Pillararene-Based Host−Guest Recognition Facilitated Magnetic Separation and Enrichment of Cell Membrane Proteins

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Electronic Supplementary Information (22 pages)

1. Materials and methods

All reagents including model proteins were commercially available and used as supplied without further purification. **DBEP5**,[S1] **WP5-P**[S2] and **G2**[S3] were synthesized according to the published methods. Water used in this manuscript was Milli Q water. ^{1}H or ^{13}C NMR spectra were recorded with a Bruker Avance DMX 400 spectrophotometer with use of the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. High-resolution mass spectrometry experiments were performed with a Bruker 7-Tesla FT-ICR mass spectrometer equipped with an electrospray source (Billerica, MA, USA). UV-vis spectra were taken on a Perkin-Elmer Lambda 35 UV-vis spectrophotometer. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan). The determination of the critical aggregation concentration (CAC) values was carried out on a DDS-307 instrument. Scanning electron microscopy investigation was carried out on a JEOL 6390LV instrument. Transmission electron microscopy investigations were carried out on a JEM-1200EX instrument. Dynamic light scattering was carried out on a Malvern Nanosizer S instrument at room temperature. The MALDI-TOF data were collected on an Ultraflextrememass spectrometer of Bruker. Thermogravimetric analysis (TGA) was carried out on a DSCQ1000 Thermal Gravimetric Analyzer. Magnetic properties were measured on a Quantum Design 3T VersaLab vibration sample magnetometer. Cells used in all experiments were Intestinal Porcine Epithelial Cells (IPEC-J2).

Preparation of MNP-P5

Bare **MNP** was prepared by a co-precipitation method of ferrous and ferric salts in alkaline medium.[S4] 0.200 g of ferric sulfate heptahydrate and 0.349 g of ferric chloride hexahydrate were dissolved in 10.0 mL of Milli Q water, and 2.50 mL of 25% NH_3 (aq.) was added under vigorous stirring. After the black precipitate formed, **MNP** was separated by NdFeB magnet and washed with water several times. The **MNP** was re-dispersed in water by sonication, and 0.200 g of **WP5-P** was added into the suspension. The mixture was stirred overnight. **MNP-P5** was separated by magnet and washed with water. Finally **MNP-P5** was suspended in 3.00 mL of water and stored in liquid nitrogen. The concentration of **MNP-P5** suspension was estimated to be 7.88 mg/mL by drying 1.00 mL of the suspension in vacuum.

Preparation of imidazolium labeled proteins (BSA-G and Hb-G)

The preparation of **BSA-G** was is as an example. To a solution of **G** (2.50 mg, 6.67 μmol), EDC•HCl (1.50 mg, 7.92 μmol) and NHS (1.00 mg, 8.77 μmol) was stirred in 20.0 μL of dry DMF for 2 hours in an ice bath. Then 5.00 mg of BSA in 2.00 mL of phosphate buffered saline (PBS, pH = 7.40) was added. The mixture was stirred for 2 hours in an ice bath. By centrifugal filtration (with molecular weight cut-off of 10.0 kDa), excess reagents were removed. The residue (**BSA-G**) was dissolved in 5.00 mL of PBS buffer for further analysis and the concentration of total proteins was determined to be 3.66 μg/mL (see section S11).

Isolation of G2 and Hb-G from water

A coumarin derivative **G2**[S3] aqueous solution (2.00×10^{-2} mM, 1.00 mL) was added to 1.00 mL of a **MNP-P5** suspension (7.88 mg/mL). The mixture was put on a shaking table for

1 hour and separated by magnet. The fluorescent intensity of the supernatant was analyzed. As a control, the fluorescent intensities of solutions of **G2** (1.00×10^{-2} mM) before and after adding **WP5-P** (5.00 equiv.) were also measured. To estimate the guest release efficiency, **MNP-P5** with captured coumarin were re-dispersed in 2.00 mL of water and heated to 80 °C followed by magnetic separation. The fluorescent intensity of supernatant was analyzed.

The procedure of separating **Hb-G** was the same as above. UV-vis absorption was measured instead of fluorescent intensity.

Separation of BSA-G from a cell lysate

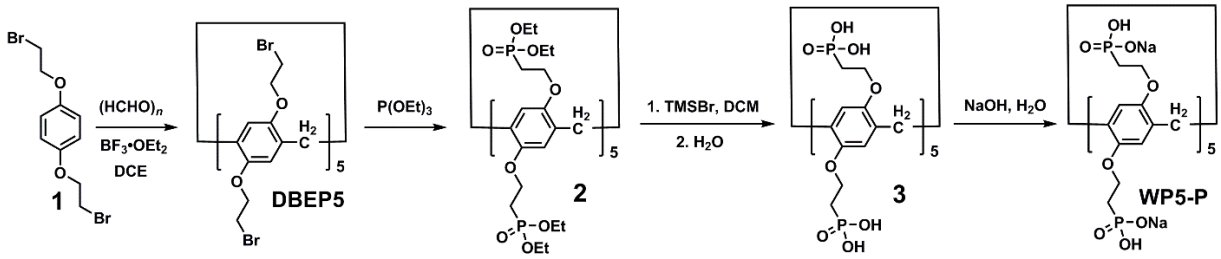
Intestinal porcine epithelial IPEC-J2 cells were cultured by Dulbecco's modified Eagle's medium/HamF12 (DMEM/F12) containing 10% fetal bovine serum (FBS) and 100 U of penicillin/streptomycin at 37 °C under a humidified atmosphere of 95% air : 5% CO_2. After washing with PBS buffer, the total proteins were extracted from cultured IPEC-J2 cells using RIPA lysis buffer (50.0 mmol/mL Tris-HCl, pH = 7.4, 150 mmol/mL NaCl, 2.00 mmol/mL EDTA, 1% SDS, 1% Triton X-100, 10% glycerol, 5.00 g of sodium deoxycholate) containing complete protease inhibitor. After removing the insoluble precipitate, the supernatant (cell lysate) was used in further studies and the concentration of total proteins was determined to be 12.2 μg/mL (see section S11). 20.0 μL of a **BSA-G** solution (3.66 μg/mL), 100 μL of cell lysate, 380 μL of 2 × PBS buffer and 400 μL of a **MNP-P5** suspension (7.88 mg/mL) were mixed together and incubated for 1 hour. After separation by magnet, the solid was washed with PBS buffer three times. The captured proteins were recovered by heating **MNP-P5** at 95 °C in 40.0 μL of protein loading buffer followed by magnetic separation. The resulting supernatant was used in SDS-PAGE analysis (Fig. 2b, lane v).

Separation of cell membrane proteins from a cell lysate

A solution containing **G** (3.75 mg, 10.0 μmol), EDC•HCl (0.328 mg, 2.00 μmol) and NHS (0.230 mg, 2.00 μmol) was stirred in 50.0 μL of water for 2 hours in an ice bath in order to activate **G**. The resulting solution was added into 4.50 mL of PBS buffer and then rapidly added into adherent monolayers of PBS washed IPEC-J2 cells. The cells were incubated for additional 30 minutes. After washing with PBS buffers three times, the cells were harvested by scraping and then lysed in 200 μL of RIPA buffer (10.0 μL of the lysate was used in SDS-PAGE, Fig. 3a, lane ii; 40.0 μL of the lysate was used in western blotting analysis, Fig. 3b, lane ii). The insoluble precipitate was discarded by centrifuging. The lysate containing intracellular proteins and **G**-labeled membrane proteins was incubated with 400 μL of a **MNP-P5** suspension (7.88 mg/mL) and 200 μL of 2 × PBS buffer for 1 hour in an ice bath. After washing, the captured proteins were recovered by heating **MNP-P5** at 95 °C in 40.0 μL of protein loading buffer. After magnetic separation, 10.0 μL and 40.0 μL of the supernatant were used in SDS-PAGE (Fig. 3a, lane iii) and western blotting analysis (Fig. 3b, lane iii), respectively.

2. *Synthesis of **WP5-P** and guest molecules*

Synthesis of WP5-P[S1,S2]



Scheme S1. Synthesis of **WP5-P**.

Synthesis of **DBEP5**:[S1] To a solution of compound **1** (20.0 g, 61.7 mmol) in 600 mL of chloroform, paraformaldehyde (3.74 g, 123 mmol) and $BF_3 \cdot OEt_2$ (7.79 mL, 61.7 mmol) were added. The mixture was stirred and monitored by TLC. After that, 500 mL of saturated $NaHCO_3$ solution was added to quench the reaction. The organic layer was separated and concentrated. The crude product was purified by silica gel column chromatography using dichloromethane:hexane = 1:1 as an eluent to obtain pure **DBEP5** as a white solid (6.01 g, 29%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 6.91 (s, 10H), 4.22 (t, J = 6 Hz, 20H), 3.84 (s, 10H), 3.64 (t, J = 6 Hz, 20H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm): 149.66, 129.06, 116.04, 68.97, 30.79, 29.41.

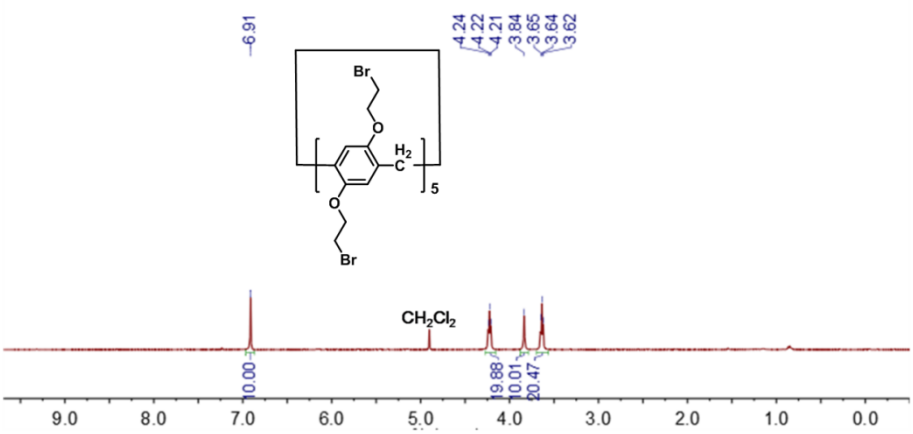


Fig. S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **DBEP5**.

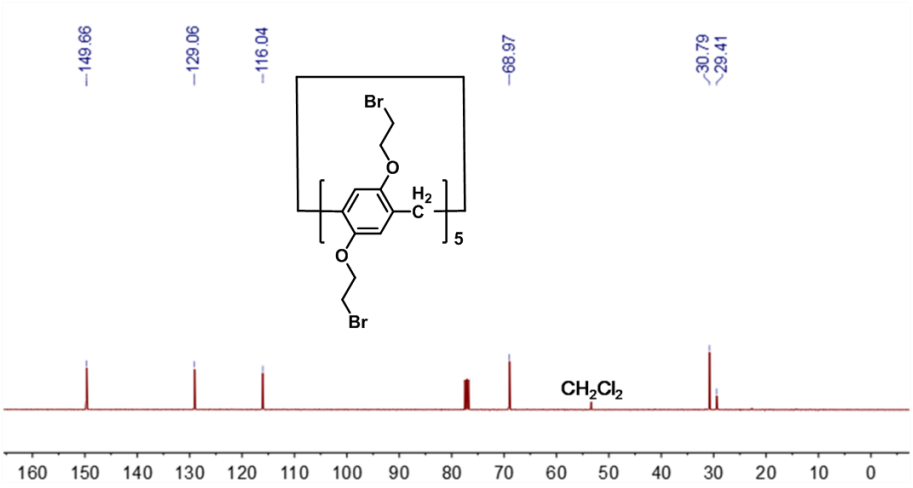


Fig. S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of **DBEP5**.

Synthesis of compound **2**:[S2] **DBEP5** (2.50 g, 1.49 mmol) was suspended in triethylphosphite (24.7 g, 149 mmol). The mixture was heated at 165 °C for 3 days. The resulting oil was purified by silica gel column chromatography using ethyl acetate:methanol = 2:1 as an eluent to obtain pure product as a yellowish oil (1.82 g, 54%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 6.85 (s, 10H), 4.10 (m, 60H), 3.73 (s, 10H), 2.31 (m, 20H), 1.26 (m, 60H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm): 149.66, 129.06, 116.04, 68.97, 30.79, 29.41.

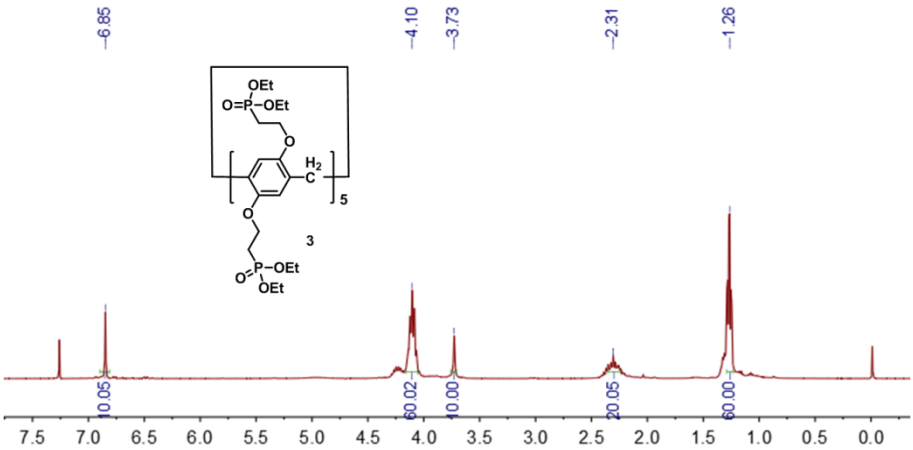


Fig. S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **2**.

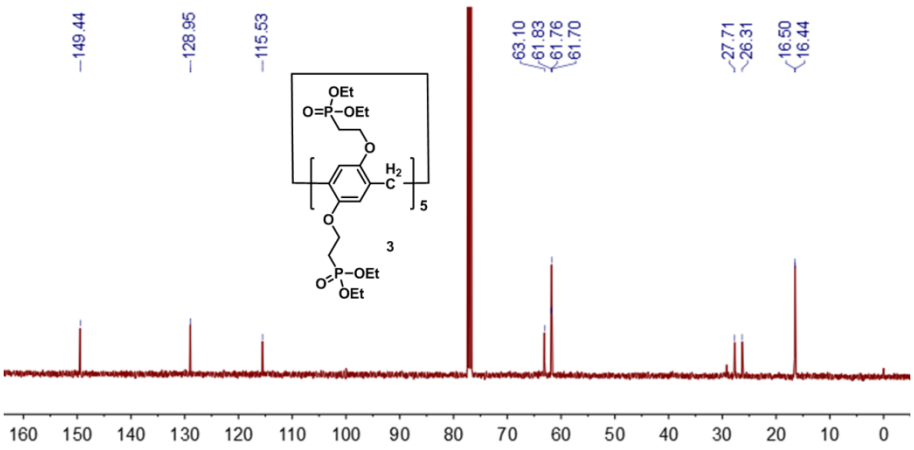


Fig. S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of **2**.

Synthesis of compound **3**:[S2] 5.50 mL (41.7 mmol) of bromotrimethylsilane (TMSBr) was added into a solution of compound **2** (1.00 g, 0.440 mmol) in dichloromethane in an ice bath. The mixture was stirred at room temperature for 3 days. After that, the solvent was removed under high vacuum. Water was added into the residue, and stirred for 30 min. After the solvent was removed, the residue was washed with acetone to obtain pure product as a pale solid (0.690 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm): 9.18 (m, 20H), 6.87 (s, 10H), 4.06 (dd, J = 43, 12 Hz, 21H), 3.68 (s, 10H), 2.25 (m, J = 6 Hz, 20H). ^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ (ppm): 148.78, 127.48, 113.81, 62.53, 30.67, 27.80.

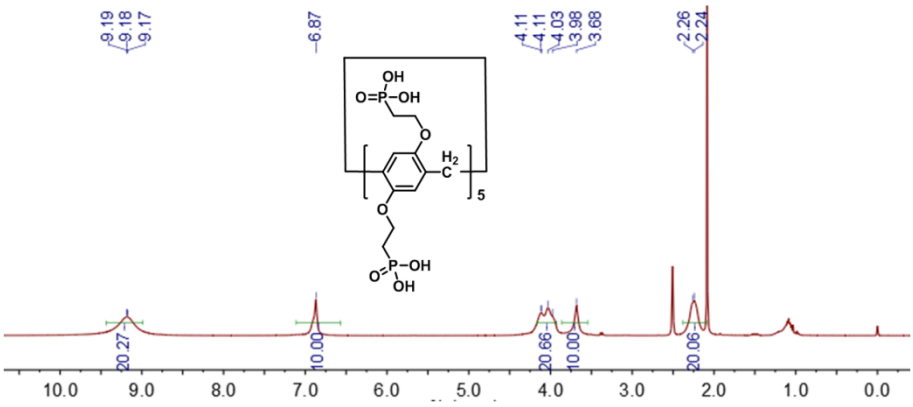


Fig. S5. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **3**.

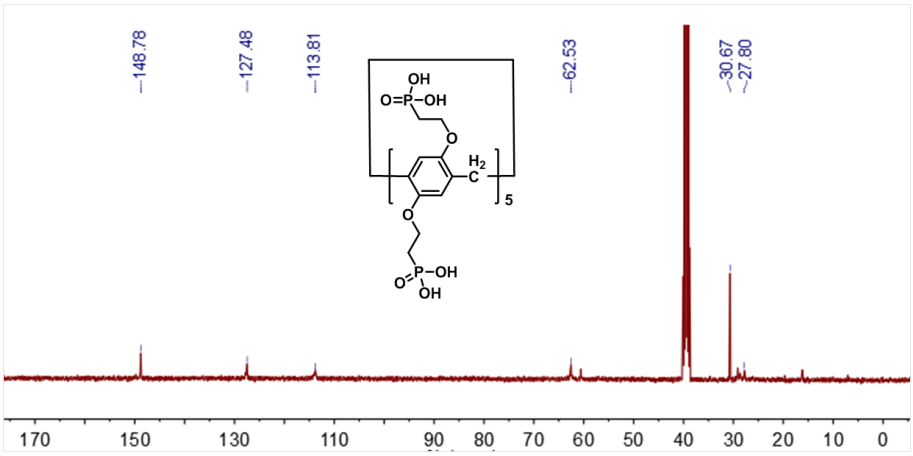


Fig. S6. ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of **3**.

Synthesis of **WP5-P**:[S2] 236 mg of NaOH (5.90 mmol) was added into an aqueous solution of **3** (1.00 g, 0.590 mmol) and stirred overnight. After the solvent was removed, **WP5-P** was obtained in quantitive yield as a yellow solid. ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm): 6.77 (br, 10H), 4.02 (m, 20H), 3.72 (m, 10H), 2.07 (br, 20H). ^{13}C NMR (100 MHz, D_2O, 298 K) δ (ppm): 149.77, 129.14, 116.01, 64.72, 32.09, 29.72.

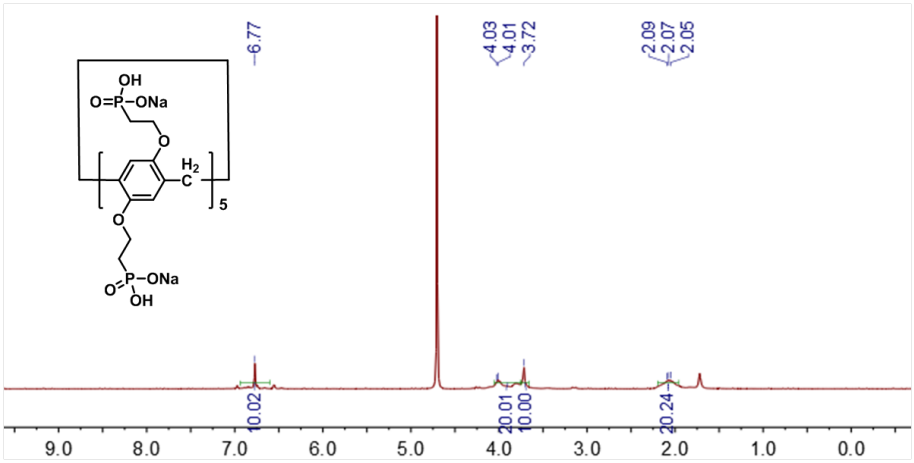


Fig. S7. ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **WP5-P**.

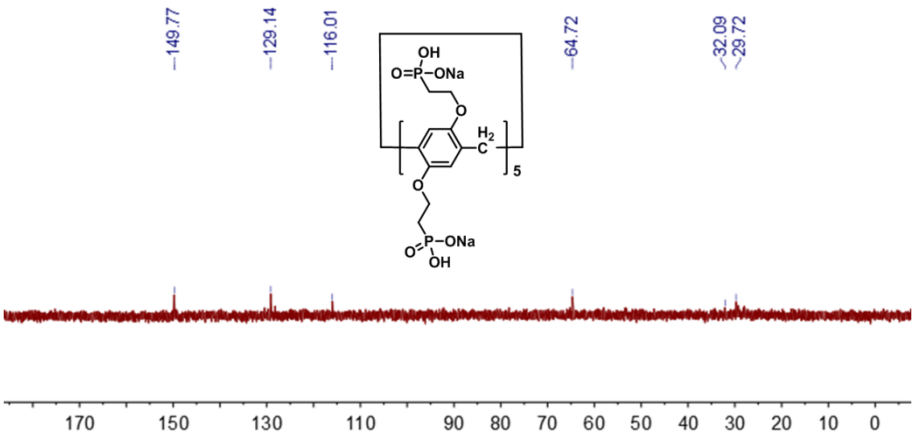
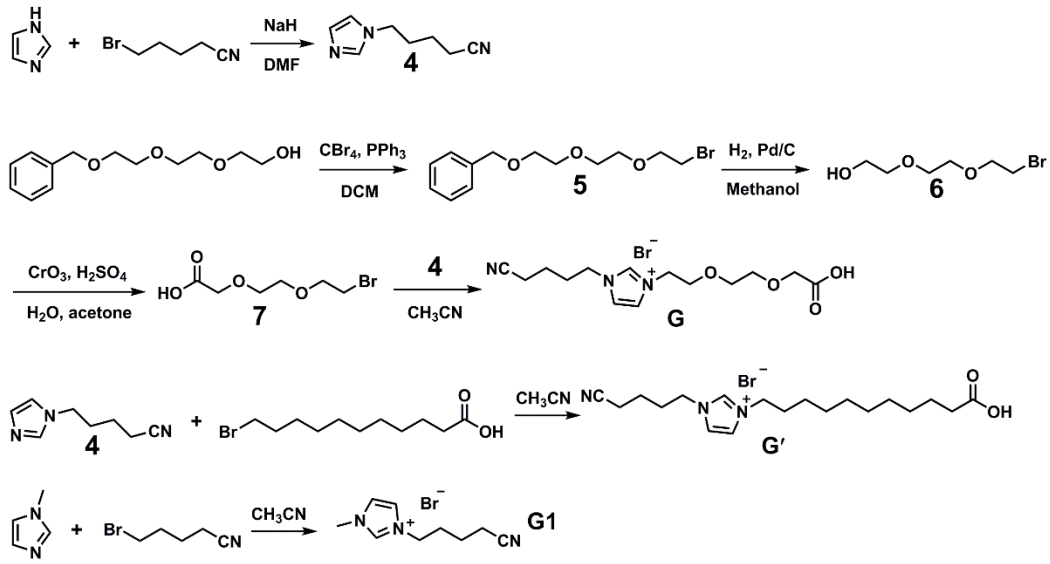


Fig. S8. ^{13}C NMR spectrum (100 MHz, D_2O, 298 K) of **WP5-P**.

Synthesis of guest molecules



Scheme S2. Synthesis of guest molecules.

Synthesis of compound **4**: To a solution of imidazole (6.80 g, 100 mmol) in 100 mL of DMF was added NaH (7.20 g, 300 mmol), the mixture was stirred at room temperature for 0.5 hour. 5-Bromopentanenitrile (20.0 g, 124 mmol) was added dropwise and reacted overnight. The solvent was removed and the crude product was purified by silica gel column chromatography using ethylacetate:methanol = 10:1 as an eluent to obtain the pure product as an orange oil (3.30 g, 22%). ^{1}H NMR (400 MHz, acetonitrile-d_3, 298 K) δ (ppm): 7.56 (s, 1H), 7.11 (s, 1H), 6.92 (s, 1H), 4.11 (t, J = 7 Hz, 2H), 2.51 (s, 2H), 1.93 (d, J = 8 Hz, 2H), 1.63 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm): 137.01, 129.80, 118.96, 118.65, 46.10,

29.99, 22.44, 16.88. HRESIMS: *m/z* calcd for $[M + H]^+$ $C_8H_{12}N_3^+$, 150.1031, found 150.1039, error 5 ppm.

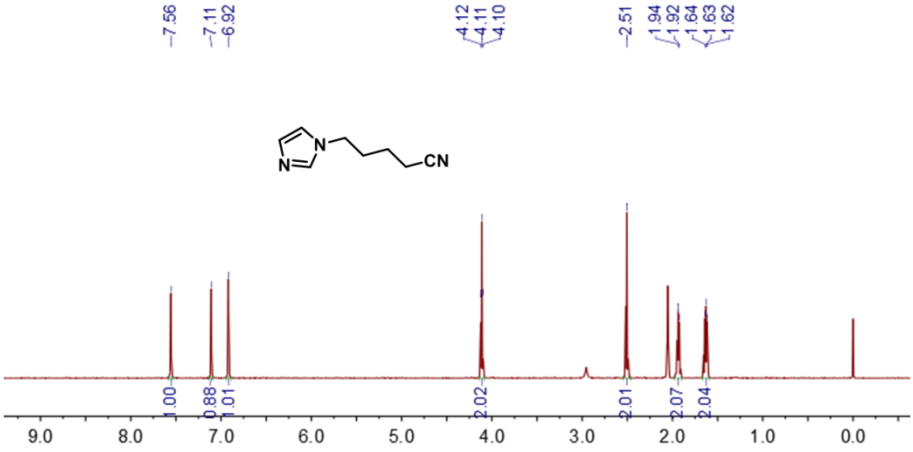


Fig. S9. ^{1}H NMR spectrum (400 MHz, acetonitrile-d_3, 298 K) of **4**.

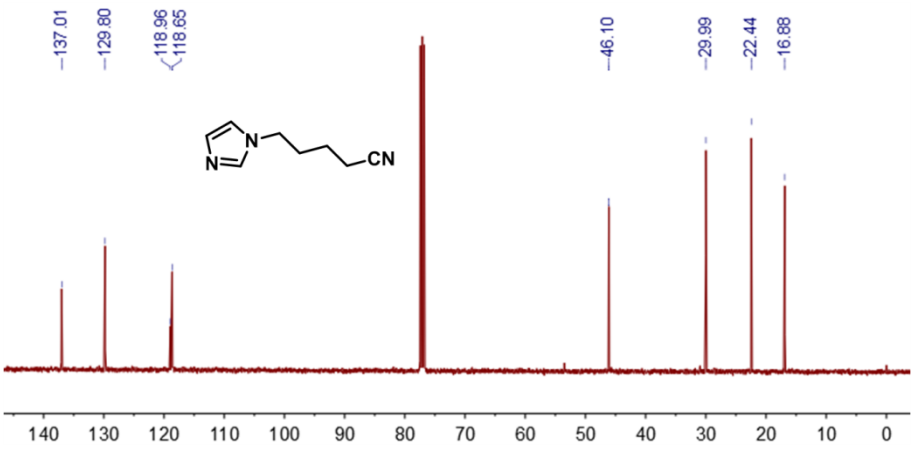


Fig. S10. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of **4**.

Synthesis of compound **5**: Carbon tetrabromide (6.62 g, 20.0 mmol) and triphenylphosphine (5.24 g, 20.0 mmol) were added into a solution of 2-(2-(2-(benzyloxy)ethoxy)ethoxy)ethan-1-ol (2.40 g, 10.0 mmol) in 200 mL of DCM and stirred for 6 hours at room temperature. The solvent was removed and the crude product was purified by silica gel column chromatography using hexane:ethyl acetate = 6:1 to obtain pure product as a pale yellow oil (3.00 g, 99%).^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 7.47–7.13 (m, 5H), 4.57 (s, 2H), 3.81 (t, J = 6.3 Hz, 2H), 3.71–3.66 (m, 6H), 3.64 (m, 2H), 3.46 (t, J = 6.3 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm): 138.24, 128.39, 127.70, 73.27, 71.24, 70.74, 70.66, 70.57, 69.45, 30.36. HRESIMS: *m/z* calcd for $[M + Na]^+$

$C_{13}H_{19}O_3BrNa^+$, 325.0410, found 325.0380, error –9.2 ppm.

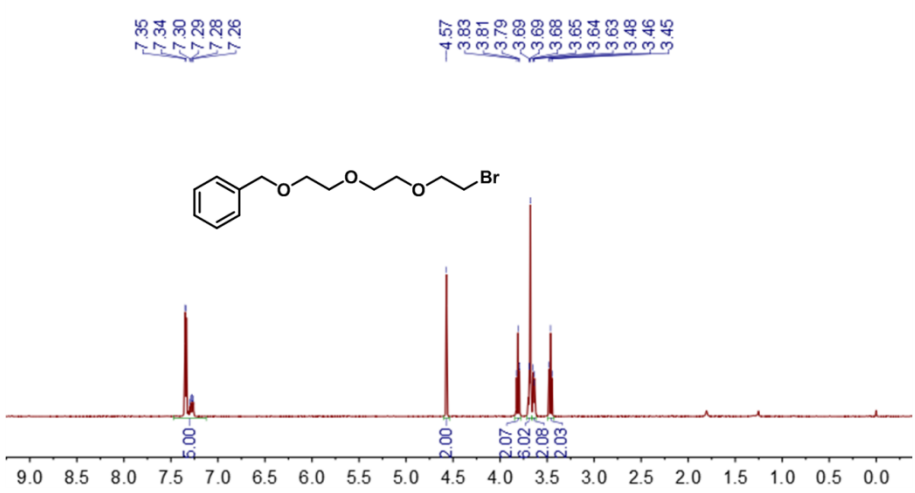


Fig. S11. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **5**.

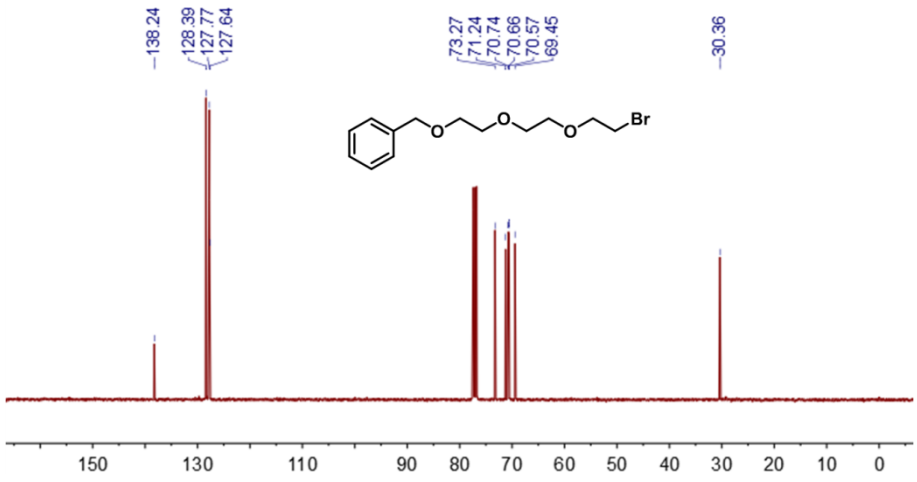


Fig. S12. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of **5**.

Synthesis of compound **6**: Compound **5** (3.00 g, 9.93 mmol) was dissolved in 100 mL of methanol. 3.00 g of Pd/C was added. The mixture was stirred in hydrogen atmosphere for 36 hours. The insoluble solid was filtered off. After the solvent was removed, the residual yellow oil (2.02 g, nearly quantitive yield) was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 3.83 (t, J = 6 Hz, 2H), 3.75 (t, 2H), 3.69 (s, 4H), 3.63 (t, 2H), 3.49 (t, J = 6 Hz, 2H), 2.24 (br, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm): 138.24, 128.39, 127.70, 73.27, 71.24, 70.74, 70.66, 70.57, 69.45, 30.36. HRESIMS: m/z calcd for $[M - H]^-$ $C_6H_{12}O_3Br^-$, 210.9975, found 210.9971, error –2 ppm.

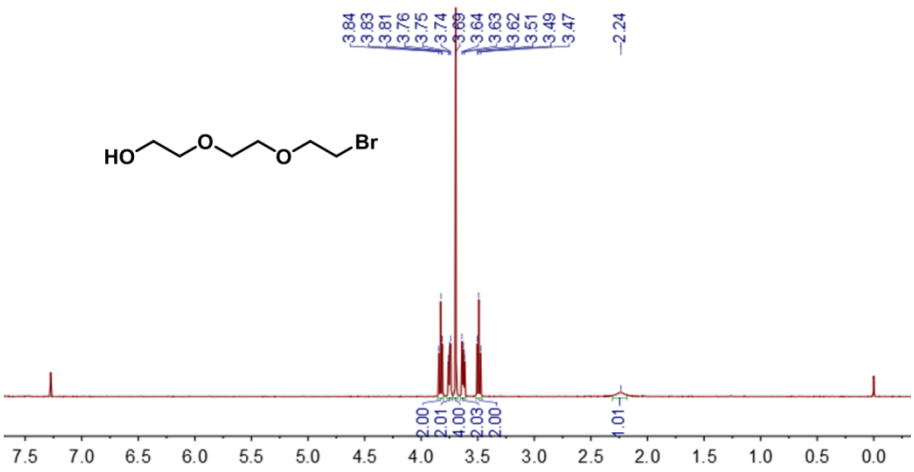


Fig. S13. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **6**.

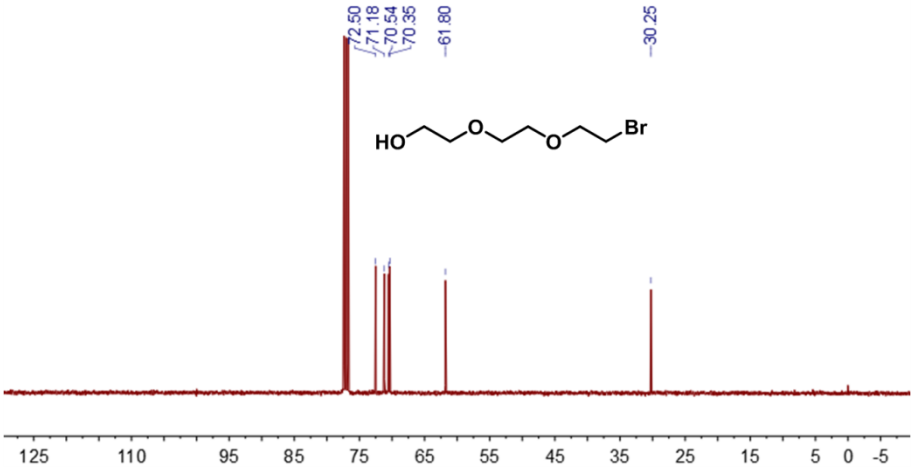


Fig. S14. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of **6**.

Synthesis of compound **7**: Freshly prepared Jones reagent (containing 5.00 g of chromium trioxide, 6.00 mL of H_2SO_4 and 50 mL of water) was added dropwise into a solution of compound **6** (2.02 g, 9.53 mmol) in 50 mL of acetone. After 3 hours, 5.00 mL of isopropanol was added to quench the reaction. After concentration in vacuum, the mixture was extracted with dichloromethane. The organic layer was concentrated and purified by silica gel column chromatography using dichloromethane:methanol = 20:1 to obtain pure product as a yellow oil (1.39 g, 62%).^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ (ppm): 4.20 (s, 2H), 3.83 (t, J = 6 Hz, 2H), 3.79–3.74 (m, 2H), 3.73–3.70 (m, 2H), 3.47 (t, J = 6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K) δ (ppm): 173.93, 71.22, 71.13, 70.30, 68.49, 30.00.

HRESIMS: *m*/*z* calcd for $[M - H]^-$ $C_6H_{10}O_4Br^-$, 224.9768, found 224.9753, error –6.6 ppm.

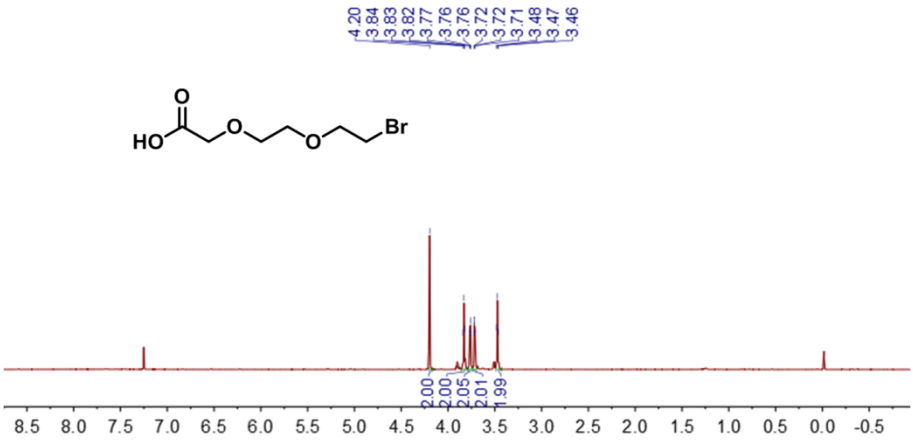


Fig. S15. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **7**.

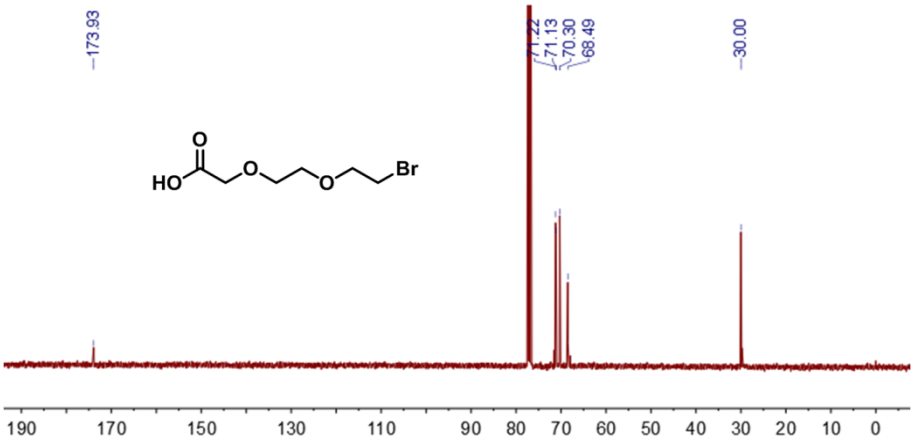


Fig. S16. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of **7**.

Synthesis of **G**: Compound **7** (0.225 g, 1.00 mmol) and compound **4** (0.119 mg, 0.800 mmol) were added to 10.0 mL of acetonitrile, and the mixture was refluxed overnight. After that, the solvent was removed under vacuum. The residue oil was dissolved in water, and washed with dichloromethane several times. After drying the aqueous layer, the residual orange oil was dissolved in minimum of methanol. Ethyl acetate and ethyl ether were added into the solution, and the mixture was cooled in liquid nitrogen until a yellow solid participate was formed. The participate was dissolved in methanol again. The procedure mentioned above was repeated ten times until the product was nearly pure. The final product (57.2 mg, 19%) was a yellow oil at room temperature. ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm): 8.92 (s, 1H), 7.61 (s, 1H), 7.58 (s, 1H), 4.45 (m, 2H), 4.31 (t, J = 7 Hz, 2H), 4.10 (s, 2H), 3.96 (m,

2H), 3.76 (dd, J = 6, 1.9 Hz, 4H), 2.58 (t, J = 7 Hz, 2H), 2.06 (t, J = 9, 7 Hz, 2H), 1.72 (t, 2H). ^{13}C NMR (100 MHz, D_2O, 298 K) δ (ppm): 172.12, 135.91, 122.89, 122.22, 121.25, 69.68, 68.54, 68.23, 48.78, 28.44, 21.45, 16.04. HRESIMS: m/z calcd for $[M–Br]^+$ $C_{14}H_{22}N_3O_4^+$, 296.1605, found 296.1599, error –2 ppm.

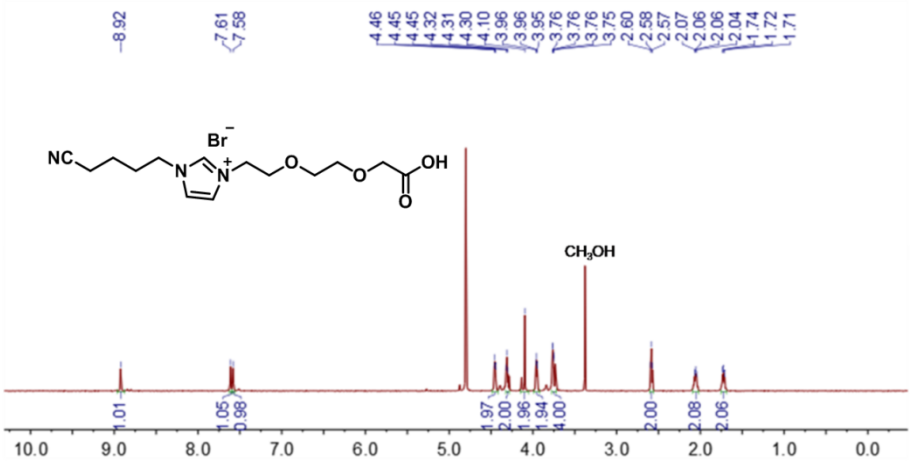


Fig. S17. ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **G**.

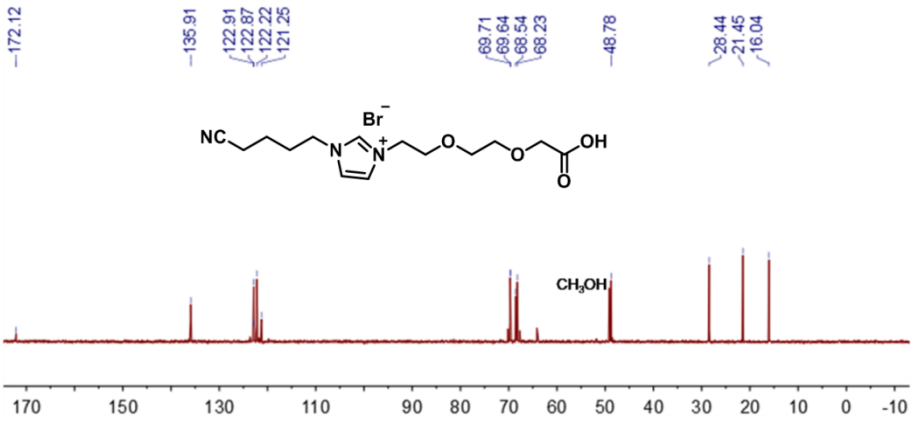


Fig. S18. ^{13}C NMR spectrum (100 MHz, D_2O, 298 K) of **G**.

Synthesis of **G′**: 11-Bromoundecanoic acid (264 mg, 1.00 mmol) and compound **4** (160 mg, 1.07 mmol) were mixed in 5.00 mL of acetonitrile and refluxed overnight. After cooling to room temperature, the product (white solid, 400 mg, yield 97%) was filtered and used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ (ppm): 11.95 (br, 1H), 9.26 (s, 1H), 7.83 (s, 2H), 4.22 (s, 2H), 4.17 (s, 2H), 2.57 (s, 2H), 2.18 (s, 2H), 1.95–1.84 (m, 2H), 1.79 (s, 2H), 1.59–1.51 (m, 2H), 1.48 (m, 2H), 1.24 (m, 12H). ^{13}C NMR (100 MHz,

DMSO-d_6, 298 K) δ (ppm): 174.47, 136.03, 122.52, 122.40, 120.35, 48.84, 47.96, 33.61, 29.22, 28.74, 28,71, 28.66, 28.49, 28.44, 28.28, 25.45, 24.44, 21.54, 15.69. HRESIMS: *m*/*z* calcd for $[M-Br]^+$ $C_{19}H_{32}N_3O_2^+$, 334.2489, found 334.2467, error –6.7 ppm.

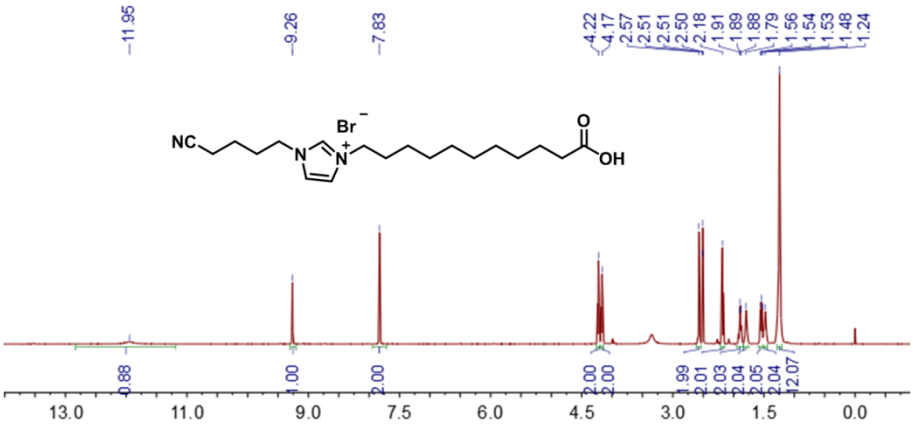


Fig. S19. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **G′**.

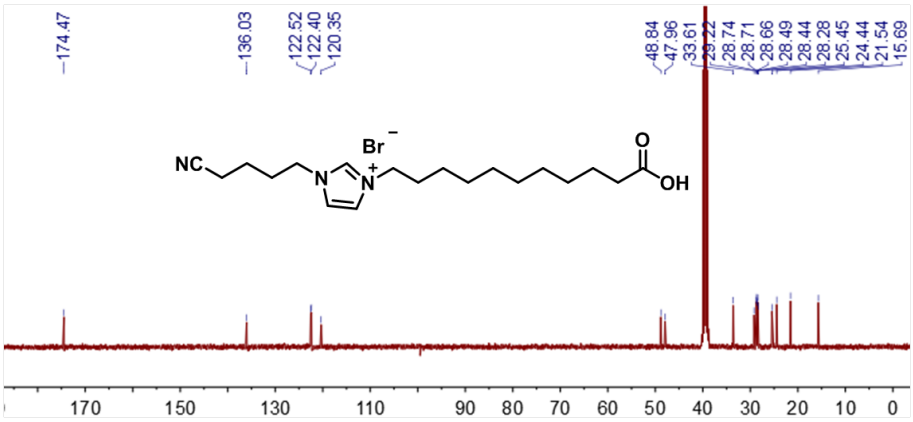


Fig. S20. ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of **G′**.

Synthesis of **G1**: 5-Bromopentanenitrile (170 mg, 1.07 mmol) was added into a solution of *N*-methylimidazole (69.0 mg, 1.00 mmol) in 5.00 mL of acetonitrile, and the mixture was refluxed overnight. After the solvent was removed, the residual oil was dissolved in water and washed with ethyl acetate three times. The product (220 mg, 91%) was obtained as a colorless oil by concentrating the aqueous layer. ^{1}H NMR (400 MHz, D_2O, 298 K) δ (ppm): 8.76 (s, 1H), 7.52 (s, 1H), 7.47 (s, 1H), 4.27 (t, J = 7 Hz, 2H), 3.91 (s, 3H), 2.57 (t, J = 7 Hz, 2H), 2.04 (m, 2H), 1.71 (m, 2H).^{13}C NMR (100 MHz, D_2O, 298 K) δ (ppm): 135.94, 123.65, 122.12, 121.23, 48.62, 35.65, 28.39, 21.39, 16.01. HRESIMS: *m*/*z* calcd for $[M-Br]^+$

$C_9H_{14}N_3^+$, 164.1182, found 164.1180, error –1 ppm.

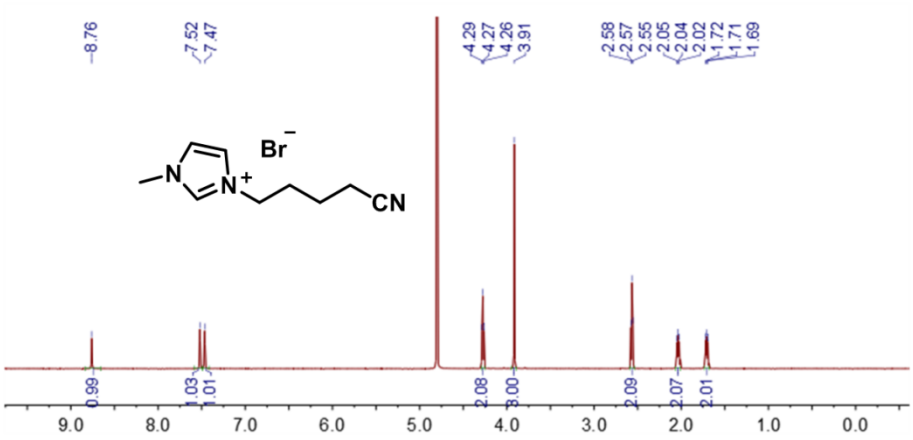


Fig. S21. ^{1}H NMR spectrum (400 MHz, D_2O, 298 K) of **G1**.

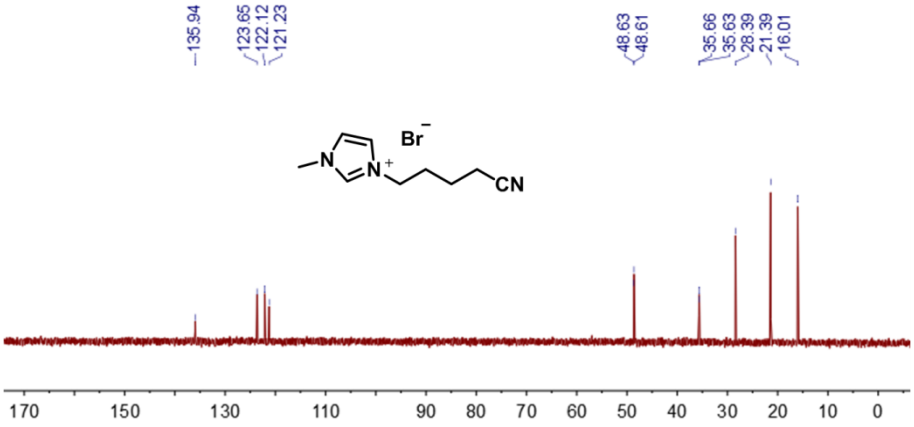


Fig. S22. ^{13}C NMR spectrum (100 MHz, D_2O, 298 K) of **G1**.

*3. Isothermal titration calorimetry (ITC) experiment of **WP5-P⊃G1***

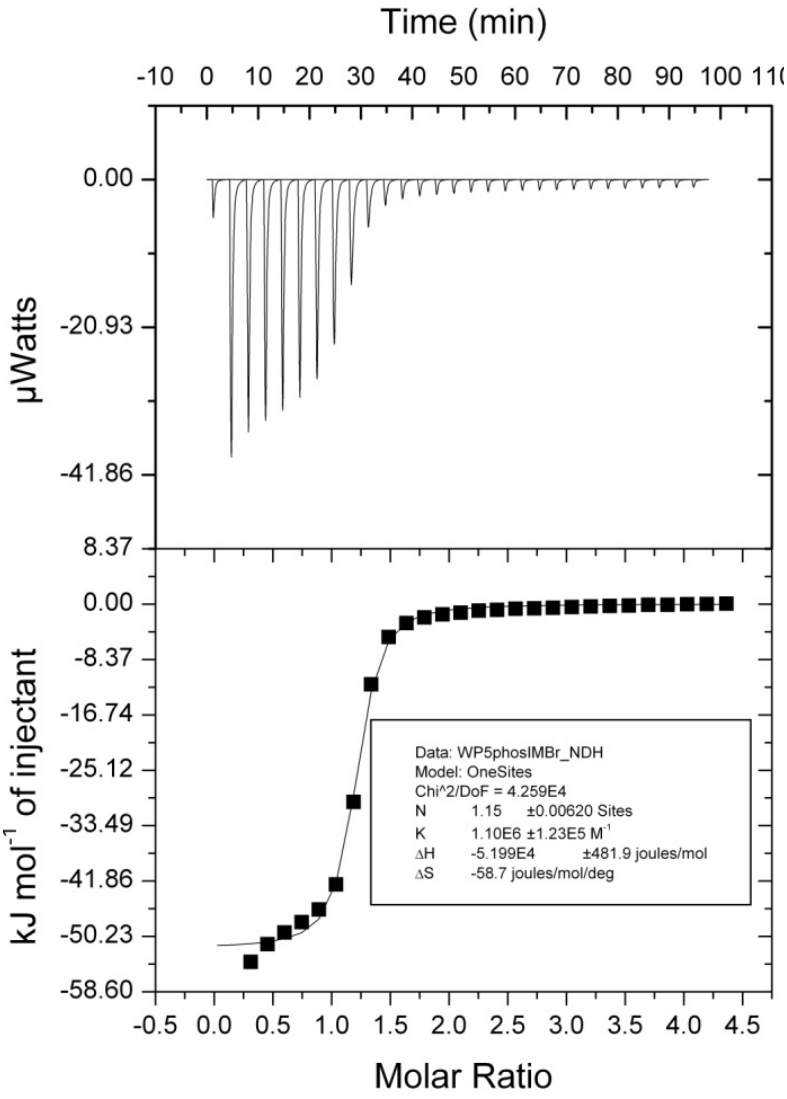
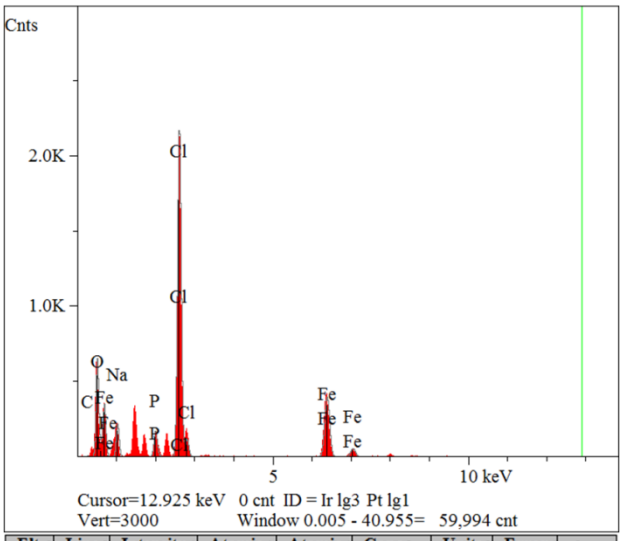


Fig. S23. ITC experiment of **WP5-P⊃G1**. From this experiment, the complexation stoichiometry between **WP5-P** and **G1** was determined to be 1:1, and the associate constant (K_a) was 1.10×10^6 M^{-1}.

4. *Energy-dispersive X-ray spectroscopy (EDS) characterization of **MNP-P5***



Elt.	Line	Intensity (c/s)	Atomic %	Atomic Ratio	Conc	Units	Error 2-sig	
C	Ka	2.11	2.323	0.0573	0.931	wt.%	1.118	
O	Ka	187.17	40.552	1.0000	21.651	wt.%	0.640	
Na	Ka	69.33	5.566	0.1373	4.270	wt.%	0.254	
P	Ka	62.39	2.332	0.0575	2.411	wt.%	0.159	
Cl	Ka	855.87	30.856	0.7609	36.504	wt.%	0.472	
Fe	Ka	210.29	18.370	0.4530	34.234	wt.%	0.907	
			100.000		100.000	wt.%		Total

Fig. S24. EDS characterization of **MNP-P5**. The spectrum (top) shows the analyzed elements, and the table (bottom) shows the approximate content of each element. The existence of C (2.32%) and P (2.33%) indicated **WP5-P** was successfully coated on **MNP**.

5. *Thermogravimetric analysis (TGA) of **MNP-P5***

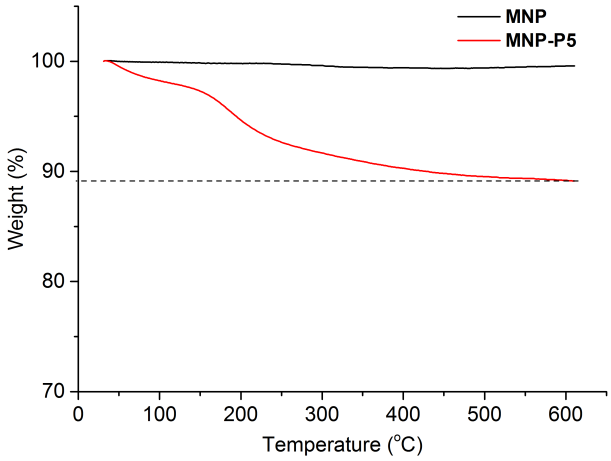


Fig. S25. TGA curves of **MNP** and **MNP-P5**.

6. *Scanning electronic microscopy (SEM) image of **MNP-P5***

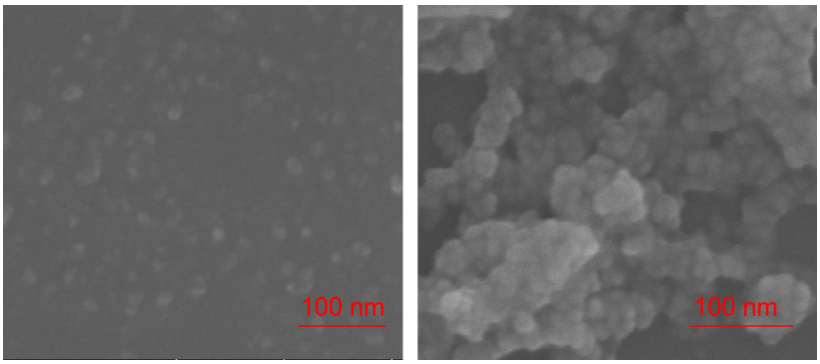
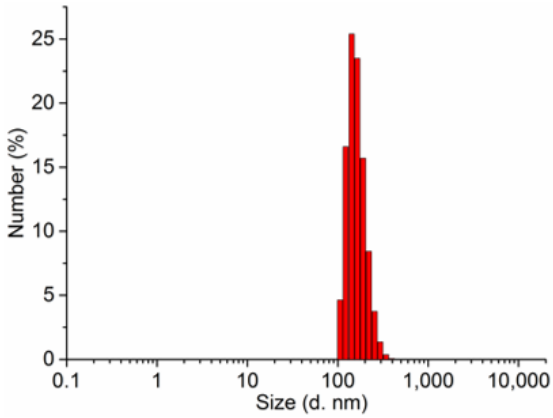


Fig. S26. SEM images of **MNP-P5**. Both of dispersed (left) and aggregated (right) **MNP-P5** existed after sonication. Even though the structures were not perfect spheres, nearly spherical nanoparticles could be observed.

7. *Dynamic light scattering (DLS) analysis and zeta potential analysis*



	WP5-P	MNP	MNP-P5
Zeta potential (mV)	−50.18	−15.68	−35.21

Fig. S27. Top: DLS analysis of **MNP-P5**. The average size was 239 nm. Bottom: zeta potential analysis of **WP5-P**, **MNP** and **MNP-P5**. The zeta potential of **MNP-P5** provided an explanation for its stability in water.

8. *Magnetic hysteresis loops of **MNP** and **MNP-P5***

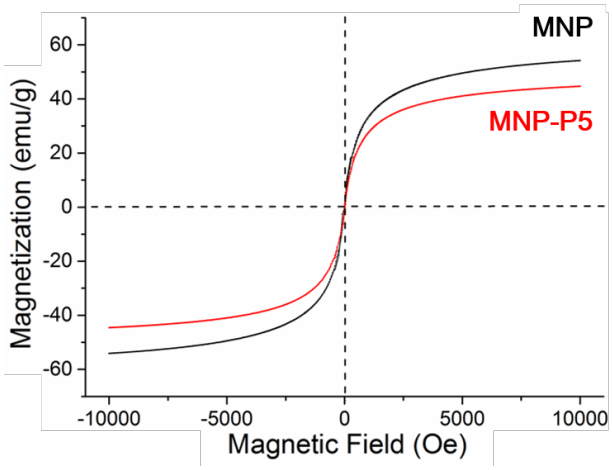


Fig. S28. Magnetic hysteresis loops of **MNP** (black line) and **MNP-P5** (red line). The saturated magnetization of **MNP** and **MNP-P5** were measured to be 54.1 emu/g and 44.6 emu/g, respectively.

9. *Fluorescence spectra of coumarin derivative **G2***

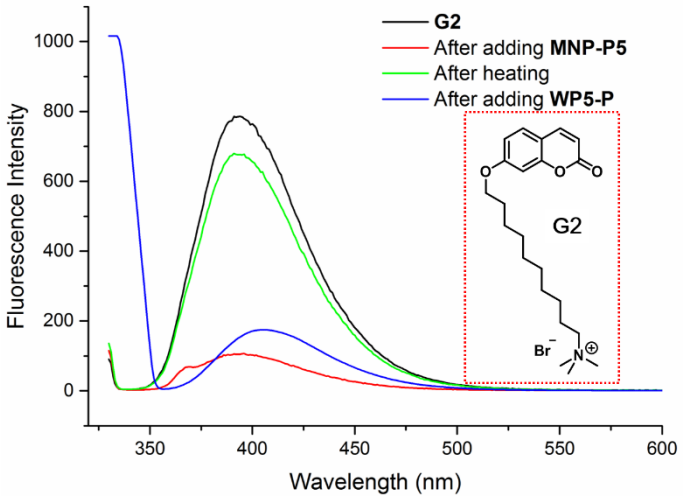


Fig. S29. Fluorescence spectra of **G2** in water. Black line: a solution of **G2** (10^{-3} mM). Red line: the supernatant of the solution after adding **MNP-P5** and separating with magnet. Green line: the supernatant of the solution after heating the suspension of **MNP-P5** and separating with magnet. Blue line: a solution of **G2** (10^{-3} mM) and **WP5-P** (5.00 equiv.). Note that a new peak appeared at 330 nm along with the fluorescence quenching after adding **WP5-P**.

*10. Mass spectra of BSA and **BSA-G***

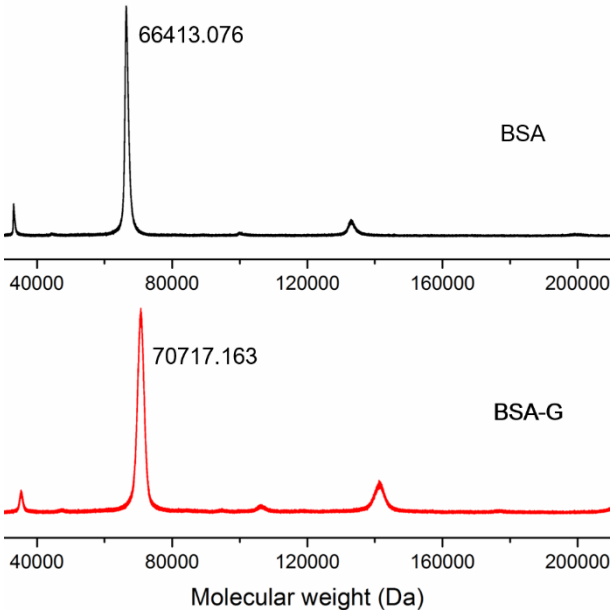


Fig. S30. The MALDI-TOF mass spectra of BSA (top) and **BSA-G** (bottom), indicating that the derivation of BSA was successful and highly efficient.

11. Measuring protein concentration using bicinchoninic acid (BCA) method

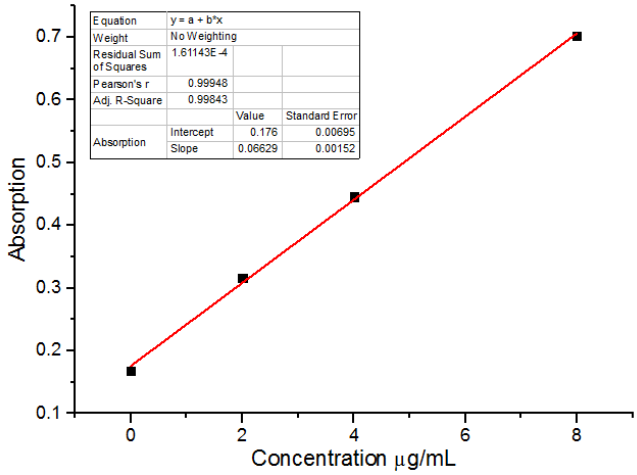


Fig. S31. The standard curve of BCA method for measuring protein concentration. The concentrations of **BSA-G** and IPEC cell lysate were determined to be 3.66 μg/mL and 12.2 μg/mL, respectively.

12. Studies of cell morphologies

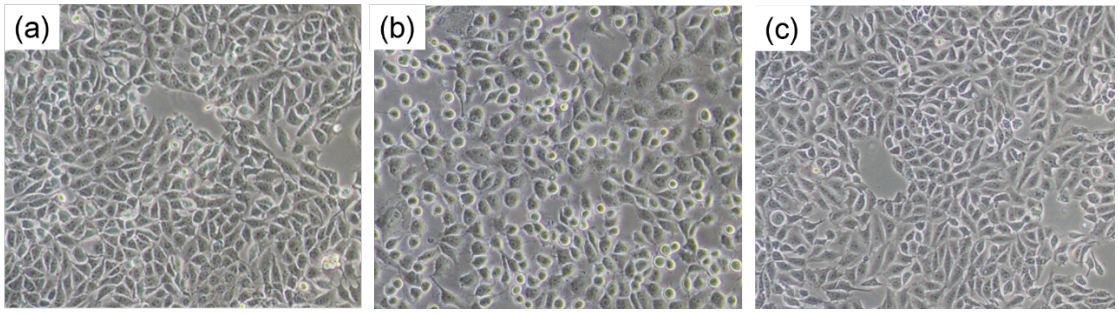


Fig. S32. Images: (a) IPEC-J2 cells; (b) IPEC-J2 cells treated with **G′** (1.00 equiv.), NHS (0.80 equiv.) and EDC (0.50 equiv.) for 30 min; (c) IPEC-J2 cells treated with **G** (1.00 equiv.), NHS (0.80 equiv.) and EDC (0.50 equiv.) for 30 min. The morphologies of IPEC-J2 cells didn't change after treatment with **G**, whereas the cell structures were destroyed and most of the cells were dead upon addition of **G′**. The explanation might be that **G′** was a surfactant which deconstructed the cell membrane. However, due to the polar polyglycol groups in **G**, the hydrophilicity of **G** increased, which rendered its membrane impermeant.

13 Comparisons between this study and literature reports

	This study	Ref. S5	Ref. S6	Ref. S7
Host	Pillar[5]arene	Cucurbit[7]uril	β-Cyclodextrin	Streptavidin
Guest	Imidazolium cation	Ferrocene	Prenyl group	Biotin
Separation method	Magnetic field	Centrifugation	Chromatography	Centrifugation
Advantages	1. Easy to synthesis 2. High affinity 3. Simple magnetic separation	1. Very high affinity and selectivity 2. Proteins are not denatured	1. Easy to synthesis 2. Large-scale separation	1. Very high affinity 2. Biocompatibility
Disadvantages	1. Oxygen sensitive 2. Proteins are denatured	1. Difficult in functionalization of CB	1. Mild affinity 2. Difficult in protein functionalization	1. Streptavidin beads are degradable 2. Interference with endogenous biotinylated proteins

14. References

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