Proton-assisted Transport of Amino Acid and Related Polycarboxylate Anions *via* Polyammonium Macrocycles

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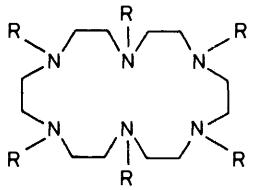
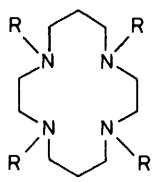
Anion-binding and transport properties of polyamine macrocycles have been examined by liquid–liquid extraction and liquid membrane transport experiments. Some lipophilic polyamine macrocycles showed excellent transport properties, especially for polycarboxylate anions, depending on their ring structure. Their anion-extraction and transport properties were significantly different from those of the quaternary ammonium cation-surfactant trioctylmethylammonium chloride, and the mechanism involves protonation of the macrocyclic nitrogen atoms.

Recognition and transportation of ionic substrates by membrane carriers are of great importance in chemistry, biology, and separation science. Model studies so far have focussed mainly on the transport of cationic substrates using macrocyclic polyethers,[1] polyamines,[2] and other synthetic carrier molecules.[3] Recently, anion transport has received much attention. The anion carriers, which possess appropriate anion-binding sites such as quaternary ammonium cations[4] and metal ions,[5] interact with anionic substrates by inclusion, electrostatic forces, co-ordination interactions, and/or hydrogen bonding.

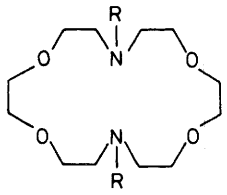
In the preceding paper,[6] we found that the 12-membered tetra-amine, 1,4,7,10-tetrabenzyl-1,4,7,10-tetra-azacyclododecane, could be an effective and pH-responsive anion carrier. Interestingly, this kind of polyamine formed a polyammonium macrocycle and effectively transported dicarboxylate anions such as glutamic acid derivatives. Here we report anion-binding and transport abilities of 14- and 18-membered polyamine macrocycles, and demonstrate that their anion-binding and transport properties are significantly controlled by the size and shape of the macrocyclic systems. Although anion complexation and related reactions with polyamines have been extensively studied,[7] their application to membrane transport systems has rarely been reported.[8] Hence, the present study may offer new insight into polyamine chemistry as well as biomimetic membrane chemistry.



(**1**) R = CH_2Ph



(**2**) R = CH_2Ph
(**3**) R = COPh



(**4**) R = CH_2Ph

$(C_8H_{17})_3N^+CH_3Cl^-$

(**5**)

Results and Discussion

Polyamine Macrocycle Carriers.—The polyamine macrocycles employed were compounds (**1**)—(**4**). The lipophilic polyamines (**1**) and (**2**), the polyamide (**3**), and the N,O-mixed donor macrocycle (**4**) were prepared by benzylation or benzoylation of the corresponding unsubstituted polyamines. They are much less soluble in water (pH > 3) than in non-polar organic solvents such as CH_2Cl_2 and $CHCl_3$, and thus offered interesting phase-transfer abilities for anionic substrates *via* protonation. The quaternary ammonium cation-type surfactant (**5**) was also examined, because it is well known to be an effective anion carrier.[4a] Since dynamic protonation is involved in anion binding, polyamine macrocycles may provide largely different anion-binding and transport phenomena from the conventional quaternary ammonium surfactants.

Protonation Profiles of Polyamine Macrocycles.—Protonation profiles of the polyamine macrocycles (**1**)—(**4**) with picric acid in CH_2Cl_2—CH_3OH solution were investigated spectroscopically (Figure 1). Addition of compounds (**1**), (**2**), or (**4**) to the solution of picric acid led to intense spectral changes indicating formation of picrate anion, while little spectral change was observed in the case of the polyamide (**3**). These observations clearly indicate that the polyamine macrocycles (**1**), (**2**), and (**4**) are easily protonated by picric acid, but polyamide (**3**) did not form the ion-pair of protonated macrocycle–picrate anion.

Plots of absorbance at 400 nm, derived from dissociated picrate anion, *vs.* molar ratio of macrocycle added to picric acid suggested that the macrocycles hold two [(**2**) and (**4**)] or three [(**1**)] protons depending on the ring size (Figure 2). pK_a Values of parent 14- and 18-membered polyamine macrocycles have been determined in aqueous solutions; for example, 10.2, 9.2, 8.7, 4.1, ~2, and ~1 for the 18-membered hexa-amine.[9] The unsubstituted macrocyclic hexa-amine acted as a multi-proton acceptor, and accommodated three protons in the macrocyclic cavity at neutral pH. Our substituted macrocycles showed parallel protonation behaviour in the CH_2Cl_2–CH_3OH solution system.

Extraction of Picrate Anion with Polyamine Macrocycles.—Anion-binding properties of protonated polyamine macrocycles were investigated by equilibrating a CH_2Cl_2 solution of a macrocycle with an aqueous solution of picric acid. Picrate anion, highly insoluble in CH_2Cl_2, was extracted *via* interaction with protonated polyamine, and the degree of extraction into the CH_2Cl_2 phase was taken to be a measure of anion-binding ability of the polyamine. Plots of the u.v.–visible absorbance of extracted picrate anion *vs.* pH value of the aqueous phase are shown in Figure 3.

When the macrocycle (**2**) was used, picrate anion was actually extracted into the CH_2Cl_2 phase from the aqueous phase under

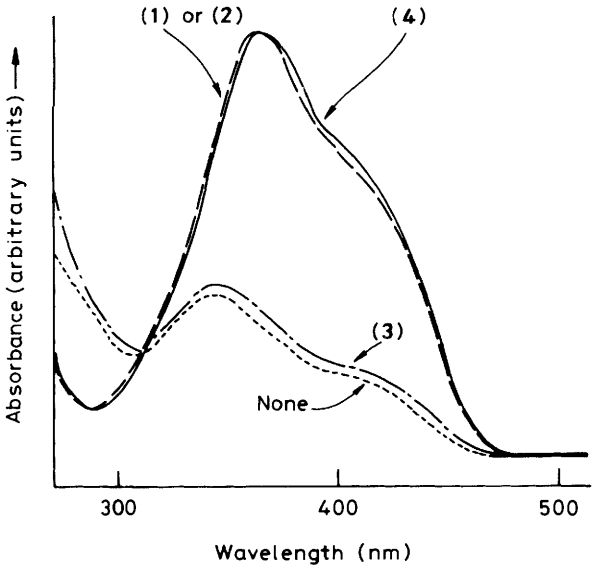


Figure 1. Changes in the u.v.–visible absorption spectrum of picric acid on the addition of the macrocycle hosts (**1**)—(**4**). 1:1 Molar ratio of macrocycle to picric acid in CH_2Cl_2–CH_3OH (9:1).

the conditions employed. The amount of extracted picrate anion was found to depend on the pH of the aqueous phases, and compound (**2**) picked up picrate anion more effectively from acidic aqueous solution than from neutral solution. The protonation of polyamine macrocycle seemed to be somewhat suppressed in the two-phase system (CH_2Cl_2–water) compared with that in the homogeneous solution systems. In other words, the anion-extraction process of polyamine macrocycles could be controlled by the pH conditions of the aqueous phase. Similar pH-dependent extraction was not observed in the case of the quaternary ammonium cation (**5**).

Liquid Membrane System.—Preliminary anion-extraction experiments show that polyamine macrocycles provide important features as pH-responsive and potential anion carriers: (i) polyamine macrocycles can bind with two or three protons, depending on their ring structure; (ii) the lipophilic macrocyclic cations formed show characteristic anion-binding abilities. Although Kimura *et al.* and Lehn and co-workers were the first to suggest the possibility of a polyamine-mediated anion-transport system, we were the first to realize such a system.[6]

Anion-transport experiments were performed by using a liquid membrane cell as described before.[5,6] After being protonated at the Aq. I/Membrane interface, the polyamine macrocycle (protonated form) binds with the anionic substrate and carries it through the membrane. At the Membrane/Aq. II interface, the substrate anion is released into the Aq. II phase, together with deprotonation of the polyamine macrocycle or with anion exchange. Hence, the concentration gradient of proton or counter-transported anion drives the anion transport.

For our choice of anionic substrates, we chose a series of amino acid and some polycarboxylate anions, and typical results are summarized in Tables 1 and 2.

Transport Properties of Polyamine Macrocycles with Amino Acid Derivative Anions.—Table 1 shows that the polyamine macrocycles (**1**) and (**2**) are pH-responsive carriers of amino acid derivative anions, whereas the polyamide (**3**) hardly transported anionic substrates at all. The transport rates of compound (**2**) for Z-Gly (Z = benzyloxycarbonyl) anion were largely

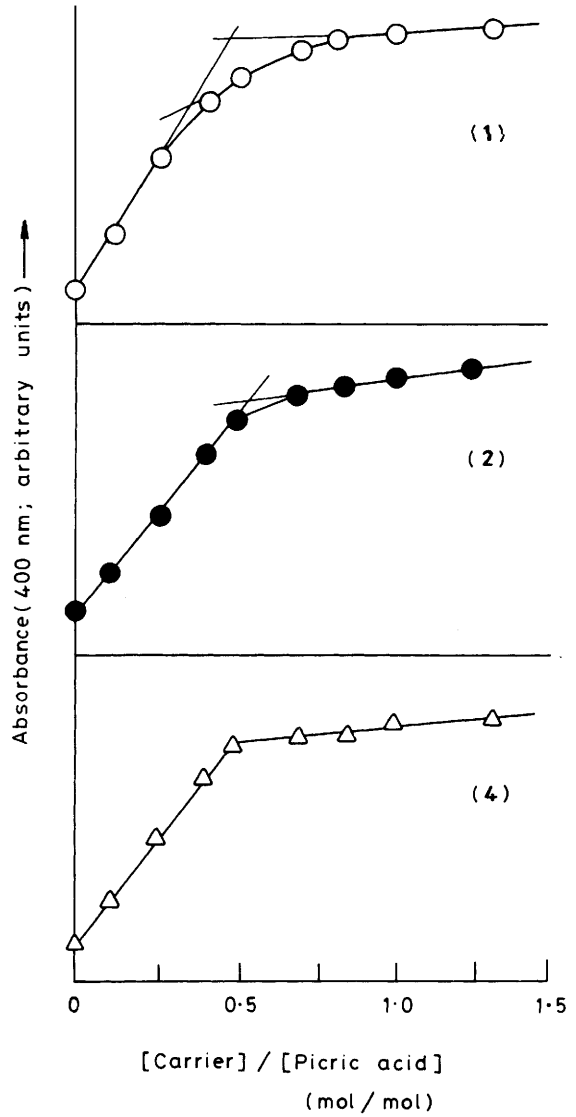


Figure 2. Plots of the u.v.–visible absorption at 400 nm *vs.* the ratio of carrier (**1**), (**2**), or (**4**): picric acid. Picric acid 4.0×10^{-7} mol, carrier 0—5.0×10^{-7} mol in CH_2Cl_2–CH_3OH (9:1)

influenced by pH conditions of the Aq.I phase, and increasing pH values were found to decrease the transport rates. On the other hand, the conventional surfactant carrier (**5**), which has been developed by Lehn and co-workers,[4a] exhibited constantly high transport efficiencies under the conditions stated in Table 1. Since such transport behaviour of carriers (**2**) and (**5**) was almost identical with those of the extraction experiments shown in Figure 3, the polyamine macrocycle-mediated anion-transport mechanism was linked to the protonation of polyamines, and is significantly regulated by proton concentrations of the aqueous phases.

The nature of the counter-transported anions was also an essential factor in determining the transport rates of polyamine systems (see Table 1). Since no diffusion was detected in the absence of counter-transported anion, the concentration gradient of the counter-transported anion could drive the fast anion transport. OH^- anion was also examined as the counter-transported anion, but it provided a lower transport rate than either Cl^- or AcO^- anion. Similar effects of counter-transported anion were confirmed in the quaternary ammonium

Table 1. Transport of amino acid derivative anions with polyamine macrocycles[a]

Substrate anion	Counter-transported anion	Transport rate × 10^6 (mol h^{-1}) (1)	(2)	(3)	(4)	(5)
Z-Gly (pH 4.50)[b]	None		0			0.06
	AcO^-, 0.5M		1.21			4.12
	OH^-, 0.5M		0.19			0.68
	Cl^-, 0.1M		0.08			1.42
	Cl^-, 0.3M		0.86			2.78
	Cl^-, 0.5M	0.71	1.17	0	0.91	2.93
	Cl^-, 0.7M		1.88			3.63
Z-Gly (pH 5.29)[b]	Cl^-, 0.5M		0.57			3.21
Z-Gly (pH 6.21)[b]	Cl^-, 0.5M		0.23			3.00
Z-Gly (pH 6.98)[b]	Cl^-, 0.5M		0.14			3.30
Z-Ala (pH 4.34)[b]	Cl^-, 0.5M	0.66	1.28	0	0.84	3.45
Z-Val (pH 4.68)[b]	Cl^-, 0.5M	0.45	0.50	0.09	0.52	1.79
Z-Gln (pH 4.50)[b]	Cl^-, 0.5M	1.57	1.96	0.02	0.56	7.31
Z-Glu (pH 4.55)[b]	Cl^-, 0.5M	1.63	2.76	0.10	*c*	1.62
Z-Asn (pH 4.54)[b]	Cl^-, 0.5M	1.70	2.69	0.09	0.53	9.54
Z-Asp (pH 4.60)[b]	Cl^-, 0.5M	2.56	2.58	0.41	*c*	1.93

[a] Initial conditions: Aq. I; Z-Amino acid (0.150 mmol)–NaOH aqueous solution (5 ml). Membrane; carrier (0.0372 mmol)–CH_2Cl_2 (12 ml). Aq. II; counter-transported anion (potassium salt) (0—3.50 mmol)–water (5 ml). The transport rates indicated were calculated from the difference in the transport rates of the carrier-containing and blank systems. [b] The values shown in parentheses were pH values of Aq. I adjusted initially. [c] Considerable amounts of carrier (4) leaked out during the experiments.

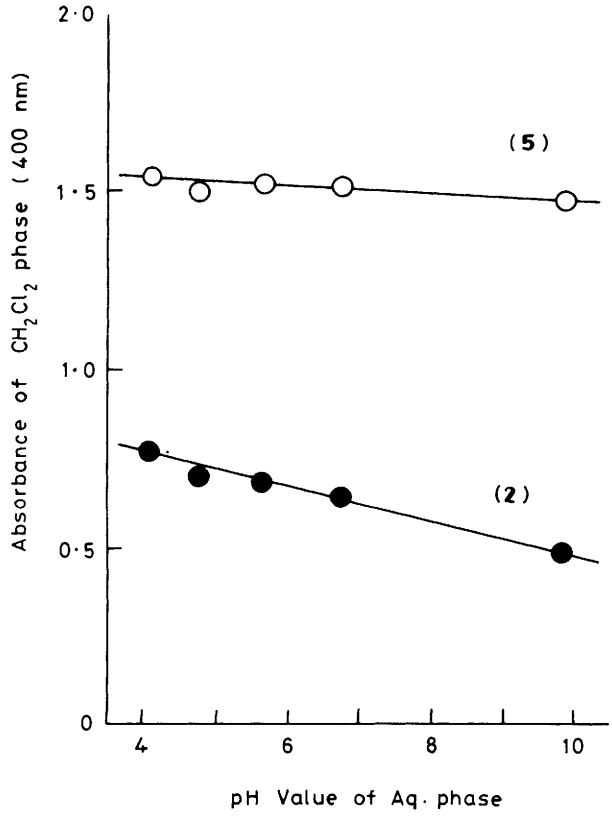


Figure 3. Plots of the 400 nm absorption of the CH_2Cl_2 phase (based on extracted picrate anion) *vs.* the pH value of the aqueous phase from the liquid–liquid extraction experiment. Extraction conditions: carrier (2) or (5) (0.010 mmol)–CH_2Cl_2 (5 ml). Picric acid 0.020 mmol–water (2 ml).

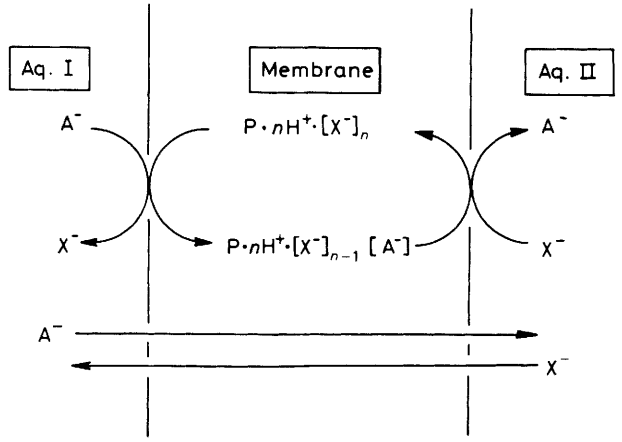


Figure 4. Liquid membrane for transport of amino acid and related anions. A^- = anionic substrate. X^- = countertransported anion. P = polyamine macrocycle-type carrier.

cation (5) system, and the same factors may control anion-releasing processes in both carrier systems.

Polyamine macrocycles (1) and (2) showed some advantages in the transport of dicarboxylate anions. They transported dicarboxylate Z-Glu and Z-Asp anions with comparable efficiencies to those of corresponding monocarboxylate Z-Gln and Z-Asn anions. In marked contrast, the quaternary ammonium cation (5) transported monocarboxylate anions much more effectively than dicarboxylate anions. Since we have already reported that the lipophilic dicationic metal complex 1,4,7,10-tetrabenzyl-1,4,7,10-tetra-azacyclododecane–$CuCl_2$ hardly transported dicarboxylate anions derived from Glu and Asp derivatives,[5b] the protonated polyamine macrocycles acted as unique polycations. The N,O-mixed donor macrocycle (4) formed a lipophilic dication *via* protonation, but showed relatively low transport abilities for a series of anionic substrates. Although pK_a values of the employed polyamine macrocycles and anionic substrates should be significantly different in the membrane phase and at the membrane/aqueous phase interface, dicarboxylate anions may effectively associate by ionic hydrogen bonds with polyamine protons packed into the macrocyclic cavities. Similar characteristic anion-binding

Table 2. Transport of polyanionic substrates with polyamine macrocycles[a]

Substrate Anion	Transport rate × 10^6 (mol h^{-1})[c] (1)	(2)	(3)	(4)	(5)
Benzenedicarboxylic acids (pH 5.42)[b]					
1,2-	1.60	2.30	0	1.15	1.47
1,3-	0.07	0.07	0	0	2.70
1,4-	0.14	0.18	0.04	0.09	2.09
Benzenetricarboxylic acids (pH 4.70)[b]					
1,2,3-	1.81	4.18	0.04	0.50	4.47
1,2,4-	1.57	3.17	0.04	0.29	5.16
1,3,5-	0.11	0.16	0	0.03	1.39
Benzenetetracarboxylic acid (pH 4.70)[b]					
1,2,4,5-	5.43	1.71	0.08	0.15	4.78

[a] Initial conditions: Aq. I; substrate acid (0.15 mmol)–NaOH aqueous solution (5 ml). Membrane; carrier (0.0372 mmol)–CH_2Cl_2 (12 ml). Aq. II; KCl (2.50 mmol)–water (5 ml). [b] The values shown in parentheses were pH values of Aq. I adjusted initially. [c] The rates indicated were calculated from the difference in the transport rates of the carrier-containing and blank systems.

modes of polyamine macrocycles have been proposed in aqueous solution systems.[7]

Transport Properties of Polyamine Macrocycles with Polycarboxylate Anions.—When polycarboxylate anions such as phthalic acid, trimellitic (benzene-1,2,4-tricarboxylic) acid, and pyromellitic (benzene-1,2,4,5-tetracarboxylic) acid derivatives and isomers were chosen as substrate anions, polyamine macrocycles showed markedly different transport properties, especially characteristic transport selectivity, from the quaternary ammonium cation (**5**) (see Table 2). For the phthalic acid anions, polyamine macrocycles (**1**), (**2**), and (**4**) transported the *o*-isomer much more effectively than the *m*- and *p*-isomers. Similarly macrocycles (**1**) and (**2**) favoured smaller anions derived from benzene-1,2,3-and 1,2,4-tricarboxylic acids rather than the larger anion from benzene-1,3,5-tricarboxylic acid. The anions effectively transported with polyamines were found to be smaller and highly charged ones. Under the same conditions, carrier (**5**) was confirmed to be a non-selective carrier for these polycarboxylate anions.

An interesting structural affinity between the substrate anions and the protonated polyamines was essentially established in the present transport system: the 18-membered hexa-amine (**1**) transported the larger pyromellitic acid anion more effectively than did the 14-membered tetra-amine (**2**), while compound (**1**) showed lower transport rates for the smaller di- and tri-carboxylic acid anions.

In biomembrane transport processes, some carrier proteins utilized for transporting dicarboxylate species are well known.[10] Although the chemical aspects of polyanion recognition and transportation mechanisms are as yet unclear, the present results strongly suggest that polyamine macrocycles may serve as a simple and primitive chemical model for carrier proteins having amine residues (*e.g.*, lysine). Therefore, further modifications of ring-size and -shape of macrocycles may offer new possibilities in the design of highly specific carrier molecules as well as in modelling biomembrane systems.

Experimental

Materials.—The polyamine macrocycles (**1**) and (**2**) were prepared in a two-phase reaction system ($CHCl_3$–water). Typically, a $CHCl_3$ solution (15 ml) of benzyl bromide (4.28 g) was added dropwise to an aqueous solution (10 ml) of 1,4,7,10,13,16-hexa-azacyclo-octadecane (hexacyclen trisulphate; Aldrich, 1.90 g) and NaOH (2.00 g). After the mixture had been vigorously stirred at room temperature for two nights, the organic layer was washed successively with dilute aqueous HCl and water, and dried over Na_2SO_4. After chromatography on alumina (eluant CH_2Cl_2), white crystals of the polyamine (**1**) were obtained by recrystallization from CH_2Cl_2–CH_3OH, m.p. 120—121 °C (yield 70%); $\nu_{max.}$ (Nujol) 1 600 cm^{-1}; δ_H ($CDCl_3$) 2.67 (24 H, ring) 3.50 (12 H, CH_2Ph), and 7.27 (30 H, Ph) (Found: C, 81.5; H, 8.1; N, 10.7. $C_{54}H_{66}N_6$ requires C, 81.16; H, 8.32; N, 10.52%). Compound (**2**) was similarly obtained, m.p. 154—155 °C (from CH_2Cl_2–MeOH) (yield 80%); $\nu_{max.}$ (Nujol) 1 602 cm^{-1}; δ_H ($CDCl_3$) 1.67—1.93 (4 H, ring), 2.40—2.73 (16 H, ring), 3.47 (8 H, CH_2Ph), and 7.27 (20 H, Ph) (Found: C, 81.1; H, 8.4; N, 10.1. $C_{38}H_{48}N_4$ requires C, 81.38; H, 8.63; N, 9.99%).

The polyamide (**3**) was prepared by the reaction of benzoyl chloride and 1,4,8,11-tetra-azacyclotetradecane (Cyclam; Alfa Products) in *N,N*-dimethylacetamide, and had m.p. 269—270 °C (yield 80%); $\nu_{max.}$ (Nujol) 1 630 cm^{-1}; δ_H ($CDCl_3$) 1.83—2.17 (4 H, ring), 3.27—3.87 (16 H, ring), and 7.17—7.57 (20 H, Ph) (Found: C, 73.3; H, 6.2; N, 9.2. $C_{38}H_{40}N_4O_4$ requires C, 74.00; H, 6.54; N, 9.08%). Compound (**4**) has previously been synthesized.[11]

Amino acid derivatives and other employed reagents were commercially available and used without further purification.

Extraction Procedure of Picrate Anion.—The extraction abilities of the carriers were estimated on the basis of the partition of picrate anion between CH_2Cl_2 (5 ml) and the aqueous phase (2 ml) adjusted to pH 4.03—9.83 with NaOH. After extraction experiments, the organic layers were separated and subjected to measurement by u.v. spectroscopy (Shimadzu UV 365).

Transport Experiments.—The transport experiments were carried out at room temperature in a U-tube glass cell (2.0 cm, i.d.) as reported before.[5] The carrier in CH_2Cl_2 (12 ml) was placed in the base of the U-tube, and two aqueous phases (5 ml) were placed in the arms of the U-tube, floating on the CH_2Cl_2 phase. The membrane phase was constantly stirred with a magnetic stirrer. The transport rates were calculated from the initial rates of appearance of substrate anions into the Aq. II phase, which were determined spectroscopically.

Reproducibilities were confirmed as ±10% or better. The details of the conditions employed are shown in Tables 1 and 2.

Acknowledgements

This research was supported in part by grants from the Nodzu Memorial Foundation of Kyoto University and also from the Ministry of Education, Science, and Culture, Japan (No. 58740230).

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Received 11*th July* 1984; *Paper* 4/1201