Thermo-Responsive Fluorescent Vesicles Assembled by Fluorescein-Functionalized Pillar[5]arene

Huacheng Zhang,a Xing Ma,b Junfei Guo,a Kim Truc Nguyen,a Quan Zhang,a Xiao-Jun Wang,a Hong Yan,a Liangliang Zhu a and Yanli Zhao*a,b

a Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, 637371, Singapore
b School of Materials Science and Engineering, Nanyang Technology University, 639798, Singapore

E-mail: zhaoyanli@ntu.edu.sg

Electronic Supplementary Information

General Methods

Organic reagents were purchased from Aldrich and used without further purifications. All the solvents and inorganic reagents were commercially available. Me2CO was dried with anhydrous MgSO4 before use. 1,4-Dimethoxy-pillar[5]arene (Dmpillar), alkynated fluorescein (S2), and azido-pillar[5]arene (S3) were synthesized according to the literature procedures. Dynamic light scattering (DLS) measurements at variable concentrations were carried out with a Zetasizer Nano ZS instrument from Malvern Instruments Ltd. at 298 K using a 633 nm ‘red’ laser. The mean hydrodynamic size was
calculated with Zetasizer software. UV-Vis spectra at variable concentrations were recorded with Shimadzu UV-3600 UV-Vis-NIR spectrophotometer at 298 K. The stock solution \((10^{-4} \text{ mol} \cdot \text{L}^{-1})\) of the compound 1 was prepared with anhydrous Me₂CO. All the sample solutions for the investigation of the critical assembly concentration were freshly prepared by diluting the stock solution according to literature procedures.\(^{[S4]}\) The variable temperature emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (Excited at 488 nm).

\(^1\text{H}\) nuclear magnetic resonance (\(^1\text{H}\) NMR) spectra were recorded at room temperature on Bruker Avance 300 with working frequencies of 300 MHz for \(^1\text{H}\) and 75 MHz for \(^{13}\text{C}\) nuclei. The \(^1\text{H}\) COSY NMR and \(^1\text{H}\) NOESY NMR spectra were recorded on a BBFO 400 spectrometer. Chemical shifts were reported in ppm relative to the signals corresponding to the residual non-deuterated solvent, and coupling constants were recorded in Hertz (Hz). All \(^{13}\text{C}\) NMR spectra were recorded with simultaneous decoupling of \(^1\text{H}\) nuclei. The following abbreviations were used to explain the multiplicities: \(s\), singlet; \(d\), doublet; \(t\), triplet; \(b\), broad peaks; \(m\), multiplet or overlapping peaks. The electronic spray ionization mass spectra (ESI-MS) were recorded on a ThermoFinnigan LCQ quadrupole ion trap mass spectrometer. The high-resolution MS were performed on a Waters Q-tof Premier MS spectrometer.

Negative-stained TEM images was recorded on a JEM 1400 electron microscope (120 kV) equipped with slow scan CCD and using cold cathode field emission as the gun. The samples for negative-stained TEM were prepared by dropping a droplet of the orange sample solution onto a TEM grid (copper grid, 300 meshes, coated with carbon film), and stained with 1% sodium phosphotungstate in H\(_2\)O (about 2 \(\mu\)L) followed by air-drying. SEM images were obtained on a JSM 6340 scanning electron microscope (0.5-30 kV) equipped with Cold cathode FEG as electron source. Fluorescent microscopy and confocal laser scattering microscopy images were recorded by using Nikon confocal microscope.
**Compounds Synthesized**

Scheme S1 The preparation of compound 1.

**Synthesis of Compound 1:** Compounds S2 (44.4 mg, 0.1 mmol), S3 (80.5 mg, 0.1 mmol), tetrakis(acetonitrile) copper(I) hexafluorophosphate (3.7 mg, 0.01 mmol), and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl] amine (TBTA) were dissolved in Me2CO (20 mL). The mixture solution was stirred under reflux for 2 d. The solution was then poured into H2O (200 mL). The aqueous phase was extracted (3 × 200 mL) with CH2Cl2. The combined organic layers were dried (Mg2SO4) and the solvent was removed in vacuum. The mixture was subjected to silica gel column chromatography (SiO2, gradient elution from 5% up to 20% MeOH in CH2Cl2) to afford the compound 1 (112.4 mg) in 90% yield. 1H NMR (300 MHz, DMSO-d6): δ = 9.99 (s, 1H), 8.44 (s, 1H), 8.13 (s, 1H), 7.72 (s, 1H), 7.15 (d, J = 6, 1H), 6.83-6.74 (m, 8H), 6.66 (d, 2H), 6.60-6.55 (m, 6H), 5.30 (s, 1H), 5.26 (s, 1H) 4.71 (s, 2H), 4.25 (s, 2H), 3.67 (s, 34H), 3.47 (s, 3H). 13C NMR (75 MHz, DMSO-d6): δ =169.2, 159.9, 152.4, 150.8, 150.4, 149.0, 129.6, 128.0, 127.9, 127.5, 124.8, 115.0, 113.8, 113.0, 110.3, 102.6, 83.4, 68.9, 67.3, 56.3, 55.8, 51.0, 50.1, 32.5, 31.1, 30.0, 29.3. HR-MS (ESI): C70H68N5O15S calcd for m/z = 1250.4433, found m/z = 1250.4453 [M + H]+.
Figures S1-S10

Fig. S1 $^1$H COSY NMR spectrum (400 MHz) of compound 1 (0.020 mM) at ambient temperature.

Fig. S2 $^1$H NOESY NMR spectrum (400 MHz) of compound 1 (0.020 mM) at ambient temperature.
**Fig. S3** DLS size distributions of compound 1 (a, 0.001 mM; b, 0.002 mM; c, 0.004 mM; d, 0.006 mM; e, 0.008 mM; f, 0.010 mM; g, 0.012 mM; h, 0.014 mM; i, 0.016 mM; j, 0.018 mM; k, 0.020 mM) in Me₂CO. Mean size changes of the assemblies in Me₂CO under variable concentrations (figure l, from 0.0001 to 0.020 mM) detected by DLS.
Fig. S4 The increase (a) and decrease (b) for the UV-Vis absorption of compound 1 in Me2CO under variable concentrations. Critical assembly concentration (CAC) of compound 1 in Me2CO was calculated by detecting the maximum absorption band in the UV-Vis spectra (c). The intensity changes of fluorescence upon various concentrations (d, e). The CAC of compound 1 in Me2CO was calculated by detecting the maximum emission band in the fluorescence measurements (f).

Fig. S5 Micro-morphologies of compound 1 in saturated MeOH by negative stained TEM: a few hollow spheres (a) and irregular blocks (b).
Fig. S6 Micro-morphologies of compound 1 in saturated MeCN by negative stained TEM: a few irregular shapes (a) and solid particles (b).

Fig. S7 Size distributions of the vesicles assembled from compound 1 in Me$_2$CO (0.020 mM) at room temperature, determined by TEM images.
Fig. S8 Confocal laser scanning microscopy image of the vesicles assembled from compound 1 in Me2CO (0.020 mM) at room temperature, scale bar = 10 µm.

Fig. S9 Negative stained TEM image (left) of compound 1 in Me2CO (0.020 mM) after heating the sample to 35 °C in a sealed vial. DLS size distributions (right) of compound 1 (0.020 mM) in Me2CO at 35 °C in a sealed vial.
Fig. S10 Variable temperature emission spectra (excited at 488 nm) of compound 1 in dilute Me₂CO (left, 0.001 mM, 10-50 °C) and compound S2 in saturated Me₂CO (right, 10-54 °C).

References


