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Different amine-functionalized poly(diphenylsubstituted acetylenes) from the same precursor†

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A series of poly(diphenyl substituted acetylenes) (PDSAs) were prepared through a post-polymerization modification strategy. Primary amine, tertiary amine and quaternized ammonium functionalities were successfully and efficiently attached onto the skeleton of poly(1,2-diphenylacetylene) *via* the precursor PDSA (P**0**) with activated ester moieties. The structures of the derived amine-functionalized PDSAs, the modification processes and the efficiency of the post-polymerization modification were characterized by multiple spectroscopic techniques. With the aid of the protection and de-protection of *tert*-butyloxyl carbonyl (P**1**), PDSA bearing terminal primary amine groups (P**2**) were obtained. The plentiful amine groups on the side-chains helped in grafting P**2** onto graphene oxide (GO) and the resultant hybrids not only showed a greatly improved dispersing ability in organic solvents, but also emitted strong yellow-greenish fluorescence. The polymer bearing tertiary amine functionalities on side chains (P**3**) could be directly derived from reacting P**0** with 3-*N*,*N*′-dimethyl-1-propylamine under mild conditions and in high yield. P**3** showed evident pH-dependent fluorescence emission behaviour. Based on P**3**, cationic PDSA P**4** was readily obtained by the transition of tertiary amine to quaternized ammonium functionalities. This transition afforded a novel fluorescent and polyelectrolytic PDSA. P**4** was tried as a water soluble fluorescent probe in calf thymus DNA (*ct*-DNA) detection. The experimental data indicated that P**4** at a low concentration of 0.1 ppm can respond to the existence of 10^{-11} g L^{-1} *ct*-DNA in aqueous solution. The working mechanism was associated with the aggregation-induced emission enhancement. The present work, together with our previous reports, suggests that the PDSA with an activated ester precursor can be employed as a broad platform to construct different functional PDSAs.

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Introduction

Polyacetylene is the prototypical conjugated polymer. In its heavily doped state, polyacetylene films show metal conductivity, which coined the era of conducting polymers.[1–3] Due to the very low stability and bad processability, the pristine and doped polyacetylene could not be applied as conducting materials in practice. Consequently, researchers turned their attention to substituted polyacetylenes.[4–20] Replacing one hydrogen atom with appropriate substituents derives mono-substituted acetylene monomers, and the polymerization of the respective monomer affords poly(mono-substituted acetylenes) (PMSAs). Owing to the large size of the substituents, the inter-chain electronic interactions have been greatly weakened and the designed PMSAs demonstrated improved processability. Meanwhile, both the inertness and the size effect of the substituents keep the unsaturated polyene main-chain out of the attack from external reactants, thereby most of the synthesized PMSAs showed better environmental and thermal

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stability in comparison with the pristine polyacetylene. Moreover, with the discovery of Rh-based catalyst systems, a variety of functional groups have been introduced into the designed PMSAs and a branch of functional polyacetylenes was established in the past two decades.[4–6] It is a pity that weakening the inter-chain electronic coupling destroyed the intrinsic electrical conductivity. As a result, PMSAs could not be deemed as good conducting polymers any more. Other functions, including photoconductivity, nonlinear optical properties, photoluminescence, liquid crystal and gas separation properties, have been investigated continuously, as described in a series of reviews.[4–20] Up to now, unfortunately, none of these properties has found practical application in the real materials world.

In recent years, the polyacetylenes derived from the polymerization of disubstituted acetylene monomers (with the two hydrogen atoms on acetylene being substituted), or poly(disubstituted acetylenes) (PDSAs) have drawn much research attention.[21–32] In comparison with PMSAs and pristine polyacetylene, the inter-chain interactions are greatly weakened and the processability of the derived PDSAs is improved substantially, due to the replacement of the two hydrogen atoms on the acetylene monomer with inert phenyl and/or alkyl groups. At the same time, the dual substitution furnishes the synthesized PDSAs with good fluorescence emission properties, as the compensation for losing the electrical conductivity. Because of the steric repulsion between the side chains, the π–π interaction between the polyene main-chains has been greatly prohibited and the fluorescence quantum yield of the poly(diphenyl-substituted acetylene) derivatives is quite high in both solution and solid states. In comparison with other kinds of conjugated polymers, PDSAs have their own uniqueness. The polymerization mechanism of PDSAs is believed to be a metathesis reaction. This is drastically different from the coupling reactions that are widely used in the preparation of other conjugated polymers. The unique polymerization mechanism affords a unique structure and polyacetylenes are the only species in the family of conjugated polymers that possess alternate double–single polymer main-chains. The intrinsic structure allows PDSAs to be a family of fluorescent polymers that can acquire chain chirality from the side chains and meanwhile to be excellent candidates for mimicking the structure of proteins. Therefore, PDSAs are promising opto/electronic materials.

However, the preparation of PDSAs is a challenging task because the catalysts that can be used for the polymerization of the disubstituted acetylene monomers are complexes containing Nb, Mo, Ta, and W chlorides. These compounds are hypersensitive to a trace amount of moisture, polar solvents and polar functionalities including amide, amine, carboxylic, hydroxyl, pyridine and so on.[5,7,15,32] Consequently, so far, only those PDSAs composed of an apolar phenyl, alkyl, silane, low polar activated ester and ether on side-chains are allowable to be obtained by direct polymerization of the corresponding disubstituted acetylene monomers.[7,12,27–39] This reality greatly limits the preparation and application of functional PDSAs. Therefore, in view of macromolecular chemistry, it is a fundamentally important task to find facile routes to functional PDSAs.

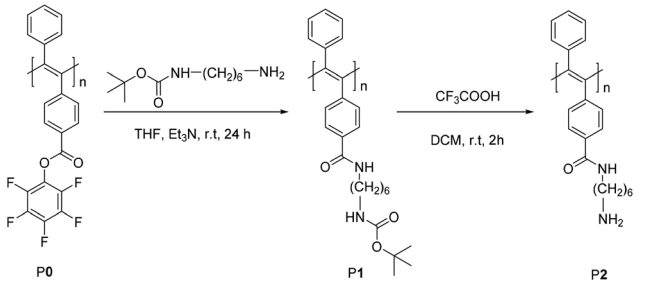
The post-polymerization modification strategy has been proved to be an alternative and efficient synthetic route towards functional PDSAs.[33–39] The basic idea of the post-polymerization modification strategy is to derive functional PDSAs from an appropriate precursor by chemically linking functional groups to the side chains of the precursor PDSA. In other words, the essence of this strategy is to graft a functional group onto the precursor polymer. Due to the characteristics of polymer reaction, high reaction efficiency and mild reaction conditions are concomitantly required. As a result, to date, only limited methods, including activated esters,[37,39] Pd-catalyzed coupling reaction,[33,34] alkyne–azide click reaction,[36] and Michael-type addition reaction,[38] have been adapted to the preparation of PDSA derivatives. In our previous work, the feasibility of the activated ester strategy for the post-polymerization modification of PDSAs was explored,[37,39] but the intended functional design has not been attempted. Rationally, the high reaction efficiency and mild reaction conditions between the activated ester and primary amine suggest that it can serve as an expanded platform for the preparation of functional PDSAs by attaching a primary amine onto the side chains of PDSAs.

In this work, we report our recent progress in the purposeful preparation of amine-functionalized PDSAs through the activated ester strategy. PDSAs with primary amine, tertiary amine and quaternary ammonium functional groups have been successfully derived and their structures were characterized with multiple spectroscopic techniques. Moreover, as highly reactive sites, primary amine groups have been used to react with the carboxyl and epoxy groups on graphene oxide (GO) to prepare hybrids of fluorescent PDSA and GO. The quaternary ammonium groups on the side chains of PDSA allow the derived polymers to be fluorescent and cationic polyelectrolytes, and we attempt to use it as a fluorescent reporter to monitor the interaction with anionic biomacromolecule DNA.

Results and discussion

Synthesis and characterization of primary amine functionalized PDSA

As displayed in Scheme 1, the key step towards the target primary amine functionalized PDSA is to prepare PDSA bearing primary amine groups at the ends of pendents. According to the literature, the first primary amine functionalized PDSA was contributed by Tang and coworkers,[40] in which the primary amine functionality was protected by a phthalimido-moiety and then was deprotected by hydrolyzing in aqueous hydrazine solution at a certain elevated temperature. The yield of the resultant was lower than 90%. In the present work, we changed the protection agent from phthalimide to *tert*-butyloxyl carbonyl (Boc), which is much more commonly used in the protection of primary amine groups in organic



Scheme 1 Synthetic route to the primary-amine functionalized PDSA (**P2**) from the precursor polymer **P0** by using an intermediate polymer (**P1**).

synthesis, because the Boc agent can be removed in high efficiency and under mild conditions. The synthetic route is shown in Scheme 1. The chemical structures of the resultants have been characterized by multiple spectroscopic techniques and the obtained data are in excellent consistency with the expected ones (see the Experimental section and ESI†).

The synthetic and experimental details of the precursor PDSA (**P0**) were described elsewhere.[37] Using a monodisperse polystyrene sample as internal calibration, the average molecular weight (M_w) and the polydispersity index (PDI) were estimated by the GPC technique to be 14.5 kDa and 1.79, respectively. The reaction of the replacement of the pentafluorophenol group by Boc-protected 1,6-hexamethylenediamine took place in very high efficiency. In the ^{19}F NMR spectrum of the precursor polymer (**P0**), three peaks appear at around −153.31, −157.77, and −162.82 ppm (Fig. S1†). After reacting at room temperature for 24 h, there are no resonant peaks in the ^{19}F NMR spectrum of the final polymer (**P2**). The absence of the resonant peaks for the F atom indicates the complete and successful substitution of the pentafluorophenol groups by Boc-protected 1,6-hexamethylenediamine in **P2**.

The successful derivation of **P2** can be further confirmed by comparing the FTIR spectra of **P0** and **P2** (Fig. 1). For **P0**, the absorption bands peaked at 3056 and 1522 cm^{-1} are assigned to the stretching and bending vibrations of the C–H bond on the phenyl groups. The bands at 763 and 687 cm^{-1} are assigned to the finger-print vibration modes of mono- and di-substituted phenyls. These features remain unchanged in the FTIR spectrum of **P2**. The strong and sharp absorption bands at around 1048 and 1013 cm^{-1} originate from the stretching modes of the C–F bonds on the pentafluorophenyl group. These two peaks vanished in the FTIR spectrum of **P2**, which is in good agreement with the ^{19}F NMR spectral information (Fig. S1†) and suggests the efficient substitution of the activated ester with amine groups. For **P2**, a broad band peaked at 3292 cm^{-1} appears in the high wavenumber region, which is assigned to the stretching of the N–H bond, suggesting the presence of amine groups. This vibration is accompanied by the band peaked at 1498 cm^{-1}. These two bands indicate the existence of primary amine groups in the resultant polymer. A group of absorption bands peaked at around 2960, 2926 and

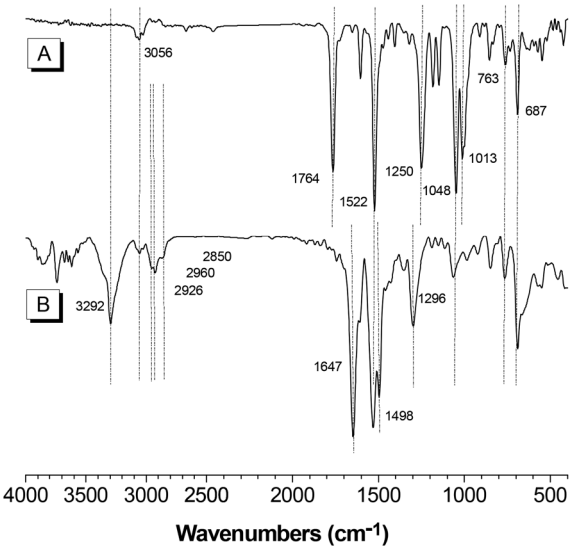


Fig. 1 FTIR spectra of (A) the precursor polymer (**P0**) and (B) the derived primary amine-functionalized PDSA (**P2**).

2850 cm^{-1} in moderate intensity, together with a stronger band peaked at 1290 cm^{-1} are the representative features of methyl groups. Moreover, the strong band peaked at 1647 cm^{-1} is assigned to the stretching vibration of carbonyl in the amide group, which is transformed from the absorption band of carbonyl in the ester group (1764 cm^{-1}, **P0**). These data confirm the efficient transition from the activated ester modified **P0** to the amine functionalized **P2**.

The ^{1}H NMR spectrum provides further evidence for the confirmation of the expected structure of **P2**. As shown in Fig. 2, a weak and broad peak at around 8.18 ppm can be assigned to the proton on the amide group. The resonant peaks centred at 7.20 to 6.30 ppm are assigned to the protons on phenyl groups. Based on the chemical shift and peak area, the peak at around 2.75 ppm is assigned to the protons on the methylene groups directly linking to the N-atoms of the

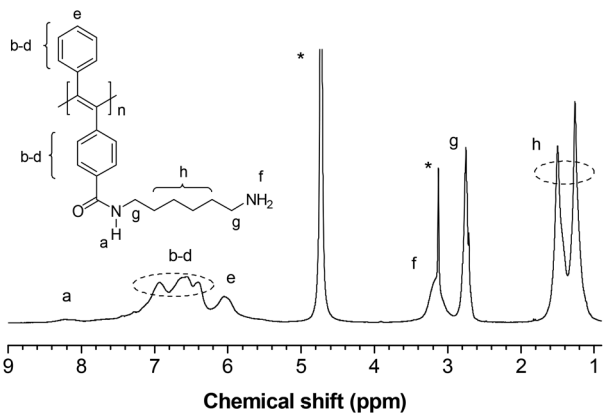


Fig. 2 ^{1}H NMR spectrum of the derived primary amine-functionalized PDSA (**P2**) in methanol-*d*. The peaks for the solvents are marked with asterisks.

1,6-hexanediamine moiety; while the peaks at around 1.50–1.26 ppm are assigned to the protons on other four methylene groups of the 1,6-hexanediamine moiety. A broad resonant feature appearing at around 3.20 ppm can be associated with the protons on the primary amine group. The spectral features in Fig. 1 and 2 validate that the detachment of the Boc protection group is highly efficient; we have obtained the high purity final product P2 by the direct and simple treatment of the intermediate polymer (P1) with trifluoromethyl acetic acid under mild conditions.

Preparation and properties of the PDSA/GO hybrid

A primary amine is a highly active functional group that can react with multiple reagents such as aldehydes, esters, aliphatic/aromatic halides, and so on. Thus the obtained primary amine functionalized PDSA P2 allows to be used as a reactive macromolecular reagent. In previous work, we once used functionalized PDSA to fabricate hybrids of PDSA and multiwalled carbon nanotubes (MWCNTs); the hybrids showed good dispersion ability in common organic solvents. Meanwhile, the hybrids emitted strong fluorescence from the PDSA component.[41] Here, we tried to prepare hybrids of P2 and graphene oxide (GO) with the aid of a reaction between the primary amine groups on P2 and the epoxy and carboxylic groups on GO.

The modification of GO with P2 proceeded smoothly. The pristine hybrid was readily obtained by stirring the reactant mixture of GO and P2 in the presence of *N*-(3-dimethyl-aminopropyl)-*N*′-ethylcarbodiimide hydrochloride and *N*-hydroxysuccinimide in *N*,*N*-dimethylformamide (DMF) for 48 h. After centrifugation and washing with DMF solvent several times till the fluorescence of P2 could not be detected in the supernatant, the hybrid of P2/GO was obtained. The amount of P2 that was grafted onto GO can be estimated by monitoring the relative weight loss on a thermogravimetric analysis (TGA) apparatus. Due to the oxidation, the pristine GO shows relatively low thermal stability and the T_5 (T_5, a temperature at which the sample lost 5% of its original weight) is only about 150 °C. But after releasing the oxygen and hydrogen composition at an elevated temperature (326 °C), the TGA curve becomes flat and the weight loss is small. The primary amine-functionalized PDSA (P2) exhibits a similar beginning decomposition temperature (T_5) at around 152 °C, but the sample shows a continuous weight loss with the increase of temperature, only about 20% of its original weight has been left at 800 °C. In comparison with pristine GO, the hybrid of P2/GO shows an elevated beginning decomposing temperature (178 °C *versus* 152 °C, Fig. 3). Before hybridization, the residual weight of GO at 800 °C is about 48%, but this value increases to 51% after the hybridization. In principle, the residual weight of the hybrid cannot be higher than pristine GO. The apparent increment of thermal stability is tentatively ascribed to the chemical reactions of the amine groups in P2 with the epoxy and carboxylic groups on GO, the cross-linking between the two components can reasonably enhance the thermal resistance property of the hybrid.

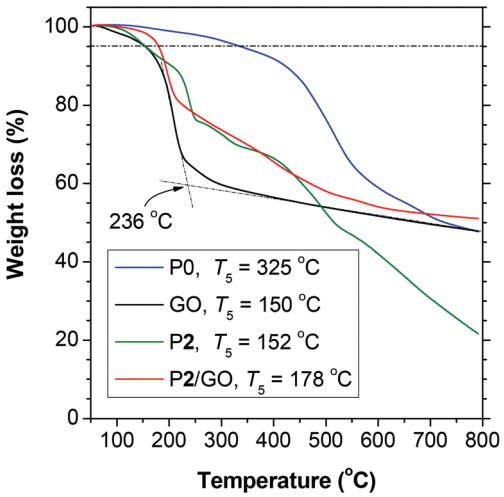


Fig. 3 Thermal gravimetric analysis (TGA) curves of pristine graphene oxide (GO) and polymer-modified GO (P2/GO) obtained under a N_2 atmosphere. Scanning rate: 20 °C min^{-1}.

To verify the successful modification, the FTIR spectra of GO and the obtained hybrid were obtained and compared (Fig. 4A and B). In the spectrum of GO, the broad band ranging from 3500 to 2000 cm^{-1} is assigned to the presence of multiple carboxylic acid and hydroxyl groups, the featureless character indicates the presence of a strong hydrogen bond. The band peaked at 1725 cm^{-1} is assigned to the absorption of the C=O group in the carboxylic acid, and the intense absorption peaked at 1580 cm^{-1} is ascribed to the phenyl-like skeleton in GO. The strong band with double peaks at around 1226 and 1054 cm^{-1} is assigned to the stretching mode of the C–O–C or ether group, and the former originates from the epoxy group. After hybridization, a pronounced peak at around 3400 cm^{-1} emerges in the broad absorption band over

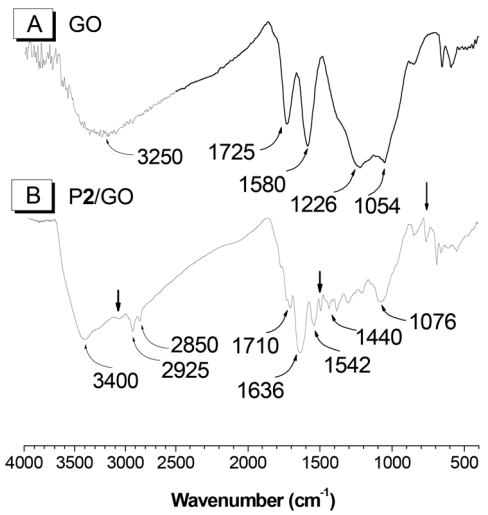


Fig. 4 FTIR spectra of (A) graphene oxide (GO) and (B) its hybrid with the primary amine functionalized PDSA (P2/GO).

3000 cm^{-1}, due to the contribution of the primary amine group. The double peaks at around 2926 and 2850 cm^{-1} are assigned to the anti-symmetric and symmetric vibration modes of the C–H bonds in methylene units. Meanwhile, the bending mode of the methylene group appears at around 1440 cm^{-1}. The strongest absorption peak at 1636 cm^{-1} coupling with a shoulder peak at around 1710 cm^{-1} is the characteristic feature of the primary amide group, indicating the formation of amide groups in the hybridization process. The absorption of the aromatic skeleton of GO shifts to about 1542 cm^{-1}. The absorption intensity of the epoxy group becomes much weaker after the reaction, suggesting that the epoxy groups are largely exhausted. The hybridization of P2 and GO grafts PDSA chains onto the surface of GO sheets; as a result, the absorption bands (3060 cm^{-1}, stretching mode for the C–H bond in a phenyl; 1500 cm^{-1}, phenyl skeleton; 765 cm^{-1}, mono-substituted phenyl group) on P2 also show up in the spectrum (Fig. 4B), as denoted by the vertical arrows.

Generally, hybrid materials possess the synthetic properties of their components. Chemically linking P2 chains onto GO provided the hybrid with good dispersion properties. In DMF solvent, the hybrid is stable and the precipitate was formed after keeping it still for months. After precipitation and drying treatment, the powder sample could be re-dispersed in DMF solvent. More importantly, as a PDSA derivative, P2 is a fluorescent polymer. Thus the P2/GO hybrid is endowed with fluorescence emitting properties. As shown by the spectral lines in Fig. 5, in solution and solid film, P2 emits greenish fluorescence with the emission maximum at 490 and 501 nm. After hybridization, the emission spectrum displays an evident red-shift. Yellow fluorescence has been observed and the emission maximum for the solution and solid film of the P2/GO hybrid appears at 528 and 545 nm, respectively (see the photographs in Fig. S3†). Since GO itself is non-emissive (Fig. S4†), the fluorescence recorded for the hybrid must originate from the P2 component. But the evident emission red-shift (38 and 44 nm for the solution and solid film, respectively) has not been observed in other hybrids containing the PDSA component.[40–45] We tentatively associate the red-shift emission with the hybridization-induced coplanarity of the polyene main-chain. Due to the steric repulsion between the adjacent phenyl groups, the PDSA main-chain and the phenyl groups directly linked to the main-chain are non-planar, and the whole polymer takes a random coil conformation in solution. After hybridization with GO, the polymer chains chemically bonded to GO's surface become partially unwound thereby the skeleton becomes coplanarity. As a result, the effective conjugation length extends and the emission exhibits a red-shift. Based on the data demonstrated by Fig. 4 and 5, we realized that the chemical modification of GO with P2 not only furnishes plentiful primary amine groups to the hybrid but also makes the hybrid moderately fluorescent.

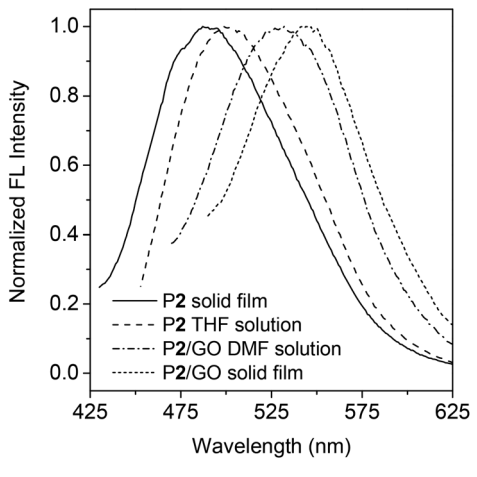
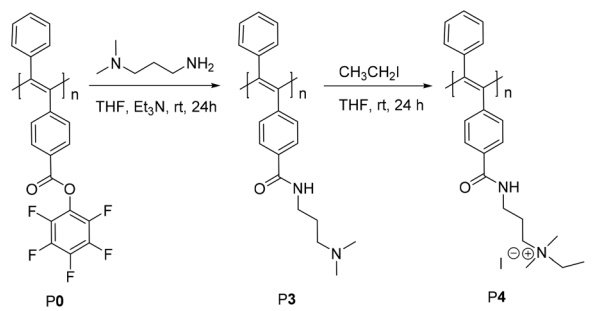


Fig. 5 Normalized fluorescence spectra of P2 and the hybrid of P2/GO in different states. Solvents: tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF). Excitation wavelength: 460 nm for the P2/GO solid film and 400 nm for other three samples.

Synthesis of tertiary amine functionalized PDSA (P3) and its pH sensitive emission property

The synthetic route to the tertiary amine functionalized PDSA (P3) is illustrated in Scheme 2. The replacement of pentafluorophenol groups by 3-(dimethyl-amino)-1-propylamine occurred smoothly at room temperature with stirring for 24 h. Using mono-dispersed polystyrene as the standard, the average molecular weight (M_w) and polydispersity index (PDI) of P3 were measured to be 11 900 and 1.52, respectively. The structure characterization data are displayed in Fig. 6 and 7 (also see the Experimental section and ESI†). In Fig. 6, the assignment of the absorption bands is similar to that discussed in the case of P0 and P2. Here we broadly categorize them into four types: red band for N–H stretching in the amide group, orange bands for C–H in unsaturated carbons (aromatic such as phenyl and ethenyl), green band for C–H in saturated alkyl groups, and blue band for carbonyl (C═O) in the amide group. The appearance of blue and green bands suggests the successful attachment of alkyl groups to P0 and the formation of the carbonyl (C═O) bond in the amide group. Meanwhile, the disappearance of the absorption band for the C–F bond suggests the successful substitution of the activated ester groups.

The ^{1}H NMR spectrum of P3 is shown in Fig. 7A, the resonant peaks for different protons on P3 are rationally recognized.



Scheme 2 Synthetic route to tertiary amine functionalized PDSA (P3) and quaternary ammonium functionalized PDSA (P4) from precursor P0.

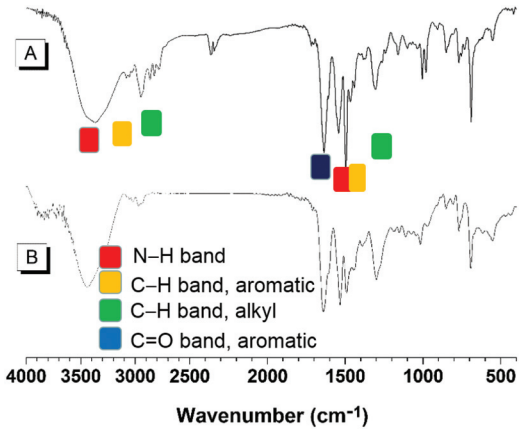


Fig. 6 FTIR spectra of the tertiary-amine functionalized PDSA (P3, A) and the derived quaternary ammonium functionalized PDSA (P4, B).

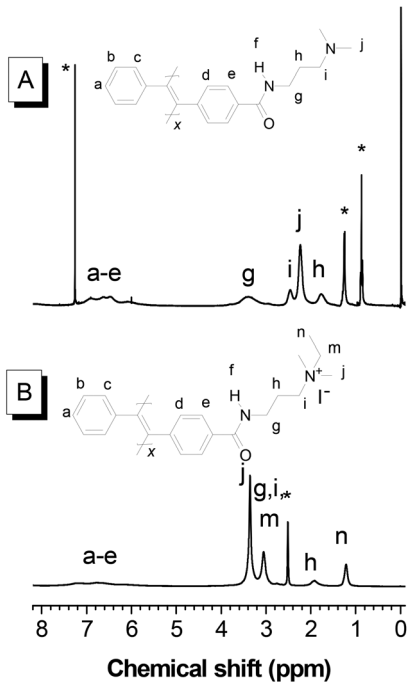


Fig. 7 ^{1}H NMR spectra of the tertiary amine functionalized PDSA (P3, A) and its derivative quaternary ammonium functionalized PDSA (P4, B). The solvent peaks are marked with asterisks.

The resonance peaks appearing in the region of δ = 7.20–6.30 ppm are assigned to the protons on the phenyl groups of P3. The chemical shifts appearing in a higher field (δ < 5 ppm) are assigned to the protons on the pendents of polymer chains. For example, the resonant peaks at 2.46 and 2.23 ppm are assigned to protons for H^i and H^j, respectively. These spectral features indicate that the expected P3 has been derived.

Since a tertiary amine is a typical organic base, P3 is expected to be a basic polymer and to show pH sensitivity. We examined this idea in a mixture solution of THF/Britton–Robinson buffer with a volume ratio of 1 : 9. The data recorded in a typical experiment are shown in Fig. 8A and B. The relative fluorescence intensity (I/I_0) fluctuates around 1.00 to 1.12 when the pH value of the mixture changes from 2.0 to 7.0, suggesting that the pH value ranging from acidic to neutral has little influence on the fluorescence emission of P3. When the pH value further increases from 7.0 to 12.0, the fluorescence intensity increases quickly and levels off at a pH value of 10.0, suggesting that the fluorescence emission of P3 was evidently influenced by the pH value under basic conditions.

This pH-dependent fluorescence emission behaviour can be explained as follows. PDSAs are a unique kind of fluorescent polymer that show aggregation-enhanced emission (AEE) properties under appropriate conditions.[22,46] AEE is referred to as a fluorescence emission behaviour in which the emission intensity increases when polymer chains are rigidified due to the collapse of the random coil or other factors which can cause the pronounced reduction of conformational changes. The mixture solution of THF and Britton–Robinson buffer (1 : 9, by volume) is an aqueous solution due to the low fraction of THF. Under acidic conditions, most of the tertiary amine groups are protonated. Under neutral conditions, part of the tertiary amine groups are protonated because of the hydrolysis effect of the tertiary amine in aqueous media. The protonation of the side groups in P3 endows the polymer with appropriate solubility in the mixture solution of THF and Britton–Robinson buffer, thereby the polymer chains take a conformation of loose and random coils. As a result, P3 shows normal fluorescence emission in this pH range.

In a basic environment, a de-protonation process occurs and the tertiary groups recover to the neutral form, thereby the polymer has a decreased solubility in basic solution in comparison with that in an acid environment. The reduction of solubility was confirmed by the experimental observation at a high pH value of 12, where a little polymer precipitated in the mixture solution and caused a decrease in fluorescence intensity (Fig. 8B). Under basic conditions, the loose and random coils of the polymer chains tend to collapse, which leads to

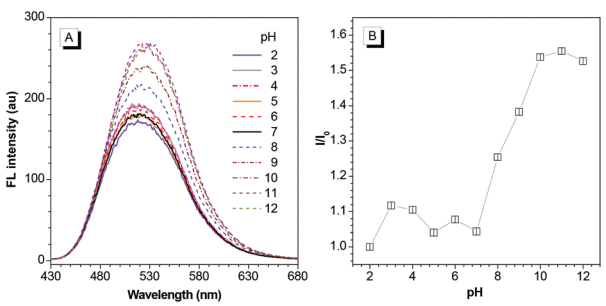


Fig. 8 (A) Fluorescence (FL) spectra of P3 in THF/Britton–Robinson buffer mixture solution (1 : 9 by volume) with different pH values. (B) Plot of relative peak FL intensity (I/I_0) of P3 *versus* pH value. The data are extracted from (A). I and I_0 are the FL intensity recorded at pH = 2.0 and another pH value, respectively. P3 concentration: 10^{-5} M, λ_{ex} = 390 nm.

evident reduction of conformational changes. Accordingly, enhanced fluorescence emission has been recorded as shown in Fig. 8. The plot in Fig. 8B is identical to a typical titration curve. It implies that PDSAs bearing basic moieties are candidates for polymeric fluorescent indicators of acid–base titration.

Synthesis of quaternary ammonium functionalized PDSA (P4) and its application in fluorescent detection of DNA

Recently, water soluble and fluorescent conjugated polymers have found versatile applications in biotechnologies. One of the promising properties of PDSAs is their efficient fluorescence emission, which allows water soluble PDSAs to be used as the active element of biosensors. Accordingly, it is conceived to be potentially useful to prepare water soluble PDSAs. To the best of our knowledge, water soluble PDSAs have been scarcely reported. The available examples are limited to primary amine functionalized PDSAs, which are dissolved in concentrated acid solution and used as a photo-active component for constructing $PbBr_2$/PDSA hybrid perovskites.[40–44] The amino acid modified PDSAs showed poor water solubility and their application in biosensors has not been reported.[37] The PDSAs dissolving in neutral aqueous solution are more biocompatible and can find more places to use in comparison with the PDSAs dissolving in acidic/basic aqueous solutions. Therefore, we tried the synthetic route of post-polymerization modification.

As shown in Scheme 2, the cationic species were realized by the reaction of a tertiary amine with iodoethane to form quaternary ammonium salt. Using mono-dispersed polystyrene as a standard, the relative molecular weight and polydispersity index of **P4** were estimated to be 27 800 and 1.97, respectively. The large difference in molecular weight between **P4** and **P3** was mainly due to the difference between the solvents used in molecular weight measurement.

The formation of functionalized PDSAs bearing quaternary ammonium salt groups (**P4**) was readily confirmed from the FTIR and ^{1}H NMR spectra of the resultants (Fig. 6 and 7). The FTIR spectra shown in Fig. 6 offer the information about the transformation of **P3** to **P4**. The co-existence of the blue, green, orange and red bands and similar spectral features in spectra A and B indicate the similarity in chemical structures between **P3** and **P4**. A subtle difference in the C–H bending modes for **P3** and **P4** can be observed in the green spectral region. The absorption bands of the C–H bending mode incidentally show up at around 1460 and 1385 cm^{-1} (Fig. 6B), which are absent in Fig. 6A. In Fig. 7A and B, the resonant peaks for different protons on **P3** and **P4** are rationally assigned. The resonance peaks appearing in a lower field (>6.30 ppm), which are assigned to the protons on the phenyl groups of the PDSAs, have variations. In a higher field (δ < 5 ppm) region, the resonant peaks at 2.46 (protons for H^i) and 2.23 ppm (protons for H^j) in Fig. 7A shift to 2.82 and 3.39 ppm in Fig. 7B, respectively, which resulted from the enhanced electronic induction-effect of the quaternized N-atom. Meanwhile, new resonant peaks appear at 4.61 ppm (protons for H^k) and 3.80 ppm (H^l) as shown in Fig. 7B. All these signal changes indicate the successful quaternization of the tertiary amine groups.

Cationic fluorescent polymers have been used for the detection of anionic bio-macromolecules such as DNA and heparins.[27–29,44,45] It is rational to assume that emission enhancement is expected upon the interaction between the cationic PDSA and anionic DNA, which can induce the rigidification of the PDSA chains and pronounced reduction of conformational changes. Here, we monitored the fluorescence response of **P4** in aqueous solution to calf thymus DNA (*ct*-DNA) and the experimental data are demonstrated in Fig. 9A and B.

The data in Fig. 9 reveal the following characteristics. Firstly, the emission intensity of **P4** enhances continuously with the increasing *ct*-DNA concentration in the range of 0 to 10 μg mL^{-1}, or 1.0 × 10^{-2} g L^{-1}. This phenomenon is in good agreement with the expectation of AEE. The enhancement of the yellowish-green fluorescence can be observed directly by the naked eye when the mixtures were illuminated with the UV-light used for thin-layer chromatography. Secondly, the fluorescence response is highly sensitive. As a low concentration of **P4** at 1.0 × 10^{-7} mol L^{-1} (about 3.0 mg L^{-1} or 3.0 μg mL^{-1}), a clear fluorescent response can be recorded for *ct*-DNA at a concentration 1.0 × 10^{-11} g L^{-1} or 1.0 × 10^{-5} μg mL^{-1}. The data shown in Fig. 9B also reveal that the *ct*-DNA-concentration dependent fluorescence response curve can be largely divided into two sections. When the *ct*-DNA concentration is in the range of 0–2 μg mL^{-1}, the emission intensity enhances quickly with the increase of *ct*-DNA concentration. When the *ct*-DNA concentration is higher than 0.2 μg mL^{-1}, the enhancement slows down and gradually levels off. This observation is associated with the "saturation effect" of the binding sites between *ct*-DNA and the polymer chain of **P4** when the *ct*-DNA concentration reaches to a certain value. When the DNA concentration is lower than 0.2 μg mL^{-1}, a Stern–Volmer detection curve can be drawn as shown in the inset of Fig. 9B and the SV curve is nearly a straight line (R^2 = 0.983). Linear simulation gives a K_{sv} of 9.506 (μg mL^{-1})$^{-1}$. All these results indicate that **P4** is a highly sensitive fluorescent probe for *ct*-DNA detection.

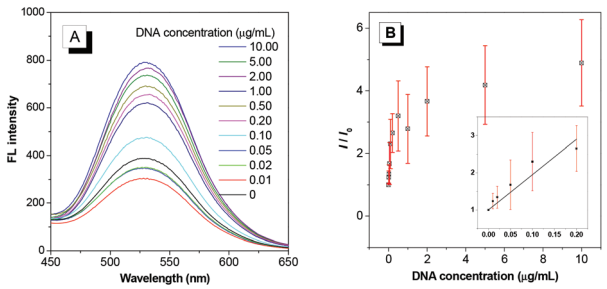


Fig. 9 (A) Fluorescence (FL) responses of **P4** to *ct*-DNA in aqueous solution. (B) Change in FL intensity with DNA concentration (μg mL^{-1}). The inset of B shows the Stern–Volmer curve in the DNA concentration range of 0–0.2 μg mL^{-1}. The error bars are based on 8 parallel experiments.

Conclusions

In summary, we have demonstrated the feasibility of the synthetic routes to functional PDSAs bearing different amines on side chains (**P1**, **P2**, **P3** and **P4**) from the same precursor polymer (**P0**). The transition from **P0** to amine functionalized PDSAs took place under mild reaction conditions and had high efficiency. The resultant polymers have been characterized with multiple spectroscopic techniques, such as FTIR, ^{1}H NMR, and ^{19}F NMR. The characterization data, on one hand, indicated the expected polymer structure, and on the other hand, suggested that the substitution of PDSA-based activated esters with amines occurred quantitatively. Specifically, the primary amine functionalized PDSA (**P2**) has been used to hybridize with graphene oxide (GO) and the obtained hybrid has shown that the polymer modification greatly enhanced the dispersion ability of GO in organic solvents. Meanwhile, PDSA has afforded the **P2**/GO hybrid with strong fluorescence emission from the PDSA component. Using **P0** as the precursor, tertiary amine functionalized PDSA (**P3**) can be readily derived by reacting **P0** with 3-(dimethyl-amino)-1-propylamine. **P3** has been used as the subsequent precursor to prepare quaternary functionalized PDSA (**P4**). The cationic polymer **P4** emits yellowish-green fluorescence and shows moderate water solubility. These properties allow it to be used as a polymeric fluorescent probe in an aqueous environment. Taking these advantages into consideration, we have examined the fluorescent response of **P4** to the addition of DNA into buffer solution, the **P4** concentration can be as low as 3.0 μg mL^{-1} and the detection limit for DNA is 1.0×10^{-5} μg mL^{-1}, which is an ultra-high sensitivity. As evidenced by the experimental results, the poly(diphenylsubstituted acetylene) bearing activated ester side groups serve as a platform-like precursor for the derivation of different amines (including primary, tertiary and quaternary). Considering that amines are one of the most abundant functionalities in organic and macromolecular chemistry, it is believed that a variety of functional PDSA-based polymers and materials can be produced on this platform.

Experimental section

Materials

Dichloromethane (DCM) was distilled under normal pressure over calcium hydride under nitrogen before use. Toluene was distilled under normal pressure from sodium benzophenone ketyl under nitrogen immediately prior to use. Triethylamine (Et_3N) was distilled and dried over potassium hydroxide. *N*,*N*-Dimethylformamide (DMF) was extra-dry grade. Other solvents like tetrahydrofuran (THF), acetone, methanol, chloroform and Britton–Robinson buffer were purchased from Sinopharm Chemical Reagent Co., Ltd and directly used without any treatment. *p*-Toluene sulfonic acid monohydrate (TsOH), *N*,*N*′-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP) and *ct*-DNA were purchased from Acros. Pentafluorophenol and WCl_6 were purchased from Aldrich. *N*-Boc-1,6-hexanediamine, trifluoroacetic acid (TFA), Ph_4Sn, and *N*-hydroxysuccinimide (NHS) were purchased from Aladdin. Iodoethane, *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDC·HCl) and 3-(dimethyl-amino)-1-propylamine were purchased from J&K. All the chemicals were used as received without any further purification.

Instrumentations

^{1}H and ^{19}F NMR spectra were recorded on a Bruker ARX 500 NMR spectrometer or Bruker ARX 400 in chloroform-d using tetramethylsilane (TMS; δ = 0 ppm) as an internal standard or in methanol-*d* and DMSO-d_6. FTIR spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer by using thin films on KBr pellets. UV-vis absorption spectra were recorded on a Varian CARY 100 Bio UV-vis spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer. Molecular weights (M_w and M_n) and polydispersity indexes (PDI, M_w/M_n) of the polymers were estimated in a suitable solvent by using a Waters PL-GPC-50 gel permeation chromatography (GPC) system equipped with a refractive index (RI) detector. A set of monodisperse polystyrene standards covering the molecular weight range of 10^3–10^7 was used for molecular weight calibration. Thermogravimetric analysis (TGA) was conducted on a Pyris 6 thermogravimetric analyzer (Perkin-Elmer) under a nitrogen atmosphere at a heating rate of 20 °C min^{-1}. The centrifugation process was carried out on the Siemensstr 25 D-78564 Z-326 type centrifugal machine.

Polymer synthesis

Polymer preparation. The precursor polymer **P0** was prepared according to the Experimental sections in our previous paper.[37] GPC data for **P0**: M_w = 14 500, PDI = 1.90. The post-modified polymers were synthesized as shown in Schemes 1 and 2. The detailed experimental procedures for the post-modification process are given below.

Preparation of P2. In a Schlenk tube were added **P0** (38.9 mg, 0.1 mmol) and *N*-Boc-1,6-hexanediamine (21.7 mg, 0.1 mmol). Then 5 mL THF and several drops of trimethylamine (TEA) were added as the solvent and catalyst. The mixture was stirred at room temperature for 24 hours. After that, the yellow polymer **P1** was precipitated in a hexane/$CHCl_3$ mixture (5 : 1 by volume, 120 mL totally) through a cotton filter under stirring and obtained with a high yield up to 95% after drying to constant weight. ^{1}H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm): 7.8–5.8 (protons on the phenyl ring), 4.7, 3.4, 3.1 (methylene proton next to the amido group), 1.8–1.2 (alkyl chain). Without any treatment, the polymer was continuously dissolved in dichloromethane (5 mL), and then about 3 ml trifluoroacetic acid was slowly added into the Schlenk tube. After 2 hours reaction at room temperature, **P2** was precipitated with the same mixture solvent. Finally, the viscous yellow **P2** was obtained with a yield of nearly 100%. (Due to the viscosity, the solvent can hardly be removed totally, leading to a higher yield.) ^{1}H NMR (400 MHz, methanol-*d*, ppm): 8.18 (1H, proton on the amido group), 7.2–6.0 (9H, phenyl part in the main chain), 3.2

(2H, amine group), 2.75 (2H, proton on the alkyl chain near the amido group), 1.5–1.26 (10 H, protons on the alkyl chain).

Preparation of P2/GO hybrid. In a two-necked round-bottom flask were added graphene oxide (GO, 50 mg), EDC·HCl (100 mg, 0.52 mmol), and NHS (100 mg, 0.87 mmol). The system was vacuumed and flushed with nitrogen 3 times. Then 12 mL dried DMF was injected into the flask and the mixture was aged for 3 h. P**2** (16 mg, 0.05 mmol) was dissolved in 3 mL dried DMF and was injected into the reaction mixture. The mixture was stirred at room temperature overnight. The obtained mixture was washed and centrifuged several times with DMF solvent until there was no obvious yellowish colour emission from the solution. That is to say, the polymer P**2** that was not reacted with GO had been removed completely. After centrifugation, the P2/GO hybrid was obtained with yellow fluorescence under a UV lamp. FTIR spectrum (thin film), ν (cm^{-1}): 3400, 2925, 2850, 1710, 1636, 1542, 1440, 1076, 765.

Preparation of P3. In a Schlenk tube were added P**0** (77.6 mg, 0.2 mmol), 3-(dimethyl-amino)-1-propylamine (20.4 mg, 0.2 mmol), 5 mL THF and several drops of Et_3N and the mixture was stirred at room temperature for 24 hours. After precipitation in hexane/chloroform solvent, 60 mg yellow polymer was obtained with a yield of 97.9%. ^1H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 8.2, 7.4–6.3, 6.1, 4.0, 3.45, 2.6–2.0, 1.9–1.6. FTIR, ν (cm^{-1}, KBr pellet): 3395, 2925, 2850, 1640, 1544, 1452, 1304, 1069, 953, 844, 752, 693. M_w = 11 900, PDI = 1.52.

Preparation of P4. In a Schlenk tube were added P**3** (30.6 mg, 0.1 mmol) and iodoethane (0.1 mmol, 15.6 mg), 3 mL THF was added as the solvent, and the mixture was stirred at room temperature for 12 hours and a yellow polymer precipitated in the tube. After filtration and washing with ethyl ether, the polymer was dried to a constant weight and the yield is 95.1%. ^1H NMR (400 MHz, DMSO-d_6): 7.3–6.1 (phenyl proton in the main chain), 3.37, 3.05, 1.91 (alkyl chain near the nitrogen atom), 1.23 (methyl group). FTIR, ν (cm^{-1}, KBr pellet): 3445, 2960, 2850, 1640, 1532, 1490, 1300, 1018, 768, 691. M_w = 27 800, PDI = 1.97.

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