# A Mild and Efficient Approach to Functional Single-Chain Polymeric Nanoparticles via Photoinduced Diels–Alder Ligation

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# A Mild and Efficient Approach to Functional Single-Chain Polymeric Nanoparticles via Photoinduced Diels–Alder Ligation

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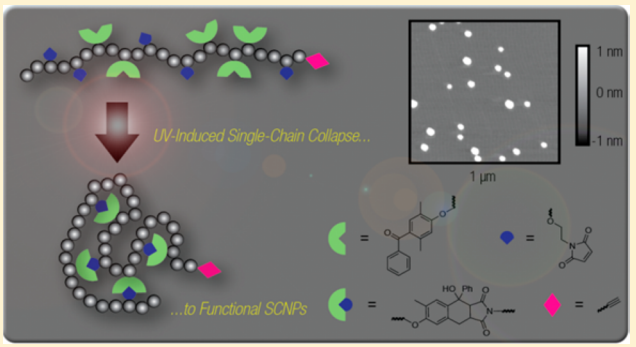
Supporting Information

**ABSTRACT:** We present a new ambient temperature synthetic approach for the preparation of single-chain polymeric nanoparticles (SCNPs) under mild conditions using a UV-light-triggered Diels–Alder (DA) reaction for the intramolecular cross-linking of single polymer chains. Well-defined random copolymers with varying contents of styrene (S) and 4-chloromethylstyrene (CMS) were synthesized employing a nitroxide-mediated radical polymerization (NMP) initiator functionalized with a terminal alkyne moiety. Postpolymerization modification with 4-hydroxy-2,5-dimethylbenzophenone (DMBP) and an *N*-maleimide (Mal) derivative led to the functional linear precursor copolymers. The intramolecular cross-linking was performed by activating the DMBP groups via irradiation with UV light of 320 nm for 30 min in diluted solution ($c_{Polymer}$ = 0.017 mg mL$^{-1}$). The ensuing DA reaction between the activated DMBP and the Mal groups resulted in well-defined single-chain polymeric nanoparticles. To control the size of the SCNPs, random copolymers with varying CMS contents (i.e., different functional group densities (FGD)) were employed for the single-chain collapse. Additionally, monotethered nanoparticles were prepared via the copper-catalyzed azide–alkyne cycloaddition between the alkyne bearing copolymer with the highest FGD and an azide-terminated poly(ethylene glycol) (PEG) prior to UV-induced cross-linking. The formation of SCNPs was followed by size exclusion chromatography (SEC), nuclear magnetic resonance (NMR) spectroscopy, dynamic light scattering (DLS), and atomic force microscopy (AFM).




## INTRODUCTION

Polymeric nanoparticles (NPs) have attracted significant attention in the nanoscience community during the past years.[1] Several techniques were developed for the preparation of polymeric NPs either from preformed polymers through solvent evaporation, salting out, dialysis, and supercritical fluid technology or by direct polymerization of monomers using classical polymerization techniques such as microemulsion, miniemulsion, surfactant-free emulsion, or interfacial polymerization.[2] However, the preparation of well-defined polymeric nanoparticles, smaller than 20 nm, is still challenging using one of the above-noted methods. Single-chain folding technology is a new avenue in polymer science to develop tailor-made materials. New promising methods for the preparation of single-chain polymeric nanoparticles (SCNPs) were developed in the past years.[3] Examples include the controlled collapse of single polymer chains by means of intramolecular covalent bonds,[4] dynamic-covalent chemistry,[5] and noncovalent[6] interactions in highly diluted solutions into polymeric nanoparticles akin to folded biomacromolecules. The folding of a polymer chain into a well-defined structure can be regarded as a very simple mimicry of biomacromolecules such as proteins,[7] which exist in a well-defined secondary, tertiary, and quaternary structure resulting from a specific amino acid sequence. SCNPs with a size range from 5 to 20 nm can be generated by a combination of controlled/living radical polymerization and modular ligation chemistries; however, the monofunctionalization of polymeric nanoparticles with such small sizes is still challenging. Functionalized polymeric nanoparticles within this size range can be considered as building blocks for nanotechnological applications such as drug and DNA delivery systems.[8] Well-defined SCNPs with a single functionality may

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**Scheme 1. General Synthetic Scheme for the Preparation of Monofunctional Single-Chain Polymeric Nanoparticles via Intramolecular UV-Induced Diels–Alder Cross-Linking**

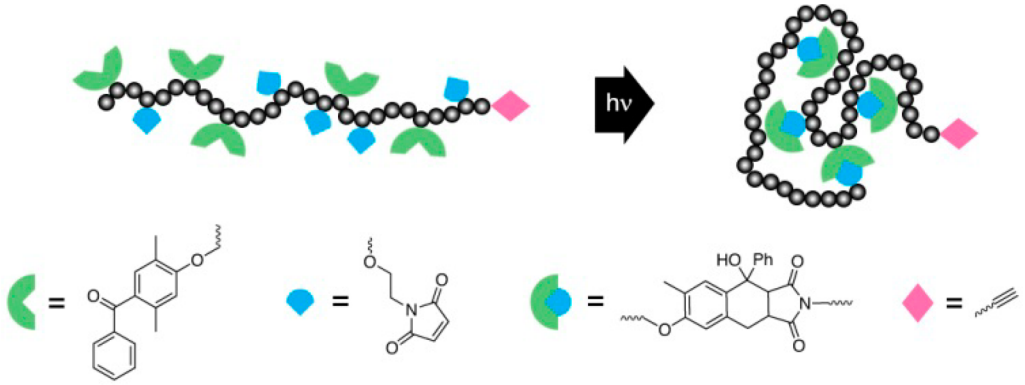


**Table 1. Molar Masses and Polydispersities for the Polymers P5, P6, P7, and P8 as Determined by SEC[a]**

| polymer | $M_n$/g mol$^{-1}$ | $M_m$/g mol$^{-1}$ | $Đ$ | polymer | $M_n$/g mol$^{-1}$ | $M_m$/g mol$^{-1}$ | $Đ$ |
|---|---|---|---|---|---|---|---|
| **P5$_a$** | 14 900 | 17 300 | 1.16 | **P7$_a$** | 16 000 | 18 400 | 1.15 |
| **P5$_b$** | 14 900 | 18 100 | 1.22 | **P7$_b$** | 16 700 | 19 700 | 1.18 |
| **P5$_c$** | 14 700 | 18 700 | 1.27 | **P7$_c$** | 16 200 | 19 300 | 1.19 |
| **P6$_a$** | 15 100 | 17 400 | 1.15 | **P8$_a$** | 15 700 | 18 750 | 1.19 |
| **P6$_b$** | 15 600 | 18 200 | 1.16 | **P8$_b$** | 16 500 | 22 100 | 1.33 |
| **P6$_c$** | 15 000 | 17 600 | 1.18 | **P8$_c$** | 20 200 | 31 500 | 1.56 |

[a]Reported relative to polystyrene equivalents.

be generated by combining controlled/living radical polymerization (CLRP) techniques[9] with recent advances in modular and orthogonal polymer ligation protocols.[10] Nitroxide-mediated polymerization (NMP) is a CLRP technique, which allows for the design of well-defined, functional, and complex macromolecular architectures.[11] Recently, Barner-Kowollik and co-workers employed an efficient modular ligation chemistry for rapid polymer–polymer conjugation[12] at ambient temperatures via a UV-light-triggered Diels–Alder reaction, which proceeds fast, atom efficient, catalyst free, and quantitative.[13] In addition, we reported examples of single-chain self-folding of well-defined linear polymers carrying various orthogonal complementary hydrogen bonding recognition motifs at preselected positions within the polymer backbone, which emulate—on a simple level—the self-folding behavior of natural biomacromolecules.[6d,h,j]

Herein, we report a new synthetic approach for the facile synthesis of monofunctional single-chain polymeric nanoparticles via a combination of the NMP technique and the above-noted photoinduced Diels–Alder reaction at ambient temperature. The common preparation of SCNPs can suffer from inconveniences such as the need to employ high temperatures, anhydrous conditions, metal catalysts, or long reaction times. These disadvantages are overcome in the current study. Linear random copolymers of styrene and 4-chloromethylstyrene carrying an alkyne functionality at the $\omega$-position of the polymer chain were obtained via NMP. The reactive groups for the UV-induced Diels–Alder reaction were grafted to the polymer backbone by an etherification and subsequently an esterification in a one-pot/two-step fashion. As a proof of principle, the linear precursor polymer—containing the highest functional group density—was conjugated with a poly(ethylene glycol) (PEG) chain, end-capped with an azide group (PEG-azide), utilizing the copper-catalyzed azide–alkyne cycloaddition (CuAAC). The monofunctional SCNPs were formed through intramolecular Diels–Alder reactions of the attached side groups triggered by UV-light irradiation. The resulting SCNPs were characterized by size exclusion chromatography (SEC), proton nuclear magnetic resonance ($^1$H NMR) spectroscopy, dynamic light scattering (DLS), and atomic force microscopy (AFM) in the collapsed state.

## EXPERIMENTAL SECTION

4-(2-(1,3-Dioxo-3a,4,7,7a-tetrahydro-1*H*-4,7-epoxyisoindol-2(3*H*)-yl)-ethoxy)-4-oxobutanoic acid (**2**),[14] PEG-azide (**3**) ($M_{n,SEC}$ = 3200 g mol$^{-1}$, $Đ$ = 1.04),[15] and 2-phenyl-3-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)propylprop-2-yn-1-yl succinate (**4**)[16] were synthesized according to literature procedures. The number-average molar mass, weight-average molar mass, and the corresponding polydispersity values of all of the following polymers (**P5** to **P8**) are collated in Table 1. In addition, full SEC traces for the polymers **P5$_a$** to **P8$_a$** are included in the Supporting Information (refer to Figure S6).

**Synthesis of Alkyne-Terminated Poly(styrene-*co*-chloromethylstyrene) (P5$_{a–c}$).** P(S-*co*-CMS) copolymers, containing one alkyne functionality, with various copolymer compositions were prepared via the NMP of styrene (S) and 4-chloromethylstyrene (CMS) in the presence of **4**. The reaction mixture was degassed via three consecutive freeze–pump–thaw cycles and left under an argon atmosphere. The tube was subsequently placed in an oil bath tempered at 125 °C for 6 h (4.5 h for **5c**). After the polymerization mixture was cooled to ambient temperature, it was diluted with THF (50 mL) and precipitated into methanol (500 mL). Next, the precipitate was filtered off and washed with methanol (2 × 50 mL). The obtained polymer was dried for 24 h under high vacuum at ambient temperature to afford a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 7.17–6.15 (Ar*H* of PS), 4.55 (s, OC$H_2$CCH), 4.44 (s, C$H_2$Cl), 3.95 (m, CHC$H_2$–OCO), 2.52–2.30 (m, OCC$H_2$C$H_2$CO), 1.78–1.18 (aliphatic protons of PS).

**Functionalization of the Random Copolymers with 4-Hydroxy-2,5-dimethylbenzophenone (P6$_{a–c}$).** Polymer **P5$_{a–c}$** (the number of CMS repeating units is 1 equiv), potassium carbonate (2.5 equiv), and 4-hydroxy-2,5-dimethylbenzophenone (**1**) (0.5 equiv) were dissolved in dry DMF (20 mL) and stirred at 70 °C for 16 h


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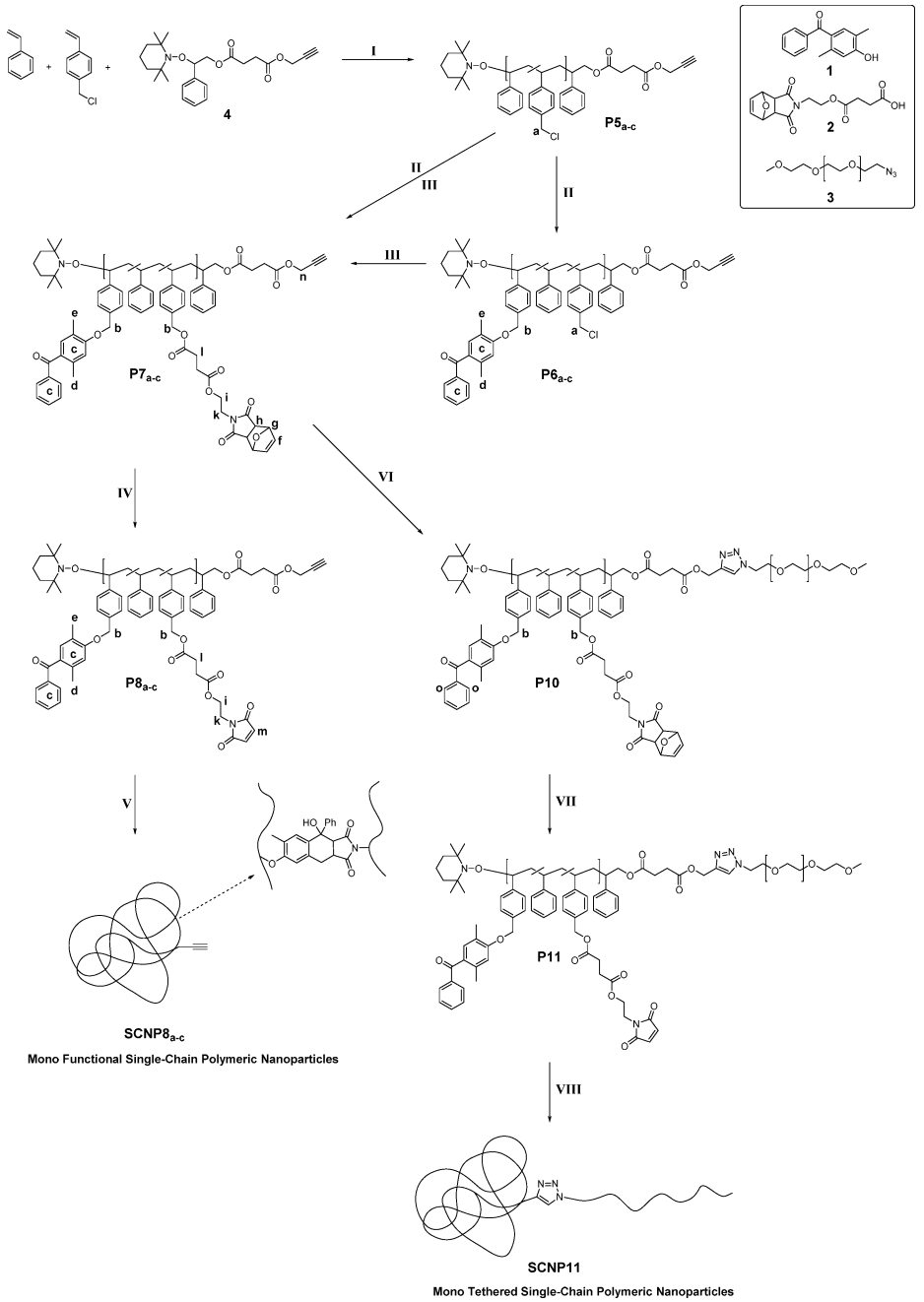
**Scheme 2. Synthetic Strategy for the Preparation of Monofunctional Single-Chain Polymeric Nanoparticles**[a]




[a]Reagents and conditions: (I) 125 °C, 6 h; (II) DMF, $K_2CO_3$, **1**, 70 °C, 15 h; (III) DMF, $K_2CO_3$, **2**, rt, 24 h; (IV) toluene, 100 °C, 15 h; (V) DCM, 320 nm, 30 min; (VI) DMF, CuBr/PMDETA, **3**, rt, 24 h; (VII) toluene, 100 °C, 15 h; (VIII) DCM, 320 nm, 30 min.

under an inert atmosphere. The reaction mixture was diluted with ethyl acetate (300 mL) and washed with brine (3 × 150 mL). The organic phase was dried over $Na_2SO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in THF (30 mL) and precipitated into methanol (300 mL). The precipitate was filtered off and washed with methanol (2 × 30 mL). The obtained polymer was dried for 24 h under high vacuum at 25 °C to afford a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 7.79 (s, Ar*H* of **1**), 7.55 (s, Ar*H* of **1**), 7.44 (s, Ar*H* of **1**), 7.17−6.15 (Ar*H* of PS), 5.02 (s, $CH_2O$), 4.55 (s, $OCH_2CCH$), 4.44 (s, $CH_2Cl$), 3.95 (m, $CHCH_2$−OCO), 2.52 (m, $OCCH_2CH_2CO$), 2.39 (s, $CH_3$), 2.22 (s, $CH_3$), 1.78−1.18 (aliphatic protons of PS).

**Functionalization of the Random Copolymer with the Protected Maleimide Compound ($P7_{a-c}$).** Polymer $P6_{a-c}$ (the number of CMS repeating units is 1 equiv), potassium carbonate (5 equiv), and **2** (0.5 equiv) were dissolved in dry DMF (15 mL) and stirred at ambient temperature for 24 h under an inert atmosphere. The reaction mixture was diluted with ethyl acetate (300 mL) and washed with brine (3 × 150 mL). The organic phase was dried over $Na_2SO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in THF (30 mL) and precipitated into methanol (300 mL). The precipitate was filtered off and washed with methanol (2 × 30 mL). The obtained polymer was dried for 24 h under high vacuum at 25 °C to afford a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 7.79 (s, Ar*H* of **1**), 7.55 (s, Ar*H* of **1**), 7.44 (s, Ar*H* of **1**), 7.17−6.15 (Ar*H* of PS), 6.45 (s, C═C*H* of **2**), 5.24 (s, OC*H* of **2**), 5.02 (s, $CH_2O$), 4.55 (s, $OCH_2CCH$), 4.25 (s, $CH_2CH_2O$), 3.95 (m, $CHCH_2$−OCO), 3.75 (s, $NCH_2CH_2$), 2.85 (s, C*H* of **2**), 2.61 (s,


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Table 2. Results of the Random Copolymerization of Styrene (S) and 4-Chloromethylstyrene (CMS) via Nitroxide-Mediated Radical Polymerization (NMP)

| polymer | Mon | $[M_0]/[I_0]$ | Init | time [h] | $M_n$[a] [g mol$^{-1}$] | $Đ$[a] | monomer feed [%CMS] | copolymer composition [%CMS][b] |
|---|---|---|---|---|---|---|---|---|
| $\mathbf{P5_a}$ | S/CMS | 200 | **4** | 6 | 14 900 | 1.16 | 8 | 9 |
| $\mathbf{P5_b}$ | S/CMS | 200 | **4** | 6 | 14 900 | 1.22 | 15 | 17 |
| $\mathbf{P5_c}$ | S/CMS | 200 | **4** | 4.5 | 14 700 | 1.27 | 30 | 34 |

[a]Determined via RI detection SEC using linear PS standards. [b]Determined by $^1$H NMR Spectroscopy.

$OCCH_2CH_2CO$), 2.39 (s, $CH_3$), 2.22 (s, $CH_3$), 1.78−1.18 (aliphatic protons of PS).

**One-Pot Functionalization of the Random Copolymer with 4-Hydroxy-2,5-dimethylbenzophenone and the Protected Maleimide Compound ($\mathbf{P7_{a-c}}$).** Polymer $\mathbf{P5_{a-c}}$ (the number of CMS repeating units is 1 equiv), potassium carbonate (10 equiv), and 4-hydroxy-2,5-dimethylbenzophenone (0.5 equiv) were dissolved in dry DMF (20 mL) and stirred at 70 °C for 16 h. After cooling to ambient temperature, **3** (0.5 equiv) was added directly to the reaction mixture, and it was stirred at ambient temperature for an additional 24 h. The reaction mixture was diluted with ethyl acetate (300 mL) and washed with brine (3 × 250 mL). The combined organic phases were dried over $Na_2SO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in THF (30 mL) and precipitated into methanol (300 mL). The precipitate was filtered off and washed with methanol (2 × 30 mL). The obtained polymer was dried for 24 h under high vacuum at ambient temperature to afford a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 7.79 (s, Ar*H* of **1**), 7.55 (s, Ar*H* of **1**), 7.44 (s, Ar*H* of **1**), 7.17−6.15 (Ar*H* of PS), 6.45 (s, C=C*H* of **2**), 5.24 (s, OC*H* of **2**), 5.02 (s, $CH_2O$), 4.55 (s, $OCH_2CCH$), 4.25 (s, $CH_2CH_2O$), 3.95 (m, $CHCH_2$−OCO), 3.75 (s, $NCH_2CH_2$), 2.85 (s, C*H* of **2**), 2.61 (s, $OCCH_2CH_2CO$), 2.39 (s, $CH_3$), 2.22 (s, $CH_3$), 1.78−1.18 (aliphatic protons of PS).

**Removal of the Furan Protective Groups from the Maleimide Compounds along the Polymeric Backbone ($\mathbf{P8_{a-c}}$).** Polymers $\mathbf{P7_{a-c}}$ were dissolved in toluene (60 mL) and subsequently nitrogen was percolated through the mixture for 30 min to remove residual oxygen. The reaction mixture was stirred at 100 °C for 15 h. The solvent was removed under reduced pressure. The residue was dissolved in THF (8 mL) and precipitated into methanol (40 mL). The precipitate was filtered off and washed with methanol (2 × 5 mL). The obtained polymer was dried for 24 h under high vacuum at ambient temperature to give a yellowish solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 7.79 (s, Ar*H* of **1**), 7.55 (s, Ar*H* of **1**), 7.44 (s, Ar*H* of **1**), 7.17−6.15 (Ar*H* of PS), 6.65 (s, C=C*H* of **2**), 5.02 (s, $CH_2O$), 4.55 (s, $OCH_2CCH$), 4.25 (s, $CH_2CH_2O$), 3.95 (m, $CHCH_2$−OCO), 3.75 (s, $NCH_2CH_2$), 2.61 (s, $OCCH_2CH_2CO$), 2.39 (s, $CH_3$), 2.22 (s, $CH_3$), 1.78−1.18 (aliphatic protons of PS).

**Functionalization of a Linear Precursor Copolymer via Copper-Catalyzed Azide/Alkyne Cycloaddition ($\mathbf{P10}$).** $\mathbf{P7_c}$ (1.00 g, 0.065 mmol), **3** (0.26 g, 0.13 mmol), CuBr (0.095 g, 0.65 mmol), and PMDETA (0.135 μL, 0.65 mmol) were dissolved in DMF (10 mL) in a Schlenk tube, which was deoxygenated by five consecutive freeze−pump−thaw cycles. The resulting mixture was stirred at ambient temperature for 24 h before the copper catalyst was removed by passing the solution over a short column of neutral alumina. The solvent was removed under reduced pressure. The residue was dissolved in THF (10 mL) and precipitated into methanol (100 mL). The precipitate was filtered off and washed with methanol (2 × 10 mL). The obtained polymer was dried for 24 h under high vacuum at ambient temperature to give a white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 7.79 (s, Ar*H* of **1**), 7.55 (s, Ar*H* of **1**), 7.44 (s, Ar*H* of **1**), 7.17−6.15 (Ar*H* of PS), 6.45 (s, C=C*H* of **2**), 5.24 (s, OC*H* of **2**), 5.02 (s, $CH_2O$), 4.51 (s, $CH_2$ next to triazole), 4.25 (s, $CH_2CH_2O$), 3.95 (m, $CHCH_2$−OCO), 3.75 (s, $NCH_2CH_2$), 3.64 (s, PEG backbone), 3.38 (s, $OCH_3$), 2.85 (s, C*H* of **2**), 2.61 (s, $OCCH_2CH_2CO$), 2.39 (s, $CH_3$), 2.22 (s, $CH_3$), 1.78−1.18 (aliphatic protons of PS). $M_{n,SEC}$ = 19 400 g mol$^{-1}$; $M_{m,SEC}$ = 22 700 g mol$^{-1}$; $Đ$ = 1.17.

**Removal of the Furan Protective Groups from the Maleimide Compounds along the Polymeric Backbone of the PEG Functionalized Polymer ($\mathbf{P11}$).** The retro-Diels−Alder reaction of **P10** was performed in the same fashion as described for polymers $\mathbf{P8_{a-c}}$. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 7.79 (s, Ar*H* of **1**), 7.55 (s, Ar*H* of **1**), 7.44 (s, Ar*H* of **1**), 7.17−6.15 (Ar*H* of PS), 6.65 (s, C=C*H* of **2**), 5.02 (s, $CH_2O$), 4.51 (s, $CH_2$ next to triazole), 4.25 (s, $CH_2CH_2O$), 3.95 (m, $CHCH_2$−OCO), 3.75 (s, $NCH_2CH_2$), 3.64 (s, PEG backbone), 3.38 (s, $OCH_3$), 2.61 (s, $OCCH_2CH_2CO$), 2.39 (s, $CH_3$), 2.22 (s, $CH_3$), 1.78−1.18 (aliphatic protons of PS). $M_{n,SEC}$ = 19 200 g mol$^{-1}$; $M_{m,SEC}$ = 24 500 g mol$^{-1}$; $Đ$ = 1.28.

**Single-Chain Polymeric Nanoparticles via Intramolecular Photoinduced Diels−Alder Cross-Linking ($\mathbf{SCNP8_{a-c}}$ and SCNP11).** The polymers $\mathbf{P8_{a-c}}$ and **P11** (4 mg) were each dissolved in 240 mL of $CH_2Cl_2$ in a round-bottom flask, sealed airtight with a septum, and flushed with nitrogen for approximately 1.5 h. The solution was subsequently irradiated for 30 min in a custom-built photoreactor with a 36 W compact low-pressure UV-A fluorescent lamp emitting at 320 nm. Afterwards, the solvent was removed under reduced pressure to yield the SCNPs, which were directly dissolved in the appropriate solvent for analysis.

## RESULTS AND DISCUSSION

The current work focuses on a new synthetic approach for the preparation of monofunctional single-chain polymeric nanoparticles through intramolecular Diels−Alder cross-linking of reactive groups along the polymeric backbone, triggered via UV-light irradiation under mild conditions.

**Synthesis of the Monofunctional Linear Photoreactive Precursor Copolymers.** For the synthesis of lateral chloromethyl functionalized copolymers, a series of poly(styrene-*co*-chloromethylstyrene)s were prepared via nitroxide-mediated polymerization (NMP) of 4-chloromethylstyrene (CMS) and styrene (S) in the presence of **4** at 125 °C. The composition of the copolymer was determined via $^1$H NMR spectroscopy. The mole fractions of CMS and S were deduced from the integrals of the peak area close to 4.51 ppm ($y$), corresponding to the two protons of the methylene unit of CMS and the total area between 6.22 and 7.28 ppm ($x$), which is associated with the aromatic protons of all repeating units. The percentage of CMS repeating units in the polymer was calculated using eq 1:

$$\%\mathrm{CMS} = \frac{5y}{2x + y} \times 100 \quad (1)$$

As evidenced in Table 2, it was possible to finely control the functional group density of the random copolymers by varying the monomer feed utilized in the polymerization processes. Owing to the nature of the NMP mechanism, the polymer chains should have the same composition due to a simultaneous initiation of all chains and also feature narrow polydispersities. The $^1$H NMR spectra of $\mathbf{P5_{a-c}}$ display the characteristic aromatic proton resonances for the polystyrene units between 7.05 and 6.54 ppm as well as the resonances of the benzylic protons of CMS ($-CH_2Cl$) and the alkyne protons of the initiator ($-OCH_2CCH$), which appear at 4.62 and 4.50 ppm (see Figure 1). The CMS moieties of copolymers $\mathbf{P5_{a-c}}$ with different mole fractions of CMS (9−34 mol %) were


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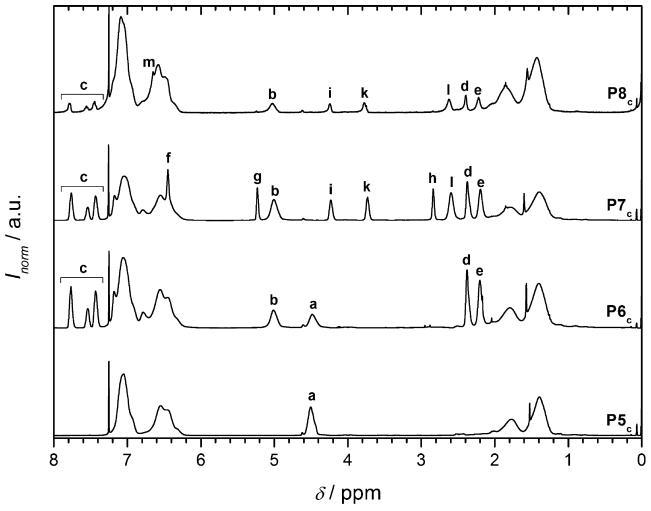


**Figure 1.** Functionalization process from poly(styrene-*co*-chloromethylstyrene) to a photoreactive single-chain polymeric nanoparticle precursor polymer followed by $^1$H NMR spectroscopy. To best showcase the course of functionalization, the spectra of polymers $\mathbf{P5_c}$ to $\mathbf{P8_c}$ (34% FGD) are depicted, as these offer the most intense resonances for the pendant reactive groups. For signal assignment refer to Scheme 2.

quantitatively—as can be judged within the bounds of the experimental uncertainty of $^1$H NMR spectroscopy—converted to the corresponding reactive groups for the UV-light triggered Diels–Alder reaction in a one-pot/two-step process. The functionalization progress of the polymer backbone could readily be monitored by $^1$H NMR spectroscopy as illustrated in Figure 1.

Initially, the lateral polymer chain was functionalized with 4-hydroxy-2,5-dimethylbenzophenone as the photoreactive precursor. Under basic conditions the hydroxy group was deprotonated followed by a quantitative—as can be judged within the bounds of the experimental uncertainty of $^1$H NMR spectroscopy—nucleophilic substitution of the chlorine atom at 70 °C. The $^1$H NMR spectrum (refer to Figure 1) of **P6** displays a decrease in intensity of the resonances associated with the $-CH_2Cl$ protons at 4.50 ppm and features new resonances corresponding to the 4-hydroxy-2,5-dimethylbenzophenone protons at 7.77–7.43, 2.38, and 3.80 ppm. To incorporate a maleimide motif into the polymer chain, a carboxyl functional maleimide compound (**2**) was synthesized following published procedures,[14] which was subsequently reacted with polymers $\mathbf{P6_{a-c}}$ in the presence of $K_2CO_3$ in DMF to obtain the functionalized copolymers $\mathbf{P7_{a-c}}$. Employing the same reaction conditions as used for the functionalization with the photoenol precursor unexpectedly led to a cross-linked material. Most likely the cross-linking process is correlated to an unintentional removal of the furan protective group and successive reaction of the maleimide with residual chlorine atoms. As a consequence, milder conditions were applied, and the reaction proceeded to high conversion at ambient temperature within 24 h. The $^1$H NMR spectrum of **P7** indicates that the signal corresponding to the $-CH_2Cl$ protons disappears and new resonances for the maleimide motif can be observed at 6.50, 5.23, 4.23, 3.73, 2.84, and 2.60 ppm, respectively (refer to Figure 1). Further investigations to improve the preparation procedure showed that a one-pot reaction with the sequential addition of 4-hydroxy-2,5-dimethylbenzophenone and the maleimide derivative, saving one work-up step and material, is the most effective synthesis route. Confirmed by $^1$H NMR spectroscopy, the resonances for the 4-hydroxy-2,5-dimethylbenzophenone and the maleimide moiety are observed with the above-discussed chemical shifts, and the resonance for the $-CH_2Cl$ protons is no longer detected at 4.50 ppm (see Figure 1). Thus, the furan protecting group was quantitatively removed at 100 °C.

**Synthesis of the Monofunctionalized Linear Photoreactive Precursor Copolymers.** As a proof of principle, copolymer $\mathbf{P7_c}$ was tethered to the PEG-azide (**3**) utilizing the CuBr/PMDETA supported copper-catalyzed azide–alkyne ligation reaction. The SEC trace of polymer **P10** is—compared to the trace of starting material $\mathbf{P7_c}$—clearly shifted to lower retention times (i.e., higher molar mass) and features a

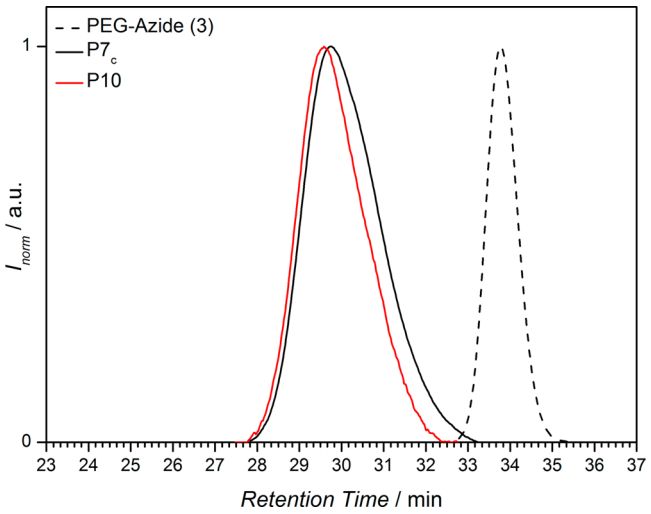


**Figure 2.** Normalized SEC traces of the alkyne bearing monofunctional polymer $\mathbf{P7_c}$, the PEG-azide **3**, and the corresponding monofunctionalized block copolymer **P10**.

monomodal distribution (refer to Figure 2). Inspection of the $^1$H NMR spectrum of **P10** reveals full conversion of the terminal alkyne as the corresponding resonance at 4.62 ppm disappears completely while new signals, corresponding to the repeating unit of PEG, emerge at 3.58 ppm (see Figure 3). The deprotection of the *N*-maleimide motif was again carried out at 100 °C and proceeded quantitatively.

**Investigation of Conditions for the Formation of the Single-Chain Polymeric Nanoparticles.** In order to identify optimum reaction conditions for the UV-triggered nanoparticle formation variable concentrations, solvents and reaction times were investigated. Polymer $\mathbf{P8_b}$ was employed for the following investigations. First, the solvent system dichloromethane/acetonitril (1/3, v/v) (concentration of $\mathbf{P8_b}$ = 1 mg mL$^{-1}$), and a reaction time of 2.5 h was applied. Under these conditions intermolecular cross-linking occurred because of the limited solubility and/or a too concentrated solution, which led to an insoluble product. Thus, all further reactions were conducted in dichloromethane (DCM) due to the good solubility of the polymer. Concentrations of 0.133, 0.067, 0.033, and 0.0117 mg mL$^{-1}$ were investigated. Only the lowest concentration of 0.0117 mg mL$^{-1}$ leads to a clean shift of the SEC trace with no shoulder at smaller retention times or bimodal distribution (see Figure S5). Having identified the optimum concentration and solvent, a kinetic study was


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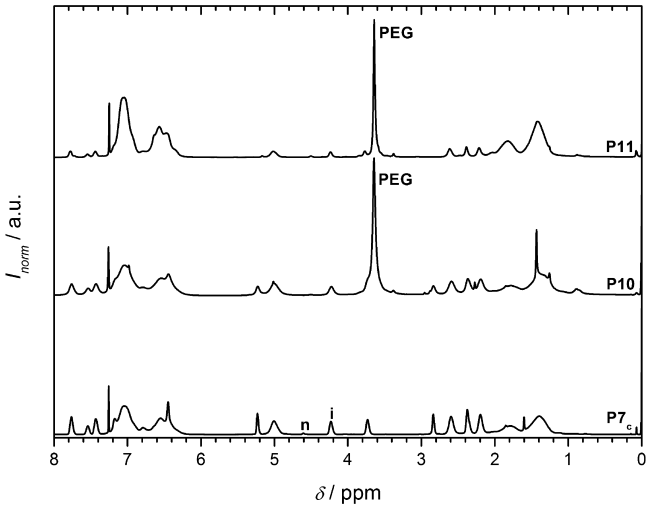


**Figure 3.** Functionalization process from the monofunctional polymer $\mathbf{P7}_c$ to the monofunctionalized linear precursor copolymer **P11** followed by $^1$H NMR spectroscopy. For the signal assignments refer to Scheme 2.

performed with polymer $\mathbf{P8}_a$ to find the optimal reaction time. Samples were taken from the reaction mixture in several time intervals. The results (refer to Figure 4) indicate that the single

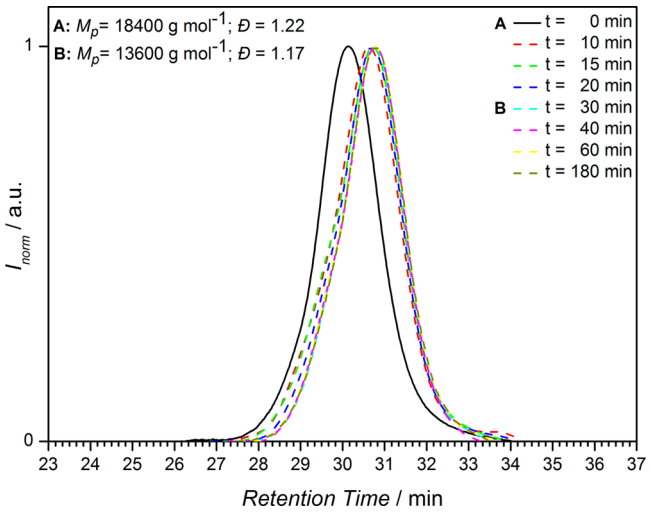


**Figure 4.** Normalized SEC traces of the single-chain cross-linking kinetics study carried out with polymer $\mathbf{P8}_a$ in DCM.

chain collapse was completed after 30 min (indicated in Figure 4 by the label "B", which is associated with the final molecular weight of 13 600 g mol$^{-1}$), since the SEC trace remains invariant at longer reaction times. For reaction times shorter than 30 min, a small shoulder in the higher molar mass region is observed, which might be attributed to remaining linear precursor copolymer. After 30 min of UV irradiation, the SEC trace is completely shifted to higher retention times (i.e., lower molar mass) and does not display a shoulder in the higher molar mass region, evidencing that no intermolecular coupling occurred during the single-chain cross-linking process.

**Characterization of the Isolated SCNPs via NMR, SEC, DLS, and AFM.** The formation of the SCNPs was monitored by $^1$H NMR spectroscopy, SEC, DLS, and AFM. For the $^1$H NMR analysis, only the polymer with the highest FGD was chosen, since these spectra show the strongest signals for the reactive side groups. The most obvious sign of SCNP formation is the quality of the spectrum. When intramolecularly cross-linked, the polymers loose degrees of freedom, which leads to a significant broadening of all signals (see Figure 5).[4f,j] Chemical evidence was gathered from the photoenol precursor's aromatic proton resonances. As discussed in previous publications,[13b,c] the aromatic protons of the photoenol precursor shift to higher field and disappear below the signal of the polystyrene aromatic protons. The functionalization process of the polymers $\mathbf{P5}_{a-c}$ to the polymers $\mathbf{P7}_{a-c}$ was quantitative as determined within the experimental error of $^1$H NMR spectroscopy, yet the ratio of the reactive groups was likely not exactly 50/50. Thus, there will always be remaining photoenol precursor and/or maleimide moieties in the SCNPs. Additionally, the intramolecular cross-linking might also separate two reactive groups in such a way that they are no longer able to react with each other, which will again lead to residual reactive groups within the SCNPs. On the basis of these facts, it was not expected that the aromatic protons of the photoenol precursor would disappear completely, but instead decrease in their intensity.

To evaluate the decrease, the integral ratios of the signals for the benzylic protons along the backbone (~5.0 ppm) and the resonances of the two aromatic protons in the *ortho*-position to the carbonyl functionality of the photoenol precursor (~7.8 ppm) are compared before and after the intramolecular cross-linking (the signals are marked with red boxes in Figure 5). The results—collated in Table 3—underpin a decrease for both the monofunctional SCNP ($\mathbf{SCNP8}_c$) and the monofunctionalized SCNP (**SCNP11**). The presented values suggest an approximate conversion of the photoenol of close to 30%.

Size exclusion chromatography (SEC) proved to be a convenient method to monitor the changes in hydrodynamic volume from the collapse of the linear precursor polymer to an intramolecular cross-linked polymeric nanoparticle, since the SEC columns separate analytes based on their hydrodynamic volume.[4e,5b] For all precursor polymers SEC demonstrates (refer to Figure 6) that the hydrodynamic volumes of the respective SCNPs are significantly smaller than the starting linear macromolecules, which results in higher retention times for the SCNPs. The change in the apparent peak molar mass ($\Delta M_p$), measured by SEC against polystyrene standards, can be correlated to the degree of collapse after intramolecular cross-linking. The fact that the precursor polymer with the lowest functional group density (FGD) has the smallest $\Delta M_p$ and the one with the highest FGD features the highest $\Delta M_p$ suggests that the size of the SCNP can indeed be controlled by varying the FGD along the polymeric backbone (see Figure 7b).

Convincingly, the same trend observed for $\Delta M_p$ is also observed when measuring the number-weighted mean hydrodynamic diameters $D_h$. Such measurements of the SCNPs and the linear precursor copolymers in THF solution were performed on a dynamic light scattering (DLS) instrument. DLS is a procedure used to determine the size distribution profile of small particles in suspension or polymers in solution, capable to detect molecules in the size range of a few nanometers up to several micrometers. The DLS results (refer to Figure 7a) show that all single polymer chains (functional and nonfunctional) undergo a size reduction upon the photochemically induced collapse. The largest change in hydrodynamic diameter ($\Delta D_h$) was recorded for the polymer with the highest FGD, the smallest accordingly for the polymer with the lowest FGD (refer to Figure 7b). The DLS results for


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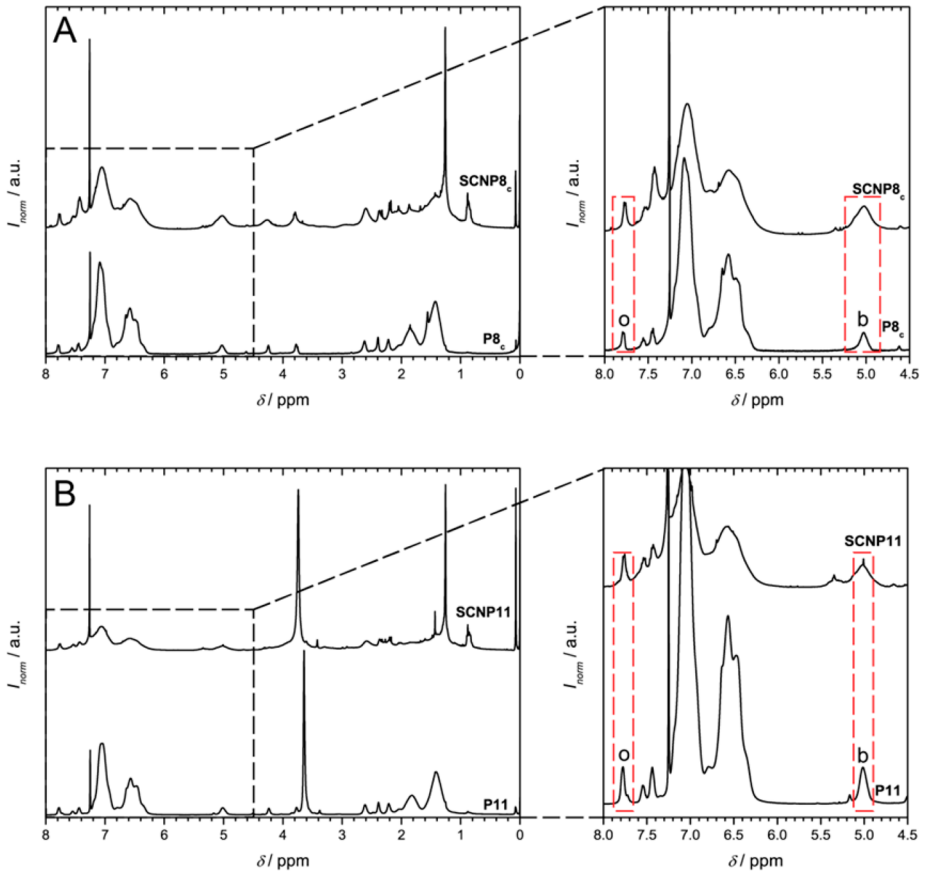


**Figure 5.** $^1$H NMR spectra of linear precursor copolymers and SCNPs. (A) Monofunctional precursor polymer **P8$_c$** and the respective **SCNP8$_c$**. (B) Monofunctionalized precursor copolymer **P11** and the respective monotethered **SCNP11**. For the signal assignment refer to Scheme 2.

**Table 3. Comparison of the Integral Ratios of the Resonances for the Benzylic Protons along the Backbone (~5.0 ppm) and the Two Aromatic Protons in the *ortho*-Position to the Carbonyl Functionality of the Photoenol Precursor (~7.8 ppm) for the Linear Precursor Polymers P8$_c$ and P11 and the Respective Resulting SCNPs SCNP8$_c$ and SCNP11**

| polymer | ratio (benzylic/photoenol) | polymer | ratio (benzylic/photoenol) |
|---|---|---|---|
| **P8$_c$** | 1/0.56 | **P11** | 1/0.57 |
| **SCNP8$_c$** | 1/0.39 | **SCNP11** | 1/0.41 |

**SCNP8$_b$** and **SCNP11** show small amounts of particles with higher $D_h$, which might be attributed to remaining linear precursor polymers or products of the intermolecular reaction of two polymer chains. A very limited amount of particles with higher $D_h$ is also observed in the DLS results for the linear precursor polymers **P8$_c$** and **P11**, which were most probably a matter of aggregation.

Finally, the single-chain polymeric nanoparticles were visualized by atomic force microscopy (AFM). Therefore, **SCNP9$_a$** was adsorbed onto freshly cleaved mica disks from diluted dichloromethane solutions. A height map image of the cast surface with a scan size of 1 $\mu$m is presented in Figure 8. Panel A shows a sample containing **SCNP9$_a$**, panel B shows the mica surface, and panel C shows a control sample, containing the linear precursor polymer P8$_a$. The AFM image of the control sample shows a thin film consisting of the linear precursor polymer and some areas where the mica surface (dark spots) is exposed. In combination with the images in panels B and C, the AFM image of **SCNP9$_a$** in panel A further supports the efficient formation of cross-linked particles, of which some could present single chain particles. A direct comparison with the diameters observed via DLS in solution is not advisible, as certainly different diameters will be observed in the solid state vs solution, and the AFM observation leads to an enhanced width of the SCNPs due to the broadness of the AFM tip. For this very reason, it is also not possible to observe different SCNP sizes for different FDGs as in Figure 7a via DLS. Nevertheless, the AFM images may suggest the presence of SCNPs as well as some larger aggregates.[17] The formation of aggregates during the casting process due to dewetting effects and evaporative self-assembly, which has been described in the literature, made the sample preparation a complex task.[17a,d] To explore whether some of the smaller particles could indeed present SCNPs (and not aggregates), a height cross-section analysis (refer to Figure S7 in the Supporting Information) with a focus on some of the smallest species in panel A of Figure 8 was carried out. Inspection of Figure S7 shows that the particles feature a height of approximately 3 nm, which are similar in size to other SCNPs reported in the literature.[17c] This observation leads to the conclusion that at least the smallest particles are indeed SCNPs. A dilution series also leads to aggregated species (alongside smaller species), which suggests that the herein produced single chain folded materials tend to aggregate when casted. Thus, the SEC and DLS data are more reliable indicators for the true single chain folded nature of the NPs than AFM, which can only serve as a supporting analysis.


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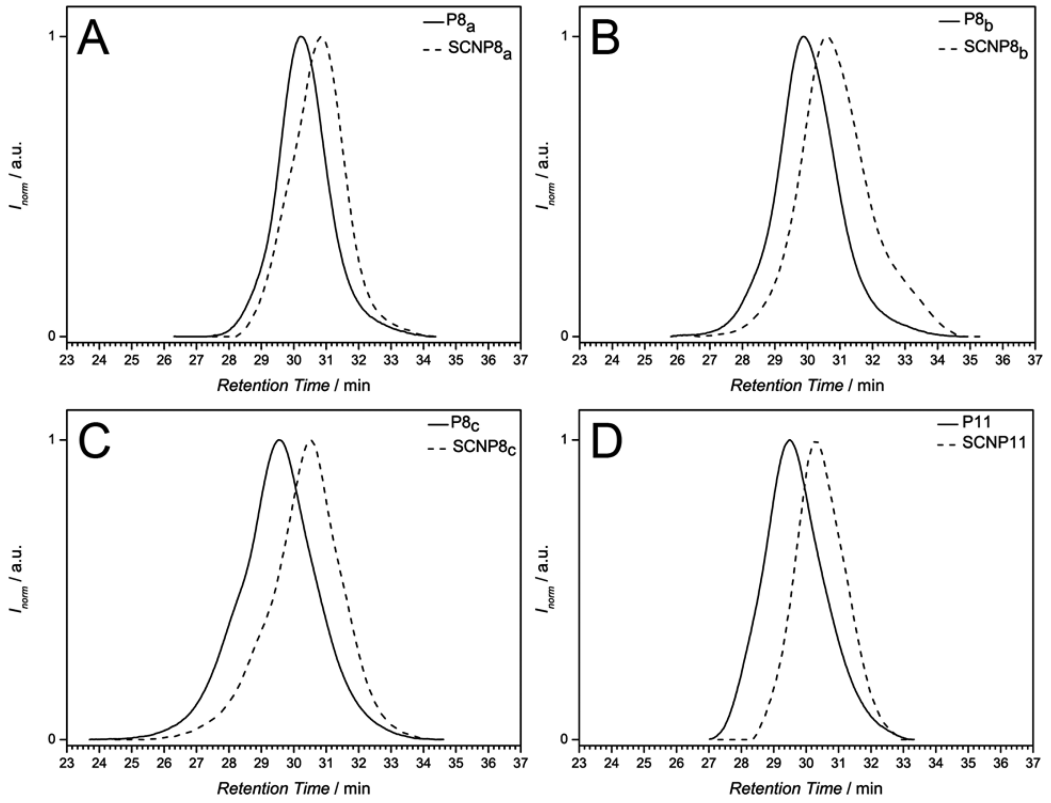


**Figure 6.** Size exclusion chromatography (SEC) traces confirming the efficient formation of single-chain polymeric nanoparticles (SCNPs). The solid curves represent the linear precursor polymers and the dashed curves the respective SCNPs. (A) Polymer with 9% functional group density (FGD). (B) Polymer with 17% FGD. (C) Polymer with 34% FGD. (D) PEG-functional polymer with 34% FGD.

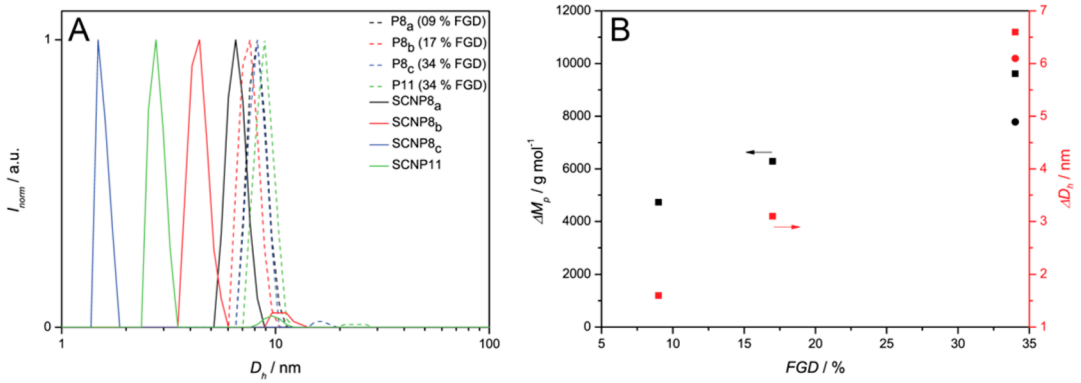


**Figure 7.** Dynamic light scattering (DLS) results and a comparison with SEC data. (A) Hydrodynamic radii ($D_h$) of the linear precursor polymers and their respective SCNPs. (B) The change in peak molar mass ($\Delta M_p$), measured by SEC, and the change in hydrodynamic volume ($\Delta D_h$), measured by DLS, plotted against the functional group density (FGD). In (B) the squares are associated with the alkyne functional polymers, whereas the circles are associated with the PEG-functional polymers.

## CONCLUSIONS

Herein a new synthetic approach for the preparation of single-chain polymeric nanoparticles under mild conditions via intramolecular UV-light-triggered Diels–Alder (DA) cross-linking is described. Nitroxide-mediated radical polymerization was utilized to prepare well-defined random copolymers of styrene and 4-chloromethylstyrene featuring lateral chlorine moieties with an alkyne terminal group. The backbone of the linear polymer chains was modified via an etherification and an esterification in a one-pot/two-step process using 4-hydroxy-2,5-dimethylbenzophenone (DMBP) and a protected *N*-maleimide (Mal) compound. The DMBP and Mal functionalized linear precursor copolymers with an alkyne terminal group were irradiated with UV light at 320 nm in diluted solution, which led to the efficient formation of SCNPs via photoinduced intramolecular cross-linking. The size of the polymeric nanoparticles could be varied by changing the functional group density along the polymeric backbone. The CuAAC conjugation of one of the monofunctional linear precursor copolymers with PEG-azide evidenced the targeted principle and led to monotethered SCNPs. The formation of the SCNPs was successfully evidenced by size-exclusion chromatography (SEC), nuclear magnetic resonance (NMR) spectroscopy, dynamic light scattering (DLS), and atomic force microscopy (AFM). The present results unambiguously prove that the formation of SCNPs on a very well-defined level is


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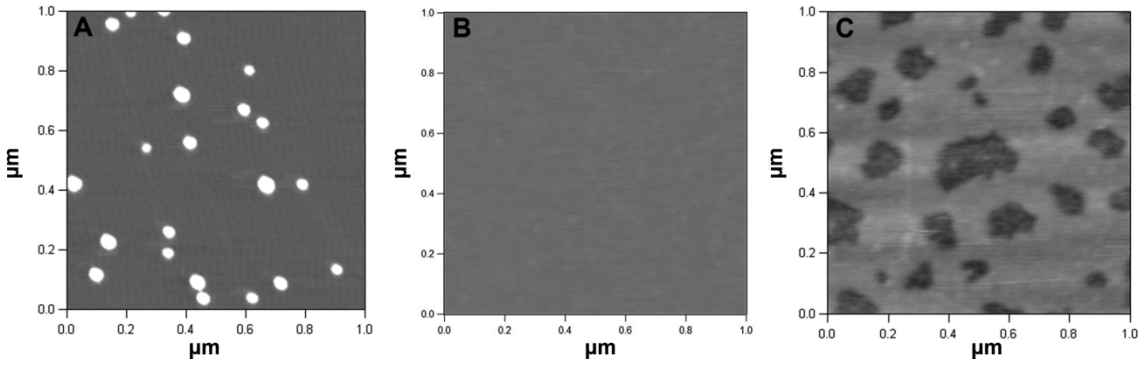


**Figure 8.** AFM topography images (1 $\mu$m × 1 $\mu$m scan size). (A) Sample containing **SCNP9$_a$**. (B) Freshly cleaved mica disk. (C) Sample containing linear precursor polymer **P8$_a$**.

indeed possible, employing high precision mild photochemical macromolecular design strategies. The construction of even more complex single-chain folded polymers and their self-folding studies in water guided by naturally occurring systems are underway in our laboratories, with the ultimate long-term aim of developing synthetic methodologies for the preparation of synthetic protein-like structures that can eventually fulfill biological functions.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Information regarding the employed materials and instrumentation as well as additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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