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Electronic Supporting Information for

Biotinylated platinum(II) metallacage towards targeted cancer theranostics

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1. General experimental procedures

1.1 Materials and instruments

All reagents and deuterated solvents were commercially available and used without further purification. NMR spectra were recorded on a Bruker Avance 400 MHz or 600 MHz spectrometer. ^{1}H NMR chemical shifts were recorded relative to residual solvent signals. $^{31}P\{^{1}H\}$ NMR chemical shifts were referenced to an external unlocked sample of 85% H_3PO_4 (δ 0.0). Mass spectra were recorded on a Micromass Quattro II triple-quadrupole mass spectrometer using electrospray ionization with a MassLynx operating system. The UV-vis experiments were conducted on a Lambd 950 absorption spectrophotometer. The fluorescent experiments were conducted on an Edinburgh FSL920 fluorescence spectrometer using a 450 W Xe lamp as the steady-state excitation source. Fluorescent images were taken by inverted fluorescence microscope (DM505, Nikon Co., Ltd., Otawara, Tochigi, Japan). Compounds **3**,[S1] **4**,[S2] **5**[S3] and **7**[S4] were prepared according to the literature procedures.

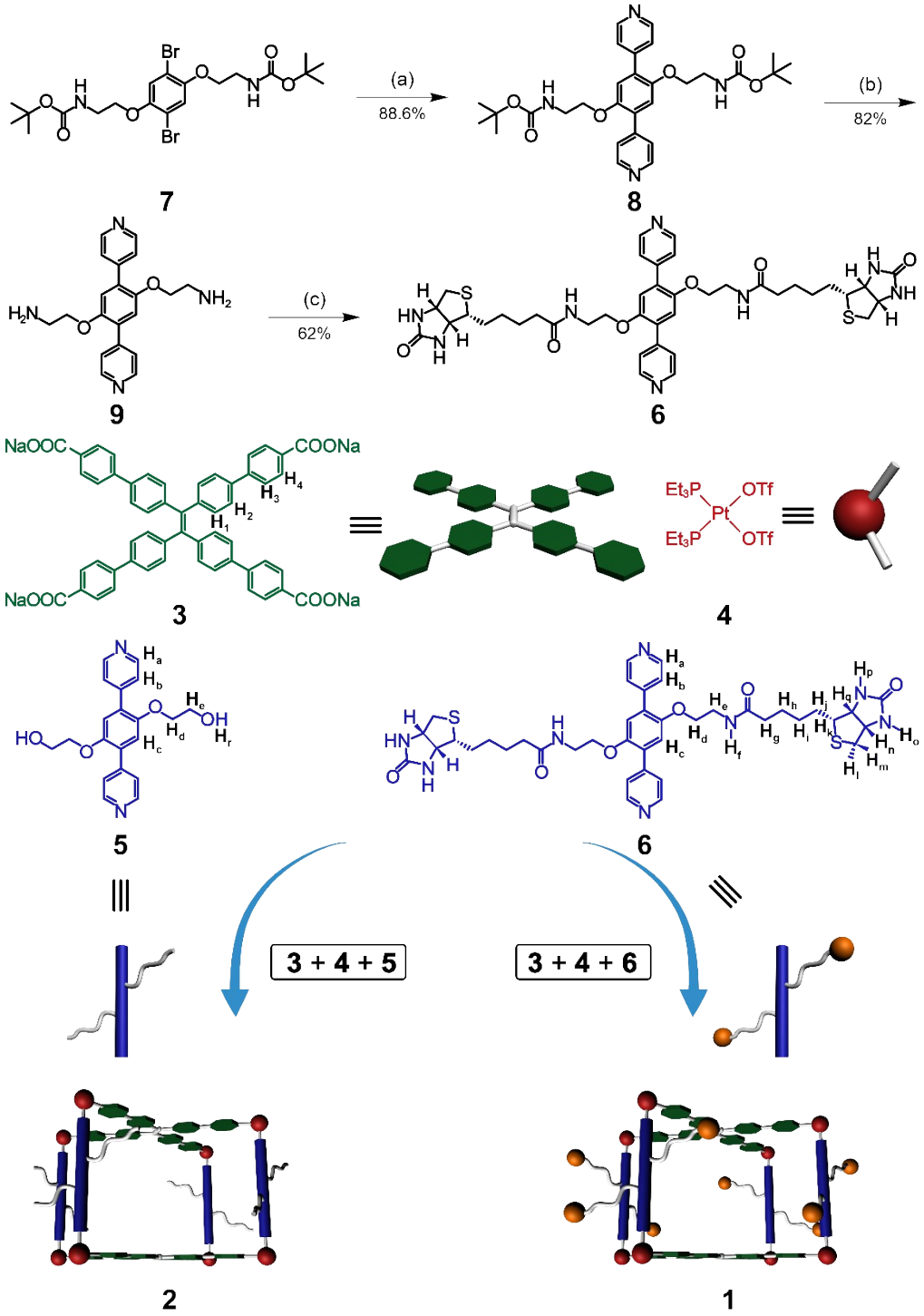
1.2 Cell culture and imaging by inverted fluorescence microscope

The HepG2 and HCT116 cancer cells were seeded at a density of 1×10^4/well in a 96-well plate in complete RPMI-1640 culture medium and incubated at 37 °C for 24 h for attachment. And then they were incubated simultaneously with the tested compounds (10 μM), DAPI for 1 h. Cells were washed twice by PBS and the photos were taken on an inverted fluorescence microscope (DM505, Nikon Co., Ltd., Otawara, Tochigi, Japan).

1.3. Cell culture for IC_{50} tests

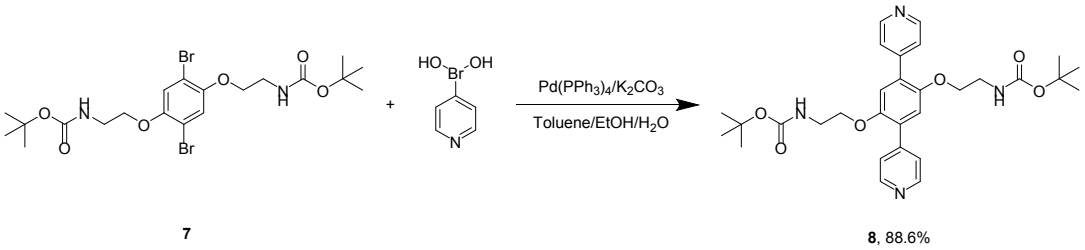
The cytotoxicity of metallacages **1**, **2** and cisplatin against different cancer cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were seeded at a density of 1.00×10^4/well in a 96-well plate and incubated for 24 h. Cells were then incubated with complete RPMI-1640 culture medium with metallacages **1**, **2** and cisplatin at various concentrations for 24 h. The medium was removed, and 40 μL MTT solution (2.00 mg/mL) was added to each well and then incubated at 37 °C for 4h.

2. *Synthetic Procedures and Characterization Data*



Scheme 1. Synthetic routes of biotinylated ligand **6** and metallacages **1** and **2**. Conditions: (a) 4-pyridylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, toluene/ethanol/H_2O (4:1:1, *v*/*v*), 85 °C, 48 h, 88.6%; (b) trifluoroacetic acid, DCM, 0°C, 3 h, 82%; (c) biotin *N*-hydroxy succinimide ester, DMF, 80 °C, 36 h, 62 %.

2.1 Synthesis of compound ***8***



Compound **7**[S4] (300 mg, 541 μmol), 4-pyridineboronic acid (159 mg, 1.30 mmol), tetrakis(triphenylphosphine)palladium (63 mg, 54.1 μmol) and K_2CO_3 (748 mg, 5.41 mmol) were dissolved in toluene (40 mL), ethanol (10 mL) and H_2O (10 mL) in a 100 mL Schlenk flask. Then the mixture was cooled by liquid nitrogen, degassed and purged with nitrogen for three times. The reaction mixture was heated at reflux for another 2 days under nitrogen. After cooling, the product was concentrated to give a crude product which was further purified by flash column chromatography with DCM:MeOH (40:1 to 10:1, *v*/*v*) as the eluent to afford compound **8** (264 mg, 88.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, 295K): 8.61 (s, 4H), 7.67 (s, 4H), 7.23 (s, 2H), 6.96 (s, 2H), 4.05 (s,4H), 3.32 (d, J = 3.3 Hz, 4H), 1.35 (d, J = 3.1 Hz, 18H).

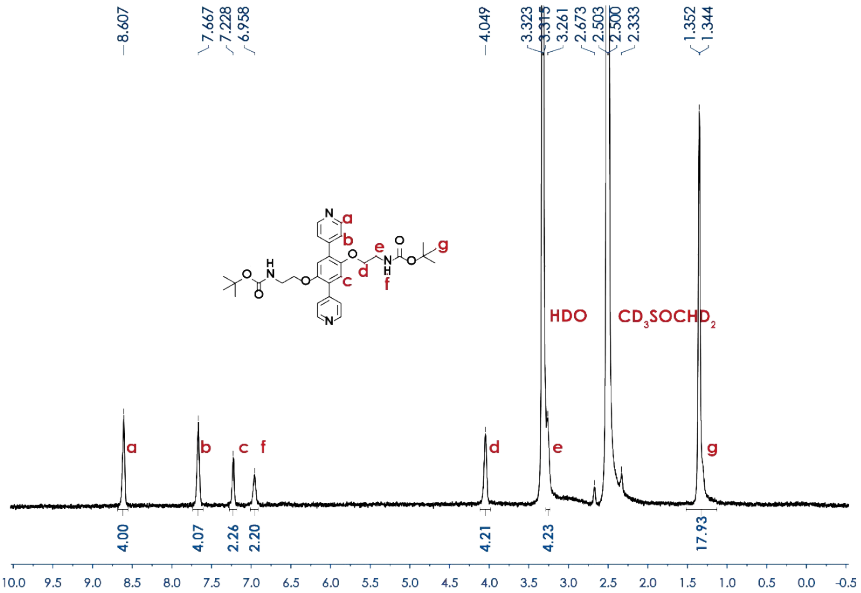
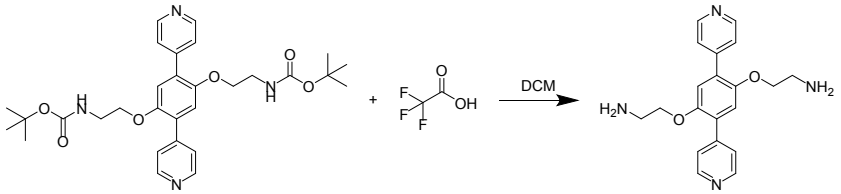


Fig. S1 ^{1}H NMR (400 MHz, DMSO-d_6, 295 K) spectrum of **8**

2.2 Synthesis of compound ***9***



8 **9**, 82%

Compound **8** (200 mg, 363.2 μmol) was dissolved into CH_2Cl_2 (20 mL) in a 50 mL-conical-flask. Then, trifluoroacetic acid (5 mL) was added dropwise to the system in ice-bath. After addition, the mixture was stirred for another 2 h. Then the solvent was removed and the residue was washed by saturated Na_2CO_3 solution (50 mL) and purified by flash chromatographic column with DCM/MeOH/$NH_3 \cdot H_2O$ (10:1:0.1, *v*/*v*) as the eluent to give compound **9** (104 mg, 82%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6, 295K): 8.63 (dd, J = 4.5, 1.6 Hz, 4H), 7.65 (dd, J = 4.5, 1.6 Hz, 4H), 7.19 (s, 2H), 4.00 (t, J = 5.7 Hz, 4H), 2.81 (t, J = 5.6 Hz, 4H), 1.94 (s, 4H).

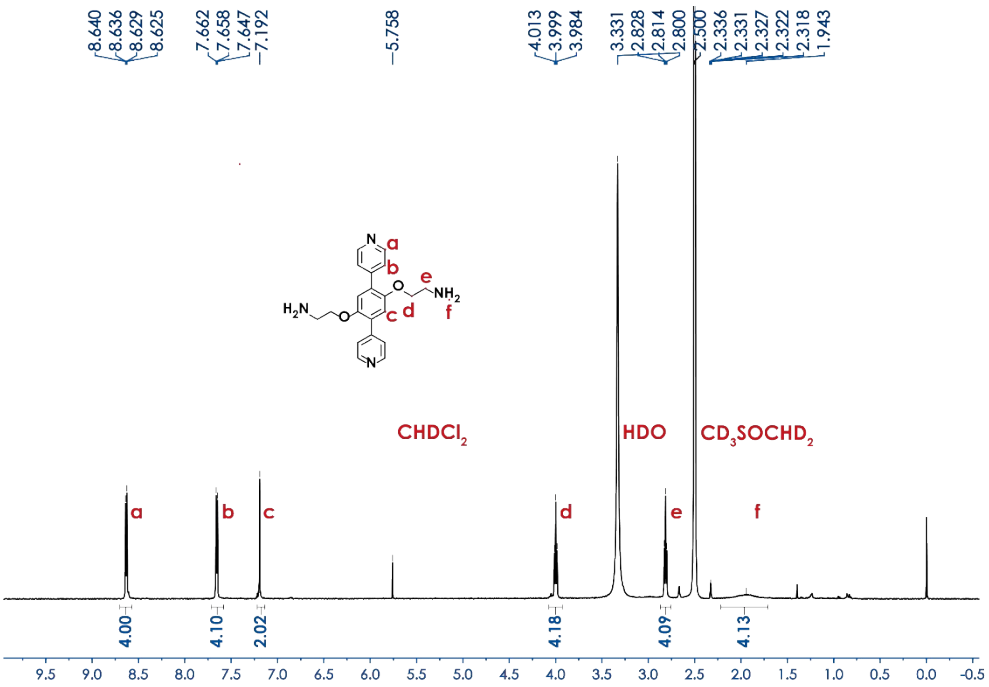
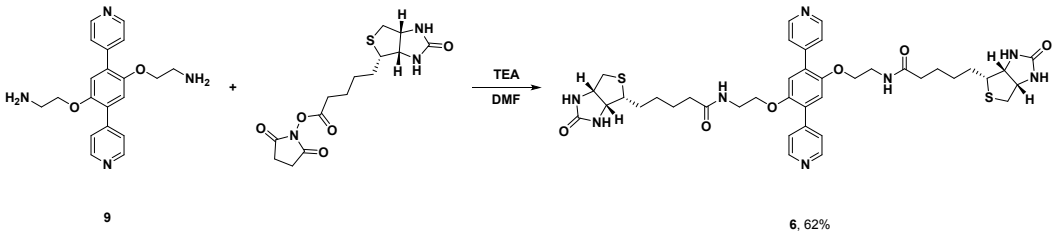


Fig. S2 ^{1}H NMR (400 MHz, DMSO-d_6, 295 K) spectrum of **9**

2.3 Synthesis of compound ***6***



9 **6**, 62%

Compound **9** (60 mg, 171 μmol) and biotin-NHS (129 mg, 377 μmol) were dissolved in anhydrous DMF (20 mL) in a 50 mL round bottom flask . Then, TEA (35 mg, 342 μmol) was added into the flask under the ice bath. The flask underwent three freeze-vacuum-thaw cycles and finally was placed in an oil bath at 60 ℃ for 24 h. After cooling, the solvent was removed. TEA should be pay attention to because it will affect the subsequent purification process. The mixture was purified by flash column chromatography with DCM/MeOH/$NH_3 \cdot H_2O$ (10:1:1, *v*/*v*) as the eluent to give compound **6** (85 mg, 62%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6, 295K): 8.61 (d, J = 5.6 Hz, 4H), 7.92 (t, J = 5.4 Hz, 2H), 7.66 (d, J = 5.7 Hz, 4H), 7.22 (s, 2H), 6.43 (s, 2H), 6.35 (s, 2H), 4.33–4.22 (m, 2H), 4.08 (dt, J = 11.0, 5.5 Hz, 6H), 3.37 (dd, J = 11.0, 5.6 Hz, 4H), 3.04 (dt, J = 8.2, 6.0 Hz, 2H), 2.78 (dd, J = 12.4, 5.1 Hz, 2H), 2.55 (d, J = 12.4 Hz, 2H), 1.65–1.19 (m, 12H).

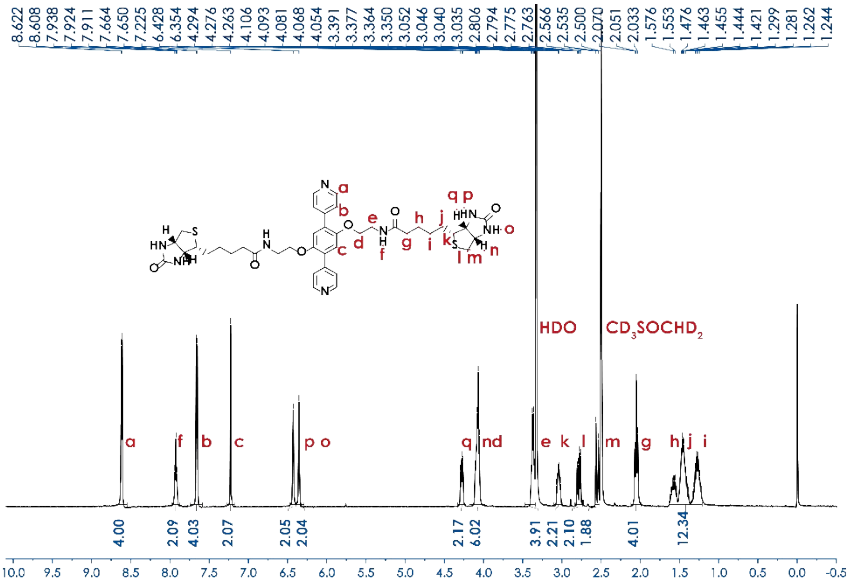


Fig. S3 ^{1}H NMR (600 MHz, DMSO-d_6, 295 K) spectrum of **6**

*2.4 Synthesis of metallacage **1***

Compound **6** (5 mg, 6.2 μmol) was added in DMSO (1 mL) in a 20 mL glass vial. A mixture of **3** (2.80 mg, 3.11 μmol) and **4** (9.08 mg, 12.45 μmol) was added in acetone (4 mL) and H_2O (1 mL) in a separated glass vial. The two vials were stirred at 50 °C until all the compounds were totally dissolved. After that, the solutions in the two vials were mixed together and the mixture was stirred in 50 °C for another 12 h. After cooling, the solvent was removed by nitrogen flow. The residue was redissolved in acetone (1.0 mL), filtered and the filtrate was poured into ethyl ether (10.0 mL) or toluene (10 mL) to give a precipitate, which was collected by centrifugation to give metallacage **1**(14.5 mg, 86%) as a light-yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6, 295K): 8.76 (d, J = 2.4 Hz, 16H), 7.94 (d, J = 5.0 Hz, 24H), 7.59 (d, J = 8.2 Hz, 16H), 7.49 (d, J = 8.3 Hz, 16H), 7.43 (d, J = 8.3 Hz, 16H), 7.06 (d, J = 8.2 Hz, 16H), 6.41 (s, 8H), 6.37 (s, 8H), 4.28 (dd, J = 7.4, 5.4 Hz, 16H), 4.09 (dd, J = 8.8, 3.1 Hz, 16H), 3.38 (dt, J = 10.2, 5.1 Hz, 16H), 3.08 – 3.03 (m, 8H), 2.78 (dd, J = 12.4, 5.0 Hz, 8H), 2.55 (d, J = 11.4 Hz, 8H), 2.10 (dd, J = 13.0, 5.6 Hz, 16H), 2.07–1.72 (m, 96H), 1.66 – 1.40 (m, 48H), 1.40–1.08 (m, 142H). ^{31}P{^{1}H} NMR (162 MHz, DMSO-d_6, 295 K): 5.94 ppm (d, $^2J_{P\text{-}P}$ = 20.7 Hz, ^{195}Pt satellites, $^1J_{Pt\text{-}P}$ = 2513 Hz), 0.97 ppm (d, $^2J_{P\text{-}P}$ = 20.7 Hz,^{195}Pt satellites, $^1J_{Pt\text{-}P}$ = 2513 Hz). ESI-TOF-MS: *m*/*z* 1034.7814 [**1** – 8OTf]$^{8+}$, 1429.7222 [**1** – 6OTf]$^{6+}$, 1745.7803 [**1** – 5OTf]$^{5+}$.

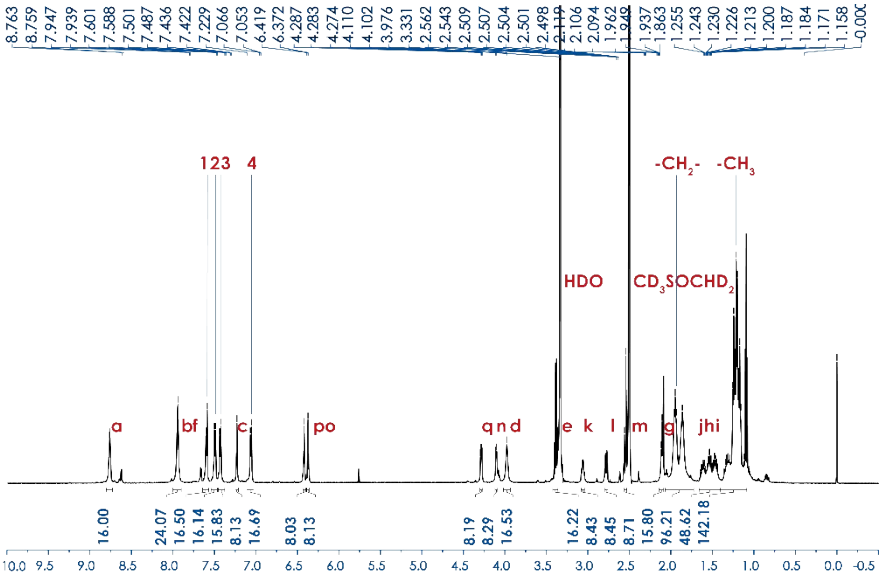


Fig. S4 ^{1}H NMR (600 MHz, DMSO-d_6, 295 K) spectrum of metallacage **1**

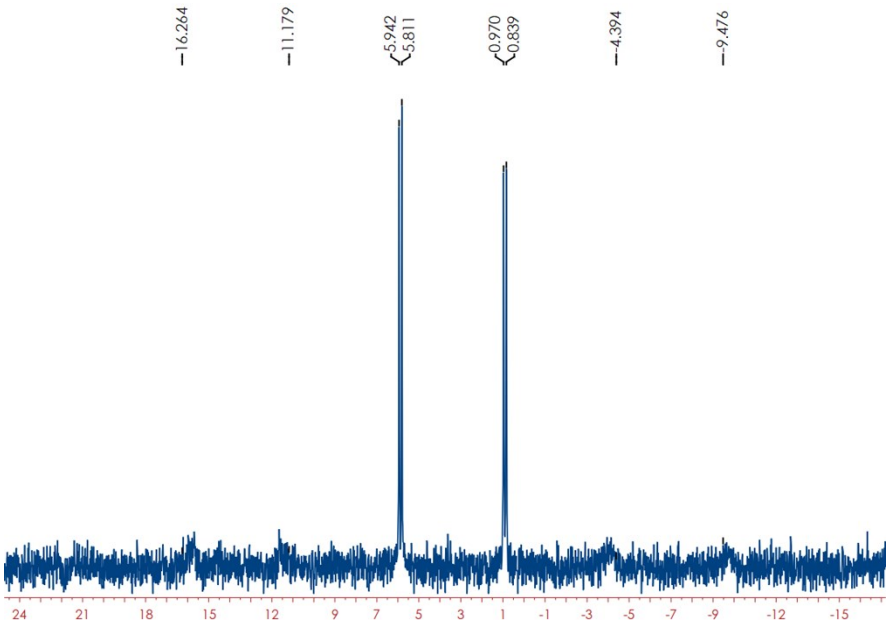


Fig. S5 $^{31}P\{^{1}H\}$ NMR (162 MHz, DMSO-d_6, 295 K) spectrum of metallacage **1**

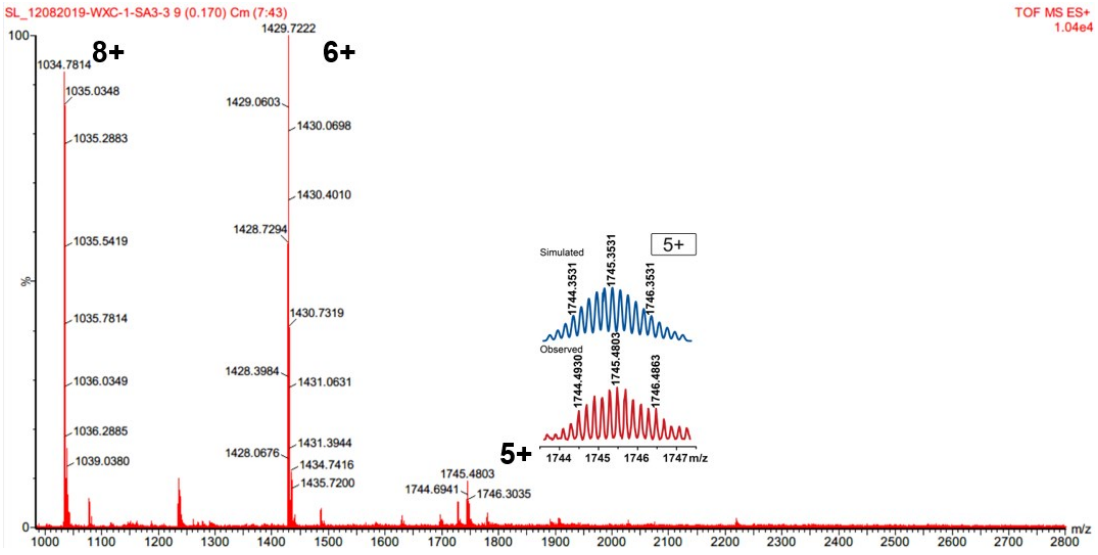


Fig. S6 ESI-TOF-MS spectrum of metallacage **1**

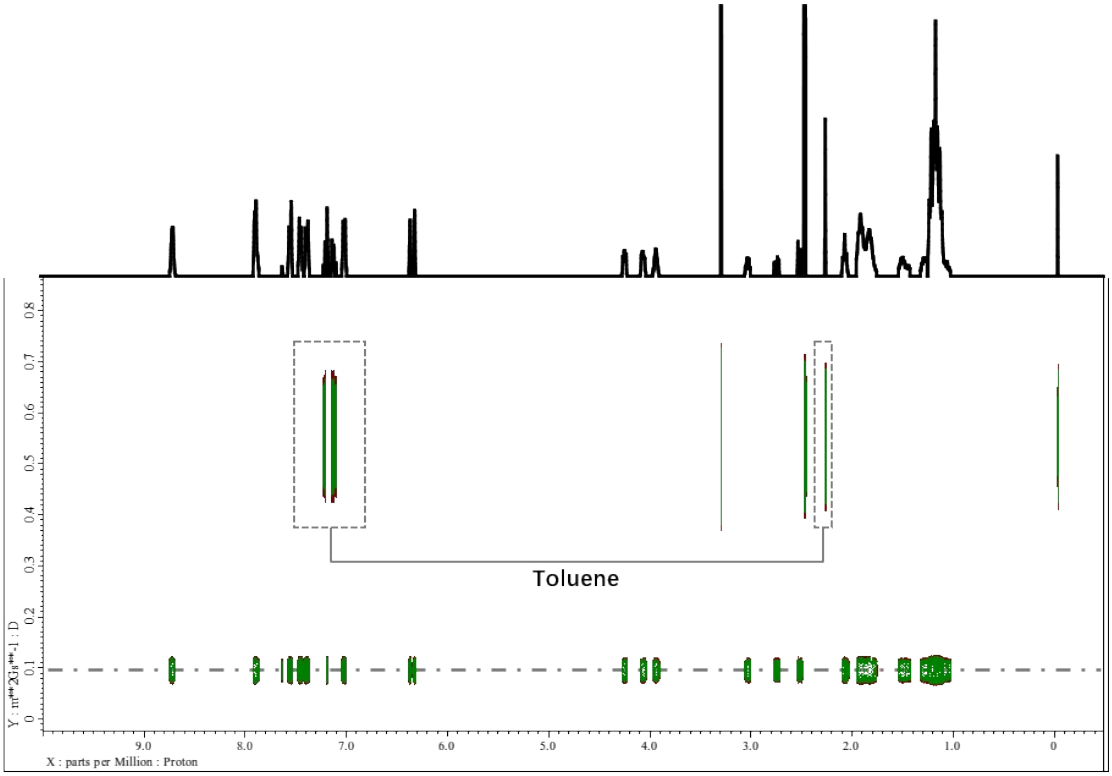


Fig. S7 2D DOSY spectra (400 MHz, 298 K, DMSO-d_6) recorded for metallacage **1**

*2.5 Synthesis of metallacage **2***

A mixture of **5** (14.10 mg, 40 μmol), **3** (18 mg, 20 μmol) and **4** (58.36 mg, 80 μmol) was dissolved in acetone (8 mL) and H_2O (2 mL) in a 20 mL glass vial. The suspension was shaken in the ultrasonic instrument and heated at 50 °C until it is evenly dispersed. The mixture was stirred at 50 °C for another 12 h. After cooling, the solvent was removed by nitrogen flow. The residue was redissolved in acetone (1.0 mL), filtered and the filtrate was poured into ethyl ether (10.0 mL) to give a precipitate, which was collected by centrifugation to give metallacage **2** (83.22 mg, 92%) as a light-yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6, 295K): 8.77 (s, 16H), 8.01 (d, J = 5.7 Hz, 16H), 7.59 (d, J = 8.2 Hz, 16H), 7.48 (d, J = 8.2 Hz, 16H), 7.42 (d, J = 8.2 Hz, 16H), 7.21 (s, 8H), 7.05 (d, J = 8.2 Hz, 16H), 4.90 (t, J = 5.3 Hz, 8H), 4.03 (s, 16H), 3.64 (d, J = 4.3 Hz, 16H), 2.04–1.73 (m, 96H), 1.36–1.09 (m, 144H). ^{31}P{^{1}H} NMR (162 MHz, DMSO-d_6, 295 K): 5.80 ppm (d, $^2J_{P\text{-}P}$ = 21.3 Hz, ^{195}Pt satellites, $^1J_{Pt\text{-}P}$ = 3355 Hz),0.84 ppm (d, $^2J_{P\text{-}P}$ = 21.2 Hz,^{195}Pt satellites, $^1J_{Pt\text{-}P}$ = 3355 Hz). ESI-TOF-MS: m/z 1129.4442 [**2** - 6OTf]$^{6+}$, 1385.1743 [**2** - 5OTf]$^{5+}$, 1768.7821 [**2** - 4OTf]$^{4+}$.

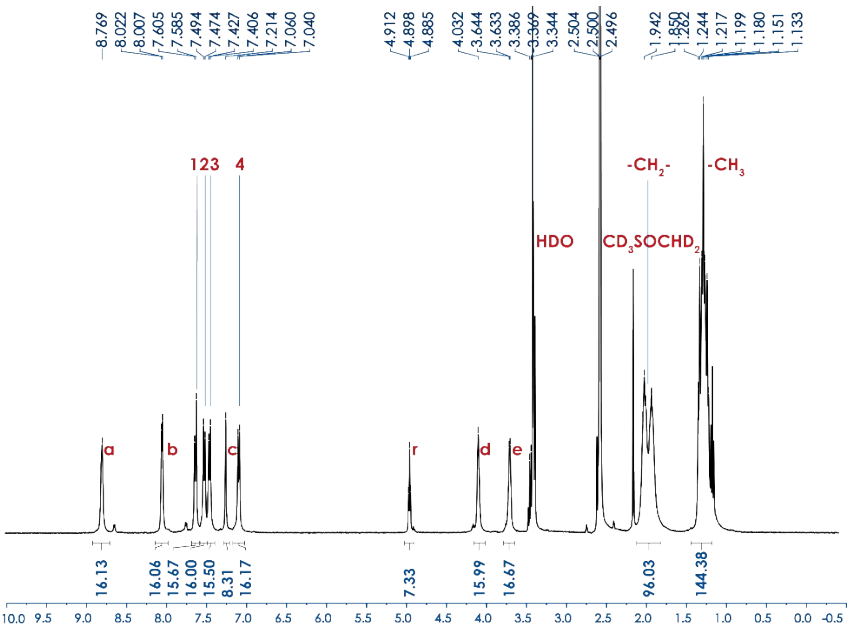


Fig. S8 ^{1}H NMR (600 MHz, DMSO-d_6, 295 K) spectrum of metallacage **2**

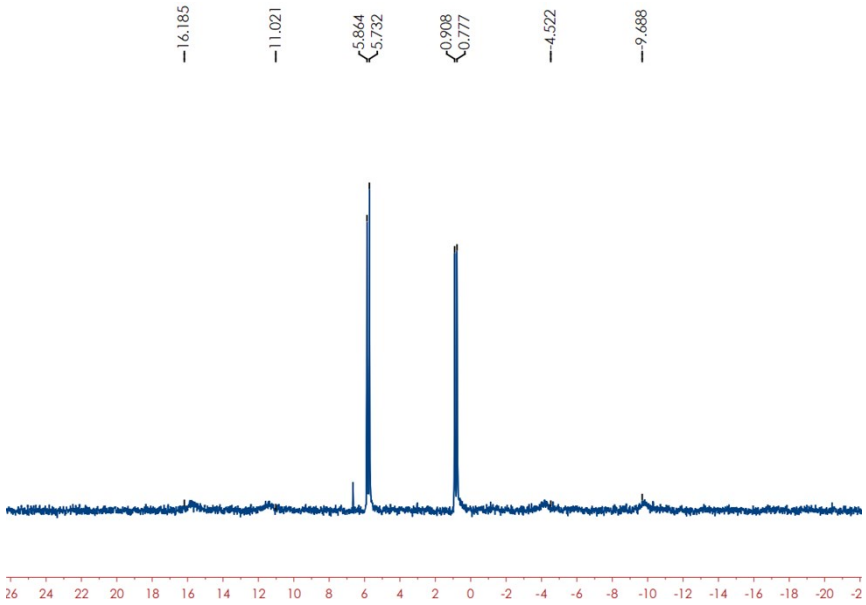


Fig. S9 $^{31}P\{^{1}H\}$ NMR (162 MHz, DMSO-d_6, 295 K) spectrum of metallacage **2**

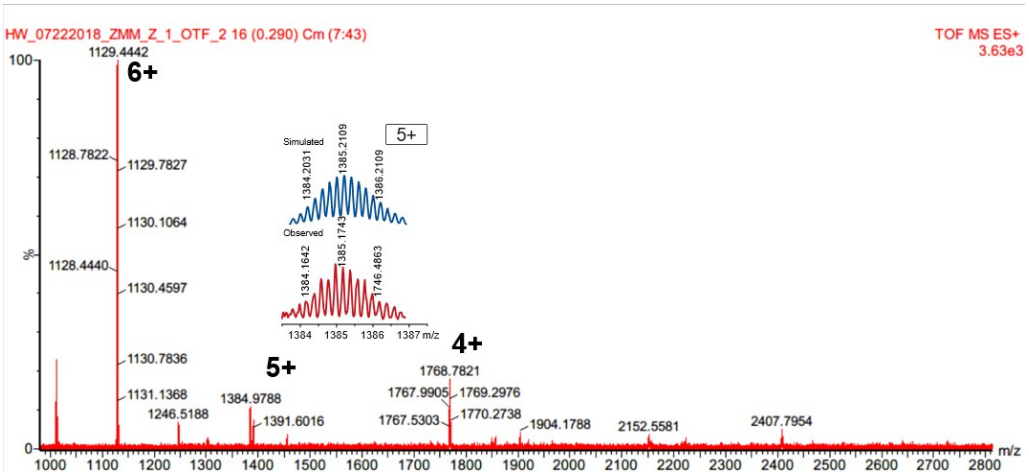


Fig. S10 ESI-TOF-MS spectrum of metallacage **2**

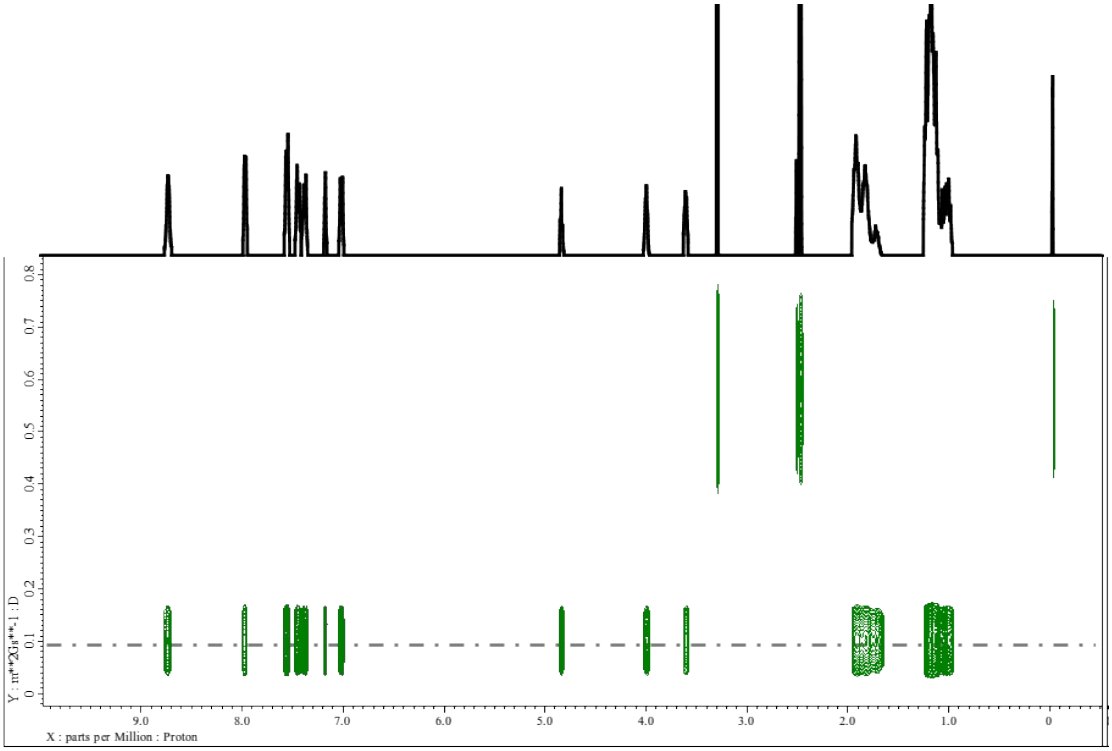


Fig. S11 2D DOSY spectra (400 MHz, 298 K, DMSO-d_6) recorded for metallacage **2**

3. Flow cytometry measurements

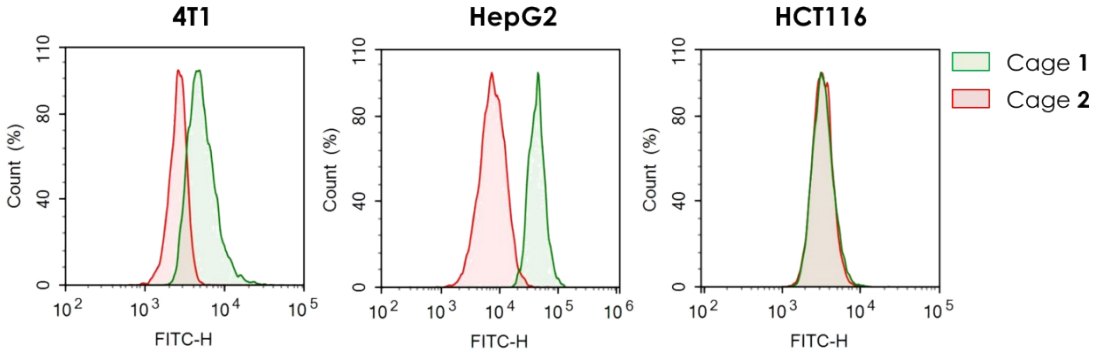


Fig. S12 Flow cytometry of 4T1, HepG2 and HCT116 cells after being incubated with 10 μM cage **1** or cage **2** for 1h. The experiment is representative of three independent repetitions.

*4. Stability of metallacage **1** and metallacage **2***

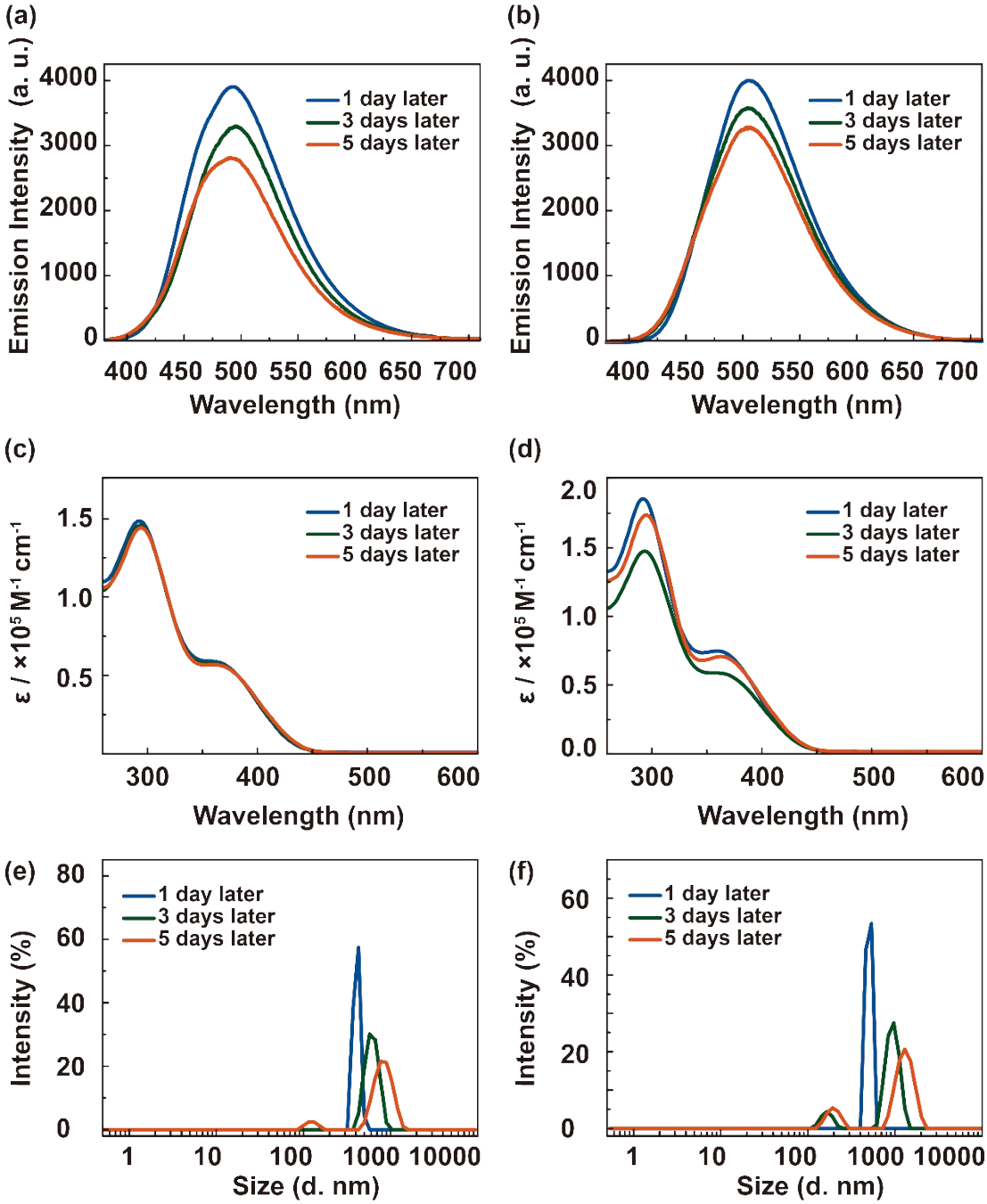


Fig. S13 Emission spectra (λ_{ex} = 365 nm, c =10.0 μM) of (a) cage **1** and (b) cage **2** in 1% DMSO/H_2O. UV-vis absorption spectra (c =10.0 μM) of (c) cage **1** and (d) cage **2** 1% DMSO/H_2O. DLS measurements (c =10.0 μM) of (e) cage **1** and (f) cage **2** 1% DMSO/H_2O.

Reference

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