Double Uphill Transport of Ca^{2+}−Na^+ or Ca^{2+}−K^+ in Opposite Directions by Lipophilic Crown Ethers with Two Carboxylic Acid Moieties

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A variety of lipophilic crown ethers containing two carboxylic acid moieties were prepared, and their transport abilities were estimated in the double uphill transport system of Ca^{2+}−Na^+ or Ca^{2+}−K^+ by pH control through a bulk liquid membrane using dichloromethane. As expected, 15-crown-5 and 18-crown-6 derivatives were found to be effective for Ca^{2+}−Na^+ and Ca^{2+}−K^+ exchange systems, respectively. As a result, the calcium ion was concentrated in the acidic phase, and the sodium ion or potassium ion was concentrated in the other phase. In addition to the structure of the carriers, a proper selection of the transport conditions was one of the crucial factors for realizing this double uphill transport system. A simple combination of the active transporter of the calcium ion and the passive carrier for the sodium ion was also found to enable the exchange between the calcium and sodium ions against their concentration gradients. When two ionophores effective for Ca^{2+}−K^+ and K^+−Na^+ exchange systems, respectively, were used at the same time, the calcium and potassium ions were concentrated in the acidic phase, whereas the sodium ion was concentrated in the basic phase.

Introduction

The enzyme Na^+−K^+ ATPase actively transports Na^+ and K^+ in opposite directions across a biological membrane.[1] The Na^+−Ca^{2+} exchange, which actually occurs in the biological membrane, plays an important role in the maintenance of an organism *in vivo*.[2,3] The concentrations of a variety of metal ions in two aqueous phases separated by a biological membrane are strictly regulated by using such a function. Thus, the active transport of metal cations by synthetic carriers across an artificial membrane has been examined for constructing an appropriate model for a biological membrane.[4−7] This active transport is also potentially effective for separation of a variety of useful metal salts. Many synthetic carriers were successfully used in the active transport system, but most of them were concerned with only one direction. In order to simultaneously transport two different cations in opposite directions against their concentration gradients as in the case of Na^+−K^+ ATPase, an additional device was needed in the design of the synthetic carriers. Recently we succeeded in preparing a Na^+−K^+ ATPase model, which possesses a bis(crown ether) structure consisting of monoaza 18-crown-6 and 15-crown-5 rings.[8] In this case, the ionophore concentrated Na^+ and K^+ cations in the basic and acidic phases, respectively. The double uphill transport of K^+ and Ca^{2+} in opposite directions was also attained using an 18-crown-6 ether dicarboxylic acid (**4**).[9] In order to apply this interesting exchange function for other combinations of metals, the relation between the structure of ionophore and the transport tendency in such transport system should be clarified in detail. From this point of view, we will describe the synthesis of some 18-crown-6 and 15-crown-5 ethers containing two carboxylic acid moieties, their transport abilities,[10] and the necessary conditions required for realization of the double uphill transport system.

Results and Discussion

Design of Ionophores. The structure of the synthetic ionophore **4** (Chart 1) was first designed on the basis of findings of Lehn *et al.*,[2] who succeeded in regulating the Ca^{2+}/K^+ selectivity in a passive transport system. As previously reported, this ionophore (**4**) was successfully applied for the K^+−Ca^{2+} exchange through an artificial membrane in opposite directions against its concentration gradient.[9] The 15-crown-5 derivative (**1a**) designed based on the structure of this ionophore, however, disappointingly showed a poor transport ability toward Na^+ as mentioned below. This result is ascribed to the difference in the stability constants of 18-crown-6 for K^+ and of 15-crown-5 for Na^+. Consequently, another structural device is needed to improve the transport ability for Na^+. Our approach to solving this problem is introduction of a secondary coordination site to the ionophore, which has often been used to raise the complexing ability of the

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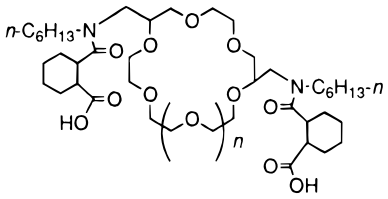
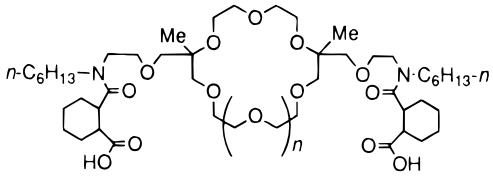
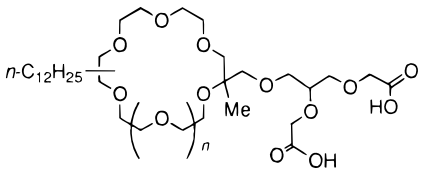
Chart 1



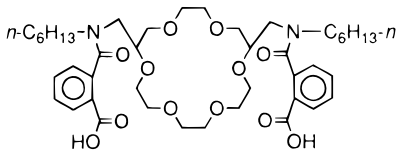
1a : *n=0*
1b : *n=1*



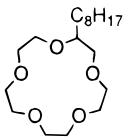
2a : *n=0*
2b : *n=1*



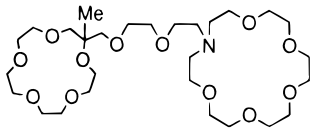
3 : *n=0*



4

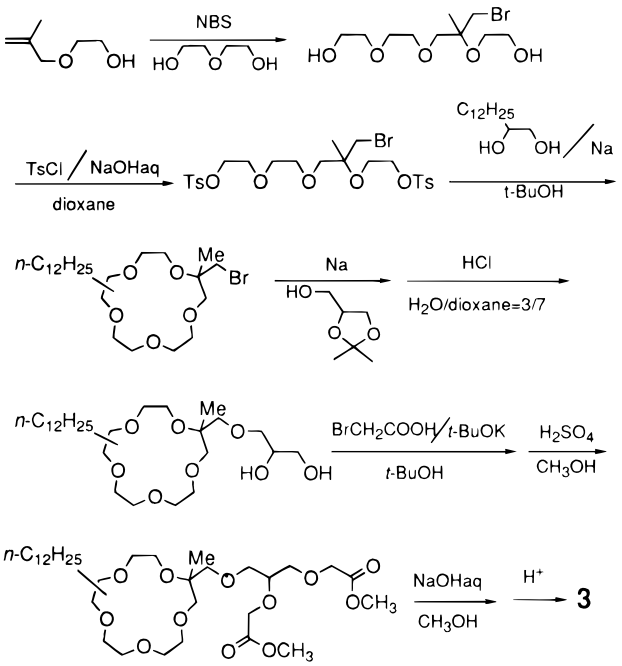


5



6

Scheme 1



crown ether.[11,12] Thus, ionophores **2** and **3** containing the ether oxygen atoms on the side arm were prepared and used for further transport experiments. Although ionophores **1** and **2** were obtained as mixtures of *syn* and *anti* isomers, the mixtures were chemically pure. Ionophore **3** was also obtained as a mixture of regio isomers according to the procedure shown in Scheme 1.

Transport Studies. Transport experiments were carried out in a U-type cell at 25 °C according to the literature.[9] Dichloromethane was used as the liquid membrane. The proton concentrations of both aqueous phases containing metal salts were adjusted by using a Tris buffer and hydrochloric acid. The concentrations of cations and picrate ions were determined by atomic absorption analysis and UV spectroscopy, respectively. The detailed transport conditions and the results in the presence of Ca^{2+} and Na^+ (or K^+) are summarized in Table 1.

Ionophore **1a** selectively transported Ca^{2+} in the passive transport from the basic (pH 10) to the acidic phase (pH 2) when $CaCl_2$ and NaCl were used in the source phase (entry 1). Ionophore **1b** also showed Ca^{2+}/K^+ selectivity (entry 4), and its transport efficiency for Ca^{2+} was much higher than that of **1a**. Both cations, however, were scarcely transported in the passive transport from the acidic to the other acidic phase in the absence of lipophilic anions such as picrate ions (entries 9 and 10).[9] When picrate anion was added to the source phase, the selective transport of Na^+ occurred (entry 11). This is reasonably explained by considering that the ionophore needs the presence of a lipophilic anion in the uptake process under acidic conditions. The extent of Na^+ ions transported (Na^+: 3%), however, was relatively small in comparison with the case of the $Ca^{2+}-K^+$ exchange by using ionophore **4** (K^+: 7%; entry 16).[9] This result is disadvantageous for the double uphill transport. In fact, the transport of Na^+ from the acidic to the basic phase was rather difficult under active transport conditions (entry 17). These findings strongly suggest that the carrier demands a much higher complexing ability in this transport system. In order to strengthen the uptake property of the carrier toward the Na^+ ion under acidic conditions, we introduced the concept of the "lariat ether" developed by Gokel *et al.*[11] to the molecular design of new ionophores. The ether oxygen atoms of the side chain of the 15-crown-5 ether derivative bearing a methyl group at the pivot carbon were known to increase the complexing ability toward Na^+.[12] As expected, ionophores **2a** and **3** having electron-donating oxygen atoms on the side arm effectively transported Na^+ from the acidic to the other acidic phase in the passive transport experiments (entries 12 and 13). Incidentally, the addition of the picrate ion in the source phase during the passive transport from the basic to the acidic phase unfortunately increased the transport velocity of the alkali metal cation (entries 7 and 8), which is undesirable for the double uphill transport. To avoid this drawback, we did not use the picrate ion in the basic phase in the double uphill transport experiments. Finally, ionophores **2a** and **3** clearly transported Na^+ and Ca^{2+} in opposite directions across the liquid membrane (entries 18 and 19). During this experiment, Ca^{2+} was transported from the basic to the acidic phase by pH control, and Na^+ was transported from the acidic to the basic phase according to the concentration gradient of picrate ions. The more than 5% of $Ca^{2+}(Na^+)$ transported after 48 h clearly demonstrates that the ionophore repeatedly carried the cations since the initial amount of cations is 20 times that of the ionophore. The exchange

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Table 1. Transport Data in the Presence of Calcium Ion and Sodium Ion (or Potassium Ion)

		initial conditions[a]				transported ions[b] (%)					
		phase 1		phase 2		phase 1			phase 2		
entry	ionophore	salt	pH[c]	salt	pH[c]	Ca^{2+}	$Na^+(K^+)$	Pic^-	Ca^{2+}	$Na^+(K^+)$	Pic^-
1	**1a**	$CaCl_2$, NaCl	10		2				8	2	
2	**2a**	$CaCl_2$, NaCl	10		2				8	7	
3	**3**	$CaCl_2$, NaCl	10		2				10	2	
4	**1b**	$CaCl_2$, KCl	10		2				30	(4)	
5	**2b**	$CaCl_2$, KCl	10		2				25	(11)	
6	**4**[d]	$CaCl_2$, KCl	10		2				11	(2)	
7	**1b**	$CaCl_2$, PicK	10		2			nd	41	(13)	nd
8	**2b**	$CaCl_2$, PicK	10		2			nd	34	(24)	nd
9	**1b**	$CaCl_2$, KCl	2		2				<1	(<1)	
10	**2b**	$CaCl_2$, KCl	2		2				<1	(<1)	
11	**1a**	$CaCl_2$, PicNa	2		2			nd	<1	3	nd
12	**2a**	$CaCl_2$, PicNa	2		2			nd	<1	12	nd
13	**3**	$CaCl_2$, PicNa	2		2			nd	<1	18	nd
14	**1b**	$CaCl_2$, PicK	2		2			nd	<1	(8)	nd
15	**2b**	$CaCl_2$, PicK	2		2			nd	<1	(17)	nd
16	**4**[d]	$CaCl_2$, PicK	2		2			nd	<1	(7)	nd
17	**1a**	$CaCl_2$, NaCl	10	$CaCl_2$, PicNa	2		2	6	22		
18	**2a**	$CaCl_2$, NaCl	10	$CaCl_2$, PicNa	2		7	9	16		
19	**3**	$CaCl_2$, NaCl	10	$CaCl_2$, PicNa	2		8	8	20		
20	**1b**	$CaCl_2$, KCl	10	$CaCl_2$, PicK	2		(6)	6	22		
21	**2b**	$CaCl_2$, KCl	10	$CaCl_2$, PicK	2		(8)	9	16		
22	**2b**	$CaCl_2$, KCl	10	$CaCl_2$, PicK	2			36	26		
23	**4**[d]	$CaCl_2$, KCl	10	$CaCl_2$, PicK	2		(6)	6	14		

[a] The initial concentrations of metal salts in the aqueous phase were arranged to be 0.01 M. In the case of active transport experiments (entries 17−23), tetramethylammonium chloride (0.1 M) was added to the phase 1 except for entry 22. [b] After 48 h. [c] [Tris] = 0.05 M (pH 10); [HCl] = 0.01 M (pH 2). [d] Reference 9.

Table 2. Passive Transport Data for Na^+, K^+, Mg^{2+}, and Ca^{2+}

		initial conditions[a]		transported cations/%[c]			
				receiving phase			
entry	ionophore	source phase pH[b]	receiving phase pH[b]	Ca^{2+}	Mg^{2+}	K^+	Na^+
24	**2a**	10	2	15	4	3	8
25	**3**	10	2	13	<1	1	9
26	**2b**	10	2	18	2	8	1
27	**2a**	2	2	1	<1	6	9
28	**3**	2	2	1	<1	4	14
29	**2b**	2	2	<1	<1	22	1

[a] PicK and PicNa denote potassium picrate and sodium picrate, respectively. In the transport from basic to acidic, $[CaCl_2] = [MgCl_2] = [KCl] = [NaCl] = 0.01$ M. In the transport from acidic to basic, $[CaCl_2] = [MgCl_2] = [PicK] = [PicNa] = 0.01$ M; source phase (H_2O, 10 mL)/membrane (CH_2Cl_2, 20 mL), [ionophore] = 0.25 mM/receiving phase (H_2O, 10 mL). [b] [Tris] = 0.05 M (pH = 10); [HCl] = 0.01 M (pH = 2). [c] After 48 h.

of Ca^{2+} and K^+ by using ionophores **1b** and **2b** showed a similar tendency observed for the exchange of Ca^{2+} and Na^+ (entries 20 and 21). In the active transport system, the presence of a tetramethylammonium ion in the basic phase is necessary as reported previously[9] (entry 22) because this cation can carry a picrate ion through the membrane as an ion pair. In the transport from the acidic to the basic phase, the ionophore needs the assistance of the lipophilic anion. Thus, the concentration of the picrate ion in the acidic phase was maintained at a higher level by the function of the tetramethylammonium ion, which enabled the double uphill transport.

The selectivities between two alkali metal cations or between two alkaline earth metal cations are also interesting in order to estimate the ability of the ionophore. The passive transport data by ionophore **2a**, **3**, and **2b** in the presence of Ca^{2+}, Mg^{2+}, K^+, and Na^+ are summarized in Table 2. The transport rate by **2a** from the basic to the acidic phase showed that the quantity of metal cations transported increases in the order Mg^{2+}, $K^+ < Na^+ < Ca^{2+}$ (entry 24). Ca^{2+} was selectively transported by using ionophore **2a**, and Ca^{2+}/Mg^{2+} selectivity was clearly observed. In the passive transport in the presence of Ca^{2+}, Mg^{2+}, K^+, and Na^+ from the one acidic phase to the other, Na^+ was transported in preference to K^+ as expected by considering the 15-crown-5 ring structure; alkaline earth metal ions were scarcely transported (entry 27). Ionophore **2b** also showed Ca^{2+}/Mg^{2+} selectivity from the basic to the acidic phase and K^+/Na^+ selectivity from the acidic to the basic phase, respectively. The cation selectivity was clearly observed in the case of the ionophore containing an 18-crown-6 ring, possibly because the 18-crown-6 ring of **2b** potentially possesses a higher complexing ability and cation selectivity than the 15-crown-5 ring of **2a**. In spite of this fact, ionophore **3** showed much better Na^+/K^+ selectivity than **2a** (entries 27 and 28). In the previous work, binding data obtained for methyl lariat ethers having an oligooxyethylene side arm at the pivot position showed that one oxyethylene unit increased the complexing ability toward Na^+ without the increase of that toward K^+.[12] On the basis of this consideration, the higher Na^+ selectivity of **3** may be ascribed to the effective coordination property of the second oxygen atom from the pivot carbon atom on the side arm, which is not present in **2a**.

As mentioned above, the transport velocity is highly dependent on concentrations of the lipophilic anion. If we are able to effectively use this concentration gradient of the picrate anion, the cation may be transported against its concentration gradient. So we examined this possibility in this transport system (Table 3). Under active transport conditions, when octyl-15-crown-5 (**5**)[13] was used as the ionophore, this ionophore transported Na^+ against its concentration gradient (entries 34 and 35). In this case, the tetramethylammonium ion produced the picrate ion gradient. Next, we attempted to use an equimolar mixture of **1a**, which is a good carrier for Ca^{2+} from the basic to the acidic phase, and **5** as the ionophore in this transport system. Interestingly, this

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Table 3. Active Transport Data between the Acidic Phase and the Basic Phase with a Mixture of 1a and Octyl-15-crown-5 (5)

			transported ions[c] (%)					
		initial conditions[a,b] phase 1	phase 1			phase 2		
entry	ionophore	salt	Ca^{2+}	Na^+	Pic^-	Ca^{2+}	Na^+	Pic^-
30	**1a** + **5**	$CaCl_2$, NaCl[a]		2	15	27		
31	**1a** + **5**	$CaCl_2$, NaCl[b]			10	15		
32	**1a** + **5**	$CaCl_2$, PicNa[a]		7		30		25
33	**1a** + **5**	$CaCl_2$, PicNa[b]		7		15		19
34	**5**	$CaCl_2$, PicNa[a]		10		<1		20
35	**5**	$CaCl_2$, PicNa[b]		3		<1		7
36	**1a** + **5**	$CaCl_2$, PicNa[a,d]			54	50	27	

[a] Phase 1 (H_2O 10 mL): $[CaCl_2] = [NaCl] = [PicNa] = 0.005$ M, [Tris] = 0.05 M (pH 10), $[Me_4N^+Cl^-] = 0.1$ M. Membrane (CH_2Cl_2 20 mL): [ionophore] = 0.25 mM. Phase 2 (H_2O 10 mL): $[CaCl_2]$ [PicNa] = 0.005 M, [HCl] = 0.01 M (pH 2). [b] $[CaCl_2] = [NaCl] = [PicNa] = 0.01$ M. [c] After 48 h. [d] Tetramethylammonium chloride was not added.

Table 4. Active Transport Data between the Acidic Phase and the Basic Phase with a Mixture of Ionophores 2b and 6

			transported ions[b] (%)							
		initial conditions[a] phase 1	phase 1				phase 2			
entrty	ionophore	salt	Ca^{2+}	Na^+	K^+	Pic^-	Ca^{2+}	Na^+	K^+	Pic^-
37	**2b** + **6**	$CaCl_2$, PicNa, PicK		5			13		11	17
38[c]	**6**	PicNa, PicK		6					35	22

[a] Phase 1 (H_2O 10 mL): $[CaCl_2] = [PicNa] = [PicK] = 0.005$ M, [Tris] = 0.05 M (pH 10), $[Me_4N^+Cl^-] = 0.1$ M. Membrane (CH_2Cl_2, 20 mL): [ionophore] = 0.25 mM, [PicH] = 0.125 mM. Phase 2 (H_2O 10 mL): $[CaCl_2] = [PicNa] = [PicK] = 0.005$ M, [HCl] = 0.01 M (pH 2). [b] After 48 h. [c] Reference 8b.

mixture worked as an efficient carrier for the $Ca^{2+}-Na^+$ exchange. Without the tetramethylammonium ion, the picrate anion was concentrated in the basic phase, and both the calcium and sodium ions were concentrated in the acidic phase (entry 36). Under the transport conditions used for entry 32, the transport of the calcium ion, sodium ion, and picrate ion and the consumption of protons were pursued over time. After 48 h, most of the protons were consumed (>99%), and then the concentration of the calcium ion in the acidic phase gradually decreased with passage of time whereas the concentration of the sodium ion in the basic phase increased. Within the time in this experiment, the concentration of the picrate ion in the acidic phase increased with the assistance of the tetramethylammonium ion. After 96 h, the concentrations of the calcium cation and the picrate anion in the acidic phase increased by 18% and 51%, respectively, and the concentration of the sodium ion in the basic phase increased by 21%. Consequently, a simple combination of the active transporter of the calcium ion and the passive selective carrier for the sodium ion was also found to make possible the $Ca^{2+}-Na^+$ exchange.

Another point of interest is the combination of $Ca^{2+}-K^+$ and K^+-Na^+ exchangers. We examined the transfer of the metal cations through an artificial membrane in the presence of Ca^{2+}, K^+, and Na^+ by using an equimolar mixture of **2b** and **6** as the ionophore (Table 4). Ionophores **2b** and **6** possess the ability to transport Ca^{2+} and K^+ cations from the basic to the acidic phase, respectively, and to transport K^+ and Na^+ in the reverse direction, respectively. As a result, Ca^{2+} and K^+ were concentrated in the acidic phase, and Na^+ was concentrated in the basic phase. This result clearly shows that both ionophores are able to play their own roles in this double uphill transport system.

Conclusion

The exchange of $Ca^{2+}-Na^+$ and $Ca^{2+}-K^+$ through an artificial membrane was successfully realized by using lipophilic crown ether derivatives containing two carboxylic acid moieties. This double uphill transport needs both the proton and picrate anion gradients. A combination of $Ca^{2+}-K^+$ and K^+-Na^+ exchangers enabled the uphill transport of two of three kinds of cations in one direction and of the other cation in the reverse direction. This double uphill transport is potentially useful for highly selective separation of metal salts because such a system makes it possible, in principle, to realize the uptake of the desired cation and the release of the undesired cation at the same time.

Experimental Section

General. ^{1}H NMR spectra were taken at 400 MHz and reported in ppm downfield from TMS. Mass spectra were obtained by fast-atom bombardment.

2,2′-[1,4,7,10,13-Pentaoxacyclopentadecane-2,9-diyl]-bis[methylene(hexylimino)carbonyl]biscyclohexanecarboxylic Acid (1a). This compound was prepared by a similar procedure to that used for **4**, which was previously reported.[9] A solution of 2,9-bis(hexylaminomethyl)-15-crown-5 (**7a**)[14] (1.34 g, 3 mmol) and *cis*-1,2-cyclohexanedicarboxylic anhydride (1.85 g, 1.2 mmol) in THF (10 mL) was stirred for 48 h at room temperature. After the solvent was evaporated off, the excess anhydride was removed by distillation in a Kugelrohr (100−110 °C/0.04 Torr). An aqueous solution of 0.1 M HCl (50 mL) was added to the residue and then extracted with dichloromethane (50 mL × 3). The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel column chromatography (methanol/dichloromethane = 1/19 to 1/4) to give a slightly yellow, viscous liquid (1.30 g, 57%): IR (neat) 3600−2300, 2920, 2860, 1710, 1630, 1350, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.1−2.5 (m, 32H), 2.6−2.9 (m, 4H), 2.95−3.95 (m, 26H); FAB-MS *m/e* (relative intensity) 755 ($M^+ + 1$, 100). Anal. Calcd for $C_{40}H_{70}O_{11}N_2 \cdot 2H_2O$: C, 60.73; H, 9.43; N, 3.54. Found: C, 61.03; H, 9.31; N, 3.54.

The synthetic procedure of 2,2′-[1,4,7,10,13,16-hexaoxacyclooctadecane-2,9-diyl]bis[methylene(hexylimino)carbonyl]biscyclohexanecarboxylic acid (**1b**) was almost the same as that used for **1a**: yield 49%; IR (neat) 3600−2400, 2920, 2850, 1720, 1640, 1300, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.2−2.0 (m, 32H), 2.3−2.8 (m, 4H), 2.9−3.9 (m, 30H); FAB-

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MS *m/e* (relative intensity) 799 (M + 1, 41). Anal. Calcd for $C_{42}H_{74}O_{13}N_2 \cdot H_2O$: C, 61.74; H, 9.38; N, 3.43. Found: C, 61.54; H, 9.35; N, 3.34.

2,2′-[2,9-Dimethyl-1,4,7,10,13-pentaoxacyclopentadecane-2,9-diyl]bis[(methyleneoxy)-2,1-ethanediyl(hexylimino)carbonyl]biscyclohexanecarboxylic Acid (2a). The precursor for **2a**, 2,9-bis[[2-(hexylamino)ethoxy]methyl]-2,9-dimethyl-15-crown-5 (**8a**) was prepared by the reaction of 2,9-bis(bromomethyl)-2,9-dimethyl-15-crown-5 (**9a**)[15] with the sodium alkoxide of *N*-hexylaminoethanol by the procedure as follows. Sodium metal (0.55 g, 24 mmol) was completely dissolved in N-hexylethanolamine (8.71 g, 60 mmol) at 60 °C. Then **9a** was added to the mixture, and the mixture was stirred for 48 h at 120 °C. After the mixture was cooled to room temperature, water (100 mL) was added to the mixture and the mixture was extracted with dichloromethane (100 mL × 2). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The unreacted alcohol was removed by Kugelrohr distillation (50−60 °C/0.04 Torr). The crude product was purified by alumina column chromatography (dioxane/hexane = 1/9 to 1/1) to afford **8a** as a slightly yellow, viscous oil (0.90 g, 27%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.13 (s, 6H), 1.29−1.50 (m, 16H), 2.15 (bs, 2H), 2.60 (t, 4H), 2.77 (t, 4H), 3.31−3.73 (m, 24H); FAB-MS *m/e* (relative intensity) 563 ($M^+ + 1$, 100).

2,12-Bis[[2-(hexylamino)ethoxy]methyl]-2,12-dimethyl-18-crown-6 (**8b**) was also prepared by the reaction of 2,12-bis(bromomethyl)-2,12-dimethyl-18-crown-6 (**9b**)[14] with the sodium alkoxide of *N*-hexylaminoethanol by a similar procedure to that for **8a**: yield 51%; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.17 (s, 6H), 1.23−1.34 (m, 12H), 1.45−1.50 (m, 4H), 2.11 (bs, 2H), 2.59 (t, 4H), 2.75 (t, 4H), 3.35−3.74 (m, 28H); FAB-MS *m/e* (relative intensity) 607 ($M^+ + 1$, 100).

The reaction of **8a** with *cis*-1,2-cyclohexanedicarboxylic anhydride in THF was carried out by almost the same procedure for **1a** to afford **2a** in 24% yield: IR (neat) 3600−2400, 2950, 1730, 1650, 1450, 1350, 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H), 1.16 (s, 6H), 1.2−2.3 (m, 32H), 2.7−2.9 (m, 4H), 3.2−4.0 (m, 32H); FAB-MS *m/e* (relative intensity) 893 ($M^+ + Na^+$, 100). Anal. Calcd for $C_{46}H_{82}O_{13}N_2 \cdot H_2O$: C, 62.14; H, 9.52; N, 3.15. Found: C, 61.89; H, 9.67; N, 3.21.

The synthetic procedure of 2,2′-[2,12-dimethyl-1,4,7,10,13,16-hexaoxacyclooctadecane-2,12-diyl]bis[(methyleneoxy)-2,1-ethanediyl(hexylimino)carbonyl]biscyclohexanecarboxylic acid (**2b**) from **8b** and *cis*-1,2-cyclohexanedicarboxylic anhydride was almost the same as that used for **1a**: yield 70%; IR (neat) 3600−2400, 2950, 1730, 1650, 1450, 1350, 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86−0.91 (m, 6H), 1.13 (s, 6H), 1.2−1.8 (m, 36H), 2.4−2.9 (m, 4H), 3.0−3.9 (m, 36H); FAB-MS *m/e* (relative intensity) 915 ($M^+ + 1$, 6). Anal. Calcd for $C_{48}H_{86}O_{14}N_2 \cdot H_2O$: C, 61.78; H, 9.50; N, 3.00. Found: C, 62.13; H, 9.42; N, 3.06.

2,2′-[[1-[[[8(or 9)-Dodecyl-2-methyl-1,4,7,10,13-pentaoxacyclopentadec-2-yl]methoxy]methyl]-1,2-ethanediyl]-bis(oxy)]bisacetic Acid (3). The key intermediate, 2-(bromomethyl)-11(12)-dodecyl-2-methyl-15-crown-5 (**10**), was prepared by the intermolecular reaction of tetradecane-1,2-diol with the tetraethylene glycol derivative containing both methyl and bromomethyl substituents under basic conditions. The latter compound was prepared by the bromoalkoxylation of ethylene glycol monomethallyl ether by using *N*-bromosuccinimide and diethylene glycol, followed by tosylation according to the literature.[12] Under a nitrogen atmosphere, sodium (0.18 g, 8 mmol) was completely dissolved in 2,2-dimethyl-1,3-dioxolane-4-methanol (5.29 g, 40 mmol) and then **10** (1.98 g, 4 mmol) was added to the mixture, which was stirred at 100 °C for 16 h. Water (100 mL) was added, and the mixture was extracted with dichloromethane (100 mL × 2). The dichloromethane layer was dried over $MgSO_4$, filtered, and concentrated. The lighter fraction was further removed by distillation in a Kugelrohr apparatus under reduced pressure (∼120 °C/0.04 Torr). The residue was purified by alumina column chromatography (dioxane/benzene = 1/99 to 5/95) to give **11** as a colorless liquid. This liquid was dissolved in 30 mL of a mixed solvent of water and dioxane (3:7) and several drops of concd HCl were added to the solution. The resulting mixture was refluxed for 4 h and then cooled to room temperature. Water (50 mL) was added, and the mixture was extracted with dichloromethane (50 mL × 3). The organic layer was dried over $MgSO_4$, filtered, and concentrated to afford 1.28 g (63%) of 11(12)-dodecyl-2-[[(2,3-dihydroxypropyl)oxy]methyl]-2-methyl-15-crown-5 (**12**) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.16 (s, 3H), 1.25−1.55 (m, 22H), 2.80 (s, 2H), 3.43−3.90 (m, 24H). To a stirred suspension of **12** (0.95 g, 1.9 mmol) and 1.90 g (15.2 mmol) of potassium *tert*-butoxide in 20 mL of *tert*-butyl alcohol was added bromoacetic acid (1.04 g, 7.5 mmol) in *tert*-butyl alcohol (10 mL) over a period of 20 min at the refluxed temperature, and the resulting mixture was refluxed for another 40 h. After being cooled to room temperature, the mixture was acidified by using concd HCl and the insoluble matter was removed by filtration through Celite and washed by benzene and concentrated. An aqueous sodium carbonate solution (5%, 70 mL) was added to the residue and washed with diethyl ether (70 mL × 2). Then the aqueous layer was extracted with dichloromethane (100 mL × 3). The dichloromethane layer was dried over $MgSO_4$, filtered and concentrated to give a viscous liquid. After this liquid was dissolved in 20 mL of methanol containing several drops of concd sulfuric acid, the solution was refluxed for 12 h. Water (60 mL) was added, and the mixture was extracted with dichloromethane (60 mL × 3), dried, filtered, and concentrated. The crude product was purified by silica gel column chromatography (methanol/dichloromethane = 0/100 to 1/99) to give 0.3 g (23%) of the dimethyl ester of **3** as a colorless viscous liquid: IR (neat) 2920, 2850, 1750, 1450, 1360, 1260, 1205, 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.15 (s, 3H), 1.2−1.5 (m, 22H), 3.41−3.82 (m, 30H), 4.15 (s, 2H), 4.33 (s, 2H); MS *m/e* (relative intensity) 651 ($M^+ + 1$, 3). Anal. Calcd for $C_{33}H_{62}O_{12}$: C, 60.90; H, 9.60. Found: C, 60.88; H, 9.70.

Compound **3** was quantitatively obtained from the hydrolysis of the dimethyl ester in methanol containing aqueous NaOH solution, followed by neutralization by concd HCl.

Transport Studies. Transport experiments were done according to the procedures reported previously, that is, in a U-type cell at 25 °C. A dichloromethane solution (20 mL) containing the ionophore was placed in the bottom of the cell, and two portions of aqueous solutions (10 mL) were carefully added on top of them. Both surface areas were 2.0 cm^2. The organic phase was magnetically stirred at 500 rpm. The details of the transport conditions were summarized in the footnotes to the tables. Both aqueous phases were sampled after 48 h and analyzed for cation concentration using a Nippon Jarrel-Ash AA-8500 atomic absorption spectrophotometer. The concentrations of the picrate anion were obtained by calculation based on the absorption at 354 nm in the UV spectrum. Each experiment was repeated at least three times, and the results are reported as the average of the three determinations. In the case of the active transport system, the data for transported ions (%) denote the mean of the increment of ions in one phase and the decrement of the ions in the other phase.

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