Syntheses of Cyclic Octapeptides and Mediation by Them of Selective Transport, Including Enantiomer Recognition, through an Organic Liquid Membrane[1)]

Hiromi KATAOKA, Tomoko HANAWA, and Toyoshi KATAGI*

Faculty of Pharmaceutical Sciences, Mukogawa University, 11-68 Koshien Kyuban-cho, Nishinomiya, Hyogo 663, Japan. *Received June 18, 1991*

Cyclic octapeptides (1—4) having analogous amino acid sequences were synthesized from the corresponding linear peptides using a conventional solution-phase method. The cyclization was performed by the EDCI/HOBt procedure using an equimolar mixture of alkaline metal cations at a high dilution (concentration of linear peptide: 1.2 mM). The selective transport of amino acid methyl ester and amine salts through an organic liquid membrane mediated by the synthetic cyclic octapeptides and the enantiomer recognition properties in the transport of racemic Phe–OMe salt were examined. The cyclic octapeptides transported D-Phe–OMe salt more efficiently than other guest salts.

Keywords cyclic peptide; cyclization; ionophore model; selective transport; enantiomer recognition

The chiral recognition properties of crown ethers containing a steric barrier and an organic ammonium salt have been studied by Cram *et al.*[2)] In our previous study, we synthesized a series of cyclic peptides and peptide-like macrocycles as ionophore models, and examined their abilities to selectively transport amino acid methyl ester and amine salts through a liquid membrane.[3-6)] We have suggested that the selective transport is based on the affinity between analogous substituents in the peptide as the carrier and in the guest, *i.e.*, the organic ammonium cation.

In this study, the sequence of the cyclic octapeptide was designed to have a localized distribution of Lys(Z) (or Glu(OBzl)) and Phe residues, because such cyclic octapeptides were expected to stereoselectively transport an organic ammonium cation. Thus the chiral recognition is not based on steric hindrance, but on the affinity between the side chains in the cyclic peptide and the organic ammonium cation. In order to change the hydrophobicity and flexibility of the cyclic octapeptide molecule, variations of the Sar and Gly sequences were designed (Fig. 1). The scheme for the syntheses of the cyclic octapeptides is shown in Figs. 2—4. The selected C-terminal residue for the fragment peptide and linear octapeptide was the Gly or Sar

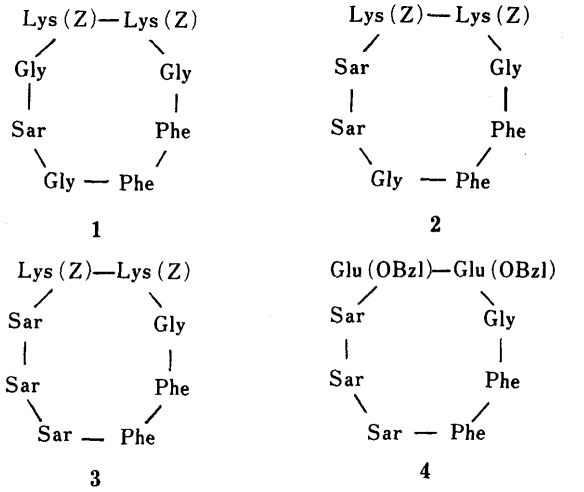

Fig. 1. Cyclic Octapeptides (1—4)

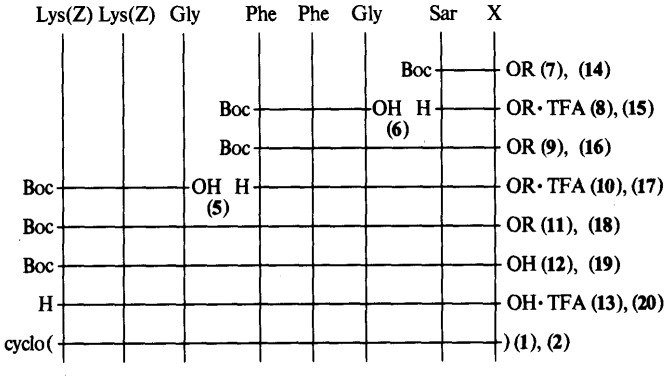

Fig. 2. Synthetic Scheme for Cyclic Octapeptides (1 and 2)
X=Gly, R=Et: 1, 7—13. X=Sar, R=Me: 2, 14—20.

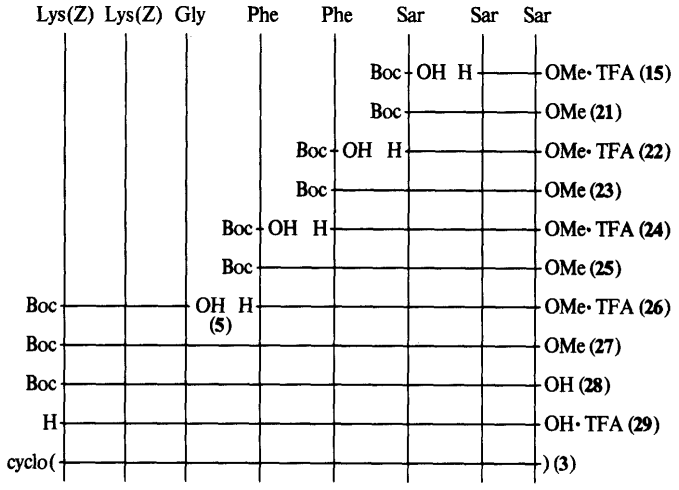

Fig. 3. Synthetic Scheme for Cyclic Octapeptide (3)

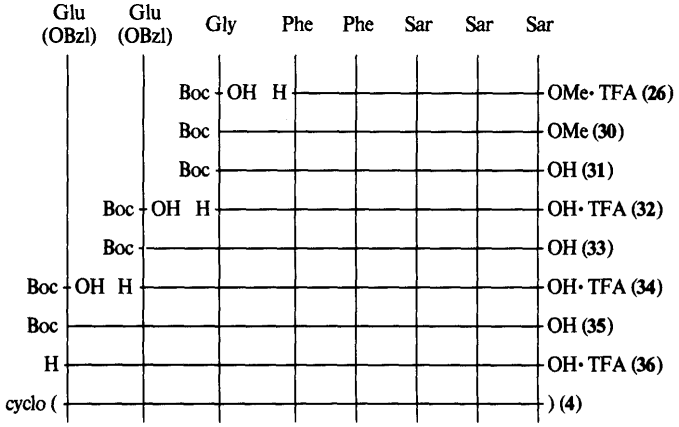

Fig. 4. Synthetic Scheme for Cyclic Octapeptide (4)

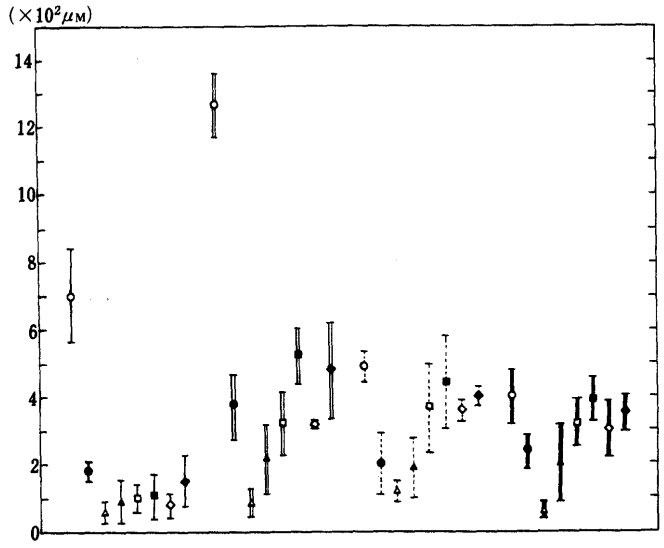

Fig. 5. Amounts of Guests Transported into the Receiving Phase Using the Cyclic Octapeptides (**1**—**4**)

Cyclic octapeptides: **1**, ⊢—⊣; **2**, ⊨⊨; **3**, ⊢--⊣; **4**, ⊨⊨. Guest salts: ○, D-Phe–OMe; ●, L-Phe–OMe; △, D-Leu–OMe; ▲, L-Leu–OMe; □, D-Phg–OMe; ■, L-Phg–OMe; ◇, (*R*)-PEA; ◆, (*S*)-PEA.

TABLE I. Association Constants (K_a) of Cyclic Octapeptides with Amino Acid Methyl Ester Salts in Chloroform

Cyclic octapeptide	K_a (M^{-1})			
	D-Phe–OMe	L-Phe–OMe	D-Leu–OMe	L-Leu–OMe
1	4.7×10^3	2.3×10^3	2.2×10^2	6.1×10^2
2	1.7×10^4	4.3×10^3	1.6×10^3	2.2×10^3
3	3.4×10^3	3.2×10^3	2.2×10^3	2.1×10^3
4	2.9×10^3	2.1×10^3	6.0×10^2	1.4×10^3

residue to avoid racemization. The octapeptides **11**, **18** and **27** were obtained by fragment condensation of the tripeptide **5** with the pentapeptide **10**, **17** or **26** by the EDCI/HOBt or mixed anhydride method. Next, the octapeptides were hydrolyzed, and their Boc groups were removed by treatment with TFA–anisole to obtain the respective linear octapeptides (**13**, **20** and **29**). The linear octapeptide **36** was synthesized by a stepwise method using CDI or a mixed anhydride method. Cyclization of the linear octapeptides was carried out by the EDCI/HOBt method, using an equimolar mixture of alkaline metal ions, such as LiCl, NaCl, KCl, and CsCl, in order to obtain a satisfactory yield.[7] The crude products were purified to homogeneity by column chromatography on silica gel, preparative thin-layer chromatography (TLC) on silica gel, and/or recrystallization with appropriate solvent systems. The structures of the cyclic octapeptides were confirmed by elemental analysis and fast atom bombardment mass spectrometry.

Transport measurements through an organic liquid membrane were performed with a double cylindrical apparatus as previously reported.[4] The amounts of the transported guest, *i.e.*, the organic ammonium cation, were determined by gas liquid chromatography (GC). The net values of the transport by each cyclic octapeptide shown in Fig. 5 are the averages of differences between the experimental measured values and blank test values in three

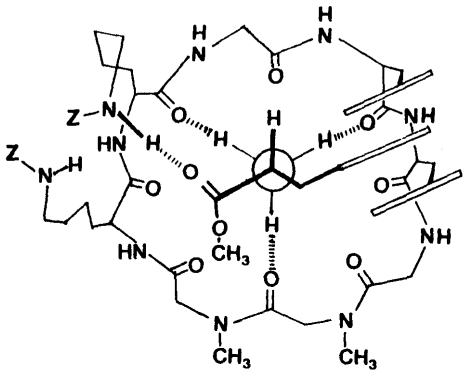

Fig. 6. Schematic Formula of the Complex of the Cyclic Octapeptide **2** and the D-Phe–OMe Salt

to five runs. Association constants were determined as described previously[4] and are listed in Table I. The cyclic octapeptides **1** and **2** could transport D-Phe–OMe salt more efficiently than L-Phe–OMe salt. When the guest was the D- or L-form of Leu–OMe or Phg–OMe, or (*R*)- or (*S*)-form of PEA, the selective transport abilities of cyclic octapeptides **1**—**4** were very small. Enantiomer recognition of these cyclic octapeptides for transporting racemic Phe–OMe·HCl was examined, and the configuration of the dominant enantiomer was always the D-form. The enantiomeric excess (ee) values of the cyclic octapeptides for racemic Phe–OMe salt were determined by high-performance liquid chromatography (HPLC) (ee values: **1**, 24.2%; **2**, 24.9%; **3**, 11.1%; **4**, 13.1%). The ee values of the cyclic octapeptides **1** and **2** exhibited a higher enantiomer selectivity than those of **3** and **4**. The ee value of the blank tests when no cyclic octapeptide was present was taken as zero percent.

The proton nuclear magnetic resonance (^{1}H-NMR) (500 MHz) spectra indicated different patterns of chemical shift between the complexes of D-Phe–OMe and L-Phe–OMe. This difference in the chemical shift suggests that the mode of interaction of the respective guests differs in the two complexes.

From these results, we hypothesize that the conformation of the cyclic octapeptide **2** undergoes conversion when a guest is present. When the guest is a D-Phe–OMe salt, the NH_3^+ group of the guest approaches the cavity of the cyclic octapeptide **2** with a three-point interaction with the carbonyl groups in the cyclic peptide. The conformation of the cyclic octapeptide changes to allow the simultaneous occurrence of hydrogen bonding and hydrophobic interaction (stacking phenomenon). The hydrogen bonding occurs between the –NH– group of the side chain of the lysine residue in the cyclic octapeptide and the carbonyl group of the ester in the guest, and the hydrophobic interaction arises between the phenyl group of the side chains of the phenylalanine residue in the cyclic octapeptide and the aromatic ring of the side chain of the guest. The schematic formula of Fig. 6 shows the structure visualized for the complex of the D-Phe–OMe salt and the cyclic octapeptide **2**, and presents the five-point binding model as the more stable complex. Probably, when the L-Phe–OMe salt is the guest, the conformation of the cyclic octapeptide **2** can not simultaneously form hydrogen bonds and cause hydrophobic interaction as described above. In the case of the cyclic octapeptides **3** and **4**, five-point interaction is unlikely

to occur due to hindrance of conformation variation (by the neighboring *N*-methyl group of the Phe residue) or to lack of the side chain containing the –NH– group of the Lys(Z) residue. On the other hand, when the guest is Leu–OMe or PEA salt, after the three-point interaction occurs, only one subsequent interaction can occur because Leu–OMe does not have an aromatic ring and PEA does not have the ester carbonyl group. The Phg–OMe salt may participate very loosely in a five-point interaction because the distance between the NH_3^+ group and the aromatic ring is shorter than that in the Phe–OMe salt. The cyclic octapeptides **1** and **2** exhibit enantiomer-selective transport towards the D-Phe–OMe salt but not the L-Phe–OMe salt.

The results of this study suggest that five-point interaction can occur between the cyclic octapeptides (**1** and **2**), having a localized sequence of Lys(Z) and Phe residues and D-Phe–OMe salt.

Experimental

All melting points were measured with a Yanaco MP-S3 apparatus and are uncorrected. Fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-DX-303 spectrometer. For measurement, each sample was dissolved in a matrix of glycerol or *m*-nitrobenzyl alcohol and the solution was bombarded with a beam of neutral Xe atoms at an energy of 3 keV. Optical rotations were determined with a Jasco polarimeter, Model DIP-360. Solvent systems for TLC were (A) chloroform : MeOH : water = 8 : 3 : 1 by vol. and (B) 1-butanol : acetic acid : pyridine : water = 4 : 1 : 1 : 1 by vol.

Preparation of the Cyclic Octapeptides Cyclo(-(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–Sar–Gly–) (**1**): The linear octapeptide **13** (1.20 g, 1.01 mmol), NMM (0.22 ml, 2.02 mmol), and HOBt·H_2O (0.77 g, 5.05 mmol) were dissolved in anhydrous DMF (211 ml). The solution was added dropwise to a mixture of EDCI·HCl (1.94 g, 10.1 mmol), an equimolar mixture of the alkaline metal cations (3.47 g, 10.1 mmol), anhydrous DMF (211 ml) and THF (421 ml) over a 5-h period at room temperature. The reaction mixture was stirred for 10 d at room temperature, then evaporated *in vacuo*. A large amount of AcOEt was added to the residue. The solution was washed with 10% $NaHCO_3$, cold 1 N HCl and water, then dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by preparative TLC on silica gel. The product was recrystallized from DMF–Et_2O; yield, 0.25 g (23.4%); mp 254—256 °C; Rf(A) = 0.60; $[\alpha]_D^{30}$ −29.1° (c = 0.6, DMF). FAB-MS m/z: 1061 $(M+H)^+$, 927 $(M+H-134)^+$. *Anal.* Calcd for $C_{55}H_{68}N_{10}O_{12}\cdot 1/2H_2O$: C, 61.73; H, 6.50; N, 13.09. Found: C, 61.86; H, 6.65; N, 13.00.

Cyclo(-(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–(Sar)$_2$–) (**2**): This cyclic peptide was obtained from the linear octapeptide **20** (1.60 g, 1.33 mmol) as described for the preparation of **1**. The crude product was purified by silica-gel column chromatography (chloroform/MeOH = 95 : 5) and preparative silica-gel TLC. The product was recrystallized from MeOH–AcOEt; yield, 0.38 g (26.7%); mp 214—215 °C; Rf(A) = 0.63; $[\alpha]_D^{30}$ −47.7° (c = 1, DMF). FAB-MS m/z: 1075 $(M+H)^+$, 941 $(M+H-134)^+$. *Anal.* Calcd for $C_{56}H_{70}N_{10}O_{12}\cdot H_2O$: C, 61.52; H, 6.64; N, 12.81. Found: C, 61.40; H, 6.78; N, 12.73.

Cyclo(-(Lys(Z))$_2$–Gly–(Phe)$_2$–(Sar)$_3$–) (**3**): This cyclic peptide was obtained from the linear octapeptide **29** (2.40 g, 1.97 mmol) as described for the preparation of **1**. The crude product was purified by column chromatography on silica gel. The eluate with chloroform/MeOH (95 : 5) afforded **3** (0.90 g, 42.0%); glassy solid (mp 122—124 °C); Rf(A) = 0.67; $[\alpha]_D^{27}$ −13.2° (c = 1, MeOH). FAB-MS m/z: 1089 $(M+H)^+$, 955 $(M+H-134)^+$. *Anal.* Calcd for $C_{57}H_{72}N_{10}O_{12}\cdot 2H_2O$: C, 60.84; H, 6.81; N, 12.45. Found: C, 61.01; H, 6.63; N, 12.16.

Cyclo(-(Glu(OBzl))$_2$–Gly–(Phe)$_2$–(Sar)$_3$–) (**4**): This cyclic peptide was obtained from the linear octapeptide **36** (1.80 g, 1.59 mmol) as described for the preparation of **1**. The crude product was purified by column chromatography on silica gel. Elution with chloroform/MeOH (95 : 5) afforded **4** (0.79 g, 49.7%); glassy solid (mp 114—116 °C); Rf(A) = 0.69; $[\alpha]_D^{27}$ −11.8° (c = 1, MeOH). FAB-MS m/z: 1003 $(M+H)^+$, 913 $(M+H-90)^+$. *Anal.* Calcd for $C_{53}H_{62}N_8O_{12}\cdot H_2O$: C, 62.34; H, 6.31; N, 10.97. Found: C, 62.60; H, 6.24; N, 10.92.

Preparation of the Linear Peptides Boc–Sar–Gly–OEt (**7**): Boc–Sar–OH (10.03 g, 53.0 mmol) was dissolved in anhydrous THF (50 ml), and CDI (8.59 g, 53.0 mmol) was added to this solution under ice-cooling. After the effervescence had ceased, the solution was stirred for 1 h and then H–Gly–OEt·HCl (7.39 g, 53.0 mmol) was added, followed by Et_3N (7.4 ml, 53.0 mmol) in anhydrous THF (40 ml) solution at 0 °C. The reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated off *in vacuo* and AcOEt was added to the residue. This solution was washed with 10% $NaHCO_3$, cold 1 N HCl and water, then dried over $MgSO_4$ and evaporated *in vacuo*. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 11.28 g (77.6%); oil; Rf(A) = 0.81. FAB-MS m/z: 275 $(M+H)^+$, 175 $(M+H-100)^+$.

H–Sar–Gly–OEt·TFA (**8**): TFA (18 ml) was added to a mixture of **7** (8.74 g, 31.9 mmol), CH_2Cl_2 (18 ml), and anisole (4.5 ml) at 0 °C. The reaction mixture was stirred until the periodic TLC inspection indicated that the removal of the Boc group was complete. The solution was evaporated, and the oily residue was crystallized by treatment with Et_2O. The crystals were collected by filtration and washed with Et_2O to give **8** (9.17 g, 99.9%). The product was shown to be homogeneous by TLC on silica gel and used for the next reaction; mp 93—94 °C, Rf(A) = 0.32. FAB-MS m/z: 175 $(M+H-TFA)^+$.

Boc–(Phe)$_2$–Gly–Sar–Gly–OEt (**9**): This peptide was obtained from **6** (4.50 g, 9.58 mmol) and **8** (2.76 g, 9.58 mmol) by the CDI method described for the preparation of **7**, except that **6** was dissolved in DMF instead of THF. Recrystallization of the residue from MeOH–AcOEt gave **9** (4.76 g, 79.4%). mp 191—192 °C, Rf(A) = 0.68, $[\alpha]_D^{26}$ −15.4° (c = 1, DMF). FAB-MS m/z: 626 $(M+H)^+$, 526 $(M+H-100)^+$. *Anal.* Calcd for $C_{32}H_{43}N_5O_8$: C, 61.43; H, 6.93; N, 11.19. Found: C, 61.28; H, 7.09; N, 11.04.

H–(Phe)$_2$–Gly–Sar–Gly–OEt·THF (**10**): The peptide **9** was deblocked with TFA–anisole as described for the preparation of **8**; yield 99.7%; mp 112—115 °C; Rf(A) = 0.65. FAB-MS m/z: 526 $(M+H-TFA)^+$.

Boc–(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–Sar–Gly–OEt (**11**): IBCF (0.1 ml, 0.79 mmol) was added to a solution of **5** (0.55 g, 0.79 mmol) and NMM (0.09 ml, 0.79 mmol) in anhydrous DMF (1 ml) under stirring at −18 ± 2 °C. After 15 min, a precooled solution of **10** (0.51 g, 0.79 mmol) and NMM (0.09 ml, 0.79 mmol) in anhydrous DMF (2.5 ml) was added, and the mixture was stirred for 1 h at −18 ± 2 °C, then overnight at room temperature. After evaporation *in vacuo*, AcOEt was added to the residue. The solution was washed with 10% $NaHCO_3$, cold 1 N HCl and water, then dried over $MgSO_4$ and evaporated *in vacuo*. The product was recrystallized from MeOH–Et_2O; yield, 0.68 g (71.7%); mp 167—168 °C; Rf(A) = 0.67; $[\alpha]_D^{26}$ −17.6° (c = 1, DMF). FAB-MS m/z: 1207 $(M+H)^+$, 1107 $(M+H-100)^+$, 1073 $(M+H-134)^+$. *Anal.* Calcd for $C_{62}H_{82}N_{10}O_{15}\cdot H_2O$: C, 60.77; H, 6.91; N, 11.43. Found: C, 60.56; H, 6.92; N, 11.37.

Boc–(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–Sar–Gly–OH (**12**): A solution of **11** (1.09 g, 0.90 mmol) in MeOH (110 ml) was treated with 1 N NaOH solution (2.7 ml, 2.70 mmol). After stirring for 3 h at 40 °C, the progress of the reaction was followed by TLC. After completion of the reaction, the solution was concentrated to half of its original volume. The solution was acidified with cold 1 N HCl under ice-cooling, and the precipitate was collected by filtration and washed with water. Recrystallization of the precipitate from MeOH–Et_2O gave **12** (1.02 g, 95.8%). mp 176—178 °C, Rf(A) = 0.26, Rf(B) = 0.48. $[\alpha]_D^{26}$ −17.8° (c = 1, DMF). FAB-MS m/z: 1179 $(M+H)^+$, 1079 $(M+H-100)^+$, 1045 $(M+H-134)^+$. *Anal.* Calcd for $C_{60}H_{78}N_{10}O_{15}$: C, 61.11; H, 6.67; N, 11.88. Found: C, 60.86; H, 6.74; N, 11.87.

H–(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–Sar–Gly–OH·TFA (**13**): The peptide **12** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 99.8%, mp 153—156 °C, Rf(A) = 0.28, Rf(B) = 0.40. FAB-MS m/z: 1079 $(M+H-TFA)^+$, 945 $(M+H-TFA-134)^+$.

Boc–(Sar)$_2$–OMe (**14**): The peptide was obtained from Boc–Sar–OH (10.01 g, 52.9 mmol) and H–Sar–OMe·HCl (7.38 g, 52.9 mmol) by the CDI method described for the preparation of **7**. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 13.11 g (90.3%); oil; Rf(A) = 0.77. FAB-MS m/z: 275 $(M+H)^+$, 175 $(M+H-100)^+$.

H–(Sar)$_2$–OMe·TFA (**15**): The peptide **14** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 90.4%; mp 105—106 °C; Rf(A) = 0.30. FAB-MS m/z: 175 $(M+H-TFA)^+$.

Boc–(Phe)$_2$–Gly–(Sar)$_2$–OMe (**16**): This peptide was prepared from **6** (1.00 g, 2.13 mmol) and **15** (0.61 g, 2.13 mmol) by the mixed anhydride method as described for the preparation of **11**. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 1.27 g (95.3%); glassy solid; Rf(A) = 0.72. FAB-MS m/z: 626 $(M+H)^+$, 526 $(M+H-100)^+$.

H–(Phe)$_2$–Gly–(Sar)$_2$–OMe·TFA (**17**): The peptide **16** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 93.2%; mp 101—102 °C (dec.); *Rf*(A) = 0.65. FAB-MS *m/z*: 526 $(M+H-TFA)^+$.

Boc–(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–(Sar)$_2$–OMe (**18**): The peptides **5** (2.18 g, 3.11 mmol) and **17** (1.99 g, 3.11 mmol), NMM (0.75 ml, 6.84 mmol), HOBt·H_2O (0.72 g, 4.67 mmol) and EDCI·HCl (0.72 g, 3.73 mmol) were added to anhydrous DMF (45 ml), and the mixture was stirred overnight at room temperature, then evaporated *in vacuo*. The residue was taken up in AcOEt, and this solution was washed with 10% $NaHCO_3$, cold 1 N HCl and water, then dried over $MgSO_4$ and evaporated *in vacuo*. Recrystallization of the residue from AcOEt–Et_2O gave **18** (3.53 g, 93.9%). mp 145—146 °C, *Rf*(A) = 0.73, $[\alpha]_D^{26}$ −19.0° (*c* = 1, DMF). FAB-MS *m/z*: 1207 $(M+H)^+$, 1107 $(M+H-100)^+$, 1073 $(M+H-134)^+$. *Anal.* Calcd for $C_{62}H_{82}N_{10}O_{15}\cdot 1/2H_2O$: C, 61.22; H, 6.88; N, 11.51. Found: C, 61.40; H, 6.90; N, 11.55.

Boc–(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–(Sar)$_2$–OH (**19**): This peptide was prepared by the same procedure employed for the preparation of **12**. Recrystallization of the precipitate from MeOH–AcOEt–Et_2O gave **19**; yield, 96.6%; mp 115—117 °C; *Rf*(A) = 0.22, *Rf*(B) = 0.42; $[\alpha]_D^{26}$ −18.6° (*c* = 1, DMF). FAB-MS *m/z*: 1193 $(M+H)^+$, 1093 $(M+H-100)^+$, 1059 $(M+H-134)^+$. *Anal.* Calcd for $C_{61}H_{80}N_{10}O_{15}\cdot H_2O$: C, 60.48; H, 6.82; N, 11.56. Found: C, 60.46; H, 6.98; N, 11.34.

H–(Lys(Z))$_2$–Gly–(Phe)$_2$–Gly–(Sar)$_2$–OH·TFA (**20**): The peptide **19** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 99.5%; mp 139—142 °C; *Rf*(A) = 0.21, *Rf*(B) = 0.35. FAB-MS *m/z*: 1093 $(M+H-TFA)^+$, 959 $(M+H-TFA-134)^+$.

Boc–(Sar)$_3$–OMe (**21**): This peptide was prepared from Boc–Sar–OH (8.63 g, 45.6 mmol) and **15** (13.14 g, 45.6 mmol) by the mixed anhydride method as described for the preparation of **11**. The resultant product was shown to be homogeneous by TLC on silica gel and was used for the next reaction; yield, 14.21 g (90.2%); oil; *Rf*(A) = 0.71. FAB-MS *m/z*: 346 $(M+H)^+$, 246 $(M+H-100)^+$.

H–(Sar)$_3$–OMe·TFA (**22**): The peptide **21** was deblocked with TFA–anisole; yield, 85.0%; oil; *Rf*(A) = 0.28. FAB-MS *m/z*: 246 $(M+H-TFA)^+$.

Boc–Phe–(Sar)$_3$–OMe (**23**): This peptide was obtained from Boc–Phe–OH (0.88 g, 3.30 mmol) and **22** (1.19 g, 3.30 mmol) by the CDI method described for the preparation of **7**. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 1.14 g (86.3%); glassy solid; $[\alpha]_D^{27}$ +2.98° (*c* = 1, MeOH); *Rf*(A) = 0.70. FAB-MS *m/z*: 493 $(M+H)^+$, 393 $(M+H-100)^+$.

H–Phe–(Sar)$_3$–OMe·TFA (**24**): The peptide **23** was deblocked with TFA–anisole; yield, 95.9%; oil; *Rf*(A) = 0.50. FAB-MS *m/z*: 393 $(M+H-TFA)^+$.

Boc–(Phe)$_2$–(Sar)$_3$–OMe (**25**): This peptide was obtained from Boc–Phe–OH (1.39 g, 5.24 mmol) and **24** (2.65 g, 5.24 mmol) by the CDI method described for the preparation of **9**. The crude product was purified by column chromatography on silica gel. The eluate with chloroform/MeOH (98 : 2) afforded **25** (3.00 g, 89.5%); glassy solid; *Rf*(A) = 0.68; $[\alpha]_D^{27}$ −18.6° (*c* = 1, MeOH). FAB-MS *m/z*: 640 $(M+H)^+$, 540 $(M+H-100)^+$.

H–(Phe)$_2$–(Sar)$_3$–OMe·TFA (**26**): The peptide **25** was deblocked with TFA–anisole; yield, 96.5%; glassy solid; *Rf*(A) = 0.60. FAB-MS *m/z*: 540 $(M+H-TFA)^+$.

Boc–(Lys(Z))$_2$–Gly–(Phe)$_2$–(Sar)$_3$–OMe (**27**): This peptide was prepared from **5** (0.84 g, 1.20 mmol) and **26** (0.78 g, 1.20 mmol) by the coupling procedure used for **18**. The product was recrystallized from AcOEt–Et_2O; yield, 1.00 g (68.2%); mp 107—108 °C; *Rf*(A) = 0.65; $[\alpha]_D^{27}$ −15.5° (*c* = 1, MeOH). FAB-MS *m/z*: 1221 $(M+H)^+$, 1121 $(M+H-100)^+$, 1087 $(M+H-134)^+$. *Anal.* Calcd for $C_{63}H_{84}N_{10}O_{15}\cdot 1/2H_2O$: C, 61.50; H, 6.96; N, 11.38. Found: C, 61.37; H, 6.99; N, 11.44.

Boc–(Lys(Z))$_2$–Gly–(Phe)$_2$–(Sar)$_3$–OH (**28**): This peptide was prepared by the procedure employed for the preparation of **12**. Recrystallization of the precipitate from AcOEt–PE gave **28** (0.52 g, 90.7%). mp 109—110 °C, *Rf*(A) = 0.30, *Rf*(B) = 0.42. $[\alpha]_D^{27}$ −15.0° (*c* = 0.9, MeOH). FAB-MS *m/z*: 1207 $(M+H)^+$, 1107 $(M+H-100)^+$, 1073 $(M+H-134)^+$. *Anal.* Calcd for $C_{62}H_{82}N_{10}O_{15}\cdot H_2O$: C, 60.77; H, 6.91; N, 11.43. Found: C, 60.73; H, 6.78; N, 11.46.

H–(Lys(Z))$_2$–Gly–(Phe)$_2$–(Sar)$_3$–OH·TFA (**29**): The peptide **28** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 95.7%; mp 132—134 °C; *Rf*(A) = 0.24, *Rf*(B) = 0.35. FAB-MS *m/z*: 1107 $(M+H-TFA)^+$, 973 $(M+H-TFA-134)^+$.

Boc–Gly–(Phe)$_2$–(Sar)$_3$–OMe (**30**): This peptide was prepared from Boc–Gly–OH (0.81 g, 4.62 mmol) and **26** (3.02 g, 4.62 mmol) by the mixed anhydride method as described for the preparation of **11**. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 2.98 g (92.5%); glassy solid; *Rf*(A) = 0.71. FAB-MS *m/z*: 697 $(M+H)^+$, 597 $(M+H-100)^+$.

Boc–Gly–(Phe)$_2$–(Sar)$_3$–OH (**31**): A solution of **30** (2.89 g, 4.15 mmol) in MeOH (29 ml) was treated with 1 N NaOH solution (6.23 ml, 6.23 mmol). After stirring for 3 h at 40 °C, the progress of the reaction was followed by TLC. After completion of the reaction, the solution was diluted with 25 ml of water, and the MeOH was evaporated off *in vacuo*. The remaining aqueous solution was extracted with AcOEt, and the aqueous layer was acidified with cold 1 N HCl under ice-cooling and extracted with AcOEt. The organic layer was washed with water, then dried over $MgSO_4$ and evaporated *in vacuo*. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 2.67 g (94.3%); glassy solid; $[\alpha]_D^{27}$ −12.4° (*c* = 1, MeOH); *Rf*(A) = 0.14, *Rf*(B) = 0.32. FAB-MS *m/z*: 683 $(M+H)^+$, 583 $(M+H-100)^+$.

H–Gly–(Phe)$_2$–(Sar)$_3$–OH·TFA (**32**): The peptide **31** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 99.5%; mp 128—131 °C; *Rf*(A) = 0.13, *Rf*(B) = 0.16. FAB-MS *m/z*: 583 $(M+H-TFA)^+$.

Boc–Glu(OBzl)–Gly–(Phe)$_2$–(Sar)$_3$–OH (**33**): IBCF (1.17 ml, 8.89 mmol) was added to a solution of Boc–Glu(OBzl)–OH (3.00 g, 8.89 mmol) and NMM (0.98 ml, 8.89 mmol) in anhydrous DMF (30 ml) under stirring at −18 ± 2 °C. After 15 min, a precooled solution of **32** (6.19 g, 8.89 mmol) and NMM (1.96 ml, 17.8 mmol) in anhydrous DMF (30 ml) was added, and the mixture was stirred for 1 h at −18 ± 2 °C, then overnight at room temperature. After evaporation *in vacuo*, 10% $NaHCO_3$ was added to the residue, and the solution was extracted with AcOEt. The aqueous layer was acidified with cold 1 N HCl under ice-cooling and extracted with AcOEt. The organic layer was washed with water, then dried over $MgSO_4$ and evaporated *in vacuo*. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 6.82 g (85.0%); glassy solid; $[\alpha]_D^{27}$ −13.8° (*c* = 1, MeOH); *Rf*(A) = 0.19, *Rf*(B) = 0.40. FAB-MS *m/z*: 902 $(M+H)^+$, 802 $(M+H-100)^+$.

H–Glu(OBzl)–Gly–(Phe)$_2$–(Sar)$_3$–OH·TFA (**34**): The peptide **33** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 96.8%; mp 120—122 °C; *Rf*(A) = 0.19, *Rf*(B) = 0.26. FAB-MS *m/z*: 802 $(M+H-TFA)^+$.

Boc–(Glu(OBzl))$_2$–Gly–(Phe)$_2$–(Sar)$_3$–OH (**35**): This peptide was prepared from Boc–Glu(OBzl)–OH (0.76 g, 2.24 mmol) and **34** (2.05 g, 2.24 mmol) by the procedure employed for the preparation of **33**. The resultant product was shown to be homogeneous by TLC on silica gel, and was used for the next reaction; yield, 2.07 g (82.0%); glassy solid; $[\alpha]_D^{27}$ −15.5° (*c* = 1, MeOH); *Rf*(A) = 0.26, *Rf*(B) = 0.42. FAB-MS *m/z*: 1121 $(M+H)^+$, 1021 $(M+H-100)^+$.

H–(Glu(OBzl))$_2$–Gly–(Phe)$_2$–(Sar)$_3$–OH·TFA (**36**): The peptide **35** was deblocked with TFA–anisole as described for the preparation of **8**; yield, 99.3%; mp 119—122 °C; *Rf*(A) = 0.30, *Rf*(B) = 0.35. FAB-MS *m/z*: 1021 $(M+H-TFA)^+$.

Transport of Organic Ammonium Cation As reported,[3] the transport experiments were performed with a double cylindrical apparatus. The organic phase contained 0.25 mM cyclic octapeptide in 10 ml of chloroform. The outer source phase contained 0.01 M amino acid methyl ester or amine hydrochloride and 0.02 M $LiPF_6$ in 2.5 ml of 0.08 M HCl and the inner receiving phase contained 2.5 ml of 0.1 M HCl. The organic phase was stirred at 400 rpm at 25 °C. After 24 h, a 1-ml aliquot of the solution in the receiving phase was harvested and lyophilized. The amounts of the organic ammonium cation were determined by GC analyses using a Shimadzu GC-14A apparatus with a column of 2% cyclohexanedimethanol succinate on Gas Chrom Q and a hydrogen flame ionization detector.

Transport of Racemic Phe–OMe·HCl The differential transport of enantiomers was examined in a manner similar to that described above. The chloroform phase contained 0.25 mM cyclic octapeptide, and the source phase contained 0.02 mM racemic Phe–OMe·HCl and 0.2 mM $LiPF_6$. After 24 h, an aliquot of the receiving phase (0.5 ml) was taken and lyophilized. The ee values of the cyclic octapeptides were determined by HPLC analyses using a chiral column, Crownpak CR(+) (Daicel Chemical Industries, Ltd.).

References and Notes

1) The amino acid residues mentioned in the experimental section with no prefix have the L-configuration. The following abbreviations are used: Sar = sarcosine, Phg = phenylglycine, PEA = α-phenylethylamine, Boc = *tert*-butoxycarbonyl, Z = benzyloxycarbonyl, CDI = *N*,*N*′-carbonyldiimidazole, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = 1-hydroxybenzotriazole, IBCF = isobutyl-

chloroformate, NMM = *N*-methylmorpholine, Et_3N = triethylamine, TFA = trifluoroacetic acid, THF = tetrahydrofuran, DMF = dimethylformamide, AcOEt = ethyl acetate, MeOH = methanol, Et_2O = diethyl ether, PE = petroleum ether.

2) D. S. Lingenfelter, R. C. Helgeson, and D. J. Cram, *J. Org. Chem.*, **46**, 393 (1981), and references cited therein.
3) T. Katagi and H. Kataoka, *Heterocycles*, **26**, 2109 (1987).
4) H. Kataoka and T. Katagi, *Tetrahedron*, **43**, 4519 (1987).
5) H. Kataoka, T. Katagi, H. Yajima, and A. Otaka, *Chem. Pharm. Bull.*, **36**, 3196 (1988).
6) H. Kataoka and T. Katagi, *Chem. Pharm. Bull.*, **36**, 3199 (1988).
7) H. Kataoka and T. Katagi, *Chem. Pharm. Bull.*, **37**, 3166 (1989).