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Bis(crown ether)s as Na^+–K^+ ATPase Model in a Liquid Membrane#

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Abstract. A new type of bis(crown ether)s containing 15-crown-5 and monoaza-18-crown-6 actively transported Na^+ and K^+ in opposite directions across a dichloromethane membrane by pH control. The effect of the structure of the ion carriers on their transport abilities was examined. A key point for the molecular design of the carriers is how to give them complexing ability toward Na^+ under acidic conditions. A proper choice of the transport conditions was found to be essential for the success of the double uphill transport.

Key words. Synthetic ionophore, active transport, alkali metal cation transport, solvent extraction.

1. Introduction

Our early attention in the field of crown chemistry created by Pedersen [1] was paid to developing facile synthetic methods for crown ethers [2]. The intramolecular cyclization method [3] developed by us for simple crown ethers [4] was successfully applied to the preparation of *N*-substituted azacrown ethers [5], thiacrown ethers [6], and polyether esters [7]. Synthetic procedures for crown ethers with a functional group such as a hydroxyl [8], aminomethyl [9], and bromomethyl [10] enabled the molecular design of highly functionalized derivatives [11–16], which recognize a specific molecule.

One of their important characteristics is the complexing ability toward hard cations such as alkali metal and alkaline earth metal cations, as first shown by Pedersen. This property is especially noteworthy from the viewpoint of the resemblance to the complexation property of naturally occurring antibiotics. In other words, crown ethers are simple model compounds for such natural ionophores [17]. The function of valinomycin and monensin has been realized in an artificial transport system using synthetic ionophores. Although a variety of proton-ionizable crowns has been documented as suitable synthetic ionophores, we succeeded in developing another active transport system using lipophilic azacrown ethers [18]. These compounds are able to selectively transport alkali metal cations against their concentration gradients based on the recognition of the ion sizes with their cavity sizes [19, 20]. The enzyme Na^+–K^+ ATPase actively transports Na^+ and K^+ in opposite directions through a biomembrane [21]. Simple lipophilic azacrown ethers,

This paper is dedicated to the memory of the late Dr C. J. Pedersen.
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however, have no ability to transport a metal ion from an acidic to a basic phase. In order to mimic the function of an enzyme in an artificial transport system, another structural device is needed for the design of the ionophore. Lipophilic monoaza-18-crown-6 can selectively transport K^+ from a basic to an acidic phase against its concentration gradient [18]. If a derivative containing monoaza-18-crown-6 possesses complexing ability toward Na^+ under acidic conditions, it has the possibility of transporting K^+ and Na^+ in opposite directions.

From this point of view, we will describe the synthesis of new bis(crown ether)s and the characterization of their transport ability as the model for the Na^+–K^+ ATPase [22].

2. Experimental

^1H NMR spectra were taken at 400 MHz on a JEOL JNM-GSX-400 spectrometer, using tetramethylsilane as the internal standard. IR and UV spectra were obtained with a Hitachi 260-10 spectrometer and a Shimadzu UV-200 spectrophotometer, respectively. Mass spectra were measured with a JEOL JMS-DX303 mass spectrometer. 2-(Bromomethyl)-2-methyl-15-crown-5 (**6**) [10], monoaza-15-crown- 5 (**7a**) [23], monoaza-18-crown-6 (**7b**) [23], *N*-(2-hydroxyethyl)-monoaza-15-crown-5 (**8a**) [13], *N*-(2-hydroxyethyl)-monoaza-18-crown-6 (**8b**) [13], *N*-[2-(2-hydroxyethoxy)ethyl]-monoaza-15-crown-5 (**9a**) [13], *N*-[2-(2-hydroxyethoxy)ethyl]-monoaza-18-crown-6 (**9b**) [13] were prepared according to the literature. *N*-(3-Hydroxypropyl)-monoaza-15-crown-5 (**10a**) was prepared by the reaction of **7a** with 3-chloropropanol in the presence of sodium carbonate at 120°C for 45 h and purified by distillation in a Kugelrohr apparatus (130°C/0.04 torr) to give a slightly yellow oil in a 60% yield. *N*-(3-Hydroxypropyl)-monoaza-18-crown-6 (**10b**) was prepared as above for **10a** from **7b** and purified by distillation in a Kugelrohr apparatus (150°C/0.05 torr) to give a slightly yellow oil in an 84% yield. *N*-Octyl-diethanolamine (**11**) was prepared by the reaction of diethanolamine with *n*-octyl bromide in the presence of sodium carbonate at 120°C for 72 h and purified by distillation in a Kugelrohr apparatus (110°C/0.04 torr) to give a slightly yellow oil in a 65% yield.

2.1. PREPARATION OF **1a**

13-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl)methyl]-1,4,7,10-tetraoxa-13-azacyclopentadecane (**1a**) was prepared by the following sequence. A mixture of 2-(bromomethyl)-2-methyl-15-crown-5 (**6**) (3.27 g, 10 mmol), monoaza-15-crown-5 (**7a**) (2.18 g, 10 mmol), and potassium carbonate (1.66 g, 12 mmol) was stirred at 140°C for 62 h under a nitrogen atmosphere. Water (100 mL) was added to the cooled reaction mixture and extracted with dichloromethane (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and concentrated. Low boiling compounds were removed under reduced pressure in a Kugelrohr apparatus (110°C/0.04 torr). The crude product was purified by chromatography over alumina (dichloromethane/dioxane = 10/1 ~ 2/1 (v/v)) to give **1a** (1.87 g, 40%) as a slightly yellow oil. The analytical data for **1a** are as follows: IR (neat) 2880, 1450, 1300, 1250, 1130 cm^{-1}; NMR: δ 1.16 (*s*, 3H), 2.75–3.01(*m*, 6H), 3.40–3.98(*m*, 34H); MS(*m*/*z*) 465(M^+, 0.8), 233(16), 232(100), 101(2), 57(3), 45(4), 43(12).

Anal. Calcd. for $C_{22}H_{43}NO_9$: C, 56.75; H, 9.31; N, 3.01. *Found*: C, 56.75; H, 9.19; N, 2.88.

2.2. PREPARATION OF **1b**

16-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl)methyl]-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (**1b**) was prepared from **6** (4.04 g, 12 mmol), **7b** (3.16 g, 12 mmol), and potassium carbonate (2.16 g, 14 mmol) at 140°C for 45 h under a nitrogen atmosphere as above for **1a**. The crude product was purified by chromatography over alumina (dichloromethane/isopropyl alcohol = 100/1.5 (v/v)) to give **1b** (1.40 g, 23%) as a slightly yellow oil. The analytical data for **1b** are as follows: IR (neat) 2870, 1460, 1300, 1250, 1120 cm^{-1}; NMR: δ 1.15(*s*, 3H), 2.80–2.84(*m*, 6H), 3.40–3.85(*m*, 38H); MS(*m*/*z*) 509(M^+, 0.8), 277(17), 276(90), 232(100), 101(4), 57(4), 45(8), 43(3).

Anal. Calcd. for $C_{24}H_{47}NO_{10}$: C, 56.56; H, 9.30; N, 2.75. *Found*: C, 56.24; H, 9.20; N, 2.94.

2.3. PREPARATION OF **2a**

13-[2-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl)methoxy]ethyl]-1,4,7,10-tetraoxa-13-azacyclopentadecane (**2a**) was prepared by the following sequence. After sodium metal (0.15 g, 6.6 mmol) was dissolved in *t*-butyl alcohol (25 mL), **8a** (1.32 g, 5 mmol) was added to the mixture, and the resultant mixture was refluxed for 1 h. **6** (1.08 g, 3.3 mmol) was added to the mixture and then the *t*-butyl alcohol was evaporated off. The residue was stirred at 130°C for 6 h. Water (100 mL) was added to the cooled reaction mixture and extracted with dichloromethane (100 mL × 3). The combined organic layer was dried over $MgSO_4$ and concentrated. Low boiling compounds were removed under reduced pressure in a Kugelrohr apparatus (150°C/0.05 Torr). The crude product was purified by chromatography over alumina (dichloromethane/isopropyl alcohol = 100/3 ~ 20/1 (v/v)) to give **2a** (0.53 g, 32%) as a slightly yellow oil. The analytical data for **2a** are as follows: IR (neat) 2850, 1450, 1300, 1250, 1120 cm^{-1}; NMR: δ 1.18 (*s*, 3H), 2.76–2.85(*m*, 6H), 3.33–3.83(*m*, 34H); fab MS(*m*/*z*) 510($M^+ + 1$, 43), 233(18), 232(100), 101(12), 57(45).

Anal. Calcd. for $C_{24}H_{47}NO_{10}$: C, 56.56; H, 9.30; N, 2.75. *Found*: C, 56.32; H, 9.21; N, 2.68.

2.4. PREPARATION OF **2b**

16-[2-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl) methoxy] ethyl] -1,4,7,10,13-pentaoxa-16-azacyclooctadecane (**2b**) was prepared from **6** (2.19 g, 6.7 mmol), **8b** (3.07 g, 10 mmol), and sodium metal (0.31 g, 14 mmol) at 130°C for 15 h as above for **2a**. The crude product was purified by chromatography over alumina (dichloromethane/dioxane = 10/1 ~ 3/2 (v/v)) to give **2b** (0.89 g, 24%) as a slightly yellow oil. The analytical data for **2b** are as follows: IR (neat) 2860, 1450, 1290, 1240, 1100 cm^{-1}; NMR: δ 1.20(*s*, 3H), 2.70–2.90(*m*, 6H), 3.41–3.92(*m*, 42H); fab MS(*m*/*z*) 554($M^+ + 1$, 82), 277(17), 276(100), 101(13), 100(21), 57(18).

Anal. Calcd. for $C_{26}H_{51}NO_{11}$: C, 56.40; H, 9.28; N, 2.53. *Found*: C, 56.58; H, 9.26; N, 2.42.

2.5. PREPARATION OF **3a**

13-[2-[2-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl) methoxy]ethoxy]ethyl]-1,4,7,10-tetraoxa-13-azacyclooctadecane (**3a**) was prepared from **6** (0.82 g, 2.5 mmol), **9a** (1.15 g, 3.75 mmol), and sodium metal (0.11 g, 5 mmol) at 130°C for 6 h as above for **2a**. The crude product was purified by chromatography over alumina (dichloromethane/dioxane = 4/1 (v/v)) to give **3a** (0.61 g, 46%) as a slightly yellow oil. The analytical data for **3a** are as follows: IR (neat) 2870, 1450, 1300, 1250, 1120 cm^{-1}; NMR: δ 1.18(*s*, 3H), 2.68–2.92(*m*, 6H), 3.40–3.88(*m*, 42H); MS(*m*/*z*) 553($M^+ + 1$, 1), 233(13), 232(100), 101(10), 45(13).

Anal. Calcd. for $C_{26}H_{51}NO_{11}$: C, 56.40; H, 9.28; N, 2.53. *Found*: C, 56.38; H, 9.38; N, 2.46.

2.6. PREPARATION OF **3b**

16-[2-[2-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl) methoxy] ethoxy] ethyl]-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (**3b**) was prepared from **6** (1.64 g, 5 mmol), **9b** (2.64 g, 7.5 mmol), and sodium metal (0.23 g, 10 mmol) at 130°C for 6 h as above for **2a**. The crude product was purified by gel permeation chromatography using chloroform as the eluent to give **3b** (1.97 g, 66%) as a slightly yellow oil. The analytical data for **3b** are as follows: IR (neat) 2870, 1460, 1300, 1250, 1120 cm^{-1}; NMR: δ 1.20(*s*, 3H), 2.62–3.00(*m*, 6H), 3.40–3.92(*m*, 46H); MS(*m*/*z*) 597(M^+, 0.8), 277(16), 276(100), 232(9), 101(15), 99(8), 57(4), 45(18), 43(7).

Anal. Calcd. for $C_{28}H_{55}NO_{12} \cdot H_2O$: C, 54.62; H, 9.33; N, 2.27. *Found*: C, 54.82; H, 9.20; N, 2.34.

2.7. PREPARATION OF **4a**

13-[3-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl)methoxy]propyl]-1,4,7,10-tetraoxa-13-azacyclooctadecane (**4a**) was prepared from **6** (2.62 g, 8 mmol), **10a** (3.33 g, 12 mmol), and sodium metal (0.55 g, 24 mmol) at 130°C for 18 h as above for **2a**. The crude product was purified by chromatography over alumina (dichloromethane/dioxane = 10/1 (v/v)) to give **4a** (0.75 g, 18%) as a slightly yellow oil. The analytical data for **4a** are as follows: IR (neat) 2880, 1460, 1300, 1260, 1100 cm^{-1}; NMR: δ 1.16(*s*, 3H), 1.52–1.92(*m*, 2H), 2.44–2.92 (*m*, 6H), 3.32–3.92 (*m*, 38H); MS(*m*/*z*) 523(M^+, 4), 233(30), 232(100), 101(34), 100(15), 57(16), 45(30).

Anal. Calcd. for $C_{25}H_{49}NO_{10}$: C, 57.34; H, 9.43; N, 2.67. *Found*: C, 57.35; H, 9.34; N, 2.36.

2.8. PREPARATION OF **4b**

16-[3-[(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl)methoxy]propyl]-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (**4b**) was prepared from **6** (0.82 g, 2.5 mmol), **10b** (1.21 g, 3.75 mmol), and sodium metal (0.11 g, 5 mmol) at 130°C for 6 h as above for **2a**. The crude product was purified by chromatography over alumina (dichloromethane/dioxane = 4/1 (v/v)) to give **4b** (0.48 g, 34%) as a slightly yellow

oil. The analytical data for **4b** are as follows: IR (neat) 2870, 1460, 1300, 1260, 1115 cm^{-1}; NMR: δ 1.16(*s*, 3H), 1.52–1.92(*m*, 2H), 2.44–2.92(*m*, 6H), 3.32–3.92(*m*, 42H); fab MS(*m/z*) 568($M^+ + 1$, 100), 277(10), 276(60), 101(22), 57(20).

Anal. Calcd. for $C_{27}H_{53}NO_{11}$: C, 57.12; H, 9.41; N, 2.47. *Found*: C, 56.82; H, 9.31; N, 2.16.

2.9. PREPARATION OF 5

N,N-[Bis[2-(2-Methyl-1,4,7,10,13-pentaoxadec-2-yl)methoxy]ethyl]octylamine (**5**) was prepared from **6** (3.27 g, 10 mmol), **11** (1.09 g, 5 mmol), and sodium metal (0.29 g, 12.5 mmol) at 130°C for 6 h as above for **2a**. The crude product was purified by chromatography over alumina (dichloromethane/dioxane = 10/1 (v/v)) to give **5** (1.73 g, 49%) as a slightly yellow oil. The analytical data for **5** are as follows: IR (neat) 2930, 2860, 1460, 1290, 1260, 1120 cm^{-1}; NMR: δ 0.88(*t*, 3H), 1.18–1.45(*m*, 18H), 2.50–2.70(*m*, 6H), 3.35–3.83(*m*,44 H); fab MS(*m/z*) 710($M^+ + 1$, 4), 596(5), 464(100), 462(44), 200(100), 154(23), 57(32).

Anal. Calcd. for $C_{36}H_{71}NO_{12} \cdot 0.5H_2O$: C, 60.14; H, 10.09; N, 1.95. *Found*: C, 59.88; H, 9.94; N, 1.81.

2.10. EXTRACTION PROCEDURE [24]

A mixture of an aqueous solution (10 mL) of alkali metal hydroxide (5×10^{-2} M) and picric acid (5×10^{-4} M) and a dichloromethane solution (10 mL) of an appropriate extractant (5×10^{-4} M) was shaken at 22°C for 9 h. The extractability was obtained from the calculation based on the absorption of picrate anion in the aqueous phase at 354 nm in the UV spectrum.

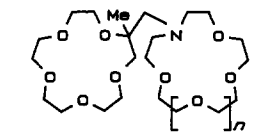
2.11. CATION TRANSPORT STUDIES

Membrane transport experiments were carried out in a U-type cell (tube diameter: 16 mm; arm height: 150 mm) at 25°C. Both surface areas were 2.0 cm^2. The details for transport conditions are summarized in the footnotes of Tables I–III and the caption of Figure 3. Each cell consisted of a 20 mL membrane phase (CH_2Cl_2, 1.25×10^{-4} M in a carrier, stirred at 500 rpm by a magnetic stirrer) interfaced to two 10 mL of aqueous phases. Both aqueous phases were sampled after appropriate times and analyzed for cation concentration using a Nippon Jarrell-Ash AA-8500 atomic absorption spectrometer. Each experiment was repeated at least three times and the results are reported as the average of the three values. The deviations from the mean were less than $\pm 15\%$ of the value given. In the case of the active transport system (Table III), the data of transported cations (%) denote the mean of the increment of cations in one phase and the decrement of cations in the other phase. The concentration of picrate anion was obtained from the calculation based on the absorption at 354 nm in the UV spectrum. The pH values were measured using a Hitachi-Horiba F7 pH Meter. The concentration of tetramethylammonium ion was determined using ^1H NMR.

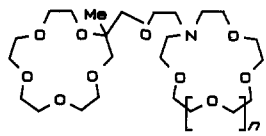
3. Results and Discussion

3.1. SYNTHESIS OF BIS(CROWN ETHER)S

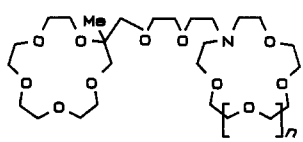
The bis(crown ether)s (**1**–**4**) were prepared by the reactions of 2-(bromomethyl)-2-methyl-15-crown-5 (**6**) [10] and the appropriate azacrown ether derivatives. Compound **5** was also prepared by the reaction of **6** and the corresponding diol. The proposed structures were consistent with the data obtained from their IR, NMR, and mass spectra and combustion analyses.



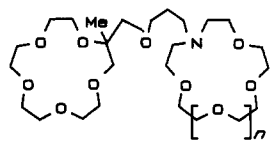
1a: *n* = 1
1b: *n* = 2



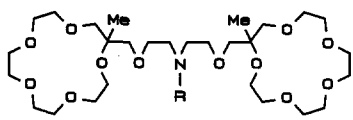
2a: *n* = 1
2b: *n* = 2



3a: *n* = 1
3b: *n* = 2



4a: *n* = 1
4b: *n* = 2



5: R = C_8H_{17}

3.2. SOLVENT EXTRACTION

The complexing ability of **1**–**5** toward alkali metal cations was assessed by the picrate extraction method with dichloromethane as the organic solvent. Figures 1 and 2 show extraction profiles of the derivatives containing two 15-crown rings (**1a**–**4a**, **5**) and the derivatives containing both 15- and 18-crown rings (**1b**–**4b**), respectively. The data are useful for estimating the cation selectivity under basic conditions. Generally speaking, compounds **1b**–**4b** possess higher K^+/Na^+ selectivity than the corresponding analogues (**1a**–**4a**). It is interesting that **4a** and **5** did not show K^+/Na^+ selectivity. This result indicates that both 15-crown rings of **4a** and **5** do not cooperatively work for the uptake of K^+. In the case of **4b**, the cooperative coordination of both crown rings toward K^+ is expected to be insignificant but the monoaza-18-crown-6 ring possesses a strong complexing ability toward K^+.

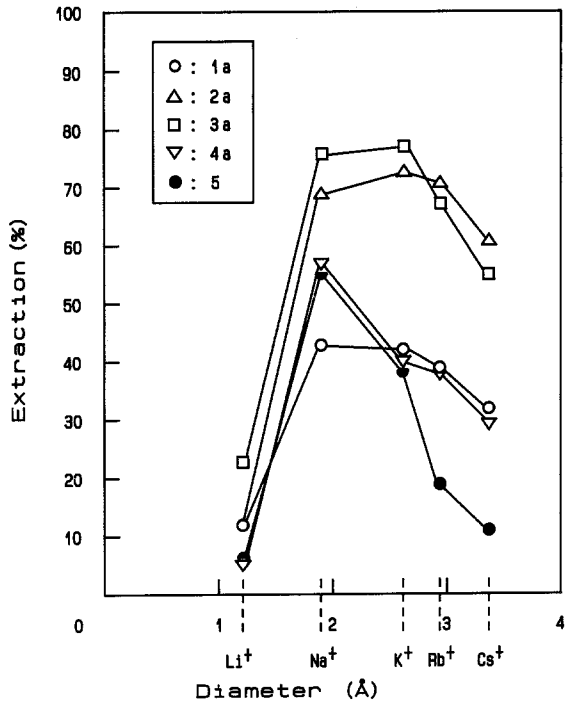


Fig. 1. Alkali-metal picrate extraction with the bis(crown ether)s containing two 15-crown rings.

3.3. PASSIVE TRANSPORT SYSTEM

Transport experiments were carried out at 25°C in a U-type cell. Dichloromethane was used as the liquid membrane. Both aqueous phases were adjusted to be basic and acidic by tetramethylammonium hydroxide and hydrochloric acid. Equimolar amounts of K^+ and Na^+ were added to either aqueous phase according to the transport conditions. The concentrations of cations, pricrate ions, and protons were determined by atomic absorption analysis, UV spectroscopy, and pH titration, respectively. The passive transport data from the basic to the acidic phase and those of the opposite direction are shown in Tables I and II. In the case of passive transport from the basic to the acidic phase, Table I clearly shows that the trend observed in transport selectivity coincides well with that expected from the extraction data. Ionophores **4a** and **5** selectively transported Na^+. The transport ability of **1a** and **1b** was rather lower than that of the corresponding analogues having the oxyethylene connecting group (**2** and **3**). On the other hand, in the passive transport from the acidic to the basic phase, all ionophores showed selectivity toward Na^+ as expected from the structure. In this case, **3** and **4** transported more cations than **1** and **2**. This finding suggests that the distance between the protonation site and the 15-crown-5 ring in the molecule remarkably affects the transport ability. Ionophore **5** carried a lot of cations possibly because each 15-crown-5 ring is able to independently uptake the cation.

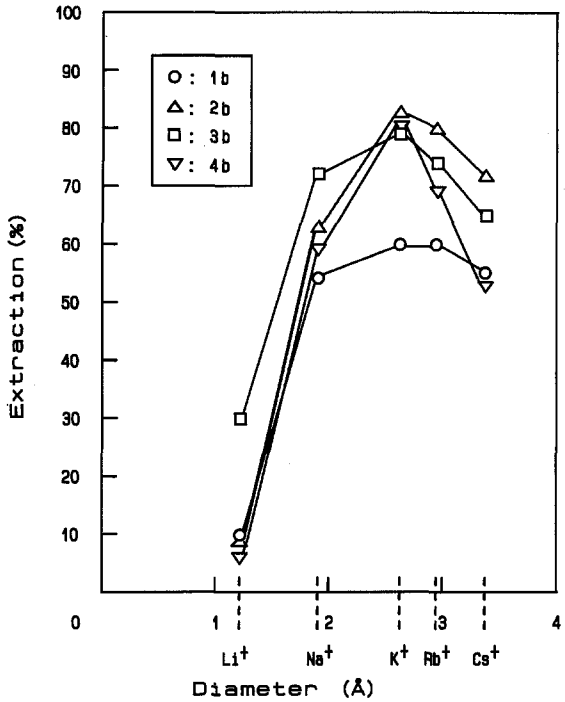


Fig. 2. Alkali-metal picrate extraction with the bis(crown ether)s containing both 15-crown and 18-crown rings.

Table I. Passive transport data[a] from the basic phase to the acidic phase.

Ionophore	Transported Cations (%)[b]		Selectivity (K^+/Na^+)
	Na^+	K^+	
1a	4	10	2.5
2a	11	28	2.5
3a	14	22	1.6
4a	13	10	0.77
5	11	7	0.64
1b	5	18	3.6
2b	6	31	5.2
3b	10	29	2.9
4b	10	26	2.6

[a]Phase 1 (basic): $[PicK] = [PicNa] = 5 \times 10^{-3}$ M, $[Me_4NOH] = 10^{-1}$ M / Membrane: [Ionophore] = [PicH] = 1.25×10^{-4} M / Phase 2(acidic): [HCl] = 5×10^{-3} M.
[b]After 48 h.

Table II. Passive transport data [a] from the acidic phase to the basic phase.

Ionophore	Transported Cations (%)[b]		Selectivity (Na^+/K^+)
	Na^+	K^+	
1a	3	<1	>3
2a	7	3	2.3
3a	15	14	1.1
4a	17	10	1.7
5	21	12	1.8
1b	3	<1	>3
2b	9	2	4.5
3b	14	4	3.5
4b	16	6	2.7

[a]Phase 1 (basic): $[Me_4NOH] = 10^{-1}$ M / Membrane: [Ionophore] = [PicH] = 1.25×10^{-4} M / Phase 2(acidic): [PicK] = [PicNa] = [HCl] = 5×10^{-3} M.
[b]After 48 h.

3.4. ACTIVE TRANSPORT SYSTEM

Table III shows the quantity of cations transported after 48 h in the active transport system. Ionophores **2a**, **2b**, **3b**, and **4b** successfully transported K^+ and Na^+ in opposite directions. The ionophores consisting of 15-crown-5, monoaza-18-crown-6, and an appropriate connecting group (**2b**, **3b**) are especially effective. Ionophores **1a** and **1b** without an oxyethylene chain as the connecting group concentrated both K^+ and Na^+ in the acidic phase. In the acidic phase, the ionophore is protonated

Table III. Active transport data.[a].

Ionophore	Transported Cations (%)[b]			
	Basic Phase		Acidic Phase	
	Na^+	K^+	Na^+	K^+
1a	–	–	2	6
2a	5	–	–	19
3a	0	–	–	13
4a	0	–	–	5
5	5	0	–	–
1b	–	–	6	12
2b	10	–	–	31
3b	13	–	–	25
4b	7	–	–	24

[a]Phase 1 (basic): [PicK] = [PicNa] = 5×10^{-3} M, $[Me_4NOH] = 10^{-1}$ M / Membrane: [Ionophore] = [PicH] = 1.25×10^{-4} M / Phase 2(acidic): [PicK] = [PicNa] = [HCl] = 5×10^{-3} M.
[b]After 48 h.

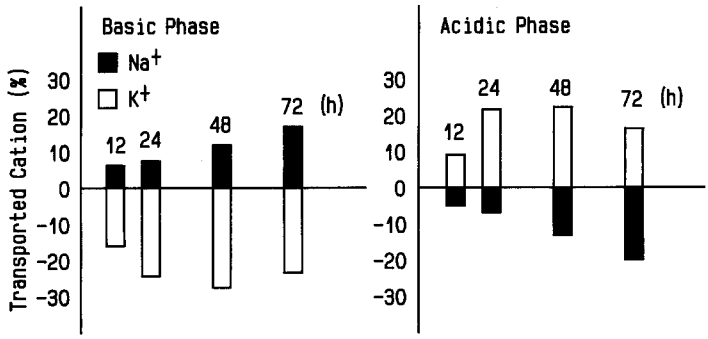


Fig. 3. Active transport of Na^+ and K^+ in opposite directions with **3b**. Standard transport conditions: Phase 1 (basic), [PicK] = [PicNa] = 5×10^{-3} M, [Me_4NOH] = 10^{-1} M / Liquid Membrane, [**3b**] = [PicH] = 1.25×10^{-4} M / Phase 2 (acidic), [PicK] = [PicNa] = [HCl] = 5×10^{-3} M.

and has to complex with Na^+ in the form of an ammonium ion. This result clearly shows that an appropriate distance between the protonation site and the coordination site for Na^+ should be needed.

Figure 3 shows the quantity of K^+ and Na^+ transported using **3b** with the passage of time. In the basic phase, the quantity of K^+ decreased and the quantity of Na^+ increased. On the other hand, in the acidic phase, the reverse trend was observed. After 48 h, most of the protons were consumed and so the transport velocity of K^+ from the acidic to the basic phase increased. As a result, the concentration of K^+ in the acidic phase gradually decreased.

When the base was changed from tetramethylammonium hydroxide to lithium hydroxide in this transport system, the double uphill transport in opposite directions disappeared as shown in Table IV (Run 2). Both K^+ and Na^+ were concentrated in the acidic phase. At this point, the behaviour of picrate anion is interesting. In this case, picrate anion was concentrated in the basic phase. On the other hand, when tetramethylammonium ion was used as the base, picrate anion was concentrated in the acidic phase (Run 1). The control experiment without the ionophore also showed that picrate ion was concentrated in the acidic phase when

Table IV. Effect of tetramethylammonium cation on the active transport.[a]

			Transported Ions (%)[b]					
			Basic Phase			Acidic Phase		
Run No.	Base	Ionophore	Na^+	K^+	Pic^-	Na^+	K^+	Pic^-
1	Me_4NOH	**3b**	13	–	–	–	25	37
2	LiOH[c]	**3b**	–	–	31	11	29	–
3	Me_4NOH	–[d]	0	0	–	0	0	20

[a] See the caption of Figure 3.
[b] After 48 h.
[c] Only base was changed from the standard transport conditions.
[d] Ionophore was not present in the system.

tetramethylammonium ion was used (Run 3). Of course, in this case, the transport of both K^+ and Na^+ was not observed. These results suggest that tetramethylammonium ion plays an important role in transporting the picrate ion from the basic to the acidic phase. The transfer of the tetramethylammonium ion from the basic to the acidic phase was determined by ^1H NMR.

The proton concentrations in the acidic phase also affect the active transport (Table V). With the increase in the proton concentration, the transport of Na^+ from the acidic to the basic phase remarkably decreased. This result suggests that the ionophore tends to transport only H^+ without Na^+ from the acidic to the basic phase when the proton concentration is high. A suitable choice of transport conditions should be of importance to attain the double uphill transport.

Although the transport mechanism is complicated, the results obtained in this study may be explained as shown in Figure 4. In the basic interface, the ionophore **3b** complexes with K^+ rather than Na^+ as expected by the extraction data. The ionophore transports K^+ with a picrate ion across the liquid membrane to the acidic phase. In the acidic interface, the nitrogen atom of the ionophore is protonated to release K^+. After protonation, the ionophore still has the binding site, consisting of the 15-crown-5 ring and an electron-donating side-arm for Na^+. Thus, the ionophore can selectively uptake sodium picrate as a form of the ammonium salt from the acidic phase to the liquid membrane and the resulting complex moves to the basic phase across the membrane. Consequently, K^+ and Na^+ are concentrated in the acidic phase and the basic phase, respectively.

When this type of ionophore transports Na^+ from the acidic to the basic phase, the ionophore demands two picrate ion. According to this cycle, picrate ions should be concentrated in the basic phase. However, tetramethylammonium ion independently transports picrate ion from the basic to the acidic phase. The higher concentration of the picrate ion in the acidic phase is advantageous for the uptake process of Na^+ in the acidic phase.

Table V. Effect of proton concentration in acidic phase on the active transport [a] with **3b**.

		Transported Ions (%)[b]					
		Basic Phase			Acidic Phase		
Run No.	[HCl] in Phase 2[c]	Na^+	K^+	Pic^-	Na^+	K^+	Pic^-
1	5.0×10^{-3}	13	–	–	–	25	37
4	1.0×10^{-2}	6	–	–	–	35	22
5	2.0×10^{-2}	–	–	–	1	40	9
6	5.0×10^{-2}	–	–	32	17	38	–
7	1.0×10^{-1}	–	–	52	21	31	–

[a] See the caption of Figure 3.
[b] After 48 h.
[c] Only proton concentration in Phase 2 was changed from the standard transport conditions (Run 1).

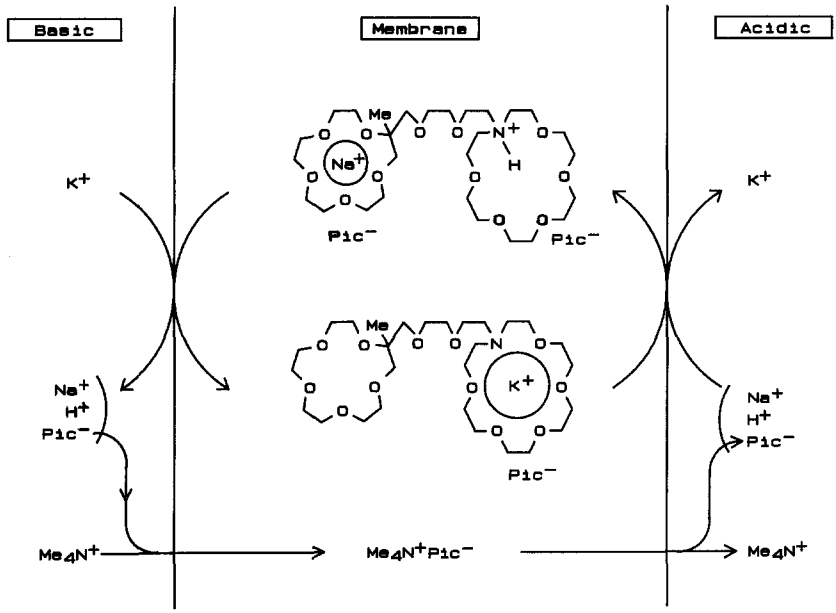


Fig. 4. Probable transport mechanism.

4. Conclusions

In this work, nine bis(crown ether)s were newly prepared and their complexing ability and transport ability were characterized. The function of the enzyme Na^+–K^+ ATPase was realized in an artificial membrane transport. To achieve the double uphill transport of Na^+ and K^+ in opposite directions, the selection of suitable transport conditions is clearly shown to be very important in addition to the design of the structure of the ionophore.

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