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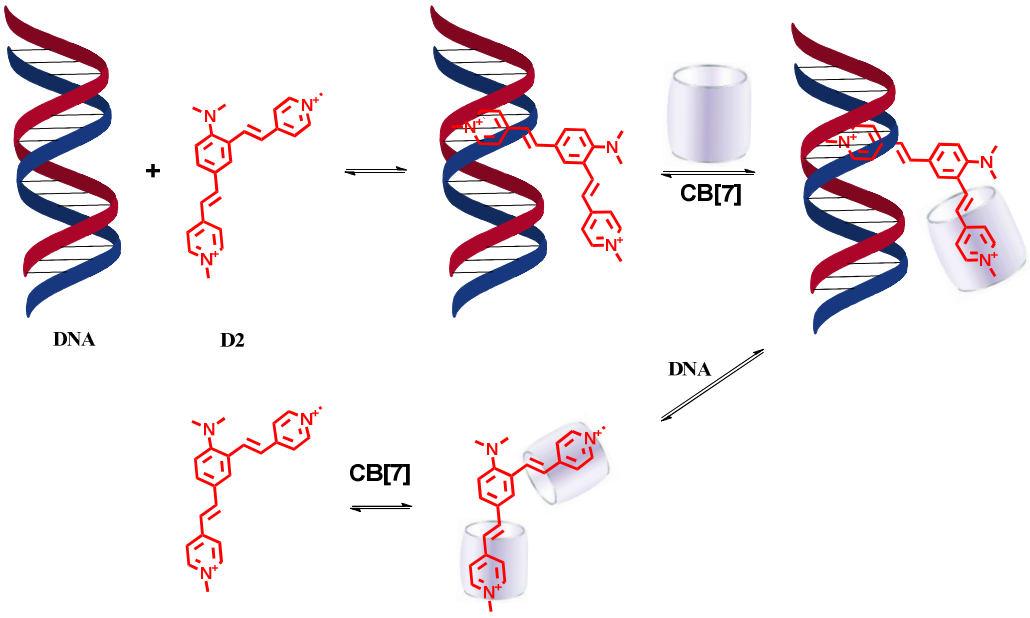


A ternary complex among **D2**, DNA and CB[7] can be formed.

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ARTICLE TYPE

# Interaction of a Hemicyanine dye and its Derivative with DNA and Cucurbit[7]uril

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The host guest interaction of trans-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (**DSMI**) with cucurbit[7]uril (CB[7]) was investigated through fluorescence, absorption, $^1$H NMR and mass spectrometry. **DSMI** can be included by CB[7] with 1:2 stoichiometry to form the **DSMI**/CB[7] complex, exhibiting a larger binding affinity than that of DNA**.** To make a comparison with **DSMI**, another pyridium vinyl group was introduced to the aniline ring of **DSMI** to get a hemicyanine derivative **D2**, and a ternary complex among **D2**, CB[7] and DNA was formed. All these provide new insights to investigate the interaction of organic dyes, CB[7] and DNA.

## Introduction

Recently, cucurbit[n]urils (CB[n]),[1,2] pumpkin-shaped macrocylic compounds comprised of n glycoluril units linked by pairs of methylene bridges with rigid cavities, have shown numerous applications ranging from molecular recognition[3-5], dye encapsulation,[6,7] drug delivery[8,9] to self-assembly molecular devices[10,11]. The negative charge density on the carbonyl groups and the inner surface of the hydrophobic cavity promote CB[n]s to include small guest molecules[12] and binding with metal cations[13] and cationic organic molecules via ion-dipole interaction[14]. The effects, such as fluorescence enhancement, fluorescent lifetime extension and photostability improving, caused by the interaction of CB[n]s and fluorescent dyes have been extensively studied.[6] However, the cooperation or competitive interaction between CB[n] and DNA has been rarely reported[15-18]. These investigations will be helpful for improving the sensitivity of fluorescent sensor[15-17], demonstrating the role of CB[n] in fluorescent sensing process. Therefore, it is meaningful to pay attentions on the interaction of organic dyes, CB[n]s and DNA.

Hemicyanine was often used as a fluorescent probe for DNA analysis,[19] cell microviscosity[20] and metal ion detection.[21] The interaction between some hemicyanine dye derivatives or styryl dyes with similar structure and CB[7] have been investigated,[22, 23, 24-26] the results demonstrated that CB[7] could bind with the dyes through ion-dipole interaction. In our previous work,[11] the chemically tunable interaction between CB[6] and trans-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (**DSMI**, Scheme 1), a common cationic hemicyanine dye always used as a fluorescent probe for DNA analysis, has been investigated. As a homologue of CB [6], the bigger cavity of CB[7] can make it easy to self-assemble with DSMI, providing a further approach to study the interaction of the dye with CB[n]s and DNA, and its good solubility in water is more suitable for biological applicatio ns. In continuation of our related research,[11] the interaction betwe en **DSMI** and CB[7] was investigated through fluorescence, absorption, $^1$H NMR and mass spectrometry, then the competitive binding interaction of **DSMI** with CB[7] and DNA was studied and the result demonstrated that CB[7] had a larger binding affinity with **DSMI** than DNA. To make a comparison with DSMI, another pyridium vinyl group was introduced to the aniline ring of **DSMI** to get a hemicyanine derivative **D2** (**Figure 1**), in the case of **D2**, one pyridium vinyl of **D2** can be included into the cavity of CB[7] and the other can be intercalated into DNA to form a ternary complex.

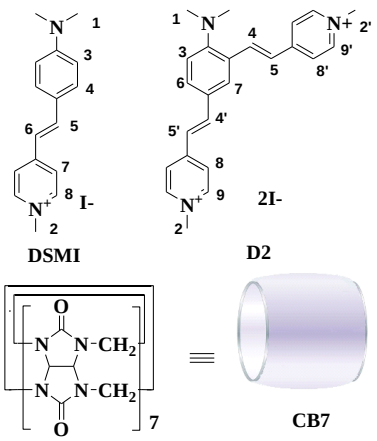



**Figure 1**. The structures of **DSMI**, **D2** and CB[7]

## Experiment section

### Materials and methods

The water used in the test was distilled water. Other solvents and reagents were of analytical grade and used without further purification. **DSMI** was synthesized as described in our previous study[11] and CB[7] was synthesized according to the literature[27]. Calf thymus DNA (ct-DNA) was obtained from Sigma Chemical Co. (USA).

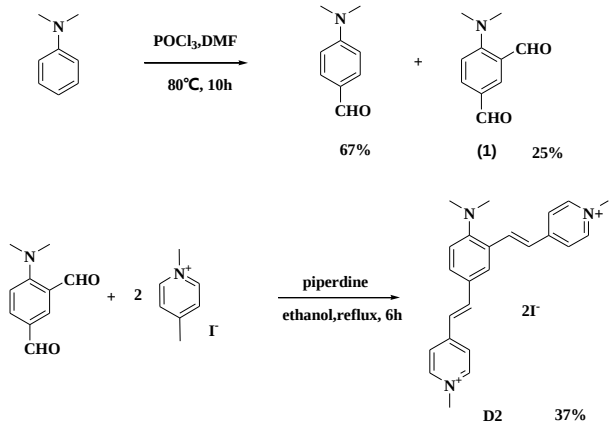
NMR data were recorded in $D_2O$ on a VARIAN INOVA-400

spectrometer. Mass spectrometric data were obtained on a Q-Tof mass spectrometer (Micromass, Manchester, England). Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer. Fluorescence measurements were performed on a VARIAN CARY Eclipse Fluorescence Spectrophotometer. The pH titration measurements were made with a Model PHS-3C meter.

The absorption and fluorescence spectra of **DSMI** with CB[7] were measured in distilled water. The fluorescence titration experiments were performed as follows: 25 μL of 1.0 × $10^{-3}$ mol/L stock solution of **DSMI** and a different amount of 1.0 × $10^{-3}$ mol/L CB[7] aqueous solution were transferred into a 5 mL volumetric flask, then the volumetric flask was filled to the final volume with distilled water. Fluorescence and absorption spectra were measured after 3 minutes of ultrasonic agitation. The NMR spectra were obtained in $D_2O$ with DSS (3-(trimethyl-silyl)-1-propanesulfonic acid sodium salt) as the internal standard; the ratio of dye versus CB[7] was calculated by the ratio of their integral areas for special peaks; the protonated form of **DSMI** and **D2** was determined firstly by addition of deuterium chloride to observe the color of the solution changing from yellow to colorless, and then checking the pH of the solution by a pH test paper.

### Synthesis

The synthetic route of **D2** is illustrated in scheme 1:




**Scheme 1** Synthetic route for the **D2**

### Compound 1 (4-dimethylamino-benzol-1, 3-dicarbaldehyde)

Compound **1** was synthesized according to the literature with little modification.[28] Phosphoryl chloride (50.0 g) was added to DMF (81.2 g) in drops. Then $Me_2NPh$ (12.1g) was added by drops into the solution, and the reaction was lasted for 10 h at 80 ℃. The reactant was poured into the mixture of 600 g ice and 150 mL 38% NaOH solution, and kept at 0 ℃ to give large amount of precipitation. The precipitation was filtered and purified by column chromatography on silica gel (eluting with petroleum ether: ethyl acetate 5:1) to give 4.5 g (25% of yield) of (**1**) $^1$H NMR (400 MHz, $CDCl_3$, $\delta$ in ppm) 10.04 (s, 1H), 9.86 (s, 1H), 7.92 (d, $J$ = 8.8, 1H), 7.03 (d, $J$ = 8.8, 1H).

### Compound D2 (2,4-bis[(E)-N-methyl-4-pyridinyl ethenyl] 1-dimethylamino benzene diiodide)

The mixture of 4-dimethylamino-benzol-1,3-dicarbaldehyde (1.77 g, 10.0 mmol), 1,4-dimethylpyridinium iodide (4.7 g, 20.0 mmol) and pyrrolidine (1 mL) in 50 mL ethanol was refluxed for 4 h, the reactant was cooled, and the precipitate was collected. Recrystallization was carried out twice to give the dark purple product 2.2 g of **D2** with yield of 37%. Anal. Calcd (found): C, 47.15 (47.03); H, 4.45 (4.40); N, 6.87 (6.81). $^1$H NMR (400 MHz, $D_2O$, $\delta$ in ppm) 8.55 (d, $J$ = 6.6, 2H), 8.50 (d, $J$ = 6.6, 2H), 8.03 (d, $J$ = 6.6, 2H), 7.98 (d, $J$ = 6.6, 2H), 7.93 (s, 1H), 7.84 (d, $J$ = 16.2, 1H), 7.72 (d, $J$ = 16.2, 1H), 7.68 (d, $J$ = 8.4, 1H), 7.31 (d, $J$ = 16.2, 1H), 7.25 (d, $J$ = 18.0, 1H), 7.21 (d, $J$ = 8.8, 1H), 4.26 (s, 3H), 4.22 (s, 3H), 2.82 (s, 6H). $^{13}$C NMR (100 MHz, $D_2O$, $\delta$ in ppm) 155.5, 154.0, 153.9, 144.8, 144.6, 140.5, 138.3, 131.1, 128.9, 128.0, 124.4, 124.0, 123.6, 121.6, 119.1, 47.5, 47.4, 44.6. HRMS m/z Found: 357.2205 $C_{24}H_{27}N_3$ ($M^+$): requires M, 357.2204. The corresponding spectra can be found in **Figure S1-S3.**

## Results and Discussion

### The host-guest interaction between DSMI and CB[7]

**DSMI** has an absorption maximum at 450 nm and a fluorescence peak at 605 nm in aqueous solution. As shown in **Figure 2**, the absorption maximum changed from 450 nm to 469 nm and the A value decreased from 0.30 to 0.12 with the addition of 10 equiv. of CB[7] in a phosphate buffer solution (pH = 7.4). Another absorption peak aroused by the protonated form of **DSMI** appeared at ~330 nm, suggesting that part of **DSMI** was protonated even at pH = 7.4 after the dye included CB[7]. The fluorescence intensity at the maximum emission wavelength was increased by ~10 times, and the fluorescence peak had a blue shift from 605 nm to 592 nm, demonstrating the interaction of the dye with CB[7]. The dye was confined in the nonpolar cavity of CB[7], decreased the nonradiative decay of the excited state and hindered the formation of the twisted intramolecular charge transfer (TICT) state, leading to a fluorescence enhancement. The fluorescence enhancing mechanism by CB[7] was similar to that of CB[6] demonstrated in our previous work.[11] However, the fluorescence intensity was less enhanced by CB[7] because of its relative larger cavity size compared with that of CB[6].

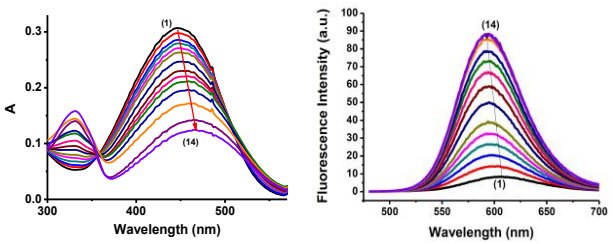



**Figure 2.** The absorption and fluorescence ($\lambda_{ex}$ = 450 nm) spectra of **DSMI** along with the addition of CB[7], from (1) to (14), 0, 0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 × $10^{-5}$ M of CB[7] was added, the concentration of the dye was 1 × $10^{-5}$ M, pH=7.4.

The pH titration on fluorescence of the complexed dye (**DSMI**/CB[7]) was obtained under conditions of virtually quantitative complexation[29] (5 $\mu$M **DSMI**, 100 $\mu$M CB[7], corresponding to >99% dye complexion, **Figure S4**), permitting a direct estimate of the pKa` value 5.6 for the complexation, while the pKa of **DSMI** was 3.1. The pKa shift of 2.5 units may be caused by the more stable complex of **DSMIH**$^+$/CB[7] compared with **DSMI**/CB[7].

The interaction between **DSMI** and CB[7] was investigated by

$^1$H NMR spectroscopy (**Figure S5)**. Because of the pKa shift from 3.1 to 5.6, the protonated form of **DSMI** could be formed when the pH was below 8, so the $^1$H NMR titration experiment was performed in $D_2O$ with a pH value of ~10. When less than 1 equiv. CB[7] was added into the alkaline solution of **DSMI** in $D_2O$, all the protons shifted to the upfield, and all the signals became broader. This indicated that **DSMI** and CB[7] formed a loosely bonded complex and the bonded CB[7] shifted back and forth on the **DSMI** molecule to undergo a fast exchanging process.[12,30,31] All the protons were under the shielding/ deshielding microenvironment, and formed a dynamic balance between the free and complexed state, leading to the broader signals. When more than 1 equiv. CB[7] was added, the signals of **DSMI** were difficult to observe due to the precipitation formed between **DSMI** and CB[7].

The interaction between the protonated form of **DSMI** (**DSMIH**$^+$) and CB[7] was also investigated by $^1$H NMR titration (**Figure S6)**. A trace amount of acid was added together with CB[7] to ensure the formation of the **DSMIH**$^+$/CB[7] complex (pH = 3). When less than 1 equiv. CB[7] was added, stable **DSMIH**$^+$/CB[7] complex was formed, and separated signals of the free **DSMI** and **DSMIH**$^+$/CB[7] complex were observed. H8 was kept fixed at 3.32 ppm and H1, H2 and H7 moved downfield, while H3, H4, H5 and H6 moved upfield, indicating that CB[7] included **DSMIH**$^+$ on the aniline ring and double bond part. When 1 equiv. CB[7] was added, the free **DSMI** signal disappeared. When more than 1 equiv. CB[7] was added into the solution, the unchanged H8 and downfield shifted H7 shifted to the upfield, and H3, H4, H5, H6 continued to shift to the upfield, while H1 and H2 shifted to the more downfield. This result suggested that the second CB[7] included **DSMI** from the pyridium ring part and the two CB[7] included **DSMI** around H1 and H2. ESI-MS confirmed the formation of (CB[7]/**DSMI** + H$^+$)$^{2+}$ (1:1) complex (**Figure S7**): *m/z* calcd. 701.2531, and found 701.2911, but the signal of 2CB[7]/**DSMI** complex was not found.

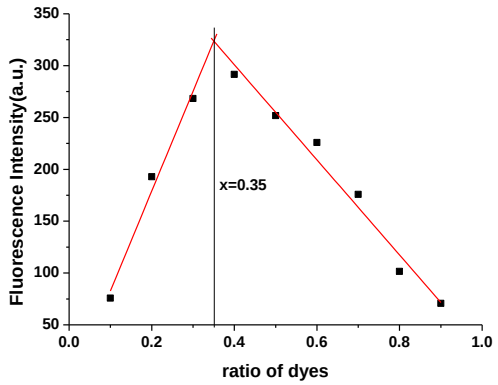



**Figure 3.** Job's plot of **DSMI**/CB [7] complexes in alkaline environment, Asymmetric plot with maximum at ~0.35 mol fraction indicating a 1:2 inclusion

From the NMR titration experiments, the stoichiometry of **DSMIH**$^+$ and CB[7] can be determined to be 1:2 under acidic condition. However, the stoichiometry of **DSMI** and CB[7] was not certain in alkaline solution. Therefore, for the complexation of **DSMI** and CB[7], the stoichiometry was determined by a continuous variation technique (Job's plot)[32] based on the fluorescence intensity of **DSMI** in the presence of CB[7]. Different concentration of **DSMI** and CB[7] solutions were prepared and mixed and the sum of the concentrations was kept constant. The typical plot for the **DSMI**/CB[7] complex is shown in **Figure 3**. The maximum of the fluorescence intensity was observed at x = 0.35, indicating that the dye/CB[7] inclusion complex in aqueous solution was mainly formed with 1:2 stoichiometry.

The binding constant values for 1:1 and 1:2 were evaluated by considering the following complexation equilibrium according to the literature,[33] where $K_1$ and $K_2$ are the binding constants for the formation of the corresponding 1:1 and 1:2 complexes.

$$\text{CB[7]+Dye} \overset{K_1}{\rightleftharpoons} \text{CB[7].Dye}$$
$$\text{CB[7].Dye+CB[7]} \overset{K_2}{\rightleftharpoons} (\text{CB[7]})_2\text{.Dye} \qquad (1)$$

At any stage, the observed fluorescence intensity $I_f$ corresponds to the sum of the fluorescence intensities from dyes, CB[7].dye and $(CB[7])_2$.dye, and is directly proportional to their corresponding concentrations in the solution. Therefore, one can write the following Equation (2):

$$I_f = I_f^0 \frac{[Dye]_{eq}}{[Dye]_0} + I_{CB7.Dye} \frac{[CB7.Dye]_{eq}}{[Dye]_0} + I_{(CB7)_2.Dye} \frac{[(CB7)_2.Dye]_{eq}}{[Dye]_0} \qquad (2)$$

Equation (2) can be rearranged to a modified Benesi-Hildebrand Equation (3).[33]

$$I_f = \frac{I_f^0 + I_{CB7.Dye} K_1[CB7]_0 + I_{(CB7)_2.Dye} K_1K_2[CB7]_0^2}{1 + K_1[CB7]_0 + K_1K_2[CB7]_0^2} \qquad (3)$$

Where $I_f^0$ is the fluorescence intensity in the absence of CB[7], $I_{CB7.Dye}$ is the fluorescence intensity when all the dye molecules are interacted with CB[7] as 1:1 complex, and $I_{(CB7)2.Dye}$ is the fluorescence intensity when all the dye molecules are complexed with CB[7] forming a 2:1 complex. And finally $[Dye]_0$ and $[CB7]_0$ are the total concentrations of the dye and the CB[7] used.

Applying equation (3) to fit the experimental data presented in **Figure S8**, the $K_1$ and $K_2$ value were estimated to be $(1.35 \pm 0.05) \times 10^5$ M$^{-1}$ and $(3.2 \pm 0.4) \times 10^3$ M$^{-1}$. The higher value of the first order binding constant and the lower value of the second order binding constant indicated that the first complexation was caused by the strong ion-dipole interaction between the carbonyl laced portals of CB[7] and the positive charge centered on **DSMI**. The second interaction was caused by the hydrophobic nature of the dye.[33] The smaller value of $K_2$ could be one of the main reasons why the MS signal for 1:2 complex was not found.

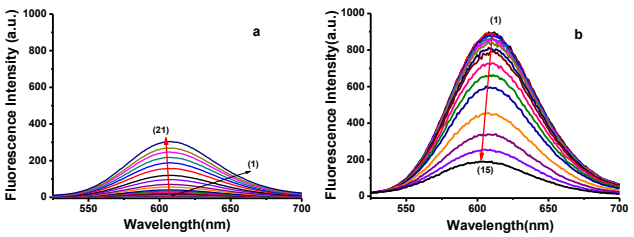


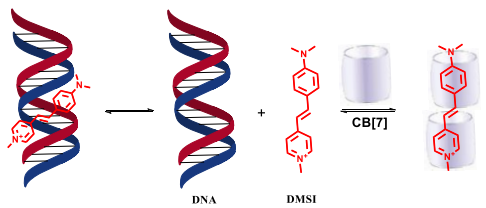

**Figure 4**. The fluorescent spectra of **DSMI** with the addition of DNA (a), from (1) to (21), 0, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, 30.0, 40.0, 60.0, 80.0, 100.0, 150.0, 200.0, 300.0 equiv. of DNA was

added; and the fluorescence spectra of **DSMI** in presence of 1000 equiv DNA with the addition of CB[7] (b), from (1) to (15), 0, 0.4, 0.8, 1.2, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 15.0, 20.0, 25.0 equiv. of CB[7] was added, the concentration of **DSMI** was $1.0 \times 10^{-5}$ M, pH=7.4.

Competitive interaction of DSMI with DNA and CB[7]

**DSMI** could bind to the double helical DNA with high affinity through a groove binding mode.[19] The groove binding inhibits the non-radiative deactivation of the excited state of **DSMI**, and results in enhanced fluorescence intensity, owing to the geometric restriction imposed by the narrow minor groove of the DNA helix. About 43-folds of fluorescence enhancement and no shift in emission maximum was observed with the addition of 300 equiv. ct-DNA (**Figure 4a**).

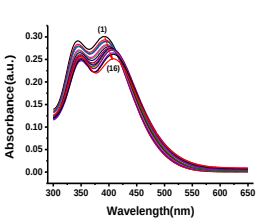
As shown in **Figure 4b**, when CB[7] was gradually added into the solution of 1000 equiv. ct-DNA and **DSMI**, the fluorescence intensity decreased dramatically to 21% of the initial fluorescence with a ~8 nm blue shift of the emission peak. Both the fluorescence intensity and the emission peak became similar to the state of **DSMI** in the presence of CB[7]. These results show that CB[7] could bind with **DSMI** competitively although **DSMI** bonded with ct-DNA at first. Therefore, it is easy to deduce that CB[7] has a larger binding affinity with **DSMI** than that of ct-DNA. The competitive process can be suggested in **Scheme 2**.




**Scheme 2** The competitive binding of **DSMI** with DNA and CB[7]

### Host-guest interaction between D2 and CB[7]

To make a comparison with **DSMI**, we introduced another pyridium vinyl group to the aniline ring of **DSMI** to generate a novel fluorescent dye **D2**. Two absorption peaks at 345 and 393 nm were observed in aqueous solution and the absorption peak at 393 nm exhibited a hypochromic shift of 57 nm compared with that of **DSMI**. It is reasonable to attribute the hypochromic shift to the reduced electron density on the aniline ring when the electron-withdrawing substituent (pyridium vinyl group) anchored.[34] The decreased electron-donating ability of the aniline ring weakened the TICT process of the parent dye. **D2** exhibited an enlarged Stokes shift up to 230 nm with very low background fluorescence ($\Phi_f$ < 0.001), and the emission maximum was located at 623 nm with a red shift of 18 nm compared with that of **DSMI**.



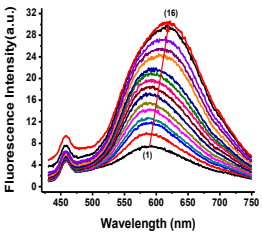




**Figure 5** The absorption and fluorescence spectra of **D2** with the addition of CB[7], from (1) to (16), 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0, 4.0, 5.0, 6.0, 8.0, $10.0 \times 10^{-5}$ M of CB[7] was added, the concentration of **D2** was $1 \times 10^{-5}$ M, pH=7.4.

Incremental addition of CB[7] to the **D2** solution resulted in a gradual decrease in the absorbance with ~6 nm and ~16 nm bathochromic shifts for the absorption peaks at 345 nm and 393 nm, respectively (**Figure 5**). Meanwhile, the emission peak shifted to 615 nm with ~8 nm red shift and the fluorescence monitored at 623 nm was enhanced about 5-fold with the gradually addition of 10 equiv. CB[7]. All these can be ascribed to CB[7] can include **D2** around the two pyridium rings, and inhibit the torsional motion of the pyridium groups.

The $^1$H NMR titration experiments were carried out in pH 10 (**Figure S9**) solution to ensure the dye was not to be protonated. H9 and H9' remained almost unchanged with the addition of CB[7], while H8, H8', H1 and H2 moved to the downfield, and the proton of vinyl group moved to the upfield. The signal changes indicated that the double bond was included in the CB[7] cavity and H1 and H2 were located outside the portal of CB[7].

The interaction between the protonated form of **D2** and CB[7] was also investigated by $^1$H NMR titration (**Figure S10**). A trace amount of acid was added together with CB[7] to ensure the formation of **D2**H$^+$/CB[7] complex (pH = 3). The signals at down field became border and difficult to be observed with the addition of CB[7]. However, it was clear to observe that H1 shifted to the downfield until 2 equiv. CB[7] was added, and H2 and H2' shifted to the upfield. These changes indicated that CB[7] interacted with **D2** around each pyridium ring. The MS spectrum further confirmed this result (**Figure S11**), *m/z* found: 506.7834 (calculated for (**D2**H$^+$+CB[7])$^{3+}$: 506.8543), 759.6782 (calculated for (**D2**+CB[7])$^{2+}$: 759.7814), 894.5886 (calculated for (**D2**H$^+$+2CB[7])/3: 894.3021).

The stoichiometry of 1:2 for the **D2**/CB[7] complex allowed the binding constant to be calculated according to the above mentioned method for **DSMI**. Applying Equation (3) to fit the experimental data presented in the fluorescent titration spectra (**Figure S12**), the $K_1$ and $K_2$ values were estimated to be (8.4 ± 0.4) × $10^4$ M$^{-1}$ and (2.3 ± 0.3) × $10^4$ M$^{-1}$ for **D2**/CB[7] complex. The difference between $K_1$ and $K_2$ of **D2** are not much, which is different from that of **DSMI**, suggesting that the first and second complexations are caused by a similar ion-dipole interaction. The pKa shift of 1 unit (**Figure S4**) with and without the presence of CB[7] can provide some further information.

### Competitive interaction of D2 with DNA and CB[7]

The fluorescence response of **D2** to DNA was investigated through optical measurement. When 30 equiv. DNA was added into the dye solution, the absorption maxima of **D2** at 345 nm and 393 nm exhibited a firstly hypochromism (with the concentration ratio [DNA]/[**D2**] < 2) and then hyperchromism (with [DNA]/[**D2**] > 2) with bathochromic shifts of 7 nm and 35 nm, respectively (**Figure S13**). The absorption spectra changes related with the concentration ratio ([DNA]/[**D2**]), demonstrating that the binding mode of **D2** with DNA was different. As suggested in the literature, **D2** may be aggregated on the surface of DNA helix at a low ratio and intercalated into DNA base pairs at a high ratio.[35] Moreover, **D2** exhibited a significant fluorescence increasing with the addition of DNA. The fluorescence intensity at 623 nm

was enhanced by ca. 35-folds with a red shift of 8 nm in the presence of 100 equiv. DNA (**Figure S13**). This may be caused by the restriction on the torsional motion of vinyl groups derived from the interactions between the cationic pyridium unit and the anionic phosphate backbone of DNA;[36] together with the water prohibiting effect in the hydrophobic environment offered by the higher-order structure of DNA.

With the presence of **D2**, the denaturation temperature of ct-DNA was increased 17.5 ℃, while; only 6.8 ℃ was increased in the case of **DSMI** (**Figure S14**), suggesting that **D2** interacted with DNA through an intercalation mode, while **DSMI** interacted with DNA through a grove binding mode.[19]

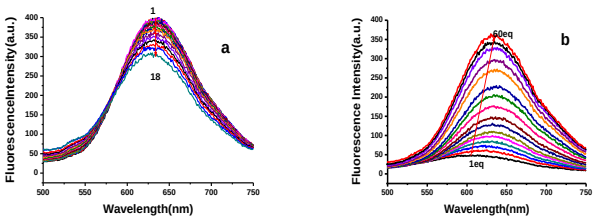
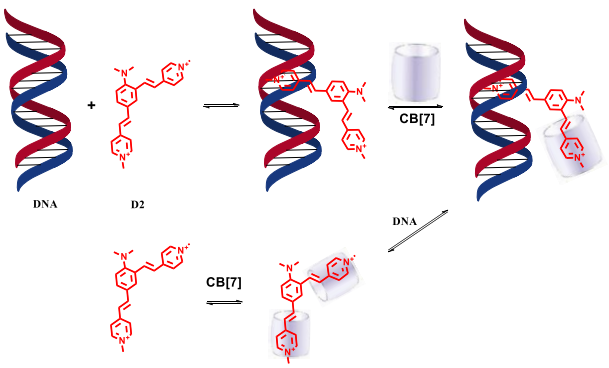



**Figure 6** a) The fluorescence spectra of **D2**/DNA with the addition of CB[7], from (1) to (18), 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 15.0, 20.0, 25.0 equiv. of CB[7] was added, the concentration of **D2** was $1 \times 10^{-5}$ M and DNA was 100 equiv. b) The fluorescence spectra of **D2**/CB[7] with the addition of 60 equiv. of DNA, CB[7] was 10.0 equiv. of **D2**, the concentration of **D2** was $1.0 \times 10^{-5}$ M, pH=7.4.

Competitive interaction among **D2**, CB[7] and DNA was performed and the results are shown in **Figure 6**. After titration of 25 equiv. CB[7] into **D2** and 100 equiv. DNA solution (**Figure 6a**), a 20% decrease was observed in the fluorescence intensity. In comparison, about a 7.5-fold fluorescence increase was observed along with the addition of 60 equiv. DNA into the solution of **D2** and 10.0 equiv. CB[7] (**Figure 6b**). It should be noted that a quite similar result was reached for the two different procedures. Taking account of the symmetric structure of **D2**, it can be proposed that one pyridium vinyl of **D2** was included into the cavity of CB[7] and the other intercalated into DNA to form a ternary complex as shown in **Scheme 3**.




**Scheme 3.** The Schematic diagram for the formation of the ternary complex among **D2**, CB[7] and DNA.

## Conclusion

In summary, the interaction between **DSMI** and CB[7] was investigated through UV-Vis, fluorescence, $^1$H NMR and MS spectrometry. It was found that **DSMI** can be included by CB[7] with a 1:2 stoichiometry. When another pyridium vinyl group was introduced to the aniline ring of **DSMI** to get a hemicyanine derivative **D2**, a ternary complex among **D2**, CB[7] and DNA was formed. **D2** interacted with DNA through a intercalation mode, while **DSMI** interacted with DNA through a grove binding mode. All these provide new insights to investigate the interaction of organic dyes, CB[7] and DNA.

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## Notes and references

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† Electronic Supplementary Information (ESI) available: NMR and Mass spectra of **D2**, MS and the fitting plot for **DSMI**/CB[7] and **D2**/CB[7] complex. See DOI: 10.1039/b000000x/

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