Nitrobenzene Functionalized Hexahomotrioxacalix[3]arene

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The synthesis and characterization of a nitrobenzene modified hexahomotrioxacalix[3]arene **1** are described. When calixarene **1** bound with ammonium ions carrying fluorescence group, the fluorescence of ammonium ions were effectively quenched and change of emission intensity provided the information of ammonium ion binding events to the calixarene **1**.

Keywords : Calixarene, Ammonium ion, Fluorescence.

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 guest molecules. They form complexes with various cations and organic molecules selectively. Therefore, their usability as host and sensor has been a matter of intensive investigations. For sensor applications, the generation of optical signal as a direct result of the complexation between host and guest is desirable. A series of chromogenic calixarene derivatives has been synthesized which exhibit a change in their UV/VIS or fluorescence spectra in the presence of metal ions or organic guest molecules.[1] Shinkai *et al.* reported functionalized hexahomotrioxacalix[3]arenes and calix[4]arenes which could detect ammonium ions or metal ions by means of fluorescence change in the pyrene functionalized calixarenes. They made use of intramolecular excimer fluorescence change of pyrene[2] and disturbance of quenching efficiency between pyrene and nitrobenzene.[3,4] As ammonium ions play important roles in both chemistry and biology,[5,6] we designed nitrobenzene modified calixarene **1** which can detect ammonium ions carrying fluorescence group. When ammonium ions with fluorescence group bind the calixarene **1**, fluorescence of the ammonium ions would be effectively quenched and change of emission intensity would provide the information of ammonium ion binding events.

Preparation of the calixarene **1** is summarized in Figure 1. 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 compound **3** in acidic condition gave the hexahomotrioxacalix[3]arene **4** in 50% yield.[7] 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 40% yield.[8]

The fluorescence change of ammonium ions with fluorescence group was monitored in the mixture of acetonitrile and chloroform (99 : 1). The fluorescence intensity of 3 μM solution of ammonium ions changed remarkably as the concentration of nitrobenzene modified calixarene **1** was increased (Figure 2). In case of pyrenemethylamine hydrochloride, the excitation wavelength was 343 nm. In Figure 3, a Benesi-Hildebrand plot[9] of the mixture of pyrenemethylamine hydrochloride and nitrobenzene modified calixarene **1** by use of change in the 378 nm fluorescence intensity is shown. Equilibrium constant K was calculated from the slope. Similar measurements for several amines with fluorescence probe were carried out and their equilibrium constants were calculated by Benesi-Hildebrand plots. The results are summarized in Figure 4.

As shown in Figure 4, the presence of ammonium ion was necessary to bind to the host molecule since pyrenemethylamine did not show any changes in its fluorescence intensity. The results implies that reducing hydrogen bonding ability of ammonium ion significantly reduces the binding affinity of ammonium ion for host molecule. The importance of hydrogen bonding was also supported from the low binding affinity of pyrenetetramethylamine hydrochloride. 1-Aminopyrene hydrochloride, 2-aminofluorene hydrochloride,[10] 2-aminonaphthalene[11] and 2-aminoanthracene[12] - aromatic ammonium ions- showed higher affinity than pyrenemethylamine hydrochloride and pyrenemethylamine perchlorate-aliphatic ammonium ions. Aromatic ammonium ions are more acidic than aliphatic ammonium ion. Therefore aromatic ammonium ions can make stronger hydrogen bonds than aliphatic ammonium ions. Pyrenemethylamine hydroperchlorate showed similar binding constants with pyrenemethylamine hydrochloride. The anion of the ammonium ion did not seem to give much effect for the binding of ammonium ion to the host molecule.

^{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 hydrochloride was insoluble in $CDCl_3$. To 1 mM solution of pyrenemethylamine hydrochloride was added nitrobenzene modified calixarene. Three kinds of protons shifted. Benzylic hydogen peak of pyrenemethylamine hydrochloride shifted to higher magnetic field (Figure 5) and two doublets (4.70 ppm and 4.13 ppm) due to OCH_2CO_2 shifted to lower 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** calculated as 950 M^{-1}. The lower value is due to the presence of CD_3OD in solution.

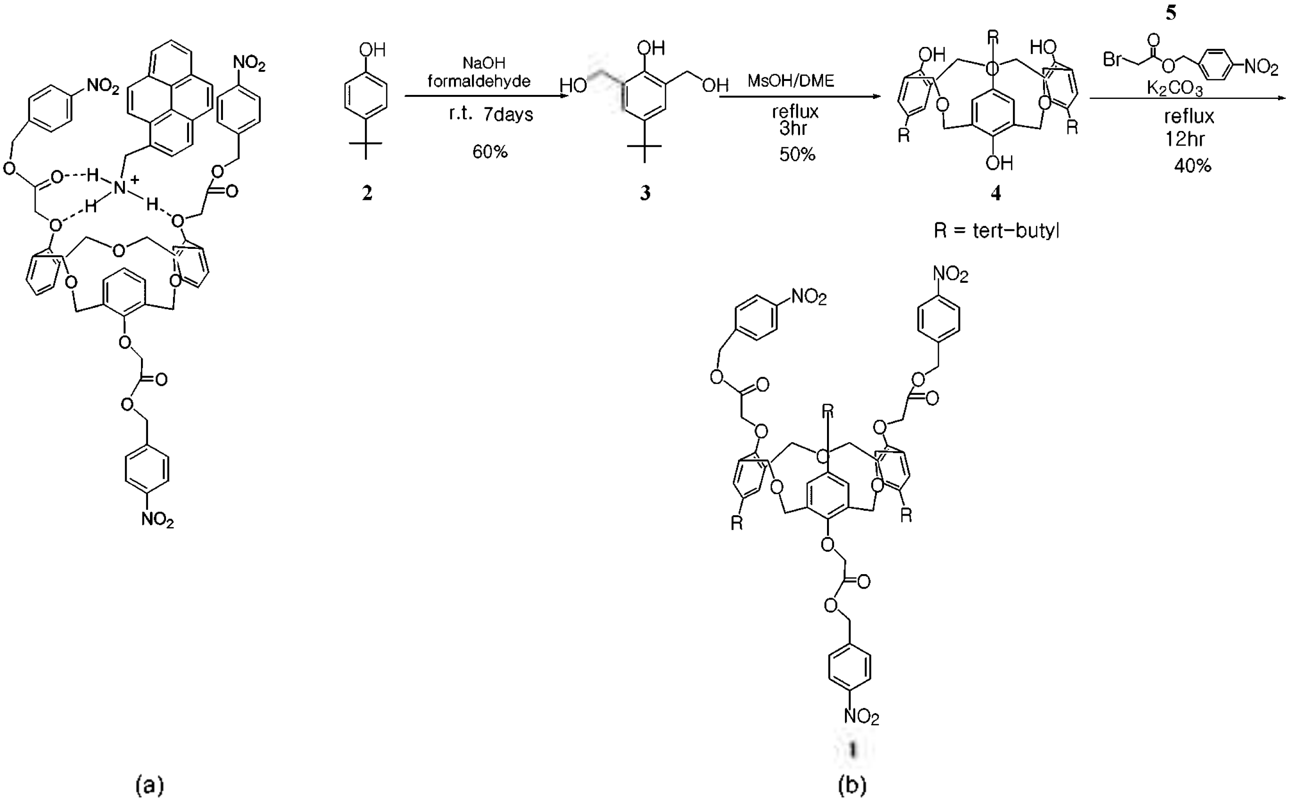

Figure 1. (a) The proposed binding mode of pyrenemethylamine hydrochloride and nitrobenzene modified calixarene **1**. The tert-butyl group was omitted for clarity. (b) The synthetic scheme for the preparation of **1**.

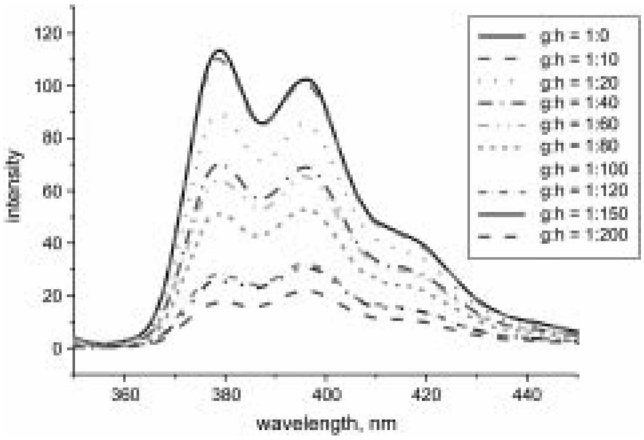

Figure 2. Fluorescence change of a mixture of pyrenemethylamine hydrochloride and calixarene **1**.

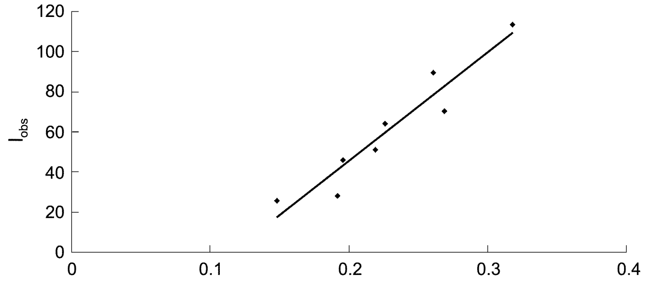

Figure 3. Benesi-Hildebrand plot for a mixture of pyrenemethylamine hydrochloride and calixarene **1**.

The complex between pyrenemethylamine hydrochloride and nitrobenzene modified calixarene **1** could be a good binary system to measure the binding constants of various ammonium ions. We are currently investigating the possibility.

Experimental Section

4-*t*-Butyl-2,6-bis(hydroxymethyl) phenol (3). To a solution of 1 g of *p*-*t*-butylphenol in 15 mL of formalin solution was added 1 g of NaOH. The mixture was stirred for 7 days at room temperature. Then the PH of solution was adjusted to 4.0 by slow addition of acetic acid. The reaction mixture was poured into 50 mL of water and the mixture was extracted with 100 mL of methylene chloride three times. After the organic layer was dried with $MgSO_4$, Evaporation of solvent and column chromatography (methylene chloride : hexane – 2 : 5) on the silicagel gave 700 mg (60%) of the compound **3** ^1H NMR (200 MHz, $CDCl_3$) 7.0 (2H, s, benzene H) 4.6 (4H, s, CH_2OH) 1.2 (9H, s, *tert*-butyl).

Hexahomotrioxacalix[3]arene (4). The solution of 1.0 g of compound **3**, 0.72 mL of methanesulfonic acid and 4 g of sodium sulfate in 100 mL of ethylene glycol dimethyl ether was refluxed for 3 hrs. After the reaction mixture was cooled

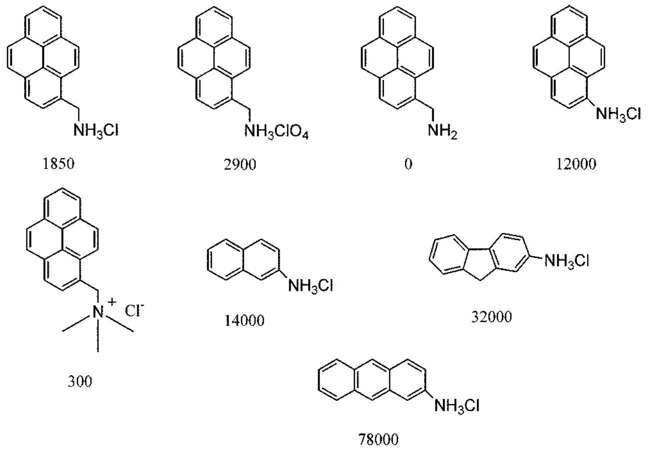

Figure 4. Equilibrium constants (K) of ammonium ions with calixarene **1**.

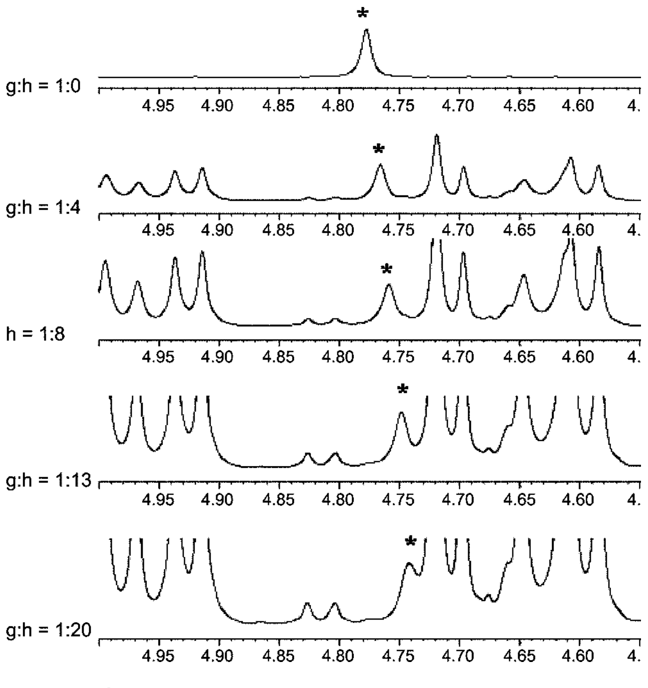

Figure 5. ^{1}H NMR spectrum of the mixture of calixarene **1** and pyrenemethylamine hydrochloride. The benzylic hydrogen peak shifts to higher field as calixarene **1** is added to the solution of pyrenemethylamine hydrochloride and the peak is marked as *.

down. 50 mL of saturated sodium bicarbonate was added to quench the acid and the solvent was evaporated *in vacuo*. 100 mL of methylene chloride was added to the residue and the solution was extracted with 100 mL of water. After the organic layer was dried with $MgSO_4$, evaporation of solvent and column chromatography (Ethyl acetate : hexane = 1 : 15) on the silicagel gave 450 mg (50%) of the compound **4**. ^{1}H NMR (200 MHz, $CDCl_3$) 8.56 (2H, s, OH) 7.11 (6H, s, benzene H) 4.71 (12H, s, CH_2OCH_2) 1.22 (27H, s, tert-butyl).

Nitrobenzene functionalized hexahomotrioxacalix[3]arene (1). To a stirred solution of 100 mg of the compound **4** in 3 mL of acetone, 84 mg of K_2CO_3 and 165 mg of 2-bromo-*p*-nitrobenzylacetate **5** was added and refluxed for 3 hrs. After 50 mL of methylene chloride was poured into the reaction mixture, it was washed with 50 mL of water 2 times. After the organic layer was dried with $MgSO_4$, evaporation of solvent and column chromatography (ethyl acetate : hexane = 1 : 3) on the silicagel gave 100 mg (40%) of the compound 1. ^{1}H NMR (200 MHz, $CDCl_3$) 8.25 (6H, m, nitrobenzene H) 7.51 (6H, m, nitrobenzene H) 7.23 (2H, s, ArH) 7.20 (2H, d, J = 2.40, ArH) 7.00 (2H, d, J = 2.4, ArH) 5.16 (8H, m) 4.75 (6H, m) 4.30 (8H, m) 3.16 (2H, s, OCH_2CO) 1.28 (9H, s, tert-butyl) 1.05 (18H, s, tert-butyl).

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10. excitation wavelength was 335 nm and emission wavelength was 368 nm.
11. excitation wavelength was 239 nm and emisssion wavelength was 396 nm.
12. excitation wavelength was 263 nm and emisssion wavelength was 483 nm.