Self-Association of Protected Newkome-Type Second-Generation Dendrimers at Nanomolar Level Concentrations in Aqueous Solution

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ABSTRACT: Protected Newkome-type second-generation dendrimers (based on Lin's amine) were synthesized with a pyrene moiety attached to the core. The photophysical property in aqueous solution of the protected dendrimers shows self-association behavior in water. Pyrene excimer emission at 475 nm is observed in water even at very low concentrations of protected dendrimer (ca. 5×10^{-8} M). This emission band is absent in other solvents even up to a concentration of 10^{-5} M. The corresponding unprotected dendrimer does not show the pyrene excimer fluorescence. The amide of pyrene butyric acid with *tert*-butylamine shows the formation of excimer, albeit with very low intensity. Quenching studies on the dendrimer with hydrophilic quencher iodide anion (I^-) reveal that there is significant quenching of fluorescence intensity in the case of *N*-*tert*-butyl-4-pyren-1-ylbutyramide as compared to that of the pyrene-attached second-generation protected dendrimer. This shows that the pyrene moiety in the case of the protected dendrimer is significantly shielded from the surrounding.

Introduction

Higher generation dendrimers assume a globular shape and as such act like micelles[1] with the core of the dendrimer completely shielded from the environment. On the other hand, lower generation dendrimers due to the partial shielding of the core from the environment reveal greater detail about the structural features of the dendrimer. Hyperbranch dendrimers[2] have been the focus of recent studies pertaining to structural morphology of dendrimers. Cardona and coworkers[3] have reported the synthesis and the photophysical properties of Newkome-type[4] dendrimers with different fluorescent chromophores (fluorophores). A fluorophore when attached to the core provides insight into the physical properties of the molecule as a whole. Pyrene,[5] due to its long fluorescence lifetime and its characteristic excimer emission, is an ideal fluorophore for such studies. Sluch and co-workers[6] reported the excitation dynamics of poly(allylcarbosilane) dendrimers with pyrene at the core at a concentration of ca. 10^{-3} M. Recently Chou and co-workers[7] reported the self-association of hyperbranched poly(sulfone−amine) dendrimers in water. This was studied by the addition of pyrene-labeled dendrimer to an aqueous solution containing free pyrene and monitoring the resultant excimer emission.

A protected Newkome-type second-generation dendrimer has a hemiellipsoid structure (Figure 1) with the core being accessible to the environment. In this article, we report the self-association of such a dendrimer in water even at nanomolar concentration (5×10^{-8} M).

Experimental Section

Synthesis of Dendrimers. Scheme 1 depicts the synthesis of Lin's amine, monomers **6** and **10**, starting from TRIS. Tris(hydroxymethyl)aminomethane was treated with *tert*-butyl acrylate in the presence of DMSO and NaOH, resulting in the formation of the corresponding triester **6**.[7] Protection of the nitrogen with benzyl chloroformate produced **7**, and hydrolysis of the *tert*-butyl groups using formic acid resulted in the formation of the triacid **8** quantitatively. Triacid **8** when refluxed with catalytic amount of pTSA in ethanol and benzene resulted in the triethyl ester **9** in quantitative yield. Triethyl ester **9** under catalytic hydrogenation yielded the amine derivative **10**. The monomers **6** and **10** were then used for the synthesis of dendrimers, tetraamide **2** and **3**, respectively (Scheme 2).

The triacid **8** was coupled with monomers **6** and **10** under peptide coupling conditions, resulting in the second-generation dendrimers **11** and **12**, respectively. Hydrogenation of the carbamates **11** and **12** gave the corresponding free amines **13** and **14**, respectively, which were then coupled to pyrenebutyric acid under similar peptide coupling conditions as was used for the preparation of the second-generation dendrimers **11** and **12** (Scheme 2). Using similar reaction conditions, monoamide **1** was synthesized from pyrenebutyric acid and *tert*-butylamine.

Reagents for Synthesis. All chemicals were reagent grade. Tetrahydrofuran (THF) used was freshly distilled from sodium benzophenone. Column chromatography was performed on silica gel (100−200 mesh) while TLC was performed on aluminum-backed plates coated with 0.25 mm silica gel.

***N*-Tris[(2-{[(tris{[2-(*tert*-butoxycarbonyl)ethoxy]methyl}methyl)amino]carbonyl}ethoxy)methyl]methyl}-4-pyren-1-ylbutyramide (Tetraamide 2)**. To a solution of 4-(1-pyrene)butyric acid (58 mg, 0.2 mmol) in dry THF (1 mL) was added EDCl (46 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol), and Et_3N (34 μL, 0.2 mmol). To the stirred solution at room temperature was added *N*-tris[(2-{[(tris{[2-(*tert*-butoxycarbonyl)ethoxy]methyl}methyl)amino]carbonyl}ethoxy)methyl]methylamine[8] (360 mg, 0.2 mmol) dissolved in dry THF (3 mL). The reaction was stirred at room temperature, and after the completion of reaction, as indicated by TLC, THF was removed under vacuum, and the reaction mixture was diluted with EtOAc (10 mL) and washed with water (5 mL), 0.5 M HCl solution (5 mL), and then with saturated solution of brine (5 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the crude compound, which was purified over silica gel using 50−80% gradient elution of EtOAc in hexane to yield the pure pyrene attached dendrimer, tetraamide **2** (200 mg), in 48% yield. IR (neat): 3440, 2912, 1728, 1667, 1523, 1369,

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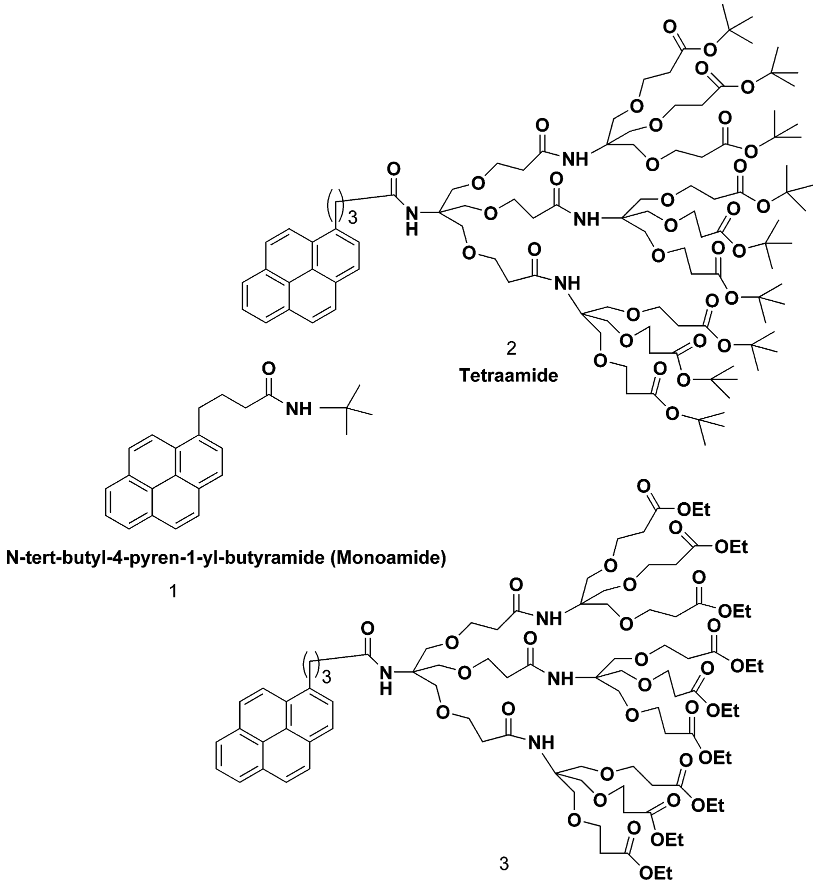


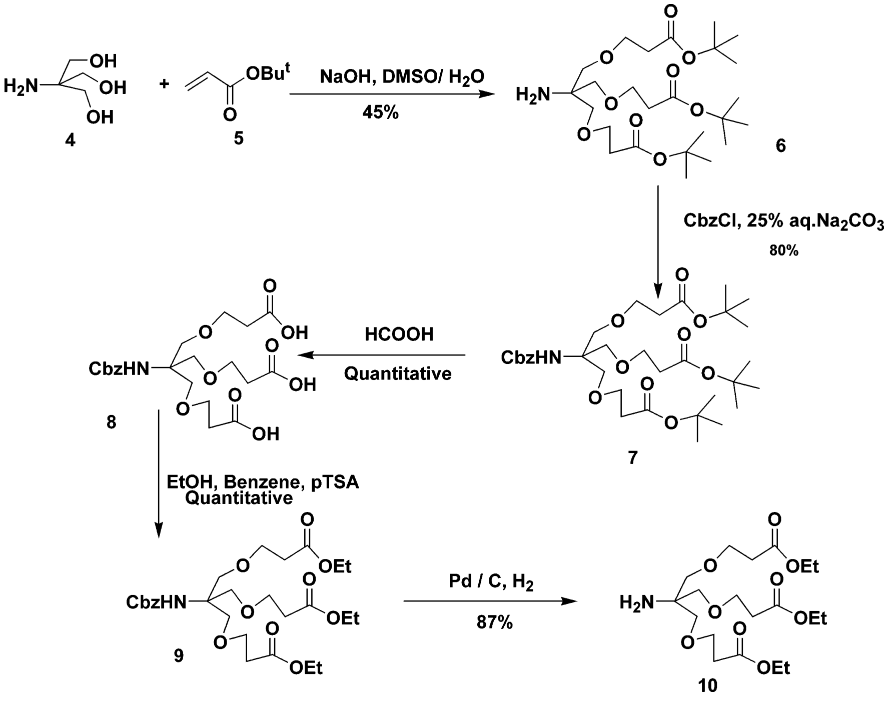
2
Tetraamide

N-tert-butyl-4-pyren-1-yl-butyramide (Monoamide)
1

3
Tetraamide

Figure 1. Second-generation dendrimer having *tert*-butyl and ethyl groups at the periphery (tetraamides **2** and **3**, respectively) and the *N*-*tert*-butyl-4-pyren-1-ylbutyramide {monoamide **1**}.

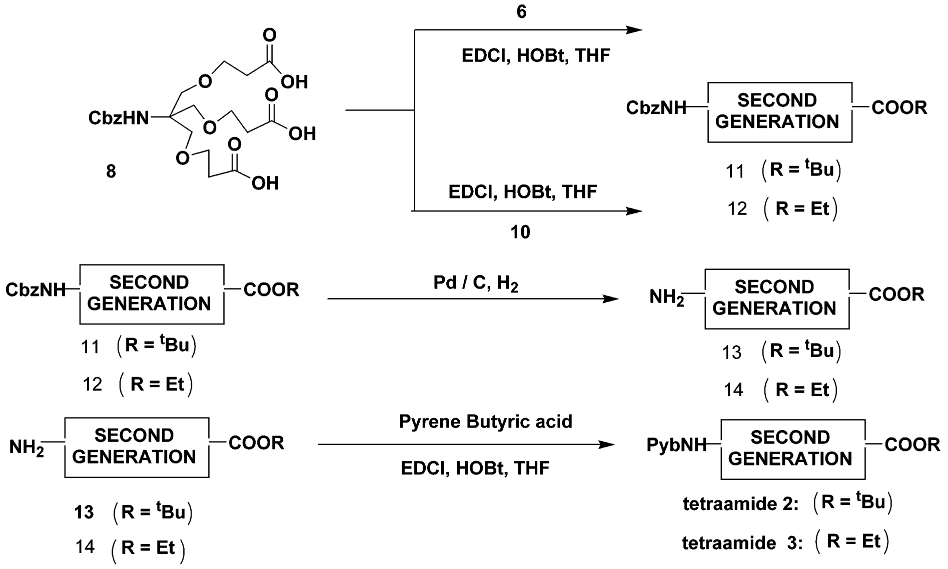
Scheme 1



1257 cm^{-1}. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.42 (s, 81H); 2.20−2.44 (m, 28H); 3.57−3.75 (m, 52H); 6.35 (bs, 3H); 7.72−8.30 (m, 9H). ^{13}C NMR (CDCl$_3$, 100 MHz): δ 27.9, 28.0, 36.0, 37.0, 59.8, 60.0, 60.3, 67.0, 67.4, 69.0, 80.4, 123.4, 124.6, 124.7, 124.8, 124.9, 125.0, 125.6, 125.7, 126.4, 127.2, 127.3, 127.4, 128.6, 129.7, 136.1, 170.8, 171.0, 173.1. MALDI−TOF MS (α-cyano-4-hydroxycinnamic acid matrix): 2069.11 (M$^+$, calcd 2069.35), 2092.89 (M + Na)$^+$, 2108.79 (M + K)$^+$.

N**-Tris[(2-{[(tris{[2carboxyethoxy]methyl}methyl)-amino]carbonyl}ethoxy)methyl]methyl}-4-pyren-1-ylbutyramide (Unprotected Dendrimer).** Tetraamide **2** (140 mg, 0.07 mmol) was stirred in 10 mL of 96% formic acid for

Scheme 2



24 h. Then the formic acid was removed at reduced pressure to produce the title compound in quantitative yield. ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 2.20−2.44 (m, 28H); 3.57−3.75 (m, 52H); 6.35 (bs, 3H); 7.72−8.30 (m, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): 28.0, 36.0, 37.0, 60.0, 60.3, 67.0, 67.4, 69.0, 80.4, 123.4, 124.6, 124.7, 124.8, 124.9, 125.0, 125.6, 125.7, 126.4, 127.2, 127.3, 127.4, 128.6, 129.7, 136.1, 170.8, 171.0 MS (ESI): 1565 (M + 1).

Synthesis of *N-tert*-Butyl-4-pyren-1-ylbutyramide (Monoamide 1). To a stirred solution of 4-(1-pyrene)butyric acid (50 mg, 0.17 mmol) in THF (1 mL) was added HOBt (24 mg, 0.17 mmol) at room temperature. The reaction mixture was stirred for 5 min, after which Et_3N (29 μL, 0.2 mmol) was added followed by EDCl (40 mg, 0.2 mol). Finally, *tert*-butylamine (22 μL, 0.2 mol) was added dropwise to the reaction mixture. The reaction mixture was then allowed to stir at room temperature. After completion of the reaction THF was removed under vacuum, and the crude mass was diluted with ethyl acetate (10 mL). The organic layer was then washed successively with 0.5 M HCl (5 mL) and saturated brine solution (5 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the crude compound, which was purified over silica gel using gradient elution of 20−30% EtOAc in hexane to afford the pure compound (28 mg) in 47% yield. IR (neat): 3392, 2998, 1721, 1657, 1257 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.2 (s, 9H), 2.10 (bs, 4H), 3.27 (t, J = 6.9 Hz, 2H), 5.15 (s, 1H), 7.16−8.20 (m, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 27.5, 28.3, 32.6, 36.8, 51.2, 123.4, 124.7, 124.9, 125.0, 125.1, 125.9, 126.7, 127.3, 127.4, 127.5, 128.8, 129.9, 130.9, 136.0, 172.0. MS (ESI): 344 (M + 1), 343 (M$^+$).

Benzyl *N*-Tris[2-(ethoxycarbonyl)ethoxy]methyl}-methylcarbamate (9). A mixture of triacid **8**[7] (1.5 g, 3.18 mmol) and a catalytic amount of pTSA in ethanol (3 mL) and benzene (7 mL) was refluxed using a Dean−Stark apparatus. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and washed with a saturated solution of $NaHCO_3$. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated to give the crude compound. The crude compound was purified by column chromatography using 20% ethyl acetate−hexane as the eluent to give the pure benzyl *N*-tris[2-(ethoxycarbonyl)ethoxy]methyl}-methylcarbamate in quantitative yield (1.7 g). IR (neat): 3392, 2928, 1734, 1507 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.73 (t, J = 6.8 Hz, 9H), 3.02 (t, J = 6.2 Hz, 6H), 4.15 (s, 6H), 4.18 (t, J = 6.4 Hz, 6H), 4.62 (q, J = 7.0 Hz, 6H), 5.53 (s, 2H), 5.75 (s, 1H), 7.77−8.03 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.2, 35.0, 58.7, 60.4, 66.1, 66.8, 69.4, 127.9, 128.0, 128.3, 136.7, 155.0, 171.4. MS (ESI): 557 (M + 1).

Tris[2-(ethoxycarbonyl)ethoxy]methyl}methylamine (10). To a solution of compound **9** (1.7 g, 3 mmol) in ethanol (15 mL) was added 10% Pd/C (170 mg), and the mixture was stirred at room temperature under an atmosphere of hydrogen (balloon). After completion of the reaction catalyst was filtered using a pad of Celite, and the filterate was concentrated under reduced pressure to yield the pure amine **10** (1.1 g, 87% yield). IR (neat): 3440, 2992, 1731 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, J = 7.0 Hz, 9H), 1.68 (bs, 2H), 2.54 (t, J = 6.3 Hz, 6H), 3.31 (s, 6H), 3.69 (t, J = 6.3 Hz, 6H), 4.14 (q, J = 7.0 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.2, 35.1, 60.4, 66.8, 66.98, 72.8, 171.5. MS (ESI): 422 (M + 1).

Benzyl *N*-Tris[(2-{[(tris{[2-(ethoxycarbonyl)ethoxy]-methyl}methyl)amino]carbonyl}ethoxy)methyl] Methylcarbamate (12). To a solution of triacid **8** in dry THF (6.0 mL) was added HOBt (115 mg, 0.85 mmol), Et_3N (0.42 mL, 3.5 mmol), and EDCl (575 mg, 3.5 mmol). The amine **10** in THF was then added, and the reaction mixture was stirred at room temperature for 24 h. After completion of the reaction, the THF was removed under vacuum and the reaction mixture was diluted with ethyl acetate. The organic layer was then washed with 0.5 M HCl and brine, separated, dried over anhydrous Na_2SO_4, and concentrated to yield the crude compound, which was then purified by column chromatography using 70−80% EtOAc in hexane as the eluent to yield the pure second-generation dendrimer, **12** (900 mg, 63% yield). IR (neat): 3360, 2944, 1731, 1664 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, J = 7.1 Hz, 27H), 2.40 (t, J = 6.3 Hz, 6H), 2.52 (t, J = 6.2 Hz, 18H), 3.64−3.69 (m, 48H), 4.13 (q, J = 7.0 Hz, 18H), 5.03 (s, 2H), 6.22 (s, 4H), 7.30−7.34 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.2, 21.0, 34.9, 37.2, 58.8, 59.7, 60.3, 60.6, 67.5, 69.0, 127.9, 128.0, 128.3, 128.4, 136.7, 151.1, 171.0, 171.7. MS (ESI): 1680 (M$^+$).

***N*-Tris[(2-{[(tris{[2-(ethoxycarbonyl)ethoxy]methyl}-methyl)amino]carbonyl}ethoxy)methyl] Methylcarbamate (14).** To a solution of ester **12** (900 mg, 0.53 mmol) in ethanol (7 mL) was added 10% Pd/C (90 mg). The reaction mixture was stirred at room temperature for 24 h under a hydrogen atmosphere (balloon). After completion of the reaction the catalyst was filtered using a pad of Celite, and the filterate was concentrated to yield the pure amine **14** (700 mg, 85% yield). IR (neat): 3360, 2944, 1731, 1664 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (t, J = 7.1 Hz, 27H), 2.43 (t, J = 6.0 Hz, 6H), 2.53 (t, J = 6.2 Hz, 18H), 3.67−3.73 (m, 48H), 4.14 (q, J = 7.0 Hz, 18H), 6.35 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.2, 18.3, 34.9, 37.2, 58.2, 59.8, 60.4, 60.5, 66.7, 66.9, 171.7, 1714.6. MS (ESI): 1547 (M + 1).

***N*-Tris[(2-{[(tris{[2-(ethoxycarbonyl)ethoxy]methyl}-methyl)amino]carbonyl}ethoxy)methyl]methyl}-4-pyren-1-ylbutyramide (Tetraamide 3).** To a stirred solution of 4-(1-pyrene)butyric acid (141 mg, 0.49 mmol) in dry THF (2 mL) at room temperature was added EDCl (94 mg, 0.49 mmol), HOBt (66 mg, 0.49 mmol), and Et_3N (68 μL, 0.49 mmol)

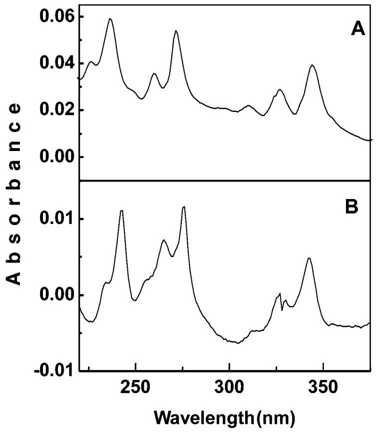


Figure 2. UV spectra of (A) tetraamide **2** and (B) monoamide **1** (both at 10^{-5} M concentration) in water.

followed by a solution of *N*-tris-[(2-{[(tris{[2-(ethoxycarbonyl)-ethoxy]methyl}methyl)amino]carbonyl}ethoxy)methyl]-methylamine (700 mg, 0.45 mmol) in dry THF (5 mL). After the completion of reaction, as indicated by TLC, THF was removed under vacuum, and the reaction mixture was diluted with EtOAc (15 mL) and washed with water (10 mL), 0.5 M HCl solution (10 mL), and then with saturated solution of brine (10 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the crude compound, which was purified over silica gel using 50−80% gradient elution of EtOAc in hexane to yield the pure pyrene attached dendrimer **3** (600 mg) in 73% yield. IR (neat): 3360, 2928, 1734, 1667, 1545, 1523, 1472, 1372, 1187 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.23 (t, $J = 7.2$ Hz, 27H), 2.04−2.18 (m, 4H), 3.36−3.40 (m, 2H), 2.42 (t, $J = 6.3$ Hz, 6H), 2.48 (t, $J = 6.2$ Hz, 18H), 3.61−3.75 (m, 48H), 4.10 (qt, $J = 7.2$ Hz, 18H), 6.20 (s, 3H), 6.66 (s, 1H), 7.22−8.35 (m, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.7, 21.0, 27.6, 34.9, 36.5, 37.1, 59.7, 59.9, 60.3, 60.4, 66.7, 67.5, 69.0, 123.5, 124.6, 124.7, 124.8, 124.9, 125.0, 125.7, 126.5, 127.4, 127.5, 128.7, 129.7, 130.9, 131.3, 136.3, 171.2, 171.5, 171.6. MS (ESI): 1840 $(M + Na)^+$.

Materials for Photophysical Studies. The stock solution (10^{-3} M) for all the studies was prepared using acetonitrile. The stock solution was diluted to an appropriate concentration using water. Aqueous solutions of dendrimer were made up by using triply distilled water. KI for quenching studies was bought locally and used as such.

Instrumentation. ^1H and ^{13}C were recorded on a 400 MHz Bruker NMR spectrometer. MALDI−TOF mass spectra of the pyrene attached protected dendrimers were recorded on an Applied Biosystems Voyager System 6316 using α-cyano-4-hydroxycinnamic acid as the matrix. Ultraviolet (UV) spectra were measured on a Perkin-Elmer Lamda 25 spectrophotometer. Fluorescence measurements were recorded on a Hitachi F-4500 spectrofluorimeter. The excitation wavelength was fixed at 342 nm, and emission spectra were recorded in the wavelength range 352−600 nm with the excitation and emission slit width 2.5 nm. For the excitation spectra, the emission wavelength was fixed at 396 nm. The value of I_E/I_M was the ratio of the emission intensity at 475 nm (excimer) to the intensity at 376 nm (monomer).

Results and Discussion

The protected second-generation dendrimers (tetraamides **2** and **3**) with pyrene in the core and the amide *N-tert*-butyl-4-pyren-1-ylbutyramide (monoamide **1**) are shown in Figure 1.

The UV spectra of the tetraamide **2** and monoamide **1** show the typical absorption spectral features of pyrene (Figure 2). The vibronic bands of the longest wavelength transition (300−350 nm) in monoamide **1** are somewhat less sharp than those of tetraamide **2**. Some protection to the chromophore offered by the comparatively larger size and hydrophobicity of the dendrimer could be a reason for the observation.

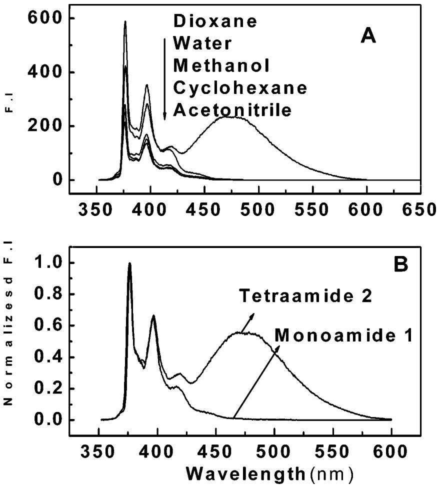


Figure 3. (A) Emission spectra of tetraamide **2** in different solvents. (B) Normalized spectra of monoamide (10^{-5} M) and tetraamide **2** (10^{-5} M) in water [λ_{ex} at 342 nm].

The emission spectra of the tetraamide **2** in different solvents are shown in Figure 3 A.

The structural features of pyrene monomer emission are very similar in all solvents. Only in water medium an intense, structureless, broad, and highly red shifted emission at 475 nm is observed, which is readily ascribable to excimer emission. This and the observation that there is a total absence of this new emission in all other solvents suggests that the excimer emission could be arising out of a water-induced hydrophobic aggregation of the molecules. Tetraamide **2** is moderately large and fairly hydrophobic. In a nonpolar solvent like acetonitrile, both monoamide **1** and tetraamide **2** showed similar fluorescence spectra, corresponding to monomer emission only. It was further noticed that, in acetonitrile, the fluorescence intensity of tetraamide is not substantially enhanced as compared to that of monoamide. Thus, in this nonaggregating solvent, pyrene moieties for both tetraamide and monoamide experience similar environments.

To ascertain that the aggregation is hydrophobic in nature, the same studies were carried out with the monoamide **1**. The monoamide **1** did not give the excimer peak with the same intensity as the tetraamide **2** (Figure 3B).

On decreasing the hydrophobicity of the dendrimer end group, from a bulky *tert*-butyl to a relatively less bulky ethyl group, tetraamide **3**, the same trend was observed as in the case of tetraamide **2**. This, coupled with the fact that the unprotected dendrimer, where there are nine acid groups instead of the esters, did not show any excimer formation (Figure 4), confirms that the excimer formation is a consequence of hydrophobic aggregation.

Figure 5 gives the normalized emission spectra at different concentrations of tetraamide **2**. It is seen that even at a very low concentration of 5×10^{-8} M there is an appreciable emission due to the excimer. With increasing concentration there is an initial increase in excimer intensity followed by a leveling effect at higher concentrations. The plot of excimer-to-monomer ratio (I_E/I_M) of the tetraamide **2** against concentration shows that there is an initial increase in the ratio of I_E/I_M.

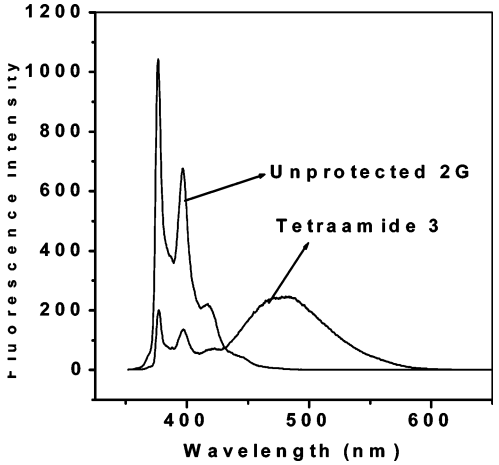


Figure 4. Comparison of the emission spectrum of the deprotected dendrimer and the tetraamide **3** in water (10^{-5} M).

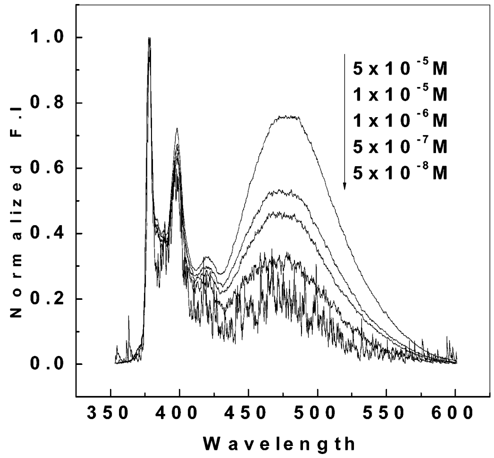


Figure 5. Emission spectra of tetraamide **2** in water at different concentrations, normalized at 0–0 vibronic band [λ_{ex} at 342 nm].

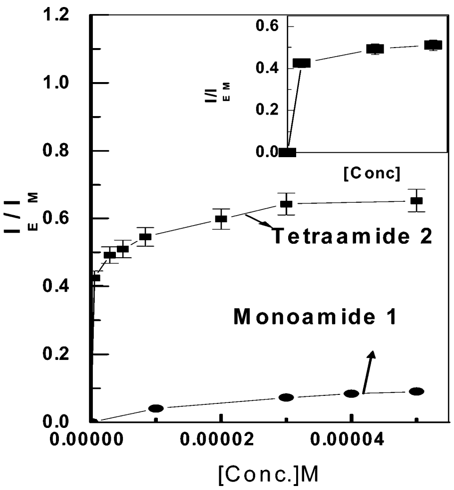


Figure 6. Plot of I_E/I_M ($\lambda_{475}/\lambda_{376}$) with concentration of tetraamide **2** and monoamide **1**. Inset depicts the variation of I_E/I_M between 5×10^{-7} and 5×10^{-6} M of tetraamide **2** [λ_{ex} at 342 nm].

Further increase of concentration results in saturation of the I_E/I_M (Figure 6). A similar plot was obtained for the tetraamide **3**. On the other hand, the ratio is not significantly affected by the change in concentration in the case of the monoamide.

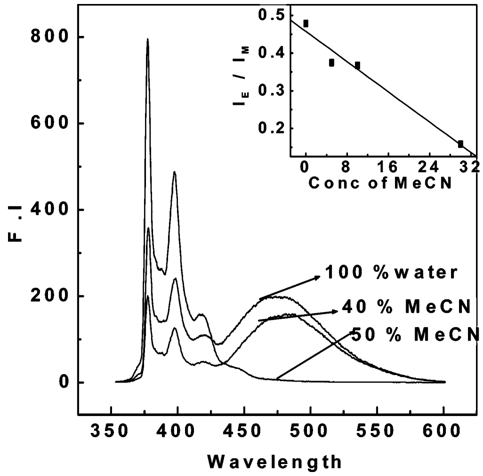


Figure 7. Change in emission spectrum with increasing vol % of acetonitrile in a water–acetonitrile mixture of tetraamide **2** at 10^{-6} M, normalized at the 0–0 vibronic band.

Since at 10^{-5} M concentration of tetraamide **2** the excimer emission is completely absent in acetonitrile, progressive addition of acetonitrile to water should decrease the relative excimer emission intensity. If the excimer formation is related to aggregation, then this emission should disappear when there is complete disaggregation.

Figure 7 shows the effect of change in the excimer intensity with increasing amount of the acetonitrile in water. It was observed that on increasing the concentration of acetonitrile there is gradual decrease in the emission intensity of the excimer, and at 50% v/v the excimer peak completely disappears (Figure 7).

A similar disggregation was also observed in the case of tetraamide **3**; the disaggregation was complete at 25% v/v of acetonitrile in water. The relatively lesser hydrophobicity of ethyl groups of tetraamide **3**, as compared to the tertiary butyl groups of tetraamide **2**, appears to be a likely reason for this observation.

Quenching studies on the tetraamide **2** in water by using hydrophilic fluorescence quencher can provide information regarding the accessibility of the pyrene core to the aqueous environment. We carried out the quenching studies with hydrophilic I^- as the quencher which essentially quenches by the heavy atom effect.[9] The quenching studies with amide revealed a significant quenching of monomer fluorescence band with 0.02 M solution of I^-. However, there was only a weak quenching in the case of tetraamide **2** even with 0.5 M solution of I^-. The variation of very weak excimer emission of monoamide **1** as a function of quencher concentration could not be monitored.

Figure 8 gives the Stern–Volmer plots for the quenching of monomer emissions of monoamide **1,** tetraamide **2**, and tetraamide **3**, as per the Stern–Volmer equation

$$I_0/I = 1 + K_{SV}[Q] = 1 + k_q\tau[Q]$$

In this expression, I_0 and I are the fluorescence intensities in the absence and presence of quencher, respectively, K_{SV} is the Stern–Volmer quenching constant, k_q is the bimolecular quenching constant, τ is the fluorescence lifetime in the absence of quencher, and [Q] is the analytical concentration of the quencher. We find that the Stern–Volmer plot is linear over the concentration range of the quencher.

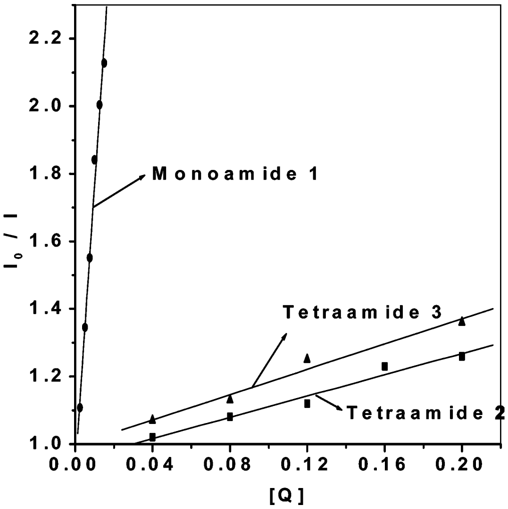


Figure 8. S−V plot of monoamide **1**, tetraamide **2**, and tetraamide **3** in water.

The K_{SV} value for monoamide **1** is 80 M^{-1}, for tetraamide **2** it is 1.3 M^{-1}, and for tetraamide **3** it is 1.8 M^{-1}. Thus, there is a very substantial reduction of quenching efficiencies in the case of tetraamide **2** and tetraamide **3**. We could not measure the fluorescence lifetime of these molecules. Taking a rough value of 200 ns for pyrene in water,[10] the corresponding bimolecular quenching constant k_q is 4.0×10^8 M^{-1} s^{-1} for monoamide **1**, 6.5×10^6 M^{-1} s^{-1} for tetraamide **2**, and 9.0×10^6 M^{-1} s^{-1} for tetraamide **3**. Since the bimolecular diffusion constant, k_{diff}, for water is 8×10^9 M^{-1} s^{-1},[11] it is seen that k_q of monoamide **1** is close to the diffusion-controlled limit. The marked reduction of k_q for tetraamide **2** and tetraamide **3** indicates that the accessibility of pyrene to the hydrophilic I^- is significantly restricted because of the aggregation of the protected dendrimers. Thus, the pyrene excimer moiety is reasonably protected from the aqueous environment in these aggregates. The slightly higher quenching efficiency in the case of tetraamide **3** as compared to the tetraamide **2** could be due to the relatively greater accessibility of the quencher to the pyrene core in the case of tetraamide **3**.

Conclusions

In this dendrimer architecture, the hydrophobicity provided by ester functional groups results in aggregation, even at nanomolar concentrations. The hydrophobic self-association of this dendrimer in aqueous medium, as reflected in the appearance of the pyrene excimer emission even at nanomolar concentration of 5×10^{-8} M, is a novel observation. Pyrene appears to be reasonably protected from the aqueous environment in the aggregated form.

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