Lipophilic Polyamine and Polyamide Macrocycles for Membrane Transport of Amino Acid Esters and Related Cations

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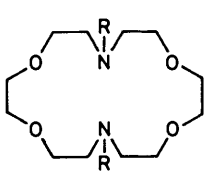
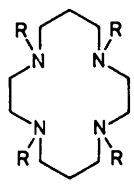
A variety of polyamine and polyamide macrocycles have been shown to be new and excellent cation-carriers for the transport of amino acid ester salts. Their transport properties were largely different from those of common polyether macrocycles, and essentially controlled by factors such as the natures of donor sites incorporated, ring sizes of macrocyclic systems, and hydrophobicities of co-transported anions. By appropriate choice of these factors, selective transport of various amino acid derivatives was successfully realized.

The recognition and transportation of cationic substrates by membrane carriers are of great importance in chemistry, biology, and separation science. As artificial carrier models, several types of macrocyclic molecules have been prepared, and demonstrated specifically to transport alkali, alkaline earth, and other cations.[1] In particular, nitrogen-containing crown ethers, so-called 'aza-crown ethers,' exhibited characteristic transport properties especially for some ammonium cations and heavy metal ions.[2] They contain nitrogen donor atoms in the macrocyclic systems, and form stable and/or selective complexes with these cations. We found previously[3] that lipophilic polyamine macrocycles were specific carriers for some organic ammonium cations. Since this kind of polyamine carrier showed markedly different transport properties from common polyether macrocyles, it was thought that the introduction of characteristic donor groups into the macrocyclic rings could offer new and unique cation-binding and transporting abilities.

Here we report cation-binding and transporting properties of a new series of aza-macrocyclic host molecules (**1**)—(**6**), in which amino or amide groups are incorporated as potential cation-binding sites. Although some types of aza-crown ethers have been well characterized,[2] we know only a few examples of macrocyclic polyamine and polyamide compounds with effective ion-transport abilities.[4] Hence, the present study may provide new insights into macrocyclic chemistry as well as biomimetic membrane chemistry.



(1) R = CH_2Ph
(2) R = COPh



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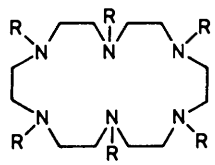

(5) R = CH_2Ph
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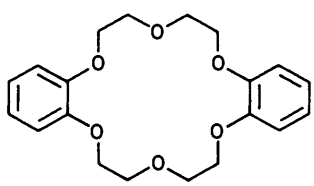

(7)

Results and Discussion

Structures of Employed Polyamine and Polyamide Macrocycles.—The polyamines and polyamides (**1**)—(**6**) were easily prepared by benzylation or benzoylation of corresponding polyamine compounds, and contain effective binding sites such as amino-nitrogen atoms and amide groups for complexing amino acid esters and other cationic substrates through various interactions: (i) charge–dipole attraction between a guest ammonium cation and electronegative donor atoms in the macrocycle; (ii) hydrogen bonding from hydrogen atoms of a guest ammonium cation to the amino nitrogen atoms or amide oxygen atoms; (iii) hydrophobic interaction between alkyl or aryl substituents on a guest ammonium cation and aromatic residues of macrocyclic carrier. Since they are much less soluble in water (pH > 3) than in $CHCl_3$, CH_2Cl_2, and other organic solvents, they are expected to act as effective phase-transfer reagents. Dibenzo-18-crown-6 (**7**) was also examined for comparison, because it is a well known carrier of primary ammonium cations and K^+ ion.[5] Recently several polyamide macrocycles have been demonstrated to form solid inclusion complexes, but their cation-binding and transporting properties have not been reported.[6]

Cation-binding and Extraction Properties of Polyamine and Polyamide Macrocycles.—The binding properties of polyamine and polyamide macrocycles for ammonium cations were first studied by the liquid–liquid extraction method. An aqueous solution of amino acid ester salt was shaken with a $CHCl_3$ solution of the macrocyclic compound. The amounts of the extracted amino acid ester and counteranion (ClO_4^-) were determined by ninhydrin colorimetry and ion-selective electrode methods, and typical results are listed in Table 1.

The polyamine macrocycle (**5**) exhibited higher extraction abilities for Phe ester salts than Gly ester salts. Especially in the presence of ClO_4^- anion, its extraction abilities were markedly enhanced. Hence, it was clear that the amino acid ester salt, composed of hydrophobic ammonium cation and counteranion, was effectively complexed and extracted by the polyamine macrocycle. Similar extraction trends were observed in the polyamide and polyether macrocycle systems, but their extraction abilities for both amino acid ester salts were much lower than those of polyamine macrocycles.

The cation-binding properties of the macrocyclic compounds were also investigated spectroscopically by using phenylazoaniline hydrochloride (PAA·HCl) as a probe (Figure 1). A solution (CH_2Cl_2–MeOH; 9:2 v/v) of PAA·HCl showed two intense absorption bands at 378 and 500 nm, and their relative intensities were varied by changes in solvent composition.

Table 1. Extraction of amino acid ester salts by macrocycles

	Extraction percentage[a]			
	GlyOEt		PheOEt	
Macrocycle	(A)	(B)	(A)	(B)
(5)	<2.0	7.5 (8.4)[b]	16.0	61.2 (66.6)[b]
(6)	<2.0	2.8	9.0	9.3 (10.8)[b]
(7)	<2.0	2.0	11.5	13.2 (13.6)[b]

Extraction conditions: (A) Amino acid ester–HCl, 0.01 mmol in 2 ml H_2O. Macrocycle, 0.02 mmol in 2 ml $CHCl_3$. (B) Amino acid ester–HCl, 0.01 mmol, $NaClO_4$, 0.01 mmol in 2 ml H_2O. Macrocycle, 0.02 mmol in 2 ml $CHCl_3$.

[a] [Amino acid ester extracted into $CHCl_3$]/[Amino acid ester added initially] × 100. [b] Values shown in parentheses mean extraction percentages of ClO_4^- anion.

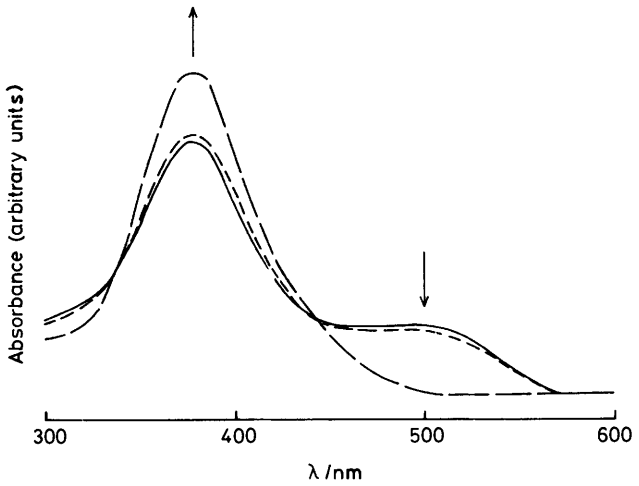

Figure 1. Spectral changes of phenylazoaniline hydrochloride (PAA·HCl) by addition of macrocycles. ——None; - - - - (6) and (7); — — (5). Conditions: PAA–HCl, 0.01 mmol. Macrocycle, 0.01 mmol in CH_2Cl_2 (9 ml) and CH_3OH (2 ml)

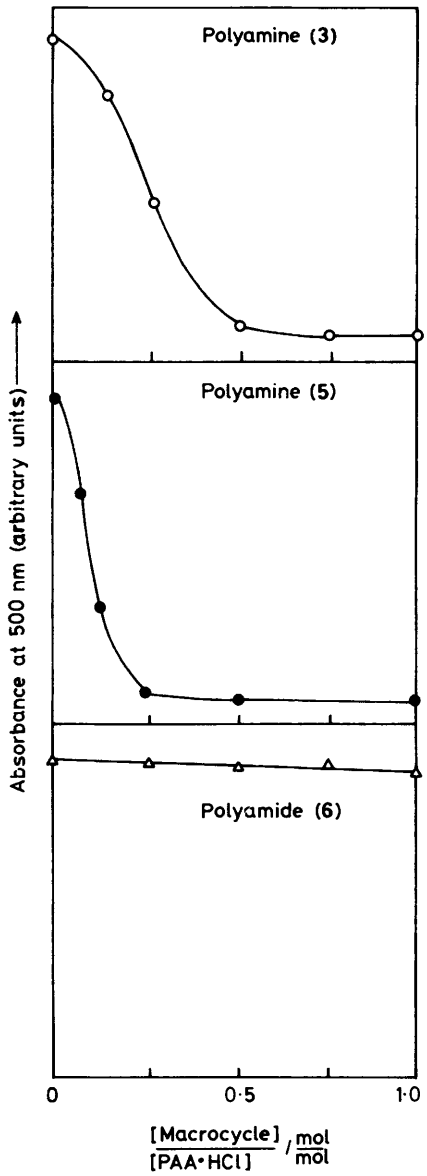

Figure 2. Plots of absorbance at 500 nm of PAA·HCl *versus* mole ratio of macrocycle added to PAA·HCl

Addition of polyamide macrocycle (6) to the solution of PAA·HCl led to only small spectral changes, though it extracted ammonium cations effectively from the aqueous to the organic phase (see Table 1). Since no spectral changes were observed in the polyether (7) system, formation of N^+–H - - - O-type hydrogen bonding seemed to induce no significant spectral changes in the PAA·HCl probe. When polyamine macrocycles (1), (3), and (5) were added to the solution of PAA·HCl, intense spectral changes were obtained. These changes strongly suggest that PAA·HCl was tightly bound to the polyamine macrocycles in a different manner from that to polyamide and polyether macrocycles.

The plots of absorbance at 500 nm *versus* mole ratio of polyamine macrocycle added to PAA·HCl added showed sharp break points (Figure 2). Although the portion of PAA in 'ammonium cation' form was not evaluated in the solvent system used, stoicheiometric interaction between polyamine macrocycle and PAA·HCl may occur in the homogeneous solution system.[7]

These results of extraction and spectroscopic investigations demonstrate that polyamine macrocycles show high extraction abilities for ammonium cations and their binding natures are largely different from those of polyamide and polyether macrocycles. Although the origin of the cation-binding process is not clear, new and unique cation-transport systems are expected to be constructed by using polyamine and related macrocycles.

Liquid Membrane System for Membrane Transport of Amino Acid Ester Salts.—Cation-transport experiments were carried out by using a liquid membrane cell as previously described (Figure 3).[8] At the aq. I–membrane interface, the macrocycle extracts substrate cation and carries it through the membrane. At the membrane–aq. II interface, the substrate cation is released into aq. II, together with co-transported anion. Hence, substrate cation and co-transported anion are moved from aq. I to aq. II across a bulk liquid membrane in the same direction.

As substrate cations, we chose a series of amino acid ester salts of biological interest as well as some inorganic cations. The transport rates were calculated from the initial rates of appearance of guest salts in the aq. II phase, and typical results are listed in Tables 2 and 3.

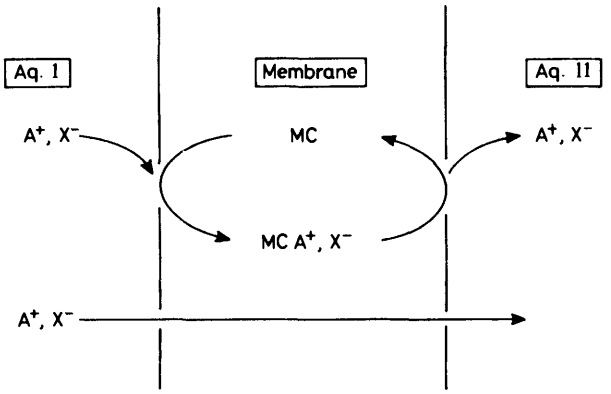

Figure 3. Liquid membrane system for transport of amino acid esters. A^+, Guest ammonium cation; X^-, co-transported anion; MC, macrocyclic carrier

Cation-transport Properties of Polyamine and Polyamide Macrocycles.—Table 2 shows that the polyamine and polyamide macrocycles are selective and effective carriers for organic ammonium cations. They exhibited high transport rates for some amino acid ester salts, while Na^+, K^+, and NH_4^+ cations were hardly transported under the same conditions. It indicates that the polyamine and polyamide macrocyles which we employed specifically discriminated organic ammonium cations from K^+ and NH_4^+ cations in the transport process. In marked contrast, polyether macrocyles (**1**) and (**7**) effectively transported both ammonium cations and K^+ ion. It is well known[5] that their cation-binding and transport properties are significantly controlled by the 'ion-cavity' size, and they cannot distinguish between cations of similar size, such as RNH_3^+ and K^+ cations. Therefore, introduction of amino and amide groups into the macrocyclic system as new binding sites successfully led to the new site-controlled cation-transport phenomena.[9]

Ring-sizes of the employed polyamine and polyamide macrocycles were found to be an essential factor in determining transport efficiencies, as frequently reported for polyether macrocyclic carrier systems. Macrocyclic hexa-amine (**5**)-mediated membrane transport of amino acid ester salts proceeded with higher efficiencies, compared with those for di- and tetra-amine macrocycles (**1**) and (**3**). Examination of space-filling molecular models of polyamine (**5**)–primary ammonium cation complexes suggests that three nitrogen atoms of the macrocyclic ring can be organized for tripod binding of ammonium cations through three N^+–H - - - N-type hydrogen bondings. Although tetra-amine (**3**) and tetra-amide (**4**) effectively transported some amino acid derivatives, only one or two binding sites seemed to be involved in the complexations and guest primary ammonium cation might be loosely bound to the macrocycles.* These results provide further possibilities that macrocycles having larger ring sizes and potential binding sites show characteristic cation-binding and transporting abilities for guanidinium, imidazolium, and other biological organic cations.[10]

Table 2 also shows that macrocyclic polyamines (**3**) and (**5**) exhibit largely different transport selectivities for a series of amino acid ester salts from macrocyclic polyamides and polyethers. The polyamines transported hydrophilic Gly and Ala esters more effectively than hydrophobic Phe and Trp esters, while polyamide and polyether macrocycles provided fast transport of Phe and Trp derivatives. Since we confirmed that polyamine macrocycle (**5**) extracted Phe ester from the aqueous to organic phase much more effectively than Gly ester (see Table 1), amino acid derivatives having higher hydrophobicities may be too tightly complexed by polyamine macrocycle to be transported, and may be accumulated into the membrane phase. In other words, the releasing process of guest amino acid ester from the membrane into the aq. II phase is rate-determining in the polyamine carrier systems.

When the polyamide macrocycles were employed as cation carriers, Phe and Trp esters were transported with higher efficiencies than those of Gly and Ala derivatives, and a 'reversed' transport selectivity was realized by alternation of donor groups incorporated into the macrocyclic carriers. Since their transport trends were almost parallel to those of extraction experiments, overall transport rates were significantly determined by the rates of the extraction process of substrate cations from the aq. I into the membrane phase. Hence, it may be possible to modify transport selectivity and efficiency at will by

Table 2. Carrier-mediated transport of amino acid derivatives

	Transport rate × 10^{-6} (mol/h)						
Guest Salt	(1)	(2)	(3)	(4)	(5)	(6)	(7)
KCl	0.7	<0.1	<0.1	<0.1	<0.1	<0.1	5.7
NH_4Cl	2.4	<0.1	0.2	<0.1	<0.1	<0.1	0.5
PheOEt·HCl*	(1.2)	(0)	(9.8)	(0)	(9.5)	(0)	(0)
PheOEt·HCl	1.9	0.1	0	2.4	4.1	5.9	10.0
			(0)	(2.2)	(5.0)	(5.7)	
TrpOEt·HCl	5.4	0	0.9	2.1	9.2	4.1	12.6
	(5.0)		(1.2)	(1.6)	(8.5)	(3.6)	
LeuOEt·HCl	5.1	0.2	1.1	1.8	8.9	4.1	11.8
	(4.6)		(1.3)	(1.6)	(7.5)	(3.6)	
ValOMe·HCl	6.8	<0.1	2.9	0.3	18.9	0.3	2.4
	(6.0)		(3.2)		(17.6)		
AlaOEt·HCl	8.1	0	4.6	0	17.6	0.3	1.3
	(7.6)		(4.9)		(13.4)		
GlyOEt·HCl	8.6	0	4.0	<0.1	14.3	<0.1	1.0
			(4.2)		(10.5)		
GlyOEt·HCl*	(1.1)	(0)	(2.8)	(0)	(1.7)	(0)	(0)
ProOMe·HCl	5.1	<0.1	0.2	0.2	10.0	0	0

Transport conditions: Aq. I; Amino acid ester·HCl, 0.5 mmol $NaClO_4$, 1.0 mmol, in 5 ml H_2O. Membrane; Macrocycle, 0.0372 mmol in 12 ml $CHCl_3$. Aq. II; H_2O, 5 ml.

Transport rates shown in Table 2 were calculated from the differences in the transport rates of ClO_4^- anion of macrocycle-containing and blank systems, which were determined by means of ion-selective electrode. The values indicated in parentheses were transport rates of amino acid esters which were determined by ninhydrin colorimetry.

* In these cases, $NaClO_4$ salt was not added to the Aq. I.

Table 3. Anion effect on transport rate of PheOEt salt

	Transport rate × 10^{-6} (mol/h)*			
Additive	(3)	(5)	(6)	(7)
None	9.8	9.5	0	0
NaCl	10.0	12.9	0.2	0.3
NaOAc	1.0	2.4	0.9	2.4
NaSCN	2.2	5.2	2.9	6.0
$NaClO_4$	0	5.0	5.7	10.0

Transport conditions: see footnotes to Table 2.

* Transport rates shown were calculated from the differences in the transport rates of macrocycle-containing and blank systems, which were determined by ninhydrin colorimetry.

* CPK molecular model building also suggests that the three amide groups of hexa-amide (**6**) could not participate in the co-operative cation binding.

appropriate choice of donor site groups attached to the macrocyclic compounds.

Co-transported anion has a pronounced effect on the cation-transport process (Table 3). For example, the cation-transport rates of polyamide macrocycle apparently depended on the natures of co-transported anions. Among the examined anions, ClO_4^- and SCN^- anions were found to provide efficient transport. As observed in the extraction experiments (Table 1), these hydrophobic anions such as ClO_4^- anion promoted the cation-extraction process and enhanced overall transport rates.[11] In the polyamine macrocycle-mediated transport systems, addition of these hydrophobic anions into the aq. I phase decreased their cation-transport efficiencies. Since polyamines showed much higher cation-extraction abilities for Gly- and Phe-esters, relatively hydrophilic anions such as Cl^- anion are required to lead the effective cation-releasing process.

Polyamine macrocycle-mediated transport systems seem to be somewhat complicated, because the possibility of interfacial protonation of basic polyamine nitrogen atoms may not be discounted even under the conditions employed (pH values of aq. I; 4—6);* however, the following experimental results strongly support the proposal that amino acid esters are extracted and transported by polyamine macrocycles as 'ammonium cation salts': (i) in the presence of an excess of $NaClO_4$, almost equimolar amounts of amino acid ester and ClO_4^- anion were extracted and transported; (ii) ClO_4^- anion was hardly transported with Na^+ and H^+ cations under the employed pH conditions; (iii) if neutral amines such as phenylethylamine were added into the membrane phase, they were maintained in the membrane phase and not released into the aq. II phase. Although the details of the structures of polyamine macrocycle–ammonium cation complexes are not yet clear, characteristic and strong cation-binding properties of polyamine macrocycles are clearly reflected in the cation-transport processes. In order to develop new and specific membrane carriers, further modifications of donor groups and ring sizes of macrocycles are currently in progress.

Experimental

Materials.—Polyamide macrocycles, (**2**), (**4**), and (**6**), were prepared by the reaction of benzoyl chloride and 1,7,10,16-tetraoxa-4,13-diazacyclo-octadecane (Merck), 1,4,8,11-tetra-azacyclotetradecane (Alfa), and 1,4,7,10,13,16-hexa-azacyclo-octadecane (Aldrich), respectively. After chromatography on alumina ($CHCl_3$ or CH_2Cl_2), recrystallization from CH_2Cl_2–hexane gave crystals.

Polyamide (**2**) (4,13-*dibenzoyl*-1,7,10,16-*tetraoxa*-4,13-*diazacyclo-octadecane*), m.p. 87—89 °C, yield 75%; $\nu_{max.}$(Nujol) 1 630 cm^{-1}; $\delta_H(CDCl_3)$ 3.63, 3.70 (24 H, ring), 7.43 (10 H, ArH) (Found: C, 66.3; H, 7.05; N, 5.8. $C_{26}H_{34}N_2O_6$ requires C, 66.4; H, 7.3; N, 5.95%).

Polyamide (**4**) (1,4,8,11-tetrabenzoyl-1,4,8,11-tetra-aza-cyclotetradecane), m.p. 269—270 °C, yield 80%, $\nu_{max.}$(Nujol) 1 630 cm^{-1}; $\delta_H(CDCl_3)$ 1.83—2.17 (4 H, ring), 3.27—3.87 (16 H, ring), 7.17—7.57 (20 H, ArH) (Found: C, 73.3; H, 6.2; N, 9.25. Calc. for $C_{38}H_{40}N_4O_4$: C, 74.0; H, 6.5; N, 9.1%).

Polyamide (**6**) (1,4,7,10,13,16-hexabenzoyl-1,4,7,10,13,16-hexa-azacyclo-octadecane), m.p. 159—161 °C, yield 45%, $\nu_{max.}$(Nujol) 1 630 cm^{-1}; $\delta_H(CDCl_3)$ 3.73 (24 H, ring), 7.40 (30 H, ArH) (Found: C, 72.3; H, 5.8; N, 9.6. Calc. for $C_{54}H_{54}N_6O_6$: C, 73.4; H, 6.2; N, 9.5%).

* Although pK_1 values of polyamine macrocycles have been reported as 9–10, our earlier works (refs. 4*d* and 7) showed that interfacial protonation of the employed lipophilic polyamine macrocycles was almost suppressed.

Polyamine macrocyles (**1**) (4,13-dibenzyl-1,7,10,16-tetraoxa-4,13-diazacyclo-octadecane), (**3**) (1,4,8,11-tetrabenzyl-1,4,8,11-tetra-azacyclotetradecane), and (**5**) (1,4,7,10,13,16-hexabenzyl-1,4,7,10,13,16-hexa-azacyclo-octadecane) were obtained by benzylation of corresponding polyamine compounds as described before.[2e,4d]

Amino acid esters and other reagents were commercially available and were used without further purifications.

Extraction Procedure of Amino Acid Ester Salts.—The extraction abilities of the amino acid derivatives were estimated on the basis of the partition of GlyOEt, PheOEt, and ClO_4^- between $CHCl_3$ (2 ml) and the aqueous phase (2 ml). After agitation for 3 h, the organic phase was separated. The amounts of amino acid esters remaining in the aqueous phase were determined by ninhydrin colorimetry. The amount of remaining ClO_4^- anion was also determined by an ion-selective electrode (Orion model 93-81). Three independent experiments were done, and reproducibilities were found to be $\pm 10\%$ or better.

Transport Experiments.—The transport experiments were performed at room temperature in a U-tube glass cell (2.0 cm, i.d.). The macrocycle in $CHCl_3$ (12 ml) was placed in the base of the U-tube, and two aqueous phases (5 ml each) were placed in the arms of the U-tube, floating on the $CHCl_3$ membrane phase. The membrane phase was constantly stirred with a magnetic stirrer.

The transport rates shown in Tables 2 and 3 were average values of three independent runs. Reproducibilities were confirmed as $\pm 15\%$ or better. The details of transport conditions are shown in each Table.

Acknowledgements

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