A Fluorescent Calix[4]arene as an Intramolecular Excimer-forming Na^+ Sensor in Nonaqueous Solution

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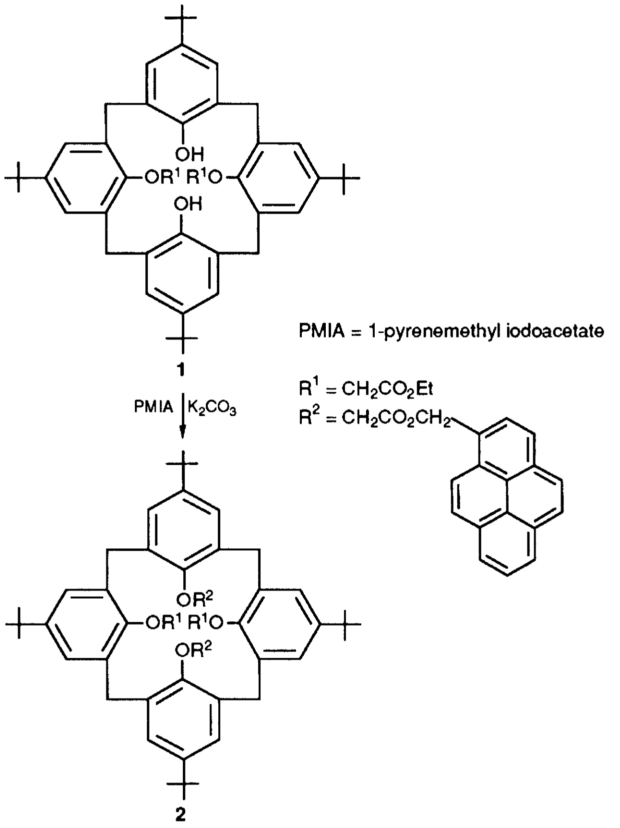
A new fluorescent calix[4]arene **2** has been synthesized as an intramolecular excimer-forming Na^+ sensor which shows a change in fluorescent characteristics specifically upon complexation with Na^+.

The development of fluorescent Na^+ sensors has become of significant importance for selective Na^+ detection in various systems, especially in biological systems.[1] Several groups have recently reported[2–5] fluorescent Na^+ sensors derived from macrocyclic compounds such as crown ethers[2,4,5] and cryptands.[3] While these sensors exhibit selective affinity toward Na^+, there are only a few sensors[2,3] in which fluorescence wavelength changes after Na^+ complexation. For the design of fluorescent Na^+ sensors, much attention has been paid to crown ethers and cryptands. Herein, we report the design and synthesis of a new fluorescent Na^+ sensor **2**, employing a calix[4]arene, which induces large shifts in



Scheme 1 The synthesis of a fluorescent calix[4]arene **2** as a Na$^+$ sensor which exhibits dual emission (pyrene monomer and excimer)

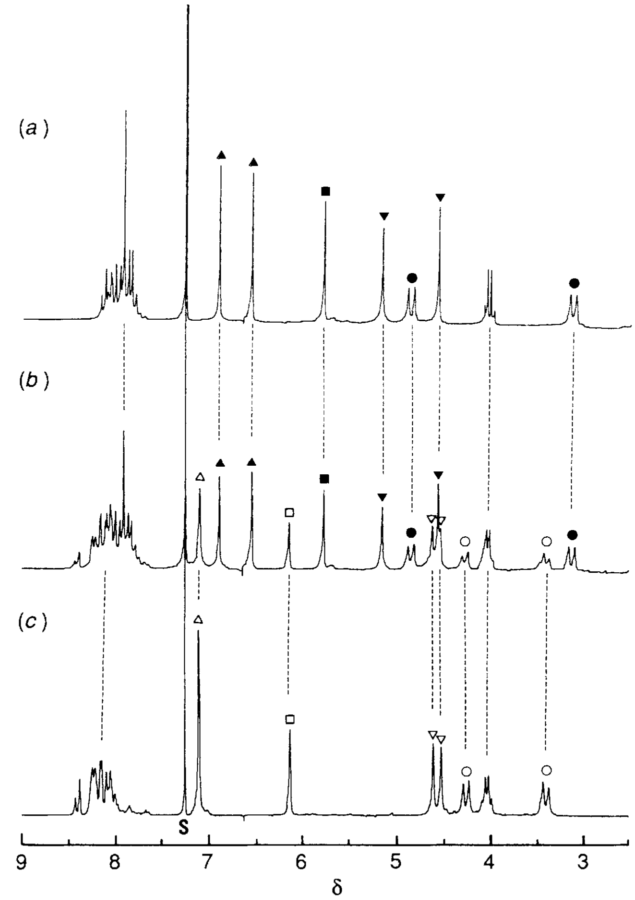


Fig. 1 Partial ^{1}H NMR spectra of the fluorescent calix[4]arene **2** in CDCl$_3$ at 25 °C: (*a*) R = [NaSCN]/[**2**] = 0, (*b*) R = 0.37, (*c*) R = 1, where [**2**] = 5.1 mmol dm^{-3}. Aliquots from a CD$_3$OD solution of 1 mol dm^{-3} NaSCN were added directly to a CDCl$_3$ solution of **2** in a NMR tube. (▲,△ = ArH; ●,○ = ArCH$_2$Ar; ▼,▽ = OCH$_2$O; ■,□ = pyrene-CH$_2$) The signal marked S is due to CHCl$_3$.

fluorescence emission spectra specifically upon complexation with Na$^+$.

In order to design an efficient Na$^+$ sensor, the following requirements must be kept in mind: (*i*) a high Na$^+$ selectivity, (*ii*) a change in fluorescence emission spectrum upon complexation with Na$^+$ and (*iii*) a high quantum yield. From the standpoint of the first requirement, we have chosen *p-tert*-butylcalix[4]arene[6] as the basic skeleton of the Na$^+$ sensor, since it has been shown that several alkoxycarbonylmethyl ethers of *p-tert*-butylcalix[4]arene exhibit a high selectivity[7–11] and complexing ability[12] toward Na$^+$ compared with crown ethers and cryptands. To satisfy the latter requirements, we have designed a new fluorescent Na$^+$ sensor **2**, employing a calix[4]arene, in which two pyrene[13] molecules are introduced as fluorophores forming an intramolecular pyrene excimer.[14] When the fluorescent calix[4]arene complexes with Na$^+$, the excimer : monomer intensity ratio will be altered by a change in the relative configuration of the two pyrene moieties, which is induced by the reorientation of the four carbonyl groups of **2** to bind a Na$^+$ ion. An examination of Corey–Pauling–Koltun (CPK) molecular model suggests that the distance between two pyrene moieties must be expanded by Na$^+$ binding. An important characteristic of the design of the fluorescent Na$^+$ sensor **2** is the combination of the fluorescence change (excimer–monomer balance) with the conformational change of a host molecule.[15]

The fluorescent calix[4]arene **2**† was synthesized by the reaction of the bis-ethoxycarbonylmethyl ether of *p-tert*-butylcalix[4]arene **1**[10] and 1-pyrenemethyl iodoacetate (Molecular Prob. Inc.) in anhydrous tetrahydrofuran in the presence of potassium carbonate (yield 10%, Scheme 1).

To examine the complexing ability of **2** towards Na$^+$, we carried out ^{1}H NMR titration experiments. In the absence of NaSCN, the ^{1}H NMR spectrum of **2** agreed with the distorted 'cone'[16] conformation with C_{2v} symmetry [Fig. 1(*a*)]: from the NOE experiments, the bridging methylene protons at δ 3.14 and 4.88 were assigned to the equatorial and axial protons, respectively.[9] When NaSCN is added to the CDCl$_3$ solution of **2**, all the signals in the ^{1}H NMR spectrum change greatly [Fig. 1(*b*)]. Upon reaching a salt: **2** molar ratio of 1:1, all the signals for the free ligand disappear [Fig. 1(*c*)] and an increase in the salt: **2** molar ratio beyond unity produces no further spectral shifts. The ^{1}H NMR titration experiment indicates a 1:1 stoichiometry for the NaSCN complex with **2**. This finding implies that the introduction of two pyrene molecules to the terminal positions (R–CH$_2$CO$_2$–) of the binding groups does not inhibit the Na$^+$ binding activity. Similar spectral behaviour as shown in Fig. 1 has been also observed in other Na$^+$ binding calix[4]arenes such as the tetrakis-butoxycarbonylmethyl ether of *p-tert*-butylcalix[4]arene[9] and tetrakis-ethoxycarbonylmethyl ether of *p-tert*-butylcalix[4]arene.[12]

Fig. 2 shows Na$^+$ titrations of the fluorescence emission spectra of **2** in methanol–tetrahydrofuran (v/v 15:1). The fluorescence spectrum of **2** is characterized by the dual emission resulting from the excimer (*ca.* 480 nm) and the monomer (*ca.* 390 nm). In the absence of Na$^+$ the excimer emission is dominant compared with the monomer emission: the intensity ratio of excimer to monomer emission is about 4. This intensity ratio is less affected by the concentration of **2** in

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† *Spectroscopic data* for **2**: ^{1}H NMR (200 MHz; CDCl$_3$) 0.92, 1.20 (s, 2 × 18H, CMe$_3$), 1.02 (t, 6H, OCH$_2$CH_3), 3.14, 4.88 (d, 2 × 4H, J 13.0 Hz, ArCH$_2$Ar), 4.04 (q, 4H, OCH_2CH$_3$), 4.59, 5.20 (s, 2 × 4H, OCH$_2$CO$_2$), 5.82 (s, 4H, pyrene-CH$_2$), 6.58, 6.94 (s, 2 × 4H, ArH), 7.72–8.20 (m, 18H, pyrene); Field desorption mass spectrum, m/z 1364(M$^+$).

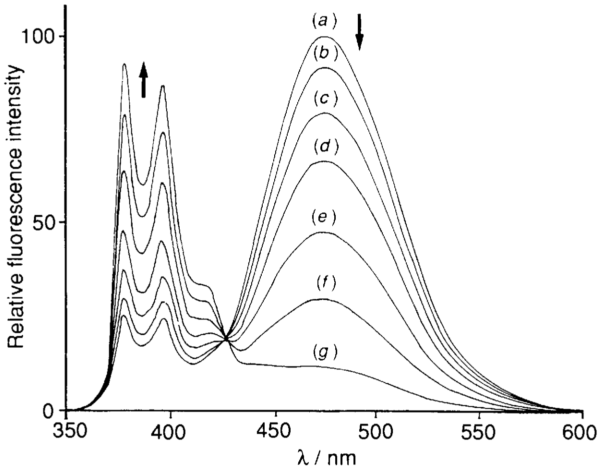

Fig. 2 Na^+ titrations of the fluorescence emission spectra of 4.8 µmol dm^{-3} of **2** in methanol–tetrahydrofuran (v/v 15:1) at 25 °C: (*a*) [NaSCN] = 0, (*b*) 2.9, (*c*) 5.8, (*d*) 12, (*e*) 33, (*f*) 82, (*g*) 428 µmol dm^{-3}. The spectra were measured with excitation at 330 nm. The excitation and emission bandwidths were set at 2 and 10 nm, respectively.

the range 10^{-4}–10^{-7} mol dm^{-3}. Thus, it is concluded that the excimer emission results from an intramolecular excimer but not from an intermolecular excimer. The addition of NaSCN to the 4.8 µmol dm^{-3} solution of **2** causes significant changes in fluorescence emission spectra with an isoemissive point at 425 nm. The fluorescence intensity of excimer emission at 480 nm decreases 9-fold in the presence of 428 µmol dm^{-3} Na^+, while the intensity of monomer emission at 390 nm increases 3.7-fold. From the data of Na^+ and K^+ titrations of the fluorescence spectra, the dissociation constants for Na^+ and K^+ complexes of **2** were determined as 3.7×10^{-5} and 5.7×10^{-3} mol dm^{-3} respectively. The Na^+/K^+ selectivity, as measured by the ratio of the dissociation constants amounts to 154. For Li^+, the affinity of **2** for Li^+ was too low to be determined accurately by fluorescence titration and the dissociation constant was estimated to be greater than 100 mmol dm^{-3}.

To verify the utility of **2** as a fluorescent Na^+ sensor, we have examined the effect of other alkali metal ions on the fluorescence intensity in the presence of Na^+. As shown in Fig. 3, the addition of NaSCN to 4.8 µmol dm^{-3} of **2** in methanol–tetrahydrofuran (v/v 15:1) causes changes in both the excimer and the monomer emission intensity. However, subsequent addition of KSCN in 10-fold excess of NaSCN gives rise to only a slight change in the fluorescence intensity. By the addition of KSCN in 100-fold excess of NaSCN, the fluorescence intensity is altered by 11%. It should be noted that the second addition of NaSCN provides the intensity change again. When another alkali metal ion such as Li^+, Rb^+ and Cs^+ in 100-fold excess of sodium ion was added to the 4.8 µmol dm^{-3} solution of **2** containing of 15 µmol dm^{-3} Na^+, the change in the fluorescence intensity at 390 or 480 nm was less than 1%. These results indicate that **2** has potential ability for use as a fluorescent sensor for selective Na^+ detection in nonaqueous solution.

This is the first example of fluorescent Na^+ sensor employing a calix[4]arene.

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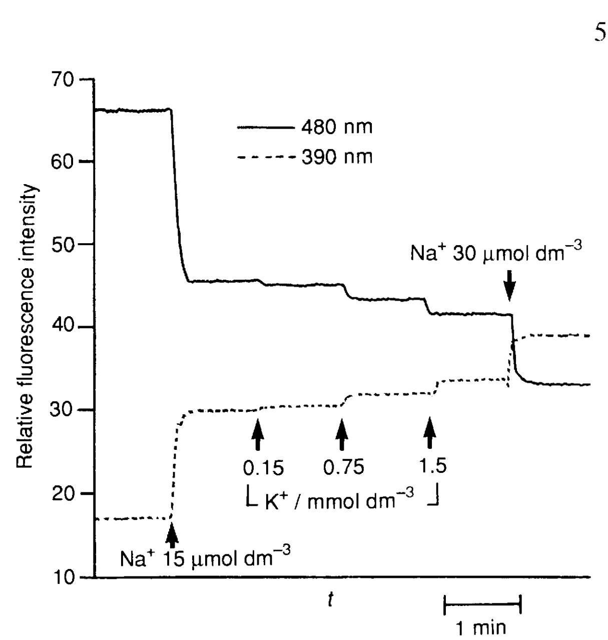

Fig. 3 The effect of Na^+ and K^+ on the fluorescence intensity of 4.8 µmol dm^{-3} of **2** in methanol–tetrahydrofuran (v/v 15:1) at 25 °C. Fluorescence intensity was monitored at 390 and 480 nm. Aliquots from 10 mmol dm^{-3} NaSCN and 1 mol dm^{-3} KSCN in methanol were added directly to the solution of **2** in a cuvette.

References

1 P. A. Negulescu, A. Harootunian, A. Minta, R. Y. Tsien and T. E. Machen, *J. Gen. Physiol.*, 1988, **92**, 26a; A. T. Harootunian, J. P. Y. Kao, B. K. Eckert, and R. Y. Tsien, *J. Biol. Chem.*, 1989, **264**, 19458; L. Ali, E. Grapengiesser, E. Gylfe, B. Hellman and P.-E. Lund, *Biochem. Biophys. Res. Commun.*, 1989, **164**, 212.

2 H. Bouas-Laurent, A. Castellan, M. Daney, J.-P. Desvergne, G. Guinand, P. Marsau and M.-H. Riffaud, *J. Am. Chem. Soc.*, 1986, **108**, 315.

3 G. A. Smith, T. R. Hesketh and J. C. Metcalfe, *Biochem. J.*, 1988, **250**, 227.

4 A. Minta and R. Y. Tsien, *J. Biol. Chem.*, 1989, **264**, 19449.

5 A. P. de Silva and K. R. A. S. Sandanayake, *J. Chem. Soc., Chem. Commun.*, 1989, 1183.

6 For a review, see C. D. Gutsche, *Prog. Macrocycl. Chem.*, 1987, **3**, 93.

7 M. A. McKervey, E. M. Seward, G. Ferguson, B. Ruhl and S. J. Harris, *J. Chem. Soc., Chem. Commun.*, 1985, 388.

8 S.-K. Chang and I. Cho, *J. Chem. Soc., Perkin Trans. 1*, 1986, 211.

9 A. Arduini, A. Pochini, S. Reverberi, R. Ungaro, G. D. Andreetti and F. Ugozzoli, *Tetrahedron*, 1986, **42**, 2089.

10 F. Arnand-Neu, E. M. Collins, M. Deasy, G. Ferguson, S. J. Harris, B. Kaitner, A. J. Lough, M. A. McKervey, E. Marques, B. L. Ruhl, M. J. Schwing-Weill and E. M. Seward, *J. Am. Chem. Soc.*, 1989, **111**, 8681.

11 T. Arimura, M. Kubota, T. Matsuda, O. Manabe and S. Shinkai, *Bull. Chem. Soc,. Jpn.*, 1989, **62**, 1674.

12 T. Jin and K. Ichikawa, *J. Phys. Chem.*, 1991, **95**, 2601.

13 The fluorescence quantum yield of pyrene monomer is fairly good (0.21–0.65 in ethanol): C. A. Parker and C. G. Hatchard, *Trans. Faraday Soc.*, 1963, **59**, 284.

14 The definition of 'excimer' may be seen in: W. R. Ware, *Time-Resolved Fluorescence Spectroscopy in Biochemistry*, ed. R. B. Cundall and R. E. Dale, Plenum, New York, 1983, p. 363.

15 The design of a crown ether type Na^+ sensor using the intensity change of intramolecular anthracene monomer–excimer has been reported by H. Bouas-Laurent *et al.*: See ref. 2. For a review, see H. Bouas-Laurent, J.-P. Desvergne, F. Fages, and P. Marsau, *Frontiers in Supramolecular Organic Chemistry and Photochemistry*, ed. H. J. Schneider and H. Dürr, VCH, Weinheim, 1991, p. 265.

16 C. D. Gutsche, B. Dhawan, J. A. Levine, K. H. No and L. J. Bauer, *Tetrahedron*, 1983, **39**, 409; C. Alfieri, E. Dradi, A. Pochini and R. Ungaro, *Gazz. Chim. Ital.*, 1989, **119**, 335.