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SUPRAMOLECULAR INTERACTION BETWEEN *p*-SULFONATED CALIXARENES WITH TYROSINE

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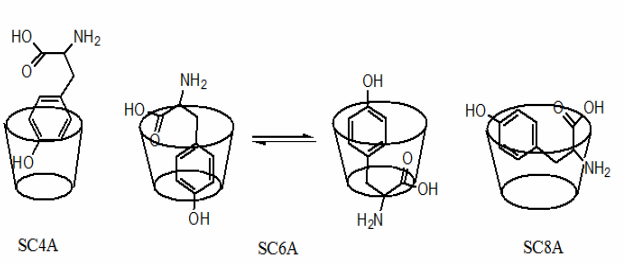
The fluorescence spectra showed the larger the cavity size was, the stronger was the inclusion ability between *p*-sulfonated calix[*n*]arenes and tyrosine. The results of ^{1}HNMR and the cavity matching effect indicated that the phenol unit of tyrosine entered the *p*-sulfonated calix[*4*]arene cavity, and the guest tyrosine molecule vertically penetrated the cavity of *p*-sulfonated calix[*6*]arene in two different inclusion modes. Tyrosine was present in the upper part of the *p*-sulfonated calix[*8*]arene cavity. The different cavity size and flexible structure of *p*-sulfonated calix[*n*]arene led to different host-guest inclusion modes. The tyrosine tended to enter the larger hydrophobic cavity of *p*-sulfonated calix[*n*]arene.



INTRODUCTION

Tyrosine is an important amino acid in the human body. A deficiency or imbalance of tyrosine leads to slow growth and mental retardation. Therefore, the accurate and sensitive determination of tyrosine in human tissues and body fluids is of great significance. It is well known that tyrosine can be obtained *in vitro*. The methods used to determine tyrosine have been widely reported, for example, fluorescence spectrophotometry,[1,2] chemi-luminescence,[3] and high performance liquid chromatography.[4] Fluorescence spectrophotometry is usually used in the quantification of drugs. The technique has the advantages of instrumental simplicity, high selectivity and good sensitivity, and suitable method for studying the inclusion process of L-tyrosine and SC*n*A.

Studies have investigated *p*-sulfonated calix[*n*] arenes, which have flexible and often defined cavities that bind positively charged species. *p*-sulfonated calix[*n*]arenes are regarded as promising watersoluble hosts for the organic amine cations,[5] pyridinium cation and viologen cation,[6,7] guanidine salt.[8] Recently, sulfonated calixarenes show broad potential application in molecular recognition, probes, enzyme mimic, and biological pharmacy.

In this research, the host selectivity differences to the tyrosine guest were discussed by fluorescence spectrometry. SC*n*A had a large quenching effect on tyrosine fluorescence in the fluorescence titration experiments. An accurate, rapid, and sensitive fluorescence probe method was established for the determination of tyrosine.

MATERIALS AND METHODS

Fluorescence spectra and intensity measurements were obtained with a Agilent Technologies Cary Eclipse. Fluorescence spectrofluorometer was equipped with a pulsed lamp. The slit

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width of both the excitation and emission monochromators was set at 5 nm. The fluorescence spectra were recorded at a scan rate of 600 nm min^{-1}. All measurements were performed in a standard 10 mm path-length quartz cell set to a temperature of 25.0 ± 0.5 ^{o}C. All pH values were measured with a pHS-3TC digital precision pH meter (Shanghai, China). ^{1}H NMR spectra were recorded using a Bruker DRX-600MHz spectrometer (Switzerland) in D_2O.

All chemicals were of analytical reagent grade, and double–distilled water was used throughout the procedures. The tyrosine (tyr, content > 98.0%) used in the experiment was obtained from the Great Britain and Tokyo Chemical industry Co., Ltd, Tokyo, Japan. The stock solution of 1.0×10^{-5} mol L^{-1} was prepared by directly dissolving in double–distilled water. SC*4*A, SC*6*A and SC*8*A were prepared according to literature [9] and identified by IR, ^{1}H NMR and element analysis. SC*n*A (*n = 4, 6, 8*) stock solution of 1.0×10^{-4} mol L^{-1} was prepared respectively in a 100 mL volumetric flask. Working solutions were obtained by dilution of the stock solution. Measurements were performed in a PBS buffer from pH 4.0 to 10.0. Different concentrations of tyrosine were injected in the PBS buffer solution to study *p*-sulfonated calix[*n*]arenes–tyrosine interactions.

RESULTS AND DISCUSSION

Formation of inclusion complexes of tyrosine and SC*n*A

The excitation spectrum and emission spectrum of the tyrosine solution changed with addition of different amounts of SC*n*A as shown in Figure 1. The maxima in the excitation spectrum of tyrosine were at 225 nm and 275 nm, respectively. When different amounts of SC*n*A were added, the excitation peak shape and peak position of tyrosine were unchanged, and the excitation peak intensity significantly decreased. Moreover the excitation peak intensity at 225 nm decreased to a greater degree than the peak at 275 nm. Thus, the excitation wavelength chosen was 225 nm and the emission wavelength of tyrosine was at 305 nm. Upon SC*n*A addition, the wavelength of the emission maximum showed a small blue shift from 305 nm to 301 nm. Significant fluorescence quenching and the blue shift indicated that the complexes were formed.

Figure 1 showed the fluorescence intensity of tyrosine decreased regularity with the concentration of sulfonated calixarenes, and the degree of fluorescence quenching increased with the number of hydroxyl units in the sulfonated calixarene, the order of fluorescence quenching degree was as follows: SC*4*A< SC*6*A < SC*8*A. This was due to the interaction between calixarene cavity and the tyrosine increased with the calixarene cavity increasing.

It is well known that calixarene and guest molecules can form inclusion complexes including internal inclusion type and external inclusion type complexes.[10,11] When internal inclusion type complexes are formed, the guest molecules will enter the hydrophobic cavity of calixarene, and the fluorescence intensity of the complex may then increase or decrease, and the fluorescence emission peak position may also red shift or blue shift. When external inclusion type complexes are formed, this is due to π-π stacking interactions between hydrophobic groups such as the phenyl group of guest molecules and the SC*n*A cavity, and the hydrogen bonding and electrostatic interactions induce them to form more stable external inclusion type complexes, which results in a marked degree of fluorescence quenching. However, the cavity size of SC*4*A (SC*6*A) did not match the size of tyrosine, therefore we speculated that SC*4*A (SC*6*A) and tyrosine formed an external inclusion type complex or partial internal inclusion of the phenyl group.

Stoichiometry and association constant of the inclusion complexes

The Benesi-Hildebrand method is a common method for the determination of the association parameters (association constants and stoichiometries) of inclusion complexes, which is generally suitable for the host system of the combination of some weak fluorescence or no fluorescence guests. In view of the weak fluorescence intensity of tyrosine, we use this method to study the fluorescence spectra of tyrosine, focusing on the inclusion constants and the inclusion ratio of the complexes.

Assuming that SC*n*A and the tyrosine forms a 1:1 ratio complex, the following expression can be written as

$$H + G \rightleftharpoons H - G \tag{1}$$

The formation constant of the complex (K) is given by

$$K = \frac{C_{H\text{-}G}}{C_H \times C_G} \tag{2}$$

An equation of inclusion constant K of the complex with guest– host was used to calculate the inclusion constant: [12]

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0) K C_{SCnA}} + \frac{1}{F_\infty - F_0} \tag{3}$$

In which, C_G is the initial concentration of tyrosine, C_H is the initial concentration of SC*n*A, F_0 is the fluorescence intensity of tyrosine in the absence of SC*n*A, F_∞ is the fluorescence intensity when all of the tyrosine molecules are essentially complexed with SC*n*A, and F is the observed fluorescence intensity at each SC*n*A concentration tested, K is the inclusion constant of the complex.

The excellent linear relationship was obtained when $1/(F–F_0)$ was plotted against $1/C_{SCnA}$ (Figure 2), which indicated the formation of a 1:1 complex. The plot will be linear if only 1:1 complexation occurs and will be nonlinear if higher-order complexes also form. The inclusion constants of the tyrosine and SC*n*A complexes at pH 7.3 were determined to be 2.357×10^4 L mol^{-1}, 3.227×10^4 L mol^{-1}, and 3.927×10^4 L mol^{-1} in the presence of SC*4*A, SC*6*A and SC*8*A. The complex inclusion constant monotonically increased with the number of phenolic units in the calixarene ring.

Generally, the Job's plot was used to calculate the association ratio. Typical Job's plots for the inclusion complexes of tyrosine and SC*n*A were shown in Figure 3. The maximum of the relative fluorescence intensity was at a molar fraction of 0.5, which confirmed that the 1:1 ratio complex assembly formed. [13]

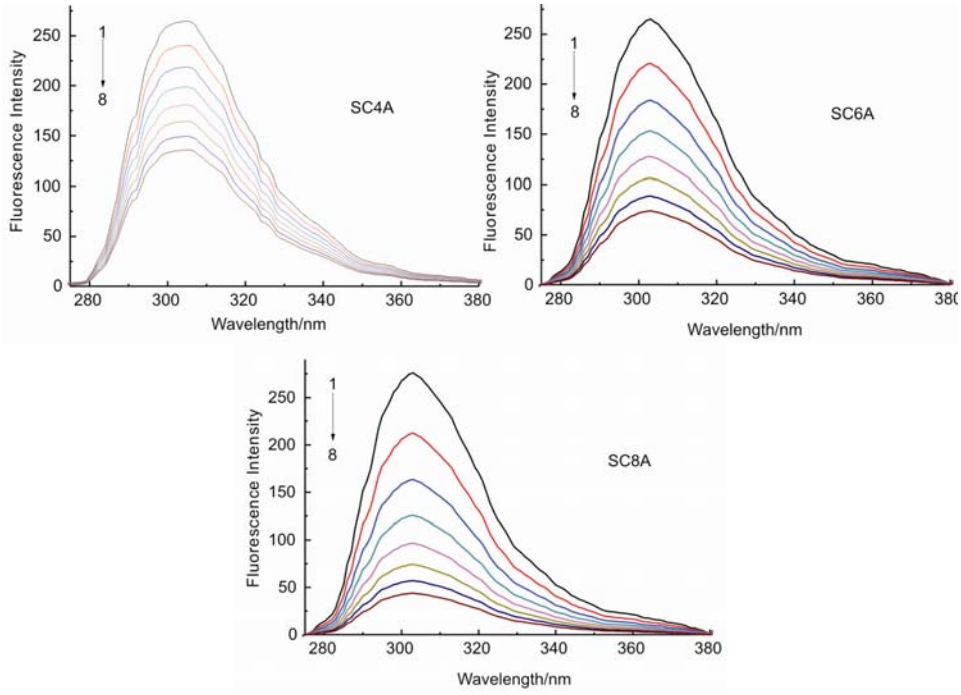

Fig. 1 – The fluorescence spectra of L-tyrosine with addition of different amounts of SC*4*A, SC*6*A, SC*8*A at 298K and pH=7.3. The concentrations of SC*n*A ($\times 10^{-5}$ mol/L): (1) 0; (2) 0.2; (3) 0.4; (4) 0.5; (5) 0.7; (6) 1; $C_{L\text{-}Tyr}=1.0\times10^{-5}$ mol/L.

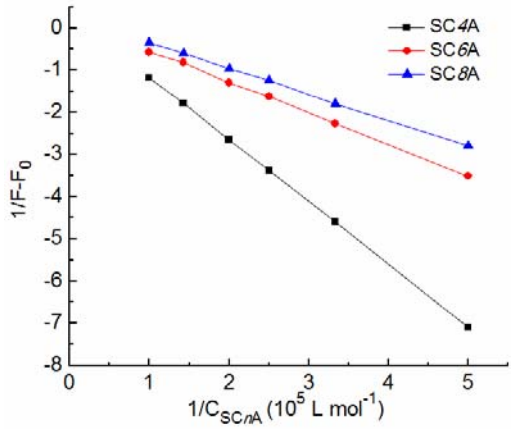

Fig. 2 – The relationship of $(F\text{-}F_0)^{-1}$ with SC*n*A^{-1} pH=7.3, T=298K. The concentrations of SC*n*A ($\times 10^{-5}$ mol/L): (1) 0; (2) 0.2; (3) 0.4; (4) 0.5; (5) 0.7; (6) 1; $C_{L\text{-}Tyr}=1.0\times10^{-5}$ mol/L.

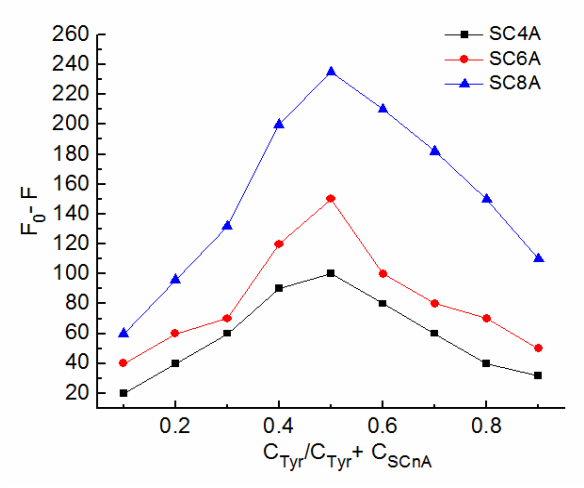

Fig. 3 – Job's plot for the complex of L-tyrosine with SC*n*A in pH 7.3 Britton–Robinson buffer solution at 25°; $C_{L\text{-}tyr} + C_{SCnA} = 1.0\times10^{-5}$ mol/L.

Thermodynamic parameters and thermodynamic origin of inclusion complexes

To explain the thermodynamic origins of the formation of inclusion complex, the stability constants at various temperatures ranging from 278 K to 318 K were obtained by the fluorescence spectra method as shown in Table 1. The relationship of inclusion constant (*K*) with temperature *T* can be described by Van't Hoff equation [14] shown as follows:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (4)$$

where *R* was the gas constant, the enthalpy change (ΔH) and the entropy change (ΔS) associated with complex formation, supposing ΔH and ΔS were constant in the range of experimental temperature. A plot of ln *K* *versus* 1/*T* was linear within experimental error, which was shown in Figure 4.

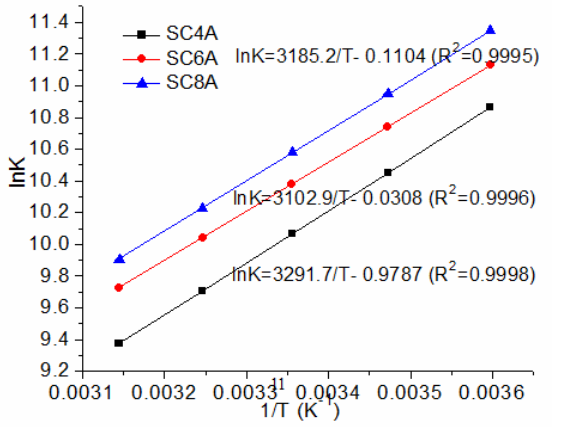

Fig. 4 – Van't Hoff plot, pH 7.3; $C_{L\text{-}Tyr}=1.0\times10^{-5}$ mol/L.

The strength of C-H…π and π-π force between SC*n*A and guest molecules depended on π electron cloud density lying in calixarene cavity. SC*4*A has a more stable framework and relatively high π electron cloud density than SC*6*A and SC*8*A, and SC*4*A had stronger C-H…π or π-π interaction with guest molecules. In theory, host-guest electrostatic interaction and hydrogen bond would have beneficial effect on enthalpy. SC*6*A and SC*8*A had one or two sulfonic acid groups more than SC*4*A, hydrogen bonding interaction with amine/carboxyl chain part of the tyrosine and charge interaction with positive charge of the tyrosine would be stronger, and enthalpy would be more favorable. But in fact, as shown in Table 1, the enthalpy change of SC*6*A and SC*8*A and tyrosine complexes had no favorable effect to SC*4*A. Obviously, C-H…π or π-π interaction played the dominant role in the host-guest inclusion complexation, not hydrogen bond or charge interaction.

As shown in Table 1, the most unfavorable entropy change appeared in SC*4*A and tyrosine complexation because of the degrees of conformation freedom loss during host-guest inclusion. Generally, the inclusion complexation of SC*n*A and tyrosine is mainly derived from the favorable enthalpy change caused by π-π and C-H…π interactions, accompanied by a small negative entropy, the order of interaction was as follows: SC*4*A< SC*6*A < SC*8*A. The result was due to cavity matching effect. Under the condition of pH=7.3, the inclusion constant of SC*n*A-tyrosine complexes were also increased with the cavity size of the SC*n*A increasing, and the order of the host selectivity was SC4A < SC6A < SC8A. Moreover, higher temperature was unfavorable to the formation of inclusion complexes. Therefore, the present experiments were conducted at room temperature.

Influence of pH

The associations of tyrosine with SC*n*A were also conducted in acidic, neutral and basic media. The pH dependence of inclusion constants of Table 2 was examined at a pH range of 4.0 to 11.0. Table 2 showed the large affinity of tyrosine to SC*n*A in the fluorescence spectra was found relevant of pH. The inclusion constants became more sensitive to pH with increasing the size of SC*n*A cavity, indicating that the structural matching effect was the dominant stabilizing factor for the host-guest complexes of the tyrosine and SC*n*A.

Deprotonation of the phenolic OH groups of SC*n*A can be further strengthened by increasing the pH to 10.5. Thus, repulsive interaction of the phenolic O^- of the lower rim of SC*n*A results in a small size of the cavity. The electrostatic interaction of the amino group of tyrosine and the sulphonate anions of SC*n*A occurred at pH 7.3. As the pH from 7.3 to 9.0 or more basic condition, the electrostatic interaction between the tyrosine and SC*n*A became much weaker. Based on these results, the inclusion constant at pH 7.3 was higher than that at pH 9.0 or 11.0.

Table 1

Complex stability constants and thermodynamic parameters for 1:1 intermolecular complex of L-tyrosine with SC*n*A in pH 7.3 buffer solution

Host	*T*(K)	$K(10^4$ L mol$^{-1})$	ΔH(kJ mol^{-1})	ΔG(kJ mol^{-1})	ΔS(J/(mol K)$^{-1}$)
SC*4*A	278	5.216	–27.356	–25.105	–8.1
	288	3.456		–25.023	
	298	2.357		–24.943	
	308	1.646		-24.861	
	318	1.176		–24.780	
SC*6*A	278	6.824	–25.795	–25.726	–0.25
	288	4.630		–25.723	
	298	3.227		–25.721	
	308	2.300		–25.718	
	318	1.676		–25.715	
SC*8*A	278	8.473	–26.476	–26.226	–0.9
	288	5.690		–26.216	
	298	3.927		–26.208	
	308	2.775		–26.198	
	318	2.005		–26.190	

Table 2

The change of the stability constants (*K*) for 1:1 inclusion complexes of L-tyrosine with SC*4*A, SC*6*A, and SC*8*A in different pH at T=298 K

$K/10^4$ (L mol^{-1})	pH					
	4.0	6.0	7.0	7.3	8	10
$K_{\text{L-tyr -SC4A}}$	1.665	1.832	1.974	2.357	1.193	1.012
$K_{\text{L-tyr-SC6A}}$	2.355	2.845	3.011	3.227	2.895	2.413
$K_{\text{L-tyr-SC8A}}$	3.057	3.638	3.752	3.927	3.110	2.846

^{1}H NMR study of the inclusion complexes

As shown in Figure 5, as tyrosine entered the SC*n*A cavity, the chemical shifts of tyrosine appeared greatly changed by the sulfonated calixarene cavity aromatic ring current. According to the different shift values, we speculated on the different inclusion modes of tyrosine-SC*n*A (see Figure 6). For the tyrosine guest, the complexation with three different SC*n*A hosts did not proceed similarly. ^{1}H NMR position shift of tyrosine following SC*6*A complexation was approximately the same as that for the SC*8*A and tyrosine complexation. The phenyl−H and phenolic−H protons exhibited

upfield shift upon SC*4*A-tyrosine complexation, and other H protons appeared downfield shift which may be attributed to the SC*4*A-tyrosine inclusion mode. It speculated that tyrosine entered the SC*4*A cavity from four H positions of the benzene ring, and the other H were out of the cavity. However, $-NH_2$, phenolic−H and −COOH protons of tyrosine exhibited downfield shift, and the phenyl−H, $-CH_2$ and −CH− protons of tyrosine presented upfield shift upon SC*6*A (SC*8*A)-tyrosine complexation. These results indicated that the inclusion modes of tyrosine-SC*6*A(SC*8*A) could be the same.

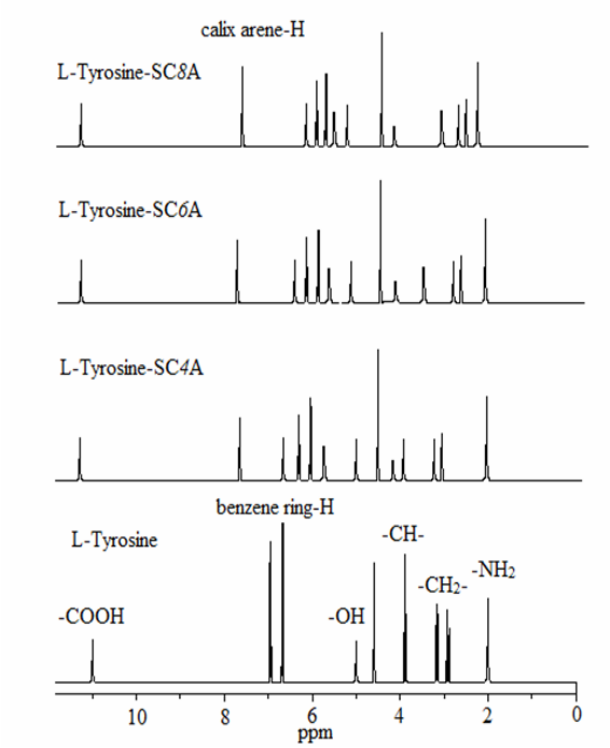

Figure 5. ^{1}H NMR spectra (600 MHz) of L-tyrosine and L-tyrosine-SC*n*A complex in D_2O.

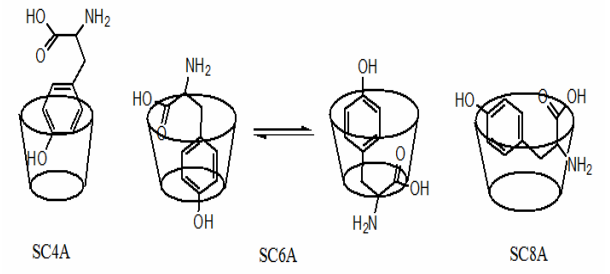

Fig. 6 – The inclsion model of L-tyrosine with SC*n*A complexes according to ^{1}H NMR spectra.

According to the optimal Gaussian available, the Onsager cavity radius of tyrosine was approximately 8 Å and the cavity inner diameter of sulfonated calix [*8*] arene was approximately 11.7 Å, thus they formed a stable internal inclusion type complex. However, the cavity size of SC*6*A was 7 Å, the cavity size of SC*8*A was 11.7 Å, and the cavity size of tyrosine was 8Å, thus this was not consistent with the above speculation. Tyrosine was in agreement with the cavity size of SC*8*A, but not of SC*6*A. According to the cavity matching effect, tyrosine entered the SC*6*A cavity vertically, and it was present in the upper part of the SC*8*A cavity due to the hydrogen bonding and charge interactions of the complex. The different cavity size and rigidity of flexible structure of SC*n*A directly led to different host-guest inclusion modes, thus the guest tyrosine molecule tended to be included into the larger hydrophobic cavity of SC*n*A.

CONCLUSIONS

The inclusion behavior of SC*n*A and tyrosine was determined by fluorescence spectra and ^{1}HNMR spectra. The maximum of the relative fluorescence intensity was at a molar fraction of 0.5, which confirmed that the 1:1 ratio complex assembly formed. The inclusion complexation was driven mainly by a favorable enthalpy change caused by a C-H…π or π…π interaction between SC*n*A and tyrosine, accompanied by a small unfavorable entropy change. The binding constants became more sensitive to pH with increasing the size of SC*n*A cavity, indicating that the structural matching effect was the dominant stabilizing factor for the host-guest complexes of tyrosine and SC*n*A. The larger the cavity size was, the stronger was the inclusion interaction. According to the results of ^{1}HNMR and the cavity matching effect, the phenol unit of tyrosine entered the SC*4*A cavity, tyrosine vertically penetrated the SC*6*A cavity by two different inclusion modes, and tyrosine was present in the upper part of the SC*8*A cavity. The different cavity size and different rigid or flexible structure of SC*n*A directly led to different host-guest inclusion modes, thus the guest tyrosine molecule tended to be included into the larger hydrophobic cavity of SC*n*A.

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