Electronic Supplementary Information

Small polyanion recognition of triazolium cyclodextrin click cluster in water

Hoa Thi Le[1], Seung Cheol Park[2], Chulhun Kang[1], Choon Woo Lim[3]*, Tae Woo Kim[1]*

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S1. Synthesis & Characterization

S1-1. Synthesis of cyclodextrin derivatives

A. Hexakis{6-chloro-6-deoxy}-α-cyclodextrin (2) and hexakis{6-azido-6-deoxy}-α-cyclodextrin (**3**)

α-Cyclodextrin was purchased from TCI (C0776) and dried in vacuum at 80 °C for at least 5 h before using. **2** and **3** were prepared by Jean-Marie Lehn's procedure and the spectra of ^{1}H, ^{13}C NMR are consistent with the literature.[1]

Compound **2**. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.76 (d, J = 5.4, 6H), 5.59 (s, 6H), 4.92 (d, J = 3.2, 6H), 4.13 – 3.89 (m, 12H), 3.90 – 3.71 (m, 12H), 3.44 (t, J = 8.9, 6H), 3.36 (s, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz): 101.8, 83.5, 72.5, 71.5, 70.7, 45.1.

Compound **3**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.60 (br, 6H, OH), 5.42 (br, 6H, OH), 4.81 (d, J = 2.5 Hz, 6H, H1′), 3.76-3.65 (m, 18H), 3.56-3.49 (m, 6H), 3.34-3.30 (m, 12H); ^{13}C NMR (DMSO-d_6, 75 MHz): 101.7, 83.4, 72.7, 71.6, 70.4, 51.3.

B. 6-amino-6-deoxy-α-cyclodextrin hydrochloride (**6**)

Compound **6** was prepared according to a literature procedure.[2] The spectra of ^{1}H, ^{13}C NMR are consistent with the literature.[3]

^{1}H NMR (300 Hz, D_2O): δ 5.18 (d, J =2.91, 6H, H_1), 4.21 (m, 6H, H_5), 4.03 (app t, 6H, H_3), 3.69 (dd, J = 3.1, 10.1, 6H, H_2), 3.61 (app t, 6H, H_4), 3.46 (dd, J =3.2, 13.7, 6H, H_6), 3.28 (dd , 6H, H_5), 3.56 (app dd, J = 6.4, 13.5, 6H, H_6); ^{13}C NMR (75 MHz, D_2O): 101.96 (C_1), 83.10 (C_4), 73.24 (C_3), 72.02 (C_2), 68.86 (C_5), 41.05 (C_6).

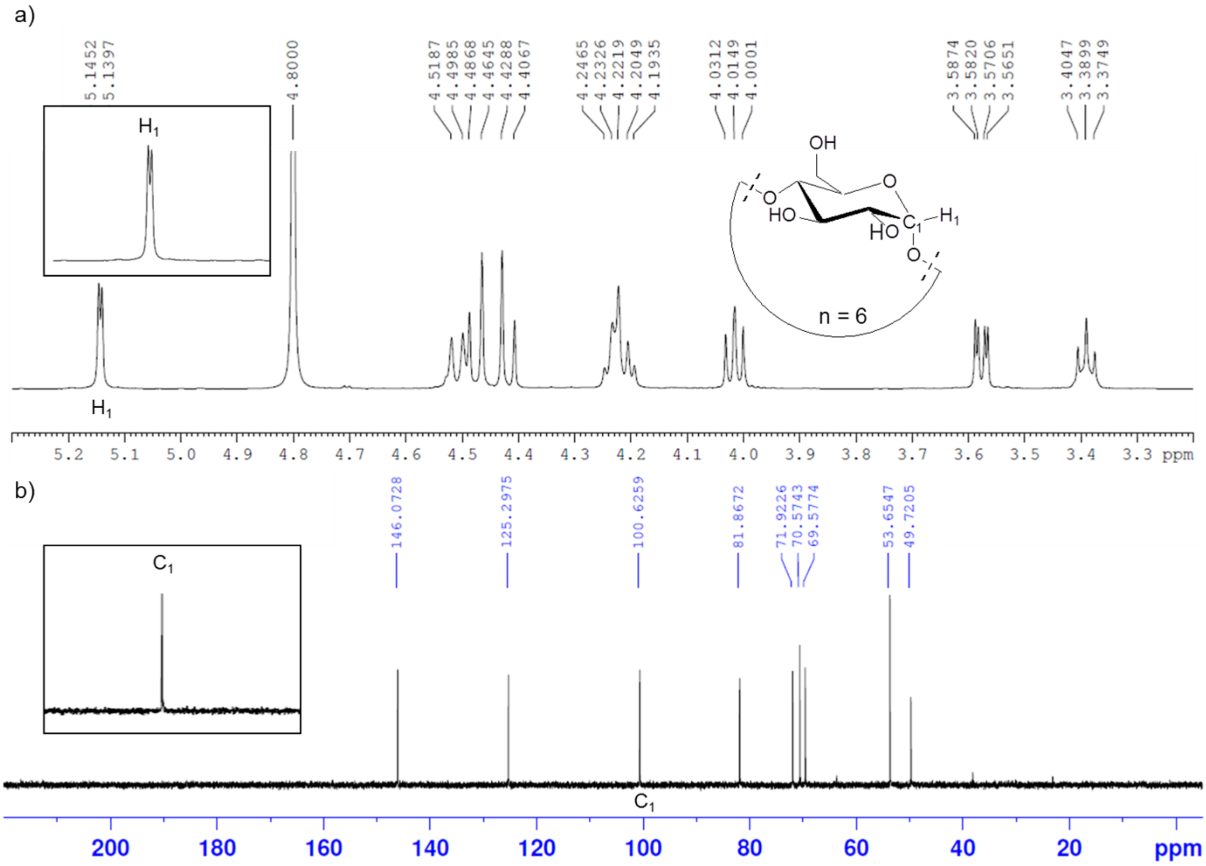


Figure S1-1. Partial NMR spectra of **4**. a) ^{1}H NMR(D_2O, 600 MHz) and b) ^{13}C NMR(D_2O, 150 MHz)

S1-2. NMR characterization of 5

Figure S1-2A. ^{1}H NMR spectrum (D_2O, 600 MHz) of **5**

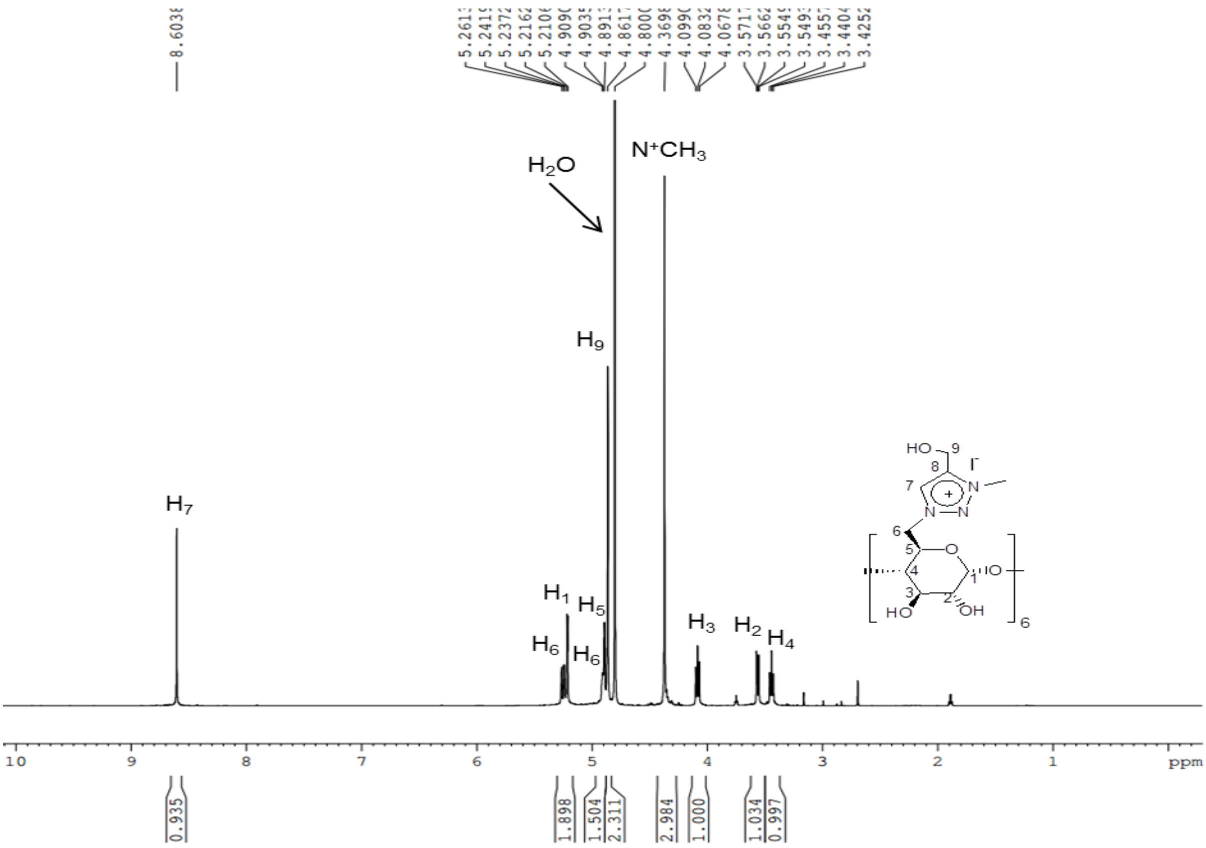


Figure S1-2B. ^{13}C NMR spectrum (D_2O, 600 MHz) of **5**

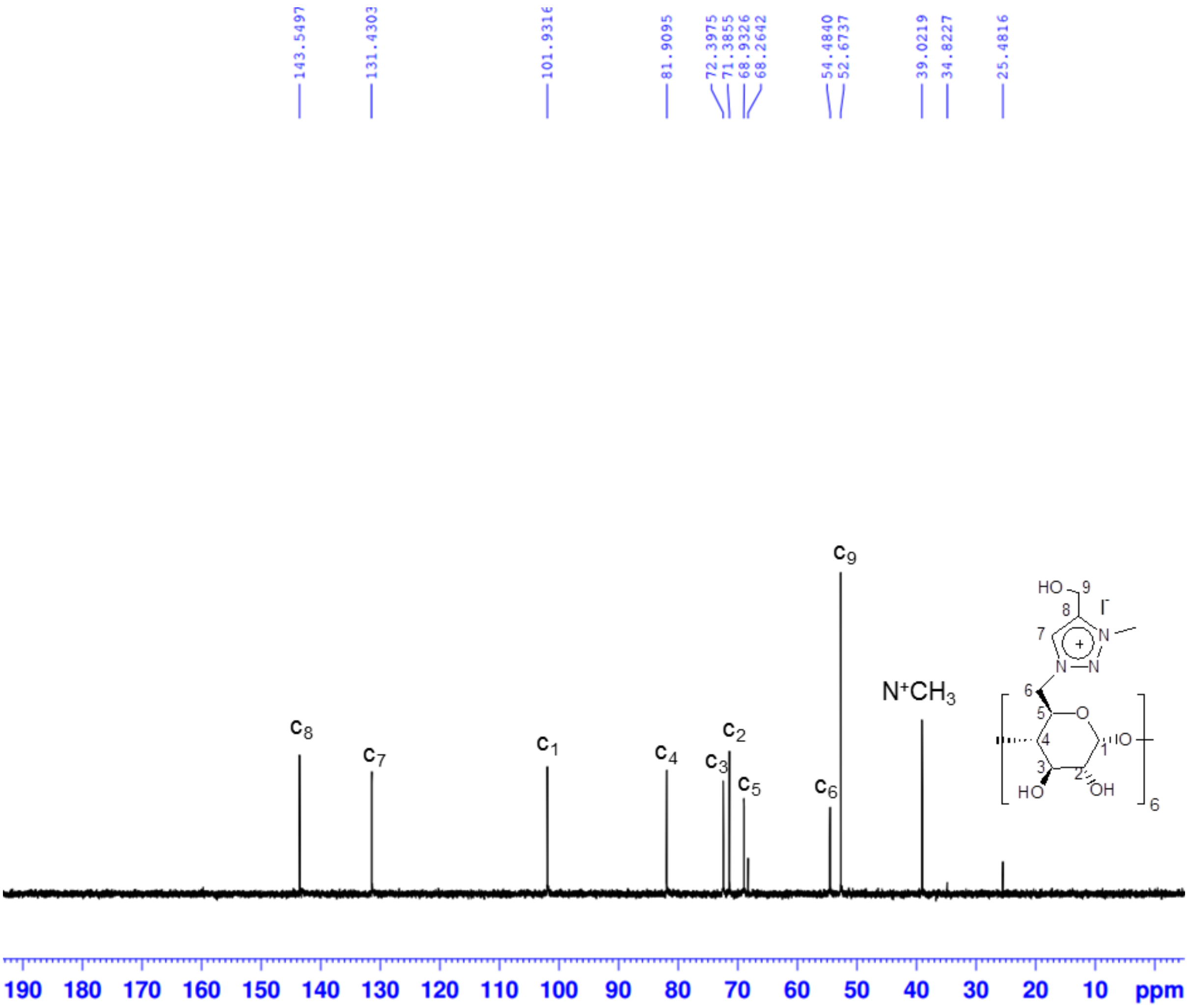


Figure S1-2C. Partial HH COSY spectrum (D_2O, 600 MHz) of the glucose part of **5**

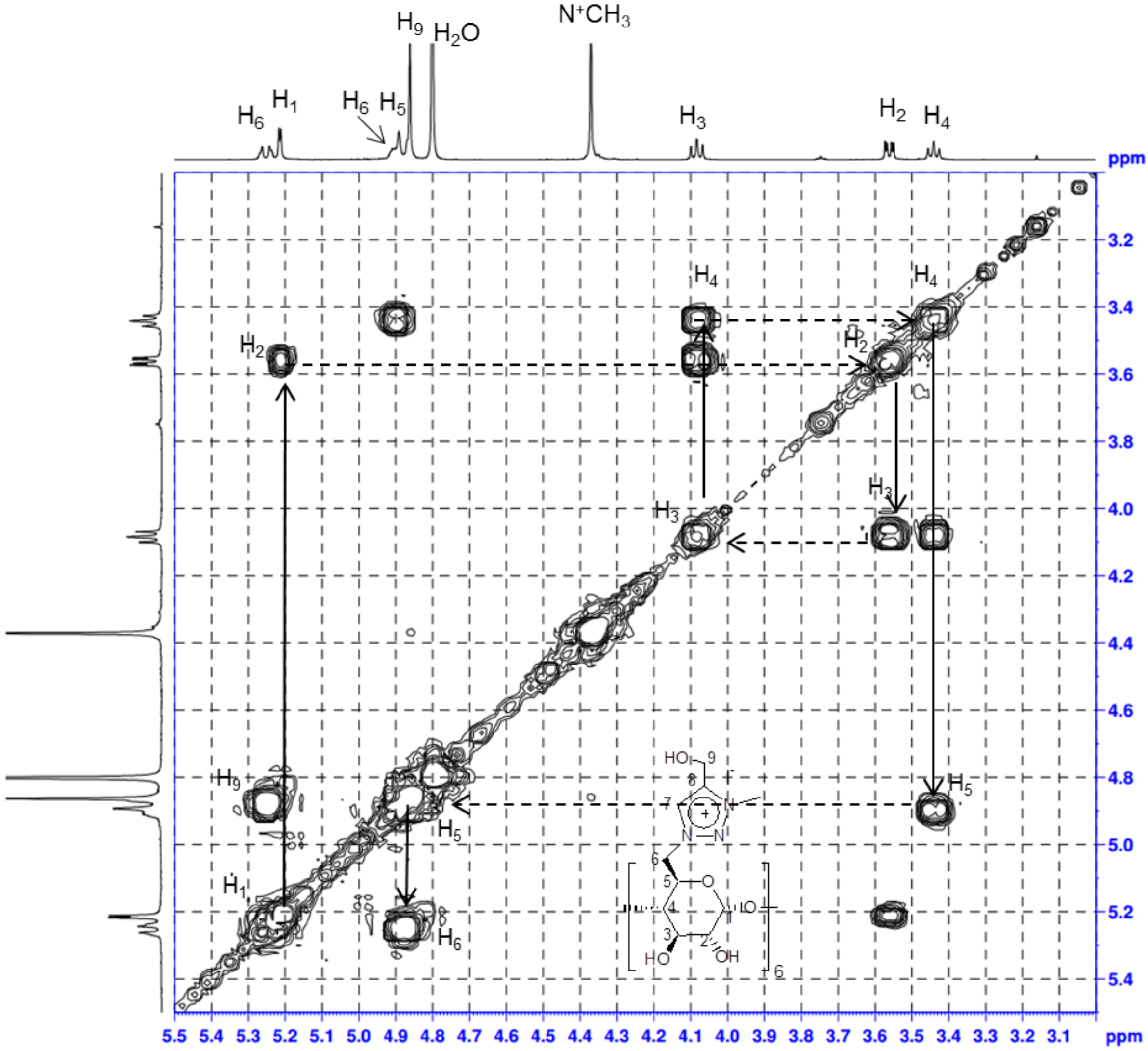


Figure S1-2D. HSQC spectrum (D_2O, 600 MHz) of **5** (↓ contour: CH_2 connectivity).

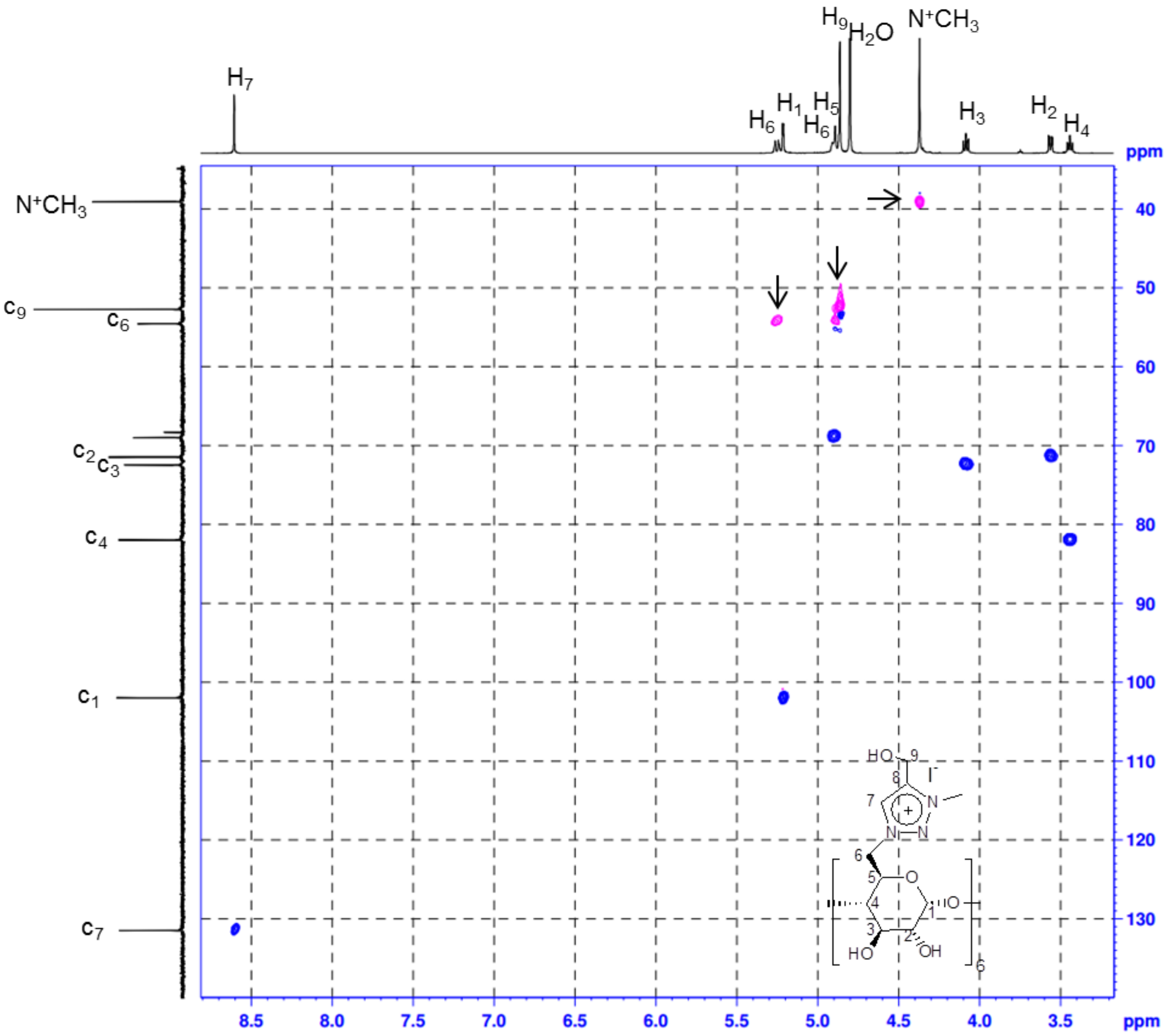
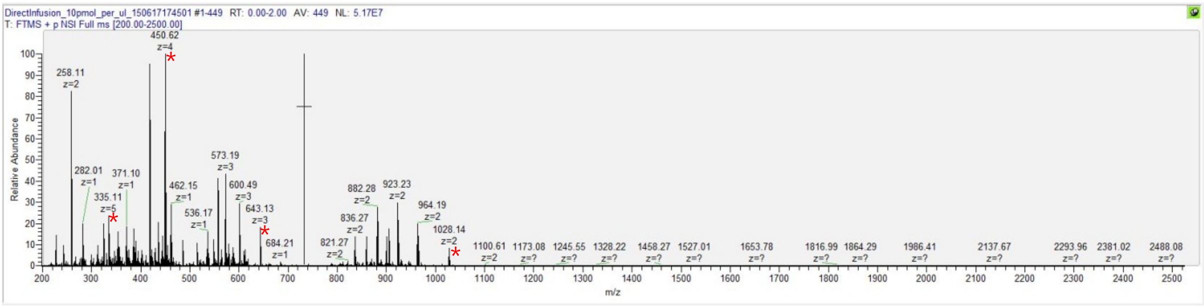


Figure S1-2E. ESI-MS of **5**

Instrument: Q-Exactive (Thermo); Injection: Direct Infusion; Sample concentration: 10 pmol/ µL

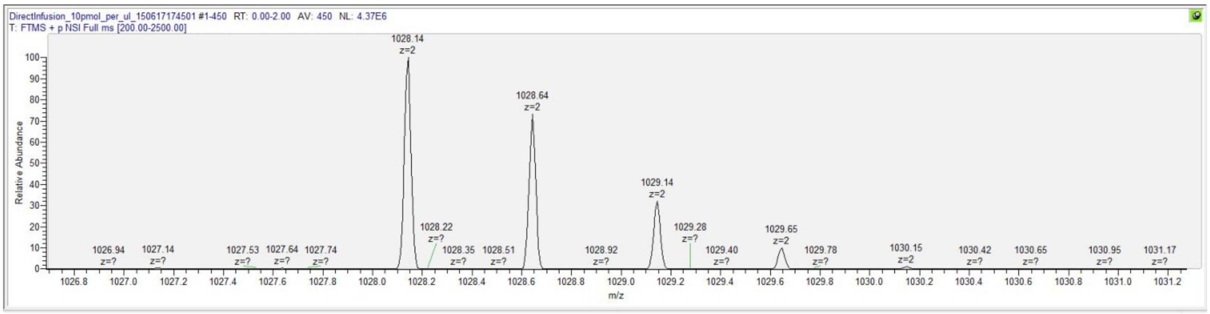
Isotope pattern was calculated from Isotope Pattern Calculator v4.0 (http://yanjunhua.tripod.com/pattern.htm)

a) Full mass spectrum



Multi-charge peak peaking (red asterisk): 1028.14 for $[M-2I]^{2+}$, 643.13 for $[M-3I]^{3+}$, 450.62 for $[M-4I]^{4+}$, 335.11 for $[M-5I]^{5+}$

b) Isotope pattern analysis of $[M-2I]^{2+}$



Calculated			Observed	
Exact mass	% abundance	Z=2 corrected mass	Peak	% abundance
2056.27	100	1028.14	1028.14	100
2057.28	65	1028.64	1028.64	73
2058.28	21	1028.14	1028.14	32

Mass peaks which relative abundances are more than 10 % are listed

S2. Fluorescence-pH dependence of cF and 5/cF complex

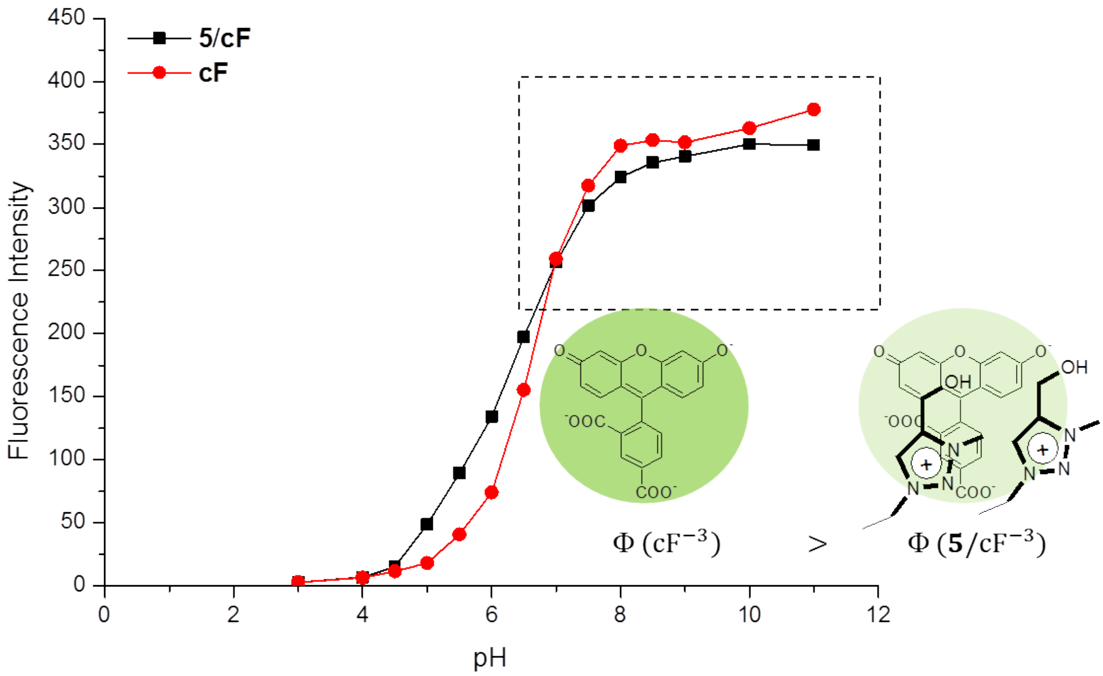


Figure S2. The Fluorescence intensity changes of cF (0.2 μM) and **5**/cF complex (cF = 0.2 μM, **5** = 20 μM) as a function of pH in 0.05 % DMSO. 10 mM HEPES buffer was adjusted pH at range 3 – 11 using HCl or NaOH by pH meter. The fluorescence was excited at 490 nm and the emission was measured at 524 nm (excitation/emission slit: 3/1.5). Φ (*i*) = quantum efficiency of species *i*.

S3. Binding constant of 5-carboxyfluorescein/cyclodextrin complex

Benesi-Hildebrand equation

The binding constant (K_1) in case of 1:1 complexation of cF to cyclodextrin (CD) and the correlation equation between K_1 and observed values are given by:

$$K_1 = \frac{[CD \cdot cF]}{[CD][cF]}$$

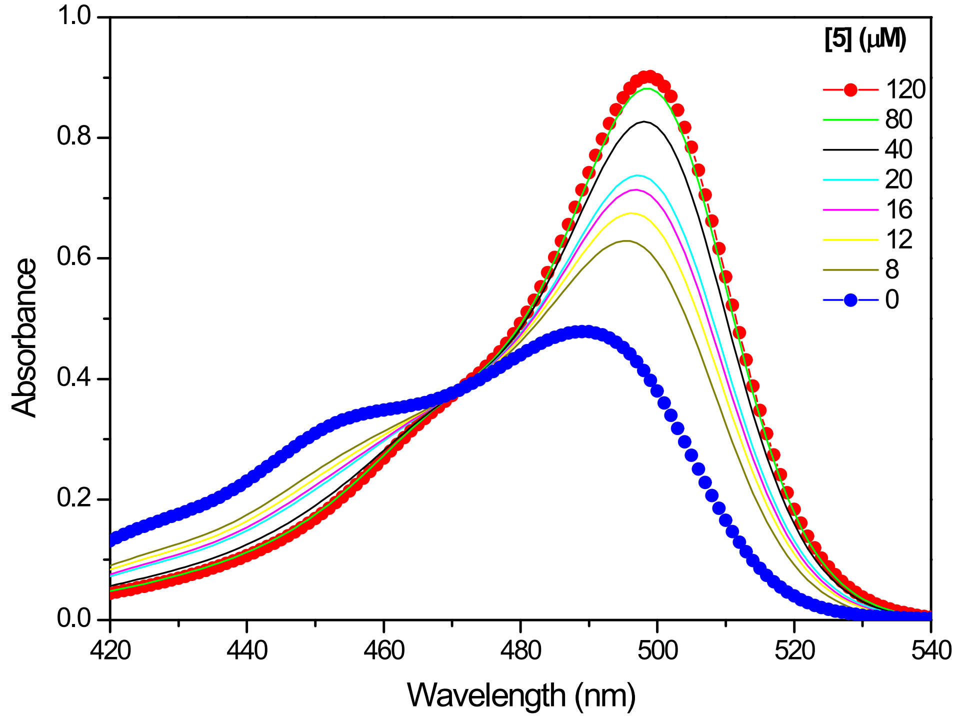
and

$$\frac{b}{\Delta A} = \frac{1}{[cF]_t K_1 \Delta\varepsilon} \frac{1}{[CD]} + \frac{1}{[cF]_t \Delta\varepsilon}$$

In this system $[CD]_t$ is much larger than $[cF]_t$, and it is appropriate to set $[CD] = [CD]_0$ (t and 0 means total and initial concentration). ΔA and Δε are the differences in the absorbance and in the molar extinction coefficient between cF and cF/CD solution.

Measurement

A working solution of cF (14 μM) was prepared in HEPES (10 mM, pH 7.4):methanol (1:1, v/v). The stock solutions of **5** (200 μM) was serially diluted with the cF working solution to give 120, 80, 40, 20, 16, 12, 8 μM. The stock solutions of **6** (200 μM) was serially diluted with cF working solution to give 120, 90, 70, 60, 35, 25, 20, 12, 8 μM. The ΔA values at various concentrations of CD were measured at λ_{max} (498 nm) of cF. The binding constants (K_1) were calculated from the slopes and y-intercepts of 1/[CD] vs. 1/[ΔA] plotting; K_1 = (y-intercept)/(slope)



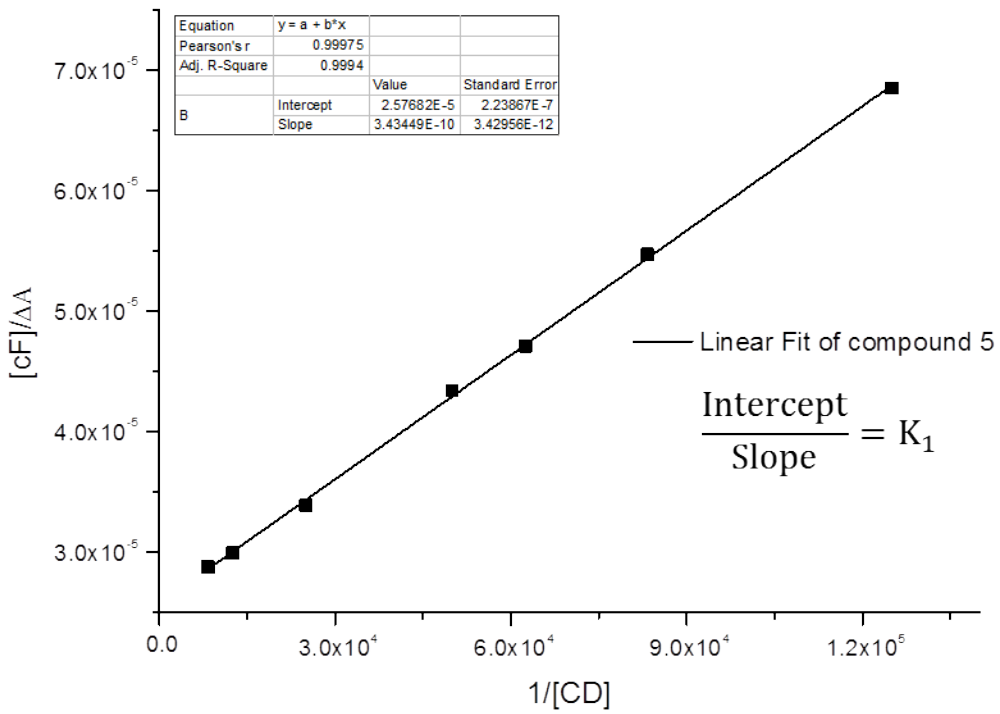
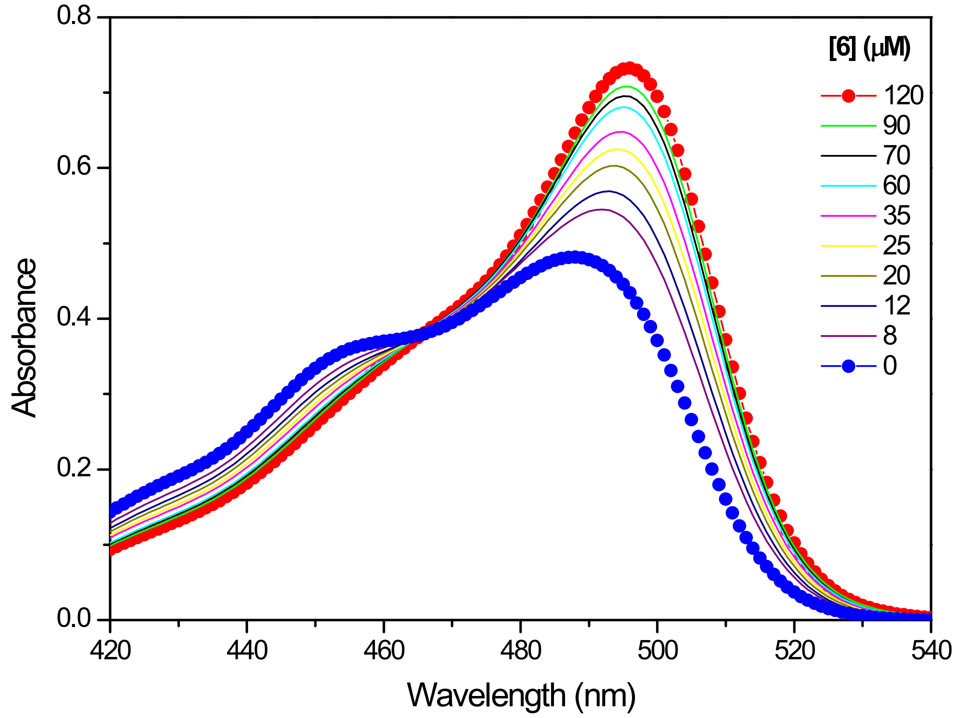


Figure S3-1. UV/vis titration of cF (14 μM) with **5**. 1/[CD] vs. 1/[ΔA] plotting at 498 nm. Inset: binding constant calculation.



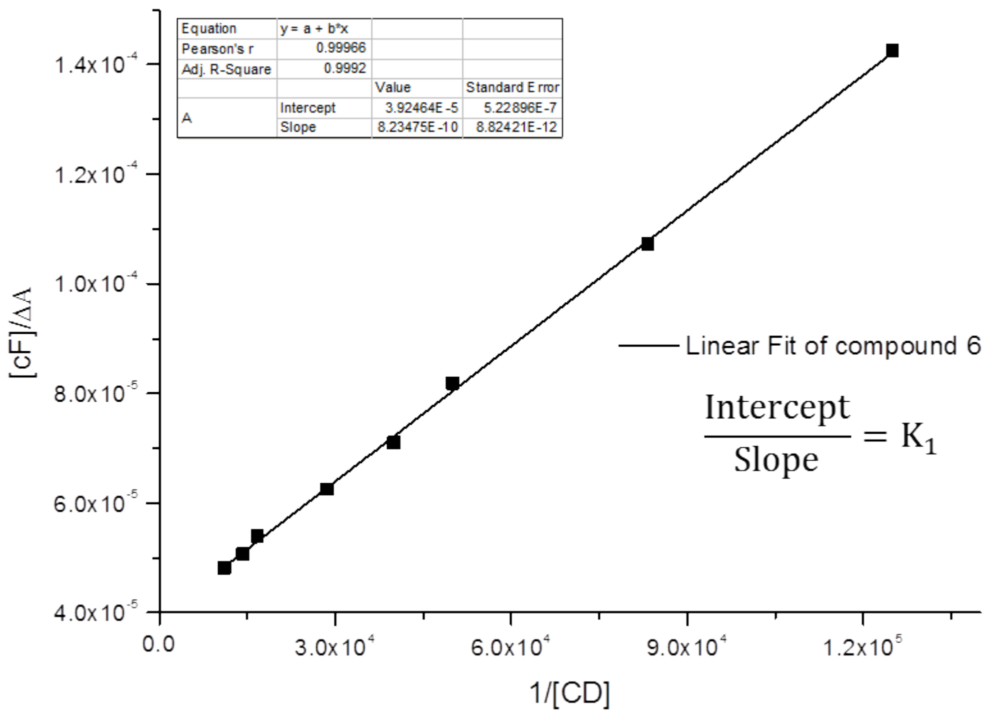
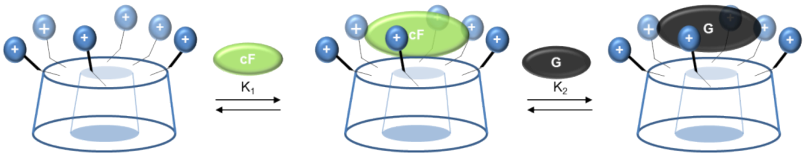


Figure S3-2. UV/vis titration of cF (14 μM) with compound **6**. 1/[CD] vs. 1/[ΔA] plotting at 498 nm. Inset: binding constant calculation.

S4. Competitive binding study

S4-1. Corner's equation for competitive binding study



Equation

The competitive binding constant (K_2) in case of 1:1 complexation of G to CD and the correlation equation between K_2 and observed values are

$$K_2 = \frac{[CD \cdot G]}{[CD][G]}$$

and

$$\frac{[G]_t}{P} = \frac{K_1}{K_2} Q + 1$$

The quantity P is defined as

$$P = [CD]_t - \frac{1}{QK_1} - \frac{[cF]_t}{Q+1}$$

The cF ratio Q can be obtained from

$$Q = \frac{\varepsilon - \varepsilon_{CD \cdot cF}}{\varepsilon_{cF} - \varepsilon}$$

The total cF concentration is constant in all solutions, thus ε can be replaced by A.

$$Q = \frac{A - A_{CD \cdot cF}}{A_{cF} - A}$$

where A_{cF} and $A_{CD \cdot cF}$ are the absorbance of free and complexed cF, respectively, and A is the apparent absorbance in any solution containing both forms. For optimum results, Q values in the range 0.3-3 were chosen

Measurement

Addition of guests (IP_3, Phytic acid, ATP, EDTA, glucose-6-phosphate, and glucose) to the complex solution formed between **5**/**6** and cF ([**5**] = [**6**] =110 μM, [cF] =14 μM) resulted in the displacement of cF by the guests. The competitive binding constants (K_2) were calculated from the slopes of Q vs. $[G]_t$/P plotting and from the K_1 of SI S4.

S4-2. Competitive binding experiment of 5

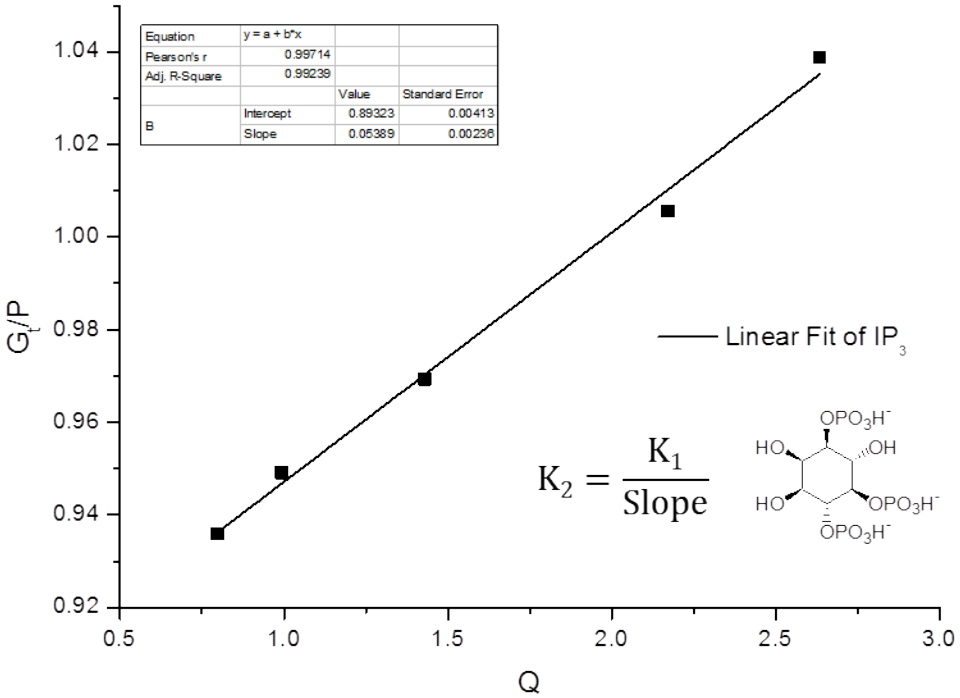
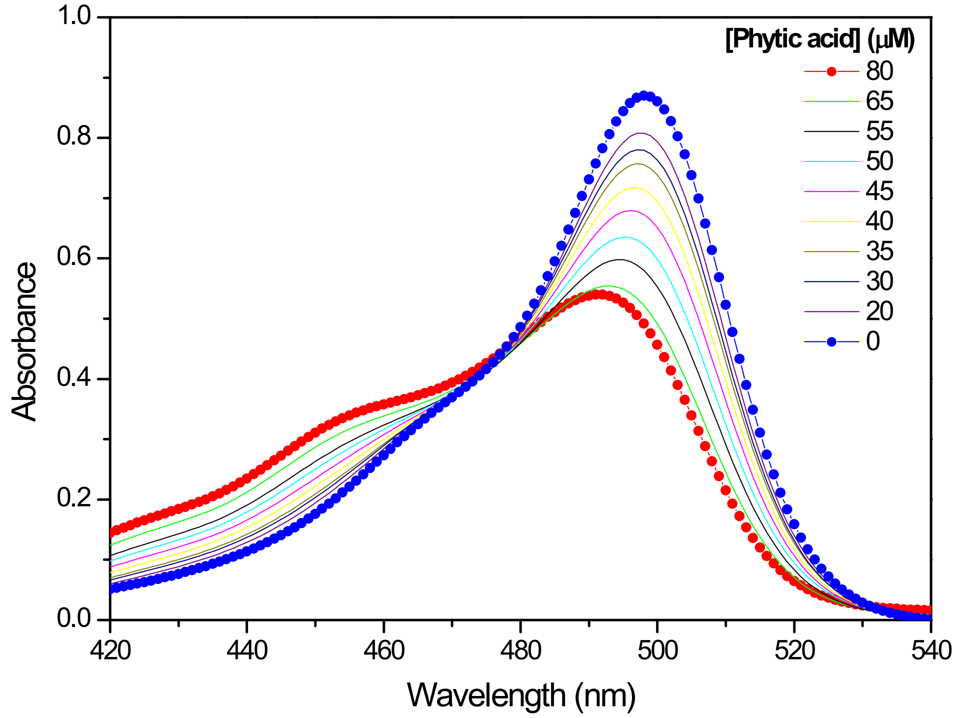


Figure S4-2A. Plotting of **5**/cF complex ([**5**] = 110 μM, [cF] =14 μM) with IP_3. Inset: 1/[CD] vs. 1/[ΔA] plotting at 498 nm and binding constant calculation.



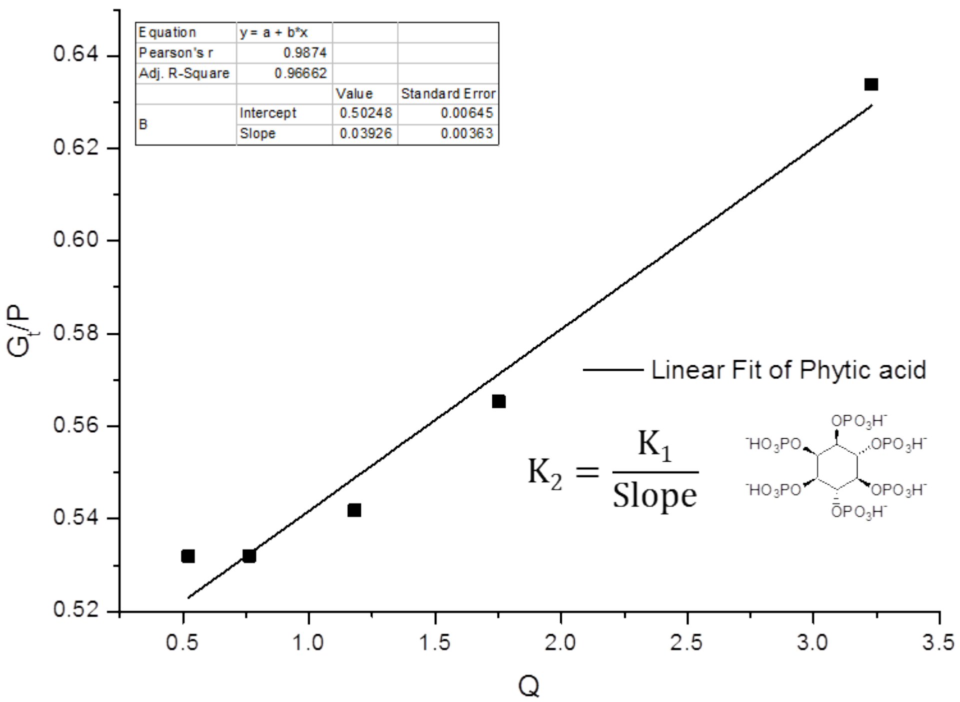
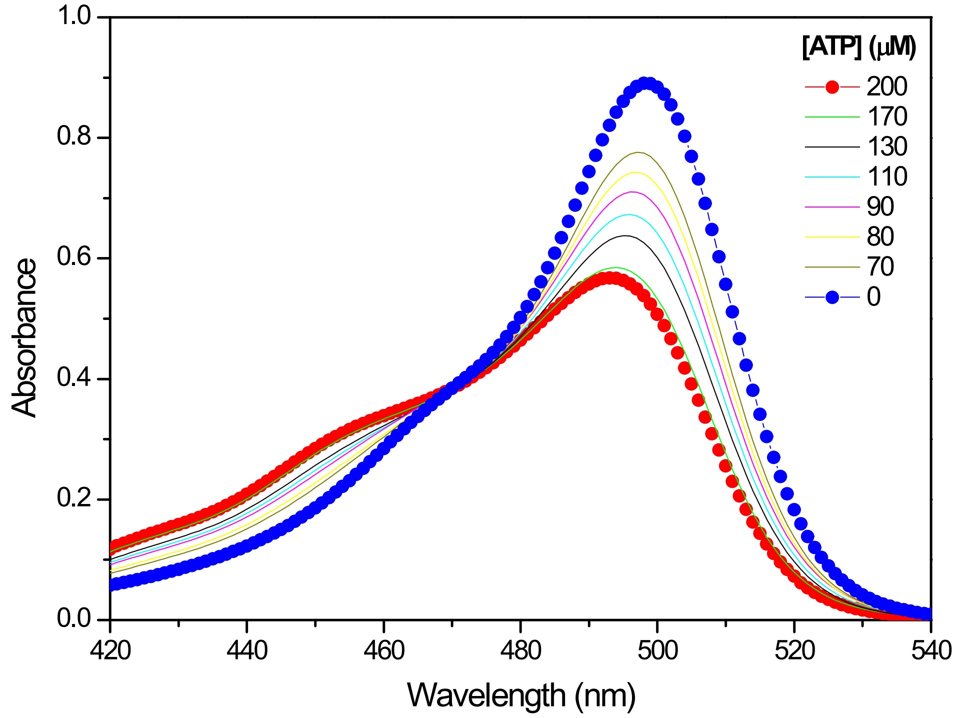


Figure S4-2B. Competitive UV/vis titration and plotting of **5**/cF complex ([**5**] = 110 μM, [cF] =14 μM) with Phytic acid. Inset: 1/[CD] vs. 1/[ΔA] plotting at 498 nm and binding constant calculation.



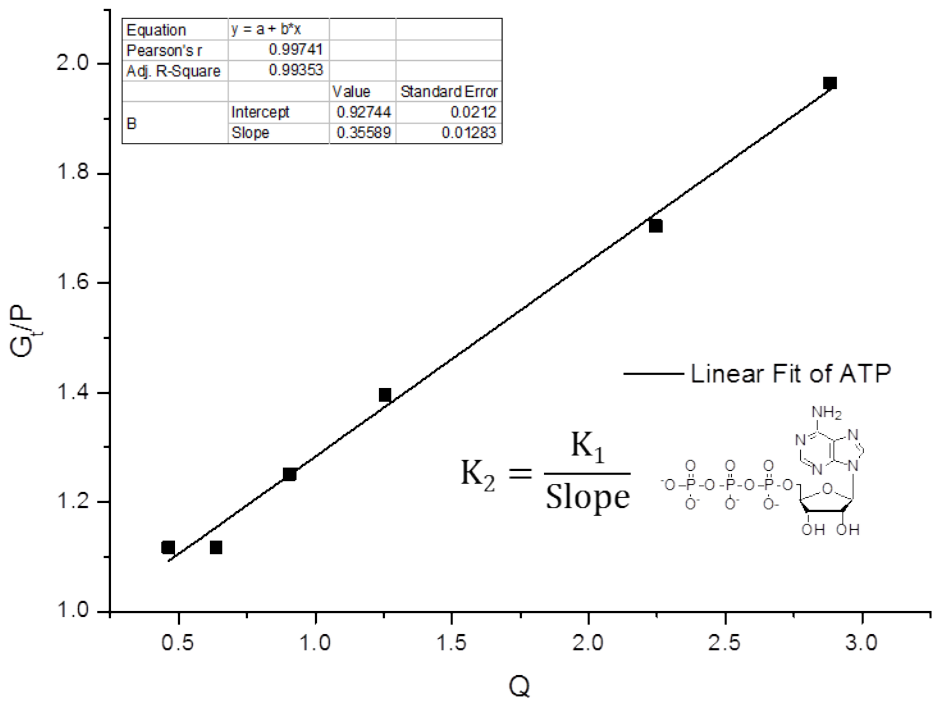
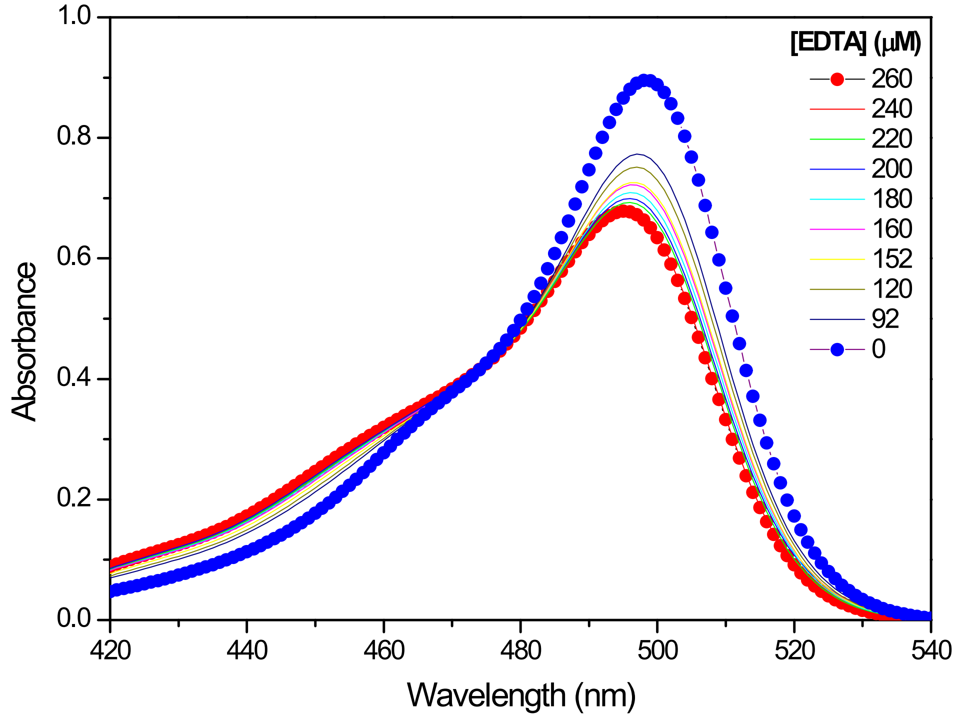


Figure S4-2C. Competitive UV/vis titration and plotting of **5**/cF complex ([**5**] = 110 μM, [cF] =14 μM) with ATP. Inset: 1/[CD] vs. 1/[ΔA] plotting at 498 nm and binding constant calculation.



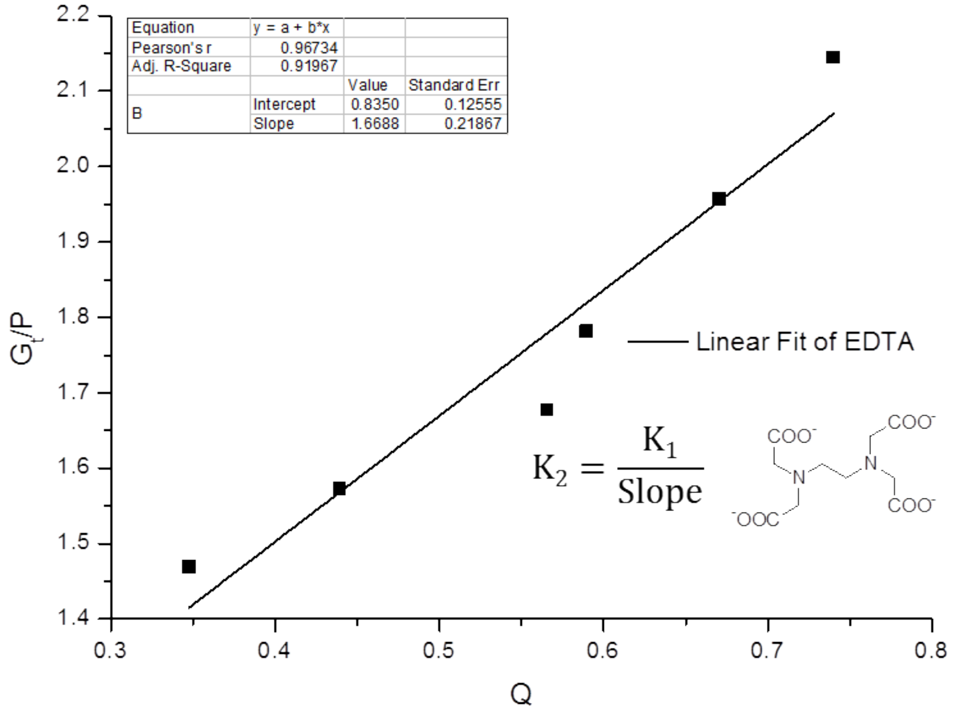


Figure S4-2D. Competitive UV/vis titration and plotting of **5**/cF complex ([**5**] = 110 μM, [cF] =14 μM) with EDTA. Inset: 1/[CD] vs. 1/[ΔA] plotting at 498 nm and binding constant calculation.

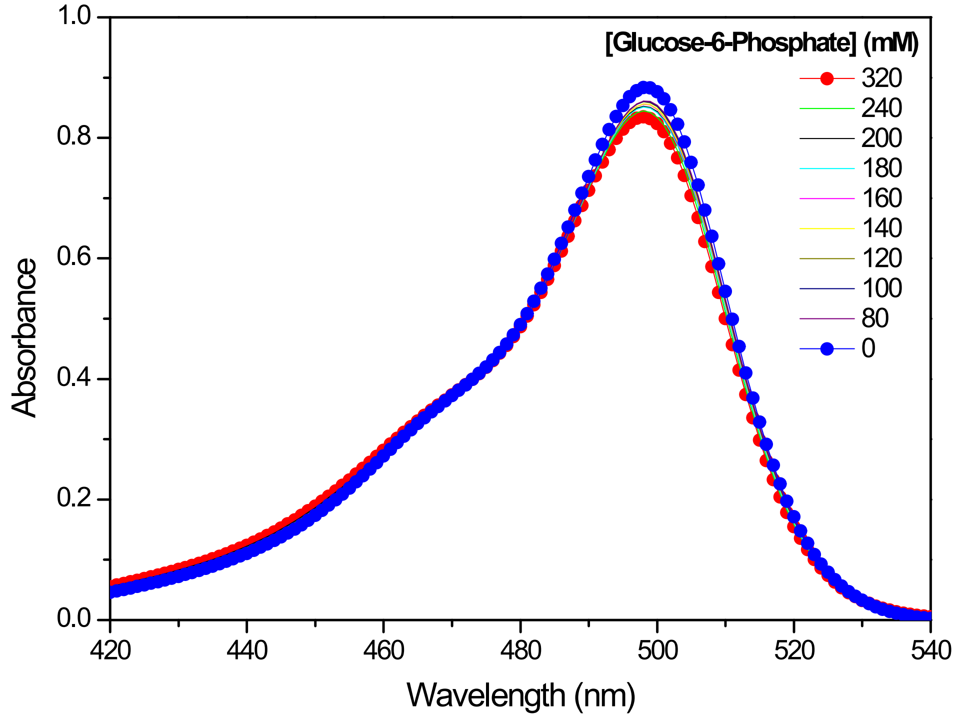


Figure S4-2E. Competitive UV/vis titration of **5**/cF complex ([**5**] = 110 μM, [cF] =14 μM) with glucose-6-phosphate.

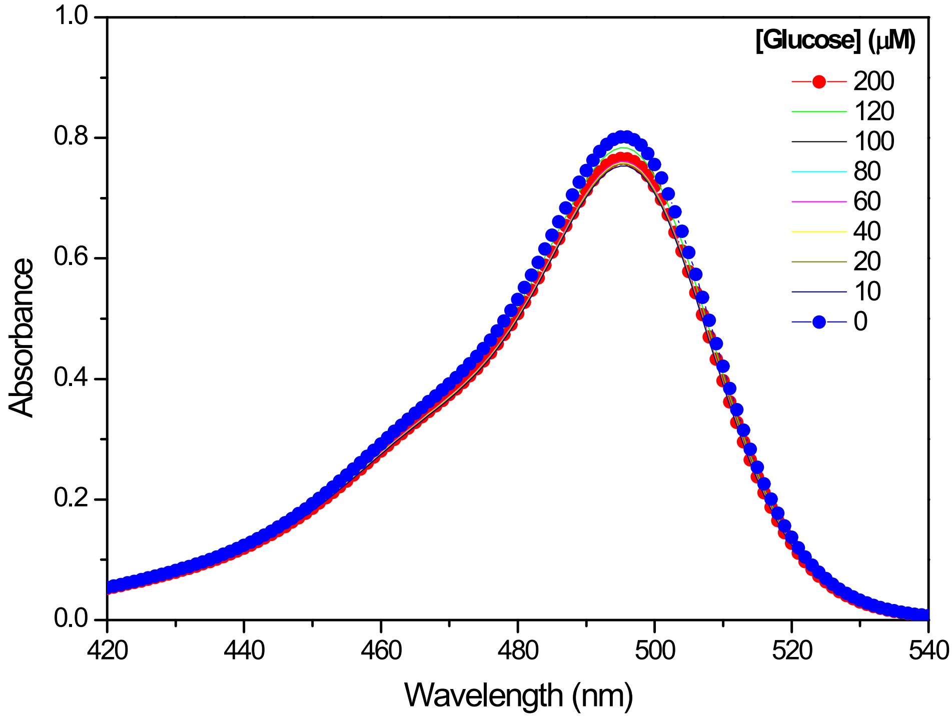
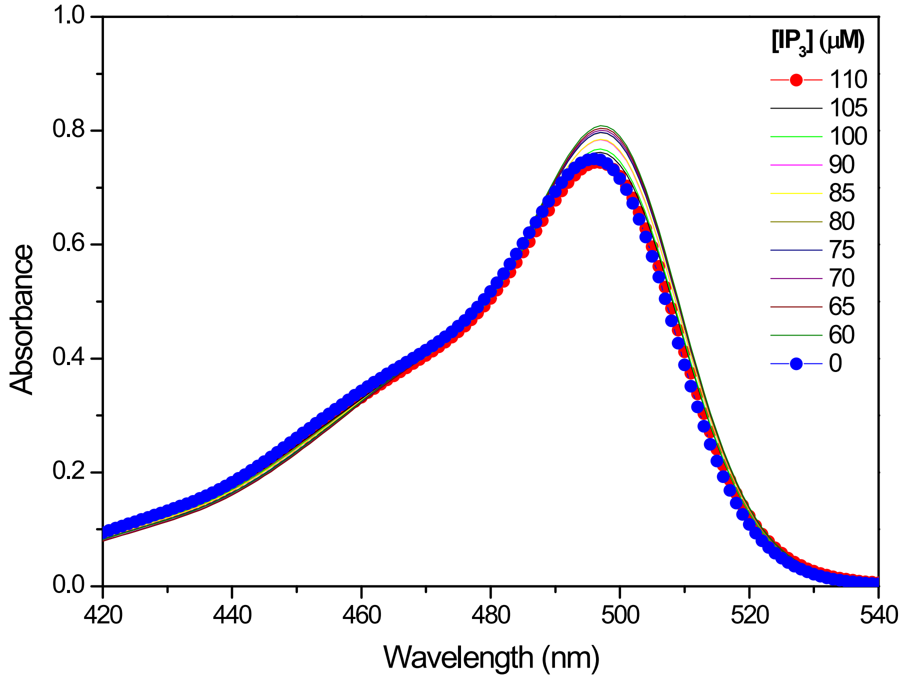


Figure S4-2F. Competitive UV/vis titration of **5**/cF complex ([**5**] = 110 μM, [cF] =14 μM) with glucose.

S4-3. Competitive binding experiment of 6



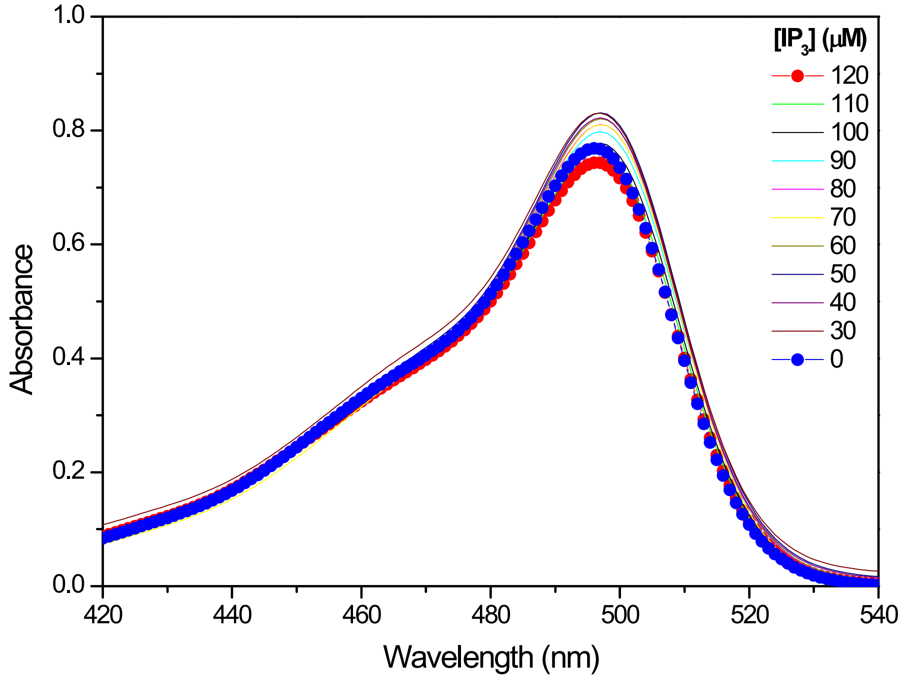
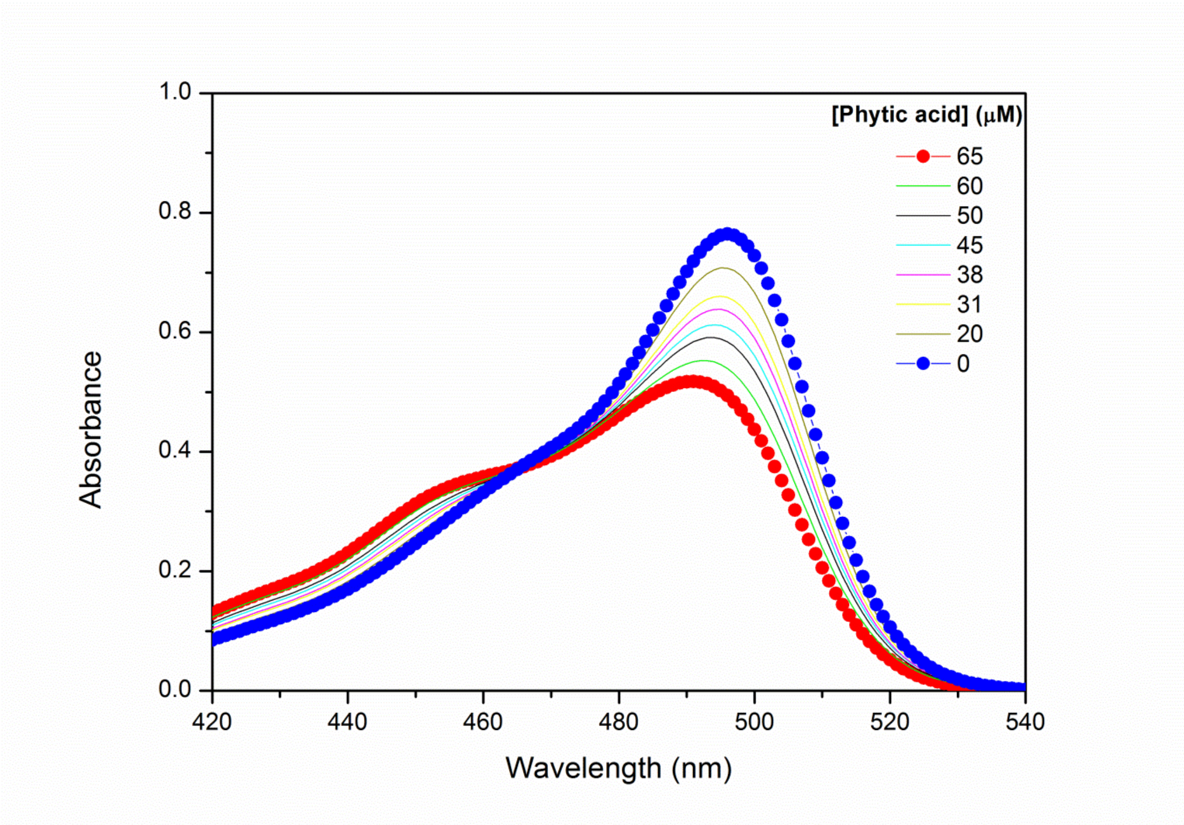


Figure S4-3A. Two independent measurements: Competitive UV/vis titration of **6**/cF complex ([**6**] = 110 μM, [cF] =14 μM) with IP_3.



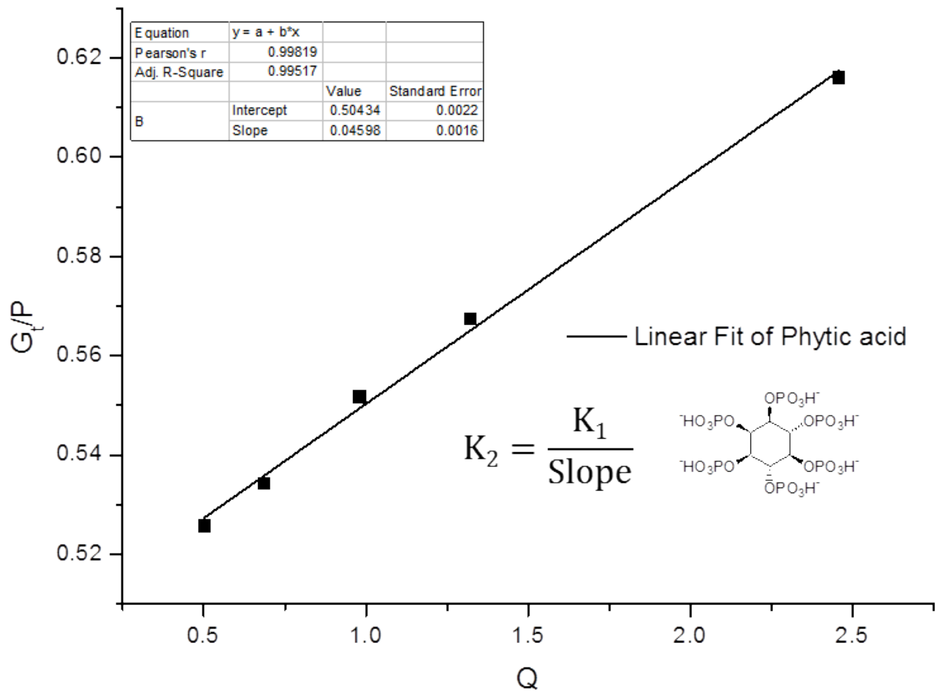
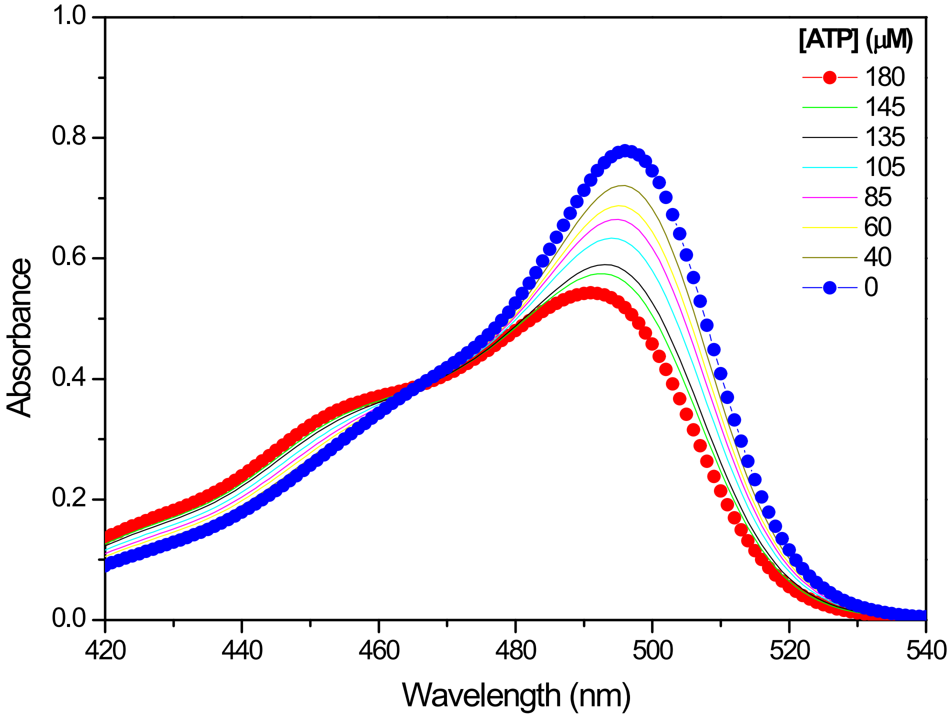


Figure S4-3B. Competitive UV/vis titration and plotting of **6**/cF complex ([**6**] = 110 μM, [cF] =14 μM) with phytic acid. Inset: 1/[CD] vs. 1/[ΔA] plotting at 498 nm and binding constant calculation.



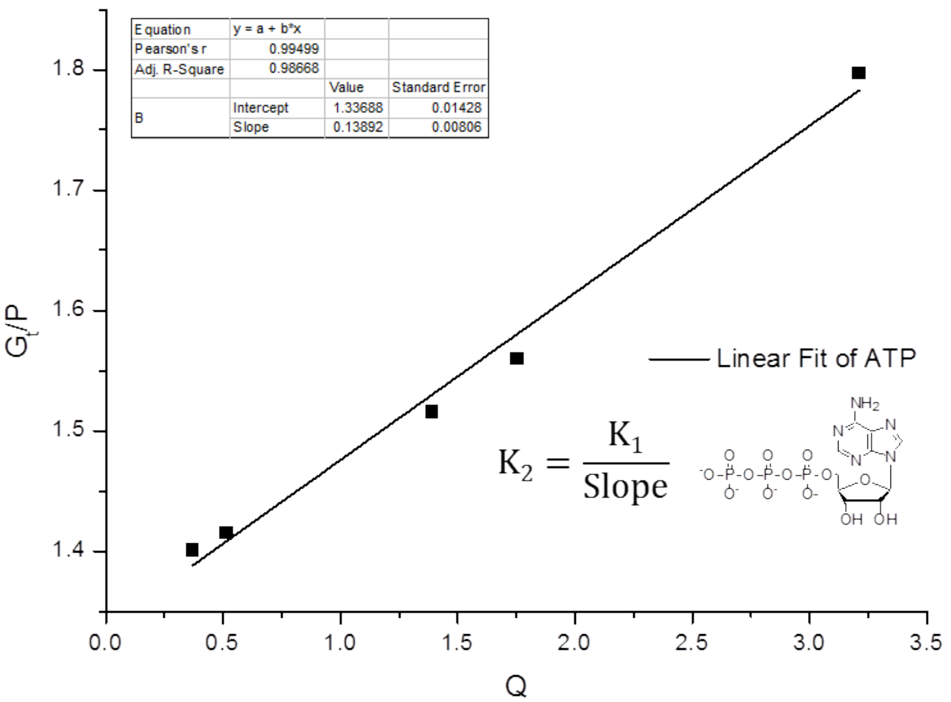
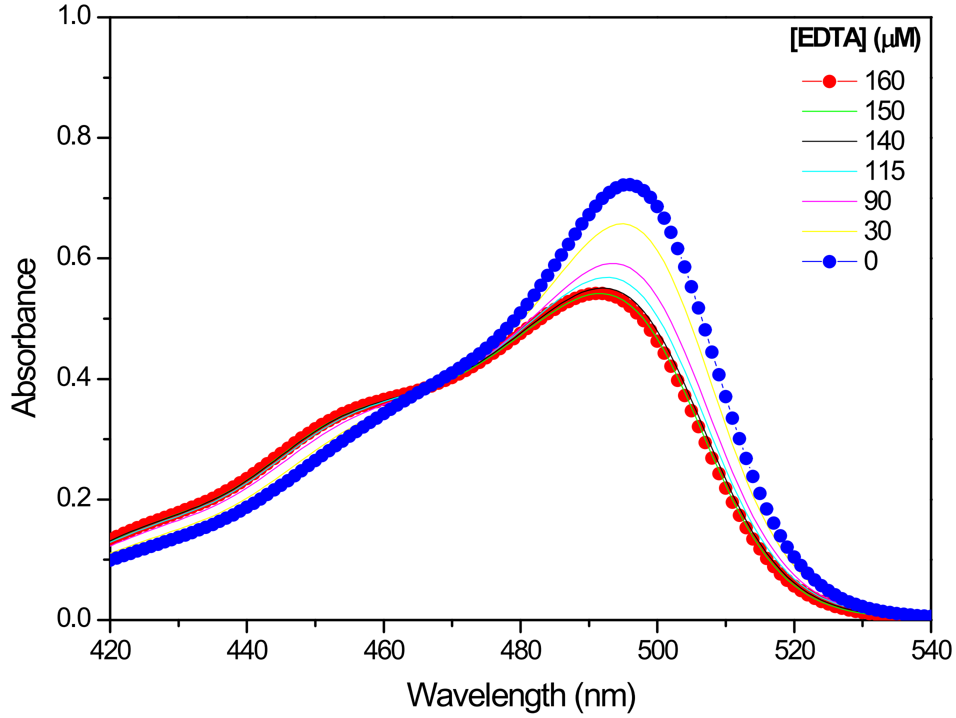


Figure S4-3C. Competitive UV/vis titration and plotting of **6**/cF complex ([**6**] = 110 μM, [cF] =14 μM) with ATP. Inset: 1/[CD] vs. 1/[ΔA] plotting at 498 nm and binding constant calculation.



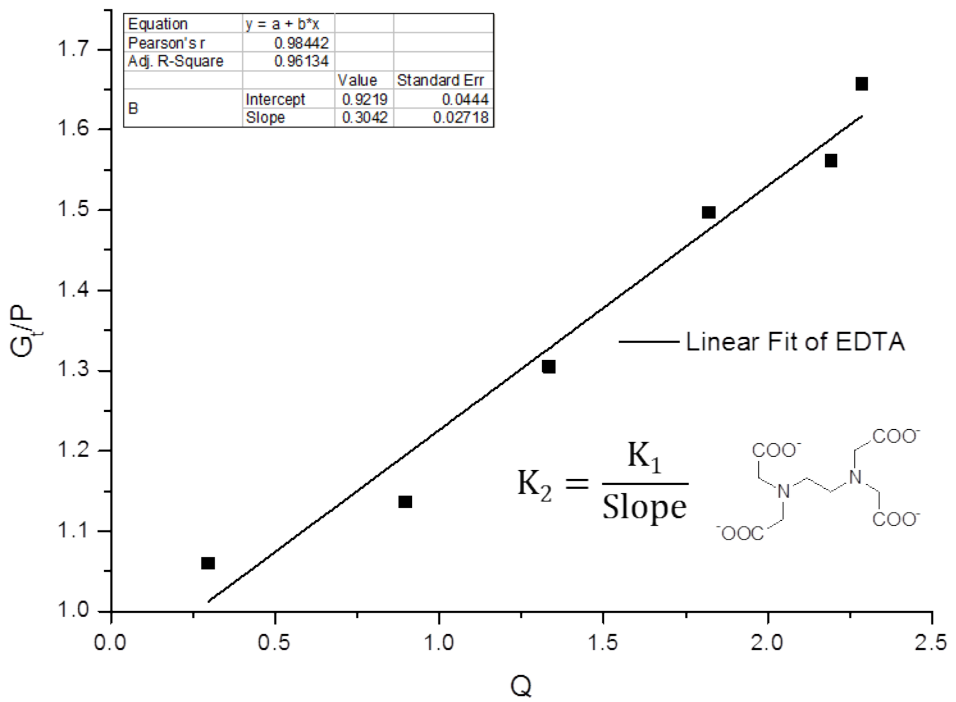


Figure S4-3D. Competitive UV/vis titration and plotting of **6**/cF complex ([**6**] = 110 μM, [cF] =14 μM) with EDTA. Inset: 1/[CD] vs. 1/[ΔA] plotting at 498 nm and binding constant calculation.

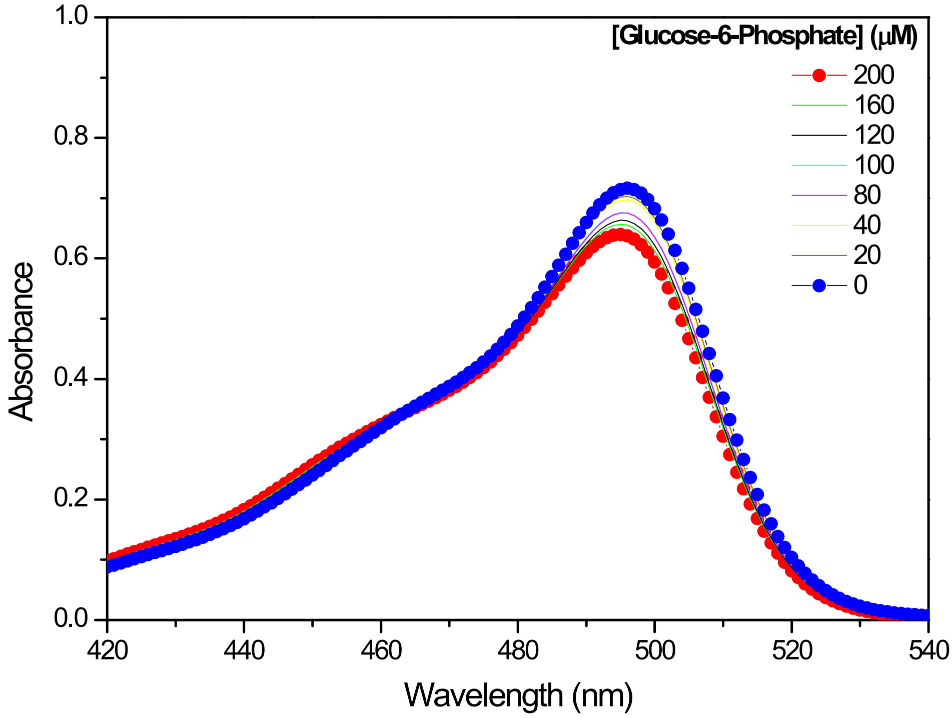


Figure S4-3E. Competitive UV/vis titration of **6**/cF complex ([**6**] = 110 μM, [cF] =14 μM) with glucose-6-phosphate.

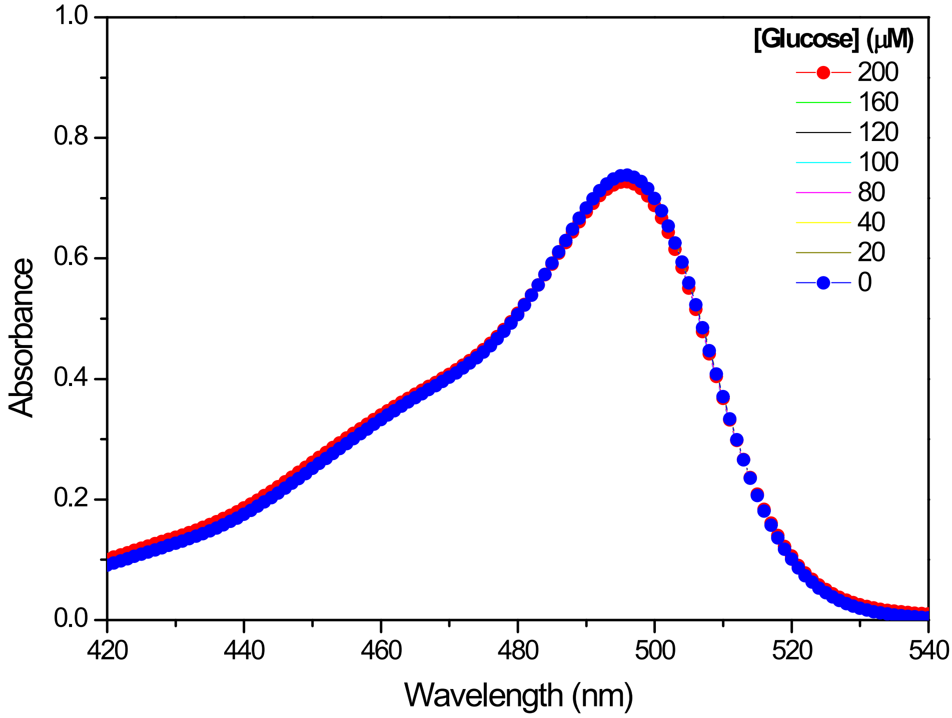


Figure S4-3F. Competitive UV/vis titration of **6**/cF complex ([**6**] = 110 µM, [cF] =14 µM) with glucose.

S5. The pH-dependent solubility of 5 and 6

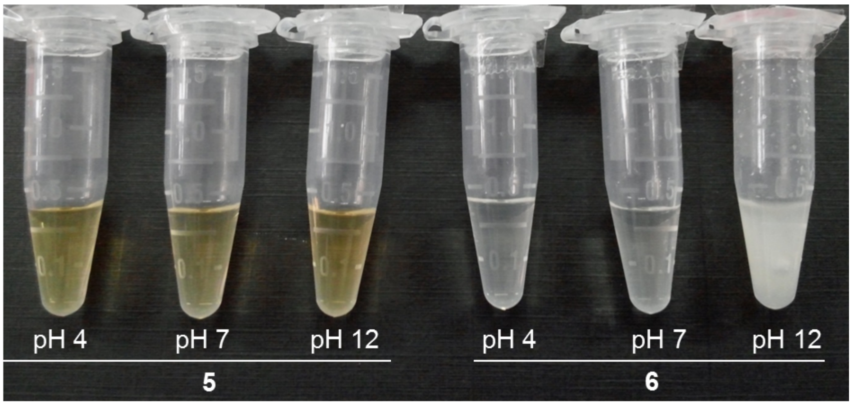
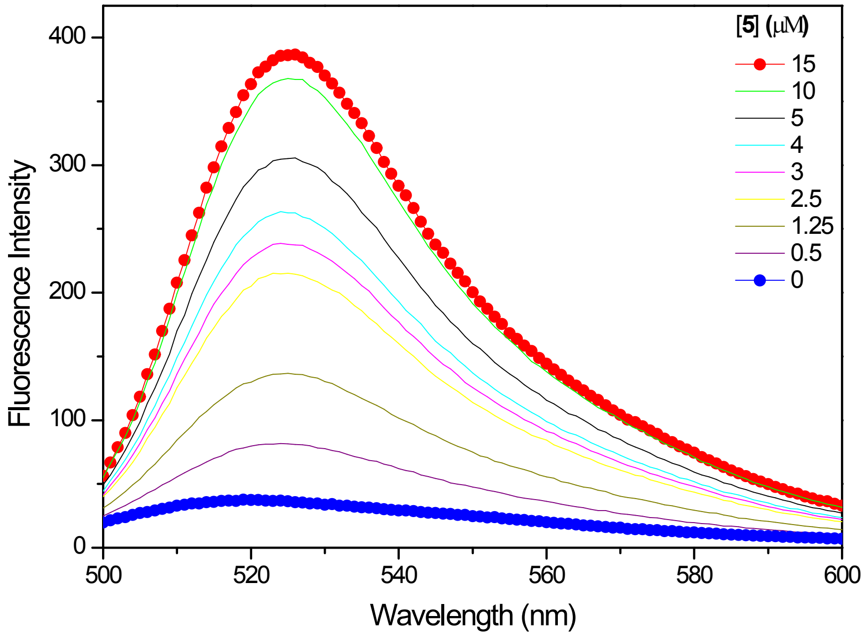


Figure S5. Photograph: Turbidity at different pH (pH 4, 7, 12) of **5** and **6**. 20 mg of CDs in 300 µL solution.

S6. Fluorescence titration of 5/cF complex



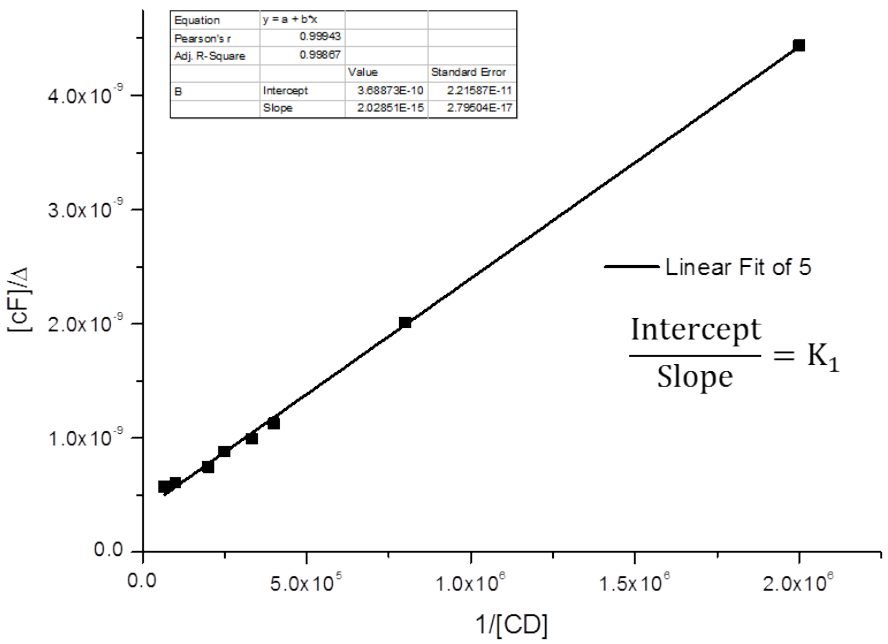


Figure S6. Fluorescence titration of cF (0.2 μM) with compound **5**. 1/[CD] vs. 1/[ΔA] plotting at 524 nm. Inset: binding constant calculation. The fluorescence was excited at 490 nm (excitation/emission slit: 3/3).

S7. Molecular Modeling of cF

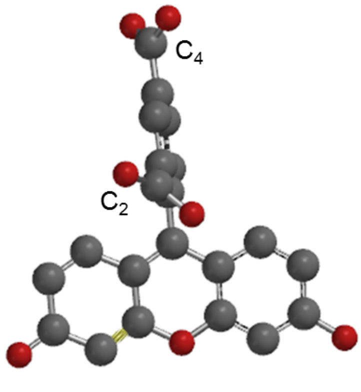


Figure S7. Molecular modeling (Equilibrium geometry at ground state with B3LYP/6-31G* basis set in vacuum, Spartan '08 v1.2.0) of cF (acidic form). The carboxylic C_2 and phenolic O distance = 7.54 Å and the carboxylic C_4 and phenolic O distance = 10.12 Å. The molecules are displayed using a ball and spoke model at the same scale.

References

1. F. Guillo, B. Hamelin, L. Jullien, J. Canceill, J-. M. Lehn, L. De Robertis, H. Driguez, *Bull. Soc. Chim. Fr.,* 1995, **132**, 857–866.

2. P. R. Ashton, R. Königer and J. F. Stoddart, *J. Org. Chem*., 1996, **61**, 903–908.

3. N. Mourtzis, K. Eliadou, C. Aggelidou, V. Sophianopoulou, I. M. Mavridis, K. Yannakopoulou, *Org. Biomol. Chem*., 2007, **5**, 125–231.