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Preparation of the Water-Soluble Pyrene-Containing Fluorescent Polymer by One-Pot Method

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Abstract: A new water-soluble pyrene-containing fluorescent polymer, 1-{3′-*S*-[poly(acryloyl ethylene diamine hydrochloride)-2′-methyl propionic acid]}propionyloxy hexyloxy pyrene (**P3**) with defined structure, was designed and synthesized using the click reaction between thiol and a carbon-carbon double bond. The intermediate products **P1** (*S*-1-dodecyl-*S*′-[poly(*N*-Boc-acryloyl ethylene diamine)-2′-methyl propionic acid]trithiocarbonate) and AHP (1-(acryloyloxy hexyloxy)pyrene) were prepared via reversible addition fragmentation chain transfer (RAFT) polymerization and Williamson synthesis, respectively. Conjugating AHP with **P1**, **P2** (1-{3′-*S*-[poly(*N*-butoxycarbonyl-acryloyl ethylene diamine)-2″-methyl propionic acid]} propionyloxy hexyloxy pyrene) was synthesized, adopting both the reduction reaction of a trithioester bond of **P1** to thiol and the click reaction between thiol and the carbon-carbon double bond of AHP simultaneously. **P3** was obtained by the deprotection of the resulting Boc-protected polymer (**P2**) with aqueous HCl. The experiment results showed that **P2** exhibited a bright blue-violet emission band at approximately 387–429 nm. After deprotection, **P3** displayed good solubility in water and not only exhibited a blue-violet fluorescence emission band at approximately 387–429 nm in aqueous solution but also had the similar photoluminescent spectra to those of AHP and **P2** in dichloromethane. The fluorescence quantum yields of **P2** in dilute tetrahydrofuran and **P3** in a dilute aqueous solution were 0.44 and 0.39, respectively. This experiment provided a novel insight into the study of water-soluble fluorescent polymers.

Keywords: pyrene; water-soluble fluorescent polymer; click-chemistry; thiol-ene reaction; reversible addition-fragmentation chain transfer (RAFT)

1. Introduction

Water-soluble fluorescent polymers have drawn much attention in recent years given their successful utility in distinguishing different proteins [1] and isozymes [2], as an effective and sensitive probe for further bio-applications differentiating tumorous and malignant cells [3,4], ranging from cytotoxicity studies to pharmacodiagnosis. Water is usually regarded as an ideal solvent in terms of its environmental impact and low cost. Water solubility is a prerequisite of those fluorescent polymers for applications in biological environments [5,6], since as long as the fluorescent polymers enjoy water solubility they can be uniformly dispersed in the aqueous medium of the system under characterization. The water-soluble fluorescent polymers in the field of applied biotechnology research exhibited unique advantages. To tackle this issue, several designed strategies to prepare water-soluble fluorescent polymers, mainly including copolymerization and chemical modification, have been well established and applied to the development of various excellent fluorescent polymers. Co-polymerization is

a family of methods that fluorochrome copolymerizes directly with a water-soluble monomer. It has been demonstrated to be a simple, effective strategy to obtain a water-soluble copolymer, but it generally enjoys several limitations due to low molecular weight, poor water-solubility and low fluorescence intensity. Chemical modification method that was also the designed strategy in this contribution, which was chemically bonded to the fluorescent dye on the polymer chain or born hydrophilic groups to fluorescent polymers. Water-soluble poly(acrylic acid) has been covalently labeled with a fluorescent hydrophobic chromophore, naphthalene (Np), randomly attached onto the polymer backbone with an amount of 3 mol % [7,8]. Curtis W. Frank and co-workers [9,10] reported that pyrene end-labeled poly(ethy1ene glycol) (PEG) was synthesized by direct esterification between PEG and l-pyrenebutyric acid (PBA). As an important and widespread tool in biomedical research and disease diagnosis [4,11], water-soluble fluorescent polymers with suitable molecular weight may be used for fluorescent detection of protein and image in the cells [12].

In order to obtain the polymer with controlled and narrow distribution of molecular weight, living radical polymerization is a conventional polymerization method. Reversible addition-fragmentation chain transfer (RAFT) polymerization is one of the most popular living radical polymerization methods for the production of well-defined polymers. After polymerization, the RAFT agent end-groups can easily be converted into thiol, opening manifold opportunities for thiol modification reactions [13]. The functional groups are effectively introduced into the polymer terminal groups by these reactions, such as thiol-ene [13], thiol-epoxy reaction [14], and so on. Such reaction of thiol and epoxy, alkenes, and alkynes is one kind of click-chemistry reactions.

The past decade has seen the emergence of "click chemistry"; the term "click chemistry" defines a series of chemical reactions respecting several criteria announced in 2001 by Sharpless and co-workers [15]. Click chemistry is addressing a set of powerful, highly-reliable, and selective reactions for the rapid synthesis of useful new compounds and combinatorial libraries [16]. Click chemistry possesses many significant advantages, such as a certain modularity [17], excellent selectivity, high purity, high efficiency, a wide scope of applications, only inoffensive byproducts, stereospecific, mild reaction conditions, and simple product isolation, *etc.* [15,18–20]. Since S–H bonds are relatively weak, thiol groups are subject to rapid oxidation during purification and storage and, thereby, result in the low conversion in the following click reaction. To circumvent this limitation, the synthesis of thiol groups and click reaction were simultaneously performed in one system (one-pot method). Highly efficient reactions of thiols with the reactive carbon-carbon double bond [20] (thiol-ene click-chemistry reaction) is one of the most mature click-chemistry reactions, in which thiol can rapidly react with the terminal C=C double bond to form a C–S bond under mild conditions, which has been applied in diverse preparation areas, such as functional polymer nanoparticles [21] and beads [22], amphipathic well-defined polymers [23], desired dendritic macromolecules [24], nanocapsules [25], substrate surface modification [20], polymer functionalization [26], and so forth.

The pyrene moiety is one of the most useful fluorophores due to its striking efficiency among excimer formation and subsequent changes in its emission properties [27]. Pyrene can emit blue fluorescence with a high fluorescence quantum yield (0.65, acetonitrile) and a long fluorescence lifetime (440 ns, a cyclohexane solution, 290 ns, ethanol) [28] responsible to the unique polycyclic aromatic hydrocarbon-conjugated structure. Therefore, pyrene is an important signaling unit, which is widely used for various analytes [29–31]. However, pyrene, or other conventional fluorescent molecules with alternating single and double bond conjugated structures, are faced with the thorny aggregation problem leading to emission spectrum red-shift and fluorescence quenching. There were several projects to avoid this phenomenon by introducing large steric groups at reactive sites of pyrene to avoid the accumulation of the conjugated plane [32,33]. However, most of the works were studies of the responses and characteristics when pyrene-containing polymers dissolved in organic solvent. For example, the fluorescence quantum yield and infrared absorption spectrum of pyrene derivatives in Robert M. Edkins′ experiment showed the dependency of the solvent [34]. This article will discuss

how the pyrenyl functional end group is introduced into a water-soluble polymer, which is applied to the light emitting properties of pyrene in aqueous solution.

In this study, *S*-1-dodecyl-*S*′-[poly(*N*-Boc-acryloyl ethylene diamine)-2′-methyl propionic acid]trithiocarbonate (**P1**) was prepared via RAFT polymerization using *N*-Boc-acryloyl ethylene diamine as a monomer. Boc-protected fluorescent polymer (**P2**) with regular structure was synthesized using the click reaction of thiol (the reduction reaction production of trithioester bond of **P1**) and the carbon-carbon double bond of AHP. Water-soluble pyrene-containing fluorescent polymer (**P3**) was obtained by the deprotection of the resulting Boc-protected polymer (**P2**). **P3** was characterized and measured by Fourier transform infrared spectrophotometry, nuclear magnetic resonance spectrometry, UV-Visible (UV-VIS) spectrophotometry, and fluorescence spectrophotometry, respectively, and the fluorescence quantum yield of **P3** in aqueous solution was obtained. The fluorescent polymer **P3** had good water solubility and also showed good optical performance.

2. Experimental Section

2.1. Reagents

1-Hydroxypyrene (HP, 98.5%) was purchased from Jiangsu Rich-chem Co., Ltd., Taizhou, China. 1,6-Dibromohexane (98%) was purchased from J&K scientific Co., Ltd. (Beijing, China). 9,10-diphenylanthracene (DPA, >95.0%) was purchased from TCI (Shanghai, China) Development Co., Ltd. (Shanghai, China) Di(*tert*-butyl) dicarbonate (Boc_2O, 97+%) was purchased from Apollo Scientific Limited (Beijing, China). Anhydrous sodium sulfate, ethylenediamine, and triethylamine (TEA) were all analytically-pure grades and were supplied by Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Sodium borohydride ($NaBH_4$), acryloyl chloride, acrylic acid, sodium hydroxide, tetrahydrofuran (THF), petroleum ether, dichloromethane (DCM), and anhydrous methanol were all analytically-pure grades and were supplied by Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). THF and DCM were distilled prior to use. Deionized water was used in the experiment.

2.2. Instruments and Measurements

Fourier transform infrared (FTIR) spectra were recorded on a Varian 640-IR FTIR spectrophotometer (Varian, Salt Lake City, UT, USA) using KBr pellets. Nuclear magnetic resonance spectroscopy (NMR) was performed on a Bruker AVANCE III 600 MHz digital NMR spectrometer (Bruker, Zurich, Switzerland) using $CDCl_3$ and D_2O as solvent at room temperature. Chemical shifts were measured using tetramethylsilane (TMS) as an internal standard. The number average molecular weight (M_n), weight average molecular weight (M_w), and polydispersity index (PDI, M_w/M_n) of polymers were determined by a Waters 515 gel permeation chromatograph (GPC) (Waters, Milford, MA, USA). Two chromatographic columns (Agilent Technologies, PLgel 5 μm 500 Å, PLgel 5 μm Mixed-C) were used in series. THF was used as eluent at a flow rate of 1.0 mL/min at 30 °C. Universal calibration was done using seven standard polystyrene samples (1600, 3790, 6000, 11,500, 44,700, 105,800, and 903,000). High-resolution atmospheric pressure-chemical ionization Fourier-transform ion cyclotron resonance mass-spectroscopy (HR-APCI-FT-ICR-MS) was performed on a Bruker Daltonik apex-Ultra 7.0 T Fourier-transform ion cyclotron resonance mass spectrometer (Bruker, Bremen, Germany) equipped with an atmospheric pressure chemical ionization source operating in the nebulizer-assisted electrospray mode. The sample was dissolved in methanol (high-performance liquid chromatography grade) solvent. The sample molecule was ionized with H^+ ions. UV-VIS spectra were recorded on a UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan). Photoluminescent (PL) spectra were recorded on a RF-5301PC fluorescence spectrophotometer (Shimadzu, Kyoto, Japan).

2.3. Synthesis of N-Butoxycarbonyl-Ethylene Diamine (N-Boc-EDA)

N-Boc-EDA was synthesized according to published literature [35]. Ethylenediamine (30 mL, 448 mmol) and THF (150 mL) were placed into a 500 mL round-bottom flask with moderate stirring

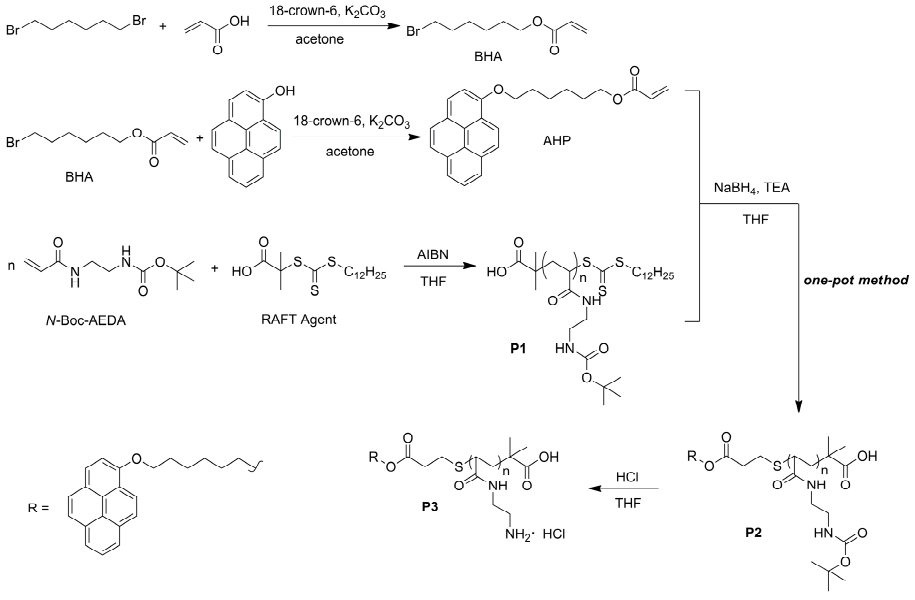
at −5 to 0 °C. Then, the THF (100 mL) solution of Boc_2O (20 mL, 87.06 mmol) was added dropwise into the cold mixture. After the addition, the reaction was continued for 24 h at room temperature, and a white suspension was obtained. The white suspension was filtered and the filtrate was rotary evaporated to give a pale yellow oily liquid. Then the liquid was washed extensively with saturated NaCl aqueous solution and filtered. The filtrate was extracted with ethyl acetate three times, and the combined organic layer was dried over anhydrous Na_2SO_4 overnight and filtered. Solvents were removed by rotary evaporation to give *N*-Boc-EDA as a pale yellow oily liquid 0.11 g (yield 73%). ^{1}H NMR (600 MHz, D_2O) δ (ppm): 3.02 (t, *J* = 6.0 Hz, 2H), 2.58 (t, *J* = 6.0 Hz, 2H), 1.34 (s, 9H).

2.4. Synthesis of N-Butoxycarbonyl-Acryloyl Ethylene Diamine (N-Boc-AEDA)S

N-Boc-AEDA was synthesized according to published literature [35]. *N*-Boc-EDA (0.72 g, 4.5 mmol), triethylamine (TEA, 0.55 g, 5.4 mmol), and DCM (20 mL) were added to a 100 mL round-bottom flask and stirred at 0 °C. Then, the solution of acryloyl chloride (0.41 g, 4.5 mmol) in DCM (15 mL) was added dropwise, and then the reaction was continued for 15 h at room temperature. After the reaction finished, saturated $NaHCO_3$ (40 mL) aqueous solution was added to the mixture. The organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. Solvents were removed by rotary evaporation to give a pale yellow solid. The pale yellow solid was chromatographed on a column of silica gel with ethyl acetate: *n*-hexane (7:3, *V*:*V*) as eluent, and white solid *N*-Boc-AEDA 0.826 g (yield 86%) was obtained. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 6.26 (d, *J* = 16.8 Hz, 1H), 6.12 (dd, *J* = 16.8 Hz, 10.8 Hz, 1H), 5.63 (d, *J* = 10.2 Hz, 1H), 3.45–3.43 (m, 2H), 3.33–3.30 (m, 2H), 1.43 (s, 9H).

*2.5. Synthesis of S-1-Dodecyl-S′-(α,α′-Dimethyl-α″-Acetic Acid)trithiocarbonate and S-1-Dodecyl-S′-[poly(N-Boc-Acryloyl Ethylene Diamine)-2′-Methyl Propionic Acid]trithiocarbonate (**P1**)*

S-1-dodecyl-*S*′-(α,α′-dimethyl-α″-acetic acid)trithocarbonate (RAFT agent) was synthesized according to published literature [36]. **P1** was synthesized according to procedures in the literature [37]. The reaction mechanism of **P1** was shown in Scheme 1. *N*-Boc-AEDA (6.000 g, 28.08 mmol) and solvent THF (30 mL) were mixed in a 100 mL eggplant-shaped flask with magnetic stirring and dissolved completely. RAFT agent (0.600 g, 1.64 mmol) and AIBN (0.048 g, 0.29 mmol) were added. The mixture was refluxed for 5 h under a nitrogen atmosphere. THF was removed by rotary evaporation to give a pale yellow solid. It was washed with water several times, dried in a vacuum oven to afford **P1** as a yellow solid (6.402 g, yield 97%).



Scheme 1. The reaction routes of BHA, AHP, **P1**–**P3**.

2.6. Synthesis of 6-Bromohexyl Acrylate (BHA)

The reaction mechanism of BHA was shown in Scheme 1. BHA was synthesized according to procedures in the literature [37].

2.7. Synthesis of 1-(Acryloyloxy Hexyloxy)pyrene (AHP)

The reaction mechanism of AHP was shown in Scheme 1. HP (0.65 g, 3 mmol), BHA (0.70 g, 3 mmol), potassium carbonate (1.38 g, 10 mmol), a small amount of catalyst 18-crown-6, and solvent acetone (20 mL) were added to a 50 mL single-neck round-bottom flask equipped with a spherical condenser. The mixture was heated at reflux and magnetically stirred throughout, overnight. Potassium carbonate was removed by suction filtration, and acetone was removed by rotary evaporation to give a white powder displaying blue-green under an ultraviolet lamp. The white powder was chromatographically purified on silica gel eluting with dichloromethane: petroleum ether (1:1, *V*:*V*) as eluent to collect the third ribbon (showing a blue fluorescence). The eluent was evaporated to dryness and the residue was dried until constant weight in a vacuum oven to give AHP as a white solid (0.374 g, 33%). Figure 1 was ^{1}H NMR spectrum of AHP in $CDCl_3$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.46 (d, *J* = 9.0 Hz, 1H), 8.11–8.08 (m, 3H, H-o, H-q, H-v), 8.03 (d, *J* = 9.0 Hz, 1H), 7.97–7.94 (m, 2H, H-p, H-t), 7.88 (d, *J* = 9.0 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 6.40 (dd, *J* = 17.4 Hz, 1.2 Hz, 1H), 6.12 (dd, *J* = 17.4 Hz, 10.2 Hz, 1H), 5.80 (dd, *J* = 10.2 Hz, 1.2 Hz, 1H), 4.33 (t, *J* = 6.6 Hz, 2H), 4.21 (t, *J* = 6.6 Hz, 2H), 2.05–2.01 (m, 2H), 1.80–1.75 (m, 2H), 1.72–1.67 (m, 2H), 1.58–1.52 (m, 2H). Figure 2 was ^{13}C NMR spectrum of AHP in $CDCl_3$. ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 166.3, 153.2, 130.3, 128.6, 127.2, 125.4, 120.5, 109.2, 131.8, 131.7, 125.9, 125.3, 125.0 (5C, C-r, C-u, C-n, C-x, C-y), 126.3, 126.0, 124.9, 121.2 (4C, C-m, C-t, C-s, C-l), 124.2, 124.1 (2C, C-o, C-q), 68.9, 64.5, 29.4, 28.7, 26.0, 25.8. Figure 3 is the HR-APCI-FT-ICR-MS spectrum of AHP. HR-APCI-FT-ICR-MS revealed the parent ion peak at *m*/*z* = 373.1797 (calcd for $C_{25}H_{25}O_3$: 373.1798, $[M + H]^+$).

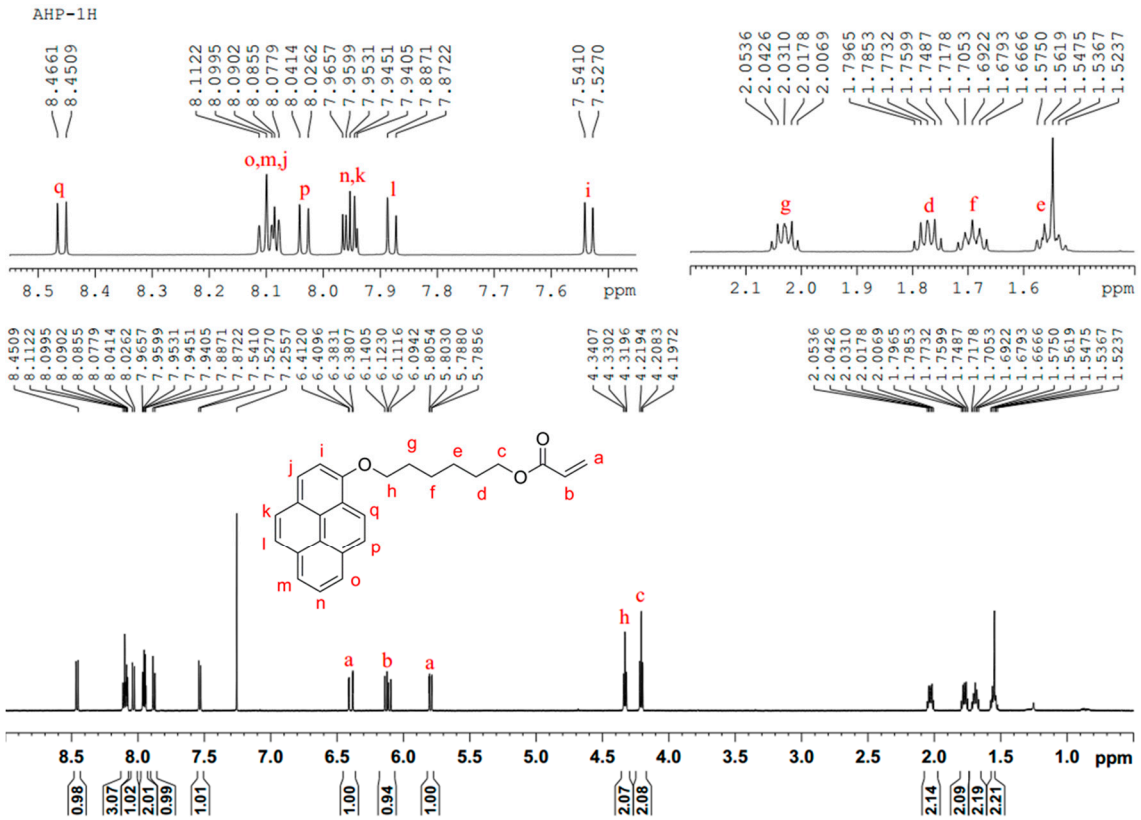


Figure 1. ^{1}H NMR spectrum of AHP in $CDCl_3$.

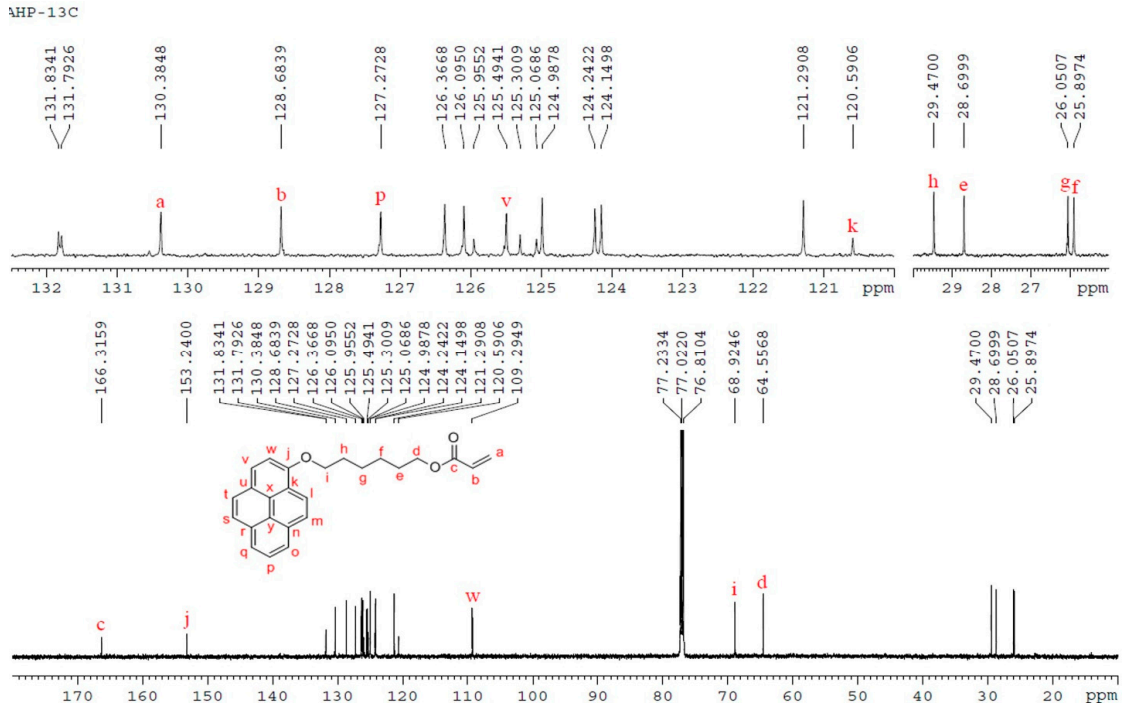


Figure 2. ^{13}C NMR spectrum of AHP in $CDCl_3$.

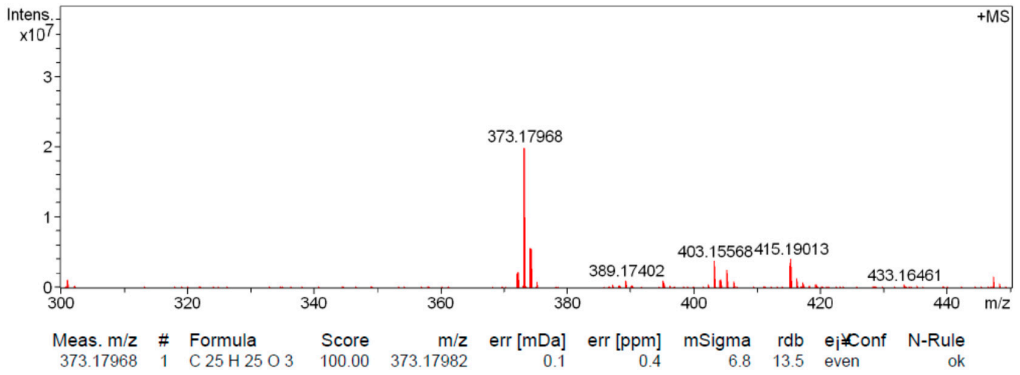


Figure 3. HR-APCI-FT-ICR-MS spectrum of AHP.

*2.8. Synthesis of 1-{3′-S-[poly(N-Butoxycarbonyl-Acryloyl Ethylene Diamine)-2″-Methyl Propionic acid]}propionyloxy Hexyloxy Pyrene (**P2**) and 1-{3′-S-[poly(Acryloyl Ethylene Diamine Hydrochloride)-2′-Methyl Propionic acid]}propionyloxy Hexyloxy Pyrene (**P3**)*

The reaction mechanism of **P2** and **P3** was shown in Scheme 1. **P1** (200.0 mg, 0.077 mmol), $NaBH_4$ (3.8 mg, 0.1 mmol), AHP (34.0 mg, 0.09 mmol), TEA (10 μL, 0.072 mmol), and solvent THF (2 mL) were added to a 5 mL eggplant-shaped flask. Then, the system was degassed for at least three cycles by pulling a vacuum and filling with nitrogen gas under magnetic stirring in an ice bath. The mixture was stirred at room temperature in a nitrogen atmosphere for 48 h. After removal of the solvent, a pale yellow solid was obtained and chromatographically purified on silica gel eluting with dichloromethane to remove the first two ribbons which were the excess AHP and the reaction byproducts. Then methanol was used as eluent to give **P2** as a wheat-colored solid (0.202g, 96%). **P2** (100 mg) was dissolved in 10 mL of THF, and then HCl gas was passed through the solution for the purpose of deprotection of the resulting Boc-protected polymer (**P2**) until an amount of precipitate formed. The formed precipitate was filtered and washed with THF, and dried in a vacuum oven to afford the desired water-soluble fluorescent polymer **P3** as a pale brown solid (71.5 mg, 95%).

3. Results and Discussion

3.1. The reaction Mechanism of the One-Pot Reaction

Scheme 1 outlines the basic reaction mechanism. Thiol is obtained by a reduction reaction of the trithioester of **P1**. The weak sulfur-hydrogen bond of thiols are readily oxidized to disulfide bonds during storage or purification, resulting in difficulty of subsequent click-chemistry reactions. The one-pot method used in this paper can solve the problem easily. In the same system, both the reduction reaction of the trithioester bond of **P1** to thiol, the click reaction of thiol, and the carbon-carbon double bond of AHP occurred simultaneously. As a result, a Boc-protected amino group fluorescent polymer (**P2**) was synthesized, then water-soluble pyrene-containing fluorescent polymer (**P3**) with a defined structure was obtained by the deprotection of the resulting Boc-protected polymer (**P2**) with aqueous HCl.

*3.2. FTIR Analysis of AHP, **P2**, and **P3***

Figure 4 was the FTIR spectra of AHP, **P2**, and **P3**. As seen from the AHP spectrum, the characteristic stretching vibration absorption peak of the ester carbonyl group C=O bond was shown at 1713 cm^{-1}. The absorption peak was shown at 1596 cm^{-1} resulting from that stretching vibration of the C=C bond in acrylic ester (–OCO–C=C). Absorption peaks at approximately 2939 and 2862 cm^{-1} were assigned to the asymmetric and symmetric stretching vibration and asymmetric stretching vibration of methylene group C–H bond, respectively. The peak at 1408 cm^{-1} responded to the C–H bond shear vibration of the vinyl end group (=CH_2). At around 1192 cm^{-1} the absorption peak was the asymmetric stretching vibration of the C–O–C bond. Two absorption peaks were shown at 1620 and 1510 cm^{-1} resulting from that stretching vibration of the aromatic ring skeleton of pyrene. There were no blockbuster absorption peaks of acid at 3300–2500 cm^{-1} in this spectrum. Comparing the spectra of **P2** and **P3**, the biggest difference was the absorption peak from 4000 to 2500 cm^{-1}. The absorption peaks shown at 3500–3300 cm^{-1} and 3330–3150 cm^{-1} were the stretching vibrations of the N–H bond in the primary amine and primary amine salt in the **P3** spectrum, respectively, which was due to the removal of the Boc group and formed a –NH_2 and –NH_2· HCl structure with HCl gas in repeating units. At around 1366 and 1390 cm^{-1} absorption peaks were the vibration of the C–H bond of *t*-butyl in the **P2** spectrum, and the former intensity was about twice as strong as the latter. The two peaks significantly reduced after removal of the Boc protection in the **P3** spectrum, at around 1693 cm^{-1} the ester carbonyl group C=O bond stretching vibration peak disappeared, remaining only at 1653 cm^{-1}, the solid amide C=O bond stretching absorption peak, which indicated that the number and morphology of the C=O bond was changed. These results clearly manifested the formation of AHP, **P2**, and **P3**.

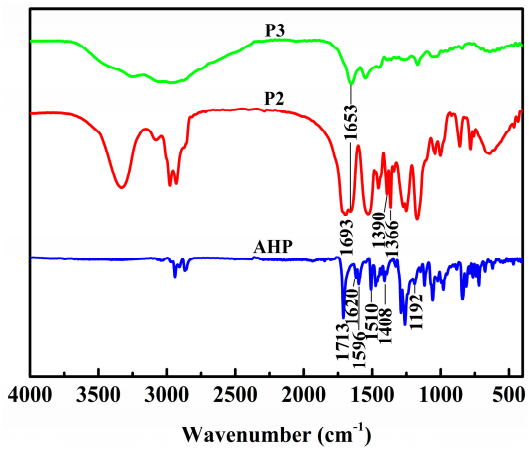


Figure 4. FTIR spectra of AHP, **P2**, and **P3**.

*3.3. NMR Analysis of **P3***

P3 is soluble in water. It is characterized by NMR, UV-VIS, and PL spectroscopy. Figure 5 showed the ^{1}H NMR spectrum of **P3**. ^{1}H NMR (600 MHz, D_2O, δ) showed a chemical shift peak from 7.60 to 8.40 ppm; these signals were assigned to the aromatic protons in pyrene units. There were two chemical shift peaks from 1.32 to 1.82 ppm and 1.94 to 2.37 ppm, which was attributed to protons of C***H***$_2$ and C***H*** groups in the polymer main chain. Two chemical shift peaks from 2.97 to 3.20 ppm and 3.21 to 3.60 ppm corresponding to protons of C***H***$_2$-NHCO and C***H***$_2$-NH_2·HCl in the polymer side chain were observed. There were two chemical shift peaks from 3.95 to 4.12 ppm and 3.75 to 3.80 ppm, which were assigned to protons of COO–C***H***$_2$ and C_6H_4O–C***H***$_2$. The ^{1}H NMR spectrum also confirmed the presence of the pyrene unit in the **P3** structure. This proved that the pyrene unit was really attached to the **P1** chain and **P2** was synthesized after the click reaction of thiol reduced from **P1** and the carbon-carbon double bond of AHP.

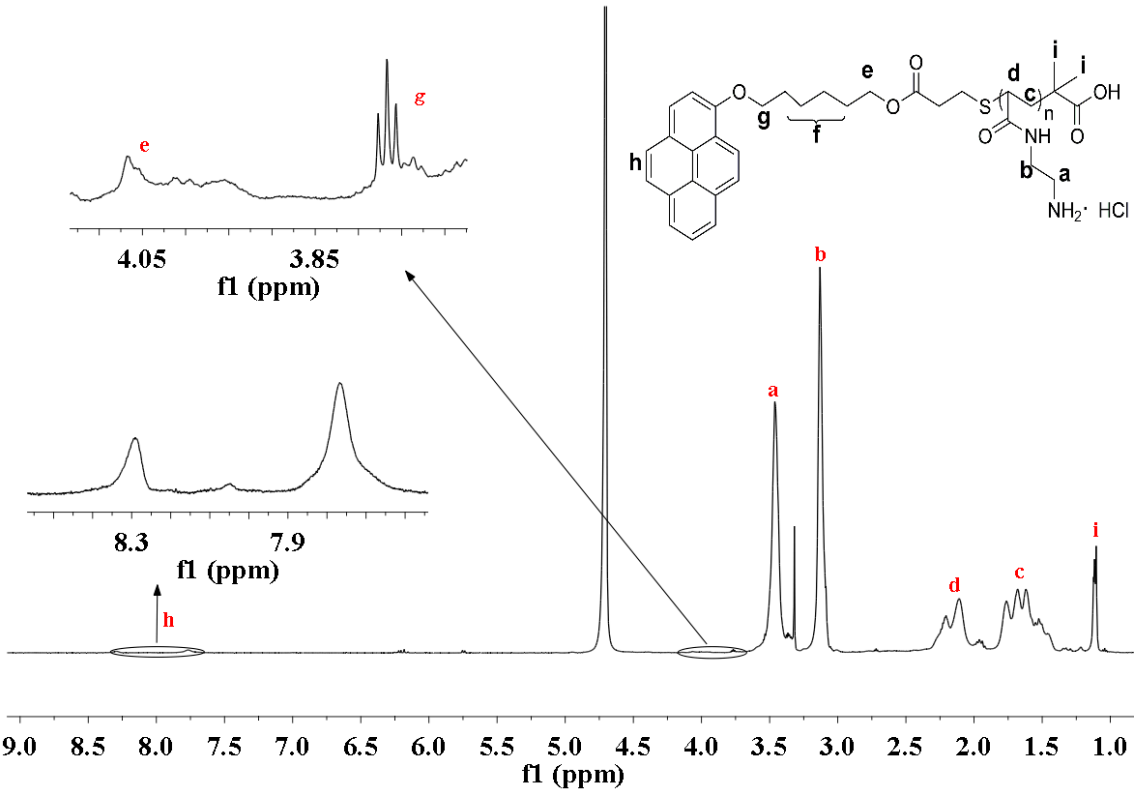


Figure 5. ^{1}H NMR spectrum of **P3** in D_2O.

3.4. GPC Analysis

Figure 6 are the GPC chromatograms for the standard sample (polystyrene), **P1**, and **P2** which were shown as a blue line, red line, and green line, respectively. The M_n of the standard sample was labeled as 3.79×10^3 g·mol^{-1}. The M_n of **P1** and **P2** were given by the software as 2.60×10^3 g·mol^{-1} and 2.50×10^3 g·mol^{-1}, respectively. PDI of **P1** is 1.39. The theoretical molecular weight of **P2** is M_n(**P2**) = M(AHP) + M_n(**P1**) − M($CS_2C_{12}H_{25}$) = 372 + 2600 − 245 = 2727 g·mol^{-1}. The analytical datum from the GPC experiment was similar with the theoretical result, which proved that AHP bonded with the **P1** chain as shown in Scheme 1.

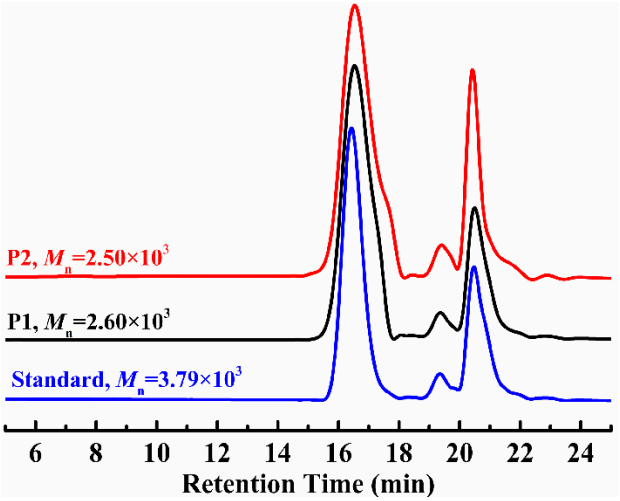


Figure 6. GPC chromatograms for **P1**, **P2**, and the standard (M_n = 3.79 × 10^3 g·mol^{-1}).

3.5. UV-VIS and PL Analysis

Figure 7 is the UV-VIS spectra of AHP, **P1**, and **P2** in CH_2Cl_2. As it can be seen from the **P1** spectrum, there was only one characteristic absorption peak at 310 nm resulting from C=S double bond [35,37]; in sharp contrast, it had disappeared in the UV-VIS spectrum of **P2**. The numbers and positions of the UV-VIS absorption peak of **P2** were consistent with those of AHP.

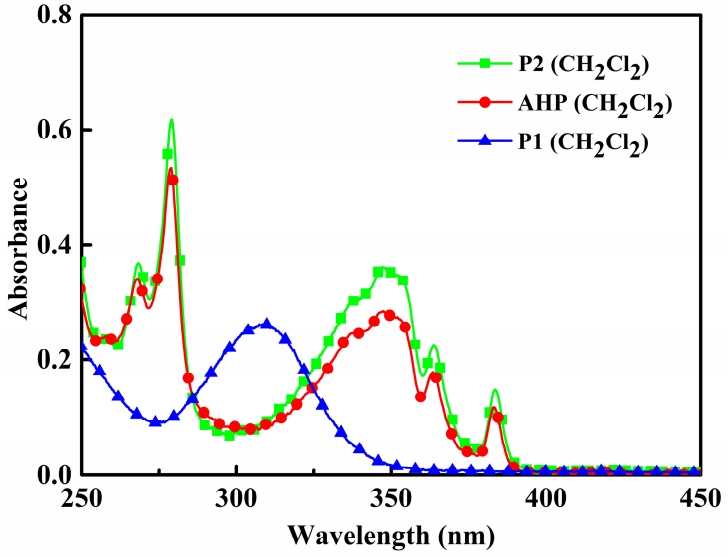


Figure 7. UV-VIS spectra of AHP, **P1**, and **P2** in CH_2Cl_2.

Figure 8 is the PL spectra of AHP, **P1**, and **P2** in CH_2Cl_2. The fluorescence emission peak of **P1** using its maximum absorption wavelength of 310 nm in the UV-VIS spectrum as the excitation wavelength was not observed. 345 nm was chosen as the excitation wavelength of AHP and **P2** according to the reference literature [34,38]. AHP and **P2** showed the fluorescence spectra of the same shape and three typical fluorescence emission peaks at 387, 407, and 429 nm, respectively. Since unreacted AHP had been removed during the processing of **P2** purified by silica gel column chromatography, these data indicated that **P2** fluorescence emission was not due to residual AHP, but from pyrene units in **P2**. Figures 7 and 8 revealed that the pyrene-containing unit AHP was really introduced to the **P1** chain and **P2** was successfully prepared after one-pot reaction.

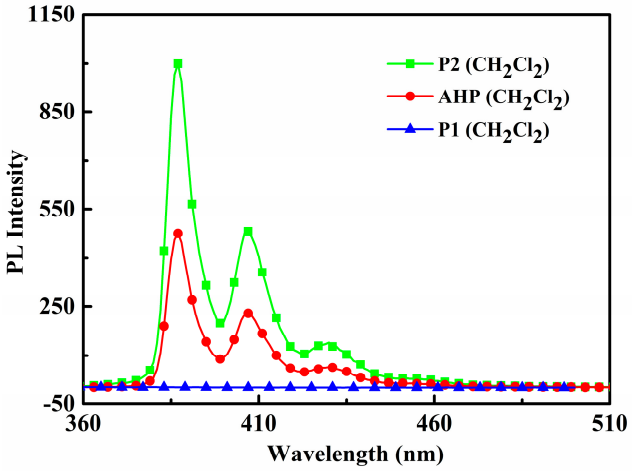


Figure 8. PL spectra of AHP, **P1**, and **P2** in CH_2Cl_2.

P2 was soluble in common organic solvents, such as dichloromethane, trichloromethane, and THF, and exhibited a bright blue-violet fluorescence emission band in solution. **P3** was soluble in water and exhibited a bright blue-violet fluorescence emission band in water, too. Figures 9 and 10 were UV-VIS and PL spectra of AHP in H_2O, **P2** in dichloromethane, and **P3** in H_2O, respectively. Since AHP was insoluble in water, there were no UV-VIS absorption peaks and fluorescence emission peaks of AHP in the aqueous solution. **P3** had good water solubility, and showed the UV-VIS characteristic absorption bands of pyrene, and were consistent with those of **P2** in dichloromethane solution. There were three fluorescence emission peaks at 387, 407, and 429 nm of **P3** in aqueous solution, respectively, which were consistent with those of **P2** in dichloromethane solution. Therefore, UV-VIS and PL measurement results showed that: **P2** was prepared by the one-pot method of thiol reduced from **P1** and the carbon-carbon double bond of AHP; water-soluble fluorescent polymer **P3** was obtained by the deprotection of the resulting Boc-protected polymer (**P2**); and it was also confirmed that UV-VIS absorption and fluorescence emission of **P2** and **P3** come from their own structure, rather than the residual AHP. The fluorescence quantum yields of **P2** (Φ_u = 0.44) in dilute THF and **P3** (Φ_u = 0.39) in dilute aqueous solution were measured using DPA as a reference substance (Φ_s=0.90) [39] according to the formula:

$$\Phi_u = \Phi_s(n_u^2 F_u / n_s^2 F_s)(A_s / A_u) \quad (1)$$

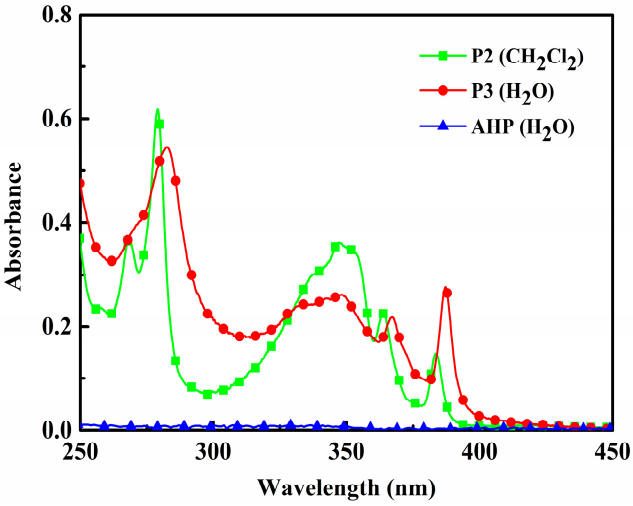


Figure 9. UV-VIS spectra of AHP, **P2**, and **P3**.

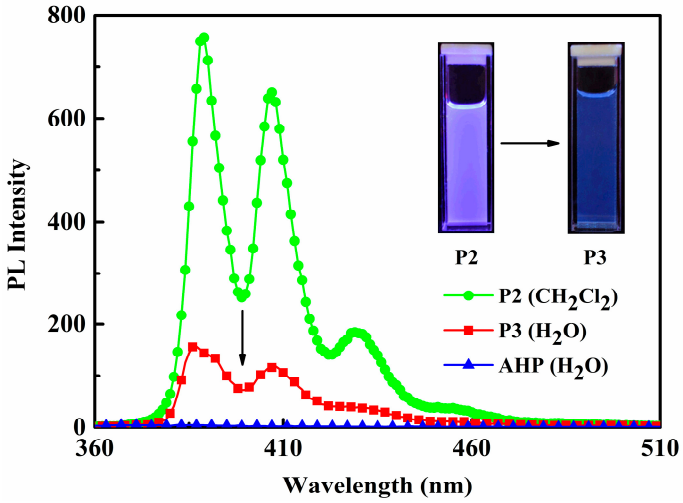


Figure 10. PL spectra of **P2** in CH_2Cl_2, AHP, and **P3** in H_2O.

4. Conclusions

Reversible addition-fragmentation chain transfer polymerization was used to obtain a Boc-protected amino group polymer (**P1**) with trithioester as a terminal group. The trithioester bond of **P1** was reduced to thiol using sodium borohydride as the reductant. The click reaction of thiol and the carbon-carbon double bond of AHP was used to prepare a Boc-protected amino group and the defined structure of a fluorescent polymer containing a pyrene unit (**P2**). A water-soluble pyrene-containing fluorescent polymer (**P3**) was synthesized by the deprotection of the resulting Boc-protected polymer (**P2**) with aqueous HCl. **P3** was soluble in water and exhibited a bright blue-violet fluorescence emission band in aqueous solution, which had the similar photoluminescent spectra to those of AHP and **P2** in dichloromethane. The fluorescent quantum yields of **P2** in dilute tetrahydrofuran and **P3** in dilute aqueous solution could reach 0.44 and 0.39, respectively.

Supplementary Materials: Supplementary materials can be accessed at: http:// www.mdpi.com/2073-4360/7/12/1538/s1.

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Author Contributions: Hongchi Zhao and Yonggang Wu designed the experiments; Xiaomeng Li, Miaomiao Wang, Haijian Tan, Qingmin Yang, Aiqing Wang, Libin Bai performed the experiments; Xiaomeng Li and Miaomiao Wang wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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