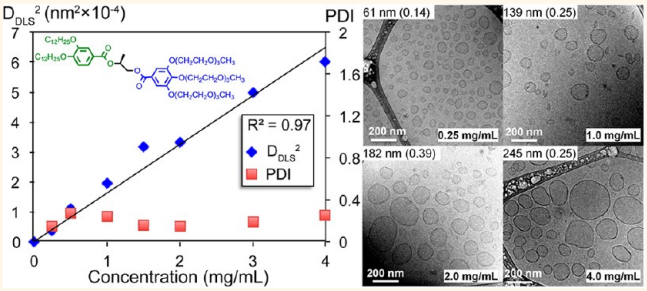
"Single–Single" Amphiphilic Janus Dendrimers Self-Assemble into Uniform Dendrimersomes with Predictable Size

Shaodong Zhang,[†] Hao-Jan Sun,[†] Andrew D. Hughes,[†] Bogdan Draghici,[†] Janis Lejnieks,[†] Pawaret Leowanawat,[†] Annabelle Bertin,[†] Lidiannie Otero De Leon,[†] Oleg V. Kulikov,[†] Yingchao Chen,[§] Darrin J. Pochan,[§] Paul A. Heiney,[‡] and Virgil Percec[†,*]

[†]Roy & Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States, [‡]Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6396, United States, and [§]Department of Materials Science & Engineering, University of Delaware, Newark, Delaware 19716, United States

ABSTRACT An accelerated modular synthesis of six libraries containing 29 amphiphilic Janus dendrimers, employed to discover and predict functions *via* primary structures, is reported. These dendrimers were constructed from a single hydrophobic and a single hydrophilic dendron, interconnected with L-Ala to form two constitutional isomeric libraries, with Gly to produce one library, and with L-propanediol ester to generate two additional constitutional isomeric libraries. They are denoted "single–single" amphiphilic Janus dendrimers. Assemblies obtained by injection of their ethanol solution into water were analyzed by dynamic light scattering and cryogenic transmission electron microscopy. A diversity of complex structures including soft and hard dendrimersomes, cubosomes, solid lamellae, and rod-like micelles were obtained in water. It was discovered that the "single–single" amphiphilic Janus dendrimers containing three triethylene glycol groups in the hydrophilic dendron favored the formation of dendrimersomes. Assemblies in bulk analyzed by differential scanning calorimetry and powder X-ray diffraction revealed that the amphiphilic Janus dendrimers with melting point or glass transition below room temperature self-assemble into soft dendrimersomes in water, while those with higher temperature transitions produce hard assemblies. In the range of concentrations where their size distribution is narrow, the diameter of the dendrimersomes is predictable by the *d*-spacing of their assemblies in bulk. These results suggested the synthesis of Library 6 containing two simpler constitutional isomeric benzyl ester based amphiphilic Janus dendrimers that self-assemble in water into soft dendrimersomes and multidendrimersome dendrimersomes with predictable dimensions.



KEYWORDS: amphiphilic Janus dendrimers · dendrimersomes · vesicles · size prediction

Vesicles are molecular nanocapsules self-assembled in water from phospholipids (liposomes),[1−3] amphiphilic block copolymers (polymersomes),[4−9] and amphiphilic Janus dendrimers (dendrimersomes)[10−12] with a diversity of applications in nanoscience and nanotechnology. They provide mimics of primitive and contemporary biological membranes[13−17] and are elaborated as biomimetic nanocapsules[18] for delivery of drugs,[19,20] genes,[21,22] and vaccines[23] and as theranostics[24] and pioneered the field of nanomedicine.[18] For all these applications, vesicles should be stable in time in various media, monodisperse, and impermeable, should exhibit good mechanical properties, and should be easily accessible both synthetically and *via* simple self-assembly processes.[19,25,26] Liposomes prepared from natural phospholipids *via* complex methodologies are unstable and permeable and exhibit poor mechanical properties. Co-assembly of phospholipids with cholesterol[2,27] and with phospholipids conjugated with poly(ethylene oxide)[28,29] provided the most successful technology currently employed to improve their mechanical properties, decrease membrane permeability, and provide stability over time. Polymersomes prepared also by tedious methods

* Address correspondence to percec@sas.upenn.edu.

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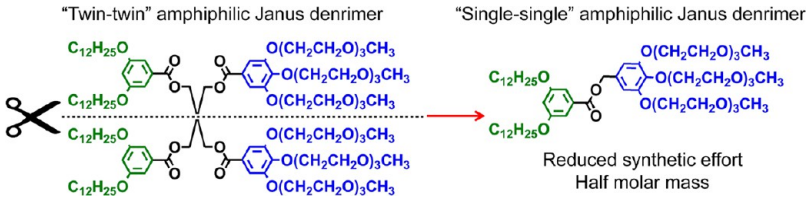


Figure 1. Comparison of "twin−twin" (left) and "single−single" (right) amphiphilic Janus dendrimers. A "twin−twin" amphiphilic Janus dendrimer is composed of twin-hydrophobic (green) and twin-hydrophilic (blue) dendrons, while the "single−single" homologue is composed of a single-hydrophobic and a single-hydrophilic dendron.

have better mechanical properties. However, they are polydisperse and often not biocompatible.[4−9] Both liposomes[1−3] and polymersomes[30,31] require multiple and difficult fractionations by extrusion[32] to generate a desirable size with narrow polydispersity.

Dendrimers with different topologies and architectures have been extensively used in biological and biomedical applications.[33−37] Our laboratory discovered that amphiphilic Janus dendrimers self-assemble into monodisperse and stable vesicles, denoted dendrimersomes, by simple injection of their solution in ethanol, THF, or other water-soluble solvents into water or buffers.[10,12] The resulting dendrimersomes are impermeable and exhibit excellent mechanical properties. With few exceptions, most of the amphiphilic Janus dendrimers used for the assembly of dendrimersomes were constructed from combinations of twin-hydrophobic and twin-hydrophilic dendrons, denoted "twin−twin" amphiphilic Janus dendrimers (Figure 1). Based on the analysis of the lamellar structure of these amphiphilic Janus dendrimers by X-ray diffraction experiments in bulk and of the dimensions of the corresponding dendrimersomes by cryo-TEM in water, a method was developed to predict the diameter and properties of dendrimersomes.[11] "Twin−twin" amphiphilic Janus dendrimers have also been elaborated to mimic the glycan ligand of biological membranes through the design of Janus glycodendrimers that self-assemble in water into glycodendrimersomes.[12] They have been shown to be extremely efficient in the binding of biomedically relevant plant, bacterial, and human sugar binding proteins known as lectins and, therefore, have great potential in nanomedicine.[18] A library approach was elaborated to discover and predict the primary structures responsible for these functions.[10−12] "Twin−twin" amphiphilic Janus dendrimers and glycodendrimers have been shown to provide a powerful strategy to produce both dendrimersomes and glycodendrimersomes. However, their synthesis from a "single" hydrophilic and a "single" hydrophobic dendron would reduce the time required for their synthesis and the number of reaction steps and produce amphiphilic Janus dendrimers with half of the molar mass of their "twin−twin" homologues (Figure 1). These "single−single" amphiphilic Janus dendrimers would therefore be of considerable interest for technological applications.

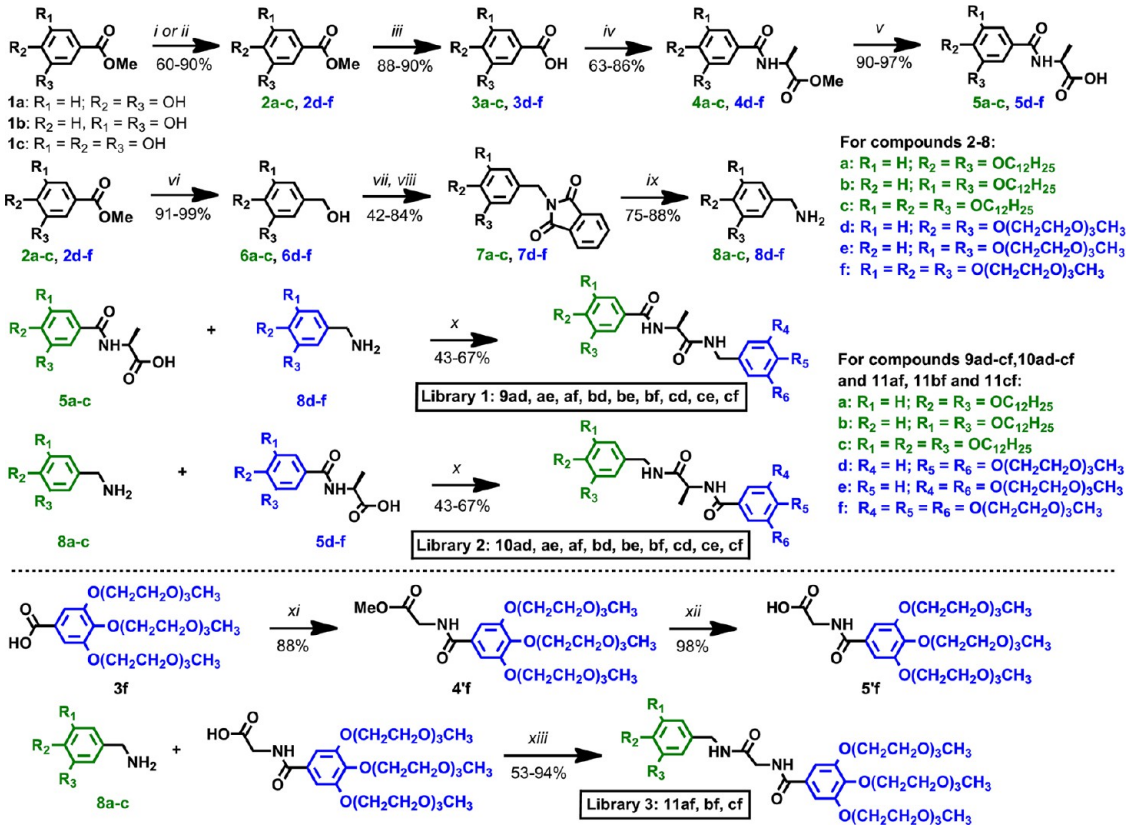
This publication reports the synthesis and analysis of six libraries of "single−single" amphiphlic Janus dendrimers, compares their properties with those of the "twin−twin" homologues,[10−12] and investigates the role of the structure of their hydrophilic and hydrophobic fragments as well as of the core of the Janus dendrimer on their self-assembly. The application of the correlation between the primary structure of "single−single" amphiphilic Janus dendrimers and their assembly in bulk and in water to the discovery of soft dendrimersomes *via* the library approach will be discussed. The method elaborated previously to predict the size of dendrimersomes at a specific concentration[11] will be expanded in this report to predict the size of "single−single" dendrimersomes over the entire range of concentrations in which their polydispersity is uniform. Finally, the ability of libraries of "single−single" amphiphilic Janus dendrimers to discover new dendrimersome architectures not accessible from "twin−twin" Janus dendrimers such as multidendrimersome dendrimersomes will be demonstrated.

RESULTS AND DISCUSSION

Modular Synthesis of "Single−Single" Amphiphilic Janus Dendrimers. Two libraries of "single−single" amphiphilic Janus dendrimers were synthesized by coupling the first-generation L-alanine-containing dendritic acids **5a**−**f** with the first-generation dendritic amines **8a**−**f** (Scheme 1). Hydrophobic building blocks (**5a**−**c**, **8a**−**c**) were synthesized by attaching dodecyl groups on their periphery, while hydrophilic building blocks were prepared by introducing triethylene glycol monomethyl ethers (**5d**−**f**, **8d**−**f**). The "single−single" amphiphilic Janus dendrimers were constructed by coupling single hydrophobic dendritic acids **5a**−**c** with single hydrophilic dendritic amines **8d**−**f** or single hydrophilic dendritic acids **5d**−**f** with single hydrophobic dendritic amines **8a**−**c**.

This modular approach elaborated for the preparation of two libraries of "single−single" amphiphilic Janus dendrimers involves three steps. The first step consists of the synthesis of a library of first-generation L-alanine-containing dendritic acids **5a**−**f**. Etherification of methyl 3,4-dihydroxybenzoate (**1a**), methyl 3,5-dihydroxybenzoate (**1b**), and methyl 3,4,5-trihydroxybenzoate (**1c**) respectively with 1-bromododecane and tosylated monomethyl ether of triethylene glycol produced first-generation dendritic esters **2a**−**f** in 60−90% yield. Saponification of their ester groups

Scheme 1. Synthesis of L-Alanine and Glycine Amide Containing Amphiphilic Janus Dendrimers[a]

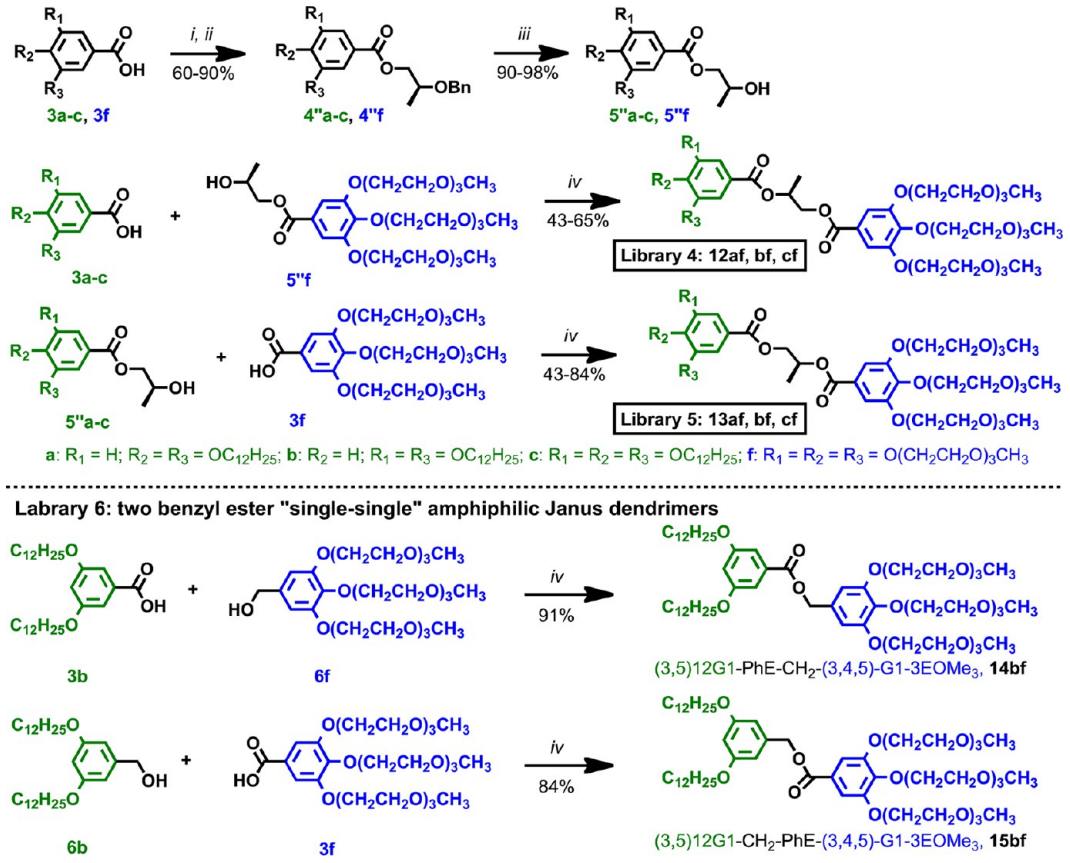


[a] Reagents and conditions: (i) $C_{12}H_{25}Br$, K_2CO_3, DMF, KI, 80 °C, 8 h; (ii) $TsO(CH_2CH_2O)_3CH_3$, K_2CO_3, DMF, KI, 80 °C, 8 h; (iii) KOH, EtOH/H_2O, reflux, 1−4 h; (iv) $CH_3CH(NH_2)COOCH_3 \cdot HCl$, CDMT, NMM, THF, 23 °C, 6−8 h; (v) KOH, EtOH/H_2O, reflux, 1−4 h; (vi) $LiAlH_4$, THF, 0 to 23 °C, 4−6 h; (vii) $SOCl_2$, DCM, 0 to 23 °C, 2 h; (viii) potassium phthalimide, THF/DMF, 0 °C, 4 h; (ix) $NH_2NH_2 \cdot H_2O$, EtOH/THF = 2:1, reflux, 8 h; (x) CDMT, NMM, THF, 23 °C, 8 h; (xi) $NH_2CH_2COOCH_3 \cdot HCl$, CDMT, NMM, THF, 23 °C, 8 h; (xii) KOH, EtOH: H_2O, reflux, 4 h; (xiii) CDMT, NMM, THF, 23 °C, 8 h.

followed by acidification generated the corresponding dendritic acids **3a**−**f** (88−90% yield). Coupling of **3a**−**f** with L-alanine methyl ester hydrochloride in the presence of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and *N*-methylmorpholine (NMM) in THF (63−86% yield), followed by hydrolysis in the presence of KOH in ethanol under reflux, yielded the first-generation L-alanine-containing dendritic acids **5a**−**f** in 90−97% yield. The second step involves the synthesis of the first-generation dendritic amines from dendritic esters **2a**−**f**. Dendritic esters **2a**−**f** were first reduced by $LiAlH_4$ in THF at 23 °C to give the corresponding alcohols **6a**−**f** (91−99% yield), which were subsequently chlorinated with $SOCl_2$ in DCM and reacted with potassium phthalimide to afford compounds **7a**−**f** in 42−84% yield. The dendritic phthalimides **7a**−**f** were deprotected with hydrazine in a mixture of ethanol and THF to yield the first-generation dendritic amines **8a**−**f** (75−88% yield). The third step of this modular synthesis involves the amidation of the hydrophobic dendritic acids **5a**−**c** with the hydrophilic dendritic amines **8d**−**f** or hydrophilic dendritic acids **5d**−**f** with hydrophobic dendritic amines **8a**−**c**. These reactions were conducted in the presence of CDMT and NMM in THF, producing 18 amphiphilic Janus dendrimers. **9ad**, **ae**, **af**, **bd**, **be**, **bf**, **cd**, **ce**, **cf** and **10ad**, **ae**, **af**, **bd**, **be**, **bf**, **cd**, **ce**, **cf**, in 43−67% yield after purification by column chromatography.

The 18 "single−single" amphiphilic Janus dendrimers from Libraries 1 and 2 were designed to elucidate the correlation between their primary structure and the structure of their assemblies in water. This will be discussed in the next section. The molecular design principles discovered with the Janus dendrimers from Libraries 1 and 2 were used to prepare three additional "single−single" amphiphilic Janus dendrimers containing only the (3,4,5)-hydrophilic pattern from Library 3 and replacing L-Ala with Gly in their amide core (Scheme 1). The hydrophilic dendritic acid **3f** was coupled with Gly methyl ester hydrochloride in the presence of CDMT/NMM in THF, providing the Gly-containing compound **4′f** in 88% yield. Saponification of **4′f** followed by acidification produced the corresponding dendritic acid **5′f** in 98% yield. The hydrophilic dendritic acid **5′f** was respectively coupled with the hydrophobic dendritic amines **8a**−**c** to afford the "single−single" amphiphilic Janus dendrimers **11af**, **11bf**, and **11cf** in 53% to 94% yield.

Scheme 2. Synthesis of Constitutional Isomeric Libraries of L-1,2-Propanediol Ester and Benzyl Ester Containing Amphiphilic Janus Dendrimers[a]



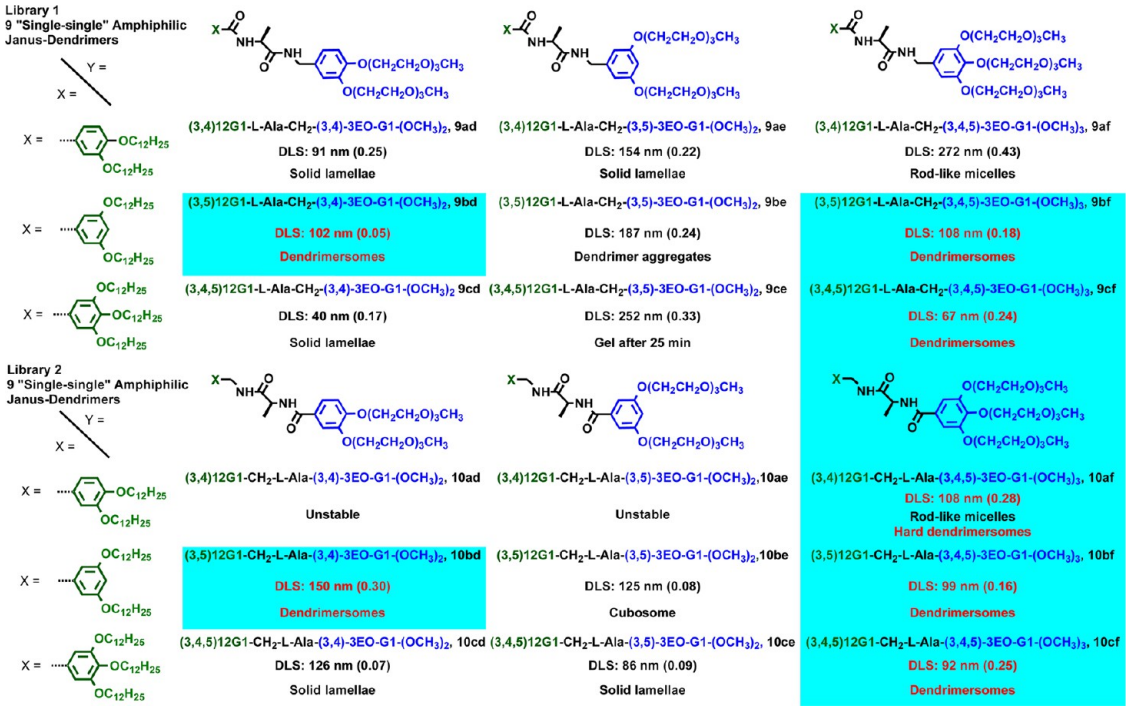
[a] Reagents and conditions: (i) $SOCl_2$, CH_2Cl_2, 0−60 °C, 2 h; (ii) (*S*)-2-(benzyloxy)-1-propanol, DCC, DPTS, CH_2Cl_2, 23 °C, 8 h; (iii) Pd/C, H_2, MeOH/CH_2Cl_2 = 1:2, 23 °C, 4 h; (iv) DCC, DPTS, CH_2Cl_2, 23 °C, 8 h.

Two more constitutional isomeric libraries of "single−single" amphiphilic Janus dendrimers with L-1,2-propanediol ester cores and (3,4,5)-hydrophilic pattern were also synthesized (Libraries 4 and 5, Scheme 2). Dendritic acids **3a**−**c** and **3f** were chlorinated with $SOCl_2$ in DCM and then coupled with (*S*)-2-(benzyloxy)-1-propanol in the presence of DCC/DPTS to give the compounds **4″a**−**c**, and **4″f** in 60% to 90% yield. Subsequently they were deprotected by hydrogenolysis on Pd/C in MeOH/DCM to yield the ester containing dendritic alcohols **5″a**−**c** and **5″f** in 90% to 98% yield. Six "single−single" amphiphilic Janus dendrimers were then constructed by coupling the single hydrophobic dendritic acids **3a**−**c** with the single hydrophilic dendritic alcohol **5″f**, or the single hydrophilic dendritic acid **3f** with the single hydrophobic dendritic alcohols **5″a**−**c**.

Two benzyl ester containing "single−single" amphiphilic Janus dendrimers form Library 6. They were designed *via* an even simpler synthetic route (Scheme 2), involving one coupling of the first-generation hydrophobic alcohol and acid with the first-generation hydrophilic acid and alcohol in 84% to 91% yields. The advantages of these two Janus dendrimers will be discussed in the last section. The purity of all Janus dendrimers is higher than 99%, as demonstrated by a combination of ^{1}H NMR, ^{13}C NMR, HPLC, and MALDI-TOF analysis.

Self-Assembly of "Single−Single" Amphiphilic Janus Dendrimers by Injection of their Ethanol Solution into Water and Analysis of the Assemblies by DLS and Cryo-TEM. The structure and short notation of "single−single" amphiphilic Janus dendrimers from Libraries 1 and 2 and the structures of their assemblies in water are summarized in Scheme 3. All Janus dendrimers are soluble in ethanol at concentrations ranging from 10 to 40 mg/mL. This concentration is in the range of the stock solution required for the self-assembly of dendrimersomes by the injection method in water.[10−12] An ethanol solution of the Janus dendrimers from Scheme 3 (100 μL of 10 mg/mL) was injected into Millipore water, followed by 5 s of vortexing. This is an extremely efficient method for the self-assembly of amphiphilic Janus dendrimers into monodisperse vesicles denoted dendrimersomes. The resulting assemblies were analyzed by dynamic light scattering (DLS) to determine their size and size distribution. Their structures were determined by cryogenic-transmission electron microscopy (cryo-TEM). The DLS data and

Scheme 3. Self-Assembly of "Single−Single" Amphiphilic Janus Dendrimers from Libraries 1 and 2[a]



[a] Short notations and the summary of the assemblies in water. The diameter (in nm) and polydispersity (in parentheses) are measured by DLS (0.5 mg/mL in water solution), and the indicated structures in water were determined by cryo-TEM. The blue background highlights the formation of dendrimersomes

the structures of their assemblies are summarized in Scheme 3.

Cryo-TEM images of dendrimersomes self-assembled by the "single−single" amphiphilic Janus dendrimers **9cf**, **10bd**, **10bf**, and **10cf** from Libraries 1 and 2 are shown in Figure 2. Other complex architectures including solid lamellae, rod-like micelles, spherical dendrimer aggregates, and cubosomes formed by other Janus dendrimers are reported in Figure SF2 in the SI. We observed that the branching pattern in the hydrophilic part provides a general guidance to the architectures assembled in water (Scheme 3). As shown in the right column highlighted with a blue background in Scheme 3, the (3,4,5)-hydrophilic pattern with three triethylene glycol monomethyl ether groups favors the formation of vesicles. When the hydrophilic part has only two triethylene glycol monomethyl ether groups in (3,4)- or (3,5)-hydrophilic patterns, the dendrimers do not self-assemble into vesicles, with the exception of the two constitutional isomers (3,5)12G1-L-Ala-CH_2-(3,4)-3EO-G1-$(OCH_3)_2$, **9bd** (Figure SF2), and (3,5)12G1-CH_2-L-Ala-(3,4)-3EO-G1-$(OCH_3)_2$, **10bd** (Figure 2b). On the other hand, when the hydrophilic part has the (3,4,5)-substitution pattern, the dendrimers self-assemble into vesicles except for the two constitutional isomers (3,4)12G1-L-Ala-CH_2-(3,4,5)-3EO-G1-$(OCH_3)_3$, **9af**, and (3,4)12G1-CH_2-L-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, **10af**, which have (3,4)-dodecyl substitution in their hydrophobic building block. We will discuss the self-assembly of the amphiphilic Janus dendrimers with a (3,4,5)-hydrophobic pattern as an example. (3,4,5)12G1-L-Ala-CH_2-(3,4)-3EO-G1-$(OCH_3)_2$, **9cd**, self-assembles into solid lamellae (Figure SF2). Gelation was observed after 25 min after injection of the ethanol solution of (3,4,5)12G1-L-Ala-CH_2-(3,5)-3EO-G1-$(OCH_3)_2$, **9ce**, into water. (3,4,5)12G1-L-Ala-CH_2-(3,4,5)-3EO-G1-$(OCH_3)_3$, **9cf**, self-assembles into dendrimersomes (Figure 2a). In the series of **10cd**, **10ce**, and **10cf** from Library 2, both (3,4,5)12G1-L-Ala-CH_2−(3,4)G1-3EOMe_2, **10cd**, and (3,4,5)12G1-L-Ala-CH_2-(3,5)G1-3EOMe_2, **10ce**, self-assemble into solid lamellae (Figure SF2). (3,4,5)-12G1-L-Ala-CH_2-(3,4,5)G1-3EOMe_2, **10cf**, with a 3,4,5-hydrophilic pattern, forms dendrimersomes (Figure 2d).

Discovering the Molecular Design Principles for "Single−Single" Amphiphilic Janus Dendrimers that Self-Assemble into Soft and Uniform Dendrimersomes. In order to elucidate the correlation between the primary structure of the "single−single" amphiphilic Janus dendrimers and their periodic arrays in bulk, the phase transitions of the supramolecular dendrimers with a (3,4,5)-hydrophilic pattern from Libraries 1 and 2 were analyzed by differential scanning calorimetry (DSC) and X-ray diffraction (XRD) (Table ST1 in the SI). When the hydrophobic side has a (3,4)-dodecyl substitution pattern, the assemblies are solid at room temperature and exhibit high melting temperatures. When the hydrophobic side has a (3,5)-hydrophobic pattern, they are liquid at room temperature. This trend can be understood by

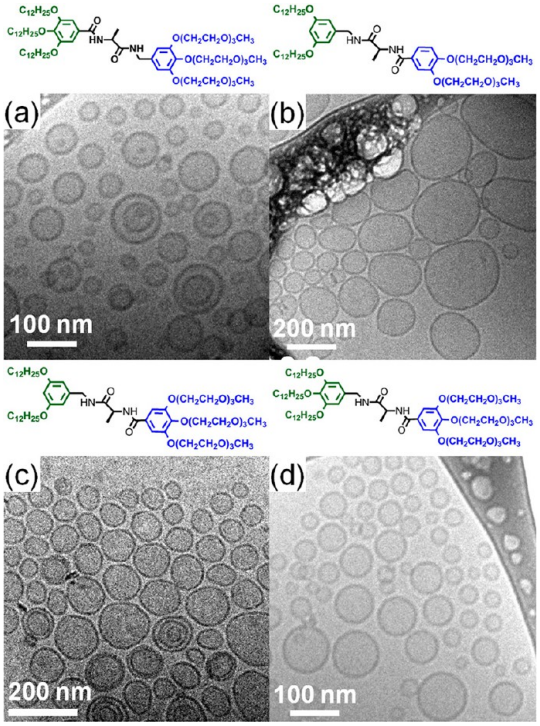


Figure 2. Selected cryo-TEM images of the structures assembled from Libraries 1 and 2. (a) (3,4,5)12G1-L-Ala-CH_2-(3,4,5)-3EO-G1-$(OCH_3)_3$, 9cf. (b) (3,5)12G1-CH_2-L-Ala-(3,4)-3EO-G1-$(OCH_3)_2$, 10bd; (c) (3,5)12G1-L-CH_2-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, 10bf; (d) (3,4,5)12G1-L-Ala-CH_2-(3,4,5)-3EO-G1-$(OCH_3)_3$, 10cf.

comparing the assemblies derived from dendrimers **10af**, **10bf**, and **10cf** from Library 2. (3,4)12G1-CH_2-L-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, **10af**, shows a transition between lamellar crystalline phase (L_1^k+L_2^k) and isotropic liquid (*i*) at 17.0 °C on the first cooling and 46.9 °C on the second heating scan. The transitions of (3,5)12G1-CH_2-L-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, **10bf**, are at −41.9 °C on the first cooling and −23.2 °C on the second heating scan. (3,4,5)12G1-CH_2-L-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, **10cf**, presents a transition between lamellar crystalline phase (L^k) and isotropic state (*i*) at 8.7 °C during the first cooling and 17.9 °C during the second heating. This trend shows that the transition temperature of the assemblies formed by the Janus dendrimers with a (3,4)-12G1-hydrophobic pattern is higher than that with (3,4,5)-12G1- and much higher than that with (3,5)-12G1-. The Janus dendrimers **9af**, **9bf**, and **9cf** from Library 1 show the same tendency for their transition temperatures as their constitutional isomers from Library 2. The assemblies of the (3,4,5)-hydrophilic-substituted "single−single" amphiphilic Janus dendrimers with (3,5)- and (3,4,5)-hydrophobic patterns exhibit lower phase transition temperatures in bulk and self-assemble into soft dendrimersomes in water. This correlation of structure and low melting temperature is important, since it allows the selection of the primary structures of "single−single" amphiphilic Janus dendrimers that are expected to self-assemble into soft dendrimersomes without screening exhaustive libraries of all possible chemical structures.

Three "single−single" amphiphilic Janus dendrimers with (3,4,5)-hydrophilic pattern from Library 3 were designed by replacing L-alanine with Gly in the amide core (Scheme 1).

Two additional constitutional isomeric libraries of "single−single" amphiphilic Janus dendrimers with L-1,2-propanediol ester cores and (3,4,5)-hydrophilic pattern were also synthesized (Scheme 2). Three amphiphilic Janus dendrimers from Library 4 containing L-1,2-propanediol ester at the core labeled with "PPD" (L-propanediol ester) are constitutional isomers of the corresponding dendrimers from Library 5.

The structure and short notation of these nine amphiphilic Janus dendrimers with a (3,4,5)-hydrophilic pattern from Libraries 1, 2, 3, 4, and 5, the structures assembled in water, their size, and polydispersities are summarized in Figure 2. The constitutional isomers from Libraries 4 and 5 are distinguished by the position of the methyl group in the "PPD" core, with prefix "I" for Library 4 and "II" for Library 5 (Figure 3).

Eight of the dendrimers self-assemble into soft or hard dendrimersomes. Only one forms a mixture of rod-like micelles and hard dendrimersomes. These results showed nearly 90% prediction accuracy for the formation of dendrimersomes as determined by the primary structure of "single−single" amphiphilic Janus dendrimers. All of these nine Janus dendrimers, except (3,4)-12G1-CH_2-Gly-(3,4,5)-3EO-G1-$(OCH_3)_3$, **11af**, self-assemble into hard or soft dendrimersomes with narrow size distribution ranging from 0.06 to 0.26 (Figure SF5 from the SI). In Library 3, (3,5)12G1-CH_2-Gly-(3,4,5)-3EO-G1-$(OCH_3)_3$, **11bf**, and (3,4,5)12G1-CH_2-Gly-(3,4,5)-3EO-G1-$(OCH_3)_3$, **11cf**, self-assemble into soft dendrimersomes with a smooth and fluid surface (Figure SF3 in the SI and Figure 4a). (3,4)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12af**, from Library 4 self-assembles into hard dendrimersomes with sharp edges and corners (Figure 4b), while (3,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12bf**, and (3,4,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12cf**, self-assemble into soft dendrimersomes (Figure SF3 in the SI). (3,4)12G1-II-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **13af**, from Library 5 also forms hard dendrimersomes, while (3,5)12G1-II-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **13bf**, and (3,4,5)12G1-II-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **13cf**, self-assemble into soft dendrimersomes (Figure SF3 in the SI).

The correlation between the transition temperatures in bulk and the branching pattern of the hydrophobic part of these nine amphiphilic Janus dendrimers is summarized in Table ST1 in the SI. The dendrimers with a Gly core present lower transition temperatures than those with an L-Ala core. The assemblies formed by the Janus dendrimers with an α-amino acid core present higher transition temperatures than their homologues with a PPD ester core. For example, (3,5)12G1-CH_2-Gly-(3,4,5)-3EO-G1-$(OCH_3)_3$, **11bf**, has a melting temperature at −20.7 °C upon the second heating, while glass

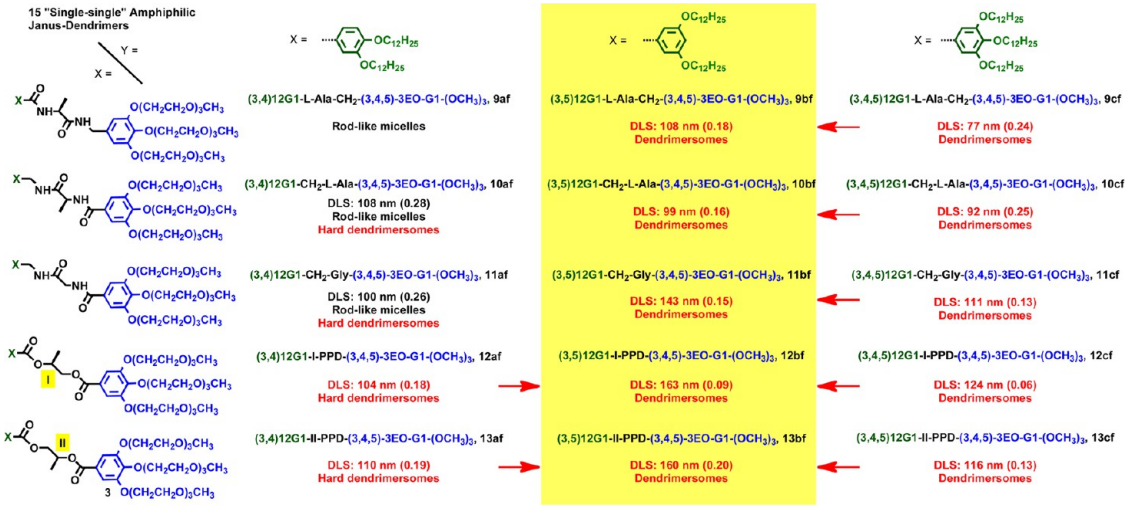


Figure 3. Structures of the amphiphilic Janus dendrimers from Libraries 1, 2, 3, 4, and 5, their short notations, and the summary of their self-assembly in water at 23 °C. The diameter (D_{DLS}, in nm) and polydispersity (in parentheses) in water are indicated. The red arrows illustrate the increase of the diameter of the dendrimersomes. The yellow background highlights the biggest dendrimerosomes formed by the Janus dendrimers with (3,5)-12G1-.

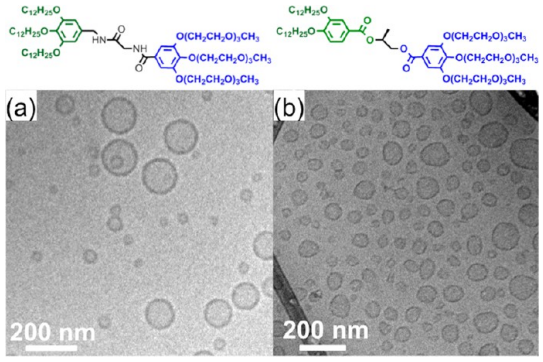


Figure 4. Representative cryo-TEM images of soft dendrimersomes assembled by (a) (3,4,5)12G1-CH_2-Gly-(3,4,5)G1-3EOMe_3, 11cf, and hard dendrimersomes assembled by (b) (3,4)12G1-I-PPD-(3,4,5)G1-3EOMe_3, 12af.

transitions of (3,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12bf**, and (3,5)12G1-II-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **13bf**, were observed respectively at −64.3 and −57.1 °C. This phenomenon is most probably due to the rigidity of the peptide bond and the hydrogen bonding between amide groups. Regardless of the Gly or PPD core, the trend of the transition temperature of the assemblies generated by these dendrimers with a (3,4,5)-hydrophilic pattern on heating and cooling is similar to that of Libraries 1 and 2. The assemblies generated from the Janus dendrimers with a (3,4)-12G1-hydrophobic pattern present the highest transition temperature, while those with (3,5)-12G1-substitution exhibit the lowest transition temperature. For example, the assembly generated by (3,4)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12af**, shows the transition from columnar-centered rectangular crystalline phase ($\Phi_{r\text{-}c}^{k}$) to isotropic liquid (*i*) at 18.3 °C on the second heating, higher than that of (3,4,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12cf**, from −1.9 °C, and much higher than the glass transition temperature of (3,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12bf**, from −64.3 °C.

Both soft and hard dendrimersomes are self-assembled from the "single−single" amphiphilic Janus dendrimers with a (3,4,5)-hydrophilic pattern. Janus dendrimers with a (3,4)-hydrophobic pattern exhibit higher phase transition temperatures and subsequently form hard dendrimersomes. On the other hand, the Janus dendrimers containing (3,5)- and (3,4,5)-hydrophobic substitutions present lower transition temperatures and, therefore, self-assemble into soft dendrimersomes.

Influence of the Hydrophobic Pattern and Dendrimer Core of "Single−Single" Amphiphilic Janus Dendrimers on the Size of the Dendrimersomes. The assemblies formed by all 15 amphiphilic Janus dendrimers with (3,4,5)-hydrophilic substitution in water at 23 °C are summarized in Figure 3, and their assemblies in bulk are given in Figure SF14 in the SI. As previously demonstrated, the vesicle-forming "twin−twin" amphiphilic Janus dendrimers with (3,5)-hydrophobic pattern present a smaller *d*-spacing in the bulk and self-assemble into larger size vesicles in water than those with (3,4)- and (3,4,5)-hydrophobic substitutions.[11] This correlation is attributed to the enhanced mechanical strength of the bilayer wall with higher interdigitation level, which favors the formation of vesicles with lower curvature and therefore larger size. The same correlation between the hydrophobic pattern and vesicle dimension was observed for "single−single" amphiphilic Janus dendrimers (Figure 3). For example, the *d*-spacing of (3,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12bf**, is 3.70 nm, thinner than those of (3,4)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12af** (d = 7.27 and 5.61 nm), and (3,4,5)12G1-I-PPD-(3,4,5-)-3EO-G1-$(OCH_3)_3$, **12cf** (d = 4.65 nm), while the vesicles assembled by **12bf** have an average diameter of 163 nm, larger than 104 nm by **12af** and 124 nm by **12cf**.

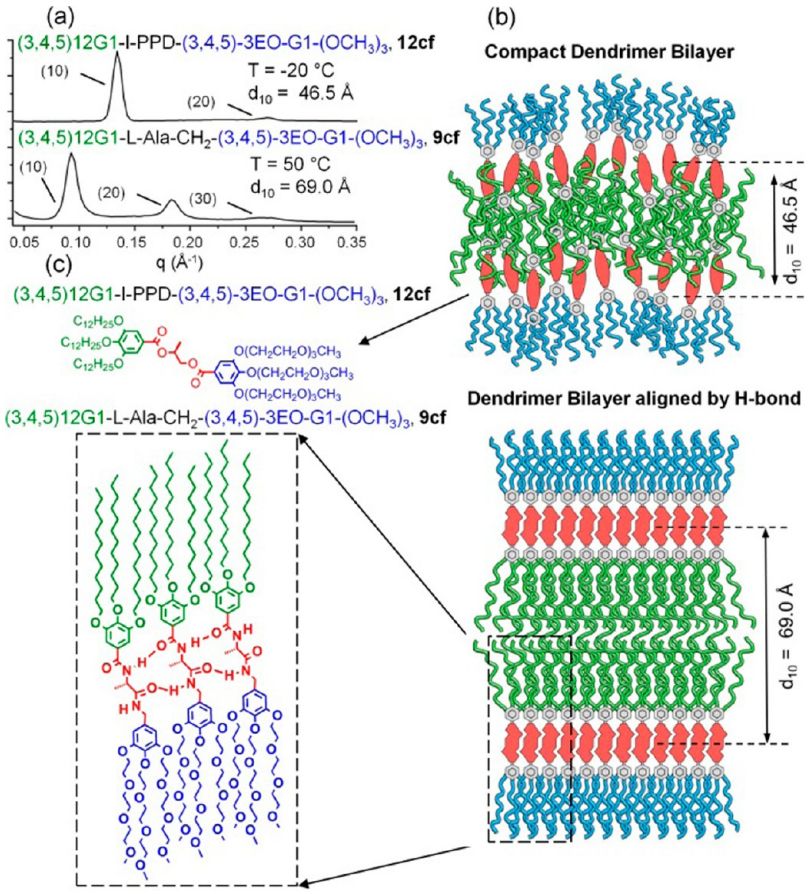


Figure 5. Powder XRD data collected in the lamellar crystalline phases of the indicated amphiphilic Janus dendrimers (a) with the corresponding molecular models (b) and molecular structures (c). Color code in (b) and (c): blue as hydrophilic fragment, green as hydrophobic fragment, and red as the dendrimer core.

As described in the previous section, the core of the Janus dendrimer determines the transition temperature, which in turn dictates the formation of soft or hard dendrimersomes. The central and right columns in Figure 3 reveal that the Janus dendrimers with PPD ester cores self-assembled into larger dendrimersomes than those with L-Ala or Gly amide cores. The left column in Figure 3 does not follow this trend, where only two Janus dendrimers form pure dendrimersomes in water. This correlation can be elucidated by comparing two amphiphilic Janus dendrimers with I-PPD-ester and L-Ala-amide cores shown in Figure 5. The *d*-spacing of (3,4,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12cf**, in the bulk is 4.65 nm, smaller than that of (3,4,5)12G1-L-Ala-CH_2-(3,4,5)-3EO-G1-$(OCH_3)_3$, **9cf**, 6.90 nm (Figure 5a). We assume that the Janus dendrimer **12cf** exhibits loose packing, which can slide up and down and interdigitate in the bilayers, leading to compact lamellar membranes (Figure 5b). On the other hand, dendrimer **9cf** is first aligned by hydrogen bonds between L-Ala-amide cores during the formation of the dendrimer monolayer. These hydrogen bonds prevent the dendrimers from interdigitating in the radius direction, resulting in lower interdigitation level of the bilayer membranes. Higher interdigitation favors formation of lower curvature and, therefore, leads to larger dendrimersome dimensions.[11]

Predicting the Size of Monodisperse Dendrimersomes Formed by "Single−Single" Amphiphilic Janus Dendrimers in Water. Assuming that the dendrimersomes are perfect hollow spheres and that their mass rather than the number of the vesicle is proportional to the concentration of the Janus dendrimers, our laboratory previously reported a simplified spherical-shell model of the dendrimersomes formed by "twin−twin" amphiphilic Janus dendrimers.[11] This model was demonstrated to agree well with the measured dependence of the radius of dendrimersomes and with the concentration of the Janus dendrimers in water. As previously reported, the thickness of the vesicle wall, d_{wall}, is almost equal to the *d*-spacing determined by XRD in bulk.[11,12] On the basis of the diameter of a reference structure determined by DLS, $D_{DLS}^{ref\ \ str}$, this model was used to predict the size of the dendrimersomes assembled from an amphiphilic Janus dendrimer by using eq 1 and eq 2.[11]

$$D_{calcd} = d + \sqrt{12d\Delta - 3d^4}/(3d) \qquad (1)$$

where

$$\Delta = [(D_{DLS}^{ref\ str}/2)^3 - (D_{DLS}^{ref\ str}/2 - d^{ref\ str})^3](M_{wt}^{ref\ str}/M_{wt}) \quad (2)$$

In eq 1, D_{calcd} is the predicted diameter of the dendrimersomes. The thickness of vesicle wall, d_{wall}, is replaced with d. As shown in eq 2, Δ is an empirical factor that correlates the volume of the vesicles self-assembled by the reference Janus dendrimer and those of the Janus dendrimer whose size is intended to be predicted. In eq 2, $d^{ref\ str}$ and $D_{DLS}^{ref\ str}$ are respectively the thickness of the vesicle wall and the diameter of the vesicles formed by the Janus dendrimer selected as reference molecule. $M_{wt}^{ref\ str}$ and M_{wt} refer respectively to the molecular weight of the reference dendrimer structure and that of the underlying Janus dendrimer structure. This methodology applies also to the prediction of the size of the dendrimersomes formed by "single−single" amphiphilic Janus dendrimers from the current study. The comparison of the predicted diameter, noted D_{calcd}, and the size measured by DLS, noted D_{DLS}, is summarized in Table 1. In order to minimize the structure effect on the size prediction, (3,5)12G1-CH_2-L-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, **10bf**, was selected as reference dendrimer for the "single−single" amphiphilic dendrimers in constitutional isomeric Libraries 1 and 2 with an L-Ala core and (3,5)12G1-II-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **13bf**, for those in Libraries 4 and 5 with a PPD core. The comparison between the predicted and measured size shows that this methodology is also applicable to the "single−single" amphiphilic Janus dendrimers.

As previously demonstrated,[11] the relationship of the radius of the dendrimersomes and the final concentration of the corresponding dendrimer in water can be calculated by eq 3.

$$c \propto \text{Volume}_{\text{vesicle shell}} = \frac{4\pi}{3}(R_{DLS}^3 - R_{In}^3) = \frac{4\pi}{3}[R_{DLS}^3 - (R_{DLS} - d)^3] \quad (3)$$

Since the thickness of vesicle wall d_{wall} is around 7 nm,[11,12] which is negligible *versus* the radius of the dendrimersome, factorization of eq 3 provides the relationship between the concentration of the Janus dendrimers and the radius R or the diameter D of the corresponding dendrimersomes, as demonstrated by eq 4.

$$c \propto 4\pi dR^2 = \pi dD^2 \quad (4)$$

Equation 4 is rearranged to give eq 5, where the constant δ depends only on the structure of the "single−single" amphiphilic Janus dendrimer. It shows that the square diameter (D_{DLS}^2) of the dendrimersome is proportional to the final concentration c of the dendrimer in water. This relationship is rewarding, since in addition to predicting the size of the dendrimersome at a specific concentration[11] it also predicts the size of the dendrimersomes at any concentration in the range of concentration where monodisperse dendrimersomes are formed.

$$D^2 = \delta c \quad (5)$$

TABLE 1. Calculation of the Predicted Radius of the Dendrimersomes

"single−single" amphiphilic Janus dendrimer	d (nm)[a]	D_{DLS} (nm)[b]	D_{calcd} (nm)[c]	error[d]
(3,5)12G1-CH_2-L-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, **10bf** (ref)	**5.80**	**99**		
(3,5)12G1-L-Ala-CH_2-(3,4,5)-3EO-G1-$(OCH_3)_3$, **9bf**	5.17	108	104	4%
(3,4,5)12G1-L-Ala-CH_2-(3,4,5)-3EO-G1-$(OCH_3)_3$, **9cf**	6.90	77	86	12%
(3,4,5)12G1-CH_2-L-Ala-(3,4,5)-3EO-G1-$(OCH_3)_3$, **10cf**	6.80	92	87	6%
(3,5)12G1-II-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **13bf** (ref)	**3.76**	**160**		
(3,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12bf**	3.70	163	161	1%
(3,4,5)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12cf**	4.65	124	135	8%
(3,4,5)12G1-II-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **13cf**	4.47	116	137	19%

[a] d-spacing determined by XRD. [b] Experimental diameter of the dendrimersome; data were determined with the concentration of dendrimers at 0.5 mg/mL after ethanol injection. [c] Diameter of the dendrimersome calculated from the combined analysis of the self-assembly process in solid state and in water: $D_{calcd} = d + (12d\Delta - 3d^4)^{1/2}/(3d)$ where $\Delta = [(D_{DLS}^{ref\ str}/2)^3 - (D_{DLS}^{ref\ str}/2 - d^{ref\ str})^3](M_{wt}^{ref\ str}/M_{wt})$. [d] Relative error was calculated by $r = [D_{calcd} - D_{DLS}]/D_{calcd} \times 100\%$.

The DLS data of the dendrimersomes assembled from (3,4)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, **12af** (Figure 6), validate eq 5 and, therefore, support the assumption of the direct proportionality between the mass of the vesicle and the concentration of the Janus dendrimers, c. At a concentration of 0.25 to 4 mg/mL, all the dendrimersomes formed by **12af** exhibit a narrow size distribution, with the polydispersity ranging between 0.14 and 0.39. The size of the vesicles increases from 61 nm to 245 nm. This size-concentration dependence follows the rule established in eq 5, where δ = 16 252. The increase of the vesicle size can also be observed by the cryo-TEM images as shown in Figure 6b−e.

Synthesis of Simple "Single−Single" Amphiphilic Janus Dendrimers Assembling into Dendrimersomes with Predictable Size. On the basis of the molecular design principles elaborated with the first five libraries containing similar hydrophilic and hydrophobic patterns but different dendrimer cores, Library 6, containing two constitutional isomeric benzyl ester "single−single" amphiphilic Janus dendrimers with even simpler primary structures, was designed and synthesized (Scheme 2). The resulting "single−single" amphiphilic Janus dendrimers are named (3,5)12G1-I-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, **14bf**, and its constitutional isomer (3,5)12G1-III-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, **15bf**. The short notations are distinguished by the position of benzyl ester core, with the prefix "I" and "III". Comparing to the "single−single" amphiphilic Janus dendrimers in Libraries 1−5 (Schemes 1 and 2 and Figure 3) and their "twin−twin" homologues[10,11] that require multiple protection and deprotection

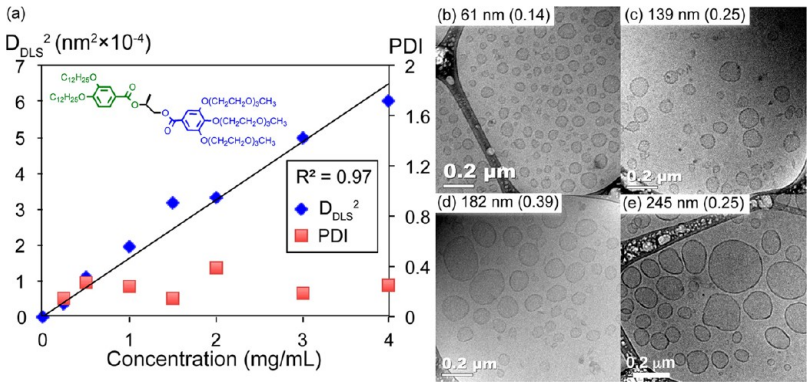


Figure 6. Concentration dependence of the diameter of dendrimersomes formed by (3,4)12G1-I-PPD-(3,4,5)-3EO-G1-$(OCH_3)_3$, 12af. (a) Square diameter (D_{DLS}^2)—concentration relationship of the dendrimersomes. Representative cryo-TEM images of the dendrimersomes with the indicated diameter (D_{DLS}, in nm) and polydispersity index (PDI, in parentheses) at a final concentration of (b) 0.25 mg/mL; (c) 1 mg/mL; (d) 2 mg/mL; (e) 4 mg/mL.

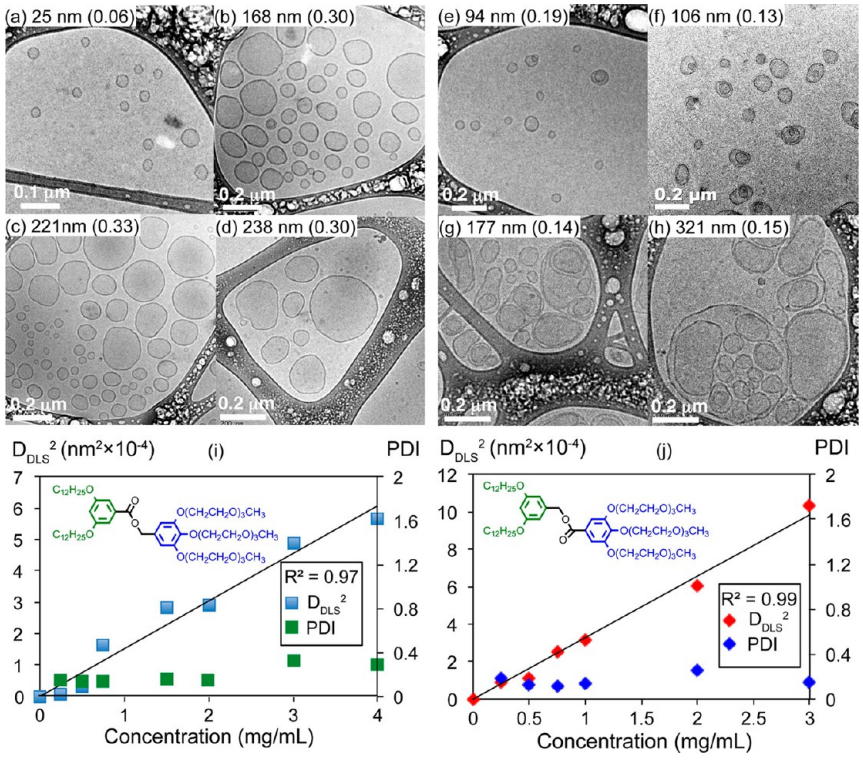


Figure 7. Cryo-TEM images of dendrimersomes formed by (3,5)12G1-I-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, 14bf, at a final concentration of (a) 0.25 mg/mL; (b) 1.5 mg/mL; (c) 3 mg/mL; and (d) 4 mg/mL and by (3,5)12G1-III-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, 15bf, at a final concentration of (e) 0.25 mg/mL; (f) 0.5 mg/mL; (g) 1 mg/mL; and (h) 3 mg/mL. The diameter (D_{DLS}, in nm) and polydispersity (in parentheses) of the dendrimersomes are indicated in the cryo-TEM images. Square diameter (D_{DLS}^2)—concentration relationship of the dendrimersomes formed by (3,5)12G1-I-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, 14bf (i), and (3,5)12G1-III-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, 15bf (j).

steps, substantial synthetic steps (up to four steps) for the synthesis of **14bf** and **15bf** have been reduced. Janus dendrimer **14bf** self-assembles into unilamellar dendrimersomes (Figure 7a—d), while **15bf** self-organizes into dendrimersomes with multicompartments (Figure 7g,h), denoted multidendrimersome dendrimersomes, a novel morphology that is not accessible with their "twin—twin" homologues. The average diameter of the dendrimersomes assembled by (3,5)12G1-I-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, **14bc**, increases from 25 nm to 238 nm with the concentration of the dendrimer from 0.25 mg/mL to 4 mg/mL (Figure 7a—d). The same tendency is observed for the multidendrimersomes formed by (3,5)12G1-III-PhE-(3,4,5)-3EO-G1-$(OCH_3)_3$, **15bf** (Figure 7e—h), as the size increases from 95 nm to 406 nm in the same range of dendrimer concentration, except that the size distribution becomes bimodal at 4.0 mg/mL (Figure SF8). Figure 7i and j reveal that the size of the dendrimersomes, regardless of the variation of shapes, is

predictable by following the proportionality between the square diameter and the concentration.

CONCLUSIONS

Six libraries containing 29 "single−single" amphiphilic Janus dendrimers were synthesized by connecting single (3,4)-, (3,5)- and (3,4,5)-12G1 hydrophobic and single (3,4)-, (3,5)-, and (3,4,5)-3EO-G1-$(OCH_3)_x$ hydrophilic dendrons *via* an accelerated modular synthetic approach. Their assemblies were obtained by injection of their ethanol solution in water, generating various architectures including soft and hard dendrimersomes, cubosomes, solid lamellae, and rod-like micelles. By using the library approach elaborated previously by our laboratory to discover and predict,[10−12] here we report the discovery that the hydrophilic part of the Janus dendrimer plays the dominat role in the formation of dendrimersomes, the (3,4,5)-3EO-G1-$(OCH_3)_3$ pattern favoring it. The vesicle-forming dendrimers with (3,5)- and (3,4,5)-hydrophobic pattern as well as the ester core presented lower transition temperatures in the bulk and self-assembled into soft dendrimersomes, while those with a (3,4)-dodecyloxy hydrophobic pattern and amide cores exhibited higher transition temperatures in the solid state and formed hard dendrimersomes. As a result of the higher interdigitation level of dodecyl chains required to maintain constant density in the hydrophobic domain,[11] these Janus dendrimers with a (3,5)-hydrophobic pattern and ester core presented a smaller lattice parameter in the bulk and formed larger dendrimersomes in water. By employing the methodology elaborated for their "twin−twin" homologues,[11] the dimensions of the dendrimersomes assembled by these "single−single" amphiphilic Janus dendrimers were predictable in a wide range of concentrations when their size distribution is narrow. This predictability provides a methodology to prepare vesicles with desired size by simply choosing a specific concentration of the amphiphiles in the applicable range. These results suggested the synthesis of two constitutional isomeric "single−single" benzyl ester amphiphilic Janus dendrimers with simple structures that self-assembled in water into monodisperse soft dendrimersomes and multidendrimersome dendrimersomes with predictable size. By comparison to their "twin−twin" homologues,[10,11] the synthesis of these "single−single" Janus dendrimers is more straightforward, requiring about half the reaction steps. Moreover, their molar mass was also reduced to half, the number of vesicle-forming amphiphilic Janus dendrimers is doubled, and their molecular efficiency is therefore enhanced by two, which would be more attractive for technological applications in nanomedicine and other fields. Last but not least, the results reported here demonstrate the power of the library approach to discover and predict functions *via* primary structures or first principles.

METHODS

Preparation of Dendrimersomes. The stock solution was prepared by dissolving the required amount of "single−single" amphiphilic Janus dendrimers in pure ethanol at 23 °C. Dendrimersomes were then generated by injection of 100 μL of the ethanol solution into 2 mL of Milli-Q water followed by 5 s vortexing to give the final dendrimer concentration of 0.5−4 mg/mL in water. Different final concentrations of dendrimersomes in water were prepared by varying the starting concentration of stock solution with the same injection volume (100 μL) into a constant 2.0 mL of ultrapure water.

Dynamic Light Scattering. DLS was performed with a Malvern Instruments particle sizer (Zetasizer Nano S, Malvern Instruments, UK) equipped with a 4 mW 633 nm He−Ne laser and avalanche photodiode positioned at 175° to the beam and temperature-controlled cuvette holder. Instrument parameters were determined automatically along with measurement times. Experiments were performed in triplicate.

Cryogenic Transmission Electron Microscopy. Cryo-TEM was performed on an FEI Technai G2 12 microscope (Hillsboro, OR, USA) at a voltage of 120 kV. A droplet of 1.2 μL of dendrimersome solution was pipetted onto a lacey carbon film coated on a copper grid loaded into an FEI Vitrobot apparatus. For some samples the droplet placement and blotting process were repeated in order to obtain suitable specimens for imaging. The sample was allowed to relax for approximately 10 s to remove any residual stresses imparted by blotting, then quickly plunged into liquefied ethane cooled by a reservoir of liquid nitrogen to ensure the vitrification of water. The vitrified samples were transferred to a Gatan 626 cryoholder in a cryo-transfer stage immersed in liquid nitrogen. During the imaging, the cryo-holder was kept below −170 °C to prevent sublimation of vitreous solvent. The digital images were recorded by a Gatan low-dose CCD camera.

X-ray Diffraction Measurements. XRD was performed using Cu Kα radiation (λ = 1.54178 Å) from a Bruker-Nonius FR-591 rotating anode X-ray source equipped with a 0.2 × 0.2 mm^2 filament operated at 3.4 kW. The radiation from the Cu target was collimated and focused with Osmic confocal optics followed by circular pinholes, and a Bruker Hi-Star multiwire (area) detector was used to detect the scattered radiation. To minimize attenuation and background scattering, an integral vacuum was maintained along the length of the flight tube and within the sample chamber. Samples were held in thin glass capillaries (1.0 mm in diameter), mounted in a temperature-controlled oven (temperature precision: ±0.1 °C, temperature range from −120 to 270 °C). The sample-to-detector distance was kept at 54.0 cm with a q-range of 0.02−0.38 $Å^{-1}$. Fiber samples were prepared for easy loading into capillaries. To prepare the fibers, about 10 mg of sample was placed in a temperature-controllable custom-made extrusion device (see Figure SF1) and extruded at RT into fibers without prior heat treatment. Typically, the fibers have a thickness of ∼0.3−0.7 mm and a length of ∼3−7 mm. All XRD measurements were done with the fiber axis perpendicular to the beam direction. XRD peak positions and interplanar d-spacing analysis were performed using Datasqueeze software (version 3.0), which allows background elimination and Gaussian, Lorentzian, Lorentzian squared, or Voigt peak-shape fitting.

Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: The detailed synthesis, ^{1}H NMR and ^{13}C NMR spectra, HPLC, MALDI-TOF and powder XRD data, DLS spectra, and selected cryo-TEM images of the assemblies generated by the "single−single" amphiphilic Janus dendrimers in water. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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