Synthesis of Cationic Diacetylene-*co*-Carbazole-*co*-Fluorene Polymers and Their Sensitive Fluorescent Quenching Properties with DNA

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Received 6 May 2010; accepted 22 June 2010 DOI: 10.1002/pola.24197 Published online in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Two neutral precursor conjugated copolymers based 2,7-diethynylfluorene and 3,6-diethynylcarbazole units in the main chain (PFC and PF2C) were prepared by Hay coupling polymerization. Their cationic copolymers (CPFC and CPF2C) were prepared by the methylation of their diethylpropylamino groups with CH₃I. For comparison, neutral conjugated homopolymers of 2,7-diethynylfluorene (PF), 3,6-diethynylcarbazole units (PC) and their cationic polymers (CPF and CPC) were also prepared with the same method. A comparative study on the optical properties of cationic polymers CPFC and CPF2C in DMF and DMF/H₂O showed that they underwent water-induced aggregation. The spectral behaviors of **CPFC** and **CPF2C** with calf thymus DNA showed that a distinct fluorescent quenching took place with minute addition of CT DNA (3.3×10^{-13} M). The results showed that the polymers would be promising biosensor materials for sensitive detection of DNA. © 2010 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 48: 4168–4177, 2010

KEYWORDS: aggregation; binding interaction; biological applications of polymers; cationic conjugated polymers; conducting polymers; CT DNA; fluorescence; photophysics; UV-Vis spectroscopy

INTRODUCTION Conjugated polymers (CPs) offer several advantages as the response basis for chemical and biological detection based on optical methods. CPs may be viewed as a collection of short, conjugated units kept in close proximity by virtue of the polymer backbone. Modification of CPs with anionic or cationic functional groups yields materials that possess the properties of conjugated polymers and water solubility which is essential for interaction with biological substrates¹ such as proteins² and DNA.³ Cationic conjugated polymers (CCPs), in particular, have been proven to be very useful for DNA sequence detection based on electrostatic interaction with the negatively charged phosphate backbone.⁴ CCPs reported previously for DNA detection were either polythiophene derivatives⁵ or poly(fluorene-*co*-phenylene) derivatives.⁶

Carbazole is a conjugated unit that has interesting optical and electronic properties such as photoconductivity and photorefractivity.⁷ Polymers containing carbazole moieties in the main chain or side chain have been widely studied because of their unique properties which allow them to become various photoelectronic materials including photoconductive, electroluminescent, and photorefractive materials.⁸ Meanwhile, various semicrystalline and amorphous polymers containing diacetylene moiety in the backbone have been reported, including diacetylene polycarbonate,⁹ diacetylene polyurethanes,¹⁰ diacetylene polyamides,¹¹ diacetylene polyesters¹² and other examples.¹³ However, to our knowledge, no efforts have been made to the development of cationic conjugated polymers based on diacetylene in the main chain for electronic and optical applications in DNA-biosensors.

In this article, novel conjugated copolymers PFxCy (PF1C and PF2C) containing diacetylene, carbazole and fluorene moieties in the main chain with tertiary amines in the side chains were synthesized as precursor polymers with different ratios of fluorene and carbazole units by oxidative coupling of acetylene groups under oxygen atmosphere with CuCl as catalyst.¹⁴ Addition of CH₃I to **PFxCy** gave the cationic polymers CPFxCy (Scheme 1). CPFC and CPF2C provided electrostatic interactions with the negatively charged analytes,15 such as DNA. The interaction between calf thymus DNA (CT DNA) and CPFC or CPF2C by means of absorption and emission spectroscopy was preliminarily investigated. The disturbance by the buffer solution was also studied. For comparison, neutral conjugated homopolymer (**PF**) of 2,7-diethynylfluorene and homopolymer (**PC**) of 3,6-diethynylcarbazole, as well as their cationic polymers (CPF and CPC) were also prepared and characterized (Scheme 1).

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Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 48, 4168-4177 (2010) © 2010 Wiley Periodicals, Inc.



SCHEME 1 Synthetic routine of polymers **PFxCy** and **CPFxCy**.

RESULTS AND DISCUSSION

Synthesis and Characterization

2,7-Diethynyl-9,9-bis[6'-(N,N-diethylamino)-hexyl]fluorene (1) was synthesized by a modified routine (Scheme 2). According to the synthetic routine in the literature,¹⁶ 9,9-bis(6-bromohexyl)-2,7-diethynyl-9*H*-fluorene was the intermediate which was prepared from 2,7-diethynyl-9H-fluorene. However, its yield was low because C-9 position of 2,7-diethynyl-9H-fluorene was easily oxidized under the strong base condition and gave 2,7-diethynyl-fluorenone as the main product. In our synthetic routine, the introduction of bromohexyl at C-9 position of fluorene was performed from 2,7-diiodo-9Hfluorene 4 with high yield (71%) (Scheme 2). The classical Sonogashira coupling reaction between 2,7-diiodo-9,9-bis(6'bromohexyl)-9H-fluorene 5 and 2-methyl-3-butyn-2-ol 6 with Pd(PPh₃)₂Cl₂ and CuI as catalysts gave 2,7-bis(2'hydroxy-2'-methylbutynyl)-9,9-bis(6'-bromohexyl)-fluorene 7 in excellent yield. Finally, monomer 1 was obtained by reaction of 7 with diethylamine following by treatment with KOH in 57% yield for two steps.

3,6-Diethynyl-N-[6'-(*N*,*N*-diethylamino)-hexyl]carbazole (2) was prepared with carbazole as starting material by using the same procedure as for the synthesis of **1** (Scheme 3). The IR spectrum of monomer **2** showed the characteristic absorption of the acetylene at 3307 cm⁻¹ for C=C–H and at 2107 cm⁻¹ for C=C. Additional structural proof was obtained from ¹H NMR and ¹³C NMR spectra. ¹H NMR showed ethynyl proton as a singlet at 3.06 ppm. Two characteristic peaks for acetylenic carbons were found at 77.8 and 85.6 ppm in ¹³C NMR spectrum.

Oxidative coupling of acetylene groups of monomer **1** and **2** with two different feed ratios under oxygen atmosphere using CuCl in 1,2-dichlorsobenzene gave the neutral precursor polymers **PFxCy** in 51–82% yields. The molar ratios of 2,7-diethynylfluorene unit to 3,6-diethynylcarbazole unit in two copolymers (**PFC** and **PF2C**) were determined by ¹H NMR spectra from the ratio of the protons of **PFC** at 0.58 ppm corresponding to monomer **1** (0.48–0.56 ppm, 4H, CH₂) and the protons of **PFC** at 4.27 ppm corresponding to monomer **2** (4.23 ppm, 2H, *N*–CH₂), as shown in Figure 1.

 $\begin{array}{l} \label{eq:scheme_2} \mbox{SCHEME 2 Synthetic routine of monomer 1.} \\ \mbox{Reagents and conditions: (i) } I_2, \ KIO_3, \ AcOH, \\ \ H_2SO_4, \ H_2O, \ 80\ ^{\circ}C, \ 10\ h, \ 79\%; \ (ii) \ BrC_6H_{12}Br, \\ \ KOH, \ H_2O, \ Bu_4NBr, \ DMSO, \ N_2, \ rt, \ 24\ h, \ 71\%; \\ \ (iii) \ (PPh_3)_2PdCI_2, \ 2\mbox{-methyl-3-butyn-2-ol}\ {\bf 6}, \ Cul, \\ \ Et_3N, \ THF, \ N_2, \ rt, \ 12\ h, \ 92\%; \ (iv) \ HNEt_2, \ reflux, \\ \ 12\ h, \ 73\%; \ (v) \ KOH, \ toluene-EtOH, \ N_2, \ 120\ ^{\circ}C, \\ \ 3\ h, \ 78\%. \end{array}$



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SCHEME 3 Synthetic routine of monomer 2.

With the calculation, the average ratios of 2,7-diethynylfluorene to 3,6-diethynylcarbazole group were estimated to be about 0.99:1 and 2.02:1 for **PFC** and **PF2C**, respectively. For comparison, the homopolymers of 2,7-diethynylfluorene (**PF**) and 3,6-diethynylcarbazole (**PC**) were also prepared with the same method. Molecular weights and polydispersity indexes are listed in Table 1. M_n and M_w ranged from 5201 to 26118 (M_n) and from 5331 to 41326 (M_w), respectively, with polydispersity indices (PDI) ranging from 1.03 to 1.64. The neutral precursor polymer **PFxCy** were isolated as yellow powder and displayed excellent solubility in chlorinated hydrocarbons, DMF, *N*-methylpyrrolidinone, *N*,*N*-dimethylacetamide, and tetrahydrofuran (THF), but insoluble in methanol and hexane.

The ¹H NMR and IR spectra of the polymers were consistent with their assigned structures. As shown in Figure 1, no residual ethynyl protons were observed in ¹H NMR of the polymers. The ¹H NMR spectra of polymers displayed the broader peaks than those of monomers. As shown in Figure 2, the characteristic absorptions bands of $C \equiv C - H$ and $C \equiv C$ for **1** (3293 and 2105 cm⁻¹) and **2** (3307 and 2107 cm⁻¹) were no longer observable in the IR spectra of polymers **PFxCy** and two new stretching bands ascribed to $C \equiv C - C \equiv C$ of copolymers appeared at 2208 and 2137 cm^{-1} for **PFC**, 2204 and 2135 cm^{-1} for **PF2C**, and four new stretching bands of homopolymers appeared at 2133, 2191 cm⁻¹ (C≡C-C≡C) and 2324, 2358 (C≡C) for PF, 2133, 2198 cm^{-1} (C=C-C=C) and 2327, 2354 cm^{-1} (C=C) for PC, respectively. This confirmed efficient polymerization of monomer 1 and 2 to afford PFxCy.

The cationic polymers CPFxCy were obtained by treating of the neutral precursor polymers PFxCy with CH_3I for 5 days. As compared with ¹H NMR spectra of PFC and CPFC in

Figure 1, the quaternization degrees of **CPFC** were nearly 100%, because after the treatment with CH_3I , all signals corresponding to $-CH_2N$ — moved to the lower field.¹⁷

Optical Properties of Polymers

The UV-vis and PL spectra of the neutral precursor polymers **PFxCy** in THF are shown in Figure 3 and their optical data are summarized in Table 1. As compared with absorption spectra with the maxima peaks at 325 nm for monomer **1** and 297nm for monomer **2**, the absorption maxima of **PFC**, **PF2C**, **PF**, **PC** were located at 381, 411, 419 and 352 nm, respectively, and were red-shifted. This fact means the extension of conjugation length upon transformation from the monomers to polymers. When **PFxCy** were excited at the absorption maxima, they showed strong emission at the bluegreen region and the maximum emission peaks of **PFC**, **PF** and **PC** at about 426, 427, 456 and 429 nm, respectively.

The solvent effect of water on quaternary ammonium polymers CPFxCy measured by UV-vis absorption and PL spectra in DMF and DMF/H $_2$ O (1:50, v/v) was shown in Figure 4. As shown in Table 1, the absorption peaks of cationic polymers **CPFxCy** in DMF and DMF/H₂O were observed at 389/412 and 392/423 nm (CPF), 390/414 and 394/424 nm (CPF2C), 420 and 421nm (CPF), 319/363/391 and 327/364/394 nm (CPC), respectively. The corresponding PL spectrum of CPFxCy in DMF and DMF/H₂O was peaked at 437/460 and 448/473 nm (CPFC), 434/458 and 446/473 nm (CPF2C), 435 and 445nm (CPF), 405 and 468 nm (CPC), respectively. With increased volume fraction of H₂O in the solvent, the absorption and emission peaks of all quaternized polymers presented obvious red-shift absorption and broadening. The absorption and emission data were consistent with a significant increase in $\pi - \pi$ stacking interactions of the conjugated polymer chains in water, which suggested that while water





was added to the polymer solution in DMF, the polymer chains most likely underwent water-induced aggregation into a morphology where their planar phenylene rings and diacetylene in adjacent main chains tightly packed face-to-face and the hydrophilic side chains extended more fully into water to optimize water-solubility. This is consistent with the previously reported water-soluble PPE-SO₃⁻¹⁸ In that case, PPE-SO₃ showed significantly decreased PL intensity, progressively red-shifted and broader absorption with increase of volume fraction of H₂O, which are prominent characteristics of conjugated polymer aggregation. Another

possible explanation for such red-shift could be explained by the increased torsion angle of the polymer main chains, due to the increased repulsion between the positive ammonium groups in the side chains and the adjacent fluorene and carbazole units in the polar solvents.¹⁹

Effect of Buffer Solution and Na₃PO₄ on UV-Vis Absorption and Photoluminescence of Cationic Conjugated Polymers

DNA is more commonly stored in buffered solutions than in pure water. The buffer ions are expected to weaken the

JOURNAL OF POLYMER SCIENCE: PART A: POLYMER CHEMISTRY DOI 10.1002/POLA

Polymer	Mw	<i>M</i> _n	PDI	UV, λ_{max} (nm)			PL, λ_{max} (nm)		
				THF	DMF	DMF/H ₂ O	THF	DMF	DMF/H ₂ O
PFC	5,331	5,201	1.03	381			426		
PF2C	8,953	8,120	1.10	411			427		
PF	38,302	26,118	1.46	419			456		
PC	41,326	25,155	1.64	352			429		
CPFC					389/412	392/423		437/460	448/473
CPF2C					390/414	394/424		434/458	446/473
CPF					420	421		435	445
CPC					319/363/391	327/264/394		405	468

TABLE 1 Molecu	ılar Weights an	d Photophysical	Properties	of Polymers
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electrostatic interactions between the positively charged polyelectrolytes and dsDNA, which is often referred to as electrostatic screening. Because the external ions decrease or collapse, the electrostatic interaction of counterions result in a decrease in the potential between the two oppositely charged pseudo surfaces.²⁰ With these considerations in mind, the absorption and PL spectral changes of **CPFC** ([RU] = 2×10^{-5} M) were firstly compared in water and in Tris-HCl (0.1 M, pH = 7.2) buffer solution. The results showed that both absorption and PL spectra still exhibited few change in terms of shape, wavelength and intensity



FIGURE 2 IR spectra of monomers 1, 2 and PFxCy.

except that the UV-vis absorption was slightly red-shifted and the PL intensity increased by less than 2% in the buffer. Then the optical response of CPFC to CTDNA (5 \times 10⁻⁵M)



FIGURE 3 (a) UV-vis absorption and (b) PL emission spectra of **PFxCy** in THF. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.].



FIGURE 4 (a) and (b) UV-vis absorption spectra; (c) and (d) PL emission spectra of **CPFxCy** in DMF or DMF/H₂O (1:50, v/v). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in Tris-HCl buffer (0.1 mol, pH = 7.2) and water was tested, the fluorescence quenching was about 37% and 38% in water and buffer, respectively. Thus, the presence of buffer ions made only a minor modification of the assay response.

More recently, energy transfer between oppositely charged polyelectrolytes has been used to obtain fluorescence superquenching.²¹ These mixtures offer possibilities of "charge reversal", where it is possible to encourage interactions between a given polyelectrolyte and a fluorescence quencher with similar charge. For example, mixing MPS \pm PPV with a cationic poly-L-lysine with appended cyanine dyes resulted in a complex where energy transfer from MPS \pm PPV to the dye took place followed by quenching with a negatively charged anthraquinone derivative.²¹ In our experiments, it was surprised that when Na₃PO₄ was added to the aqueous solution of **CPFC** and the molar ratio of [Na₃PO₄]:[**CPFC**] went up to 10:1, Na₃PO₄ caused amplified fluorescence increase (fivefold) concomitant with almost unchanged shape of absorption spectra as shown in Figure 5. When Na₃PO₄ was added to the copolymer solution of CPF2C, the similar phenomenon was observed. This result was just opposite to the usually fluorescence quenching of cationic conjugated polymers with the addition of negatively charged substances.

The remarkably amplified fluorescence increase upon addition of Na₃PO₄ might be a characteristic of **CPFC** as sensitive fluorescent sensors for detection of DNA. For comparison, Na₃PO₄ was added to the homopolymers solution of **CPF** and **CPC**. It was found that the usual fluorescence quenching of **CPF** and **CPC** when $[Na_3PO_4] = 2 \times 10^{-5}$ M.

UV-Vis Absorption and Photoluminescence of Copolymers CPFC and CPF2C with DNA

The preliminary study of the interaction between copolymers and CT DNA was monitored by UV-Vis absorption spectra by fixing polymer concentration ($[RU]_0 = 2.0 \times 10^{-5}$ M) upon addition of CT DNA (4.3×10^{-9} M to 8.3×10^{-5} M). The representative absorption spectra of **CPFC** in the absence and presence of CT DNA were shown in Figure 6. When DNA concentration was lower than that of polymer repeat units, an absorption increase of polymers was observed with no significant changes in the spectral shapes. When DNA concentration was near or above the polymer concentration, a dramatic decrease of absorbance was observed, accompanied by slight red shift and broadening. The maximum decrease of the peak in the absorbance at 396 nm was found to be 37.4% (**CPFC**), 53.1% (**CPF2C**), with the CT DNA concentrations of 2.7 × 10⁻⁵ M and 2.0 × 10⁻⁵ M, respectively.



FIGURE 5 (a) UV-vis absorption and (b) PL spectra of **CPFC** ([RU] = 2×10^{-5} M) in DMF/H₂O with addition of PO₄³⁻ ([PO₄³⁻] = 0, 4.5 × $10^{-8} \sim 2 \times 10^{-4}$ M, from bottom to top), λ_{exc} = 390 nm.

From the quenching experiments of **CPFC** a decrease of fluorescence upon the addition of CT DNA ([DNA] = 3.0×10^{-13} to 8.3×10^{-5} M) was monitored [Fig. 7(a)]. Because the maxima and shapes of the fluorescence spectra did not change and no new peaks appeared, possible mechanism for the quenching of **CPFC** was attributed to its aggregation near the negatively charged dsDNA, leading to self-quenching.²² Similar results were obtained for **CPF2C**.

CPFC and **CPF2C** exhibited high sensitivity with CT DNA. Addition of a very small amount of DNA ([CT DNA] = 3.3×10^{-13} M) could induce distinct fluorescent quenching (ca. 2.7% and 1.6% quenching respectively for **CPFC** and **CPF2C**, $\lambda_{\text{max}} = 449$ nm). Electrostatic attractions between positively charged conjugated polymer and negatively charged phosphate groups in DNA play an important role in their close proximity. Therefore, the interaction between DNA and cationic conjugated polyelectrolyte (CPE) is important for detection. From the previous studies, the interaction of opposite charged analytes influences chain conformation, aggregation, and thereby fluorescence of CPE. Herein, a possible mechanism is that the multidimensions from the interchain aggregation of polymers may touch DNA in different directions and conduce to enhancement of the quenching ability of tertiary amino group on conjugated backbone, resulting in more fluorescence quenching.²³ Another reason is that interchain aggregation could lead to further amplification of fluorescence quenching through the occurrence of interchain exciton migration.²⁴

The Stern-Volmer quenching constant (K_{SV}) was obtained from the initial linear part of a plot of the ratio of fluorescence intensity in the presence and the absence of quencher as a function of quencher concentration.²⁵ The Stern-Volmer plot in Figure 7(b) is typical for those obtained with the mixtures of **CPFC** and **CPF2C** and CT DNA. A linear region was observed at low quencher concentrations, followed by



FIGURE 6 Absorption spectra of CPFC in the absence and presence of CT DNA ([RU]₀ = 2 × 10⁻⁵ M): (a) [CT DNA] = 6.5×10^{-7} to 3.2×10^{-6} M; (b) [CT DNA] = 1.3×10^{-5} to 8.3×10^{-5} M.



FIGURE 7 (a) Emission of **CPFC** decreased in intensity upon complexation with additions of CT DNA ([CT DNA] = 0 to 3×10^{-5} M); (b) Stern-Volmer plot of **CPFC** and **CPF2C** quenched by CT DNA (1×10^{-9} to 1×10^{-8} M) in Tris-HCl buffer at pH = 7.2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

an up-sloping nonlinear "superlinear" region where the energy transfer may be best described by a "sphere-of-action" mechanism.²⁶ The non-linear parts were originated from the coexistence of static and dynamic quenching processes for aggregation of DNA with chromophore.

The Stern-Volmer quenching constants calculated for **CPFC** and **CPF2C** by CT DNA in DMF/Tris-HCl (1:600, v/v, pH = 7.2) were 1.9×10^8 , 5.2×10^7 M⁻¹ respectively, indicating **CPFC** has better sensitivity of CCP fluorescence toward quenching by DNA than that of **CPFC**. Such high *Ksv* values indicated an efficient fluorescence quenching of **CPFC** and **CPF2C** when they were bound to CT DNA through electrostatic interaction. Therefore, it is possible to detect DNA easily at very low concentrations with **CPFC** and **CPF2C**. However, the maximum fluorescence quenching was only 59% (**CPFC**) and 56% (**CPF2C**). The fluorescence intensity

decreased at a slower rate with higher DNA concentrations. Thus, the fluorescence could not be completely quenched even at high DNA concentrations. Similar behavior was observed with other CCPs.²⁷ This phenomenon was consistent with the changes of UV-vis spectra.

EXPERIMENTAL

Materials

CT DNA was purchased from Sigma-American Biotechnology Company. Double distilled water was used to prepare the buffer solutions. Tris(hydroxymethyl)-aminomethane buffer (Tris) was prepared with Tris (0.1 M) and adjusted to pH of 7.2 with hydrochloric acid. All other reagents and chemicals, unless otherwise stated, were purchased from commercial sources and used without further purification. Solvents were dried and distilled. 2,7-Bis(2'-hydroxy-2'-methylbutynyl)-9,9bis(6'-bromohexyl)-fluorene (7) and 3,6-bis(2'-hydroxy-2'methylbutynyl)-N-(6'-(*N*,*N*-diethylamino)-hexyl]-carbazole (**12**) were prepared by a typical procedure of Sonogashira coupling reaction.²⁸

Measurements

¹H NMR and ¹³C NMR spectra were recorded on a Bruker-400 MHz instrument using tetramethylsilane as an internal reference. Infrared (IR) spectra were obtained with a Nicolet 380 FT-IR spectrometer. Elemental microanalyses were carried out on a Vario EL III CHNS Elemental Analyzer. The molecular weights of the polymers were determined by gel permeation chromatography (GPC) relative to polystyrene standards, using THF at room temperature at a flow rate of 1.0 mL/min. UV-vis absorption and PL spectra were recorded on an UV-2450 and F-4500 spectrometer respectively.

Synthesis of 2,7-Diethynyl-9,9-bis[6'-(*N*,*N*-diethylamino)hexyl]fluorene (1)

Compound 7 (1.65 g, 2.5 mmol) was dissolved in 20 mL of diethylamine by heating and the solution was refluxed for 12 h. After the excess diethylamine was removed, KOH (0.84 g, 15 mmol), toluene (10 mL) and methanol (5 mL) were added. The mixture was refluxed under N₂ at 120 °C for 3 h, extracted with CH₂Cl₂ (3 × 30 mL). The combined organic layer was washed with water by three times, dried over anhydrous Na₂SO₄. After removing solvent, the residue was purified by silica gel column chromatography (EtOAc/ petroleum ether/Et₃N = 1:1:0.01, v/v/v) to give **1** as yellow oil (0.69 g, 57%).

¹H NMR (400 MHz, CDCl₃, δ , ppm): 0.48–0.56 (m, 4H), 0.93 (t, J = 7.2 Hz, 12H), 1.00–1.03 (m, 8H), 1.20–1.22 (m, 4H), 1.88–1.92 (m, 4H), 2.24 (m, 4H), 2.42 (q, J = 7.2 Hz, 8H), 3.12 (s, 2H), 7.41–7.45 (m, 4H), 7.59 (d, J = 7.6 Hz, 2H). IR (cm⁻¹): 3293 (C=C–H), 2105 (–C=C–).

Synthesis of 3,6-Diethynyl-N-[6'-(*N*,*N*-diethylamino)hexyl]carbazole (2)

To a stirred solution of **13** (2.71 g, 5.6 mmol) in toluene (20 mL) and methanol (10 mL) was added KOH (1. 91 g, 34.1 mmol). The mixture was refluxed under N₂ at 120 °C for 12 h, extracted with CH₂Cl₂ (3 × 60 mL). The combined organic layer was washed with water three times, dried over

anhydrous Na₂SO₄, and evaporated to dryness. The crude product was purified by silica gel column chromatography using EtOAc/petroleum ether/Et₃N (1:1:0.01, v/v/v) as eluent to afford **2** as yellow viscous liquid (1.86 g, 90%).

¹H NMR (400 MHz, CDCl₃, δ , ppm): 0.96 (t, J = 7.2 Hz, 6H), 1.27–1.38 (m, 6H), 1.80–1.84 (m, 2H), 2.33 (t, J = 7.2 Hz, 2H), 2.46 (q, J = 7.2 Hz, 4H), 3.06 (s, 2H), 4.23 (t, J = 7.2Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 7.57 (dd, ³J = 8.4 Hz, ⁴J =1.2 Hz, 2H), 8.18 (d, ⁴J = 1.2 Hz, 2H); ¹³C NMR (C₅D₅N, 100 MHz, δ , ppm): 12.2, 27.3, 27.4, 27.6, 29.3, 43.3, 47.1, 53.0, 77.8, 85.6, 110.1, 113.6, 122.7, 125.3, 130.6, 141.1. ACPI: 371 [M⁺]. IR (cm⁻¹): 3307 (C=C-H), 2107 (-C=C-).

General Procedure of Synthesis of Neutral Precusor Polymers (PFxCy)

To a dried three-neck round-bottomed flask attached an inlet and outlet for oxygen were added CuCl (10 mol %), N,N,N',N'-tetramethylethylenediamine (TMEDA) 0.1mL and 6 mL of anhydrous 1,2-dichlorobenzene. The mixture was warmed to 70°C while oxygen was bubbled through. After 20 min, a solution of 3,6-diethynylcarbazole, **1**, 2,7-diethynyl-fluorene, **2** in 10 mL of 1,2-dichlorobenzene was added to the mixture. After 3 h, the solid was precipitated in 10 mL of methanol. The precipitate was filtered, washed several times with methanol, and dried under vacuum at room temperature to afford **PFC** and **PF2C** as yellow solids.

PFC: yield 78%; ¹H NMR (400 MHz, CDCl₃, δ , ppm): 0.58 (m, 4H), 0.97–1.05 (m, 26H), 1.27–1.37 (m, 12H), 1.86–1.94 (m, 6H), 2.30–2.35 (m, 6H), 2.48 (m, 12H), 4.27 (s, 2H), 7.34–7.36 (m, 2H), 7.48–7.50 (m, 4H), 7.65–7.63 (m, 4H), 8.26 (m, 2H). $M_{\rm w}$: 5331; $M_{\rm w}/M_{\rm n}$: 1.03 (GPC). IR (cm⁻¹): 2208, 2137(C=C-C=C).

PF2C: yield 82%. ¹H NMR (400 MHz, CDCl_3 , δ , ppm): 0.58 (m, 8H), 1.01–1.04 (m, 46H), 1.32–1.43 (m, 16H), 1.83–1.94 (m, 10H), 2.39 (m, 10H), 2.55–2.55 (sm, 20H), 4.29 (m, 2H), 7.35–7.37 (m, 2H), 7.48–7.52 (m, 8H), 7.64–7.66 (m, 6H), 8.26–8.27 (m, 2H). M_w : 8953; M_w/M_n : 1.10 (GPC). IR (cm⁻¹): 2204, 2135(C=C–C=C).

Synthesis of Polymer PF

The above procedure was followed using CuCl (2 mg, 0.01 mmol), TMEDA 0.1mL, monomer **1** (55 mg, 0.1 mmol), and 4 mL of 1,2-dichlorobenzene. The polymerization was carried out at 70 $^{\circ}$ C. The light yellow fibrous was filtered and dried overnight under vacuum at room temperature to give **PF** (65% yield).

¹H NMR (400 MHz, CDCl₃, δ , ppm): 0.57 (m, 4H), 0.95 (m, 12H), 1.04 (m, 8H), 1.23 (m, 4H), 1.92 (m, 4H), 2.27 (m, 4H), 2.43–2.45 (m, 8H), 7.48 (m, 4H), 7.63–7.65 (m, 2H). IR (cm⁻¹): 2324, 2358 (C=C), 2133, 2191 (C=C–C=C).

Synthesis of Polymer PC

The above procedure was followed using CuCl (2 mg, 0.01 mmol), TMEDA (0.1 mL), monomer **2** (46 mg, 0.1 mmol), and 4 mL of 1,2-dichlorobenzene. The polymerization was carried out at 70 $^{\circ}$ C. The light yellow fibrous was filtered

and dried overnight under vacuum at room temperature to give **PC** (51% yield).

¹H NMR (400 MHz, CDCl₃, δ , ppm): 1.01 (m, 6H), 1.34–1.42 (m, 6H), 1.84 (m, 2H), 2.39 (m, 2H), 2.51 (m, 4H), 4.12 (m, 2H), 7.22–7.29 (m, 2H), 7.61–7.63 (m, 2H), 8.11–8.19 (m, 2H). IR (cm⁻¹): 2327, 2354 (C=C), 2133, 2198 (C=C-C=C).

General Procedure of Synthesis of Cationic Quaternary Ammonium Polymers CPFxCy

 $CH_{3}I$ (0.5 mL) was added to a solution of neutral precusor polymers **PFxCy** in 10 mL of THF at room temperature. After 5 min stirring, some precipitate was observed, which was dissolved by addition of 2 mL of DMF. Additional $CH_{3}I$ (1 mL) was added and the mixture was stirred for 5 d at room temperature. The precipitated solid was filtered and washed with THF several times to afford cationic polymers **CPFxCy** as yellow solids.

CPFC: yield 90%. ¹H NMR (400 MHz, DMSO- d_6 , δ , ppm): 0.49 (br, 4H), 1.08–1.16 (m, 26H), 1.30–1.79 (m, 12H), 2.49–3.24 (m, 33H), 4.47 (br, 2H), 7.64 (m, 2H), 7.79–7.76 (m, 6H), 7.99 (m, 2H), 8.58 (m, 2H).

CPF2C: yield 94%. ¹H NMR (400 MHz, DMSO- d_6 , δ , ppm): 0.49 (br, 8H), 1.08–3.35 (m, 102H), 4.49 (br, 2H), 7.65 (m, 4H), 7.80 (m, 8H), 7.99 (m, 4H), 8.59–8.60 (m, 2H).

CPF: yield 85%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 0.46 (br, 4H), 1.06–1.14 (m, 20H), 1.41 (m, 4H), 2.07 (m, 2H), 2.83–2.85 (m, 6H), 3.04 (m, 8H), 3.21–3.22 (m, 8H), 7.60–7.64 (m, 2H), 7.80 (m, 2H), 7.99–8.00 (m, 2H).

CPC: yield 88%. ¹H NMR (400 MHz, DMSO-*d*₆, δ, ppm): 1.15 (m, 6H), 1.30 (m, 4H), 1.52 (m, 2H), 1.80 (m, 2H), 2.86–2.88 (m, 2H), 3.09 (m, 3H), 3.21–3.23 (m, 4H), 4.48 (m, 2H), 7.75 (m, 4H), 8.57 (m, 2H).

DNA Binding Study

All experiments involving the interaction of copolymers **CPFC** and **CPF2C** with CT DNA were performed in Tris buffer. The experiment was performed by fixing the concentrations of the polymer (the concentration of **CPFC** and **CPF2C** were calculated in terms of repeat units (RU), [RU] = 2.0×10^{-5} M) while increasing amount of DNA. DNA concentrations ranged from 0, 3.0×10^{-13} M to 8.3×10^{-5} M in base pairs. DNA concentration was determined by absorption spectrometry at 260 nm using a molar extinction coefficient 6600 M⁻¹·cm⁻¹ (A₂₆₀/A₂₈₀ > 1.80).²⁹

CPFC and **CPF2C** can be dissolved in DMF/H₂O mixtures well, but they have a low solubility in water. Stock polymer solutions with concentrations of around 4×10^{-3} M in repeat units were prepared in 10 mL of DMF/H₂O (1:3, v/v). Aliquots of this solution were diluted to give solutions with polymer concentrations of 8×10^{-7} to 4×10^{-4} M in terms of repeat units that were used for the photometric measurements. In DNA binding experiments, stock polymer solutions were diluted with Tris-HCl (0.1 M, pH = 7.2) to give the test polymer concentrations of 2×10^{-5} M in DMF/Tris-HCl (1:600, v/v).

CONCLUSIONS

In conclusion, two novel cationic quaternary ammonium conjugated copolymers **CPFC** and **CPF2C** containing fluorenyl, diacetylene and carbazolyl units were successfully synthesized via an oxidative coupling approach. The polymers were characterized by IR, ¹H NMR, UV-vis, and fluorescence. The water-induced aggregation of the polymers in DMF/H₂O was confirmed by spectroscopy. The polymers emitted intense blue fluorescence and exhibited high quenching efficiency with DNA, which indicated that the polymers might be applied as sensitive fluorescent materials for detection of DNA.

This work was supported by the National Natural Science Foundation of China (20872038, 20904010) and the Fundamental Research Funds for the Central Universities (2009ZM0170).

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