*Tetrahedron* Vol. 43, No. 20, pp. 4519 to 4530, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00


# SYNTHESES OF MACROCYCLES FROM L-AMINO ACID AND THEIR SELECTIVE TRANSPORT OF AMINO ESTER SALTS THROUGH AN ORGANIC LIQUID MEMBRANE

Hiromi Kataoka and Toyoshi Katagi

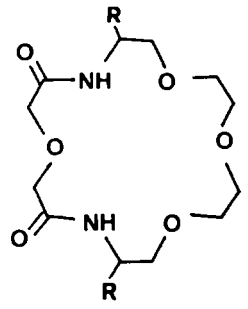
Faculty of Pharmaceutical Science, Mukogawa University
Edagawa-cho, Nishinomiya 663, Japan

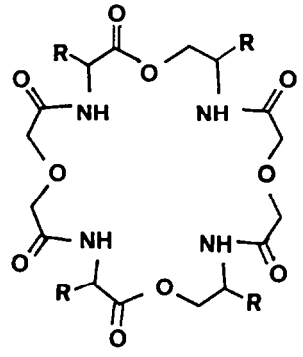
(*Received in Japan* 9 *July* 1987)

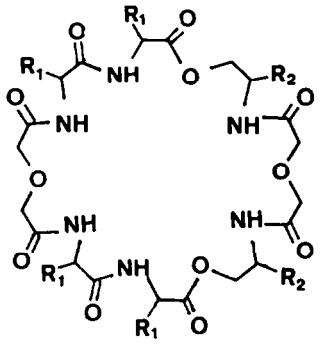
Abstract: Syntheses of 18-membered macrocycle 17, 24-membered macrocycles 18, 19, and 30-membered macrocycles 20, 21, 22, 23, 25, were achieved by using diglycolic acid and amino acids as constituents. Selective transport of amino ester salts through an organic liquid membrane was mediated by their macrocycles and intermediate compounds. The transfer abilities of these carriers containing amino acid moieties were different from that of 18-crown-6.

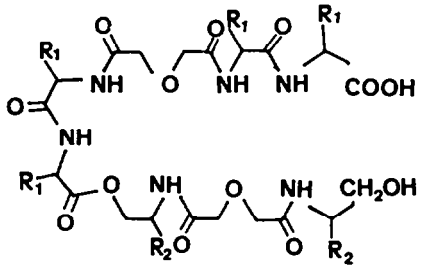
Transport phenomena through organic membranes have been extensively developed in recent years. Many synthetic carriers for metal ions, such as cyclic polyethers, cryptates, cyclic polyamines and other macrocycles, have been studied. Amino acid derivatives have been effectively transported as ammonium cations by using a variety of crowns[1-5] and other types of carriers.[6] The transport of acylamino acids by macrocyclic polyamine-transition metal complexes has recently been reported.[7] Macrocycles which contain L-amino acids with modifiable functionalities in the side chain should be able to serve as hosts in host-guest complexation. We have been interested in the selective transport of amino ester salts by interaction between a substituent in the side chain of amino esters and one in that of macrocycles. Recently, we reported syntheses of macrocycles containing the L-amino acid moiety.[8,9] At the same time, reports appeared on syntheses of diacids constituted of L-phenylalanine with oligoetyleneoxide and their complexation with metal cations.[10,11] In the present paper, we report on some of our work concerning the syntheses of macrocycles, one of which contains L-phenylalanine moieties and the others L-leucine moieties, and the ability of these synthetic macrocycles to transport L-amino ester salts through a chloroform membrane separating two aqueous phases.

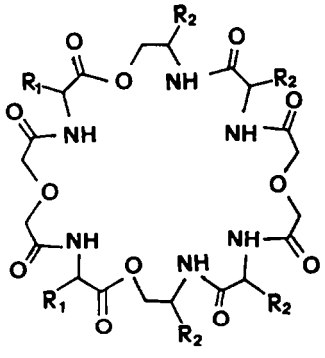
The macrocycles were synthesized as shown in Chart 1 and Scheme 1. Some of the diesters containing two amino acid moieties (2, P-$CO_2$Bzl[10]; 3, P-$CO_2$Et[10]; 4, L-$CO_2$Et), which were obtained by condensation of diglycolic acid (1)[12] with the corresponding L-amino esters by use of N, N'-tionyldiimidazole (TDI),[13,14] were hydrolyzed to a diacid (8, P-$CO_2$H[10]; 9, L-$CO_2$H) and/or reduced to a diol (13, P-$CH_2$OH; 14, L-$CH_2$OH) employing $LiAlH_4$ (Table 1). An attempt to treat diol 13 with ethylene dibromide in the presence of NaH gave not the











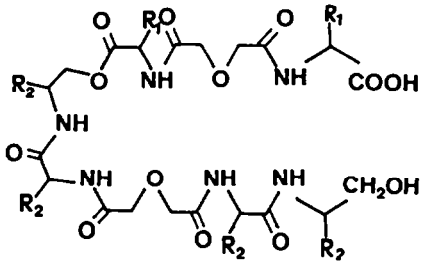
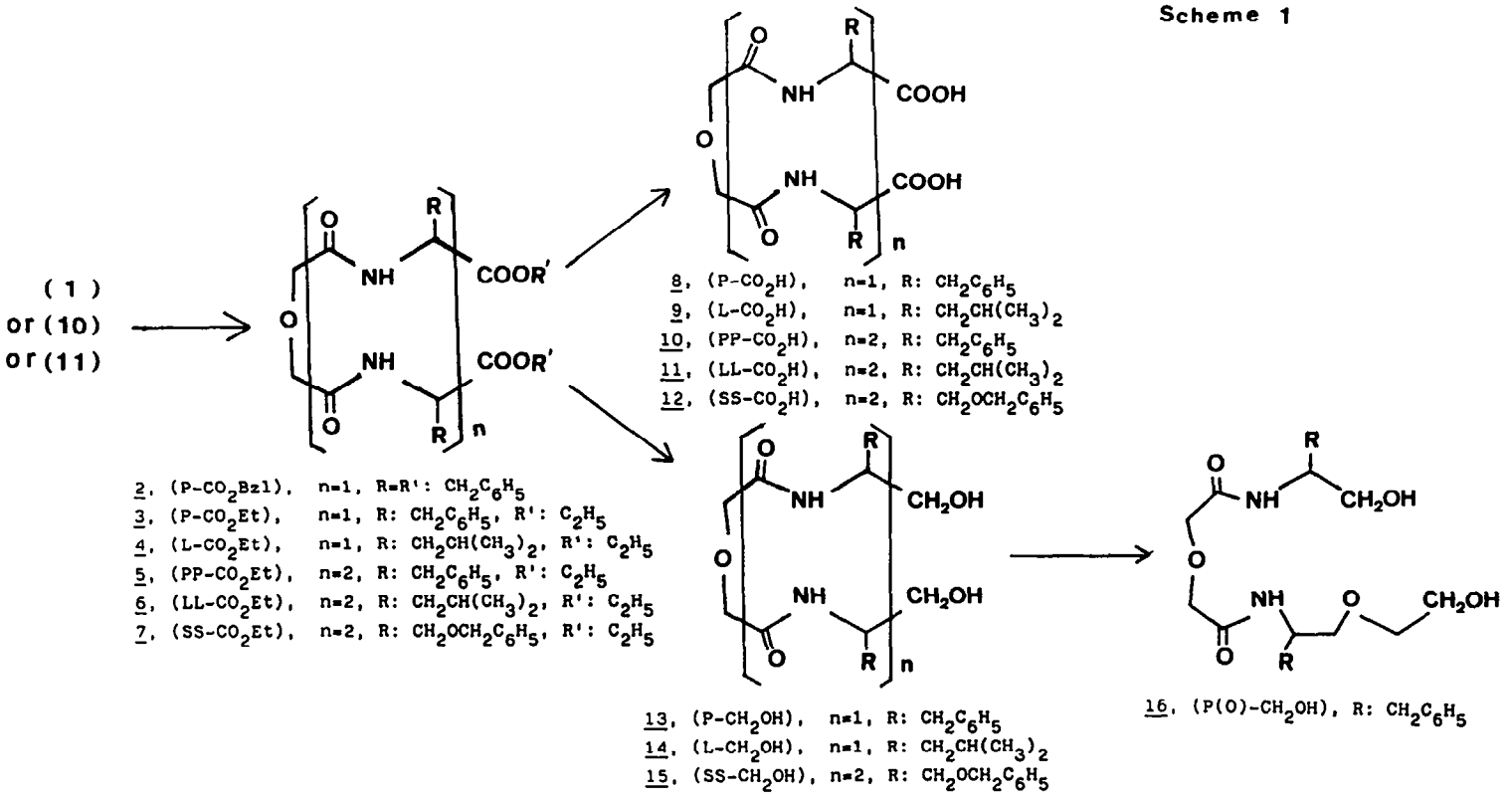


Chart 1

Scheme 1




2, (P-$CO_2$Bzl), n=1, R=R': $CH_2C_6H_5$
3, (P-$CO_2$Et), n=1, R: $CH_2C_6H_5$, R': $C_2H_5$
4, (L-$CO_2$Et), n=1, R: $CH_2CH(CH_3)_2$, R': $C_2H_5$
5, (PP-$CO_2$Et), n=2, R: $CH_2C_6H_5$, R': $C_2H_5$
6, (LL-$CO_2$Et), n=2, R: $CH_2CH(CH_3)_2$, R': $C_2H_5$
7, (SS-$CO_2$Et), n=2, R: $CH_2OCH_2C_6H_5$, R': $C_2H_5$

8, (P-$CO_2$H), n=1, R: $CH_2C_6H_5$
9, (L-$CO_2$H), n=1, R: $CH_2CH(CH_3)_2$
10, (PP-$CO_2$H), n=2, R: $CH_2C_6H_5$
11, (LL-$CO_2$H), n=2, R: $CH_2CH(CH_3)_2$
12, (SS-$CO_2$H), n=2, R: $CH_2OCH_2C_6H_5$

13, (P-$CH_2$OH), n=1, R: $CH_2C_6H_5$
14, (L-$CH_2$OH), n=1, R: $CH_2CH(CH_3)_2$
15, (SS-$CH_2$OH), n=2, R: $CH_2OCH_2C_6H_5$

16, (P(O)-$CH_2$OH), R: $CH_2C_6H_5$

Table 1. Physical and spectral data of diesters (2, 3, 4, 5 and 6), diacids (8, 9, 10 and 11) and diols (13 and 14)

| Compd. | Mp (°C) | Yield (%) | Recrystallization solvent | MS (m/z) | Formula | Elemental Analysis Calcd. (%) C | H | N | Found. (%) C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 83.5-84.0 | 65.3 | AcOEt-P.E. | 608($M^+$) | $C_{36}H_{36}N_2O_7$ | 71.03 | 5.96 | 4.60 | 70.97 | 5.95 | 4.57 |
| 3 | 129.5-130.5 | 71.7 | EtOH-$Et_2O$ | 484($M^+$) | $C_{26}H_{32}N_2O_7$ | 64.45 | 6.66 | 5.78 | 64.49 | 6.73 | 5.71 |
| 4 | 65.0-65.5 | 75.8 | AcOEt-P.E. | 416($M^+$) | $C_{20}H_{36}N_2O_7$ | 57.67 | 8.71 | 6.73 | 57.60 | 9.01 | 6.73 |
| 5 | 175.0-177.0 | 85.0 | EtOH | 778($M^+$) | $C_{44}H_{50}N_4O_9$ | 67.85 | 6.47 | 7.19 | 67.45 | 6.50 | 7.02 |
| 6 | oil | 100.0[a] | | 642($M^+$) | | | | | | | |
| 8 | 151.5-152.5 | 98.0[a] | AcOEt | 428($M^+$) | $C_{22}H_{24}N_2O_7$ | 61.67 | 5.65 | 6.54 | 61.43 | 5.60 | 6.48 |
| 9 | 129.0 | 89.9 | AcOEt-P.E. | 270($M^+$-2COOH) | $C_{16}H_{28}N_2O_7$ | 53.32 | 7.83 | 7.77 | 53.15 | 7.94 | 7.56 |
| 10 | 166.0-168.0 | 99.1[a] | THF-benzene | 723($MH^+$)[b] | $C_{40}H_{42}N_4O_9$ | 66.47 | 5.86 | 7.75 | 66.28 | 5.92 | 7.46 |
| 11 | 110.0-112.0 | 65.2 | AcOEt | 586($M^+$) | $C_{28}H_{50}N_4O_9$ | 57.32 | 8.59 | 9.55 | 57.27 | 8.96 | 9.42 |
| 13 | 128.0-130.0 | 87.8 | AcOEt | 400($M^+$) | $C_{22}H_{28}N_2O_5$ | 65.98 | 7.05 | 7.00 | 66.03 | 7.26 | 6.98 |
| 14 | oil | 77.6[a] | | 332($M^+$) | | | | | | | |
| 14*[c] | 52.5-53.5 | | $Et_2O$ | 416($M^+$) | $C_{20}H_{36}N_2O_7$ | 57.67 | 8.71 | 6.73 | 57.50 | 8.94 | 6.37 |

a) crude yield. b) FAB-MS. c) Diacetate of compound 14.

expected cyclic compound, but the diol 16 (P(O)-$CH_2OH$). On the other hand, when diol 13 was reacted with diethyleneglycol ditosylate[15] in the presence of sodium methylsulfinylmethide,[16,17] an 18-membered cyclic compound 17 (P-18) resulted. The 24-membered cyclic compounds 18 (PP-24) and 19 (LL-24) were prepared by condensation between the corresponding diacid (8 or 9) and diol (13 or 14) by using N, N'-carbonyldiimidazole (CDI). Diesters containing four amino acid moieties (5, PP-$CO_2Et$; 6, LL-$CO_2Et$), which were prepared from a diacid (8 or 9) and the corresponding amino esters using CDI, were hydrolyzed to a diacid (10, PP-$CO_2H$; 11, LL-$CO_2H$) (Table 1). But the diester containing four serine moieties 7 (SS-$CO_2Et$) was obtained by condensation of 1 with H-$(Ser(Bzl))_2$-OEt, which was followed by hydrolysis to a diacid 12 (SS-$CO_2H$) or by reduction to a diol 15 (SS-$CH_2OH$).

Table 2. Template effect of some alkaline metals in the preparation of PPL-30.

| Alkaline metal | Yield (%) |
|---|---|
| none | 1.7 |
| LiCl | 3.1 |
| NaCl | 9.8 |
| KCl | 6.1 |
| | 11.4[a] |
| CsCl | 11.4 |
| | 17.4[a] |

a) During the reaction, an additional condensation reagent was added to the mixture.

The procedure used to prepare 24-membered cyclic compounds easily gave 20 (LLL-30), but 21 (LLP-30) was obtained in lower yield with the linear type product 25 (LLP-30L). Cyclic compound 22 (PPL-30) was obtained in lowest yield. When the template effect of some alkaline metal ions was examined, $Cs^+$ gave the best result as shown in Table 2, and addition of CDI during the reaction increased the yield of 22. Condensation of diacid 12, which contains four O-Bzl-serine components, and diol 13 by use of CDI was not successful in the presence of CsCl, but gave cyclic compound 23 (SSP-30) in low yield when a mixture of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI) and dimethylaminopyridine (DMAP)[18] was used instead of CDI. Synthesis of a cyclic compound 25 (LSS-30) was achieved by the same procedure with a large amount of linear product 26 (LSS-30L).

When preparing cyclic compounds by condensation of diacids with diols, the larger the group in the diacid side chain, the lower was the yield of the cyclic compounds, and the larger that of the diol, with increased yield of linear-type compounds.

Association constants were determined as described in the references,[19,20] but they could be calculated by considering the partition coefficient because the binding ability of the synthetic macrocycles are smaller than that of crown ethers. The salts of hydrophobic amino esters $RCH(CO_2Me)NH_3 \cdot PF_6$ were distributed between chloroform and water in the absence of host, while less than 0.15% of the hosts used was detected in the aqueous phase, except when 18-crown-6 was used. The amount of the amino ester salts in the aqueous phase was determined by gas chromatography. Association constants determined for

Table 3. Association constants (Ka) and partition coefficients (P) of synthetic macrocycles in chloroform at 25°C.[a]

| Host | Ka($M^{-1}$) | | | P[b] | Recovery (%) |
|---|---|---|---|---|---|
| | Phe-OMe | Leu-OMe | Val-OMe | | |
| PPL-30 | 3050 | 450 | 170 | 1430 | 99 |
| LLL-30 | 1730 | 1980 | 240 | 1380 | 99 |
| PP-24 | 250 | 50 | 40 | 1650 | 99 |
| LL-24 | 300 | 60 | 30 | 670 | 101 |

a) Each value is the average of two or more independent determinations.
b) $P = [Host]_{org} / [Host]_{aq}$.

Table 4. Rate of transport of amino acid methyl ester through a liquid membrane(mM).[a]

| Carrier | Phe-OMe | Leu-OMe | Val-OMe | Tyr-OMe | Gly-OMe | Ser-OMe |
|---|---|---|---|---|---|---|
| PPL-30 | 57.4 | 29.5 | 14.8 | 2.6 | 0.2 | 0 |
| LLP-30 | 46.6 | | | | | |
| LLL-30 | 43.3 | 48.2 | 17.1 | 2.5 | 0.2 | 0 |
| LSS-30 | 25.1 | | | | | |
| SSP-30 | 24.2 | | | 0.1 | | |
| LLP30L | 23.9 | | | 2.5 | | |
| LL-24 | 21.9 | 5.9 | 2.1 | 0.3 | 0 | 0 |
| PP-24 | 17.9 | 4.9 | 2.5 | 0.2 | 0 | 0 |
| LL-$CO_2$Et | 23.6 | 50.4 | 4.5 | 0.5 | 0.2 | 0 |
| PP-$CO_2$Et | 18.0 | 10.8 | 1.2 | 0.1 | 0 | 0 |
| SS-$CO_2$Et | 8.7 | 3.4 | 0.8 | 0 | 0 | 0 |
| P-$CO_2$Et | 3.5 | 1.9 | 0.3 | 0.1 | 0 | 0 |
| P-$CO_2$Bzl | 3.1 | 1.0 | 0.1 | 0 | 0 | 0 |
| L-$CO_2$Et | 0.3 | 0.7 | 0 | 0 | 0 | 0 |
| P(O)-$CH_2$OH | 29.5 | 7.0 | 1.6 | 0.3 | | 0.1 |
| P-$CH_2$OH | 17.3 | 10.9 | 1.8 | 0.3 | 0 | 0.1 |
| L-$CH_2$OH | 10.6 | 12.1 | 2.1 | 0.2 | 0 | 0.1 |
| LL-$CO_2H$[b] | 3.2 | 5.9 | 0.3 | 0.1 | 0 | 0 |
| SS-$CO_2H$[b] | 2.6 | 0.4 | 0.2 | 0 | 0 | 0 |
| PP-$CO_2H$[b] | 0 | 0.2 | 0 | 0.1 | 0 | 0 |
| P-$CO_2H$[b] | 0.8 | 0.7 | 0.2 | 0 | 0 | 0 |
| L-$CO_2H$[b] | 0.6 | 0.1 | 0.1 | 0 | 0 | 0 |
| 18-crown-6 | 11.2 | 13.7 | 14.5 | 45.0 | 46.5 | 55.2 |

a) Transport condition: Aq. source phase: 0.2 M amino acid methyl ester hydrochloride and 0.4 M $LiPF_6$ in 5 ml of 0.08M HCl; membrane: carrier[b] (5 mM) in 20 ml of $CHCl_3$; Aq. receiving phase: 5 ml of 0.1 M HCl. b) Concentration of this carrier was one-tenth (0.5 mM) of the other carriers.

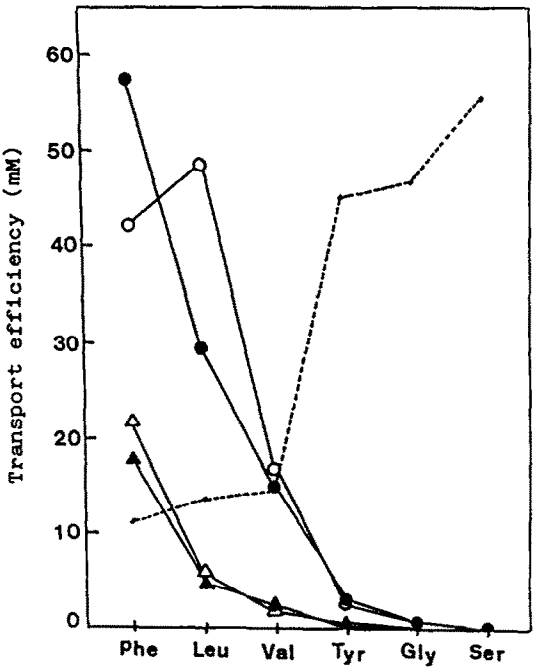



Figure 1. Graphical representation of transport of various amino acid methyl ester through a liquid membrane(mM). ● = PPL-30, ○ = LLL-30, ▲ = PP-24, △ = LL-24, • = 18-crown-6.

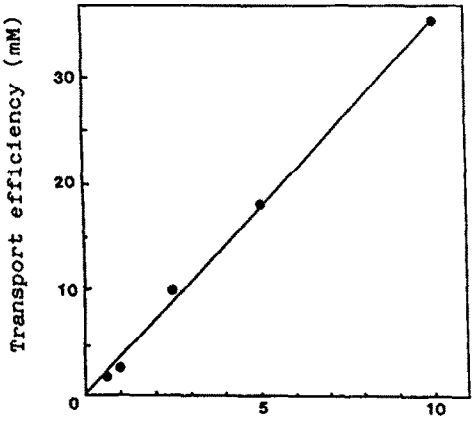



Figure 2. Relationship between the concentration of carrier (PP-$CO_2$Et) and transport efficiency of transferred guest (Phe-OMe)

hosts PPL-30, LLL-30, PP-24, and LL-24 at 25°C are reported in Table 3.

$$Ka = \frac{[Complex]_{org}}{[H]_{org}\,[G]_{org}}$$

$$= \frac{R[Hi]_{org}\,(1+P\,V_{org}/V_{aq})-P[Gi]_{aq}}{P\{[Gi]_{aq}-R[Hi]_{org}\,V_{org}/V_{aq}\}\{[Hi]_{org}\{1-R(1+P\,V_{org}/V_{aq})\}+P[Gi]_{aq}\}}$$

In equation, R is the molar ratio of guest to host in the chloroform phase, P is partition coefficient ($P = [G]_{org}/[G]_{aq}$), $[Hi]_{org}$ is the initial concentration of the host in the chloroform phase, $[Gi]_{aq}$ is the initial concentration of the amino ester salts in the aqueous phase, and $V_{org}$ and $V_{aq}$ are the volumes of the two phases.

Transport The transport of L-amino ester hydrochlorides through a liquid membrane was examined using these macrocyclic compounds and their synthetic intermediates (diols, diesters and diacids) as carriers in the apparatus described by Kobuke et al.[21] The source phase contained a mixture of an amino ester hydrochoride and $LiPF_6$ in 0.08 M HCl aqueous solution. This amino ester cation was transferred into chloroform by complexation with a synthetic macrocycle and was released into the receiving phase containing 0.1 M HCl. The concentration of the amino ester in the receiving phase was determined by the absorbance at 570 nm by means of the reaction with ninhydrin.[22,23] The transfer ability of these carriers was compared with that of 18-crown-6 (Table 4). The net value of the transport by each carrier shown in Table 4 was the average of differences of apparent values from blank test values in several runs. The transfer abilities of 30- and 24-membered-cycles and of 18-crown-6 are compared in Table 4 and Figure 1. Cycles containing amino acid moieties showed different tendencies from that of 18-crown-6. The crown was not very efficient for transporting Phe-OMe and Leu-OMe, but was for Tyr-OMe, Ser-OMe and Gly-OMe. On the other hand, synthetic macrocycles could transfer amino esters containing the lipophilic side chain and distributing to the chloroform phase (Phe-OMe·HCl; $P = 1.1\times10^{-2}$, Leu-OMe·HCl; $P = 1.0\times10^{-2}$, and Val-OMe·HCl; $P = 4.2\times10^{-3}$). However, they were not very useful for the guests, leading to little distribution to the chloroform phase (Gly-OMe·HCl; $P = 9.0\times10^{-4}$).

Among the synthetic macrocycles, 30-membered cycles were more efficient carriers than 24-membered cycles. It was presumed that the hole size of the 30-membered ring was suitable for binding the ammonium group in the guest molecule. Also, as affinity between analogous substituents in hosts and guests promotes transport, PPL-30 was the most efficient carrier for the transport of Phe-OMe and LLL-30 gave the best results for Leu-OMe. As for the 24-membered rings, it was assumed that their hole size was not be able to accommodate the guest molecule, thus weakening the binding and the affinity of the substituents. The same affinity effects were observed with linear diesters and diols. In the transport experiments using diacids as carriers (Table 4), the concentration of diacid, which is slightly soluble in chloroform, was one-tenth (0.5 mM) that of the other carriers. As the relationship between the concentration of the carrier (PP-$CO_2$Et) and the transferred guest (Phe-OMe) was almost linear (Figure 2), LL-$CO_2$H was almost as efficient a

carrier as LL-$CO_2$Et.

This study showed that the affinity between the analogous substituents in host and guest affects the selective transport of a particular amino acid by a carrier-the carrier displays affinity for the amino acid having analogous substituents.

## EXPERIMENTAL

All melting points were measured with a Yanaco MP-S3 apparatus and are uncorrected. Mass spectra were taken with a Hitachi RMU-6MG spectrometer (MS), and a JEOL mass spectrometer Model JMS DX-303, computer system JMS DA-5000 (MS). Fast atom bombardment mass spectra were recorded on a JEOL mass spectrometer Model LMS DX-303, JMS FAB-09, computer system JMS DA-5000 (FAB-MS). Samples were dissolved in a matrix of glycerol. The solution was bombarded with a beam of neutral Xe atoms at an energy of 3 KeV.

### Preparation of diesters (2, 3 and 4)

An anhydrous tetrahydrofuran (THF) solution of the TDI (2.5 eq.) was prepared in the usual way.[13,14] To the solution was added 1 (1 eq.), with stirring for 30 min. at room temperature. Next, the corresponding amino acid ester hydrochloride (2 eq.) and triethylamine ($Et_3N$) (2 eq.) were added to this solution. The reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo, and ethyl acetate (AcOEt) was added to the residue. This solution was washed with 10% HCl, 10% $NaHCO_3$ and brine, then dried over anhydrous $MgSO_4$ and evaporated in vacuo. The resultant product was purified by recrystallization or column chromatography on silica gel (Table 1). Preparation of 3 by dicyclohexylcarbodiimide (DCC) gave poor yields (13%).

### Preparation of diesters (5 and 6)

Using the above procedure, diacids (10 or 11) were reacted with the corresponding amino acid ethyl ester using CDI as a condensing agent instead of TDI. The resultant product 5 was purified by recrystallization (Table 1). A portion of 6 was purified by column chromatography on silica gel; elution with AcOEt afforded the pure product. Usually the crude product of 6 was used for the next step without further purification.

### Preparation of dicarboxylic acids (8, 9, 10 and 11)

To the solution of diester (1 eq) in methanol (MeOH), 1 N NaOH solution (3 eq) was added, and the mixture was stirred for 6 h at 40°C. The solution was diluted with 30 ml of water, and the MeOH was evaporated in vacuo. The remaining aqueous solution was washed with AcOEt, and the aqueous layer was acidified with 10% HCl and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The resultant product was purified by recrystallization (Table 1). The 8 and 10 were found to be sufficiently pure and were used for the next reaction without recrystallization.

### Preparation of diols (13 and 14)

To a solution of diester in anhydrous THF was added excess $LiAlH_4$ at -50°C. The reaction mixture was stirred at -30°C until it showed one spot and the absence of any starting material by t.l.c. (silica gel, MeOH:AcOEt:acetic acid = 1:9:0.1). The reaction mixture was made acidic with 10% HCl and NaCl was added. The THF layer was separated, and then the organic layer was extracted with AcOEt. The organic extract was washed with 10% $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and evaporated in vacuo. The resultant product 13 was purified by recrystallization. A portion of 14 was acetylated and recrystallized for preparation of analytical sample (Table 1). Usually the crude product of 14 was used for the next step without purification.

### Boc-(Ser(Bzl))$_2$-OEt

Boc-Ser(Bzl)-OH (4.55 g) was dissolved in anhydrous THF (50 ml) and CDI (2.50 g) was added to this solution under ice-cooling. After the effervescence had ceased, the solution was stirred for 1 h and then the Ser(Bzl)-OEt·HCl[24] (3.99 g) was added, followed by $Et_3N$ (2.2 ml) in anhydrous THF (10 ml) solution at 0°C. The reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo and then AcOEt was added to the residue. This solution was washed with 10% citric acid solution, 10% $NaHCO_3$ solution and brine, then dried over anhydrous $MgSO_4$ and evaporated in vacuo. Recrystallization of the residue from i-propyl ether gave Boc-(Ser(Bzl))$_2$-OEt (6.48 g, 84.0%), mp 71.0-73.0°C. MS m/z: 500($M^+$). Anal. Calcd for $C_{27}H_{36}N_2O_7$: C, 64.78; H, 7.25; N, 5.60. Found: C, 64.63; H, 7.46; N, 5.61.

### H-(Ser(Bzl))$_2$-OEt·HCl

To the Boc-(Ser(Bzl))$_2$-OEt (2.10 g) was added a solution of saturated dry HCl in anhydrous AcOEt (20 ml) at 0°C, and the mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo. Recrystallization of the residue from AcOEt gave the deprotected peptide (1.81 g, 98.8%), mp 120.0-121.5°C. MS m/z: 400($M^+$-HCl). Anal. Calcd for $C_{22}H_{29}N_2O_5Cl$·1/6 $H_2O$: C, 60.06; H, 6.72; N, 6.37. Found: C, 60.15; H, 6.87; N, 6.33.

SS-$CO_2$Et (7)

7 (0.64 g) was dissolved in anhydrous THF (20 ml) and CDI (1.49 g) was added to this solution under ice-cooling. After the effervescence had ceased, the solution was stirred for 1 h and then H-(Ser(Bzl))$_2$-OEt·HCl (4.01 g) was added, followed by a solution of $Et_3N$ (1.28 ml) in anhydrous THF at 0°C. The reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo and then AcOEt was added to the residue. This solution was washed with 10% HCl, 10% $NaHCO_3$ solution and brine, then dried over anhydrous $MgSO_4$ and evaporated in vacuo. Recrystallization of the residue from ethanol-water gave 9 (3.19 g, 77.1%), mp 98.0-100.0°C. MS m/z: 898($M^+$). Anal. Calcd for $C_{48}H_{58}N_4O_{13}$: C, 64.13; H, 6.50; N, 6.23. Found: C, 63.93; H, 6.56; N, 6.28.

SS-$CO_2$H (12)

To the solution of 7 (3.05 g) in hot MeOH (70 ml), 1 N NaOH solution (13.4 ml) was added, and the mixture was stirred for 1 h at 50°C. The solution was diluted with 70 ml of water, and the MeOH was evaporated in vacuo. The remaining aqueous solution was washed with AcOEt, and then the aqueous layer was slowly added under ice-cooling to 1 N HCl (40 ml), and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and evaporated in vacuo to dryness, giving a glass-like solid (2.57 g, 89.9%). FAB-MS m/z: 843($MH^+$).

SS-$CH_2$OH (15)

To a solution of 7 (3.01 g) in anhydrous THF (300 ml) was added $LiAlH_4$ (4.0 g) at -30°C. The reaction mixture was stirred at -20°C until it showed one spot and the absence of any starting material by t.l.c. (silica gel, MeOH:AcOEt:acetic acid = 1:9:0.1). The reaction mixture was made acidic with 10% HCl and NaCl was added. The THF layer was separated, and then the organic layer was extracted with AcoEt. The organic extract was washed with 10% $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel. The residue obtained from the fraction eluted with AcOEt was recrystallized from AcOEt to give 15 (0.29 g, 10.6%), mp 129.0-131.0°C. FAB-MS m/z: 815($MH^+$). Anal. Calcd for $C_{44}H_{54}N_4O_{11}$: C, 64.85; H, 6.68; N, 6.88. Found: C, 64.45; H, 6.76; N, 6.77.

P(O)-$CH_2$OH (16)

NaH (50% mineral oil dispersion) (0.24 g) was washed with anhydrous petroleum ether (P. E.) and THF by swirling, allowing the hydride to settle, and decanting the liquid portion in order to remove the mineral oil. To the hydride in anhydrous THF was added 13 (1.0 g), with stirring for 2 h at room temperature, and then ethylene dibromide (0.47 g) and 18-crown-6 (0.66 g) were added. And the reaction mixture was stirred for 1 week at room temperature. The reaction mixture was made acidic with 10% HCl and salting out by adding NaCl. The THF layer was separated, and then the organic layer was extracted with AcOEt. The organic extract was washed with 10% $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and evaporated in vacuo. Recrystallization of the residue from AcOEt gave 16 (0.47 g, 90.5%), mp 102.0-103.0°C. MS m/z: 442($M^+$). Anal. Calcd for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.17; H, 7.00; N, 6.56.

P-18 (17)

NaH (50% mineral oil dispersion) (115.2 mg) was washed with anhydrous P. E. by swirling, allowing the hydride to settle, and decanting the liquid portion in order to remove the mineral oil. Anhydrous dimethyl sulfoxide (DMSO) (2 ml) was added to the hydride with stirring for 1 h at 70-75°C in an atmosphere of dry nitrogen. Next, 13 (500 mg) was added to the resulting methylsulfinylmethide solution at room temperature. The mixture was stirred for 1 h at 45-50°C, and then diethyleneglycol ditosylate[15] (546 mg) was added at room temperature. After the reaction mixture had been stirred for 7 h, brine was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and evaporated in vacuo. Recrystallization of the residue from acetone gave 17 (22 mg, 3.74%), mp 122.0-123.2°C. MS m/z: 470($M^+$). Anal. Calcd for $C_{26}H_{34}N_2O_6$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.25; H, 7.44; N, 5.92.

PP-24 (18)

8 (12.20 g) was dissolved in anhydrous THF (400 ml) and CDI (10.22 g) was added to this solution under ice-cooling. After the effervescence had ceased, the solution was stirred for 1 h and then 13 (10.37 g) in anhydrous N, N'-dimethylformamide (DMF) (20 ml) and THF (100 ml) solution was added at room temperature. This reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo and then a large amount of AcOEt was added to the residue. This solution was washed with 10% HCl, 10% $NaHCO_3$ solution and brine, then dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel. Elution with AcOEt afforded the pure material 18. Recrystallization of the residue from AcOEt gave 18 (3.74 g, 18.2%), mp 173.0-174.5°C. MS m/z: 792($M^+$). Anal. Calcd for $C_{44}H_{48}N_4O_{10}$: C, 66.65; H, 6.10; N, 7.07. Found: C, 66.46; H, 6.15; N, 6.87.

LL-24 (19)

9 (4.90 g) was dissolved in anhydrous THF (150 ml), and CDI (4.85 g) was added to this solution under ice-cooling. After the effervescence had ceased, the solution was stirred for 1 h and then 14 (4.13 g) in anhydrous THF (50 ml) solution was added at room temperature. This reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo and then AcOEt was added to the residue. This solution was washed with 10% HCl, 10% $NaHCO_3$ solution and brine, then dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel. Elution with diethyl ether ($Et_2O$) afforded the pure material 19, a glass-like solid (2.13 g, 23.9%), mp 81.0-83.0°C. MS m/z: 656($M^+$). Anal. Calcd for $C_{32}H_{56}N_4O_{10}$: C, 58.52; H, 8.59; N, 8.53. Found: C, 58.45; H, 8.84; N, 8.58.

LLL-30 (20)

To a solution of 11 (5.00 g) in anhydrous THF (85 ml) was added CDI (2.76 g) at 0°C. The solution was left standing for 1 h after the effervescence had subsided, and then a solution of 14 (2.83 g) in anhydrous THF (40 ml) was added. After stirring for 24 h, the solvent was evaporated in vacuo and the residue was taken up in AcOEt. The mixture was washed with 10% HCl, 10% $NaHCO_3$ solution and brine dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using $Et_2O$ as an eluent to afford the pure 30-membered macrocycle 20 (1.65 g, 22.0%). mp 104.5-106.5°C. MS m/z: 883($M^+$). Anal. Calcd for $C_{44}H_{78}N_6O_{12}\cdot 1/2\ H_2O$: C, 59.24; H, 8.93; N, 9.42. Found: C, 59.22; H, 9.23; N, 9.31.

LLP-30 and LLP-30L (21 and 24)

To a solution of 11 (2.00 g) in anhydrous THF (30 ml) was added CDI (1.10 g) at 0°C. The solution was left standing for 1 h after the effervescence had subsided, and then a solution of 13 (1.36 g) in anhydrous DMF (10 ml) and THF (10 ml) was added. After stirring for 3 days, the solvent was evaporated in vacuo and the residue was taken up in AcOEt. The mixture was washed with 10% HCl, 10% $NaHCO_3$ solution and brine dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using AcOEt/benzene (5:95) as an eluent to afford the pure 30-membered macrocycle 21 (0.22 g, 6.8%), a glass-like solid, FAB-MS m/z: 951($MH^+$). Anal. Calcd for $C_{50}H_{74}N_6O_{12}\cdot 1/2\ H_2O$: C, 62.55; H, 7.87; N, 8.75. Found: C, 62.63; H, 8.12; N, 8.53. The linear-type compound 24 was isolated from a fraction of MeOH/AcOEt (5:95). Recrystallization from AcOEt gave 24 (0.58 g, 17.6%), mp 121.0-122.0°C. FAB-MS m/z: 969($MH^+$). Anal. Calcd for $C_{50}H_{76}N_6O_{13}$: C, 61.96; H, 7.90; N, 8.67. Found: C, 62.03; H, 7.91; N, 8.46.

PPL-30 (22)

To a solution of 10 (6.17 g) in anhydrous THF (85 ml) was added CDI (2.76 g) at 0°C. The solution was permitted to stand for 1 h after the effervescence had subsided, and then CsCl (14.3 g) and a solution of 14 (3.12 g) in anhydrous THF (40 ml) were added. The reaction mixture was stirred for 3 days. Next, CDI (1.38 g) was added, and the mixture was stirred for an additional day. The solution was filtered, the filtrate was evaporated in vacuo, and a large amount of AcOEt was added to the residue. The mixture was washed with 10% HCl, 10% $NaHCO_3$ solution and brine, then dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel. Elution with AcOEt afforded the pure 30-membered macrocycle 22 as a glass-like solid (1.51 g, 17.4%). It crystallized from AcOEt when the solution was kept at room temperature over a period of three months. mp 178.0-180.0°C. FAB-MS m/z: 1019($MH^+$), Anal. Calcd for $C_{56}H_{70}N_6O_{12}\cdot 1/2\ H_2O$: C, 65.42; H, 6.96; N, 8.17. Found: C, 65.39; H, 7.08; N, 8.26.

SSP-30 (23)

12 (6.53 g), 13 (3.10 g), DMAP (3.76 g), CsCl (12.9 g), and EDCI·HCl (2.95 g) were added successively to anhydrous DMF (80 ml). The reaction mixture was stirred for 4 days at room temperature. Next, EDCI·HCl (1.48 g) was added, and the mixture was stirred for an additional day. The solvent was evaporated in vacuo and the residue was taken up in AcOEt. The mixture was washed with 10% HCl, 10% $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using AcOEt as an eluent to afford the pure 30-membered macrocycle 23 (0.50 g, 5.3%). mp 112.5-113.5°C. (Recrystallized from AcOEt-$Et_2O$) FAB-MS m/z: 1207($MH^+$). Anal. Calcd for $C_{66}H_{74}N_6O_{16}\cdot(C_2H_5)_2O\cdot H_2O$: C, 64.70; H, 6.67; N, 6.47. Found: C, 64.53; H, 6.87; N, 6.36.

LSS-30 and LSS-30L (25 and 26)

9 (0.34 g), 15 (0.70 g), DMAP (0.46 g), CsCl (1.5 g) and EDCI·HCl (0.36 g) were added in this order to anhydrous DMF (20 ml). The reaction mixture was stirred for 4 days at room temperature. Next, EDCI·HCl (0.18 g) was added, and the mixture was stirred for an additional day. The solvent was evaporated in vacuo and the residue was taken up in AcOEt. The mixture was washed with 10% HCl, 10% $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using AcOEt as an eluent to afford the 30-membered macrocycle 25 (0.13 g, 13.2%), FAB-MS m/z: 1139($MH^+$). Anal. Calcd for $C_{60}H_{78}N_6O_{16}\cdot 1/2\ H_2O$: C, 62.76; H, 6.93; N, 7.32. Found: C, 62.73; H, 7.06; N,

7.06. MeOH/AcOEt (1:4) as an eluent afforded the linear-type compound 26 (0.52 g, 47.6%), FAB-MS m/z: 1157($MH^+$).

Transport of amino acid methyl ester salt

A glass tube (1.6 cm i.d.) was placed in a cylindical tube (2.6 cm i.d.) to separate two aqueous phases. The outer source phase contained 0.2 M amino acid methyl ester hydrochloride and 0.4 M $LiPF_6$ in 5 ml of 0.08 M HCl. The inner receiving phase contained 5 ml of 0.1 M HCl. The organic phase was placed at the bottom of the cylindical tube. This contained 5 mM carrier in 20 ml of $CHCl_3$ and was stirred at 400 r.p.m. by a magnetic stirrer at 25-26°C. After 24 h, a 0.1-ml sample of the receiving phase was withdrawn, and the concentration of the solution of amino acid methyl ester salt was determined (570 nm) by the procedure of E. W. Yemm, and E. C. Cocking[22,23] using on a Shimazu spectrophotometer UV-100-02.

The association constants were determined at the same concentration for the transport. A 0.1-ml sample of the aqueous phase was lyophilized, and the residue was trifluoroacetylated at room temperature. The amount of N-trifluoroacethyl amino esters were determined by gas chromatographic analyses using Shimazu GC-7AG with a column of 2% cyclohexanedimethanol succinate on Gas Chrom Q. Quantitative analysis was performed by using n-paraffin as an internal standard (TFA-Phe-OMe; n-docosane, TFA-Leu-OMe; n-octadecane, and TFA-Val-OMe; n-cetane).

ACKNOWLEDGEMENT

The authors wish to thank the staff of the Analysis Center of Mukogawa University for the elemental analyses and MS spectra.

REFERENCES AND FOOTNOTE

1. R. C. Helgeson, J. M. Timko, P. Moreau, S. C. Peacock, J. M. Mayer, and D. J. Cram, J. Am. Chem. Soc., 96, 6762 (1974).
2. S. C. Peacock, L. A. Domeier, F. C. A. Gaeta, R. C. Helgeson, J. M. Timko, and D. J. Cram, J. Am. Chem. Soc., 100, 8190 (1978).
3. R. M. Izatt, J. D. Lamb, N. E. Izatt, B. E. Rossiter, Jr., J. J. Christensen, and B. L. Haymore, J. Am. Chem. Soc., 101, 6273 (1979).
4. M. Newcomb, J. L. Toner, R. C. Helgeson, and D. J. Cram, J. Am. Chem. Soc., 101, 4941 (1979).
5. H. Tsukube, K.Takagi, T. Higashiyama, Y. Iwachido, and N. Hayama, J. Chem. Soc., Chem. Commun., 1986, 448.
6. K. Maruyama and H. Tsukube, J. Chem. Soc., Chem. Commun., 1980, 1222.
7. H. Tukube, J. Chem. Soc., Perkin Trans. 1, 1983, 29.
8. T. Katagi and H. Kuriyama, Heterocycles, 19, 1681 (1982).
9. T. Katagi and H. Kuriyama-Kataoka, Heterocycles, 23, 2663 (1985).
10. T. Lodi, R. Marchelli, A. Dossena, E. Dradi, and G. Casnati, Tetrahedron, 38, 2055 (1982).
11. R. Marchelli, E. Dradi, A. Dossena, and G. Casnati, Tetrahedron, 38, 2061 (1982).
12. T. Kiyoura, T. Takashi, and H. Matsuda, C. A., 79, 41955q (1973).
13. H. A. Staab and K. Wendel, Angew. Chem., 73, 26 (1961).
14. T. H. Wieland and K. Vogeler, Angew. Chem., 73, 435 (1961).
15. J. Dale and P. O. Kristiansen, Acta. Chem. Scand., 26, 1471 (1972).
16. E. J. Corey and M. Chaykovsky, J. Am. Chem. Soc., 84, 866 (1962).
17. E. J. Corey and M. Chaykovsky, J. Am. Chem. Soc., 87, 1345 (1965).
18. C. Gilom and Y. Klausner, Tetrehedron Lett., 1979 3811.
19. J. M. Timko, S. S.Moore, D. M. Walba, P. C. Hiberty, D. J. Cram, J. Am. Chem. Soc., 99, 4207 (1977).
20. M. Newcomb, J. M. Timko, D. M. Walba, D. J. Cram, J. Am. Chem. Soc., 99, 6392 (1977).
21. Y. Kobuke, K.Hanji, K. Horiguchi, M. Asada, Y. Nakayama, and J. Furukawa, J. Am. Chem. Soc., 98, 7414 (1976).
22. E. W. Yemm and E. C. Cocking, Analyst., 80, 209 (1955).
23. E. C. Cocking and E.W. Yemm, Biochem. J. (London), 58, xii (1954).
24. W. Göhring and G.Jung, Liebigs Ann. Chem., 1975, 1781.
25. All amino acid residues were of the L-configuration. Abbreviations used in this paper include: Boc = t-butyloxycarbonyl, OBzl = benzyl ester, Bzl = benzyl. Boc-Ser(Bzl)-OH and Ser(Bzl)-OH was obtaind from peptide Institute, Inc., (Osaka, Japan).