

ELCTRONIC SUPPORTING INFORMATION

Controllable self-assembly of amphiphilic macrocycles into closed-shell and open-shell vesicles, nanotubes, and fibers

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Experimental Section

Synthesis and Characterization. Starting materials and reagents were procured from Sigma Aldrich and TCI America and used as received. The synthesis of **MC1** and **MC2** macrocycles is illustrated in **Scheme S1**. Reactions were performed in dry solvents under N₂ atmosphere unless otherwise specified. Analytical thin layer chromatography (TLC) was performed on silica gel 60-F254 (Merck) plates and detected under a UV lamp. Column chromatography was performed on silica gel 60 (SorbTech). ¹H NMR spectra were recorded at 298 K in appropriate deuterated solvents using Bruker Avance 400 MHz spectrometer. Electrospray Ionization mass spectra (ESI-MS) were recorded on a JEOL AccuTOF JMS-T100LC ESI mass spectrometer. High-resolution MALDI-TOF mass spectra were recorded using a Bruker MALDI-TOF mass spectrometer.

Compound 1. This compound was synthesized following a literature procedure.^{S1} To a solution of *m*-phenylenediamine (4.08g, 37.7 mmol) in anhydrous MeOH (100 mL) (Boc)₂O (9.05 g, 44.7 mmol) and Et₃N (0.6 mL, 4.15 mmol) were added at 0 °C and the reaction mixture was stirred at room temperature for 16 h. Solvent was removed under reduced pressure and the crude reaction mixture was purified by column chromatography (SiO₂, CHCl₃/MeOH) to obtain compound **1** as a white solid (5.49g, 26.4 mmol, 70%). ¹H NMR (400 MHz, CDCl₃, 25°C): δ = 7.01 – 7.05 (*t*, 1H, *J* = 8 Hz Ar-H), 6.97 (*s*, 1H, N-H), 6.53 – 6.55 (*d*, 1H, *J* = 8 Hz, Ar-H), 6.45 (*s*, 1H, Ar-H), 6.34 – 6.36 (*d*, 1H, *J* = 8 Hz, Ar-H), 3.51 (*s*, 2H, NH₂), 1.50 (*s*, 9H, CH₃) ppm.

Compound 2. 5-Nitroisophthalic acid (1.02g, 4.8 mmol) was first converted to the corresponding di-acid chloride by treating it with SOCl₂ (5 mL, excess) and DMF (5 drops, catalytic) under refluxing conditions for 6 h. After evaporating the liquid phase under reduced pressure, small portions of PhMe (5 mL x 3) were added to the reaction mixture and evaporated under reduced pressure to completely remove SOCl₂ from the medium. The resulting di-acid chloride was then dissolved in dry THF (10 mL) and added dropwise to a solution of compound **1** (2.51 g, 12.07 mmol), DMAP (1.47 g, 12 mmol), and DIPEA (2 ml, 12 mmol) in dry THF (40 mL). After stirring this reaction mixture at room temperature for 16 h, solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (SiO₂, CH₂Cl₂/EtOAc) to obtain compound **2** as a white solid (1.37 g, 2.9 mmol, 61%). ¹H NMR (400 MHz, CD₃OD, 25°C): δ = 8.95 (*s*, 2H, Ar-H), 8.88 (*s*, 1H, Ar-H), 7.92 (*s*, 2H, Ar-H), 7.38 – 7.39 (*d*, 2H, *J* = 8.38 Hz, Ar-H), 7.25 – 7.29 (*t*, 2H, *J* = 8.38 Hz, Ar-H), 7.18 – 7.20 (*d*, 2H, *J* = 8.38 Hz, Ar-H), 1.53 (*s*, 18H, CH₃) ppm. ESIMS (*m/z*): [M]⁺_{observed} = 591.42, [M]⁺_{calcd} = 591.61.

Compound 3. Compound **2** (0.2 g, 0.4 mmol) was treated with trifluoroacetic acid (TFA, 5 mL) at room temperature for 6 h. After evaporating TFA under reduced pressure, H₂O (30 mL) was added to the residue and the pH was adjusted to 9 by adding an aqueous ammonia solution dropwise into the medium. The aqueous medium was then extracted with EtOAc (3 x 100 mL). The combined organic phase

containing the product was then dried over Na₂SO₄, evaporated under vacuum, and the resulting solid crude product washed with CH₂Cl₂ to obtain pure diamine **3** as a white solid (0.15 g, 0.38 mmol, 89%). ¹H NMR (400 MHz, DMSO-*d*₆, 25°C): δ = 10.43 (*s*, 2H, -CONH), 8.88 (*s*, 3H, Ar-H), 7.10 – 7.11 (*t*, 2H, *J* = 2 Hz, Ar-H), 6.98 – 7.02 (*t*, 2H, *J* = 7.99 Hz, Ar-H), 6.87 – 6.90 (*m*, 2H, Ar-H), 6.34 – 6.36 (*m*, 2H, *J* = 8.11 Hz, Ar-H), 5.17 (*s*, 4H, NH₂) ppm. ¹³C NMR (600 MHz, DMSO-*d*₆, 25°C): δ = 165.36, 151.65, 150.49, 141.89, 139.55, 135.67, 131.67, 127.54, 113.07, 111.18 and 108.9 ppm. ESIMS (*m/z*): [M+Na]⁺_{observed} = 414.16, [M]⁺_{calcd} = 391.38.

Compound 4. This compound was synthesized by following a literature procedure.^{S2} A suspension of dimethyl-5-hydroxyisophthalate (1.99 g, 9.5 mmol) and K₂CO₃ (3.99 g, 28.5 mmol) in dry DMF (60 mL) was heated under reflux for 1 h. Octylbromide (1.67 ml, 9.6 mmol) was added to the reaction mixture, which was heated under reflux for another 24 h. After cooling the reaction mixture to room temperature, it was poured into cold water (150 mL) and extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with water, dried over Na₂SO₄, and removed under reduced pressure to obtain a viscous colorless oil, which upon standing overnight yielded pure compound **4** as a white crystalline solid (2.5 g, 7.7 mmol, 82%). ¹H NMR (400 MHz, CDCl₃, 25°C): δ = 8.25 – 8.26 (*t*, 1H, *J* = 1.5 Hz, Ar-H), 7.73 – 7.74 (*d*, 2H, *J* = 1.5 Hz, Ar-H), 4.01 – 4.04 (*t*, 2H, *J* = 6.6 Hz, O-CH₂), 3.95 (*s*, 6H, -COOCH₃), 0.86 – 1.83 (*m*, 15H, alkyl-H) ppm. ESIMS (*m/z*): [M+H]⁺_{observed} = 323.07, [M]⁺_{calcd} = 322.40.

Compound 5. A suspension of compound **4** (0.4 g, 1.26 mmol) in a solution of NaOH (0.04 g, 1.13 mmol) in MeOH (30 mL) and H₂O (5 mL) was heated under reflux for 5 h. After cooling the reaction mixture to room temperature, it was poured into cold water (100 mL) and the pH of the solution was adjusted to 1 with concentrated HCl. The crude product was extracted with EtOAc (3 x 100 mL) and the combined organic phase was washed with water, dried over Na₂SO₄, and removed under vacuum. After column chromatography (SiO₂, CHCl₃/MeOH) compound **5** was obtained as a white solid (0.26 g, 0.84 mmol, 68%). ¹H NMR (400 MHz, CDCl₃, 25°C): δ = 8.34 – 8.33 (*t*, 1H, *J* = 1.5 Hz, Ar-H), 7.79 – 7.80 (*d*, 2H, *J* = 1.5 Hz, Ar-H), 4.03 – 4.07 (*t*, 2H, *J* = 6.5 Hz, O-CH₂), 3.93 (*s*, 3H, -COOCH₃), 0.87 – 1.85 (*m*, 15H, alkyl-H) ppm. ¹³C NMR (600 MHz, CDCl₃, 25°C): 166.11, 159.34, 131.88, 130.77, 123.38, 120.71, 120.28, 68.71, 52.45, 31.79, 29.30, 29.21, 29.08, 25.97, 22.65, 14.08 ppm. ESIMS (*m/z*): [M+Na]⁺_{observed} = 331.24, [M]⁺_{calcd} = 308.16.

Compound 6. To a solution of compound **5** (0.7g, 2.3 mmol) and 4-methylmorpholine (1.25 mL, 11.5 mmol) in dry THF (15 mL) was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (0.42 g, 2.4 mmol) at 0 °C. Upon stirring the reaction mixture at room temperature for 3 h compound **5** was fully converted to the corresponding activated ester, as confirmed by TLC (CH₂Cl₂). A solution of 1,3-phenylenediamine (0.11 g, 1.05 mmol), DMAP (0.3 g, 2.3 mmol), and DIPEA (2 ml) in THF (15 mL) was added dropwise to the solution of activated ester of compound **5** and the resulting reaction mixture was stirred at room temperature for 16 h. After MeOH (2 mL) was added to the reaction mixture to convert unreacted activated ester to the methyl-ester (**4**), solvent was removed under reduced pressure. The crude product was purified by column chromatography (SiO₂, CHCl₃/MeOH) to obtain compound **6** as a white powder (1.11 g, 1.6 mmol, 71%). ¹H NMR (400 MHz, DMSO-*d*₆, 25°C): δ = 10.47 (*s*, 2H, amide-NH), 8.31 – 8.32 (*t*, 1H, *J* = 2 Hz, Ar-H), 8.12 – 8.13 (*t*, 2H, *J* = 1.5 Hz, Ar-H), 7.78 – 7.79 (*m*, 2H, *J* = 1.5 Hz, Ar-H), 7.61 – 7.62 (*m*, 2H, *J* = 1.5 Hz, Ar-H), 7.50 – 7.53 (*dd*, 2H, *J* = 2 Hz, Ar-H), 7.32 – 7.36 (*t*, 1H, *J* = 8 Hz, Ar-H), 4.09 – 4.13 (*t*, 4H, *J* = 6.4 Hz, -OCH₂), 3.90 (*s*, 6H, -COOCH₃), 0.84 – 1.79 (*m*, 30H, alkyl-H). ESIMS (*m/z*): [M+H]⁺_{observed} = 689.39, [M]⁺_{calcd} = 688.37.

Compound 7. To a solution of diester **6** (0.16 g, 0.23 mmol) in THF (20 mL) and MeOH (10 mL) was added a solution of LiOH (0.017 g, 0.69 mmol) in H₂O (1.5 mL). After stirring the reaction mixture at room temperature for 24 h, it was poured into cold water and acidified with aqueous 1 M HCl solution to obtain a white precipitate, which was filtered and washed thoroughly with H₂O (3 x 100 mL) and MeOH (3 x 100 mL) and dried in air to obtain pure dicarboxylic acid **7** as a white solid (0.13 g, 0.2 mmol, 83%).

^1H NMR (400 MHz, DMSO- d_6 , 25°C): δ = 13.32 (*s*, 2H, -COOH), 10.45 (*s*, 2H, -CONH, **1**), 8.31 – 8.32 (*t*, 1H, J = 2 Hz, Ar-H, **2**), 8.11 – 8.12 (*t*, 2H, J = 1.5 Hz, Ar-H, **5**), 7.74 – 7.75 (*m*, 2H, J = 1.5 Hz, Ar-H, **6**), 7.59 – 7.60 (*m*, 2H, J = 1.5 Hz, Ar-H, **7**), 7.50 – 7.52 (*dd*, 2H, J = 2 Hz, Ar-H, **3**), 7.31 – 7.35 (*t*, 1H, J = 8 Hz, Ar-H, **4**), 4.08 – 4.11 (*t*, 4H, J = 6.5 Hz, -OCH₂), 0.84 – 1.79 (*m*, 30H, alkyl-H) ppm. ^{13}C NMR (600 MHz, DMSO- d_6 , 25°C): δ = 167.15, 164.87, 159.13, 139.59, 137, 132.89, 128.9, 121.27, 118.68, 117.83, 116.84, 68.55, 31.7, 29.18, 25.93, 22.55 and 14.41 ppm. ESIMS (m/z): $[\text{M}+\text{H}]^+$ _{observed} = 661.41, $[\text{M}]^+$ _{calcd} = 660.34.

MC1. To a solution of dicarboxylic acid **7** (0.58 g, 0.88 mmol) and 4-methylmorpholine (0.5 ml, 4.4 mmol) in dry THF (20 mL) 2-chloro-4,6-dimethoxy-1,3,5-triazine (0.39 g, 2.2 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 6 h. Once all diacid **7** was fully converted to the corresponding activated diester, as confirmed by TLC (CH₂Cl₂), it was diluted with dry THF (50 mL). A solution of compound **3** (0.34 g, 0.88 mmol), DMAP (0.27 g, 2.2 mmol), and DIPEA (0.38 mL, 2.2 mmol) in dry THF (20 mL) was added quickly to the diluted solution of activated ester through an addition funnel. The reaction mixture was stirred at room temperature for 48 h. After filtering the slightly cloudy reaction mixture, the clear solution was evaporated under reduced pressure to obtain a viscous residue, which precipitated as a white solid upon addition of MeOH (50 mL). After filtering and washing this solid material with MeOH and CH₂Cl₂ thoroughly, it was dissolved in THF (100 mL) under ultrasonication and a trace amount of suspended particles was removed by filtration. The filtrate was evaporated to obtain pure **MC1** as a yellowish white solid (0.8 g, 0.78 mmol, 90%). ^1H NMR (400 MHz, DMSO- d_6 , 25°C): δ = 10.87 (*s*, 1H, -CONH), 10.80 (*s*, 1H, -CONH), 10.44 – 10.51 (4H, -CONH₂s), 9.02 – 9.03 (*d*, H, J = 1.4 Hz, ArH), 8.96 – 8.98 (2s, 2H, Ar-H), 8.40 – 8.41 (*m*, 2H, Ar-H), 8.13 – 8.35 (3m, 3H, Ar-H), 7.35 – 7.75 (4m, 13H, Ar-H), 4.10 – 4.17 (*m*, 4H, -OCH₂s), 0.83 – 1.83 (4m, 30H, alkyl-H) ppm. ^{13}C NMR (600 MHz, DMSO- d_6 , 25°C): δ = 165.32, 163.81, 159.36, 148.13, 137, 14, 134.91, 131.1, 129.57, 126.72, 117, 113.34, 68.95, 32.05, 29.56, 26.35, 22.92 and 14.73 ppm. HR-MALDI-TOF (m/z): $[\text{M}+\text{H}]^+$ _{observed} = 1016.915, $[\text{M}]^+$ _{calcd} = 1015.448.

MC2. To a solution of **MC1** (0.41g, 0.4 mmol) in dry THF (20 mL) and dry MeOH (20 mL) in a round-bottom flask under N₂ atmosphere 10% Pd-C (0.41 g) was added and the reaction mixture was heated to 70 °C. Then hydrazine (0.38 mL, 12 mmol) was added to the reaction mixture, which was heated under reflux for another 6 h. After cooling the reaction mixture to room temperature, Pd-C was removed by filtering it through celite and the filtrate was evaporated under vacuum. The resulting solid was washed with MeOH to obtain **MC2** as a yellowish white solid (0.32 g, 0.31 mmol, 82%). ^1H NMR (400 MHz, DMSO- d_6 , 25°C): δ = 10.44 – 10.47 (2s, 4H, 4 NHC(O)), 10.30 - 10.33 (2s, 2H, 2NHC(O)), 8.13 – 8.36 (6H), 7.26 – 7.73 (15H, Ar-H and Ar-H *ortho* to -NH₂), 5.63 (broad *s*, 2H, NH₂), 4.13 – 4.17 (*m*, 4H, O-CH₂s), 0.82 – 1.79 (4m, 30H, alkyl-H) ppm. ^{13}C NMR (600 MHz, DMSO- d_6 , 25°C): δ = 166.92, 165.61, 159.37, 157.8, 140.13, 137.36, 136.88, 129.52, 117, 116.6, 115.27, 113.31, 68.7, 32.04, 29.55, 29.48, 26.33 and 14.74 ppm. HR-MALDI-TOF (m/z): $[\text{M}+\text{H}]^+$ _{observed} = 986.972, $[\text{M}]^+$ _{calcd} = 985.474.

Preparation of Samples for AFM, FE-SEM, TEM, and DLS Measurements:

Vesicles were obtained from 0.1 mM, 0.5 mM, and 1.0 mM solutions of **MC1** and **MC2** macrocycles in (a) THF, (b) 9:1 THF/H₂O, and (c) 4:1 THF/H₂O. No vesicle was found in 1 mM **MC1** solutions in 9:1 THF/PhMe. Addition of (a) 10% AcOH and (b) 10% TFA into 1 mM solutions of **MC1** and **MC2** in 9:1 THF/H₂O converted vesicles into nanotubes. These solutions were kept at room temperature for 1 h. before analyzing with DLS, and preparing SEM, TEM, and AFM samples on surfaces.

Samples for AFM and SEM analyses were prepared by drop-casting abovementioned solutions of **MC1** and **MC2** on cleaved mica surfaces. After initially evaporating solvents at room temperature, mica-surfaces were kept in an oven at 45 °C for 16 h. For SEM analysis, sample-coated mica surfaces were sputtered with Ir (thickness = ca. 4 nm) to deposit a conducting layer.

TEM samples were prepared by drop-casting a **MC1** and **MC2** solutions (1 mM) on lacey formvar/carbon-copper grid (200 mesh) and keeping it in an oven at 45 °C for 16 h.

Instruments.

Dynamic light scattering (DLS). The average size distribution of **MC1** and **MC2** vesicles in solutions was measured by Wyatt Technologies DynaPro Dynamic Light Scattering instrument at 20 °C. Data were analyzed by Dynamic™ version 7.0.0.94. For DLS measurements, solutions of **MC1** and **MC2** vesicles in THF, 9:1 THF/H₂O, and 4:1 THF/H₂O were used. To minimize scattering of light from contaminants such as dust particles, these solutions were centrifuged for 5 minutes at 10,000 rpm prior to DLS measurements.

AFM. Tapping-mode AFM measurements were conducted on a Bruker Icon AFM instrument with Olympus silicon visible apex probe; model-OTESP.

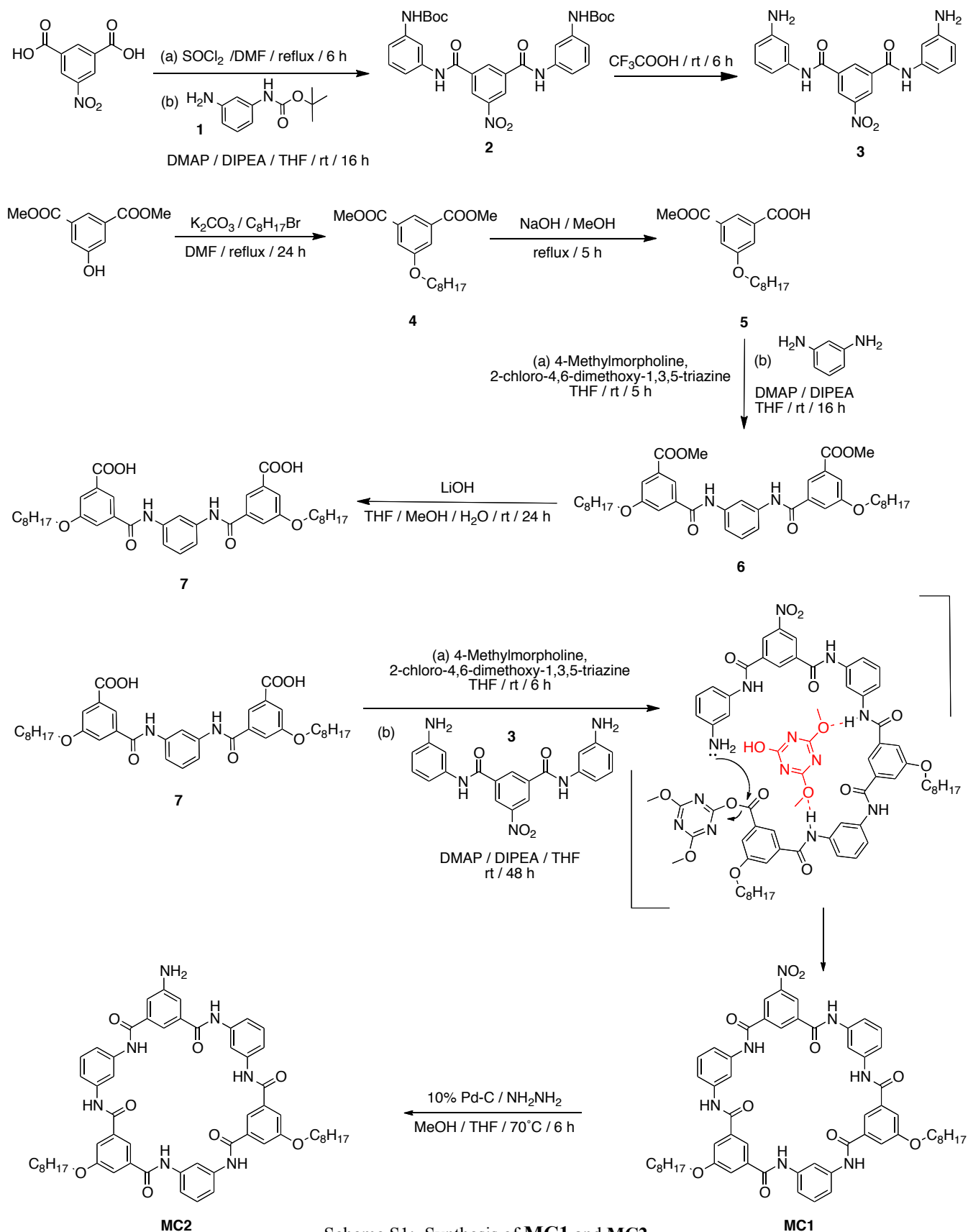
SEM. SEM samples were analyzed with Field-Emission Scanning Electron Microscope (FE-SEM): FEI NOVA NANO SEM 400.

TEM. TEM samples were analyzed using JEOL JEM-2010 Transmission Electron Microscope. All TEM images were recorded under bright field TEM mode (200 KV accelerating voltage).

Reference:

(S1) Suda, Y.; Arano, A.; Fukui, Y.; Koshida, S.; Wakao, M.; Nishimura, T.; Kusumoto, S.; Sobel, M. *Bioconjugate Chem.* **2006**, *17*, 1125–1135.

(S2) Yang, Y.; Xue, M.; Xiang, J.-F.; Chen, C.-F. *J. Am. Chem. Soc.* **2009**, *131*, 12657–12663.



Scheme S1: Synthesis of **MC1** and **MC2**.

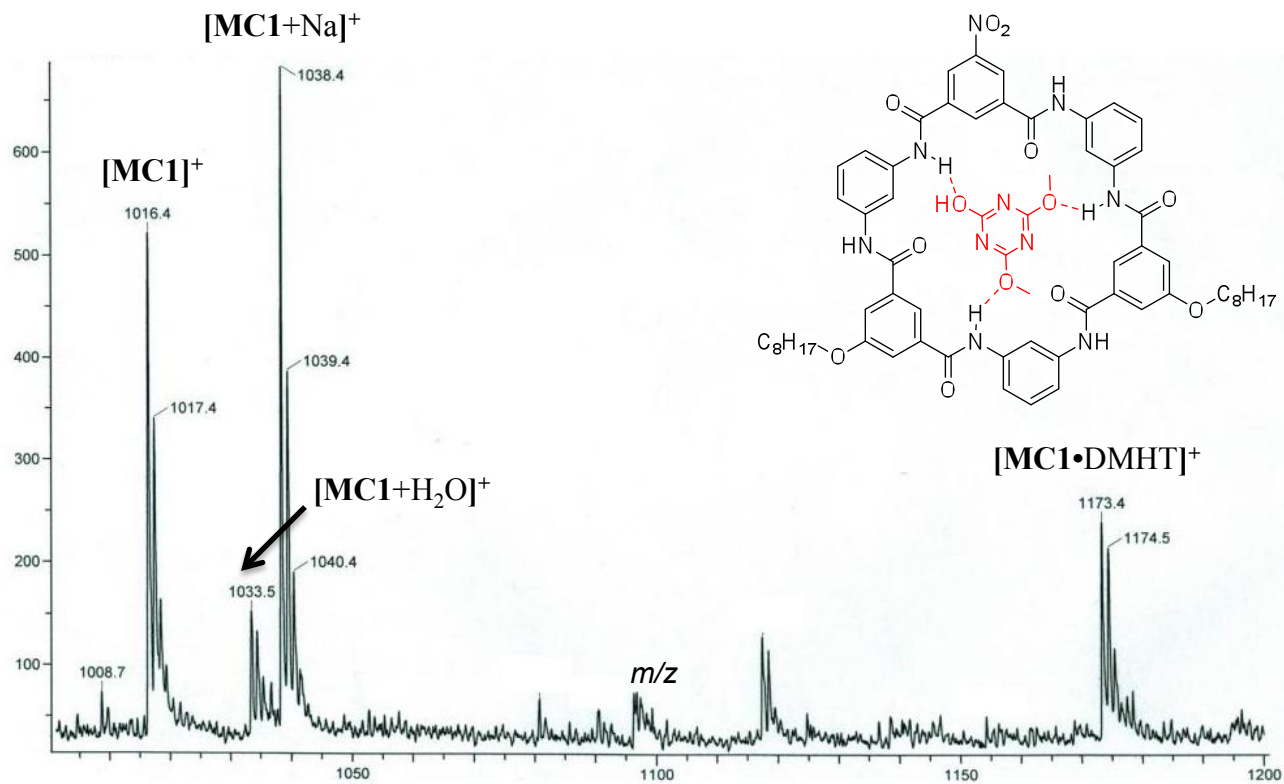


Fig S1: ESI-MS of crude reaction mixture shows m/z signals corresponding to **MC1** (1016.4), $[\text{MC1} + \text{H}_2\text{O}]^+$ (1033.5), $[\text{MC1} + \text{Na}]^+$ (1038.4), and $[\text{MC1} + \text{DMHT}]^+$ (1173.4).

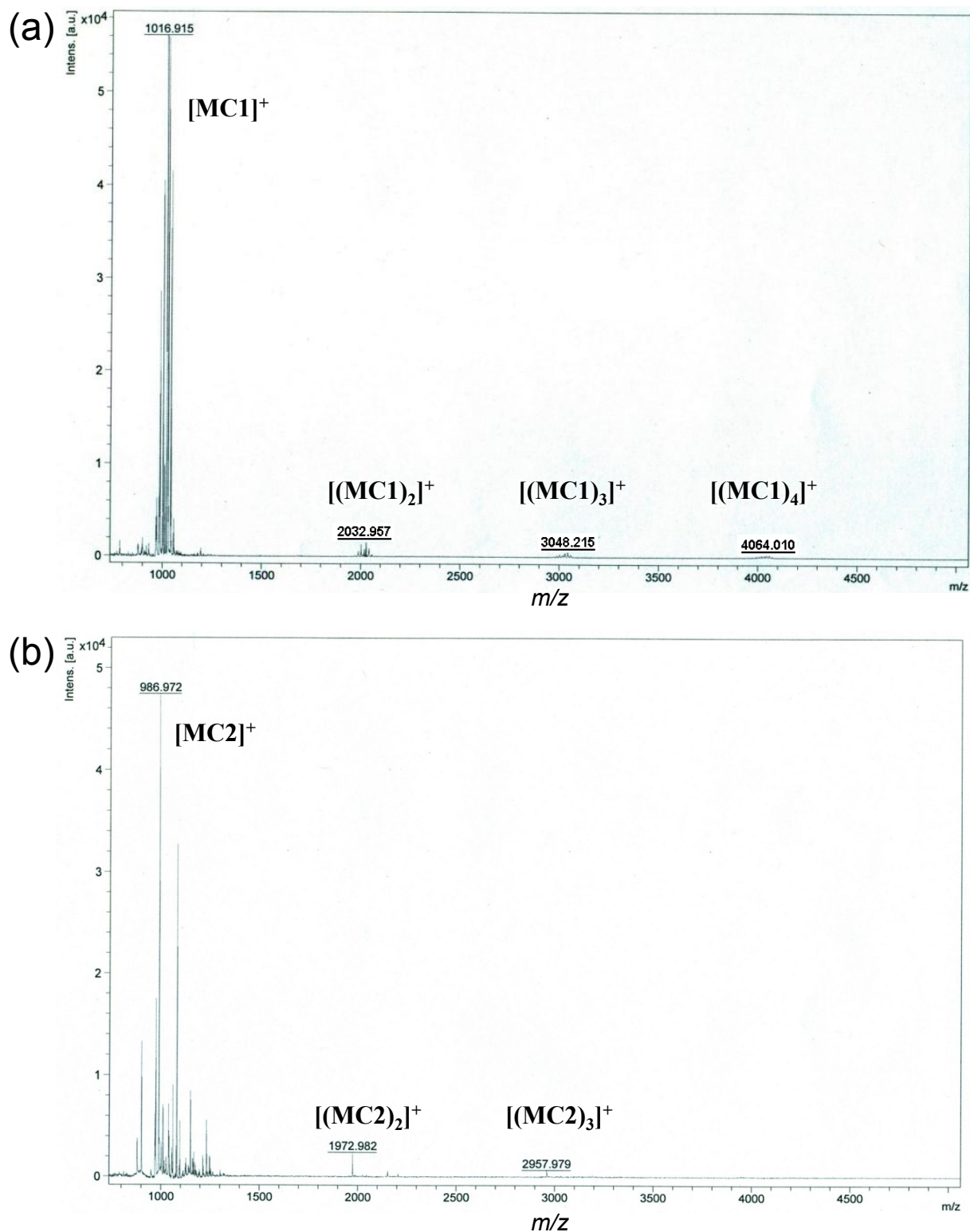


Fig. S2: High Resolution MALDI-TOF MS analysis of (a) **MC1** and (b) **MC2** shows molecular ion peaks as well as self-assembled dimer, trimer, and tetramer.

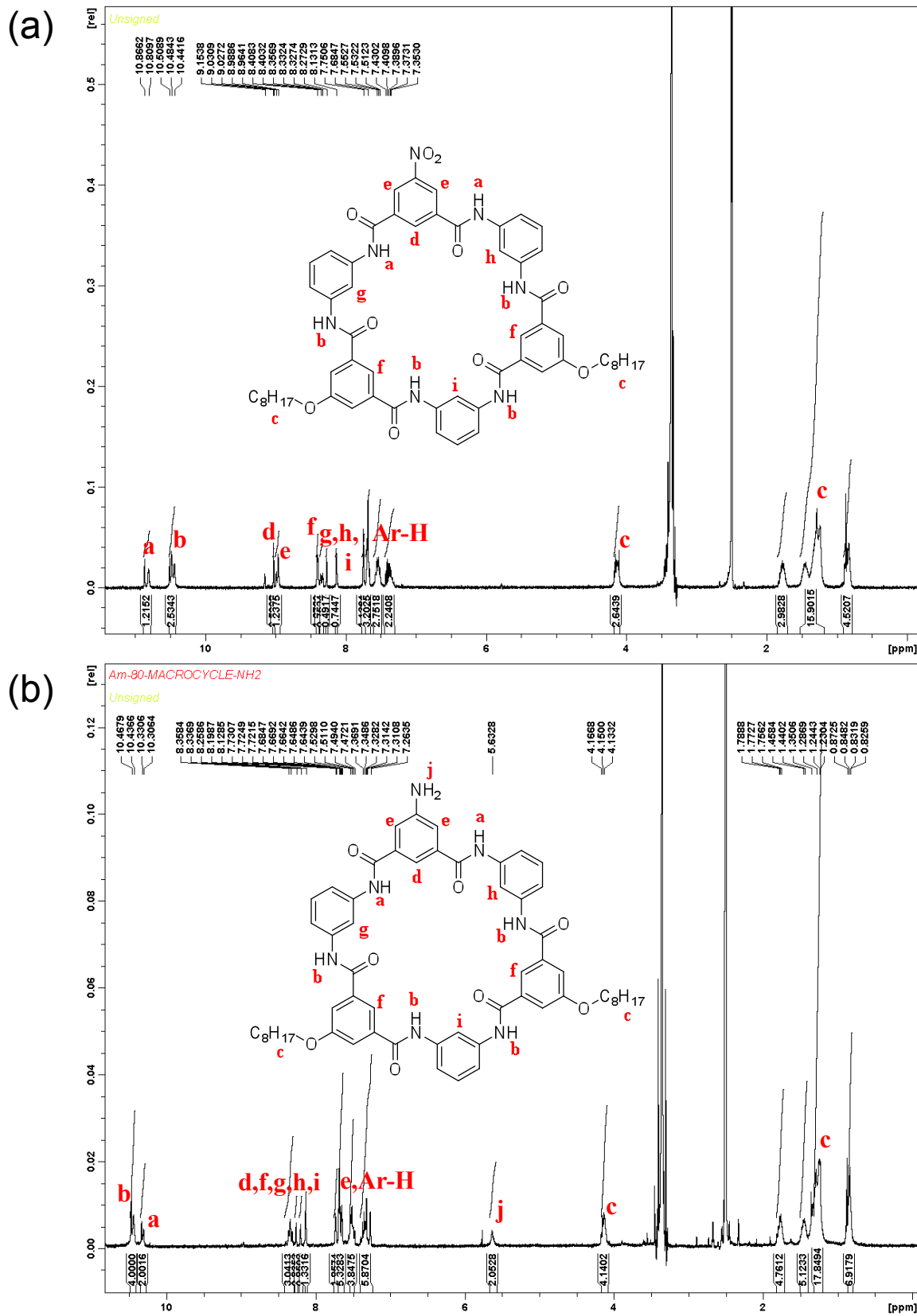


Fig S3: $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$, 25 °C) spectra of (a) MC1 and (b) MC2.

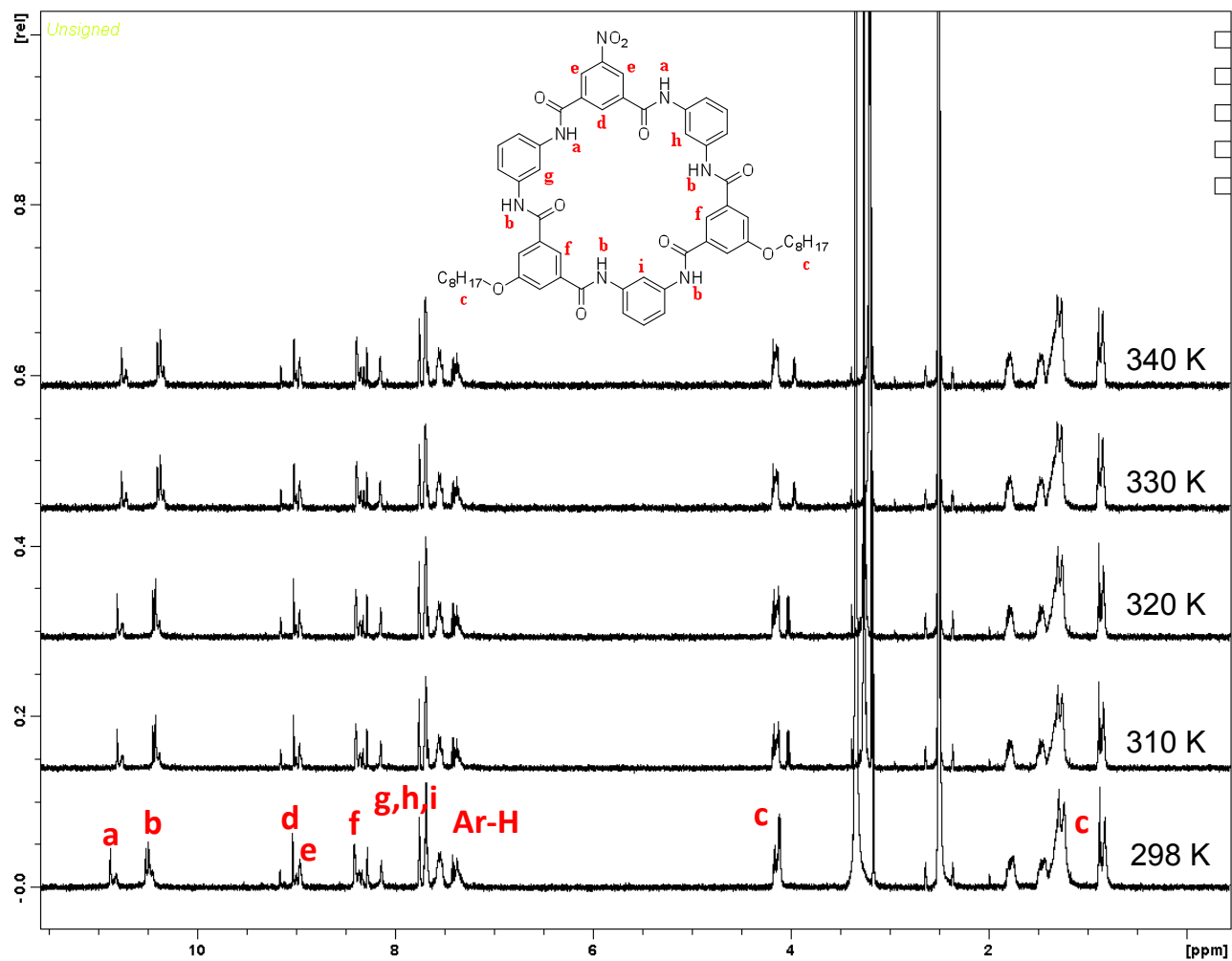


Fig S4: VT-NMR study (^1H NMR, 200 MHz, $\text{DMSO-}d_6$) of **MC1** from 298 – 340 K.

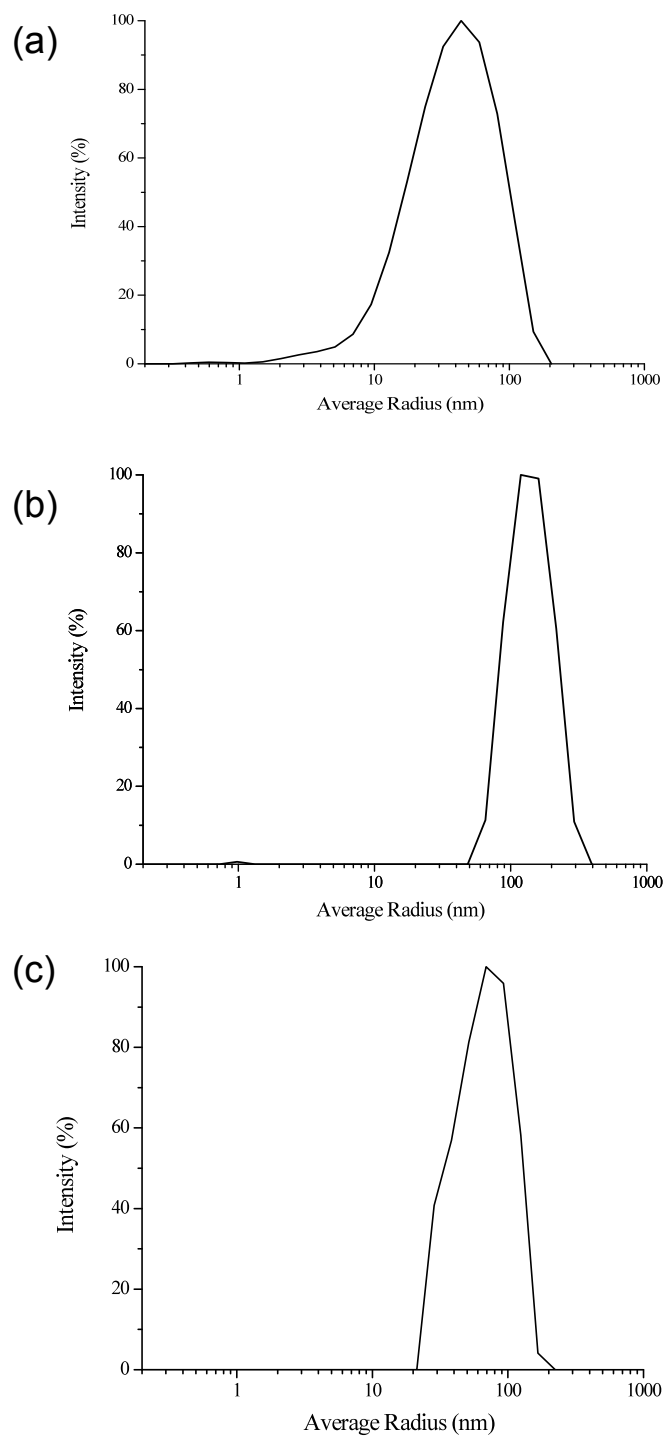


Fig S5: DLS data (20 °C) of vesicles obtained from (a) 1 mM **MC1** solution in THF, (b) 1 mM **MC1** solution in 4:1 THF/H₂O, and (c) 1 mM **MC2** solution in THF.

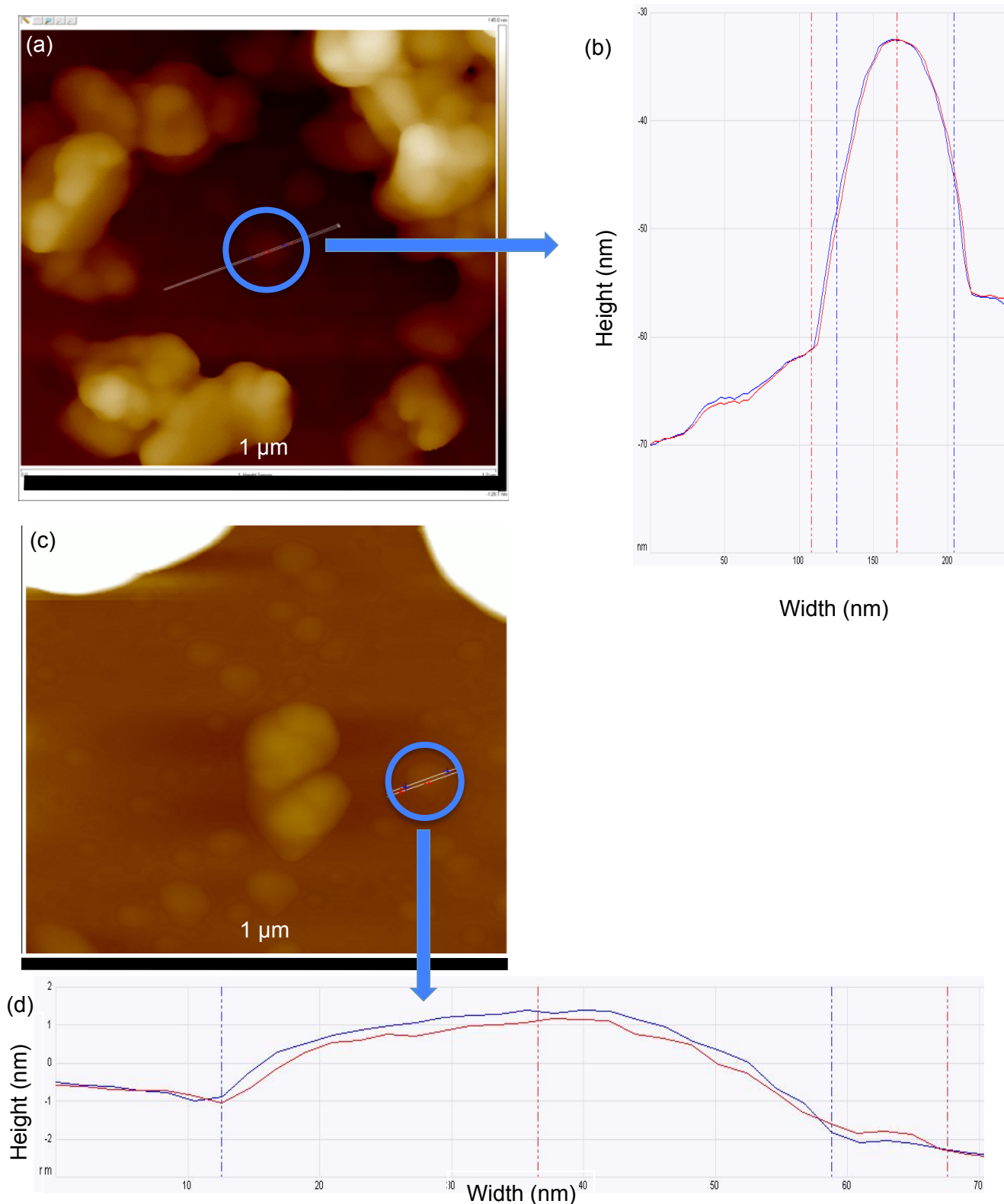


Fig S6: AFM cross-sectional analysis of selected closed-shell vesicles obtained from (a) 1 mM **MC1** in THF and (c) 1 mM **MC2** in 4:1 THF/H₂O. (b and d) The height vs. width plot (the width/height ratio is ca. 5 for the selected **MC1** vesicle (a) and ca.10 for the selected **MC2** vesicle (b)) show that dry vesicles are flattened on mica surfaces, indicating the hollowness of these vesicles. If these vesicles were not hollow and had solid cores, their width/height ratio would have been close to 1, as they would not be flattened upon solvent evaporation.

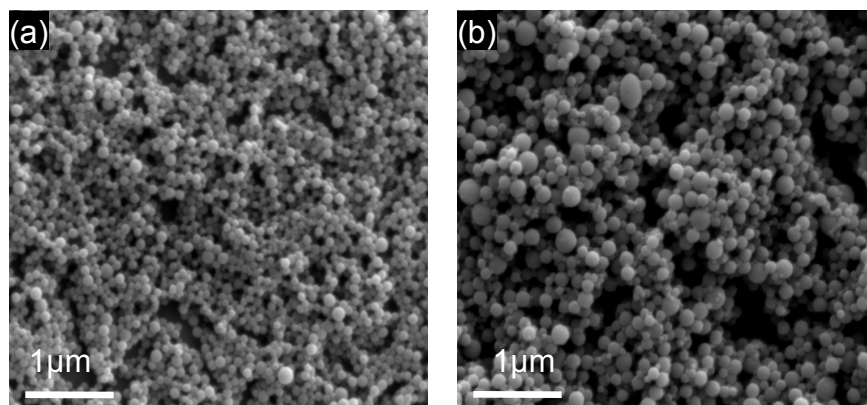


Fig S7: FE-SEM images of closed vesicles obtained from 1 mM **MC1** solutions in (a) THF and (b) 4:1 THF/H₂O drop-casted on cleaved mica surfaces. Larger vesicles (ca. 5 times) are formed in the presence of H₂O (b).

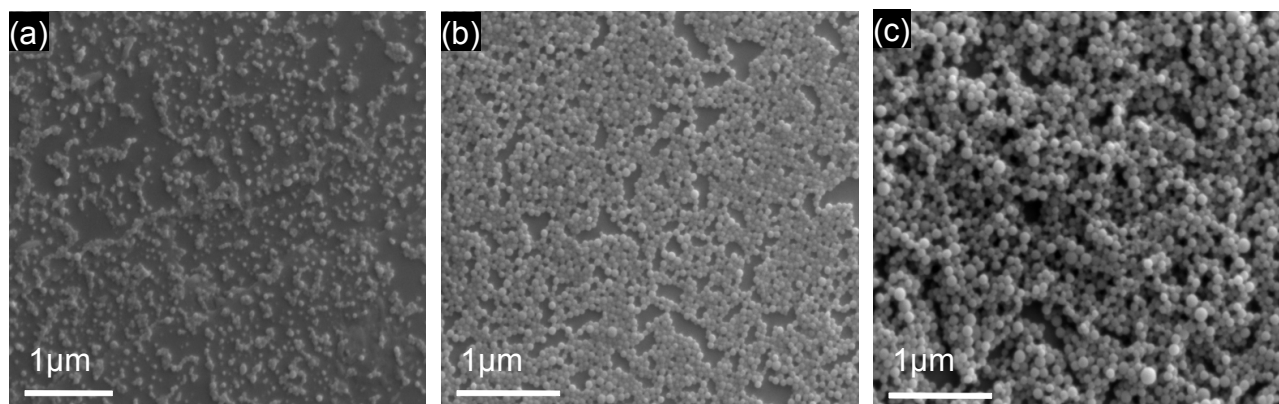


Fig S8: FE-SEM images of closed-shell **MC1** vesicles obtained from its THF solutions at different concentrations: (a) 0.1 mM, (b) 0.5 mM, and (c) 1.0 mM. The size and population of vesicles increase with increasing **MC1** concentrations in a given polar solvent (THF).

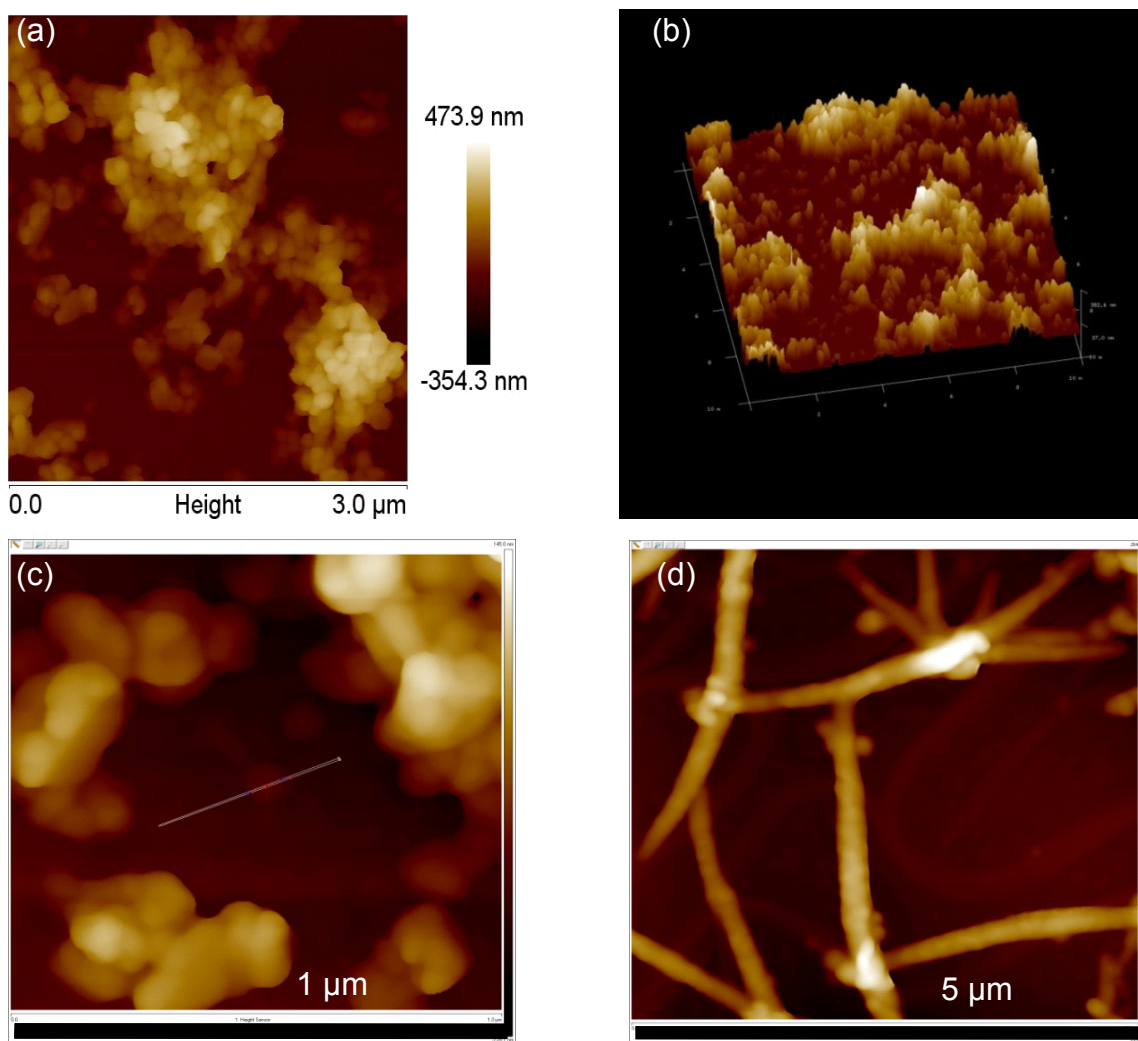


Fig S9: Tapping-mode AFM images of different **MC1** nanostructures formed in different solvents (on cleaved mica surfaces): (a) height image, (b) 3D image, (c) an enlarged section of a surface covered with vesicles obtained from 1 mM **MC1** in THF; (d) height image of **MC1** nanotubes formed in 9:1 THF/H₂O in the presence of 10% TFA.