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Supramolecular hydrophobic guest transport system based on pillar[5]arene

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Electronic Supplementary Information (13 pages)

1. General methods

All reagents were commercially available and used as supplied without further purification. The water-soluble pillar[5]arene derivatives **1** and **2** were prepared according to a previous report.[1] Solvents were either used as purchased or dried prior to use by usual laboratory methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ECX 400 MHz, Jeol Eclipse 500 MHz, or Bruker AVANCE III 700 MHz NMR spectrometers. UV-Vis spectra were measured using a Varian Cary 50 Bio UV/Vis spectrophotometer at 20 ℃. All the fluorescence spectra were recorded with a PERKIN ELMER LS 50B fluorescence spectrometer. Quartz cells (1 cm path length) were used throughout.

Methods for the *in vitro* biological experiments

Cell culture: HEK 293 (Human Embryonic Kidney, ATCC # CRL-1573) and A549 cell (ATCC xxxx) were grown in DMEM (GIBCO media Invitrogen) with 10% heat inactivated fetal calf serum (FCS, Hyclone), and 1% Penicillin/Streptomycin (Invitrogen). HepG2 (Heptacellular carcinoma, Human, ATCC #HB-8065), HeLa (provided by Dr. David Mosser) and MCF-7 (Mammary Gland Adenocarcinoma, ATCC # HTB-22) were grown in Minimum Essential Media (MEM, ATCC # 30-2003) with 10% FCS and 1% Penicillin/Streptomycin. THP-1 (Blood Monocytes, ATCC # TIB-202) cells were grown in Roswell Park Memorial Institute medium (RPMI) media (ATCC # 30-2001) with 10% FCS and 1% Penicillin/Streptomycin. SK-OV-3 (Ovarian Adenocarcinoma, ATCC # HTB-77) cells were grown in McCoy's (ATCC # 30-2007) with 10% FCS and 1% Penicillin/Streptomycin.

Toxicology. HEK293 cells (2.5×106), HepG2 cells (4×105) and THP-1 cells (2.5×106) were seeded in a 96 well plate (Corning) at 200 µl/well. After the cells were allowed to adhere for 24 h, they were treated with 0.010, 0.1, 1 and 10 mM of erythromycin, erythromycin estolate, and **1** over a 48 h period. Samples were tested in triplicates for each run. Three independent runs were performed for each sample. Cells were assayed using the CellTiter 96 AQueous Kit® (Promega), an MTS-based assay, which quantifies cell viability by measuring cellular metabolism. A549 cells were assayed by MTT assay. In short, cells were seeded into 96-well

plates at 10, 000 cells per well. After 24 h incubation, the culture medium was removed and replaced with 100 μL sample containing solution at different concentrations. The cells were incubated for further 48 h, and then 20 μL MTT solution (5 mg/mL in PBS) was added to each well. After 2 h incubation, the culture medium as well as unreacted MTT was removed carefully and 100 μL DMSO was added to each well to dissolve the blue formazan crystals. The optical density was measured by a microplate reader at 490 nm. PBS treated cells were used as control.

Solubility determination of NHM **with 1**

Into a solution of **1** at a known concentration, excess amount of NHM was added. The suspended mixture was sonicated at room temperature for 1 h. The mixture was then centrifuged (4000 rpm, 30 min). The concentration of NHM in the supernatant solution was measured by ^{1}H NMR (400 MHz) spectroscopy by comparing the integral of a known concentration of acetonitrile as internal standard with selected ^{1}H NMR resonances for the NHM.

2. Solubility enhancement evaluation of NHM with 2

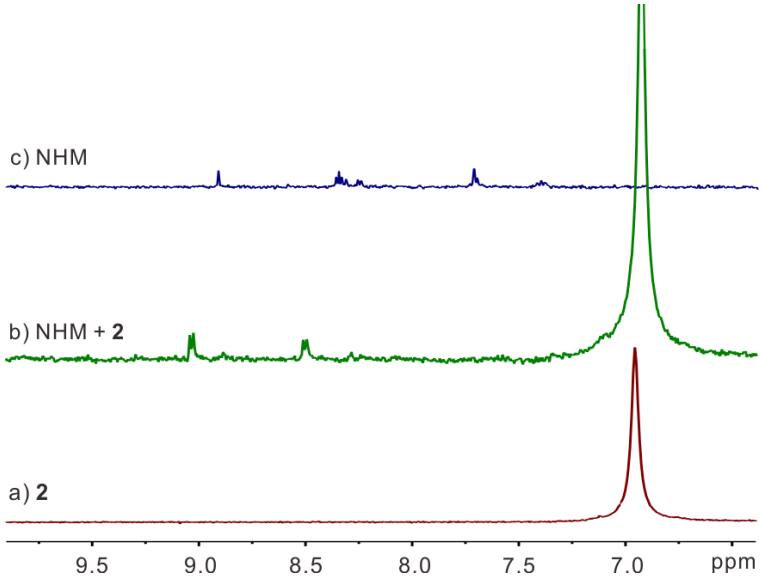

Figure S1. Partial ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of a) **2**, b) suspensions of fixed amount NHM (6.0 mg) after addition of 60 mg **2**, and c) the saturated solution of NHM. As shown in Figure S1b, there is only a set of undefined peaks of NHM (9.0-7.0 ppm), after the addition of 60 mg **2**, indicating no noteworthy improvement for the solubility of **2**, which is consistent with visual observations.

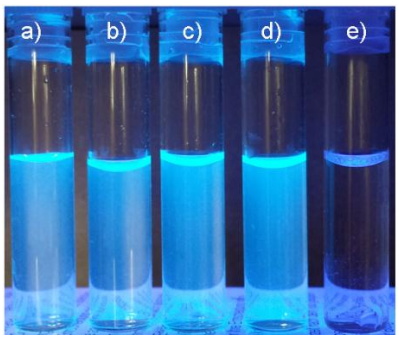

Figure S2. Photos of NHM in water after adding different concentration of **2** (under UV light with 365nm). a) NHM, b) NHM and **2** (1 mg/mL), c) NHM and **2** (5 mg/mL), d) NHM and **2** (10 mg/mL), e) **2** (10 mg/mL). In samples a-d, the concentration of NHM was 0.044mg/mL. According to this fluorescence evaluation, it is confirmed that host **2** was unable to induce the distinguished quench of fluorescence.

3. Detailed phase solubility diagrams for NHM with 1

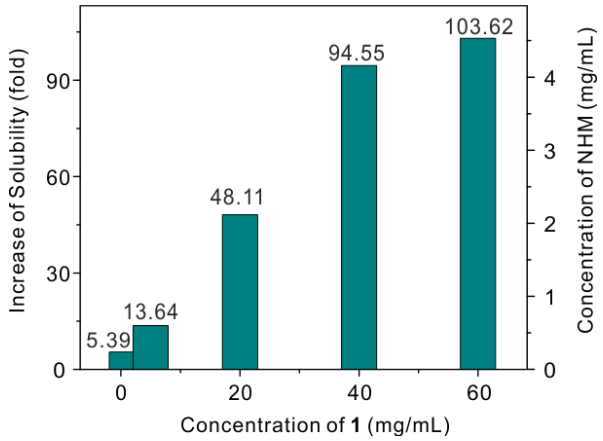

Figure S3. The diagrams of supramolecularly increased aqueous solubilities of NHM, displaying in multiples of intrinsic solubility of free NHM (left axle), and in concentration of weight between NHM and **1** (right axle) and

4. Determination of the fluorescence quenching efficiency upon addition of 1 towards NHM

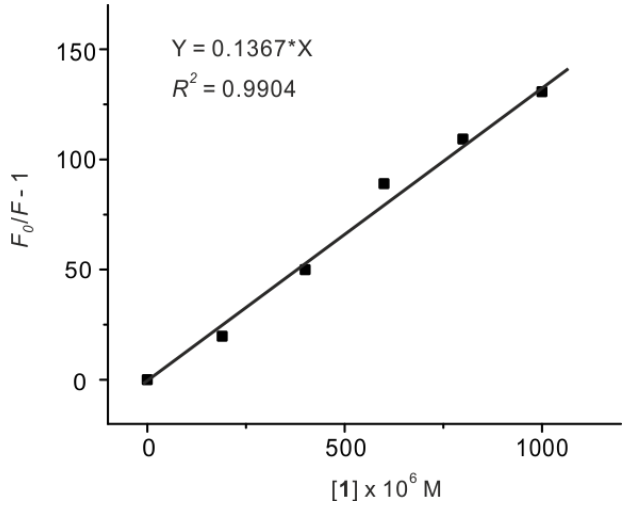

Figure S4. Variation of the relative fluorescence intensity (F_0/F-1) of NHM (262 μM) as a function of [**1**]. The slope gives the Stern-Volmer quenching constant (K_{sv}).

The fluorescence quenching process follows the Stern–Volmer relationship (Eq. 1):

Eq. 1 $$F_0/F = 1 + K_{sv}[\mathbf{1}] = 1 + k_q\tau_0[\mathbf{1}]$$

Here, F_0 and F are the fluorescence intensities in the absence and presence of quencher **1**, respectively. K_{sv} is the Stern-Volmer constant. k_q is the bimolecular quenching constant, and τ_0

is the lifetime of the probe molecule in the absence of the quencher. The quenching process can be usually induced by a collision process (dynamic quenching mechanism) or by formation of a complex between quencher and fluorophore (static quenching mechanism). As shown in Figure S4, the slope K_{sv} obtained was 1.367×10^5 M^{-1} for the experiment performed in aqueous medium (pH 7.0). Taking into account the fluorescence lifetime of each acidic and basic form of NHM (22 ns and 6 ns, respectively),[2] we can estimate the bimolecular quenching constant (k_q) has a value of ~ 10^{12} M^{-1} s^{-1}. These values are two orders larger than the diffusion-limited rate constant (~10^{10} M^{-1} s^{-1}),[3] which clearly exclude the possibility that the fluorescence quenching we observed is owing to a collision process (dynamic quenching mechanism). As a result, the static quenching mechanism dominates in our case, confirming the molecular recognition between NHM and host **1**.

5. Determination of stoichiometry and association constant for complex NHM•1

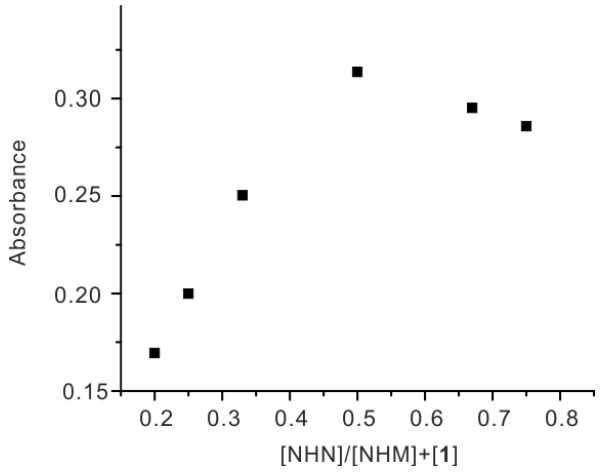

Figure S5. Job plot showing the 1:1 stoichiometry of the complexation between **1** and NHM in water using the UV absorbance at 372 nm. $[\mathbf{1}]_0 + [NHM]_0 = 240$ μM. $[\mathbf{1}]_0$ and $[NHM]_0$ are initial concentrations of **1** and NHM.

Determination of the binding constants for NHM•**1** was carried out using Benesi-Hildebrand equations for 1:1 complexes (Eq.2):

Eq.2

Here, K is the equilibrium constant for the formation of the complex, A_0 is the absorbance intensity of free NHM at wavelength 372 nm, A' is the absorbance intensity of the [NHM•**1**] complexes and $A_{obs.}$ is the observed absorbance intensity. A typical double reciprocal plot is shown in Figure S6. The binding constant K of NHM•**1** was calculated as 3.4×10^4 M^{-1}.

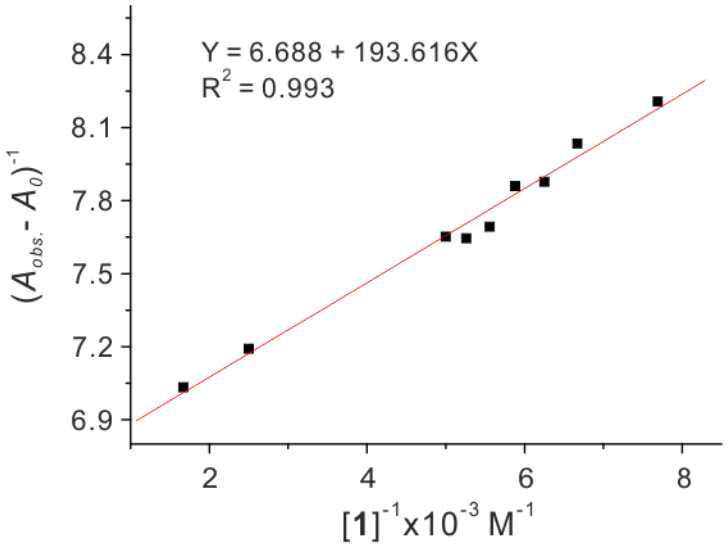

Figure S6. Linear fit for a double reciprocal plot for complexation between NHM and **1** in aqueous solution.

6. Fluorescence emission spectra of the complexation and pH stimuli-release experiments.

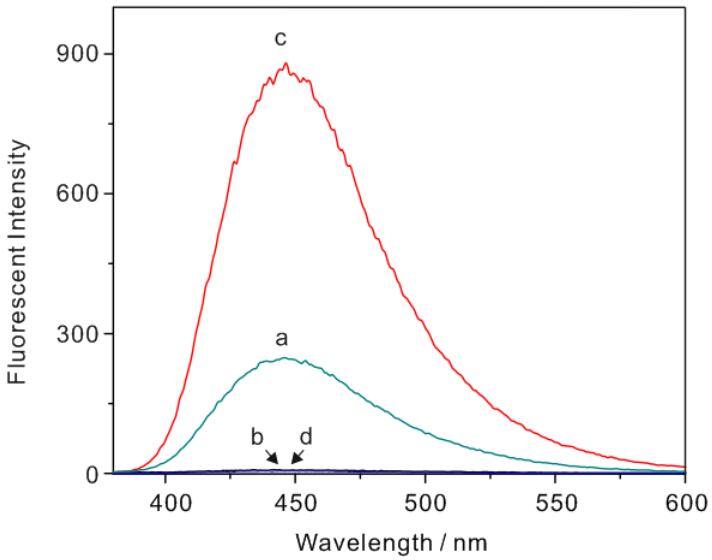

Figure S7. The fluorescence emission spectra of the pH-triggered reversible complexation and decomplexation processes between **1** and NHM (λ_{ex} = 365 nm): a) saturated solution of NHM (4.4×10^{-2} mg/mL); b) addition of excess **1** (54.4×10^{-2} mg/mL) to a; c) adding a drop of concentrated hydrochloric acid to b (the precipitate was removed by centrifugation, pH = 1.5); d) adding a little excess of ammonia to c until the pH = 7.0.

A previous study on the pH-dependent of fluorescence emission has shown that the fluorescent intensity of NHM will be increased approximately three times in comparison to than in neutral medium.[4] Here the fluorescent intensity of curve c) at 450 nm is I_c = 855.7, the value at this wavelength for curve a) is I_a = 243.3, therefore, I_c/I_a = 3.51. Accordingly, we can roughly estimate that over 90% of NHM is dissociated with **1**.

7. MTS results of pillar[5]arene derivatives 1 and 2

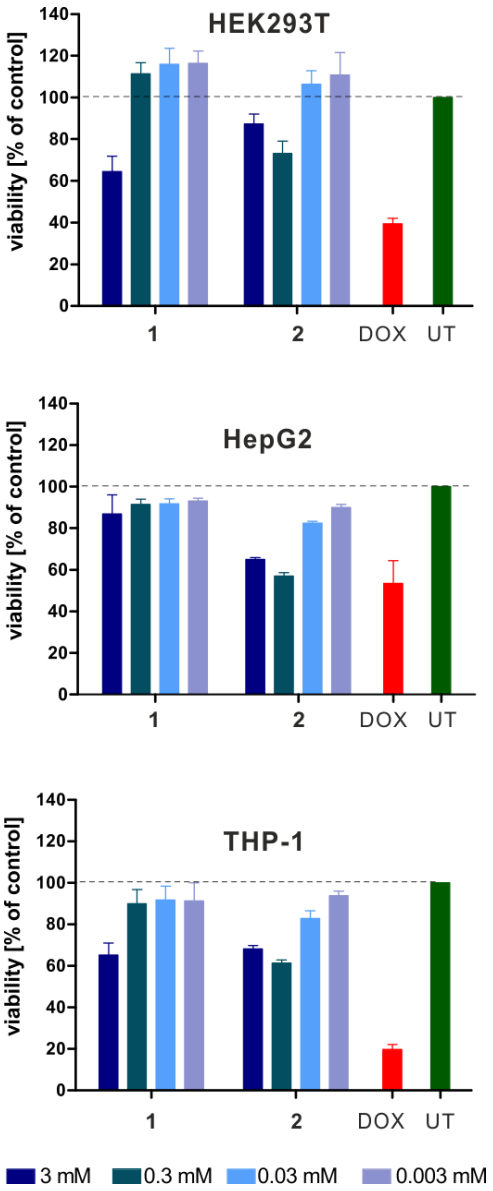

Figure S8. In vitro cell viability (MTS assay) performed with pillararenes **1**-**2** obtained for (0.003 mM, 0.03 mM, 0.3 mM, 3 mM) after 48 h incubation with three cell lines: HEK 293T cells (top), HepG2 cells (middle) and THP-1 cells (bottom). Each bar presents the average value obtained from three independent experiments and the corresponding standard error of the mean. DOX is the anti-tumor drug doxorubicin dissolved in methanol. UT means untreated.

In order to examine the biocompatibility of pillar[5]arene derivatives **1** and **2**, we used the cell viability (MTS) assay to evaluate the toxicity of uncomplexed **1** or **2** in three cell lines: human

kidney cells (HEK 293T), human liver cells (HepG2) and human monocyte cells (THP-1). We conducted a dose escalation experiment (Figure S8). Cells were treated with varying concentrations of **1** or **2** (3, 30, 300 and 3000 μM) for 48 h.

8. Cell morphology observation after adding different dose of NMH with 1

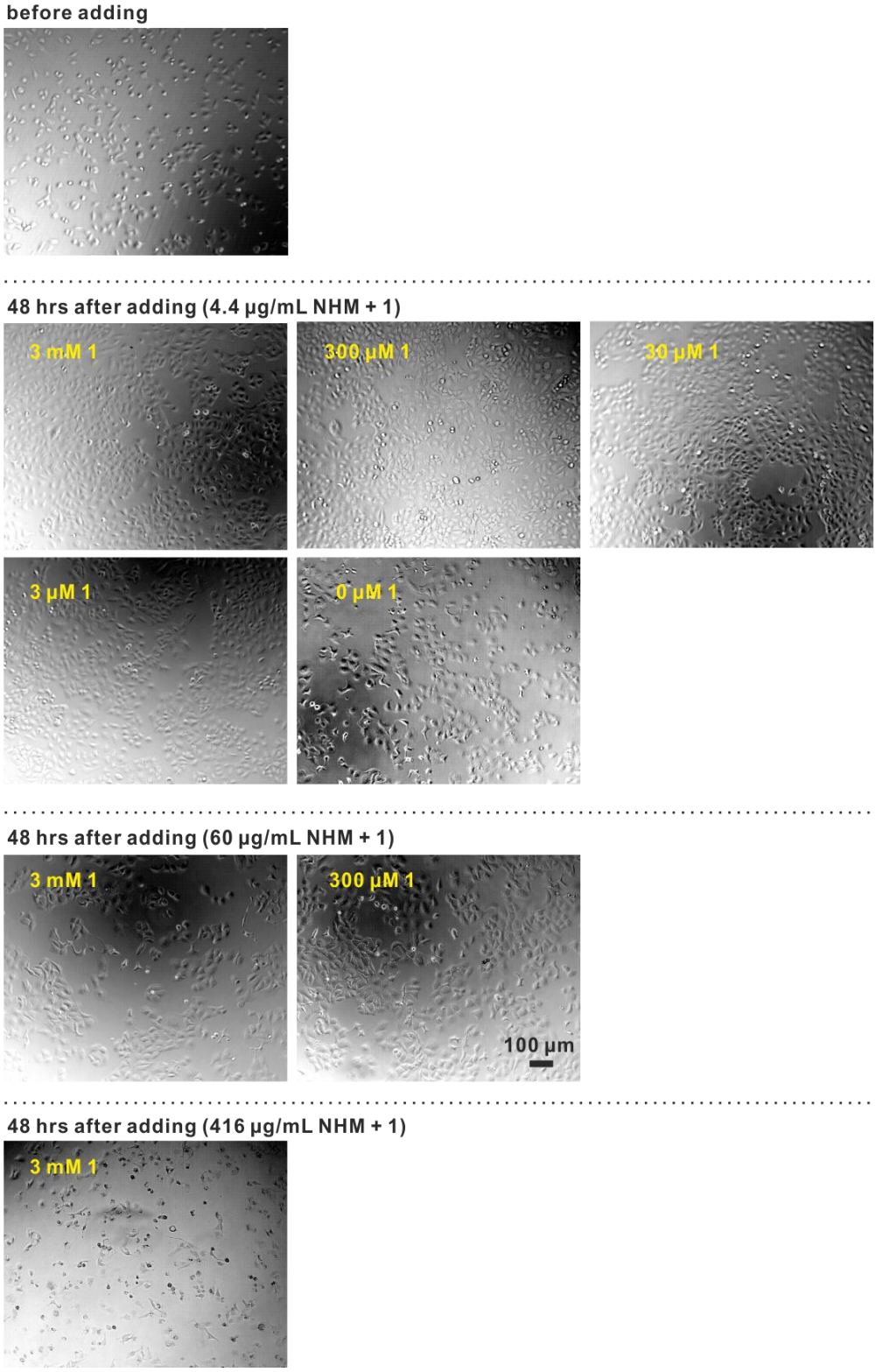

Figure S9 Change in A549 cell morphology after exposure to NHM•**1** complex (the concentration of NHM was increased from 26 μM to 2.488 mM). In comparison with the untreated cells, there is no significantly morphological changed of cells upon the addition of NHM to 60 μg/mL NHM.

9. Reference

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