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# Synthesis and Aqueous Aggregation Properties of Amphiphilic Surface-Block Dendrimers

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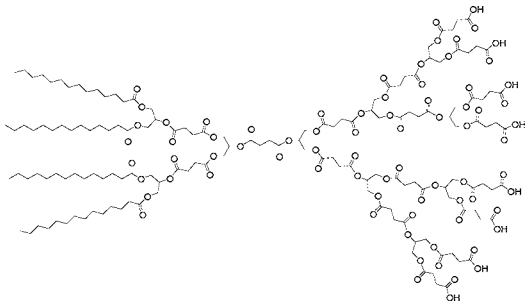
## ABSTRACT



**A family of dendritic amphiphiles were synthesized from the natural metabolites of glycerol, succinic acid, and myristic acid. The surfaces of these dendrimers display different numbers of alkyl chains and carboxylic acids, varying the hydrophobic-to-hydrophilic ratio over a relatively broad range. In solution these dendritic amphiphiles form supramolecular structures, and these aggregates have been characterized by light microscopy, transmission electron microscopy, and tensiometry. These aggregates can entrap the hydrophobic species pyrene.**

Amphiphiles are prevalent throughout the physical and life sciences and, because of their self-assembly properties, are investigated for various applications from model membranes to drug delivery.[1] The defining characteristic of amphiphilic molecules is the simultaneous presence of both hydrophilic and hydrophobic regions. In aqueous solution these molecules often spontaneously assemble into ordered structures such as micelles and vesicles. In addition to traditional small molecular weight amphiphiles such as fatty acids and phospholipids, there is significant interest in synthetic amphiphilic polymers.[2] In general, synthetic polymers possess lower critical aggregation concentrations and the aggregation structures tend to be more stable than those formed with low molecular weight amphiphiles.[2a] However, it can be difficult to systematically vary and obtain consistent aggregate size and/or morphology with polymeric systems because these properties are dependent on composition. Dendritic polymer amphiphiles possess the favorable attributes associated with large amphiphiles yet maintain the monodispersity found in naturally occurring and synthetic low molecular weight amphiphiles. Amphiphilic dendrimer polymers have been reported, most notably "unimolecular" dendritic amphiphiles or dendritic-linear polymers possessing a hydrophilic dendritic component and a hydrophobic linear

(1) (a) Zana, R. *Colloids Surf., A* **1997**, *123*−*124*, 27−35. (b) Fuhrhop, J. H.; Helfrich, W. *Chem. Rev.* **1993**, *93*, 1565−1582. (c) Israelachvili, J. N. *Intermolecular and Surface Forces: With Applications to Colloidal and Biological Systems*; Academic Press: New York, 1985. (d) Silvius, J. R. *Annu. Rev. Biophys. Biomol. Struct.* **1992**, *21*, 323−348. (e) Cevc, G.; Marsh, D. *Phospholipid Bilayers: Physical Principles and Models*; Wiley: New York, 1987.

(2) (a) Yang, L.; Alexandridis, P. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 132−143. (b) Discher, B. M.; Hammer, D. A.; Bates, F. S.; Discher, D. E. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 125−131. (c) Torchilin, V. P. *J. Controlled Release* **2001**, *73*, 137−172. (d) Bates, F. S. *Science* **1991**, *251*, 898−905. (e) Velonia, K.; Rowan, A. E.; Nolte, R. J. M. *J. Am. Chem. Soc.* **2002**, *124*, 4224−4225. (f) Cameron, N. S.; Corbierre, M. K.; Eisenberg, A. *Can. J. Chem.* **1999**, *77*, 1311−1326.

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polymer (or vice versa).[3] The dendritic amphiphiles presented in this manuscript have different numbers of both hydrophilic and hydrophobic surface moieties. Specifically, we have synthesized and characterized a family of amphiphilic surface-block dendrimers as shown in Figure 1.

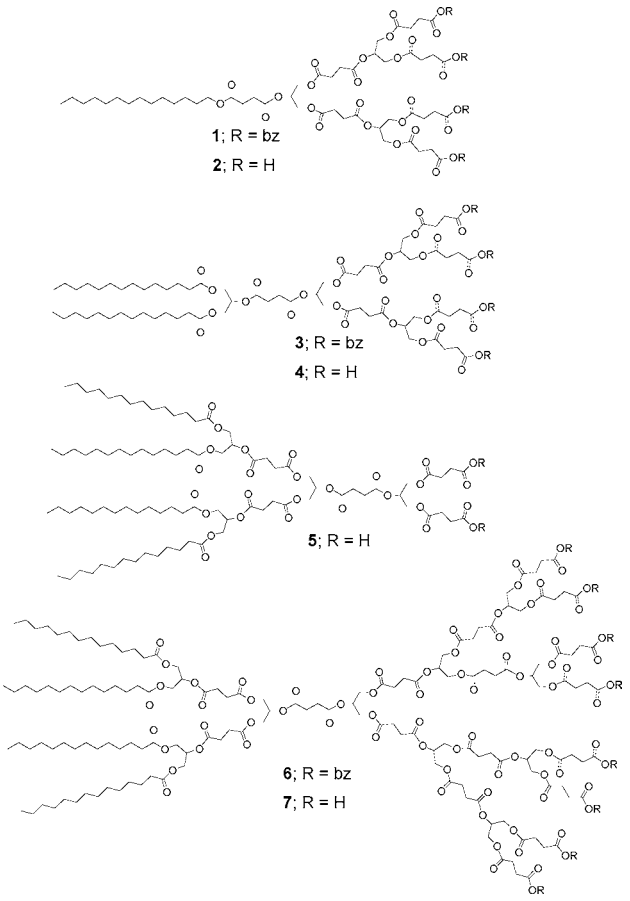
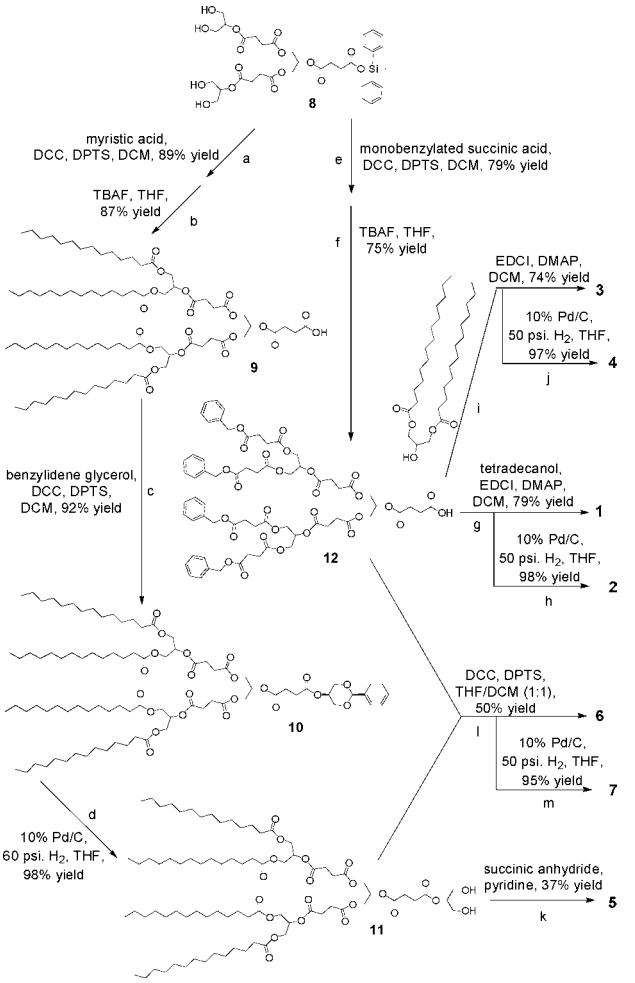



**Figure 1.** Four amphiphilic surface-block dendrimers.

An advantage of working with dendritic polymers[4] is the stepwise preparation methods, which proceed in either a divergent or convergent fashion. The convergent approach allows for a large degree of chemical diversity such that functional groups can be incorporated at nearly any position in the dendritic architecture with degrees of precision highly unusual for traditional linear polymers. Our laboratory is particularly interested in dendritic macromolecules composed of biocompatible building blocks for drug delivery and tissue engineering applications.[5] The amphiphilic surface-block dendrimers **2**, **4**, **5**, and **7** were synthesized as shown in Scheme 1 using a convergent approach. Surface-block

**Scheme 1.** Synthesis of Dendritic Compounds




dendrimers contain at least two different types of peripheral end groups confined to definite areas, while the interior branching structure of the dendrimer remains constant.[6]

These poly(glycerol-succinic acid) (PGLSA) dendrimer derivatives are similar with respect to their interior composition, which consists of glycerol and succinic acid. However, the surface of these PGLSA dendrimers has been modified

(3) (a) Imae, T. *Surfactant Sci. Ser.* **2003**, *112*, 525−545. (b) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Johnson, A. L.; Behera, R. K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1178−1180. (c) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, *12*, 1287−1297. (d) Percec, V.; Cho, W.; Ungar, G.; Yeardley, D. J. P. *J. Am. Chem. Soc.* **2001**, *123*, 1302−1315. (e) van Hest, J. C. M.; Delnoye, D. A. P.; Baars, M. W. P. L.; Elissen-Roman, C.; van Genderen, M. H. P.; Meijer, E. W. *Chem. Eur. J.* **1996**, *2*, 1616−1626.

(4) (a) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665−1688. (b) Fischer, M.; Vögtle, F. *Angew. Chem., Int.* Ed. **1999**, *38*, 885−905. (c) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendritic Molecules: Concepts, Syntheses*, Perspectives; VCH: New York, 1996. (d) Tomalia, D. A.; Fréchet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 2719−2728. (e) Fréchet, J. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4782−4787. (f) Luman, N. R.; Kim, T.; Grinstaff, M. W. *Pure Appl. Chem.* **2004**, *76*, 1375−1385. (g) Grinstaff, M. W. *Chem. Eur. J.* **2002**, *8*, 2838−2846.

(5) (a) Wathier, M.; Jung, P. J.; Carnahan, M. A.; Kim, T.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2004**, *126*, 12744−12745. (b) Morgan, M. T.; Carnahan, M. A.; Immoos, C. E.; Ribeiro, A. A.; Finkelstein, S.; Lee, S. J.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2003**, *125*, 15485−15489. (c) Carnahan, M. A.; Middleton, C.; Kim, J.; Kim, T.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2002**, *124*, 5291−5293. (d) Carnahan, M. A.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2001**, *123*, 2905−2906. (e) Carnahan, M. A.; Grinstaff, M. W. *Macromolecules* **2001**, *34*, 7648−7655.

(6) (a) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1991**, *5*, 1059−1076. (b) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *Macromol. Symp.* **1994**, *77*, 11−20.

**Table 1.** Calculated Molecular Weights (MW), Mass Spectrometry (MALDI-MS), Size Exclusion Chromatography (SEC), Thermal Transition Temperature, Critical Aggregation Concentration (cac), and Area Per Molecule (ApM) for Surface-Block Dendrimers

| compd | MW (g/mol) | MALDI ($M+Na^+$) | SEC ($M_w$) | PDI | transition temp (°C) | cac (mol/L) | ApM (Å$^2$) |
|---|---|---|---|---|---|---|---|
| **2** | 1137.13 | 1158.72 | 1482 | 1.02 | −22 | $2.0 \times 10^{-4}$ | 440 |
| **4** | 1435.55 | 1458.34 | 1872 | 1.02 | −10 | $1.1 \times 10^{-5}$ | 210 |
| **5** | 1656.12 | 1678.37 | 2160 | 1.01 | 18 | | |
| **7** | 3301.46 | 3322.85 | 3944 | 1.03 | 47 | $1.1 \times 10^{-5}$ | 360 |

with various numbers of alkyl chains and carboxylic acids in a controlled fashion. The 14-carbon chain of myristic acid adds sufficient hydrophobicity to the macromolecules while the carboxylic acid groups are hydrophilic. Compounds **2** and **4** both display four carboxyl groups, whereas **2** contains a single myristic chain and **4** possesses two. Compounds **5** and **7** both display four myristic chains; **7** contains eight carboxyl groups and **5** possesses two. All compounds shown in Figure 1 and Scheme 1 have been fully characterized by $^1$H and $^{13}$C NMR, MALDI-MS, size exclusion chromatography (SEC), and elemental analysis (EA) as reported in Supporting Information.

The following description of the synthesis for compound **4** is a representative example, and using slight modifications, each of the macromolecules shown in Figure 1 can be prepared in high yield (details in Supporting Information). First, compound **8**[7] was coupled with 4 equiv of monobenzylated succinic acid in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) in 79% yield. Treatment of this intermediate with tetrabutylammonium fluoride (TBAF) afforded compound **12** in 75% yield. Compound **12** was then coupled with 1,3-di-*O*-tetradecanoylglycerol,[8] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 4-(dimethylamino)pyridine (DMAP), to afford **3** in 74% yield. Finally, compound **3** was subjected to hydrogenolysis conditions to cleave the benzyl esters and afford compound **4** in 97% yield.

The data presented in Table 1 show good correlation between calculated molecular weight and the MW observed by MALDI-MS. The low polydispersity indices obtained by SEC indicate a nearly monodisperse system. The masses obtained by SEC were calibrated against linear polystyrene standards and show an increase in MW from **2** to **7** for the synthesized compounds. The transition temperatures were determined by differential scanning calorimetry (DSC) and show a trend that correlates increased MW with increased transition temperatures. The data were collected using 2 mg of sample in an aluminum pan, and the temperature was equilibrated for 10 min at −60 °C. The temperature was increased at 3 °C/min to 100 °C where it was held for 5 min. The heating−cooling cycle was repeated two times, and the data on the third cycle was analyzed.

(7) Luman, N. R.; Smeds, K. A.; Grinstaff, M. W. *Chem. Eur. J.* **2003**, *9*, 5618−5626.
(8) Cockman, S. J.; Joll, C. A.; Mortimer, B. C.; Redgrave, T. G.; Stick, R. V. *Aust. J. Chem.* **1990**, *43*, 2093−2097.

When dissolved in aqueous solution, dendrimers **2**, **4**, and **7** spontaneously form supramolecular aggregates. Because of the poor solubility of **5**, no hydration structures are observed. The morphology of the structures formed by **2**, **4**, and **7** can be altered depending upon external factors such as temperature, concentration, and sample preparation. A selection of the supramolecular assemblies formed from these amphiphiles is shown in Figure 2 (additional pictures are in Supporting Information). The micrographs displayed in Figure 2A and 2B are obtained via transmission electron microscopy (TEM) with a negative staining solution of 2% uranyl acetate and a concentration of $1 \times 10^{-3}$ M of dendritic amphiphile dissolved in 200 mM buffered HEPES solution (pH = 7.4). Figure 2A shows multilamellar aggregates of compound **7** after sonication with an intralamellar distance of approximately 7 nm. Figure 2B shows vesicles of compound **4** with an intralamellar distance of approximately 18 nm following sonication and extrusion through a 0.1 $\mu$m nylon filter. Preparation of compound **4** without extrusion provided results similar to those shown in Figure 2A. Multilamellar aggregates of compound **7** are observed by TEM for both extruded and nonextruded solutions. Compound **2** does not display aggregation structures via TEM.

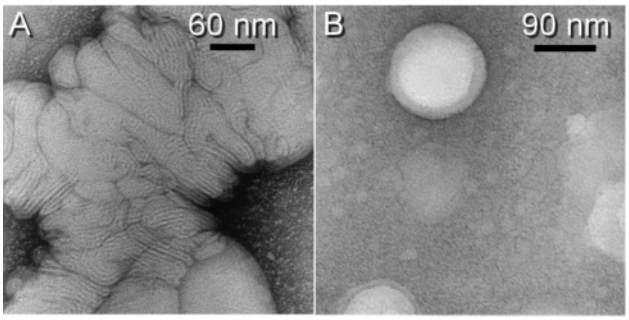



**Figure 2.** (A) TEM image of compound **7**. (B) TEM image of compound **4** after extrusion.

Additional studies were performed to quantify the solution aggregation behavior of these macromolecules. Tensiometric determination of the critical aggregation concentration (cac) for compounds **2**, **4**, and **7** was performed under isothermal conditions (24 °C). The surface tension ($\sigma$, mN/m) was plotted as a logarithmic function of surfactant concentration in PBS buffer (8.3 mmol, pH = 7.2), and a break in the

curve occurs at the threshold aggregation concentration.[9] As shown in Table 1, the critical aggregation concentrations for compounds **2**, **4**, and **7** are $2.0 \times 10^{-4}$, $1.1 \times 10^{-5}$, and $1.1 \times 10^{-5}$ M, respectively. Compounds **4** and **7** have double the ratio of hydrophobic alkyl chains to hydrophilic carboxyl groups as compound **2**. The larger degree of hydrophobicity likely plays a significant role in the lower cac's observed for compounds **4** and **7** as compared with **2**. Dendritic-linear hybrids and modified poly(propylene imine) dendrimers have been reported to have cac's of $10^{-6}-10^{-7}$ M and modified poly(amido amine) dendrimers have been reported with cacs of $2-6 \times 10^{-4}$ M.[10]

The area per molecule (ApM) values of 440, 210, and 360 Å$^2$ were calculated from the Gibbs absorption equation[11] for compounds **2**, **4**, and **7**, respectively (Table 1). Compound **2** forms the most densely packed monolayers, followed by **7** and **4**, respectively. The ApM values for these dendritic amphiphiles are roughly 10 times the reported values for myristic acid[12] and coincide with the range of values reported for other amphiphilic species.[11,13]

A particularly interesting functional aspect of surfactant systems is the ability to solubilize hydrophobic molecules within the nonpolar environments of multimolecular aggregates. To demonstrate the solubilization capability of these amphiphilic dendrimers, we selected the hydrophobic fluorescent probe pyrene. The photophysical properties of pyrene are well documented, and the fluorescence spectrum is highly dependent on the local environment. For example, the intensity of the first vibrational band (0−0 band, $I_1$ ~373 nm) of pyrene increases when pyrene transitions from bulk aqueous solution to the hydrophobic regions of surfactant aggregates.[14] The fluorescence intensity of pyrene ($5 \times 10^{-7}$ M, $\lambda_{ex} = 320$ nm, $\lambda_{em} = 373$ nm) in buffered HEPES solutions (200 mM, pH = 7.4) with dendritic amphiphiles **2**, **4**, or **7** was measured above and below the cac. Fluorescence intensities of 20, 23, and 32 au were observed with compounds **2**, **4**, and **7** at concentrations below the cac ($1 \times 10^{-5}$ M), respectively. Above the cac ($1 \times 10^{-3}$ M), the intensity of the 373 nm peak increases to 246, 289, and 361 au with compounds **2**, **4**, and **7**, respectively. Additionally, the change in emission intensity ratio of the first ($I_1 =$ 373 nm) and third ($I_3 =$ 383 nm) vibrational bands is regarded as a reliable indicator of the surrounding polarity of pyrene. The $I_1/I_3$ ratio of pyrene in aqueous solution is ~1.6; however, when pyrene is incorporated within the interior of surfactant aggregates, this value typically decreases by ~30−40%.[14] Below the cac of compounds **2**, **4**, and **7** ($1 \times 10^{-5}$ M) the $I_1/I_3$ ratio of pyrene was determined to be 1.7, 1.5, and 1.4, respectively. Above the cac of compounds **2**, **4**, and **7** ($1 \times 10^{-3}$ M) the $I_1/I_3$ ratio decreased to 0.8, 1.2, and 1.1, respectively. The pyrene concentration was $5 \times 10^{-7}$ M in all solutions. The observed changes in the $I_1$ and $I_1/I_3$ emission intensity are consistent with pyrene being located in a more nonpolar environment supporting pyrene incorporation within the dendritic aggregates.

In conclusion, a family of myristylated poly(glycerol-succinic acid) dendritic amphiphiles were synthesized that show a wide range of aqueous aggregation behavior. The synthetic approach reported can be applied to the preparation of a range of amphiphilic molecules and allows control over the hydrophilic-to-hydrophobic ratio. The thermal transition temperatures and critical aggregation concentrations were determined, which provide quantitative information on the relatively few reported examples of dendritic amphiphiles. Additionally, dendritic amphiphile aggregates were able to entrap a hydrophobic dye, pyrene. Such dendritic polyesters are likely to be of interest for medical, biotechnological, and biological applications due to their biocompatible building blocks and the interesting structures formed in aqueous solution.

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**Supporting Information Available:** Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL051583Q

(9) (a) Birdi, K. S. *Handbook of Surface and Colloid Chemistry*; CRC Press: Boca Ranton, FL, 1997. (b) Hiemenz, P. C. *Principles of Colloid and Surface Chemistry*; Marcel Dekker: New York, 1997.

(10) (a) 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. (b) Schenning, A. P. H. J.; Elissen-Roman, C.; Weener, J.; Baars, M. W. P. L.; van der Gaast, S. J.; Meijer, E. W. *J. Am. Chem. Soc.* **1998**, *120*, 8199−8208. (c) Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1997**, *30*, 8072−8074. (d) Aoi, K.; Motoda, A.; Okada, M.; Imae, T. *Macromol. Rapid Commun.* **1997**, *18*, 945−952.

(11) Rosen, M. J. *Surfactants and Interfacial Phenomena*; Wiley: New York, 1978.

(12) Patil, G. S.; Matthews, R. H.; Cornwell, D. G. *J. Lipid Res.* **1976**, *17*, 197−202.

(13) (a) Sui, G.; Micic, M.; Huo, Q.; Leblanc, R. M. *Langmuir* **2000**, *16*, 7847−7851. (b) Felder, D.; Gallani, J.; Guillon, D.; Heinrich, B.; Nicoud, J.; Nierengarten J. *Angew. Chem., Int. Ed.* **2000**, *39*, 201−204.

(14) (a) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339−7352. (b) 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. (c) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039−2044.