Supporting Information

Pro-guest and acyclic-cucurbit[n]uril conjugated polymers for controlled release of anti-tumor drugs

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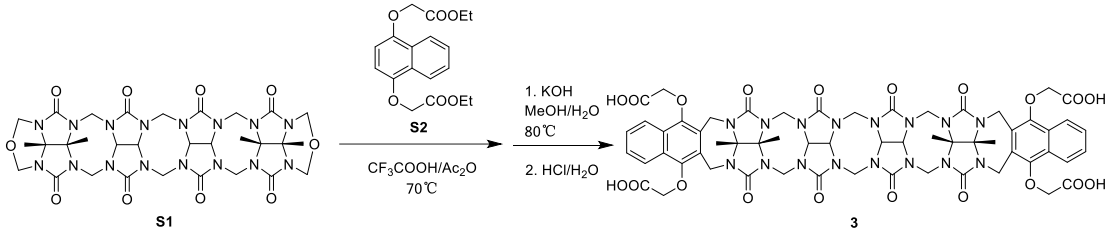
General Experimental

All reagents and solvents were purchased from commercial suppliers and used without further purification. Compound **S1** and **S2** were synthesized according to literature procedures.[1-2] NMR spectra were recorded on a Bruker AVANCE III HD. FT-IR spectra were recorded on a ThermoFisher Nicolet iS10 spectrometer. UV-Vis spectroscopy was recorded on a Cary 100 spectrometer (Agilent). Fluorescence spectroscopy was conducted on a RF-6000 fluorescence spectrometer (Shimadzu). High resolution mass spectrometry (HR-MS) was acquired using a Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (ThermoFisher) equipped with a Dionex Ultimate 3000 HPLC system (ThermoFisher). Melting point was determined by a microscopic melting point apparatus (Shanghai Jingke). Fluorescence microscopy was carried out on a Leica DMi 8 microscope (Leica, Germany).

Synthetic Procedures

Container **3**：Compound **S1** (1.0 g, 1.28 mmol) and compound **S2** (3.0 g, 9.03 mmol) were dissolved in TFA (5 ml) and acetic anhydride (5 ml). The solution was heated at 70 ℃ for 3 hours. Solvent was removed by rotary evaporation, and washed with MeCN (70 mL × 2). The residue and KOH (670 mg, 11.96 mmol) were suspended in a mixture of MeOH (30 ml) and H_2O (25 ml), and heated at 80 ℃ for 48 hours. Solvent was removed by rotary evaporation. The residue was dissolved in water (150 mL) and acidified with 1 M HCl to get precipitate. The precipitate was filtered and dried under high vacuum to yield container **3** as a white solid (332 mg, 0.26 mmol, 20.3%).



^{1}H NMR (400 MHz, D_2O): 7.56 (s, 4H), 6.92 (s, 4H), 5.44 (d, *J* = 15.6 Hz, 4H), 5.38 (d, *J* = 15.4 Hz, 2H), 5.28 (d, *J* = 8.8 Hz, 2H), 5.23 (d, *J* = 16.0 Hz, 4H), 5.15 (d, *J* = 8.8 Hz, 2H), 4.42 (d, *J* = 15.6 Hz, 4H), 4.39 (d, *J* = 16.0 Hz, 4H), 4.14 - 4.07 (m, 8H), 3.84 (d, *J* = 15.4 Hz, 2H), 1.70 (s, 12H). ^{13}C NMR (100MHz, D_2O, 1, 4-dioxane as external reference): δ 175.94, 156.64, 155.63, 147.77, 127.39, 126.37, 122.48, 78.36, 77.06, 73.70, 71.55, 71.30, 53.05, 48.46, 35.89, 15.59, 15.00. FT-IR (cm^{-1}): 3450m, 2995w, 2928w, 1724s, 1473s, 1379w, 1314m, 1230m, 1085m, 799m. HR-MS (ESI): 1297.3957 ($[M + H]^+$), calculated 1297.3890. M.P. > 300 ℃ (decomposed).

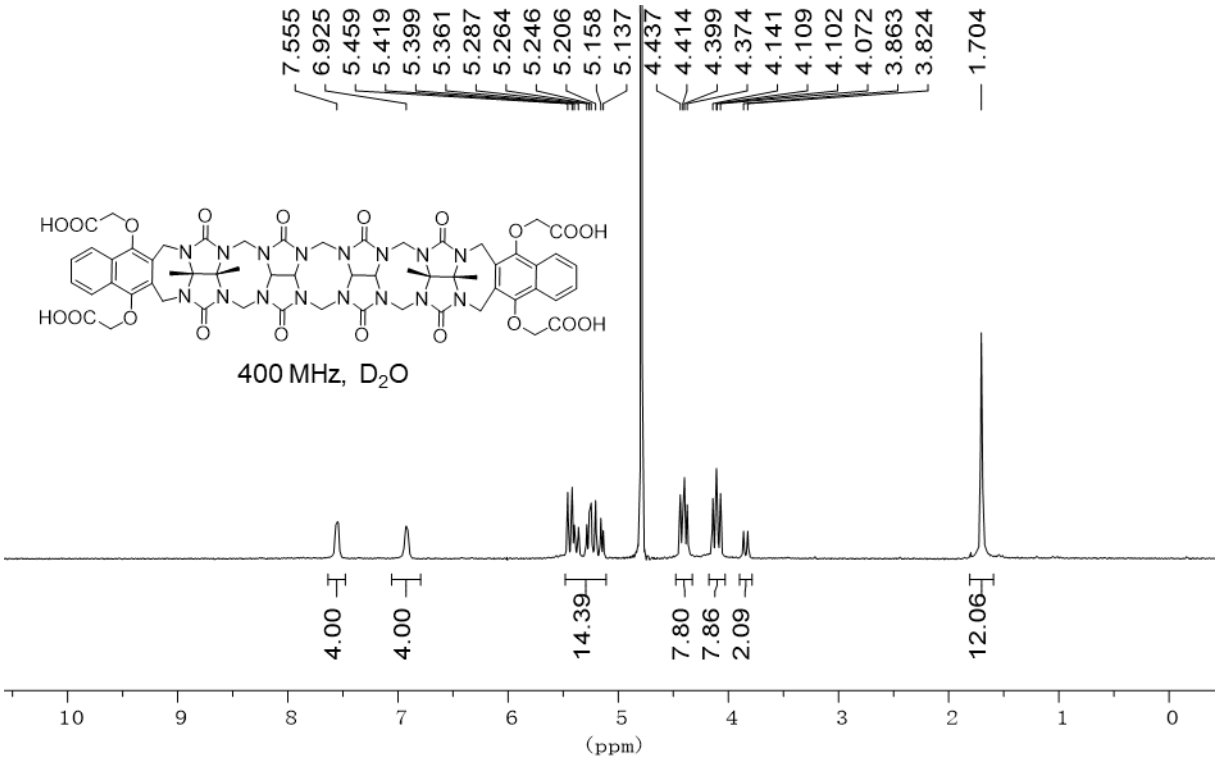


Figure S1. ^{1}H NMR spectrum (400 MHz, D_2O, r.t.) recorded for container **3**.

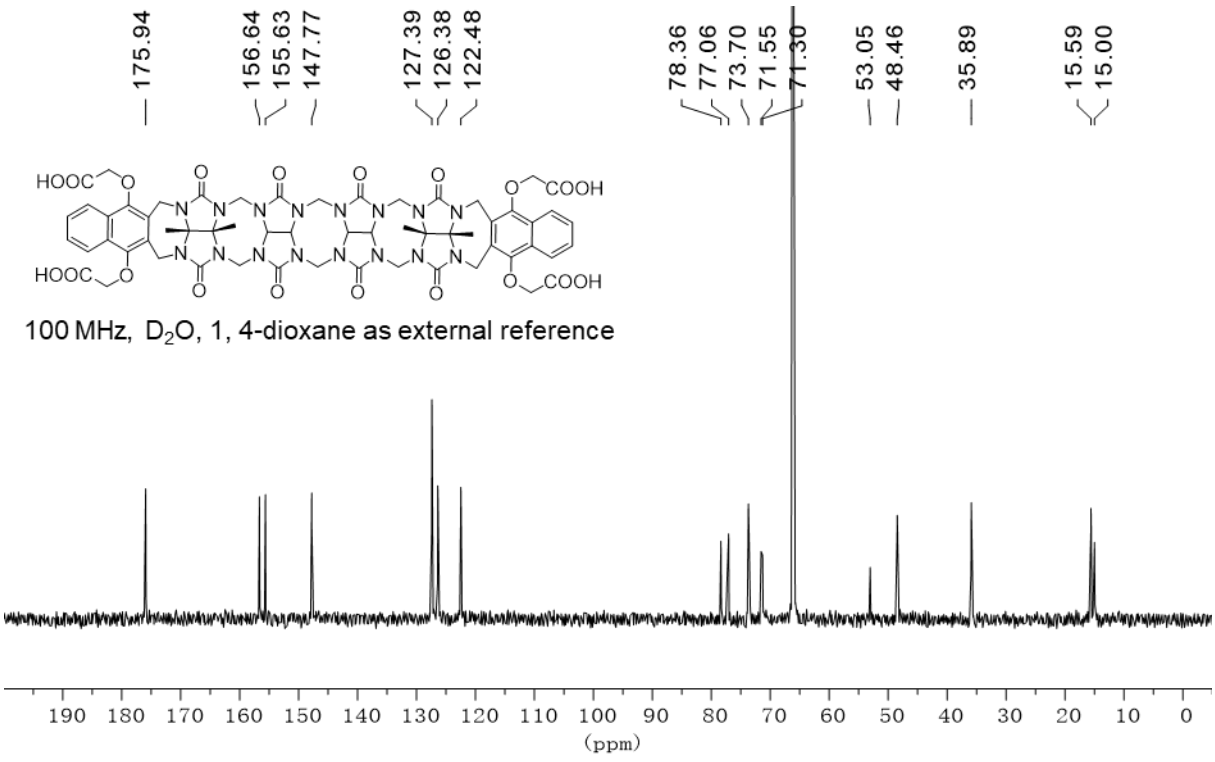


Figure S2. ^{13}C NMR spectrum (100 MHz, D_2O, 1, 4-dioxane as external reference) recorded for container **3**.

Polymer **1**： A solution of container **3** (30 mg, 23 μmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (4.4 mg, 23 μmol) and N-hydroxysulfosuccinimide (12.5 mg, 58 μmol) in PBS (50 mL) was stirred for 15 min at room temperature. A solution of polyallylamine hydrochloride (M_w = 40 - 60 kD, 120 mg) in water (75 ml) was added, and pH was adjusted to 8-9 by 1 M NaOH. The solution was stirred at r.t. for 12 h, and dialyzed (MWCO = 12 – 14 kD) against water for 48 hours and lyophilized to yield polymer **1** as a white solid (130 mg, 87%).
^{1}H NMR (400 MHz, D_2O): 7.53 (s, 4H), 6.90 (s, 4H), 5.43 (d, *J* = 15.4 Hz, 4H), 5.36 (d, *J* = 15.4 Hz, 2H), 5.26 (d, *J* = 8.8 Hz, 2H), 5.22 (d, *J* = 15.8 Hz, 4H), 5.13 (d, *J* = 9.2 Hz, 2H), 4.41 (d, *J* = 15.4 Hz, 4H), 4.39 (d, *J* = 15.8 Hz, 4H), 4.13 - 4.06 (m, 8H), 3.83 (d, *J* = 15.4 Hz, 2H), 2.89 (s, 2H), 2.43 (s, 160H),1.70 (s, 12H), 1.35 - 0.97 (m, 240H). FT-IR (cm^{-1}): 3396s, 3083m, 2924s, 1723s, 1629m, 1561s, 1477m, 1315s.

Polymer **2-CA**： Polymer **1** (30 mg) and triethylamine (300 mg, 296 μmol) were dissolved in water (20 mL). Citraconic anhydride (112 mg, 100 μmol) was added, and the solution was stirred at r.t. for 12 h. At the reaction duration, triethylamine was added if necessary to maintain the pH at 8. Solution was dialyzed (MWCO = 12 – 14 kD) against water for 72 h, and lyophilized to yield polymer **2-CA** as a yellow solid (40 mg, 75%).
^{1}H NMR (400 MHz, D_2O): 7.72 (s, 4H), 7.11 (s, 4H), 5.85 (s, 40H), 5.61 (s, 20H), 5.57 – 5.26 (m, 14H) , 4.56 - 4.50 (m, 8H), 4.26 – 4.22 (m, 8H), 3.97 (d, *J* = 15.2 Hz, 2H), 3.21 (s, 120H), 2.49 (s, 40H),1.96 – 1.93 (m, 180H), 1.75 – 1.22 (m, 240H). FT-IR (cm^{-1}): 3431m, 3083w, 2922m, 1724w, 1653m,1617m, 1560s, 1443m, 1408m, 1262m.

Polymer **2-MA**： Polymer **1** (30 mg) and triethylamine (300 mg, 296 μmol) were dissolved in water (20 mL). Maleic anhydride (98 mg, 100 μmol) was added, and the solution was stirred overnight at at r.t. for 12 h. At the reaction duration, triethylamine was added if necessary to maintain the pH at 8. Solution was dialyzed (MWCO = 12 – 14 kD) against water for 72 h, and lyophilized to yield polymer **2-MA** as a white solid (35 mg, 70%).
^{1}H NMR (400 MHz, D_2O): 7.70 (s, 4H), 7.09 (s, 4H), 6.29 (d, *J* = 12.0 Hz, 70H), 5.90 (d, *J* = 12.0 Hz, 70H), 5.56 – 5.32 (m, 14H) , 4.53 - 4.47 (m, 8H), 4.23 – 4.17 (m, 8H), 3.95 (d, *J* = 15.2 Hz, 2H), 3.14 (s, 140H), 2.53 (s, 20H), 1.80 (s, 12H), 1.67 – 1.20 (m, 240H). FT-IR (cm^{-1}): 3411m, 3085m, 2927m, 1724w, 1653m, 1561s, 1438m, 1351m.

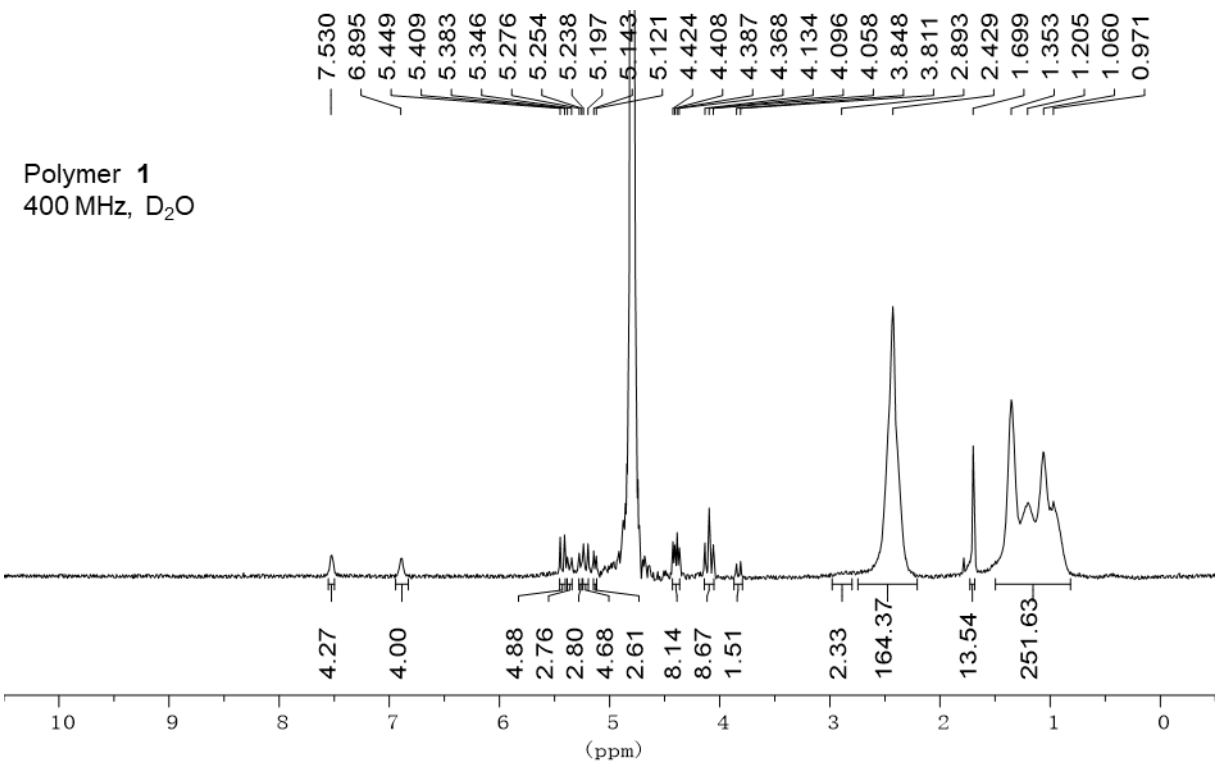


Figure S3. ^{1}H NMR spectrum (400MHz, D_2O, r.t.) recorded for polymer **1**.

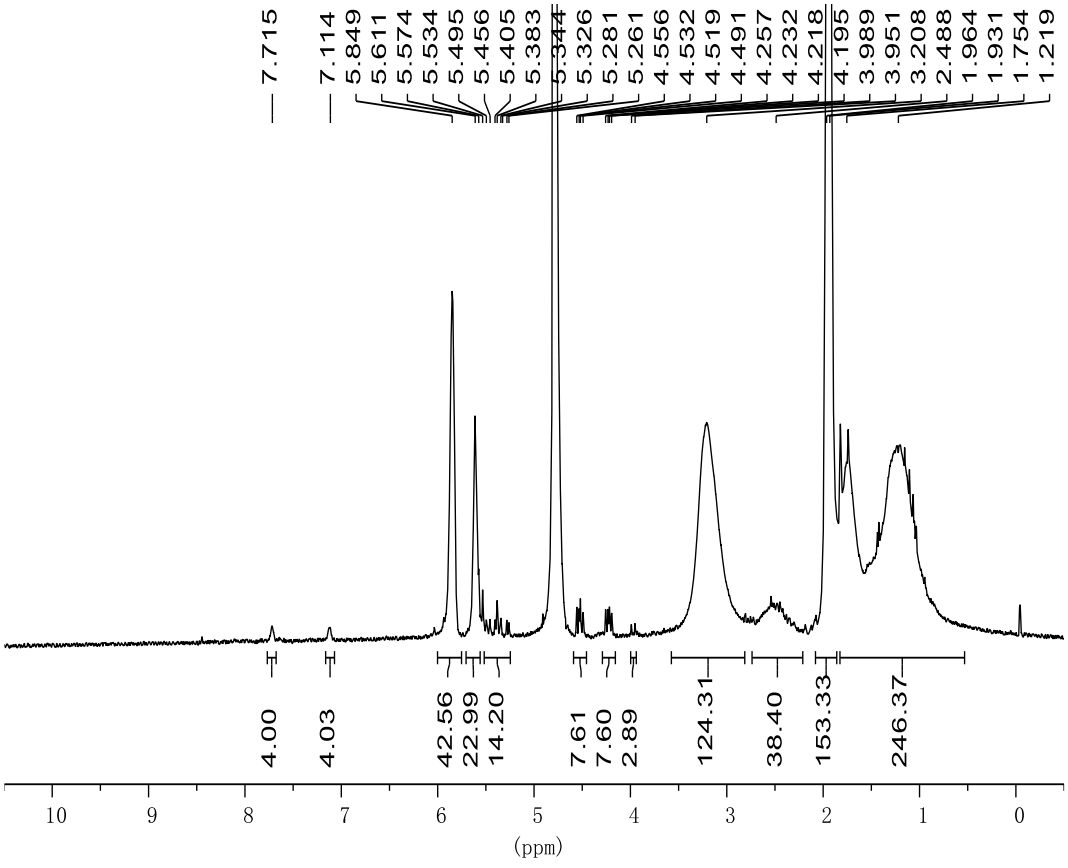


Figure S4. ^{1}H NMR spectrum (400MHz, D_2O, r.t.) recorded for polymer **2-CA**.

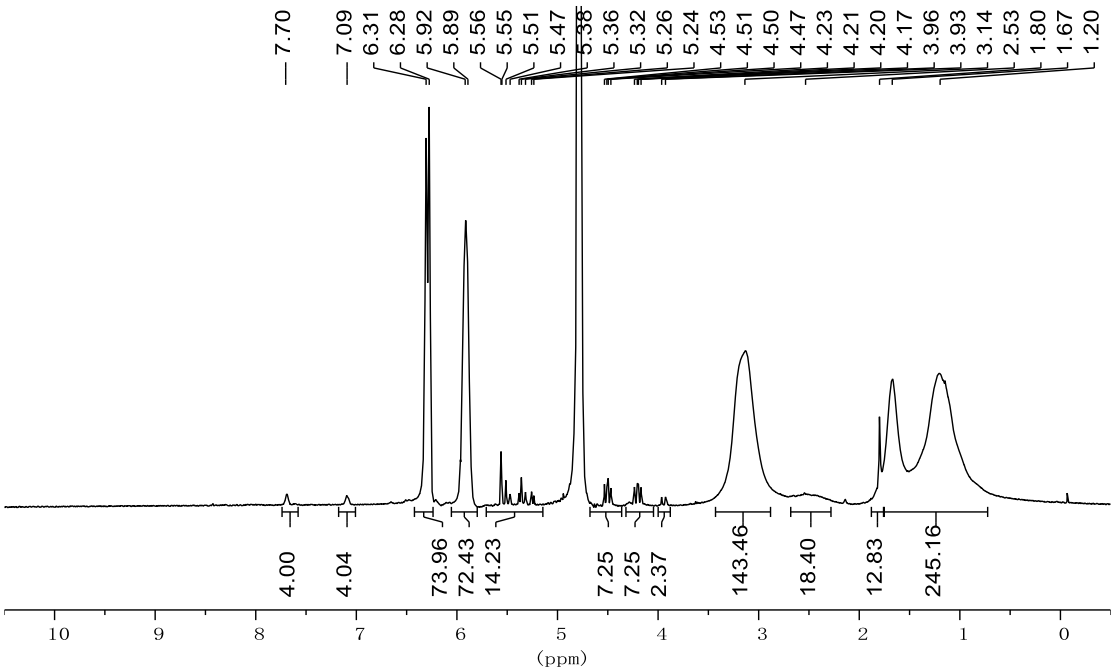


Figure S5. ^{1}H NMR spectrum (400MHz, D_2O, RT) recorded for polymer **2-MA**.

Study for Host-guest Chemistry

Binding constant K_a determined by direct titration was calculated with nonlinear fitting with Origin 9 software based on 1:1 binding and the following equation: $[H]^0$: initial host concentration; $[G]^0$: initial guest concentration; Δ_A: change of absorbance; Δ_ε: change of coefficiency; K_a: binding constant

$$\Delta_A = \Delta_\varepsilon \cdot \{\frac{1}{2}([H]^0 + [G]^0 + \frac{1}{K_a}) - \sqrt{\frac{1}{4}([H]^0 + [G]^0 + \frac{1}{K_a}) - [H]^0 \cdot [G]^0}\}$$

Binding constant K_a determined by displacement assay was calculated with nonlinear fitting with Scientist software based on the following Model:

```
// MicroMath Scientist Model File
IndVars: ConcAntot
DepVars: Absorb
Params: ConcHtot, ConcGtot, Khg, Kha, AbsorbMax, AbsorbMin
Khg = ConcHG / (ConcH * ConcG)
Kha = ConcHAn / (ConcH * ConcAn)
Absorb = AbsorbMin + (AbsorbMax-AbsorbMin)*(ConcHG/ConcGtot)
ConcHtot = ConcH + ConcHG + ConcHAn
ConcGtot = ConcHG + ConcG
ConcAntot = ConcAn + ConcHAn
0 < ConcHG < ConcHtot
0 < ConcH < ConcHtot
0 < ConcG < ConcGtot
0 < ConcAn < ConcAntot
***
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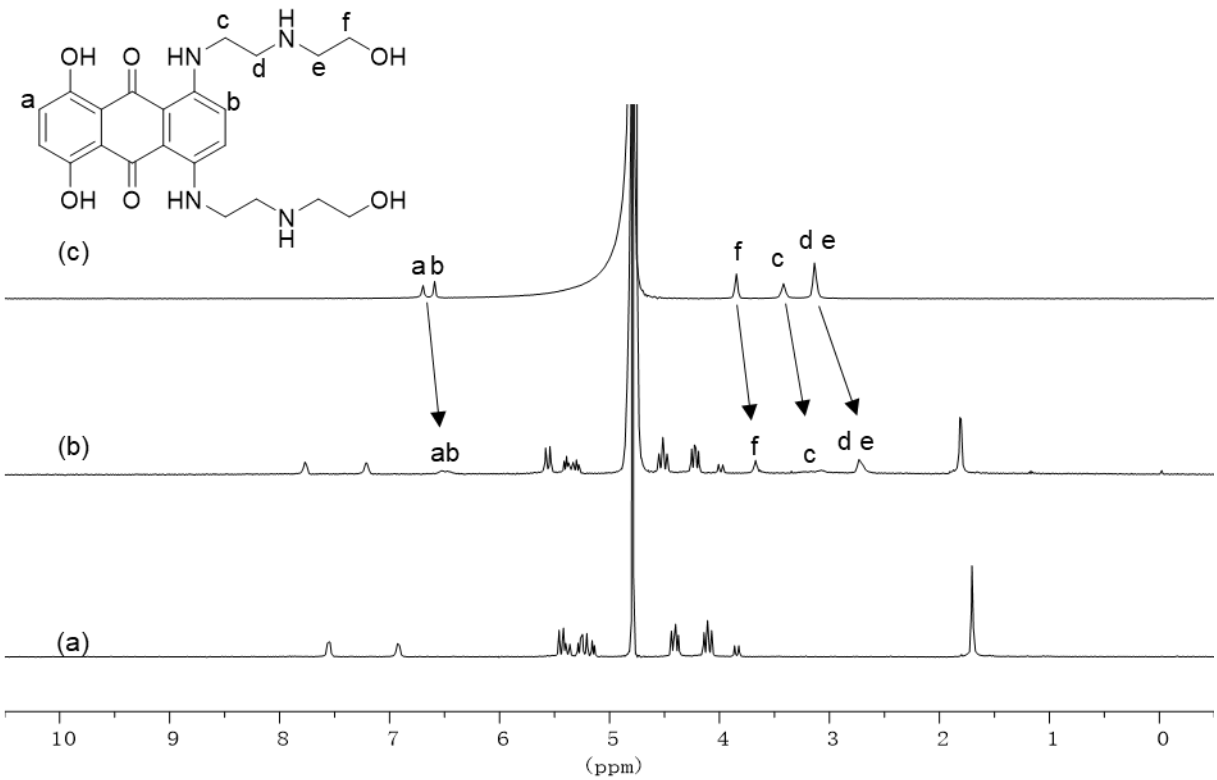


Figure S6. ^{1}H NMR spectra recorded for (a) container **3**, (b) container **3** + mitoxantrone ([**3**]:[mitoxantrone] = 1 : 1), (c) mitoxantrone in NaD_2PO_4 buffer (10 mM, pD 7.4).

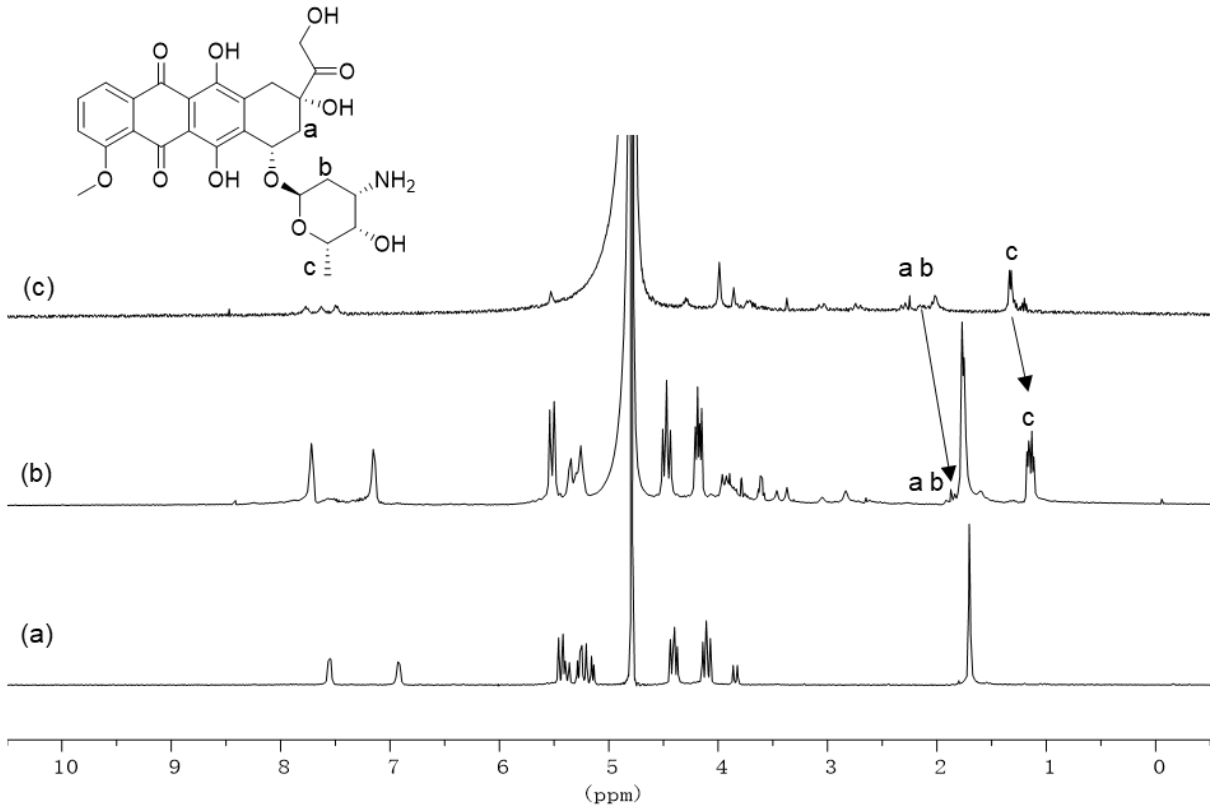


Figure S7. ^{1}H NMR spectra recorded for (a) container **3**, (b) container **3** + doxorubicin ([**3**]:[doxorubicin] = 1 : 1), (c) doxorubicin in NaD_2PO_4 buffer (10 mM, pD 7.4).

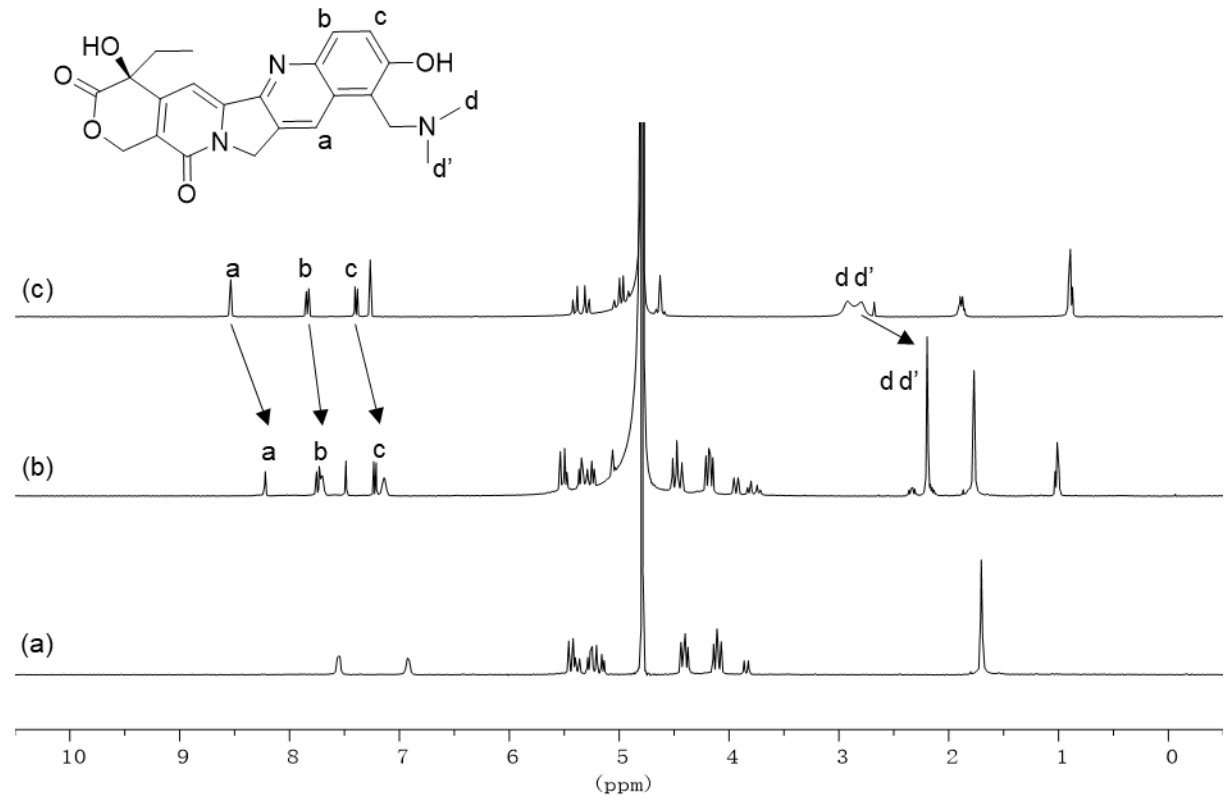


Figure S8. ^{1}H NMR spectra recorded for (a) container **3**, (b) container **3** + topotecan ([**3**]:[topotecan] = 1 : 1), (c) topotecan in NaD_2PO_4 buffer (10 mM, pD 7.4).

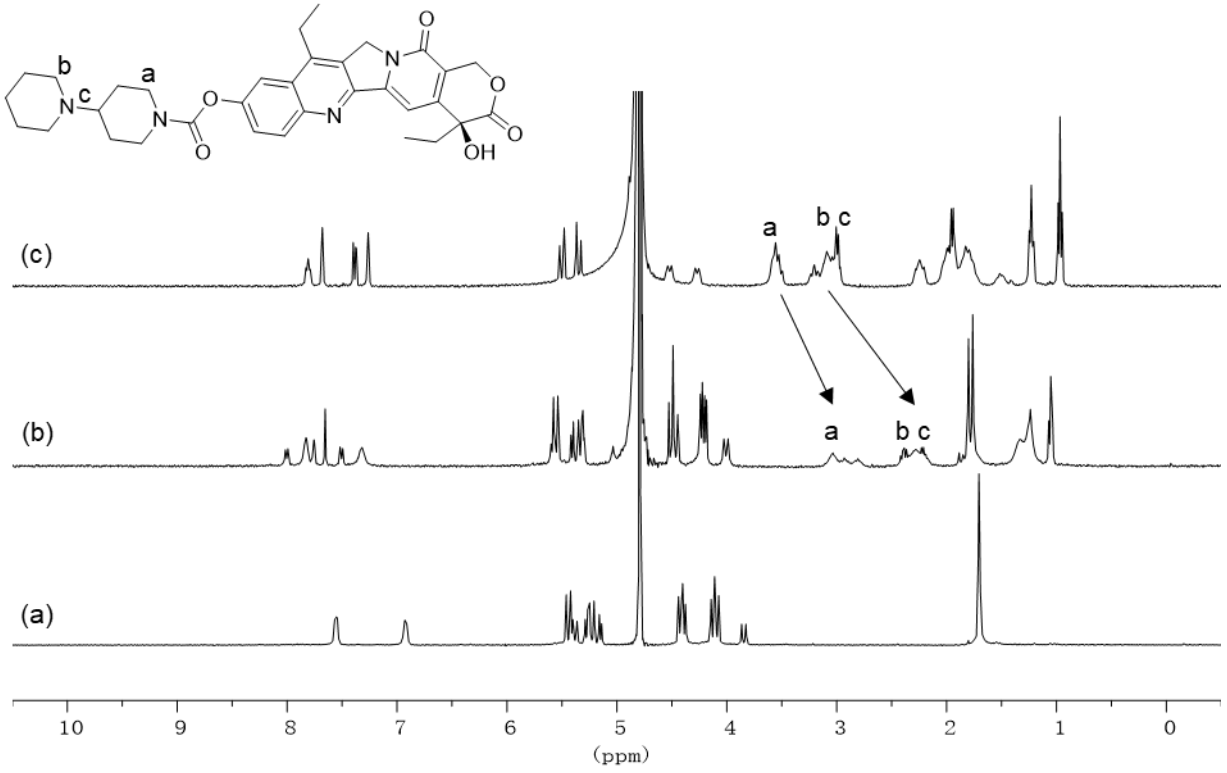


Figure S9. ^{1}H NMR spectra recorded for (a) container **3**, (b) container **3** + irinotecan ([**3**]:[irinotecan] = 1 : 1), (c) irinotecan in NaD_2PO_4 buffer (10 mM, pD 7.4).

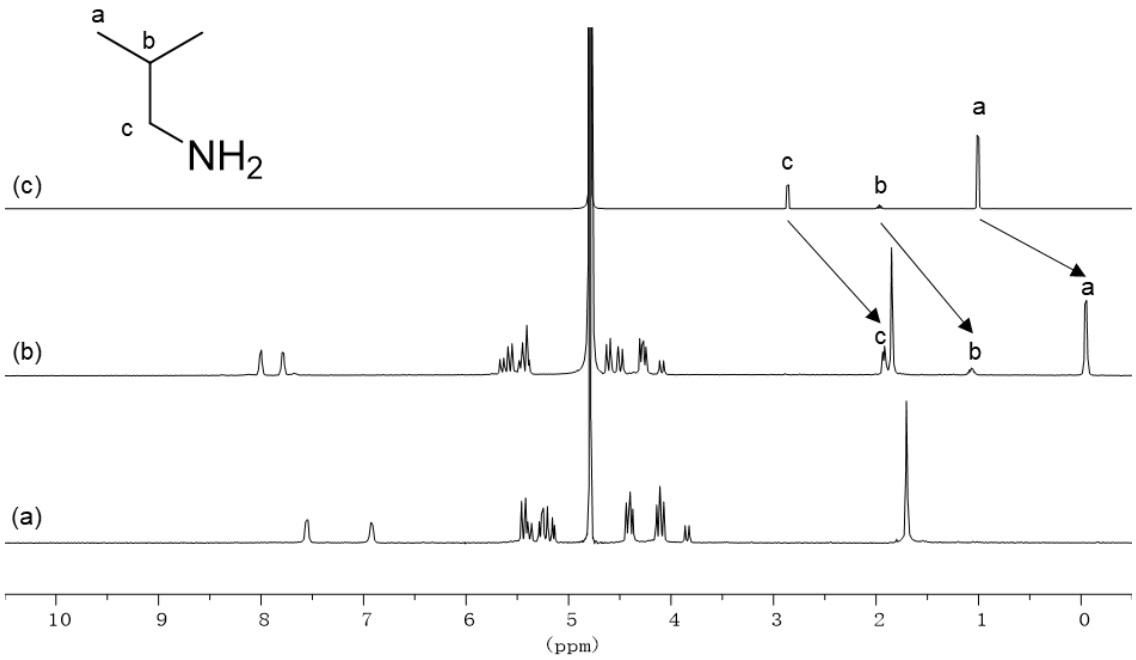
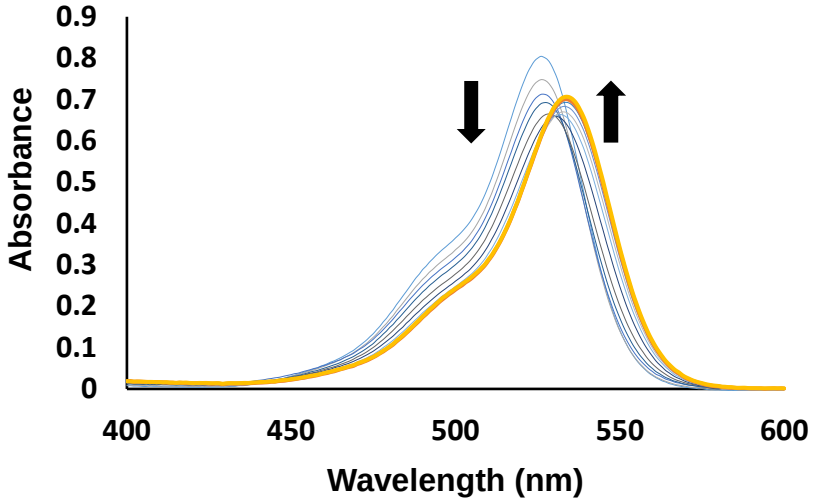


Figure S10. ^{1}H NMR spectra recorded for (a) container **3**, (b) container **3** + guest **4** ([**3**]:[**4**] = 1:1), (c) guest **4** in NaD_2PO_4 buffer (10 mM, pD 7.4).

(A)



(B)

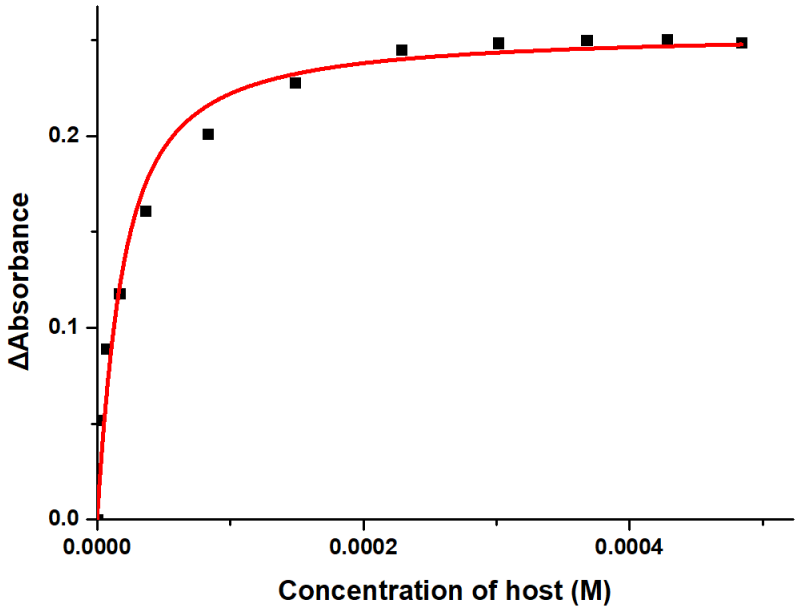
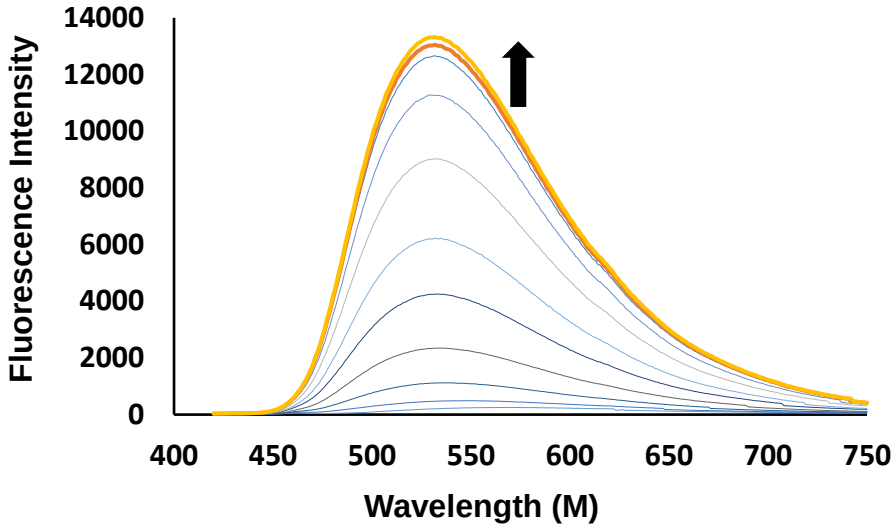


Figure S11. (A) UV/Vis spectra from the titration of rhodamine 6G (10 μM) with container **3** (0 – 430 μM) in PBS (pH 7.4); (B) plot of absorbance at 520 nm as a function of container **3** concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (7.4 ± 1.7) × 10^4 M^{-1}).

(A)



(B)

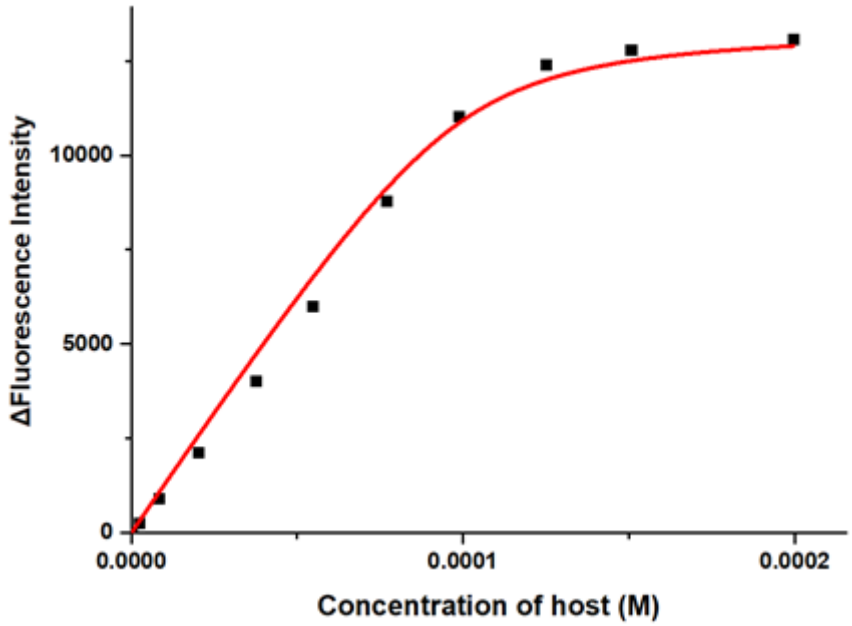
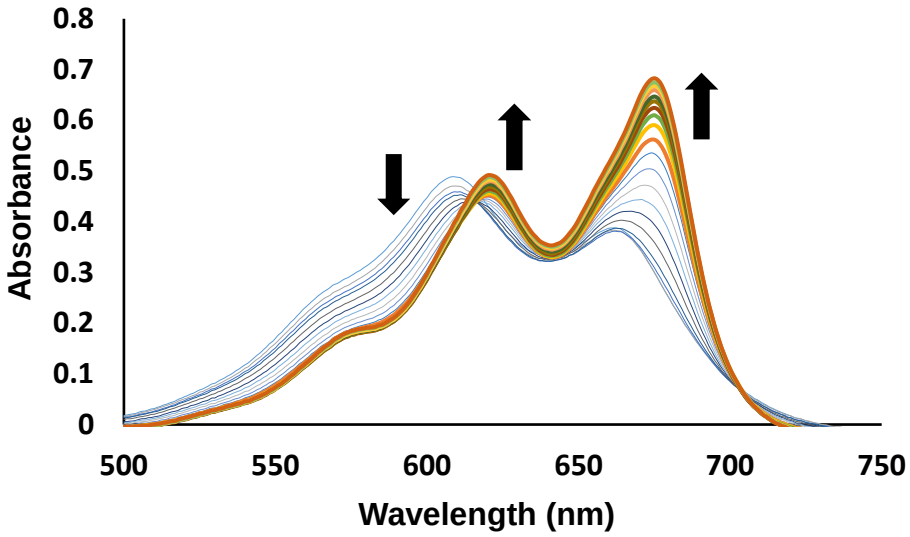


Figure S12. (A) Fluorescence spectra from the titration of berberine (100 μM) with container **3** (0 – 200 μM) in PBS (pH 7.4), ex 400 nm; (B) plot of the fluorescence intensity at 535 nm as a function of container **3** concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (2.4 ± 1.6) × 10^5 M^{-1}).

(A)



(B)

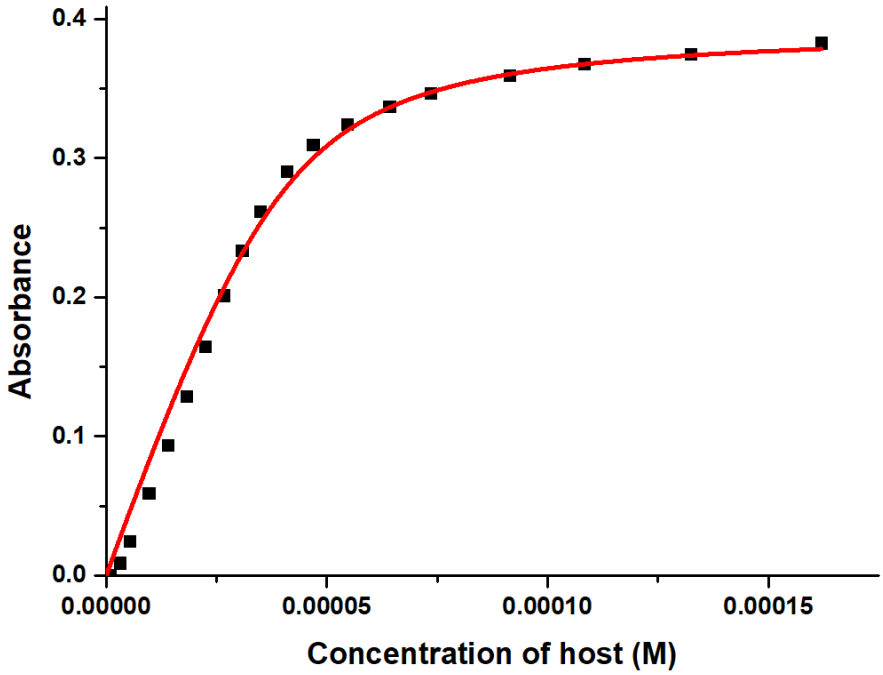
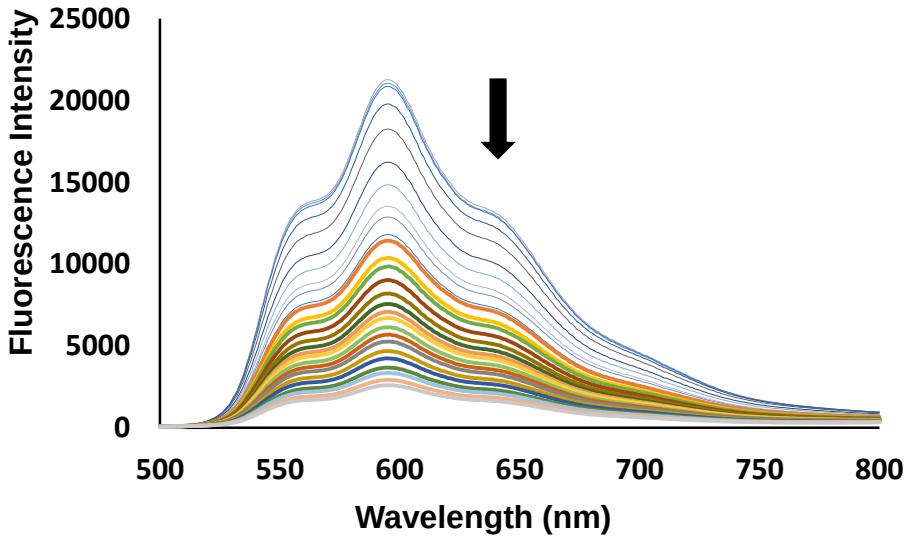


Figure S13. (A) UV/Vis spectra from the titration of mitoxantrone (40 μM) with container **3** (0 – 160 μM) in PBS (pH 7.4); (B) plot of absorbance at 675 nm as a function of container **3** concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (1.9 ± 0.4) × 10^5 M^{-1}).

(A)



(B)

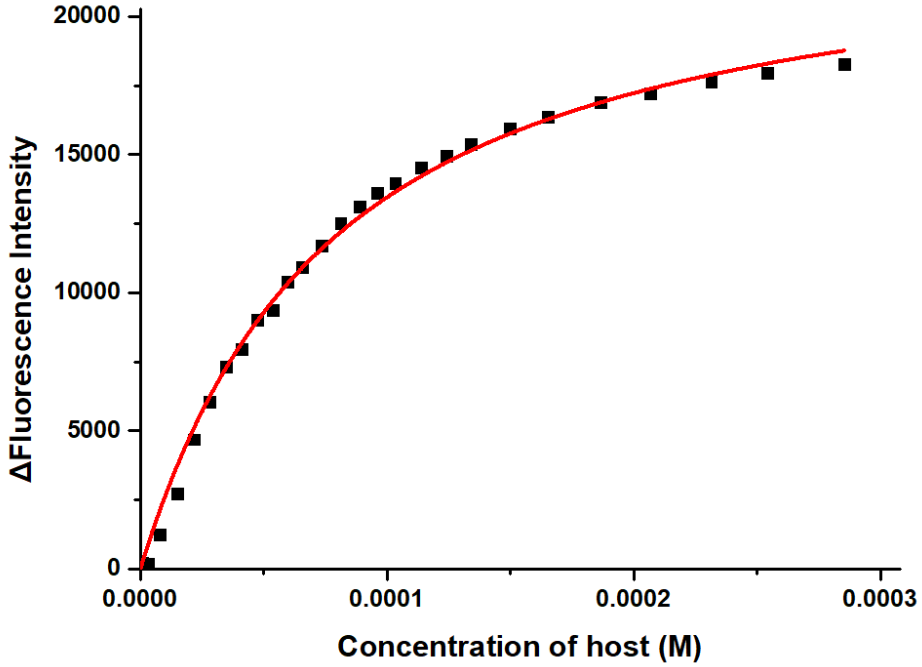
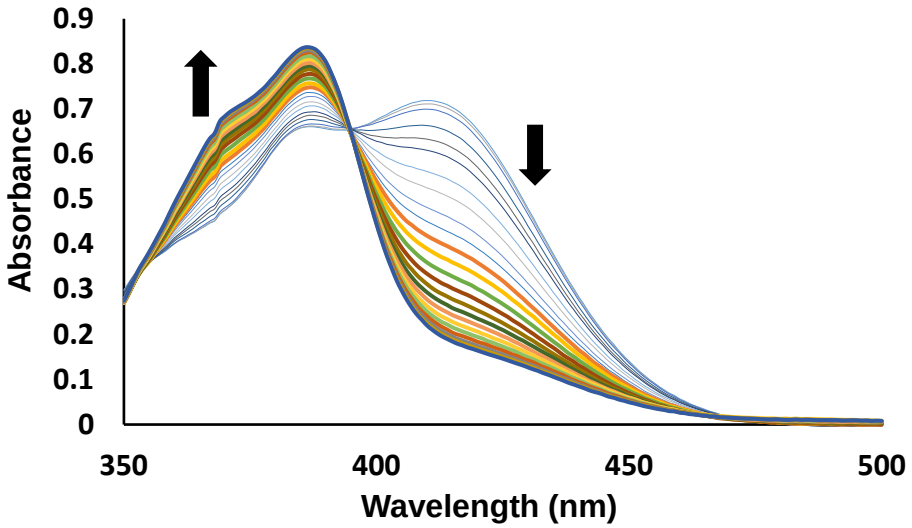


Figure S14. (A) Fluorescence spectra from the titration of doxorubicin hydrochloride (10 μM) with container **3** (0 – 285 μM) in 10 mM NaH_2PO_4 buffer (pH 7.4), ex 494 nm; (B) plot of fluorescence intensity at 600 nm as a function of container **3** concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (1.4 ± 0.1) × 10^4 M^{-1}).

(A)



(B)

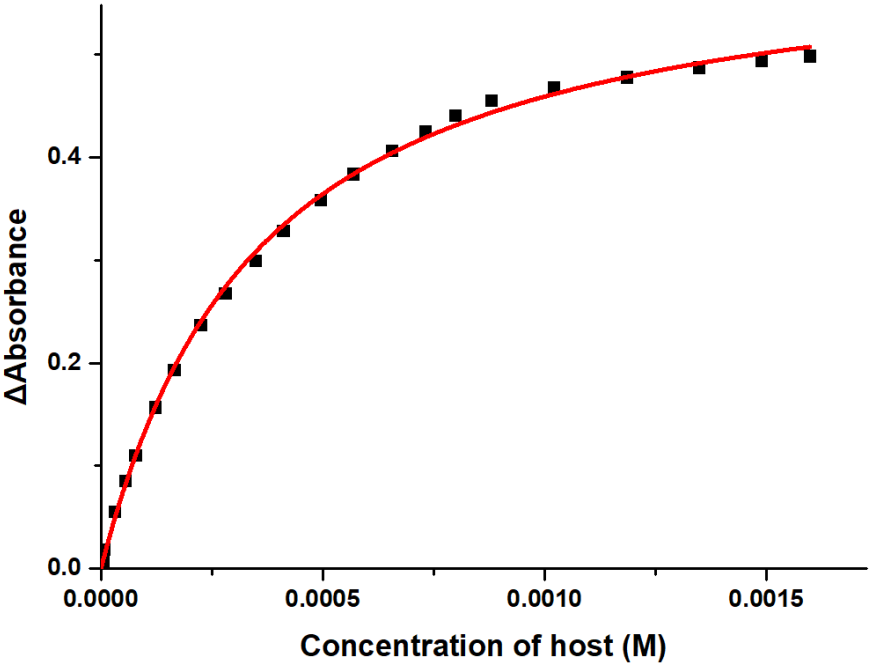
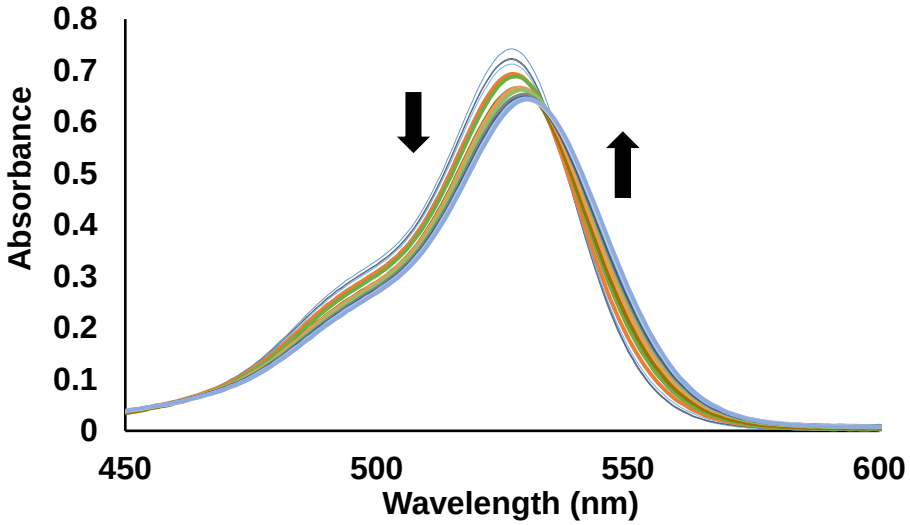


Figure S15. (A) UV/Vis spectra from the titration of topotecan (40 μM) with container **3** (0 – 1020 μM) in PBS (pH 7.4); (B) plot of absorbance at 410 nm as a function of container **3** concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (3.1 ± 0.1) × 10^3 M^{-1}).

(A)



(B)

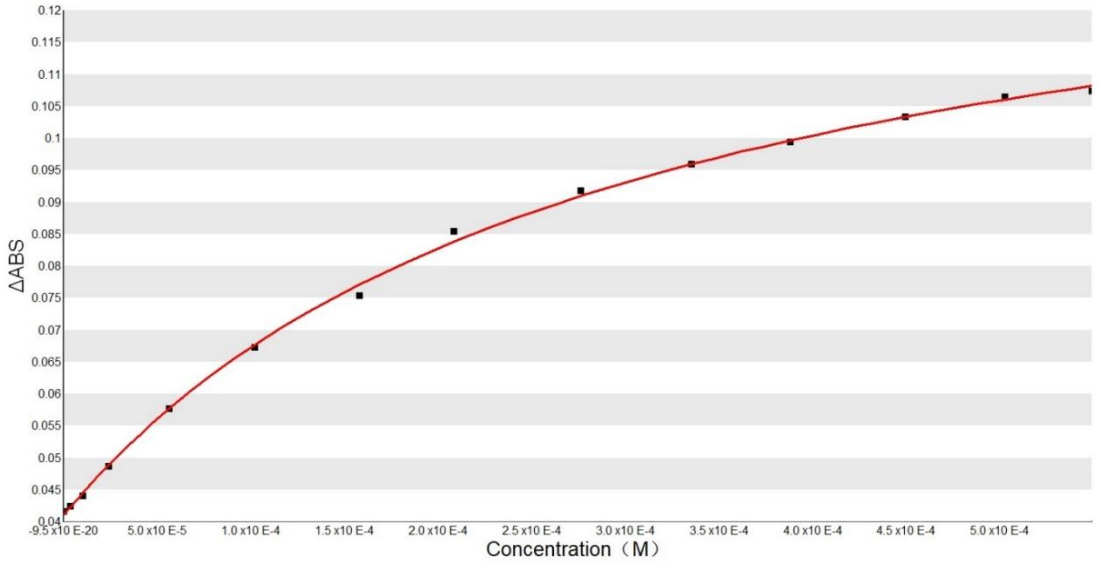
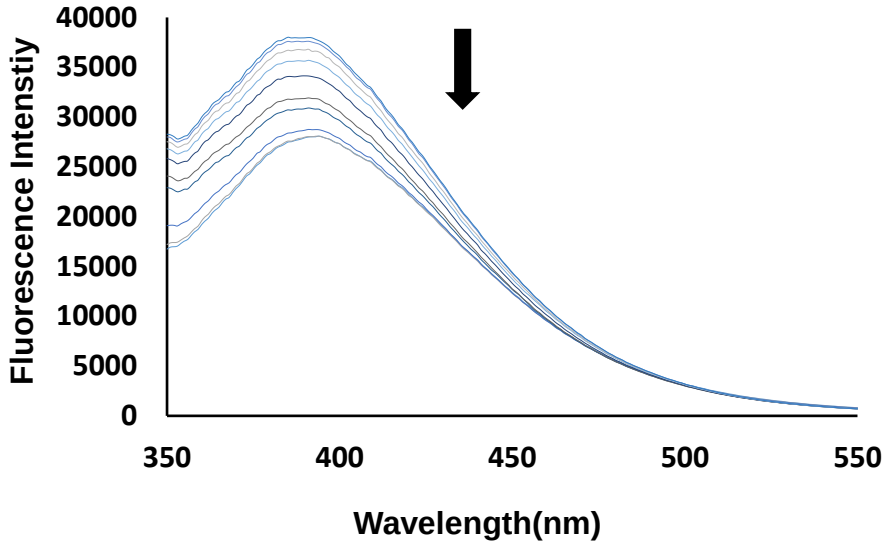


Figure S16. Displacement titration of a solution of rhodamine 6G (10 μM) and container **3** (10 μM) solution with irinotecan (0 - 550 μM) (PBS, pH 7.4): (A) spectral change, (B) Nonlinear fitting plot of absorbance at 560 nm versus concentration for the displacement titration of rhodamine 6G with Scientist™. K_a value was determined to be (6.6 ± 0.4) × 10^3 M^{-1}.

(A)



(B)

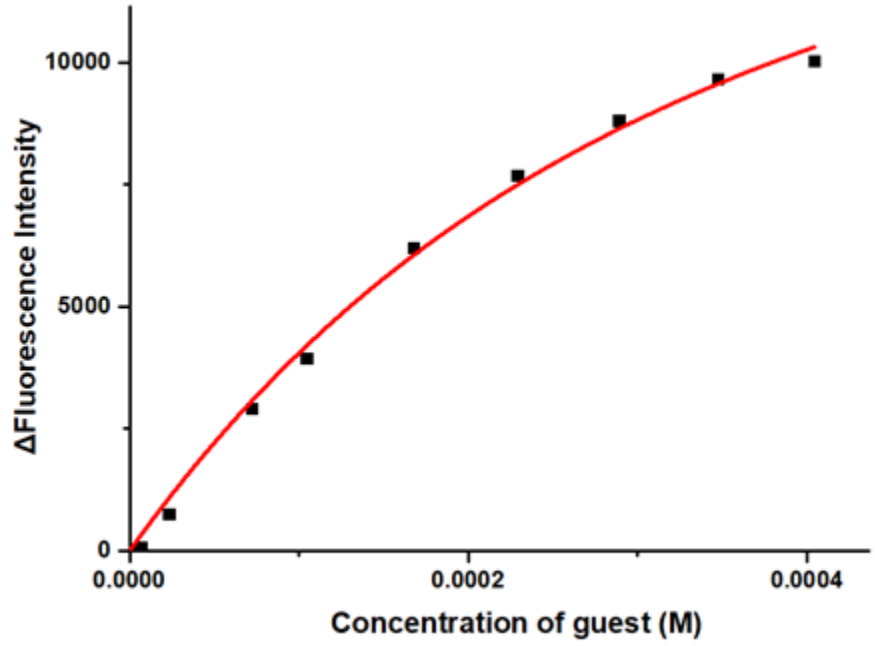


Figure S17. (A) Fluorescence spectra from the titration of container **3** (120 μM) with guest **4** (0 – 400 μM) in PBS (pH 7.4), ex 335 nm; (B) plot of the fluorescence intensity change at 395 nm as a function of container **3** concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (3.9 ± 0.7) × 10^3 M^{-1}).

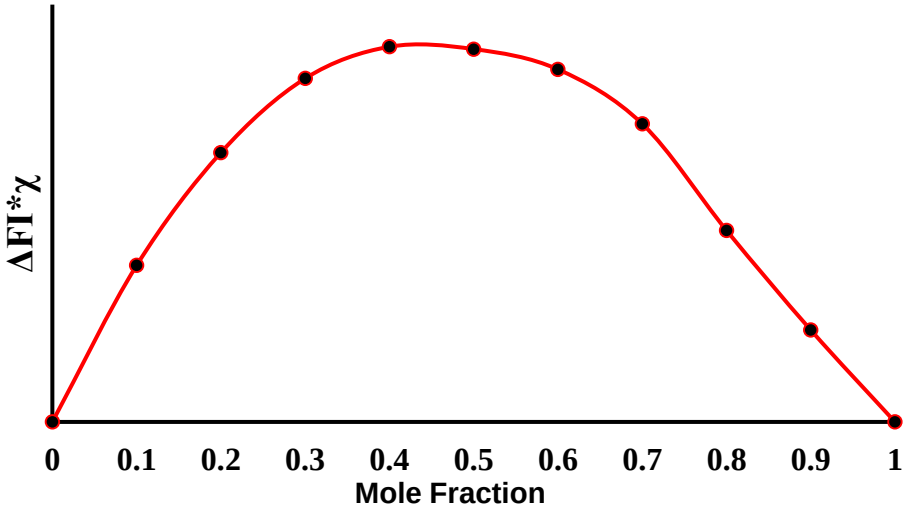
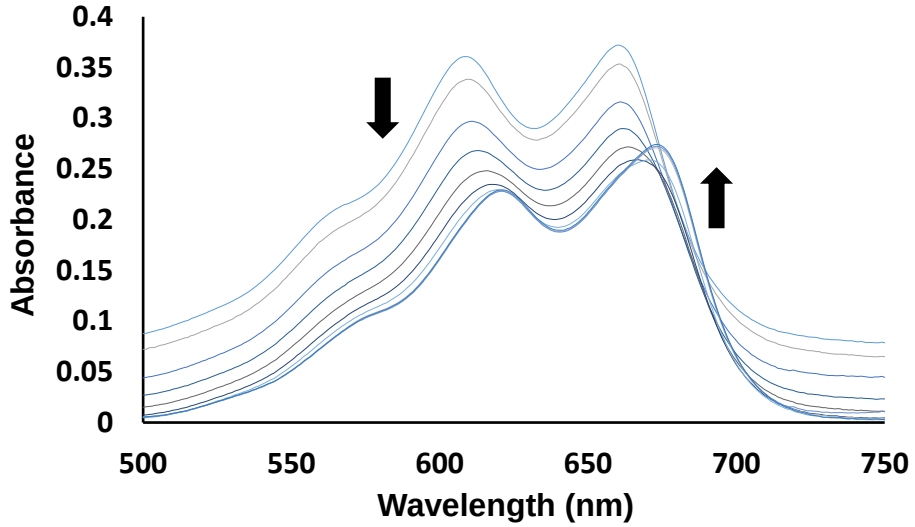


Figure S18. Job plot for doxorubicin and container **3** from fluorescence spectra in PBS ([**3**] + [doxorubicin] =13.3 μM). **χ :** doxorubicin molar fraction; ΔFI: fluorescence intensity change. Ex 494 nm, em 600 nm.

(A)



(B)

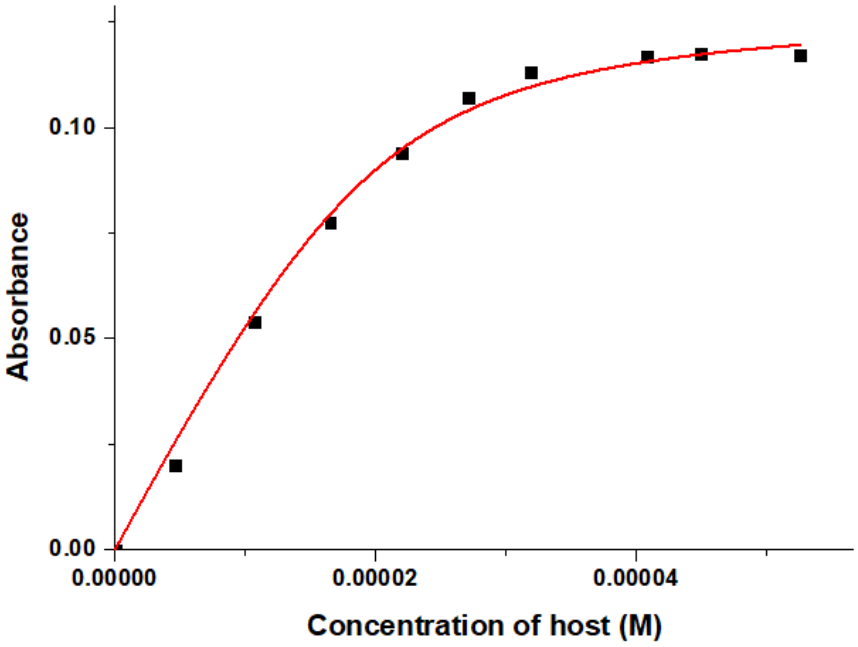
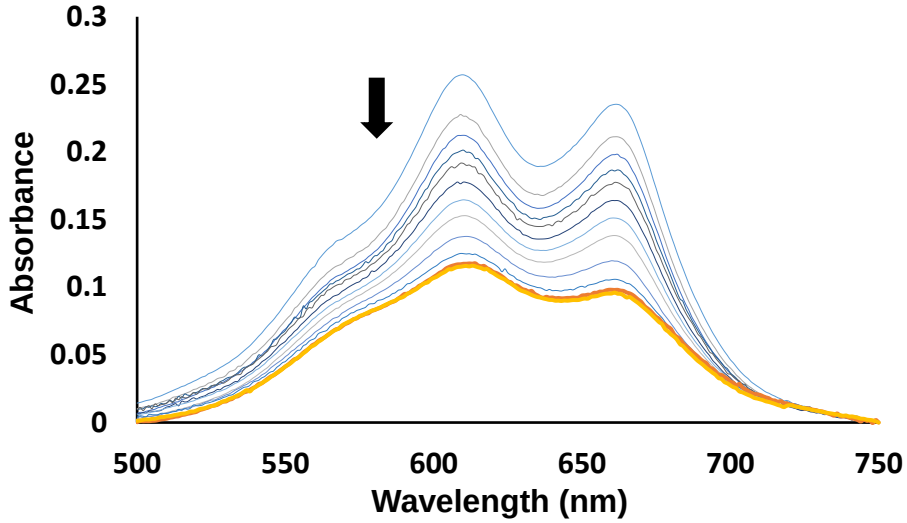


Figure S19. (A) UV/Vis spectra from the titration of mitoxantrone (20 μM) with container **3** (0 – 60 μM) in sodium acetate buffer (pH 4.6, 200 mM); (B) plot of the ΔA_{550} as a function of container **3** concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (3.8 ± 1.0) × 10^5 M^{-1}).

(A)



(B)

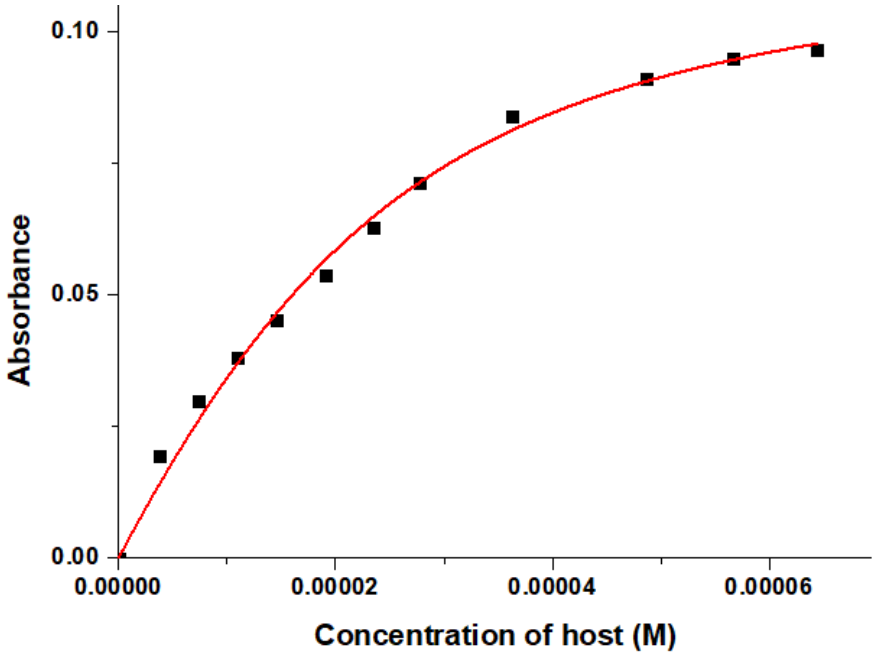


Figure S20. (A) UV/Vis spectra from the titration of mitoxantrone (20 μM) with polymer **2-CA** (calculated for container **3** 0 – 65 μM) in PBS (pH 7.4); (B) plot of the ΔA_{675} as a function of container **3 (**polymer **2-CA** concentration). The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (1.0 ± 0.2) × 10^5 M^{-1}).

Method for controlled release of berberine. Berberine (25 μM) and polymer **2-CA** or **2-MA** (1 mg / mL, 70 μM calculated by container **3**) was dissolved in buffered solution of pH 4.6 (100 mM acetate buffer solution), 6.0 (100 mM phosphate buffer solution) or 7.4 (PBS). Solution was incubated at 37 ℃, and fluorescence intensity (ex 400 nm, em 535 nm) was monitored. Release percentage was calculated based on the following equation:

$\Delta I/I_0 = (I_i-I_t)/(I_i-I_b)$

I_i: fluorescence intensity at time 0 h. I_t: fluorescence intensity at time t. I_b: fluorescence intensity of berberine only solution (25 μM).

Method for controlled release of doxorubicin. Doxorubicin hydrochloride (10 μM) and polymer **2-CA** or polymer **2-MA** (1 mg / mL, 70 μM calculated by container **3**) was dissolved in buffered solution of pH 4.6 (100 mM acetate buffer solution) or pH 7.4 (PBS). Solution was incubated at 37℃, and fluorescence intensity (ex 494 nm, em 600 nm) was mintored. Release percentage was calculated based on the following equation:

$\Delta I/I_0 = (I_t-I_i)/(I_d-Ii)$

I_i: fluorescence intensity at time 0 h. I: fluorescence intensity at time t. I_d: fluorescence intensity of doxorubicin hydrochloride only solution (10 μM).

Cell experiment

Cell uptake assays. HeLa cells were seeded in 96-plate wells (10000 cells / well, *n* = 6) and cultured for 24 h. Cells were incubated with doxorubicin hydrochloride alone (10 μM), doxorubicin hydrochloride with polymer **2-CA** (1 mg/mL) or polymer doxorubicin hydrochloride with polymer **2-MA** (1 mg/mL) at pH 7.4 or pH 6.0. After 4 h incubation, cells were washed by PBS. Cells were imaged by fluorescence microscopy. Fluorescence intensity was determined by Microplate Reader (Biotek Synergy H1) to quantify cell uptake of doxorubicin (ex 494 nm, em 600 nm).

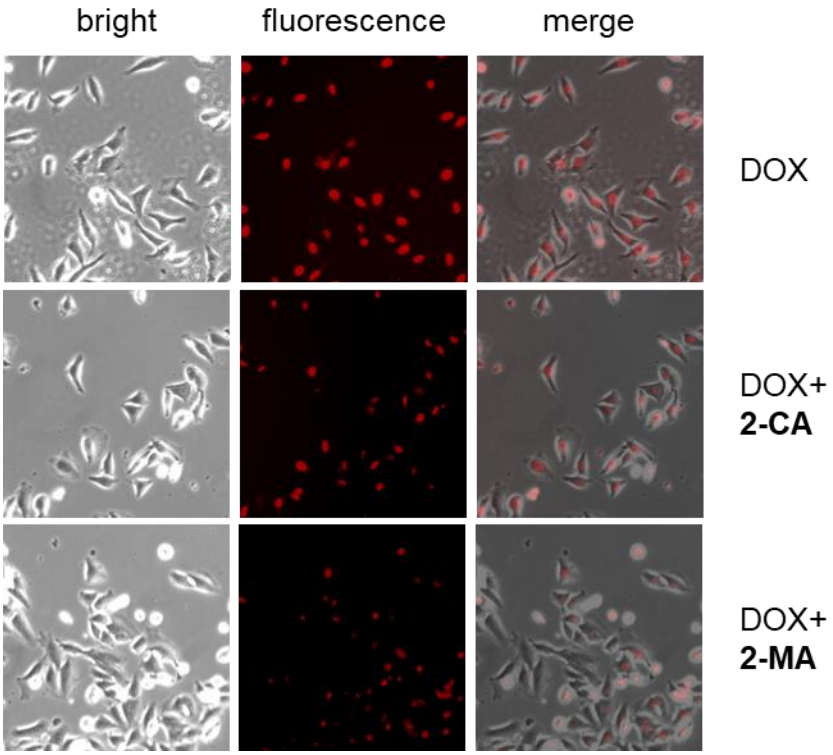


Figure S21. Fluorescence microscopic images for HeLa cells treated with doxorubicin hydrochloride (top row), doxorubicin + polymer **2-CA** (middle row), and doxorubicin + polymer **2-MA** (bottom row) at pH 7.4. Incubation time: 4 h.

References

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