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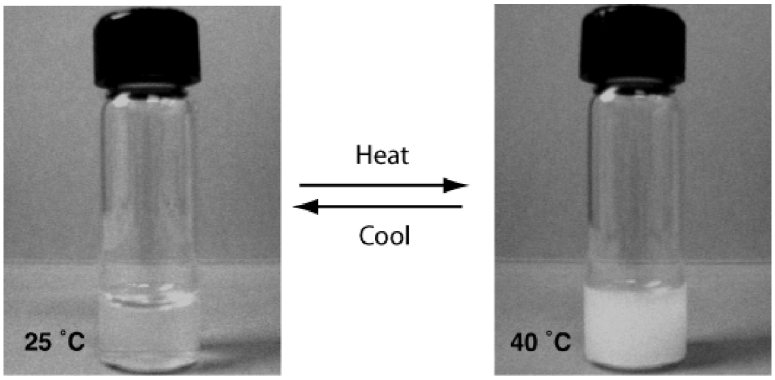
J. Am. Chem. Soc., **2005**, 127 (42), 14922-14929• DOI: 10.1021/ja054542y • Publication Date (Web): 30 September 2005



Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

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Temperature-Sensitive Dendritic Micelles

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Abstract: Syntheses up to three generations have been achieved of biaryl-based amphiphilic dendrons with a charge-neutral pentaethylene glycol as the hydrophilic part and a decyl chain as the hydrophobic part. Studies on the temperature-dependent characteristics revealed that these dendrons exhibit a generation-dependent lower critical solution temperature (LCST). This behavior is attributed to the combination of the amphipathic nature of the hydrophilic pentaethylene glycol side chain and dendritic effect. Interestingly, this biaryl-based scaffold also maintains the ability to form a micelle-like assembly in polar solvents and an inverted micelle-like assembly in apolar solvents. Polarity of the dendritic interior was investigated using dye-based microenvironment studies. The aggregation behavior of these micelles was analyzed by fluorescence spectroscopy and dynamic light scattering. Critical micelle concentrations (CMC) of these assemblies were investigated using fluorescence excitation spectra of the sequestered guest molecule, pyrene.

Introduction

The globular shape, excellent control over polydispersity, and the ability to display a high density of functionalities on its surface are among the factors that have made dendrimers attractive scaffolds for a variety of applications.[1] Examples of where dendrimers have been explored for applications include drug delivery,[2] gene therapy,[3] magnetic resonance imaging,[4] catalysis,[5] and light-harvesting antennae.[6] The diversity of these applications arises from the fact that one was able to instill a variety of physical properties in dendritic structures through functional group modifications at the core, branches, and the periphery of these highly branched macromolecules. Incorporation of stimuli-sensitive character into dendrimers[7] could significantly expand the scope of these molecules in applications such as the ones mentioned above. A feature that is significantly under-explored with dendrimers involves temperature-sensitive behavior.[8] Macromolecules with temperature-sensitive properties have advantages in applications such as controlled drug release,[9] molecular separations,[10] and tissue culture substrates.[11] We have been interested in eliciting thermally responsive properties into our amphiphilic dendrimers,[12] since such a system combines the complementary advantages of temperature sensitivity and

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(1) (a) Fréchet, J. M. J.; Tomalia, D. A. In *Dendrimers and Other Dendritic Polymers*; John Wiley & Sons: New York, 2001. (b) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendrimers and Dendrons: Concepts, Syntheses, Applications*, 2nd ed.; Wiley-VCH: Weinheim, 2001. (c) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665. (d) Fréchet, J. M. J. *Science* **1994**, *263*, 1710. (e) Tomalia, D. A.; Fréchet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 2719. (f) Majoral, J.-P.; Caminade, A.-N. *Acc. Chem. Res.* **2004**, *37*, 341.

(2) (a) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347. (b) Gillies, E. R.; Fréchet, J. M. J. *Drug Discovery Today* **2005**, *10*, 35. (c) Aulenta, F.; Hayes, W.; Rannard, S. *Eur. Polym. J.* **2003**, *39*, 1741. (d) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742. (e) Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev.* **2004**, *33*, 43. (f) Zhang, W.; Jiang, J.; Qin, C.; Thomson, L. M.; Pérez, L. M.; Parrish, A. R.; Safe, S. H.; Simanek, E. E. *Supramol. Chem.* **2003**, *15*, 607. (g) Ambade, A. V.; Savariar, E. N.; Thayumanavan, S. *Mol. Pharm.* **2005**, *2*, 264.

(3) (a) Haensler, J.; Szoka, F. C. *Bioconjugate Chem*. **1993**, *4*, 372. (b) Bielinska, A.; Kukowska-Latallo, J. F.; Johnson, J.; Tomalia, D. A.; Baker, J. R., Jr. *Nucleic Acids Res.* **1996**, *24*, 2176. (c) Loup, C.; Zanta, M.-A.; Caminade, A.-M.; Majoral, J.-P.; Meunier, B. *Chem. Eur. J.* **1999**, *5*, 3644. (d) Joester, D.; Losson, M.; Pugin, R.; Heinzelmann, H.; Walter, E.; Merkle, H. P.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1486.

(4) (a) Kim, Y.; Zimmerman, S. C. *Curr. Opin. Chem. Biol.* **1998**, *2*, 733. (b) Stiriba, S.-E.; Frey, H.; Haag, R. *Angew. Chem., Int. Ed*. **2002**, *41*, 1329. (c) Kobayashi, H.; Brechbiel, M. W. *Mol. Imaging* **2003**, *2*, 1.

(5) (a) Knapen, J. W. J.; van der Made, A. W.; de Wilde, J. C.; van Leeuwen, P. W. N. M.; Wijkens, P.; Grove, D. M.; van Koten, G. *Nature* **1994**, *372*, 659. (b) Astruc, D.; Chardac, F. *Chem. Rev.* **2001**, *101*, 2991. (c) Oosterom, G. E.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1828. (d) Schmitzer, A. R.; Franceschi, S.; Perez, E.; Rico-Lattes, I.; Lattes, A.; Thion, L.; Erad, M.; Vidal, C. *J. Am. Chem. Soc.* **2001**, *123*, 5956. (e) Hecht, S.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2001**, *123*, 6959.

(6) (a) Devadoss, C.; Bharathi, P.; Moore, J. S. *J. Am. Chem. Soc.* **1996**, *118*, 9635. (b) Adronov, A.; Fréchet, J. M. J. *Chem. Commun.* **2000**, 1701. (c) Choi, M.-S.; Aida, T.; Yamazaki, T.; Yamazaki, I. *Angew. Chem., Int. Ed.* **2001**, *40*, 3194. (d) Balzani, V.; Ceroni, P.; Maestri, M.; Vicinelli, V. *Curr. Opin. Chem. Biol*. **2003**, *7*, 657. (e) Imahori, H. *J. Phys. Chem. B* **2004**, *108*, 6130. (f) Thomas, K. R. J.; Thompson, A. L.; Sivakumar, A. V.; Bardeen, C. J.; Thayumanavan, S. *J. Am. Chem. Soc.* **2005**, *127*, 373 and references therein. (g) Nantalaksakul, A.; Reddy, D. R.; Bardeen, C. J.; Thayumanavan, S. *Photosynth. Res.* In press.

(7) For example, see: (a) Jiang, D.-L.; Aida, T. *Nature* **1997**, *338*, 454. (b) Sebastián, R.-M.; Blias, J.-C.; Caminade, A.-M.; Majoral, J.-P. *Chem. Eur. J.* **2002**, *8*, 2172. (c) Dirksen, A.; Zuidema, E.; Williams, R. M.; De Cola, L.; Kauffmann, C.; Vögtle. F.; Roque, A.; Pina, E. *Macromolecules* **2002**, *35*, 2743. (d) Kojima, C.; Haba, Y.; Fukui, T.; Kono, K.; Takagishi, T. *Macromolecules* **2003**, *36*, 2183. (e) Gillies, E. R.; Jonsson, T. B.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 1193.

(8) (a) Haba, Y.; Harada, A.; Takagishi, T.; Kono, K. *J. Am. Chem. Soc*. **2004**, *126*, 12760. (b) Hofacker, A. L.; Parquette, J. R. *Angew. Chem., Int. Ed.* **2005**, *44*, 1053.

(9) Piskin, E. *Int. J. Pharm.* **2004**, *277*, 105 and references therein.

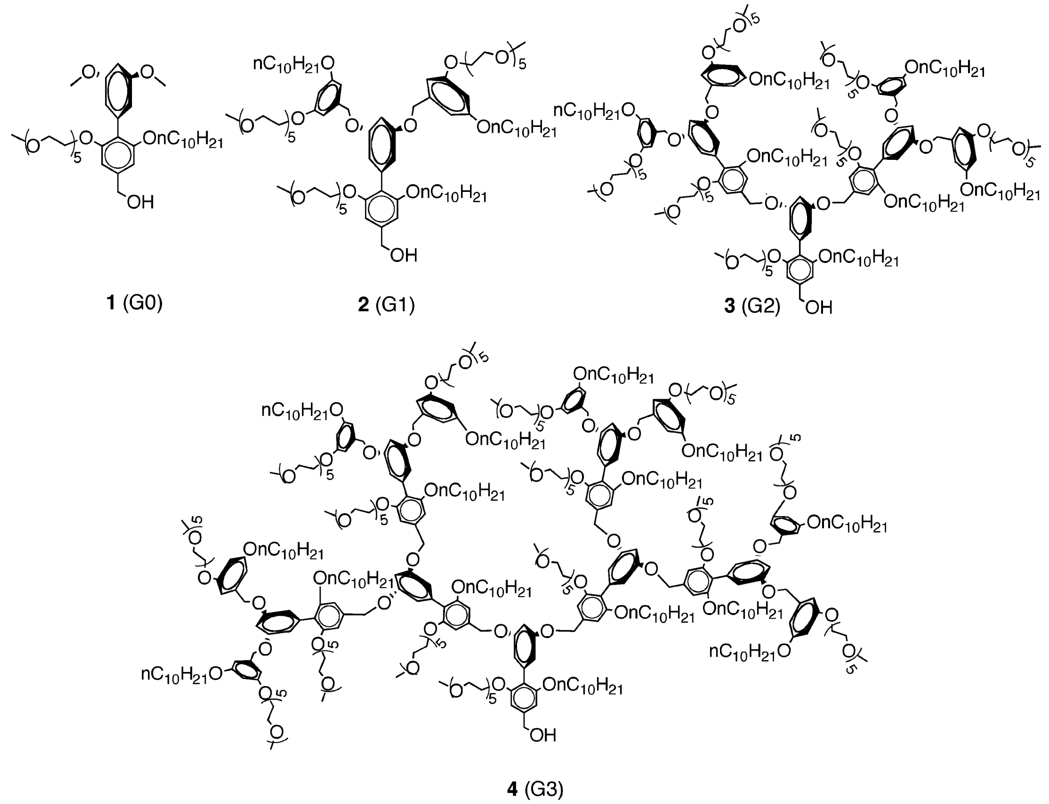
(10) Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. *J. Membr. Sci.* **1991**, *64*, 283.

(11) (a) Takezawa, T.; Mori, Y.; Yoshizato, K. *Biotechnology* **1990**, *8*, 854. (b) Nakano, Y. In *Function of Polymer Gels*; Shima, K., Ed.; CMC: Tokyo, 1999; p 61.

(12) (a) Vutukuri, D. R.; Basu, S.; Thayumanavan, S. *J. Am. Chem. Soc.* **2004**, *126*, 15636. (b) Bharathi, P.; Zhao, H.; Thayumanavan, S. *Org. Lett.* **2001**, *3*, 1961.

10.1021/ja054542y CCC: $30.25 © 2005 American Chemical Society

Chart 1. Structures of Dendrons **1**−**4**



1 (G0) **2** (G1) **3** (G2) **4** (G3)

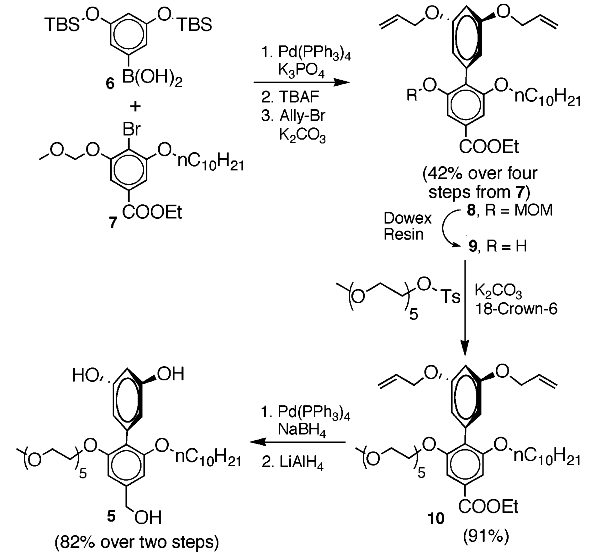
micellar properties for applications such as controlled release and catalysis.

We have recently introduced a new class of biaryl-based facially amphiphilic dendrimers that exhibit a unique functionality switch, depending on the solvent environment.[12] In our preliminary communication, we have reported on dendrimers with carboxylate as hydrophilic functionalities that exhibited a micelle-like assembly in water and an inverted micelle-like assembly in apolar solvents such as toluene.[12a] Our interest in utilizing a charge-neutral oligoethylene glycol as the hydrophilic side chain in this work stems from the possibility of incorporating temperature sensitivity into these dendritic structures. We have previously attempted triethylene glycol monomethyl ether as the hydrophilic moiety and the butyl group as the hydrophobic functionality in these dendrimers.[12b] Those dendrimers did not exhibit the appropriate solubility characteristics to investigate a solvent-sensitive or a temperature-sensitive behavior. In this contribution, we describe the syntheses and study of the biaryl-based dendrimers with pentaethylene glycol monomethyl ether as the hydrophilic unit and a decyl group as the hydrophobic functionality. The resultant dendrimers exhibit generation-dependent temperature sensitivity in the form of the lower critical solution temperature (LCST), while maintaining the ability to form micelles or inverted micelles, depending on the solvent environment. Syntheses and details of the physical organic characterization of these assemblies are reported here.

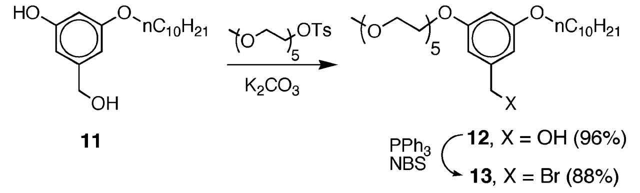
Results and Discussion

Synthesis. Structures of the targeted generation-zero to generation-three (G0−G3) dendrons **1**−**4** are shown in Chart 1. The dendrons were assembled from a basic biaryl building block unit, represented by structure **5** in Scheme 1. Synthesis of the monomer **5** was achieved in seven steps, with Suzuki coupling as the key biaryl coupling reaction, from the aryl boronic acid **6**[12b] and the bromo aryl ester **7** (Scheme 1). Compounds **6** and **7** were reacted with Pd(PPh$_3$)$_4$ in DME in the presence of potassium phosphate to afford the corresponding *tert*-butyldimethylsilyl protected biaryl compound. Since the separation of the coupling product from the bromoaryl ester **7** proved to be difficult, the mixture was taken to the next step without further purification. The *tert*-butyldimethylsilyl groups were deprotected using TBAF to afford the corresponding diphenolic compound in 47% yield. Note that the hydrophilic part was not installed in **7** prior to the coupling reaction, because installation of the long-chain oligoethylene glycol moiety

Scheme 1. Synthesis of Repeating Monomer Unit



Scheme 2. Synthesis of Peripheral Monomer Unit



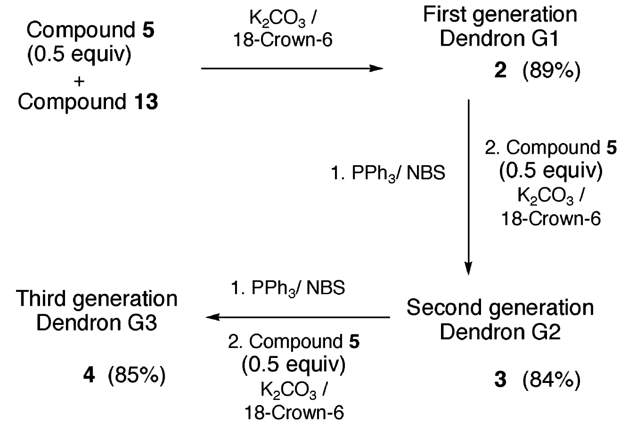
hampered the Suzuki coupling, resulting in products with less than 5% yield. Since the hydrophilic chain was to be installed in the place of the MOM group, the two free phenolic functionalities were reprotected with the allyl group to obtain compound **8**. Then, deprotection of the MOM group by using a Dowex-acidic resin yielded the compound **9**. The hydrophilic moiety was now introduced onto compound **9** by using the tosylate of pentaethylene glycol monomethyl ether[13] under the alkylation conditions to obtain compound **10**. Deprotection of the TBS group and protection with the allyl group had to be carried out, since the TBS group proved to be unstable to the alkylation conditions and the allyl group is unstable to the Suzuki coupling conditions.[14] Deprotection of the allyl groups by using $Pd(PPh_3)_4/NaBH_4$ followed by a reduction reaction with $LiAlH_4$ afforded the required building block monomer **5**.

The periphery of the dendrons was synthesized from compound **11**[12a] by alkylating with tosylate of pentaethylene glycol monomethyl ether in the presence of potassium carbonate and 18-crown-6 (Scheme 2). The resultant hydroxymethyl derivative **12** was converted to the corresponding bromomethyl derivative **13** using NBS and PPh_3. Compound **13** with hydrophobic *n*-decyl group and a hydrophilic pentaethylene glycol monomethyl ether group was used as the peripheral monomer.

The first-generation dendron G1 (**2**) was synthesized by reacting **5** and **13** in the presence of potassium carbonate and 18-crown-6 with 89% isolated yield. Conversion of this hydroxymethyl compound to the corresponding bromomethyl compound using a PPh_3/NBS reagent system followed by an alkylation reaction with the repeat unit **5** afforded the second-generation dendron **3** in 84% yield. Another iteration of these two steps using compounds **3** and **5** afforded the third-generation dendron **4** in 85% yield (Scheme 3). All dendrons were characterized by ^1H NMR, ^{13}C NMR, and MALDI-ToF mass spectrometry. GPC was used as an additional check of purity.

Temperature Sensitivity of Dendrons. The primary purpose of incorporating oligoethylene glycol units within these amphiphilic dendrimers is to bring about a temperature-sensitive behavior to these macromolecules. It has been reported that polymers containing oligo- or polyethylene glycol functionalities exhibit LCST behavior, which has been attributed to a temperature-sensitive hydrogen bonding ability of these moieties with the solvent, water.[15] It is suggested that the degree of hydration of the ethylene oxide chains decreases with increasing temperature. Such a reduced hydration of the oligoethylene glycol unit increases its interfacial energy with water, which results in precipitation of the molecule.

Scheme 3. Synthesis of Dendrons



In the amphiphilic assemblies based on our biaryl dendrimers, the hydrophilic functionalities are presented on the convex exterior in water, while the hydrophobic functionalities are presented in the concave interior.[12] In the assemblies formed from dendrons **1**−**4**, the hydrophilic functionalities presented on the exterior involve the pentaethylene glycol units. We envisaged the possibility that, upon increasing the temperature of the solution, the solvation of the pentaethylene glycol units by water would decrease, resulting in an LCST behavior. This was indeed observed, as evident from the formation of a precipitate from an aqueous solution upon heating, as shown in Figure 1 with the G3-dendron **4**. This process is thermally reversible.

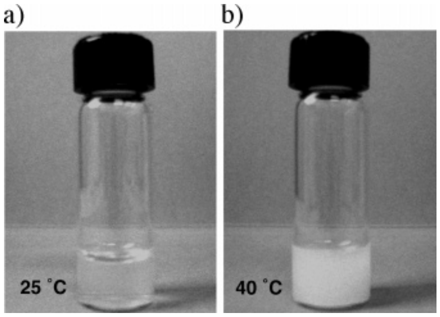


Figure 1. Photographs show an aqueous solution of G3 (**4**), (a) before heating, (b) after heating.

To identify the point at which the LCST transformation occurs in these dendrimers, we carried out turbidity measurements at various temperatures. This was achieved by monitoring the high tension (HT) voltage in the CD spectrophotometer. In conventional CD, the voltage at the photomultiplier tube increases with absorbance and can be used for monitoring turbidity.[16] HT voltage of the solution was monitored at 600 nm while the temperature was slowly varied (2 °C/min**)**. The LCST of G2 and G3 dendrons (**3** and **4**) were found to be around 32 and 31 °C, respectively, whereas that of G1 (**2**) is around 42 °C (Figure 2a). Interestingly, the compound **1** (G0) did not exhibit any sharp transition point that resembles an LCST behavior (Figure 2b). These results suggest that the temperature-sensitive character of the dendrons is dependent on the generation.

This observation is intriguing because all these dendrons could form multimolecular assemblies through aggregation. Our expectation that these dendrons are likely to form amphiphilic

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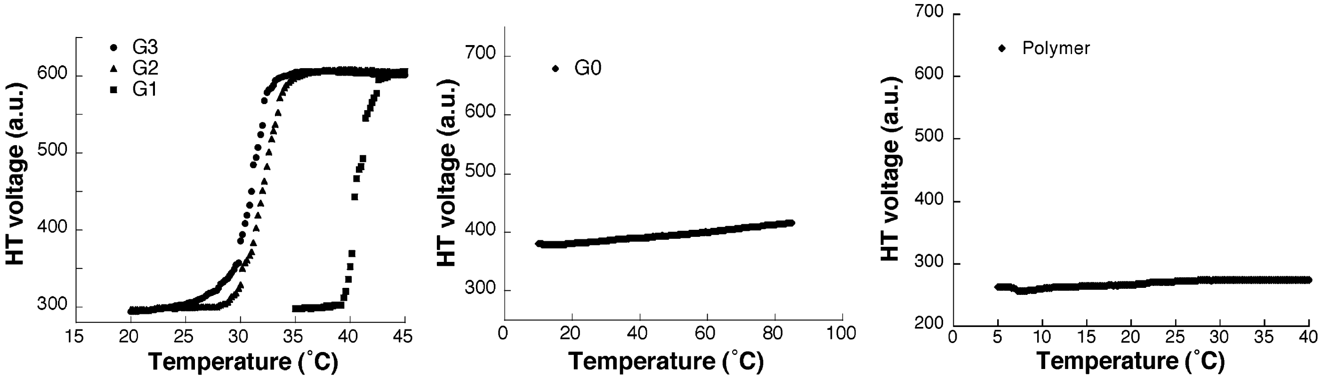
(13) Pentaethylene glycol derivative was synthesized according to the reported procedure: Lauter, U.; Meyer, W. H.; Wegner, G. *Macromolecules* **1997**, *30*, 2092.

(14) Vutukuri, D. R.; Bharathi, P.; Yu, Z.; Rajasekaran, K.; Tran, M.-H.; Thayumanavan, S. *J. Org. Chem.* **2003**, *68*, 1146.

(15) Saeki, S.; Kuwahara, N.; Nakata, M.; Kaneko, M. *Polymer* **1976**, *17*, 685. (b) Aoshima, S.; Oda, H.; Kobayashi, E. *J. Polym. Sci., Part A: Polym. Chem.* **1992**, *30*, 2407.

(16) Arvinte, T.; Bui, T. T. T.; Dahab, A. A.; Demeule, B.; Drake, A. F.; Elhag, D.; King, P. *Anal. Biochem.* **2004**, *332*, 46.



(a) (b) (c)

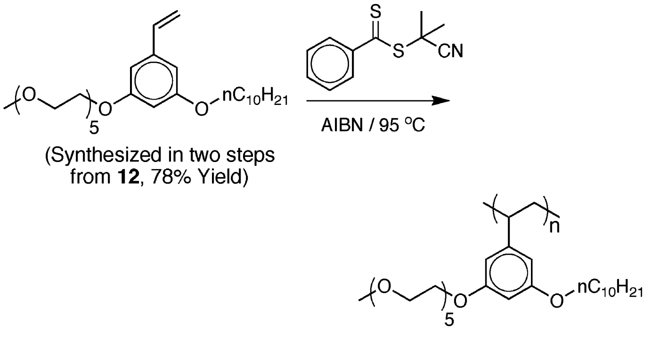
Figure 2. Dependence of changes in HT voltage with temperature for (a) G1, G2, and G3; (b) G0; and (c) the polymer **15**.

assemblies through aggregation is based on the fact that the analogous carboxylate dendrons do form aggregates in water.[12a] To examine whether a similar aggregation behavior is observed with the dendrons **1**−**4**, we probed the aqueous solutions of these molecules using dynamic light scattering (DLS). The hydrodynamic radius (R_H) for G1 was found to be about 75 nm, while those of G2 and G3 were around 125 nm. The size of the aggregate formed from G0 is also around 125 nm. Since all these assemblies are rather large and are formed from very similar building blocks, it is reasonable to expect that the generation of the dendron should have very little effect on the LCST behavior. However, we notice a significant generation dependence. Since the sizes of the assemblies are different, one could attribute the difference in LCST as a simple manifestation of the aggregate size. However, the reason seems to be more complicated. The size of the assembly formed from compounds **1** and **4** (G0 and G3) are very similar, whereas the LCST behaviors of the assemblies from these molecules are very different. Presentation of the pentaethylene glycol units in a dendritic architecture at a critical generation seems to be important for the observed temperature-sensitive behavior, the so-called dendritic effect.

We were also interested in identifying further whether this property is unique to dendritic architectures. For this purpose, we synthesized a styrene-derivative containing a pentaethylene glycol moiety as the hydrophilic functionality and a decyl group as the hydrophobic functionality. Compound **12** from the peripheral monomer synthesis was oxidized using PCC, and the resultant aldehyde derivative under Wittig reaction conditions afforded compound **14**. Polymerization of the styrene-derivative **14** under a controlled radical polymerization condition, reversible addition−fragmentation chain transfer (RAFT) polymerization, afforded the polymer **15** with an M_n of 11 kDa (molecular weight of G3 is 8225 Da) and a PDI of 1.1 (Scheme 4). Examination of the change in HT voltage of an aqueous solution of this molecule at different temperatures showed that the polymer **15** does not exhibit any LCST characteristics (Figure 2c). This result in combination with the generation-dependent behavior above suggests that dendritic structures do provide certain architectural advantages in the observed LCST behavior. Thus, the obtained trend in LCST behavior is attributed to the dendritic effect.

Micelle and Aggregation Behavior of Dendrons. As mentioned above, we were also interested in studying whether these biaryl dendrons still preserve the ability to form micellar assemblies. To investigate this aspect, we used the encapsulation of pyrene as the hydrophobic probe in aqueous solutions. The combination of absorption, emission, and excitation spectra provides valuable information about the micellar assemblies obtained from these dendrons.

Scheme 4. Synthesis of Styrene-Based Amphiphilic Polymer **15**



(Synthesized in two steps from **12**, 78% Yield)

15 (80%)

The absorption spectra of pyrene in water in the presence of dendrons **2**−**4** are shown in Figure 3. In the absence of any dendron, no pyrene could be detected in water as evident from the fact that the absorption spectrum of this solution is practically indistinguishable from the solvent baseline. These results are taken to indicate that the dendrons are capable of providing an apolar microenvironment that sequesters the hydrophobic pyrene molecule within its interior. As noted above, DLS studies

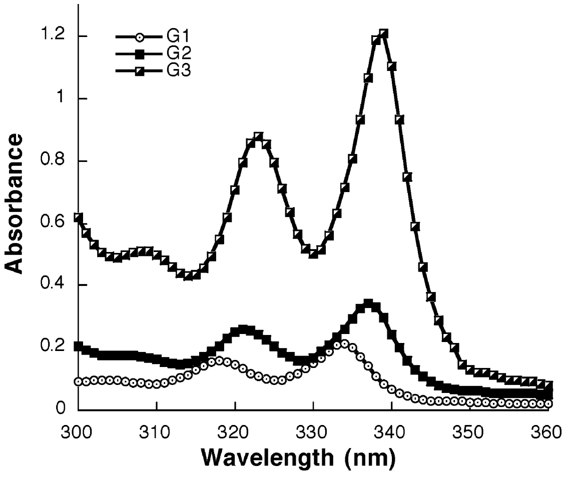


Figure 3. Absorption spectra of pyrene in aqueous solutions of dendrons (10^{-5} M).

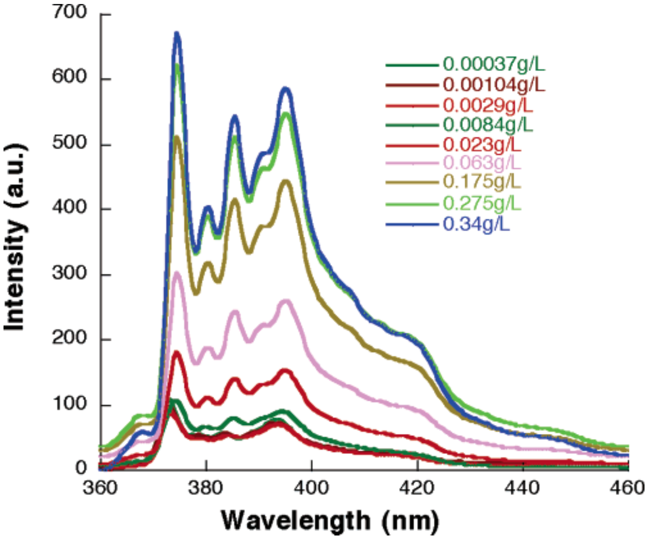


Figure 4. Emission spectra of pyrene in water at different concentrations of G3 (**4**).

show that the assembly in water involves the formation of a 75−125-nm particle. The generation dependence of the sequestering ability is clearly evident from the absorption spectra. On the basis of the absorption spectra, one could also quantitatively estimate the number of pyrene molecules sequestered per dendron. The G-1 dendron **2** is able to take up 1.6 molecules per dendron, while the G-2 and G-3 dendrons **3** and **4** were able to bind to 3.3 and 7 pyrene molecules per dendron, respectively.

Since the fluorescence spectrum of pyrene is sensitive to changes in its microenvironment, this technique is useful in probing the polarity of the dendritic interior that binds the hydrophobic guest molecule. The relative intensity of the first and the third emission peaks (I_1/I_3) in the emission spectrum of pyrene is known to be sensitive to the polarity of its microenvironment.[17] The fluorescence behavior is exemplified using the spectra of pyrene at different concentrations of the G-3 dendron **4** in Figure 4. The I_1/I_3 value obtained for all the compounds **1**−**4** is about 1.2, which is similar to that observed in PS−PEO block copolymer micelles where PS forms the core of the micelle.[18] Interestingly, the I_1/I_3 ratio was found to vary with the concentration of the dendrons in water.[19] The trend observed in Figure 5, upon plotting this ratio against the log [dendron], allows us to note the presence of a critical micelle concentration (CMC) for these dendrons. It is not surprising that a finite CMC would be observed for these molecules, unlike those reported with the classical amphiphilic dendrimers,[20] since the current dendrons are known to afford large aggregates (see the DLS studies above).

A more common way of measuring CMC involves a plot of I_{338}/I_{333} ratio as a function of log [dendron] from the excitation spectra of the pyrene at different concentrations of dendrons, as shown in Figure 6. The red-shift of the low-energy band from 333 to 338 nm in the excitation spectra, in combination with decrease in I_1/I_3 in emission spectra at a higher concentration of dendrons, indicates the partitioning of pyrene from an aqueous environment to a hydrophobic environment. At lower concentrations, the I_{338}/I_{333} takes the value of pyrene in water as shown in Figure 7. The plot of I_{338}/I_{333} as a function of log *c* is steady at low concentration and becomes sigmoidal in the crossover region. The CMC value can be taken from the intersection of the tangent to the curve at the inflection point with the horizontal tangent through the points at low dendron concentrations. The

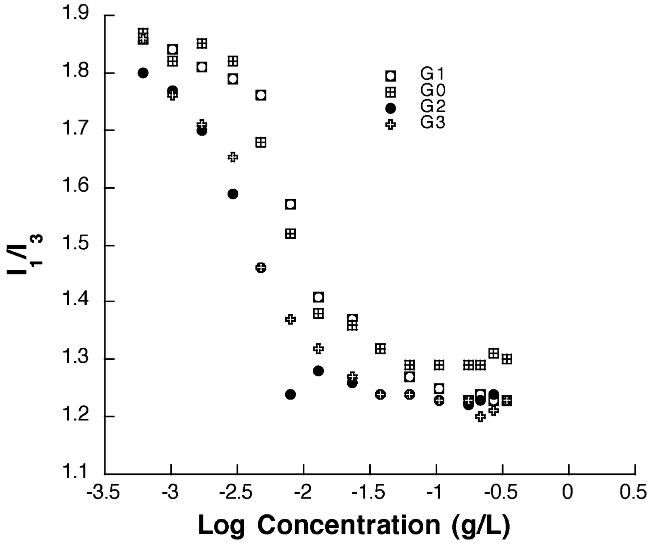


Figure 5. Plot of I_1/I_3 in the emission spectra vs concentrations of dendrons.

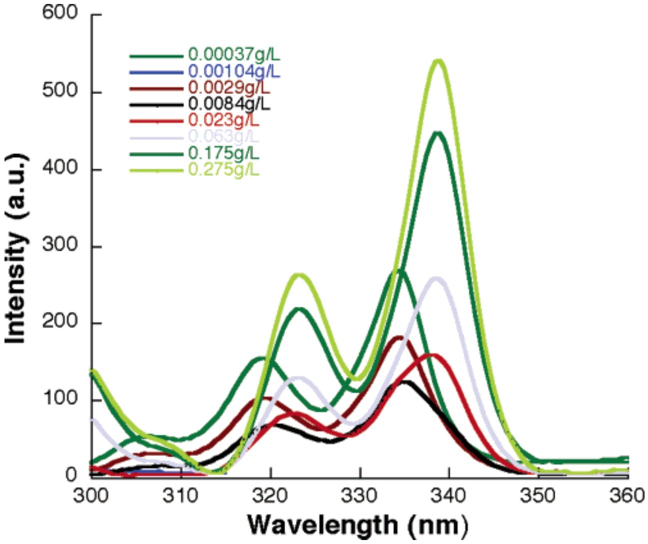


Figure 6. Excitation spectra of pyrene in water at different concentrations of G3 (**4**).

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(17) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.

(18) Wilhelm, M.; Zhao, C.-L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J.-L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033.

(19) For example, see: (a) Lee, S. C.; Chang, Y.; Yoon, J,-S.; Kim, C.; Kwon, I. C.; Kim, Y.-H.; Jeong, S. Y. *Macromolecules* **1999**, *32*, 1847. (b) Lysenko, E. A.; Bronich, T. K.; Slonkina, E. V.; Eisenberg, A.; Kabanov, V. A.; Kabanov, A. V. *Macromolecules* **2002**, *35*, 6351. (c) Gitsov, I.; Lambrych, K. R.; Remnant, V. A.; Pracitto, R. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 2711.

(20) (a) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Johnson, A. L.; Behera, R. K. *Angew. Chem., Int. Ed. Engl*. **1991**, *30*, 1176. (b) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Saunders: M. J.; Grossman, S. H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1178. (c) Tomalia, D. A.; Berry, V.; Hall, M.; Hedstrand, D. M. *Macromolecules* **1987**, *20*, 1164. (d) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1287. (e) Newkome, G. R.; Young, J. K.; Baker, G. R.; Potter, R. L.; Audoly, L.; Cooper, D.; Weis, C. D.; Morris, K.; Johnson, C. S., Jr. *Macromolecules* **1993**, *26*, 2394. (f) Kuzdzal, S. A.; Monnig, C. A.; Newkome, G. R.; Moorefield, C. N. *J. Chem. Soc., Chem. Commun*. **1994**, 2139 (g) Schmitzer, A.; Perez, E.; Rico-Latters, I.; Lattes, A.; Rosca, S. *Langmuir* **1999**, *15*, 4397. (h) Gopidas, K. R.; Whitesell, J. K.; Fox, M. A. *J. Am. Chem. Soc*. **2003**, *125*, 14168. (i) For dendritic polymer micelle see: Fréchet, J. M. J. Gitsov, I.; Monteil, T.; Rochat, S.; Sassi, J.-F.; Vergelati, C.; Yu, D. *Chem. Mater.* **1999**, *11*, 1267.

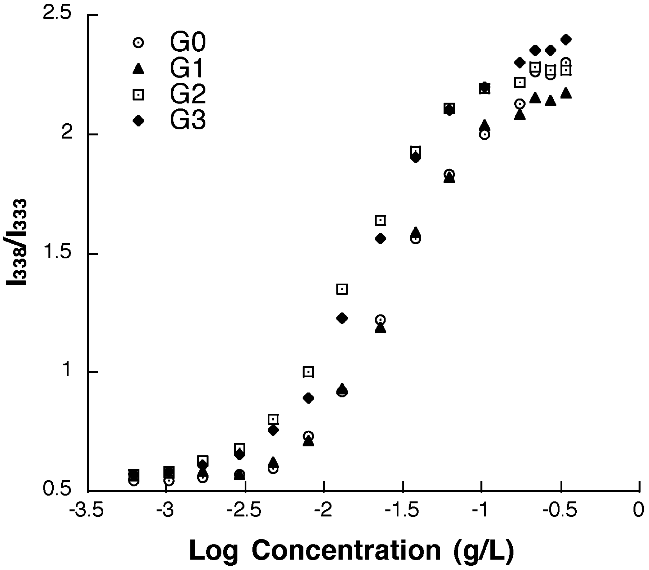

Figure 7. Plot of I_{338}/I_{333} in the excitation spectra vs concentration of dendrons.

Table 1. CMC Value of Dendrons **1**−**4**

	CMC (M)	CMC (mg/L)
G0 (**1**)	7×10^{-6}	8
G1 (**2**)	4×10^{-6}	8
G2(**3**)	8×10^{-7}	3
G3(**4**)	3×10^{-7}	3

obtained CMC values are shown in Table 1. When one looks at the CMC value in terms of molarity, the G2 and G3 dendrons (**3** and **4**) exhibit a CMC of an order of magnitude compared to that of G0 and G1 dendrons. However, since the number of amphiphilic repeat units in these dendrons are different, the more appropriate value would involve mg/L of these molecules. The CMC, in terms of wt % is about 3 mg/L for G2 and G3, whereas it is about 8 mg/L for G1 and G0 (Table 1). Thus, the gain in CMC upon increasing to higher generations is only about 2.7.

At first glance, it could be surprising that the generation of the dendrons only has a minor effect on the CMC. However, this observation could be based on the size of the micellar assemblies. All these dendrons form fairly large micellar structures (75−125 nm). For example, both G0 and G3 dendrons (**1** and **4**) afford the same size micellar particle of about 125 nm. However, the number of G0 molecules that need to come together to make the assembly would be much greater than that of G3, since G0 as a molecule is much smaller than G3 as a molecule. Therefore, it is likely that G0 would pay a greater entropic penalty for the formation of the 125-nm assembly. On the other hand, the flexible linkages between the repeat units allow for several conformations within the 15-mer G3 dendron. To form the micellar assembly from these possible conformations, the G3 dendron has to pay significant entropic penalty to intramolecularly organize and present the oligoethylene glycols on the solvent-exposed exterior surface, while tucking the hydrophobic decyl groups in the interior. In the case of G0, the intramolecular requirement does not exist but is compensated by the need for organizing a greater number of molecules intermolecularly. We *speculate* that these opposing effects of generation dependence vs entropy are the reasons for the small differences in CMC values for the dendrons.

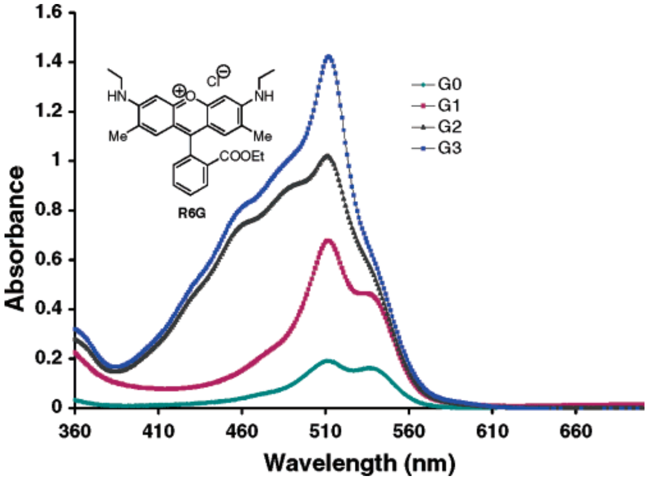

Figure 8. Absorption spectra of R6G in G0 (**1**), G1 (**2**), G2 (**3**), and G3 (**4**).

We were also interested in identifying whether these dendrons could exhibit reverse-micelle characteristics in an apolar solvent. This could be interesting for certain applications. For example, it is imaginable that dendrimers containing neutral functionalities showing reverse micelle characteristics will have significant implications on stabilizing a protein in nonaqueous solvent and monitoring the enzyme kinetics in organic solvent. We chose toluene as the apolar solvent for this purpose. Since, both the decyl chain and the pentaethylene glycol unit are soluble in toluene, it is difficult to imagine the formation of a reverse micelle. However, when one introduces a small amount of water that is a selective solvent for the pentaethylene glycol unit, this functionality could phase-separate from the decyl moiety. Accordingly, we added 20 equiv of water with respect to each of pentaethylene glycol unit in toluene. The water-soluble dye rhodamine 6G (R6G) was taken as a fluorescent probe to examine the formation of reverse-micelle dendrimer. The solutions of dendrons with R6G were analyzed by absorption and fluorescence spectroscopy. When we used only 3 equiv of water or no water, the extent of the dye sequestration was very small.

The dye was well taken up by these dendrons and could be seen from the absorption spectra as shown in Figure 8. The absorbances of all the dendrons were normalized to 0.2, and fluorescence spectra were taken in comparison with absorbance-matched aqueous solution of R6G. The absorbance matching of both solutions states that we have an equal amount of R6G in both solutions, but the fluorescence emissions from the solutions containing the dendrons were significantly less than that from the aqueous control solution, as indicated in Figure 9. The quenching was high in G3, followed by G2, G1, and G0, which can be directly interpreted from the absorbance spectra where G3 has taken up more number of dye molecules followed by G2, G1, and G0. It is known that R6G exhibits self-quenching with an increase in concentration, due to the increased proximity between two R6G molecules.[21] The observed reduction of fluorescence emission in this case suggests that the R6G is trapped in a confined space, which in turn suggests the formation of reverse-micelle-like assembly.

To further characterize the morphology of these micelles, we utilized TEM, and the particle size was also analyzed using

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[21] Jung, H. M.; Price, K. E.; McQuade, D. T. *J. Am. Chem. Soc.* **2003**, *125*, 5351.

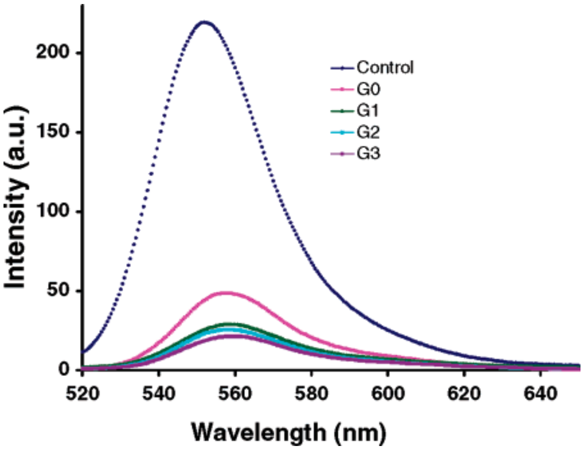

Figure 9. Relative emissions of R6G in the toluene solution of dendrons. The control spectrum is from an aqueous solution of R6G, which is absorbance matched with the toluene solutions of dendrons.

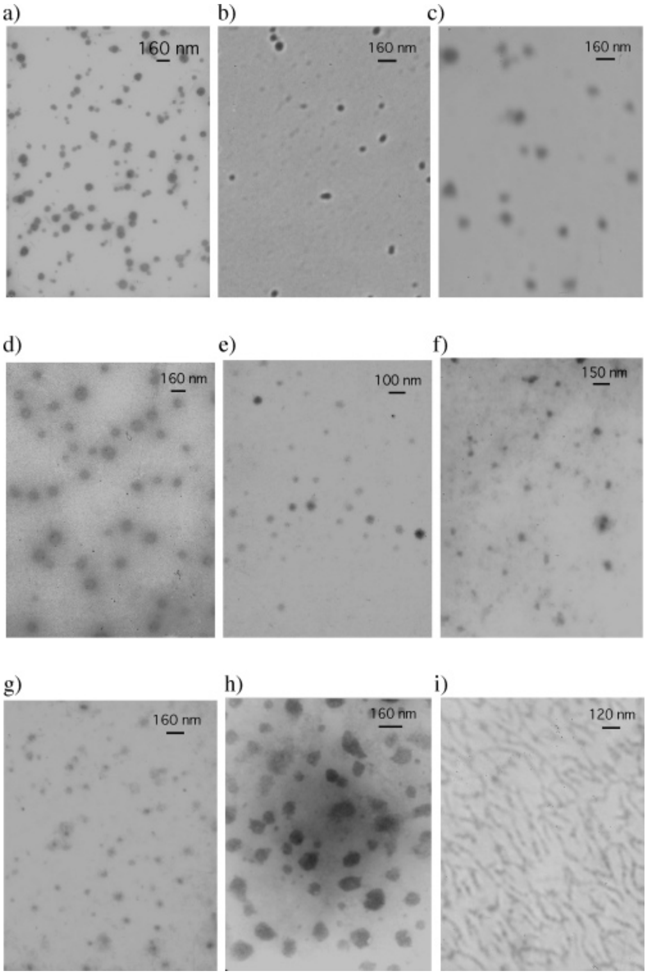

Figure 10. Micelles formed in water from (a) G0 (**1**), (b) G1 (**2**), (c) G2 (**3**), (d) G3 (**4**). Reverse micelle formed in toluene solution with 20 equiv of water per pentaethylene glycol unit (e) G0 (**1**), (f) G1 (**2**), (g) G2 (**3**), (h) G3 (**4**). (i) Wormlike assemblies from toluene solution of G2 with 3 equiv of water per pentaethylene glycol unit.

TEM. These particles seem to be spherical micelles, and G0, G2, and G3 were similar in size as shown in Figure 10a, c, and d respectively, while the size of G1 (Figure 10b) was slightly smaller, consistent with the DLS results above.

To investigate the morphology of the reverse micelles, we analyzed the TEM of the dendrons from the toluene solutions. It was observed that these assemblies were spherical reverse micelles as shown in Figure 10e, f, g, and h for G0, G1, G2, and G3, respectively. The particle size (R_{TEM}) was around 60 nm for G3; for G2 and G1, it was around 40 nm, and G0 had a size around 30 nm. We attempted to analyze the size of the inverse-micelle-type assemblies in toluene solutions with the use of DLS. We were able to obtain a size (R_{H}) of 55 nm for G3 dendron **4**, whereas reliable data could not be obtained for other dendrons with DLS. Also, as mentioned above, when the pentaethylene glycol side chains are not fully hydrated due to the deficient amount of water in the toluene solution, the ability of these dendrimers to sequester R6G was significantly reduced. To investigate the type of morphology that is formed under these conditions, we obtained TEM pictures of the dendrons in toluene with only 3 equiv of water per pentaethylene glycol unit. As can be seen from Figure 10i, wormlike assemblies are obtained when a deficient amount of water is used in toluene solution.

Summary

We have synthesized biaryl-based neutral amphiphilic dendrons up to three generations with pentaethylene glycol as the hydrophilic functionality and decyl chain as the hydrophobic functionality. The synthesized dendrons are capable of forming micelles and reverse micelles in polar and apolar solvents, respectively. The CMC and the polarity of the microenvironment of these dendrimer micelles were analyzed by using pyrene as fluorescent probe. The dendritic architecture was found to be responsible for the obtained LCST behavior of these dendrons and changes significantly as we go from G1 to G2 and stays almost constant at G3. The LCST and micellar behavior of these molecules open up the possibility of utilizing these dendrons for applications such as drug delivery,[22] catalysis,[23] cloud point extraction,[24] and microbial biotransformation.[25]

Experimental Section

^{1}H NMR spectra were recorded on a 400 MHz NMR spectrometer using the residual proton resonance of the solvent as the internal standard. Chemical shifts are reported in parts per million (ppm). When peak multiplicities are given, the following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet; b, broad. ^{13}C NMR spectra were proton decoupled and recorded on a 100 MHz NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. EI mass spectra were obtained at the Molecular Weight Characterization Facility at University of Massachusetts. Flash chromatography was performed with 37−75 μm silica gel. Analytical thin-layer chromatography was performed on silica plates with F-254 indicator and the visualization was accomplished by UV

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(22) (a) Hoffman, A. S. *J. Controlled Release* **1987**, *6*, 297. (b) Chung, J. E.; Yokoyama, M.; Yamato, M.; Aoyagi, T.; Sakurai, Y.; Okano, T. *J. Controlled Release* **1999**, *62*, 115. (c) Chung, J. E.; Yokoyama, M.; Okano, T. *J. Controlled Release* **2000**, *65*, 93. (d) Kim, I. S.; Jeong, Y. I.; Cho, C. S.; Kim, S. H. *Int. J. Pharm.* **2000**, *205*, 165. (e) Liu, S. Q.; Tong, Y. W.; Yang, Y.-Y. *Biomaterials* **2005**, *26*, 5064.

(23) (a) Mariagnanam, V. M.; Zhang, L.; Bergbreiter, D. E. *Adv. Mater.* **1995**, *7*, 69. (b) Komori, K.; Matsui, H.; Tatsuma, T. *Bioelectrochemistry* **2005**, *65*, 129.

(24) The hydrophobic substance can be removed from water through cloud point extraction. As a proof-of-principle, the pyrene was encapsulated G3 aqueous solution and measured the absorbance; it was 0.3 (10^{-6} M of G3). The same solution was heated in a glass syringe to precipitate the dendrimer and then filtered through the 0.22 μm filter and then the absorbance was measured. The absorbance was decreased from 0.3 to 0.07. The complete removal of pyrene could not be obtained, which is likely due to the inability to maintain a warmer temperature during filtration. For examples of cloud point extraction, see: (a) Lee, C.-K.; Su, W.-D. *Sep. Sci. Technol.* **1999**, *34*, 3267. (b) Li, J.-L.; Bai, R.; Chen, B.-H. *Langmuir* **2004**, *20*, 6068.

(25) Wang, Z.; Zhao, F.; Chen, D.; Li, D. *Enzyme Microb. Technol.* **2005**, *36*, 589.

lamp or using an iodine chamber. THF and toluene were distilled over Na/Ph_2CO. All other chemicals were obtained from commercial sources and used as received, unless otherwise mentioned. Synthetic procedures and the characterization data for all the compounds are given in Supporting Information.

Sample Preparation for Micelle Study. The solution for the micelle study was prepared initially by dissolving the dendrons in acetone (1 mL) and water (10 mL) was added dropwise (50 μL/min) to that solution. Then acetone was evaporated on a rotary evaporator, and the resultant solution was dialyzed against water using 1000-Da cut off dialysis membrane. Excess of pyrene was added to the aqueous solution of these dendrons, and stirred at room temperature for 5h. Then the solution was filtered through a 0.22 μm filter. The absorption spectra of these solutions were recorded using quartz cells.

Emission and Excitation Spectra. The pyrene stock solution (5×10^{-7} M) was made in acetone. Required amount of this solution was transferred into a vial, and the acetone was evaporated. Then necessary amount of the dendrimer solution was added to make the pyrene concentration 5×10^{-7} M. Emission spectra were obtained by exciting the pyrene solution at 339 nm. For excitation spectra λ_{em} was fixed at 374 nm. The scanning speed was set at 50 nm/min.

Sample Preparation for Reverse Micelle. A solution of dendrons (10^{-4} M) was prepared in toluene, twenty equivalents of water was added to this solution to induce the phase segregation and sonicated for 2 h at 40 °C. Then excess of rhodamine 6G was added and sonicated for further 1 h and the solutions were filtered through a 0.2 μm filter. Absorption spectra of these solutions were recorded on a UV−Vis spectrophotometer using quartz cells.

Dynamic Light-Scattering (DLS) Experiments. DLS experiments were performed in a digital correlator and goniometer. The light source was a solid-state laser system, operating at 514 nm. The temperature was kept constant at 25 °C throughout the experiments. Dust was eliminated by filtering the solution through a 0.22 μm filter. All measurements were done at a correlation time of 2 min. The particle size was analyzed using both nonnegative nonlinear least-squares (NNLS) and constrained regularized continuous inversion (CONTIN) algorithm program.

Turbidity Measurements. Turbidity measurements were carried out for all dendrons (G0−G3) and the polymer using a CD spectrophotometer. The same dialyzed aqueous solutions prepared for micelle study were taken in quartz cell and the HT voltage was monitored at different temperature.

TEM Measurements. TEM measurements were performed using a JEOL 100CX 100KV TEM. Samples were prepared by dipping copper EM grids (precoated with the thin film of Formvar and then coated with carbon) in aqueous or toluene solutions of the dendrons and dried at room temperature and visualized under TEM.

Acknowledgment. Partial support for this work was obtained from the NIGMS of the National Institutes of Health (R01 GM65255-01). We are also grateful to the NSF for the equipment facilities through the MRSEC and for a Career award to S.T.

Supporting Information Available: Synthesis and characterization of compounds; fluorescence spectra of pyrene in G0 (**1**), G1 (**2**), and G2 (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

JA054542Y