Design and Synthesis of New Cationic Water-Soluble Pyrene Containing Dendrons for DNA Sensory Applications

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ABSTRACT: A series of new water-soluble cationic pyrene-dendron derivatives, **G1**, **G2**, and **G3,** was successfully synthesized and characterized. These new dendrons were designed with the quaternized amino moieties at the periphery of the dendrons for DNA detection and functionalized with pyrene as a fluorescent probe. The electrostatic interactions between the plasmid DNA (pDNA) and cationic charged dendrons in an aqueous solution resulted in a change in the photophysical properties of pyrene, which could be shown in the UV-vis and fluorescence spectra. Pyrene dendrons showed a high and rapid fluorescence response upon the addition of pDNA, which was strongly dependent on the size and hydrophobicity of the dendrons. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 50: 297–305, 2012

KEYWORDS: dendrons; DNA; polyamide; pyrene; sensor; water-soluble polymers

INTRODUCTION Dendrimers are well-defined, three-dimensional hyperbranched macromolecules with a large number of terminal groups that can be easily utilized for introducing functionalities at the periphery of the dendrimers. Such a structural specificity has received great interests as new polymeric materials for applications in the fields of molecular light harvesting,[1] catalysts,[2] liquid crystals,[3,4] molecular encapsulation,[5,6] and drug delivery systems.[7–10] Dendrimers containing fluorescent components not only facilitate the detailed investigation of the self-assembly process and molecular interaction in the dendrimers, but also extend their applications in fluorescence-based sensing materials.[11–15] Pyrene is a good candidate as a fluorescent probe because its fluorescence properties are well known and highly sensitive to the microenvironment.[16,17] In addition, it has a strong tendency to form excimers via intermolecular π–π stacking, which exhibit a broad and structure-less fluorescent emission red-shifted with respect to that of monomeric pyrene. In the past decades, several pyrene-labeled dendrimers have been developed by a noncovalent incorporation of pyrene into the cavities of the dendrimers, or covalent attachment of pyrene to the core or periphery of the dendrimers.[18–26] However, few of them have been reported for use in sensory applications.[21]

For sensory applications, electrostatic interactions between cationic moieties and negatively charged analyte targets (e.g., DNA or RNA) are commonly coordinated when designing fluorescence-based sensors. Various cationic fluorescence-based sensors, such as cationic amphiphilic molecules,[27,28] cationic conjugated polymers,[29–31] or cationic charged dendrimers,[12] have been designed and synthesized for DNA or RNA detection. Recently, we also reported the aligned electrospun nanofibers formed from cationic polyfluorene that showed an enhanced sensitivity to plasmid DNA (pDNA).[32] Although cationic fluorescent conjugated polymers have been proven to have a high sensitivity due to the excitation energy transfer mechanism resulting in amplification of the fluorescent signal, their rigid conformation led to its poor response to various secondary structures naturally present in biological macromolecules, such as the double helices of DNA.[33] In addition, the uncontrolled molecular weight variation of conjugated polymers caused reproduction of their sensitivity difficult with different batches of conjugated polymers. Dendrimers have more conformational freedom and precise molecular structures that allows them to be more suitable for DNA detection. However, the synthesis of cationic

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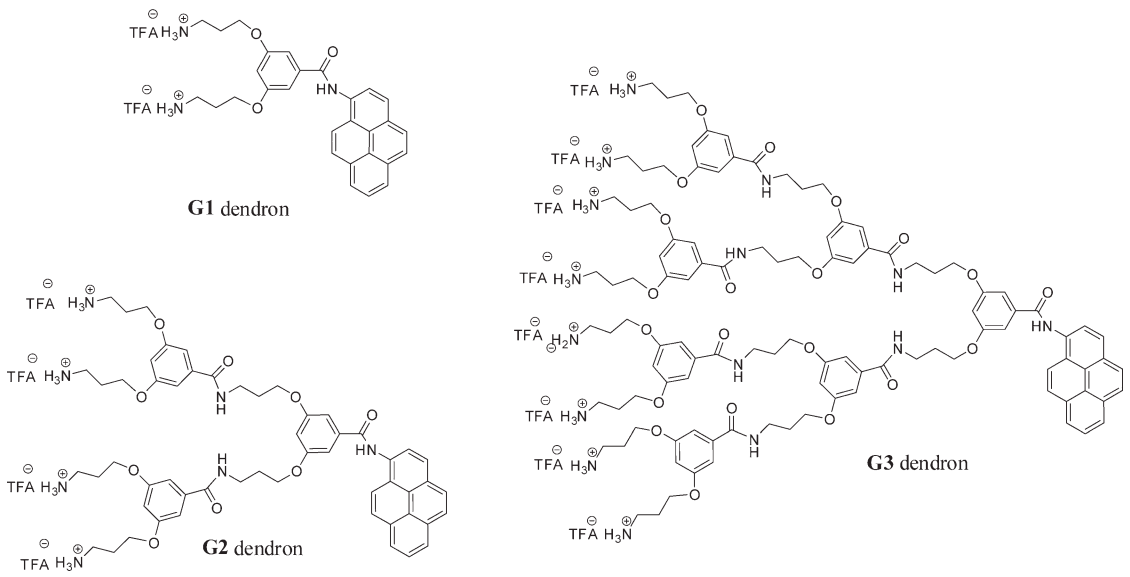


SCHEME 1 Chemical structures of G1, G2, and G3 dendrons.

dendrimers might require a tedious multistep procedure with a repetitive protection–deprotection and purification process for each generation. Recently, we demonstrated the simple and rapid synthesis of dendritic polyamides and successfully obtained third generation dendrimers.[34–37]

Hence, we now report the synthesis and investigation of a different generation of new cationic charged dendrons with functional pyrene as a fluorescent probe (G1, G2, and G3 dendrons) for sensing DNA molecules (Scheme 1).

The quaternized amino moieties at the periphery of dendrons were designed for favorable electrostatic attraction with negatively charged nucleic acids. The number of charged moieties, the distance between the cationic groups and fluorescent units, as well as the hydrophobicity of the dendron generation were also examined to reveal their sensitivity to a pDNA, since it has been reported that the binding affinity of the cationic molecules to pDNA was strongly dependent on the structures of the cationic molecules.[38,39]

EXPERIMENTAL

Materials

N-Methyl-2-pyrrolidinone (NMP) was distilled under reduced pressure from calcium hydride, and then stored under nitrogen. Triethylamine (TEA) was distilled from calcium hydride under nitrogen, and then stored under nitrogen. Diphenyl(2,3-dihydro-2-thioxo-3-benzoxazolyl)phosphonate (DBOP) was supplied from KYOCERA Chemical Corporation and recrystallized from hexane, then stored under nitrogen in a refrigerator. The pDNA molecules (base pair: 700) used for studying the responsive properties of the pyrene-dendrons were purchased from Sigma (Milwaukee). The stock solutions of pDNA were prepared by dissolving certain amount of solid pDNA in double distilled water and stored at 4 °C in the dark. Other reagents and solvents were obtained commercially and used as received unless otherwise noted.

Instrumentation

^{1}H NMR spectra were recorded in deuterated dimethylsulfoxide (DMSO-d_6) or chloroform ($CDCl_3$) on a BRUKER DPX-300 spectrometer at 300 MHz. Infrared spectra were recorded on a Horiba FT-720 spectrophotometer. Matrix-assisted laser desorption ionization with time of flight (MALDI-TOF) mass spectra were recorded on a Kratos Kompact MALDI instrument operated in linear detection mode to generate positive ion spectra using dithranol as a matrix, Tetrahydrofuran (THF) as a solvent, sodium trifluoroacetate as an additive agent. Size exclusion chromatography (SEC) was performed on a Jasco GULLIVER 1500 system equipped with two polystyrene gel columns (Plgel 5-mm MIXED-C) eluted with $CHCl_3$ at a flow rate of 1.0 mL min^{-1} calibrated by standard polystyrene samples. Absorption and photoluminescence spectra were measured with a Jasco V-550 spectrophotometer. Transmission electron microscopy (TEM) images were obtained with a Philips TEM (CM 100) instrument operating at a voltage of 80 kV with a Morada CCD camera. The samples of G1–G3 dendrons were prepared with concentration of 100 μM.

Synthesis

The pyrene-dendrons (G1, G2, and G3) were synthesized by a divergent method as shown in Schemes 2 and 3.

Preparation of 3-Bromo-propyl-1-NHBoc (2)[40]

1M NaOH aq. (10 mL) was added to a solution of 3-bromopropylamine (1.00 g, 4.56 mmol) and di-*tert*-butyl dicarbonate (0.945 g, 4.10 mmol) in dioxane/water (20 mL/10 mL). The mixture was stirred at room temperature for 1 h, and then diluted with ethyl acetate and water. The organic layer was washed successively with 1N HCl aq., 5 wt % of $NaHCO_3$ aq., and brine, and then dried with $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The product was obtained as colorless oil (0.898 g, 92% yield). ^{1}H NMR ($CDCl_3$, δ, ppm): 1.44 (s, 9H), 2.05 (triplet–triplet appearing as a quintet, 2H, $J = 6.6$ Hz), 3.27 (triplet–doublet

appearing as a quartet, 2H, $J = 6.6$ Hz), 3.45 (t, 2H, $J = 6.6$ Hz), 4.67 (s, 1H).

Preparation of Protected-AB_2 Building Block (3)

To a mixture of compound **2** (3.63 g, 15.0 mmol) and K_2CO_3 (2.07 g, 15.0 mmol) in DMF (10 mL) was added methyl 3, 5-dihydorxybenzoate (0.84 g, 5.00 mmol) at room temperature under nitrogen. The reaction mixture was stirred at 60 °C overnight, and then poured into water. The precipitate was filtered and dried under reduced pressure to give a white solid (2.24 g, 93% yield). M.p. 123–124 °C. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.44 (s, 18H), 1.99 (triplet–triplet appearing as a quintet, 4H, $J = 6.6$ Hz), 3.32 (triplet–doublet appearing as a quartet, 4H, $J = 6.6$ Hz), 3.90 (s, 3H), 4.04 (t, 4H, $J = 6.6$ Hz) 4.73 (s, 2H), 6.64 (t, 1H), 7.17 (d, 2H).

Preparation of AB_2 Building Block (4)[41]

A mixture of compound **3** (3.38 g, 7.00 mmol) and KOH (0.550 g, 9.80 mmol) in methanol/water (42 mL/14 mL) was refluxed for 2 h. The reaction solution was cooled to room temperature and acidified with acetic acid. Then, the organic layer was diluted with ethyl acetate and washed with water three times, and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure to afford a white solid (2.85 g, 87% yield). M.p. 128–129 °C. ^{1}H NMR (DMSO-d_6, δ, ppm, 40 °C): 1.37 (s, 18H), 1.82 (triplet–triplet appearing as a quintet, 4H, $J = 6.5$ Hz), 3.08 (triplet–doublet appearing as a quartet, 4H, $J = 6.5$ Hz), 4.00 (t, 4H, $J = 6.5$ Hz), 6.69 (t, 1H, $J = 2.2$ Hz), 6.77 (s, 2H), 7.04 (d, 2H, $J = 2.2$ Hz).

Synthesis of Protected-First Generation Dendron (protected-G1)

To a solution of **4** (1.48 g, 3.15 mmol) and 1-aminopyrene (0.652 g, 3.00 mmol) in NMP (3 mL) were added DBOP (1.21 g, 3.15 mmol) and TEA (0.440 mL, 3.15 mmol) under nitrogen. The reaction solution was stirred at room temperature for 3 h, and then poured into water. The precipitate was collected, washed with methanol, and dried *in vacuo* at 80 °C to give a gray powder (1.60 g, 80% yield). M.p. 161–162 °C. IR (KBr, cm^{-1}): 1180 (Ar-O-alkyl), 1585 (C═O, amide and carbamate), 3039, 3058 (Ar-H), 3421, 3532 (N—H, amide, and carbamate). ^{1}H NMR (DMSO-d_6, δ, ppm, 40 °C): 1.38 (s, 18H), 1.89 (triplet-triplet appearing as a quintet, 4H, $J =$ 6.5 Hz), 3.13 (triplet–doublet appearing as a quartet, 4H, $J =$ 6.5 Hz), 3.22 (s, 3H), 4.10 (t, 4H, $J = 6.5$ Hz), 6.74 (t, 1H, $J =$ 2.0 Hz), 6.80 (s, 2H), 7.32 (d, 2H, $J = 2.0$ Hz), 8.05- 8.35 (m, 9H), 10.65 (s, —CON*H*—, 1H). Anal. calcd for $C_{39}H_{45}N_3O_7$: C, 70.14; H, 6.79; N, 6.29. Found: C, 70.04; H, 6.68; N, 6.37.

Synthesis of First Generation Dendron (G1)

Protected-G1 (0.500 g, 0.749 mmol) was dissolved in trifluoroacetic acid (TFA) (5 mL) and stirred at room temperature for 1.5 h. The solvent was evaporated to dryness and ether was added. The precipitate was collected and dried *in vacuo* at 80 °C to give a gray powder (0.485 g, 93% yield). M.p. 176–185 °C. IR (KBr, cm^{-1}): 1176 (Ar-O-Alkyl), 1203 (C-F), 1592 (C═O, carboxylate), 1677 (C═O, amide), 2700–3200 (N—H, ammonium), 3404, 3432 (N—H, amide). ^{1}H NMR (DMSO-d_6, δ, ppm, 40 °C): 2.07(triplet–triplet appering as a quintet, 4H, $J = 6.9$ Hz), 3.20 (t, 4H, $J = 6.9$ Hz), 4.19 (t, 4H, $J = 6.9$ Hz), 6.79 (s, 1H), 7.38 (s, 2H), 8.04–8.40 (m, 9H), 10.68 (s, -CON*H*-, 1H). Anal. calcd for $C_{33}H_{31}F_6N_3O_7$ $1.08H_2O$: C, 55.43; H, 4.67; N, 5.88. Found: C, 55.20; H, 4.54; N, 6.11.

Synthesis of Protected-Second Generation Dendrons (protected-G2)

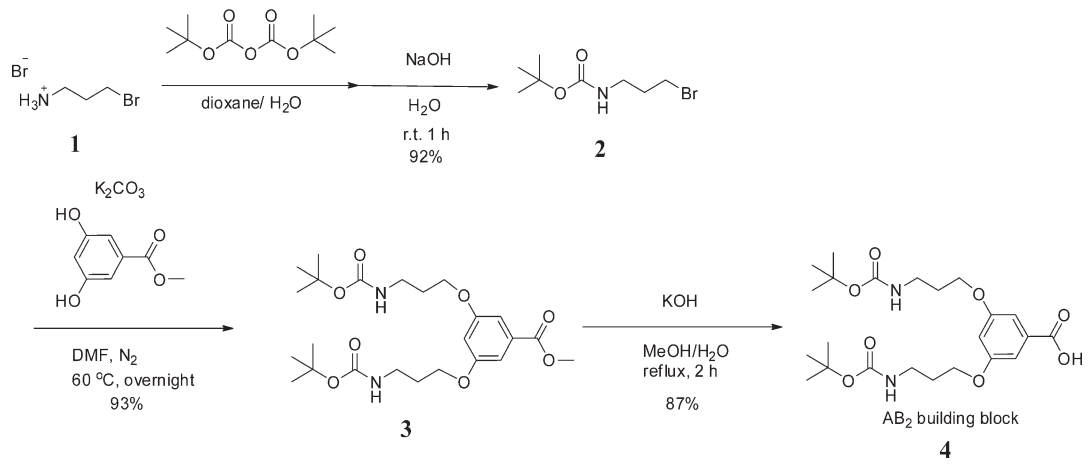
To a solution of **4** (0.539 g, 1.15 mmol) in NMP (5 mL) were added DBOP (0.422 g, 1.10 mmol) and TEA (0.154 mL, 1.10 mmol) under nitrogen. The reaction solution was stirred at room temperature for 1 h. Then, **G1** (0.347 g, 0.500 mmol) and TEA (0.280 mL, 2.00 mmol) were added to the solution and the solution was stirred at room temperature for 6 h. The reaction solution was poured into water, and the precipitate was collected and dried. The crude product was dissolved in methanol (100 mL) and water (25 mL) was added to this solution. The precipitate was collected and dried *in vacuo* at 80 °C to give a gray powder (0.636 g, 93% yield). M.p. 118–121 °C. IR (KBr, cm^{-1}): 1164 (Ar-O-alkyl), 1689 (C═O, amide, and carbamate), 2935, 2973 (Ar-H), 3313, 3355 (N—H, amide, and carbamate). ^{1}H NMR (DMSO-d_6, δ, ppm, 40 °C): 1.36 (s, 36H), 1.81 (triplet–triplet appearing as a quintet, 8H, $J = 6.8$ Hz), 2.03 (triplet–triplet appearing as a quintet, 4H, $J = 6.8$ Hz), 3.07 (triplet–doublet appearing as a quartet, 8H, $J = 6.8$ Hz), 3.45 (triplet–doublet appearing as a quartet, 4H, $J = 6.8$ Hz), 3.99 (t, 8H, $J = 6.8$ Hz), 4.16 (t, 4H, $J = 6.8$ Hz), 6.59 (s, 2H), 6.69–6.80 (m, 5H), 7.00 (s, 4H), 7.35 (s, 2H), 8.03–8.37 (m, 9H), 8.43 (t, 2H, $J = 5.0$ Hz), 10.7 (s, 1H). Anal. calcd for $C_{75}H_{97}N_7O_{17}$: C,65.82; H, 7.14; N, 7.16. Found: C, 65.38; H,6.93; N,7.05. MALDI-TOF MS: Calcd.: $[M]^+ = 1367.7$, Found: $[M+Na]^+ = 1389.5$.

Synthesis of Second Generation Dendron (G2)

Protected-G2 (0.550 g, 0.402 mmol) was dissolved in 5 mL of TFA and stirred for 1.5 h. The solvent was evaporated to dryness and ether was added. The precipitate was collected and dried *in vacuo* at 80°C to give a gray powder (0.544 g, 95% yield). IR (KBr, cm^{-1}): 1172 (Ar-O-Alkyl), 1203 (C-F), 1592 (C═O, carboxylate), 1677 (C═O, amide), 2700–3200 (N—H, ammonium), 3370, 3432 (N—H, amide). ^{1}H NMR (DMSO-d_6, δ, ppm, 40 °C): 1.94–2.12 (m, 12H), 2.96 (t, 8H, $J =$ 6.6 Hz), 3.46 (triplet–triplet appearing as a quintet, 4H, $J =$ 6.8 Hz), 4.08 (t, 8H, $J = 6.6$ Hz), 4.16 (t, 4H, $J = 6.8$ Hz), 6.65 (s, 2H), 6.77 (s, 1H), 7.07 (s, 4H), 7.36 (s, 2H) 8.04-8.36 (m, 9H), 8.48 (t, 2H, $J = 4.9$ Hz) 10.70 (s, —CON*H*—, 1H). Anal. calcd for $C_{63}H_{69}F_{12}N_7O_{17}$ $1.18H_2O$: C, 52.35; H,4.98; N, 6.78. Found: C, 51.89; H, 4.90; N, 7.24.

Synthesis of Protected-Third Generation Dendron (protected-G3)

DBOP (0.248 g, 0.644 mmol) and TEA (90.0 μL, 0.644 mmol) were added to a solution of **4** (0.316 g, 0.672 mmol) in NMP (1 mL) under nitrogen and the solution was stirred at room temperature for 1 h. To this solution were added **G2** (0.200 g, 0.140 mmol) and TEA (0.240 mL, 1.68 mmol) and the solution was stirred at room temperature overnight. The reaction solution was poured into water, and the precipitate was collected and dried. The crude product was dispersed in



SCHEME 2 Synthesis of AB_2 building block (**4**).

methanol (20 mL), and water (5 mL) was added to this mixture. The precipitate was collected and dried *in vacuo* at 80 °C to give a gray powder (0.345 g, 89% yield). IR (KBr, cm^{-1}): 1164 (Ar-O-alkyl), 1693 (C═O, amide and carbamate), 2935, 2973 (Ar-H), 3343 (N—H, amide and carbamate). ^{1}H NMR (DMSO-d_6, δ, ppm, 40 °C): 1.25 (s, 72H), 1.71 (triplet–triplet appearing as a quintet, 16H, J = 6.6 Hz), 1.79–1.99 (m, 12H), 2.97 (triplet–doublet appearing as a quartet, 16H, J = 6.6 Hz), 3.21–3.40 (m, 12H), 3.87 (t, 16H, J = 6.6 Hz), 3.94 (t, 8H, J = 6.3 Hz), 4.06 (t, 4H, J = 6.6 Hz), 6.48 (s, 4H), 6.52 (s, 2H), 6.56–6.71 (m, 7H), 6.88 (s, 8H), 6.94 (s, 4H) 7.92–8.24 (m, 9H), 8.24–8.39 (m, 6H), 10.6 (s, 1H). Anal. calcd for $C_{147}H_{261}N_{15}O_{37}$: C, 63.73; H,7.31; N,7.58. Found: C,63.40; H,7.25; N,7.59. MALDI-TOF MS: Calcd.: $[M]^+$ = 2768.4, Found: $[M+Na]^+$ = 2790.5.

Synthesis of Third Generation Dendron (G3)

Protected-G3 (0.260 g, 0. 0939 mmol) was dissolved in TFA (5 mL) and stirred at room temperature for 1.5 h. The solvent was evaporated to dryness and ether was added. The precipitate was collected and dried *in vacuo* at 80 °C to give a gray powder (0.254 g, 94% yield). IR (KBr, cm^{-1}): 1172 (Ar-O-Alkyl), 1203 (C-F), 1592 (C═O, carboxylate), 1681 (C═O, amide), 2700–3200 (N—H, ammonium), 3440 (N—H, amide). ^{1}H NMR (DMSO-d_6, δ, ppm, 40 °C): 1.88–2.12 (m, 28H), 2.96 (t, 16H, J = 6.7 Hz), 3.36–3.52 (m, 12H), 4.00–4.20 (m, 28H), 6.60–6.66 (m, 6H), 6.78 (s, 1H), 7.04 (s, 12H), 8.03–8.34 (m, 9H), 8.42–8.55 (m, 6H), 10.70 (s, -CON*H*-, 1H). Anal. calcd for $C_{123}H_{145}F_{24}N_{15}O_{37}$ 2.39 H_2O: C,50.51; H, 5.16; N,7.18. Found: C,50.07; H, 5.09; N, 7.63.

Preparation of G1–G3 Solutions and pDNA Sensing Studies

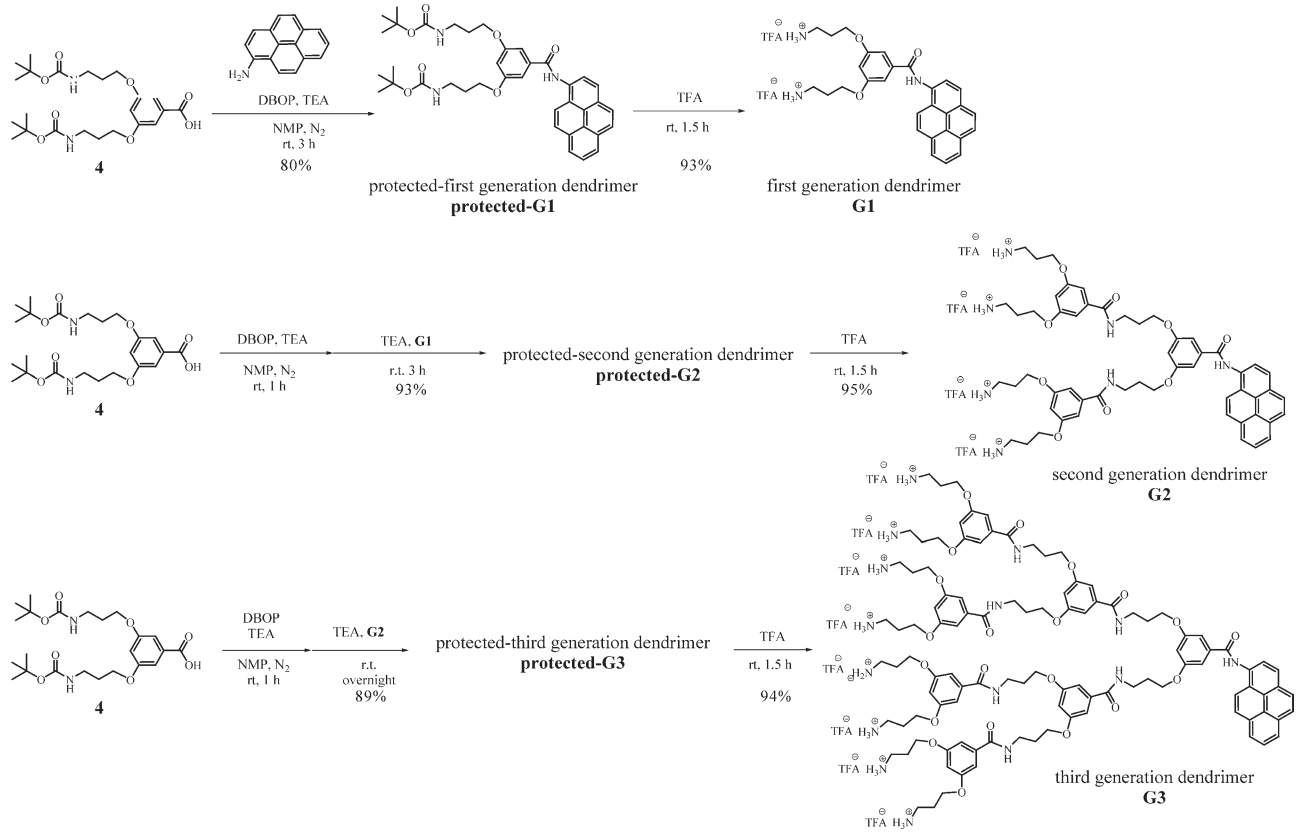
The sample solutions were prepared by dissolving certain amount of pyrene-dendrons in double distilled water with gentle shaking and stored at 4 °C in the dark before titration. For the pDNA titration, the sample solutions were prepared to 5 μM. To 2 mL of sample solution was added 0.5- to 5-μL aliquots of pDNA stock solutions. Upon each addition, the solution was stirred for 2 min to reach equilibrium. UV-vis spectrum and fluorescence spectrum were subsequently monitored. The fluorescence spectra were excited at 340 nm for G1 and G2 and 347 nm for G3. No obvious influence on both UV-vis and fluorescence spectra was observed due to the limited water volume added.

RESULTS AND DISCUSSION

Synthesis of AB_2 Building Block and Dendrons

Scheme 2 shows the synthetic route for an AB_2 building block. Compound **2** was prepared according to a previous paper.[40] Methyl 3,5-dihydroxybenzoate reacted with **2** in the presence of K_2CO_3 to yield a protected AB_2 building block precursor (**3**), which was converted into an AB_2 building block (**4**) by the hydrolysis of the methyl ester group of **3**. The ^{1}H NMR spectrum of the AB_2 building block **4** is shown in the supporting information (Fig. S1). All peaks are well assigned to the corresponding structure.

The dendrons were grown from the 1-aminopyrene core by a divergent approach using DBOP as the condensing agent.[42] Coupling reactions for the synthesis of the protected-G1, G2, and G3 were conducted by a two-step method[36] consisting of (i) the activation of a carboxylic acid moiety of the AB_2 building block by DBOP to generate an active amide moiety and (ii) the condensation of the active amide with 1-aminopyrene, G1 or G2. The *tert*-butyl carbamate group of the protected-G1, G2, and G3 was deprotected with TFA to afford the cationic charged dendrons (G1, G2, and G3) (Scheme 3). It should be noted that this new synthetic method is the first example of the synthesis of hemi-aliphatic polyamide dendrons by two-step method using DBOP. All products were characterized by IR, ^{1}H NMR spectroscopy, and elemental analysis. The IR spectrum of G3 showed strong absorptions at 3440 and 1681 cm^{-1} due to the characteristic N—H and C═O stretchings of the amide groups, respectively. Furthermore, the characteristic carboxylate and ether stretching were observed at 1592 and 1172 cm^{-1}, respectively (Fig. S2). The ^{1}H NMR spectrum of G3 showed signals corresponding to the amide protons (f, l, and r) at 8.42–8.55 and 10.69 ppm, pyrene protons (s) at 8.04–8.40 ppm, and alkyl protons of the end unit (a) at 2.96 ppm, respectively (Fig. 1).



SCHEME 3 Synthesis of first–third generation water-soluble polyamide dendrons (G1, G2, and G3).

The ^{1}H NMR spectra of G1 and G2 are shown in the supporting information (Fig. S3). Moreover, Figure 2 shows the MALDI-TOF MS spectra of the protected-G2 and G3, which exhibited peaks observed at M/Z ($[M+Na]^+$) = 1389.5 and 2790.5 and well agreed with the calculated mass (1389.7 and 2790.4), together with minor peaks, which could be assignable to partially deprotected dendrons. The deprotection probably occurred during the measurement. Moreover, no peaks derived from dendrons having defect structures were observed in Figure 2. The SEC curves for protected-G1, G2, and G3 showed quite narrow polydispersities (Fig. S4). These findings clearly indicated the formation of the target dendrons.

Photophysical Properties of pDNA Sensibility

The sensing ability of this new series of cationic charged dendrons as a function of the pDNA molar concentration was investigated in an aqueous solution. Figure 3 shows the UV-vis spectra of G1, G2, and G3 with various concentrations of pDNA in an aqueous solution. The dendron concentration was fixed at 5.0 μM. The absorption peak attributed to pyrene at 340 nm was observed in the UV-vis spectra of G1, but it gradually decreased and red-shifted when pDNA was added to the G1 aqueous solution [Fig. 3(A)]. The absorption spectra of G2 and G3 showed the absorption peak of pyrene at 347 nm and slightly red-shifted to 350 nm with the increasing pDNA concentration [Fig. 3(B,C)]. The red shift due to pyrene groups absorption may be caused by the formation of the dendron/pDNA complex. Although the pyrene absorption peak of G2 kept increasing during the addition of pDNA, the pyrene absorption peak of G3 increased up to a 15 mM pDNA solution addition and then became saturated. This is probably because the larger molecular size and more charged terminal groups of G3 compared with the G2 ones induced a higher affinity with pDNA and formed a stiffer aggregation. The formation of the G3/pDNA complex aggregation did not change with the further addition of pDNA.

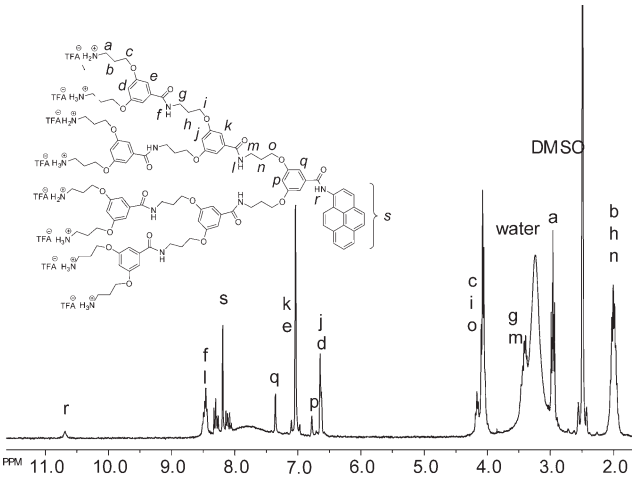


FIGURE 1 ^{1}H NMR spectrum of third generation dendron (G3).

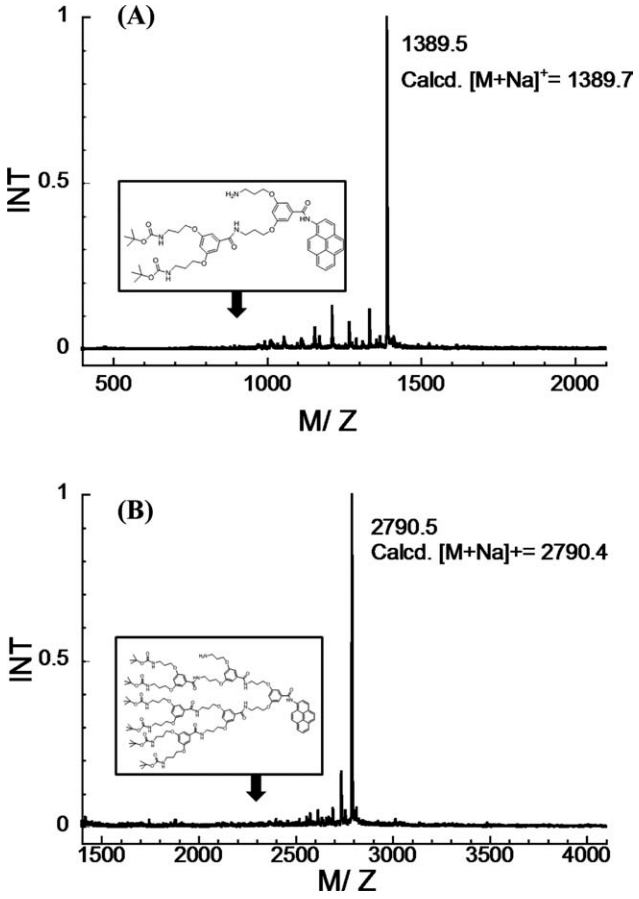


FIGURE 2 MALDI-TOF-MS spectrum of (A) protected-second generation dendron (protected-G2), and (B) protected-third generation dendron (protected-G3).

The fluorescence emission spectra of the G1, G2, and G3 dendrons with the increasing pDNA concentration are shown in Figure 4. The emission at 385 nm corresponds to the monomeric pyrene emission. Without pDNA, G1 showed a strong excimer fluorescence at 442 nm due to intermolecular π–π stacking, however, no excimer emission was observed for G2 and G3. This is attributed to the large dendron size and more highly charged groups of G2 and G3 that resulted in the increased steric hindrance and charge repulsion to form the pyrene excimer. When the pDNA concentration increases, G1 gradually showed a decrease in the fluorescent emission from the pyrene excimer. However, G2 and G3 initially showed an enhancement of the fluorescent emission, and then a decrease in its fluorescence intensity with an increase in the pDNA concentration, which is different from the behavior of the G1/pDNA complexes. This result indicates that the excimer formation of pyrene might be strongly dependent on the size and hydrophobicity of the dendrons because G2 and G3 have a larger size and more hydrophobic units than G1.[27] In addition, the formation of the G2/pDNA and G3/pDNA complexes reduces the electrostatic repulsion between the cationic charges of G2 and G3, which gives rise to the formation of more pyrene excimers. To illustrate the effect of the ratios of the positive charge to negative charge on the emission of the pDNA/dendrons in detail, the fluorescence intensities for the G1–G3 dendrons are plotted versus the various ratios of the pDNA/dendrons (Fig. 5).

As shown in the figure, the G1–G3 dendrons exhibited a different fluorescence tendency and maximum emission intensities at the different pDNA/dendrons charge ratios. Initially,

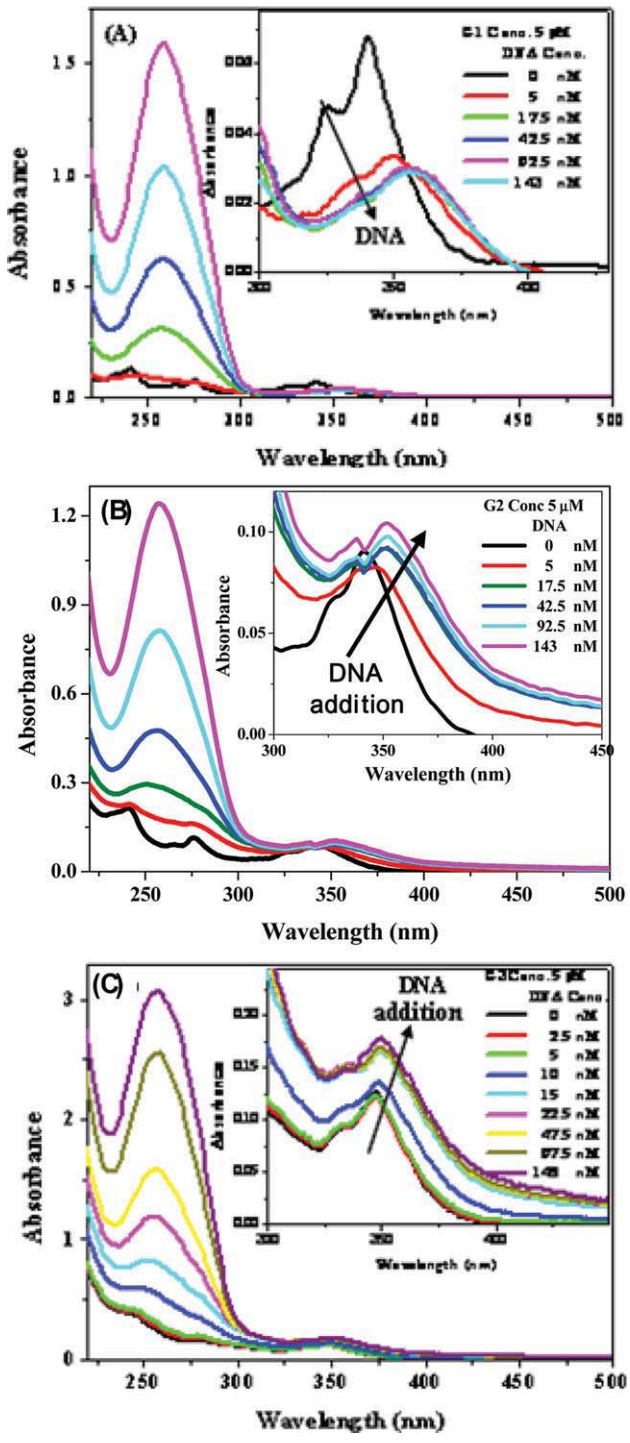


FIGURE 3 UV-vis spectra of (A) G1, (B) G2 and (C) G3 as a function of added pDNA.

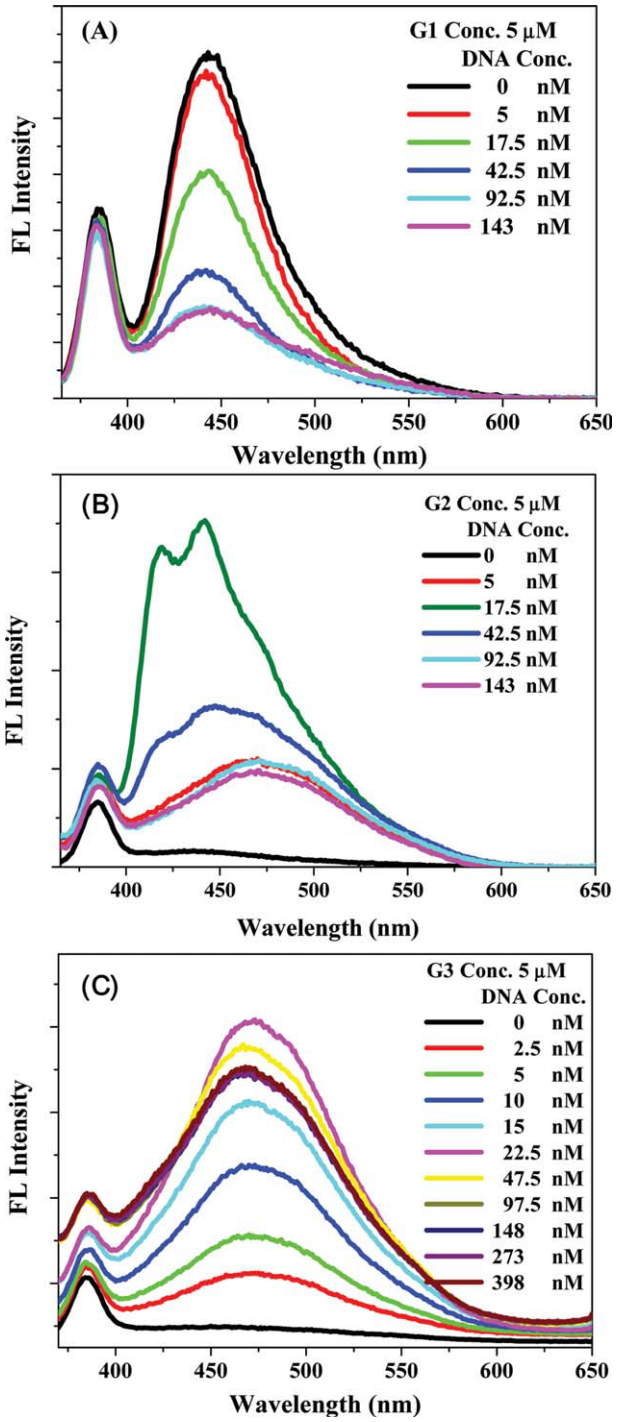


FIGURE 4 Fluorescence spectra of (A) G1, (B) G2, and (C) G3 as a function of added pDNA.

with the increasing pDNA/dendrons ratios, pDNA plays a role in either the disruption or helps with the formation of the pyrene excimers, which gradually decrease or increase the emission intensities of dendrons. This result also indicates the interactions between the pDNA and cationic dendrons are significantly dependent on the cationic charged moieties and hydrophobicity of the dendron generation. Actually, the fluorescent emission intensities of G1 kept decreasing after the addition of pDNA, suggesting that the pyrene excimer formation was disrupted by the electrostatic interactions between the negatively charged pDNA and positively charged amino groups in the dendrons. When excess pDNA was added (charge ratio > 1) to the G2 aqueous solution, the fluorescence intensities decreased and reached saturation. This might be because an excessive amount of pDNA reduces the number of dendrons attached to each pDNA strand and thus prevents formation of the pyrene excimers. On the other hand, G3 showed only a slight decrease in the fluorescence intensities after the charge ratio of 1. G3, which possesses a greater number of charged terminal groups probably has a stronger interaction with pDNA and formed aggregates during the addition. The aggregates might not collapse with the further addition of pDNA. These results agree with the results of UV-VIS spectra [Fig. 3(B,C)].

Morphologies of Self-assembled Pyrene Dendrons and pDNA Complexes

The morphologies of G1–G3 dendrons and dendron/pDNA complexes were also investigated by TEM with a fixed concentration of dendrons and the pDNA/dendrons ratio of 3.0. (Fig. 6). Without addition of pDNA, interesting morphologies of G1–G3 dendrons are observed. G1 dendron showed the aggregation of the sphere-like nanostructures with diameter around 20–60 nm [Fig. 6(A)]. G2 and G3 dendrons exhibit entangled fibrous network with widths around 20–100 nm for G2 and 15–80 nm for G3, respectively [Fig. 6(B,C)]. On the other hand, when addition of the pDNA into the dendron solutions, the large lamellar-like sheet of G1/pDNA complexes is observed in Figure 6(D), however, the small lamellar aggregations of G2/pDNA and G3/pDNA complexes are shown in Figure 6(E,F), respectively. The morphologies of the dendron/pDNA complexes are significantly different from

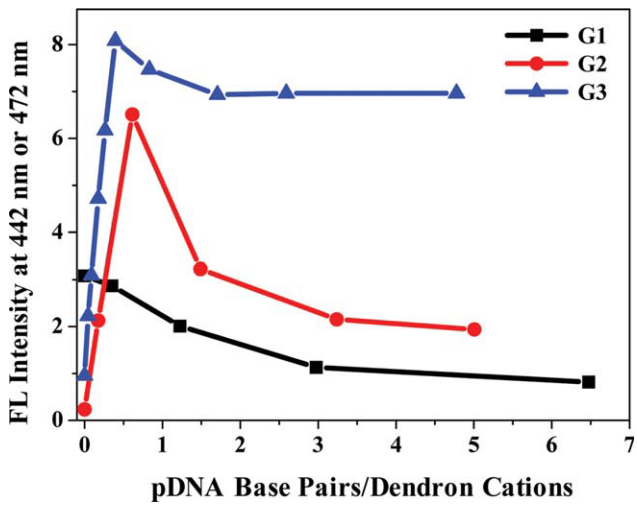


FIGURE 5 Fluorescence emissions at 442 nm for the G1 dendron and 472 nm for G2 and G3 dendrons with concentration of 5 μM and various ratios of the pDNA base pairs (negative charge)/dendron cations in aqueous solution.

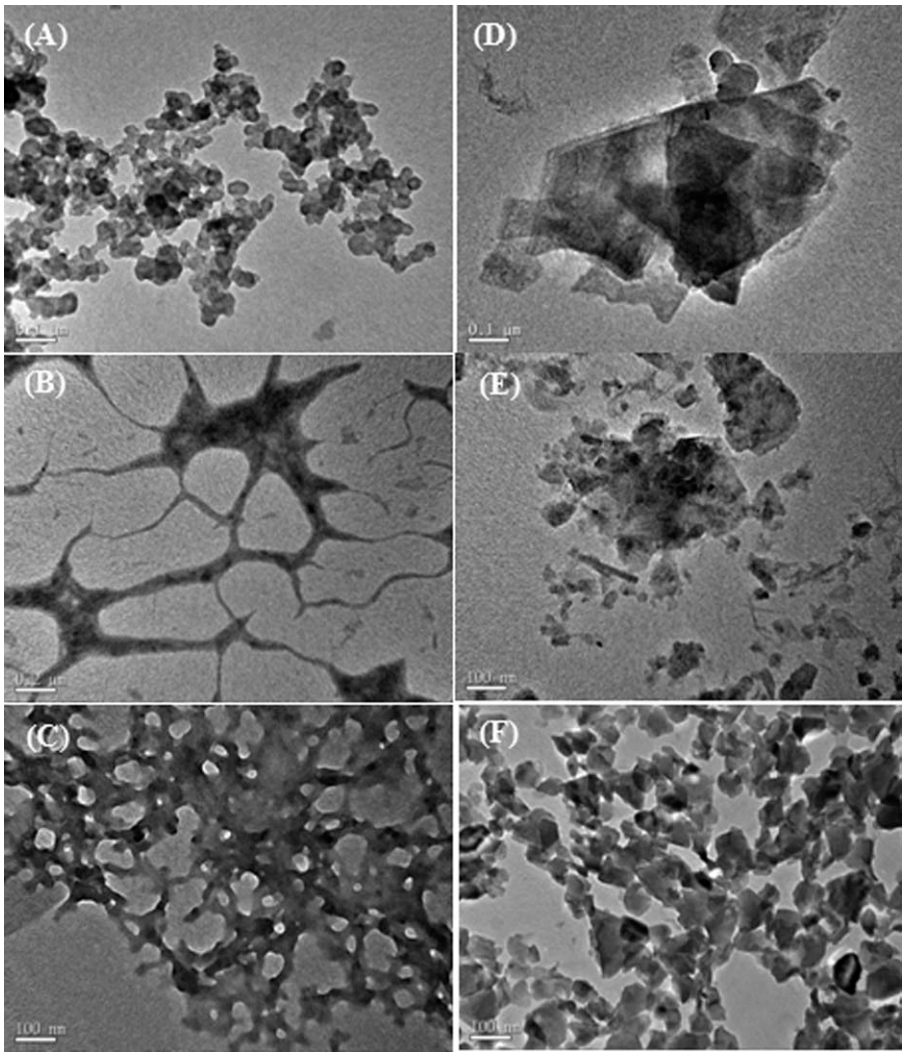


FIGURE 6 TEM images of self-assembled (A–C) G1–G3 dendrons, and (D–F) G1–G3 dendrons/pDNA complexes, respectively.

those of G1–G3 dendrons, which indicated the existence of electrostatic interactions between the negatively charged pDNA and positively charged amino groups in the dendrons. The larger lamellar aggregation of G1/pDNA complexes compared to those of G2/pDNA and G3/pDNA complexes might be attributed to the number of the cationic charged moieties in the dendron. Since G1 has the less cationic charges, the more G1 may interact on each pDNA strand. In addition, G2 and G3 with large size may reduce the number of dendrons attached to each pDNA strand due to steric hindrance effect and prevent the formation of large aggregates.

CONCLUSIONS

We have designed and successfully synthesized a new series of water-soluble cationic pyrene-dendrons, G1, G2, and G3. The UV-vis and fluorescence spectral investigation demonstrated that the electrostatic interactions between the pDNA and cationic charged dendrons in an aqueous solution caused a change in the photophysical properties of pyrene due to the formation or disassembly of the pyrene excimers. The fluorescence responses of the pyrene-dendrons upon addition of pDNA were strongly dependent on the size, hydrophobicity and number of cationic charges of the dendrons. The TEM images of dendron/pDNA complexes also demonstrated the existence of electrostatic interactions between the negatively charged pDNA and positively charged amino groups in the dendrons.

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