Reverse vesicles formed by hydrogen bonded arylamide-derived triammonium cyclophanes and hexaammonium capsule†

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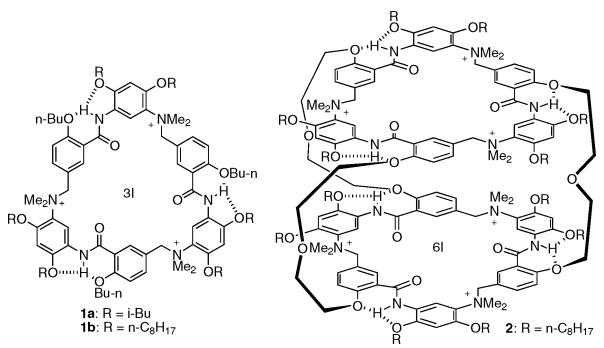
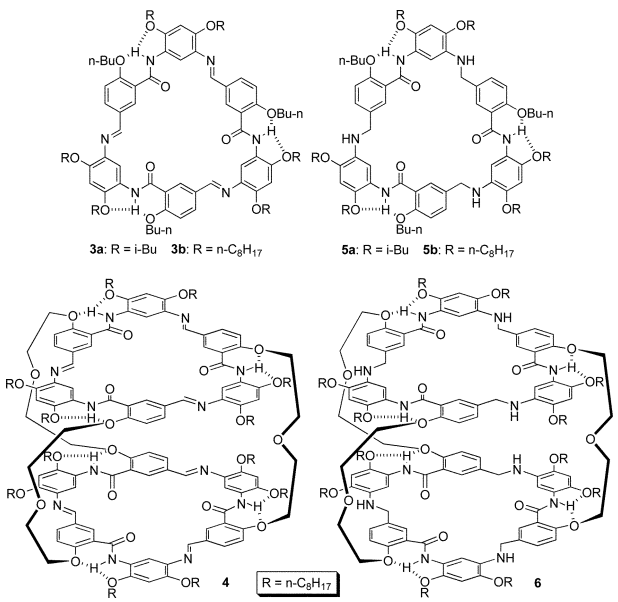
Hydrogen bonded rigid imine-based macrocycles and capsules have been reduced to cyclic triamines and two-layered hexaamines, the triammonium and hexaammonium derivatives of which are revealed to form reverse vesicles in organic liquids of low polarity, which are characterized by SEM, AFM, (HR)TEM, DLS and XRD.

Vesicles,[1,2] microscopic capsules that enclose an aqueous volume with thin membranes of specific molecules or polymers, have drawn great attention due to their potential applications in drug and gene delivery, as nanoreactors, and as artificial cell membranes.[3] Reverse vesicles consist of a solvent core of low polarity that is surrounded by a reverse membrane shell.[4] As the organic counterparts to the "normal" vesicles in aqueous solution, reverse vesicles may also be potentially endowed with similar functions. However, examples of this class of vesicles are quite limited. Moreover, reverse vesicles have been assembled mainly from aliphatic amphiphiles in nonpolar hydrocarbons, in which the hydrophobic interaction of the polar segments is maximized.[4b–h] To the best of our knowledge, there is only one other example that utilizes dimeric resorcinarenes to form reverse vesicles.[4i] Herein, we report a new approach to reverse vesicles, which are based on the aggregation of hydrogen bonded arylamide-derived triammonium macrocycles **1a** and **1b** and hexaammonium capsule **2**.



We previously reported the quantitative synthesis of imine-based macrocycles **3a** and **3b** and capsule **4** from precursors that bear amino and aldehyde units by using the dynamic covalent chemistry.[5] The key for this approach was that the intramolecular N–H···O hydrogen bonds induced the arylamide segments to adopt a preorganized conformation,[6] leading to the exclusive formation of the macrocycles after the reactions reached equilibrium. Compounds **1a**, **1b**, **2**, **5a**, **5b** and **6** were thus further prepared from them (see ESI†).



SEM images showed that in chloroform both **1a** and **1b** self-assembled into spherical vesicles (Fig. 1), the average diameter of which was estimated to be *ca.* 2.4 (**1a**) and 6.0 (**1b**) μm. The size of the vesicles was decreased with the dilution of the solution (see ESI†), which was similar to many of the classic vesicles formed in polar media.[7] However, no twins, triplets or more complicated aggregates were observed within the investigated concentration range, implying that the aliphatic side chains did not form important van der Waals interactions across the vesicles. Similar vesicles were also generated in dichloromethane (Fig. 1b), but not in polar solvents, such as methanol, acetonitrile or acetone. Moreover, adding methanol to the chloroform solutions gradually inhibited the formation of the vesicular structures. The solubility of **1a** and **1b** in hydrocarbons was low. However, vesicles could be observed from the binary solutions (1 : 1, v/v) of chloroform and *n*-hexane, cyclohexane and decalin (see ESI†). All these results supported that a new class of reverse vesicles was generated.

The morphology of the aggregates of **1a** and **1b** was also investigated by tapping-mode AFM (Fig. 2, also see ESI†),

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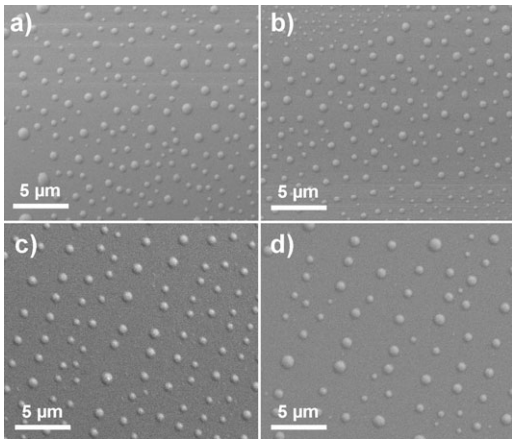


Fig. 1 SEM images of (a) **1a** ($CHCl_3$), (b) **1a** (CH_2Cl_2), (c) **1b** ($CHCl_3$) and (d) **2** ($CHCl_3$), obtained by evaporation of the solutions (3.0 mM) on mica.

which also showed the formation of spherical aggregates. Cross-section analysis of the typical structures revealed large ratios of diameter to height (11 and 14, respectively), indicating their flattened shape and the hollow feature of the original vesicles,[8] which were evaporated on the mica surface. All the samples in dichloromethane also exhibited similar spherical vesicles (see ESI†).

The formation of vesicles by **1a** and **1b** in solution was further confirmed by the dynamic light scattering study (Fig. 3). The mean hydrodynamic diameters of the vesicles was 295 and 342 nm, respectively. The number-averaged distribution of **1b** (0.3 mM) was 140–340 nm, which was narrower than its surface size distributions of 200–900 nm, determined by SEM and AFM, and 100–700 nm, determined by TEM. The larger sizes exhibited on the surface may be attributed to the flattening of the vesicles upon evaporation of the encapsulated solvent. TEM revealed a clear contrast between the peripheral and central areas of the spherical aggregates (Fig. 4), which further supported the hollow nature. The thickness of the vesicles was estimated to be *ca.* 2.0 nm. Considering that the diameter of the macrocyclic framework is *ca.* 1.6 nm, this thickness suggested that the wall

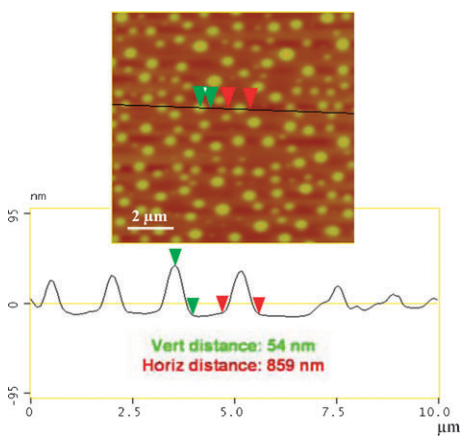


Fig. 2 AFM image of **1b**, obtained by evaporation of the solution in chloroform (0.3 mM) on mica.

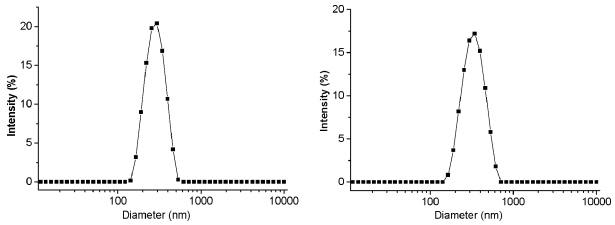


Fig. 3 The intensity-weighted distribution of the vesicles obtained from the DLS measurements of **1a** and **1b** from chloroform (0.3 mM) at 25 °C.

of the vesicles had a monolayer structure. We tentatively propose that the macrocyclic frameworks stacked to form cylindrical assemblies, which further aggregated into extended layers and finally generated the vesicles (Fig. 5), as reported for the formation of the conventional vesicles formed from macrocycles in polar solvents.[8a,9] The X-ray diffraction (XRD) of the dried samples of both **1a** and **1b** (see ESI) revealed a broad peak at 0.40 nm and a sharp peak at 1.7 nm, respectively. The former might be attributed to a less compact π stacking,[10] while the latter was close to the size of the cyclic framework (1.6 nm) and thus supported the monolayer mode shown in Fig. 5. SEM images of **1b** (0.3 mM) showed that some of the vesicles contained holes (see ESI†), also evidencing their hollow nature.[11]

To get insight into the mechanism for the formation of the vesicles, compound **7** was prepared. SEM images showed that **7** did not give rise to vesicular structures in all the above solvents (Fig. 6), indicating that the cooperative stacking of the aromatic segments of **1a** and **1b** played a key role in the formation of the vesicles. Such stacking should be mainly driven by the aggregation of the ionic segments in the center of the cylinder formed by the macrocycles, because in this way, they could be shielded from the solvent molecules of low polarity. Several additional experiments supported this mechanism: when the iodide anion of **1b** was exchanged with nitrate, it still formed vesicles. In contrast, with the larger perchloride as the anion, the salt did not form vesicles. Compound **8**, which bears three larger ammonium moieties, also did not generate vesicles (Fig. 6). These results can be readily rationalized by considering that the large perchlorate and ammonium units weakened the stacking of the macrocycles and in turn the formation of the vesicles. Diluting the solution of **1b** in $CDCl_3$ from 40 mM to 1 mM caused the H-1 and H-2 signals (see the structure) in ^1H NMR to shift downfield by 0.07 ppm. In contrast, no similar shifting

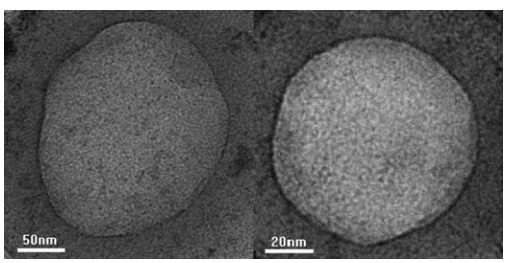


Fig. 4 TEM images of **1a** (left) and **1b** (right), obtained by evaporation the $CHCl_3$ solution (0.3 mM) on copper grids.

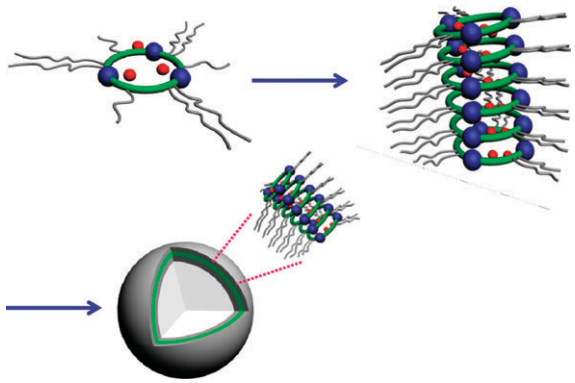
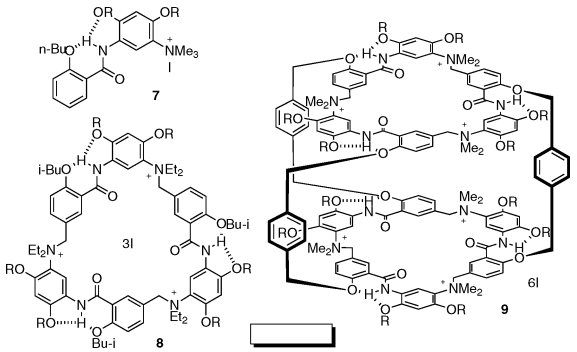


Fig. 5 A schematic illustration for the formation of the mono-layered vesicles from the tricationic macrocycles.

(< 0.01 ppm) was exhibited for **3b**, **5b**, **7** or **8**. These observations also revealed that **1b** stacked at high concentrations.



SEM images showed that both imine- (**3b**) and amine-based (**5b**) cyclophanes did not form vesicles in chloroform or dichloromethane. However, in the presence of an excess of hydrogen iodide (10 eq.), vesicular structures could be observed (see ESI†). This result should be attributed to the protonation of their imino and amino units. We propose that the resulting salts were like **1b** to stack to lead to the formation of the vesicles.

Interestingly, SEM and AFM showed that **2** also generated vesicles in chloroform, with a mean diameter of *ca.* 1.5 μm (Fig. 1d, also see ESI†). Because the two macrocyclic segments are connected with three flexible linkers, they should stack intramolecularly. As a result, intermolecular stacking could occur efficiently to lead to the formation of vesicles. When the two macrocyclic units are connected with the rigid *p*-xylene linkers, no vesicles were observed on the SEM images for the corresponding capsule **9** (Fig. 6). It was expected that

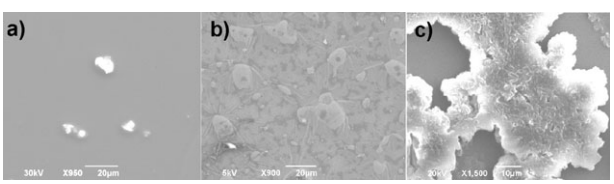


Fig. 6 SEM images of (a) **7** (3.0 mM), (b) **8** (3.0 mM), and (c) **9** (1.5 mM), obtained by evaporation of the solutions in $CHCl_3$ on mica.

intramolecular stacking was relatively weaker for **9**, because it should had a more flexible conformation owing to the relative movement of the two macrocycles, which might disable its ordered stacking and the formation of the vesicles.

In conclusion, we have demonstrated a new approach for the construction of reverse vesicles. The vesicles formed by **2** may be further used as a model system to study the cross-membrane transformation of planar aromatic molecules. For the vesicles formed by macrocyclic salts, one future study will focus on the modification of the side chains. Vinyl, ethynyl or azido groups will be introduced. In this way, the macrocycles may be cross-linked through the metathesis or click reaction to generate vesicles that are stable in polar media, which may display new interesting properties due to the electrostatic repulsion of the ammonium units.

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