Agricultural and Biological Chemistry

ISSN: 0002-1369 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/tbbb19

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To cite this article: Michio Kondo, Koujiro Miyazaki, Yukihiro Yada, Hideaki Horimoto, Masahiko Samoto & Akira Murata (1984) Synthesis of Higher Homologs of Bifunctional l-Lysine Derivatives with Phage-inactivating Effect, Agricultural and Biological Chemistry, 48:5, 1263-1267, DOI: 10.1080/00021369.1984.10866288

To link to this article: http://dx.doi.org/10.1080/00021369.1984.10866288

Synthesis of Higher Homologs of Bifunctional L-Lysine Derivatives with Phage-inactivating Effect

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Received October 20, 1983

Higher homologs of lysine derivatives consisting of L-lysine and dicarboxylic acids were synthesized. The acids ranged from carbon atoms C_{11} to C_{17} and C_{20} and were coupled with ε-benzyloxycarbonyl-lysine ethyl ester (Lys (Z)-OEt) by conventional methods of peptide synthesis. The removal of the Z-group from the protected compounds by hydrogenation gave the final products as bifunctional agents: undecanedioyl-Lys-OEt, dodecanedioyl-Lys-OEt, tridecanedioyl-Lys-OEt, tetradecanedioyl-Lys-OEt, pentadecanedioyl-Lys-OEt, hexadecanedioyl-Lys-OEt, heptadecanedioyl-Lys-OEt and eicosanedioyl-Lys-OEt. All the products showed a greater inactivating effect on several phages than azelaoyl-Lys-OEt, which showed the highest phage-inactivating effect among the compounds reported in our previous paper.

In the previous paper,[1] a new type of lysine derivative linked with dibasic carboxylic acid was synthesized and the influence of elongation of the methylene chain ($n=2\sim8$) between the two ε-amino-functional groups on the phage-inactivating activity was investigated. Among the seven products synthesized as bifunctional agents, azelaoyl-Lys-OEt ($n=7$) showed the highest inactivating effect on several phages and the high phage-inactivating characteristic of this reagent seemed to be due to the optimum distance and orientation of the two ε-amino groups for interaction with the phosphate residues of the phage-DNA.

In this paper, as an extension of our studies, we report the synthesis of higher homologs of a bifunctional lysine derivative (**1**) in which the dicarboxylic acids ranged from carbon atoms C_{11} to C_{17} and C_{20} (Fig. 1), and the influence on the phage-inactivating activity of methylene chain-elongation above sebacoyl-Lys-OEt ($n=8$).

The conventional methods of peptide synthesis have been employed as simple and mild reaction processes to couple dicarboxylic acids (undecanedioic acid, dodecanedioic acid, tridecanedioic acid, tetradecanedioic acid, pentadecanedioic acid, hexadecanedioic acid, heptadecanedioic acid and eicosanedioic acid) with a ε-benzyloxycarbonyl(Z)-lysine ethyl ester (OEt). The coupling of Lys-OEt with undecanedioic acid, activated with the formation of *N*-hydroxysuccinimide ester (-ONSu), gave undecanedioyl-Lys(Z)-OEt (**2P**). A dicyclohexylcarbodiimide (DCC) condensation of Lys(Z)-OEt with dodecanedioic acid was carried out in the presence of 1-hydroxybenzotriazole (HOBt), and the reaction gave dodecanedioly-Lys(Z)-OEt (**3P**). A mixed anhydride method, using ethyl chloroformate, was applied for the formation of tridecanedioyl-Lys(Z)-OEt (**4P**), tetradecanedioyl-Lys(Z)-OEt (**5P**), hexadecane-

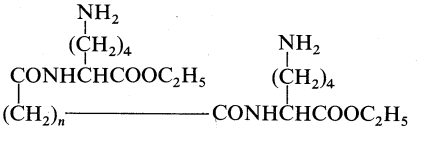
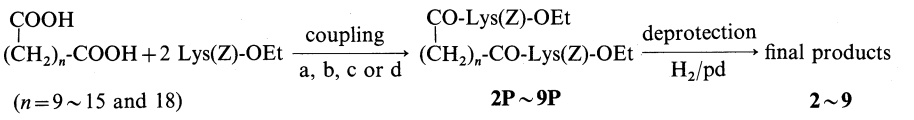


FIG. 1. Chemical Structure of Higher Homolog of Bifunctional Lysine Derivative (**1**), ($n=9\sim15$ and 18).



SCHEME 1. Synthetic Process for Final Products (**2**~**9**).

Procedures of the coupling reaction: a, DCC+HONSu; b, DCC+HOBt; c, mixed anhydride; d, WSCD+HOBt.

TABLE I. PHYSICOCHEMICAL PROPERTIES OF PROTECTED COMPOUNDS

Protected compound	mp (°C)	$[\alpha]_D^{23}$ c*, 1.2~2.0 in $CHCl_3$	Elemental analysis, Found (Calcd.)		
			C%	H%	N%
Undecanedioyl-Lys(Z)-OEt (**2P**)	124	+11.3	64.72 (64.80)	8.32 (8.09)	7.39 (7.03)
Dodecanedioyl-Lys(Z)-OEt (**3P**)	131	+9.5	65.77 (65.16)	8.82 (8.20)	6.90 (6.91)
Tridecanedioyl-Lys (Z)-OEt (**4P**)	113	+9.9	64.96 (65.51)	8.32 (8.31)	6.62 (6.79)
Tetradecanedioyl-Lys(Z)-OEt (**5P**)	129	+8.9	65.27 (65.84)	8.43 (8.41)	6.45 (6.68)
Pentadecanedioyl-Lys(Z)-OEt (**6P**)	120	+9.9	65.24 (66.16)	8.32 (8.52)	6.78 (6.57)
Hexadecanedioyl-Lys(Z)-OEt (**7P**)	133	+10.2	65.71 (66.49)	8.68 (8.60)	6.62 (6.42)
Heptadecanedioyl-Lys(Z)-OEt (**8P**)	120	+9.9	66.43 (66.78)	8.61 (8.71)	6.67 (6.36)
Eicosanedioyl-Lys(Z)-OEt (**9P**)	140	+11.1	67.39 (67.66)	9.03 (8.96)	6.06 (6.07)

* Concentration: *c*, g/100 ml.

TABLE II. PHYSICOCHEMICAL PROPERTIES OF FINAL PRODUCTS

Final product[a]	mp (°C)	$[\alpha]_D^{20}$ c*, 1.5~2.0 in $CHCl_3$	Elemental analysis, Found (Calcd.)		
			C%	H%	N%
Tridecanedioyl-Lys-OEt·2HCl (**4**)	92	−15.0	53.50 (53.78)	9.15 (9.34)	8.35 (8.65)
Pentadecanedioyl-Lys-OEt·2HCl (**6**)	114	−15.4	54.96 (55.10)	9.31 (9.55)	8.22 (8.29)
Hexadecanedioyl-Lys-OEt·2HCl (**7**)	109	−17.2	56.03 (55.72)	9.64 (9.64)	8.48 (8.21)
Heptadecanedioyl-Lys-OEt·2HCl (**8**)	123	−17.3	56.66 (56.32)	9.34 (9.74)	7.93 (7.96)
Eicosanedioyl-Lys-OEt·2HCl (**9**)	126	−17.5	57.90 (57.97)	9.95 (10.00)	7.42 (7.51)

* Concentration: *c*, g/100 ml.

[a] Final products **2**, **3** and **5** were obtained as oils or hygroscopic powders.

dioyl-Lys(Z)-OEt (**7P**) and eicosanedioyl-Lys-(Z)-OEt (**9P**). A water-soluble carbodiimide (WSCD) condensation of Lys(Z)-OEt with pentadecanedioic acid or heptadecanedioic acid in the presence of HOBt yielded the protected compounds, pentadecanedioyl-Lys-(Z)-OEt(**6P**) and heptadecanedioyl-Lys(Z)-OEt(**8P**). The selective removal of the Z-group from the protected compounds (**2P**~**9P**) by hydrogenation afforded the final products as bifunctional agents as illustrated in Scheme 1. All intermediate compounds were characterized by elemental analysis, melting point and optical rotation measurements.

The physicochemical properties of the protected compounds and the final products are summarized in Tables I and II.

The inactivating effect of the final products on selected phages was examined, the results being shown in Table III. With the compound **3** no data are available due to its poor solubility. As shown in Table IV, a comparative test of compounds **2** and **3** at lower concentrations shows that compound **3** has a greater inactivating effect than compound **2** on each phage except $\phi 6$. The results shown in

TABLE III. INACTIVATING EFFECT OF SYNTHETIC COMPOUNDS ON SELECTED PHAGES

A phage ($1 \sim 4 \times 10^7$ PFU/ml) was incubated with each of the compounds (1 mM) in a 0.02 M Tris-HCl buffer, pH 7.4, for 30 min at 37°C. The phage survival of the control without the compound is represented as 100%.

Phage	Survival (%)								
	Compounds								
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
J1	0	—[b]	0	0	0	0	0	0	(0)[c]
T5	15	—	0	0	0	0	0	0	(45)
T4	3	—	0	0	0	0	0	0	(30)
M2	20	—	0	0	0	0	0	0	(30)
T3	4	—	0	0	0	0	0	0	(0)
ϕX174	4	—	0	0	0	0	0	0	(0)
δA	0	—	0	0	0	0	0	0	(10)
MS2	0	—	0	0	40	0	10	3	(0)
ϕ6[a]	0	—	0	0	0	0	0	0	(60)

[a] Phage ϕ6 was incubated for 20 min at 25°C because of its relative instability at 37°C.
[b] No data were available due to the poor solubility of the compound **3**.
[c] Parentheses indicate the data of azelaoyl-Lys-OEt[1] for reference.

TABLE IV. INACTIVATING EFFECT OF COMPOUNDS **2** AND **3** ON SELECTED PHAGES

See the legend for Table III, except that the concentration of the compounds was 0.1 mM.

Phage	Survival (%)	
	Compounds	
	2	**3**
J1	20	0
T5	100	50
T4	100	55
M2	100	50
T3	70	50
ϕX174	100	50
δA	60	15
MS2	0	1
ϕ6	15	55

Tables III and IV indicate that all the final products have a high inactivating effect on all the phages tested. In the previous paper,[1] we have reported that among seven compounds ($n=2\sim8$), azelaoyl-Lys-OEt ($n=7$) exhibited the highest inactivating effect on each phage except ϕ6. However, each of the synthetic compounds (**2**, $n=9\sim9$, $n=18$) of higher homologs shows a greater inactivating effect on the phages than azelaoyl-Lys-OEt. A few exceptions are compound **2** *vs.* phages T3 and ϕX174, and the compounds **6**, **8** and **9** *vs.* phage MS2. A detailed study on the phage-inactivating effect of these bifunctional agents and on the mechanism of phage-inactivation by the agents will be published elsewhere.

EXPERIMENTAL*

All melting points are uncorrected. Thin layer chromatography was carried out on silica gel GF_{254} (Merck) with the following solvent systems: R_f^1, $CHCl_3$–MeOH (5:1, v/v), R_f^2, $CHCl_3$–MeOH–AcOH (8:1:1) and R_f^3, $CHCl_3$–MeOH–NH_3 (8:4:1). Paper chromatography was performed on Toyo Roshi No. 50 paper with the following solvent systems: R_f^4, *n*-BuOH–AcOH–H_2O (4:1:2) and R_f^5, *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2). Optical rotations were measured on a Yanagimoto polarimeter OR-50. Infrared (IR) spectra were recorded on a Hitachi 260-10 instrument using an NaCl plate. Di-

* All optically active amino acids are of the L configuration. Abbreviations according to the IUPAC-IUB Commission are used throughout. Additional abbreviations: DMF, *N,N'*-dimethylformamide; NMM, *N*-methyl morpholine; HONSu, *N*-hydroxysuccinimide; THF, tetrahydrofuran; TEA, triethylamine; and WSCD, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.

carboxylic acids were commercially available except for pentadecanedioic acid and heptadecanedioic acid which were prepared.

Undecanedioyl-Lys(Z)-OEt (**2P**). To a solution of undecanedioic acid (433 mg, 2 mmol) in THF (9 ml) were added HONSu (690 mg, 6 mmol) and DCC (825 mg, 4 mmol) at 0°C. After stirring for 5 hr, the 2120 cm^{-1} diimide band of the DCC in the IR spectra had almost disappeared and the active ester (–ONSu) of undecanedioic acid was formed. A solution of Lys(Z)-OEt·*p*-TsOH[2] (2.11 g, 4.4 mmol) and Et_3N (0.607 ml, 4.4 mmol) in $CHCl_3$ (9 ml) was added to the active ester. After 2 days, the *N,N'*-dicyclohexylurea (DCU) was filtered off and the filtrate was diluted with $CHCl_3$ (200 ml). The $CHCl_3$ solution was washed successively with 0.5 N HCl, sat. NaCl and 0.5 N $NaHCO_3$, dried with Na_2SO_4 and evaporated under reduced pressure. The residual oil was solidified by the addition of petroleum ether and the product finally recrystallized from hot ethyl acetate–ether–petroleum ether; yield, 1.04 g (65%); R_f^1 0.78, R_f^2 0.52, R_f^3 0.89.

Dodecanedioyl-Lys(Z)-OEt (**3P**). To a solution of Lys(Z)-OEt·*p*-TsOH (980 mg, 2 mmol) and Et_3N (0.28 ml, 2 mmol) in $CHCl_3$ (4 ml) was added a solution of dodecanedioic acid (230 mg, 1 mmol) and HOBt (324 mg, 2.4 mmol) in a mixture of THF (4 ml), $CHCl_3$ (2 ml) and DMF (0.5 ml), DCC finally being added (412 mg, 2 mmol) at 0°C. The mixture was stirred for 4 hr at 0°C and then for 2 days at room temperature. After stirring for 2 days, the DCC band in the IR spectra had disappeared. The DCU was filtered off and the filtrate was treated as described for **2P**. The product was finally recrystallized from a mixture of ethyl acetate–$CHCl_3$–ether; yield, 705 mg (87%); R_f^1 0.79, R_f^2 0.54, R_f^3 0.97.

Tridecanedioyl-Lys(Z)-OEt (**4P**). To a solution of tridecanedioic acid (1.222 g, 5 mmol) in THF (20 ml) were added NMM (1.10 ml, 10 mmol) and ethyl chloroformate (1.14 ml, 10 mmol) at 0°C. The mixture was stirred for 15 min at 0°C and to this was added a solution of Lys(Z)-OEt·*p*-TsOH (4.810 g, 10 mmol) and NMM (1.10 ml, 10 mmol) in $CHCl_3$ (25 ml). The reaction mixture was stirred overnight at room temperature and then evaporated. The residue was taken up in ethyl acetate and the solution was treated as described for **2P**; yield, 3.527 g (85%); R_f^1 0.82, R_f^2 0.87, R_f^3 0.92.

Tetradecanedioyl-Lys(Z)-OEt (**5P**). This was prepared from tetradecanedioic acid (258 mg, 1 mmol) and Lys(Z)-OEt·*p*-TsOH (961 mg, 2 mmol) by a mixed anhydride method similar to **4P**; yield, 460 mg (55%); R_f^1 0.80, R_f^2 0.55, R_f^3 0.90.

Pentadecanedioyl-Lys(Z)-OEt (**6P**). To a solution of Lys(Z)-OEt·*p*-TsOH (2.21 g, 4.3 mmol) and NMM (0.51 ml, 4.2 mmol) in DMF (15 ml) was added a solution of pentadecanedioic acid (601 mg, 2 mmol) and HOBt (594 mg, 4.4 mmol) at 0°C. The mixture was stirred for 4 hr at 0°C and then for 2 days at room temperature. The mixture was evaporated *in vacuo* and the residue was taken up to $CHCl_3$ (300 ml). The solution was treated as described for **2P**; yield, 1.51 g (86%); R_f^1 0.75, R_f^2 0.90, R_f^3 0.92.

Hexadecanedioyl-Lys(Z)-OEt (**7P**). this was prepared from hexadecanedioic acid (572 mg, 2 mmol) and Lys(Z)-OEt·*p*-TsOH (1.924 g, 4 mmol) by a mixed anhydride method similar to **4P**; yield, 1.41 g (82%); R_f^1 0.71, R_f^2 0.92, R_f^3 0.89.

Heptadecanedioyl-Lys(Z)-OEt (**8P**). This was prepared from heptadecanedioic acid (545 mg, 2 mmol) and Lys(Z)-OEt·*p*-TsOH (2.115 g, 4.4 mmol) in a similar manner to the preparation of **6P**; yield, 1.34 g (79%); R_f^1 0.77, R_f^2 0.80, R_f^3 0.93.

Eicosanedioyl-Lys(Z)-OEt (**9P**). this was prepared from eicosanedioic acid (684 mg, 2 mmol) and Lys(Z)-OEt·*p*-TsOH (1.924 g, 4 mmol) by a mixed anhydride method similar to **4P**; yield, 1.33 g (72%); R_f^1 0.70, R_f^2 0.57, R_f^3 0.93.

Undecanedioyl-Lys-OEt·2HCl (**2**). Compound **2P** (318 mg, 0.4 mmol) was hydrogenated in a mixture of EtOH (6.6 ml) and 1.04 N ethanolic hydrogen chloride (0.84 ml) in the presence of palladium black for 8 hr. The solution filtered from the catalyst was evaporated to dryness. The NMR spectrum showed an absence of the Z-group; yield, 254 mg (98%) as a chromatographycally homogenous oil; R_f^4 0.84, R_f^5 0.83.

Dodecanedioyl-Lys-OEt·2HCl (**3**). Compound **3P** (260 mg, 0.32 mmol) was hydrogenated by a similar method as that described for **2**, except that an ethanol–$CHCl_3$ solution was used in this case; yield of oil, 148 mg (83%); R_f^4 0.85, R_f^5 0.94.

Tridecanedioyl-Lys-OEt·2HCl (**4**). The deprotection of **4P** (825 mg, 1 mmol) by hydrogenolysis in the same manner as for **3** gave **4** as a hydroscopic powder; yield, 629 mg (99%); R_f^4 0.87, R_f^5 0.79.

Tetradecanedioyl-Lys-OEt·2HCl (**5**). The deprotection of **5P** (420 mg, 0.5 mmol) by hydrogenolysis in the same manner as for **3** gave **5** as an oil; yield, 310 mg (98%); R_f^4 0.86, R_f^5 0.82.

Pentadecanedioyl-Lys-OEt·2HCl (**6**). The deprotection of **6P** (512 mg, 0.6 mmol) by hydrogenolysis in the same manner as for **2** gave **6** as crystals; yield, 380 mg (94%); R_f^4 0.86, R_f^5 0.80.

Hexadecanedioyl-Lys-OEt·2HCl (**7**). Compound **7P** (867 mg, 1 mmol) was hydrogenated by a similar method to that for **3**. The product was recrystallized from hot ethyl acetate–ether–petroleum ether; yield, 593 mg (88%); R_f^4 0.88, R_f^5 0.81.

Heptadecanedioyl-Lys-OEt·2HCl (**8**). Compound **8P** (881 mg, 1 mmol) was hydrogenated by a similar method to that for **3**; yield, 604 mg (86%); R_f^4 0.88, R_f^5 0.84.

Eicosanedioyl-Lys-OEt·2HCl (**9**). Compound **9P** (923 mg, 1 mmol) was hydrogenated by a similar method to that for **3**. The product was recrystallized from hot ethyl acetate–petroleum ether; yield, 627 mg (86%); R_f^4 0.89, R_f^5 0.81.

Penta- and hepta-decanedioic acids. A mixture of ethyl hydrogen suberate (23.9 g) and thionyl chloride (10.6 ml) was heated to give the ester acid chloride (23.0 g, 88%), which was treated with Et_3N (14.4 ml) followed by hydrolysis[3] to afford 7-oxopentadecanedioic acid (11.7 g, 79%). The ketonic diacid (11.7 g) was reduced to give pentadecanedioic acid (9.5 g, 85%, mp 110～111°C; lit.[4a] mp 113～114°C) by the Wolff–Kishner procedure. Heptadecanedioic acid was prepared from ethyl hydrogen azelaate by the same method as that above; yields, ester acid chloride 93%, ketonic diacid 51%, heptadecanedioic acid 66% (mp 113～114°C; lit.[4b] mp 116～117°C).

Electrophoresis. Electropholysis, using Toyo Roshi No. 51 A paper, was carried out with a solvent system of formic acid-AcOH–MeOH–H_2O (1 : 3 : 6 : 10, v/v, pH 1.8) at 500 V/40 cm for 3 hr. All the synthetic compounds migrated more slowly towards the cathode than the control Lys (10 cm); 2 (7.7 cm), 3 (7.6 cm), 4 (7.5 cm), 5 (7.4 cm), 6 (7.1 cm), 7 (7.0 cm), 8 (6.9 cm), 9 (6.8 cm).

Amino acid analysis. For amino acid analyses, samples were hydrolyzed as described in the previous paper.[1] The Lys in the hydrolysate of the synthetic compounds (**2**～**9**) was detected on a thin layer or paper chromatogram.

Assay of synthetic compounds in vitro.

1) Phages. The nine phages listed in the previous paper[1] were used; Jl of *Lactobacillus casei*, T3, T4, T5, ϕX174, δA and MS2 of *Escherichia coli*, M2 of *Bacillus subtilis*, and ϕ6 of *Pseudomonas phaseolicola*. These phages represent a wide variety in nucleic acid type, host strain, size and morphology, serological grouping and some other chracteristics.

2) Assay of phages. The phages were assayed by the standard double agar layer technique.[5]

3) Inactivation of phages. 0.5 ml of a sample solution and 0.5 ml of each phage solution were mixed with 4.0 ml of a prewarmed 0.02 M Tris-HCl buffer, pH 7.4. The reaction was carried out in the same manner as described in the previous paper.[1]

Acknowledgments. We wish to thank Miss Y. Ueda for her assistance in part of the synthesis work and Mr. H. Fukada and Miss S. Nishi for the biological assay.

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