Synthesis and Characterization of Polyphenylenes with Polypeptide and Poly(ethylene glycol) Side Chains

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ABSTRACT: We report a novel approach for fabrication of multifunctional conjugated polymers, namely poly(*p*-phenylene)s (PPPs) possessing polypeptide (poly-L-lysine, PLL) and hydrophilic poly(ethylene glycol) (PEG) side chains. The approach is comprised of the combination of Suzuki coupling and *in situ* *N*-carboxyanhydride (NCA) ring-opening polymerization (ROP) processes. First, polypeptide macromonomer was prepared by ROP of the corresponding NCA precursor using (2,5-dibromophenyl)methanamine as an initiator. Suzuki coupling reaction of the obtained polypeptide and PEG macromonomers both having dibromobenzene end functionality using 1,4-benzenediboronic acid as the coupling partner in the presence of palladium catalyst gave the desired polymer. A different sequence of the same procedure was also employed to yield polymer with essentially identical structure. In the reverse sequence mode, low molar mass monomer (2,5-dibromophenyl)methanamine, and PEG macromonomer were coupled with 1,4-benzenediboronic acid in a similar way followed by ROP of the L-Lysine NCA precursor through the primary amino groups of the resulting polyphenylene. © 2015 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2015**, *53*, 1785–1793

KEYWORDS: conjugated polymer; *N*-carboxyanhydride; poly-L-lysine; poly(ethylene glycol); poly(*p*-phenylene)s; polypeptide; ring-opening polymerization; Suzuki coupling; biopolymers; peptides; water-soluble polymers

INTRODUCTION The extensive role of conjugated polymers (CPs) in emerging synthetic bio-applications,[1,2] optical,[3] electronic,[4–6] diodes,[7,8] and display technologies,[9] represents the promising way in which considerable endeavors have been directed towards the realization of many aspects of conducting organic polymers. The conducting properties of CPs arise from having delocalized π-electron bonding along the polymer backbone bearing a framework of alternating double and single carbon-carbon bonds along which electrons can flow.[1,10–14] The mechanical flexibility, tunable optical properties, excellent conductivity, high mechanical strengths and processability of some conducting polymers make them potentially useful materials for new applications such as high-contrast organic light-emitting displays and low-cost organic photovoltaic solar cells.[15–21] Conjugated structure of CPs can create excellent one-dimensional surface for energy transport of electrons and strong UV absorption.[22–26] Especially, their fluorescence feature is one of the most susceptible to environmental change and this allows for eminent selectivity in signaling reporter group materials and provides advantages for CPs to be used in sensor technologies including pH sensors, temperature sensors and recently developed biosensors.[27–29] In CPs, the adjusting molecular morphology can lead to high surface area and extensive stability of the enzymes incorporated. Variations at surface properties depend on types of pendent groups, allowing for the production of polymers which are water soluble or exceptionally compatible with biomolecules.[30,31]

Among vast number of CPs, poly(*p*-pheneylene)s (PPP)s are the most promising class of polymer in terms of relatively high photoluminescence, electroluminescence quantum efficiencies and thermoxidative stability.[32–34] PPPs can easily be functionalized to give a wide range of derivatives due to the fact that their syntheses generally include phenyl compound monomers readily allowing for many chemical modifications. Unfortunately, PPPs suffer from insolubility in many solvents, which limit their processability.[35–37] However, the addition of conformational alkyl or water soluble pendants or polymers as side chains to the backbone can lead to the obtainment of

Additional Supporting Information may be found in the online version of this article.

soluble PPPs with high molecular weight. In related studies in the authors' laboratory, it has been shown that PPPs can be combined with conventional synthetic polymers through the combination of various chain polymerizations with coupling processes to give soluble graft copolymers while preserving the conjugated backbone properties.[31,38,39]

Innovative approaches including the combination of polypeptides with polymeric structures have received enormous interest in the fields of biomedicine,[40] drug delivery,[41,42] tissue engineering,[43] and they are regarded as impressive biomaterial mimicking natural proteins.[44] Such conjugation systems can also open new perspectives for the fabrication of novel biomaterials that possess antibody/antigen, biocatalyst activity, compatibility with blood and/or tissue properties.[45–47] Polypeptides, existing in three-dimensional conformations such as α-helix, β-sheet, and random coil shapes, exhibit intriguing morphological surface and excellent properties, such as low toxicity, biodegradability, and biocompatibility.[48,49] Recent novel studies involving the synthesis of polymers such as polyether, polyester, chitosan, polysiloxane etc., combined with polypeptide segments, have the potential to revolutionize today's morphological structures and biomedical applications.[50] Considering conventional methods which have some drawbacks such as, multistep reactions, the use of hazardous reagents such as phosgene, lack of sufficient monomer purity and sensitivity to water contamination or heating conditions, the ideal synthetic pathway for polypeptides is the ring-opening polymerization (ROP) of the corresponding α-amino acids of *N*-carboxyanhydrides (NCAs).[51–57] In this technique, the polymerization can readily be induced with primary or secondary amines and their living nature allows the decisive control of molecular weight and terminal structure. The precursor urethane derivatives as monomer are readily synthesized by a two-step procedure: (i) transformation of α-amino acids into the corresponding ammonium salts and (ii) *N*-carbamylation of the salts with diphenyl carbonates. These urethane derivatives of α-amino acids are stable for several months at room temperature.[51] It should be noted that high stability and ease of polymerization of these molecules with amines provides the possibility to form polypeptide-polythiophene copolymers as bio-based covering materials and ethanol biosensors.[39]

Although polymers bearing bound polypeptides provide unique properties and advantage as regards biofunctionalization, the use of soluble support is a more essential requirement in biological application.[58–63] Water solubility can be achieved by the incorporation of polyethers, such as poly(ethylene glycol) (PEG). Polypeptide-PEG combinations as well as glycopeptides[64–67] with various topological structures receive great attention due to their excellent solubility in both water and organic solvents, and their biocompatibility. Moreover, they exhibit no antigenicity, immunogenicity, or toxicity towards the human body and is approved by the US American Food and Drug Administration (FDA).[68,69]

Herein, we report a novel approach for the synthesis of PPP copolymers bearing both PEG units and polypeptide groups by the combination of Suzuki coupling and *in situ* NCA-ROP processes. This amine compound was used as initiator for NCA polymerization in the synthesis of polypeptide macromonomers and also used as monomer for the synthesis of PPP copolymer bearing primary amine groups and PEG. We provide two strategies to obtain PPP polymers bearing PEG and poly-L-lysine (PLL) side chains; (1) Suzuki coupling polymerization between PEG and polypeptide macromonomers, (2) NCA graft polymerization over the PPP backbone bearing the primary amine and PEG groups. To investigate the structure of the polymers and monomers, ^{1}H-NMR was applied. UV-Vis and fluorescence spectra of PPP polymers are accepted as evidence of the formation of π-conjugated backbone. The polymers obtained with the characteristic features they possess could in the future be applied to various bio mimicking and other bio applications.

EXPERIMENTAL

Materials

Tetrakis (triphenylphosphine) palladium(0) ($Pd(PPh_3)_4$), poly(ethylene glycol) mono methyl ether ($M_{n,RI} = 550$ g mol^{-1}) (Me-PEG550), *N*-boc-L-lysine, tetrabutylammonium hydroxide (37% in methanol), 2,5-dibromo-benzoic acid, *N*,*N*-dimethylacetamide (DMAc) 1,4-benzene-diboronic acid, *N*-bromosuccinimide (NBS), 4-(*N*,*N*'-dimethyl) amino pyridine (DMAP), dicyclohexylcarbodiimide (DCC), poly(ethylene glycol) mono methyl ether (PEG_{2000}), Phthalimide potassium salt, hydrazine monohydrate (98%), and benzoyl peroxide are from Sigma-Aldrich and used without any further purification. All solvents were purified and dried.

Measurements

^{1}H-NMR spectra were performed with an Agilent VNMRS 500 MHz, and chemical shifts were recorded in ppm units using tetramethylsilane as an internal standard. UV/Vis absorbance measurements were recorded on a Shimadzu UV-1601 spectrometer. Fluorescence measurements were performed with a model LS-50 spectrometer from PerkinElmer at room temperature. The fluorescence quantum yields were determined according to IUPAC technical report using coumarine 1 (7-diethylamino-4-methylcoumarin) as standard.[70] Gel permeation chromatography (GPC) measurements were carried out in Viscotek GPCmax auto sampler system. Instrument was equipped with a pump (GPCmax, Viscotek Corp., Houston, TX), light-scattering detector ($\lambda_0 = 670$ nm, Model 270 dual detector, Viscotek Corp.) consisting of two scattering angles: 7° and 90° and the refractive (RI) index detector (VE 3580, Viscotek Corp.). Both detectors were calibrated with PS standards in the narrow molecular weight distribution. Three ViscoGEL GPC columns (G2000HHR, G3000HHR and G4000HHR) employed with THF in the 1.0 mL min^{-1} flow rate at 30 °C. All data were analyzed using Viscotek OmniSEC Omni-01 software.

Synthesis of 1,4-Dibromo-2-(bromomethyl) benzene (1)

2,5-Dibromotoluene (2.55 mL, 18.5 mmol), NBS (6.2 g, 24.8 mmol) and 0.1 g of benzoyl peroxide were added to dry

40 mL of CCl_4 under nitrogen atmosphere. The solution was refluxed with condenser under nitrogen and 200 W light for 4 h. Heating started from 65 °C and temperature was increased by 10 °C per hour and in the last 1 h temperature was kept at 95 °C. After that, the precipitate was filtered and washed with a supplementary amount of CCl_4 and finally with a small quantity of CH_2Cl_2. The organic layer was washed with water several times, and then dried over $NaSO_4$. The solvent was evaporated in *vacuo*. Then the remaining crude material was purified by flash column chromatography (SiO_2, Et_2O) and recrystallized in petroleum ether to yield (**1**) (3.04 g, 50%, white crystal). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.54 (s, 2H), 7.30 (dd, $J = 8.5$, 2.4 Hz, 1H), 7.45 (d, $J = 8.5$ Hz, 1H), 7.60 (d, $J = 2.3$ Hz, 1H).

Synthesis of 2-(2,5-Dibromobenzyl)isoindoline-1,3-dione (2)

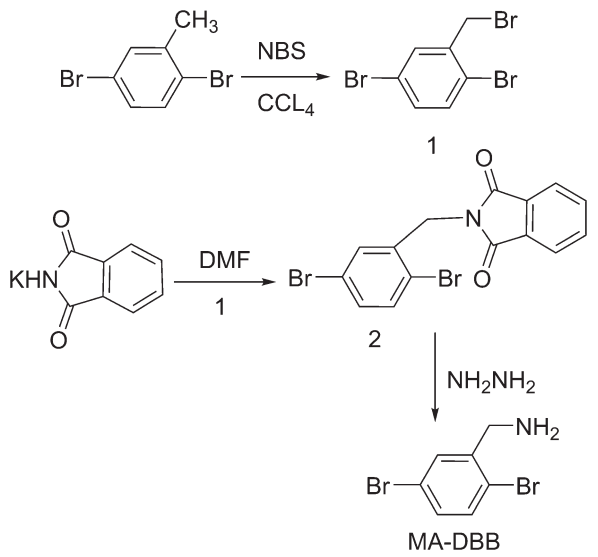
To the solution of (**1**) (2.9 g, 8.8 mmol) in 10 mL dry DMF, potassium phthalimide (2.45 g, 13.22 mmol) were added. After stirring for 12 h at 160 °C in a schlenck tube under nitrogen atmosphere, the reaction mixture was poured into water. The precipitate was washed with water, purified by flash column chromatography (SiO_2, CH_2Cl_2), precipitated in ethanol and dried under vacuum to yield (2) (2.56 g, 74%, white solid). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.94 (s, 2H), 7.26 (dd, $J = 5.7$, 2.5 Hz, 1H), 7.28 (d, $J = 2.3$ Hz, 1H), 7.45 (d, $J = 8.3$ Hz, 1H), 7.79 (dd, $J = 5.5$, 3.0 Hz, 2H), 7.92 (dd, $J = 5.5$, 3.1 Hz, 2H).

Synthesis of (2,5-Dibromophenyl) Methane Amine (MA-DBB)

The solution of (**2**) (0.381 g, 0.95 mmol) in 25 mL absolute ethanol with 3 mL hydrazine were stirred at 90 °C for 5 h. After allowing to cool to room temperature, the mixture was extracted with CH_2Cl_2 and 10% $NaHCO_3$. The organic layer was dried over Na_2SO_4 and reduced in *vacuo*. The residue was purified by column chromatography (Al_2O_3 (neutral), THF) to yield (**3**) (145 mg, 57%, yellow oil). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.59 (br, 2H), 3.87 (s, 2H), 7.23 (dd, $J = 8.4$, 2.4 Hz, 1H), 7.38 (d, $J = 8.4$ Hz, 1H), 7.54 (d, $J = 2.4$ Hz, 1H).

Synthesis of Urethane Derivatives of N_ε-Carbobenzoxy-L-Lysine (NCA-LL)

To a stirred solution of N_ε-carbobenzoxy-L-lysine (3.5 g, 12.5 mmol) in methanol (35 mL) and distilled water (5 mL), tetrabutylammonium hydroxide (37% in methanol) (8.77 g, 12.5 mmol) was slowly added at room temperature. After stirring for 1 h, the mixture was concentrated under reduced pressure. The residues were dissolved in acetonitrile (25 mL). The solution was added dropwise over 10 min to a stirred solution of DPC (2.68 g, 12.5 mmol) in acetonitrile (25 mL) at room temperature, and then the mixture was stirred overnight. To the reaction mixture, 1N of HCl aqueous solution (20 mL) was added. The mixture was put into a separating funnel containing distilled water (30 mL) and ethyl acetate (30 mL). The aqueous layer was extracted with ethyl acetate (3 × 30 mL) and the organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude products were purified by flash column chromatography (SiO_2, chloroform:acetone:acetic acid- = 7:3:0.1, as eluent), and then recrystallized with ethyl acetate/*n*-hexane to yield 3.4 g (68%), as white solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34–1.58 (m, 4H), 1.67–1.83 (m, 1H), 1.84–1.96 (m, 1H), 3.15 (m, 2H), 4.39 (m, 1H), 4.86(s, 1H), 5.08 (s, 2H), 5.84 (d, 1H, $J = 8.1$ Hz), 7.09 (d, 2H, J =7.8 Hz), 7.15 (t, 1H, J =7.4 Hz), 7.28–7.37 (m, 7H).



SCHEME 1 Synthesis of MA-DBB via Gabriel reaction.

Synthesis of Polypeptide Macromonomer (DBB-PLL)

To the solution of N_ε-carbobenzoxy-L-lysine (0.4 mg in 1 mL DMAc) in a flame-dried Schlenk tube, the amine initiator, MA-DBB, (5.3 mg in 0.5 mL DMAc) was added. The reaction mixture was stirred at 60 °C for 120 h under argon atmosphere. After the polymerization, mixture was cooled to room temperature and poured into diethyl ether. The precipitates were filtrated, and then dried under vacuum to yield 230 mg (80%), as white solids. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.29–1.77 (broad), 1.78–2.01 (broad), 2.93–3.27 (broad), 3.49 (dd, $J = 14.0$, 7.0 Hz, 2H), 3.78–3.98 (broad), 4.85–5.25 (broad), 5.32–5.64 (broad), 6.84 (d, $J = 7.9$ Hz, 1H), 7.06 (s, 1H), 7.17–7.36 (broad), 7.39 (d, $J = 8.5$ Hz, 1H).

Synthesis of PEG Macromonomer (DBB-PEG)

2,5-Dibromo-benzoic acid (4 g, 14.3 mmol), DMAP (174.7 mg, 1.43 mmol), Me-PEG_{2000}-OH (25.74 g, 12.87 mmol) were dissolved in 80 mL of dry CH_2Cl_2 under nitrogen atmosphere. To this solution was added DCC (3.246 g, 15.7 mmol) in 20 mL dry CH_2Cl_2 drop-wise under nitrogen. Then, the reaction mixture was stirred for 5 days at room temperature. After that, the mixture was filtered and washed with 250 mL of CH_2Cl_2. The organic phase was quenched with 10% $NaHCO_3$ and brine, and then dried over Na_2SO_4. The solution was concentrated and passed through a silica-gel column using CH_2Cl_2 as eluent. After removal of solvent in *vacuo*, the polymer was precipitated in cold diethyl ether to yield 26 g (95%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.85–3.46

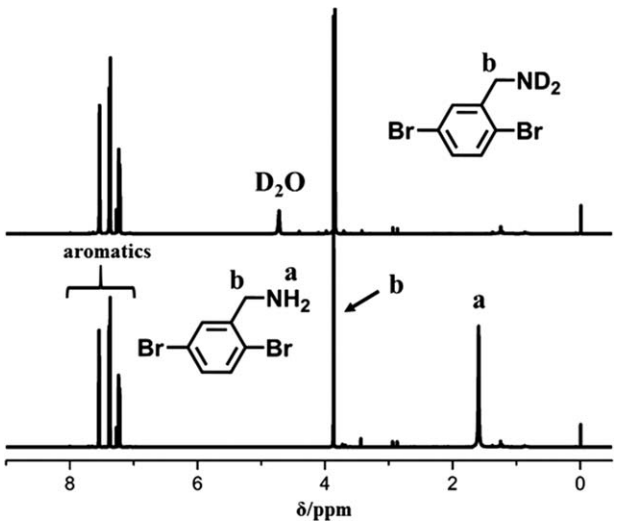


FIGURE 1 ^{1}H-NMR spectra of MA-DBB before (bottom) and after (top) proton exchange.

(broad), 3.38 (broad), 7.45 (dd, $J = 8.5$, 2.4 Hz, 1H), 7.52 (d, $J = 8.5$ Hz, 1H), 7.93 (d, $J = 2.4$ Hz, 1H).

Synthesis of PPP Conjugated Polymer Bearing Poly-L-Lysine and PEG (PPP-g-PEG-PLL)

DBB-PLL (0.18 g), DBB-PEG (0.8 g), and benzene-1,4-diboronic acid (74.58 mg, 0.45 mmol) was dissolved in a solution of 2 mL of 2.0 M K_2CO_3 (aq.) and 3 mL of THF in a Schlenk tube. The mixture was bubbled with nitrogen over a period of 30 min and then, $Pd(PPh_3)_4$ (26 mg, 0.226 mmol) was added. The reaction mixture was stirred at 70 °C for 4 days in the absence of oxygen and light. After polymerization, the reaction mixture was extracted with water and CH_2Cl_2. Organic phase was collected and dried over Na_2SO_4. After the concentration of mixture under reduced pressure, the final polymer was precipitated in cold diethyl ether and dried under vacuum to yield 0.556 g (55%). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.01–1.68 (broad), 1.69–2.09 (broad), 2.97–3.24 (broad), 3.40 (d, $J = 15.2$ Hz, 3H), 3.41–3.71 (broad), 3.81–3.76 (m, 2H), 3.82-3.92 (broad), 4.19–4.36 (broad), 4.93–5.12 (broad), 5.37–5.52 (broad), 7.12–7.36 (broad), 7.35–8.36 (broad).

Synthesis of PPP Conjugated Polymer Bearing Primary Amine and PEG (PPP-NH_2-g-PEG)

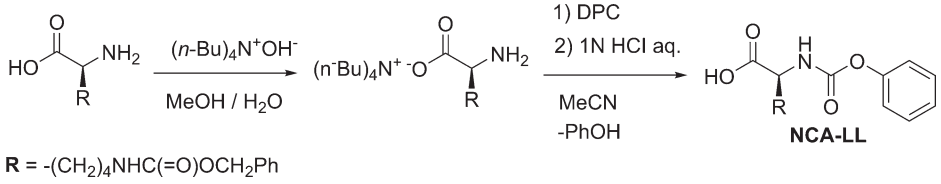
The reaction solution was prepared with 30 mL THF and 20 mL of aqueous K_2CO_3 solution under nitrogen. Prior use, this solution was degassed by bubbling nitrogen over a period of 30 min. In the 10 mL of the reaction solution, MA-DBB (0.8 g, 3 mmol), DBB-PEG (2.3 g), benzene-1,4-diboronic acid (0.663 g, 4 mmol), $Pd(PPh_3)_4$) (0.092 g, 0.08 mmol) was dissolved under nitrogen and stirred at 70 °C for 4 days in the Schlenk tube under vacuum. After stirring, the reaction mixture extracted with CH_2Cl_2 and small amount of water. Organic phase was concentrated and precipitated in excess diethyl ether to yield 1.85 g, white powder. ^{1}H-NMR (CDCl3, 500 MHz): δ 2.06 (broad), 3.38 (broad), 3.45–3.8 (broad), 7.92–8.20 (broad), 7.6–7.9 (broad), 7.2–7.6 (broad).

Synthesis of PPP Conjugated Polymer Bearing Poly-L-Lysine and PEG (PPP-g-PEG-PLL) by Route 2

PPP-NH_2-*g*-PEG (0.9 g) was charged into flame-dried Schlenk tube and then dissolved in 1 mL of DMAc, followed by addition of NCA precursor (NCA-LL)/DMAc solution (0.3 g/1 mL). The reaction mixture was stirred at 60 °C for 48 h under argon atmosphere. After the NCA ROP polymerization, the mixture was cooled to room temperature and then precipitated in cold diethyl ether. The resulting precipitates were filtered and dried under vacuum to yield 0.523 g (95%). ^{1}H-NMR (CDCl3, 500 MHz): δ 1.07–1.66 (road), 1.75–2.15 (broad), 2.91–3.20 (broad), 3.55 (t, 3H), 3.56–3.68 (broad), 3.77–3.72 (m, 2H), 3.81–4.07 (broad), 4.18–4.37 (broad), 4.91–5.11 (broad), 5.43–5.60 (broad), 7.08–7.36 (broad), 7.34–8.39 (broad).

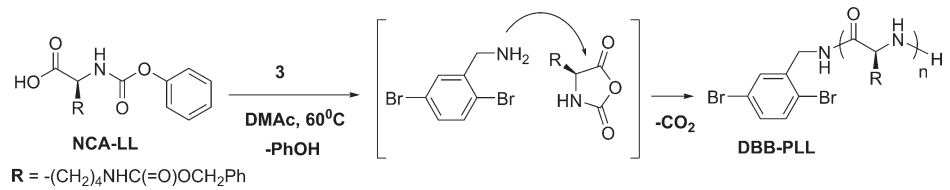
RESULTS AND DISCUSSION

The crucial part of the approach for the synthesis of PPPs with macromolecular side chains is to obtain dibromobenzene functionalized polymers or low molar mass related components since applied Suzuki coupling reactions utilize dibromobenzenes in conjunction with the benzene diboronic acids and derivatives. Thus, primary amino functional dibromobenzene possessing suitable functionalities for both polypeptide incorporation and Suzuki coupling processes was synthesized via Gabriel reaction.[71–76] 2,5-Dibromotoluene was chosen as starting material because it can readily react with *N*-bromosuccinimide (NBS) to afford the allylic bromination of the methyl group in the presence of peroxide. After obtaining **1** in good yield, the substitution reaction with the conjugate base of phthalimide gave the corresponding *N*-alkyl phthalimide, 2-(2,5-dibromobenzyl)isoindoline-1,3-dione, (**2**). The efficiency of the reaction was increased by using preformed potassium salt of phthalimide, instead of phthalimide, facilitating the reaction to be conducted in a polar protic solvent such as DMF under homogenous conditions. Treatment of the imide derivative, **2** with hydrazine in methanol results in the formation of phthalhydrazide and (2,5-dibromophenyl) methanamine (MA-DBB) through



R = -$(CH_2)_4NHC(=O)OCH_2Ph$

SCHEME 2 Synthetic route for the urethane derivative of α-amino acid, NCA-LL.



SCHEME 3 Synthesis of DBB-PLL by *in situ* NCA ring opening polymerization.

intramolecular substitution. By using column chromatography, the desired amine compound MA-DBB could be separated from the side product phthalhydrazide. The overall synthetic pathway is outlined in Scheme 1. The structure of MA-DBB was confirmed by ^{1}H-NMR analysis (Fig. 1). Singlet peak at 1.59 ppm was assigned to $-NH_2$ protons, which was further confirmed by the examination of the hydrogen–deuterium exchange reaction. After the deuterium exchange using D_2O, the peak at 1.59 ppm disappeared and a new singlet peak at 4.72 ppm appeared.

As stated previously, polypeptides can be synthesized from the corresponding NCA precursors. For our convenience, we have selected L-lysine based NCA precursor, L-lysine-*N* (phenyloxycarbonyl) amino acid (NCA-LL). This urethane derivatives of α-amino acid, was synthesized in two reaction steps; (i) the transformation of α-amino acid into the corresponding ammonium salt by an ionic exchange reaction and (ii) *N*-carbamylation of the ammonium salt-protected amino acid via nucleophilic attack of the active primary amino group to the carbonyl group of diphenyl carbonate (Scheme 2).

The final product NCA-LL is stable and can be stored for several months at room temperature. Starting material of *N*-(phenoxycarbonyl) amino acid was synthesized according to the described procedure.

Synthesis of PPPs with Polypeptide and PEG Side Chains

In order to develop the protocol for the synthesis of the final polypeptide bioconjugate, Suzuki coupling, and *in situ* NCA ROP were combined in two different sequences. In the first route, polypeptide macromonomer (DBB-PLL) was prepared by ROP of NCA-LL using MA-DBB as an initiator (Scheme 3). In earlier studies, we have shown that at high amine concentration, the monomer is rapidly consumed and polymerization proceeds in a controlled manner.[51] In the polymerization process, the NCA ring is formed by the elimination of phenol. Then, the primary amine group of the initiator attacks to the carbonyl carbon of NCA ring to form an amide bond between initiator and monomer with the evolution of CO_2, and the other activated amine side of the opened NCA ring ensues polypeptide chain growth.

The structure of the polypeptide and presence of the terminal dibromobenzene group were confirmed by ^{1}H-NMR analysis. As can be seen from Figure 2, aromatic protons of the end group resonate at 7.47, 7.06, and 6.84 ppm.

Characteristic aromatic protons corresponding to the repeating unit appear between 7.13 and 7.42 ppm.

The other polymeric coupling component, PEG macromonomer was synthesized by the well-known Steglich esterification method under mild conditions with the favorable catalytic action of 4-dimethylaminopyridine (DMAP)[31] (Scheme 4). ^{1}H-NMR spectral analysis of DBB-PEG evidenced the presence of both PEG repeating units and end group (Fig. S1, Supporting Information).

The assembling of PEG and polypeptide side chains on the PPP backbone was achieved by Suzuki condensation polymerization that provides molecular design flexibility. In this connection, it should be pointed out that Yamamoto coupling of only macromonomers without any spacer units is expected to yield much lower chain growth due to the steric hindrance of the bulky polymer chains. In the applied strategy, hydrophilic and biorelated segments can be incorporated to the PPP structure through the sequence adjustment using a spacer benzene ring. In the Suzuki polymerization, macromonomers and the coupling partner 1,4-benzenediboronic acid in homogeneous suspension of

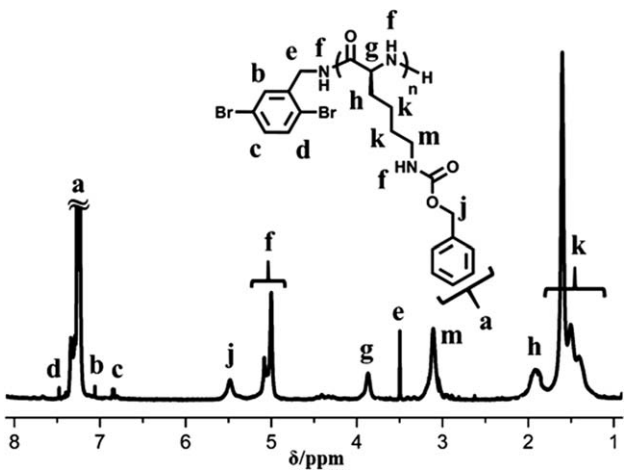
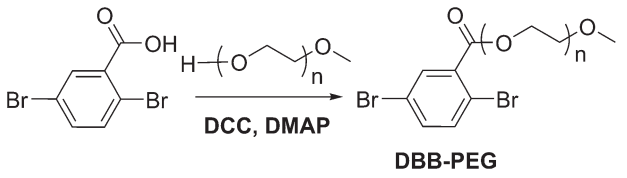
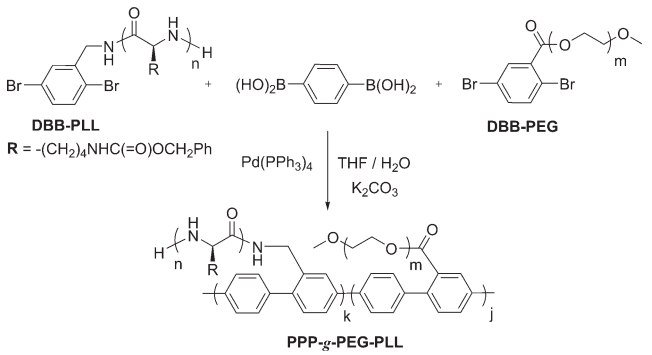


FIGURE 2 ^{1}H-NMR spectrum of polypeptide macromonomer, DBB-PLL.



SCHEME 4 Synthesis of PEG macromonomer via Steglich esterification reaction.



SCHEME 5 Synthesis of PPP polymer bearing poly-L-lysine and PEG side chains.

THF/water with potassium carbonate were reacted in the presence of palladium catalyst (Scheme 5). The polymerization time was deliberately prolonged to 4 days to overcome steric limitations of the macromonomers and obtain graft copolymers with adequate molecular weight. As polypeptides have tendency to form helical structures affecting the solubility properties, the molar ratio of PEG macromonomer to polypeptide macromonomer was deliberately kept low (mol/mol; 6/1) so as to provide sufficient solubility (Supporting Information, Table S1). ^{1}H-NMR spectrum of the resulting graft copolymer, PPP-*g*-PEG-PLL, presented in Figure 3, clearly verifies the presence of both PPP backbone and side chains. Conjugated phenyl protons of PPP backbone appear as broad signals at 8.3 to 7.34 ppm. While phenyl protons of PLL segment are detected at 7.32 and 7.16, the broad signals at 3.74 and 3.42 are attributed to the CH_2 protons of PEG side chain.

Photophysical characteristics of the graft copolymer were also investigated. UV-Vis absorption of spectra of PPP-*g*-PEG-PLL and its precursors, PEG and PLL macromonomers registered in DMF with the same concentration are presented in Figure 4. As can be seen, DBB-PLL macromonomer has relatively stronger absorption than that of the DBB-PEG macromonomer due to the aromatic phenyl groups in the structure.

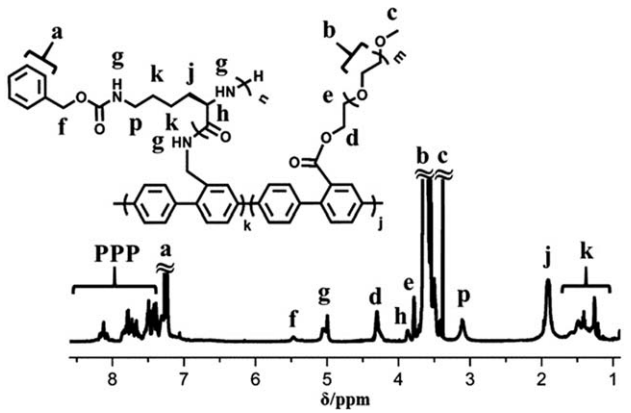


FIGURE 3 ^{1}H-NMR spectrum of PPP-*g*-PEG-PLL in $CDCl_3$.

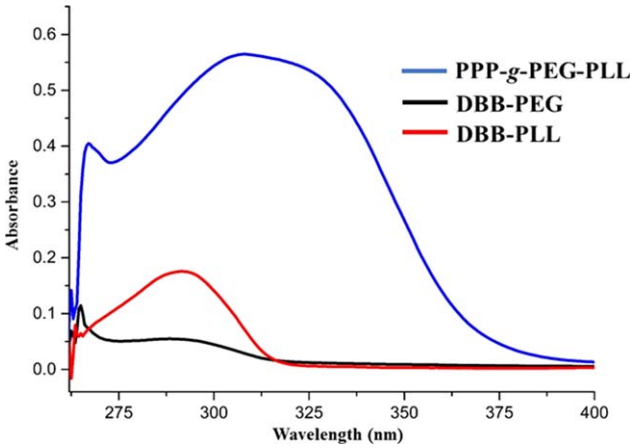


FIGURE 4 UV absorption spectra of DBB-PEG, DBB-PLL, and PPP-*g*-PEG-PLL in DMF solution (0.1 mg m**L**$^{-1}$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Expected strong absorption band of PPP-*g*-PEG-PLL was due to the presence of supplementary conjugated phenylene rings in the main chain.

Figure 5 shows the fluorescence spectra of PPP-*g*-PEG-PLL. The influence of starting macromonomers was very low in the observed spectrum of the graft copolymer, as their independent fluorescence intensities are rather weak and a nearly mirror-image-like relation between absorption and emission spectra was observed. It should also be noted that the fluorescence spectrum has a small shoulder emission at lower wavelength which may arise from the random placement of the macromonomer sequences in the backbone. Moreover, the aggregations that are taking place during the nanoparticles formation through self-assembling of PPP backbones in solution create the red shifted shoulders in asymmetric shape.[77,78] In the second

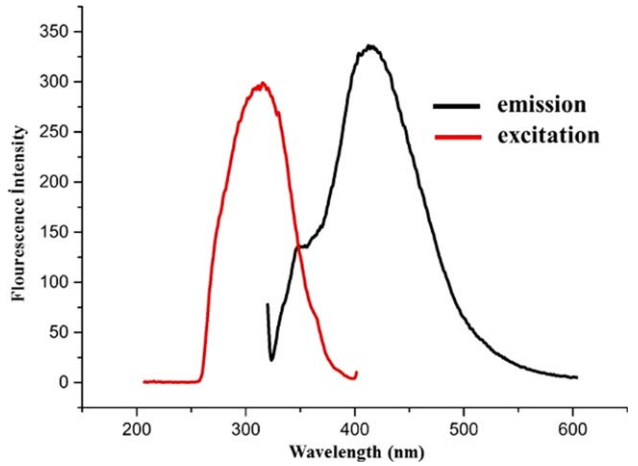
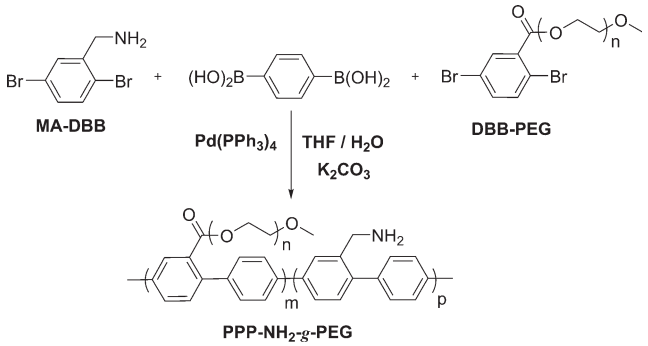


FIGURE 5 Fluorescence excitation and emission spectra of PPP-*g*-PEG-PLL in DMF (1.43 mg **mL**$^{-1}$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



SCHEME 6 The synthesis of PPP-NH_2-*g*-PEG by Suzuki coupling polymerization.

route, the sequence of the processes of the already described strategy was reversed. The usual Suzuki coupling reaction described in the first route using MA-DBB, DBB-PEG, and *p*-benzenediboronic acid under similar experimental conditions yielded PPP with amino groups in the structure (Scheme 6).

In the process, the mole ratio of MA-DBB to DBB-PEG was 3/1 so as to obtain polymers with longer chain length. As the relative amount of DBB-PEG is increased, due to its polymeric nature and steric hindrance, limited chain growth is expected.

The structure of the amino-functional PEG grafted PPP (PPP-NH_2-*g*-PEG) was confirmed by ^{1}H-NMR analysis and Figure 6 exhibits the typical proton signals of PEG and PPP units as well as that of the primary amine at 1.96 ppm. Notably, the latter protons disappear after D_2O proton exchange reaction. Compared with the precursors, PPP-NH_2-*g*-PEG shows strong UV absorbance and fluorescence emission at higher wavelengths due to the extended conjugation arising from the PPP structure (Supporting Information, Figs. S2 and S3) Photophysical properties of the polymers investigated in dilute DMF and water solutions are depicted in Table S2 (Supporting Information).

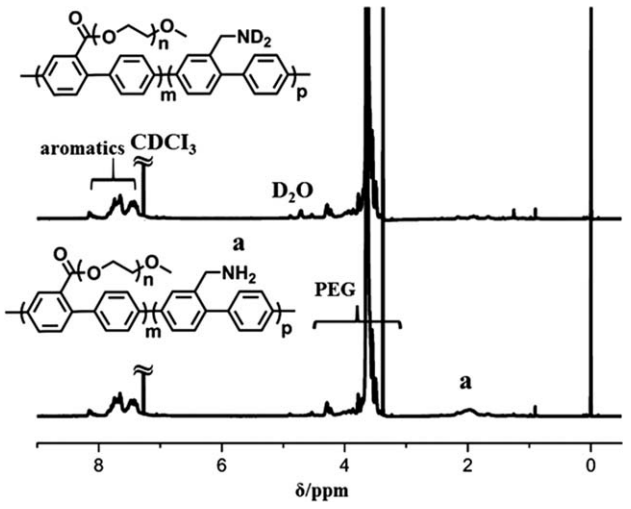
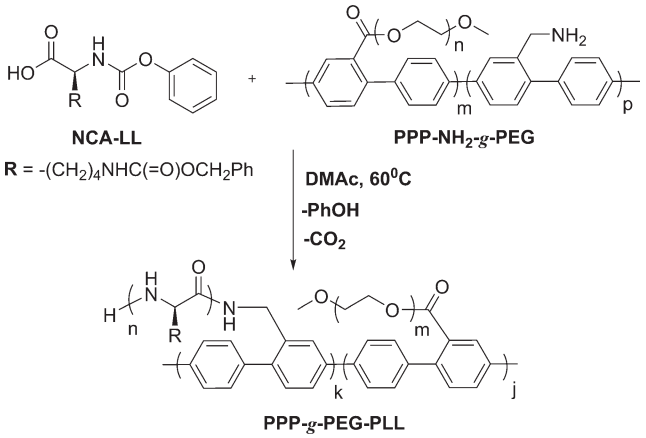


FIGURE 6 ^{1}H-NMR spectra of PPP-NH_2-*g*-PEG before (bottom) and after (top) proton exchange.



SCHEME 7 The synthesis of PPP-*g*-PEG-PLL by NCA ROP of NCA-LL by using PPP-NH_2-*g*-PEG.

In the final step for the synthesis of PPP bearing PEG and PLL side chains, NCA ROP of NCA-LL was conducted through the primary amino groups of PPP-NH_2-*g*-PEG exactly under the same polymerization conditions described in the first route. The overall reaction is depicted in Scheme 7. The ^{1}H-NMR spectrum of the graft copolymer exhibited almost the same spectral characteristics to that obtained by the first route since both methods yield essentially structurally identical polymers.

However, the aromatic protons corresponding to the PPP backbone were broader and more intense (Supplementary Information, Fig. S4). This is expected since the Suzuki coupling reaction was carried out with low molar mass monomer, MA-DBB, facilitates longer PPA chain growth. This was further confirmed by the molecular weight measurements. As can be seen from Table 1, where molecular weight characteristics of the graft copolymers obtained by the two routes and intermediate polymers formed at various stages are compiled.

TABLE 1 Molecular Weight Characteristics of the Graft Copolymers and Precursors

Polymer	M_w[a] (g mol^{-1})	M_w/M_n[a]	DP[a]
DBB-PLL	86,660	2.01	328
DBB-PEG	6,880	1.24	150
PPP-*g*-PEG-PLL (route 1)	140,450	1.85	–
PPP-NH_2-*g*-PEG	34,430	1.41	–
PPP-*g*-PEG-PLL (route 2)	41,810	2.41	–

[a] Weight-average molecular weight (M_w), molecular weight distribution (M_w/M_n), and degree of polymerization (DP) were determined by GPC with light scattering detector according to polystyrene standards.

CONCLUSIONS

In conclusion, in this work we presented a design principle to fabricate conjugated PPP graft copolymers with hydrophilic PEG and polypeptide side chains by the combination of NCA ROP and Suzuki polycondensation processes via two routes. In the first route, the dibromo benzene functional PEG and PLL macromonomer obtained by *in situ* NCA ROP were used in a Suzuki coupling reaction using benzene diboronic acid as the antagonist Suzuki coupling agent. In the other route, the same reactions were conducted in a different sequence. First, PPP with primary amine and PEG side groups was formed by Suzuki reaction. The NCA ROP through the pendant amino groups yielded the desired PPP graft copolymers. The described approach via Suzuki coupling and NCA ROP provides a versatile two-stage method applicable for obtaining conjugated polymers and polypeptides. In principle, it should not matter which route is employed first as both methods essentially yield structurally identical polymers as evidenced by spectral characterization. Highly emissive nature of the backbone and hydrophilic and biocharacter of the side chains make these polymers an interesting class of bio-related emitting materials to be further evaluated in biosensor and functional materials. Such applications were previously demonstrated for electrochemically formed polythiophene derivatives.[30,39] Further studies in this line are now in progress.

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