Chemical Science

Supporting Information

for

Stimuli-Responsive Metal-Organic Frameworks Gated by Pillar[5]arene Supramolecular Switches

Li-Li Tan, Haiwei Li, Yu-Chen Qiu, Dai-Xiong Chen, Xin Wang, Rui-Yi Pan, Yan Wang, Sean Xiao-An Zhang, Bo Wang* and Ying-Wei Yang*

L.-L. Tan, Y.-C. Qiu, D.-X. Chen, X. Wang, R.-Y. Pan, Prof. Dr. Y. Wang, Prof. Dr. S. X.-A. Zhang, Prof. Dr. Y.-W. Yang

State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, International Joint Research Laboratory of Nano-Micro Architecture Chemistry (NMAC), Jilin University, 2699 Qianjin Street, Changchun 130012, P. R. China

E-mail: ywyang@jlu.edu.cn or yyang@chem.ucla.edu

H. Li, Prof. Dr. B. Wang

Key Laboratory of Cluster Science, Ministry of Education of China, School of Chemistry, Beijing Institute of Technology, 5 South Zhongguancun Street, Beijing, 100081, P.R. China

E-mail: bowang@bit.edu.cn

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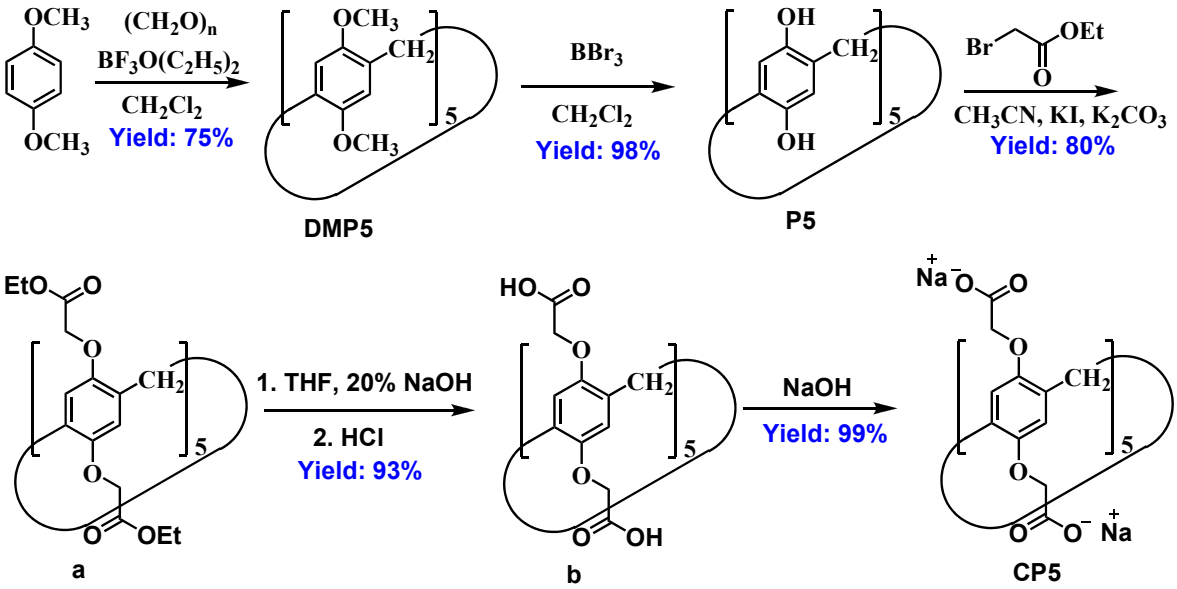
1. Materials and Methods

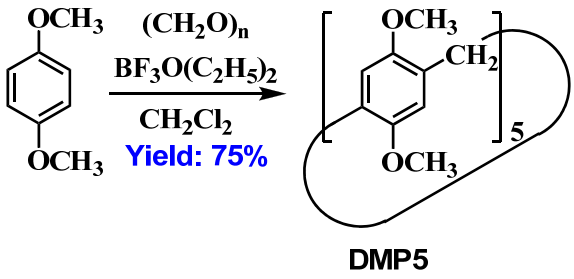
Starting materials and reagents were purchased from Aladdin, and used as received. All reagents were purchased from commercial sources and used without further purification, unless otherwise noted. Deionized water was used in all relevant experiments. A series of phosphate buffer solutions (PBS) were prepared according to the Appendix XV of the Chinese Pharmacopeia (the Second Part, 2010 Edition). PBS (pH = 7.4) was prepared as follows: $Na_2HPO_4 \cdot 12H_2O$ (5.8 g) and $NaH_2PO_4 \cdot 2H_2O$ (0.6 g) were dissolved in deionized H_2O (100 mL). ^1H NMR spectra were recorded at 25 ºC on a Bruker AVANCE III 300 MHz NMR and 500 MHz, TMS was used as internal standard. Chemical shifts were reported in ppm relative to the signals corresponding to the residual non-deuterated solvents, and coupling constants were recorded in Hertz (Hz). Electro-spray ionization mass spectrometry (ESI-MS) was performed using Liquid Chromatography Mass Spectrometry Instrument (Agilent1290-micrOTOF Q II). TGA were carried out on a TGA/Q500 from room temperature to 900 ºC at a heating rate of 10 ºC/min in nitrogen flow. N_2 sorption isotherms at 77 K were measured on a Quantachrome instruments ASiQMVH002-5 after pretreatment by heating the samples. The pore size distribution was estimated by the DFT method from a N_2 sorption experiment at 77 K. Scanning electron microscopy (SEM) were performed on a JEOL JSM6700F. Transmission electron microscopy (TEM) images were collected on a JEM 2100F instrument at an accelerating voltage of 200 kV. Fourier transform infrared (FTIR) spectra were recorded on a Vertex 80 V spectrometer. Ultraviolet-visible (UV-vis) spectra were recorded on a Shimadzu UV-2550 instrument. Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS instrument. Zeta potential measurements were tested on a Zetasizer Nano 9300 instrument.

2. Syntheses and Preparation

2.1 Synthesis of CP5 [S1, S2, S3]

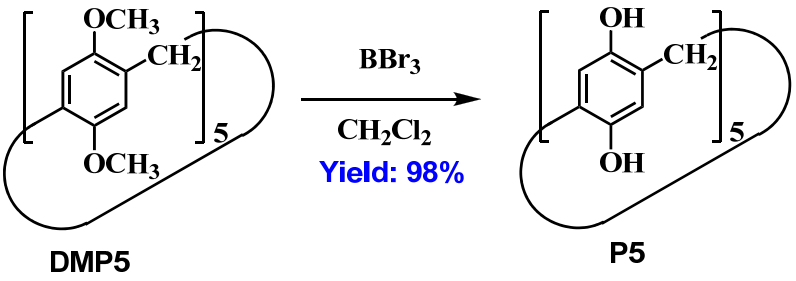


2.1.1 Synthesis of dimethoxypillar[5]arene (DMP5)



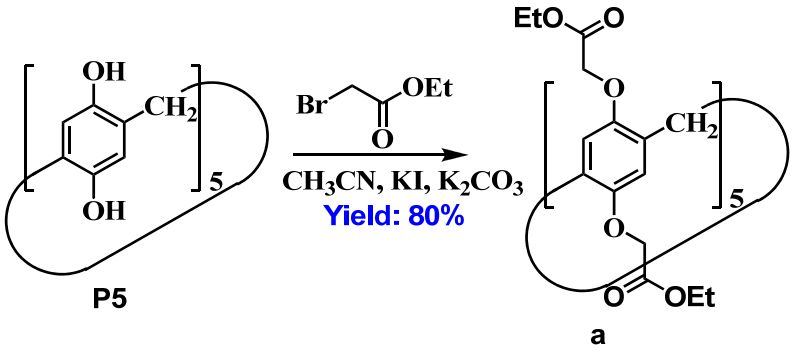
To a solution of 1,4-dimethoxybenzene (0.691 g, 5 mmol) in dichloromethane (60 mL) paraformaldehyde (0.450 g, 15 mmol) was added under nitrogen atmosphere. Then, boron trifluoride diethyl etherate ($BF_3O(C_2H_5)_2$, 0.75 mL, 6 mmol) was added to the solution, and the mixture was stirred at 0 °C for 130min. The mixture obtained was washed with water, sodium bicarbonate solution and again with water, then with brine, and then dried with $MgSO_4$. The residue after rotary evaporation was purified by column chromatography on silica gel using a solvent mixture (petroleum/ethyl acetate = 10:1) as the eluent to obtain the DMP5 as a white solid. Yield: 75%. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 6.76 (s, 10H), 3.77 (s, 10H), 3.64 (s, 30H).

2.1.2 Synthesis of pillar[5]arene (P5)



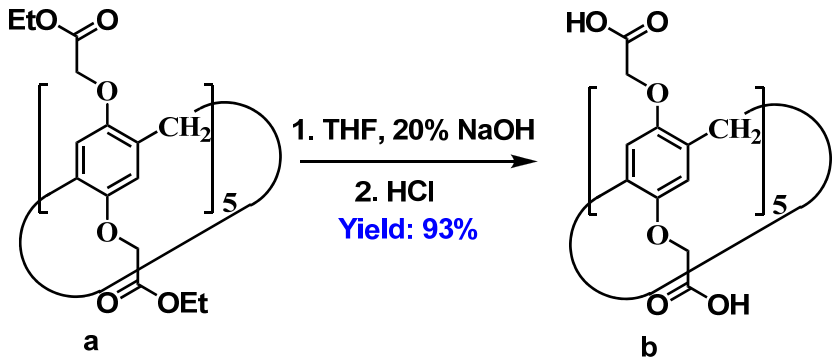
To a solution of DMP5 (0.33 g, 0.44 mmol) in CH_2Cl_2, a solution of boron tribromide (2 mL, 21.50 mmol) in CH_2Cl_2 (1.80 mol/L) was added. The mixture was stirred at room temperature over 72 h. Then water was added to the mixture and stir for 48 h under N_2 atmosphere at room temperature. P5 was obtained by filtration as an oyster white solid. Yield: 98%. ^{1}H NMR (300 MHz, acetone-d_6, 298 K): δ 7.96 (s, 9H), 6.66 (s, 10H), 3.59 (s, 10H).

2.1.3 Synthesis of ethoxycarbonyl-substituted P5 (a)



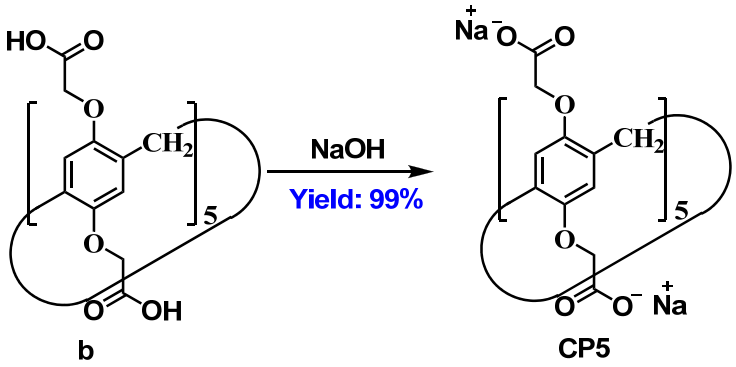
P5 (1.22 g, 2 mmol) was dispersed in acetonitrile (60 mL) and K_2CO_3 (3.5 g) was added. The mixture was stirred for 30 min at room temperature, then a small amount of KI and excess amount of ethyl bromoacetate (5 mL, 45 mmol) were added. The mixture was heated under reflux at a nitrogen atmosphere for 18 h, then filtered and washed with chloroform after cooling down. The filtrate was concentrated, and the residue was subjected to column chromatography (silica gel, dichloromethane: acetone= 100:0 to 30:1). The crude product was crystallized by slow diffusion of n-hexane into a chloroform solution to result in ethoxycarbonyl-substituted P5 (a). Yield: 80%. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K): δ 7.05 (s, 10H), 4.54 (q, J = 15 Hz, 20H), 4.09 (m, J = 6 Hz, 20H). 3.86 (s, 10H), 0.98 (t, J = 6 Hz, 30H).

2.1.4 Synthesis of carboxylic acid substituted P5 (b)



Sodium hydroxyl solution (20%, 30 mL) was added to the solution of compound a (1.47 g, 1 mmol) in of THF (60 mL). The mixture was heated under reflux for 15 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The residue was diluted into 100 mL of water, and then acidified with HCl. The resulting precipitate was collected by filtration, washed with water, and dried under vacuum to give carboxylic acid substituted P5 (b). Yield: 93%. ^{1}H NMR (300 MHz, d_6-DMSO, 298 K): δ 12.93 (s, 10H), 7.11 (s, 10H), 4.69 (d, J = 15 Hz, 10H). 4.41 (d, J = 15 Hz, 10H), 3.74 (s, 10H).

2.1.5 Synthesis of carboxylatopillar[5]arene (CP5)



Compound b (0.24 g) was dispersed in 3 mL of deionized water. Then, sodium hydroxide solution (80 mg sodium hydroxide in 1 mL of deionized water) was added dropwise into the mixture until the reaction mixture is clear. Final product CP5 was obtained by drying under vacuum. Yield: 99%. ^{1}H NMR (500 MHz, D_2O, 298 K): δ 6.72 (s, 10H), 4.46 (d, J = 16 Hz, 10H), 4.21 (d, J = 16 Hz, 10H), 3.86 (s, 10H).

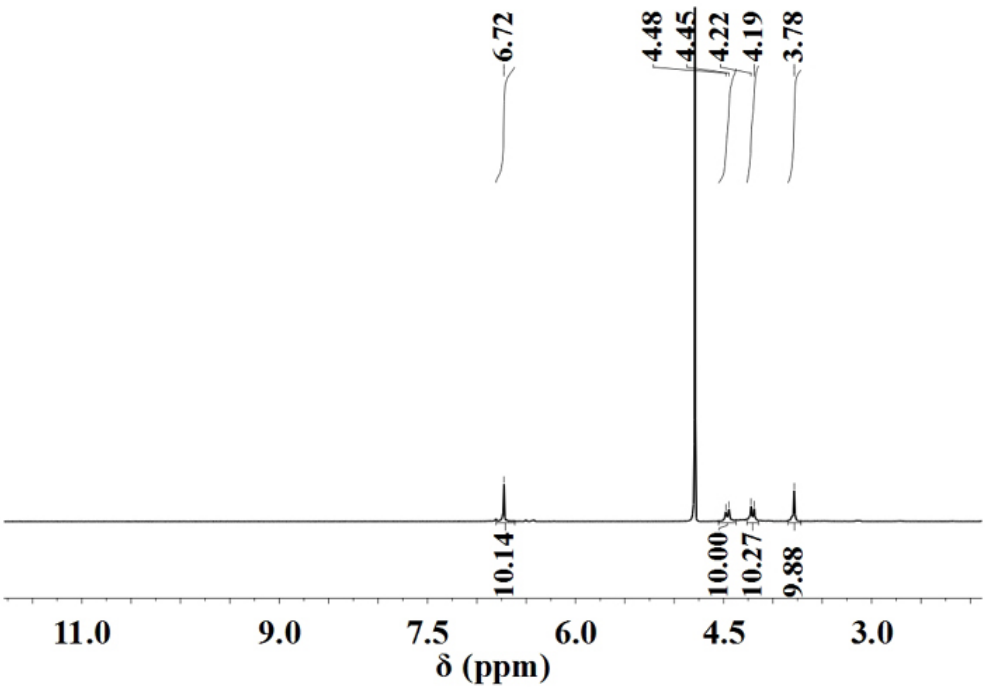
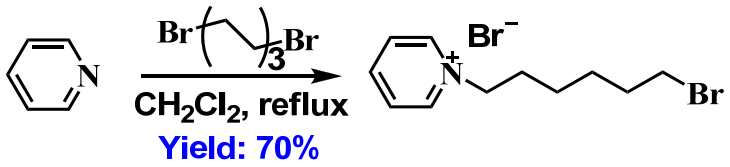


Figure S1. ^{1}H NMR spectrum of CP5 in D_2O.

2.2 Synthesis of 1-(6-bromohexyl)pyridinium bromide (Py)



Pyridine (0.2 mL) was slowly added to the solution of 1,6-dibromohexane (2 mL) in CH_2Cl_2 (30 mL), then the reaction was heated under reflux for 36 hours. Final product was collected by filtration. Yield: 70%. ^{1}H NMR (300 MHz, D_2O, 298 K): δ 8.84 (t, J = 5.6 Hz, 2H), 8.54 (dd, J = 11.2, 4.5 Hz, 1H), 8.07 (t, J = 6.9 Hz, 2H), 4.63 (t, J = 7.3 Hz, 2H), 3.49 (t, J = 6.7 Hz, 2H), 2.08 – 2.01 (m, 2H), 1.83 (dd, J = 14.5, 6.8 Hz, 2H), 1.48 (dd, J = 15.2, 7.5 Hz, 2H), 1.41 – 1.33 (m, 2H).

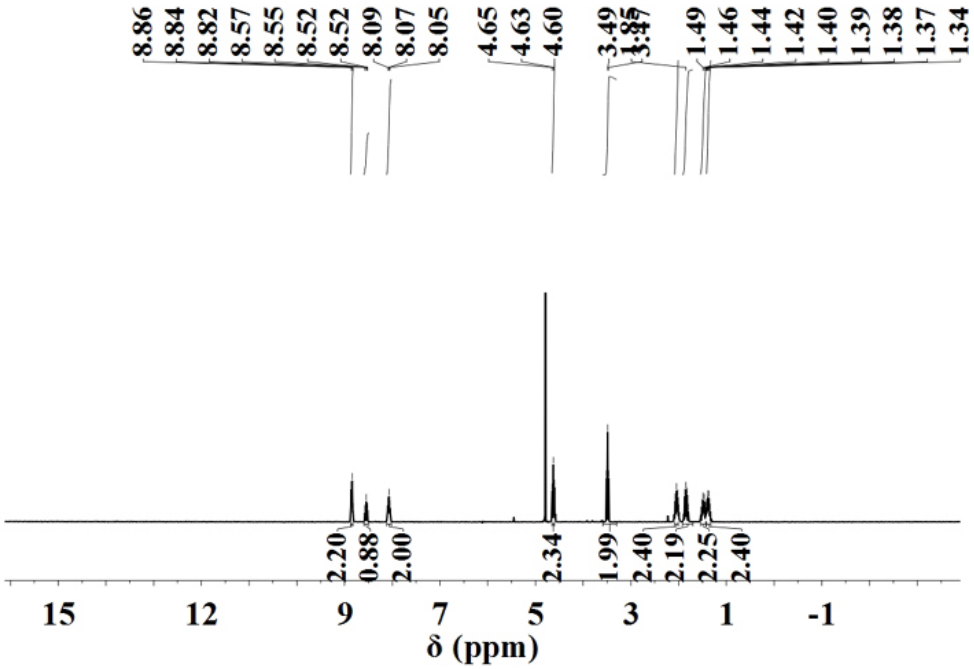
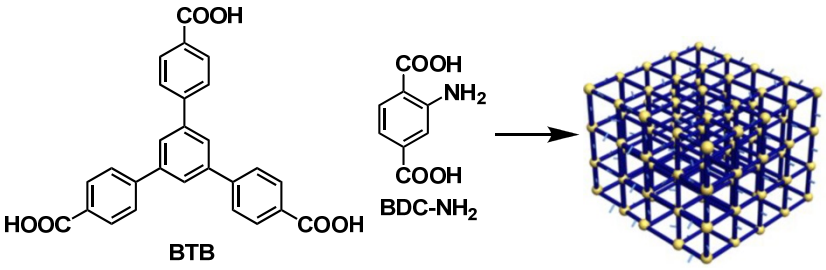


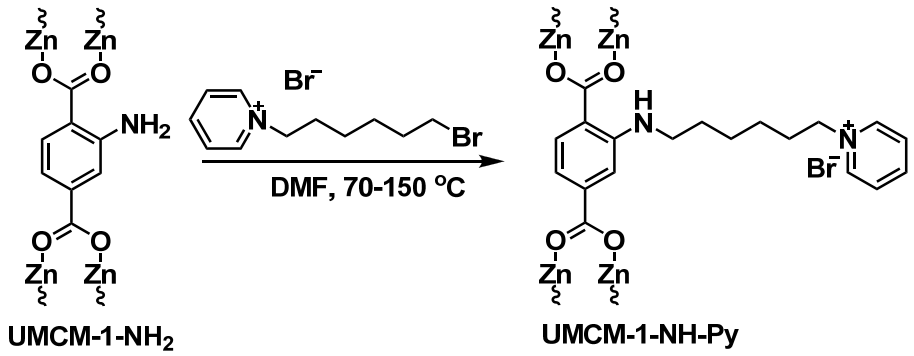
Figure S2. ^{1}H NMR spectrum of Py in D_2O.

2.3 Preparation of UMCM-1-NH_2



$Zn(NO_3)_2 \cdot 6H_2O$ (3.21 g, 10.8 mmol), NH_2-BDC (0.490 g, 2.7 mmol), and 4,4',4″-benzene-1,3,5-triyl-tribenzoic acid (BTB, 0.424 g, 0.97 mmol) were dissolved in DMF (100 mL). The solution was divided into 10 mL portions and transferred to 10 scintillation vials (20 mL capacity each). The vials were placed in an isothermal oven heated at 85 °C. After 48 hours, the vials were removed from the oven and left to cool to room temperature. Beige, crystalline needle clusters were present in every vial. The mother liquor was decanted, and crystals were washed with DMF (3 × 12 mL) and soaked in CH_2Cl_2 for 24 hours. The crystals were then rinsed with CH_2Cl_2 (3 × 10 mL) and left to soak for 3 days with fresh CH_2Cl_2 added every 24 hours.

2.4 Post-synthetic modification of UMCM-1-NH_2



UMCM-1-NH_2 UMCM-1-NH-Py

UMCM-1-NH_2 was dried at 75 °C under vacuum for at least 12 h. The freshly dried UMCM-1-NH_2 56 mg (ca. 0.054 mmol equiv. of $–NH_2$) was suspended in DMF (10 mL). To this suspension 1-(6-bromohexyl)pyridinium bromide (0.0349 g, 0.108 mmol) was added. The solution was stirred at 70-150 °C for 55 hours. The solid was isolated by centrifugation and washed three times with DMF, then washed three times with ethanol. Then the product was dried under vaccum. The crystal were washed with $CHCl_3$ (3 × 6 mL) before soaking in pure $CHCl_3$ (10 mL) for 3 days, with fresh $CHCl_3$ added every 24 hours. UMCM-1-NH-Py was characterized by NMR, TEM, SEM, BET, DLS, FT-IR, TGA and ESI-MS.

3. Cargo Loading and Controlled Release Experiments

3.1 Cargo loading and CP5 capping

The Rh6G-loaded, CP5-capped MOFs: MOFs (10 mg) were suspended in PBS (pH = 7.4) of Rh6G (15 mL, 1 mM) for 12 h at room temperature. An excess amount of CP5 (30 mg) was added to the above mixture. The resulting reaction mixture was stirred for 2 days at room temperature. The Rh6G-loaded, CP5-capped MOFs were washed with deionized H_2O by centrifugation and dried under vacuum.

The doxorubicin hydrochloride (DOX)-loaded, CP5-capped MOFs: MOFs (10 mg) were suspended in PBS (pH = 7.4) of DOX (15 mL, 1 mM) for 12 h at room temperature. An excess amount of CP5 (30 mg) was added to the above mixture. The resulting reaction mixture was stirred for 2 days at room temperature. The DOX-loaded, CP5-capped MOFs were washed with deionized H_2O by centrifugation and dried under vacuum.

The Rh6G-loaded MOFs: MOFs (10 mg) were suspended in PBS (pH = 7.4) of Rh6G (15 mL, 1 mM) for 12 h at room temperature. The Rh6G-loaded MOFs were washed with deionized H_2O by centrifugation and dried under vacuum.

3.2 Controlled release experiments

The cargo-loaded, CP5-capped MOFs (1 mg) were suspended in PBS then putted into dialysis bag, which was immersed into the cuvette that was stirred gently with PBS (3 mL). Activation of the nanovalves was accomplished by changing pH of PBS or adding competitive binding agent. During this period of time, UV-vis absorption spectra of the solution were recorded at predetermined times. The amount of released cargo was quantified by plotting the absorption curve with cargo solutions of different concentrations as a function of time. Control experiments were carried out with the Rh6G-loaded MOFs (1 mg). These cargo-loaded MOFs were suspended in solutions and then stirred for 3 days to result in complete release.

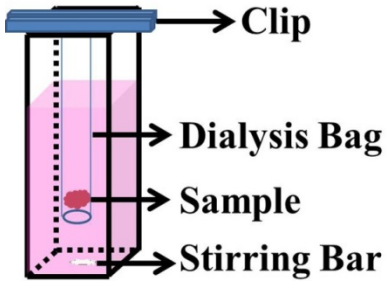


Figure S3. Spectroscopic setup for controlled release experiments.

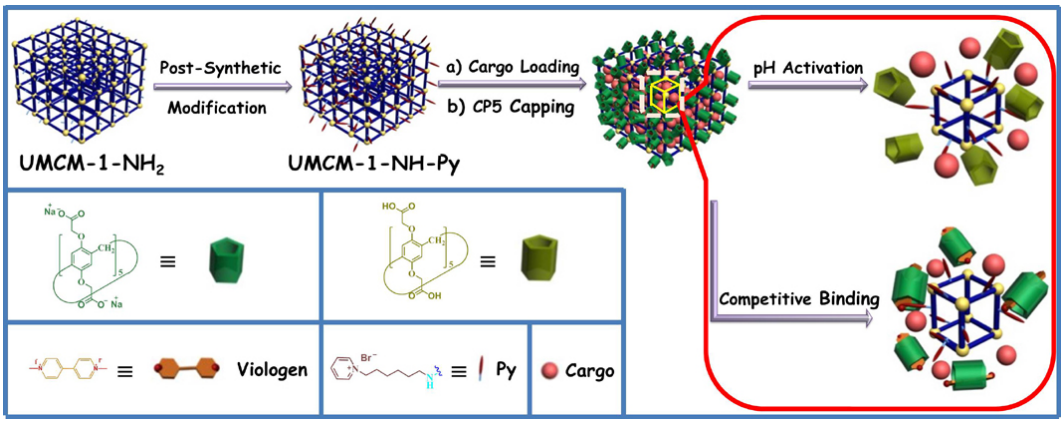


Figure S4. Schematic representation of stimuli-responsive valves based on mechanized nanoMOFs (UMCM-1-NH_2) with positive charged pyridinium units (Py) stalks encircled by pillarenes on the surfaces. The mechanized UMCM-1-NH_2 nanovalves can be operated either by pH changes or by competitive binding to regulate the release of cargo molecules, i.e., rhodamine 6G (Rh6G) and doxorubicin hydrochloride (DOX).

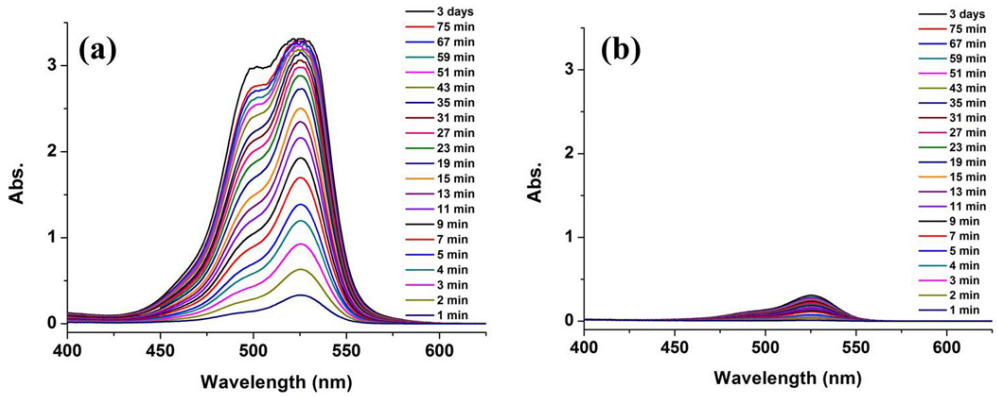


Figure S5. The UV absorbance of Rh6G solution after the complete release in PBS (pH = 2): (a) the Rh6G-loaded, CP5-capped UMCM-1-NH-Py; (b) the Rh6G-loaded UMCM-1-NH_2.

Encapsulation efficiency. For the calculation of encapsulation efficiency, Rh6G release was triggered by changing pH (Figure S5). MOFs (1 mg, the Rh6G-loaded, CP5-capped UMCM-1-NH-Py or the Rh6G-loaded UMCM-1-NH_2) were putted into dialysis bag, which was immersed into the cuvette that was stirred gently with PBS (3 mL, pH = 2). During this period of time, UV-vis absorption spectra of the solution were recorded at predetermined times. According to Lambert-Beer Law, it can be calculated that for 1 g of the Rh6G-loaded, CP5-capped UMCM-1-NH-Py / the Rh6G-loaded UMCM-1-NH_2, 61 μmol / 5 μmol of Rh6G molecules can be released. The results showed that the encapsulation efficiency of MOFs without attaching the CP5 molecule was significantly lower than that with attaching the CP5 molecule. This reveals the important role of CP5 supramolecular switches in our system.

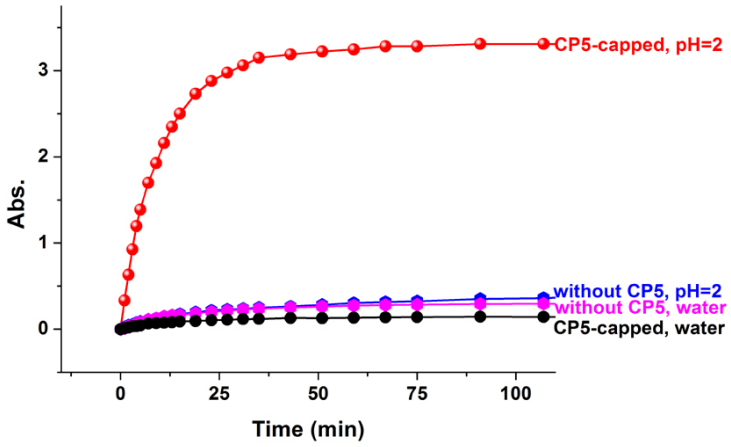


Figure S6. The release profiles of Rh6G-loaded, CP5-capped UMCM-1-NH-Py and Rh6G-loaded UMCM-1-NH_2 without CP5 capped caused by changing pH.

From Figure S6 we could reach the following conclusions: (1) premature leakage without attaching the CP5 molecules was more obvious than with attaching the CP5 molecules. It indicated the important role of CP5 supramolecular switches in our system which effectively prevented premature leakage of cargo. (2) The encapsulation efficiency of MOFs without attaching the CP5 molecules was significantly lower than with attaching the CP5 molecules. This is because, without CP5 supramolecular switches on the surface of MOFs, not only Rh6G dyes from physisorption but also Rh6G dyes partially from pore interiors of materials can be washed away in the progress of cleaning and centrifuge.

4. Material Characterization

4.1 ^{1}H NMR spectra

^{1}H NMR spectra were recorded at 25 ºC on a Bruker AVANCE III 300 MHz NMR. During measurement, approximately 5 mg of MOF (UMCM-1-NH-Py or UMCM-1-NH_2) was dried under vacuum at 100 °C overnight and digested with sonication in DMSO-d_6 (500 μL) and D_2SO_4 (50 μL). The conversion of the modification of UMCM-1-NH_2 was calculated to be 50%.

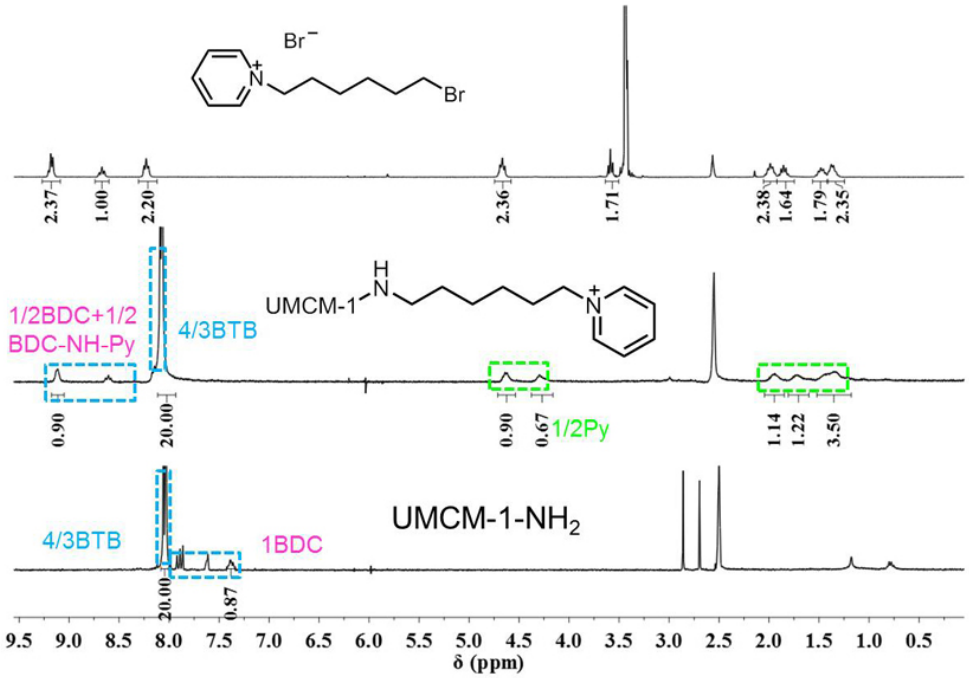


Figure S7. ^{1}H NMR spectra of Py, UMCM-1-NH-Py and UMCM-1-NH_2.

4.2 Electrospray ionization mass spectrometry (ESI-MS)

ESI-MS was performed using Liquid Chromatography Mass Spectrometry Instrument (Agilent1290-micrOTOF Q II). For measuring ESI-MS, UMCM-1-NH-Py (1 mg) were digested in DMSO (0.1 mL) and concentrated H_2SO_4 (0.01 mL) with sonication, then K_2CO_3 was added to the solution to remove the excess H_2SO_4, and methanol (0.5 mL) was added to dilute the solution. The peak of BDC-NH-Py indicated that the generation of the UMCM-1-NH-Py as shown in Figure S8.

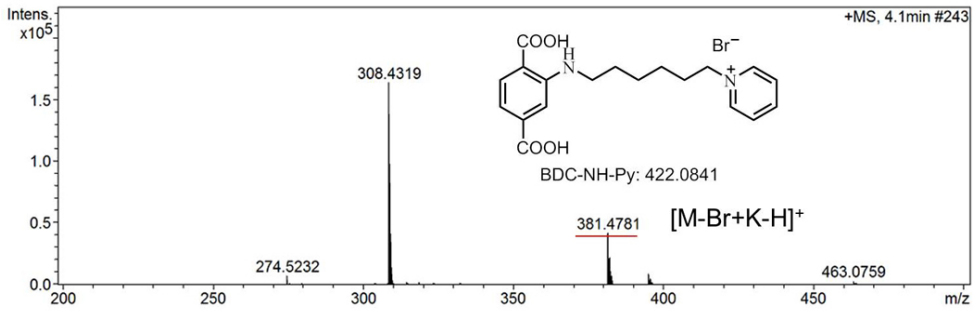


Figure S8. ESI-HRMS spectrum of BDC-NH-Py.

4.3 Fourier transform infrared (FTIR) spectra

FTIR spectra can be used to verify and monitor the functionalization of UMCM-1-NH_2. Compared to UMCM-1-NH_2, the presence of the-CH_2- (~2859 and ~2928 cm^{-1}), -NH- (~1283 cm^{-1}) and -C=N- peaks (~1708 cm^{-1}) indicates the Py was successfully attached to UMCM-1-NH_2. After loading Rh6G and capping CP5, the shift and the intensity changes of peaks appeared around 2900 cm^{-1} and peaks from 1700 cm^{-1} to 1000 cm^{-1} (Figure S9c) have taken place.

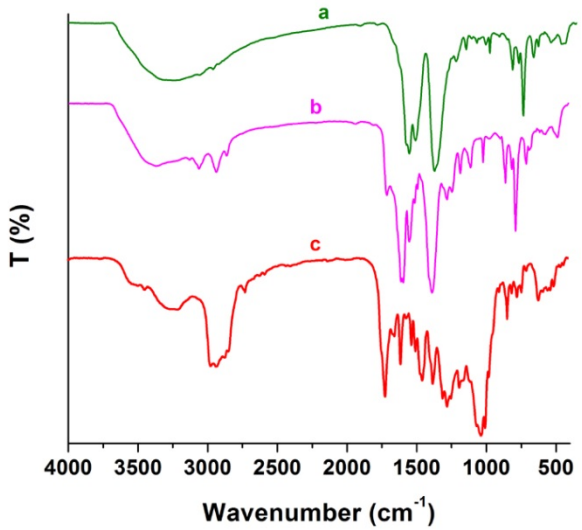


Figure S9. FTIR spectra of (a) UMCM-1-NH_2, (b) UMCM-1-NH-Py, (c) the Rh6G-loaded, CP5-capped UMCM-1-NH-Py.

4.4 Thermogravimetric analysis

Thermogravimetric analysis (TGA) of UMCM-1-NH_2 showed rapid weight loss (15%) first (Figure S10) which corresponds to the liberation of DMF molecules entrapped inside the cavity,

followed by a plateau region until 430 ºC, where the material began decomposing. Since UMCM-1-NH_2 is thermally stable, the lost weight of UMCM-1-NH-Py in TGA curves (Figure S10) at 325 ºC was ascribed to the loss of Py.

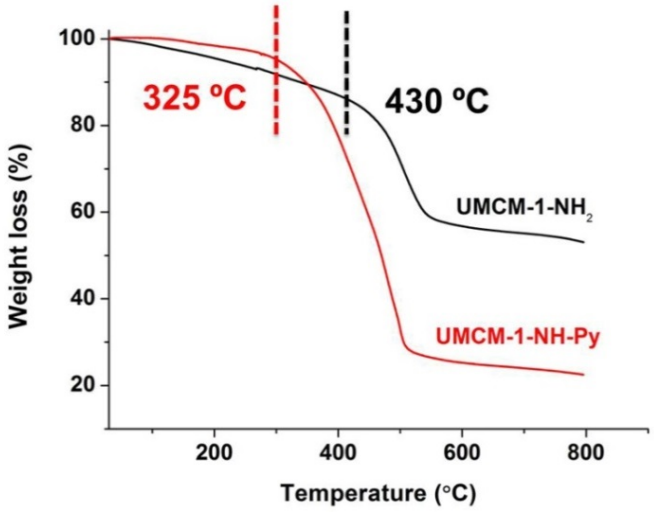


Figure S10. Thermogravimetric analysis of UMCM-1-NH-Py and UMCM-1-NH_2

4.5 Pore size distribution

Pore size distribution calculated by NLDFT method from N_2 sorption isotherms for activated MOFs at 77 K. UMCM-1-NH-Py also shows two main sharp peaks at approximately 1.7 nm, 4.6 nm, which is consistent with UMCM-1-NH_2.

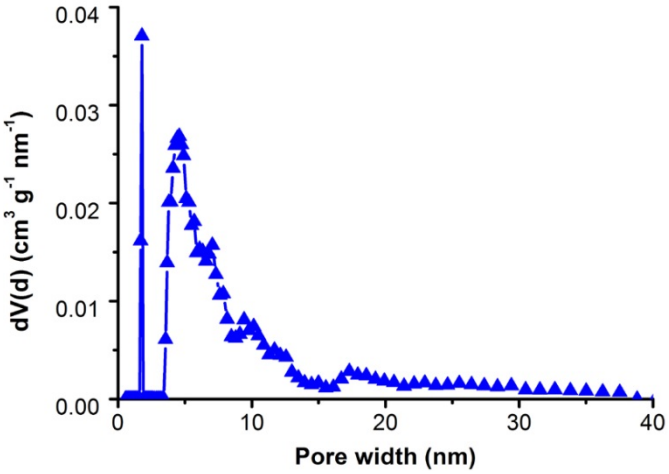


Figure S11. Pore size distribution calculated by NLDFT method from N_2 sorption isotherms for activated UMCM-1-NH_2 at 77 K. It shows two main sharp peaks at ca. 1.7 nm and 4.6 nm.

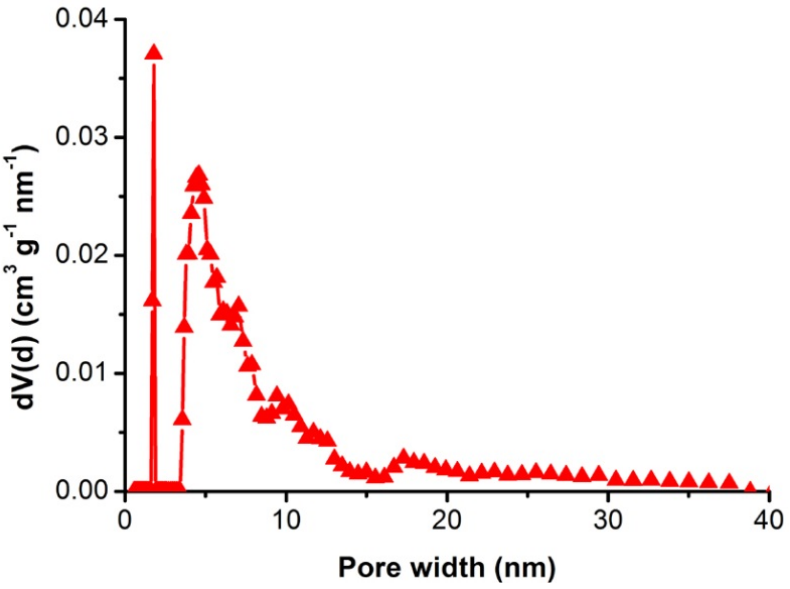


Figure S12. Pore size distribution calculated by NLDFT method from N_2 sorption isotherms for activated UMCM-1-NH-Py at 77 K. It shows two main sharp peaks at ca. 1.7 nm and 4.6 nm.

4.6 Zeta potentials and particle diameter determination in water

Suspensions of each material in ultrapure H_2O were prepared and tested three times. The sample concentration was 0.1 mg/mL and the experiments were done at 25 °C. Zeta potentials of MOFs were displayed in Table S1. According to the analysis of the testing result, UMCM-1-NH-Py has positive surface charges, Rh6G-loaded, CP5-capped UMCM-1-NH-Py has negative surface charges, which indicated the successful modification and capping. The zeta potential of Rh6G-loaded, CP5-capped UMCM-1-NH-Py was measured to be ca. -16.4 mV which also indicates that the newly synthesized drug delivery system can maintain certain stability and is strong enough to transport drugs successfully in biological media.

Dynamic light scattering (DLS) was used to calculate the average diameter of the MOFs (0.1 mg mL^{-1}) in water. It has been found (Table S1) that the average particle size in solution was getting smaller after modification and capping due to the good solubility of Py and CP5 in water. The average particle diameter of Rh6G-loaded, CP5-capped UMCM-1-NH-Py was calculated to be 102.9 nm, which was within the size range can be easily taken up by cells.

Table S1. Zeta potential and average particle diameter obtained by DLS.

	Zeta potential (mV)		Particle diameter (nm)	
UMCM-1-NH_2	-20.9 -23 -22.2	-22.0 ±0.9	597.9 560.1 603.9	587.3 ±23
UMCM-1-NH-Py	24.9 25.2 31.3	27.1 ±3.5	307.3 339 350.1	332.1 ±22
Rh6G-loaded, CP5-capped UMCM-1-NH-Py	-16.4 -14.5 -18.4	-16.4 ±1.9	101.4 99.9 107.5	102.9 ±4

4.7 Scanning electron microscopy (SEM) image and transmission electron microscopy (TEM) image

Electron-diffraction pattern revealed the single-crystalline nature of UMCM-1-NH-Py.

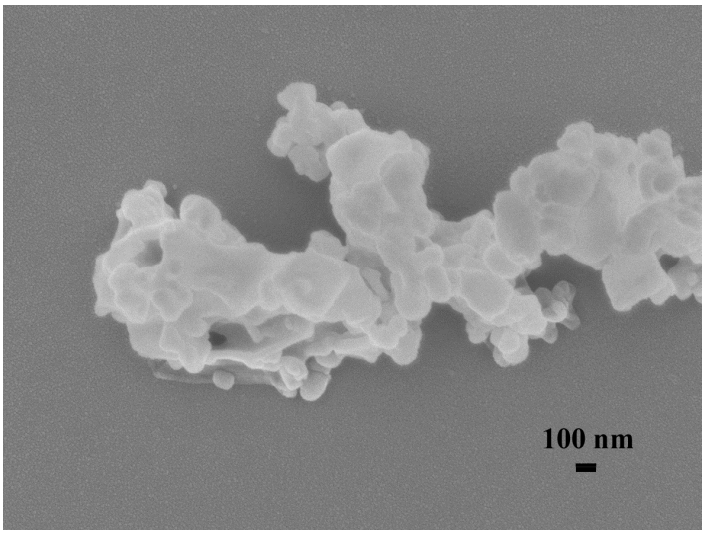


Figure S13. SEM image of UMCM-1-NH-Py.

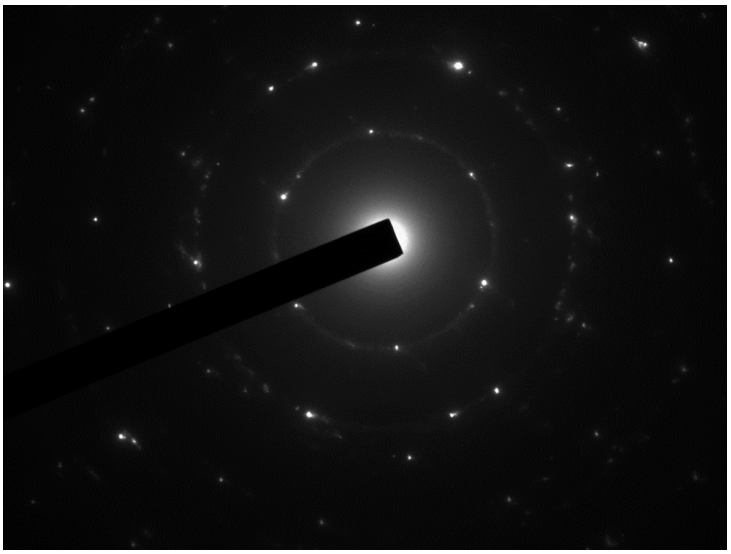


Figure S14. Electron diffraction patterns of UMCM-1-NH-Py.

4.8 Crystal structure analysis [S4]

UMCM-1-NH_2 (UMCM = University of Michigan Crystal-line Material) is a MOF 'co-polymer' constructed by combining both a two-fold symmetric (2-amino-1,4-benzenedicarboxylate, BDC-NH_2) and three fold symmetric (1,3,5-benzene-tri-p-benzoate, BTB) organic linker, this produces a hexagonal mesopore with a 1D hexagonal channel of 27 × 32 Å surrounded by six smaller polygons micropore with a dimension of 14 × 17 Å.

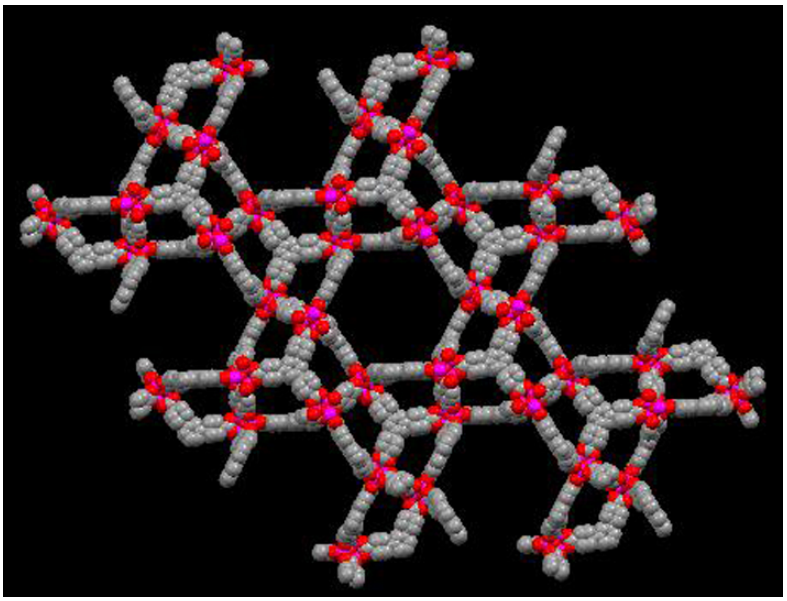


Figure S15. X-ray single crystal structure of UMCM-1-NH_2.

4.9 *In vitro* cytotoxicity study

The cytotoxicities of UMCM-1-NH-Py and CP5-capped UMCM-1-NH-Py in vitro were evaluated by the standard MTT assay. Normal human embryonic kidney (HEK) 293 cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 units per mL), and streptomycin (100 mg mL^{-1}) at 37 °C in tissue culture flasks in a moist atmosphere (5% CO_2 / 95% O_2). After three generations, they were sowed into 96-well plates with 4000 cells per well and incubated for 24 h under the same conditions as before. For toxicity studies, the materials before and after assembly were added into 96-well plates at different gradient concentrations, *i.e.*, 3.125, 6.25, 12.5, 25, 50 μg mL^{-1}, and cells were allowed to grow for 44 h. Then MTT solution (20 μL, 5 mg mL^{-1}) was added to each well and cells were incubated for 4 h. The resulting formazone crystals were solubilized by adding DMSO (150 μL) to each well. The extent of cell survival was determined at 490 nm using an automated microplate reader. The cell viability was calculated by using the following formula: viability (%) = (mean absorbance value of treatment group/mean absorbance value of control) × 100.

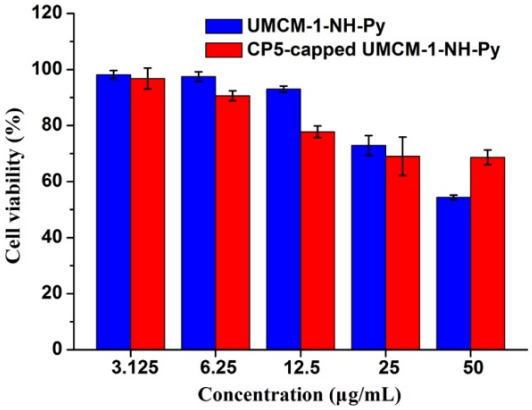


Figure S16. MTT cytotoxicity assay of 293 cells treated with UMCM-1-NH-Py and CP5-capped UMCM-1-NH-Py at various concentrations.

5 References

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S3. D.-X. Chen, Y.-L. Sun, Y. Zhang, J.-Y. Cui, F.-Z. Shen and Y.-W. Yang, *RSC Adv.* 2013, **3**, 5765-5768.

S4. Z. Wang, K. K. Tanabe and S. M. Cohen, *Inorg. Chem.* 2009, **48**, 296-306.