Comb-Dendritic Block Copolymers as Tree-Shaped Macromolecular Amphiphiles for Nanoparticle Self-Assembly

Lu Tian and Paula T. Hammond*

Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139

Received January 30, 2006. Revised Manuscript Received June 10, 2006

An amphiphilic comb-dendritic block copolymer was designed and synthesized based on poly (γ-*n*-dodecyl-L-glutamate) as a hydrophobic comb block and a hydrophilic polyester dendron block modified with poly(ethylene glycol); the high persistence length of the rodlike helical polypeptide backbone within the comb block resulted in a "tree-like" architecture for this comb-dendritic block copolymer. In aqueous solution, the macromolecules self-assemble into spherical micelles with the hydrophobic comb blocks forming the inner core and the hydrophilic dendritic blocks forming the exterior shell. The cone-shaped morphology and the alkyl pendant groups enhance the stability of the micelles, resulting in unusually low critical micelle concentrations of approximately 10^{-8} M. The particle size of these micelles was pH dependent due to the presence of the high-density carboxylic acid groups at the surfaces of micelles. The drug loading capacity of the polymeric micelles is about 30 wt % for the hydrophobic bactericide, triclosan, used as a model drug. In addition, the nanoscale clustering of the peripheral acid groups are potential substrates for subsequent functionalization with biospecific ligands to improve biologically relevant affinity enhancement in targeting drug delivery applications.

Introduction

Through the manipulation of intermolecular noncovalent interactions, block copolymers can self-assemble into a wide variety of nano- to microscaled structures with versatile potential applications.[1] In addition to the chemical composition and functionality, the molecular architecture of polymeric systems is a key factor in the resulting self-assembled structures.[2] Among the numerous reported architectures of block copolymers, the introduction of a dendritic structure as a building block is particularly attractive;[3] by covalently linking different linear polymer blocks to a dendron, one can incorporate the advantages of traditional spherical dendrimers and the phase-segregated morphological behavior of traditional block copolymers. A number of such systems have been explored since they were first introduced in the previous decade.[4−16] In particular, Lee and co-workers[14,15] and Stupp and co-workers[16] recently reported dendron-rod hybrid block copolymers with well-defined molecular structures and shape persistence, which yield interesting hierarchical self-assembled nanostructures including ribbon structures.

* To whom correspondence should be addressed. E-mail: Hammond@mit.edu. Phone: (617)258-7577. Fax: (617)258-5766.

(1) (a) Thomas, E. L. *Science* **1999**, *286*, 1307. (b) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418−2421. (c) Service, R. F. *Science* **2005**, *309*, 95.

(2) (a) Bucknall, D. G.; Anderson, H. L. *Science* **2003**, *302*, 1904−1905. (b) Stupp, S. I.; LeBonheur, V.; Walker, K.; Li, L. S.; Huggins, K. E.; Keser, M.; Amstutz, A. *Science* **1997**, *276*, 384−389. (c) Cornelissen, J. J. L. M.; Fischer, M.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Science* **1998**, *280*, 1427−1430. (d) Jenekhe, S. A.; Chen, X. L. *Science* **1999**, *283*, 372−375. (e) Li, Z.; Kesselman, E.; Talmon, Y.; Hillmyer, M. A.; Lodge, T. P. *Science* **2004**, *306*, 98−101.

(3) (a) Pyun, J.; Zhou, X.-Z.; Drockenmuller, E.; Hawker, C. J. *J. Mater. Chem.* **2003**, *13*, 2653−2660. (b) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200−1204. (c) Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. *Nature Biotechnol.* **2005**, *23*, 1517−1526.

(4) (a) Gitsov, I.; Wooley, K. L.; Fréchet, J. M. J. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1200−1202. (b) Gitsov, I.; Wooley, K. L.; Hawker, C. J.; Ivanova, P. T.; Fréchet, J. M. J. *Macromolecules* **1993**, *26*, 5621−5627. (c) Gitsov, I.; Fréchet, J. M. J. *Macromolecules* **1993**, *26*, 6536−6546.

(5) (a) Gitsov, I.; Fréchet, J. M. J. *Macromolecules* **1994**, *27*, 7309−7315. (b) Matyjaszewski, K.; Shigemoto, T.; Fréchet, J. M. J.; Leduc, M. *Macromolecules* **1996**, *29*, 4167−4171. (c) Leduc, M.; Hawker, C. J.; Dao, J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1996**, *118*, 11111−11118. (d) Leduc, M. R.; Hayes, W.; Fréchet, J. M. J. *J. Polym. Sci. Polym. Chem.* **1998**, *36*, 1−10.

(6) (a) Chapman, T. M.; Hillyer, G. L.; Mahan, E. J.; Shaffer, K. A. *J. Am. Chem. Soc.* **1994**, *116*, 11195−11196. (b) Chapman, T. M.; Mahan, E. J. *Polym. Mater. Sci. Eng.* **1995**, *73*, 275−276.

(7) (a) van Hest, J. C. M.; Delnoye, D. A. P.; Baars, M. W. P. L.; van Genderen, M. H. P.; Meijer, E. W. *Science* **1995**, *268*, 1592−1595. (b) van Hest, J. C. M.; Baars, M. W. P. L.; Elissen-Roman, C.; van Genderen, M. H. P.; Meijer, E. W. *Macromolecules* **1995**, *28*, 6689−6691.

(8) Aoi, K.; Motoda, A.; Okada, M. *Macromol. Rapid. Commun.* **1997**, *18*, 945−952.

(9) (a) Iyer, J.; Fleming, K.; Hammond, P. T. *Macromolecules* **1998**, *31*, 8757−8765. (b) Iyer, J.; Hammond, P. T. *Langmuir* **1999**, *15*, 1299−1306.

(10) Gitsov, I.; Lambrych, K. R.; Remnant, V. A.; Pracitto, R. *J. Polym. Sci. Polym. Chem.* **2000**, *38*, 2711−2727.

(11) Ihre, H.; Padilla De Jesus, O. L.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2001**, *123*, 5908−5917.

(12) Istratov, V.; Kautz, H.; Kim, Y.-K.; Schubert, R.; Frey, H. *Tetrahedron* **2003**, *59*, 4017−4024.

(13) (a) Gillies, E. R.; Fréchet, J. M. J. *Chem. Commun.* **2003**, *14*, 1640−1641. (b) Gillies, E. R.; Jonsson, T. B.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 11936−11943.

(14) (a) Yoo, Y.-S.; Choi, J.-H.; Song, J.-H.; Oh, N.-K.; Zin, W.-C.; Park, S.; Chang, T.; Lee, M. *J. Am. Chem. Soc.* **2004**, *126*, 6294−6300. (b) Jang, C.-J.; Ryu, J.-H.; Lee, J.-D.; Sohn, D.; Lee, M. *Chem. Mater.* **2004**, *16*, 4226−4231.

(15) Holzmueller, J.; Genson, K. L.; Park, Y.; Yoo, Y.-S.; Park, M.-H.; Lee, M.; Tsukruk, V. *Langmuir* **2005**, *21*, 6392−6398.

(16) (a) Zubarev, E. R.; Stupp, S. I. *J. Am. Chem. Soc.* **2002**, *124*, 5762−5773. (b) de Gans, B. J.; Wiegand, S.; Zubarev, E. R.; Stupp, S. I. *J. Phys. Chem. B* **2002**, *106*, 9730−9736.

10.1021/cm060232i CCC: $33.50
Published on Web 07/28/2006

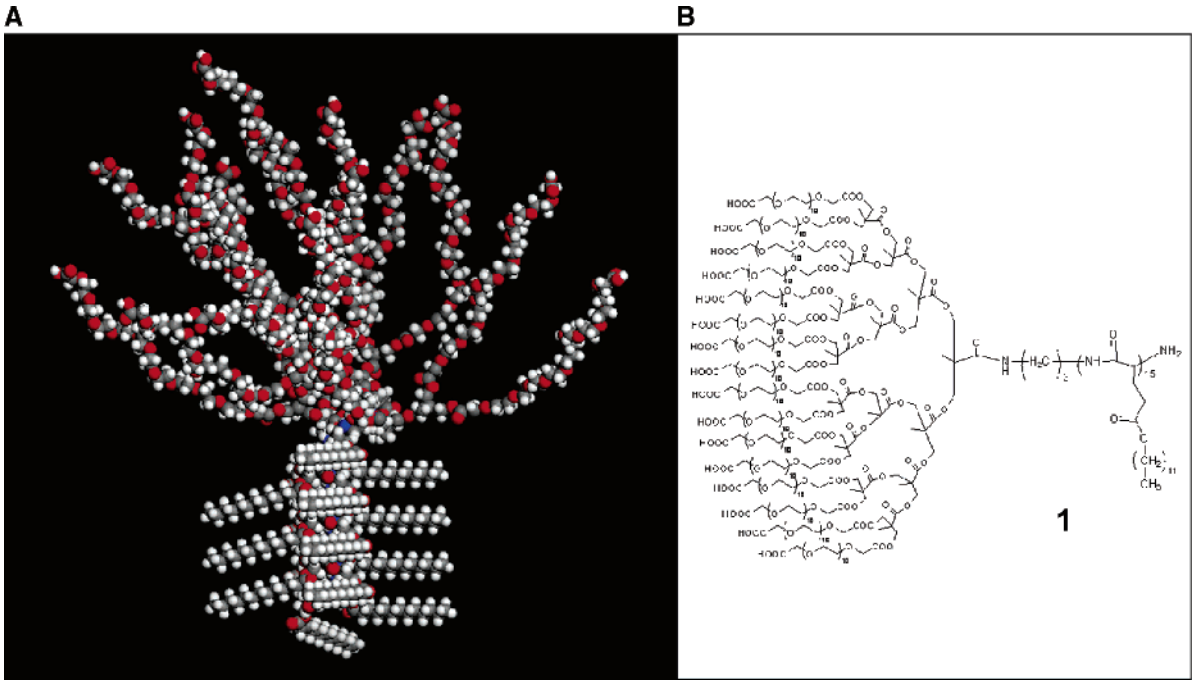


Figure 1. (A) Space filling molecular model of the amphiphilic tree-like macromolecule (**1**). The three-dimensional image was obtained with Materials Studio. Gray atoms, carbon; white atoms, hydrogen; red atoms, carbon; blue atoms, nitrogen. (B) Chemical structure of macromolecule **1**.

Among the many supramolecular structures possible, micellar nanoparticles are promising drug carriers for delivery hydrophobic therapeutic reagents.[17] Self-assembly of linear-dendritic hybrid block copolymers in solution provides a potential route to core−shell micellar nanostructures with desirable dendritic functional groups. Thus far, most of the amphiphilic linear-dendrimer systems studied have involved the use of the dendritic block as the more hydrophobic system, thus acting as the nano-encapsulating core of micelles.[10,13] In the few cases where the inverse arrangement has been studied, the linear block was not biocompatible, making the system unsuitable for biological or medical applications.[14,15] This arrangement is particularly interesting in creation a high ligand density functional exterior for targeted drug delivery. Finally, a challenge with such linear-dendrimer systems is the formation of extremely stable micelles. Most linear-dendrimer amphiphiles have critical micelle concentrations in the range of 10^{-6} to 10^{-4} M; however, there are great advantages to the ability to create more stable micellar structures that withstand dilution in the bloodstream or in other delivery conditions.

In this paper, a new amphiphilic biocompatible comb-dendritic block copolymer (**1**) rationally designed as a "tree-shaped" self-assembly building block was developed for advanced micellar drug carriers (Figure 1). This system uses design concepts to yield micellar nanoparticles that contain a highly functional dendritic exterior shell and a highly stable hydrophobic core.

There are four unique characteristics that make these new systems particularly relevant for the design of nanoscale building blocks. First, the comb block is a rigid, helical polypeptide with alkyl side groups, resulting in the formation of extremely stable micelles with an unusually low critical micelle concentration. Second, the micelle structure formed incorporates the comb block as the interior core, leaving the dendritic block as exterior groups on the shell of the nanoparticles, providing a highly functional surface that can be used to cluster biocompatible ligands for efficient targeting and sensing capabilities. Third, we also present a macromolecular structure that takes on a unique cone shape due to the semirigid rod nature of the comb block and the hydration and repulsive charge of the acid functional end groups of the dendritic block, yielding self-assembly behavior that enables direct application of theory relating molecular geometric shape to final micellar form; the conical shapes of these macroamphiphiles further enhance micelle stability. Last, it is important to note that the entire macromolecular system is biocompatible and biodegradable, thus making it suitable for various biomedical applications.

It is anticipated that these systems will present a new approach to the design of targeted micellar drug delivery vehicles, particularly for cancer drug delivery and biomarkers. Herein, we report the synthesis and physiochemical characterization of micelle-forming amphiphilic comb-dendritic block copolymers, including their micellar behavior and hydrophobic drug encapsulation behavior.

Experimental Section

Materials. Palladium (10 wt % on activated carbon) and poly(ethylene glycol) bis(carboxymethyl) ether (HOOC−PEG−COOH) were obtained from Aldrich and used as received. Dowex 50WX2-100 H^+ resin was purchased from Lancaster Synthesis Inc. 4-(Dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) and *N*-carboxyanhydride of *ω*-*n*-dodecyl-L-glutamate were synthesized according to the literature procedures.[18−20] The bis-MPA-based fourth generation dendron (**2**) was prepared based on the double-stage convergent approach introduced by Ihre et al.[21] All other

(17) (a) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113−131. (b) Rösler, A.; Vandermeulen, G. W. M.; Klok, H.-A. *Adv. Drug Delivery Rev.* **2001**, *53*, 95−108. (c) Lavasanifar, A.; Samuel. J.; Kwon, G. S. *Adv. Drug Delivery Rev.* **2002**, *54*, 169−190. (d) Hubbell, J. A. *Science* **2003**, *300*, 595−596.

chemicals used in this work were purchased from Alfa Aesar and used without further purification.

Methods. ^{1}H NMR were recorded on Varian VXR 300 MHz spectrometers. Samples (~5−10 mg/mL) were dissolved in $CDCl_3$, with TMS as an internal reference. Fourier transform infrared (FTIR) spectra were recorded on a Thermo Nicolet NEXUS 870 series spectrophotometer by solvent casting samples onto a KBr pellet. MALDI-TOF mass spectrometry was done on Bruker Omniflex system with a reflectron accessory.

Gel permeation chromatography (GPC) measurements were carried out to monitor trends in the variation of molecular weight and polydispersity as measured against polystyrene standards. These measurements were performed in tetrahydrofuran (THF) solution using a Water Breeze 1525 HPLC system equipped with two Styragel HT columns operated at 35 °C, series 2414 refractive index detector, series 1525 binary HPLC pump, and 717plus autosampler. Water Breeze Chromatography Software Version 3.30 was used for data collection as well as data processing. THF was used all the time as eluent for analysis and as solvent for sample preparation. Appropriate amount of sample (~5 mg/mL) was dissolved into THF and filtered using a 0.45-μm PTFE syringe filter (Whatman, Clifton, NJ) before injection into the column. A flow rate of 1.0 mL/min was used. The average molecular weight of the sample was calibrated against narrow molecular weight polystyrene standards (Polysciences, Warrington, PA).

Circular dichroism (CD) spectroscopy of polymer solution was carried out by using an Aviv model 202 CD spectrometer. Measurements were performed at 25 ± 0.1 °C, sampling every 0.2 nm with a 10-s averaging time over the range of 200−250 nm (bandwidth = 1.0 nm). Polymer concentration was 200 μg/mL.

CMC measurements of the macromolecule **1** in aqueous were performed by fluorescence spectroscopy using pyrene as the probe because pyrene preferentially partitions into a hydrophobic micellar core from water.[22] Upon changing the polarity of its microenvironment, pyrene undergoes a red shift (from 337 to 341 nm) in the excitation spectrum. Fluorescence peak intensity ratios ($I_{341\ nm}/I_{337\ nm}$) were plotted against the logarithm of polymer concentrations to determine CMC as the onset of micellization.[22,23] Fluorescence spectroscopy was carried out on a Spex FluoroMax-2 spectrofluorometer (Edison, NJ) at 25 °C. A stock solution of pyrene at 5.00×10^{-7} M in water was prepared. Polymer samples were dissolved in the stock pyrene solution, diluted to specific concentrations. Excitation was performed from 300 to 360 nm, with 390 nm as the emission wavelength.

Tapping-mode atomic force microscopy (AFM) measurements were conducted in air with a Dimension 3100 system (Digital Instruments, Santa Barbara, CA) operated under ambient conditions. The samples were prepared for AFM analysis by depositing a 25-μL drop of the aqueous solution (50 μg/mL) onto the plasma-treated silicon surface and allowing it to dry freely in air for 3 days. Transmission electron microscopy (TEM) was performed on a JEOL JEM 200CX electron microscope (JEOL Ltd., Japan). The samples were prepared for TEM analysis by depositing a 5-μL drop of the polymer aqueous solutions onto the FORMVAR-coated copper grids and allowing it to dry freely in air for 3 days. Before analysis, the samples were stained with 1.0 wt % phosphotungstic acid.

Multi-angle dynamic light scattering (DLS) study was performed by photon correlation spectroscopy[24] using a Brookhaven Instruments, Co. (Holtsville, NY) system consisting of a model BI-200SM goniometer, a model EMI-9865 photomultiplier, a model BI-9000AT digital correlator, and a Coherent Innova 90C Series ion laser (Santa Clara, CA) operated at 514 nm. All measurements were made at 25 ± 0.1 °C; 1.0 mg/mL of polymer was dissolved into water and filtered using a 0.45-μm Acrodisc syringe filter (Pall Co., Ann Arbor, MI) before measurements. The DLS measurements were taken at angles varying from 45° to 105°. All determinations were made in triplicate. The resulting autocorrelation functions were single exponential decays, described by eq 1. The value Γ can be calculated through an exponential fit to this equation. By plotting Γ against the square of the scattering vector **q**, the slope as a function of the diffusion coefficient D can be calculated according to eq 2. Consequently, the average hydrodynamic diameter d can then be determined from the Stokes−Einstein relationship:

$$C(\tau) = Ae^{-2\Gamma t} + B \tag{1}$$

where $C(\tau)$ is the autocorrelation function, A is an instrumental constant, B is the baseline, and

$$\Gamma = q^2 D \tag{2}$$

where q is the scattering vector and D is the diffusion coefficient and where

$$q = 4\pi n \sin(\theta/2)/\lambda \quad \text{and} \quad D = k_B T/3\eta\pi d \tag{3}$$

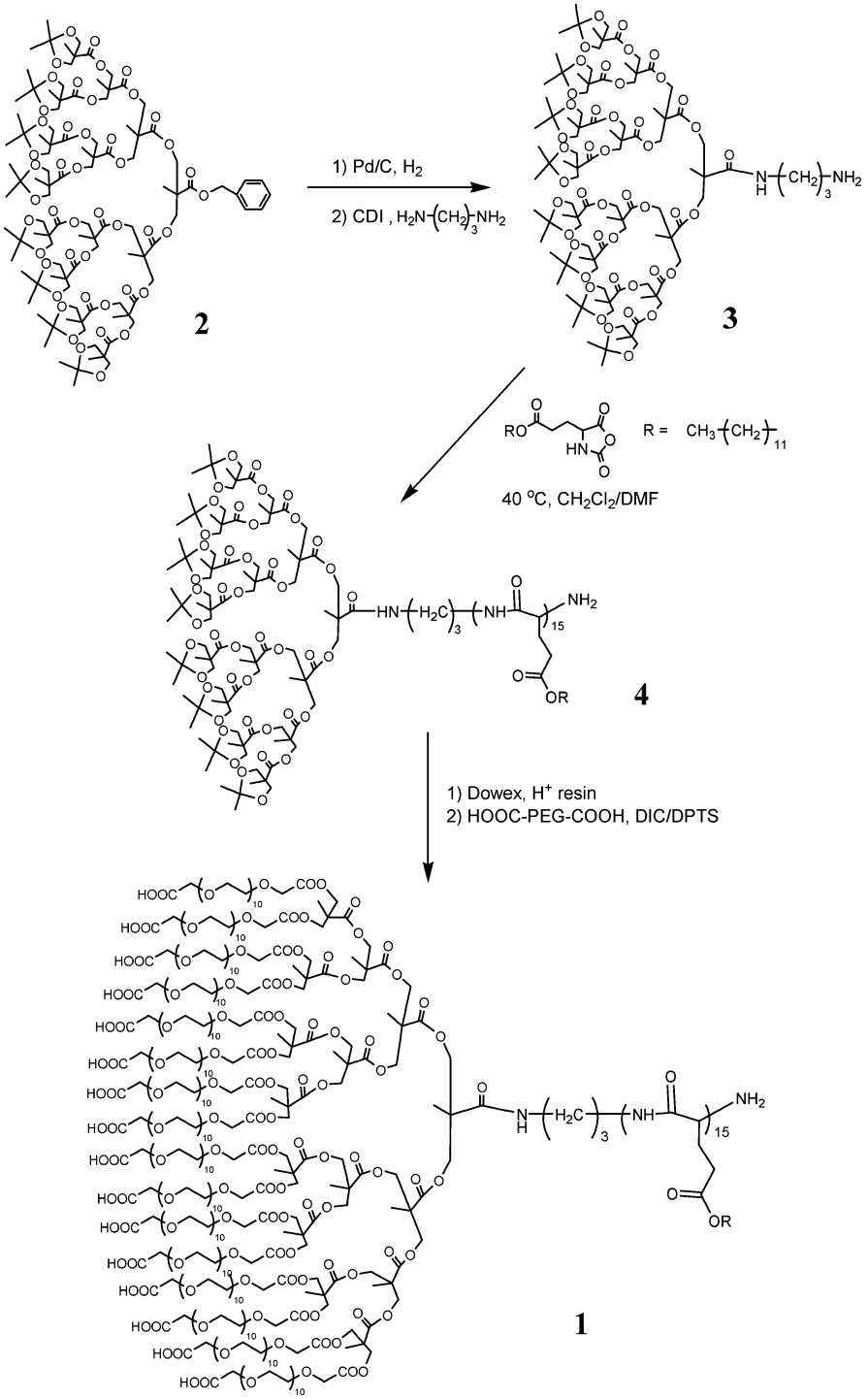
where n is the refractive index, θ is the scattering angle, λ is the laser wavelength, k_B is Boltzmann's constant, T is the temperature in Kelvin, η is the liquid viscosity, and d is the particle diameter.

Drug loading experiments were performed based on O/W emulsion method with triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol).[25] A 5.0 mL triclosan solution (2.0 mg/mL) in methylene chloride was added dropwise into 10.0 mL of polymer aqueous solution (1.0 mg/mL) under vigorous stirring at room temperature. The mixture was stirred in an air open system overnight to remove methylene chloride by evaporation. The resulting solution was centrifuged at 5000 rpm for 30 min and then filtered by a 0.45-μm polyterafluoroethylene (PTFE) syringe filter to remove the unbound triclosan. The actual amount of triclosan loaded into micelles was determined by measuring the UV absorbance at 282 nm after complete disruption of the micelles by addition of DMF (9:1). The calibration curve was obtained by measuring the UV absorbance at 282 nm of standard solutions (DMF/water = 9/1) containing 0−100 μg/mL of triclosan ($Y = 0.0195X - 0.00475$, $r^2 = 0.9999$). The linear calibration curve shows a direct proportional relationship between the UV absorbance (λ = 282 nm) and the triclosan concentration. The entire drug loading experiments have been done four times.

Synthesis. As shown in Scheme 1, the polyester dendritic initiator (**3**) was synthesized from 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) based polyester dendrons (**2**) by transferring the benzyl ester group at the root of dendron to a primary amino group through catalytic hydrogenolysis followed by an amidation reaction with an excess amount of 1,3-diaminopropane. A ring-opening polymerization of *N*-carboxyanhydride of γ-*n*-dodecyl-L-glutamate was initiated with dendron **3** to form a linear-dendritic block copolymer

(18) Moore, J. S.; Stupp, S. I. *Macromolecules* **1990**, *23*, 65−70.
(19) Wassermann, D.; Garber, J. D.; Meigs, F. M. U.S. Patent 3,285,953, 1966.
(20) Daly, W. H.; Poche, D. *Tetrahedron Lett.* **1988**, *29*, 5859−5862.
(21) Ihre, H.; Hult, A.; Fréchet, J. M. J. Gitsov, I. *Macromolecules* **1998**, *31*, 4061−4068.
(22) Wilhelm, M.; Zhao, C.-L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J.-L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033−1040.
(23) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339−7352.
(24) Tscharnuter, W. In *Encyclopedia of Analytic Chemistry*; Meyers, R. A., Ed.; John Wiley & Sons Ltd.: Chichester, 2000; p 5469.
(25) La, S. B.; Okano, T.; Kataoka, K. *J. Pharm. Sci.* **1996**, *85*, 85−90.

Scheme 1. Synthetic Route toward the Tree-shaped Amphiphilic Comb-Dendritic Block Copolymer



(**4**). Finally, the dendritic block was conjugated with PEG through esterification using the carbodiimine method after deprotection of acetonide groups. IR, mass spectroscopy, NMR, and gel-permeation chromatography were applied to confirm the synthesized structures.

*Synthesis of Polyester Dendritic Initiator (**3**).* The fourth generation polyester dendron (**2**) (1.0 g, 0.48 mmol) and 1,1′-carbonyldiimidazole (CDI) (0.16 g, 0.97 mmol) were dissolved into anhydrous THF (30 mL). After stirring for 3 h under argon at room temperature, 1,3-diaminopropane (0.29 g, 3.9 mmol) was added dropwise. The reaction mixture was stirred for 1 day under argon at room temperature. After the solvent was evaporated at room temperature, the residue was then dissolved in dichloromethane (150 mL) and washed with sodium bicarbonate saturated aqueous solutions (30 mL) and with 50-mL portions of brine (3×), dried over anhydrous sodium sulfate, and evaporated to dryness. The crude product was purified by chromatography using dichloromethane/methanol (9/1, v/v) as eluent. Product **3** was obtained as a colorless oil (0.78 g, 73% yield). ^{1}H NMR ($CDCl_3$) (δ): 4.31 (s, 24H, CH_2), 4.24 (s, 4H, CH_2), 4.14 (d, 16H, CH_2), 3.61 (d, 16H, CH_2), 3.40 (m, 2H, CH_2), 2.96 (m, 2H, CH_2), 1.82 (m, 2H, CH_2), 1.41 (s, 24H, CH_3), 1.34 (s, 24H, CH_3), 1.27 (s, 21H, CH_3), 1.17 (s, 24H, CH_3). IR (KBr, cm^{-1}): 3363, 1539 (N−H), 2990, 2877 (C−H), 1738, 1653 (C═O), 1240, 1155, 1123, 1082 (C−O). MALDI-TOF MS: 2159.5 ([M + Na]$^+$).

*Synthesis of Linear-Dendritic Copolymer (**4**).* The polyester dendritic initiator (**3**) (0.9 g, 0.42 mmol) and *N*-carboxyanhydride (NCA) of γ-*n*-dodecyl-L-glutamate (2.2 g, 6.4 mmol) were dissolved in a mixture of dichloromethane/DMF (5/1, v/v). The ring-opening

polymerization proceeded homogeneously at 40 °C for 24 h under argon. The solvents were then removed under vacuum. The crude product was purified by precipitation into methanol (250 mL) from dichloromethane (15 mL). The product **4** was obtained as white solid (2.3 g, 84% yield). ^{1}H NMR ($CDCl_3$) (δ): 4.31 (s, 24H, CH_2), 4.27 (s, 4H, CH_2), 4.15 (d, 16H, CH_2), 4.03 (m, 15H, CH), 3.97 (t, 30H, CH_2), 3.62 (d, 16H, CH_2), 3.18 (m, 4H, CH_2), 2.62 (m, 30H, CH_2), 2.21 (m, 30H, CH_2), 1.85 (m, 2H, CH_2), 1.59 (m, 30H, CH_2), 1.41 (s, 24H, CH_3), 1.36 (s, 24H, CH_3), 1.25 (m, 21H, CH_3; 270H, CH_2), 1.16 (s, 24H, CH_3), 0.87 (t, 45H, CH_3). IR (KBr, cm^{-1}): 3292, 1549 (N−H), 2925, 2855 (C−H), 1737, 1656 (C═O), 1246, 1173, 1123, 1083 (C−O). GPC: MW = 6.7 kDa, PDI = 1.08.

*Synthesis of Amphiphilic Tree-like Macromolecule (**1**).* The acetonide protective groups of the linear-dendritic copolymer (**4**) were deprotected by stirring in methanol/THF (1:3, v/v) in the presence of an acidic Dowex 50W-X2 resin for 18 h. The deprotected product (2.0 g, 0.32 mmol), HOOC−PEG−COOH (MW = 600 Da) (9.1 g, 15 mmol) (dehydrated by azeotropic distillation in toluene), and DPTS (1.1 g, 3.7 mmol) were dissolved in dichloromethane (100 mL). After 10 min flushing with argon, 1,3-diisopropylcarbodiimide (DIC) (1.9 g, 15 mmol) was added dropwise. After 48 h, the DIC side product was removed by suction filtration. The filtrate was diluted by dichloromethane (400 mL), washed with 50-mL portions of brine (3×), dried over anhydrous sodium sulfate, and evaporated to dryness. The resulted polymer was dialyzed against distilled water for 24 h by a Spectra/Por cellulose ester membrane with a MW cutoff of 15 kDa from Spectrum Laboratories. The dialyzed solution was dried by vacuum to make the crude product, which is purified by precipitation into ethyl ether (500 mL) from methanol (20 mL). The final product was obtained as white waxy solid (3.3 g, 67% yield). ^{1}H NMR ($CDCl_3$) (δ): 4.27 (m, 92H, CH_2), 4.16 (d, 32H, CH_2), 4.03 (m, 15H, CH), 3.97 (t, 30H, CH_2), 3.65 (m, ∼640H, CH_2), 2.64 (m, 30H, CH_2), 2.20 (m, 30H, CH_2), 1.58 (m, 30H, CH_2), 1.24 (m, 21H, CH_3; 270H, CH_2), 1.17 (s, 24H, CH_3), 0.86 (t, 45H, CH_3). IR (KBr, cm^{-1}): 3294, 1548 (N−H), 2922, 2871 (C−H), 1739, 1656 (C═O), 1250, 1202, 1115, 1036 (C−O). GPC: MW = 15.5 kDa, PDI = 1.12.

Results and Discussion

Design and Synthesis. The well-defined structure of macromolecule **1** consists of poly(γ-*n*-dodecyl-L-glutamate) (PDLG) as a linear hydrophobic comb block and a polyester dendron modified at the endgroups with poly(ethylene glycol) (PEG) as a hydrophilic dendritic block. The α-helical conformation of PDLG, which imparts a rodlike character to the hydrophobic block, coupled with the presence of an aliphatic side chain emanating from each repeat unit, creates a novel hydrophobic semirigid comb-rod.[26] The alkyl pendant groups of PDLG intercalate and further enhance the hydrophobic interactions between the blocks.[27] Such multibranched hydrophobic domains are proposed to be more efficient in self-assembly in aqueous media than a single hydrophobic block, resulting highly stable polymer micellar structures.[10,28] The dendritic structure of the hydrophilic block provides high-density peripheral functional groups for subsequent functionalization with biospecific ligands, which can be used to form nanoscale ligand clusters. The dendrons are modified with short-chain PEG because of its well-known biological compatibility and stealth properties.[29] Additionally, the free carboxylic acid groups at the end of the PEG segments provide the possibility for further chemical surface modification with a ligand. All the building blocks chosen for macromolecule **1** are biocompatible and consist of biodegradable ester and amide bonds. Initial in vitro cytotoxicity study of macromolecule **1** has been conducted with Hep G2 human hepatocellular carcinoma cells. On the basis of cell viability relative to the control (no polymer added), the cytotoxicity of the polymer was negligible at 24 h of incubation for all polymer test concentrations (data not shown here).

The synthesis of macromolecule **1** consists of three steps: (1) preparation of bis-MPA-based polyester dendrons with a primary amino group at the root of dendron; (2) ring-opening polymerization of *N*-carboxyanhydride of γ-*n*-dodecyl-L-glutamate initiated with the amino dendron molecule, and (3) PEG modification of the dendritic block.

The bis-MPA-based fourth generation dendron (**2**) was prepared based on the double-stage convergent approach introduced by Ihre et al.,[21] and the structure was confirmed by TLC, IR, and NMR. After deprotection of the benzyl ester group by catalytic hydrogenolysis at the root of dendron **2**, the resulting free carboxylic acid was reacted with an excess amount of 1,3-diaminopropane in the presence of CDI as the dehydration reagent to provide a primary amino group. The structure of dendron **3** was confirmed with NMR data and compared to dendron **2**, specifically by observing three methylene proton resonance peaks at 3.40, 2.96, and 1.82 ppm from diaminopropane. This amidation reaction can also be verified by observing the absorptions at 1653 cm^{-1} (C═O) and 1539 cm^{-1} (N−H) at IR spectra. Finally, MALDI-TOF MS was used to measure the molecular mass of dendron **3**, which is 2159.5 ($[M + Na]^+$), well nearly equivalent to the calculated value of 2159.4 ($[M + Na]^+$).

By using dendron **3** as a primary aliphatic amine initiator, the NCA of γ-*n*-dodecyl-L-glutamate was polymerized to make a comb-dendritic block copolymer (**4**). Because the primary aliphatic amines are more nucleophilic than the active chain ends, the initiation is faster than the propagation of monomer;[30] all initiating dendron molecules remain attached to the growing polypeptide chain and the process has a living character, which can give nearly monodisperse polypeptides. The polymerization was monitored by ^{1}H NMR by measuring the integration ratio between a typical proton peak at 3.62 ppm (16H per dendron) from the dendritic initiator and one proton peak at 1.59 ppm (2H per glutamate) from the poly (γ-*n*-dodecyl-L-glutamate). Consequently, the polymerization degree can be calculated, which is matched with the theoretical value of monomer feed ratio to initiator (15:1). GPC also gives a reasonable molecular weight of

(26) Daly, W. H.; Poche, D.; Negulescu, I. I. *Prog. Polym. Sci.* **1994**, *19*, 79−135.
(27) Lavasanifar, A.; Samuel, J.; Kwon, G. S. *Colloids Surf. B* **2001**, *22*, 115−126.
(28) Kreig, A.; Lefebvre, A.; Hahn, H.; Balsara, N.; Qi, S.; Chakrabort, A.; Xenidou, M.; Hadjichristidis, N. *J. Chem. Phys.* **2001**, *115*, 6243−6251.
(29) (a) Otsuka, H.; Nagasaki, Y.; Kataoka, K. *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 3−10. (b) Otsuka, H.; Nagasaki, Y.; Kataoka, K. *Adv. Drug Delivery Rev.* **2003**, *55*, 403−419.
(30) Daly, W. H.; Poche, D.; Negulescu, I. I. *Prog. Polym. Sci.* **1994**, *19*, 79−135.

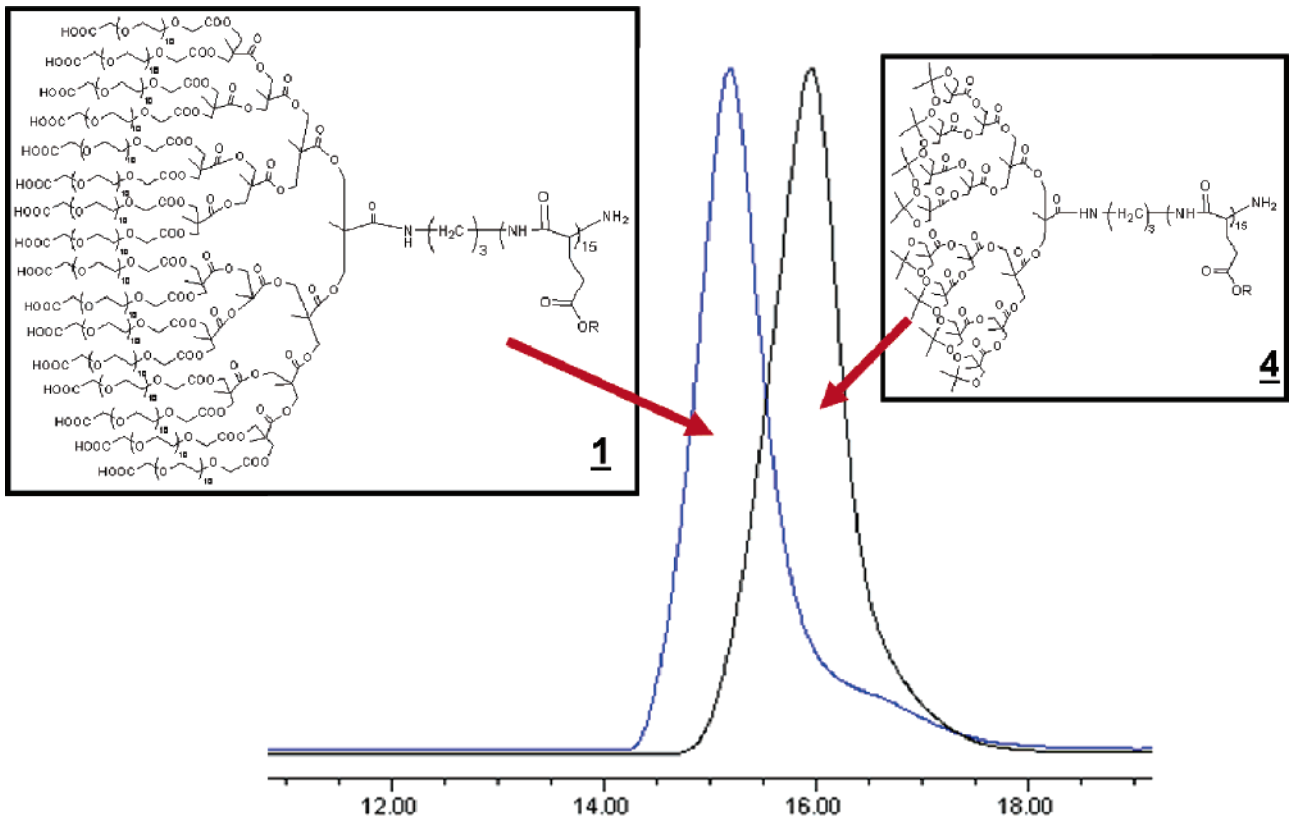


Figure 2. GPC chromatograms illustrating the difference in retention times between macromolecules **1** and **4**.

around 6.7 kDa with PDI at 1.08. The polypeptide backbone assumes a rigid α-helical conformation, with the long hydrocarbon side chains propagating from each repeating unit. The α-helical conformation can be identified by the strong C═O amide I absorption at 1656 cm^{-1} and N−H amide II absorption at 1549 cm^{-1}. Also in aqueous solutions, the α-helical conformation of the comb block within polymer micelles was verified by observing characteristic negative ellipticity at 209 and 222 nm.

The final step of the synthesis of macromolecule **1** is the modification of dendritic blocks by PEG bis(carboxymethyl) ether (HOOC−PEG−COOH) through esterification reaction using DIC/DPTS method.[18] First, the acetonide protective groups of the linear-dendritic copolymer (**4**) were deprotected through trans-etherification of the acetonide groups with methanol under acidic conditions. Then a large excess amount of HOOC−PEG−COOH and 1,3-diisopropylcarbodiimde (equivalent to PEG) were introduced for esterification of the free hydroxyl groups at the dendron periphery to avoid the intra- and intermolecular cross-linking side reactions. The amino group at the end of PDLG was not reactive for this coupling reaction from our experience of failure to conjugate HOOC−PEG−COOH block with the end amino group of two PDLG blocks to make a triblock copolymer. It was probably due to the steric hindrance effect of the α-helix rod and long alkyl side chains. The crude reaction mixture was monitored by GPC for the formation of the final product (**1**). Upon the reaction completion, clear shifts from the characteristic peak of the starting materials (**4**) to the products (**1**) were noted in the GPC trace with no significant polydispersity changes (Figure 2). The crude product was purified by dialysis method and precipitation.

Polymeric Micelles. In aqueous solution, macromolecule **1** self-assembles into micellar structures with the comb-like hydrophobic block forming the core and hydrophilic dendritic blocks forming the exterior shell (Figure 3). The secondary structure (i.e., α-helical conformation) of comb block PDLG within polymer micelles was verified by observing characteristic negative ellipticity at 209 and 222 nm via CD spectroscopy in water. Unlike typical linear blocks, the rigidity of the PDLG block allows its treatment as an alkyl fragment with l_c = ideal polymer chain length. At a PDLG block length of 15 and dendrimer generation 4.0, the cone-shaped molecular architecture favors spherical micellar aggregates according to the theory of Israelachvili[31] (i.e., the critical packing parameter $v/a_0l_c < 1/3$; v is hydrocarbon volume; a_0 is optimal headgroup area; l_c is critical chain length). The resulting polymeric micelles possess a functional dendritic surface for the placement of nanoscale ligand clusters. The distance between each ligand and the scale of the clustering can be controlled by alternating the dendron generation and the degree of functionalization.

The critical micelle concentration (CMC) is most commonly employed to evaluate the thermodynamic stability of the polymeric micelles in aqueous solutions.[17] In this work, CMC measurements were performed using the fluorescence method with pyrene as the probe because pyrene preferentially partitions into the hydrophobic core of micelles from water[22,23] (Figure 4). Compared to traditional surfactants (such as SDS, CMC = 8.6×10^{-3} M), amphiphilic polymers generally have lowered CMC values (usually at $\sim 10^{-6}$ M).[27]

(31) Israelachvili, J. N. *Intermolecular and Surface Forces*; Academic Press: San Diego, 1995.

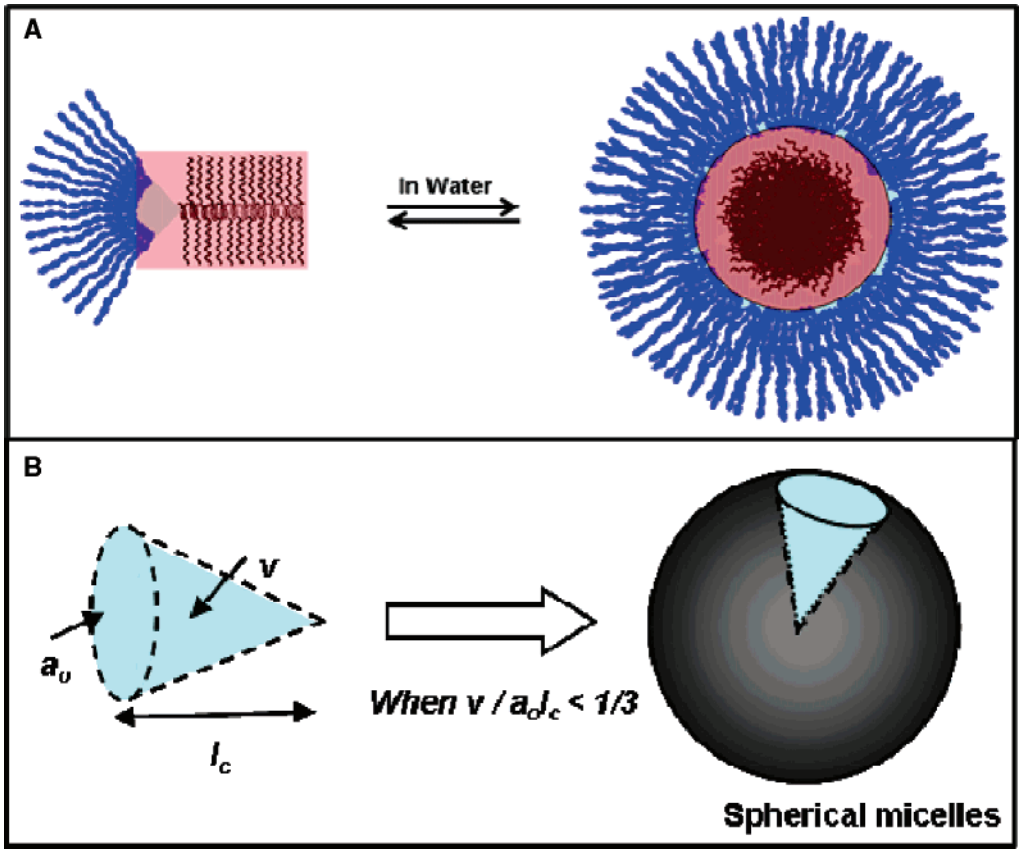


Figure 3. (A) Cartoon illustration of self-assembly of tree-shaped macromolecules to form core−shell spherical micelles. (B) Schematic of critical packing shapes for spherical micelles based on the theory of Israelachvili et al.[36]

With such low CMC values, amphiphilic polymers can form a stable micellar aggregates with low rates of dissociation in vivo.[32] Furthermore, amphiphilic polymers can stabilize the hydrophobic drugs inside the micelle core to achieve the sustained release and higher accumulation at specific physiological sites.[33] Macromolecule **1** has a remarkably low CMC value at 3.4×10^{-8} M. This result illustrates the effectiveness of the cone-shaped arrangement of the amphiphiles and the multi-branched alkyl structure for stabilizing micellar aggregates. Other amphiphilic polymers with branched core-forming architectures, such as dendritic molecules[10] and pendant alkyl chains,[27] also show low CMC values around 10^{-6} M levels. To determine the aggregation number of polymer micelles from macromolecule **1**, static light scattering (SLS) measurements were done on 1.0 mg/mL polymer aqueous solution at 25 °C. The aggregation number is averagely 25 molecules per micelle. Ongoing studies will be done to determine the aggregation numbers with different conditions (e.g., temperature and pH).

The high level of stability of polymer micelles allows us to image these structures as 3D spherical nanoparticles on surfaces using both AFM and TEM without any further stabilization. Shown in Figure 5, monodisperse spherical particles around 30 nm in diameter were found in both AFM and TEM images. These stable, narrowly distributed nanoscale micelles are expected to show extravasation efficacy

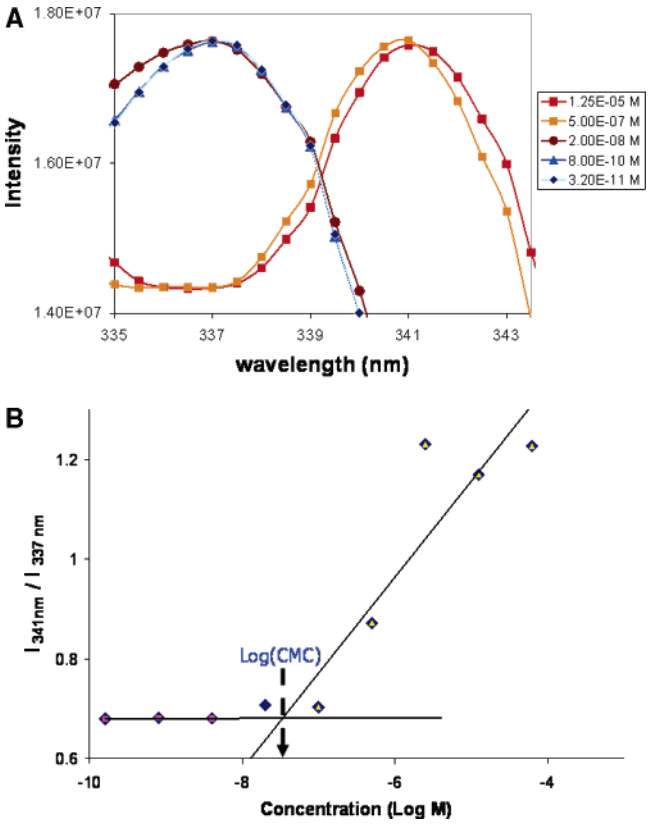


Figure 4. (A) Pyrene fluorescence excitation spectra of polymer aqueous solutions ($\lambda_{emission} = 390$ nm). (B) Fluorescence intensity ratios of pyrene excitation bands ($I_{341\ nm}/I_{337\ nm}$) as a function of the concentration of polymer aqueous solutions.

(32) Allen, C.; Maysinger, D.; Eisenberg, A. *Colloids Surf. B* **1999**, 16, 3−27.
(33) Kataoka, K.; Kwon, K.; Yokoyama, M.; Okano, T.; Sakurai, Y. *J. Controlled Release* **1993**, *24*, 119−132.

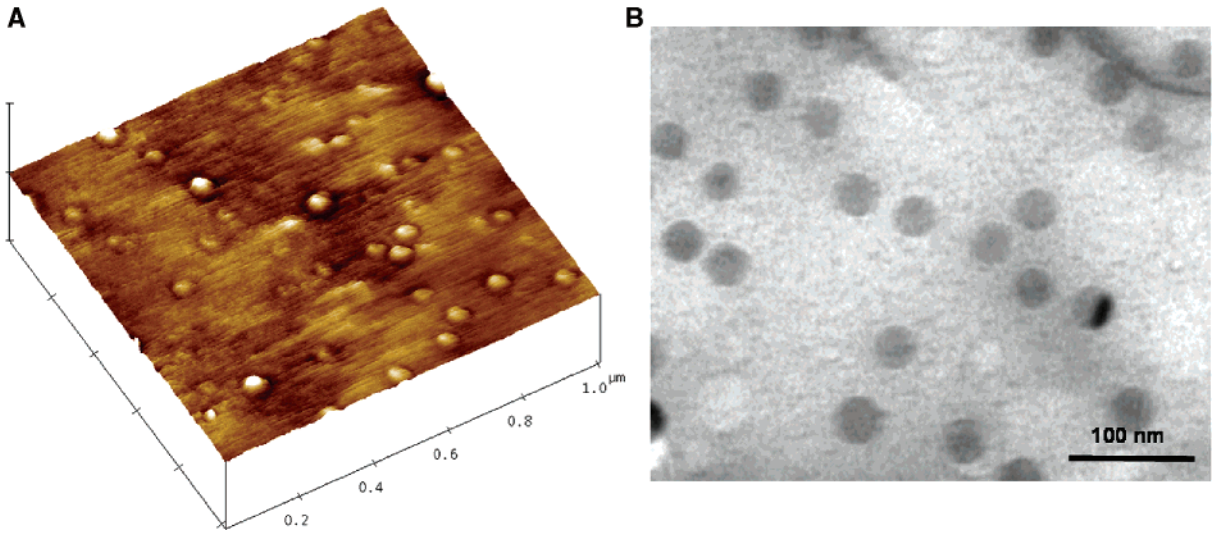


Figure 5. Tapping mode AFM image (phase) (A) and TEM images (B) of spherical structures of micellar aggregates from macromolecule **1**.

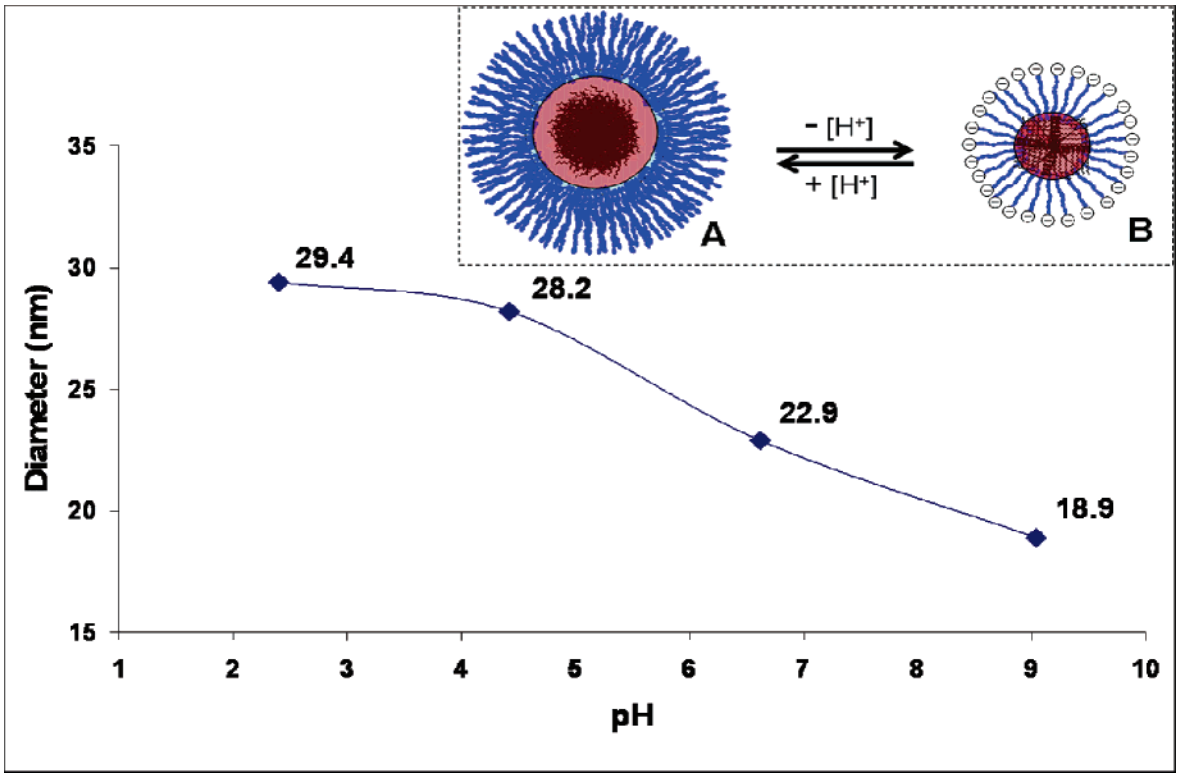


Figure 6. Particle sizes of polymeric micelles from macromolecule **1** in different pH aqueous solutions at 1.0 mg/mL. In the inserted cartoon illustration, at low pH, noncharged macromolecular amphiphiles with smaller headgroups form larger micelles (A) with a higher aggregation number; at high pH, charged macromolecular amphiphiles with larger headgroups form smaller micelles (B) with a lower aggregation number.

for solid tumor tissues[34] and evade reticuloendothelial system up-take, when considered as anti-cancer drug delivery vehicles.[35] These systems may also prove relevant as micellar carriers for environmental remediation or bioseparations applications as well.

To assess the particle size of the micellar aggregates, multi-angle dynamic light scattering (DLS) measurements were carried out using photon correlation spectroscopy.[24] All the autocorrelation functions resemble single exponential decays, which represent the narrowly dispersed populations of particle sizes. As shown in Figure 6, the micelle size shifts from 29.4 to 18.9 nm upon changing the solution pH from acidic to basic. The size of polymer micelles can be changed upon stretching and compressing of the hydrophilic blocks of amphiphilic block copolymers. For macromolecule **1**, the

(34) (a) Hobbs, S. K.; Monsky, W. L.; Yuan, F.; Roberts, W. G.; Griffith, L.; Torchilin, V. P.; Jain, R. K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4607−4612. (b) Moghimi, S. M.; Hunter, A. C.; Murray, J. C. *Pharmacol. Rev.* **2001**, *53*, 283−318.

(35) (a) Papisov, M. I. *Adv. Drug Delivery Rev.* **1995**, *16*, 127−139. (b) Yokoyama, M.; Fukushima, S.; Uehara, R.; Okamoto, K.; Kataoka, K.; Sakurai, Y.; Okana, T. *J. Controlled Release* **1998**, *50*, 79−92. (c) Nishiyama, N.; Kataoka, K. *J. Controlled Release* **2001**, *74*, 83−94.

surface carboxylic acids of the hydrophilic dendritic block become deprotonated with increased pH, which induces negative charge. Due to charge repulsion between the PEG−COOH chains, the hydrodynamic volume of each individual polymer, particularly the hydrophilic charged dendritic block, will increase. We observed that the micelle size decreases upon increasing the pH of the solutions. A possible explanation is that due to the persistent molecular shape and high density of ionizable COOH groups on the dendron, the headgroups area (a_o) of the hydrophilic dendritic block will increase markedly due to charge repulsion between end groups. Such repulsion can lead to smaller micelles with fewer polymer amphiphiles, thus lowering the repulsive energetic penalty of spherical micellar packing.[36]

Regarding the potential applications for lipophilic drug carriers, the drug loading capacity of these micelles has been evaluated using triclosan as a model or example drug. Triclosan is an extremely hydrophobic bactericide ($\log P = 4.76$), for which the low aqueous solubility ($\sim$10 μg/mL) hampers its biological activity.[37] Triclosan-loaded micelles were obtained by O/W emulsion methods.[25] The actual amount of triclosan loaded into the micelles was determined by measuring the UV absorption at 281 nm after complete disruption of the micelles upon addition of DMF. A substantial drug loading level of 30.4 $\pm$ 1.2% (w/w %, drug/polymer) was achieved, which is about 30 times higher than the actual drug solubility in water. Additionally we anticipate that these polymer micelles can be used as controlled release vehicles for hydrophobic anticancer therapeutics, and the relevant work is in progress.

(36) Israelachvili, J. N.; Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 1525−1568.
(37) Loftsson, T.; Leeves, N.; Bjornsdottir, B.; Duffy, L.; Masson, M. *J. Pharm. Sci.* **1999**, *88*, 1254−1258.

Conclusion

In summary, we have shown a novel example of biocompatible amphiphilic comb-dendritic block copolymers that combine the diblock copolymer framework with the nanoscale multivalent functionality of dendrimers at the hydrophilic periphery. In aqueous solution, the tree-like macromolecules can self-assemble into spherical micelles with unimodal size distributions. The cone-type morphology and the alkyl pendant groups enhance stability of the micelles, resulting in an unusually low CMC of 10^{-8} M. The presence of the carboxylic acid group at the surfaces of micelles makes the particle size of these micelles pH dependent. The drug loading capacity of the polymeric micelles is about 30 wt % for the hydrophobic bactericide, triclosan. In addition, the nanoscale clustering of the peripheral acid groups are potential substrates for subsequent functionalization with biospecific ligands to improve biologically relevant affinity enhancement.

Acknowledgment. The authors acknowledge the National Science Foundation (DMR-0413524) and the Institute for Soldier Nanotechnology at MIT for financial support. We also thank Dr. Pil J. Yoo (Chemical Engineering, MIT) and Dr. Hongwei Gu (Chemistry, MIT) for their assistance.

CM060232I