

Poly(pyridinium) salts containing calix[4]arene segments in the main chain as potential biosensors

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Novel poly(pyridinium) salts **4** were synthesized by polycondensation of 4,4'-(1,4-phenylene)bis(2,6-diphenylpyrylium tetrafluoroborate) with calix[4]arene diamines for the purpose of biosensing to meet the shape adaptability with the secondary structures of biological macromolecules. The polymers were characterized by NMR, FT-IR, elemental analysis, inherent viscosity, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), and wide-angle X-ray diffraction. Both the absorption and photoluminescence (PL) spectra of the polymers were also studied. Moreover, the interactions between polymer **4b** and *Pseudomonas fluorescens* DNA were investigated primarily by a fluorescent titration method and transmission electron microscopy (TEM).

Introduction

Conjugated polymers have received increasing attention because of their potential applications as electroluminescence (EL) materials in light-emitting diodes (LEDs).¹ Recently, there is an increasing trend of employing conjugated polymers as optically sensitive materials for increasing the sensitivity of fluorescent biological sensors because they provide the advantage of collective response, compared with noninteracting small molecules. Particularly, cationic conjugated polymers have been proven useful for strand-specific DNA sensing. Bazan and co-workers^{2–4} recently reported a fluorescence energy transfer (FRET) approach that employed conjugated polymers as photoluminescence signal amplifiers to detect specific DNA sequences. To improve the shape adaptability with the secondary structures presented by biological macromolecules, Liu and Bazan *et al.* have developed zigzag-shaped cationic conjugated polymers and tetrahedral-shaped cationic conjugated oligomers for DNA sensing,⁵ which proved more FRET efficient to labelled double strand DNA (dsDNA) and single strand DNA (ssDNA) together with improved selectivity between dsDNA and ssDNA. Herein, the objective is to develop a new class of cationic polymers for the purpose of biosensing, which contain both conjugated segments as polymeric chromophores and flexible segments for increasing their solubility and flexibility to meet the shape adaptability with the secondary structure of biological macromolecules.

Polymers bearing quaternary nitrogen atom in the backbone are usually known as ionenes⁶ or poly(pyridinium) salts. Cationic

pyridinium segments incorporated in the main chain of the polymers not only increase the aqueous solubility of the resultant polymers, but also provide the fluorescent chromophores for the copolymers to detect the interaction between these polymers and biological macromolecules. These polymers themselves, however, are either indissoluble or unstable under certain conditions being susceptible to substitution and elimination reaction. The introduction of aliphatic moieties into poly(pyridinium) salts backbone is an alternative method to solve these problems.⁷ Different from previous reports, herein we have successfully attempted the incorporation of calix[4]arene segments into the backbone to develop a new type of poly(pyridinium) salt.

As we know, calixarenes, cyclic phenolic oligomers wherein two adjacent phenol rings are linked together by an ortho–ortho CH₂ bridge or an analogous homo CH₂ bridge, are a large and mature family of macrocyclic receptors.^{8,9} All kinds of host compounds for a wide range of guests such as cationic and neutral molecules can be synthesized by substitution on the upper- or lower-rim of the central annulus. Benefiting from their versatile bowl-shaped platforms and their wide range of molecular recognition properties, calixarene hosts have been introduced into polymers as sensing elements for various applications. For example, calix[4]arene derivatives were grafted onto Merrifield's resin resulting in their extraction abilities for transitional metal ions or dichromate.¹⁰ Also, calixarene was incorporated in the main chains by copolymerization to provide a straightforward preparation of polymeric materials as cationic sensors or non-linear optical materials.^{11,12} More recently, attention has been paid for the use of calixarene derivatives in biological systems as mimics of ion channels, agents for surface recognition, enzyme mimics, gene transfection vectors and in drug delivery systems.^{13–24} Thus, in present paper calix[4]arene diamines were utilized for polycondensation aiming to enhance the solubility and flexibility of the resultant polymer together with providing the bonding sites with bio-affinitive cations, such as sodium, potassium *etc.*

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The present investigation deals with the synthesis and characterization of a new class of poly(pyridinium) salts containing calix[4]arene segments in the main chain for the goal of biosensing to meet the shape adaptability with the secondary structures of biological macromolecules. Moreover, the interactions of these kinds of polymers with label-free *Pseudomonas fluorescens* DNA were also investigated primarily by a fluorescent titration method and transmission electron microscopy (TEM).

Experimental section

Characterization methods

The ^1H and ^{13}C NMR spectra were recorded with a Varian Unity-plus 400 MHz spectrometer in CDCl_3 using TMS as an internal standard. IR spectra were recorded on a Bio-Rad FTS-6000 FT-IR spectrometer with KBr pellets. Elemental analysis was performed on a Perkin-Elmer 2400C instrument. Gel permeation chromatography (GPC) was conducted with a Waters Breeze 1515 series liquid chromatograph equipped with a differential refractometer (Waters 2410) as a detector with polystyrene as a standard and CHCl_3 as an eluent. The inherent viscosity, $\text{IV} (= \ln(t/t_0)/c)$, where t and t_0 are the flow times for the polymer solution and solvent, respectively, and c is the polymer concentration at a constant temperature, IV values of the polymers were measured in CHCl_3 at various concentrations with a Cannon Ubbelohde-type viscometer at 35°C . Glass transition temperatures (T_g) were measured with a NETZSCH DSC 204 differential scanning calorimeter under a nitrogen (N_2) flow at heating rates of $10^\circ\text{C}/\text{min}$. Polymers usually weighing 10 mg were using for this analysis. The thermal gravimetric analysis (TGA) was performed with a NETZSCH TG 209 instrument at a heating rate of $10^\circ\text{C}/\text{min}$ in atmospheres of air and N_2 , respectively. The wide-angle X-ray diffraction patterns were obtained for powder specimens on an X-ray PW-1840 Philips diffractometer.

Absorption spectra of polymers in CH_2Cl_2 were recorded with a Shimadzu UV-2101PC ultraviolet-visible (UV-vis) spectrophotometer at ambient temperature. Their photoluminescence (PL) spectra in solutions were recorded with a Hitachi F-4500 FL Spectrophotometer with a xenon lamp light source. The DNA extracted from *Pseudomonas fluorescens* was provided kindly by Tianjin Research Center for Agricultural Biotechnology (Tianjin, China). The DNA purity was detected by absorption spectra until UV absorbance $260\text{ nm}/280\text{ nm}$ was >1.9 .

Preparation of starting materials

25,27-Bis(phthalimidoalkoxy)-26,28-dihydroxy-*p*-tert-butylcalix[4]arene (1). Compounds **1** were synthesized from a procedure described as reported in the literature.²⁵ The ^1H NMR data are as following. **1a**: ^1H NMR (200 MHz, CDCl_3) δ 0.94 (s, 18H), 1.26 (s, 18 H), 2.42 (m, 4H), 3.28 (d, $J = 13.0\text{ Hz}$, 4H), 3.40 (t, $J = 6.8\text{ Hz}$, 4H), 4.09 (t, $J = 6.2\text{ Hz}$, 4H), 4.28 (d, $J = 13.0\text{ Hz}$, 4H), 6.78 (s, 4H), 7.02 (s, 4H), 7.44 (s, 2H), 7.61 (m, 4H), 7.73 (m, 4H). **1b**: ^1H NMR (200 MHz, CDCl_3) δ 0.94 (s, 18H), 1.26 (s, 18H), 2.08 (m, 8H), 3.27 (d, $J = 13.0\text{ Hz}$, 4H), 3.40 (t, $J = 6.8\text{ Hz}$, 4H), 3.87 (t, $J = 5.2\text{ Hz}$, 4H), 4.01 (t, $J = 5.6\text{ Hz}$, 4H), 4.22 (d, $J = 13.0\text{ Hz}$, 4H), 6.78 (s, 4H), 7.00 (s, 4H), 7.53 (s, 2H), 7.65 (m, 4H), 7.78 (m, 4H).

25,27-Bis(aminoalkoxy)-26,28-dihydroxy-*p*-tert-butylcalix[4]arene (2). Compounds **2** were synthesized according to a reported method²⁶ and characterized as follows. **2a**: ^1H NMR (300 MHz, CDCl_3): 7.06(s, 4H, ArH), 6.93 (s, 2H, OH), 6.79(s, 4H, ArH), 6.20 (bs, 4H, NH_2), 4.14 (d, $J = 12.9\text{ Hz}$, 4H, ArCH_2Ar), 3.40–3.20 (m, 12H, ArCH_2Ar , OCH_2 , CH_2N), 2.23 (m, 4H, CH_2), 1.29 (s, 18H, *t*-BuH), 1.20 (s, 18H, *t*-BuH). **2b**: ^1H NMR (300 MHz, CDCl_3): 7.05–7.04(m, 8H, ArH), 6.98 (s, 2H, OH), 5.6 (bs, 4H, NH_2), 4.35 (d, $J = 12.9\text{ Hz}$, 2 H, ArCH_2Ar), 4.26 (d, $J = 13.5\text{ Hz}$, 2H, ArCH_2Ar), 4.10 (t, $J = 6.3\text{ Hz}$, 4H, OCH_2), 3.43 (d, $J = 12.9\text{ Hz}$, 2H, ArCH_2Ar), 3.39 (d, $J = 13.5\text{ Hz}$, 2H, ArCH_2Ar), 2.99 (t, $J = 6.9\text{ Hz}$, 2H, CH_2N), 2.18 (m, 4H, CH_2), 1.87 (m, 4H, CH_2), 1.22 (s, 36H, *t*-BuH).

***p*-Bis[4-(2,6-diphenylpyrylium)]benzene ditetrfluoroborate (3).** Compound **3** was prepared according to previous literature.²⁷ A flask was charged with a mixture of 1,4-benzenedicarboxaldehyde (0.30 g, 2.24 mmol), acetophenone (1.96 g, 8.96 mmol), and 1,2-dichloroethane (15 mL). Boron trifluoride etherate (1.4 mL) diluted with 1,2-dichloroethane (5 mL) was added portionwise to the stirred mixture at room temperature, and was refluxed for 5 h under N_2 . The solution was concentrated under reduced pressure, and ether was added to the concentrate. The dark red solid obtained was filtered, washed with ether, then washed with water, and finally dried to afford **3**. It was recrystallized from a mixture of 1,4-dioxane–ether (1:1 v/v) (1.1 g, 70%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): 9.21 (s, 4H), 8.72–7.61(m, 24H).

Preparation of polymers

As a typical example, the preparation of poly(pyridinium) salt **4a** is given: a flask was charged with a solution of **3** (357 mg, 0.5 mmol) and calix[4]arene diamine **2a** (382 mg, 0.5 mmol) in DMF (10 mL) and toluene (20 mL). The mixture was stirred and refluxed under N_2 for 17 h. The mixture was subsequently concentrated under reduced pressure, and water was added to the concentrate. The brown solid obtained was filtered and triturated from methanol. The product was collected by filtration, and washed with methanol. It was further purified by dissolution in CH_2Cl_2 and precipitated from methanol to afford **4a** as a yellow powder (0.32 g, 45%). ^1H NMR (400 MHz, CDCl_3): δ 8.05–7.38 (m, 28H, Ar-H of pyridium), 6.99 (s, 4H, Ar-H of calix), 6.48 (s, 4H, Ar-H of calix), 4.72 (bs, 2H, OCH_2), 4.61 (bs, 2H, OCH_2), 3.58 (d, 4H, $J = 12.8\text{ Hz}$, ArCH_2Ar), 3.38 (bs, 2H, N^+CH_2), 3.31 (bs, 2H, N^+CH_2), 2.98 (d, 4H, $J = 12.8\text{ Hz}$, ArCH_2Ar), 2.10 (bs, 4H, OCH_2CH_2), 1.34 (s, 27 H, *t*-Bu-H), 0.77 (s, 27 H, *t*-Bu-H). ^{13}C NMR (400 MHz, CDCl_3): δ 156.47, 156.28, 150.16, 149.83, 146.87, 141.65, 137.96, 132.96, 131.29, 130.99, 129.83, 129.71, 129.38, 129.23, 129.17, 129.02, 128.84, 128.73, 128.37, 128.26, 127.77, 125.37, 125.03, 33.96, 33.79, 32.91, 32.84, 32.36, 32.15, 32.02, 31.92, 31.81, 31.60, 31.41, 31.17, 30.95, 30.87, 30.80, 30.54, 30.41, 29.80. IR (KBr, cm^{-1}): 2967, 2903, 1612, 1476, 1060.

Polymer **4b** was similarly prepared from the polycondensation of compound **3** and compound **2b** (0.38 g, 51%). ^1H NMR (400 MHz, CDCl_3): δ 8.03–7.41 (m, 28H, Ar-H of pyridium), 7.05, 7.02, 7.00, 6.96 (s_{each} , 8H, Ar-H of calix), 4.72 (bs, 2H, OCH_2), 4.40 (bs, 2H, OCH_2), 4.26–3.13 (m, 12m, N^+CH_2 , ArCH_2Ar), 1.99–1.70 (m, 8H, CH_2CH_2), 1.29, 1.22, 1.16, 0.92,

0.91 (s_{each} , 36H, t -Bu). ^{13}C NMR (400 MHz, CDCl_3): δ 156.51, 149.68, 146.57, 141.45, 137.61, 133.10, 132.85, 131.40, 130.52, 129.56, 129.47, 129.23, 129.15, 128.74, 128.40, 128.32, 128.13, 127.90, 127.76, 126.02, 125.85, 34.12, 34.02, 33.35, 32.50, 32.15, 31.86, 31.61, 31.55, 31.08, 30.67, 30.29, 29.91. IR (KBr, cm^{-1}): 2960, 2903, 1612, 1476, 1088, 1053.

Results and discussion

Synthesis and structural characterization of polymers

Scheme 1 outlines the reaction sequence applied for the synthesis of the poly(pyridinium) salts **4**. 1,4-Benzenedicarboxaldehyde was reacted with four equivalents of acetophenone in the presence of boron trifluoride etherate to afford the bispyrylium salts **3**. Numerous pyrylium salts have been prepared by reacting an aromatic aldehyde with acetophenone in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.²⁸ The polycondensation of **3** with an equimolar amount of a diamine such as calix[4]arene diamine **2a** or **2b** gave the poly(pyridinium tetrafluoroborate)s **4**. The polymerization reaction was carried out in a mixture solvents of DMF and toluene, where toluene was used to remove the evolved water. A transformation of the pyrylium ring to that of pyridinium occurred during this reaction, and therefore the removal of the evolved water could be useful. As previously reported, Harris and colleagues²⁹ showed that the removal of the evolved water as an azeotrope from the reaction mixture increased the degree of polymerization of the obtained poly(pyridinium) salts. Because the reaction conditions have been investigated in detail by Harris and colleagues,²⁹ no optimization of the reaction was undertaken. The polycondensation took place in a homogenous reaction mixture, and the polymer was isolated by pouring the mixture into a nonsolvent.

Structural characterization of the polymers was accomplished by IR and NMR spectra. The FT-IR spectra of typical polymer **4a** showed characteristic absorptions around 2967, 2903 (C–H stretching of aliphatic); 1612, 1476 (aromatic and pyridinium structure); 1060 (BF_4^-). The ^1H NMR signals of polymers at room temperature are broad but distinguishable, especially for the calix[4]arene units. The ^1H NMR spectrum of the representative **4a** in CDCl_3 solution displayed characteristic peaks at 8.05–7.38 (m, 28 H, Ar-H of pyrylium), 6.99 (s, 4H, Ar-H of calix), 6.48 (s, 4H, Ar-H of calix), 3.38 (bs, 2H, N^+CH_2),

3.31 (bs, 2H, N^+CH_2), 2.98 (d, 4H, ArCH_2Ar). Two singlets of the t -butyl protons of **4a** reveal that the calix[4]arene moiety keeps in a pinched cone conformation in the cationic polymer backbone. Alternatively, one main singlet of the t -butyl protons of **4b** indicates that the calix[4]arene moiety keeps in a cone conformation in the polymer structure. The ^{13}C NMR spectra of **4a** and **4b** seem that the carbons of the aromatic moieties resonated between 160 and 120 ppm, whereas those of the aliphatic segments resonated around 30 ppm.

Yields, number-average molecular weight, inherent viscosities and elemental analyses of polymers are summarized in Table 1. Polymers **4a** and **4b** were obtained in 45 and 51% yields, respectively. The number-average molecular weight (M_n) for **4a** and **4b** was rather low (5700 and 10 900) with a polydispersity index of 1.19 and 1.14, respectively, identical to what has been observed in other polymerizations of this type. Their inherent viscosities ranged from 1.13 to 1.69 dL g^{-1} , which also strongly suggested that the polymers were of low molecular weights, consistent with those determined by GPC.

Crystallinity and solubility of polymers

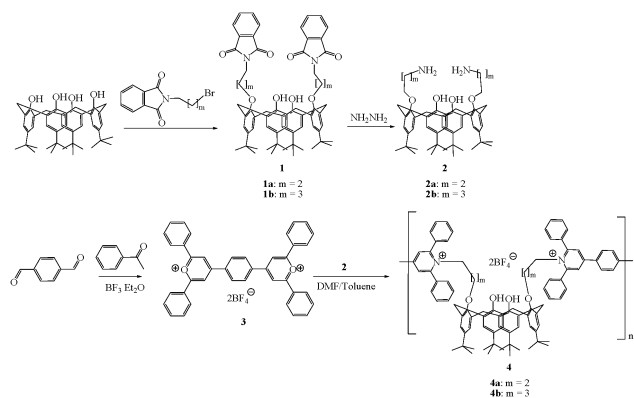
The crystallinity of the polymers was estimated by their wide-angle X-ray diffraction curves of powder specimens obtained at room temperature. The polymers displayed amorphous X-ray diffraction patterns. The amorphous character of the polymers was confirmed by the lack of melting, as was shown by DSC. This is reasonable due to the stiffness of the rigid blocks as well as the flexibility of the bulky calix[4]arene segments in their backbone that significantly increased the disorder in the chain. Moreover, the amorphous nature of the polymers reflects their enhanced solubility.

The polymers were readily soluble at room temperature in common organic solvents such as CH_2Cl_2 , CHCl_3 , acetone, acetonitrile or tetrahydrofuran (THF), which may be attributed to good hydrophobic calix[4]arene units in their backbone, whereas the polymers also had poor solubility in polar protic solvents such as methanol, ethanol and so forth probably because of polymeric ionic nature.

Thermal properties of polymers

DSC was used to determine the glass transition temperature (T_g) and the results are presented in Table 2. No significant endotherms associated with melting were observed in the DSC thermograms. Both polymers showed weak endotherms around 67 °C that could be attributed to the t -butyls melting or the side phenyls melting within the pyridinium units. It was noted that the T_g values of these polymers ranging from 190.9 to 159.3 °C for polymer **4a** and **4b** respectively, are drastically influenced by the bridging tethers within calixarene units. With the increase of tether length, the T_g values of the polymers decreased significantly. The differences are over 30 °C between **4a** and **4b** even though the tether length increased only one methylene unit. The results can be rationalized that the methylene spacer within the cationic polymer backbone contributes an obvious effect on the thermal properties of the polymers.

The thermal stability of the polymers was evaluated by means of TGA and Fig. 1 shows the TGA curves of the polymers. The



Scheme 1 Synthesis route for poly(pyridinium) salts **4**

Table 1 Yields, number-average molecular weight, inherent viscosities and elemental analyses of polymers

Polymer	Yield in %	GPC <i>Mn</i>	η_{inh} (dL g ⁻¹)	Empirical formula	Elemental analyses (in %)			
					C	H	N	
4a	45%	5700	1.13	(C ₉₂ H ₁₀₀ B ₂ F ₈ N ₂ O ₄) _n (1471.4) _n	Calc.	75.10	6.85	1.90
					Found	75.09	6.62	1.83
4b	51%	10 900	1.69	(C ₉₄ H ₁₀₄ B ₂ F ₈ N ₂ O ₄) _n (1499.45) _n	Calc.	75.29	6.99	1.87
					Found	75.20	6.72	1.91

Table 2 Thermal stabilities of the polymers

Polymer	TGA				
	DSC <i>T_g</i> (°C)	In N ₂		In Air	
		DT ₅ ^a (°C)	DT ₅₀ ^a (°C)	DT ₅ ^a (°C)	DT ₅₀ ^a (°C)
4a	190.9	293	476	293	539
4b	159.3	263	500	276	534

^a DT₅ and DT₅₀ are the temperatures (°C) at which weight losses of 5 and 50%, respectively, are observed.

temperatures (DT), at which weight losses of 5 and 50% were obtained both in N₂ and in air for the polymers, are also summarized in Table 2. They were stable up about 260 °C and the difference in the onset of thermal decomposition between polymers **4a** and **4b** was insignificant. It is interesting to note that when the temperatures were lower than about 550 °C the weight losses of polymers **4a** and **4b** in air was less than those in N₂ as could be seen from Fig. 1. It might be caused by the partial oxidation of the aromatic rings within the calix[4]arene skeleton (oxygen absorption). While the temperatures were higher than 550 °C, the oxidated aromatic rings decomposed quickly with a rapid loss of weight.

Optical properties of polymers

Because the polymers involved 4,4'-(1,4-phenylene)-bis(2,6-diphenylpyridinium) ions in the main chain as chromophores, their optical properties in solution were investigated by UV-vis and photoluminescence spectroscopy. The UV-vis maxima and the optical energy gaps (*E_g*) for both polymers are listed in Table 3. The *E_g* values were determined from the onset wavelength of

the UV-vis spectra. The UV-vis spectra of polymers in CH₂Cl₂ solution are shown in Fig. 2. It seems that **4a** and **4b** exhibited two maxima around 333 nm and 338 nm, respectively, associated with the closely spaced π - π^* transitions common to aromatic rings. Literature documented that the salt absorption consists of two electronic transitions, *i.e.*, intramolecular charge transfer complexes of the 2,6- and the 4-substituent with the positively charged nitrogen center.³⁰ Therefore, it was reasonable to state that the absorption band of polymers **4a** and **4b** arose from the same electronic transitions as those in closely related pyridinium salts.³¹ The absorptions of calix[4]arene units showed weak peaks at around 230 nm. The *E_g* values ranged from 3.18 to 3.15 eV comparable to those of other related polymers. It appears that the UV-vis spectra of **4a** and **4b** were slightly dependent of the structure of the non-conjugated chain, namely, the fragment derived from diamine.

The polymers showed yellow-green photoluminescence. The PL spectra of polymers in CH₂Cl₂ solution are presented in Fig. 2. Polymers **4a** and **4b** showed PL maxima at around 550 nm with excitation at 435 nm. The wavelengths at peak maxima are summarized in Table 3. Specifically, the polymers **4a** and **4b**, bearing different tether lengths between the repeated bispyridinium units and different calix[4]arene frameworks, had similar PL curves with a close maximum at around 550 nm. This clearly suggests that the calix[4]arene diamine structure did not influence the emission spectra. Compared with other saturated alkyl chain bridged bispyridinium polymers with blue PL with PL maxima at around 460 nm³² and conjugated aromatic bridged bispyridinium polymers with violet-green PL with PL maxima at around 515 nm,³³ the PL maxima of polymers **4a** and **4b** were remarkably red-shifted by 90 and 35 nm, respectively. This feature strongly indicates that the introduction of the calix[4]arene segments in the polymer backbone influenced considerably the PL emission maximum.

To understand the influence of the solvent polarity on the shape of the PL spectrum and its maximum, PL spectra of polymers **4a** and **4b** in various common organic solvents with different polarities were obtained from dilute solutions by photoexcitation at 435 nm. Emission spectra of the representative polymer **4b** in different solvents such as THF, CH₂Cl₂, acetone and acetonitrile are shown in Fig. 3. From Fig. 3, no significant differentiation was observed in the shape of spectra. However, the intensities of the PL of **4b** in these solvents decreased quickly with the increase of the solvent polarity together with a small red-shift, except the PL intensity in THF was higher than that in the less polar CH₂Cl₂. It could be explained as follows: CH₂Cl₂, acetone and acetonitrile are good guests for the cavity of the calix[4]arene moiety and the local polarity of the polymer chain changed with the uptake of the

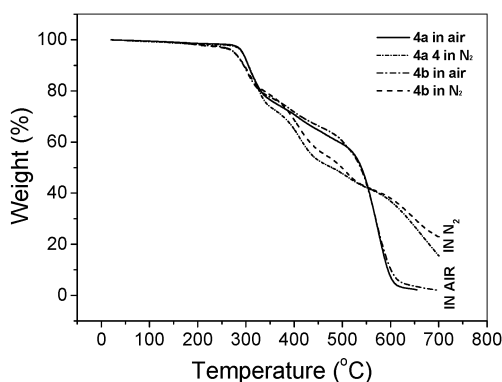


Fig. 1 TGA plots of polymers **4a** and **4b** in atmospheres of air and N₂ at a heating rate of 10 °C/min.

Table 3 Absorption, the optical energy gaps and PL data for the polymers

Polymer	$\lambda_{a, \max}$ in solution (nm) ^a	E_g (eV) ^b	$\lambda_{ex, \max}$ in solution (nm) ^c	$\lambda_{f, \max}$ in solution (nm) ^d
4a	333	3.18	435	554
4b	338	3.15	435	553

^a $\lambda_{a, \max}$ is the absorption maximum from UV-vis spectra in a CH_2Cl_2 solution. ^b E_g is the optical energy gap calculated from UV-vis spectra in a CH_2Cl_2 solution. ^c $\lambda_{ex, \max}$ is the PL excitation maximum in a CH_2Cl_2 solution. ^d $\lambda_{f, \max}$ is the PL maximum in a CH_2Cl_2 solution.

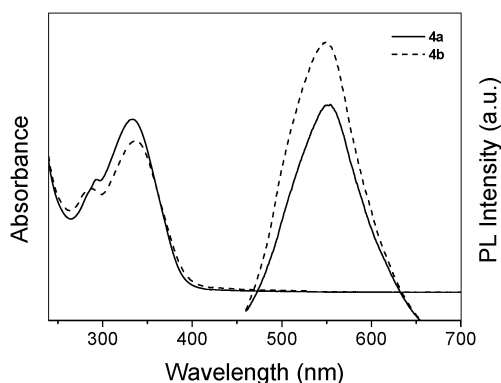


Fig. 2 UV-vis and PL spectra of polymers **4a** (solid lines) and **4b** (dashed lines) in CH_2Cl_2 solution. The intensities were arbitrarily chosen in order to optimally fit the curves.

solvent molecule in the calix[4]rene cavity. However, THF can not be included in the calix[4]arene cavity. Thus, the intensity of the PL of **4b** doesn't obey the order of the solvent polarity in THF.

The maximum emission wavelengths ($\lambda_{f, \max}$) of the polymers in solution were affected by the excitation wavelength. For example, polymer **4b** exhibited $\lambda_{f, \max}$ values of 527, 547, and 553 nm upon excitation at 400, 420 and 435 nm, respectively (Fig. 4). As can be seen from Fig. 4, the $\lambda_{f, \max}$ values increased gradually with the increase of excitation wavelengths and then decreased rapidly with the increase of excitation wavelengths. The optimized excitation wavelength of polymer **4b** can be easily selected.

The concentration of the polymer in CH_2Cl_2 influenced the PL curve of the solution, thereby supporting the existence of chain association. Fig. 5 presents the PL spectra of **4b** at various concentrations in CH_2Cl_2 solutions. It seems that with

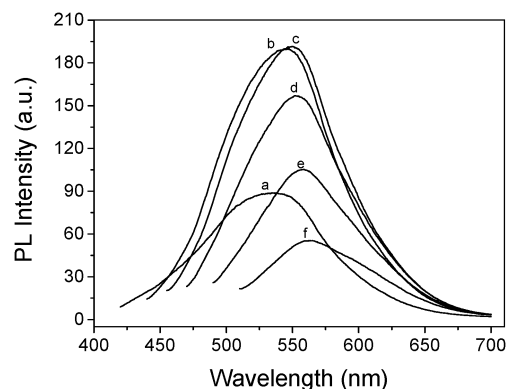


Fig. 4 Emission spectra of polymer **4b** at various excitation wavelengths: (a) 400, (b) 420, (c) 435, (d) 450, (e) 470 and (f) 490 nm in 1 mg/mL CH_2Cl_2 solution at 25 °C.

concentration increasing, the PL curves became less intense with red-shifts of their maximum emission wavelengths. The results reveal that with high concentrations of the polymer in solution, the intramolecular or intermolecular interactions were significant. It is reasonable to accept that these interactions took place in concentrated solutions between the adjacent phenyl groups and formed an excimer or aggregate complex that acted as an emitting center. A similar behavior was observed in the other synthesized polymers.³⁴

Interactions of polymer **4b** with DNA

The DNA binding behavior of polymer **4b** is investigated by fluorescence titrations, where the *Pseudomonas fluorescens* DNA with different concentrations in Tris buffer solution (pH 7.2) are

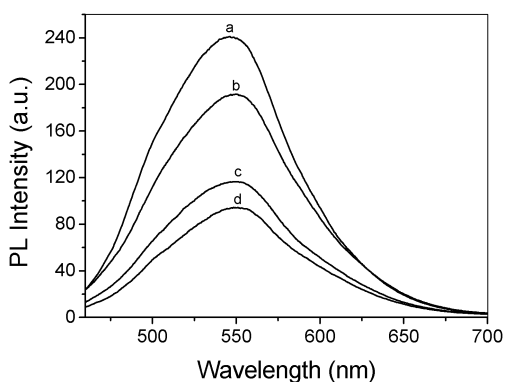


Fig. 3 Emission spectra of polymer **4b** in various solvents (a) THF, (b) CH_2Cl_2 , (c) acetone and (d) acetonitrile under the concentration of 1 mg/mL excited at 435 nm at 25 °C.

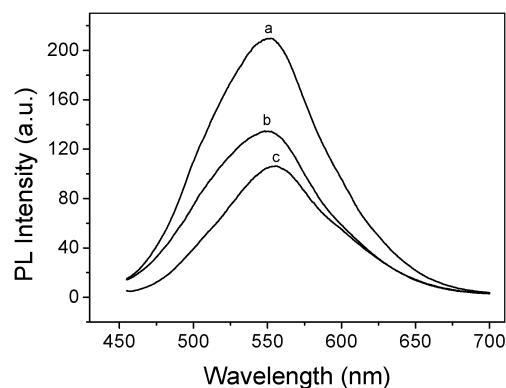


Fig. 5 Emission spectra of polymer **4b** at various concentrations (a) 0.05, (b) 0.1 and (c) 0.5 g/dL in CH_2Cl_2 under excitation wavelength of 435 nm at 25 °C.

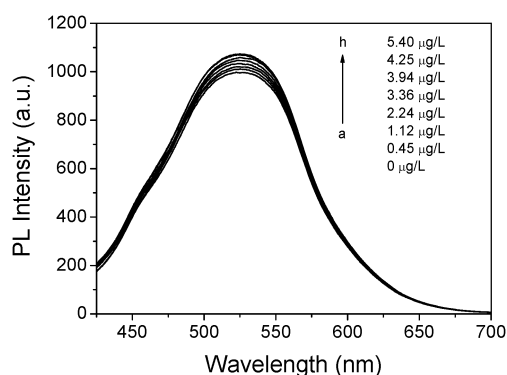


Fig. 6 PL spectra of polymer **4b** (0.29 mg/L) with increasing of *Pseudomonas fluorescens* DNA (0–5.40 µg/L from *a* to *h*). The PL spectra were measured by direct excitation of polymer **4b** at 435 nm.

gradually added to a solution of polymer **4b**. In this experiment, **4b** was dissolved in the mixed solvents of ethanol and Tris buffer solution (pH 7.2) at 25 °C (1: 4 V/V) due to its poor solubility in water or buffer aqueous solution. The *Pseudomonas fluorescens* DNA sample was prepared by initially determining DNA strand concentration (1.0 mg/L) based on 260 nm and 280 nm absorbency measurements done on a Shimadzu UV-2101PC spectrometer. Considering the rather low molecular weight of polymer **4b**, the higher concentration (0.29 mg/L) than that of *Pseudomonas fluorescens* DNA was used. Fig. 6 shows the typical fluorescence titration curves of polymer **4b** with *Pseudomonas fluorescens* DNA.

On the basis of well-known interpolymer interactions between oppositely charged polyelectrolytes,³⁵ we anticipated that complexation of polymer **4b** with *Pseudomonas fluorescens* DNA in solution would lead to contraction and aggregation of polymer chains, resulting in fluorescence intensity change of polymer. From Fig. 6, it could be found that PL intensity of polymer **4b** gradually enhanced with the increasing amounts of DNA, although the change seemed to be not obvious compared with that of labelled small DNA.³⁶

It is interesting to note that the maximum emission intensity of each addition arrived equilibration within about 10 min, suggesting that there was a spatial adaptation between the DNA chain and the polymer chain to interact. However, there wasn't any obvious blue or red shift of the maximum emission wavelengths ($\lambda_{f, \max}$) with the increasing of DNA, indicating that there wasn't any electronic coupling through the internal carbon linkage of the structure of emission bispyridinium moieties. These results can be explained roughly as follows: before interaction with cationic polymer **4b**, single strand DNA interacts with its complementary strand to keep the classic double strand conformation. When the cationic polymer **4b** is added, the higher local charge density of the double strand DNA (dsDNA) should result in a strong interaction with the polymer chain by electrostatic attraction, hydrophobic drive and so forth. Additionally, the lower-rim oxygen binding site in the main chain of polymer **4b** would coordinate the counter ion on the surface of the dsDNA to enhance the interaction between dsDNA and the polymer chains. On the other hand, the cationic polymer chains can easily adapt their conformation to the local conformation of

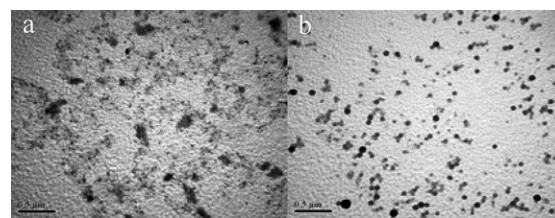


Fig. 7 TEM images of (a) *Pseudomonas fluorescens* DNA (1 mg/L) in Tris buffer solution (pH 7.2) and (b) polymer **4b** (1 mg/L) with *Pseudomonas fluorescens* DNA (1 mg/L) in THF/ Tris buffer solution (pH 7.2) (1:4 V/V).

the dsDNA because of the flexibility of the backbone of the polymer chain to produce the strongest interaction.

To get further evidence about the interactions between polymer **4b** and *Pseudomonas fluorescens* DNA, transmission electron microscopy (TEM) studies were performed. Fig. 7 shows the typical TEM images of *Pseudomonas fluorescens* DNA in the absence and presence of polymer **4b**. Without polymer **4b**, the free DNA existed as loose clews (Fig. 7a). After the addition of polymer **4b** (W/W = 1/1), the originally loose DNA clews turn to the solid particles with an average diameter of *ca.* 80 nm (Fig. 7b). The phenomenon clearly demonstrated the good DNA condensation ability of polymer **4b**, and the electrostatic interactions of the positively charged polymer **4b** with the negatively charged DNA should be one of the predominant driving forces.

Conclusions

In summary, novel fluorescent poly(pyridinium) salts **4** were synthesized by copolymerization of 4,4'-(1,4-phenylene)bis(2,6-diphenylpyrylium tetrafluoroborate) with calix[4]arene diamines for the purpose of biosensing to meet the shape adaptability with secondary structures of biological macromolecules. Their thermal and optical properties have been elucidated to obtain a full understand of this type of polymer to exploit their application in biosensing. The interaction of polymer **4b** with label-free *Pseudomonas fluorescens* DNA has been investigated by fluorescent titration method and TEM, which demonstrates that *Pseudomonas fluorescens* DNA has a strong interaction with this type of polymer predominantly by electrostatic interactions. To get a better understanding of the force controlling the association between the cationic poly(pyridinium) salts and DNA, further optimization of the interaction between this type of polymers with labeled ssDNA and dsDNA is now in progress aiming to yield practical detection platforms and will be reported in the coming work. Additional work is also being done to use this type of fluorescent polymers as a cell imaging sensor.

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