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Title:

Small polyanion recognition of triazolium cyclodextrin click cluster in water

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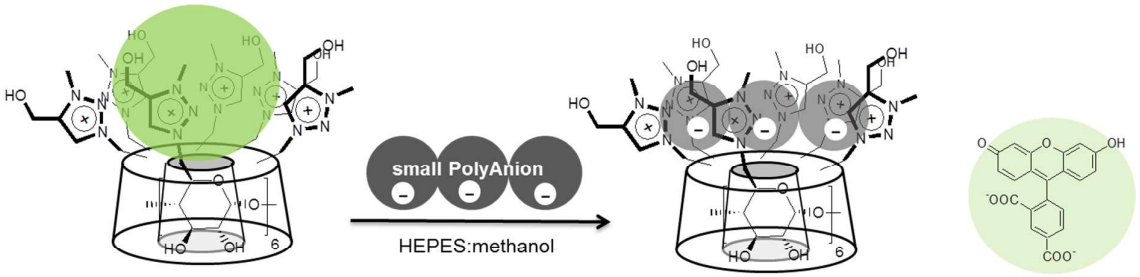
Graphic Abstract



Keywords:

Small polyanion, triazolium, cyclodextrin click cluster, copper(I)-catalyzed azide–alkyne cycloaddition, anion recognition

Abstract

In order to detect small polyanions (sPAs), which play important roles in many biological systems, a triazolium cyclodextrin click cluster (**5**, hexakis{6-(3-methyl-4-hydroxymethyl-1H-1,2,3-triazolium-1-yl)-6-deoxy}-α-cyclodextrin iodide) was synthesized and characterized. The competition binding to **5** occupied by 5-carboxyfluorescein, of inositol-1,4,5-trisphosphate (IP_3), phytic acid, adenosine triphosphate (ATP), ethylenediaminetetraacetic acid (EDTA), glucose, and glucose-6-phosphate was evaluated by UV/vis titration in HEPES (10 mM, pH 7.4):methanol (1:1, v/v). We obtained the binding constants of IP_3 and phytic acid to **5** (1.4×10^6 and 1.9×10^6 M^{-1}, respectively); however, the binding of ATP and EDTA were significantly lower (2.1×10^5 and 4.5×10^4 M^{-1}, respectively). Moreover, glucose and glucose-6-phosphate did not show any detectable binding. In addition, the sPA recognition of the triazolium cyclodextrin click cluster in water was confirmed by fluorescence titration.

Introduction

Small polyanions (sPAs), such as inositol triphosphate (IP_3), nicotinamide adenine dinucleotide phosphate, and polycarboxylic acids, play versatile and critical roles in many biological systems. For instance, IP_3, a product of the hydrolysis of phosphatidylinositol phosphates, acts as an intracellular messenger in signal transduction and lipid signaling,[1] and phytic acid is the principal storage form of phosphorus in many plant tissues.[2] Accordingly, many studies have sought to develop the receptors for sPAs, yet they have been hampered by many limitations, including weak binding and low selectivity.[3] Therefore, introduction of selective receptors to sPAs in aqueous environments is of paramount importance, and remains a challenge in the field of host-guest chemistry.

Anion recognition in the solution involves various noncovalent interactions such as electrostatic interaction, hydrogen bonding, and metal-coordination.[4] In the aqueous environment, electrostatic interaction-based anion recognition is particularly important because hydrogen bonding between anions and their receptors can be hampered by hydrogen bonding with surrounding water molecules.[5] Therefore, a common strategy of sPA recognition in water has been the adoption of multivalent electrostatic interactions between sPAs and highly positively charged receptors, where the positive charges include ammonium and guanidinium moieties.[6] Indeed,

the number of positive charges of receptors has been a crucial parameter for the achievement of high binding constants to sPAs, along with their spatial orientation.[7] The synergistic action of these two factors in water is efficiently provided by scaffold structures of receptors: polyaza macrocycles, symmetric clefts, and cyclodextrin.[8] However, there is still a need for the development of novel sPA receptors based on new scaffold structures and binding motifs.

Cyclodextrins (CDs), cyclic oligosaccharides consisting of 6–8 glucose units, have been used as a directional, multivalent scaffold for supramolecular assembly, as a gene delivery system, and for chiral separation, among other uses.[9] The copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction is known to be modular, specific, wide in scope, and provides high yields.[10] The resulting 1,2,3-triazoles can be regiospecifically and efficiently alkylated at the N-3 position.[11] Recently, cyclodextrin click clusters (CCCs, perfunctionalized CDs with 1,2,3-triazole moieties) have been frequently explored by CuAAC.[12] The CCC approach has been proven to be a convenient way to conjugate multiple moieties on a CD molecule with high efficiency.

Utilizing CuAAC chemistry and methylation, the triazolium motif has been employed for the recognition of anions in organic solvents by Pandey[13], Schubert[14] and Beer.[15] Recently Lim and Beer reported that acyclic bis-triazolium receptors exhibit enhanced perrhenate anion recognition in water. However, the anion binding of triazolium receptors in water is fairly weak (K_a = 10 ~ 51 M^{-1}).[16] The addition of positively charged triazolium moieties to CCCs would provide the CCCs with sPA recognition ability. Additionally, multiple triazoliums on CD will have limited conformational flexibility,[17] which acts as an obvious advantage for high-affinity sPA recognition. Herein, we suggest a novel sPA receptor based on triazolium CCC and investigate sPA recognition by the receptor in water.

Results and discussion

Scheme 1

6-Chloro-6-deoxy-α-cyclodextrin (**2**) and 6-azido-6-deoxy-α-cyclodextrin (**3**) were synthesized by following Lehn's procedure (ESI S1-1A).[18] The synthesis and characterization of hexakis{6-(4-hydroxymethyl-1H-1,2,3-

triazol-1-yl)-6-deoxy}-α-cyclodextrin (**4**) from **3** was reported by our group,[19] but we modified the procedure to scale-up without Diaion™ HP-20 column chromatography. Hexakis{6-(3-methyl-4-hydroxymethyl-1H-1,2,3-triazolium-1-yl)-6-deoxy}-α-cyclodextrin iodide (**5**) was synthesized from **4** by microwave assisted methylation (Scheme 1). **5** was fully characterized by ^{1}H, ^{13}C NMR, HH COSY (correlation spectroscopy) and CH HSQC (heteronuclear single quantum coherence) spectroscopy (ESI S1-2). In addition, in order to compare triazolium with ammonium (a known anion recognition element), amino-6-deoxy-α-cyclodextrin hydrochloride (**6**) was synthesized from **3** by adapting published procedures (ESI S1-1B).[20]

Complete triazole formation by CuAAC was confirmed by the presence of axial symmetry in the NMR spectra of **4.** A single doublet for anomeric protons (H_1) appeared in the ^{1}H NMR spectra (δ = 5.14 ppm, J = 3.3 Hz, characteristic J value of α configuration) and the corresponding C_1 atoms also appeared as one singlet (δ = 100.6 ppm) in the ^{1}H decoupled ^{13}C NMR spectrum (ESI Fig. S1-1).

Figure 1

The ^{1}H NMR spectrum of **4** in D_2O shows the unique germinal coupling of two diastereomeric H_9 (J = 13.3 Hz) (Fig. 1a). After methylation, H_6, H_9 of **5** showed downfield shift and the H_9 showed a different germinal coupling pattern (Fig. 1b). In addition, H_7 also downshifted from δ 7.94 to δ 8.60 after methylation. The downfield shift of H_6, H_7, H_9 after methylation at N_3 position resulted from the permanent positive charge of triazolium, which deshielded the hydrogens through inductive effect.

Figure 2

Molecular modeling of **5** and **5**/5-carboxyfluorescein (cF) complex are showed in Fig. 2. The average distance between $N_{3,i}$ and $N_{3,(i+1)}$ is 8.9 Å and the average distance between $N_{3,i}$ and $N_{3,(i+3)}$ is 17.3 Å in **5**. The distance between phenolic O and carboxylic C_2 and the distance between phenolic O and carboxylic C_4 in cF are 7.5 Å and 10.0 Å, respectively (ESI Fig. S7). From this calculation, we can infer that the positive charges of **5** are sufficiently separated and provide room for cF to bind by electrostatic interaction. In addition the modeling of **5**/cF complex shows the structural complementarity of cationic host (**5**) and anionic guest (cF).

Scheme 2

Based on the structural features of triazolium CCCs, pre-organization, multivalent positive charges, and suitable orientation, we devised a way for **5** to bind a series of sPAs, as depicted in Scheme 2a. However, **5** and the anionic guests in this experiment have very weak UV absorption in the visible range. Thus, an indicator displacement assay was employed, as this type of assay is useful for monitoring a guest that has no chromophore.[21] Indicator cF was already used for anion recognition of cationic receptors because cF has a relatively sensitive absorption/emission response depending on the receptor-guest complexation in physiological pH range.

Two dianion and trianion prototropic forms of cF (cF^{2-} and cF^{3-}) are the principal ground-state species under physiological conditions (Scheme 2b).[22] The cF^{2-}/cF^{3-} equilibrium depends on the pH. The known pK_a of the phenolic proton of cF is ~ 6.5. The trianionic form is responsible for the strong visible absorption band and potent fluorescence emission. However, the dianionic form has less intense absorption and fluorescence emission.

Figure 3

First, we measured the pH dependence of cF and **5**/cF complexes by UV/vis spectrometer (Fig. 3). The pH-dependent absorption of cF showed an equivalence point at approximately 6.5 and this value is consistent with the known pK_a (~ 6.5). Interestingly, the pH-dependent absorption of **5**/cF complex parallel-downshifted to 0.5 pH unit in the pH range of 5.5 ~ 7.5. At a fixed pH (e.g. 6.5), the addition of **5** increased the absorbance (ΔA = +0.25). These phenomena can be explained by the binding constant difference between the **5**/cF^{2-} and **5**/cF^{3-} complexes. In host-guest chemistry, electrostatic interaction is supposed to be proportional to the +/– charge-pairs participating in a binding event. **5** (+6 charged triazolium CCC) would prefer to form a stronger complex with cF^{3-} than cF^{2-}. As a result, the cF^{2-}/cF^{3-} equilibrium of cF was reorganized into an increasing trianionic contribution by the addition of **5**.

We also measured the pH dependence of cF and **5**/cF complex by fluorescence spectrometer (ESI Fig. S2). The relationship between the pH-dependent emission curves of cF and **5**/cF are a little bit different from those of the

pH-dependent absorption curves. The abnormality that occurs at pH > 7 range are a result of the fluorescence shielding effect of triazolium moieties (Φ (cF^{3-}) > Φ (**5**/cF^{3-})).

Figure 4

Second, we tested the effect of solvent composition (HEPES (10 mM, pH 7.4)-methanol) on absorption and emission of cF and **5**/cF complex. We observed that the absorbance and fluorescence intensity of cF proportionally decreases according to increasing methanol percentage (Fig. 4, black circle for absorbance, red circle for fluorescence intensity). Interestingly, the addition of **5** reduced methanol- dependence on absorption and emission of cF **(**Fig. 4, black square for absorbance, red square for fluorescence intensity). We could not measure the absorbance and fluorescence intensity of **5**/cF in methanol (100%) because **5** was insoluble in methanol. Accurate binding constant measurements require a HEPES-methanol composition to maximize the signal difference between free cF and the **5**/cF complex. Thus, for the next binding experiment, we used 50% methanol for UV/vis titration and 80% methanol for fluorescence titration with HEPES (10mM, pH 7.4).

Figure 5

The binding constants of **5** and **6** to cF were determined using the Benesi-Hildebrand equation by UV/vis titration (ESI S3). The addition of **5** or **6** to constant [cF] resulted in bathochromic shifts in the absorption of cF (Fig. 5a for **5** and ESI Fig. S3-2 for **6**). Both of them showed isosbestic points around 470 nm, which supported the 1:1 binding mode between positively charged receptors and **cF**. The binding constants of **5** and **6** to cF were measured in HEPES (10 mM, pH 7.4):methanol (1:1, v/v) and summarized in Table 1 (7.5×10^4 $M^{-1}$for **5** and 4.8×10^4 M^{-1} for **6**). A competition assay, using an ensemble of cF and cationic receptors, was used to measure the IP_3 (inositol-1,4,5-trisphosphate) binding constant by Anslyn.[23] This color change is afforded by a shift in the dianion-trianion equilibrium of cF when the chromophore is either bound to the receptor or free in solution at a physiological pH.

The binding constants of the competition assay were calculated using Corner's equation (ESI S4-1) and summarized in Table 1. Competition binding titration of cF and IP_3 to **5** showed a characteristic absorption decrease ($K_a = 1.4 \times 10^6$ M^{-1}, Fig. 5b, ESI Fig. S4-2A). Phytic acid showed the largest binding constant to **5** (K_a

= 1.9×10^6 M^{-1}, ESI Fig. S4-2B). Competition binding titrations of cF and adenosine triphosphate (ATP)/ ethylenediaminetetraacetic acid (EDTA) to **5** were performed (K_a (ATP) = 2.1×10^5 M^{-1}, ESI Fig. S4-2C; K_a (EDTA) = 4.5×10^4 M^{-1}, ESI Fig. S4-2D). However, the competition assays of mono-phosphate (glucose-6-phosphate) and a neutral guest (glucose) did not show any detectable absorption changes (ESI Fig. S4-2E, Fig. S4-2F).

The binding study suggests that main driving force of **5**-sPA binding is multivalent electrostatic interaction (IP_3 vs. glucose-6-phosphate). However, the spatial orientation of negative charges on a guest is also important to explain the binding selectivity of **5** (IP_3 vs. ATP/EDTA). The high binding constant of **5**-sPA complex would result from the synergistic action of these two factors in water.

Competition titrations using an ensemble of cF and **6** were performed with the same guests (ESI S4-3). The triazolium-based receptor (**5**) shows stronger binding for complementary anionic guests (e.g. K_a (phytic acid) = 19×10^5 M^{-1} for **5** vs. 10×10^5 M^{-1} for **6**), but the ammonium-based receptor (**6**) shows stronger binding with non-complementary anionic guests (e.g. K_a (ATP) = 3.5×10^5 M^{-1} for **6** vs. 2.1×10^5 M^{-1} for **5**). The same trend was reported by Lehn in guanidinium- and ammonium-based receptors.[24] Interestingly, the competition titration of IP_3 to **6** did not result in expected titration pattern in two independent measurements (ESI Fig. S4-3A). These phenomena have been explained by nonspecific complex formation between ammoniums and anionic guests. In addition, **6** has a serious drawback, which is its low solubility in basic conditions (ESI S5).

Figure 6

Fluorescence spectroscopy is a much more sensitive technique than UV/vis spectroscopy. High methanol percentages enforce electrostatic interactions resulting in stronger binding between **5** and anion guests. The binding constant of cF to **5** in HEPES (10 mM, pH 7.4):methanol (2:8, v/v) were determined using the Benesi-Hildebrand equation by fluorescence titration. The binding constant in 80% methanol ($K_a = 1.8 \times 10^5$, Fig. 6a and ESI Fig. S6) is 2.4 times higher than the binding constant in 50% methanol ($K_a = 7.5 \times 10^4$).

Conclusions

In summary, a novel triazolium cyclodextrin click cluster (**5**) was synthesized using CuAAC and methylation. A competition assay employing an ensemble of 5-carboxyfluorescein (cF) and receptor **5** was used to measure binding constants by UV/vis titration in co-solvent (HEPES (10 mM, pH 7.4):methanol, 1: 1, v/v) for guests, such as, inositol-1,4,5-trisphosphate (IP_3), phytic acid, ATP, EDTA, glucose, and glucose-6-phosphate. The competition binding constants of **5** decreased in the following order: phytic acid ~ IP_3 > ATP > EDTA. Glucose and glucose-6-phosphate did not give off any detectable UV/vis change. To our knowledge, our report is the first trial to utilize triazolium moieties on cyclodextrin click clusters to achieve small polyanion recognition in water.

Experimental

General

Analytical thin-layer chromatography (TLC) was carried out on a Merck 60 F254 precoated silica gel plate (0.2 mm thickness). Visualization was performed using a UV lamp or 10% H_2SO_4 in ethanol. ^1H, ^{13}C NMR, HH COSY, and CH HSQC spectra were measured on a Bruker Avance III 600 NMR spectrometer equipped with a PABBO BB-1H Z GRD probe head. The other NMR spectra were measured on a JEOL JNM-AL300 spectrometer. Chemical shifts were reported as δ in units of parts per million (ppm), and J-values were noted in Hz. Microwave syntheses were conducted in a 10 mL sealed tube on a CEM Discover™ microwave reactor using external IR temperature control. ESI MS spectrum was measured on a Thermo Scientific Liquid Chromatography/LTQ-Orbitrap Mass Spectrometer.

UV-visible spectra were measured by JASCO V-560 UV-visible spectrophotometer at ambient temperature. Cuvette with 1cm path length (Quarts SUPRASIL 105B-QS) was used. Fluorescence spectra were measured by Shimadzu RF-5301PC spectrophotometer at ambient temperature. Cuvette with 3.5 mL volume (Standard Quartz Cell, CQ-001) was used.

α-cyclodextrin (PN C0776) and 5-carboxyfluorescein (PN C2477), and phytic acid (PN P0409) were purchased from TCI. D-myo-inositol 1,4,5-tris-phosphate trisodium salt (PN I9766), EDTA (ethylenediaminetetraacetic

acid, PN E9884), glucose (PN G8270), and iodomethane (PN 289566) were purchased from Sigma/Aldrich. ATP (Adenosine triphosphate, PN 126888), and glucose-6-phosphate (PN127027) were obtained from Boehringer Mannheim (Germany). All solvents are HPLC grade.

Hexakis{6-(4-hydroxymethyl-1H-1,2,3-triazol-1-yl)-6-deoxy}-α-cyclodextrin (**4**)

A mixture of 6-azido-6-deoxy-α-cyclodextrin (800 mg, 0.712 mmol), propargyl alcohol (370 µL, 6.41 mmol, eqv. 1.5/N_3 group), 0.8 mL $CuSO_4.5H_2O$ / THPTA (0.1 M, ratio 1:5), and sodium ascorbate (80 mg, 0.40 mmol) were mixed with 110 mL of THF/0.1 M phosphate buffer/ethanol (6:5:2). The mixture was stirred at 80 ℃ for 20 h. The reaction was complete and checked by TLC (1-propanol/ethyl acetate/water/28% ammonia = 6/1/3/1) (azido starting spot was not observed on TLC plate). After the reaction, the mixture was shaken with CupriSorb^{TM} resins for 1 day to remove the copper ion. The supernatant was evaporated and precipitated by chilled methanol (50 mL, x 3 times). After centrifugation, the solid was washed with chilled methanol for 3 times. The solid was dissolved in water (8 mL) and lyophilized using a freeze dryer (Ilshin Europe B.V, model FD8512) to yield a pale yellowish powder (843 mg, 0.578 mmol, yield 81%).

^{1}H NMR (600 MHz, D_2O): d 7.94 (s, 6H, H_7), 5.14 (d, 6H, J = 3.3 Hz, H_1), 4.50 (app t, 6H, H_6), 4.43 (app t, 6H, H_9), 4.23 (app t, 6H, H_5), 4.20 (d, 6H, J = 6.84 Hz, H_6), 4.01 (app t, 6H, H_3), 3.58 (app d, 6H, H_2), 3.39 (app t, 6H, H_4); ^{13}C NMR (150 MHz, D_2O): 146.1 (C_8), 125.3 (C_7), 100.6 (C_1), 81.9 (C_4), 71.9 (C_3), 70.6 (C_2), 69.6 (C_5), 53.7 (C_9), 49.7 (C_6); LR MALDI-TOF (CHCA, positive): Calculated 1458.5 for $C_{54}H_{78}N_{18}O_{30}$, observed 1481.1 for $[M + Na]^+$.

Hexakis{6-(3-methyl-4-hydroxymethyl-1H-1,2,3-triazolium-1-yl)-6-deoxy}-α-cyclodextrin iodide (**5**)

To a solution of **4** (500 mg, 0.343 mmol) in DMF (1 mL) was added iodomethane (0.576 mL, 9.26 mmol, eqv. 4.5/triazole group). The reaction mixture was irradiated at 30 W and 90 °C in a microwave reactor (CEM Discover, Dynamic mode, closed vessel) for 30 min. After the reaction was complete, half of the reaction volume was evaporated *in vacuo* and the residue was triturated using chilled acetone. The precipitate was washed four times with acetone and the solid was dried to give the desired product (570 mg, 0.247 mmol, yield: 72 %). ^{1}H NMR (D_2O, 600 MHz): δ 8.60 (s, 6H, H_7), 5.24 (app t, 6H, H_6),5.21 (d, 6H, J = 3.4, H_1), 4.91 (d, 6H, J = 3.3 Hz, H_9), 4.88 (d, 6H, J = 17.8 Hz, H_5), 4.37 (s, 18H, N^+CH_3), 4.08 (app t, 6H, H_3), 3.56 (app dd, 6H, H_2),

3.44 (app t, 6H, J = 9.1, H_4); ^{13}C NMR (D_2O, 150 MHz): 143.5 (C_8), 131.4 (C_7), 101.9 (C_1), 81.9 (C_4), 72.4 (C_3), 71.4 (C_2), 68.9 (C_5), 54.5 (C_6), 52.7 (C_9), 39.0 (N^+CH_3); HR ESI-MS: Calculated 2310.08 for $C_{60}H_{96}N_{18}O_{30}I_6$ (M), observed 1028.14 for $[M - 2I]^{2+}$; high Z = + 3, +4, +5 peaks: observed 643.13 for $[M - 3I]^{3+}$, 450.62 for $[M - 4I]^{4+}$, 335.11 for $[M - 5I]^{5+}$ (ESI Fig. S1-2E).

Acknowledgements

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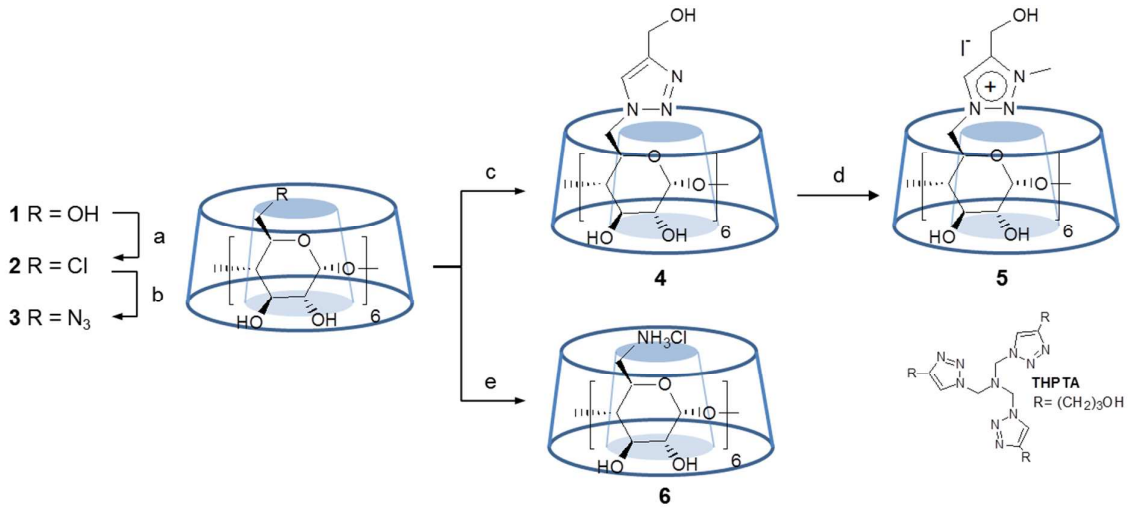
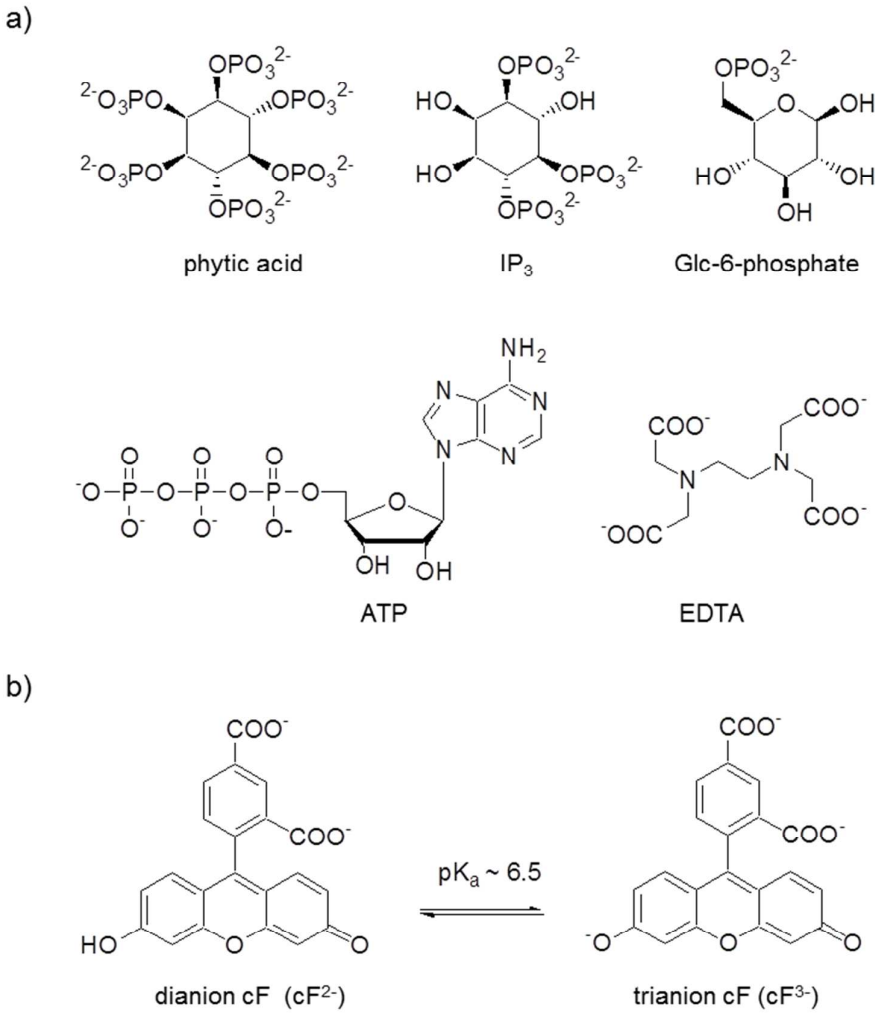
Scheme, Figure, and Table

Scheme 1. Reactions and reagents. a) CH_3SO_2Cl, DMF, 65 °C; b) NaN_3, DMF, 75 °C; c) propargyl alcohol, $CuSO_4$/THPTA, sodium ascorbate, THF/phosphate buffer (pH 7, 0.1 M); d) CH_3I, DMF, MW; e) i. PPh_3, DMF, NH_4OH, ii. HCl. THPTA = tris(3-hydroxypropyltriazolylmethyl)amine, MW= microwave irradiation.



Scheme 2. a) The molecular structures of anion guests used in this experiment. The charge states of the anions represent the major ionic species in physiological pH. b) Dianion-trianion equilibrium of 5-carboxy fluorescein.



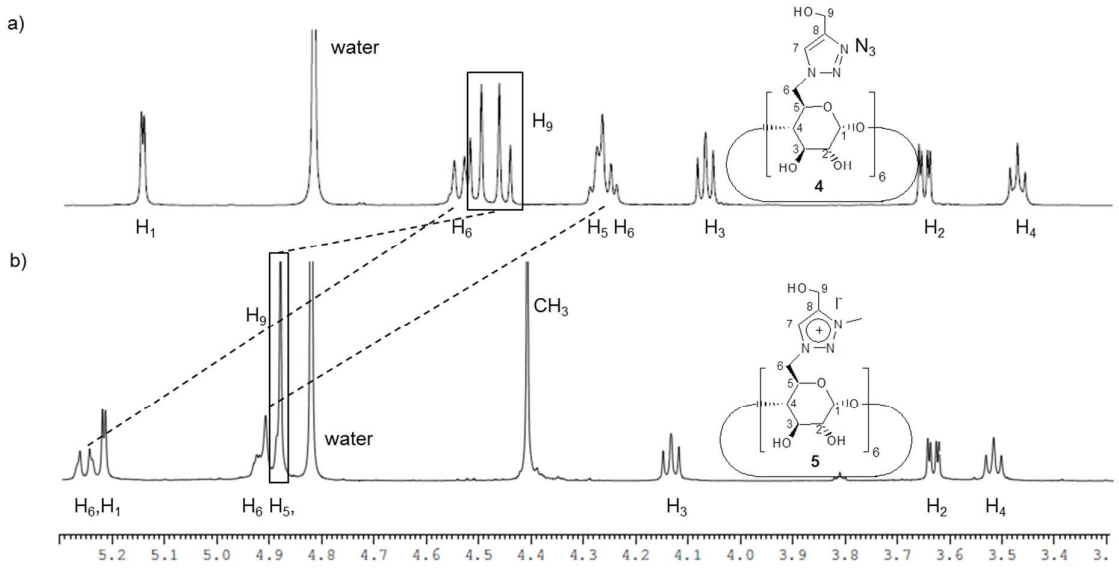


Figure 1. Partial ^{1}H NMR spectra (600 MHz, D_2O) of **4** (a) and **5** (b).

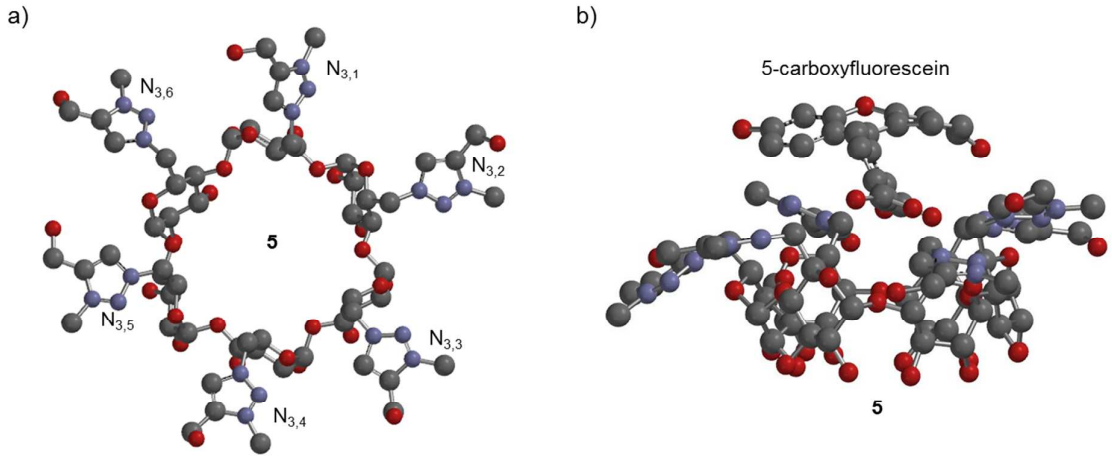


Figure 2. a) Molecular modeling (B3LYP calculation with 6-31G* basis set in vacuum, GAUSSIAN 09' rev. 03) of **5**. The average distance between $N_{3,i}$ and $N_{3,(i+1)}$ (± STDEVA) = 8.85 (± 0.61) Å and the average distance between $N_{3,i}$ and $N_{3,(i+3)}$ (± STDEVA) = 17.25 (± 0.97) Å. b) Molecular modeling (Molecular Mechanics MMFF94 calculation, Spartan '08 v1.2.0) of **5**/cF. The hydrogen atoms were omitted for clarity.

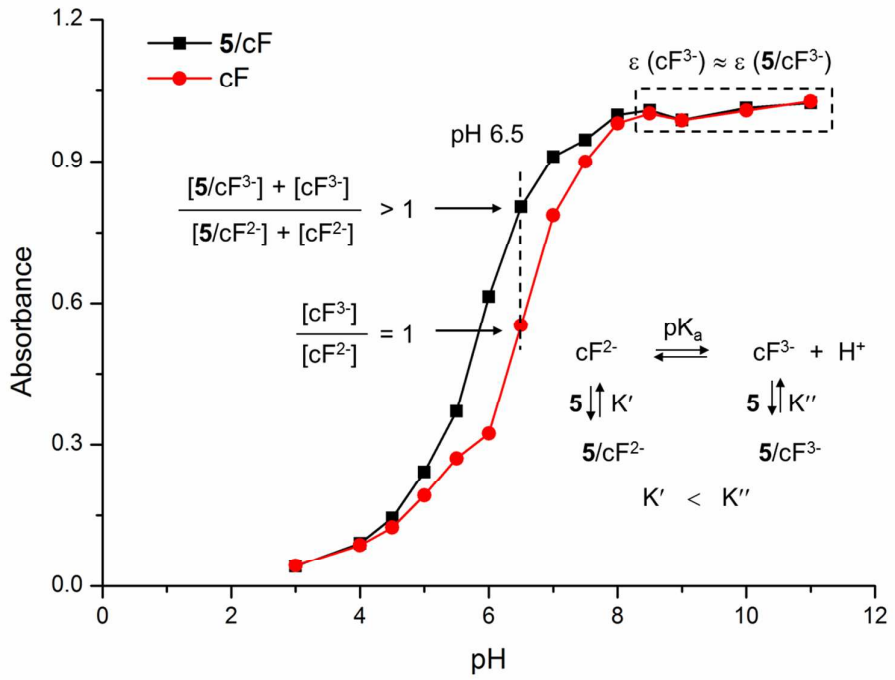


Figure 3. UV/vis absorption .changes at 498 nm of cF (14 μM) and **5**/cF complex (cF = 14 μM, **5** = 100 μM) as a function of pH in 0.1 % DMSO. HEPES buffer (10 mM) was adjusted to a pH range of 3-11 using HCl or NaOH by pH meter. K' = binding constant of **5**/cF^{2-} complex, K'' = binding constant of **5**/cF^{3-} complex, ε(*i*) = extinction coefficient of species *i*.

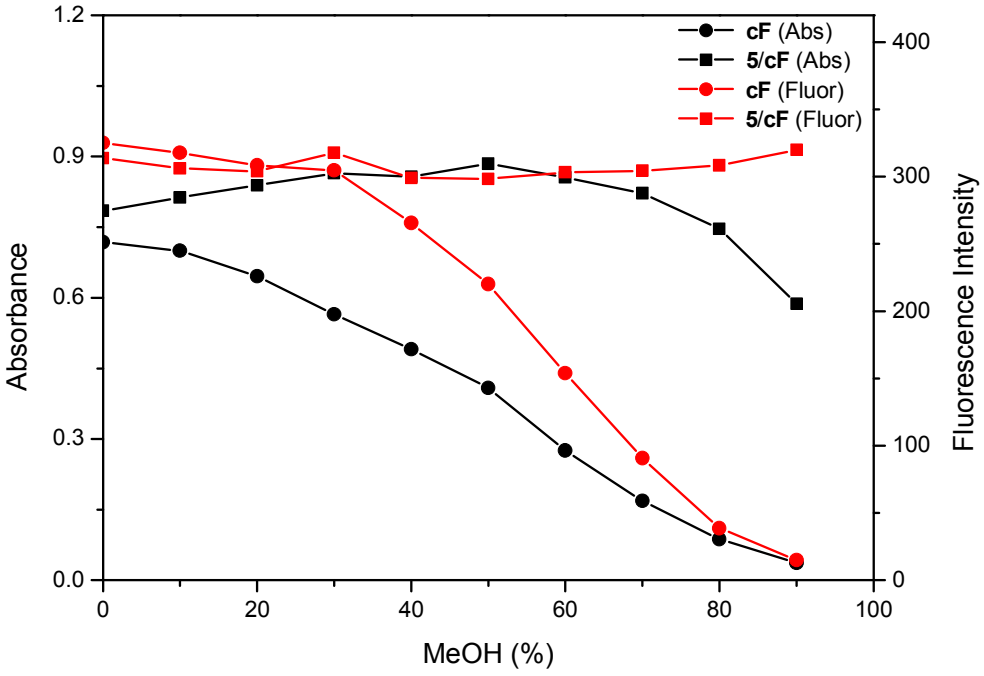


Figure 4. UV/vis absorption and emission changes of cF and **5**/cF complex depending on HEPES (10 mM, pH 7.4)-methanol composition. [cF] = 14 μM, [**5**] = 100 μM for UV/vis and [cF] = 0.2 μM, [**5**] = 20 μM for fluorescence measurement. The absorbance was observed at 498 nm. The fluorescence was excited at 490 nm and the emission was measured at 524 nm (excitation/emission slit: 3/3).

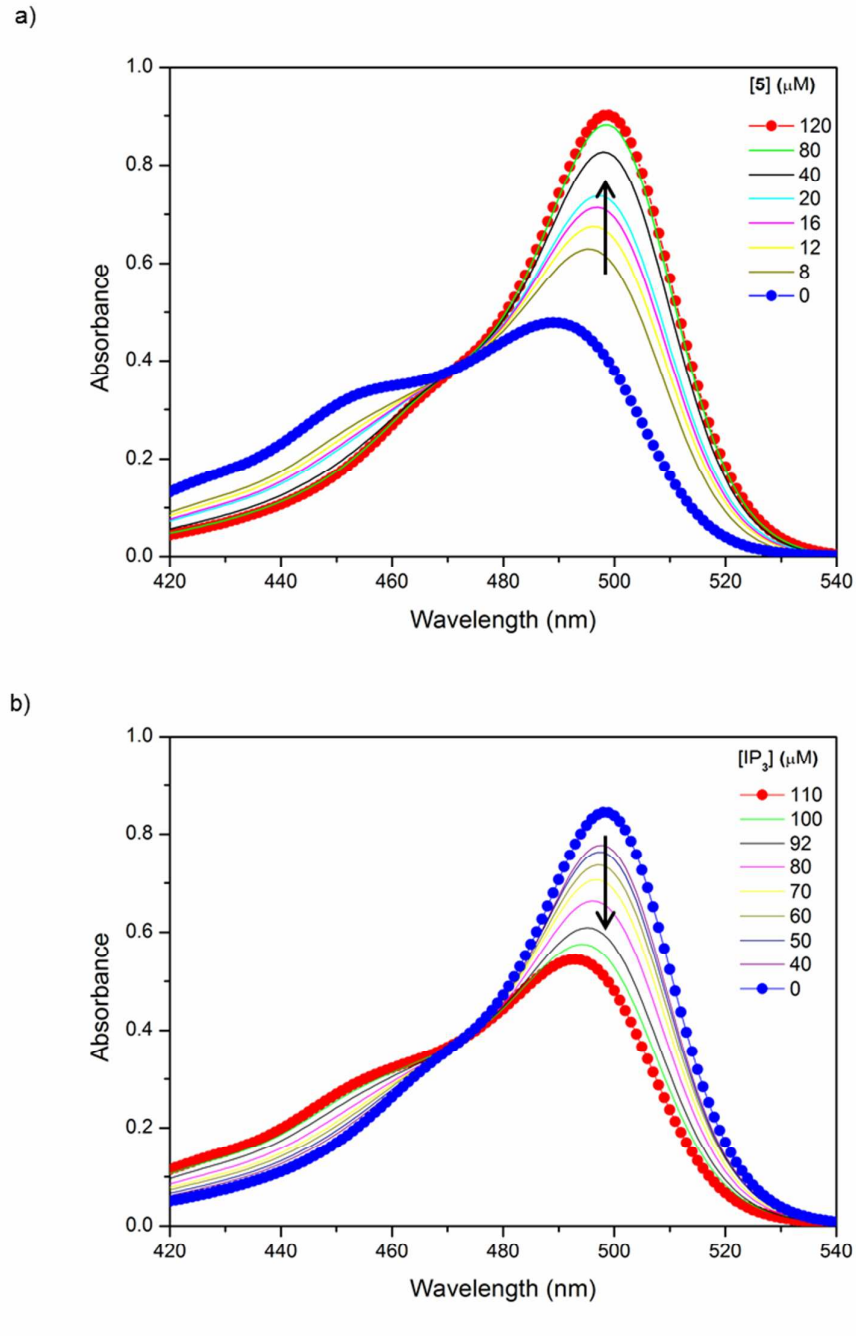


Figure 5. UV/vis absorption spectra for binding constant measurement in HEPES (10 mM, pH 7.4):methanol (1:1, v/v). a) UV/vis titration of **5** (0 to 120 μM) at a fixed [cF**]** **(**14 μM), b) UV/vis titration of IP_3 (0 to 110 μM) at a fixed [**5**] (110 μM) and [cF**]** **(**14 μM).

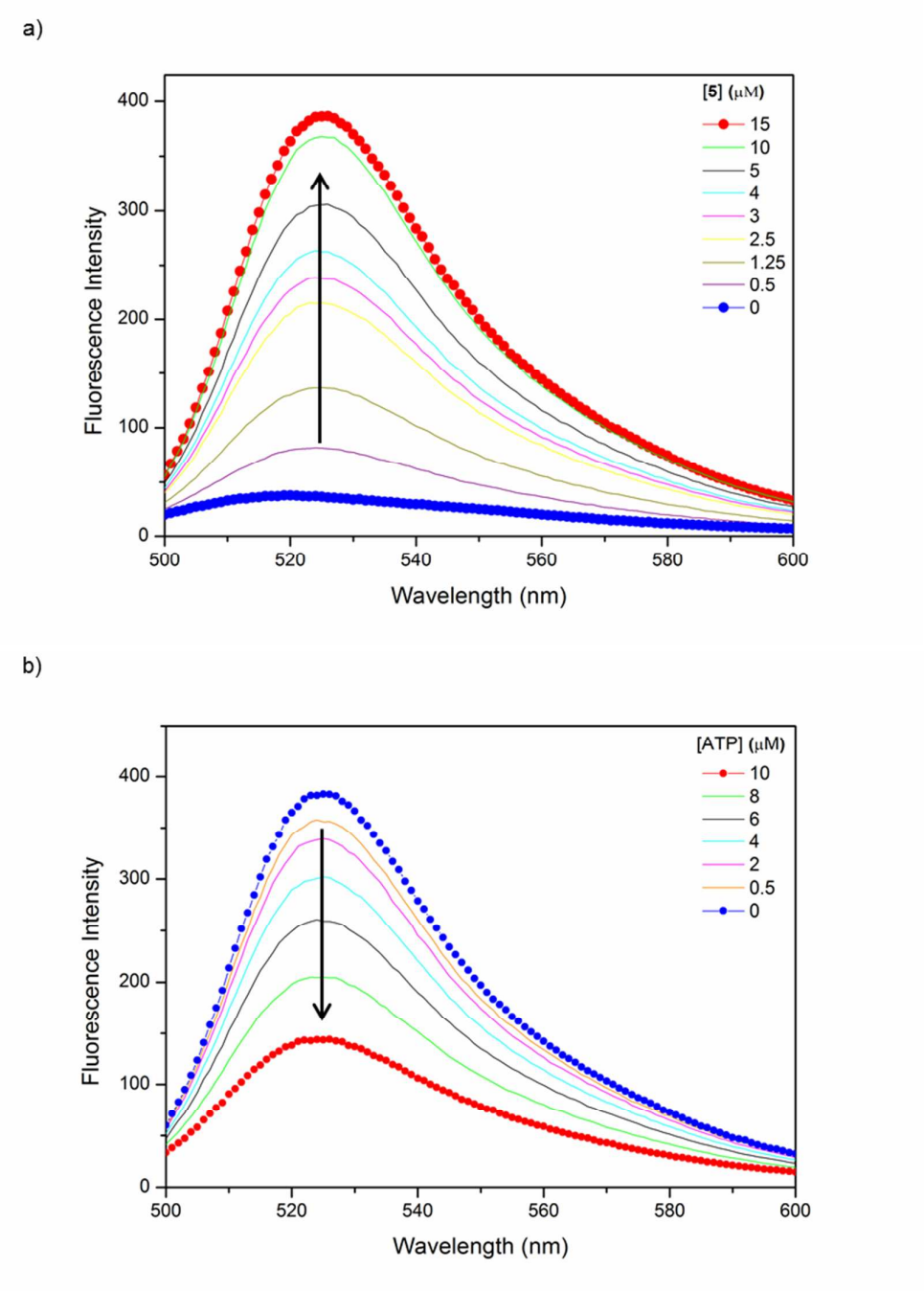


Figure 6. Fluorescence spectra for binding constant measurement in HEPES (10 mM, pH 7.4):methanol (2:8, v/v), excited at 490 nm. a) Fluorescent titration of **5** (0 to 15 μM) at fixed [cF] (0.2 μM), b) Fluorescent titration of ATP (0 to 10 μM) at fixed [cF] (0.2 μM) and [**5**] (10 μM).

Table 1. Association constants (K_a) of anionic guests for **5** or **6** at ambient temperature in HEPES (10 mM, pH 7.4):methanol (1:1, v/v).*

Guest	**5**	**6**
	$K_a/10^5$ (M^{-1})	$K_a/10^5$ (M^{-1})
cF (5-carboxyfluorescein)	0.75	0.48
IP_3 (inositol-1,4,5-trisphosphate)	14	-[a]
phytic acid (inositol hexakisphosphate)	19	10
ATP	2.1	3.5
EDTA	0.45	1.6
glucose-6-phosphate	ND[b]	ND[b]
glucose	ND[b]	ND[b]

* All K_a except cF were obtained by a UV/vis competition method between cF and guests at [**5** or **6**] = 110 μM, [cF] = 14 μM. The K_a of cF was obtained using the Benesi-Hildebrand equation. The UV/vis titrations show single isosbestic points. See SI S4.

[a] The titration data from two independent measurements were unable to be fitted. See SI Fig. S4-3A.

[b] ND = Not detectable UV/vis spectrum change

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