

## Discrimination of Primary Alkylamines Using Azobenzene-appended Calix[4]arene Derivatives in Solution and in the Solid State

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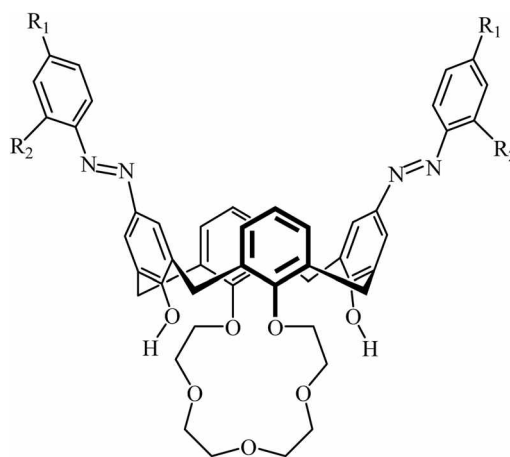
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Received August 27, 2007

**Key Words :** Amines, Calix[4]arene, Chemosensor, Discrimination, Silica nanotubes

A pressing challenge in the design of chemosensors is the discrimination among different types of biomolecules. A simple monitoring system that distinguishes among the various molecular species of amines, amino acids, or proteins would be extremely useful in environmental and biological technologies.<sup>1</sup> The Hinsberg test, based on an organic reaction that converts amines to sulfonamides, has been used over the past century for selective characterization of 1°, 2°, and 3° amines.<sup>2,3</sup> For recognition of amines, macrocyclic polyethers have been reported to bind 1° ammonium ions by anchoring the R-NH<sub>3</sub><sup>+</sup> group into their circular cavities *via* three H bonds (N-H...O).<sup>4</sup> However, the selective recognition of specific alkylamines has not been achieved due to the lack of discrimination.

With recent advances in nanomaterials and nanotechnologies, new methods have emerged for the design of optical sensors and biosensors, and for development of highly sensitive solid-state sensors.<sup>5-7</sup> Recently, the porous silicates SBA-15 and MCM-41 have attracted significant interest for the development of optical chemical sensors.<sup>8-12</sup> Although these materials hold great promise in the area of sensor technologies, the need for nano-optical sensors that rapidly detect environmentally important toxic species at low concentrations has not been met. Particularly, receptor-immobilized nanotubes have some important advantages as solid chemosensors in heterogeneous solid-liquid phases. Nanotubes, as opposed to spherical nanostructures, have large surface areas and well-defined pores and would be useful as selective and efficient adsorbents for specific guest molecules found in environmental pollutants. Nanotubes can also be easily isolated from pollutants by simple filtration. Additionally, the single nanotube is advantageous in the development of a nano-scaled sensing device. Based on these ideas, we synthesized the azo-coupled calix[4]arene receptors **1** and **2**, and then grafted receptor **2** onto the surface of silica nanotubes (SNT) as a new approach for the development of nanomaterial chemosensors. Herein, we report the appearance of selective color changes that occur upon the addition of primary amines to receptor **1** in solution and to solid SNTs that have receptor **2** incorporated into their structure (SNT-2).

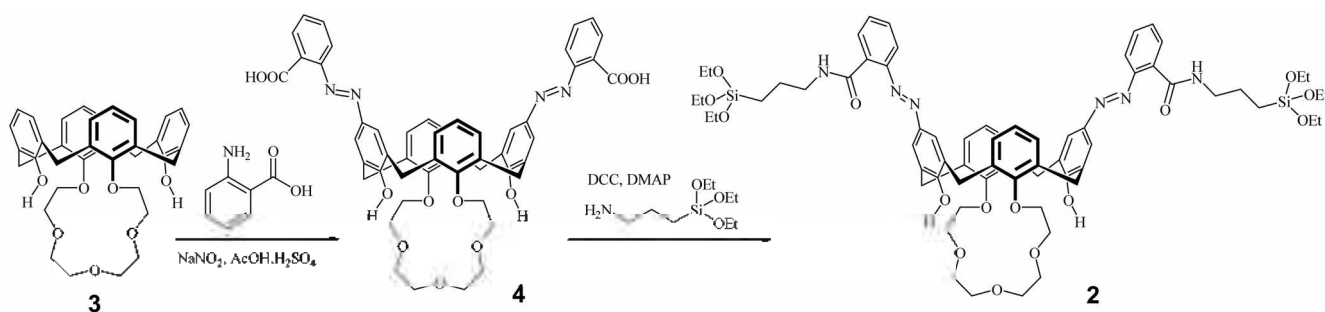


**1.** R<sub>1</sub> = NO<sub>2</sub>, R<sub>2</sub> = H  
**2.** R<sub>1</sub> = H, R<sub>2</sub> = CONH(CH<sub>2</sub>)<sub>3</sub>Si(OEt)<sub>3</sub>

The synthesis was initiated with calix[4]arene as a starting material, which was reacted with butaethyleneglycol ditosylate in the presence of Cs<sub>2</sub>CO<sub>3</sub>, followed by the azo-coupling reaction to yield **1**.<sup>13</sup> From <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, **1** was confirmed to have a cone conformation. Compound **2** was synthesized in a manner similar to that for **1** (Scheme 1). The triethoxyl silane group was introduced as a precursor to the sol-gel reaction onto the SNT surface.

The binding behaviors of **1** toward various amines were investigated by UV/Vis spectroscopy. Association constants<sup>14,15</sup> were also determined from the UV-Vis band changes upon addition of 1°, 2°, and 3° amines to chloroform solutions of **1** (Table S1). Compound **1** responded remarkably to the 1° amines, exhibiting a distinct change in the absorption band with maximum at 401 nm (corresponding to orange color) to an absorption band with λ<sub>max</sub> at 567 nm (corresponding to blue color) with an isosbestic point at 450 nm (Figures 1, 2 and S1-6). Notably, a single isosbestic point appears in the spectra of **1** with amines in CHCl<sub>3</sub>, supporting the idea that the stoichiometry for the complex of **1** with amine is 1:1 (Figure S1). The log K<sub>a</sub> of **1** for 1° amines is ~4.07-4.57, considerably larger than the values for the 2° and 3° amines.

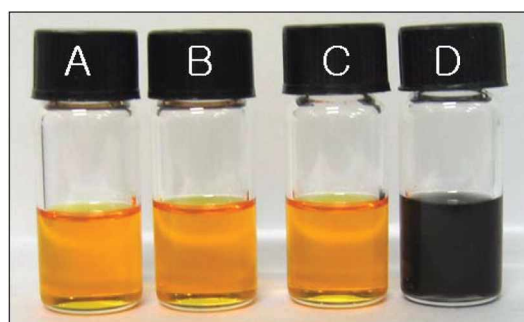
Compound **1**, however, did not respond to either 2° or 3°



**Scheme 1.** Synthetic route of azo-coupled calix[4]arene **2**.

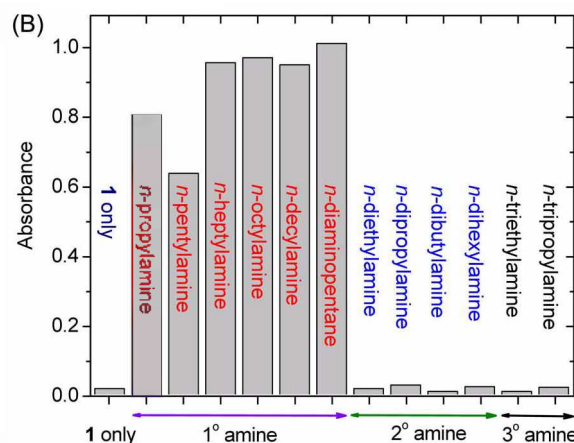
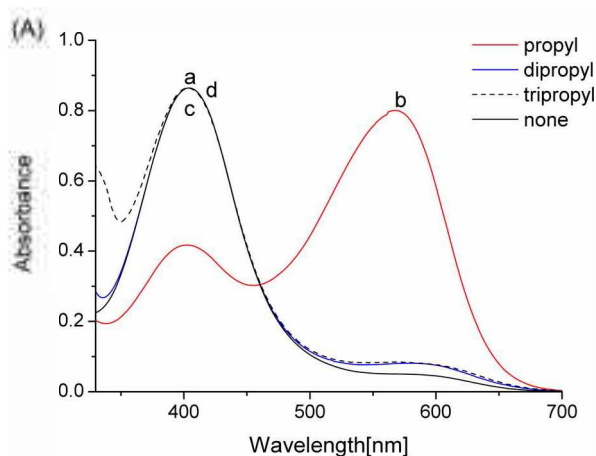
amines, producing no color changes (Figures 1B, 2, and S7). The bathochromic shifts of **1** upon the addition of 1° amine are believed to arise from the phenolic oxygen atoms of the calix[4]arene, which are highly polarized when this ligand forms a complex with the 1° amine. This polarization results in greater stabilization of the excited states relative to the ground states.<sup>16</sup> The proton transfer from the phenolic OH to the nitrogen atom of the 1° amine may provide a stable H-bonding pattern between three hydrogen atoms in the ammonium salt and the oxygen atoms of crown-5.

As a first approach toward the preparation of a selective chromogenic nanomaterial system, **2** was anchored onto the **SNT**. Then, incorporation of **2** into the **SNT** was carried out in toluene under reflux conditions for 24 h. After cooling to room temperature, the solid yellow product was isolated by filtration, washed with THF, and then dried. The purified

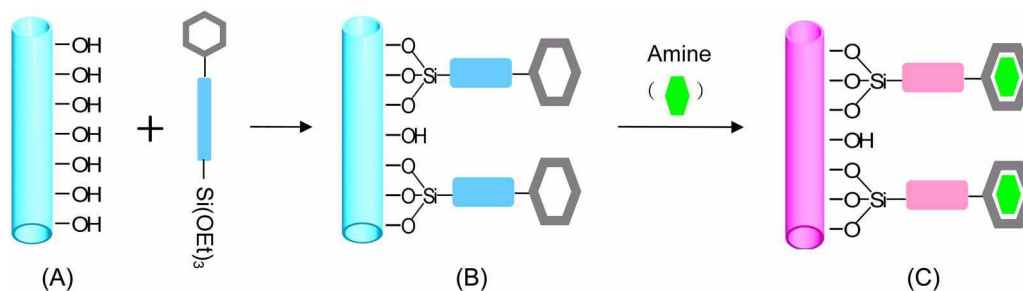


**Figure 2.** Color changes of **1** (0.005 mM) upon the addition of 10,000 equiv of 1°, 2°, and 3° amines in CHCl<sub>3</sub>: (A) **1** only; (B) **1** + 3° amine; (C) **1** + 2° amine; (D) **1** + 1° amine.

product was characterized by transmission electron microscopy (TEM), and UV-Vis spectroscopy.



**Figure 1.** (A) Spectral changes of (a) **1** (0.01 mM) upon the addition of (b) *n*-propylamine, (c) dipropylamine, and (d) tripropylamine (10,000 equiv) in CHCl<sub>3</sub>. (B) Absorbance of **1** (0.01 mM) at 567 nm upon the addition of various amines (10,000 equiv) in CHCl<sub>3</sub>.



**Scheme 2.** Synthetic route of **SNT-2**: (A) **SNT**, (B) immobilization of receptor **2** by sol-gel reaction, and (C) discrimination of 1° amine using **SNT-2**.

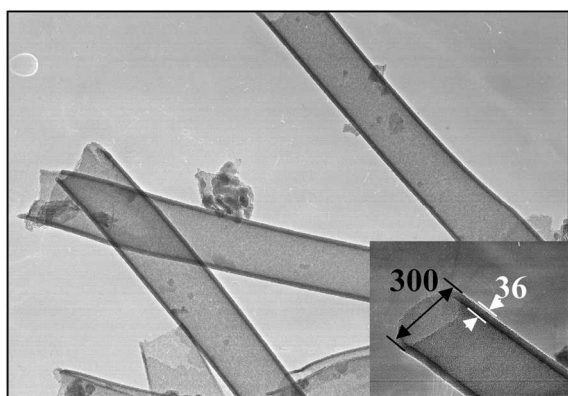


Figure 3. TEM image of SNT-2.

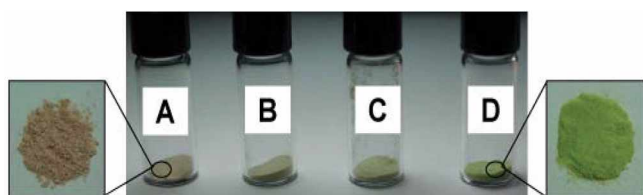


Figure 4. Color changes of SNT-2 upon the addition of 1°, 2°, and 3° amines (10,000 equiv): (A) SNT-2 only, (B) SNT-2 + 3° amine, (C) SNT-2 + 2° amine, and (D) SNT-2 + 1° amine.

The silica product was a well defined nanotube, *ca.* 300 nm in diameter and several micrometers in length (Figure 3). The TEM image of the colored silica displayed a hollow structure with uniform size dimensions, *i.e.*, inner diameter of *ca.* 228 nm and wall thickness of 36 nm, indicating that **2** had been successfully immobilized onto the surface of SNT by post sol-gel reaction.

The coloration response of SNT-2 was examined by addition of amines to the  $\text{CHCl}_3$  suspension, followed by the isolation of the amine-loaded SNT-2 (Figure 4). Interestingly, upon the addition of 1° amine in suspension, the color of the SNT-2 changed from slight pink to green (Figure 4). As observed for **1** in solution, only 1° amines were selectively bound to the calix[4]arene moiety attached onto the SNT surface. These findings confirm that SNT-2 can also be useful as a colorimetric sensing material for selective detection of 1° amine in the presence of other amines. To our best understanding, this is a rare example of chromogenic sensing for a 1° amine by functional inorganic nanotubes. However, this color change observed in the solid state differs slightly from that observed in the solution state. This observation may be attributed to the acidic properties exhibited by the SNT from unreacted  $-\text{SiOH}$  groups.

To further examine the coloration of SNT-2 with 1° amine, we observed the UV/Vis spectrum of the solid SNT-2 in complex with 1° amine. Upon the addition of 1° amine, the  $\lambda_{\text{max}}$  of the UV/Vis spectrum of SNT-2 shifted from 520 nm to 690 nm (Figure 5), giving clear evidence that the 1° amine was bound to the receptor **2** attached onto the surface of the SNT.

Additionally, we also characterized **2** by IR spectroscopy in the absence and the presence of amines (Figure S8). The

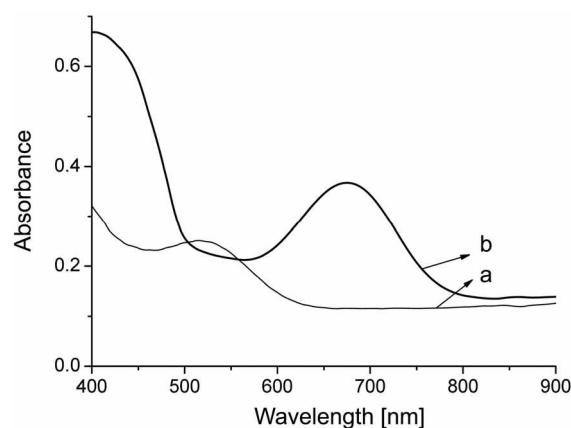


Figure 5. Solid UV/Vis spectra of SNT-2 in the (a) absence and (b) the presence of 1° amine (10,000 equiv).

$-\text{C}=\text{O}$  peak ( $1639\text{ cm}^{-1}$ ) in free **2** shifted to  $1634\text{ cm}^{-1}$  upon complexation with 1° amine (Figure S8). The resulting shift to a smaller wavenumber is attributed to the binding of 1° amine to the crown ether group. On the other hand, no significant changes were observed in the presence of either 2° or 3° amines.

In summary, a combination of indicators **1** and SNT-2 provides a key to discrimination of 1° amine. This simple and straightforward qualitative analysis with diazo-calix[4]-crown **1** and SNT-2 parallels the classical Hinsberg test and can be considered as an innovative tool in discriminating 1° amines from 2° and 3° amines. Furthermore, these findings may lead to development of new types of tailor-made, selective molecular sensing systems built through fine control of the properties of both the molecular receptor and the solid support.

## Experimental Section

**Apparatus for spectroscopy measurement:**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were measured by a Bruker ARX 300 apparatus. IR spectra (KBr pellets) were obtained using a Shimadzu FT-IR 8400S, and MS spectrum was obtained by a JEOL JMS-700 mass spectrometer. The optical absorption spectra of the samples were obtained in the room temperature and in the wavelength region of 250–750 nm using an UV-VIS spectrophotometer (Jasco V-530).

**Fabrication of SNT-2.** Compound **2** (100 mg) was dissolved in toluene (10 mL). The silica nano tube (SNT, 100 mg) was added as solid. The suspension of SNT was stirred in reflux condition for 24 h. Then, the collected solid was washed copiously with toluene 50 mL to rinse away any surplus **2** and then dried under vacuum.

**Solid UV-Vis changes and color change of SNT-2 upon addition of amine.** The SNT-2 (5 mg) was added to the amine solution (10,000 eq). Then, solid UV-VIS change of SNT-2 were measured by UV-VIS-NIR spectrophotometer (Hitachi U-3501).

**Compound 1.** Compound **1** was prepared from the adaptation of the reported procedures.<sup>17</sup>

**Compound 2.** A solution of **4** (0.86 mg, 0.98 mmol) was dissolved in THF (15 mL) and the solution cooled to 0 °C. 3-Aminopropyltriethoxysilane (0.65 g, 2.93 mmol), DCC (0.60 g, 2.93 mmol), and DMAP (0.5 mL) were then added successively. The reaction mixture was refluxed for 20 h. After removing the solvent, the residue was taken up in CHCl<sub>3</sub> and washed with water. The organic solvent was evaporated to dryness. The crude product was purified by flash column chromatography on aluminium oxide eluting with dichloromethane:ethyl acetate:*n*-Hex (6:3:1) as eluent (*R<sub>f</sub>* = 0.53) provided **2** as a yellow crystalline solid in 88% yield. m.p. 280 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.06 (s, 4H, ArH), 7.75 (s, 2H, OH), 7.00–6.62 (m, 14H, ArH), 4.42 (d, 4H, -CH<sub>2</sub>-, *J* = 13.4 Hz), 4.09 (m, 8H, -OCH<sub>2</sub>CH<sub>2</sub>O-), 3.85 (m, 12H, -OCH<sub>2</sub>CH<sub>2</sub>O-), 3.54 (m, 6H, -OCH<sub>2</sub>CH<sub>2</sub>O-). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 169.3 (-CO) 154.1, 153.8, 152.4, 146.2, 134.3, 130.3, 130.2, 128.9, 128.5, 128.3, 125.7, 122.9, 119.2 (ArH), 77.2, 71.5, 70.6, 58.5, 45.3, 31.5, 24.8, 18.5, 7.5 (-CH<sub>2</sub>- or -CH<sub>3</sub>). IR (KBr pellet, cm<sup>-1</sup>): 3418, 3115, 2945, 2869, 1649, 1569, 1089, 807. MS (FAB-MS) *m/z* 1284.5 (*M* + 1, calcd 1284.6).

**Compound 3.** To a stirred solution of 5.0 g (11.3 mmol) of calix[4]arene and 1.6 g (11.3 mmol) of K<sub>2</sub>CO<sub>3</sub> in 150 mL of CH<sub>3</sub>CN, 11.9 g (23.7 mmol) of tetraethylene glycol ditosylate was added and the reaction mixture was left to stir at room temperature for 20 hours. The solvent was removed and the residue was treated with CHCl<sub>3</sub> (100 mL) and water (100 mL). The organic layer was separated and the solvent was removed. The crude products were purified by column chromatography (eluent, CHCl<sub>3</sub>:*n*-hexane = 1:1) to give the product **3** (4.81 g, 74%), m.p. 253 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.75 (s, 2H, OH), 7.08–6.64 (m, 12H, ArH), 4.44 and 3.33 (pair of d, 8H, ArCH<sub>2</sub>Ar, *J* = 16.2 Hz), 4.08 (tt, 8H, -OCH<sub>2</sub>CH<sub>2</sub>O-), 3.94 (t, 4H, ArOCH<sub>2</sub>CH<sub>2</sub>-), 3.84 (t, 4H, ArOCH<sub>2</sub>CH<sub>2</sub>-). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 153.8, 152.4, 133.5, 130.3, 129.4, 128.9, 128.5, 128.3, 125.7, 119.2, 77.2, 71.5, 70.6, 31.5. IR (KBr pellet, cm<sup>-1</sup>): 3490, 2933, 1560, 1174, 787.

**Compound 4.** The diazonium salt of 4-nitroaniline (1.4 g, 10.2 mmol) was prepared by adding an aqueous solution of sodium nitrite (0.7 g, 10.2 mmol) dropwise into a homogeneous mixture of 2.7 mL of sulfuric acid and 48 mL of glacial acetic acid. The mixture was stirred at 0 °C for 5 min. The diazonium salt solution was added dropwise into a solution of **3** (2.0 g, 3.4 mmol) in 50 mL of DMF at 0 °C. The solution was stirred for an additional 12 h at 0 °C. CHCl<sub>3</sub> (50 mL) and water (50 mL) were added, and the organic layer was separated and dried over MgSO<sub>4</sub>. The organic solvent was evaporated to dryness. The crude product was purified by flash column chromatography on aluminium oxide eluting with dichloromethane:MeOH (100:1) as eluent (*R<sub>f</sub>* = 0.64) provided **4** as a red crystalline solid in 34% yield. m.p. 260 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub> + DMSO-*d*<sub>6</sub>) δ 8.06 (s, 4H, ArH), 7.75 (s, 2H, OH), 7.00–6.62 (m, 14H, ArH), 4.44 (d, 4H, -CH<sub>2</sub>-, *J* = 13.4 Hz), 4.09 (m, 8H, -OCH<sub>2</sub>CH<sub>2</sub>O-), 3.85 (m, 12H, -OCH<sub>2</sub>CH<sub>2</sub>O-), 3.54 (m, 6H, -OCH<sub>2</sub>CH<sub>2</sub>O-). <sup>13</sup>C NMR (CDCl<sub>3</sub>+DMSO-*d*<sub>6</sub>) δ 169.3 (-CO) 154.1, 153.8, 152.4, 146.2, 134.3, 130.3, 130.2, 128.9, 128.5,

128.3, 125.7, 122.9, 119.2 (ArH), 77.2, 71.5, 70.6, 31.5. IR (KBr pellet, cm<sup>-1</sup>): 3447, 2920, 1750, 1511, 1219, 771.

**Acknowledgment.** The first two authors contributed approximately equally to this work. Also, this work was supported by the KOSEF (M20702000050-07B-0200-05000) and the Korea Research Foundation (KRF-2005-005-J09703).

**Supporting Information Available:** Association constants and absorption spectra of **1** with amines and FT-IR spectra of **SNT-2** with amines are available on request from the correspondence author. E-mail: jonghwa@gnu.ac.kr. Fax: +82-55-758-6027.

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