SYNTHESIS OF A CYCLIC OCTAPEPTIDE

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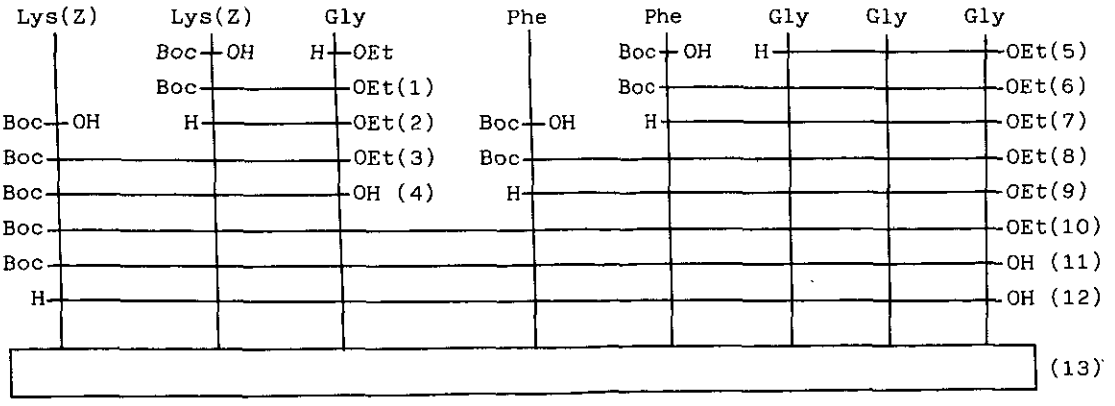
Abstract - The cyclic octapeptide cyclo-((L-Lys(Z))$_2$-Gly-(L-Phe)$_2$-(Gly)$_3$) (13) was synthesized as an ionophore model. And transport of L-Phe-OMe·HCl through an organic liquid membrane mediated by (13) is reported.

Many natural biologically active cyclic peptides or depsipeptides, such as valinomycin[1], gratisin[2], gramicidin S[3], and amidomycin[4] have been known to possess repeated amino acid sequences in their molecule, and some of these peptides were reported to form lipophilic complexes with cations. In order to examine the selectivity of macrocycles towards metal cations or ammonium cations, we have previously prepared some macrocycles containing diglycolic acid, L-phenylalanine and L-leucine as constituents.[5,6] In the present study, which will be valuable for we report the synthesis of novel cyclic octapeptide (13) containing two lysine and two phenylalanine residues, the study on the binding with substituted ammonium cations.



The scheme which we employed for preparing cyclic octapeptide (13) is summarized in the flow chart. Peptides (1), (3), (6), (8), and (10) were prepared using N, N'- carbonyldiimidazole as condensation agent. Peptides (3) and (10) were hydrolyzed with 1 N NaOH in methanol, giving (4) and (11). Compound (5)·HCl was obtained by esterification of H-$(Gly)_3$-OH.[7] On the other hand, deprotected peptides (2)·HCl, (7)·HCl, and (9)·HCl were obtained by treatment of the corresponding protected peptides (1), (6), and (8) with dry hydrogen chloride in anhydrous ethyl acetate. The Boc group was removed from (11) using trifluoroacetic acid containing anisole to obtain (12)·TFA (white crystals, quantitative yield). The cyclization of (12) was carried out using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole monohydrate. The crude product thus obtained was purified by column chromatography on silica gel using chloroform/methanol (95:5) as eluent to obtain (13). The structures of all the synthetic peptides were supported by their MS spectra (EI or FAB). FAB-MS spectra of Boc or Z-protected peptide showed a strong (MH^+-100) ion and (MH^+-134) ion, respectively, together with the (MH^+) ion.

Transport The transport of L-Phe-OMe·HCl through a liquid membrane was examined using synthetic cyclic octapeptide (13) as carrier in an apparatus

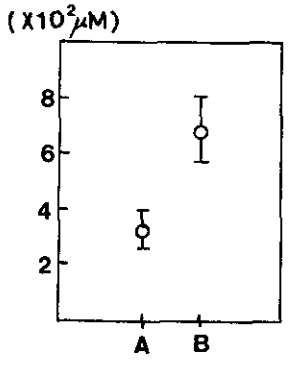


Figure 1
Graphical representation of transport of L-Phe-OMe·HCl through a liquid membrane (μM).
A = cyclic octapeptide (13), B = 18-crown-6.

described in literture.[8] The mixture of a L-Phe-OMe·HCl and $LiPF_6$ in 0.08 M HCl aqueous solution was in the source phase, and the ammonium cation transfer into chloroform by complexation with a cyclic octapeptide, and is released to the receiving phase containing 0.1 M HCl aqueous solution. The concentration of a L-Phe-OMe·HCl in the receiving phase was determined by gas-liquid

chromatographic analysis of N-trifluoroacethyl ester derivative. The transfer ability of cyclic octapeptide (13) was compared with that of 18-crown-6, and the transported value of (13) and 18-crown-6 was 308 μM and 695 μM, respectively. (Figure 1). The net value of transport was the average of differences of apparent values from blank test values in several runs. Thus transfer ability of (13) was indicated about one half of 18-crown-6.

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 Nihondenshi mass spectrometer Model JMS DX-303, computer system JMS DA-5000 (MS^1). Fast atom bombardment (FAB) mass spectra were recorded on a Nihondenshi mass spectrometer Model JMS DX-303, JMS FAB-09, computer system JMS DA-5000 (MS^2). 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. Optical rotations were determined with a Jasco polarimeter Model DIP-360. Solvent systems for thin-layer chromatography (t.l.c.) were (A) chloroform: metanol:water = 8:3:1 by vol. and (B) 1-butanol:acetic acid:pyridine:water = 4:1:1:1 by vol.

H-L-Lys(Z)-Gly-OEt·HCl (2·HCl)[9]

To Boc L-Lys(Z)-Gly-OEt (1)[9,10] (5.07 g, 0.01 mol), 2.6 N dry hydrogen chloride in anhydrous ethyl acetate (42 ml, 0.1 mol) was added at 0°C, and the mixture was stirred for 50 min, at 0°C. The ethyl acetate was evaporated in vacuo. Recrystallization of the residue from ethanol-diethyl ether gave the pure compound (2·HCl) (4.22 g, 96.4%), mp 160.5-161.5°C, Rf(A) = 0.59, $[\alpha]_D^{23}$ +14.0° (C 1, methanol). MS m/z: 365 (M^+-HCl). Anal. Calcd for $C_{18}H_{28}N_3O_5Cl$: C, 53.80; H, 7.02; N, 10.46. Found: C, 53.66; H, 7.12; N, 10.48.

Boc-(L-Lys(Z))$_2$-Gly-OEt (3)

To a solution of Boc-L-Lys(Z)-OH (5.06 g, 0.013 mol) in anhydrous tetrahydrofuran (100 ml), N, N'-carbonyldiimidazole (2.18 g, 0.013 mol) was added under ice-cooling. After the effervescence had ceased, the solution was stirred for 1 h and then (2·HCl) (5.30 g, 0.013 mol) was added, followed by

triethylamine (1.83 ml, 0.013 mol) at 0°C. The reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo and then ethyl acetate was added to the residue. This solution was successively washed with 10% citric acid, 10% sodium bicarbonate and brine, and then dried over anhydrous magnesium sulfate, the solvent was evaporated in vacuo. Recrystallization of the residue from ethyl acetate-petroleum ether gave (3) (6.92 g, 71.5%), mp 144.0-146.0°C, Rf(A) = 0.78, $[\alpha]_D^{22}$ +20.3° (C 1, methanol). MS^2 m/z: 728 (MH^+), 628 (MH^+-100), 594 (MH^+-134). Anal. Calcd for $C_{37}H_{53}N_5O_{10}$: C, 61.06; H, 7.34; N, 9.62. Found: C, 60.89; H, 7.55; N, 9.55.

Boc-(L-Lys(Z))$_2$-Gly-OH (4)

To the solution of (3) (1.93 g, 2.7 mmol) in methanol (30 ml), 1 N NaOH solution (4.1 ml, 4.1 mmol) was added, and the mixture was stirred for 6 h at 40°C. The solution was diluted with 30 ml of water, and the methanol was evaporated in vacuo. The remaining aqueous solution was extracted with ethyl acetate, and the aqueous layer was acidified with 10% citric acid and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate, and the solvent evaporated in vacuo. Recrystallization of the residue from ethyl acetate-petroleum ether gave (4) (1.68 g, 90.5%), mp 99.0-101.0°C, Rf(A) = 0.26, $[\alpha]_D^{22}$ +19.4° (C 1, methanol). MS^2 m/z: 700 (MH^+), 600 (MH^+-100), 566 (MH^+-134). Anal. Calcd for $C_{35}H_{49}N_5O_{10}$: C, 60.07; H, 7.06; N, 10.01. Found: C, 60.07; H, 7.14; N, 10.17.

H-(Gly)$_3$-OEt·HCl (5·HCl)[11]

To H-(Gly)$_3$-OH[7] (25.19 g, 0.13 mol), anhydrous ethanol was added, and the suspension was saturated with dry hydrogen chloride at 0°C. The reaction mixture was allowed to stand for 3 days, then the solvent was evaporated in vacuo. Recrystallization of the residue from methanol gave (5·HCl) (17.86 g, 52.9%), mp 217°C (dec.), R_f(B) = 0.41. MS m/z: 218 (M^++1-HCl), 217 (M^+-HCl). Anal. Calcd for $C_8H_{16}N_3O_4Cl$: C, 37.88; H, 6.36; N, 16.56. Found: C, 37.71; H, 6.40; N, 16.41.

Boc-L-Phe-(Gly)$_3$-OEt (6)

This was prepared by the same procedure employed for the preparation of (2).

Recrystallization of the residue from ethyl acetate gave (6) (58.7%), mp 148.0-148.5°C, Rf(A) = 0.64, $[\alpha]_D^{22}$ +7.1° (C 1, methanol). MS m/z: 465 (M^++1), 464 (M^+). Anal. Calcd for $C_{22}H_{32}N_4O_7$: C, 56.89; H, 6.94; N, 12.06. Found: C, 56.96; H, 7.09; N, 11.96.

H-L-Phe-$(Gly)_3$-OEt·HCl (7·HCl)

To (6) (6.22 g, 1.3 mmol) was added a solution of saturated dry hydrogen chloride in anhydrous ethyl acetate (75 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 methanol-diethyl ether gave (7·HCl) (5.12 g, 95.4%), mp 199.0-200.0°C, Rf(A) = 0.47, $[\alpha]_D^{22}$ +40.0° (C 1, methanol). MS m/z: 365 (M^++1-HCl), 364 (M^+-HCl). Anal. Calcd for $C_{17}H_{25}N_4O_5Cl$: C, 50.94; H, 6.29; N, 13.98. Found: C, 50.70; H, 6.17; N, 13.92.

Boc-$(L\text{-}Phe)_2$-$(Gly)_3$-OEt (8)

A solution of Boc-L-Phe-OH (5.05 g, 1.1 mmol) in an anhydrous mixture of tetrahydrofuran (20 ml) and N, N'-dimethylformamide (15 ml) was treated as described for (1). Recrystallization of the residue from ethyl acetate-diethyl ether gave (8) (10.35 g, 88.9%), mp 184.0-185.0°C, Rf(A) = 0.56, $[\alpha]_D^{24}$ -12.8° (C 1, dimethylformamide). MS[1] m/z: 611 (M^+). Anal. Calcd for $C_{31}H_{41}N_5O_8$: C, 60.87; H, 6.76; N, 11.45. Found: C, 60.49; H, 6.89; N, 11.38.

H-$(L\text{-}Phe)_2$-$(Gly)_3$-OEt·HCl (9·HCl)

This was prepared by the same procedure employed for the preparation of (7·HCl). Recrystallization of the residue from methanol-diethyl ether gave (9·HCl) (98.9%), mp 232.0-234.0°C (dec.), Rf(A) = 0.58, $[\alpha]_D^{24}$ -39.5° (C 1, dimethylformamide). MS m/z: 511 (M^+-HCl). Anal. Calcd for $C_{26}H_{34}N_5O_6Cl \cdot 1/2H_2O$: C, 56.06; H, 6.33; N, 12.57. Found: C, 56.12; H, 6.37; N, 12.63.

Boc-$(L\text{-}Lys(Z))_2$-Gly-$(L\text{-}Phe)_2$-$(Gly)_3$-OEt (10)

N, N'-Carbonyldiimidazole (0.49 g, 3.0 mmol) was added to a solution of (4) (2.10 g, 3.0mmol) in anhydrous tetrahydrofuran (20 ml) and N, N'-dimethylformamide (15 ml) under ice-cooling. After the effervescence had ceased, the solution was stirred for 1 h. Next, to this mixture (9·HCl)

(1.65 g, 3.0 mmol) was added, followed by triethylamine (0.4 ml) at 0°C. The reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo, and a small amount of ethyl acetate, diethyl ether and 10% citric acid was added to the residue. The solution was filtered, and the precipitate was washed with 10% citric acid, 10% sodium bicarbonate and water. Recrystallization of the precipitate from methanol-water gave (10) (2.40 g, 67.0%), mp 182.0-184.0°C, Rf(A) = 0.60, $[\alpha]_D^{24}$ -19.3° (C 1, dimethylformamide). MS^2 m/z: 1193 (MH^+), 1093 (MH^+-100), 1059 (MH^+-134). Anal. Calcd for $C_{61}H_{80}N_{10}O_{15}\cdot 1/2H_2O$: C, 60.94; H, 6.79; N, 11.65. Found: C, 60.95; H, 6.67; N, 11.82.

Boc-(L-Lys(Z))$_2$-Gly-(L-Phe)$_2$-(Gly)$_3$-OH (11)

To a solution of (10) (1.61 g, 1.4 mmol) in methanol (30 ml) was added 1 N NaOH solution (2.7 ml, 2.7 mmol), followed by stirring for 6 h at 45°C. The solution was concentrated to one half of its original volume. The solution was acidified with 10% citric acid and the precipitate was collected by filtration and washed with water. Recrystallization of the precipitate from methanol-diethyl ether gave (11) (1.52 g, 96.7%), mp 155.0-156.0°C, Rf(A) = 0.17, $[\alpha]_D^{24}$ -16.6° (C 1, dimethylformamide). MS^2 m/z: 1165 (MH^+), 1065 (MH^+-100), 1031 (MH^+-134). Anal. Calcd for $C_{59}H_{76}N_{10}O_{15}\cdot 1/2H_2O$: C, 60.35; H, 6.61; N, 11.93. Found: C, 60.39; H, 6.86; N, 11.79.

H-(L-Lys(Z))$_2$-Gly-(L-Phe)$_2$-(Gly)$_3$-OH·TFA (12·TFA)

The Boc group of the above acid (11) (0.49 g, 0.4 mmol) was removed by exposure to trifluoroacetic acid (1.0 ml, 13.5 mmol) containing anisole (0.25 ml, 2.3 mmol) at 0°C for 1 h. The trifuroroacetic acid was evaporated at 0°C. The oily residue was crystallized by treatment with anhydrous diethyl ether and the crystals were collected by filtration and washed with anhydrous diethyl ether to give (12·TFA) (0.50 g, an almost quantitative yield), mp 204.0-207.0°C (dec.), Rf(A) = 0.13, $[\alpha]_D^{24}$ -7.5° (C 1, dimethylformamide). MS^2 m/z: 1065 (MH^+-TFA), 931 (MH^+-TFA-134). The crude material was used for the next step.

Cyclo-((L-Lys(Z))$_2$-Gly-(L-Phe)$_2$-(Gly)$_3$) (13)

Compound (12·TFA) (0.45 g, 0.38 mmol), N-methylmorpholine (0.08 g, 0.76 mmol)

and 1-hydroxybenzotriazole monohydrate (0.58 g, 3.8 mmol) were dissolved in anhydrous tetrahydrofuran (200 ml) and N, N'-dimethylformamide (200 ml). The solution was added to 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.73 g, 3.8 mmol) in a mixture of anhydrous tetrahydrofuran (200 ml) and N, N'-dimethylformamide (100 ml) over a 5 h period at 0°C. The reaction mixture was stirred for 10 days at room temperature. The solvent was evaporated in vacuo, and the residue was treated with a small amount of ethyl acetate, diethyl ether and 10% citric acid. The insoluble matter was collected by filtration and washed with 10% citric acid, 10% sodium bicarbonate and water. The crude product was purified by column chromatography on silica gel. Elution with chloroform/methanol (95:5) afforded cyclic octapeptide (13). This material was recrystallized from methanol-diethyl ether, yielding (13) (0.20 g, 50.0%), mp 250.0-254.0°C, Rf(A) = 0.71, $[\alpha]_D^{24}$ -39.0° (C 1, dimethylformamide). MS^2 m/z: 1047 (MH^+), 913 (MH^+-134). Anal. Calcd for $C_{54}H_{66}N_{10}O_{12}\cdot 3/2H_2O$: C, 60.38; H, 6.47; N, 13.04. Found: C, 60.29; H, 6.39; N, 12.98.

Transport of L-Phe-OMe·HCl

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 0.02 M L-Phe-OMe·HCl and 0.04 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 0.01 mM carrier in 20 ml of $CHCl_3$ and was stirred at 600 r.p.m. by a magnetic stirrer at 37-38°C. After 24 h, a 0.5 ml sample of the receiving phase was withdrawn, and the solution was lyophilization. To a solution of the residue in trifluoroacetic acid (0.2 ml) was added trifluoroacetic anhydride (0.5 ml) at room temperature and the solution was permitted to stand for 1.5 h and evaporated in vacuo at about 15°C. The oily residue was dissolved in ethyl acetate (1 ml), and concentration of TFA-L-Phe-OMe was determined by gas liquid chromatographical analyses using Shimazu GC-7AG (hydrogen flame ionization detecter) with a column (L = 1.6 m, I.D. = 3 mm) of 2% cyclohexanedimethanol succinate on Gas Chrom Q (80-100 mesh), carrier gas; N_2, flow rate; 50 ml/min, oven temperature; 160°C. Quantitative analysis was performed by using n-docosane as an internal standard (I.S.).

Retention time (min); TFA-L-Phe-OMe = 7.09, I.S. = 9.59. That quantitative value was calculated by the following regression equation:

$$y = 0.558443x - 0.00207043$$

y = area ratio (sample/I.S.)

x = weight ratio (sample/I.S.)

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