Anal. Chem. **1995,** *67,* 324–334

Design and Synthesis of Calcium and Magnesium Ionophores Based on Double-Armed Diazacrown Ether Compounds and Their Application to an Ion-Sensing Component for an Ion-Selective Electrode†

Koji Suzuki,* Kazuhiko Watanabe, Yukihiro Matsumoto, Mitsuru Kobayashi, Sayaka Sato, Dwi Siswanta, and Hideaki Hisamoto

Department of Applied Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223, Japan

The double-armed diazacrown ethers, which have a base diazacrown ether ring with two diamide-type side chains, were designed and synthesized on the basis of the proposed molecular model for the novel neutral Ca^{2+} and Mg^{2+} ionophores. The potentiometric ion-selective electrodes were prepared with over 20 kinds of systematically synthesized diazacrown ether derivatives. The relationship between the molecular structures of the ionophores and the ion selectivities was fully discussed. The electrodes based on the 21- and 18-membered diazacrown ether derivatives possessing a glycolic diamide and malonic diamide in their side chains (K23E1 and K22B5) exhibited excellent Ca^{2+} and Mg^{2+} selectivities, respectively. The ion-selectivity features of the novel Ca^{2+} and Mg^{2+} ionophores supply important structural information about the design of host molecules for alkaline earth metal cations.

Calcium is one of the essential electrolytes in the human body, and its biological actions nave been actively investigated.[1,2] For monitoring this important electrolyte, a calcium ion-selective electrode, which is a convenient chemical sensor, has been successfully developed and utilized.[3–5] Magnesium is also an important blood electrolyte. Nevertheless, despite the large demand for monitoring Mg^{2+} in human and animal bodies, a Mg^{2+}-selective electrode with adequate characteristics satisfying the requirement for blood analysis has not been developed to date. In the past, several Mg^{2+}-selective ionophores for an ion-selective electrode, which were mostly β-diketone types, were investigated.[4–9] However, their Mg^{2+} selectivity is not good enough for blood analysis.

To obtain excellent alkaline earth metal divalent cation-selective ionophores, especially a Mg^{2+} ionophore for ISE, the ionophore molecule must have high selectivity and high sensitivity to Mg^{2+}. The former requirement can be simply understood by considering the well-designed ionophore chemical structure as the selective coordinating ligand for Mg^{2+}. On the other hand, the latter requirement is a difficult subject and the stability of the complex formed by the ionophore and the cation must be considered, in which the ion complex should not have too high or too low a stability constant. In fact, during the first stage of our investigation, we attempted to use some lipophilic cryptand derivatives such as [2.2.1] and [2.2.2] types for the divalent cation-selective ionophore; however, the electrodes using these cryptands did not exhibit any divalent cation selectivity, since these types of macrobicycles have high stability for alkaline earth metal ions and, therefore, the electrodes do not have a reversibile response to divalent cations.[10]

The ionic binding features are generally discussed using the hardsoft and acidbase (HSAB) concept.[11] The ionic binding order of nonionic functional groups is roughly ester < ether < phosphate < amide < amine, which is based on the donicity values as one of the Lewis acidity–basicity factors of many simple organic molecules.[12] A water molecule has donicity value similar to those of some ester- and ether-type compounds. Thus, when considering the coordinating feature of alkaline earth metal ions such as Ca^{2+} or Mg^{2+} in an aqueous medium, an ionophore possessing a phosphate, amide, or amine group, which offers a higher donicity than those of ester and ether groups, is more preferable as a binding site for alkaline earth metal cations, because the ionophore has a stronger electrostatic coordinating power for oxygen or nitrogen than an ester or ether group. The ionophore can coordinate alkaline earth metal divalent cations strongly because these divalent cations have a higher surface charge density than alkali metal monovalent cations. However, an ionophore having an amine group is not suitable for the ion-sensing component of

† This report is dedicated to the late Professor Wilhelm Simon of ETH, Zürich, Switzerland.

(1) *Calcium Transport and Intracellular Calcium Homeostasis*; Panser, D., Bronner, F., Eds.; NATO ASI Series H, *Cell Biology*, Vol. 48; Springr-Verlag: New York, 1990.
(2) *Calcium Ion and Cell Functions*; Endo, M., Nishizawa, Y., Yagi, K., Miyamoto, E., Eds.; Kyoritsu-Shuppan: Tokyo, Japan, 1988.
(3) Gasser, R. *Sel. Electrode Rev.* **1988**, *10*, 49.
(4) Byrne, T. P. *Sel. Electrode Rev.* **1988**, *10*, 107.
(5) Umezawa, Y. *Handbook of Ion-Selective Electrodes: Selectivity Coefficients*; CRC Press: Boca Raton, FL, 1990.
(6) Erne, D.; Morf, W. E.; Arvanitics, S.; Cimerman, Z.; Amman, D.; Simon W. *Helv. Chim. Acta* **1985**, *68*, 110.
(7) Zhongmin, H.; Buhrer, T.; Muller, M.; Rusterholz, B.; Rouilly, M.; Simon, W. *Anal. Chem.* **1989**, *61*, 574.
(8) Nagashima, H.; Tohda, K.; Matsunari, Y.; Tsunekawa, Y.; Watanabe, K.; Inoue, H.; Suzuki, *Anal. Lett.* **1990**, *23*, 1993.
(9) Rouilly, M.; Rusterholz, B.; Spichger, U. E.; Simon, W. *Clin. Chim.* **1990**, *36*, 466.
(10) Aruga, H.; Suzuki, K.; Shirai, T. Presented at the 47th Spring Meeting of the Chemical Society of Japan, April 1983, Tokyo, Japan, 1983; Abstr. 3W05.
(11) Pearson, R. G. *J. Chem. Educ.* **1968**, *45*, 643.
(12) Jensen, W. B. *The Lewis Acid–Base Concepts*; John Wiley & Sons: New York, 1980, Chapter 7.

0003-2700/95/0367-0324$9.00/0

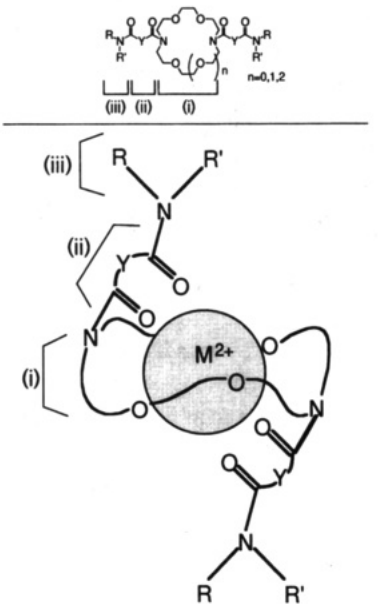

Figure 1. Ionophore molecular model for alkaline earth metal cations such as Ca^{2+} and Mg^{2+}: (i) base backbone ring (azacrown ether) section; (ii) side binding arm (side chain) section; (iii) lipophilic terminal (bulky subunit) section.

a potentiometric sensor since its binding power to divalent cations is very high, as was mentioned previously for the cryptand case. In addition, this type of ionophore also has a strong proton affinity, which causes interference from H^+ in the sample, so that an electrode based on such an ionophore would be hard to use in practical measurements without a pH-adjusting buffer.

Here we report the design and preparation of novel highly Ca^{2+}- and Mg^{2+}-selective ionophores based on the proposed model, which takes into consideration the above-mentioned binding features as well as the tridimensional coordination space design for the ionophore–cation complex formation. The proposed ionophore model shown in Figure 1, which is a double-armed diazacrown ether possessing diamides in its side arms, has the following structural features: (i) base azacrown ring, which offers hydrophilic space for cations to fit into the cavity size of the ring; (ii) diamide side chains, which offer effective binding sites for divalent cations and also produce the tridimensional coordination space formed together with the base azacrown; and (iii) lipophilic subunits at the terminals of the side chains, which offer adequate lipophilicity for the complex formed with the ionophore molecule and the divalent cation.

Ion-selective features are examined with the electrodes using over 20 kinds of newly prepared diazacrown derivatives that were systematically synthesized in accord with the three structural factors involved in the proposed ionophore model. As a result, the electrodes based on 21- and 18-membered ring diazacrown ether derivatives having a glycolic diamide and malonic diamide side chains exhibited excellent Ca^{2+} and Mg^{2+} selectivities, respectively (K23E1, K23E5, and K22B; see Figures 5 and 8). The ion-selective electrode based on K23E1 has sufficient sensitivity and lipophilicity that the determination of Ca^{2+} for blood analysis and Ca^{2+} concentrations in human serum was successfully accomplished with this electrode. The novel molecules K23E1 and K22B5 are two of the best Ca^{2+} and Mg^{2+} ionophores developed to date compared to other Ca^{2+} and Mg^{2+} ionophores because of their ion selectivity features. The obtained results concerning the structural selectivity features for alkaline earth metal ions supply important knowledge of host–guest complex chemistry.

EXPERIMENTAL SECTION

Reagents. The highest grade, commercially available reagents were used for the syntheses of neutral ionophores and for the preparation of the aqueous sample electrolytes. The distilled and deionized water had resistivity of more than 1.5×10^7 Ω cm at 25 °C. Dioctyl sebacate (DOS; (dielectric constant $\epsilon = 4$) and 2-nitrophenyl octyl ether (NPOE; $\epsilon = 24$), used as membrane solvents (plasticizers), were purchased from Fluka AG (Buchs, Switzerland) and Dojindo Laboratories (Kumamoto, Japan), respectively. Poly(vinyl chloride) (PVC; high molecular weight type) used as the electrode membrane material was obtained from Sigma Chemical Co. (St. Louis, MO). Potassium tetrakis(*p*-chlorophenyl)borate (K-TCPB), used as the anionic additive for the polymeric membrane, was obtained from Dojindo Laboratories. The test serum samples were supplied from Ortho Diagnostics Inc. (Raritan, NJ). 1,4,10-Trioxa-7,13-diazacyclopentadecane, 1,7,10,16-tetraoxa-4,13-diazacyclooctadecane, and 1,7,10,13,19-pentaoxa-4,16-diazacyclohenicosane (Kryptofix 21, 22, and 23, respectively), which were used for the base azacrown ring for the synthesized ionophores, were purchased from Merck AG (Darmstadt, Germany). Monomethyl malonate potassium salt, used as the starting material for the B-type side chain (a side chain including malonamide) for azacrown derivatives, was purchased from Fluka AG. All other chemicals for the synthesis of ionophores were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Ionophore Synthesis. The chemical structures of the synthesized ionophores are indicated in Figures 2–9. The structures of all synthesized compounds were confirmed by ^1H NMR, IR, and elemental analysis. These analytical data for all new compounds are described in the supplemental material.

The Basic-AM-I (azelaic acid *N,N,N',N'*-tetraphenylamine) was synthesized by dissolving 1.5 g (8.0 mmol) of azelaic acid in 5 mL of thionyl chloride, followed by evaporation of the excess thionyl chloride, and mixing with 2.0 mg of diphenylamine in 10 mL of absolute pyridine. The reaction product was extracted three times with 100 mL of chloroform/water (5/1). After evaporation of the chloroform layer, the product was purified by silica gel column chromatography (eluent, hexane/ethyl acetate, 4/1) which gave 0.9 g of Basic-AM-I (yield, 31%). Using the same procedures for the synthesis of Basic-AM-I with benzyl acid and 1,7-diaminoheptane instead of azelaic acid *N,N,N',N'*-tetraphenylamine and diphenylamine, respectively, Basic-AM-II (1,7-dibenzoyl-1,7-diaminoheptane) was prepared (yield, 40%).

The Basic-ES and Basic-ET were synthesized as follows. Azelaic acid (1.5 g, 8.0 mmol) and phenol (1.5 g, 16.0 mmol) were dissolved in 8 mL of absolute dimethylformamide (DMF) at 0 °C, and 3.1 g (1.1 equiv) of diethylthianosulfonic acid was added. After the addition of 3.7 g (2.1 equiv) of trimethylamine into the DMF solution, the reaction mixture was allowed to stand for 4 h and then the DMF was evaporated. The product was extracted three times with 100 mL of chloroform/water (5/1). After evaporation of the chloroform, the product was purified by silica gel column chromatography (eluent, hexane/ethyl acetate 5/1) thus giving Basic-ES (azelaic acid diphenyl ether; yield, 65%). Benzohydrol,

1.5 g was dissolved in absolute DMF and 0.3 g of NaH (1.5 equiv) and 0.9 g of 1.7-dibromoheptane (0.5 equiv) were added, and the mixture was heated at 100 °C for 1 h. The DMF solution was then evaporated and the product extracted three times with 100 mL of chloroform/water (1/1). The product was purified by silica gel column chromatography (eluent, hexane/ethyl acetate 5/1), which gave Basic-ET (1,1,9,9-tetraphenyl-2,8-dioxanonane; yield, 64%).

Ionophore K22A (4,13-didodecanoyl-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane) was synthesized by dissolving 180 mg of 1,7,-10,16-tetraoxa-4,13-diazacyclooctadecane (Kryptofix 22; K22, 0.7 mmol) and 300 mg of *n*-dodecanoyl chloride (1.4 mmol) into 0.9 mL of absolute pyridine, which was then allowed to stand for 1 day at room temperature. The reaction mixture was then mixed with chloroform and 5 wt % $NaHCO_3$ followed by evaporation of the chloroform layer to yield 133 mg (31%) of K22A as a white powder. The product was purified by silica gel column chromatography with hexane/ethyl acetate (1/2) as the eluent. K22B (4,13-[bis(*N*-dodecylcarbamoyl)acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane) was synthesized according to the following procedures. Monomethyl malonate potassium salt (3.0 g, 19.2 mmol) was suspended in 20 mL of CH_2Cl_2. Thionyl chloride was then added to the suspension at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The precipitated white crystals were filtered off from the mixture, and the filtrate was concentrated to yield monomethyl malonyl chloride as a yellowish liquid. The dodecylamine (3.56 g, 19.2 mmol) was dissolved in 20 mL of CH_2Cl_2, and monomethyl malonyl chloride was slowly added to the CH_2Cl_2 solution at 0 °C. The reaction mixture was stirred at room temperature for 1 day and then dissolved in chloroform. After the mixture was washed twice with HCl (pH 2) and water, respectively, the organic phase was evaporated. The residue was purified by silica gel column chromatography with hexane/ethyl acetate (1/1) as the eluent to yield 540 mg (10%) of (*N*-dodecylcarbamoyl)methyl acetate as a white powder. This powder (540 mg, 1.9 mmol) was added to a mixture of methanol/water (15 mL/5 mL), and 0.16 g of LiOH (3.8 mmol) was then added together with 5 mL of water. After the reaction mixture was stirred for 15 h, the methanol/water was evaporated and the residue was dissolved in water in which the solution was adjusted to pH 1 with concentrated HCl. The solution was then extracted three times with ethyl acetate. The ethyl acetate was evaporated to yield 486 mg (95%) of (*N*-dodecylcarbamoyl)acetic acid as a white powder. This powder (207 mg, 0.8 mmol) was dissolved in 7 mL of CH_2Cl_2, and 0.32 mL (2.2 mmol) of triethylamine was added to the CH_2Cl_2 solution at 0 °C. [*N*,*N*-Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (194 mg, 0.76 mmol) and 100 mg (0.38 mmol) of 1,7,10,16-tetraoxa-4,13-diazacyclooctadecane were added to the reaction mixture and the resultant mixture was stirred for 1 day at room temperature. After 100 mL of chloroform/water (1/1) was added to the reaction mixture, the chloroform layer was evaporated and the residue was purified by reversed-phase HPLC (ODS column; eluent, methanol) to yield 128 mg (44%) of K22B as a white powder. K21B and K23B were synthesized according to the same procedures for obtaining K22B, in which 1,4,10-trioxa-7,13-diazacyclopentadecane (Kryptofix 21, K21) and 1,7,10,13,19-pentaoxa-4,16-diazacyclohenicosane (Kryptofix 23, K23), respectively, were used in place of K22 as the base crown ring section of the double-armed azacrown derivatives. K22B1 (4,13-[bis(*N*-octadecylcarbamoyl)acetyl]1,7,10,16-tetraoxa-4,13-diazacyclooctadecane), K22B4 (4,13-[bis(*N*,*N*-dicyclohexylcarbamoyl)-acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane), K22B5 (4,13-[bis(*N*-adamantylcarbamoyl)acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane), K22B6 (4,13-[bis(*tert*-butylcyclohexyl)-carbamoyl)acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane), K22B7 (4,13-[bis(*N*-phenylcarbamoyl)acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane), and K22B8 (4,13-[bis(*N*,*N*-diphenyl-carbamoyl)acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane) were synthesized according to the same procedures used for the preparation of K22B, in which octadecylamine, dicyclohexylamine, adamantylamine, 4-*tert*-butylcyclohexylamine, aniline, and diphenylamine were used for obtaining K22B1, K22B4, K22B5, K22B6, K22B7, and K22B8, respectively, in place of the dodecylamine used for the synthesis of the arm section of K22B.

K22C (4,13-[bis(*N*-dodecylcarbamoyl)propionyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane) was synthesized by the following procedures. Succinic anhydride (2.0 g, 20 mmol) and 3.7 g (20 mmol) of dodecylamine were added to 30 mL of pyridine and the resultant mixture was refluxed for 3 days. The pyridine was then evaporated, and the residue was dissolved in 30 mL of diethyl ether and 30 mL of HCl aqueous solution (pH 1.5). After the diethyl ether layer was evaporated, the recrystallization was performed with methanol/water (1/1) to obtain 5.4 g (95%) of (*N*-dodecylcarbamoyl)methyl propionate as a white powder. This powder (218 mg, 0.8 mmol) was dissolved in 7 mL of CH_2Cl_2, and 0.2 mL (1.6 mmol) of triethylamine and 194 mg (0.8 mmol) of [*N*,*N*-bis(2-oxo-3-oxazolidinyl)]phosphinic chloride were added to the mixture at 0 °C. After standing for 15 min, 1,7,10,16-tetraoxa-4,13-diazacyclooctadecane (100 mg, 0.4 mmol) was added to the reaction mixture, and the resultant mixture was stirred for 1 day at room temperature. The reaction mixture was dissolved in chloroform and then extracted three times with chloroform and with the same amount of water. The chloroform was evaporated, and the residue was dissolved in methanol. The purification was performed with reversed-phase HPLC (ODS column; eluent, methanol) to yield 122 mg (40%) of K22C as a white powder. K22D (4,13-[bis(*N*-dodecylcarbamoyl butyryl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane) and K22E (4,13-[bis(*N*-dodecylcarbamoyl)-3-oxabutyryl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane) were synthesized according to the same procedures for obtaining K22C, in which glutalic anhydride and diglycolic anhydride were used for the preparation of K22D and K22E (yields, 41−45%), respectively, in place of succinic anhydride to synthesize the side-arm section of K22C. K21E, K23E, and K50E (yield, 45−53%) were prepared by the same procedures for obtaining K22E, in which 1,4,10-trioxa-7,13-diazacyclopentadecane (K21), 1,7,10,13,19-pentaoxa-4,16-diazacyclohenicosane (K23), and 1,4,7,10,13-pentaoxa-16-azacyclooctadecane (K50) were used for the base azacrown ring section of K21E, K23E, and K50E, respectively. In addition, five additional kinds of 18-membered diazacrown derivatives of K22E1, K22E2, K22E3, K22E4, and K22E5 (yield, 42−65%), which all possess glycolic diamide side chains, were synthesized by the same procedures for obtaining K22E, in which *n*-octadecylamine, di-*n*-octylamine, 3-aza-bicyclo[3,2,2]nonane, dicyclohexylamine, or 1-adamantylamine was used instead of using *n*-laurylamine for the synthesis of K22E that corresponds to the terminal of the arm section of the 18-membered diazacrown derivatives (K22-type compounds). In addition, K23E1 and K23E5 were also prepared

via the same procedures for the synthesis of K22E1 and K22E5 (yield, 45−52%) using the 21-membered diazacrown (K23) as the base diazacrown ring section. Furthermore, a noncyclic glycolic diamide derivative, Basic-E (*N,N*-didodecyl-3-oxapentanediamide), was prepared via the same procedures for obtaining K22E where *n*-laurylamine was used instead of K22.

Ionophore 6A2B5 (1,4-[bis(*N*-adamantylcarbamoyl)acetyl]-1,4-diazacyclohexane) was synthesized according to the following procedures. (*N*-Adamantylcarbamoyl)acetic acid, the side-arm section of 6A2B5, was synthesized via the same procedures for obtaining the arm section of K22B described above. (*N*-Adamantylcarbamoyl)acetic acid (83 mg, 0.35 mmol) was dissolved in 10 mL of CH_2Cl_2. Triethylamine (0.10 mL, 0.69 mmol), [*N,N*-bis(2-oxo-3-oxazolidinyl)]phosphinic chloride (88 mg, 0.35 mmol) and 1,4-diazacyclohexane (15 mg, 0.17 mmol) were then added to the CH_2Cl_2 solution, and the resultant mixture stirred for 1 day at room temperature. The reaction mixture was introduced to 50 mL of chloroform/water (1/1) solution, and the chloroform layer was evaporated. The product was purified by reversed-phase HPLC (ODS column; eluent, methanol) to yield 64 mg (73%) of 6A2B5 as a white powder. Ionophores of 12A3B5 (1,5,9-[tris(*N*-adamantylcarbamoyl)acetyl]-1,5,9-triazacyclododecane), 14A4B5 (1,4,8,11-[tetrakis(*N*-adamantylcarbamoyl)acetyl]-1,4,8,11-tetraazacyclotetradecane), 18A6B5 (1,4,7,10,13,16-[hexakis(*N*-adamantylcarbamoyl)acetyl]-1,4,7,10,13,16-hexaazacyclooctadecane), and K2B5 (*N,N'*-3,7-dioxaoctamethylenebis(*N'*-adamantylmalonamide)) were synthesized (yields, 65−78%) according to the same method for the synthesis of 6A2B5, in which 1,5,9-triazacyclododecane, 1,4,8,-11-tetraazacyclotetradecane, 1,4,7,10,13,16-hexaazacyclooctadecane, and 2,2'-(ethylenedioxy)diethylamine was used for 12A3B5, 14A4B5, 18A6B5, and K2B5, respectively, instead of using 1,4-diazacyclohexane, which corresponds to the base ring section of 6A2B5.

Ionophore 18A4O2B5 (4,7,13,16-[tetrakis(*N*-adamantylcarbamoyl)acetyl]-1,10-dioxa-4,7,13,16-tetraazacyclooctadecane) was synthesized using the following procedures. Ethylenediamine (5 g, 83 mmol) and 40 g (210 mmol) of *p*-toluenesulfonyl chloride were added to 80 mL of pyridine at 0 °C and the resultant mixture stirred for 4 h at room temperature. The pyridine was then evaporated, and the residue was mixed with 100 mL of water/chloroform (1/1). Recrystallization from chloroform produced 23 g (75%) of ethylendiamine ditosylate as yellowish crystals. These crystals (12.8 g, 35 mmol) and 5 g (35 mmol) of bis(2-chloroethyl) ether were added to 72 mL of DMF, and 7.2 g (52 mmol) of K_2CO_3 was suspended in the mixture, which was then refluxed for 5 h. After the solution was cooled to room temperature, a large amount of water (>200 mL) was added to the reaction mixture. The yellowish gummy product was separated by filtration and washed three times with water. The product was then dissolved in chloroform, and the solution was dried by adding Na_2SO_4. The chloroform was evaporated, and the residue was dried under reduced pressure to yield 13.9 g (78%) of 1,10-dioxa-4,7,13,16-tetraazacyclooctadicane tetratsylate as white crystals. These crystals (13.9 g, 15.8 mmol) were dissolved in a mixture of 48% HBr/acetic acid (1/1) solution and refluxed for 6 days. The mixture was then cooled to room temperature, and diethyl ether was added to the mixture until a white crystal precipitated. After the white crystals were separated by filtration, the crystals were dissolved in 50 mL of 30% KOH aqueous solution and extracted three times with CH_2Cl_2. The organic solution was concentrated to yield 123 mg (3%) of 1,10-dioxa-4,7,13,16-tetraazacyclooctadicane as white crystals. (*N*-Adamantylcarbamoyl)acetic acid (145 mg, 0.61 mmol) was dissolved in CH_2Cl_2 (20 mL), and 0.18 mL (1.2 mmol) of triethylamine was added to the CH_2Cl_2 solution at 0 °C and stirred. Then, 156 mg (0.61 mmol) of [*N,N*-bis(2-oxo-3-oxazolidinyl]phosphinic chloride and 40 mg (0.15 mmol) of 1,10-dioxa-4,7,13,16-tetraazacyclooctadicane were added to the reaction mixture and the resultant mixture stirred for 1 day at room temperature. After the reaction mixture was dissolved in chloroform/water (1/1), the chloroform was evaporated and the residue was purified by reversed-phase HPLC (ODS column; eluent, methanol) to yield 90 mg (53%) of 18A4O2B5 as a white powder.

Basic-B5 (*N,N'*-diadamantylmalonamide) was synthesized according to the following procedures. Adamantylamine (47 mg, 0.31 mmol) and (*N*-adamantylcarbamoyl)acetic acid (75 mg, 0.31 mmol) were dissolved in 20 mL of CH_2Cl_2 and the resultant mixture stirred for 30 min at room temperature. Then, 70 mg (0.31 mmol) of 2,2'-dipyridyl disulfide and 83 mg (0.31 mmol) of triphenylphosphine were added to the mixture and the resultant mixture stirred for 1 day at room temperature. After the reaction mixture was combined with 50 mL of chloroform/water (1/1), the organic layer was concentrated and purified by silica gel column chromatography using ethyl acetate as the eluent, followed by reversed-phase HPLC (ODS column; eluent, methanol) to yield 67 mg (58%) of Basic-B5 as a white powder.

Electrode Preparation and emf Measurements. Ion-sensitive electrode membranes of the PVC matrix type were prepared according to a previously reported procedure.[13] The membrane compositions were basically 2 wt % ionophore, 66 wt % membrane solvent (NPOE or DOS) and ~32 wt % PVC, in which 100 mol % K-TCPB relative to the ionophore content was added to the membrane. The prepared ion-sensitive membrane was cut as a 6 mm circle (~200 μm in thick) and placed into the tip of the ion-selective electrode body assembly (Liquid Electrode Membrane Kit, DKK Co., Ltd., Tokyo, Japan). The prepared electrodes were immersed in 0.1 M $MgCl_2$ or $CaCl_2$ solution for over 12 h of preconditioning before use. The electrode response potential (emf) measurements were performed at 25 ± 0.5 °C with the reported procedures using the electrochemical cell system

$$\text{Ag; AgCl, 3 M KCl}|\text{0.3 M } NH_4NO_3|\text{test solution}|\text{membrane}|\text{0.1 M } MCl_2\text{, AgCl; Ag} \quad (M = \text{Mg or Ca})$$

All sample solutions were prepared with chloride cations, but without any pH adjusting buffer. The selectivity coefficients were calculated from response potentials in 0.1 M cation chloride test solutions using the separate solution method (SSM), which was performed according to the IUPAC recommendation.[14]

Determination of Lipophilicities of Ionophores. The lipophilicities of synthesized ionophores, log $P_{o/w}$ (distribution coefficient between organic liquid and water), were determined from the R_f values of the reversed-phase thin-layer chromatography (RP-TLC) according to the method reported by Simon et al.[15] The

(13) Suzuki, K.; Tohda, K.; Aruga, H.; Matsuzoe, M.; Inoue, H.; Shirai, T. *Anal. Chem.* **1988**, *60*, 1714.
(14) IUPAC Recommendation for Nomenclature of Ion-Selective Electrodes. *Pure Appl. Chem.* **1976**, *48*, 129.
(15) Laubli, M. W.; Dinton, O.; Pretsch, E.; Simon, W. *Anal. Chem.* **1985**, *57*, 2756.

RP-TLC consisted of octadecylsilane-modified reversed-phase silca plates (KC18F, Whatman) that were to 18 × 20 cm and developed chromatographically with ethanol/water (9/1) as the mobile phase.

Complex Stoichiometry Study. The stoichiometry for the Ca^{2+}−K22E complex was determined using the following procedures with ^1H NMR spectroscopy. Ionophore K22E (30 mg, 3.6 × 10^{-5} mol) was dissolved in 1 mL of $CDCl_3$ and $^1/_5$−5 times mole amounts of $CaCl_2$ dissolved in 1 mL of D_2O were added. After vigorously shaking the mixture for 10 min, the ^1H NMR spectra ranging from δ = 0 ppm (TMS base proton value) to 10 ppm of the $CDCl_3$ phase were measured with a 220 MHz NMR spectrophotometer (GX-220, JEOL, Tokyo, Japan). The chemical shift value of the methylene proton (δ = 4.05 ppm for ion-free K22E) at CH_2CO in the arm section of K22E was monitored in the ^1H NMR spectra, and the varied chemical shift value ($\Delta\delta$ measured $\delta(CH_2CO)$ value in ppm minus 4.05) was plotted as a function of the mole ratio of $CaCl_2$/K22E in the test sample in order to determine the stoichiometry for the Ca^{2+}−K22E complex.

RESULTS AND DISCUSSION

Alkaline Earth Metal Ionophore Design. Before discussion of the structural features of the newly synthesized Ca^{2+} and Mg^{2+} ionophore molecules based on the ionophore model shown in Figure 1, ion-selective features of functional groups used as a binding site for cations were examined with the electrodes by using four basic compounds that include an amide, diester, or diether group as the binding site for cations. The chemical structures and ion-selective features of these basic compounds are shown in Figure 2 (Basic-AM-I, Basic-AM-II, Basic-ES, Basic-ET). These are diamides (Basic-AM-I and Basic-AM-II; Basic-AM-II has amide groups different from Basic-AM-I in the carbonyl and amine positions), diester (Basic-ES), and diether (Basic-ET), which are all lipophilic, noncyclic compounds having seven methylene chains between functional groups as binding sites for cations. If the number of methylene chains is small (probably one to three), the ion-selective features must primarily depend on the distance between the coordination sites. Hence, the methylene number of seven (more than four) was chosen for these basic compounds. In Figure 2, the ion-selectivity coefficients were determined based on Ca^{2+} as the primary ion. Though all four basic compounds exhibited high Cs^+ selectivity, amide-type compounds (Basic-AM-I and Basic-AM-II) apparently are more selective for alkaline earth metal ions against alkali metal ions compared with those of the ester- and ether-type compounds (Basic-ES and Basic-ET), as we expected. The latter two compounds showed both very high Cs^+ selectivity and exhibited poor alkaline earth metal cation selectivity, as demonstrated in Figure 2. Using the basic amide compound Basic-AM-I, the effect of polarity of the electrode membrane on its ion-selective feature was also examined with a typical relatively high polarity membrane solvent, NPOE (ϵ = 24), and a low-polarity membrane solvent, DOS (ϵ = 4), which are typical plasticizers for PVC membrane electrodes. In Figure 2, the selectivity factors for the blank membranes based on NPOE or DOS without any ionophores are also demonstrated, which indicate the ion-selective features of the TCPB anion itself as an anionic additive in the electrode membrane. As shown in the first column (for NPOE-based membrane) and the second column (for DOS-based membrane) of Figure 2, the ion selectivity of the electrode using Basic-AM-I for alkaline earth metal ions against alkali metal ions was preferable in the

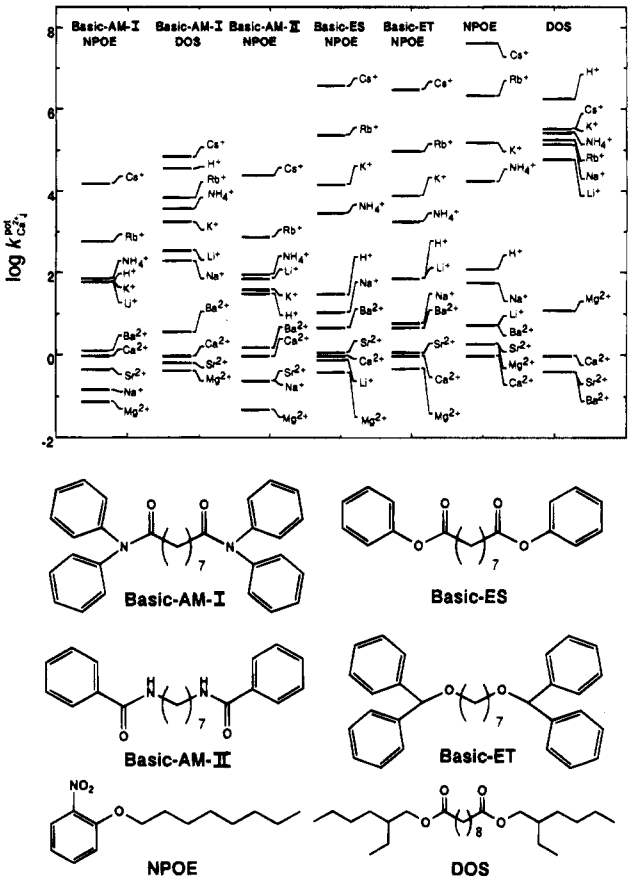

Figure 2. Selectivity factors for electrodes based on two basic diamides (Basic-AM-I and Basic-AM-II), a basic diester (Basic-ES), a basic diether (Basic-ET), NPOE (a blank membrane), and DOS (a blank membrane). Electrode membrane composition: For ionophore-based membranes, 2 wt % ionophore, 66 wt % NPOE or DOS, and 32 wt % PVC including 50 mol % K-TCPB against the ionophore content. For blank membranes, 66 wt % NPOE or DOS, 1 wt % K-TCPB, and 33 wt % PVC.

case where a high-polarity solvent such as NPOE was used as the electrode membrane solvent. Consequently, the following investigation was performed with the PVC matrix membrane electrodes using NPOE as the membrane solvent.

In consideration with these basic results on binding features of some important functional groups for alkaline earth metal ions, the ionophore model for Ca^{2+} and Mg^{2+} was designed as shown in Figure 1. This model basically consists of three functional sections: (i) base backbone ring, (ii) side arms, and (iii) lipophilic terminals in the side arms. As briefly described in the introduction, these all play important roles for selective coordination to the alkaline earth metal divalent cations: (i) the base azacrown ring offers a tridimensional as well as hydrophilic coordination space for a cation which fits into the cavity size of the ring. Hence, this section is important for the size fit concept concerning the cavity. (ii) Since the amide group is effective for the selective coordination of alkaline earth metal cations, side arms possessing diamides offer suitable coordination site fits for the cation, in which the distance between amide groups is also an important factor for ion selectivity, because the carbonyl oxygens in the amide groups as coordination sites in the side arms produce a specific configuration together with the base crown ring that is important for selective coordination to alkaline earth cations. (iii) High lipophilicity is required for the ionophore since it is used as a

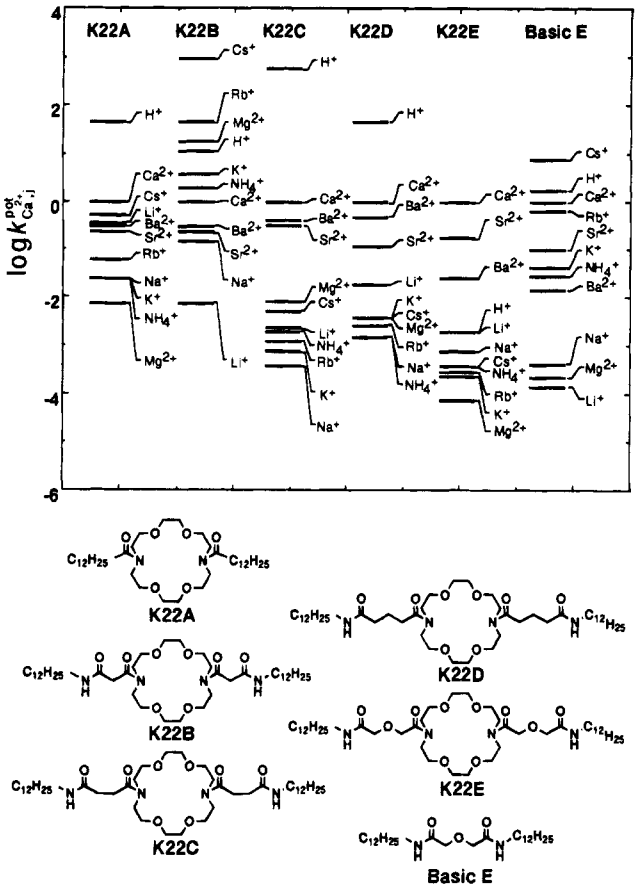

Figure 3. Selectivity factors for the electrodes based on five types of 18-membered diazacrown ether derivatives with different binding arms (K22A, K22B, K22C, K22D, K22E) and a noncyclic diamide (Basic-E). Electrode membrane composition: 2 wt % ionophore, 66 wt % NPOE, and 32 wt % PVC including 100 mol % K-TCPB (or 50 mol % K-TCPB for Basic-E) against the ionophore content.

component for a sensor membrane with a long lifetime. Introduction of a lipophilic group such as an alkyl or adamantyl group into an ionophore molecule often varies its selectivity feature. In addition, introducing a bulky lipophilic group or subunit into the ionophore molecule brings a lipophilicity as well as blocking wall effect into the molecule, making it hard to form a stable complex with a large-size cation.

Based on the ionophore model shown in Figure 1, over 20 kinds of novel ionophore molecules were synthesized in which the chemical structures were systematically varied by changing the structures of the above-mentioned three functional sections.

Calcium Ionophores. First, we investigated the arm section of the azacrowns on the structural ion-selectivity feature. In this case, the base azacrowns were all 18-membered rings (K22) and the binding arm side chains were varied with the A–E types. As shown in Figure 3, the electrodes based on all these ionophores, except K22B, exhibited high Ca^{2+} selectivity. Especially, K22E showed that best Ca^{2+} selectivity as well as favorable poor proton affinity compared to those of all the other ionophores.

As one of the high Ca^{2+}-selective ionophores, *N,N,N',N'*-tetracyclohexyl-3-oxapentanediamide (ETH 129) was developed, which also has glycolic diamides that are reported to form a 3/1 ionophore–Ca^{2+} complex.[16] For comparing the ion-selective features of the double-armed azacrown, K22E and the simple noncyclic diamide *N,N*-didodecyl-3-oxapentanediamide (Basic E), which also has a structure similar to that of ETH129, were prepared. As shown in Figure 3, the Ca^{2+} selectivity was not very high compared with K22E, which has a structure similar to basic E in the arm section of the azacrown derivative. Furthermore, the Ca^{2+} complex stoichiometry was investigated with the double-armed azacrown (K22E) using proton NMR spectroscopy. From the ^1H NMR spectrum of this compound in $CDCl_3$, the methylene hydrogens next to the ether oxygen in the side arms (E-chain; glycolic diamide) appeared at $\delta = 4.05$ ppm versus the base value of the TMS proton ($\delta = 0$ ppm). With the addition of $CaCl_2$ to the $CDCl_3$ solution, the chemical shift value of the methylene protons shifted downfield. In the case where the downfield shift value ($\Delta\delta$: δ_{CH_2CO}(observed) − 4.05) was plotted as a function of the additive content of $CaCl_2$, the resulting $\Delta\delta$ values were almost saturated at Ca^{2+}/K22E = 1/1.[17] This indicates that the stoichiometry of K22E and Ca^{2+} is 1/1, as we expected during the ionophore designing stage.

For a Ca^{2+} ionophore based on the 18-membered diazacrown (K22), K22E is the most favorable compared among all others, as shown in Figure 3. The base crown ring size was then investigated with an E-type side chain (glycolic diamide) as the side arms and prepared different ring size azacrown derivatives having 15- and 21-membered ring with E-chains that are K21E and K23E, respectively. In addition, K50E, which is an 18-membered monoazacrown derivative with only one arm, was prepared to compare its ion-selectivity feature with other azacrown derivatives possessing double arms. As shown in Figure 4, these three ionophores (K21E, K23E, K50E) exhibited high Ca^{2+} selectivities but showed different selectivity behaviors against alkali metal monovalent cations. The best Ca^{2+} selectivity was obtained with K23E in which Ca^{2+} selectivity against monovalent cations such as Na^+ and K^+ was improved compared with the result with K22E (see Figure 3). The worst Ca^{2+}-selective ionophore was K50E, which is a monoazacrown derivative having one binding arm. This indicates that the double-armed azacrowns are apparently effective for divalent cation selectivity. Ring size is clearly affected in the monovalent alkali metal cation selectivity of the diazacrown derivative, which is the same as in cases of other simple crown ethers reported to date. The larger azacrown ring size gave better results for Ca^{2+} selectivity versus the small-size alkali metal ions such as Li^+ and Na^+ (see Figure 4).

The glycolic diamide as the side chain in K22- or K23-type diazacrowns is the most effective for Ca^{2+} selectivity. Hence, the different lipophilic subunits in the terminal of the side arms were examined with 18-membered diazacrown (K22) having glycolic diamide side chains (E-type side chain). The bulky lipophilic subunits used were *n*-octadecyl (E1), di-*n*-octyl (E2), azabicyclononyl (E3), dicyclohexyl (E4), and adamantyl (E5), which were introduced at the terminal of the glycolic diamide side chains in the K22-based diazacrown. Five types of compounds, K22E1, K22E2, K22E3, K22E4, and K22E5 were prepared and are shown in Figure 5. As shown in Figure 5, they all exhibited high Ca^{2+} selectivity. Regarding normal alkyl chains as the lipophilic subunit, the long alkyl chains introducing larger carbon numbers gave better results in Ca^{2+} selectivity than that of the similar

(16) Schefer, U.; Amman, D.; Pretsch, E.; Oesch, U.; Simon, W. *Anal. Chem.* **1988**, *58*, 2282.

(17) Watanabe, K. Ph.D. Thesis, Faculty of Science and Technology, Keio University, Yokohama, Japan, 1992.

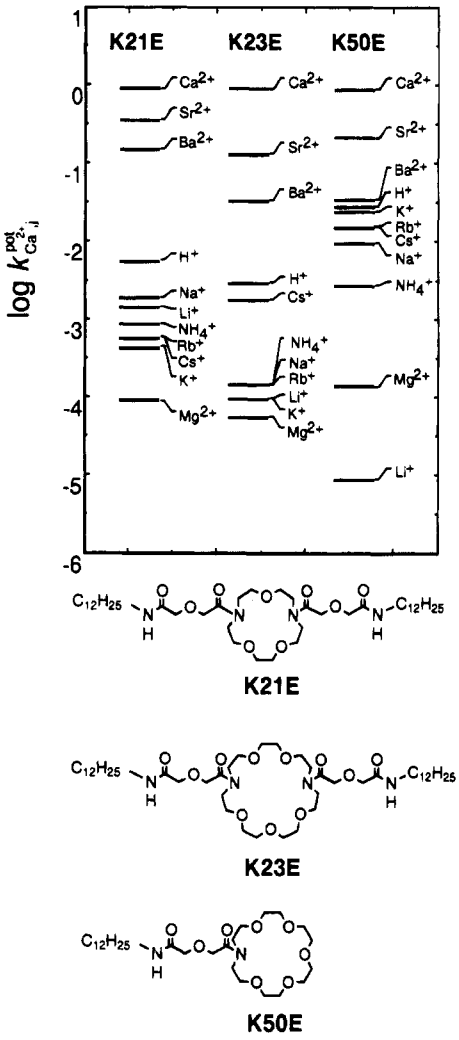

Figure 4. Selectivity factors for the electrodes based on three types of 15- (K21E), 18- (K50E), and 21-membered diazacrown ether derivatives (K23E). Electrode membrane composition: 2 wt % ionophore, 66 wt % NPOE, and 32 wt % PVC including 100 mol % K-TCPB (or 50 mol % K-TCPB for K50E) against the ionophore content.

azacrown derivatives having short alkyl chains (K22E1 > K22E in Ca^{2+} selectivity against other tested cations; see also Figure 3) or having two alkyl chains of a secondary amine type (K22E > K22E2 in Ca^{2+} selectivity). Though K22E3 and K22E4, with hexyl rings, showed very high Ca^{2+} selectivity, the best Ca^{2+} selectivity was observed with the electrode based on K22E5, which possessed an adamantyl group as the lipophilic subunit. Thus, the large base ring size diazacrown compounds, K23E5 and K23E1, which possess a bulky adamantyl or a long alkyl chain of the *n*-octadecyl group at the terminal of the side arms were prepared and their ion-selectivity features examined. As shown in Figure 4, the diazacrown derivative having a large ring size produces better Ca^{2+} selectivity versus small-size ions such as Li^+ and Na^+ (K23E > K22E > K21E in Ca^{2+} selectivity against other cations). The two 21-membered diazacrowns of K23E5 and K23E1 also exhibited much better Ca^{2+} selectivity compared with those of a similar K22-type azacrown derivatives K22E5 and K22E1 which have a smaller ring size than those of the K23-based compounds. K23E1 is judged the best Ca^{2+} ionophore developed to date, because it has an excellent Ca^{2+} selectivity of over 10 000 times versus all other alkaline earth metal and alkali metal ions

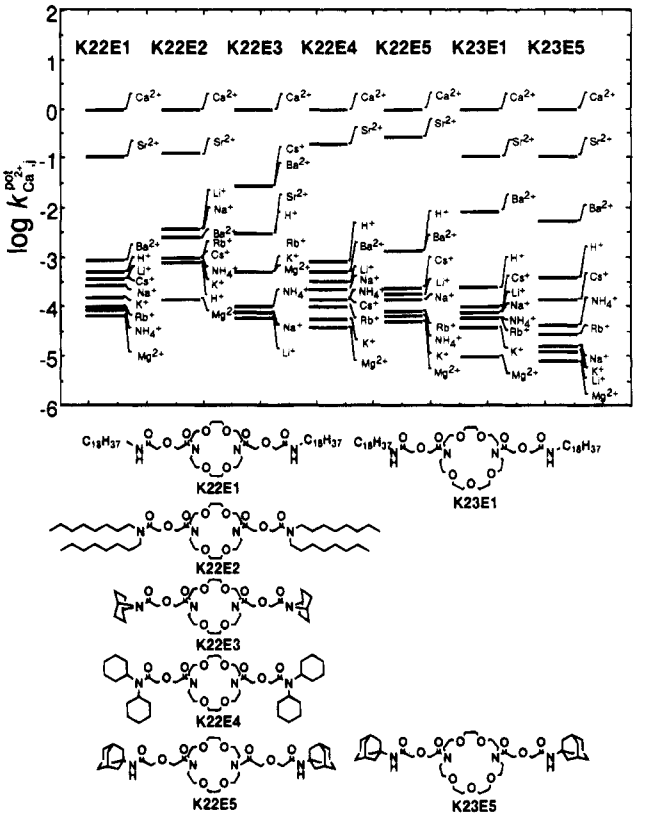

Figure 5. Selectivity factors for the electrodes based on seven types of 18- (K22E1, K22E2, K22E3, K22E4, K22E5) and 21-membered diazacrown ether derivatives (K23E1, K23E5). Electrode membrane composition: 2 wt % ionophore, 66 wt % NPOE, and 32 wt % PVC including 100 mol % K-TCPB against the ionophore content.

(its selectivity coefficient values, log $k^{pot}_{Ca,j} < -4.0$; *j*, interfering cation), as shown in Figure 5. In addition, this ionophore has a very high lipophilicity value (log $P_{o/w} = 14.6 \pm 0.4$; 3.1 ± 0.2 for K23E5), which is an important characteristic for practical use as an ion-sensing component for the ion-selective electrode membrane.

The effect of the amount of K-TCPB as the anionic additive in the electrode membrane on the ion-selective feature was examined with PVC matrix membrane electrodes based on K23E1 as the Ca^{2+} ionophore and NPOE as the membrane solvent. Typical results are shown in Figure 6. In general, the addition of the anionic additive causes positive emf response behavior, for instance, reduction of the anion interference at high sample activities, reduction of membrane resistance, improving the detection limit, and so on. On the other hand, the addition of the anionic additive affects the ion selectivity of the electrode. The best Ca^{2+} selectivity was experimentally observed in the case where 100 mol % K-TCPB against the ionophore content was incorporated in the electrode membrane. The lower concentration range of less than 100 mol % K-TCPB against the ionophore content is effective in improving the Ca^{2+} selectivity against alkali metal monovalent cations, but less effective against alkaline earth metal divalent cations, except Ca^{2+}. On the other hand, an amount of the anionic additive of more than 100 mol % K-TCPB against the ionophore content caused significant reduction in Ca^{2+} selectivity against other cations, because the cation selectivity is strongly governed by the ion-selective feature of the TCPB anion itself. This ion-selective behavior on the content of the anionic additive was similar in all cases where the other synthesized

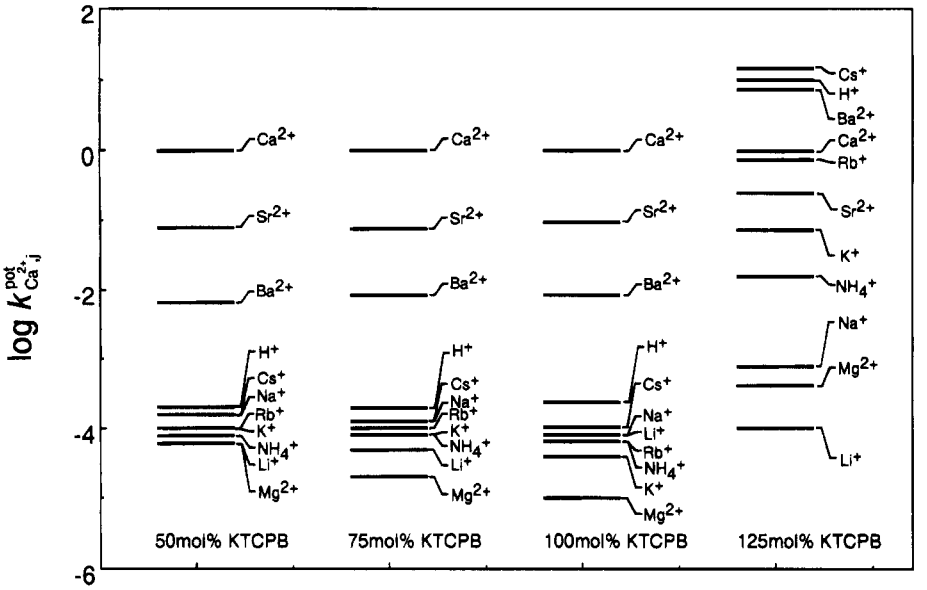

Figure 6. Effect of the content of the anionic additive (K-TCPB) in the Ca^{2+} electrode membrane on its ion-selectivity factor. Electrode membrane composition: 2 wt % ionophore, 66 wt % NPOE, and 32 wt % PVC including 50–125 mol % K-TCPB versus the ionophore content.

ionophores of the double-armed azacrown type were used as the ion-sensing component for the electrode membrane. On the other hand, when a single-armed azacrown or noncyclic diamide-type ionophore was used as the ion-sensing component for the electrode membrane, the optimum Ca^{2+} ion selectivity was experimentally observed in the case where K-TCPB content is 50 mol % versus the ionophore content in the electrode membrane. These facts suggest that the stoichiometry of the ionophore–Ca^{2+} in the electrode membrane is 1/1 and 2/1 in the case where the ionophore is a double-armed azacrown possessing two diamides and is a single-armed azacrown or noncyclic compound possessing one diamide, respectively.

Using the best Ca^{2+}-selective ionophore, K23E1, a Ca^{2+} response curve was obtained with the test samples containing interfering ions similar to the upper normal ion concentration level of human extracellular fluid, which incorporated 150 mM Na^+, 5.0 mM K^+, and 0.8 mM Mg^{2+} (all chlorides). The electrode response was almost Nernstian (29 mV/pCa^{2+}) in the range from 1×10^{-5} to 1×10^{-1} M Ca^{2+} even when the test sample contained the extracellular background ions as shown in Figure 7. The Ca^{2+} concentration in real human serum samples were also measured with this electrode. The results were satisfactory; the Ca^{2+} value was 2.46 ± 0.04 mM (n = 5) as compared to the expected value of 2.5 ± 0.1 mM.

Magnesium Ionophores. Regarding a magnesium ionophore, malonamide in the side arms of a diazacrown compound such as K22B is an effective candidate for the basic structure of the ionophore molecule. The Mg^{2+} selectivity of this molecule is apparently higher than those for similar diazacrown derivatives as shown in Figure 3, in which the other K22-based compounds possessing a simple diamide in the side arms, K22A, K22C, and K22D, exhibited higher selectivity to Ca^{2+} than those to Mg^{2+}. This fact suggests that the malonic diamide derivative is suitable for Mg^{2+} coordination. In this case, the distance between the two ketones (β-diketone section) in the malonamide is important for Mg^{2+} coordination while glutamide and adipamide, which have much longer distances between the two ketones, are not suitable for Mg^{2+} coordination. Oxamide is also not suitable for Mg^{2+}

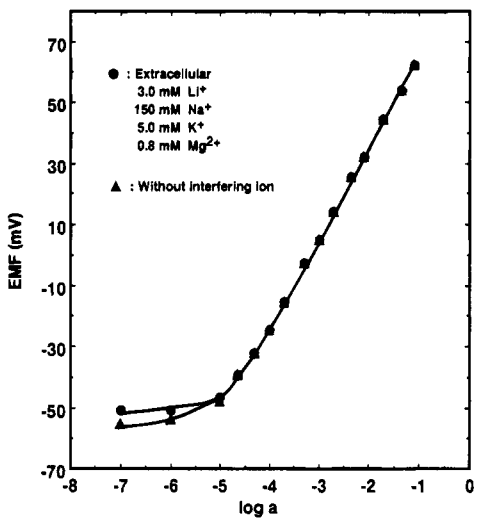

Figure 7. Typical response curves for Ca^{2+} obtained with the electrodes based on K23E1 and NPOE. Circles and triangles indicate the response values for Ca^{2+} with and without interfering ions, respectively, similar to the upper normal ion concentration levels of human serum (150 mM Na^+, 5.0 mM K^+, 0.8 mM Mg^{2+}). Electrode membrane composition: 2 wt % K23E1, 66 wt % NPOE, and 32 wt % PVC including 100 mol % K-TCPB against the ionophore content.

coordination, because the two carbonyl oxygens in the α-diketone in oxamide are in opposite directions to each other, so that it is not anymore effective as a binding site for Mg^{2+} than was concluded for our previous investigation using α- and β-diketone type ionophores.[8]

The base ring size of the azacrown compounds seriously affected their ion selectivity features (see Figure 4 as an example for Ca^{2+} ionophores). Hence, two different ring size diazacrowns possessing B-type side chains based on malonic diamide were prepared. These are K21B and K23B, which have 15- or 21-membered ring diazacrowns, respectively. Compared with the ion-selective feature of K22B, which has an 18-membered base ring (see Figure 3), these two azacrowns were less effective for Mg^{2+} selectivity against both alkali metal ions and alkaline earth metal ions, as shown in the first and second columns of Figure 8.

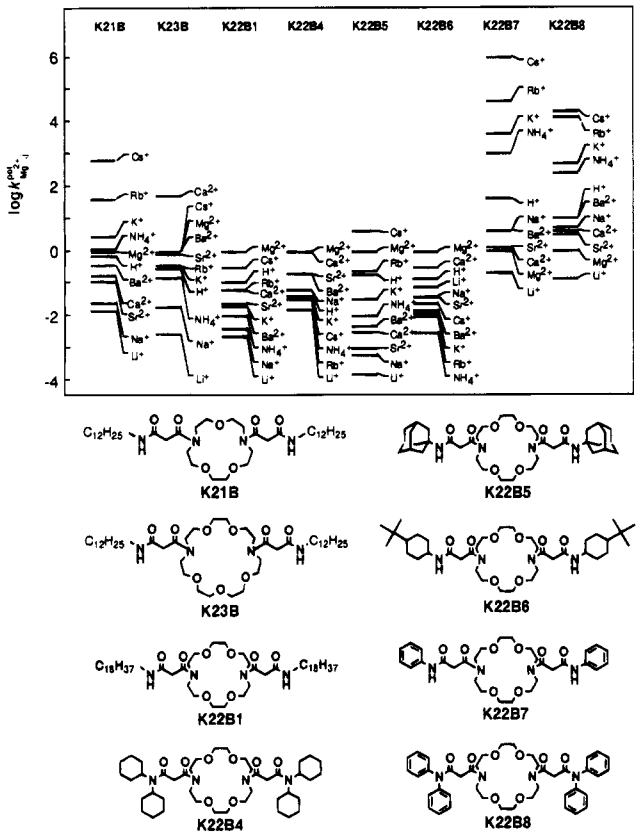

Figure 8. Selectivity factors for the electrodes based on eight types of 15- (K21B), 18- (K22B1, K22B4, K22B5, K22B6, K22B7, K22B8) and 21-membered diazacrown ether derivatives (K23B). Electrode membrane composition: 2 wt % ionophore, 66 wt % NPOE, and 32 wt % PVC including 100 mol % K-TCPB against the ionophore content.

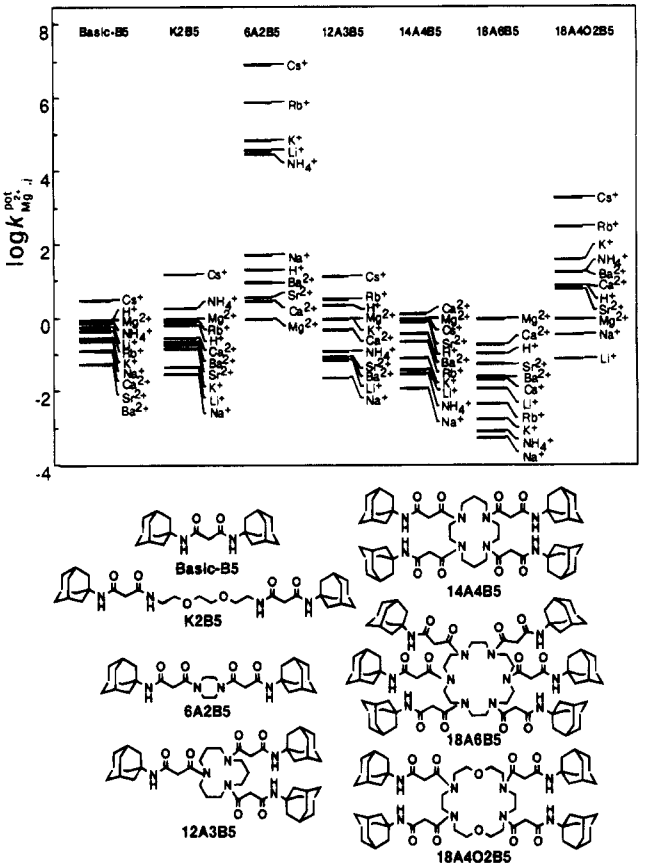

Figure 9. Selectivity factors for the electrodes based on two types of noncyclic malonic amide derivatives (K2B5, Basic-B5) and five types of azacrown derivatives with side arms possessing malonic amides (6A2B5, 12A3B5, 14A4B5, 18A6B5, 18A4O2B5). Electrode membrane composition: 2 wt % ionophore, 66 wt % NPOE, and 32 wt % PVC including 100 mol % K-TCPB (50 mol % K-TCPB for noncyclic-type ionophores) against the ionophore content.

The estimated cavity diameters of K21 (15-membered ring), K22 (18-membered ring), and K23 (21-membered ring) based on their corresponding space-filling models are 1.5–1.8, 2.3–2.6, and 3.1–3.4 Å, respectively, when they are assumed to have simple planar structures. However, in the case where the ring was distorted, the cavity size is reduced tridimensionally. The diameter of a magnesium ion is ~1.3-1.4 Å, and though the nearest size is that of the cavity of the K21 ring, K22B as an 18-membered ring diazacrown gave the best Mg^{2+} selectivity result. This result can be understood based on the fact that a large distortion is caused in the base ring when the ionophore molecule coordinates to Mg^{2+}. On the other hand, the cavity size of the 21-membered azacrown derivative K23B is too large to fit the Mg^{2+} cavity size even if the cyclic backbone section is distorted by coordination with the cation, so that the electrode based on this ionophore exhibited poor Mg^{2+} selectivity, as shown in Figure 8.

Based on the good results for Mg^{2+} selectivity with the 18-membered diazacrown having a K22-type base ring, six more K22B-type derivatives, K22B1, K22B4, K22B5, K22B6, K22B7, and K22B8, were prepared which possessed an *n*-octadecyl, a dicyclohexyl, an adamantyl, a *p-tert*-butylcyclohexyl, a phenyl, or a diphenyl group, respectively, at the terminal of the malonamide-type side chains of the diazacrown. The selectivity factors for the electrodes using these six derivatives were also shown in Figure 8. Compared with the ion-selectivity feature for the K22B-based electrode, the electrodes using K22B7 and K22B8, which possessed phenyl amide groups, had poor Mg^{2+} selectivity. On the other hand, the electrodes based on K22B1, K22B4, K22B5, and K22B6 showed apparently better Mg^{2+} selectivity against large-size alkali metal cations such as Cs^+, Rb^+, and K^+ than that based on K22B (see Figure 3), but they exhibited poor Mg^{2+} selectivity against Ca^{2+}, except for the one based on K22B5. The electrode using K22B5 showed high Mg^{2+} selectivity and its logarithmic selectivity coefficients of Mg^{2+} to Na^+, K^+, and Ca^{2+} ($\log k^{pot}_{Mg,Na}$, $\log k^{pot}_{Mg,K}$, and $\log k^{pot}_{Mg,Ca}$) were −3.2, −1.5 and −2.4, respectively, that is, the highest Mg^{2+}/Ca^{2+} selectivity developed to date. The diazacrown derivatives with two side arms possessing bulky adamantyl groups are very effective in both Mg^{2+} and Ca^{2+} ionophore design (K22B5 and K23E5; see Figure 5).

In order to understand the structural feature for the Mg^{2+} selectivity of K22B5, two further noncyclic derivatives possessing adamantyl amide groups, Basic B5 and K2B5, were prepared. The former compound is a simple diamide (*N,N*-adamantylmalonamide), which has a structure similar to the arm section of K22B5, and the latter one is similar to the K22B5 without one 4,7-dioxahexane bridge which is equal to a half-section of the K22 base ring. As shown in the first and second columns of Figure 9, these noncyclic adamantylmalonamide derivatives show relatively high Mg^{2+} selectivity against other cations, but the Mg^{2+} selectivity is poorer than that of K22B5, which has a cyclic backbone (a 18-membered azacrown derivative). This fact indicates that the azacrown ring is an important structural factor for the selective Mg^{2+} coordination.

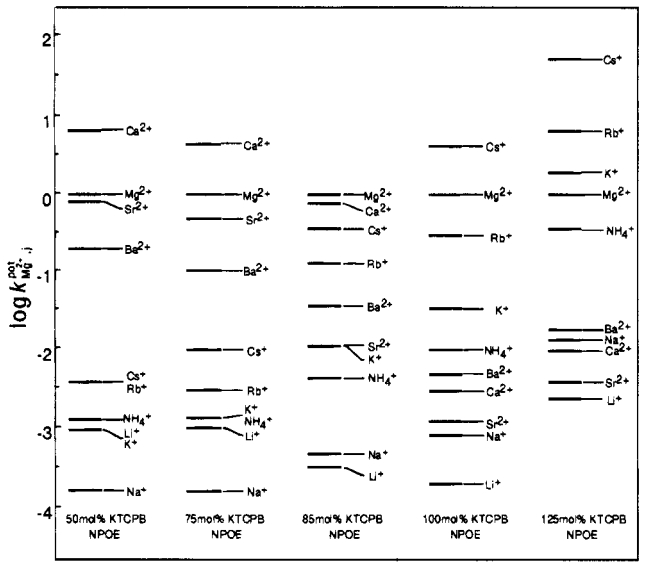

Figure 10. Effect of the content of the anionic additive (K-TCPB) in the Mg^{2+} electrode membrane on its selectivity factor. Electrode membrane composition: 2 wt % K22B5, 66 wt % NPOE, and 32 wt % PVC including 50–125 mol % K-TCPB versus the ionophore content.

To further develop the excellent Mg^{2+} selectivity shown with the ionophore K22B5, five additional azacrown derivatives possessing plural numbers of adamantylmalonamides were synthesized. These are 6A2B5, 12A3B5, 14A4B5, 18A6B5, and 18A4O2B5. Their chemical structures and ion-selective features are also shown in Figure 9. These azacrown derivatives have a 6–8-membered azacrown base ring and also possess two to six adamantylmalonamides in their side chains. As shown in Figure 9, these five azacrown derivatives did not show very high Mg^{2+} selectivity compared to that exhibited by K22B5. The reasons are that the binding arms of these compounds do not necessarily play an effective role in Mg^{2+} coordination, because these binding arms are tridimensionally too crowded to coordinate Mg^{2+} cooperatively. In addition, the base azacrown ring of these compounds is very distorted by the cation coordination with the plural arm sections, so that the base ring no longer offers effective sites and space for Mg^{2+} coordination. These facts suggest that the most important parameter for obtaining an excellent Mg^{2+}-selective ionophore is how well the design fits the malonic diamide sections tridimensionally in the ionophore molecule when it coordinates with Mg^{2+}.

Using the best Mg^{2+}-selective ionophore, K22B5, the optimum amount of K-TCPB as an anionic additive in the polymeric electrode membrane based on PVC and NPOE was examined. As shown in Figure 10, the best Mg^{2+} selectivity was observed when the content of the anionic additive in the electrode membrane was 100 mol % against the ionophore content that gave similar behavior in the case where the Ca^{2+} ionophore K23E1 was used in the Ca^{2+}-selective electrode membrane (see Figure 6). The polymeric membrane electrode based on K22B showed Mg^{2+} selectivity against Ca^{2+} when the K-TCPB content was more than 85 mol % against the ionophore content in the electrode membrane, while the electrode exhibited no greater Mg^{2+} selectivity when the K-TCPB content was less than 85 mol %, and in the range over 100 mol % K-TCPB, the strong effect of the TCPB anion itself appears in its ion selectivity behavior of the electrode. The K-TCPB content in the electrode membrane containing the Mg^{2+} ionophore is significantly affected by its Mg^{2+}-selectivity behavior compared with that in the case for the electrode membrane containing the Ca^{2+} ionophore (see Figure 7). This fact suggests that the extraction of Mg^{2+} from the aqueous phase into the lipophilic membrane phase requires a stronger coordinating power compared with the case where Ca^{2+} is extracted. The anionic additive, therefore, has an effective role in increasing polarity and anionic coordinating power to Mg^{2+} cooperatively with the ionophore.

Utilizing the best Mg^{2+}-selective polymeric membrane electrode based on K22B5, the Mg^{2+} response curve was obtained with interfering ions similar to the upper normal concentration level of human extracellular fluid which incorporated 150 mM Na^+, 5.0 mM K^+, and 3.0 mM Ca^{2+}. Though the electrode based on K22B5 shows an almost Nernstian response to Mg^{2+} ranging from 2×10^{-5} to 1×10^{-1} M (29.3 mV/pMg^{2+} at 25 °C) when the sample contains no interfering ion, it response was almost Nernstian (29.8 mV/pMg^{2+}) in the range from 2×10^{-3} to 1×10^{-1} M Mg^{2+} in the case when the test sample contained the extracellular background ions. Required selectivities for a Mg^{2+}-selective electrode for human serum analysis are −3.9, −0.9, and −2.4 for Na^+, K^+, and Ca^{2+}, respectively.[18] The ion selectivity of an electrode using K22B5 is, therefore, not slightly sufficiently for Na^+. Hence, better Mg^{2+} selectivity against these interfering ions is required for human serum analysis. Thus, further design and synthesis is still in progress to obtain much more Mg^{2+}-selective ionophores.

CONCLUSIONS

Here we report the structural features of ionophore molecules that are designed and prepared for obtaining highly Ca^{2+}- and Mg^{2+}-selective electrodes. The ion-selectivity factors (log k^{pot}_{ij}; i, Ca^{2+} or Mg^{2+}; j, interfering ion) of the electrodes based on all ionophores discussed here are summarized in Table 1. Our proposed ionophore model for divalent alkaline earth metal ions is shown in Figure 1, which has three different functional sections: (i) base azacrown ring, (ii) diamide binding arms, and (iii) lipophilic terminal. As a result of the investigations on the structural ion-selective features of over 20 ionophores, in which their structures including these three functional sections were systematically varied with several molecular subunits, excellent Ca^{2+} and Mg^{2+} ionophores were finally obtained. The 21-membered diazacrown having two glycolic amide side chains with an adamantyl or octadecyl group (K23B5 or K23E1; see Figure 5) exhibited the best Ca^{2+} selectivity. The 18-membered diazacrown having two malonamide side chains with an adamantyl group (K22B5; see Figure 8) exhibited the best Mg^{2+} selectivity. We previously reported the design and synthesis of highly Li^+ and Na^+ selective ionophores for an ion-selective electrode, which are also prepared based on the proposed ionophore models, and successful results were obtained by investigating the ion-selective features of several synthesized crown compounds that were also systematically prepared with some functional subunits.[19–21] These

(18) Ammann, D.; Oesch, U.; Buhrer, T.; Simon, W. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 879.
(19) Suzuki, K.; Hayashi, K.; Tohda, K.; Watanabe, K.; Ouchi, M.; Hakushi, T.; Inoue, Y. *Anal. Lett.* **1991**, *24*, 1085.
(20) Kobiro, K.; Tobe, Y.; Watanabe, K.; Yamada, H.; Suzuki, K. *Anal. Lett.* **1993**, *26*, 49.
(21) Suzuki, K.; Yamada, H.; Sato, K.; Watanabe, K.; Hisamoto, H.; Tobe, Y.; Kobiro, K. *Anal. Chem.* **1993**, *65*, 3404.

Table 1. Ion Selectivity Factors (k_{ij}^{pot}; j = interfering ion) of the Electrodes Based on the Synthesized Ionophores and Lipophilicity ($P_{o/w}$) of the Ionophores[a]

ionophore	membrane solvent	log k_{ij}^{pot} (i = Ca^{2+} or Mg^{2+})											log $P_{o/w}$
		H^+	Li^+	Na^+	K^+	Rb^+	Cs^+	NH_4^+	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	
none	NPOE	2.1	0.7	1.7	5.1	6.3	7.6	4.2	0.3	0.0	0.3	0.7	5.7 ± 0.3
none	DOS	6.2	4.7	5.1	5.5	5.3	5.5	5.4	1.1	0.0	−0.4	−0.4	9.4 ± 0.3
Basic AM-I	NPOE	1.8	1.8	−0.8	1.8	2.7	4.2	1.9	−1.1	0.0	−0.4	−0.4	2.9 ± 0.2
Basic AM-I	DOS	4.9	2.5	2.3	3.3	3.8	4.8	3.6	−0.4	0.0	−0.2	0.5	2.9 ± 0.2
Basic AM-II	NPOE	1.5	1.8	−0.6	1.6	2.9	4.4	2.0	−1.3	0.0	−0.6	0.2	2.0 ± 0.2
Basic-ES	NPOE	1.4	−0.1	1.0	4.1	5.3	6.6	3.4	−0.4	0.0	0.1	1.7	2.6 ± 0.2
Basic-ET	NPOE	1.8	1.8	0.8	3.9	5.0	6.4	3.3	−0.3	0.0	0.1	0.6	3.1 ± 0.3
K22A	NPOE	1.7	−0.5	−1.6	−1.6	−1.2	−0.3	−1.6	−2.1	0.0	−0.7	−0.5	8.1 ± 0.4
K22B	NPOE	1.1	−1.6	−0.8	0.6	1.7	3.0	0.3	1.3	0.0	−0.7	−0.5	7.1 ± 0.4
K22C	NPOE	2.8	−2.6	−3.4	−3.1	−2.9	−2.3	−2.7	−2.1	0.0	−0.5	0.4	6.9 ± 0.4
K22D	NPOE	1.7	−1.7	−2.8	−2.1	−2.6	−2.1	−2.8	−2.1	0.0	−0.9	−0.3	6.8 ± 0.4
K21E	NPOE	−2.2	−2.8	−2.7	−3.3	−3.2	−3.2	−3.0	−4.0	0.0	−0.4	−0.8	7.4 ± 0.4
K22E	NPOE	−2.7	−2.7	−3.1	−3.6	−3.5	−3.4	−3.4	−4.1	0.0	−0.8	−1.6	7.0 ± 0.3
K23E	NPOE	−2.5	−4.0	−3.8	−4.0	−3.8	−2.7	−3.8	−4.2	0.0	−0.8	−1.4	6.9 ± 0.3
K50E	NPOE	−1.5	−5.0	−2.0	−1.5	−1.7	−1.7	−2.5	−3.8	0.0	−0.6	−1.4	4.1 ± 0.3
Basic E	NPOE	0.2	−3.8	−3.4	−1.4	−0.2	0.9	−1.5	−3.6	0.0	−1.0	−1.8	7.7 ± 0.4
K22E1	NPOE	−3.3	−3.5	−3.6	−3.8	−4.0	−3.5	−4.1	−4.2	0.0	−1.0	−3.0	14.4 ± 0.4
K23E1	NPOE	−3.6	−4.1	−4.1	−4.4	−4.2	−4.0	−4.2	−5.0	0.0	−1.0	−2.1	14.6 ± 0.4
K22E2	NPOE	−3.1	−2.4	−2.4	−3.1	−3.0	−3.0	−3.0	−3.9	0.0	−0.9	−2.6	9.5 ± 0.4
K22E3	NPOE	−2.6	−4.2	−4.1	−3.3	−3.3	−1.6	−4.0	−3.3	0.0	−1.6	−1.6	2.9 ± 0.2
K22E4	NPOE	−3.1	−3.5	−3.7	−4.3	−4.0	−3.9	−3.9	−4.5	0.0	−1.0	−3.3	5.2 ± 0.2
K22E5	NPOE	−2.9	−3.8	−3.9	−4.3	−4.1	−3.6	−4.2	−4.3	0.0	−0.6	−2.9	3.3 ± 0.2
K23E5	NPOE	−3.4	−4.9	−4.8	−4.8	−4.6	−3.9	−4.4	−5.1	0.0	−1.0	−2.3	3.1 ± 0.2
K21B	NPOE	−0.2	−1.8	−1.6	0.5	1.6	2.8	0.1	0.0	−0.8	−1.0	−0.4	7.4 ± 0.4
K23B	NPOE	−0.6	−2.6	−1.8	−0.4	−0.4	0.0	0.8	0.0	1.7	0.1	0.0	6.9 ± 0.4
K22B1	NPOE	−1.0	−2.7	−2.4	−1.7	−1.2	−0.5	−2.0	0.0	−1.2	−1.6	−1.7	15.0 ± 0.3
K22B4	NPOE	−1.2	−1.8	−1.2	−1.4	−1.5	−1.5	−1.5	0.0	0.0	−0.7	−0.7	4.5 ± 0.2
K22B5	NPOE	−0.7	−3.8	−3.2	−1.5	−0.6	0.7	−2.0	0.0	−2.5	−3.0	−2.3	3.0 ± 0.4
K22B6	NPOE	−0.9	−1.1	−1.4	−1.9	−2.0	−1.6	−2.5	0.0	−0.5	−1.4	−1.8	3.4 ± 0.4
K22B7	NPOE	1.6	−0.7	−0.6	3.7	4.7	6.1	3.1	0.0	0.0	0.2	0.6	1.8 ± 0.2
K22B8	NPOE	1.1	−2.8	0.8	2.8	4.1	4.3	2.5	0.0	0.7	0.6	1.1	2.3 ± 0.2
Basic-B5	NPOE	0.0	−0.2	−0.6	−0.5	−0.3	0.5	−0.1	0.0	−0.9	−1.2	−1.2	5.1 ± 0.4
K2B5	NPOE	−0.2	−1.3	−1.5	−0.8	−0.1	1.3	0.3	0.0	−0.5	−0.7	−0.6	3.2 ± 0.3
6A2B5	NPOE	1.3	4.6	1.7	4.9	5.9	7.1	4.5	0.0	0.5	0.6	1.1	4.0 ± 0.3
12A3B5	NPOE	0.3	−1.1	−1.6	0.0	0.5	1.2	−0.9	0.0	−0.3	−1.0	−1.0	4.6 ± 0.4
14A4B5	NPOE	−0.1	−1.3	−1.9	−1.0	−0.6	0.0	−1.4	0.0	0.2	−0.1	−0.4	6.1 ± 0.4
18A6B5	NPOE	−1.0	−1.9	−3.2	−2.6	−2.3	−1.6	−3.0	0.0	−0.7	−1.2	−1.5	7.6 ± 0.4
18A4O2B5	NPOE	0.9	−1.1	−0.4	1.6	2.5	3.5	1.3	0.0	0.9	0.8	1.3	6.2 ± 0.4

[a] For electrode membrane compositions, see Experimental Section.

basic discussions on the structural ion-selective features of the ionophores and their systematic investigations are important and effective for obtaining good ionophores. The discussions of the present work are applicable as general knowledge related to host–guest complex chemistry and contribute to obtaining excellent ion-selective chemical ion sensors.

ACKNOWLEDGMENT

This investigation was partially discussed with the late Professor W. Simon of ETH–Zurich. The authors are grateful for this kind suggestions. Partial support of this work by a Kurata Research Grant, The Iketani Science and Technology Foundation, and The Asahi Glass Foundation is acknowledged.

SUPPLEMENTARY MATERIAL AVAILABLE

Analytical data for the new compounds (6 pages). Ordering information is given on any current masthead page.

Received for review June 2, 1994. Accepted October 28, 1994.[⊗]

AC9405720

[⊗] Abstract published in *Advance ACS Abstracts*, December 1, 1994.