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# Synthesis of Basic Tripeptide Derivatives with Phage-inactivating Activity

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**Summary**

A new type of basic tripeptide derivatives, Boc-Lys-Xxx-Lys-OEt (Xxx=Leu, Pro, Trp, Phe, Gly and Ser), were synthesized by the conventional solution method. Five derivatives (Xxx=Leu, Pro, Phe, Gly and Ser) were synthesized by step-wise method started from C-terminal. Condensation was performed by DCC-HOBt method, and the reaction gave protected compounds. Synthesis of Trp-containing derivatives was carried out without strong acid treatment. The removal of Z-group from the protected compounds gave the final products. Purity of the final products were assessed by thin layer chromatography, paper chromatography, electrophoresis and high performance liquid chromatography. They exhibited phage-inactivating activity.

Key words: tripeptide, phage, synthesis

## Introduction

We have so far studied the effects of nutritional substances on phages in relation to the control of phage in the fermentation industries[1-9].

As to amino acids, the following results were obtained: (i) Cysteine exerts an inactivating effect on phages[10, 11]. (ii) Basic amino acids, lysine, arginine, histidine and ornithine, exert inactivating effects on phages[12, 13]. (iii) In lysine, the basic character of $\varepsilon$-amino group plays an important role in the phage-inactivating effect[14]. (iv) Lysine derivatives with two amino groups exert no phage-inactivating effect, indicating the importance of the distance between two amino groups[14].

We therefore designed a new series of bifunctional lysine derivatives linked with dicarboxylic acid [EtO-Lys-CO-$(CH_2)_n$-CO-Lys-OEt][15, 16] and examined for phage-inactivating effect to know the influence of elongation of the methylene chain between two $\varepsilon$-amino groups. The results showed that the longer the methylene chain, the greater the phage-inactivating effect becames[17, 18].

In order to obtain further information on the phage-inactivating and other biological

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Symbols and abbreviations used follow the IUPAC-IUB recommendations; other abbreviations used are as follows: $Et_3N$, triethylamine; DCC, dicyclohexylcarbodi-imide; HOBt, 1-hydroxybenzotriazole; DMF, dimethylformamide; NMM, N-methylmorpholine; EtOAc, ethyl acetate.

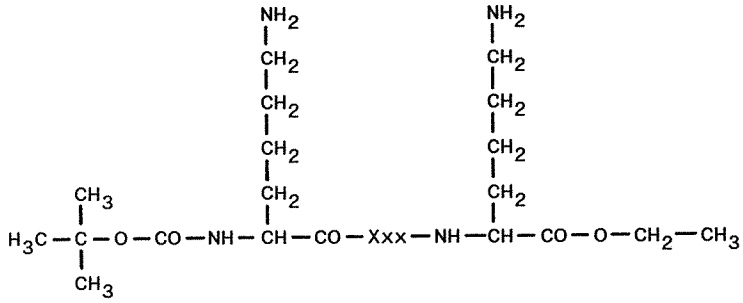

Fig. 1. Structure of Basic Tripeptide Derivatives.
(Xxx=Leu, Pro, Trp, Phe, Gly and Ser)

effects of the basic character of ε-amino group, we designed a new type of basic tripeptide derivatives which are inserted a neutral amino acid between two lysines (Fig. 1). t-Butyloxycarbonyl group in N-terminal and ethyl ester in C-terminal were necessary to maintain the bifunctional nature of basic tripeptide derivatives. The neutral amino acids inserted were selected due to their side chains: leucine and proline with aliphatic side chains, tryptophan and phenylalanine with aromatic side chains, serine with hydrophilic side chain and glycine with no side chain.

We have been found that the synthesized basic tripeptide derivatives have phage-inactivating activity. Little has been reported as to the effects on phages of tripeptides, oligopeptides and their derivatives.

In this paper, as a part I of series of studies, we describe the synthesis of six basic tripeptide derivatives.

## Materials and Methods

**Analytical methods** Thin-layer chromatography was carried out on silica gel (Merck 60 $GF_{254}$) with the following solvent systems: (1) $CHCl_3$-MeOH (5:1, v/v), (2) $CHCl_3$-MeOH (9:1), (3) $CHCl_3$-MeOH-AcOH (8:1:1) and (4) n-BuOH-AcOH-pyridine-$H_2O$ (4:1:1:2). Paper chromatography was performed on Toyo Roshi No. 50 paper with the solvent system, n-BuOH-AcOH-$H_2O$ (4:1:2). Electrophoresis was carried out using Toyo Roshi No. 51A paper with the solvent system, HCOOH-AcOH-MeOH-$H_2O$ (1:3:6:10, v/v, pH 1.8) at 500V/40cm for 3 hr. High performance liquid chromatography was carried out on a Merck Lichrospher RP-18(e) ODS column (0.4x12.5cm) at a flow rate of 1ml/min by a linear gradient of 5-95% $CH_3CN$ in 0.05% TFA over 60 min.

**Synthesis of Intermediate dipeptide derivatives**

**Boc-Leu-Lys-OEt (1)** To a solution of H-Lys(Z)-OEt•pTosOH (1.92g, 4.0mmol) in DMF (8ml) were added NMM (0.44ml, 4.0mmol), Boc-Leu-OH (0.93g, 4.0mmol), HOBt (0.74g, 4.8mmol) and DCC (0.83g, 4.0mmol) at 0°C. The mixture was stirred for 4 hr at 0°C and for 12 hr at room temperature. Insoluble material was filtered off and the filtrate was evaporated. The residue was dissolved in EtOAc and the solution was washed with $H_2O$, 10% citric acid and 4% $NaHCO_3$, successively. The organic phase was dried

($Na_2SO_4$), filtered, and evaporated to dryness. The residue was recrystallized from EtOAc-ether.

**Boc-Pro-Lys(Z)-OEt (2)** This was prepared from Boc-Pro-OH (0.86g, 4.0mmol) and H-Lys(Z)-OEt•pTosOH (1.92g, 4.0mmol) in the same manner as described for **1**.

**Boc-Lys(Z)-Trp-OEt (3)** This was prepared from Boc-Lys(Z)-OH (1.52g, 4.0mmol) and H-Trp-OEt (0.93g, 4.0mmol) as described for **1**.

**Boc-Phe-Lys(Z)-OEt (4)** This was prepared from Boc-Phe-OH (1.06g, 4.0mmol) and H-Lys(Z)-OEt•pTosOH (1.92g, 4.0mmol) as described for **1**. The product was recrystallized from EtOAc-ether.

**Boc-Gly-Lys(Z)-OEt (5)** This was prepared from H-Lys(Z)-OEt•pTosOH (1.92g, 4.0mmol) and Boc-Gly-OH (0.70g, 4.0mmol) as described for **1**.

**Boc-Ser-Lys(Z)-OEt (6)** This was prepared from Boc-Ser-OH (821mg, 4.0mmol) and H-Lys(Z)-OEt•pTosOH (1.92g, 4.0mmol) as described for **1**.

**Synthesis of intermediate tripeptide derivatives**

**Boc-Lys(Z)-Leu-Lys(Z)-OEt (7)** Compound (**1**) (1.17g, 2.24mmol) was dissolved in 4N HCl/dioxane (1.1ml) and formic acid (12ml) at 0°C. After being left to stand for 2 hr at 0°C, the solution was evaporated to dryness. The foamy residue obtained was used without further purification. To the solution of Boc-Lys(Z)-OH (851mg, 2.24mmol) and the foamy residue of H-Leu-Lys(Z)-OEt•HCl (1.02g, 2.24mmol) in DMF (10ml) were added NMM (0.25ml, 2.24mmol), HOBt (413mg, 2.69mmol) and DCC (462mg, 2.24mmol) at 0°C. The reaction mixture was stirred for 4 hr at 0°C and for 24 hr at room temperature. This solution was worked up as described for **1**.

**Boc-Lys(Z)-Pro-Lys(Z)-OEt (8)** This was prepared from Boc-Lys(Z)-OH (1.33g, 3.50mmol) and **2** (1.77g, 3.50mmol) as described for **7**.

**Boc-Lys(Z)-Trp-Lys(Z)-OEt (9)** Hydrazine hydrate (2.2ml) was added to a solution of **3** (1.31g, 2.20mmol) in methanol (12ml) and the reaction mixture was kept for 24 hr at room temperature. The solvent was removed *in vacuo*. The residue was solidified on the addition of water. The dipeptide hydrazide was collected by filtration and washed with water. The solidified peptide hydrazide was dried over $P_2O_5$. The product was recrystallized from MeOH-petroleum ether. A solution of Boc-Lys(Z)-Trp-$N_2H_3$ (1.02g, 1.76mmol) in DMF (30ml) was cooled at −20°C. To this solution, 4N HCl/dioxane (0.88ml, 3.52mmol) and isopentyl nitrite (0.27ml) were added. After 10 min, the solution was neutralized with $Et_3N$ (0.49ml). To this solution were added a chilled solution of H-Lys(Z)-OEt•pTosOH (846mg, 1.76mmol) and $Et_3N$ (0.25ml, 1.76mmol) in DMF (30ml). The reaction mixture was stirred for 3 days at 0°C and was evaporated *in vacuo*. The residual oil was treated as described for **1**. The product was recrystallized from EtOAc-ether-petroleum ether.

**Boc-Lys(Z)-Phe-Lys(Z)-OEt (10)** This was prepared from Boc-Lys(Z)-OH (974mg, 2.56mmol) and **4** (1.42g, 2.56mmol) as described for **7**. The product was recrystallized from EtOAc-ether-petroleum ether.

**Boc-Lys(Z)-Gly-Lys(Z)-OEt (11)** This was prepared from Boc-Lys(Z)-OH (1.16g, 3.05mmol) and **5** (1.42g, 3.05mmol) as described for **7**. The product was recrystallized from

acetone-ether.

**Boc-Lys(Z)-Ser-Lys(Z)-OEt (12)** This was prepared from Boc-Lys(Z)-OH (1.11g, 2.93mmol) and **6** (1.45g, 2.93mmol) as described for **7**. The product was recrystallized from EtOAc-ether-petroleum ether.

**Deprotection for final tripeptide derivatives**

**Boc-Lys-Leu-Lys-OEt (13)** Compound (**7**) (236mg, 0.30mmol) was hydrogenated in a mixed solution of EtOH (4ml), AcOH (0.8ml) and $H_2O$ (0.4ml) in the presence of palladium black for 2 hr. The solution was separated from the catalyst by filtration. The filtrate was evaporated to dryness. The product was yielded as a hygroscopic powder.

**Boc-Lys-Pro-Lys-OEt (14)** This was prepared from **8** (154mg, 0.20mmol) by hydrogenolysis as described for **13**. The product was obtained as an oil.

**Boc-Lys-Trp-Lys-OEt (15)** This was prepared from **9** (206mg, 0.24mmol) by hydrogenolysis as described for **13**. The product was obtained as a foam.

**Boc-Lys-Phe-Lys-OEt (16)** This was prepared from **10** (176mg, 0.22mmol) by hydrogenolysis as described for **13**. The product was obtained as hygroscopic powder.

**Boc-Lys-Gly-Lys-OEt (17)** This was prepared from **11** (152mg, 0.21mmol) by hydrogenolysis as described for **13**. The product was obtained as a foam.

**Boc-Lys-Ser-Lys-OEt (18)** This was prepared from **12** (151mg, 0.20mmol) by hydrogenolysis as described for **13**. The product was obtained as a foam.

## Results and Discussion

Synthesis of the basic tripeptide derivatives was carried out by the conventional solution method. Five derivatives containing Leu, Pro, Phe, Gly and Ser were synthesized by stepwise method started from C-terminal (Fig. 2).

Condensation of ε-benzyloxycarbonyl(Z)-lysine ethyl ester (OEt) with N-t-butyloxycarbonyl(Boc)-Xxx-OH was performed by dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (DCC-HOBt) method, and the reaction gave protected dipeptide ester, Boc-Xxx-Lys(Z)-OEt. The protected dipeptide ester was treated with hydrogen chloride in formic acid and the deprotected peptide, H-Xxx-Lys(Z)-OEt, was subsequently condensed with Boc-Lys(Z)-OH by DCC-HOBt method to yield the protected tripeptide ester, Boc-Lys(Z)-Xxx-Lys(Z)-OEt.

Synthesis of Trp-containing peptide was carried out without strong acid treatment to prevent the probable side reactions of indole ring in Trp (Fig. 3). Intermediate dipeptide ester

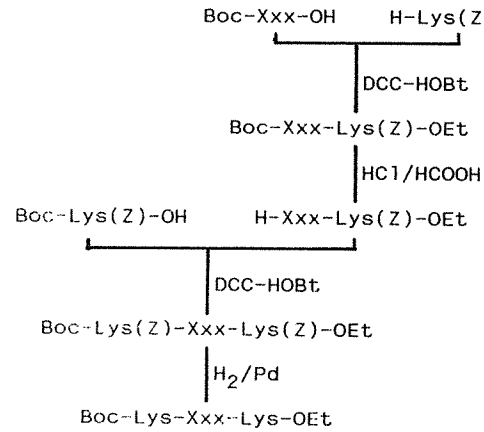


Fig. 2. Synthesis of Boc-Lys-Xxx-Lys-OEt. (Xxx=Leu, Pro, Phe, Gly and Ser)

(**3**) was synthesized by DCC- HOBt method. The C-terminal ethyl ester of **3** was converted into a hydrazide. The reaction of the azide derived from Boc-Lys(Z)-Trp-$N_2H_3$ with H-Lys(Z)-OEt gave the protected tripeptide ester (**9**) in a good yield.

The selective removal of the Z-group from protected compounds by catalytic hydrogenation afforded the desired products, Boc- Lys- Xxx- Lys- OEt. The final products were obtained in nearly quantitative yields as an oily material or hygroscopic powder.

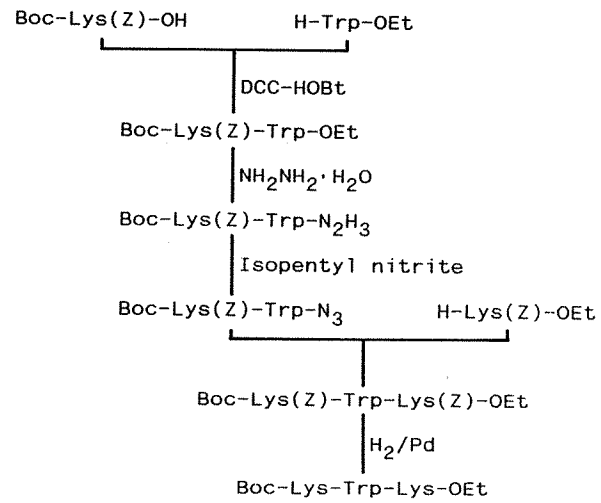


Fig. 3. Synthesis of Boc-Lys-Trp-Lys-OEt.

All intermediates were characterized by elemental analysis, melting point and optical rotation (Table 1). Purity of the final products was assessed by thin layer chromatography, paper chromatography, electrophoresis and high performance liquid chromatography (Table 2).

The effect of synthesized basic tripeptide derivatives on a wide variety of phages was examined. All the derivatives exerted inactivating effects on all the phages examined, although the phages exhibited differential sensitivities to the inactivation. Details on the phage-inactivating effect of these derivatives will be published elsewhere.

Table 1. Physicochemical and analytical data of intermediate peptide derivatives

| Compound | (No.) | Yield (%) | m.p. (°C) | $[\alpha]_D$ (concn., solv.) | $R_f$(Solv.)[a] | Found (%) (Calcd. C | H | N) | Formula |
|---|---|---|---|---|---|---|---|---|---|
| Boc-Leu-Lys(Z)-OEt | (1) | 73 | 88-90 | −23.2 (1.07,$CHCl_3$) | 0.34(1), 0.85(2) | 61.93 (62.17) | 8.26 (8.31) | 8.22 (8.06) | $C_{27}H_{43}N_3O_7$ |
| Boc-Pro-Lys(Z)-OEt | (2) | 92 | Oil | | 0.92(1), 0.98(3) | | | | |
| Boc-Lys(Z)-Trp-OEt | (3) | 80 | 47-51 | +20.8 (1.00,$CHCl_3$) | 0.85(1), 0.98(3) | 64.29 (64.63) | 7.22 (7.12) | 9.48 (9.42) | $C_{32}H_{42}N_4O_7$ |
| Boc-Phe-Lys(Z)-OEt | (4) | 73 | 99-102 | − 5.92(0.52,$CHCl_3$) | 0.91(1), 0.85(2) | 64.89 (64.85) | 7.48 (7.44) | 7.85 (7.56) | $C_{30}H_{41}N_3O_7$ |
| Boc-Gly-Lys(Z)-OEt | (5) | 82 | Oil | | 0.68(1), 0.70(3) | | | | |
| Boc-Ser-Lys(Z)-OEt | (6) | 82 | 104-108 | − 8.59(2.02,DMF) | 0.74(1), 0.86(3) | 57.97 (58.17) | 7.53 (7.53) | 8.65 (8.48) | $C_{24}H_{37}N_3O_8$ |
| Boc-Lys(Z)-Leu-Lys(Z)-OEt | (7) | 75 | 99-101 | −18.2 (0.90,$CHCl_3$) | 0.79(1), 0.50(2) | 64.24 (61.81) | 7.95 (7.84) | 9.08 (8.93) | $C_{41}H_{61}N_5O_{10}$ |
| Boc-Lys(Z)-Pro-Lys(Z)-OEt | (8) | 69 | Oil | | 0.83(1), 0.57(2) | | | | |
| Boc-Lys(Z)-Trp-Lys(Z)-OEt | (9) | 76 | 146-148 | −39.4 (0.99,$CHCl_3$) | 0.82(1), 0.79(3) | 64.22 (64.47) | 7.00 (7.06) | 9.87 (9.81) | $C_{46}H_{60}N_6O_{10}$ |
| Boc-Lys(Z)-Phe-Lys(Z)-OEt | (10) | 74 | 118-120 | −14.4 (1.00,$CHCl_3$) | 0.84(1), 0.58(2) | 64.48 (64.61) | 7.31 (7.27) | 8.57 (8.56) | $C_{44}H_{59}N_5O_{10}$ |
| Boc-Lys(Z)-Gly-Lys(Z)-OEt | (11) | 76 | 91-92 | − 2.88(0.51,$CHCl_3$) | 0.63(1), 0.52(2) | 60.85 (61.06) | 7.31 (7.34) | 9.74 (9.62) | $C_{37}H_{53}N_5O_{10}$ |
| Boc-Lys(Z)-Ser-Lys(Z)-OEt | (12) | 78 | 102-104 | −17.2 (1.00,$CHCl_3$) | 0.56(2), 0.77(3) | 58.95 (58.82) | 7.24 (7.40) | 9.29 (9.03) | $C_{38}H_{55}N_5O_{11}$ $H_2O$ |

[a]Thin-layer chromatography.

Table 2. Analytical data of basic tripeptide derivatives

| Compound | (No.) | $R_f$(solv.)[a] | $R_f$[b] | M.v.[c] | R.t.[d] |
|---|---|---|---|---|---|
| Boc-Lys-Leu-Lys-OEt | (13) | 0.32(1), 0.71(4) | 0.80 | 0.83 | 22.0 |
| Boc-Lys-Pro-Lys-OEt | (14) | 0.35(1), 0.69(4) | 0.76 | 0.74 | 17.0 |
| Boc-Lys-Trp-Lys-OEt | (15) | 0.06(1), 0.63(4) | 0.81 | 0.81 | 22.3 |
| Boc-Lys-Phe-Lys-OEt | (16) | 0.26(1), 0.64(4) | 0.79 | 0.75 | 20.9 |
| Boc-Lys-Gly-Lys-OEt | (17) | 0.07(1), 0.63(4) | 0.65 | 0.78 | 24.0 |
| Boc-Lys-Ser-Lys-OEt | (18) | 0.05(1), 0.43(4) | 0.64 | 0.86 | 14.6 |

[a]Thin-layer chromatography.
[b]Paper chromatography.
[c]Migration value of paper electrophoresis. Value for lysine is represented as 1.00.
[d]Retention time (min) of high performance liquid chromatography.

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# ファージ不活化活性をもつ塩基性トリペプチド誘導体の合成

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## 摘　　要

リジン，リジン誘導体，2官能性リジン誘導体のファージ不活化活性に関する研究の流れに沿って，6種の塩基性トリペプチド誘導体 Boc-Lys-Xxx-Lys-OEt（Xxx＝Leu, Pro, Trp, Phe, Gly, Ser）をデザインし，通常の液相法によって合成した．うち，5種の誘導体（Xxx＝Leu, Pro, Phe, Gly, Ser）は，DCC 縮合剤を用いて逐次合成した．Trp を挿入した誘導体は，酸処理を避けるために，アジド法を用いた．合成した誘導体の純度検定は，薄層クロマトグラフィー，ペーパークロマトグラフィー，濾紙電気泳動および高速液体クロマトグラフィーにより行った．なお，これら6種の塩基性トリペプチド誘導体は，ファージ不活化活性を有していた．