CHEMISTRY LETTERS, pp. 125-128, 1994.

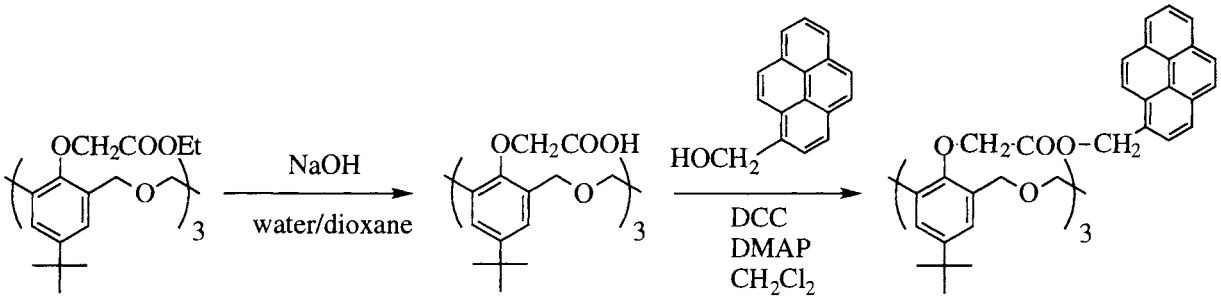
Novel Fluorometric Sensing of Ammonium Ions by Pyrene Functionalized Homotrioxacalix[3]arenes

Michinori TAKESHITA and Seiji SHINKAI*
Chemirecognics Project, ERATO, Research Development Corporation of Japan,
Aikawa 2432-3, Kurume, Fukuoka 830

Pyrene functionalized homotrioxacalix[3]arenes selectively recognize primary ammonium ions detected by an intramolecular excimer fluorescence change of *partial cone* conformer. This is a novel sensing system for primary ammonium ions.

The development of ammonium ion recognition systems has been of much concern because ammonium ions play important roles in both chemistry and biology.[1, 2] For example, Lehn *et al.* reported that C_3 symmetry crown ethers bearing ester groups form very stable complexes with primary ammonium ions.[2a] Kaneda *et al.* reported that crown ethers with a functionalized dye moiety are useful as a sensory system for recognition of amines by a color change.[3] More recently, it was reported that calix[n]arene derivatives with C_3 or C_6 symmetry can selectively bind with ammonium ions.[4, 5] To the best of our knowledge, the detection of the binding processes was carried out by NMR spectroscopy or indirectly by two-phase solvent-extraction which required high concentration solutions of host molecules and guest ammonium ions. Meanwhile, detections of alkali metal cations or organic molecules by use of fluorescence changes in intramolecular excimer of the pyrene or anthracene functionalized calix[4]arenes[6] and cyclodextrins[7] were reported. This method has a great advantage in detecting the complexes at a very low concentration. Here, we report the detection of ammonium ion by means of fluorescence change in the pyrene functionalized homotrioxacalix[3]arenes which are expected to enable us to quantitatively microanalyze ammonium ions in solutions.

Preparation of the title compounds is summarized in Scheme 1. The starting materials **1** and **2** were reported previously.[4] Esterification of tricarboxylic acids **3** and **4** with 1-pyrenylmethanol[8] afforded the desired esters **5** and **6**.[9]



1: *cone*
2: *partial cone*
3: *cone*
4: *partial cone*
5: *cone*
6: *partial cone*

Scheme 1.

As a fluorescence detection system, *partial cone* **6** was more useful than *cone* **5**: the intensity of the intra-

molecular excimer fluorescence (in acetonitrile/chloroform (99:1), excitation 343 nm) of **5** did not change remarkably upon addition of *n*-hexNH$_3$ClO$_4$ (Fig. 1), whereas the fluorescence of intensity of **6** changed remarkably as the guest concentration was increased (Fig. 2).

In Figure 3, a Benesi-Hildebrand plot[10] of the mixture of **6** and *n*-hexNH$_3$ClO$_4$ by use of change in the 480 nm fluorescence intensity (excitation: 343 nm) in MeCN/CHCl$_3$ (99:1) is shown. Since the plot assuming the formation of a 1:1 complex is linear, the stoichiometry of the complex should be 1:1. Equilibrium constant K was calculated from the slope. Similar measurements for several primary and secondary ammonium ions, amines and alkali metal cations were carried out and their equilibrium constants were calculated by Benesi-Hildebrand plots. The results are summarized in Table 1.

As shown in Table 1, equilibrium constants K for primary ammonium ions are much greater than those for secondary ammonium ions and alkali metal cations. However, we could not find any significant relationship between K and the size of the alkyl group. The perceptible fluorescence change was not observed for free amines such as 1-adamantamine. The K values of **5**-primary ammonium ions were about 4 to 5 times larger than those of **6**-series. The result implies that loss of one of the three hydrogen bondings reduces the binding ability of host molecule. There was no change in fluorescence of **6** with addition of Hex$_2$NH$_2$ClO$_4$ in MeCN/CHCl$_3$ (99:1), but only in less polar solvent such as

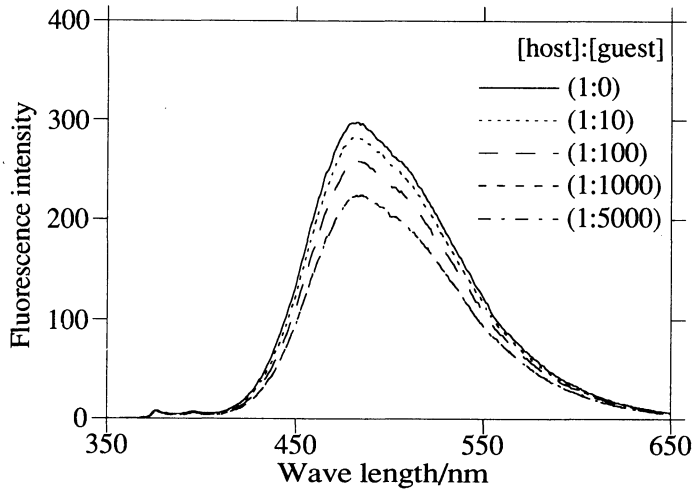


Fig. 1. Fluorescence change of a mixture of **5** and *n*-HexNH$_3$ClO$_4$.

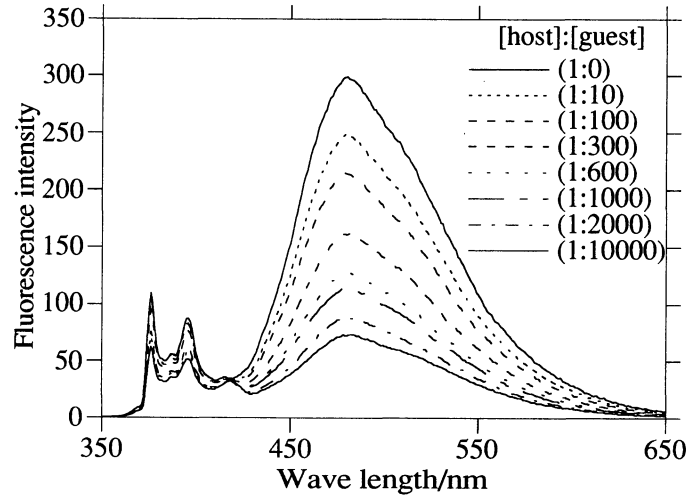


Fig. 2. Fluorescence change of a mixture of **6** and *n*-HexNH$_3$ClO$_4$.

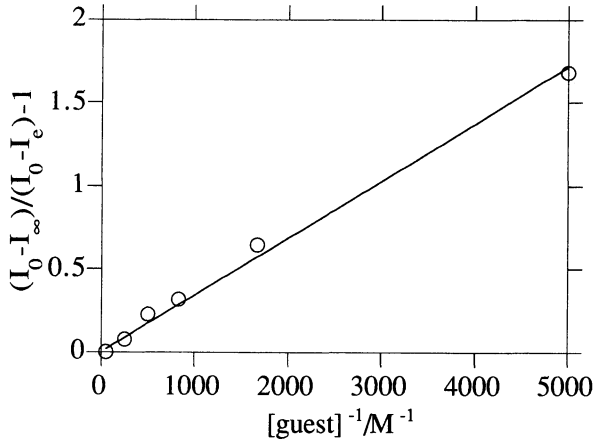


Fig. 3. Benesi-Hildebrand plot for a mixture of **6** and *n*-HexNH$_3$ClO$_4$.

Table 1. Equilibrium constants of **5** and **6** for ammonium ions calculated from fluorescence intensities at 480nm[a)]

Host	Ammonium ion	Equilibrium const (K)[b)]
5	*n*-$HexNH_3ClO_4$	12000
6	*n*-$HexNH_3ClO_4$	2920
6	*n*-$BuNH_3Cl$	1790
5	*tert*-$BuNH_3ClO_4$	3600
6	*tert*-$BuNH_3ClO_4$	750
6	1-AdamantylNH_3ClO_4	1800
6	1-Adamantamine	≈0[c)]
6	L-PheOMe$HClO_4$[d)]	3950
6	*n*-$Hex_2NH_2ClO_4$	≈0,[c)] (1050)[e)]
6	$NaClO_4$	25
6	$KClO_4$	330

a) [host]=2×10^{-6} M, excitation: 343 nm, Benesi-Hildebrand equation was used. b) in MeCN/$CHCl_3$ (99:1). c) No change was observed. d) L-phenylalanine methyl ester hydroperchlorate. e) in $CHCl_3$(100).

$CHCl_3$ (100) the fluorescence change was observed. The low K value of dihexylammonium ion is ascribed to loss of one hydrogen-bond or steric hindrance of dihexyl moiety. On the other hand, L-phenylalanine methyl ester hydroperchlorate was also detectable in this system. These results mean that **6** can selectively recognize primary ammonium ions and is useful for quantitative analysis of primary ammonium ions in the presence of secondary ammonium ions and alkali metal cations.

To determine the binding site of **6**, ^1H NMR and ^{13}C NMR spectra of a mixture of **6** and *tert*-$BuNH_3ClO_4$ were measured in $CDCl_3$. Three kinds of carbons (each 2C) and two kinds of protons (each 2H, doublet) shifted to lower magnetic field remarkably as shown in Fig. 4. From these results, we can specify the binding site as such that two oxygens in two OCH_2CO groups act as acceptors for RNH_3^+ protons. It was difficult to specify which oxygen acts as the third acceptor from NMR spectral studies, but we presume (from CPK model buildings) that one of the ring oxygens ($ArCH_2O$) contributes to the binding of the ammonium guest.

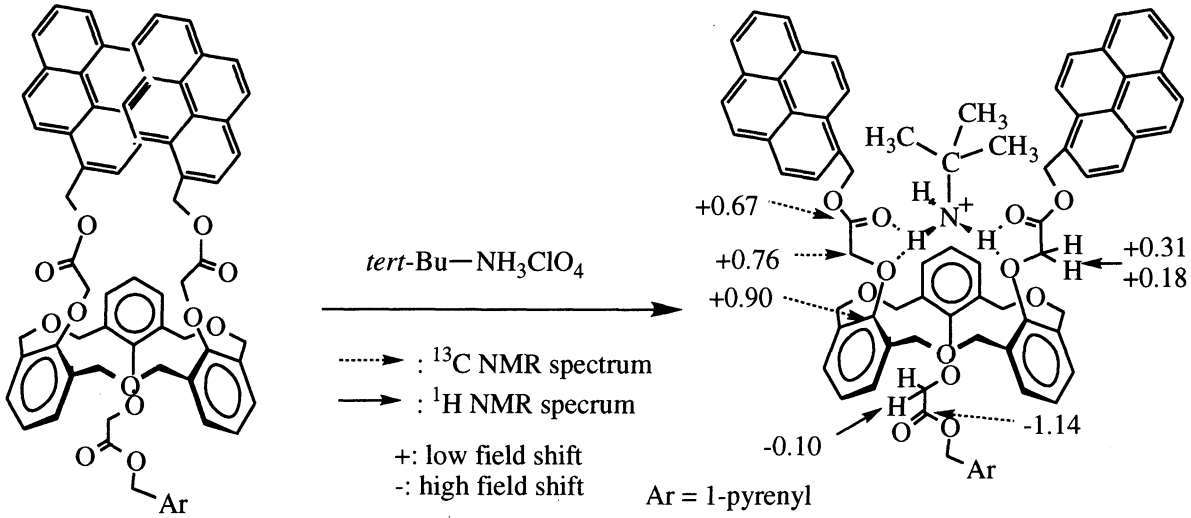


Fig. 4. NMR spectral change of **6** after formation of a complex in $CDCl_3$ at 295 K (*tert*-Butyl residues are omitted for clarity).

Partial cone **6** with *pseudo*-C_3 symmetry has two pyrenes at the same side of the calix[3]arene ring, so that the excimer emission is strongly affected by bound primary ammonium ions. On the other hand, *cone* **5** with neat C_3 symmetry is rather insensible to the guest-binding in spite of large K value for ammonium ions, because two of three pyrenes can probably still interact with each other even in forming the complex. The foregoing finding

provide important guiding principles for designing RNH_3^+ receptors. To sensitively detect RNH_3^+, a cone ester derivative with two pyrenes may be ideal.[11] It is expected, however, that the binding process would be competitively inhibited by co-existing K^+ and $R_2NH_2^+$.[4] In contrast, *partial cone* **6** features the large K for RNH_3^+ and the relatively small K for K^+ and $R_2NH_2^+$, which enables us to selectively detect primary ammonium ions.

References

1) Reviews: "Crown Ethers & Cryptands," ed by G. Gokel, Royal Society of Chemistry, Cambridge, UK (1991), and the references cited therein; L. C. Hodgkinson, S. J. Leigh, and I. O. Sutherland, *J. Chem. Soc., Chem. Commun.*, **1976**, 639.

2) Reviews: J. -M. Lehn, *Angew. Chem., Int. Ed. Engl.*, **27**, 89 (1988); D. J. Cram, *ibid.*, **27**, 1009 (1988).

3) T. Kaneda, K. Hirose, and S. Misumi, *J. Am. Chem. Soc.*, **111**, 742 (1989); T. Kaneda, S. Umeda, Y. Ishizaki, H.-S. Kuo, S. Misumi, Y. Kai, N. Kanehisa, and N. Kasai, *ibid.*, **111**, 1881 (1989).

4) K. Araki, N. Hashimoto, H. Otsuka, and S. Shinkai, *J. Org. Chem.*, in press.

5) S. Chang, M. Jang, S. Han, J. Lee, M. Kang, and K. No, *Chem. Lett.*, **1992**, 1937.

6) I. Aoki, H. Kawabata, K. Nakashima, and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, **1991**, 1771; I. Aoki, T. Sakaki, S. Tsutsui, and S. Shinkai, *Tetrahedron Lett.*, **33**, 89 (1992); T. Jin, K. Ichikawa, and T. Koyama, *J. Chem. Soc., Chem. Commun.*, **1992**, 499; C. Pérez-Jiménez, S. J. Harris, and D. Diamond, *ibid.*, **1993**, 480.

7) A. Ueno, I. Suzuki, and T. Osa, *J. Am. Chem. Soc.*, **111**, 6391 (1989); I. Suzuki, M. Ohkubo, A. Ueno, and T. Osa, *Chem. Lett.*, **1992**, 269.

8) K. W. Bair, R. L. Tuttle, V. C. Knick, M. Cory, and D. D. McKee, *J. Med. Chem.*, **33**, 2385 (1990).

9) **5**: white powder; mp 141.8-143.5 °C; ^{1}H NMR ($CDCl_3$) δ = 1.03 (27H, s, *tert*-butyl), 4.43 (6H, d, *J* = 12.9 Hz, $ArCH_2OCH_2Ar$), 4.46 (6H,s), 4.82 (6H, d, *J* = 12.9 Hz), 5.54 (6H, s), 6.87 (6H, s, benzene ring), 7.68 - 7.98 (27H, m, pyrene ring); **6**: white powder; mp 206.5 - 208.0; ^{1}H NMR ($CDCl_3$) δ = 0.75 (18H, s, *tert*-butyl), 1.23 (9H, s, *tert*-butyl), 3.20 (2H, s), 4.15 (2H, d, *J* = 11.4 Hz), 4.16 (2H, d, *J* = 11.8 Hz), 4.18 (2H, d, *J* = 16.5 Hz), 4.29 (2H, d, *J* = 9.2 Hz), 4.63 (2H, d, *J* = 11.8 Hz), 4.69 (2H, d, *J* = 16.5 Hz), 4.96 (2H, d, *J* = 11.4 Hz), 5.11 (2H, d, *J* = 9.2 Hz), 5.39 (2H, s), 5.57 (2H, d, *J* = 12.5 Hz), 5.67 (2H, d, *J* = 12.5 Hz), 6.91 (2H, d, *J* = 2.6 Hz), 7.14 (2H, d, *J* = 2.6 Hz), 7.35 (2H,s), 7.86 - 8.26 (27H, m, pyrene ring); ^{13}C NMR ($CDCl_3$) δ = 30.77, 31.36, 33.69, 34.28, 63.54, 64.70, 65.18, 67.20, 67.42, 69.67, 71.42, 122.76, 122.94, 124.38, 124.41, 124.58, 124.62, 124.67, 125.27, 125.32, 125.89, 125.96, 126.71, 127.17, 127.26, 127.33, 127.46, 127.60, 127.79, 128.02, 128.32, 128.58, 128.65, 128.68, 128.87, 129.21, 129.75, 130.51, 130.63, 130.65, 131.02, 131.17, 131.27, 131.43, 131.50, 146.14, 146.65, 153.22, 154.46, 169.61, 170.08.

10) H. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).

11) The preparation of this compound is still unsuccessful because of the synthetic difficulty.

(Received September 20, 1993)