

## Supplementary Information

### **Self-Constructing Giant Vesicles for Mimicking Biomembrane Fusion and Acting as Enzymatic Catalysis Microreactors**

Shengda Liu,<sup>a</sup> Guo An,<sup>a</sup> Jiayun Xu,<sup>a</sup> Xiumei Li,<sup>a</sup> Tingting Wang,<sup>a</sup> Xiaotong Fan,<sup>a</sup>  
Chunxi Hou,<sup>a</sup> Quan Luo,<sup>a</sup> Junqiu Liu\*<sup>a</sup> and Yanqiu Han\*<sup>b</sup>

- a. State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China
- b. Department of Neurology, The Second Hospital, Jilin University, Changchun 130041, PR China.

## Contents

1. General Information
2. Experimental Section
3. Fig. S1.  $^1\text{H}$  NMR spectra of biPEG-benzaldehyde.
4. Fig. S2. ESI-MS analysis of biPEG-benzaldehyde.
5. Fig. S3.  $^1\text{H}$  NMR spectra of dimethoxy-pillar[5]arene.
6. Fig. S4. MALDI-TOF mass analysis of dimethoxy-pillar[5]arene.
7. Fig. S5.  $^1\text{H}$ -NMR spectra of pillar[5]arene.
8. Fig. S6. MALDI-TOF mass analysis of pillar[5]arene.
9. Fig. S7.  $^1\text{H}$ -NMR analysis of ethoxycarbonylmethoxy-substituted-pillar[5]arene.
10. Fig. S8. MALDI-TOF mass analysis of ethoxycarbonylmethoxy-substituted-pillar[5]arene.
11. Fig. S9.  $^1\text{H}$  NMR spectra of hydrazide-pillar[5]arenes.
12. Fig. S10. ESI-MS analysis of hydrazide-pillar[5]arenes.
13. Fig. S11.  $^1\text{H}$ -NMR spectra of HP5 generated by hydrazide-pillar[5]arene.
14. Fig. S12. MALDI-TOF mass spectra of hydrzone-pillar[5]arenes.
15. Fig S13. DLS of HP5 with different concentrations.
16. Fig S14. DLS of HP5 with stir and sonication.
17. Fig S15. DLS of HP5 with aqueous solution and PBS solution.
18. Fig S16. DLS of HP5 with different pH.
19. Fig S17. CMC of HP5 vesicles.
20. Fig S18. Simulation diagram of the process of fusion and broken hydrogen bonds by

sonicate.

**21.** Fig S19. OM of HPR-loaded vesicles, TMB-loaded vesicles and fusion vesicles.

**22.** References.

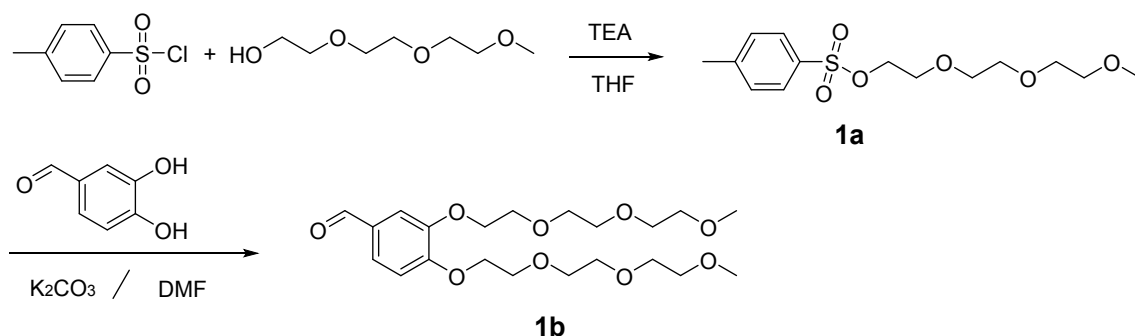
## General Information

<sup>1</sup>H-NMR spectra were measured on Bruker AVANCE III 500 instrument using a tetramethylsilane (TMS) as a reference. ESI-MS spectra were conducted on a Thermo Finnigan-LCQ Advantage Mass Spectrometer. MALDI-TOF mass spectra were performed by Bruker Autoflex Speed TOF/TOF Mass Spectrometer. The Dynamic Light Scattering (DLS) experiments were performed by Malvern Instruments Zetasizer Nano ZS. Optical Microscopy (OM) images were obtained by the Olympus BX61. Scanning Electron Microscope (SEM) images were recorded on a JEOL JSM-6700F scanning electron microscope with primary electron energy of 3kV. Transmission electron microscopy (TEM) images were performed on a JEM-2100F instrument with an accelerating voltage of 200 kV.

## Experimental Section

### Syntheses of biPEG-benzaldehyde

3, 4-Bis [2-[2-(2-methoxyethoxy)ethoxy]ethoxy]benzaldehyde (biPEG-benzaldehyde) was prepared according to previously reported and the method was illustrated as follow.<sup>1</sup>



**Scheme S1.** Synthetic route of the biPEG-benzaldehyde.

### Synthesis of tosyltriethyleneglycolmono-methylether (1a)

Triethyleneglycol monomethyl ether (8.21 mg, 0.05 mol) and triethylamine (20 ml, 0.15 mol) were stirred in tetrahydrofuran solution (30 ml) at 0 °C in the case of ice bath. Then another tetrahydrofuran solution (30 ml) of p-toluenesulfonyl chloride (11.4 g, 0.06 mol) was added dropwise to the reaction system by a dropping funnel in 2 h. Then the mixture continued to react for 12 h at room temperature. The product was poured into aqueous hydrochloric acid and extracted with chloroform. The yellow liquid crude product was obtained by evaporating the solvent with a total yield around 58.5%. The product was used to the subsequent synthesis without any other purification.

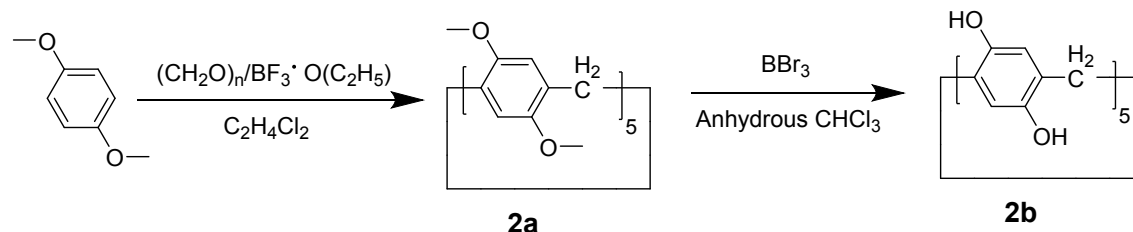
### Synthesis of 3, 4-bis[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]benzaldehyde (1b)

3, 4-Dihydroxybenzaldehyde (0.5 g, 3.6 mmol), tosyltriethyleneglycolmono-methylether (2.56 g, 8 mmol) and potassium carbonate (2.05 g, 15 mmol) were stirred at 100 °C overnight in N, N-dimethylformamide (DMF) (5 ml) under a nitrogen atmosphere. The reaction mixture was extracted with alcohol and water after cooling to room temperature. The organic phase was dried over sodium sulfate and removed by evaporation under reduced pressure, yielding a yellow oil product with a total yield around 84%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 25 °C)

$\delta$ (ppm):  $\delta$  9.84 (d, 1H), 7.44 (d, 1H), 7.43 (s, 1H), 6.99 (t, 1H), 4.27–4.20 (m, 4H), 3.90 (t, 4H), 3.77–3.73 (m, 4H), 3.69–3.63 (m, 8H), 3.57–3.53 (m, 4H), 3.38 (d, 6H). ESI MS:  $m/z$  431.2  $[M+H]^+$ .

### Syntheses of pillar[5]arene

Pillar[5]arene was prepared according to previously reported method.<sup>2</sup>



**Scheme S2.** Synthetic route for pillar[5]arene.

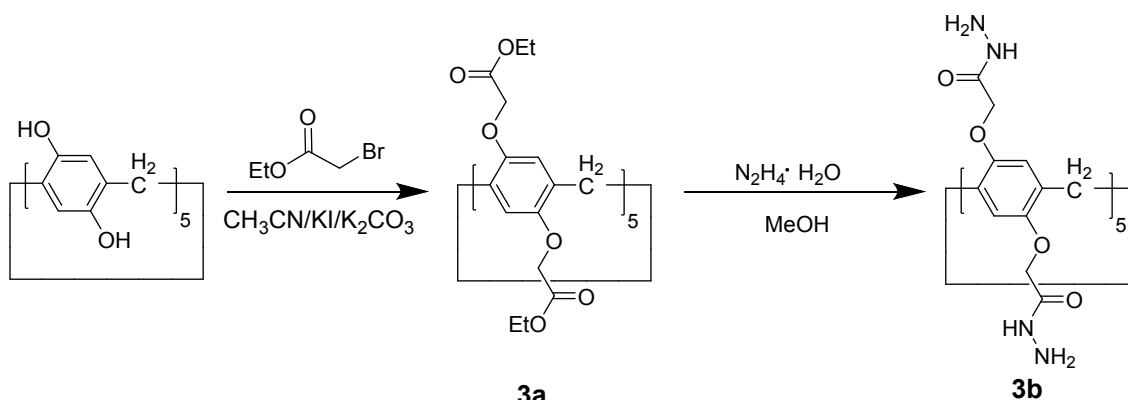
### Synthesis of dimethoxy-pillar[5]arene (2a)

Paraformaldehyde (1.96 g, 65 mmol) with a solution of 1, 4-dimethoxybenzene (2.79 g, 20 mmol) was stirred in 1, 2-dichloroethane (180 ml) for 10 minutes. Boron trifluoride diethyl etherate  $[BF_3 \cdot O(C_2H_5)_2]$ , 2.4 ml, 20 mmol] was added to the solution and the mixture was stirred at room temperature for 1.5 h. Then the mixture was poured into methanol and filtered. The filter cake was collected and poured into acetone. The mixture in acetone was filtered again. The final white solid of the dimethoxy-pillar[5]arene was obtained by collecting the filter cake and drying under vacuum with a yield around 45%.  $^1H$  NMR (500 MHz,  $CDCl_3$ , 25 °C)  $\delta$ (ppm):  $\delta$  6.91 (s, 10H), 3.79 (s, 10H), 3.76 (s, 30H). MALDI-TOF MS:  $m/z$  750.28  $[M]^+$ , 773.28  $[M+Na]^+$ , 789.25  $[M+K]^+$ .

### Synthesis of pillar[5]arene (2b)

Boron tribromide (13.25 g, 53 mmol) with a solution of dimethoxy-pillar[5]arene (2 g, 2.7 mmol) was stirred in dry chloroform (150 ml) at room temperature for 72 h. Then the mixture was poured into deionized water and reacted for another 3 h. The mixture was evaporated under reduced pressure and washed with chloroform. The final white solid of pillar[5]arene was obtained by drying under vacuum with a yield around 50%.  $^1H$  NMR (500 MHz, DMSO, 25 °C)  $\delta$ (ppm):  $\delta$  8.46 (s, 10H), 6.58 (s, 10H), 3.43 (s, 10H). MALDI-TOF MS:  $m/z$  610.04  $[M]^+$ , 633.03  $[M+Na]^+$ , 649.04  $[M+K]^+$ .

## Synthesis of hydrazide-pillar[5]arenes



**Scheme S3.** Synthetic route for hydrazide-pillar[5]arene.

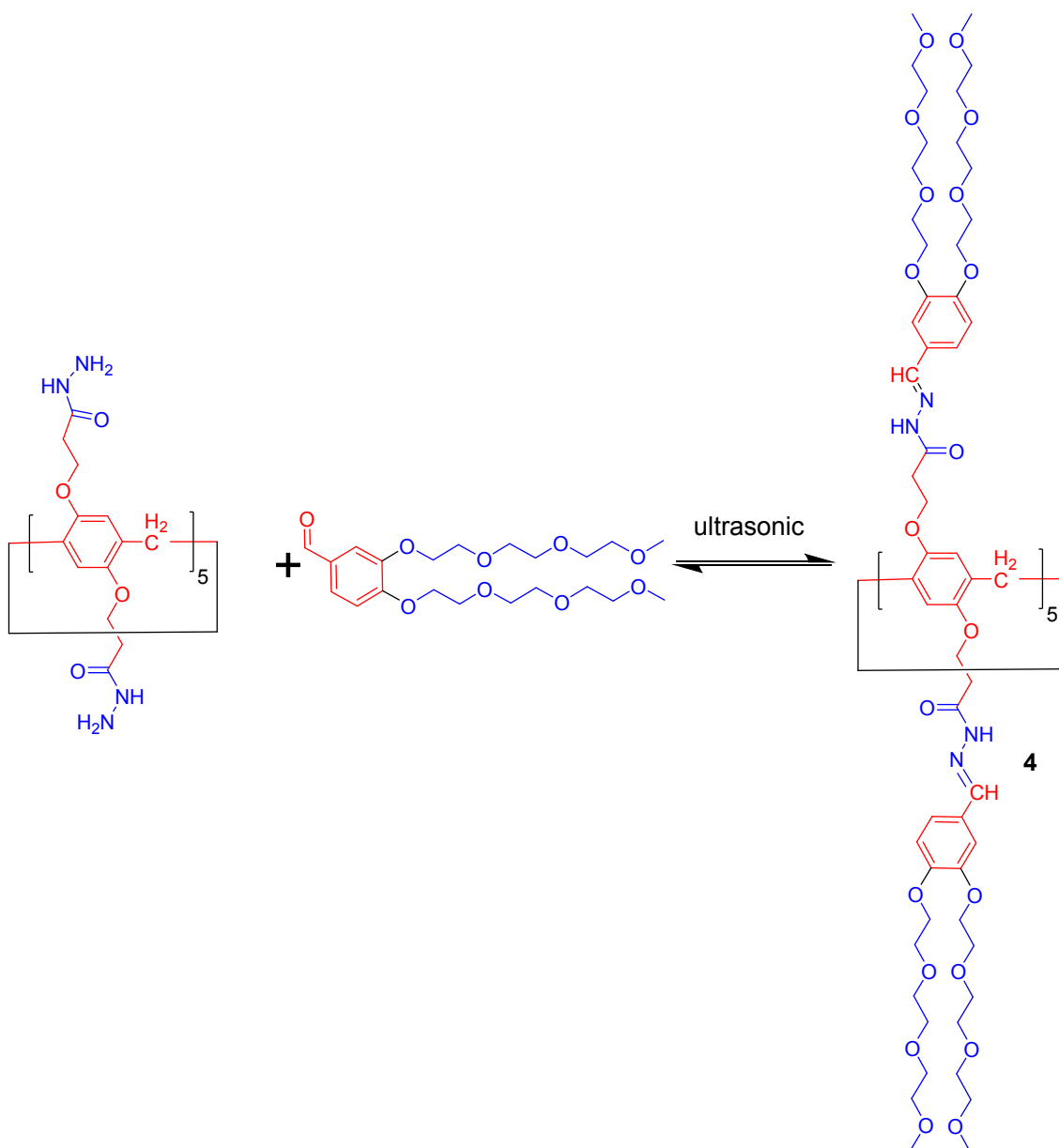
### Ethoxycarbonylmethoxy-substituted-pillar[5]arene (3a)

Pillar[5]arene (2.50 g, 4.1 mmol) was firstly dissolved in acetonitrile ( $\text{CH}_3\text{CN}$ ) (30 ml) under a nitrogen atmosphere. Potassium carbonate (7.00 g, 50 mmol) and potassium iodide as the catalyzer (30 mg, 0.2 mmol) were added and the reaction mixture was stirred for 20 minutes. Then ethyl bromoacetate (11.00 ml, 99 mmol) was added and the reaction mixture was heated at 86 °C to reflux for 24 h. After removal of the solvent, the resulting solid was dissolved in chloroform. Then methanol (500 ml) was poured into the solution under -20 °C. After filtration, the remained solid was purified by column chromatography. The product was white solid with a total yield around 60%.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta$ (ppm):  $\delta$  7.04 (s, 10H), 4.60–4.48 (m, 20H), 4.14–4.01 (m, 20H), 3.86 (s, 10H), 0.99–0.91 (m, 30H). MALDI-TOF MS:  $m/z$  1470.71 $[\text{M}+\text{H}]^+$ .

### Hydrazide-pillar[5]arenes (3b)

Hydrazine hydrate (5.0 ml, 100 mmol) was added into a solution of ethoxycarbonylmethoxy-substituted-pillar[5]arene (0.76 g, 0.52 mmol) in methanol (40 ml) under a nitrogen atmosphere. The solution was heated at 70 °C to reflux for 4 h and then cooled to room temperature. The precipitate was collected by filtration and washed with methanol and dichloromethane. The white solid product was obtained by vacuum distillation to remove the hydrazine hydrate with a yield around 87%.  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}$ , 25 °C)  $\delta$ (ppm):  $\delta$  9.29 (s, 10H), 6.89 (s, 10H), 4.42 (s, 20H), 4.38 (s, 20H), 3.75 (d, 10H). ESI MS:  $m/z$  1332.4  $[\text{M}+\text{H}]^+$ .<sup>3</sup>

## Formation of hydrzone-pillar[5]arenes



**Scheme S4.** Synthetic route for hydrzone-pillar[5]arene.

### Synthesis and assembly of hydrzone-pillar[5]arenes (4)

Hydrzone-pillar[5]arenes (HP5) was prepared and isolated by adding hydrazide-pillar[5]arene (1.33 mg,  $1 \times 10^{-3}$  mmol) and biPEG-benzaldehyde (5.16 mg,  $1.2 \times 10^{-2}$  mmol) into PBS solution (100 mM, 1 ml, pH= 6.0), so that the final ratio of hydrazide-pillar[5]arene to biPEG-benzaldehyde was 1: 12. This solution was stewed overnight after reaction in ultrasonic oscillator (30 °C) for 80 minutes. Then a disperse system of giant vesicles was formed. Due to the reversibility of the hydrazone bond, the product's <sup>1</sup>H-NMR spectra is a little messy, but the chemical shift of hydrazone bond can be seen at 8.63 ppm (Figure S11). MALDI-TOF MS: m/z 5491.24 [M+K]<sup>+</sup>.

### **Preparation of HRP-loaded vesicles**

Hydrazide-pillar[5]arene (6.65 mg), biPEG-benzaldehyde (21.4 mg) and horseradish peroxidase (HRP) (0.5 mg) were mixed into PBS solution (100 mM, 5ml, pH= 6.0) in 80 minutes with ultrasonic oscillator, and then the non-encapsulated HRP was removed by ultrafiltration (Molecular Weight Cut-off: 100K) against PBS solution.

### **Preparation of TMB-loaded vesicles**

Hydrazide-pillar[5]arene (6.65 mg), biPEG-benzaldehyde (21.4 mg) and 3, 3', 5, 5'-tetramethylbenzidine (TMB) (0.5 mg) were mixed into PBS solution (100 mM, 5ml, pH= 6.0) containing 10% alcohol in 80 minutes with ultrasonic oscillator, and then the non-encapsulated HRP was removed by ultrafiltration (Molecular Weight Cut-off: 3K). Alcohol was helpful to solute TMB.

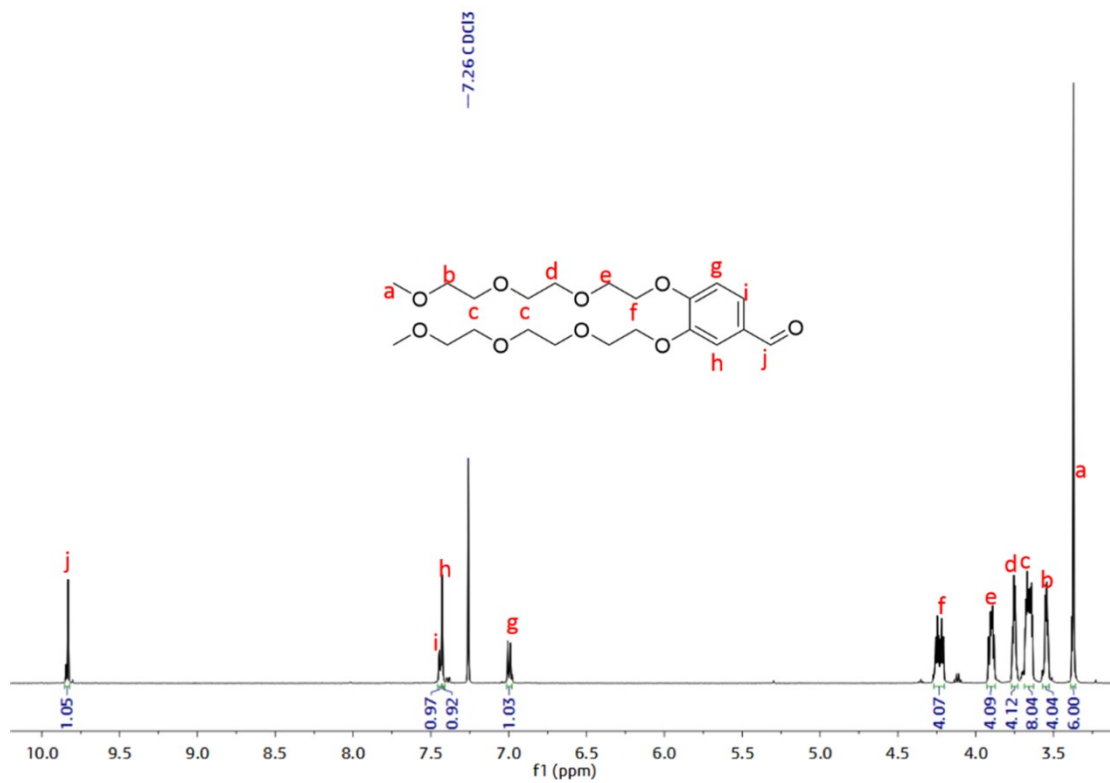
### **Preparation of fusion vesicles**

The solution of HRP-loaded vesicles (1 ml) and the solution of TMB-loaded vesicles (1 ml) were mixed in a volume ratio 1 to 1 in 80 minutes with ultrasonic oscillator.

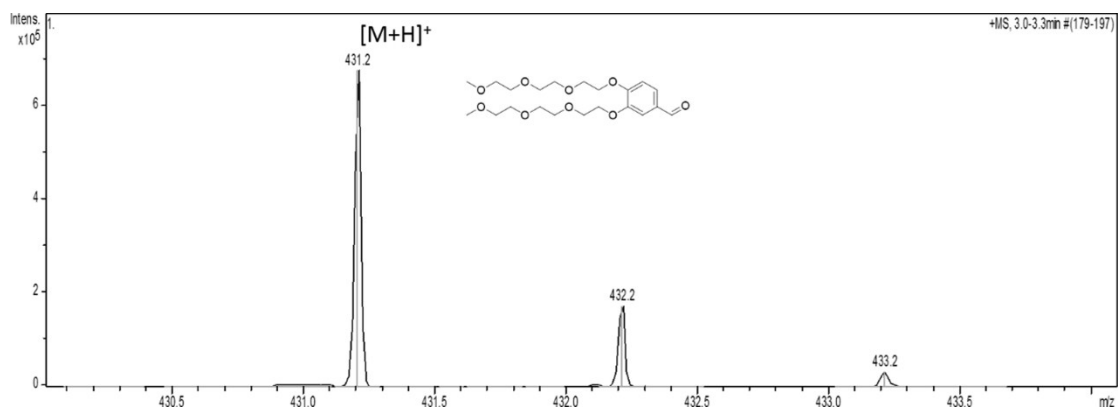
### **The process of enzymatic catalysis by vesicle fusion**

HRP and TMB were as model enzyme and substrate. After vesicles fusion, HRP and TMB were in the fusion vesicles. We added H<sub>2</sub>O<sub>2</sub> (1 mM) to the solution of fusion vesicles of HRP and TMB, TMB can be oxidized with the maximum absorption at 652 nm.

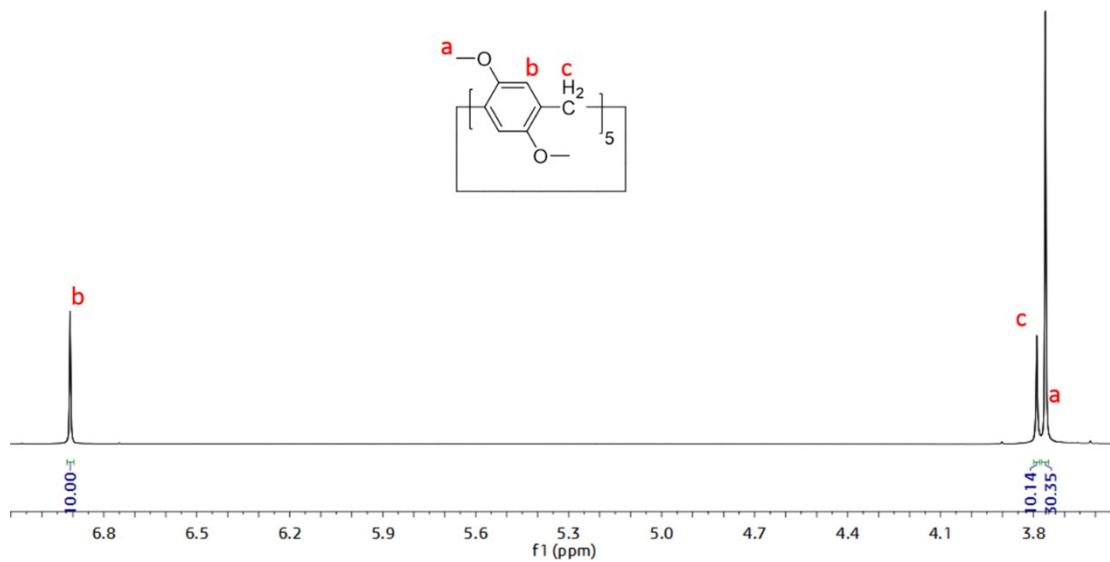




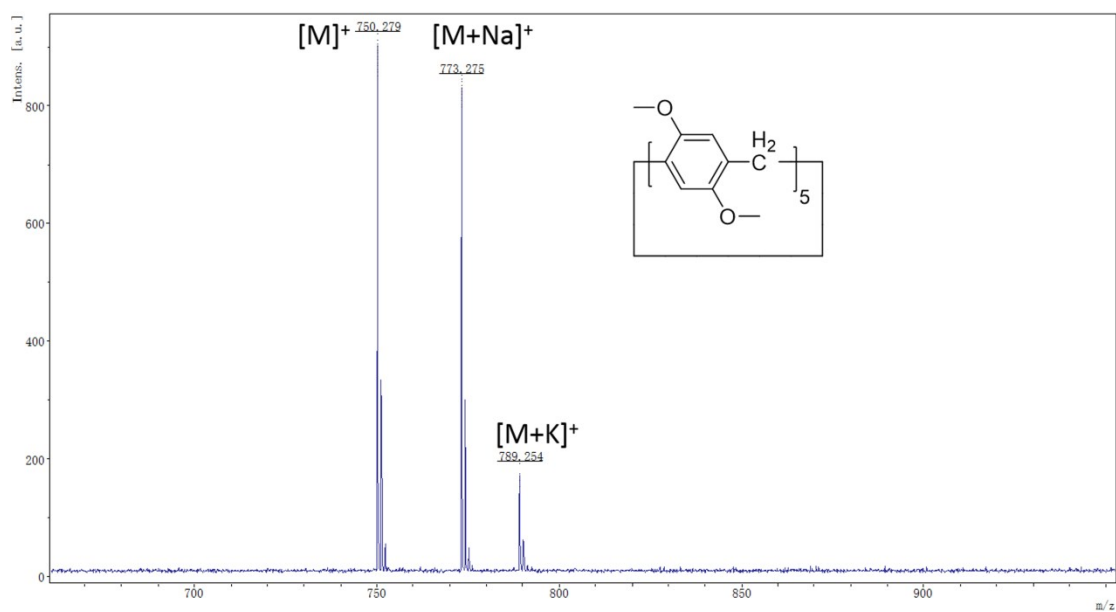
**Figure S1.** <sup>1</sup>H-NMR spectra of biPEG-benzaldehyde.



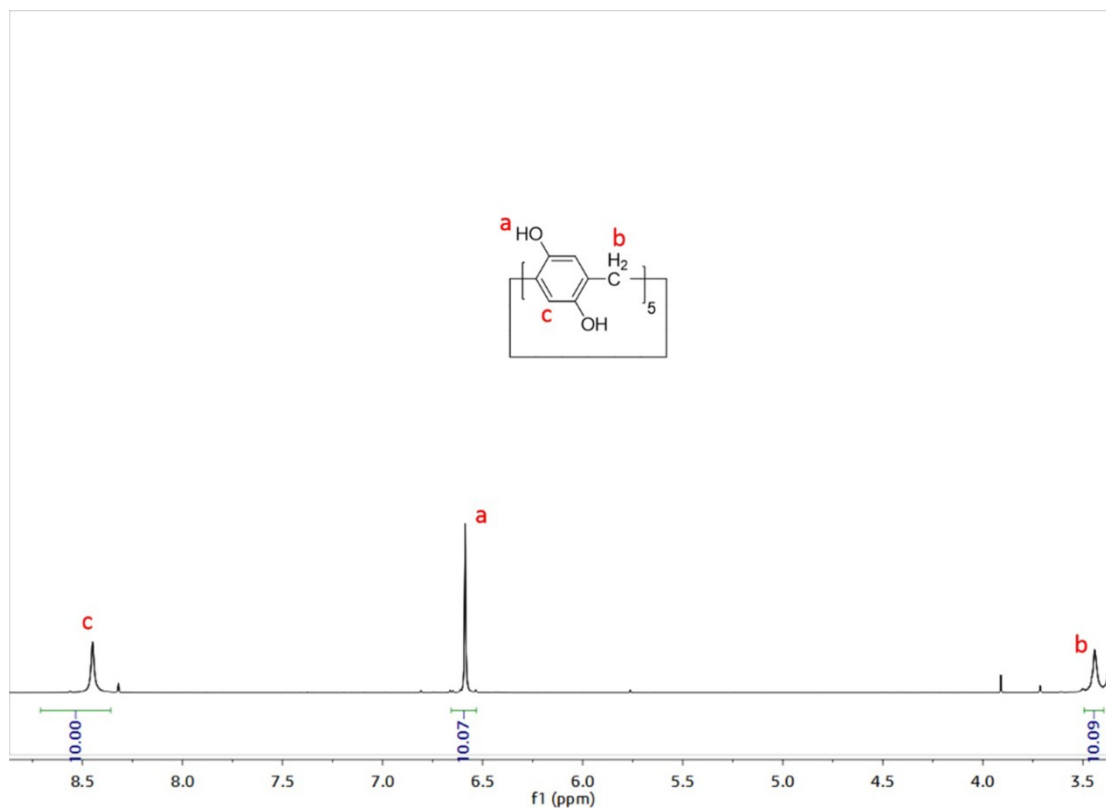
**Figure S2.** ESI-MS analysis of biPEG-benzaldehyde.



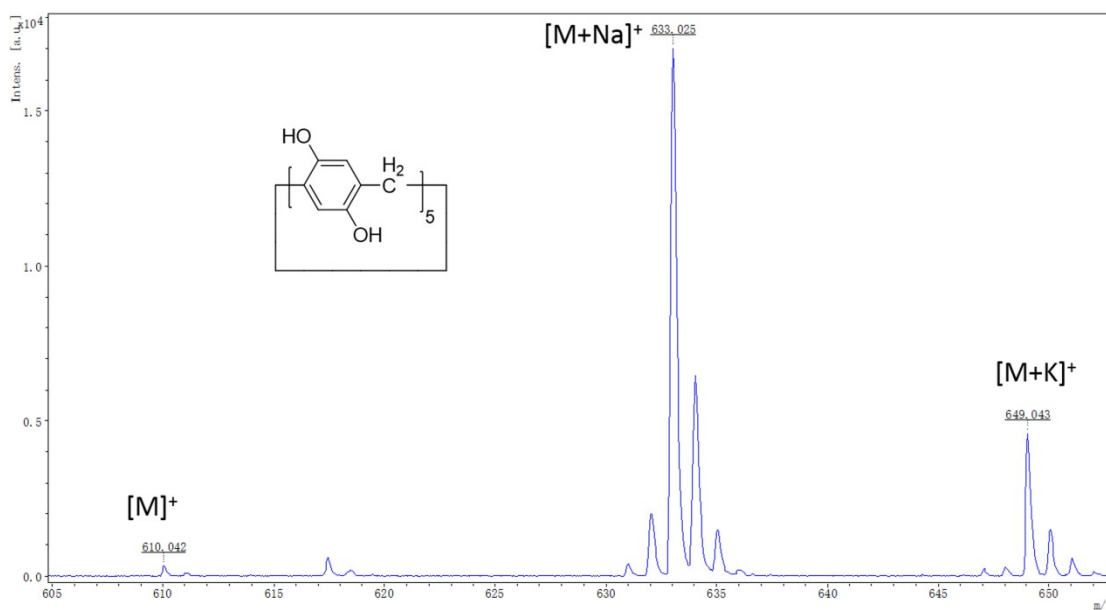
**Figure S3.**  $^1\text{H}$  NMR spectra of dimethoxy-pillar[5]arene.



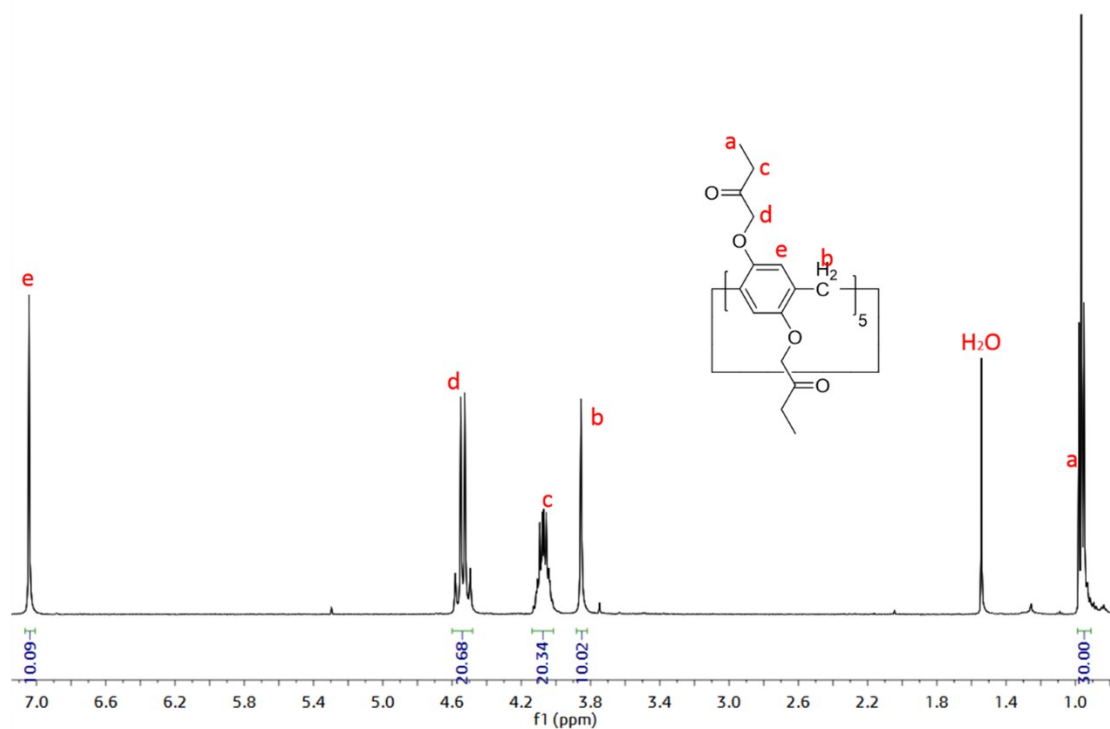
**Figure S4.** MALDI-TOF mass analysis of dimethoxy-pillar[5]arene.



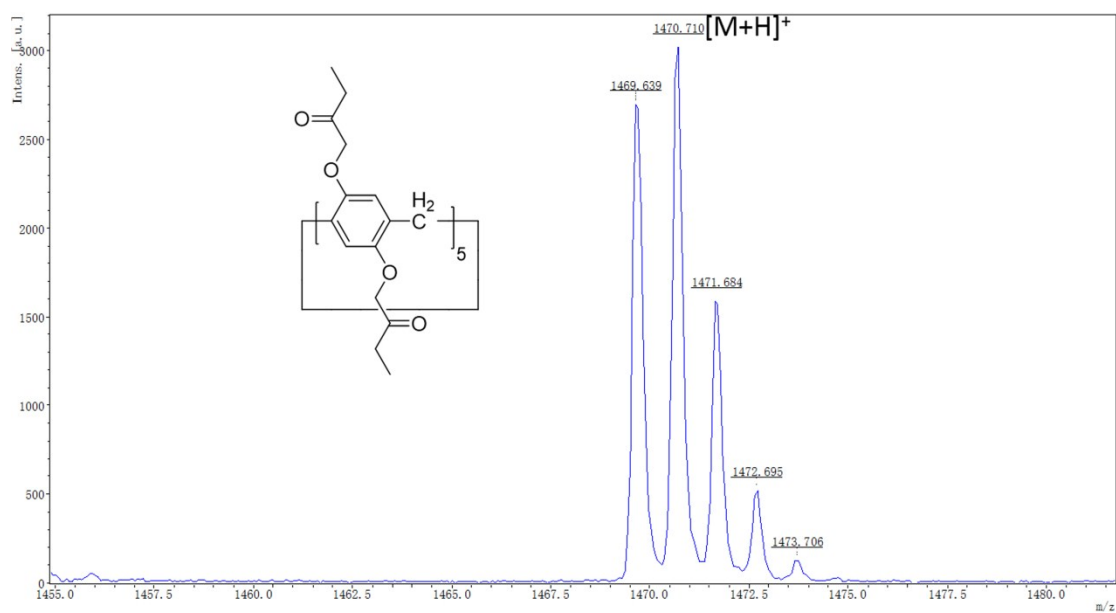
**Figure S5.**  $^1\text{H-NMR}$  spectra of pillar[5]arene.



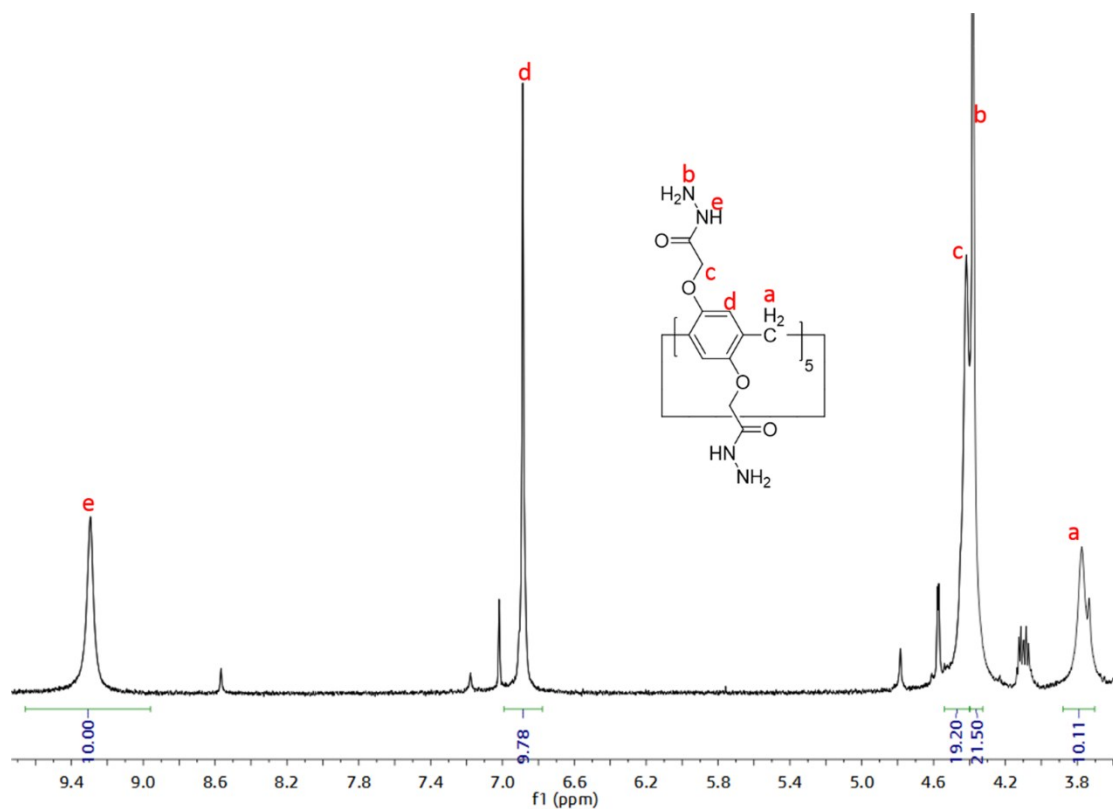
**Figure S6.** MALDI-TOF mass analysis of pillar[5]arene.



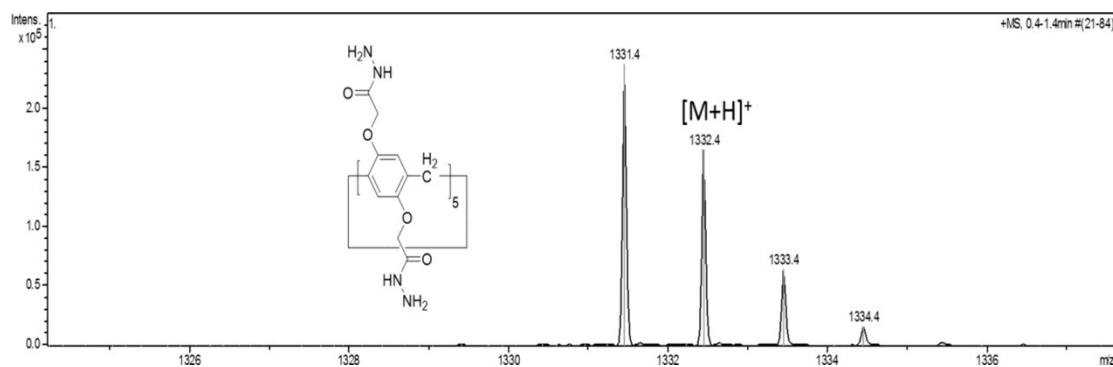
**Figure S7.** <sup>1</sup>H NMR spectra of ethoxycarbonylmethoxy-substituted-pillar[5]arene.



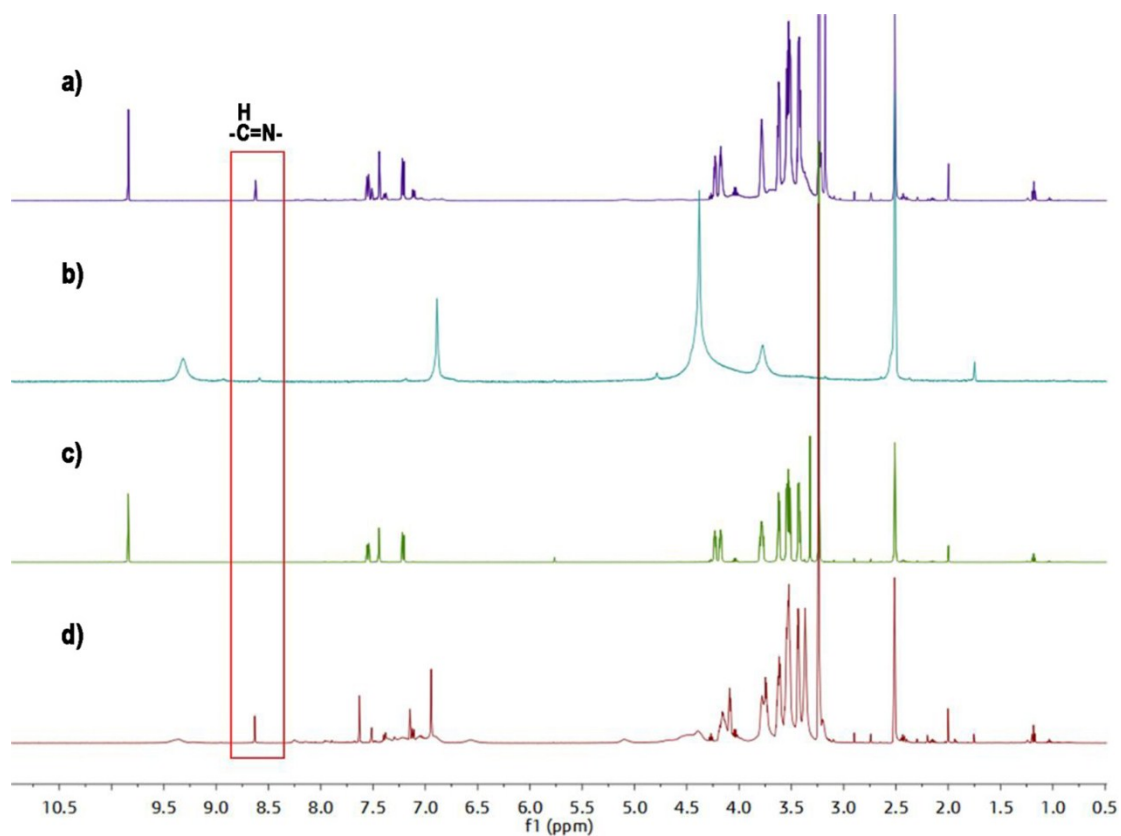
**Figure S8.** MALDI-TOF mass analysis of ethoxycarbonylmethoxy-substituted-pillar[5]arene.



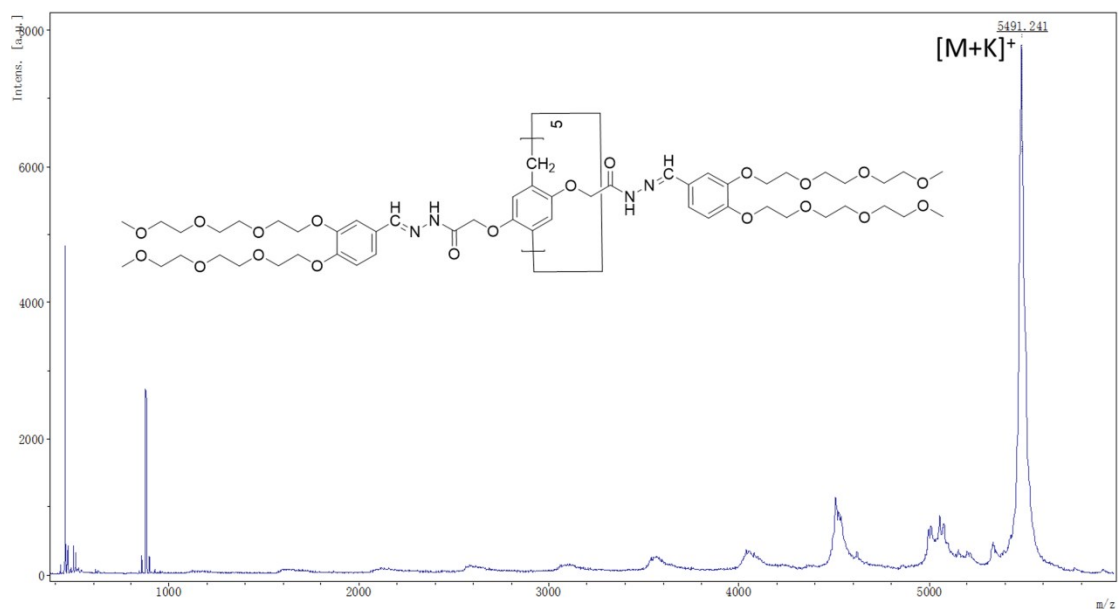
**Figure S9.**  $^1\text{H}$  NMR spectra of hydrazide-pillar[5]arenes.



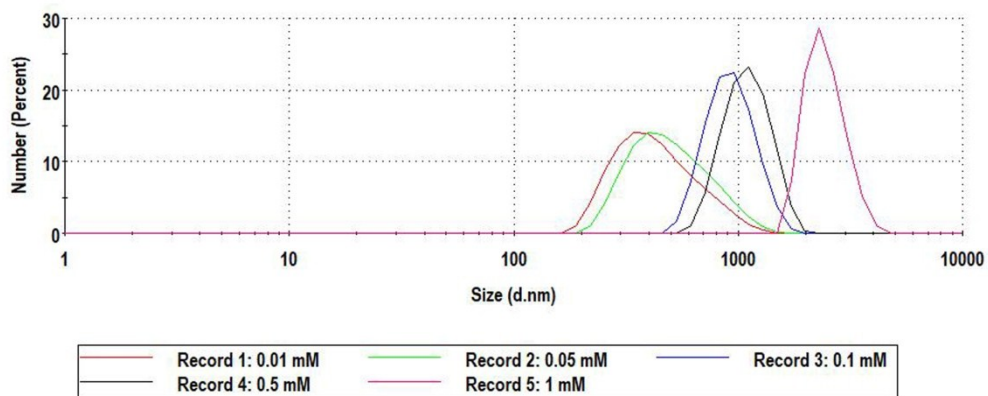
**Figure S10.** MALDI-TOF mass analysis of hydrazide-pillar[5]arenes.



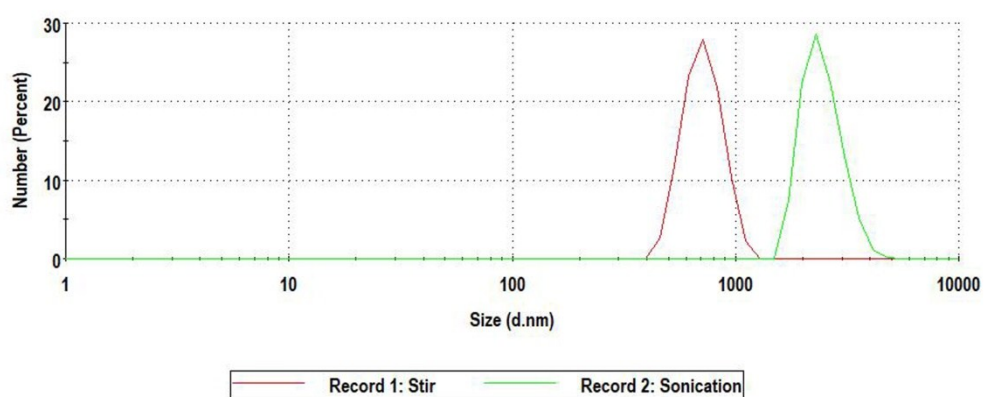
**Figure S11.**  $^1\text{H-NMR}$  spectra of (a) HP5 generated by hydrazide-pillar[5]arene: biPEG-benzaldehyde=1: 12, (b) biPEG-benzaldehyde, (c) hydrazide-pillar[5]arene, (d) HP5 generated by hydrazide-pillar[5]arene: biPEG-benzaldehyde=1: 5.



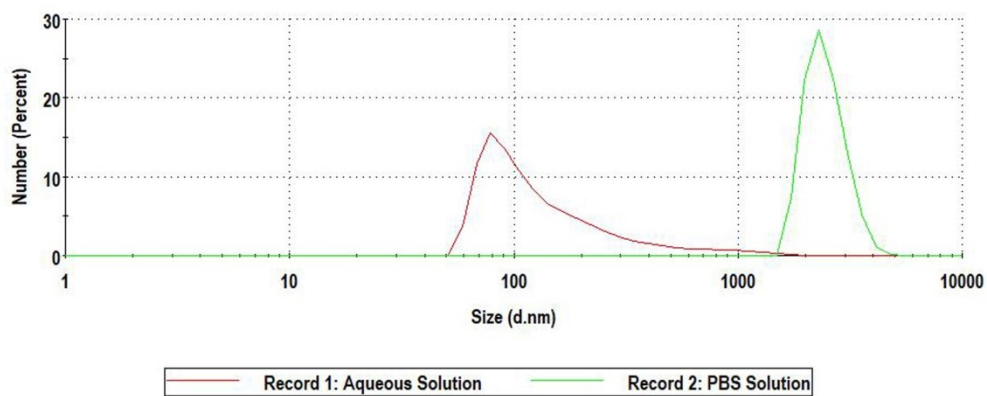
**Figure S12.** MALDI-TOF mass analysis of hydrazone-pillar[5]arenes.



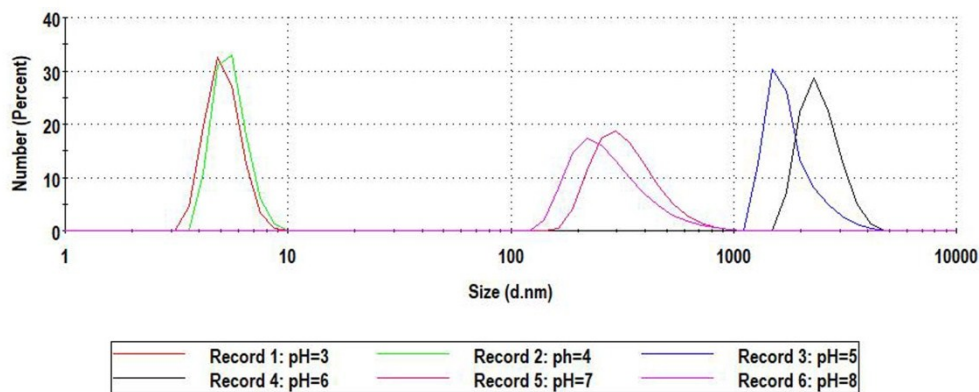
**Figure S13.** DLS of HP5 with different concentrations.



**Figure S14.** DLS of HP5 with stir and sonication.

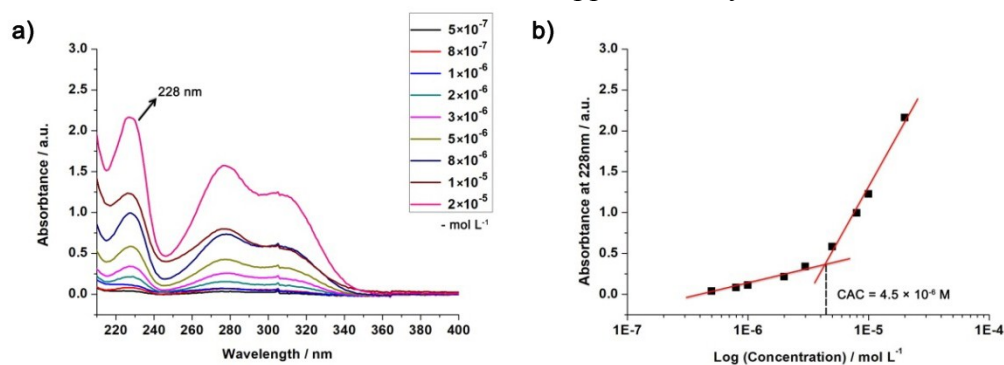


**Figure S15.** DLS of HP5 with aqueous solution and PBS solution.



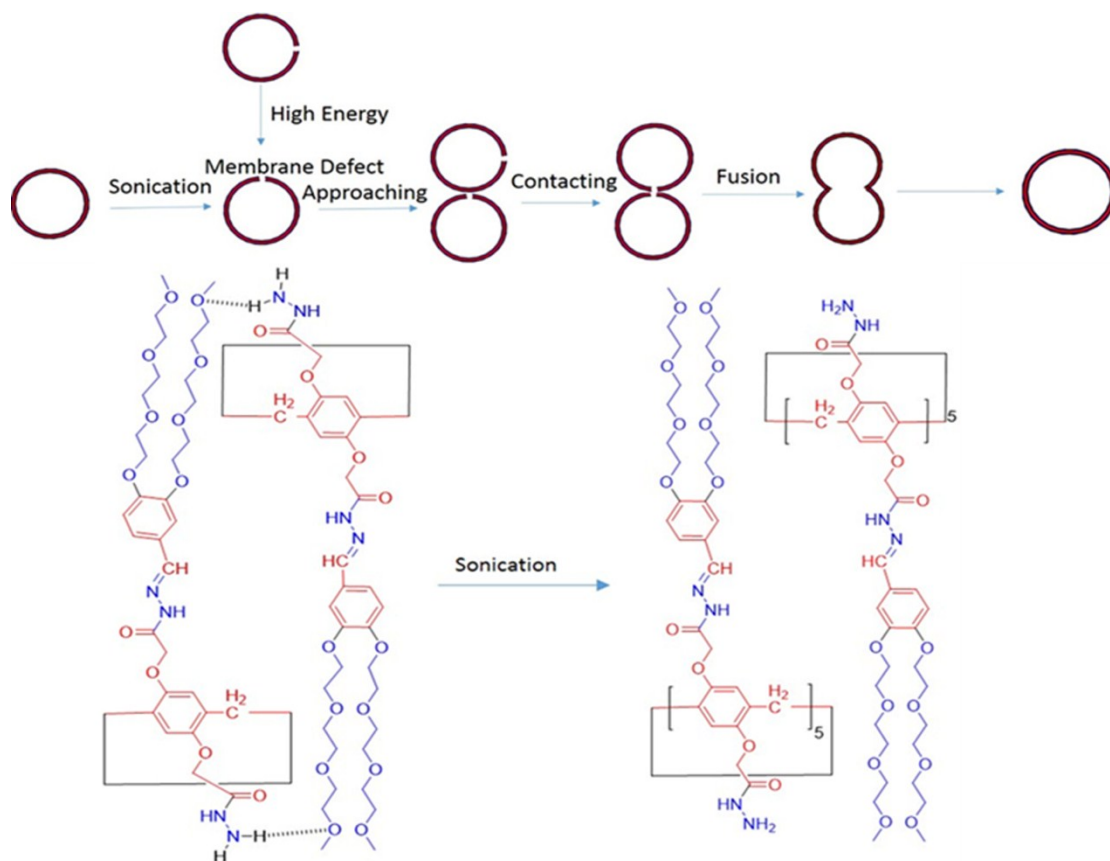
**Figure S16.** DLS of HP5 with different pH.

Critical aggregation concentration (CMC) can be determined by UV-Vis method by the previous report.<sup>5</sup> We obtained the CMC by measuring the UV-Vis absorption spectra of the HP5 with increasing concentrations, and the isolated absorption peaks at 228 nm were used for the measurement. The CMC is approximately  $4.5 \times 10^{-6}$  M.

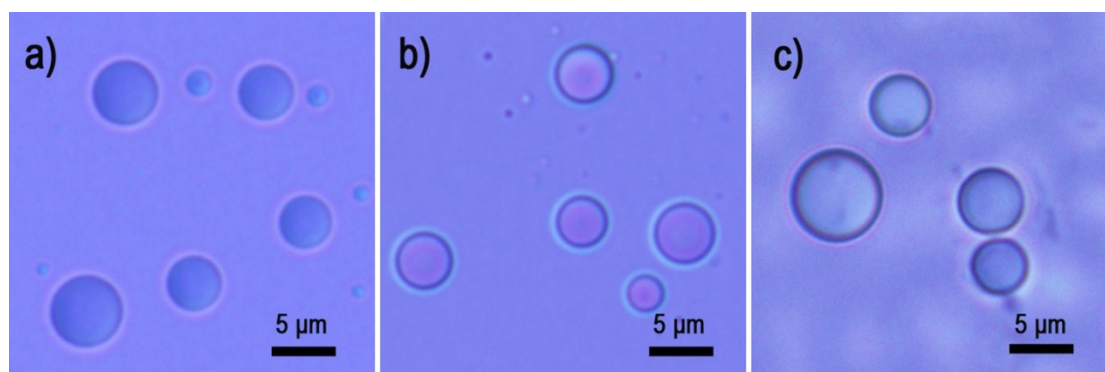


**Figure S17.** CMC of HP5 vesicles. A) UV-Vis spectra of vesicular solution with different concentrations (from  $5 \times 10^{-7}$  M to  $2 \times 10^{-5}$  M) and B) its absorbance at 228 nm as a function of concentration of vesicular solution.





**Figure S18.** Simulation diagram of the process of fusion and broken hydrogen bonds by sonicate.



**Figure S19.** OM of HPR-loaded vesicles, TMB-loaded vesicles and fusion vesicles.

#### References:

1. J. Boekhoven, J. M. Poolman, C. Maity, F. Li, L. van der Mee, C. B. Minkenberg, E. Mendes, J. H. van Esch and R. Eelkema, *Nat. Chem.*, 2013, **5**, 433-437.
2. T. Ogoshi, S. Kanai, S. Fujinami, T. Yamagishi and Y. Nakamoto, *J. Am. Chem. Soc.*, 2008, **130**, 5022-5023.
3. X. B. Hu, Z. Chen, G. Tang, J. L. Hou and Z. T. Li, *J. Am. Chem. Soc.*, 2012, **134**, 8384-8387.
4. Y. F. Zhou and D. Y. Yan, *J. Am. Chem. Soc.*, 2005, **127**, 10468-10469.
5. R. Dong, B. Zhu, Y. Zhou, D. Yan, X. Zhu, *Angew. Chem. Int. Ed.* 2012, **51**, 11633 –11637.