



Cite this: *Chem. Commun.*, 2015, 51, 8648

Received 28th November 2014,  
Accepted 18th March 2015

DOI: 10.1039/c4cc09513h

www.rsc.org/chemcomm

## Towards engineering of self-assembled nanostructures using non-ionic dendritic amphiphiles†

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**Engineering nanostructures of defined size and morphology is a great challenge in the field of self-assembly. Herein we report on the formation of supramolecular nanostructures of defined morphologies with subtle structural changes for a new series of dendritic amphiphiles. Subsequently, we studied their application as nanocarriers for guest molecules.**

In recent years, self-assembled nanostructures have emerged as a new class of functional materials suitable for applications in nanomedicine and nanobiotechnology.<sup>1–4</sup> The functional efficacy of these materials depends on the morphology of the nanostructures. In this regard, self-assembly of amphiphilic molecules has gained considerable attention due to the formation of a wide range of morphologies, starting from simple micelles and vesicles to more complex hierarchical structures such as fibers, ribbons, helices, tubes, etc.<sup>5,6</sup> However, engineering such structures with defined sizes and morphologies has been a persistent challenge in the field of self-assembly, especially for non-ionic amphiphiles. Non-ionic amphiphiles are of particular interest for biomedical applications.<sup>7–10</sup> In recent times much attention has been focused on a new category of amphiphilic systems, dendritic amphiphiles, for use as functional supramolecular materials.<sup>11,12</sup> Dendritic amphiphiles with well-defined structures lie at the interface of classical surfactants and amphiphilic polymers in terms of their structure, and their self-assembly result in well-defined and persistent<sup>13–15</sup> nanostructures.<sup>16</sup> Multiple functional groups at the hydrophilic end of dendritic amphiphiles make them unique building blocks for the construction of functional materials compared to linear amphiphiles.<sup>17</sup> They have been investigated for biomedical applications as nanocarriers for hydrophobic drug molecules, gene therapy etc.<sup>18–27</sup> However, studies on parameters which influence

their self-assembly have not been attempted very much. In general, there are several factors that govern the self-assembly of amphiphiles, for example, hydrophilic–lipophilic balance (HLB), choice of solvent, etc.<sup>28–32</sup> Understanding the influence of these factors offers a way to engineer the morphologies in controlled fashion. Herein, we report on the synthesis and self-assembly of a new series of dendritic amphiphiles and study the influence of HLB and shape of the amphiphiles. Our studies emphasize that one can engineer functional supramolecular architectures of different morphologies by rationally designing dendritic amphiphiles.

Our molecular design involves glycerol based G2 and G3 dendrons as head groups and lipophilic tail segments that have two alkyl chains (Fig. 1). These unique molecular structures can be used to evaluate the influence of HLB and geometry of the

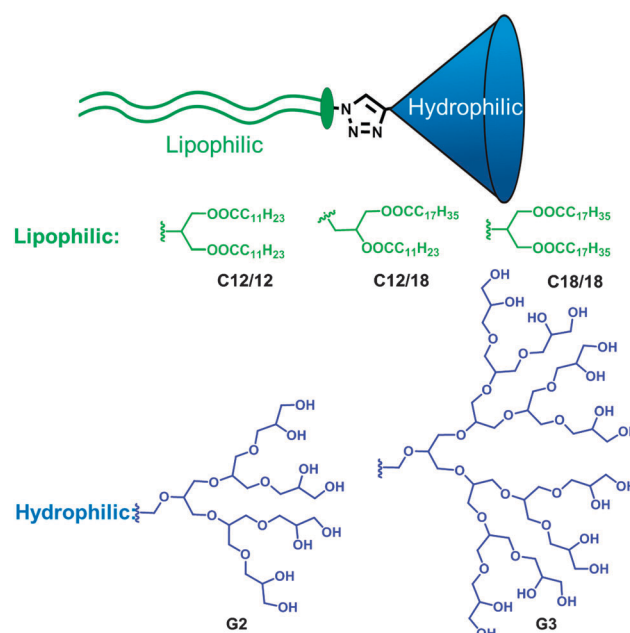


Fig. 1 Structure of the dendritic amphiphiles. (1: G2C12/12, 2: G2C12/18, 3: G2C18/18, 4: G3C12/12, 5: G3C12/18, and 6: G3C18/18).

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c4cc09513h

dendritic amphiphiles upon their self-assembly by varying the generation of the head group and the length and geometry of the lipophilic tail segment. A modular approach has been chosen for the synthesis of our new series of dendritic click amphiphiles, which involves an alkyne–azide based ‘cycloaddition’ as a key step for the conjugation of hydrophobic tails to the protected dendrons (ESI†). Self-assembly of these molecules (1–6) in water was investigated using surface tension measurements, dynamic light scattering (DLS), and cryogenic electron microscopy (cryo-TEM). All compounds were sufficiently soluble in water except compound 3. While compounds 1–6 (except 3) were studied for self-assembly behavior and for their ability to act as nanocarriers for hydrophobic molecules, compound 3 was solely examined in terms of its assembly behavior because of its insolubility.

First the critical aggregation concentrations (CACs) of all the compounds were determined. Of all the established methods, the non-invasive methods are the most advantageous for avoiding the influence of external probes in the measurements. Surface tension measurements for all the amphiphiles (except 3) were performed at different concentrations using the pendant drop method. A decrease of the surface tension with a corresponding increase in concentration of the amphiphiles and the attainment of a plateau at CAC was observed (Fig. S1, ESI†). The experimental data clearly indicate that the higher generation G3 amphiphiles (4–6) had a much lower CAC compared to the lower generation G2 analogs. The CAC of all G3 derivatives varied between 2–4  $\mu\text{M}$ , which indicates the lipophilic part did not significantly influence the aggregation process. For G2 derivatives (1 and 2), however, the lipophilic part did significantly affect the CAC as indicated by 16  $\mu\text{M}$  for 1 and 130  $\mu\text{M}$  for 2, a difference in the order of magnitude. Most interestingly, the more hydrophobic dissymmetric compound 2 showed a significantly higher CAC value compared to the less hydrophobic symmetric amphiphile 1, which is in stark contrast expected from the HLB. We certainly have to attribute the higher CAC for compound 2 as an influence of the dissymmetric lipophilic part for 2; it is also worth mentioning that compound 2 dissolved more readily in water than compound 1. Molecular symmetry factors are known to affect the assembly's behavior. For instance, the study of the symmetric and dissymmetric tail structures' effect upon gemini surfactants<sup>33</sup> showed that the tails' structure had a significant effect on the phase transition temperatures. Unlike in gemini surfactants, where the CAC mainly depends on the overall hydrophobicity of the two alkyl chains, in the case of dendritic amphiphiles, the CAC depends on both the head group generation as well as on the structure/geometry of the hydrophobic tail.

The sizes of the nanostructures/aggregates were measured using dynamic light scattering. The DLS data predicted the existence of small micelles for all G3 compounds (4–6) with mean hydrodynamic diameters of about 8 nm. The narrow intensity distributions indicated that the micelles could be expected to be monodisperse. Unlike for G3 derivatives, G2 amphiphiles revealed aggregates of a markedly larger size (Table 1) by DLS. Also the distributions were wider and the average sizes considerably differed depending on the structure of the molecules. No significant difference in the size

Table 1 Physicochemical properties of amphiphiles 1–6

| Amphiphile     | CAC (M)              | DLS ( $D_h$ , nm) | Cryo-TEM <sup>a</sup> (nm)                 |
|----------------|----------------------|-------------------|--|
| 1              | $1.6 \times 10^{-5}$ | — <sup>b</sup>    | $6 \pm 0.5$                                |
| 2              | $1.3 \times 10^{-4}$ | 10.2 & 35.0       | $8.2 \pm 0.5$ & $6.0 \pm 1.0$ <sup>c</sup> |
| 3              | —                    | 125               | ~125                                       |
| 4              | $4 \times 10^{-6}$   | 8.0               | $4 \pm 0.5$                                |
| 5 <sup>d</sup> | $4 \times 10^{-6}$   | 7.9               | $6.5 \pm 0.5$                              |
| 6 <sup>d</sup> | $2-4 \times 10^{-6}$ | 8.0               | $6.9 \pm 0.5$                              |

The CACs were calculated using surface tension measurements. DLS data were measured at 1% w/v concentration for all the amphiphiles, except for amphiphile 3 which was done at 0.1% w/v. Cryo-TEM measurements were performed at 0.5% w/v for all the amphiphiles except for amphiphile 3, which was done at 0.1% w/v. <sup>a</sup> The diameters of the self-assemblies were reported in nm. <sup>b</sup> The DLS data is not considered for worm-like micelles. <sup>c</sup> Diameters of both spherical and worm-like micelles were reported. <sup>d</sup> CAC was measured by surface tension and fluorescence methods.

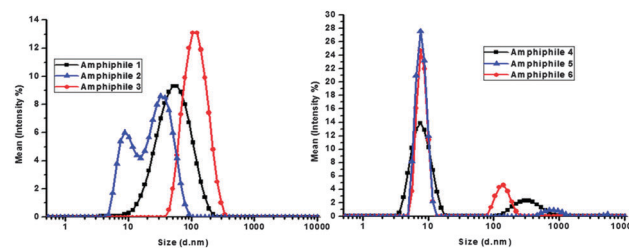
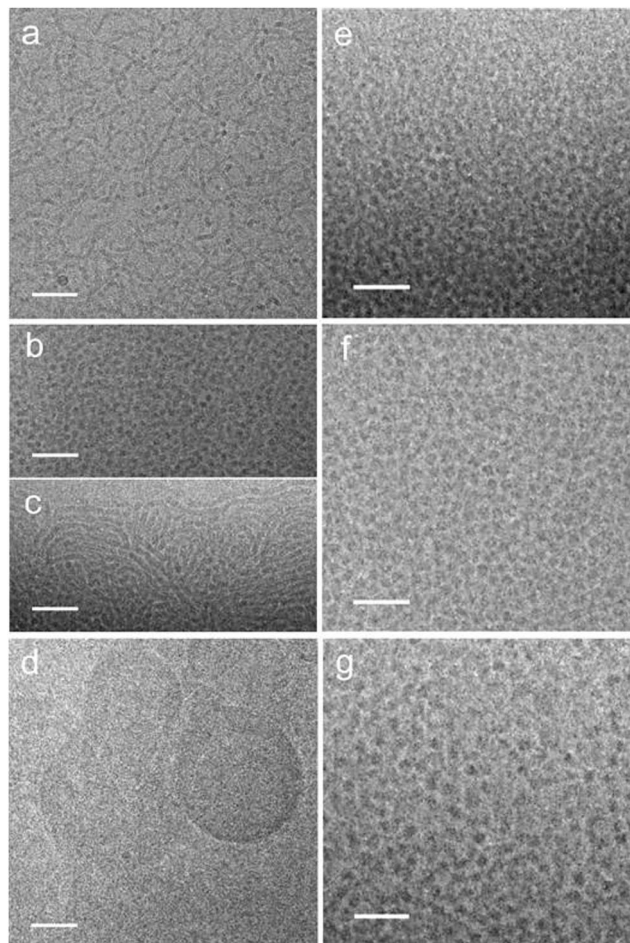


Fig. 2 DLS profile of the amphiphiles 1–6 in water. The data were recorded at a concentration of 0.1% w/v for 3, and 1% w/v for all the other amphiphiles.

distribution profiles was observed by varying the concentration (0.1% and 1% w/v) of the amphiphiles. Not only did the molecular structure of amphiphile 2 result in an unusual assembly behavior in terms of CAC but it also affected the size distribution profile (Fig. 2).

DLS is generally used to determine the hydrodynamic size of the particles and their distribution profile in a sample, assuming that the morphologies of the particles are spherical. But this does not give any information on the actual morphology of the nanostructures. Therefore, direct complementary morphological investigations were performed using cryo-TEM for compounds 1–6. Representative micrographs are displayed in Fig. 3 and the estimated assembly dimensions are given in Table 1. All the G3 derivatives (4–6), irrespective of the structure of the hydrophobic tail, showed populations of small and nearly monodisperse micelles with diameters less than 10 nm, in good agreement with the DLS data. As one would expect, the estimated average diameter of micelles increased with the length of the amphiphiles hydrophobic chain. The finding that DLS gave slightly larger micelle diameters can be ascribed to hydration effects (DLS measures the hydrodynamic diameter of the particles). The G2 derivatives, on the other hand, showed different types of self-assembled morphologies ranging from spherical to worm-like micelles and vesicles, depending on the structure of the lipophilic part. For the symmetric G2 amphiphiles 1 and 3, increasing the alkyl chain length led to a morphological transition from worm-like micelles to vesicles (Fig. 3a and d and Fig. S3a, ESI†). Although the



**Fig. 3** Representative cryo-TEM micrographs of aqueous solutions of amphiphiles **1–6**. The left column (scale bar: 50 nm) shows micrographs of the G2 compounds, **1** (a), **2** (b and c), and **3** (d). The right column (scale bar: 30 nm) micrographs of the G3 compounds, **4** (e), **5** (f), and **6** (g).

hydrophobic volume to chain length is a constant ( $ESI^+$ ), the surfactant tail proved to play a morphology determining role. Such an implicit effect of chain length on the aggregate structure is known and has recently rationalized by thermodynamic considerations.<sup>34</sup>

In contrast, the dissymmetric amphiphile **2** formed spherical as well as worm-like micelles. The observation of two coexisting morphologies for compound **2** explains the bimodal distribution in the DLS profile. These observations, including the higher CAC and coexistence of two morphologies, indicate that amphiphile **2** is the most dynamic of all the G2 derivatives due to the lipophilic part's dissymmetry, which imparted a more cone-like shape for this molecule. Reports in the literature that the shape of a dendritic block influences supramolecular self-assembly<sup>35–37</sup> in the bulk phase clearly show that cone-like amphiphiles lead to a spherical supramolecular arrangement and that tapered amphiphiles arrange themselves in columns. In the case of amphiphile **2**, a simple packing argument could explain our observations. Note that the dissymmetry in the chain lengths of double-tailed amphiphiles results in a reduced hydrophobic volume to chain length ratio compared to symmetric case. Thus, in an identical

head group area (G2 dendron), the molecular packing parameter becomes smaller, favoring an aggregate morphology with larger curvature ( $ESI^+$ ).<sup>28</sup> To verify whether the coexistence of spherical and worm-like micelles is a kinetic effect, we carried out additional tempering experiments. However, no significant difference in DLS (not shown) was observed; cryo-TEM studies (Fig. S3b,  $ESI^+$ ) revealed that the equilibrium quantitatively shifted towards worm-like micelles without any change in their cross-sectional diameter.

The major advantages of non-ionic amphiphilic systems are their biocompatibility and non-toxicity compared to their ionic counterparts, which is a primary requirement for biomedical applications such as drug delivery. To illustrate the applicability of these new non-ionic amphiphilic systems as nanocarriers, dye solubilization experiments were performed. Two chromophores, Nile red and Nimodipine, were studied for their encapsulation using amphiphiles **1–6** with the exclusion of amphiphile **3**. Both chromophores were efficiently encapsulated with a solid phase extraction protocol ( $ESI^+$ ), and the resultant calculations of the transport capacity for these different amphiphiles are presented in Table 2. A clear increase in the encapsulation capacity was observed with an increase in the hydrophobic part of the same generation amphiphiles. To understand the encapsulation process, the chromophore encapsulated samples, especially of G3 derivatives, were studied by DLS. The size distribution profile indicated the existence of larger aggregates in the Nile red encapsulated samples, and no significant change in the size was observed for the Nimodipine ones. We already reported on this kind of behavior for Nile red encapsulation<sup>38</sup> and therefore decided to compare the transport capacity of G3 and G2 amphiphiles with a similar lipophilic part using Nimodipine encapsulation data, which ensured better encapsulation in the case of the G2 derivatives. The head group generation proved to have a more pronounced influence upon the transport efficiency in the C12/12 amphiphiles. This possibly morphological effect upon the self-assemblies highlights the importance of the nanostructure's morphology for such applications.

However the encapsulation efficiency for these systems is rather low ( $\sim 2$  wt%). Our studies have also demonstrated that it is possible to control the nanostructures by rationally designing the dendritic amphiphiles. The process of guest encapsulation can influence the supramolecular self-assembly of amphiphilic systems. To demonstrate the stability of the nanostructures, cryo-TEM images were recorded for the Nimodipine encapsulated sample for amphiphile **2**. Most surprisingly, the most dynamic system, amphiphile **2**, showed

**Table 2** Dye encapsulation data for amphiphiles **1–6** (except for **3**)

| Amphiphile | Transport capacity (mg g <sup>-1</sup> ) |            |
|------------|--|------------|
|            | Nile red                                 | Nimodipine |
| <b>1</b>   | 3.0                                      | 15.0       |
| <b>2</b>   | 3.2                                      | 19.3       |
| <b>4</b>   | 2.2                                      | 8.3        |
| <b>5</b>   | 3.4                                      | 14.8       |
| <b>6</b>   | 5.0                                      | 17.2       |

Dye encapsulation experiments were performed at 0.5% w/v concentration of the amphiphiles.

coexistence of spherical and worm-like micelles after encapsulation emphasizing the stability of our structures during encapsulation protocol (Fig. S4, ESI†).

In conclusion, we have reported on the engineering of self-assembled nanostructures by subtle changes in the structural parameter for non-ionic dendritic amphiphiles. Our results emphasize that the dendritic amphiphiles self-assembly is mainly dictated by the head group generation. Studies on G2 amphiphiles revealed that the shape plays a vital role compared to the HLB and fine tuning the structural parameters results in different morphologies. In the case of G3 molecules, the larger head group (G3 dendron) facilitates the formation of globular micelles, which are well defined in size. Such higher generation molecules are useful for engineering monodisperse micelles for further applications, e.g., as nanocarriers for hydrophobic guest molecules. This was evaluated using the dye Nile red and the drug Nimodipine. More interestingly, the newly designed dendritic amphiphiles showed that defined morphologies formed, even after guest encapsulation, which is relevant for drug delivery systems.

We would like to thank the Focus Area Nanoscale of the Freie Universität, Berlin ([www.fu-berlin-nanoscale.de](http://www.fu-berlin-nanoscale.de)) for financial support and the DFG for funding the core facility Biosupramol.

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