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Development of a Binary Sensory System Based on Nitrobenzene Functionalized Homotrioxacalix[3]arene and Pyrenemethylamine Hydrochloride

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Abstract

A novel binary system which reads binding processes of cationic guests such as primary ammonium ions to the hexahomotrioxacalix[3]arene is developed. In this system, pyrenemethylamine hydrochloride binds to the nitrobenzene modified hexahomotrioxacalix[3]arene **1**, which quenches the fluorescence of pyrenemethylamine hydrochloride when they bind each other. However, when the pyrenemethylamine hydrochloride is replaced with other cationic guests, it gives strong fluorescence depending on the binding affinity of cationic guests, which enables us to estimate the affinity of cationic species.

Introduction

Calixarenes are homologous cyclic compounds, which are synthesized by the condensation reactions between para-substituted phenols and formaldehydes. They usually take bowl-shaped conformation, which could bind with guest molecules. They form complexes with various cations and organic molecules selectively. As ammonium ions play important roles in both chemistry and biology [1–2], calixarenes as ammonium ion receptors have been a matter of intensive investigations. While there are abundant examples of interaction between quaternary ammonium ions and calixarene through cation-π interactions [3–8], relatively few examples are known between primary ammonium ions and calixarenes. While cation-π interactions are a major driving force in the recognition of quaternary ammonium ions with calixarenes, the recognition of primary alkyl ammonium ions by calixarenes utilizes cation-π inteactions [9], hydrogen bonding [10–13] and shape of ammonium ions [14, 15].

The binding process of ammonium ions has been monitored by NMR spectroscopy or change of optical signal as a direct result of the complexation between ammonium ions and calixarenes. In most of the cases the change of intramolecular conformation or the way of electronic transition in host molecules as a source of optical signal was utilized when guest molecules bind to the host molecules. Another novel idea to monitor the binding process of ammonium ion was utilizing the binary system which was developed by Inoue and Shinkai [16, 17]. They utilized an ammonium ion fluorescent reporter molecule, which was quenched pseudo-intramolecularly when it was bound to the π- basic cavity of resorcin[4]arene. When it was replaced with another ammonium ion such as acetylcholine, it gave strong fluorescence [18–23].

To enlarge the scope of utilizing novel binary system, we designed another binary system, nitrobenzene modified hexahomotrioxacalix[3]arene and pyrenemethylamine hydrochloride, which was able to read binding processes of cationic guests such as primary ammonium ions to the calixarene. In this system, pyrenemethylamine hydrochloride binds to the nitrobenzene modified hexahomotrioxacalix[3]arene, which quenches the fluorescence of pyrenemethylamine hydrochloride. However, when the pyrenemethylamine hydrochloride is replaced with other cationic guests, it gives strong fluorescence, which enables us to estimate the affinity of cationic species (Figure 1).

Experimental

Instrumentation

Fluorescence spectra were measured on a Perkin-Elmer fluorescence spectroscopy LS-50B. All fluorescence measurements were monitored in the mixture of acetonitrile and chloroform (99 : 1).

^{1}H NMR spectra were measured on a 200 MHz Bruker ASPECT 3000 spectroscopy. All measurements were carried out in 7% CD_3OD in $CDCl_3$.

Syntheses and characterization

Preparation of the calixarene **1** is summarized in Figure 2. The synthesis started from the reaction between p-*tert*-butylphenol **2** and formaldehyde in basic condition. The compound **3** was obtained in 60% yield. Refluxing the

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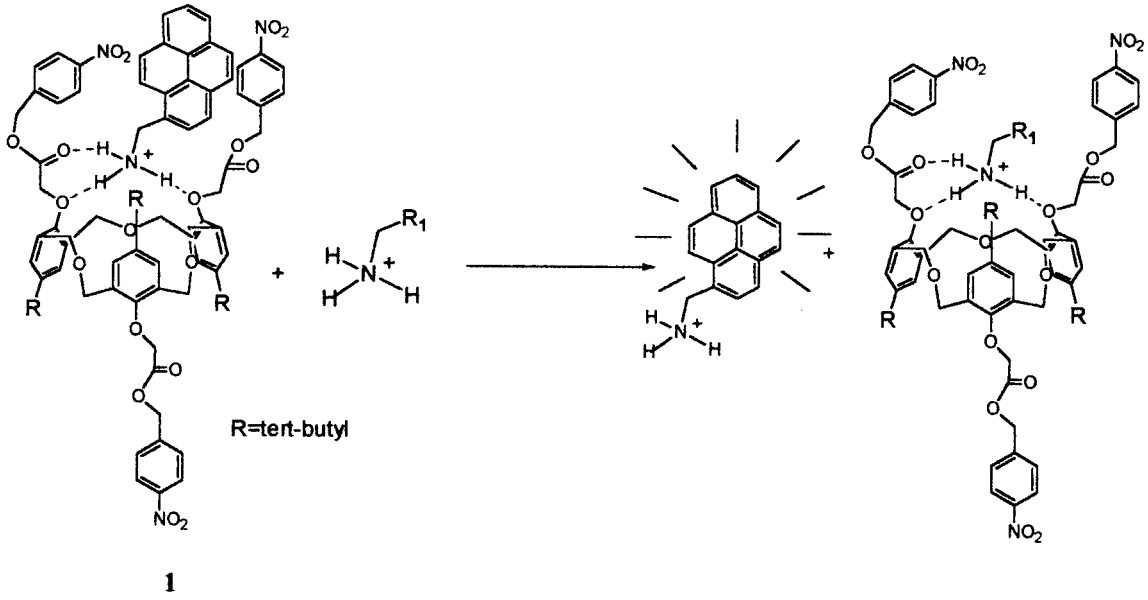

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Figure 1. The fluorescence of pyrenemethylamine hydrochloride was quenched when it bound to the nitrobenzene modified calixarene **1**. When the pyrenemethylamine hydrochloride was replaced with other cationic guests, it gave strong fluorescence.

compound **3** in acidic condition gave the hexahomotrioxacalix[3]arene **4** in 65% yield[24]. In this condition only partial cone calixarene was obtained. This was followed by substitution reaction with the compound **5** to give the final compound **1** in 60% yield [25].

Para-tert-butyl-2,6-bis-hydroxymethyl phenol **3**

To a stirred solution of 15 ml of 35% formalin solution were added 2.7 ml of 5N aqueous NaOH, 20 ml water and 1 g (6.7 mmol) of para-*tert*-butylphenol. After stirring 7 day, acetic acid was added until PH reached 4. The reaction mixture was extracted with 100 ml dichloromethane 3 times and the dichloromethane layer was washed with 50 ml of water 3 times. The organic layer was dried with $MgSO_4$ and filtered. Evaporation of the dichloromethane and silicagel chromatography (Hexane : Ethyl acetate = 2 : 5) gave 839 mg of compound **3** in 60% yield.

^{1}H NMR(200 MHz ; $CDCl_3$) 7.0(s, 2H) 4.6(s, 4H) 1.2(s, 9H).

5,13,21-tris-tert-butyl-homotrioxacalix[3]arene **4**

To a solution of 1.0 g (4.8 mmol) of compound **3** in 100 ml ethylene glycol dimethylether were added 0.72 ml (9.6 mmol) of methane sulfonic acid and 4 g of sodium sulfate. The reaction mixture was refluxed for 3 hours. Then the reaction mixture was treated with 50 ml of saturated aqueous sodium bicarbonate and the reaction mixture was extracted with 100 ml of dichloromethane 3 times. The organic layer was dried with $MgSO_4$. Evaporation of dichloromethane and silicagel chromatography (hexane : ethyl acetate = 15 : 1) gave 1.78 g of product **4** in 65% yield.

^{1}H NMR(200 MHz; $CDCl_3$) 8.56 (s, 3H) 7.11(s, 6H) 4.71(s, 12H) 1.22(s, 27H).

5, 13, 21-tris-tert-butyl-tris-para-nitrobenzyloxycarbonylmethoxyhomotrioxacalix[3]arene **1**

To a solution of 100 mg (0.17mmol) of compound **4** and 84 mg (0.52 mmol) of K_2CO_3 in 3 ml acetone was added 165 mg (0.52 mmol) of 4-nitro-benzyl bromoacetic acid **5**. After the reaction mixture was refluxed for 3 hours, the reaction mixture was poured into 100 ml of water and extracted with 100 ml of dichloromethane 3 times. The organic layer was dried with $MgSO_4$. Evaporation of dichloromethane and silicagel chromatography(hexane : ethylactate = 3 : 1) gave 120 mg of desired product **1** in 60% yield.

^{1}H NMR(200 MHz; $CDCl_3$) 8.25(m, 6H) 7.51(m, 6H) 7.23(s, 2H) 7.20(d, J = 2.4, 2H) 7.00(d, J = 2.4, 2H) 5.16(m, 8H) 4.75(m, 6H) 4.30(m, 8H) 3.16(s, 2H) 1.28(s, 9H) 1.05(s, 18H) HRMS calculated for $C_{63}H_{69}N_3O_{18}Na^+$ 1178.5 found for 1178.7.

Fluorescence spectroscopy study

The association of calixarene **1** and pyrenemethylamine hydrochloride: To a 6 μM, 1 ml solution of pyrenemethylamine hydrochloride was added 1 ml of various concentration of nitrobenzene modified hexahomotrioxacalix[3]arene **1**.

Exchange experiments: 1 ml of 1200 μM nitrobenzene modified hexahomotrioxacalix[3]arene **1** and 1 ml of 6 μM pyrenemethylamine hydrochloride were mixed. To this solution was added 1 ml of various concentration of ammonium salts

In both cases, fluorescence spectrum was measured at the excitation wavelength 343 nm and emission wavelength 378 nm.

NMR study

The NMR titration of calixarene **1** and pyrenemethylamine hydrochloride: To a 0.5 ml, 1 mM solution ($CDCl_3$: CD_3OD

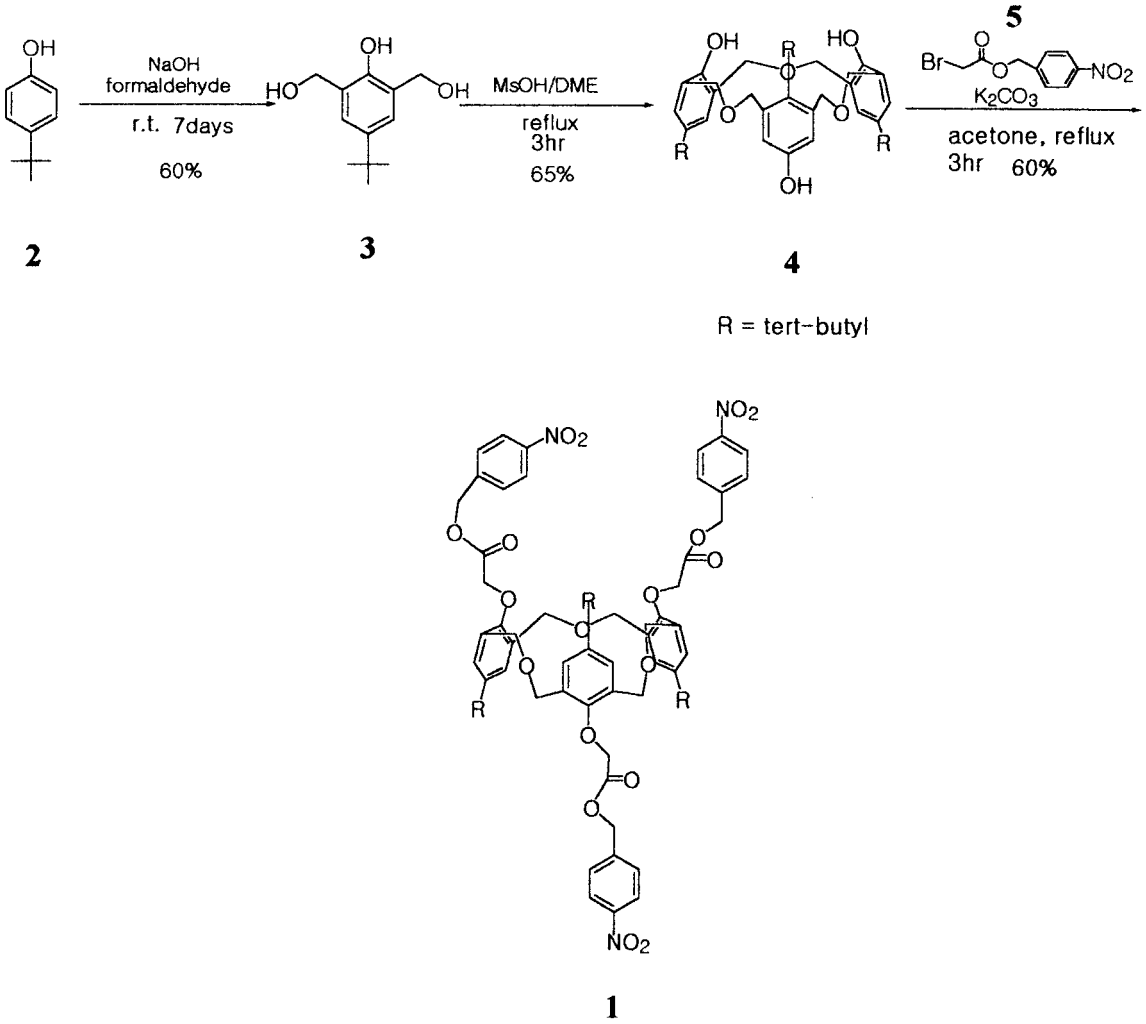


Figure 2. The synthetic scheme for the preparation of **1**.

= 93 : 7) of pyrenemethylamine hydrochloride was added 10 μl of various concentration of nitrobenzene modified hexahomotrioxacalix[3]arene **1**. The chemical shift of benzylic hydrogen in the pyrenemethylamine hydrochloride was measured.

The NMR titration of calixarene **1** and benzylamine hydrochloride: To a 0.5 ml, 1 mM solution ($CDCl_3 : CD_3OD$ = 93 : 7) of benzylamine hydrochloride was added 10 μl of various concentration of nitrobenzene modified hexahomotrioxacalix[3]arene **1**. The chemical shift of benzylic hydrogen in the benzylamine hydrochloride was measured.

Exchange experiments: To a mixture of 0.5 ml of 1 mM pyrenemethylamine hydrochloride and 9 mM calixarene **1** was added 10 μl of various concentration of ammonium ions.

Results and discussion

Fluorescence spectroscopy study

The association between pyrenemethylamine hydrochloride and nitrobenzene modified hexahomotrioxacalix[3]arene **1** was investigated first. The emission fluorescence spectrum of 3 μM pyrenemethylamine hydrochloride solution was shown in Figure 3 (pyrene : calixarene = 1 : 0). The excitation wavelength was 343 nm. The intensity of the emission spectrum from 3 μM solution of pyrenemethylamine hydrochloride decreased remarkably as the concentration of nitrobenzene modified hexahomotrioxacalix[3]arene **1** was increased, which indicates the association between the two species. Assuming 1 : 1 binding stoichiometry, a Benesi–Hildebrand plot [26] of the mixture of pyrenemethylamine hydrochloride and nitrobenzene modified calixarene **1** by use of change in fluorescence intensity at 378 nm gave the association constant K as 1850 M^{-1}. This result established that the binary system between pyrenemethylamine hydrochloride and nitrobenzene modified hexahomotrioxacalix[3]arene **1** could satisfy the first requirement that the quenching of fluorescence from pyrenemethylamine hydrochloride through association.

The second requirement is regeneration of fluorescence of pyrenemethylamine hydrochloride by selective substitution of pyrenemethylamine hydrochloride with other cationic species. Therefore, the solution of 3 μM pyrenemethylamine hydrochloride and 600 μM nitrobenzene modified hexahomotrioxacalix[3]arene was prepared

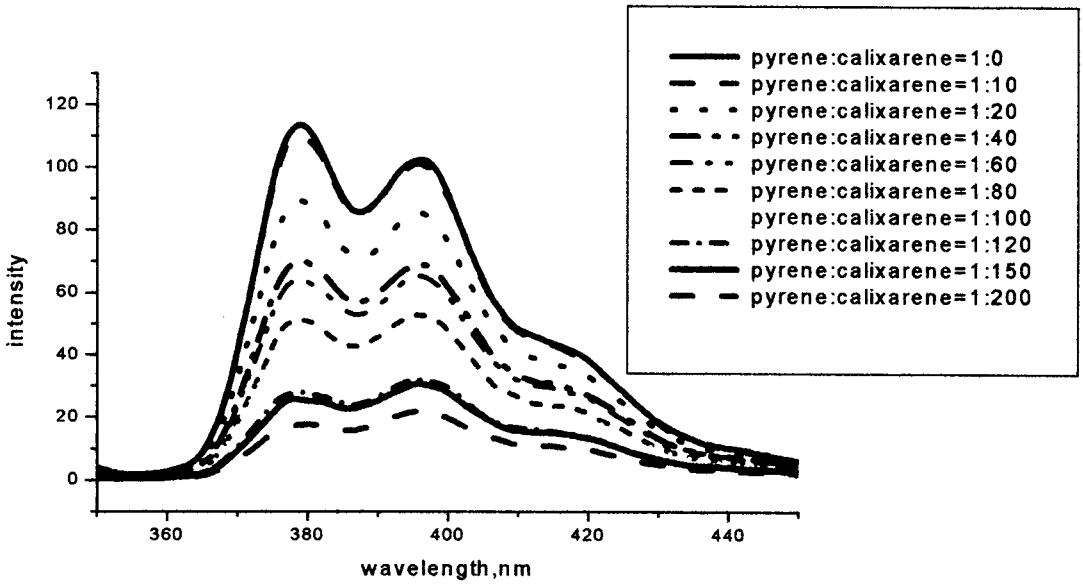


Figure 3. Fluorescence change of a mixture of pyrenemethylamine hydrochloride and calixarene **1**.

and its emission fluorescence spectrum was measured (Figure 4a). The excitation wavelength was 343 nm. In the other vial, the solution of 3 μM pyrene methylamine hydrochloride, 600 μM nitrobenzene modified hexahomotrioxacalix[3]arene and 3 μM benzylamine hydrochloride was prepared. When the fluorescence spectrum was measured, the intensity of fluorescence spectra was increased as expected (Figure 4b). As the equivalents of benzylamine hydrochloride were increased, the intensity of fluorescence spectra was increased more (Figure 4c–4f). These results indicated that benzylamine hydrochloride substituted pyrenemethylamine hydrochloride from the nitrobenzene modified hexahomotrioxacalix[3]arene. The binding constant of benzylamine hydrochloride for nitrobenzene modified hexahomotrioxacalix[3]arene **1** was determined by following a literature method [27, 28], which gave the association constant of benzylamine hydrochloride as 2300 M^{-1}.

Similar measurements for several other amine salts were carried out and their association constants were calculated. The results are summarized in Table 1. As shown in Table 1, the presence of ammonium ion was necessary to bind to the host molecule since benzyl amine did not show any changes in its fluorescence intensity. The result implies that reducing the ability of hydrogen bonding in ammonium ion significantly reduces the binding affinity for host molecule. The importance of hydrogen bonding was also supported from the low binding affinity of benzyltrimethylammonium chloride, which showed its association constant only 340 M^{-1}. Benzylamine hydroperchlorate showed association constant as 3000 M^{-1}, which was similar with benzylamine hydrochloride. The anion of the ammonium ion did not seem to give much effect for the binding of ammonium ion to the host molecule.

Since primary ammonium ions are important not only in chemistry but also on biology, we measured association constants of several amino acids. As the carboxylic acid group in the amino acid could participate in hydrogen bonding with nitrobenzene modified hexahomotrioxacalix[3]arene **1**, the carboxylic group was protected with methyl ester. The results are also summarized in Table 1. From these analyses of primary alkyl ammonium ions and amino acids, any perceptible relationship between the size of alkyl groups and the association constants was not found.

Table 1. Association constants for various ammonium ions and amino acids

Ammonium salts	Association constants (K)
6	0
7	2300 ± 110
8	3000 ± 254
9	340 ± 10
10	2000 ± 352
11	1750 ± 50
12	4400 ± 100
13	400 ± 163
14	3200 ± 326
15	3500 ± 100
16	2600 ± 356
17	2400 ± 300
Phe	1800 ± 453
Leu	1400 ± 240
Ala	1350 ± 84
Pro	550 ± 80

NMR study

The binding stoichiometry of calixarene **1** and pyrenemethylamine hydrochloride or calixarene **1** and benzylamine hydrochloride was studied by Job plot using NMR. From the Job plot, 1 : 1 binding was confirmed.

^{1}H NMR spectra of a mixture of nitrobenzene calixarene **1** and pyrenemethylamine hydrochloride were measured in 7% CD_3OD in $CDCl_3$ as pyrenemethylamine hydrochlor-

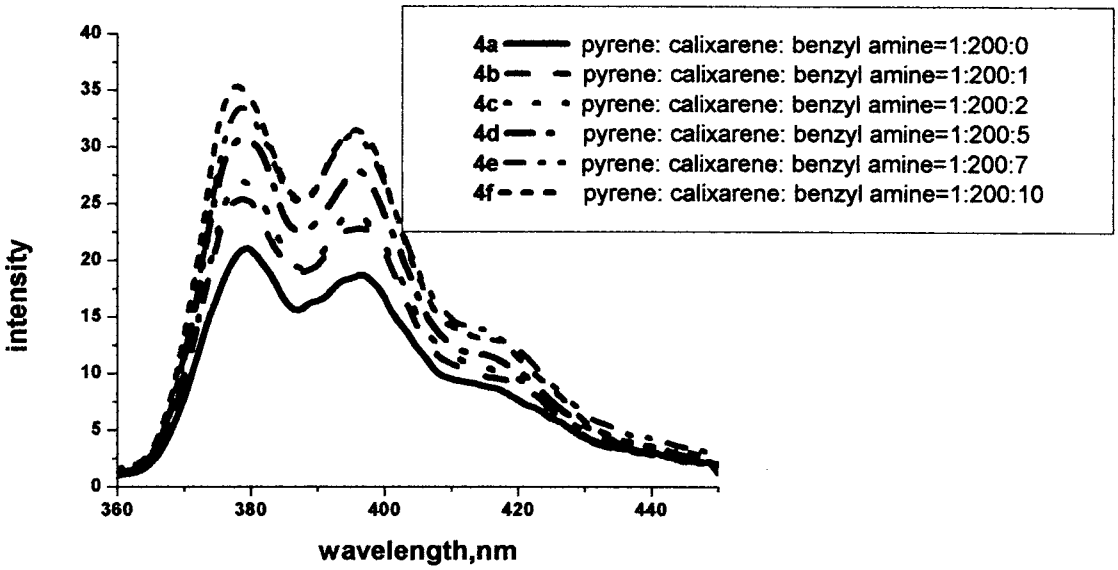
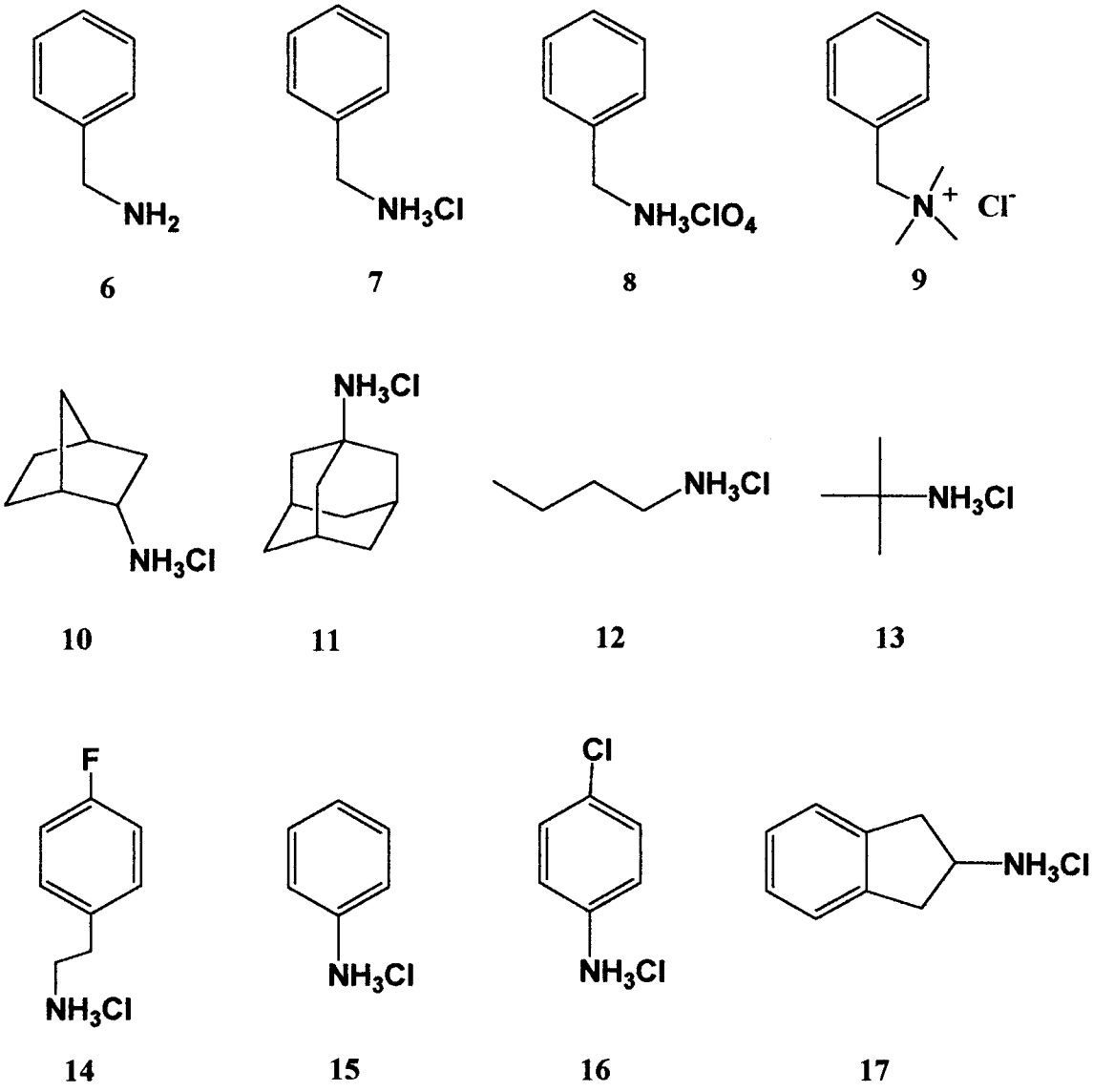


Figure 4. The change of fluorescence spectra in the mixture of pyrenemethylamine hydrochloride and calixarene **1** when benzylamine hydrochloride was added.



Scheme 1.

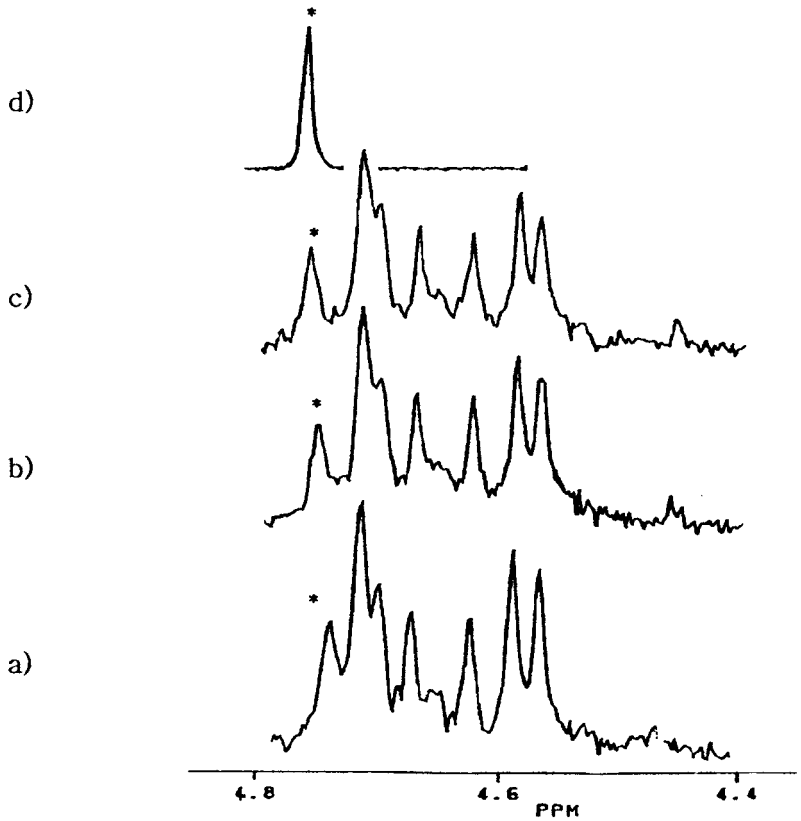


Figure 5. ^{1}H NMR spectra of: (a) the mixture of 1 mM pyrenemethylamine hydrochloride and 4 mM nitrobenzene modified hexahomotrioxacalix[3]arene; (b) when 5 equivalents of benzylamine hydrochloride was added; (c) 15 equivalents of benzylamine hydrochloride was added; (d) pyrenemethylamine hydrochloride only. Benzylic peak of pyrenemethylamine hydrochloride was marked as star.

ide was insoluble in $CDCl_3$ or 1% $CDCl_3$ in CD_3CN. To a 1 mM solution of pyrenemethylamine hydrochloride was added 10 μl of various concentration of nitrobenzene modified calixarene **1**. Three kinds of protons shifted. Benzylic hydrogen peak of pyrenemethylamine hydrochloride shifted to higher magnetic field and two doublets (4.70 ppm and 4.13 ppm) due to OCH_2CO_2 shifted to lower magnetic field. These results imply that the two OCH_2CO_2 groups act as acceptors for RNH_3^+ protons. From the NMR titration study, the binding constant of pyrenemethylamine hydrochloride to nitrobenzene modified calixarene **1** was calculated as 100 M^{-1}. The lower value is due to the presence of CD_3OD in solution.

The substitution of pyrenemethylamine hydrochloride with other ammonium ions also supported by ^{1}H NMR spectra. As ammonium ion salts were insoluble in $CDCl_3$ or 1% $CDCl_3$ in CD_3CN, all NMR measurements were carried out in 7% CD3OD in $CDCl_3$. In the mixture of 1 mM pyrenemethylamine hydrochloride and 9 mM nitrobenzene modified hexahomotrioxacalix[3]arene **1**, the benzylic peak of pyrenemethylamine hydrochloride appeared at 4.72 ppm (Figure 5a). When we added 5 equivalents of benzylamine hydrochloride to this solution, the benzylic peak of pyrenemethylamine hydrochloride moved to downfield (Figure 5b). As we increased the concentration of benzylamine hydrochloride to 15 equivalents, the benzylic peak of pyrenemethylamine hydrochloride moved to the position close to the free pyrenemethylamine hydrochloride, which appeared at 4.77 ppm (Figure 5c and 5d). From the NMR titration of substitution phenomena, the binding constant of benzylamine hydrochloride to nitrobenzene modified calixarene **1** was calculated as 120 M^{-1}.

Direct titration of benzylamine hydrochloride with nitrobenzene modified calixarene **1** employing the same condition with pyrenemethylamine hydrochloride gave the association constant as 190 M^{-1}, which is similar value obtained from exchange method.

The binding constants of pyrenemethylamine hydrochloride and benzylamine hydrochloride obtained from NMR study cannot be directly compared with binding constants obtained from fluorescence study as they were measured in different solvent. However, both data support the binding of pyrenemethylamine hydrochloride to the calixarene **1** and substitution phenomena of other ammonium ion with pyrenemethylamine hydrochloride.

Conclusion

We have shown that a mixture of calixarene **1** and pyrenemethylamine hydrochloride could be a novel binary system, which is able to read binding processes of cationic guests such as primary ammonium ions to the homotrioxacalix[3]arene. The association constants we obtained through this method are in good agreement with association constants which were obtained through intramolecular fluorescence change [29].

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