Highly Selective Membrane Transport of Copper(II) Ion by Synthetic Linear Oligomer Carriers

By **Kazuhiro Maruyama** * and **Hiroshi Tsukube**, Department of Chemistry, Faculty of Science, Kyoto University, Kyoto 606, Japan
Takeo Araki, Department of Chemistry, Faculty of Science, Shimane University, Matsue 690, Japan

A new class of synthetic carriers having urea or thiourea units has been shown to extract and to transport transition- and heavy-metal ions with high selectivities. The rates and specificities in this transport system are largely dependent on the carrier structure, the nature of co-transported anions, and other additives. The best carrier, a urea-containing octameric oligomer $[-CH_2CH_2N(CONHPh)-]_8$ (1), provided the first example of completely selective transport of copper ion through a liquid membrane, and a chemical analogue to biological copper transport.

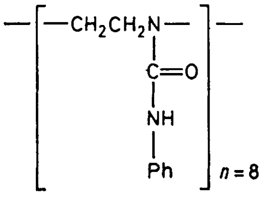
SPECIFIC transport of a metal ion across a membrane plays an essential role in many biochemical processes.[1] It is important to make a model for biological transport systems not only to simulate a biochemical process, but also for the development of new methodology in separation science. Some artificial ligand molecules [2] such as macrocyclic compounds have already been presented as model carriers, and shown to transport alkali-metal and organic ammonium cations with high selectivities. Their use led to the separation and concentration of valuable and undesirable species from environmental and biological systems. In marked contrast, only a few synthetic carriers [3] for selective transport of transition- and heavy-metal ions, which are also important from biochemical and medical points of view,[4] have been reported. Therefore, development of specific and effective carriers for transport of these metal ions is still a challenging problem from both practical and theoretical aspects.

Among the many types of ligand molecules reported, linear ligands [5] are of significance in relation to some bio-ionophores [6] such as monensin, gramicidin, nigericin, and salinomycin. The intrinsic flexible nature of a linear ligand may permit dynamic conformational change in the binding and releasing processes of metal ions, and enhance the overall transport rate, as suggested by Vögtle and co-workers.[7]

We recently reported that a new class of linear multidentate ligands such as (1) (so-called podand-urea receptor [5]) transported copper ion with high efficiency and excellent selectivity.[8] This is a full detailed paper of the work. Although some membrane systems [3] have been attempted, we believe that the present case is the first successful example of liquid membrane transport in which copper ion was completely selected. Moreover, the results obtained revealed that the introduction of a definite structure in the carrier may lead to the efficient and selective transport of a given chemical species 'at will'.

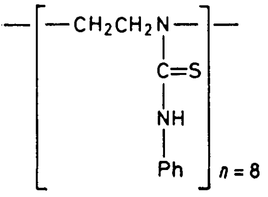
RESULTS AND DISCUSSION

Structural Features of New Synthetic Oligomer Carriers.—Many bio-ionophores are composed of metal-binding sites, rigid–flexible portions, and a hydrophobic group, the elegant combination of which leads to the remarkably high specificity and efficiency in biological transport. For this reason, we examined a new series of carrier molecules having a regular pattern of urea or thiourea units which are specific and potential co-ordination sites for metal ions. We previously developed a one-step synthesis of linear oligomers having the structure $[CH_2CH_2N(CXNHPh)]_{n\approx 8}$ (1: X = O, 2: X = S).[9] They have a defined molecular weight (degree of polymerization = 8),



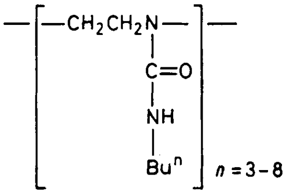
$-\left[-CH_2CH_2N(C{=}O)(NH)(Ph)-\right]_{n=8}-$

(1)



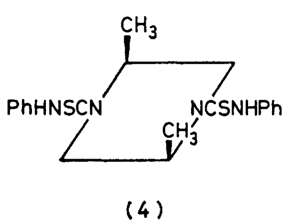
$-\left[-CH_2CH_2N(C{=}S)(NH)(Ph)-\right]_{n=8}-$

(2)

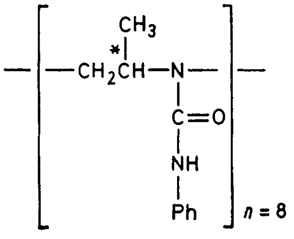


$-\left[-CH_2CH_2N(C{=}O)(NH)(Bu^n)-\right]_{n=3-8}-$

(3)



(4)



$-\left[-CH_2\overset{*}{C}H(CH_3)-N(C{=}O)(NH)(Ph)-\right]_{n=8}-$

(5)

$PhCOCH_2COCH_3$

(6)

and are prepared in good yields by ring-opening oligomerization of the corresponding aziridine monomers. These oligomers consist of three functionalized regions: (*i*) a binding site (⟩NCXNH–) that can co-ordinate to some kinds of metal ions, (*ii*) a hydrophobic region (–Ph) that can produce a hydrophobic microenvironment and partition into the lipid-like membrane, and (*iii*) a polyethyleneimine main chain which is essentially flexible and keeps its functional groups inseparable. In addition to these factors, the oligomeric nature of the ligands (1), (2), and (3) may impart characteristic properties not found with the more usual ligands.[10a,b] n-Butyl-carrying oligomer (3) [9b] was similarly prepared to examine the effect of pendant groups. The rotation of pendant groups in this oligomer is freer than in the ⟩NCXNH–Ph-carrying oligomers. Although its molecular-weight distribution was found not to be restricted to an octamer, the recurrence of n-butyl groups in a single chain provides sufficiently high solubility in the liquid membrane used. The thiourea-containing dimer analogue (4) was also compared. It was expected to complex with some metal ions as a bidentate ligand, but its co-ordination chemistry is somewhat different from those of previously mentioned carriers.

Each ligand used has a novel type of binding site (⟩NCXNH–) and arrangement of them in a single molecule. These structural features seem to fit the requirements for an effective carrier, *i.e.* cation-binding property, hydrophobicity, and mobility. Although a few urea-containing macrocyclic ligands [11] have been prepared, they have not been employed as carriers for transport of transition- or heavy-metal ions.

Extraction Properties of Urea- and Thiourea-containing Ligands.—The ability of a new series of ligands to bind heavy- and transition-metal ions such as copper(II), nickel(II), cobalt(II), cadmium(II), mercury(II), and iron(III) ions was examined by equilibrating a methylene chloride solution of each ligand with an aqueous metal perchlorate solution.[12] Metal perchlorates, nearly insoluble in methylene chloride, are extracted *via* complex formation, and the degree of extraction into the methylene chloride phase was taken to be a measure of efficiency of the ligand as a complexing agent for each metal ion. The results are listed in Table 1.

Urea- and thiourea-containing ligands, (1)—(4), had similar metal-ion extraction properties. They generally complexed more strongly, with copper, mercury, and iron ions, than with nickel, cobalt, cadmium, and zinc ions. This trend follows the 'Irving–Williams order' of stability,[13] which has been observed in the related heterogeneous adsorption experiments.[9d,10a] Urea-containing oligomers, (1) and (3), showed high degrees of extraction, but their selectivities for copper ion were not so high. The introduction of a sulphur atom into the ligand molecules has a significant effect on the extraction behaviour. Among the ligands used, the thiourea-containing oligomer (2) had excellent complexing ability and selectivity for copper and mercury ions. In addition, a greater number of ⟩NCSNH– units in a single chain is an important factor in enhancing the ligand's ability, since the thiourea-containing dimer analogue (4) exhibited only modest ability.

TABLE 1

Metal-ion extraction with urea and thiourea ligands [a]

	Extraction (%)			
Metal ion	(1)	(2) [b]	(3)	(4) [b]
Cu^{2+}	19	30	26	25
Ni^{2+}	12	7	15	10
Co^{2+}	6	4	9	7
Zn^{2+}	8	6	10	6
Cd^{2+}	12	5	14	11
Hg^{2+}	15	58	5	32
Fe^{3+}	16	*c*	11	*c*

[a] Organic phase: 1.2×10^{-4} mol monomeric unit in the ligand, 3 cm^3 CH_2Cl_2. Aqueous phase: 3.0×10^{-5} mol metal perchlorate, 1.5×10^{-3} sodium perchlorate, 3 cm^3. The two phases were stirred for 1 h at ambient temperature. [b] See ref. 9*c*. [c] A yellow precipitate was formed in each case.

The stoicheiometries of the copper complexes of (1) and (2) were confirmed by means of electronic spectroscopy. The spectra of these complexes exhibited *d–d* transition bands at 830 and 670 nm respectively, in methylene chloride–methanol solution (Figure 1). Variations in the *d–d* transition absorptions with mol ratio of ⟩NCXNH– to copper ion are shown in Figure 2, which clearly indicates 4 : 1 complex formation. Previously reported laser-Raman and far-i.r. spectroscopic studies on the complexes [9d,10a] have revealed that the CX (X = O or S) group is directly involved in co-ordination.† A circular dichroism spectrum of the related optically active octameric ligand (5), $[CH_2C^*H(CH_3)N(CONHPh)]_{n=8}$, complexed to copper [14] showed typical copper bands with diffused fine structures, indicating that the copper ion was co-ordinated in a 'square-planar' configuration. In such a complex, the octameric ligand wraps itself around the copper ion as a pseudocyclic ligand. This enclosure of the copper ion occurs in such a way that the polar ⟩NCXNH– groups of the ligand are directed inwards (*endo*-hydrophilic) towards the copper ion; the periphery of the complex therefore consists of hydrophobic moieties making an *exo*-hydrophobic complex (Figure 3). Such a complex structure is very similar to those of acyclic antibiotics.[6]

The stoicheiometry of the copper complex of the dimer analogue (4) was very different from those observed in the (1)– and (2)–copper complexes. The plot shown in Figure 2 indicates that two thiourea units co-ordinate to one copper ion, suggesting that the hydrophobic ligand molecule does not wrap around the copper ion in this complex. The equilibrium constants of these complexes were not as large (2 000—3 500 dm^3 mol^{-1}), as suggested by Kobuke *et al.*[2a] and Kirch and Lehn,[2c] but still suitable for carrying metal ions efficiently.

Transport of Transition- and Heavy-metal Ions by Novel Synthetic Carriers.—The structural features,

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† Although complexation of these ligands is followed by deprotonation from ⟩NCXN*H*– groups under the basic conditions, they act as neutral ligands under the conditions employed in this paper.

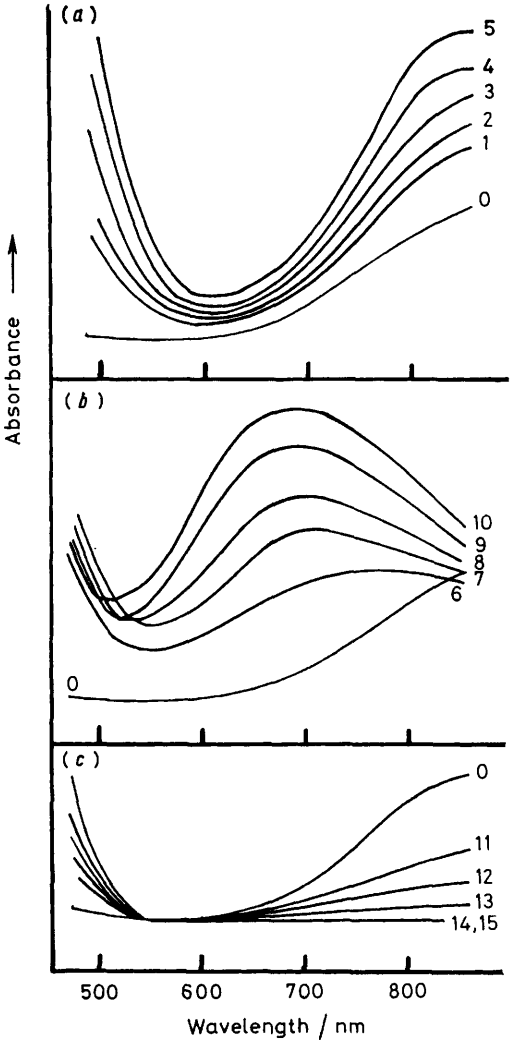

FIGURE 1 Electronic spectra of $CuCl_2$–urea or –thiourea ligand systems: (*a*) (1)–$CuCl_2$; (*b*) (2)–$CuCl_2$; (*c*) (4)–$CuCl_2$; $[CuCl_2] = 2.0 \times 10^{-3}$ mol dm^{-3}; solvent, CH_2Cl_2 (4 cm^3)–MeOH (1 cm^3). The mol ratio of urea unit in the ligand to copper ion is varied as follows: 0, 0; 1, 2.0; 2, 3.0; 3, 4.0; 4, 6.0; 5, 8.0; 6, 2.0; 7, 3.0; 8, 4.0; 9, 6.0; 10, 8.0; 11, 1.0; 12, 2.0; 13, 3.0; 14, 4.0; 15, 8.0

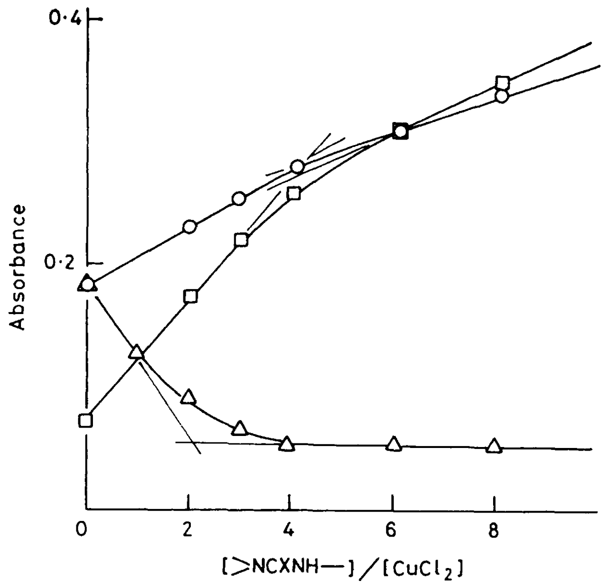

FIGURE 2 Plot of *d–d* transition absorbance against ratio of [>NCXNH–] to [copper ion]: (1)–$CuCl_2$ system (at 850 nm) (○); (2)–$CuCl_2$ system (at 670 nm) (□); and (4)–$CuCl_2$ system (at 850 nm) (△). $[CuCl_2] = 2.0 \times 10^{-3}$ mol dm^{-3}; [urea unit in ligand] = 0—1.59×10^{-2} mol dm^{-3}; solvent, CH_2Cl_2 (4 cm^3)–MeOH (1 cm^3)

mentioned above, of this type of ligand are expected to show new transport phenomena. Using these ligands, the passive transport of transition- and heavy-metal ions was examined. The liquid membrane system used in this study is shown in Figure 4. The ligands are much less soluble in aqueous phases I and II than in the methylene chloride membrane. After complexation of a carrier with a metal ion on the left side of the membrane, the complex formed slowly diffuses down its gradient. On the right side of the membrane, the metal ion is extracted into aqueous phase II, *via* formation of a ' ligand–metal ion–amino-acid ' type ternary complex. The freed carrier diffuses back across the membrane. The net result is that the metal ion is transported from aqueous phase I (left) to aqueous phase II (right) across the liquid membrane. Some chelating reagents,[3] such as β-diketone and oxyquinoline, have been shown to transport some metal ions. Most of them were anionic chelating reagents, but our ligands are neutral and carry the metal ion, together with the counter anion.

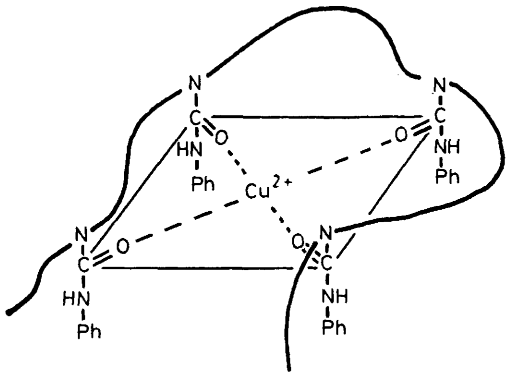

FIGURE 3 Possible structure of (1)–copper complex

The copper ion was transported with surprisingly high selectivity by the new carriers (1) and (2). Typical results are shown in Table 2. Using urea-containing carrier (1), we confirmed that 37% of the total copper ion was transported from aqueous phase I to II after 24 h, together with perchlorate anion,[15,*] under the conditions stated. Finally (after 72 h), the concentrations of both aqueous phases were almost equal. On the other hand, other metal ions such as nickel, cobalt, zinc, cadmium, and iron were hardly transported, and mercury ion

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[*] The amount of transported perchlorate anion was determined by the methylene blue method (see ref. 15).

only slightly moved. Thiourea-containing octameric carrier (2) has a higher overall ability, especially for iron, with the result that its selectivity for copper transport is lower than that of the urea carrier (1). In other words, the carrier (1) seemed to fulfil the requirements of a selective carrier; it had suitable binding ability and high complexation selectivity.

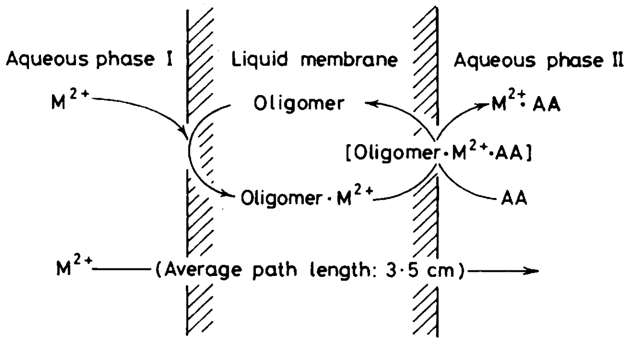

FIGURE 4 Liquid membrane system for transport of metal ions (AA = amino-acid)

The transport selectivities of these carriers are almost parallel to those observed in extraction experiments as shown in Table 1, except for mercury transport. These selectivities, therefore, seemed to be mainly determined by the same factors governing the metal extraction process into the methylene chloride membrane. In the mercury transport system, the transport rate could be controlled by other factors. Although it was effectively extracted with carriers (1) and (2), its transport rates were slightly suppressed. We studied the time–concentration profiles for extraction and release of this ion by the carrier (1) from water to methylene chloride and *vice versa*. The results showed that the mercury ion was rapidly extracted into methylene chloride, but that its release occurred quite slowly in this system. This suggests that the overall transport rate of the mercury ion, which was strongly bound to the carrier (1), is dependent on the rate of the releasing process rather than that of the extraction process. This indicates that a metal ion complexed rather loosely can be transported with higher efficiency. Similar results have been reported in other systems.[2]

The transport rates of copper ion using these carriers largely depends on the nature of the amino-acid which acts as metal acceptor in aqueous phase II. In the absence of amino-acid, copper ion accumulated in the membrane, resulting in a lower transport rate. When an amino-acid such as as alanine or histidine was added to aqueous phase II, the transport rate was remarkably enhanced. Since it was confirmed that the amino-acids used were relatively insoluble in the methylene chloride membrane and scarcely transported, they were believed to play an essential role in the metal releasing process *via* formation of a ternary complex as shown in Figure 4. Recently such a ternary complex (' ligand–metal ion–amino-acid ') has been recognized as being an important transient species in biological transport such as copper transport of human serum albumin.[16]

The nature of the counter-anion species, which is also transported with the metal ion complex through the membrane, should be considered. Christensen and co-workers [17] have demonstrated that larger unipositive anions transfer more rapidly than smaller halides in the crown ether-mediated transport system. A similar anion effect

TABLE 2

Transport rate of various metal perchlorates [a]

Membrane system		10^6 Transport rate/mol h^{-1}						
Carrier	Amino-acid	Cu^{2+}	Ni^{2+}	Co^{2+}	Zn^{2+}	Cd^{2+}	Hg^{2+}	Fe^{3+}
(1) (0.15 mmol) [b]	His	6.20	0	0	0	0	0.68	0
(1) (0.10 mmol)	His	3.72	0	0	0.04	0.04	0.40	0
	Ala	1.20	0	0	0.04	0.04	0.08	0
	Ala (pH = 7) [c]	0.88	0	0	0	0	0.08	0
	None	0.52	0	0	0	0	0.08	0
(1) (0.05 mmol)	His	1.08	0	0	0	0	0.08	0
(2) (0.10 mmol)	His	4.84	0.20	0	0	0	2.24	1.32
	Ala	3.40	0.08	0	0.04	0	1.40	0.72
	None	1.56	0	0	0	0	0.56	0.32
(3) (0.10 mmol)	His	1.10	0	0.64	0	0	1.10	0
	Ala	0.81	0	0.41	0	0	0.68	0
	None	0.29	0	0.08	0	0	0.21	0
(4) (0.15 mmol)	His	2.56	0	0	0	0	0.24	0.24
	Ala	0.72	0	0	0	0	0.04	0.08
	None	0.20	0	0	0	0	0	0
(6) (0.30 mmol)	His	0	0	0				
	Ala	0	0	0				
	d	7.52	7.84	7.16				
None	His	0	0	0	0	0	0	0
	Ala	0	0	0	0	0	0	0
	None	0	0	0	0	0	0	0

[a] Aqueous phase I: 4×10^{-4} mol metal perchlorate, 4 cm^3 H_2O. Organic phase: carrier, 8 cm^3 CH_2Cl_2. Aqueous phase II: 2×10^{-3} mol amino-acid, 10 cm^3 H_2O. His = Histidine, Ala = alanine. [b] Values in parentheses indicate the amount of the monomeric unit of carrier added in the organic phase. [c] Acetate buffer (0.1 mol dm^{-3}). [d] Aqueous phase I: 0.5N aqueous NH_3. Aqueous phase II: 0.5N aqueous HCl. Other conditions were the same as given in (*a*). See ref. 3*c*.

was observed in the present system. We examined three copper salts, perchlorate, acetate, and chloride; when the carrier (2) was employed, the transport rates obtained for respective anions were as follows: 4.84×10^{-6} mol h^{-1} (perchlorate) $> 1.81 \times 10^{-6}$ mol h^{-1} (acetate) $> 1.52 \times 10^{-6}$ mol h^{-1} (chloride). This trend supports the anion effect mentioned above, and is also the same as that of the extraction experiment reported before.[9c]

Similar transport properties were observed using the n-butyl carrying oligomer (3) and the thiourea-containing dimer analogue (4). Although extraction experiments (Table 1) indicate that the complexing abilities were comparable to those of corresponding octameric oligomers, the transport efficiencies were relatively lower. As reported by several investigators,[18,*] the balance of rigid-flexible properties of the carrier molecule is another important factor determining the efficiency and specificity of the metal-binding process; a rigid carrier shows higher selectivity but lower efficiency, while a flexible carrier has lower selectivity but higher efficiency. Consideration of the Corey-Pauling-Koltun molecular model of the carrier–copper complexes suggests that octameric carriers (1) and (2) have a good balance of flexible and rigid portions; *i.e.* the urea unit linked to a benzene ring and the polyethyleneimine chain. On the other hand, the thiourea-containing dimer analogue (4) which contains a rigid piperazine ring instead of a flexible polyethyleneimine chain has the disadvantage of repeating complexation and decomplexation, resulting in a lower transport rate. The n-butyl carrying oligomer (3) has too flexible a side chain to keep its shape during transport, where rotation of $>$NCXNH–Ph in the oligomers (1) and (2) is considerably restricted.

Benzoylacetone (6), which is a strong and anionic chelating reagent for transition-metal ions, was examined for comparison. Evans and co-workers [3c] have demonstrated that it could transport copper ion selectively over nickel and cobalt ions, followed by simultaneous counter transport of a proton. When (6) was used as a carrier under the same conditions, it did not transport metal ions. In coupled transport like this, more drastic conditions are needed for promotion of membrane potential differences. A pH gradient across the membrane was found to drive the metal ions, but we observed no ' selective ' transport of copper ion by using benzoylacetone (6).

EXPERIMENTAL

The inorganic salts were reagent grade and employed as received. The reagents for metal-ion analysis were all analytical grade.

Carrier Synthesis.—Oligomeric carriers, (1)—(5), were prepared by ring-opening oligomerization of corresponding aziridine monomers in good yields.

1-(*N*-Phenylcarbamoyl)aziridine, which was obtained quantitatively from aziridine and phenyl isocyanate, gave the octameric oligo[1-(*N*-phenylcarbamoyl)aziridine] (1) in 40—60% yield.[9a] Under an argon atmosphere, to a monomer solution in dried ethyl acetate (0.2 mol dm^{-3}), an equimolar amount of diethylsulphate was added dropwise with stirring, and the resulting solution was allowed to stand at 60 °C for 20 days. An oily viscous product was deposited gradually from the solution. This product was isolated, washed with ethyl acetate, dissolved in tetrahydrofuran (thf), and then reprecipitated from thf solution by adding diethyl ether. After the oligomer powder was immersed in a 10% aqueous ammonia solution at room temperature overnight, it was collected, washed with water repeatedly, and dried *in vacuo*. The resulting white powder was purified by Amberlite 400 column chromatography. The thus obtained oligomer was found to be an octameric material including trace amounts of lower analogues by gel permeation chromatography.

Other urea- and thiourea-containing oligomers (2), (3), and (5) were similarly obtained in satisfactory yields according to previously reported methods: (2), 80%; [9c] (3), 60%; [9a] (5), 70%.[14a] Although (2) and (5) were also found to be octameric oligomers, (3) has somewhat broader molecular weight distributions (degree of polymerization = 3—8).

The thiourea-containing dimer analogue (4) was prepared by the reaction of anhydrous *cis*-2,5-dimethylpiperazine and phenyl isothiocyanate (80%).[9d] The detailed spectroscopic data and elemental analysis values of these compounds have already been reported.[9a-d]

Ion Extraction Experiments.—A methylene chloride solution of each ligand was allowed to come into contact with an aqueous solution of metal perchlorate in the presence of 0.5 mol dm^{-3} sodium perchlorate. After a given period, the aqueous phase was separated, and the amount of remaining metal ion was determined by absorptiometry.[19] The degree of extraction was calculated from the difference between the initial and final amounts of metal ion in the aqueous phase. A similar extraction was performed with pure methylene chloride. We confirmed that a negligible amount of metal ion was extracted in each case.

Metal-ion Transport Experiment.—All transport experiments were carried out at ambient temperature. A cylindrical glass cell (inside diameter, 4.0 cm) holding a glass tube (inside diameter, 2.0 cm), thus separating the two aqueous phases, was used. The inner aqueous phase I contained metal perchlorate in water (4 cm^3). The outer aqueous phase II included an amino-acid (metal-ion acceptor) such as histidine and alanine in water (10 cm^3). The methylene chloride phase (8 cm^3) lay below these aqueous phases, and bridged the two aqueous phases. This phase was stirred by a magnetic stirrer. The transport rate was obtained from the initial rate of appearance of metal ion in aqueous phase II, which was determined by the absorptiometry method (usually after 7 h). Reproducibility was confirmed as ±15% or better.

A similar transport experiment was carried out in the absence of a carrier molecule for reference. The detailed conditions are included in Table 2.

In the coupled transport experiment, the methylene chloride membrane containing benzoylacetone (6) as carrier separated a 0.5 mol dm^{-3} aqueous ammonia solution (aqueous phase I) of metal perchlorate from a 0.5 mol dm^{-3} aqueous hydrochloric acid solution (aqueous phase II).[3c] Other conditions were the same as those above.

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[*] The relationship between ligand rigidity and complexing properties has been widely discussed in the macrocyclic polyether and oligopeptide systems (see ref. 18).

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