Electronic Supplementary Information

A guanidiniocarbonyl-pyrrole functionalized cucurbit[7]uril derivative as a cytomembrane disruptor for synergistic antibacterial therapy

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1. General information and experimental procedures

1) Materials

The commercially available reagents and solvents were either employed as purchased or dried according to procedures described in the literature. All reactions were performed under N_2 atmosphere unless otherwise stated. Analytical thin layer chromatography (TLC) was performed using 0.25 mm silica gel plates. Column chromatography was performed with silica gel (200-300 mesh) produced by Titan Scientific Co. (Shanghai, China), Ltd. Live/Dead bacterial staining kit with DMAO & PI and BCA protein assay kit was obtained from Beyotime Biotechnology (Shanghai, China). Phosphate buffered saline (PBS), yeast extract, agar, and tryptic soy broth (TSB) were purchased from Biosharp Life Sciences (Hefei, Anhui, China).

2) Bacterial strains

Escherichia coli (*E. coli,* ATCC 8739) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538) involved in this study were obtained from the American Type Culture Collection (ATCC).

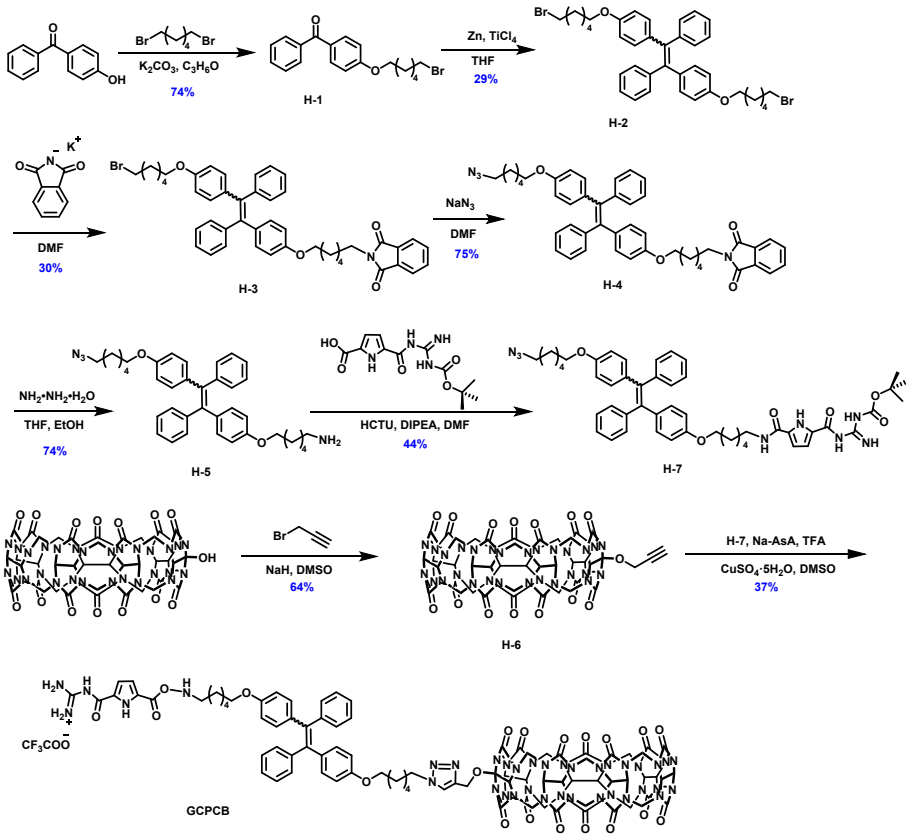
3) Measurements

NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer with internal standard tetramethylsilane (TMS) and solvent signals as internal references at room temperature, and the chemical shifts (δ) were expressed in ppm and *J* values were given in Hz. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on an Agilent 6540Q-TOF LCMS equipped with an electrospray ionization (ESI) probe operating in the positive-ion mode with direct infusion. High-resolution MALDI-TOF was performed using a Bruker Ultraflextreme MALDI TOF-TOF. Transmission electron microscope (TEM) investigations were performed on a Talos F200X G2 instrument. Dynamic light scattering (DLS) measurements were carried out on a Malvern Nano ZS90 system (Malvern Instruments Ltd., UK). Zeta-potential measurements were performed at 25 °C on a Zetasizer Nano Z apparatus (Malvern Instruments Ltd., UK) using the Smoluchowski model to calculate the Zeta-potential from the measured electrophoretic mobility. UV-vis spectra were recorded on a Shimadzu UV-1700 spectrophotometer. The excitation and emission spectra were recorded on a Hitachi F-7000 Fluorescence Spectrometer. The confocal laser scanning microscopy (CLSM) investigations were carried out on an Olympus FV1000 instrument. Scanning electron microscopy (SEM) images were captured with an SU8010 instrument.

2. Synthesis of host GCPCB, guest BH, model host molecule CB[7], and control molecules H-9 and H-10

1) Synthesis of host GCPCB

GCPCB was designed and synthesized according to the following procedures (Scheme S1).



Scheme S1. Synthesis route of host **GCPCB**.

Synthesis of compound H-1

Under N_2 atmosphere, the hydroxybenzophenone (2.45 g, 12.4 mmol) and K_2CO_3 (4.25 g, 31 mmol) were dissolved in acetone (30 mL) and stirred for 1 h. Then, the solution of 1,6-dibromohexane (5 mL, 31 mmol) in acetone (10 mL) was added dropwise into the above mixture. The mixture was refluxed overnight. After the reaction mixture was cooled to room temperature, the inorganic salt was filtered and washed with acetone three times. The solvent was then removed under reduced pressure and the residue was extracted with chloroform (3 × 60 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography (petroleum ether/ethyl acetate = 20:1, *v/v*) to afford compound **H-1** as a white solid (3.36 g, 9.3 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm) = 7.82 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.47 (t, *J* = 8.0 Hz, 2H), 6.95 (d, *J* = 12.0 Hz, 2H), 4.04 (t, *J* = 8.0 Hz, 2H), 3.43 (t, *J* = 8.0 Hz, 2H), 1.87 (dt, *J* = 28.0, 4.0 Hz, 4H), 1.53 (t, *J* =4.0 Hz, 4H).

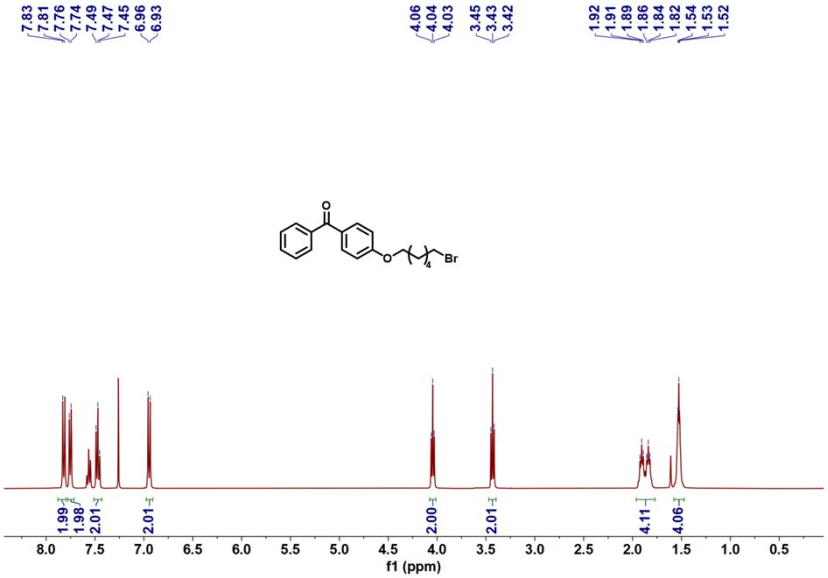


Fig. S1 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **H-1**.

Synthesis of compound H-2

Compound **H-1** (361 mg, 1 mmol) and zinc dust (327 mg, 5 mmol) were placed in THF (50 mL). Under N_2 atmosphere, the mixture was cooled to −20 °C and $TiCl_4$ (0.22 mL, 2 mmol) was added slowly. The mixture was refluxed overnight, and then quenched with saturated aqueous $NaHCO_3$ solution before being filtered. The filtrate was extracted with dichloromethane. The organic layer was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (petroleum ether/dichloromethane = 1:1, *v/v*) to afford compound **H-2** as a yellow oil (200 mg, 0.29 mmol, 29%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm) = 7.13 – 7.04 (m, 10H), 6.96 – 6.90 (m, 4H), 6.67 – 6.61 (m, 4H), 3.92 – 3.87 (m, 4H), 3.43 (td, *J* = 4.0, 4.0 Hz, 4H), 1.92 – 1.88 (m, 4H), 1.80 – 1.75 (m, 4H), 1.52 – 1.48 (m, 8H).

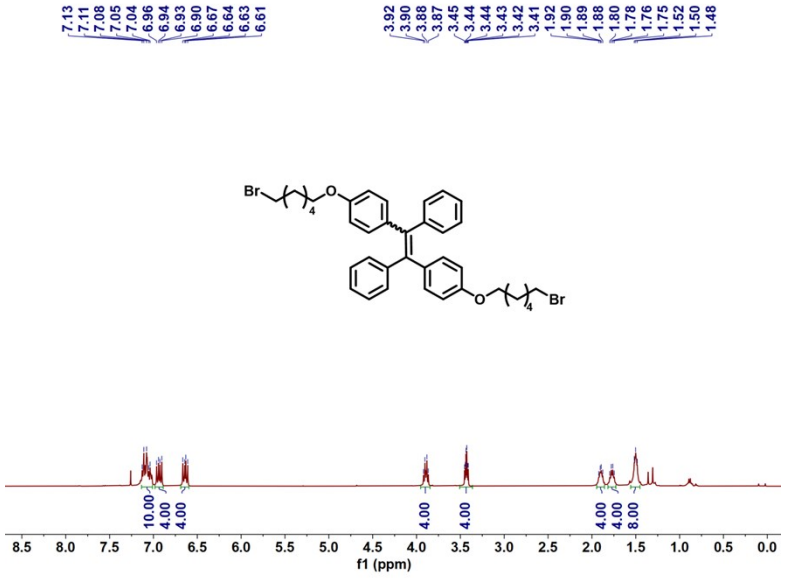


Fig. S2 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **H-2**.

Synthesis of compound H-3

Compound **H-2** (200 mg, 0.29 mmol) and potassium phthalimide (8.89 mg, 0.048 mmol) were dissolved in DMF (10 mL) and stirred. Under N_2 atmosphere, the mixture was stirred at 40 °C for 12 h. After cooling the reaction mixture to room temperature, it was extracted with dichloromethane. The organic layer was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 10:1, *v/v*) to afford compound **H-3** as a yellow solid (65.6 mg, 0.087 mmol, 30%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm) = 7.84 (dd, *J* = 4.0, 4.0 Hz, 2H), 7.71 (dd, *J* = 4.0, 4.0 Hz, 2H), 7.11 – 7.01 (m, 10H), 6.94 – 6.87 (m, 4H), 6.65 – 6.57 (m, 4H), 3.89 – 3.85 (m, 4H), 3.69 (td, *J* = 4.0, 4.0 Hz, 2H), 3.42 (td, *J* = 8.0, 4.0 Hz, 2H), 1.88 – 1.87 (m, 2H), 1.76 – 1.69 (m, 6H), 1.49 – 1.40 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm) = 168.50, 157.47, 144.35, 139.64, 136.35, 133.91, 132.54, 132.19, 131.45, 127.55, 126.16, 123.22, 113.52, 67.53, 37.97, 33.88, 33.86, 32.71, 29.18, 28.57, 27.98, 26.68, 25.75, 25.35. HR-ESI-MS: m/z $[M + Na]^+$ calcd for $[C_{46}H_{46}BrNO_4Na]^+$ 778.2508, found 778.2514.

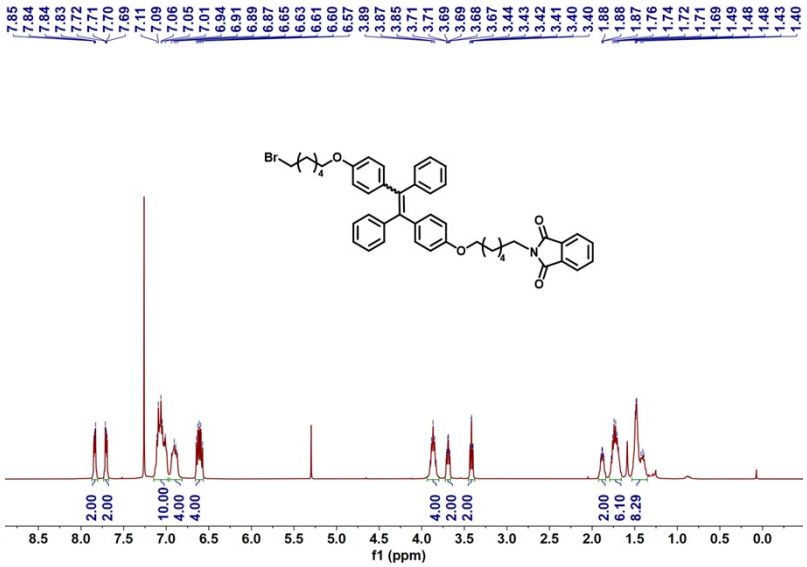


Fig. S3 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **H-3**.

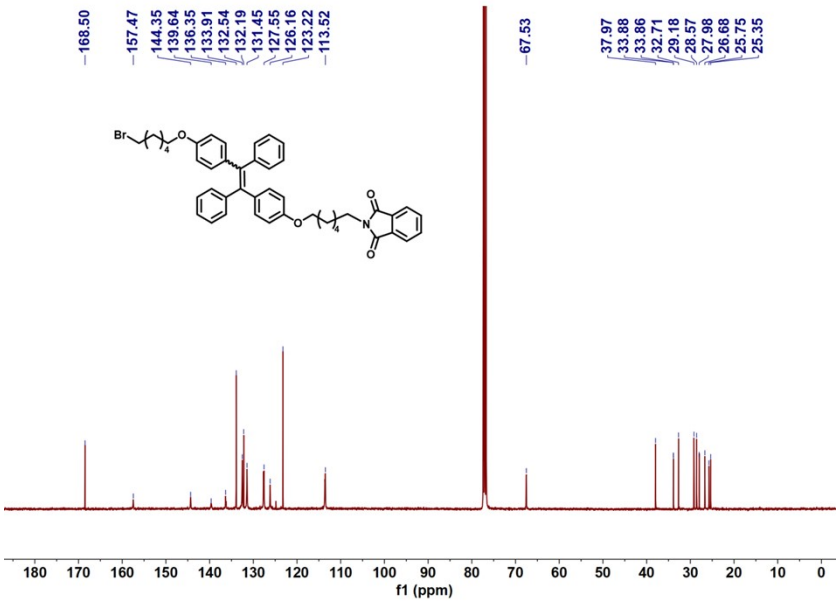


Fig. S4 ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of compound **H-3**.

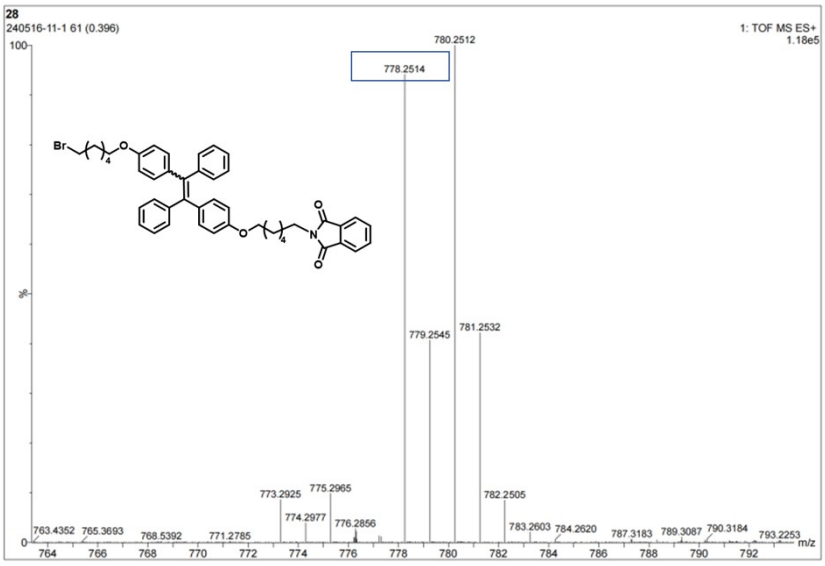


Fig. S5 HR-ESI-MS spectrum of compound **H-3**.

Synthesis of compound H-4

Compound **H-3** (86 mg, 0.114 mmol) and NaN_3 (8.1 mg, 0.125 mmol) were dissolved in DMF (2 mL) and stirred. Under N_2 atmosphere, the mixture was stirred at 60 °C for 12 h. After cooling the mixture to room temperature, it was extracted with dichloromethane. The organic layer was then washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford compound **H-4** as a yellow oil (61.8 mg, 0.086 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm) = 7.84 (dd, *J* = 4.0, 4.0 Hz, 2H), 7.71 (dd, *J* = 4.0, 4.0 Hz, 2H), 7.11 – 7.01 (m, 10H), 6.92 – 6.87 (m, 4H), 6.63 – 6.59 (m, 4H), 3.88 – 3.85 (m, 4H), 3.69 (td, *J* = 4.0, 12.0 Hz, 2H), 3.27 (td, *J* = 4.0, 12.0 Hz, 2H), 1.76 – 1.71 (m, 6H), 1.48 – 1.46 (m, 2H), 1.45 – 1.42 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm) = 168.44, 157.48, 144.36, 139.70, 136.33, 133.86, 132.49, 132.19, 131.42, 127.52, 126.12, 123.17, 113.54, 67.50, 51.40, 37.93, 31.58, 29.17, 29.14, 28.79, 28.52, 26.64, 26.52, 25.69. HR-ESI-MS: m/z [M + Na]$^+$ calcd for $[C_{46}H_{46}N_4O_4Na]^+$ 741.3417, found 741.3422.

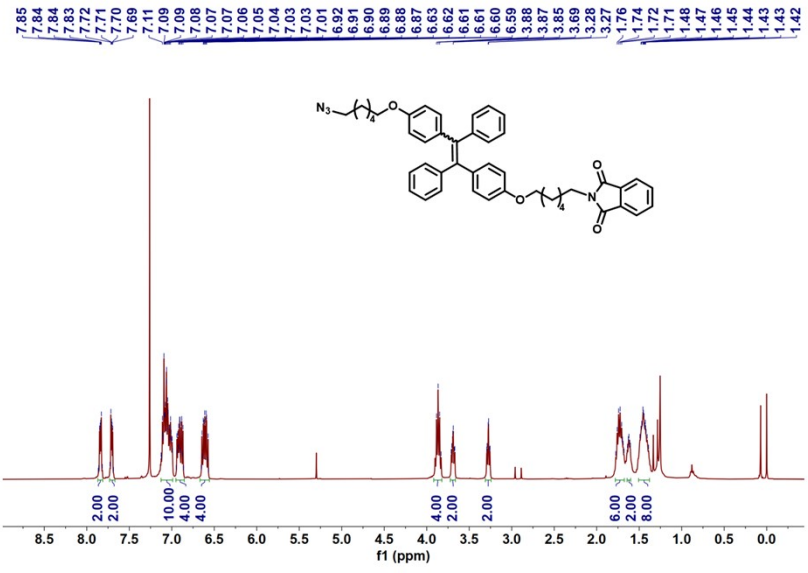


Fig. S6 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **H-4**.

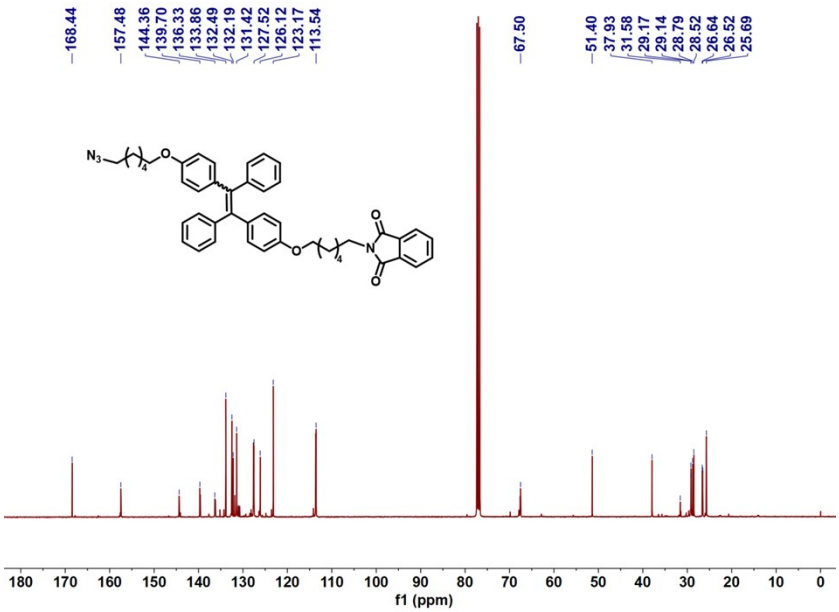


Fig. S7 ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of compound **H-4**.

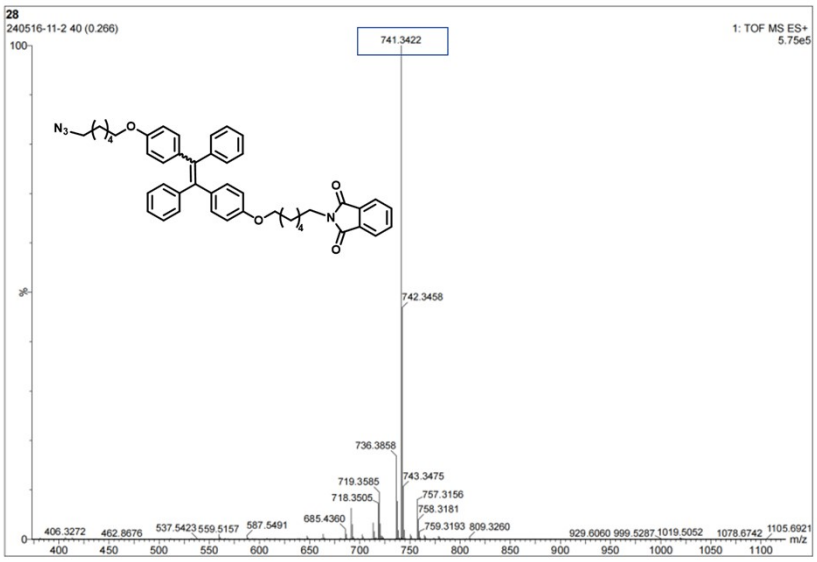


Fig. S8 HR-ESI-MS spectrum of compound **H-4**.

Synthesis of compound H-5

Compound **H-4** (297 mg, 0.413 mmol) was dissolved in THF (4 mL) and EtOH (4 mL). Under N_2 atmosphere, the hydrazine hydrate (41.3 mg, 0.83 mmol) was added to the above mixture, and the mixture was stirred at 60 °C for 12 h. After cooling the reaction mixture to room temperature, 2 N KOH (15 mL) was added and extracted with dichloromethane. The organic layer was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford compound **H-5** as a yellow oil (184 mg, 0.31 mmol, 74%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm) = 7.11 – 7.07 (m, 6H), 7.05 – 6.96 (m, 4H), 6.94 – 6.80 (m, 4H), 6.66 – 6.60 (m, 4H), 3.85 – 3.80 (m, 4H), 3.28 (t, *J* = 4.0 Hz, 2H), 2.37 (s, 2H), 1.64 (s, 4H), 1.53 – 1.50 (m, 2H), 1.37 – 1.33 (m, 10H). ^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ (ppm) = 157.54, 144.31, 139.74, 136.00, 132.38, 131.20, 128.25, 126.76, 114.10, 67.68, 51.04, 41.96, 33.55, 29.21, 29.04, 28.65, 26.63, 26.37, 25.94, 25.55. HR-ESI-MS: m/z $[M + H]^+$ calcd for $[C_{38}H_{45}N_4O_2]^+$ 589.3545, found 589.3548.

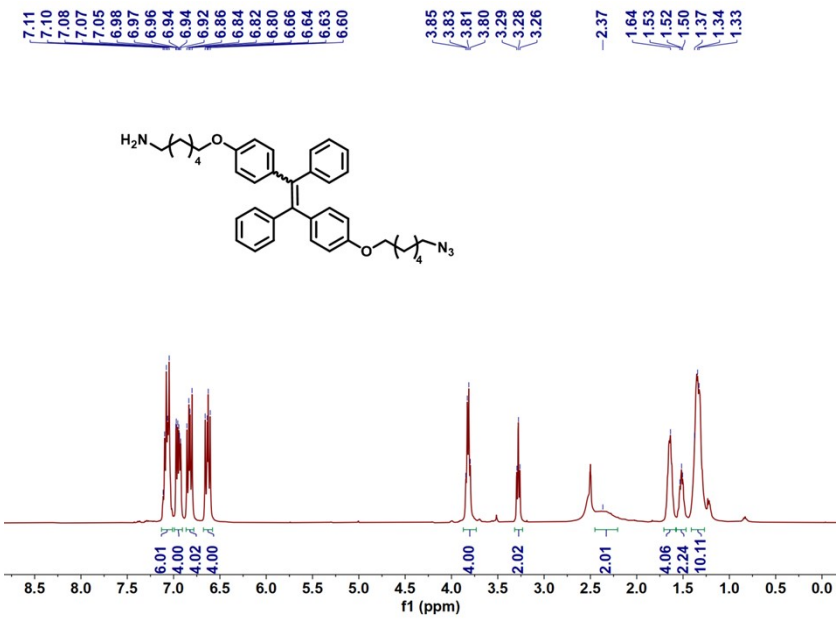


Fig. S9 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **H-5**.

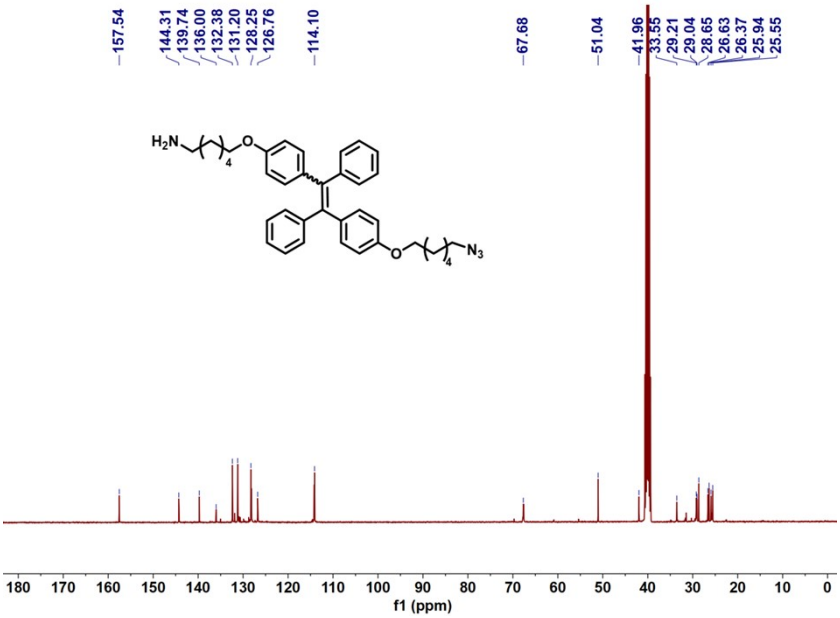


Fig. S10 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of compound **H-5**.

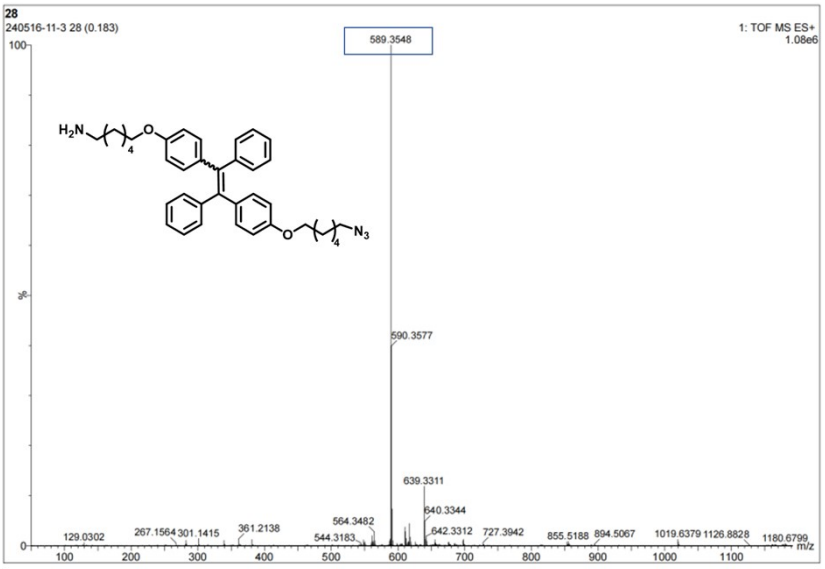


Fig. S11 HR-ESI-MS spectrum of compound **H-5**.

Synthesis of compound H-6

Compound **CB[7]-OH** (30 mg, 0.0254 mmol) and NaH (10 mg, 0.25 mmol) were dissolved in DMSO (3 mL) and stirred for 2 h. Under N_2 atmosphere, the propargyl bromide (12.1 mg, 0.1 mmol) was added to the above mixture at 0 °C. Then the mixture was stirred for 12 h at room temperature. The mixture was poured into methanol, and the precipitate was collected and washed with methanol 4 times to remove the impurities thoroughly. Finally, the residue was dried affording the desired compound **H-6** as a light brown solid (19.5 mg, 0.016 mmol, 64%). ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm) = 5.81 (d, *J* = 16.0 Hz, 2H), 5.75 – 5.66 (m, 12H), 5.51 – 5.36 (m, 14H), 4.43 (d, *J* = 16.0 Hz, 2H), 4.25 – 4.17 (m, 13H), 2.46 (s, 1H).

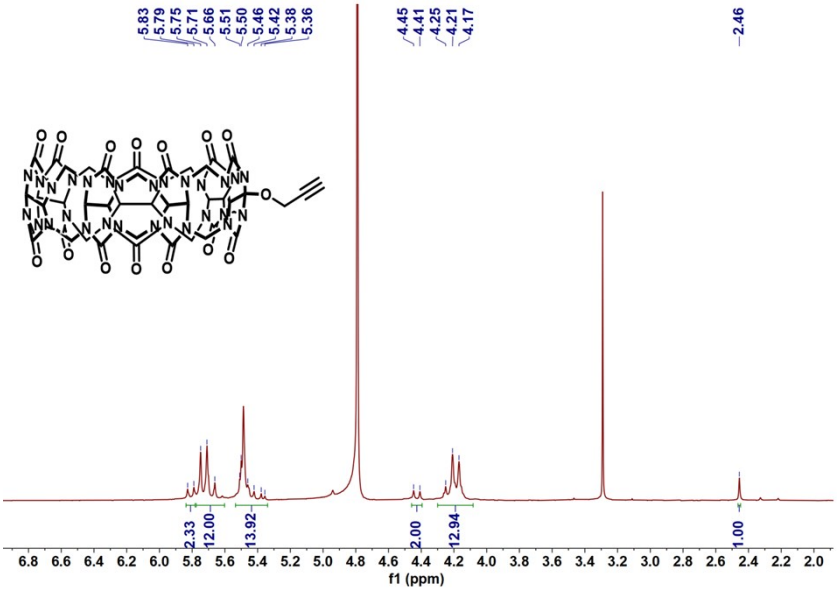


Fig. S12 ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of compound **H-6**.

Synthesis of compound H-7

Compound **GCP**[S1] (45.3 mg, 0.622 mmol), HCTU (70.7 mg, 0.684 mmol), and DIPEA (24.6 mg, 0.76 mmol) were dissolved in DMF (4 mL) and stirred for 40 min. Then, the **H-5** (90 mg, 0.16 mmol) in DMF (1 mL) was added to the above mixture. Under N_2 atmosphere, the mixture was stirred for 12 h. Then, the mixture was extracted with dichloromethane. The organic layer was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 1:1, *v/v*) to afford compound **H-7** as a brown solid (58 mg, 0.07 mmol, 44%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm) = 11.03 (s, 1H), 9.34 (s, 1H), 8.59 (s, 1H), 8.32 (s, 1H), 7.95 (s, 1H), 7.16 – 7.06 (m, 6H), 6.97 – 6.92 (m, 4H), 6.86 – 6.76 (m, 6H), 6.71 – 6.63 (m, 4H), 3.89 – 3.83 (m, 4H), 3.31 (d, *J* = 8.0 Hz, 2H), 3.22 (d, *J* = 4.0 Hz, 2H), 1.67 – 1.64 (m, 4H), 1.57 – 1.50 (m, 4H), 1.46 (s, 9H), 1.37 – 1.35 (m, 8H). ^{13}C NMR (100 MHz, DMSO-d_6, 298K) δ (ppm) = 165.70, 162.05, 161.70, 158.74, 157.53, 144.29, 140.36, 136.07, 132.35, 131.18, 128.25, 126.74, 124.87, 123.25, 115.40, 114.13, 110.23, 81.95, 67.61, 51.06, 29.47, 29.03, 28.63, 28.24, 28.12, 26.36, 25.75, 25.54, 24.92. HR-ESI-MS: m/z [M + H]$^+$ calcd for $[C_{50}H_{59}N_8O_6]^+$ 867.4558, found 867.4562.

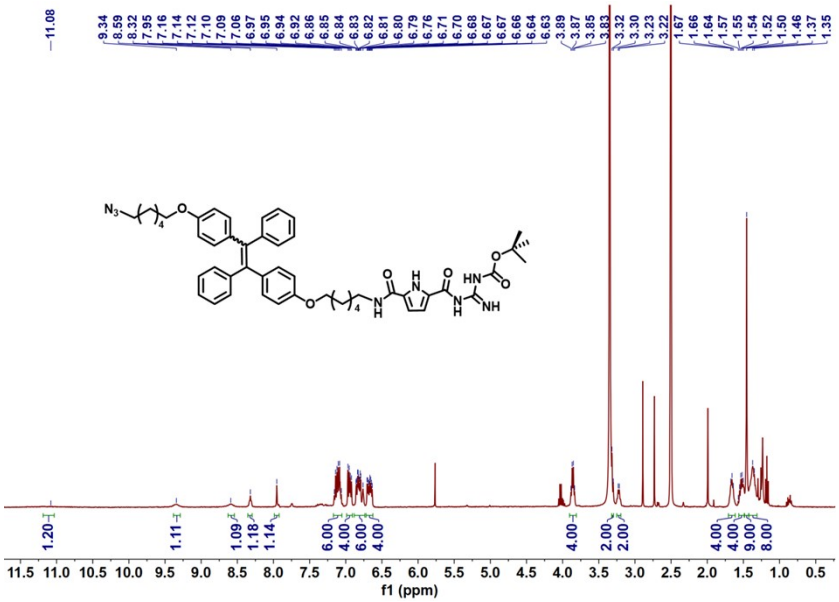


Fig. S13 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **H-7**.

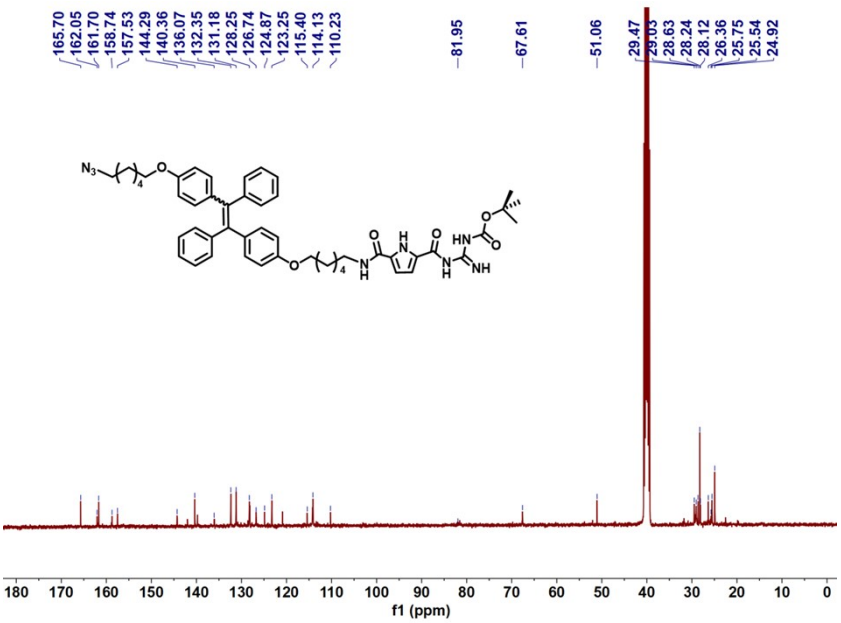


Fig. S14 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of compound **H-7**.

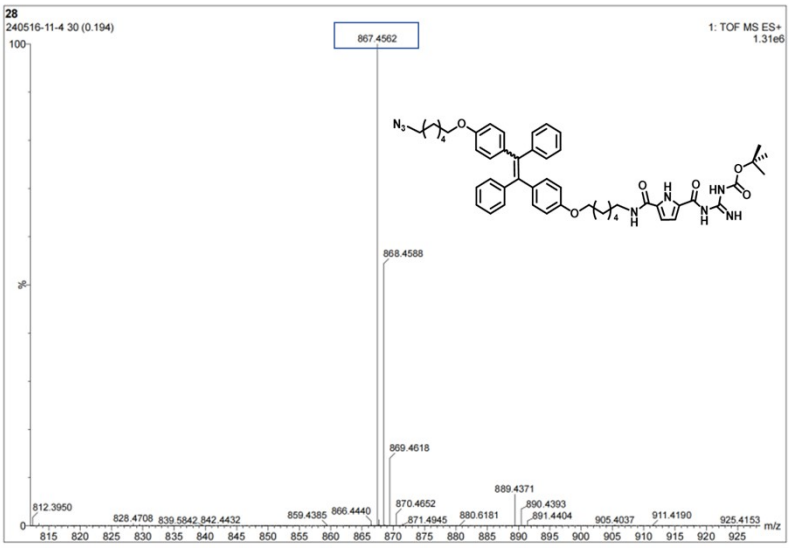


Fig. S15 HR-ESI-MS spectrum of compound **H-7**.

Synthesis of compound GCPCB

Compound **H-6** (30 mg, 0.025 mmol), **H-7** (27 mg, 0.03 mmol), and $CuSO_4 \cdot 5H_2O$ (8 mg, 0.028 mmol), were dissolved in DMSO (2 mL) and stirred. Under N_2 atmosphere, the Na-AsA (8.92 mg, 0.045 mmol) was added to the above mixture and stirred at 80 °C for 24 h. After cooling down, the mixture underwent centrifugation to remove the precipitates of Cu compounds. The mixture in DMSO was dispersed in 7 mL water and dialyzed against Milli-Q water using a 1k Da molecular weight cut-off dialysis membrane for a total of 5 times throughout 72 h. During these five dialysis cycles, the pH values of the dialysis solvent were gradually adjusted from acid to neutral in order to further remove the Cu-based catalyst, respectively. The substance in the dialysis tube was lyophilized and the fluffy solid obtained was dissolved in dimethyl sulfoxide (2 mL) and dichloromethane (0.5 mL), trifluoroacetic acid (0.18 mL, 0.0024 mmol) was added to the above mixture and stirred for 12 h. The mixture was then added dropwise to methanol (8 mL) and a solid was precipitated, the supernatant was removed by centrifugation and dried to obtain a tan-colored solid (22 mg, 0.011 mmol, 37%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm) = 12.40 (s, 1H), 11.68 (s, 1H), 8.23 (s, 1H), 7.13 – 7.08 (m, 7H), 6.97 – 6.92 (m, 4H), 6.84 – 6.80 (m, 4H), 6.73 – 6.67 (m, 5H), 5.65 (d, *J* = 12.0 Hz, 14H), 5.41 (s, 13H), 4.50 (s, 2H), 4.27 (d, *J* = 56.0 Hz, 14H), 3.85 (s, 4H), 3.21 (d, *J* = 4.0 Hz, 4H), 1.64 (s, 6H), 1.50 (s, 4H), 1.35 (d, *J* = 12.0 Hz, 6H). Due to the complex structure and relatively lower solubility in solvents, a precise characterization of ^{13}C NMR was not possible. MALDI-TOF-MS: m/z $[M - CF_3CO_2]^+$ calcd for $[C_{90}H_{95}N_{36}O_{20}]^+$ 1999.7518, found 1999.1118.

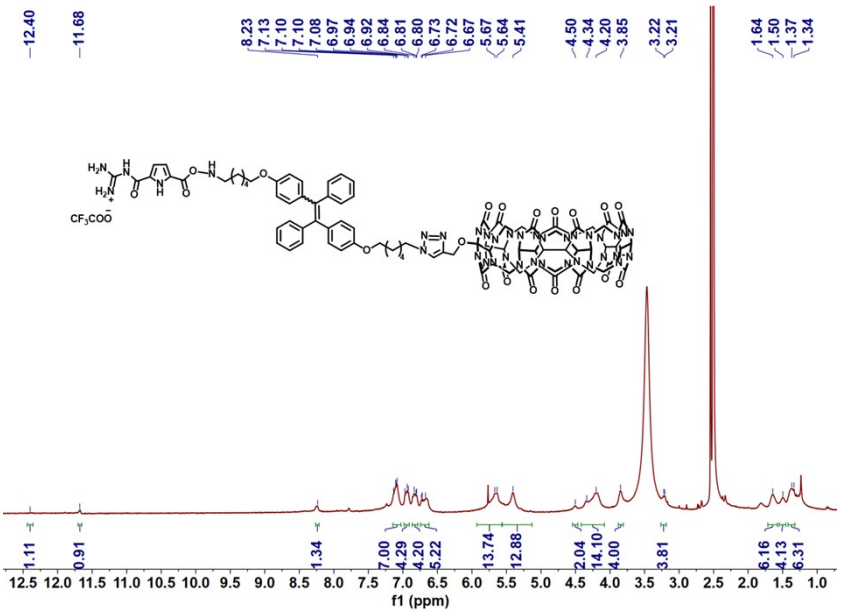


Fig. S16 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **GCPCB**.

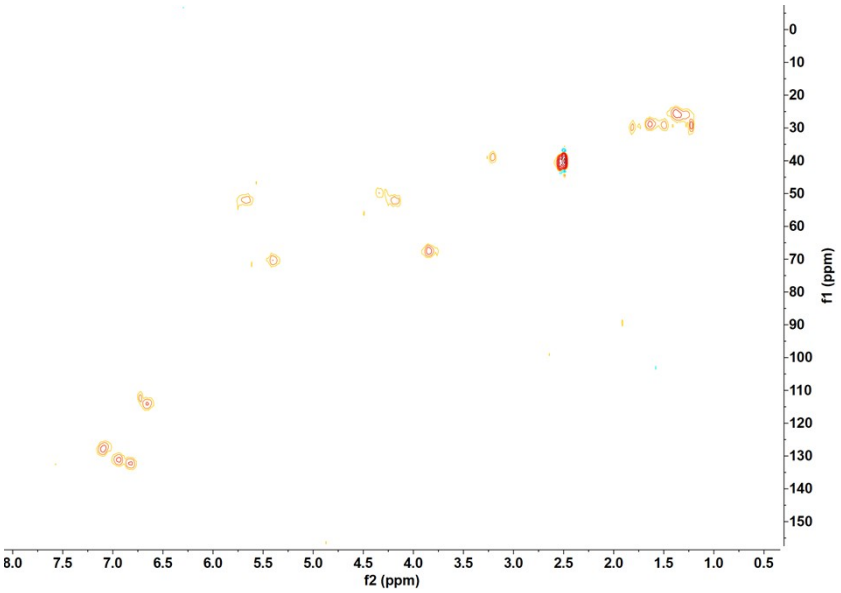
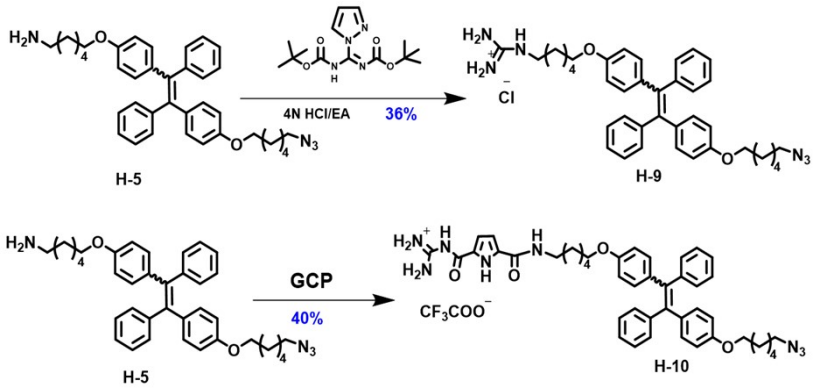


Fig. S17 The ^{1}H-^{13}C 2D HSQC NMR spectrum of compound **GCPCB**.

2) Synthesis of control molecules H-9 and H-10

H-9 and **H-10** were designed and synthesized according to the following procedures (Scheme S2).



Scheme S2. Synthesis route of control molecules **H-9** and **H-10**.

Synthesis of compound H-9

Under N_2 protection, *N*, *N*′-Boc$_2$-1H-pyrazole-1-carboxamidine (683 mg, 2.2 mmol) was added to a stirred solution of **H-5** (652 mg, 1.1 mmol) in DMF (20 mL). The resulting solution was stirred at room temperature for 24 hours. Then, the reaction mixture was concentrated under reduced pressure and the obtained residue was dissolved in CH_2Cl_2 (30 mL) and the solution was washed with water (3 × 80 mL). The organic phase was concentrated under reduced pressure. Then it was dissolved in a mixed solution the mixture solution of MeOH (20 mL) and $CHCl_3$ (10 mL) and 4N HCl/EA (0.3 mL) was added to the solution. The resulting solution was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and the obtained residue was dissolved in a small amount of anhydrous MeOH (0.5 mL), which was then added dropwise to plenty of diethyl ether (100 mL), the precipitates were collected by filtration, washed by diethyl ether and dried in vacuum, and the target compound **H-9** was obtained as a yellowish solid (249 mg, 0.4 mmol, 36%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm) = 8.44 (s,1H), 7.66 (s, 1H), 7.14 – 7.09 (m, 6H), 6.97 – 6.92 (m, 4H), 6.87 – 6.81 (m, 4H), 6.71 – 6.64 (m, 4H), 3.89 – 3.84 (m, 4H), 3.08 (d, *J* = 8.0 Hz, 2H), 2.86 (s, 2H), 1.66 (d, *J* = 8.0 Hz, 4H), 1.58 – 1.54 (m, 2H), 1.49 – 1.45 (m, 2H), 1.36 (s, 8H). ^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ (ppm) = 158.65, 157.51, 144.30, 139.74, 136.01, 132.38, 131.19, 128.28, 126.79, 114.09, 67.60, 48.62, 41.14, 32.91, 29.09, 28.86, 26.28, 26.01, 25.73, 25.64, 25.50. HR-ESI-MS: m/z [M-Cl]$^+$ calcd for $[C_{39}H_{47}N_6O_2]^+$ 631.3755, found 631.3764.

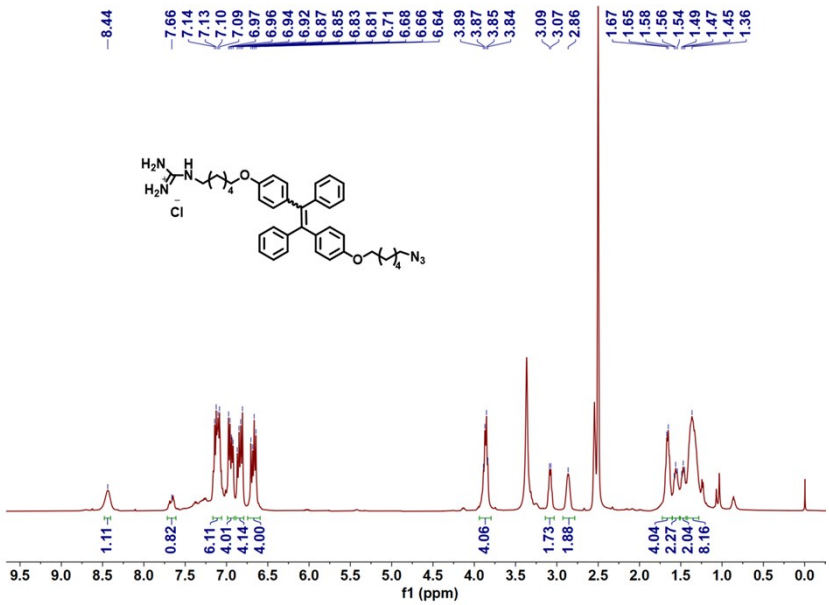


Fig. S18 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **H-9**.

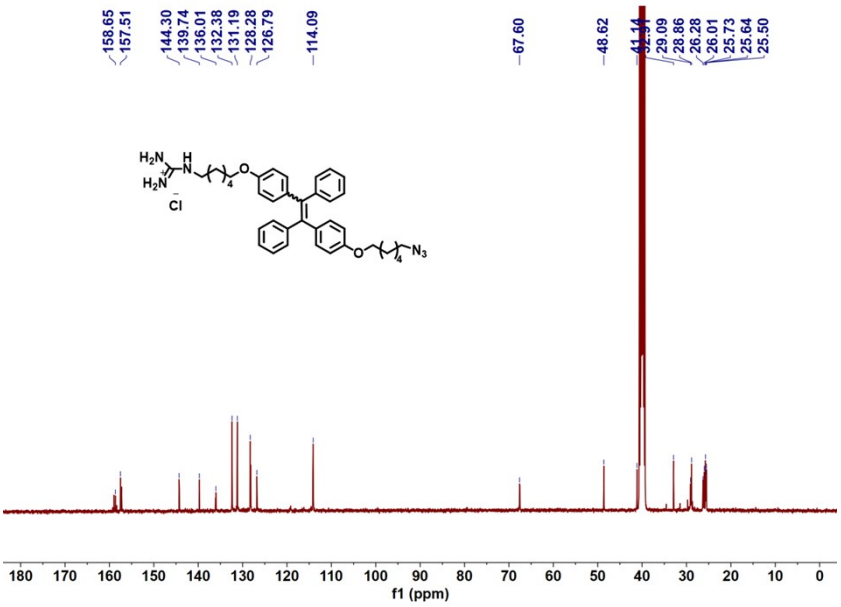


Fig. 19 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of compound **H-9**.

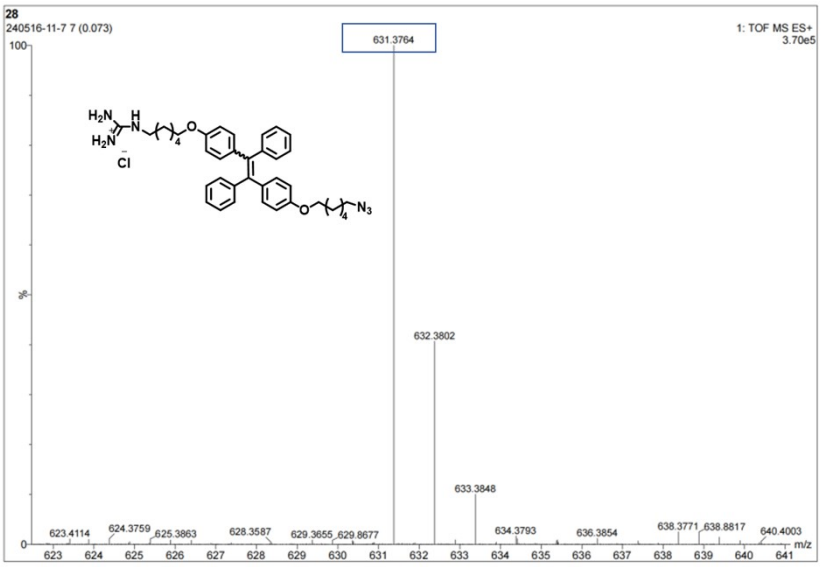


Fig. S20 HR-ESI-MS spectrum of compound **H-9**.

Synthesis of compound H-10

Compound **H-7** (80 mg, 0.1 mmol) was dissolved in DCM (3 mL) under N_2 protection, then trifluoroacetic acid (0.15 mL, 1.8 mmol) was added to the solution. The resulting solution was stirred at room temperature for 1 day. The mixture was concentrated under reduced pressure, and the resulting residue was dissolved in a small amount of DCM, the solution was then added dropwise to a large amount of diethyl ether. The precipitate was collected by filtration, washed with diethyl ether, and dried under vacuum to yield compound **H-10** as a light brown solid (28.3 mg, 0.04 mmol, 40%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm) = 12.35 (s, 1H), 11.12 (s, 1H), 8.43 (s, 1H), 8.29 (s, 4H), 7.15 – 7.08 (m, 7H), 6.97 – 6.91 (m, 4H), 6.85 – 6.79 (m, 5H), 6.69 – 6.63 (m, 4H), 3.88 – 3.83 (m, 4H), 3.30 (d, J = 8.0 Hz, 2H), 3.24 (d, J = 8.0 Hz, 2H), 1.65 (s, 4H), 1.55 – 1.49 (m, 4H), 1.36 (s, 8H).^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ (ppm) = 159.45, 157.55, 157.52, 155.56, 144.30, 139.77, 136.06, 133.35, 132.36, 131.18, 128.25, 126.75, 125.82, 115.89, 114.13, 112.71, 67.70, 51.06, 39.20, 31.45, 29.41, 29.12, 28.64, 26.68, 26.36, 25.76, 25.54. HR-ESI-MS: m/z [M-CF_3CO_2]$^+$ calcd for [$C_{45}H_{51}N_8O_4$]$^+$ 767.4028, found 767.4033.

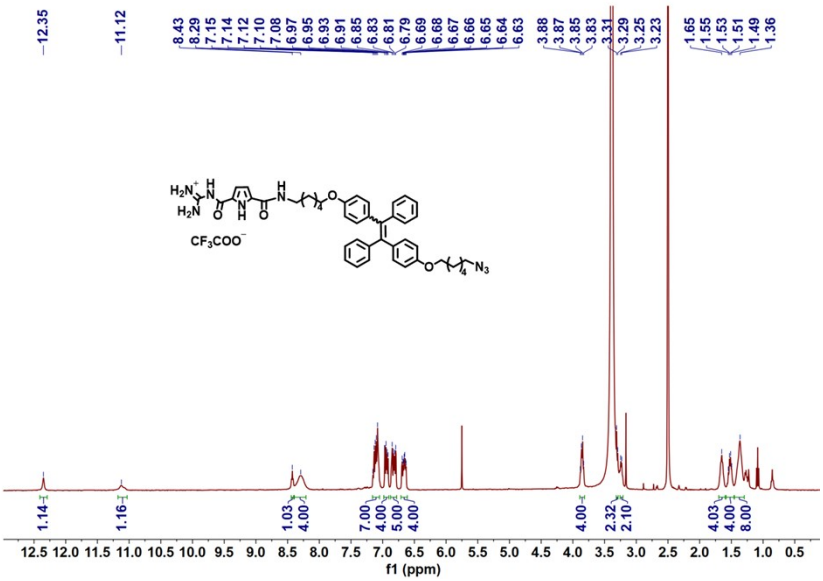


Fig. S21 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **H-10**.

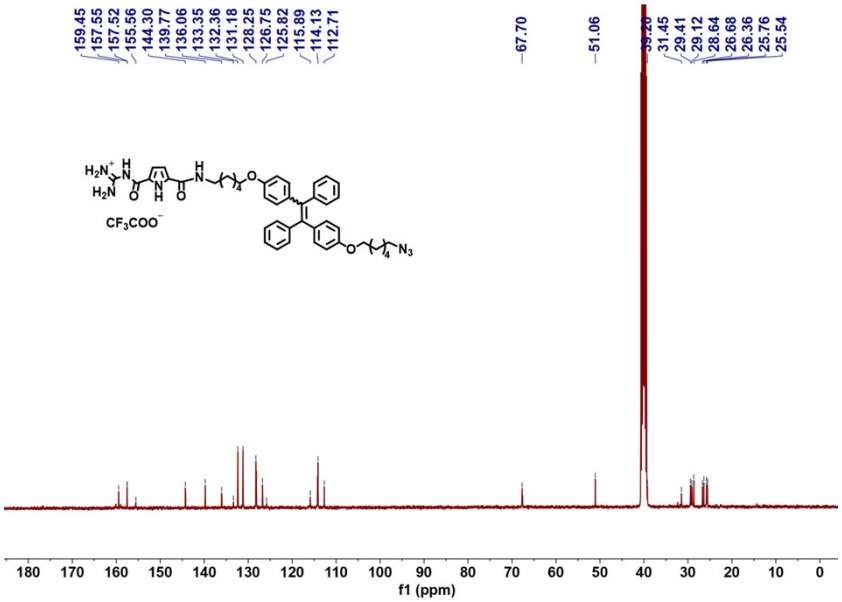


Fig. S22 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of compound **H-10**.

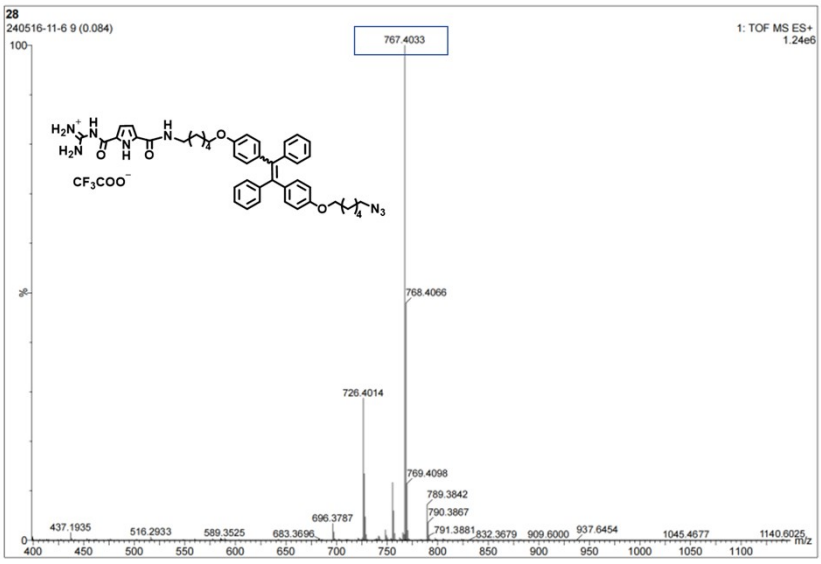


Fig. S23 HR-ESI-MS spectrum of compound **H-10**.

3. Job's plot of complex CB[7]⊃BH

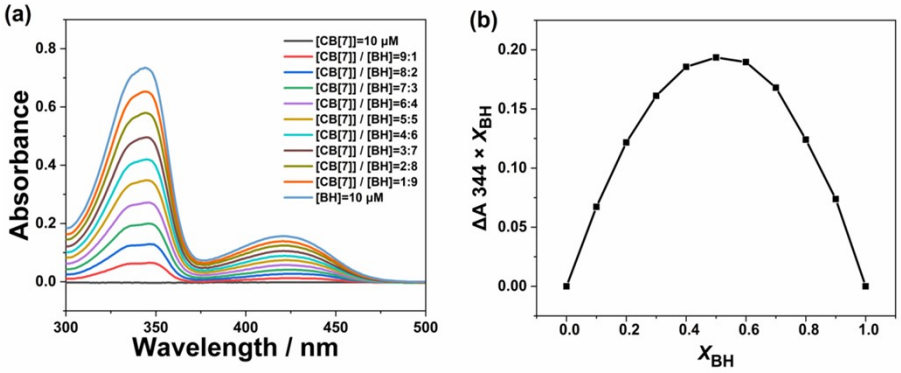


Fig. S24 (a) UV-Vis absorption spectra of complex **CB[7]⊃BH** with different molar ratios in water while **CB[7]** + **[BH]** = 10 μM. (b) Job's plot of complex **CB[7]⊃BH** shows a 1:1 stoichiometry between **CB[7]** and **BH** by plotting the absorbance difference at 344 nm against the mole fraction of **CB[7]**.

4. Investigation of the binding constant between CB[7] and BH

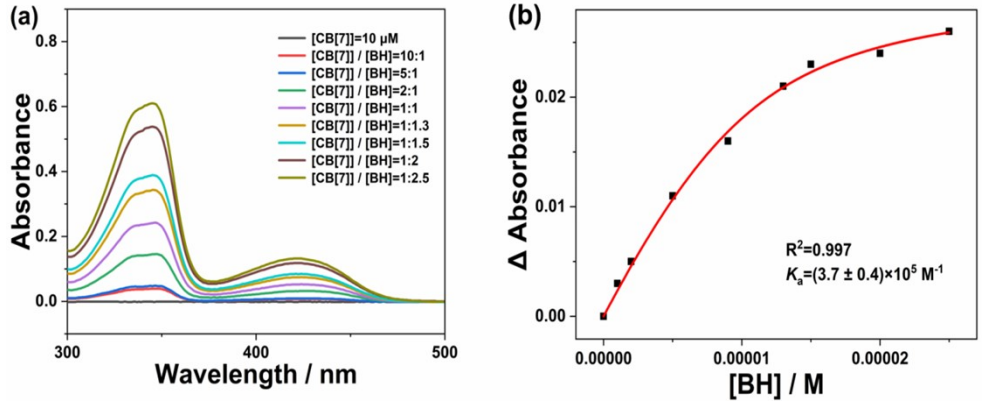


Fig. S25 Determination of the association constants between **CB[7]** and **BH**: UV−Vis absorption changes of **BH** with varied concentrations of **CB[7]**; (b) Dependence of the UV−vis absorption at 344 nm on **BH** with varied concentrations of **CB[7]**.

To determine the binding affinity between **CB[7]** and **BH**, UV-vis absorption titration experiments were performed. The solution had a constant concentration of **BH** and varied concentration of **CB[7]**. The binding constant (K_a) between **CB[7]** and **BH** was calculated via the non-linear curve-fitting method.

For fitting equation under a 1:1 stoichiometry for **BH** to **CB[7]**:

$$\Delta A = (\Delta A_\infty/[H]_0)\,(0.5[G]_0 + 0.5([H]_0 + 1/K_a) - (0.5\,([G]_0^2 + (2[G]_0(1/K_a - [H]_0)) + (1/K_a + [H]_0)^2)^{0.5}))$$

Where ΔA is the UV-vis absorption changes at 344 nm at $[H]_0$, ΔA_∞ is the UV-vis absorption changes at 344 nm when **BH** and **CB[7]** are completely complexed, $[G]_0$ is the initial concentration of **BH**, and $[H]_0$ is the fixed initial concentration of **CB[7]**.

5. Tyndall effects of GCPCB, GCPCB⊃BH, H-9, and H-10 solution

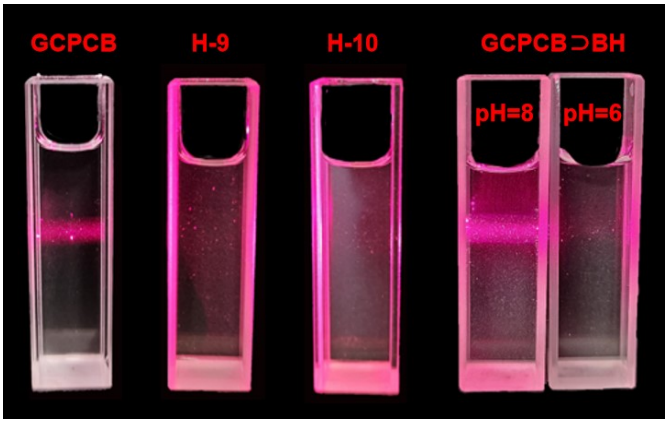


Fig. S26 Tyndall effects of **GCPCB**, **H-9**, and **H-10** solution, and Tyndall effect of **GCPCB⊃BH** aggregates after the solution pH was adjusted to 8.0 and 6.0, respectively.

6. AIE properties of GCPCB

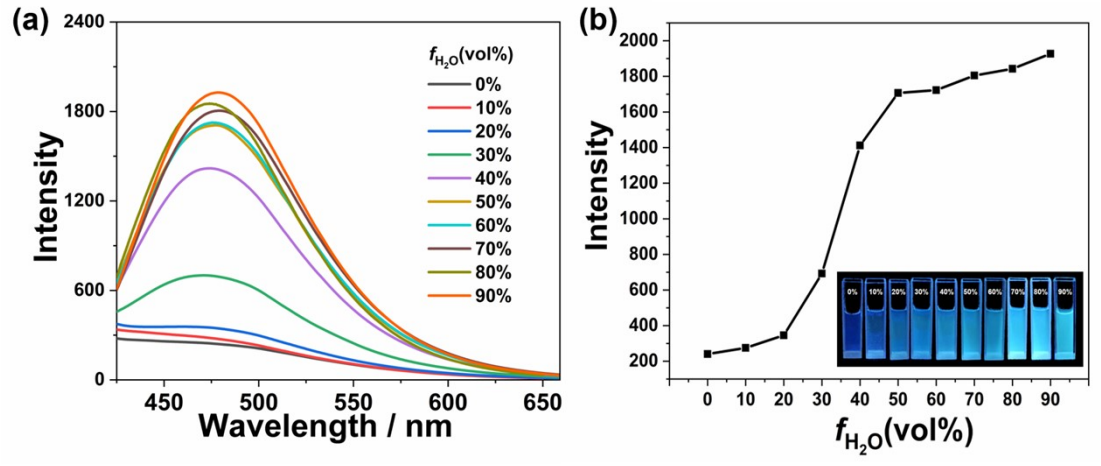


Fig. S27 (a) Fluorescence spectra of **GCPCB** in water-methanol mixture with different water contents; (b) Plot of maximum emission intensity of **GCPCB**. [**GCPCB**] = 5×10^{-5} M.

7. Critical aggregation concentration (CAC) determination of GCPCB and GCPCB⊃BH

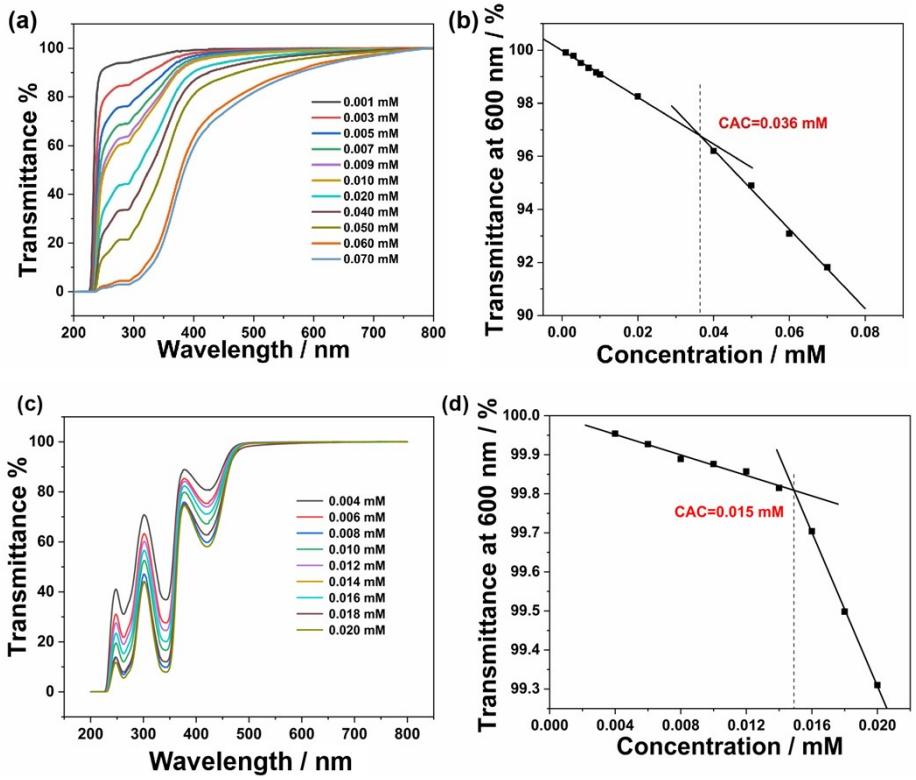


Fig. S28 Optical transmittance of **GCPCB** (a and b) and **GCPCB⊃BH** (c and d) in water on increasing the concentration at 25 °C.

8. Zeta-potentials of aggregates formed by GCPCB and GCPCB⊃BH

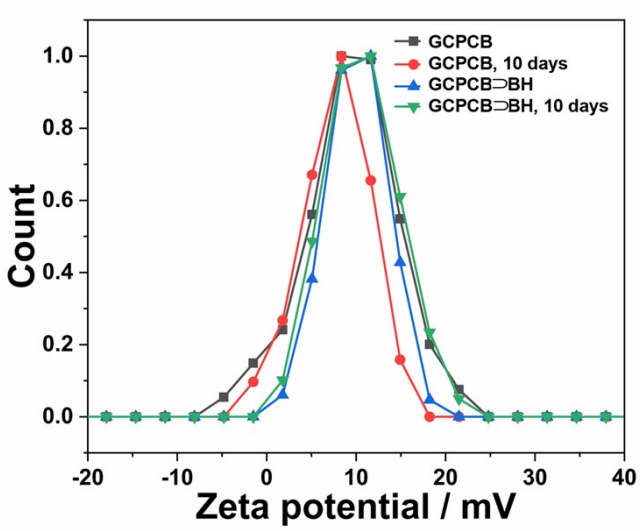


Fig. S29 The zeta potentials of the aggregates formed by **GCPCB**, **GCPCB⊃BH**, and after incubation at room temperature for 10 days were measured as +8.33 mV, +10.91 mV, +7.76 mV, and +10.77 mV, respectively.

9. TEM images of GCPCB⊃BH

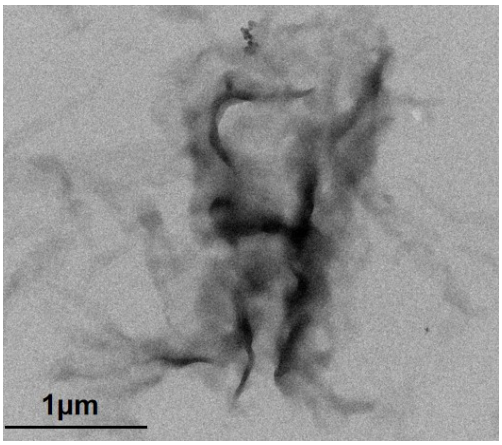


Fig. S30 TEM images of **GCPCB⊃BH** aggregates after the solution pH was adjusted to 6.0.

10. Antibacterial activity assays

Bacteria were cultured in an LB medium at 37 °C and 275 rpm until reaching a stationary phase. The cultures were then harvested by centrifugation and washed with 0.85% sodium chloride solution three times. Concentrations of re-suspended bacteria were determined by optical density measured at 600 nm. M9 medium was used to make dilutions of bacterial solution to a concentration of 1×10^6 cfu/mL. A volume of 50 μL of these solutions was added into a 96-well plate and mixed with 50 μL of testing solutions in M9, giving a final bacterial concentration of 5×10^5 cfu/mL for incubation for 16 h. Testing concentration varied half-fold per a standard protocol. A growth control group with only bacterial solution and a sterile control group with only growth medium were carried out at the same time. All experiments were performed in triplicates, and at least two independent experiments were repeated on different days. The MIC is defined as the lowest concentration of testing chemical that inhibits the visible growth of bacteria as observed with the unaided eye.

Tab. S1 Antibacterial activity of compounds against *E. coli* and *S. aureus*

Compounds	Minimum inhibitory concentration / mM	
	E. coli	*S. aureus*
GCPCB	5	0.625
H-9	＞5	＞5
H-10	＞5	＞5

11. *In vitro* antimicrobial activity assay

To evaluate the antimicrobial activity of host **GCPCB**, guest **BH,** and host-guest complexes **GCPCB⊃BH** *in vitro*, 100 μL bacterial solution was first incubated with an equal volume of each group of materials for 2 hours. Subsequently, the bacterial suspension was diluted 100 times with Luria-Bertani (LB) medium to stop killing. Finally, 50 μL of each group was incubated on LB AGAR plates. After 16 h of culture, the number of colonies was counted by plate counting method and the survival rate was calculated.

12. Bacterial live/dead staining assay

The integrity of the bacterial membrane was analyzed with a live/dead bacterial staining kit with DMAO & PI. Logarithmic growth stage *E. coli* and *S. aureus* were collected and diluted to 1×10^9 CFU/mL in PBS. The nanoparticles and bacterial suspension were mixed 1:1 (*v/v*) in 96-well plates, DMAO dye, and PI dye were added, and incubated at 37 °C for 15 minutes. Images were captured using confocal laser scanning microscopy (CLSM) (FV1000, Olympus, Japan).

13. MTT assays

Staphylococcus aureus and *Escherichia coli* were cultured overnight in Luria-Bertani (LB) liquid medium at 37 °C, and then a small amount of bacterial suspension was added to LB liquid medium for activation, and finally the activated bacterial solution was diluted to 2×10^6 CFU mL^{-1} for use. The MIC of the host molecule to different microorganisms was determined by broth microdilution method. In other words, 100 μL of LB liquid medium was added into each hole of the 96-well plate, and the host molecule was added by double dilution method to obtain the host molecule solution with concentration gradient. Subsequently, 100 μL bacterial suspension was added and incubated for 20 h. The absorbance at 600 nm ($OD_{600\ nm}$) was determined by an enzyme labeler (Infinite F50, TECAN, Switzerland). Bacteria suspension treated with LB liquid medium was the positive control. The MIC was defined as the lowest concentration of testing chemical that inhibits the visible growth of bacteria as observed with the unaided eye.

14. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM): Morphological changes of bacteria treated with nanoparticles were recorded by SEM. 1×10^9 CFU/mL suspension of *S. aureus* and *E. coli* was added to a 24-well plate with cell crawlers at the bottom. The nanoparticles were then incubated with the bacterial suspension and cultured for 12 h. After the bacterial suspension was sucked out, 2.5% (*w/v*) glutaraldehyde was added and bacteria were fixed at 4 °C overnight. Finally, it was rinsed twice with PBS, dehydrated with ethanol gradient (30, 50, 70, 80, 90 and 100% *v/v* for 10 minutes each time) and dried in a drying oven. The surface morphology of bacteria and the integrity of the membrane were observed by SEM (SU8010, Hitachi, Japan).

15. Leakage of cellular nucleic acids and proteins

The integrity of the bacterial membrane was detected by the leakage of bacterial nucleic acids and proteins into the bacterial suspension. The logarithmic bacteria were diluted to 1×10^9 CFU/mL in LB medium, centrifuged at 3000 rpm/min for 3 min, and then re-suspended in PBS. The bacterial suspension was then cultured with nanoparticles at 37 °C for 12 h and centrifuged at 8000 rpm/min for 5 minutes. Finally, the supernatant was collected and the absorbance of the supernatant was measured with a microplate reader (Infinite F50, TECAN, Switzerland) at the wavelength of 260 nm to monitor the leakage of nucleic acids. Protein leakage was detected with the BCA protein assay kit.

16. References

S1. C. Schmuck and M. Schwegmann, *J. Am*. *Chem*. *Soc*., 2005, **127**, 3373-3379.