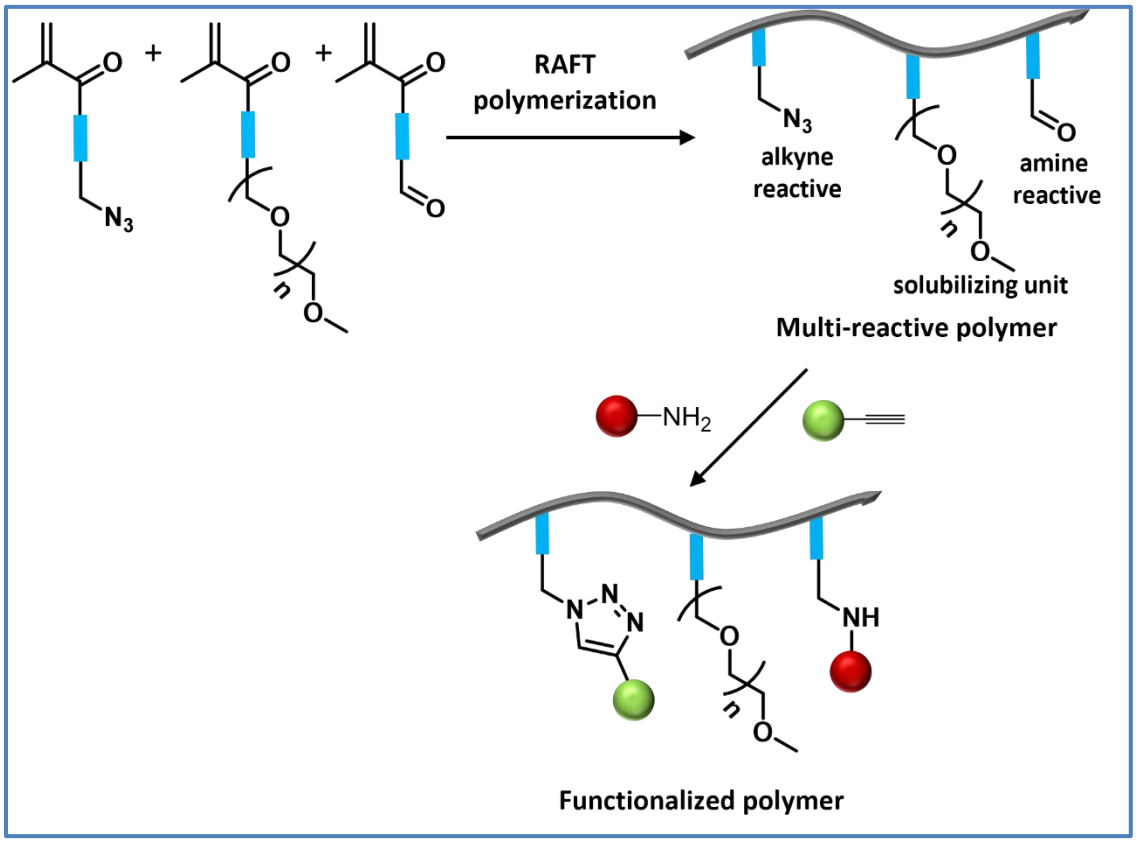

Fig. 1 A general schema for the dual-reactive polymer synthesis and functionalization.

3. Results and Discussion

The main objective of this study is to synthesize a well-defined hydrophilic polymer with low dispersity that can be readily modified with different reactive groups to introduce various functionalities to the polymeric architecture. For this purpose, aldehyde- and azide-containing methacrylate monomers were successfully synthesized according to the literature procedures mentioned earlier [17, 26]. The inclusion of PEGMEMA in the polymer structure was also demanding since PEG possesses hydrophilic and antifouling properties. It can impart water solubility to the polymer and decrease bacterial adhesion, making it a good choice for bioapplications [28].

RAFT is an appropriate living polymerization technique for the direct synthesis of well-defined polymeric architectures and 2-cyano-2-propyl benzodithioate is known as a compatible chain transfer agent for the polymerization of methacrylate monomers [29, 30]. The polymerization was carried out using AHMA, OHMA, and PEGMEMA monomers with 1:5:40 mol ratio with the use of 2-cyano-2-propyl benzodithioate RAFT agent and AIBN radical initiator, at 70 ºC for 5 hours. The polymerization reaction achieved a high yield of 90%, resulting in a polymer with Mn of 20 kDa and PDI of 1.30. GPC result shows the polymer still has a narrow size distribution for a yield of 90%. Figure 2. shows the polymer synthesis reaction and GPC diagram of P1. To demonstrate the dual-reactivity, the azide side chains of P1 were first functionalized with an alkyne-bearing molecule; ((prop-2-yn-1-yloxy)methyl)benzene to obtain P2, and then aldehyde side chains were functionalized with amyl amine molecule to obtain P3. Figure 3. shows the functionalization sequence of P1 in detail. For this purpose, ((prop-2-yn-1-yloxy)methyl)benzene was synthesized by alkylating phenol with propargyl bromide and P1 was reacted with this molecule through azide-alkyne Huisgen cycloaddition reaction to yield P2. Figure 4. shows the H-NMR spectra of P1, before and after functionalization reactions. In the bottom spectrum which belongs to P1 peaks at 9.77 ppm, 3.42 ppm, and 3.36 ppm correspond to aldehyde (CO-***H***), azide (N_3-C***H₂***), and polyethylene glycol (O-C***H₃***) protons respectively. After the first functionalization step, the disappearance of **c** peak at 3.42 ppm corresponds to azide (N_3-C***H₂***) protons, and the rise of new peaks **d** at 4.66 ppm and **e** at 4.58 ppm corresponds to C***H₂***-O-C***H₂*** protons between the benzene and triazole part proves the successful functionalization of P1 through Huisgen click reaction to get P2. Then aldehyde side chains of P2 were functionalized via reductive amination reaction of aldehydes. Reductive amination of aldehydes with amines in the presence of $NaBH_4$ is a well-known and useful method [31-33]. Once the imine intermediate is formed between an aldehyde and an amine compound, C=N bond is known to be reduced with a reducing agent such as $NaBH_4$ to carbon-nitrogen bond eliminating other side reactions. Herein, to functionalize aldehyde side chains of P2 via reductive amination, amyl amine was used as a model molecule in the presence of $NaBH_4$ reducing agent and obtained P3. H-NMR analysis of P3 in Figure 5. shows a loss in the peaks at 9.77 ppm corresponding to the aldehyde proton and the emergence of new peaks at 2,44 ppm (**h)** and 2,29 ppm (**g)** attributed to C***H₂***-NH-C***H₂***, indicating successful functionalization.

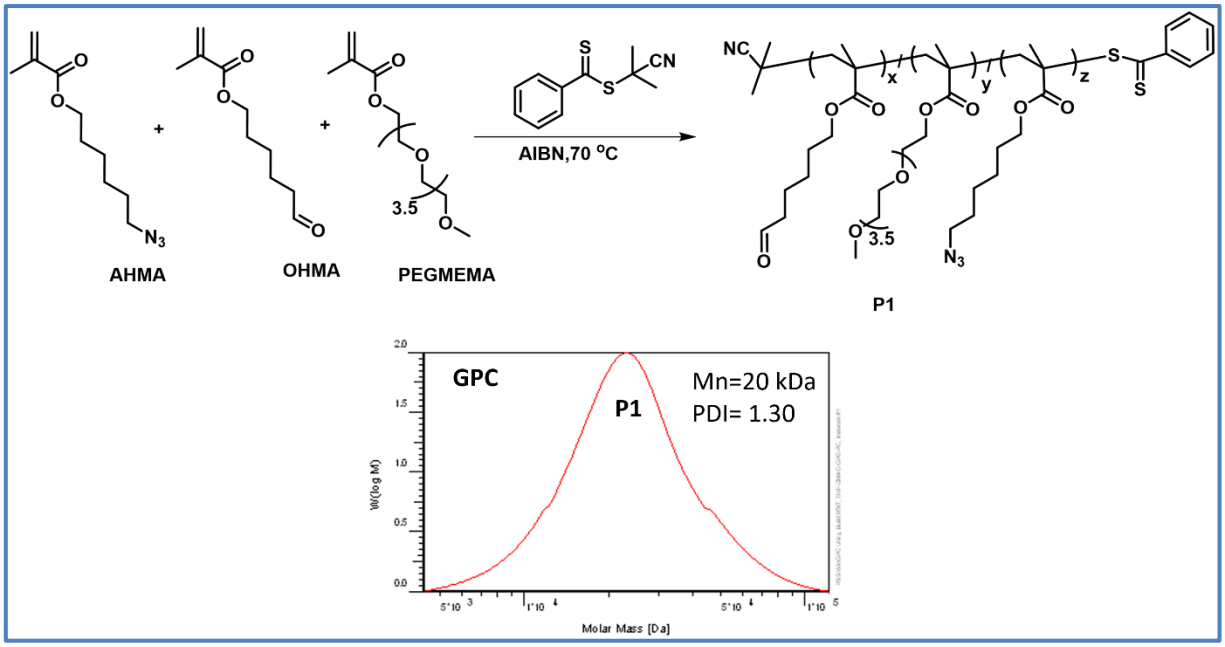

Fig. 2 Dual-reactive polymer synthesis and the GPC curve of P1.

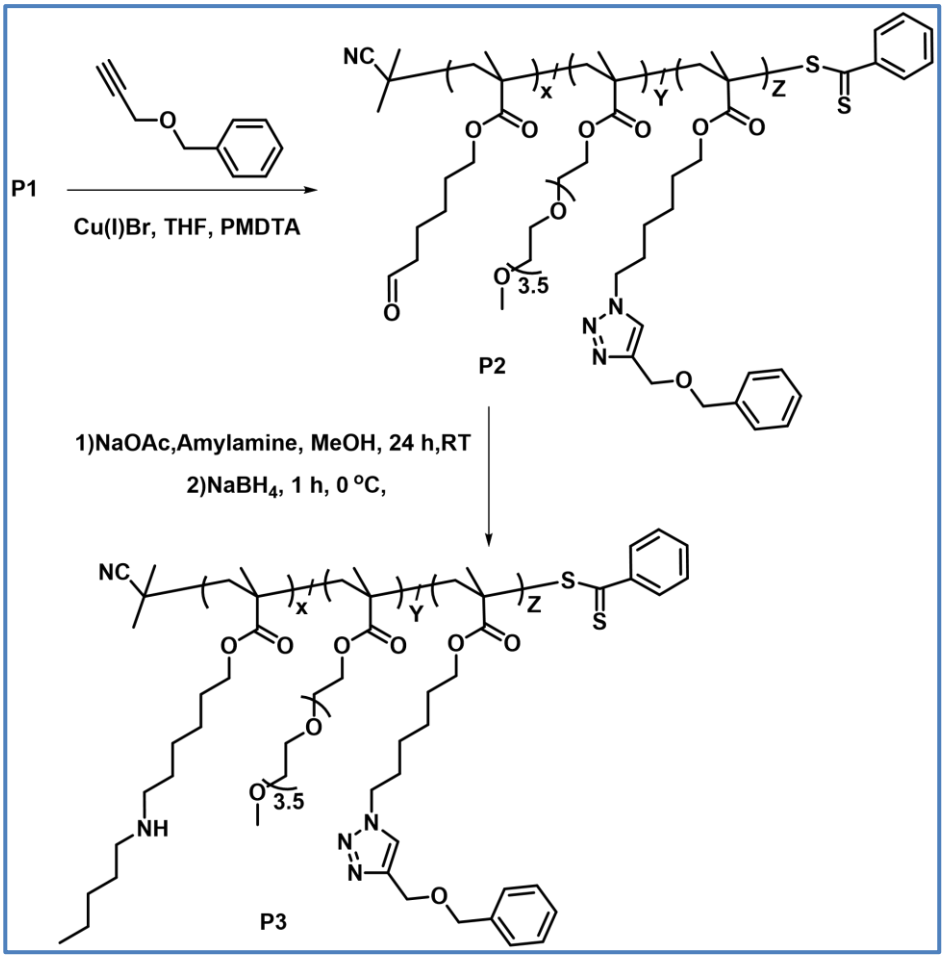

Fig. 3 Functionalization sequence of P1 to obtain P2 and P3.

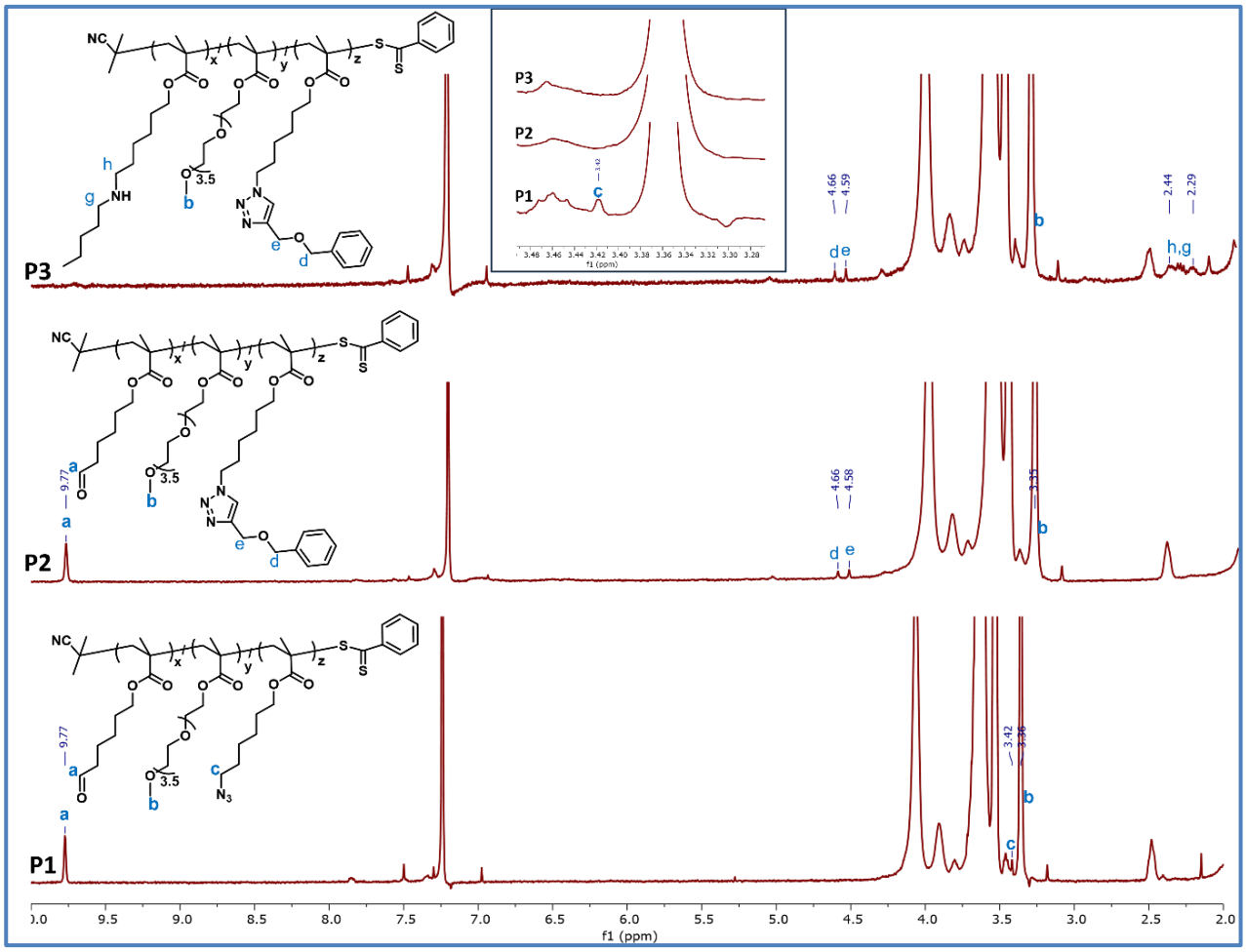

Fig. 4 H-NMR spectra of P1 before functionalization, after functionalization with alkyne molecule (P2), and after functionalization with amyl amine (P3).

4. Conclusion

In conclusion, a dual-reactive hydrophilic polymer with aldehyde and azide side chains of 20 kDa molecular weight was synthesized with narrow molecular weight distribution and high yield via RAFT polymerization. The molecular weight of polymers is known to affect the solubility of polymer-drug conjugate and the drug release rate, and it also influences the viscosity of the polymer which is important in vivo applications [34-35]. In this dual-reactive hydrophilic polymer system, it is possible to play with the molar ratio of monomers to initiator and RAFT agents to determine the molecular weight of the polymer for the desired application. Using the synthesized 20 kDa P1 polymer, the functionalization of the pendant azide groups of the polymer was demonstrated with a model alkyne molecule via Huisgen click reaction. Further functionalization of the polymer was carried out with a model amine molecule via reductive amination of pendant aldehyde chains. These types of narrow-distributed hydrophilic dual-reactive polymers have the potential to be functionalized with various agents, such as imaging agents, drug molecules, or targeting moieties, making them highly promising for polymer-conjugated targeted drug delivery systems.

Declaration

Author Contribution: Conceive– N Cengiz; Design– N Cengiz; Experimental Performance, Data Collection and Processing– N Cengiz; Analysis and Interpretation– N Cengiz; Literature Review– N Cengiz; Writer– N Cengiz; Critical Review– N Cengiz.

Conflict of interests: There are no conflicts to declare.

Acknowledgements

This work is supported by Tekirdağ Namık Kemal University Research Fund (project no. NKUBAP.00.10.AR.15.02).

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