tochem. **1972,** *20,* 527.
5. Nakane, P. K.; Kawaoi, A. *J. Histochem. Cytochem.* **1974,** *22,* 1084.
6. Schramm, W.; Yang, T.; Midgley, A. R. *Clin. Chem.* **1987,** *33,* 1331.
7. Sanderson, C. J.; Wilson, D. V. *Immunology* **1971,** *20,* 1061.
8. Schramm, W.; Pack, S. H. *Anal. Biochem.* **1992,** *205,* 47.
9. Bos, E. S.; van der Doelen, A. A.; van Rooy, N.; Schuurs, A. H. *J. Immunoassays* **1981,** *2,* 187.
10. Zettner, A. *Clin. Chem.* **1973,** *19,* 699.
11. Kemp, H. A.; Morgan, M. R. *J. Immunol. Meth.* **1986,** *94,* 65.
12. Laurence, D. J.; Wilkinson, G. *Anal. Chem.* **1974,** *46,* 1132.
13. Bachas, L. G.; Meyerhoff, M. E. *Anal. Biochem.* **1986,** *156,* 223.

Synthesis and Ion Binding Properties of Rebek's Cleft-Type Ionophores Bearing Two Convergent Carboxylic Acid Functions

Nam Yee Kim, Sung Woo Park [†], and Suk-Kyu Chang*

Department of Chemistry, Chung-Ang University, Seoul 156-756, Korea
[†]National Institute of Scientific Investigation, Seoul 158-097, Korea
Received February 18, 1997

A series of new ligands having convergent dicarboxylic acid functions, based-upon Rebek's cleft-type ionophore, have been prepared and their ion binding properties were investigated by the competitive extraction and transport experiments. The main purpose of the modification was to increase the lipophilicity of the Rebek's ionophore, which was attempted by utilizing propyl analog of Kemp's triacid or by changing the bridging unit. Ionophores **5** and **6** were found to have a pronounced ion-binding property toward Ca^{2+} ion. The selectivity in competitive extraction of ionophore **5** at pH 9 for Ca^{2+} over Mg^{2+} and Sr^{2+} is 2.0 and 59.3, respectively. The selectivity in competitive transport of ionophore **5** for Ca^{2+} over Mg^{2+} and Sr^{2+} is 29.8 and 99.3, and that of ionophore **6** is 10.0 and 23.2, respectively.

Introduction

The design of a molecular recognition system for a chemically and biologically important guest is a very attractive research area.[1~3] A great number of studies to develop selective completing agents have been performed aiming for the applications in the fields of separation and analytical sciences as well as biological and medical studies.[4~7] Recently, chemists have a strong interest in Kemp's triacid as a key motif for the design of organized structures as well as molecular recognition devices.[8] The Kemp's triacid has three axial carboxylic acid groups on a cyclohexane ring framework and the triaxial conformation of the three carboxylic acids forces a U-shaped relationship between any two of them. Rebek *et al.* [9,10] have synthesized a variety of interesting molecules having a cleft-like shape from Kemp's triacid for the recognition of many biologically interesting guests. The design principle is the formation of new structures by utilizing the more basic syn lone pairs of carboxylate, along with the convergent functionality of the carboxylate functions. Among them, they prepared a new type of ionophore based-upon the Kemp's triacid as shown in Figure 1 and found that the ionophore showed an extraordinary selectivity in binding calcium and magnesium ions.[11]

In contrast to its great potential as a specific ionophore, however, there are few reports on the application to chelating agents or carriers for metal ions.[12,13] We prepared a series of new carriers based on the Rebek's original structure, having a purpose to develop a simple but efficient ligand or carrier for calcium ion. The main strategy is to increase the lipophilicity with the molecular framework intact: that is the substitution of equatorial methyl group with more lipophilic propyl group and the utilization of methylene dianiline or more lipophilic aromatic ring instead of simple *m*-xylidine diamine of the Rebek's ionophore. The ionophoric behavior of the prepared compounds was investigated by means of the competitive extraction and competitive transport through liquid membrane of alkali and alkaline earth metal cations.

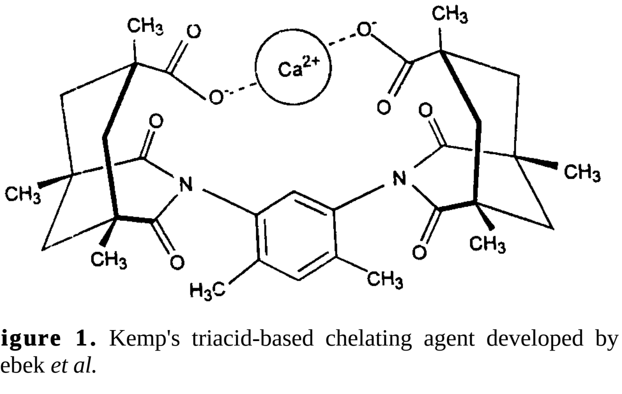


Figure 1. Kemp's triacid-based chelating agent developed by Rebek *et al.*

Experimental

The ^{1}H NMR spectra were recorded with a Varian MSL-300 (300 MHz) spectrometer. The chemical shifts in $CDCl_3$ solution were reported relative to TMS as an internal standard. The IR and UV spectra were recorded with a Nicolet 510P FT-IR spectrometer and a Shimadzu UV 2100 spectrophotometer, respectively. Melting points were determined on a Mettler FP 61 melting point apparatus. The ion chromatography (IC) and inductively coupled plasma (ICP) spectrometric analyses were performed with a Dionex 2000i Ion Chromatography using a conductivity detector and a Jobin Yvon JY 24 ICP spectrometer, respectively. Fast atom bombardment (FAB) mass spectra were obtained with a JEOL JMS AX505WA spectrometer. Chemicals (Kemp's triacid, 6'-butoxy-2,6-diamino-3,3'-azodipyridine, and 4,4'-methylene dianiline) were purchased from Aldrich Chemical Co., and used without further purification. 2,6-Diaminopyridine was purified by column chromatography. Kemp's propyltriacid was prepared as described in the literature.[14] Analytical TLC was done on aluminum plate precoated with silica gel 60 F_{254} and column chromatography was performed on silica gel 60 (230-340 mesh, ASTM).

General procedure for the preparation of ionophores.[15] A mixture of Kemp's triacid or Kemp's propyltriacid (3 mmol) and the corresponding diamine (1.5 mmol) was grounded and heated at 200 °C for 4 h under the nitrogen atmosphere. After cooling, the crude product was dissolved in 500 mL of CH_2Cl_2 and filtered, and the filtrate was evaporated under reduced pressure. Purification by column chromatography on silica gel (15% MeOH/ethyl acetate) and subsequent crystallization from CH_2Cl_2/hexane yielded **3-6** as a white or slightly brown powder.

2,6-Diaminopyridine derivative 3. White solid; yield 89%; mp >300 °C; ^{1}H NMR ($CDCl_3$) 7.83 (t, J=7.8 Hz, 1 H), 6.99 (d, J=7.8 Hz, 2 H), 2.81 (d, J=13.1 Hz, 4 H), 2.13 (d, J=13.3 Hz, 2 H), 1.50 (d, J=13.3 Hz, 2 H), 1.30 (s, 18 H), 1.24 (d, J=13.2 Hz, 4 H); IR (KBr) 3420, 2974, 1742, 1709, 1450, 1381, 1190 cm^{-1}; MS (FAB): m/z calcd for $C_{29}H_{36}O_8N_3$(M+H)$^+$ 554.2, found 554.

Methylenedianiline derivative 4. Light brown solid; yield 60%; mp >300 °C; ^{1}H NMR ($CDCl_3$) 7.19 (br, 4 H), 7.04 (br, 4 H), 3.98 (s, 2 H), 2.69 (d, J=13.2 Hz, 4 H), 1.97 (d, J=13.2 Hz, 2 H), 1.36 (d, J=13.2 Hz, 2 H), 1.18 (s, 18 H), 1.03 (d, J=13.2 Hz, 4 H); IR (KBr) 3439, 2968, 1734, 1689, 1464, 1381, 1182 cm^{-1}; MS (FAB): m/z calcd for $C_{37}H_{43}O_8N_2$ (M+H)$^+$ 643.3, found 643.

Butoxy-azodipyridine derivative 5. Yellow solid; yield 89%; mp >300 °C; ^{1}H NMR ($CDCl_3$) 8.71 (s, 1 H), 8.19 (d, J=8.3 Hz, 1 H), 7.82 (d, J=8.9 Hz, 1 H), 7.12 (d, J=8.3 Hz, 1 H), 6.77 (d, J=8.9 Hz, 1 H), 4.44 (t, J=6.7 Hz, 2 H), 2.83 (d, J=13.8 Hz, 4 H), 2.14 (d, J=13.4 Hz, 2 H), 1.80-1.77 (m, 2 H), 1.56-1.46 (m, 4 H), 1.34-1.23 (m, 22 H), 0.98 (t, J=7.4 Hz, 3 H); IR (KBr) 3433, 2966, 1742, 1711, 1489, 1464, 1381, 1186 cm^{-1}; MS (FAB): m/z calcd for $C_{38}H_{47}O_9N_6$(M+H)$^+$ 731.3, found 731.

Kemp's propylacid derivative 6. White solid; yield 49%; mp >300 °C; ^{1}H NMR ($CDCl_3$), 7.80 (t, J=7.8 Hz, 1 H), 6.91 (d, J=7.8 Hz, 2 H), 2.70 (d, J=13.6 Hz, 4 H), 2.29 (d, J=13.1 Hz, 2 H), 1.94 (t, J=11.4 Hz, 4 H), 1.51-1.47 (m, 10 H), 1.42-1.22 (m, 16 H), 0.93-0.85 (m, 18 H) ; IR (KBr) 3420, 2961, 1740, 1713, 1446, 1333, 1192 cm^{-1}; MS (FAB): m/z calcd for $C_{41}H_{60}O_8N_3$ (M+H)$^+$ 722.4, found 722.

Distribution experiments.[16~18] The distribution of ionophores **3-6** between $CHCl_3$ and aqueous buffer solution[19] was measured. Aqueous phase was a buffer solution of the pH 4-11 (2.0 mL): pH of the aqueous phase was adjusted with succinic acid-NH_4OH for pH 4-6, Tris-HCl for pH 7-9, and 3-cyclohexylaminopropanesulfonic acid (CAPS)-NH_4OH for pH 10-11. Organic phase was 0.08-0.3 mM of ionophores **3-6** in $CHCl_3$ (2.0 mL). The mixture of the aqueous and organic solution was shaken for 30 sec by vortex-genie. After 30 min at 25 ± 2 °C, it was shaken again for 30 sec and thermostated for further 1 h. The concentration of the ligands in the organic layer was measured using a UV/Vis spectrophotometer at their λ_{max} (258 nm for **3** and **6,** 244 nm for **4,** and 353 nm for **5).** The distribution coefficient, K_d was calculated according to the following equation, where, A_i and A_f stand for the absorbance of organic phase before and after the extraction, respectively.

$$K_d = \frac{A_f}{(A_i - A_f)}$$

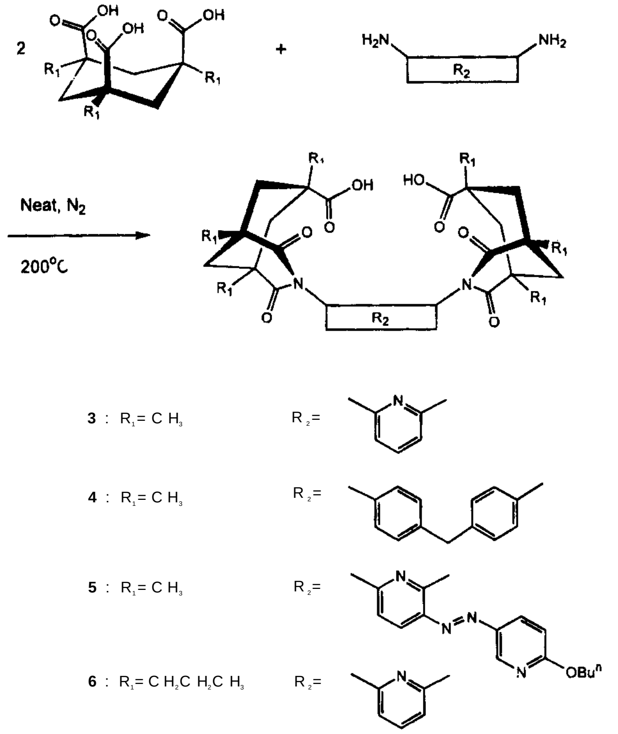
Competitive extraction experiments.[19,20] The competitive extraction experiments were performed at 25 ± 2 °C. Extraction condition is as follows: aqueous phase was a buffer solution (2.0 mL) containing 1.0 mM each of alkali metal chlorides or alkaline earth metal chlorides ($MgCl_2$, $CaCl_2$, $SrCl_2$ and $BaCl_2$) in buffered solution of varying pH (pH 5-10) and organic phase was 5.0 mM of ionophores **3-6** in $CHCl_3$ (2.0 mL). Extraction procedure was exactly the same as the distribution experiment. After extraction, the organic phase was back extracted with 0.1 M-HCl and then the amounts of alkali or alkaline earth metal ions in aqueous phase were determined by the IC or ICP method, respectively.

Competitive transport experiments.[12,21~23] The transport experiments were carried out using a U-tube (inner diameter=1.8 cm) at 25 ± 2 °C for the time period of 2 days. Transport condition is as follows: source phase was 5.0 mL of Tris-buffer solution (pH=9) containing 0.1 M each of alkali metal chlorides or alkaline earth metal chlorides ($MgCl_2$, $CaCl_2$, $SrCl_2$ and $BaCl_2$); membrane phase was 5.0 mM of ionophores **3-6** in $CHCl_3$ (15 mL); receiving phase was 1.0 M of HCl (5.0 mL). The organic membrane phase was stirred at 200 rpm carefully so as not to disturb the phase boundaries. The concentrations of the transported ions in receiving phase were determined by IC or ICP technique.

Results and Discussion

Synthesis of the ionophore. The syntheses of ionophores **3-6** were performed by merely heating the two components in the absence of solvent as shown in Scheme 1. Ionophores **3, 4,** and **5** were prepared by the condensation reaction of 2,6-diaminopyridine, methylenedianiline, or 6'-butoxy-2,6-diamino-3,3'-azopyridine with Kemp's triacid in good yield (60-89%). Ionophore **6** was synthesized by the condensation of 2,6-diaminopyridine with propyl analog of Kemp's triacid in 49% yield.

The widely used chelators such as EDTA and related compounds complex many metal ions by forming non-co-



Scheme 1.

valent bonds utilizing the anti-lone pairs of the carboxylate oxygen.[10] However, ionophores **3-6** as well as Rebek's chelating agent are designed to form complexes with the target metal ions using the more basic syn-lone pairs.[11]

Distribution coefficient as a function of pH. The lipophilicity of the ionophores is a very important factor for their applications as analytical tools or carriers. In order to increase the lipophilicity of Rebek's original ionophore having strong binding affinity toward Ca^{2+} ion, more lipophilic groups were introduced to the backbone of the bridging unit, or methyl groups in Kemp's triacid were replaced with propyl groups. The increases in lipophilicity increment π_x for the ionophores **5** and **6** compared with **3** are estimated to be larger than 2.5 and 6.0, respectively, which are large enough to endow sufficient lipophilicity into the ligands.[24] The variations in distribution coefficient (K_d) as a function of pH values are shown in Figure 2. As expectedly, ionophore **6** which is derivative of propyl analog of Kemp's triacid exhibited sufficient lipophilicity at weakly basic pH region (pH 8-9). In contrast to this, the ionophores **3** and **4** which are derivatives of Kemp's triacid did not show any significant lipophilicity even at neutral pH region (pH 6-8). Ionophore **5** having long chain substituted pyridine ring also was found to be sufficiently lipophilic at weakly basic pH region (pH 8-9).

Competitive extraction of metal cations. The completing abilities of ionophores **3-6** were examined by competitive extraction experiment using mixtures of alkali or alkaline earth metal salts. As shown in the Table 1, ionophores **3-6** showed low extraction ability toward alkali metal cations with a slight selectivity toward Na^+ and K^+ ions under basic aqueous conditions. From the results of the extraction of alkali metal ions, it is suggested that the derivatives of Kemp's triacid or their propyl analogs do not have an efficient completing ability toward alkali metal ions.

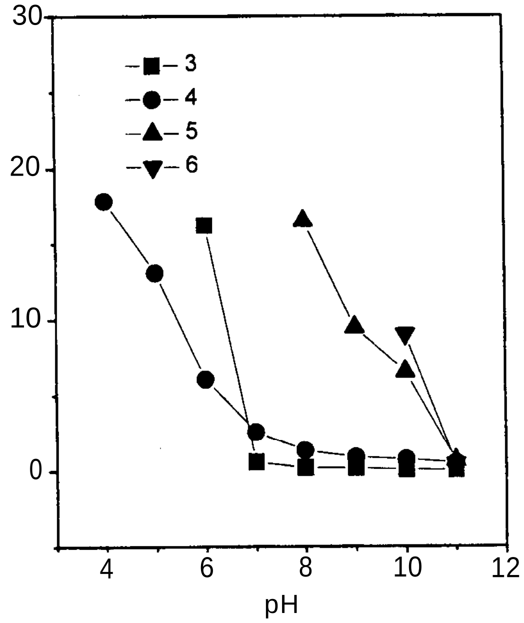


Figure 2. pH dependence of distribution coefficient of ionophores **3-6**.

Table 1. Competitive Extraction of Alkali Metal Ions from Water into Chloroform at 25 ± 2 °C [a]

pH	Ionophore	Extraction ability [b](%)				
		Li^+	Na^+	K^+	Rb^+	Cs^+
5	**3**	-	1.2	-	-	-
6	**3**	-	1.0	0.8	-	-
	4	-	3.8	1.3	-	-
	5	-	1.0	1.6	-	-
7	**3**	-	1.0	1.0	-	-
	4	-	6.8	1.5	-	-
	5	-	1.0	1.4	-	-
8	**3**	-	1.0	0.7	-	-
	4	-	6.3	2.0	-	1.8
	5	-	1.5	1.4	-	-
	6	-	4.4	1.7	-	-
9	**3**	-	0.5	0.5	-	-
	4	-	6.9	2.1	-	2.5
	5	-	1.5	1.5	-	-
	6	0.8	4.7	2.0	-	-
10	**3**	-	-	-	-	-
	4	-	5.0	1.1	-	2.2
	5	2.2	1.1	1.1	-	-
	6	3.9	0.8	2.5	1.0	-

[a] Determined by IC. Column: HPIC-CS1, eluant: 5 mM HCl, flow rate: 1 mL/min, detection: suppressed conductivity. [b] "-" means less than 0.5%.

As expectedly, ionophores **5** and **6** exhibited an efficient extraction ability toward alkaline earth metal cations (Table 2). Especially, the extraction efficiency of ionophore **6** toward Ca^{2+} ion was up to 80% at pH 9-10. The selectivity pattern decreases in the following order: $Ca^{2+}>Mg^{2+}>Ba^{2+}>Sr^{2+}$. The Ca^{2+} ion selectivity of ionophores **5** and **6** at pH 9 was as follows: $Ca^{2+}/Mg^{2+}=2.0$, 7.0 and $Ca^{2+}/Sr^{2+}=59.3$, 14.0,

Table 2. Competitive Extraction and Selectivity of Alkaline Earth Metal Ions from Water into Chloroform at 25 ± 2 ℃

pH	Ionophore	Extraction ability[a] (%)				Selectivity	
		Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Ca^{2+}/Mg^{2+}	Ca^{2+}/Sr^{2+}
5	**3**	-	0.9	-	-	-[b]	-[b]
6	**3**	-	1.1	-	-	-[b]	-[b]
	4	-	0.8	-	-	-[b]	-[b]
	5	-	0.8	-	-	-[b]	-[b]
7	**3**	-	1.8	-	-	-[b]	-[b]
	4	-	0.8	-	-	-[b]	-[b]
	5	-	2.2	-	-	-[b]	-[b]
8	**3**	0.1	0.8	-	-	8.0	-[b]
	4	0.5	9.8	2.0	2.4	20.9	5.0
	5	9.6	32.3	-	1.2	3.3	-[b]
	6	2.8	44.2	0.5	1.2	16.0	98.3
9	**3**	0.7	1.0	-	-	1.4	-[b]
	4	1.3	19.4	5.4	4.0	15.0	3.6
	5	31.9	64.7	1.1	6.6	2.0	59.3
	6	11.2	78.8	5.6	10.1	7.0	14.0
10	**3**	0.7	1.3	-	-	1.9	-[b]
	4	1.7	22.6	8.1	7.3	13.6	2.8
	5	49.5	75.7	3.0	14.2	1.5	25.6
	6	24.0	80.0	20.7	30.4	3.3	3.9

[a] "-" means less than 0.5%. [b] Can not be estimated.

Table 3. Competitive Transport and Selectivity of Alkaline Earth Metal Cations through Chloroform Liquid Membrane at pH 9[a]

Ionophore	Transport rate (x 10^{-8} mol/h)				Selectivity	
	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Ca^{2+}/Mg^{2+}	Ca^{2+}/Sr^{2+}
3	0.2	5.7	-	-	28.5	-[b]
4	-	0.2	-	-	-[b]	-[b]
5	1.0	29.8	0.3	0.4	29.8	99.3
6	2.8	27.8	1.2	2.6	10.0	23.2

[a] "-" means less than 0.1 x 10^{-8} mol/h, [b] Can not be estimated.

respectively. The ionophores **3** and **4** showed poor extraction ability toward alkaline earth metal ions even under basic aqueous conditions that can be partly explained by the low lipophilicity of **3** and **4.** The extraction efficiency of **5** and 6 is much larger than that of **3** having similar but simplified structure of the Rebek's ionophore.

Competitive transport across liquid membrane. The results of competitive transport experiments of alkaline earth metal cations after 2 days are summarized in Table 3. The transport of alkali metal cations is in general not efficient to observe any significant transport of guests even after two days of transport experiments. As can be seen in the Table, the transport rates of ionophores **5** and **6** for Ca^{2+} ion were 29.8 x 10^{-8} mol/h and 27.8 x 10^{-8} mol/h, respectively, which are also larger than 3 of the simple model compound of Rebek's ionophore. The cleft or cavity size of ionophores **5** and **6** is similar to that of the original chelating agents developed by Rebek *et al.* but the molecular flexibility is larger than that of Rebek's molecule due to the absence of the two methyl groups at **3** and **5** position. The flexibility of ionophores **5** and **6** might be beneficial for these molecules to work as an efficient carrier. Ionophores **5** and **6** showed high Ca^{2+} selectivity in transport over Mg^{2+} and Sr^{2+}, and the selectivity ratio of **5** and **6** for Ca^{2+}/Mg^{2+} was. 29.8 and 10.0 and for Ca^{2+}/Sr^{2+} was 99.3 and 23.2, respectively. The methylenedianiline-based ionophores **4** has lower transport ability than the diaminopyridine-based ionophores **5** and **6** for Ca^{2+} ion.

Acknowledgment. This work was supported by the Ministry of Education (BSRI-96-3450).

References

1. Lehn, J. M. *Angew. Chem. Int. Ed. Engl.* **1988,** *27,* 89.
2. Cram, D. J. *Angew. Chem. Int. Ed. Engl.* **1988,** *27,* 1009.
3. Balzani, V.; Cola, L. D. *Supramolecular Chemistry;* Kluwer Dordrecht, 1992; p 17.
4. Schierbaum, K. D.; Weiss, T.; van Velzen, E. U. T.; Engberson, J. F. J.; Reinhoudt, D. N.; Gopel, W. *Science* **1994,** *265,* 1413.
5. Tsukube, H.; Inoue, T.; Hori, K. *J. Org. Chem.* **1994,** *59, 8047.*
6. Kasuga, K.; Hirose, T.; Takahashi, T.; Hiratani, K. *Chem. Lett.* **1993,** 2183.
7. Cadogan, A.; Gao, Z.; Lewenstam, A.; Ivaska, A. *Anal. Chem.* **1992,** *64,* 2496.
8. Rebek, J., Jr. *Angew. Chem. Int. Ed. Engl.* **1990,** *29,* 245.
9. Rebek, J., Jr. *Pure& Appl. Chem.* **1989,** *61,* 1517.
10. Zurer, P. *C&ENews* **1988,** *Aug. 1,* 21.
11. Marshall, L.; Parris, K.; Rebek, J., Jr.; Luis, S. V.; Burguete, M. I. *J. Am. Chem. Soc.* **1988,** *110,* 5192.
12. Hirose, T.; Baldwin, B. W.; Uchimaru, T.; Tsuzuki, S.; Uebaysahi, M.; Taira, K. *Chem. Lett.* **1995,** *231.*
13. Hirose, J.; Baldwin, B. W.; Wang, Z. H.; Kasuga, K.; Uchimaru T.; Yliniemela, A. *Chem. Commun.* **1996,** 391.
14. Jeong, K. S.; Tjivikua, T.; Muehldorf, A.; Deslongchamps, G.; Famulok, M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991,** *113,* 201.
15. Rebek, J., Jr.; Marshall, L.; Wolak, R.; Parris, K.; Killoran, M.; Askew, B.; Nemeth, D.; Islam, N. *J. Am. Chem. Soc.* **1985,** *107,* 7476.
16. Sadakane, A.; Iwachido, T.; Toei, K. *Bull. Chem. Soc. Jpn.* **1975,** *48,* 60.
17. Hasegawa, Y.; Nakano, T.; Odori, Y.; Ishikawa, Y. *Bull. Chem. Soc. Jpn.* **1984,** *57,* 8.
18. Takeda, Y.; Nishida, M. *Bull. Chem. Soc. Jpn.* **1989,** *62,* 1468.
19. Ogata, M.; Fujimoto, K.; Shinkai, S. *J. Am. Chem. Soc.* **1994,** *116,* 4505.
20. Walkowiak, W.; Kang, S. L; Stewart, L. E.; Ndip, G.; Bartsch, R. A. *Anal. Chem.* **1990,** *62,* 2022.
21. Habata, Y.; Ugajin, H.; Akabori, S. *J. Org. Chem.* **1994,** *59,* 676.
22. Habata, Y.; Ikeda, M.; Akabori, S. *J. Chem. Soc., Perkin Trans. 1* **1992,** 2651.
23. Charewicz, W. A.; Heo, G. S.; Bartsch, R. A. *Anal. Chem.* **1982,** *54,* 2094.
24. Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971,** *71,* 525.