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Synthesis and Characterization of Anionic Triazine Dendrimers with a Labile Disulfide Core

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Abstract

Anionic dendrimers based on melamine with disulfide bonds at the core were prepared to investigate the solubility of these architectures, the ability of these molecules to solubilize pyrene as a model drug, and the ability of these architectures to undergo thiol–disulfide exchange. The ability to solubilize pyrene is directly correlated with molecular weight of the dendrimer—aggregation of dendrons does not occur. Thiol–disulfide exchange occurs rapidly using dithiothreitol as the reductant to yield dendrimers with thiol cores that can undergo oxidation in air to yield the original dendrimer.

INTRODUCTION

The incorporation of thiols into dendrimers has been exploited to prepare monolayers on gold,¹ encapsulate metal centers,² metal clusters,³ and nanoparticles,⁴ and to end-functionalize polymers.⁵ In addition to these materials applications, we and others are exploring the use of dendrimers in drug delivery. Disulfides can facilitate attachment⁶ and/or release⁷ of small molecules or breakdown of the dendrimer vehicle.⁸ These events commence on encountering a reductant, such as glutathione, cysteine, or homocysteine, present at low concentrations in the vasculature⁹ but at markedly higher concentrations in hypoxic tumors or intracellularly.^{10–11}

In a series of reports on a dendrimer with a disulfide core, Takaguchi and coworkers showed that polyamidoamine (PAMAM) dendrimers undergo reduction with dithiothreitol (DTT) and can be reoxidized in air.^{8b} Interestingly, they note an increased solubilizing potential of the thiol-containing dendron over the larger disulfide dendrimer using phenol blue as a guest. This difference is attributed to the formation of micellar-type architectures of the thiol-containing dendron. Our interest in using dendrimers based on melamine for drug delivery led us to explore related molecules. We chose to investigate architectures with carboxylates on the surface to compare the solubility and sequestration potential to cationic dendrimers of similar structure.¹³ The targets explored in this study are shown in Chart 1.

The guest for these studies is pyrene, a common choice for such studies, and accordingly, one that allows for comparisons across a range of dendrimer families including our triazines, PAMAM,¹⁴ the polypropyleneimines (PPI),¹⁵ and Frechet's aryloethers.¹⁶ The extinction coefficient to environment.¹⁷

The goals of these studies were (i) to develop an efficient route for incorporating a disulfide group at the core of dendrimer, (ii) to investigate the solubility properties of these anionic dendrimer hosts, (iii) to quantify and compare the solubilization of pyrene in these architectures to related cationic triazine dendrimers and other dendrimer families, (iv) to evaluate thiol–

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disulfide exchange in these dendrimers and potential differences in solubilization efficiency of the thiol-containing dendron and disulfide-containing dendrimer, and (v) to probe for evidence of micellar assembly by either the dendrimer or dendrons.

SYNTHESIS OF DENDRONS

Scheme 1 shows the convergent synthetic route applied to these dendrons prior to incorporation of the disulfide core. A procedure similar to our previous convergent synthesis was used.^{13, 18} The *t*-butyl ester of glycine offers an inexpensive route to the desired carboxylate periphery. Iterative reactions with piperazine and triazine trichloride provide subsequent generation of the dendrons in reasonable yields. As with previously described melamine dendrimers, the iterative nature of the synthesis of these dendrons is reflected in ¹H and ¹³C NMR spectra.^{19, 20} For monochlorotriazines **9** and **11**, the protons and carbons of the piperazine group on the interior of the dendrimer appear at ~3.8 ppm and ~44 ppm, respectively. Upon reaction with piperazine to yield **8**, **10**, and **12**, new lines in the ¹H and ¹³C spectra appear that correspond to the piperazine at the focal point; notably peaks at ~2.5 ppm in the ¹H spectrum that correspond to methylenes of piperazine distant from the side of substitution. The appearance and disappearance of the chlorinated carbon of the monochlorotriazine is also a good indicator of iterative synthesis.

INSTALLATION OF THE DISULFIDE CORE AND DEPROTECTION

The disulfide core of **1–4** was installed in a two-step procedure (Scheme 2). First, the monochlorotriazine dendrons **7**, **9**, **11**, and **13** were reacted with excess ethylenediamine at 100 °C to yield amines **14**, **15**, **16**, and **17**, respectively. These amines were incubated with 3,3'-dithiodipropionic acid di(*N*-succinimidyl ester) at room temperature to afford the protected dendrimers **18**, **19**, **20**, and **21** in 70–80% yields. Subsequent deprotection with TFA proceeds quantitatively to give four generations of water-soluble dendrimers **1–4**. Installation of the core is reflected by new peaks in the ¹H and ¹³C NMR spectra.

CHARACTERIZATION

The purity suggested by these traces and corroborated by NMR analyses is also supported by the MALDI-TOF traces of the intermediates and final products. The latter show a single species with the lines corresponding to the expected adducts with H⁺, Na⁺, and K⁺. The resolution of these spectra provided a convenient opportunity to monitor the reduction of these species upon adding dithiothreitol.

The size exclusion chromatograms (SEC) of the protected disulfide dendrimers are shown in Fig. 1. Unlike other melamine dendrimers comprising *p*-aminobenzylamine as a linking group, these piperazine-based dendrimers do not appear to aggregate, and show better solubility on the SEC column as evident from the absence of significant species (Fig. 1).

ANALYSIS BY CAPILLARY ELECTROPHORESIS

Analytical samples aged five years at room temperature in capped vials were subjected to further characterization by capillary electrophoresis (CE). CE traces for the deprotected disulfide dendrimers are shown in Fig. 2. Though the anionic dendrimer molecules are attracted to the anode (placed at the injection end of the capillary), detection of the analytes was enabled by the significant magnitude of the electroosmotic flow at pH 9.0 (typically between 65 and 46 × 10⁻⁵ cm² V⁻¹s⁻¹). Electropherograms for samples of **1–3** that were not subjected to chromatographic purification steps following a microscale deprotection, showed a peak corresponding to TFA (μ^{eff} ~ -36.5 × 10⁻⁵ cm² V⁻¹s⁻¹). With the high resolving power typical of CE separations, signals for other minor impurities with lower anionic effective mobilities

(i.e., slower anions) were also detected. Analysis of the sample for compound **1** by ESI-TOF-MS in negative ion mode suggests these minor impurities correspond to partially deprotected dendrimers. It is unlikely that these species would have been detected by SEC. Regardless, these data provide compelling evidence for the long-term stability of these materials as well as corroborative data as to the purity of these samples up to generation four.

REDUCING THE DISULFIDE BOND

Disulfide exchange with dithiothreitol (DTT) is an S_N2 reaction with a rate that is very dependent on pH. The high pH required for solubilization of these dendrimers makes evaluating the relative rates of exchange for **1–4** difficult. Qualitatively, when the dendrimer is dissolved at 0.3 mM concentration in a 20 mM phosphate buffer (pH 10) containing 0.9 mM DTT, the exchange reaction is complete within 30 min. The MALDI-TOF spectra are representative of the data produced. Comparison of the relative rates of exchange proved untenable. Neither resolution of these species by chromatography nor assignment of the relative populations through mass spectrometry proved successful.

SOLUBILIZATION OF PYRENE

To eliminate difficulties associated with reoxidation of the thiol-containing dendrons, the corresponding monochlorotriazines were used in place of the products of the reduced dendrimers. The solubilization experiment was performed using an excess of pyrene (deposited as a thin film on the wall of a flask). Following sonication in a phosphate buffer (20 mM, pH 10) with the dendron or dendrimer for 5 minutes, the solution was allowed to equilibrate overnight. The concentration of pyrene in this solution was determined spectrophotometrically (340 nm).

Figure 3a shows that none of the architectures appear to exhibit a critical micelle concentration (CMC) over the concentration range investigated. As expected, the solubilizing ability increases as the size of the architecture increases. Solubilization efficiency appears to be independent of whether the solubilizing agent is a disulfide-containing dendrimer or monochlorotriazine dendron. Figure 3b shows the relationship between molecular weight of the architecture and the solubilizing efficiency of these architectures. This relationship has been observed in many different dendrimer families, including PAMAM, PPI, and Fréchet's aryether dendrimers.^{14–16} Accordingly, cleavage of the dendrimer reduces the solubilization efficiency by a factor of two.

In conclusion, consistent with PAMAM,¹⁴ PPI,¹⁵ and Fréchet's aryether¹⁶ dendrimer families, we observe a linear relationship between solubilization efficiency and size. Accordingly, we do not believe that fragmentation of the dendrimer leads to micelle formation that might trap guest. The use of anionic dendrimers as vehicles for drug delivery does appear to be limited by solubility limitations. However, more recent studies on a different architecture show that substoichiometric esterification with polyols remedies this shortcoming. These dendrimers require pH > 9 for solubility in water. Fréchet's initial reports of dendrimers with *p*-substituted benzoic acids on the periphery showed similar solubility limitations.²⁰ With toxicity associated with some cationic dendrimers,²¹ a survey of the solubility of dendrimers with varying functional group peripheries appears to be in order, although oligo- or poly(ethyleneglycol) may emerge as a convenient alternative due to biocompatibility and related issues.²²

EXPERIMENTAL

General TFA Deprotection (1)

Intermediate **18** (69 mg, 0.02 mmol) was dissolved in 2 mL of CH₂Cl₂–TFA (1:1). After stirring for 12 h, the solvent was removed and an oil was obtained. ¹H NMR (300 MHz, CD₃OD) δ 4.01 (br, 8 H), 3.42 (m, 4 H), 3.29 (m, 4 H), 2.80 (m, 4 H), 2.48 (m, 4 H); ¹³C NMR (75 MHz, CD₃OD) δ 171.50, 170.00, 164.20, 40.43, 38.48, 35.24, 33.73. MS: Calcd 744 (M⁺). Found (ESI-TOF): 745 (M+H⁺).

Dendrimer 2

¹H NMR (300 MHz, CD₃OD) δ 4.07 (br, 16 H), 3.88 (br, 32 H), 3.49 (m, 4 H), 3.34 (m, 4 H), 2.81 (m, 4 H), 2.48 (m, 4 H). ¹³C NMR (75 MHz, CD₃OD) δ 171.42, 169.80, 164.40, 40.43, 38.48, 35.24, 33.73. MS: Calcd 1689.63 (M⁺). Found (ESI-TOF): 845 (M+2H)²⁺.

Dendrimer 3

¹H NMR (300 MHz, DMSO) δ 8.59 (br, 16 H), 4.06 (br, 32 H), 3.79 (br, 96 H), 3.39 (br, 4 H), 3.25 (br, 4 H), 2.89 (m, 4 H), 2.48 (br, 4 H). ¹³C NMR (75 MHz, DMSO) δ 171.49, 171.11, 165.39, 161.47, 44.15, 42.86, 35.80, 34.40. MS: Calcd 3579.53 (M⁺). Found (MALDI-TOF): 3579.21 (M⁺).

Dendrimer 4

¹H NMR (300 MHz, DMSO) δ 8.56 (br, 32 H), 4.02 (br, 64 H), 3.76 (br, 224 H), 3.37 (br, 4 H), 3.23 (br, 4 H), 2.87 (m, 4 H), 2.47 (br, 4 H). ¹³C NMR (75 MHz, DMSO) δ 171.50, 171.21, 165.59, 44.17, 42.96, 35.90, 34.48. MS: Calcd 7355.08 (M⁺). Found (MALDI-TOF): 7373.01 (M+Na⁺).

Dendron 5

¹H NMR (300 MHz, DMSO) δ 8.57 (br, 8 H), 4.07 (br, 16 H), 3.79 (br, 32 H). ¹³C NMR (75 MHz, DMSO) δ 171.11, 165.34, 164.59, 44.10, 42.88. MS: Calcd 1677.64 (M⁺). Found (MALDI-TOF): 1678.58 (M+H⁺).

Dendron 6

¹H NMR (300 MHz, DMSO) δ 8.57 (br, 16 H), 4.07 (br, 32 H), 3.79 (br, 80 H). ¹³C NMR (75 MHz, DMSO) δ 171.15, 165.35, 44.08, 42.88. MS: Calcd 3568.85 (M⁺). Found (MALDI-TOF): 3576.37.

General Triazine Trichloride Reaction (7)

H-Gly-OBu·HCl (3.35 g, 20 mmol) was dissolved in 50 mL of THF before a solution of triazine trichloride (1.84 g, 10 mmol) and *N,N*-diisopropylethylamine (5.3 mL, 30 mmol) in 50 mL THF was added. The mixture was stirred for 12 h at room temperature. After removing solvent, the residue was dissolved in 200 mL CHCl₃ and extracted with water (3 × 50 mL). The organic phase was dried over Na₂SO₄. After removing solvent, 3.18 g white solid was obtained (85%). ¹H NMR (300 MHz, CDCl₃) δ 6.81 (br, 2 H), 4.05 (m, 4 H), 1.47 (s, 18 H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 169.73, 168.68, 165.62, 82.48, 43.79, 28.30. MS: Calcd 374 (M⁺). Found (+LSIMS): 374 (M⁺).

General Piperazine Reaction (8)

Intermediate **7** (2.2 g, 5.88 mmol) was dissolved in 100 mL of THF before piperazine (0.01 g; 11.76 mmol) was added. The solution was bubbled with nitrogen and sealed in a Parr vessel. After stirring at 70 °C for 12 h, the reaction mixture was cooled to room temperature, the solid

was removed, and solvent was evaporated. The residue was dissolved in CHCl_3 , extracted with water ($3 \times 50 \text{ mL}$) and then dried over Na_2SO_4 . Upon removing solvent, the crude product was directly used in the next reaction.

Intermediate 9

The residue was purified by chromatography with $\text{CH}_2\text{Cl}_2/\text{CHOH}$ (50:1, 25:1) to give a white solid (2.19 g, 84%). ^1H NMR (300 MHz, CDCl_3) δ 3.97 (br, 8 H), 3.77 (br, 16 H), 1.44 (s, 36 H). ^{13}C NMR (75 MHz, CDCl_3) δ 170.10, 165.00, 164.66, 81.73, 43.77, 42.87, 28.29. MS: Calcd 959 (M^+). Found ($^+\text{FAB-DP}$): 959 (M^+).

Intermediate 10

The residue was dissolved in a solution of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (30:1) and then purified by column chromatography using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (20:1, 16:1) to afford the product as a white solid (1.42 g, 91%). ^1H NMR (300 MHz, CDCl_3) δ 3.99 (br, 8 H), 3.85 (br, 4 H), 3.75 (m, 16 H), 3.35 (br, 1 H), 3.02 (br, 4 H), 1.45 (s, 36 H). ^{13}C NMR (75 MHz, CDCl_3) δ 170.14, 166.23, 165.24, 8184, 43.88, 43.16, 28.31. MS: Calcd 1008 (M^+). Found ($^+\text{FAB-DP}$): 1009 ($\text{M}+\text{H}^+$).

Intermediate 11

The residue was purified by chromatography with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (30:1, 20:1) to give a white solid (771 mg, 79%). ^1H NMR (300 MHz, CDCl_3) δ 4.02 (d, 16 H), 3.85 (br, 16 H), 3.79 (br, 32 H), 1.47 (s, 72 H). ^{13}C NMR (75 MHz, CDCl_3) δ 169.89, 165.64, 164.84, 81.86, 43.89, 43.28, 28.31. MS: Calcd 2126 (M^+). Found (ESI-TOF): 2127 ($\text{M}+\text{H}^+$).

Intermediate 12

The residue was purified by column chromatography using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (20:1, 16:1) to afford the product as a white solid (282 mg, 81%).

^1H NMR (300 MHz, CDCl_3) δ 4.03 (br, 16 H), 3.81 (br, 50 H), 2.94 (br, 4 H), 1.48 (s, 72 H). ^{13}C NMR (75 MHz, CDCl_3) δ 170.22, 166.28, 165.67, 81.77, 43.91, 43.39, 43.21, 28.35. MS: Calcd 2177 (M^+). Found (ESI-TOF): 2178 ($\text{M}+\text{H}^+$).

Intermediate 13

The residue was purified by chromatography with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (30:1, 20:1) to give a white solid (172 mg, 77%). ^1H NMR (300 MHz, CDCl_3) δ 4.02 (br, 32 H), 3.80 (br, 112 H), 1.47 (s, 114 H). ^{13}C NMR (75 MHz, CDCl_3) δ 170.25, 166.20, 165.66, 81.73, 43.30, 43.38, 28.35. MS: Calcd 4466.56 (M^+). Found (ESI-TOF): 2234.3 ($\text{M}+2\text{H}^+$)/2, 1489.9 ($\text{M}+3\text{H}^+$)/3, 1117.6 ($\text{M}+4\text{H}^+$)/4, 894.3 ($\text{M}+5\text{H}^+$)/5.

General Ethylenediamine Reaction (14)

Intermediate 7 (501 mg, 1.34 mmol) was dissolved in 10 mL of dioxane before ethylenediamine (201 mg, 3.35 mmol) was added. The solution was bubbled with nitrogen and sealed in a Parr vessel. After stirring at 100 °C for 12 h, the reaction mixture was cooled to room temperature, the solid was filtered, and solvent was evaporated. The residue was purified by column chromatography using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (10:1, 5:1) to afford a white solid (468 mg, 88%). ^1H NMR (300 MHz, CDCl_3) δ 3.88 (br, 4 H), 3.29 (br, 2 H), 2.74 (br, 2 H), 2.01 (br, 2 H), 1.36 (s, 18 H); ^{13}C NMR (75 MHz, CDCl_3) δ 170.40, 166.43, 166.10, 81.44, 43.58, 41.67, 28.21. MS: Calcd 397 (M^+). Found ($^+\text{FAB-DP}$): 398 ($\text{M}+\text{H}^+$).

Intermediate 15

The residue was purified by column chromatography using CH₂Cl₂/CH₃OH (10:1, 5:1) to afford the product as a white solid (412 mg, 84%). ¹H NMR (300 MHz, CDCl₃) δ 4.01 (br, 8 H), 3.75 (br, 16 H), 3.62 (br, 2 H), 3.18 (br, 2 H), 1.47 (s, 36 H). ¹³C NMR (75 MHz, CDCl₃) δ 170.36, 167.20, 166.19, 165.26, 81.83, 43.85, 43.15, 41.50, 40.68, 28.35. MS: Calcd 982 (M⁺). Found (+FAB-DP): 982 (M⁺).

Intermediate 16

The residue was purified by column chromatography using CH₂Cl₂/CH₃OH (10:1, 5:1) to afford the product as a white solid (261 mg, 81%). ¹H NMR (300 MHz, CDCl₃) δ 3.99 (br, 16 H), 3.76 (br, 50 H), 3.32 (br, 2 H), 1.45 (s, 72 H). ¹³C NMR (75 MHz, CDCl₃) δ 169.95, 167.20, 165.58, 164.10, 82.00, 43.85, 43.32, 28.35. MS: Calcd 2151 (M⁺). Found (MALDI-TOF): 2150 (M⁺).

Intermediate 17

Intermediate **13** (161 mg, 0.036 mmol) was dissolved in 5 mL of dioxane. To the solution 5.4 mg (0.09 mmol) of ethylenediamine was added. The solution was bubbled with nitrogen and sealed in a Parr vessel. The mixture was heated to 100 °C for 12 h. The reaction mixture was cooled to room temperature, the solid was filtered, was removed. The residue was purified by column chromatography using CH₂Cl₂/CH₃OH (20:1, 10:1) to afford the product as a white solid (126 mg, 78%).

¹H NMR (300 MHz, CDCl₃) δ 4.01 (br, 32H), 3.81 (br, 98H), 3.32 (br, 2 H), 1.48 (s, 144 H). ¹³C NMR (75 MHz, CDCl₃) δ 170.28, 166.19, 165.70, 164.20, 81.79, 43.93, 43.38, 28.35. MS: Calcd 4490 (M⁺). Found (ESI-TOF): 2246 (M+2)²⁺, 1498 (M+3)³⁺, 1124 (M+4)⁴⁺, 899 (M+5)⁵⁺.

General Esterification Reaction (18)

Intermediate **14** (80 mg, 0.20 mmol) was dissolved in 2 mL of THF before 3,3'-dithiodipropionic acid di(N-succinimidyl ester) was added. After stirring at room temperature for 12 h, the solvent was removed and residue was purified by column chromatography using CH₂Cl₂/CH₃OH (20:1, 16:1) to afford a white solid (77.5 mg, 80%). ¹H NMR (300 MHz, CDCl₃) δ 3.98 (br, 8 H), 3.48 (br, 4 H), 3.36 (br, 4 H), 2.91 (br, 4 H), 2.48 (br, 4 H), 1.45 (s, 36 H); ¹³C NMR (75 MHz, CDCl₃) δ 172.32, 170.28, 165.73, 82.04, 43.70, 40.23, 35.91, by chromatog 35.36, 28.44. MS: Calcd 969 (M⁺). Found (ESI-TOF): 969 (M⁺).

Intermediate 19

The residue was purified by column chromatography using CH₂Cl₂/CH₃OH (20:1) to afford a white solid (63 mg, 74%). ¹H NMR (300 MHz, CDCl₃) δ 4.02 (m, 16 H), 3.80 (br, 32 H), 3.58 (br, 4 H), 3.40 (br, 4 H), 2.85 (br, 4 H), 2.40 (br, 4 H), 1.48 (s, 72 H). ¹³C NMR (75 MHz, CDCl₃) δ 171.50, 170.16, 166.69, 166.11, 165.10, 81.93, 43.86, 43.38, 43.18, 41.41, 40.07, 35.86, 34.18, 28.36. MS: Calcd 2137.13 (M⁺). Found (ESI-TOF): 2138.13 (M+H⁺), 1069.57 (M+2H)²⁺, 713.36 (M+3H)³⁺.

Intermediate 20

The residue was purified by column chromatography using CH₂Cl₂/CH₃OH (20:1, 15:1) to afford a white solid (83 mg, 74%). ¹H NMR (300 MHz, CDCl₃) δ 4.02 (br, 32 H), 3.80 (br, 96 H), 3.57 (br, 4 H), 3.41 (br, 4 H), 2.87 (br, 4 H), 2.44 (br, 4 H), 1.48 (s, 144 H). ¹³C NMR (75 MHz, CDCl₃) δ 171.49, 170.19, 166.10, 165.63, 165.14, 81.81, 43.88, 43.38, 41.90, 40.05, 35.86, 33.95, 28.35. MS: Calcd 4477.23 (M⁺). Found (ESI-TOF): 2239.69 (M+2H)²⁺, 1493.10 (M+3H)³⁺, 1120.08 (M+4H)⁴⁺, 896.27 (M+5H)⁵⁺.

Intermediate 21

The residue was purified by column chromatography using $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (20:1) to afford the product as a white solid (57.7 mg, 70%). ^1H NMR (300 MHz, CDCl_3) δ 4.00 (br, 64 H), 3.78 (br, 224 H), 3.57 (br, 4 H), 3.41 (br, 4 H), 2.87 (br, 4 H), 2.45 (br, 4 H), 1.48 (s, 288 H). ^{13}C NMR (75 MHz, CDCl_3) δ 171.48, 179.95, 165.84, 165.56, 164.88, 81.87, 44.00, 43.46, 41.40, 40.40, 36.06, 33.95, 28.46. MS: Calcd 9154.63 (M^+). Found (ESI-TOF): 2289.63 ($\text{M} + 4\text{H}$) $^{4+}$, 1831.97 ($\text{M} + 5\text{H}$) $^{5+}$, 1526.77 ($\text{M} + 6\text{H}$) $^{6+}$, 1308.85 ($\text{M} + 7\text{H}$) $^{7+}$. Found (MALDI-TOF): 9155 ($\text{M} + \text{H}^+$).

Capillary Electrophoresis (CE)—A Beckman P/ACE MDQ Capillary Electrophoresis System was used. A Capillary Electro-phoresis System equipped with a photodiode array detector was used. Bare fused silica capillary (Polymicro Technologies, Inc., Phoenix, AZ) with an internal diameter of 50 μm was used. The conditions used for all CE experiments were as follows: applied potential, 20 kV; detection wavelength, 214 nm; total length of the capillary, 31.1 cm; length of the capillary from injection point to the detection window, 20.9 cm; temperature, 25 $^\circ\text{C}$. Background electrolyte (BGE) used to analyze all of the samples was prepared using a solution of 100 mM Boric acid titrated to pH 9.0 using lithium hydroxide (ionic strength = 40 mM). An aqueous solution of dimethyl sulfoxide (DMSO) was used as the neutral marker. Sequence for analysis was as follows: 0.5 min rinse of the capillary with the BGE at 20 psi; 3 second injection of the neutral marker solution at 0.5 psi; 3 second injection of the sample solution at 0.5 psi; electrophoresis under positive-to-negative polarity (positively charged electrode at the injection end and negatively charged electrode at the outlet end of the capillary). The time scale on the electropherograms was converted to an effective mobility scale using the Origin 8 software package.

Size Exclusion Chromatography (SEC)—Traces were obtained on a Waters 600 Chromatograph System by monitoring at $\lambda = 254$ nm with a Waters 2487 Dual Absorbance Detector using a Waters Styragel HR 5E column. THF was used as the carrier solvent at a flow rate of 0.6 mL/min. All dendrimers were analyzed at 0.06 mM using an injection volume of 10 μL .

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Fig. 1.
SEC traces (min) of the protected dendrimers **18–21**.

Fig. 2. Capillary electrophoresis of **1–4** either as crude reaction products or after purification. Peaks corresponding to trifluoroacetic acid (TFA) and impurities resulting from incomplete deprotection (*) are indicated.

Fig. 3. Solubilization of pyrene. (a) A linear relationship between $[dendrimer]$ and $[pyrene]$ suggests no CMC for these architectures. (b) A linear relationship between molecular weight and $[pyrene]$ recorded at 6×10^{-4} M (dashed line in 3b).

Scheme 1.
Synthesis of dendrons.

Scheme 2.

Installation of the core and deprotection. (a) Excess ethylenediamine, dioxane, 100 °C, 12 h. (b) BisNHS ester, THF, 12 h. (c) 1:1 Trifluoroacetic acid:CH₂Cl₂, 12 h.

Chart 1.