[Chem. Pharm. Bull. 32(8)2925—2931(1984)]

Studies on Structure–Activity Relationships of FK-156, an Immunostimulating Peptide, and Related Compounds. I. Synthesis of Stereoisomeric Analogues of FK-156

HIDEKAZU TAKENO, SATOSHI OKADA, KEIJI HEMMI, MATSUHIKO ARATANI, YOSHIHIKO KITAURA, and MASASHI HASHIMOTO*

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2–1–6, Kashima, Yodogawa-ku, Osaka 532, Japan

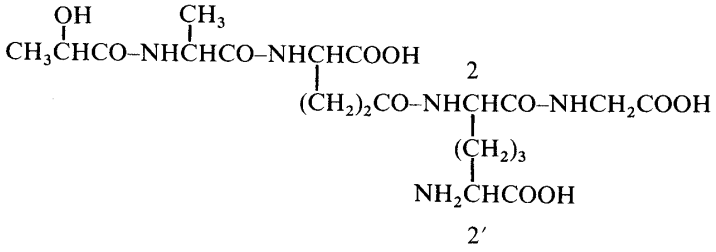
(Received October 31, 1983)

Five stereoisomeric analogues, **2**—**6**, of FK-156 (**1**), an immunostimulating microbial metabolite, have been synthesized and screened for immunostimulating activity. The results indicate that the D-configuration of glutamic acid and L-configuration of diaminopimelic acid at the site combining with other amino acids are essential, while the configurations of lactic acid and alanine are of little importance for the biological activity of the FK-156 series.

Keywords——immunostimulant; peptide synthesis; FK-156; structure–activity relationship; acylpeptide

The chemistry of the peptidoglycans of the bacterial cell-wall has been a subject of much recent interest because of the unique immunostimulating activity of these compounds.[1] In the previous paper,[2] we reported the synthesis of FK-156 (**1**), an immunostimulating microbial metabolite with a structurally close resemblance to the peptidoglycan peptide. As part of a program of reserch on this natural product, we were interested in studying the structure–activity relationships with the aim of finding more active compounds in this series. As is the case in many other biologically active compounds, stereochemistry of the amino acids which constitute the FK-156 molecule might be a factor of major importance for the biological activity. Thus, we synthesized some stereoisomers of FK-156. This paper is concerned with the syntheses of these stereoisomers, **2**—**6**, and their biological activities.

For the synthesis of the isomer (**2**) with the 2(L),2′(L)-diaminopimelyl moiety, 2(L),2′(L)-dicarbobenzyloxy diaminopimelic acid (**7**), prepared according to the literature,[3] was first coupled to glycine as follows. Preactivation of **7** with 1.3 eq of isobutyl chloroformate in the presence of *N*-methylmorpholine (2 eq), followed by condensation with 1.3 eq of benzyl glycinate gave **8** in 55% yield. The protective groups in **8** were removed by hydrogenolysis over palladium charcoal to give L-2,2′-diaminopimelyl glycine (**9**) in 96% yield. Regioselective protection of the two amino functions in **9** was performed by using a copper chelate procedure[2] as follows. In the presence of 1 eq of cupric chloride, **9** was allowed to react with 7 eq of carbobenzoxychloride at pH 10—12. After treatment with hydrogen sulfide, the reaction mixture was subjected to Diaion HP-20 column chromatography, and elution with aqueous methanol gave **10** in 67% yield.[4] The structural assignment of **10** was based on its pK_a values, 3.2 and 3.7, corresponding to those of the carboxy groups of acylated α-amino acids, and 8.7, corresponding to the amino group of amidated α-amino acids.[3,5] The protected lactoyldipeptide (**11**)[2] was converted to the *N*-hydroxysuccinimide ester, which was allowed to react with **10** to give **12** in 73% yield. Removal of the protecting groups of **12** was performed by hydrogenolysis over 10% palladium charcoal for the benzyl and the carboben-



2

2′

Compd.	Configurations of component				
	Lactoyl	Ala	Glu	A_2pm 2	A_2pm 2′
FK-156 (**1**)	D	L	D	L	D
2	D	L	D	L	L
3	D	L	D	D	L
4	L	L	D	L	D
5	D	D	D	L	D
6	D	L	L	L	D

Fig. 1

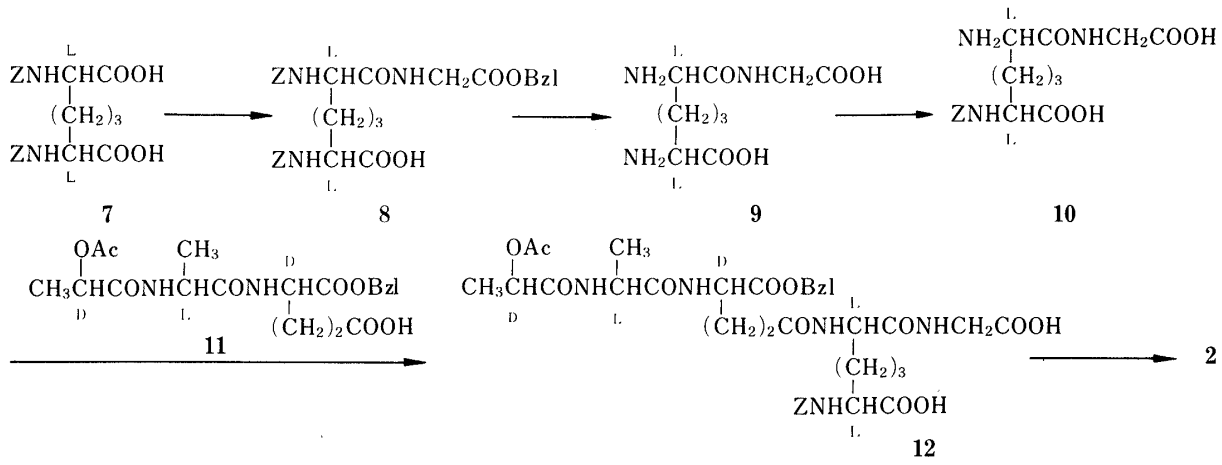


Chart 1

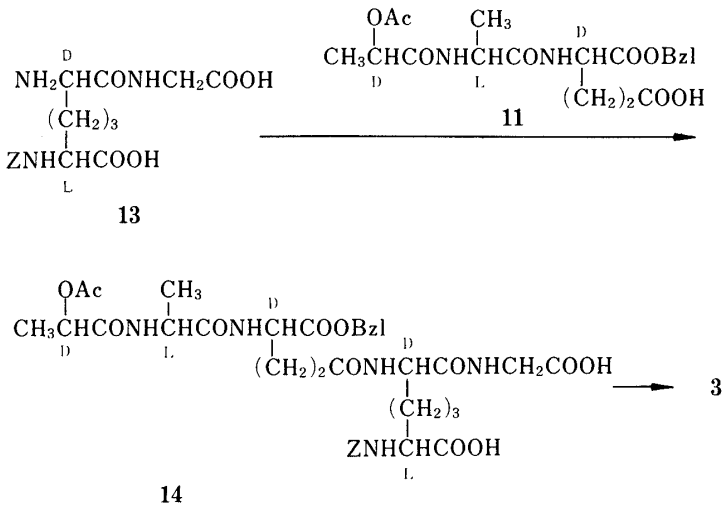


Chart 2

zyloxy groups, followed by alkaline hydrolysis for the acetyl group to afford **2** in 68% yield.

The 2(D),2′(L)-diaminopimelyl isomer (**3**) was prepared from **13** as outlined in Chart 2. Compound **13** was previously prepared in the synthesis of a geometric isomer of FK-156.[2b] The lactoyldipeptide fragment (**11**) was coupled to **13** by the active-ester procedure, affording the condensation product **14** in 88% yield. Hydrogenation and subsequent alkaline hydrolysis of **14** gave **3** in 52% yield.

Compounds **4**, **5**, and **6** were prepared from **15a—c**, respectively, as shown in Chart 3. The general procedures were as follows. α-Benzyl *tert*-butyloxycarbonyl-L-alanyl-D-glutamate (**15a**), prepared from *tert*-butyloxycarbonyl-L-alanine and α-benzyl D-glutamate according to the procedure described in the literature,[6] was treated with hydrogen chloride and the resulting product was acylated with *O*-acetyl L-lactoyl chloride[7] to afford **16a**. The fragment **16a** was led to the mixed-anhydride *in situ* by treatment with isobutyl chloroformate and allowed to react with the silylated meso 2,2′-diaminopimelylglycine moiety,[2] prepared *in situ* by treatment with bis(trimethylsilyl)acetamide, giving the condensation product **18a**. For the removal of the protecting groups, the following procedures were successively employed. Hydrogenolysis was first carried out for removal of the benzyl group to give **19a**, which in turn was treated with aqueous potassium carbonate for removal of the acetyl group to yield **20a**. Treatment with trifluoroacetic acid for removal of the *tert*-butyloxycarbonyl group and oxidation of the hydrazine group with sodium metaperiodate in dilute sulfuric acid, which was followed by spontaneous hydrolysis to the carboxylic acid, afforded **4** in 44% yield.

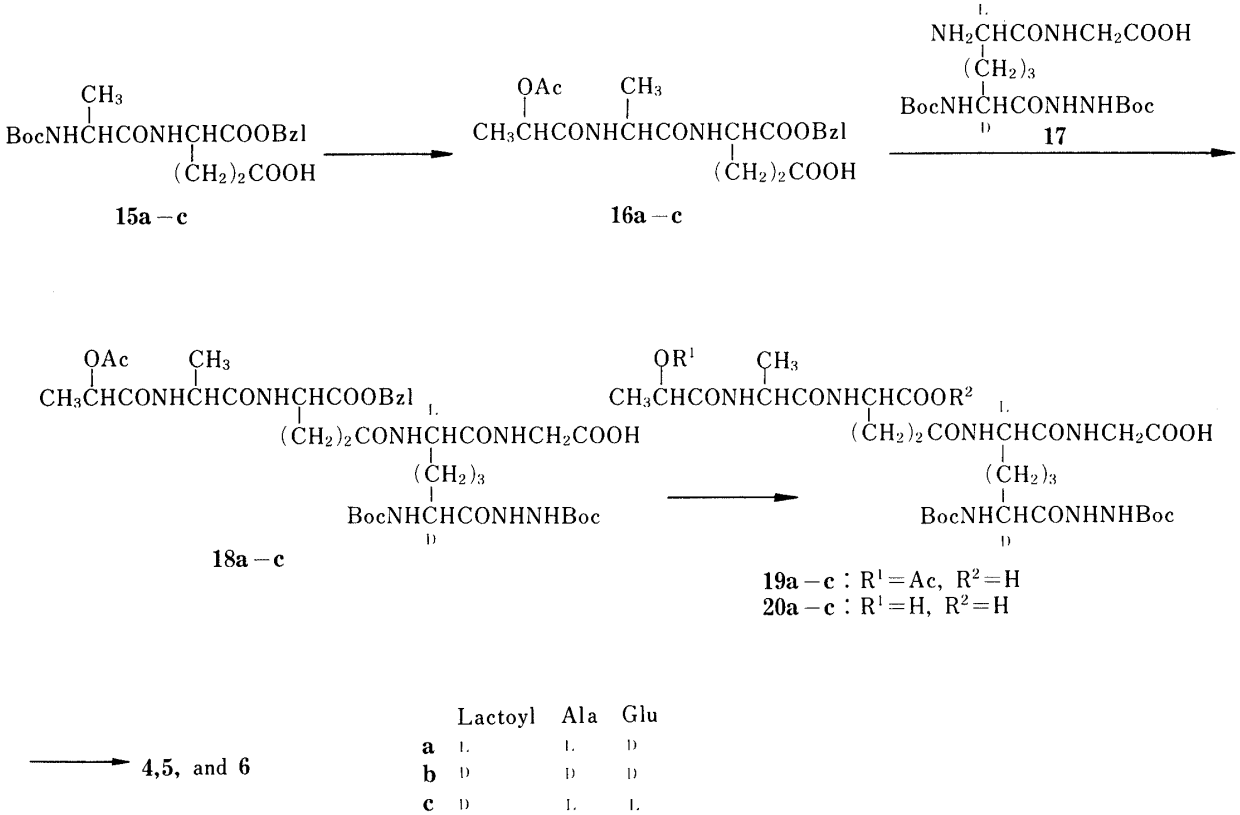


Chart 3

The biological activities of compounds **2—6** were evaluated by measuring the phagocytic activity in carbon clearance assay in mice, protective effect against *E. coli* infection in mice, and induction of delayed-type hypersensitivity against egg albumin in guinea pigs. As shown in Table I, the L-lactoyl isomer (**4**) and the D-alanyl isomer (**5**) showed potent phagocytic

TABLE I. Biological Activities of Compounds **1**—**6**

Compd.	Carbon clearance[a] K (mean ± S.E.)	K_t/K_c	Protective effect[b] survival/tested	DTH to egg albumin[c] skin response (mm)
Control	0.031 ± 0.003	1.0	0/10	0
1	0.059 ± 0.002[d]	1.9	9/10	10.2 ± 1.0[e]
2	0.039 ± 0.003	1.3	7/10	6.3 ± 0.4[e]
3	0.028 ± 0.002	0.9	0/10	1.6 ± 1.2
4	0.053 ± 0.003[d]	1.7	8/10	6.7 ± 0.7[e]
5	0.053 ± 0.002[d]	1.7	8/10	5.3 ± 0.5[e]
6	0.032 ± 0.003	1.0	1/10	2.4 ± 1.5

a) Clearance from the blood was measured according to the method described by Biozzi *et al.*[10] Compounds were administered at a dose of 1 mg/kg to DDY male mice.
b) Compounds were administered *i.p.* at a dose of 1 mg/kg to ICR mice on the 4th day before challenge with *E. coli* 22 by the same route. Results were obtained on the 3rd day after the bacterial challenge.
c) Hartley guinea pigs were immunized in both hind footpads with 1 mg of egg albumin and 1 μg/site of test compound in FIA. After 2 weeks, 5 μg of egg albumin was given as a challenge and the skin reaction (diameter of induration) was measured after 48 h.
d) $p<0.005$. *e*) $p<0.01$.

activities (comparable to that of **1**), while **3** and **6** were inactive in the carbon clearance assay. In the antiinfection assay, compounds **2**, **4**, and **5** again showed significant activities, though slightly less than that of **1**, while **3** and **6** were entirely inactive.

These results showed that the D-configuration of glutamic acid and the L-configuration of diaminopimelic acid at the site combining with glutamic acid and glycine are essential, while the configurations of lactic acid and alanine are of little importance for the biological activities of the FK-156 series. These conclusions were also supported by the fact that N^2-(γ-D-glutamyl)-2(L),2′(D)-diaminopimelic acid is the minimal structural unit capable of eliciting the biological properties characteristic of FK-156, as will be discussed in the following paper of this series.[8]

Experimental[9]

Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) and nuclear magnetic resonance (NMR) spectra were recorded on a Hitachi 260-10 spectrophotometer and a JEOL PS-100 spectrophotometer, respectively. Optical rotations were measured on a JASCO automatic polarimeter. Thin-layer chromatography (TLC) was carried out on Silica gel 60-F_{254} (E. Merck AG) using the following solvent systems: A, *n*-BuOH–AcOH–H_2O (5 : 2 : 3); B, *n*-PrOH–H_2O (7 : 3). The spots were detected by visualization under ultraviolet (UV) light or spraying with 0.5% ninhydrin solution in EtOH. High-pressure liquid chromatography (HPLC) was carried out on a Waters Associates chromatograph equipped with a model 6000A pump and a Shimadzu SPD-2A spectrophotometric detector (210 nm) using a column (4 × 150 mm) packed with Lichrosorb RP-18 (7 μm, E. Merck) and 1% CH_3CN–phosphate buffer (pH 2) was used as a mobile phase.

Di-Z-L-A_2pm-GlyOBzl (8)——A solution of **7** (2.93 g, 6.39 mmol) and *N*-methylmorpholine (1.29 g, 12.78 mmol) in CH_2Cl_2 (30 ml) was cooled to −15 °C and isobutyl chloroformate (1.14 g, 8.31 mmol) was added dropwise with stirring at −17——15 °C. After stirring for 30 min at the same temperature, a solution of GlyOBzl·*p*-TsOH (2.80 g, 8.31 mmol) and *N*-methylmorpholine (0.84 g, 8.31 mmol) in CH_2Cl_2 (15 ml) was added dropwise and the mixture was stirred at −25——10 °C for 1 h and at 0 °C for 30 min. After concentration of the reaction mixture, a mixture of 2% HCl (20 ml) and AcOEt (50 ml) was added to the residue. The resulting precipitate was removed by filtration. The organic layer was washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent gave an oily residue, which was crystallized from AcOEt to give a crystalline solid (2.26 g). Further purification was performed by column chromatography on silica gel (Silica gel 60, 70—230 mesh, E. Merck) with a $CHCl_3$–MeOH system (50 : 1—10 : 1). The fractions containing the product were collected and evaporated to give 2.13 g (55%) of **8** as white crystals: mp 165 °C (dec). $[\alpha]_D$ −6.1 ° ($c=0.2$, AcOH). IR (Nujol): 3320, 1765, 1740, 1720 (sh), 1680, 1645 cm^{-1}. NMR (DMSO-d_6) δ: 1.1—1.9 (6H, m), 3.89 (2H, d, $J=5$ Hz), 3.7—4.4 (2H, m), 5.00 (4H, s), 5.10 (2H, s), 7.1—7.6 (2H, m), 7.32 (15H, s), 8.31 (1H, t, $J=5$ Hz). *Anal.* Calcd for $C_{32}H_{35}N_3O_9$: C, 63.46; H, 5.83; N, 6.94. Found: C, 63.16; H, 5.94; N,

6.73.

L-A_2pm-Gly (9)——A solution of **8** (1.43 g, 2.36 mmol) in AcOH (20 ml) was hydrogenated over 10% Pd–C (290 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was crystallized from a mixture of H_2O (10 ml) and MeOH (30 ml) to give 600 mg (96%) of **9**: mp 257—258 °C (dec.). $[\alpha]_D$ 50.8° ($c=0.13$, H_2O). *Rf*, 0.24 (B). IR (Nujol): 3350, 1660, 1640 (sh), 1590 (br) cm^{-1}. NMR (D_2O) δ: 1.2—2.3 (6H, m), 3.5—4.3 (2H, m), 3.87 (2H, s). *Anal.* Calcd for $C_9H_{17}N_3O_5$: C, 40.75; H, 7.22; N, 15.84. Found: C, 40.61; H, 7.26; N, 15.63.

Z-2′-L,L-A_2pm-2-Gly (10)——A solution of **9** (403 mg, 1.52 mmol) and $CuCl_2 \cdot 2H_2O$ (259 mg, 1.52 mmol) in H_2O (30 ml) was cooled to 0 °C and pH was brought to 12.5 with 3 N NaOH. Carbobenzoxy chloride (1.30 g, 7.6 mmol) was added with stirring and the mixture was maintained at pH 10—12 with 3 N NaOH. After 2 h, additional carbobenzoxy chloride (0.56 g, 3 mmol) was added and the mixture was stirred for 2 h at 0 °C. The reaction mixture was acidified to pH 2 with 1 N HCl and washed with ether. The aqueous layer was brought to pH 5 and treated with H_2S gas for 5 min at 0 °C. The resulting precipitate was filtered off and the filtrate was concentrated to about 5 ml. The concentrate was subjected to Diaion HP-20 column chromatography and eluted with aqueous MeOH to give 387 mg (67%) of **10** as an amorphous powder: $[\alpha]_D$ 20.0° ($c=0.4$, AcOH). *Rf*, 0.62 (A). IR (Nujol): 1690 (br), 1540 (br) cm^{-1}. NMR (D_2O) δ: 1.2—2.2 (6H, m), 3.88 (2H, d, $J=2.5$ Hz), 3.8—4.3 (2H, m), 5.10 (2H, s), 7.43 (5H, s). *Anal.* Calcd for $C_{17}H_{23}N_3O_7$: C, 52.30; H, 6.20; N, 10.77. Found: C, 52.14; H, 6.05; N, 10.87.

D-Lactoyl(OAc)-L-Ala-γ-D-Glu(α-OBzl)-2-Z-2′-L,L-A_2pm-2-Gly (12)——*N*-Hydroxysuccinimide (84 mg, 0.73 mmol) and dicyclohexylcarbodiimide (150 mg, 0.73 mmol) were added successively to a solution of **11** (308 mg, 0.73 mmol) in dioxan (4 ml) at 0 °C with stirring. After stirring for 10 h at the same temperature, the precipitate was filtered off and the filtrate was evaporated. The residue was dissolved in dioxane (2 ml) and the solution was used for the next reaction without further purification. Triethylamine (221 mg, 2.19 mmol) and active-ester solution were added to a solution of **10** (278 mg, 0.73 mmol) in a mixture of H_2O (5 ml) and dioxan (5 ml) at room temperature. After being stirred for 6 h at the same temperature, the reaction mixture was concentrated. Then AcOEt (30 ml) and 2% HCl (15 ml) were added to the residue. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to give an amorphous material, which was crystallized from ether to give 478 mg (73%) of **12**: mp 102—108 °C. $[\alpha]_D$ −22° ($c=0.4$, AcOH). IR (Nujol): 1730, 1650 cm^{-1}. NMR ($CDCl_3$–CD_3OD) δ: 1.37 (3H, d, $J=7$ Hz), 1.46 (3H, d, $J=7$ Hz), 2.11 (3H, s), 1.2—2.6 (10H, m), 3.95 (2H, s), 4.0—4.6 (4H, m), 4.96 (1H, q, $J=7$ Hz), 5.08 (2H, s), 5.15 (2H, s), 7.33 (10H, s). *Anal.* Calcd for $C_{37}H_{47}N_5O_{14}$: C, 56.55; H, 6.03; N, 8.91. Found: C, 56.28; H, 6.05; N, 8.76.

D-Lactoyl-L-Ala-γ-D-Glu(α-OH)-2-L,L-A_2pm-2-Gly (2)——A solution of **12** (393 mg, 0.5 mmol) in AcOH (10 ml) was hydrogenated over 10% Pd–C (80 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was dissolved in MeOH (6 ml). A solution of K_2CO_3 (414 mg, 3 mmol) in H_2O (10 ml) was added at 0 °C with stirring. After being stirred for 16 h at room temperature, the reaction mixture was concentrated. The residue was dissolved in H_2O (5 ml) and the solution was brought to pH 2 with 3% HCl, then subjected to Diaion HP-20 column chromatography with H_2O. The eluate was concentrated and lyophilized to give 183 mg (68%) of **2**: mp 130 °C. $[\alpha]_D$ −23.3° ($c=0.3$, H_2O). *Rf*, 0.18 (A), 0.34 (B). HPLC (t_R), 9.32 min (FK-156, 10.19 min). IR (Nujol): 1730, 1650 cm^{-1}. NMR (D_2O) δ: 1.39 (3H, d, $J=7$ Hz), 1.45 (3H, d, $J=7$ Hz), 1.1—2.6 (10H, m), 3.86 (1H, t, $J=6$ Hz), 4.00 (2H, s), 4.1—4.6 (4H, m). Amino acid anal., Ala 1.00, Glu 1.03, Gly 0.98, A_2pm 1.05. *Anal.* Calcd for $C_{20}H_{33}N_5O_{11} \cdot H_2O$: C, 44.69; H, 6.56; N, 13.03. Found: C, 44.60; H, 6.62; N, 12.85.

D-Lactoyl(OAc)-L-Ala-γ-D-Glu(α-OBzl)-(D)-Z-(L)*meso*-A_2pm(D)-Gly (14)——Triethylamine (470 mg, 4.65 mmol) and the succinimide ester of **11** [prepared from **11** (653 mg, 1.55 mmol)] in dioxan (5 ml) were added to a solution of **13** (528 mg, 1.55 mmol) in a mixture of H_2O (10 ml) and dioxan (10 ml). After being stirred for 6 h at room temperature, the reaction mixture was concentrated and AcOEt (50 ml) and 2% HCl (30 ml) were added to the residue. The organic layer was washed with brine (20 ml), dried over $MgSO_4$ and evaporated to give an amorphous solid, which was crystallized from $CHCl_3$–ether to give 1.10 g (88%) of **14**: mp 136 °C (dec.). $[\alpha]_D$ 3.60° ($c=0.2$, THF). IR (Nujol): 1730 (br), 1650 (br) cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ: 1.37 (3H, d, $J=7$ Hz), 1.45 (3H, d, $J=7$ Hz), 1.2—1.9 (6H, m), 2.10 (3H, s), 2.0—2.5 (4H, m), 3.93 (2H, s), 4.1—4.7 (3H, m), 5.10 (2H, s), 5.17 (2H, s), 7.30 (10H, s). *Anal.* Calcd for $C_{37}H_{47}N_5O_{14}$: C, 56.55; H, 6.03; N, 8.91. Found: C, 56.41; H, 6.21; N, 8.68.

D-Lactoyl-L-Ala-γ-D-Glu(α-OH)-(D)*meso*-A_2pm(D)-Gly (3)——A solution of **14** (403 mg, 0.5 mmol) in AcOH (10 ml) was hydrogenated over 10% Pd–C (100 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was dissolved in MeOH (10 ml). A solution of K_2CO_3 (414 mg, 3 mmol) in H_2O (10 ml) was added at 0 °C with stirring. After being stirred for 16 h at room temperature, the reaction mixture was concentrated. The residue was dissolved in H_2O (5 ml) and brought to pH 2 with 3% HCl. The solution was applied to a column of Diaion HP-20 and eluted with H_2O. The eluate was lyophilized to give 137 mg (52%) of **3**: mp 142 °C (dec.). $[\alpha]_D$ 3.46° ($c=0.4$, H_2O). *Rf*, 0.18 (A), 0.34 (B). HPLC t_R, 8.57 min. IR (Nujol): 1720, 1650 cm^{-1}. NMR (D_2O) δ: 1.38 (3H, d, $J=7$ Hz), 1.45 (3H, d, $J=7$ Hz), 1.1—2.6 (10H, m), 3.80 (1H, t, $J=7$ Hz), 3.95 (2H, s), 4.0—4.5 (4H, m). Amino acid anal., Ala 1.00, Glu 1.04, Gly 1.01, A_2pm 1.03. *Anal.* Calcd for $C_{20}H_{33}N_5O_{11} \cdot 1/2H_2O$: C, 45.45; H, 6.48; N, 13.25. Found: C, 45.26; H, 6.52; N, 13.23.

L-Lactoyl(OAc)-L-Ala-D-Glu(OH)OBzl (16a)——Hydrogen chloride gas was bubbled into a cooled and stirred solution of **15a** (2.04 g, 5 mmol) in AcOEt (20 ml) during a period of 30 min. The reaction mixture was purged with N_2 gas and evaporated to leave an oily residue, which was dissolved in CH_2Cl_2 (10 ml). Bis(trimethylsilyl)acetamide (3.36 g, 16.5 mmol) was added, and the mixture was cooled to −40 °C. *O*-Acetyl-L-lactoyl chloride (1.05 g, 7 mmol) was then added with stirring and the whole was stirred at −20——10 °C for 1 h and allowed to warm to room temperature. After evaporation of the reaction mixture, the residue was dissolved in AcOEt (60 ml) and washed successively with 3% HCl and brine. The organic layer was dried over $MgSO_4$ and evaporated to give an oil, which was crystallized from isopropylether to give 1.84 g (87%) of **16a**: mp 119—121 °C. $[\alpha]_D$ −22.7° (c=0.5, MeOH). IR (Nujol): 3260, 1740 (sh), 1720, 1695, 1665, 1660 cm^{-1}. NMR ($CDCl_3$) δ: 1.39 (3H, d, J=7 Hz), 1.46 (3H, d, J=7 Hz), 2.15 (3H, s), 2.00—2.65 (4H, m), 4.00—4.90 (2H, m), 5.20 (2H, s), 5.21 (1H, q, J=7 Hz), 7.14 (1H, d, J=8 Hz), 7.37 (5H, s), 7.45 (1H, m), 9.57 (1H, s). *Anal.* Calcd for $C_{20}H_{26}N_2O_8$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.58; H, 6.11; N, 6.40.

D-Lactoyl(OAc)-D-Ala-D-Glu(OH)OBzl (16b)——Compound **16b** was prepared from *tert*-Boc-D-Ala-D-Glu-(OH)OBzl (**15a**) in the same manner as described for the preparation of **16a** and isolated as the dicyclohexylamine (DCHA) salt. Isolation: The oily product (**16b**), prepared from 3.1 g (5.26 mmol) of **15b**, was dissolved in ether (40 ml). DCHA (953 mg, 5.26 mmol) was added with stirring at 0 °C. The resulting precipitate was collected by filtration to give 2.12 g (66% from **15b**) of the DCHA salt of **16b**. Physical data for the DCHA salt of **16b**: mp 132.5—134 °C. $[\alpha]_D$ 39.8° (c=0.5, MeOH). IR (Nujol): 3300, 1740, 1670, 1650, 1630 cm^{-1}. NMR ($CDCl_3$) δ: 1.40 (3H, d, J=7 Hz), 1.46 (3H, d, J=7 Hz), 2.16 (3H, s), 2.72—3.29 (2H, m), 4.21—4.65 (2H, m), 5.00—5.39 (1H, m), 5.19 (2H, s), 7.24 (1H, m), 7.38 (5H, s), 8.16 (2H, s), 9.25 (1H, d, J=6 Hz). *Anal.* Calcd for $C_{32}H_{49}N_3O_8 \cdot 1/2H_2O$: C, 62.72; H, 8.22; N, 6.86. Found: C, 62.48; H, 7.99; N, 6.87.

D-Lactoyl(OAc)-L-Ala-L-Glu(OH)OBzl (16c)——The DCHA salt of **16c** was prepared in 76% yield in the same manner as described for the preparation of **16b**. Physical data for the DCHA salt of **16c**: mp 150—152 °C. $[\alpha]_D$ −22.6° (c=0.5, MeOH). IR (Nujol): 3340, 1735, 1650, 1630 cm^{-1}. NMR ($CDCl_3$) δ: 1.39 (3H, d, J=7 Hz), 1.48 (3H, d, J=7 Hz), 2.14 (3H, s), 2.75—3.18 (2H, m), 4.26—4.70 (2H, m), 5.00—5.38 (1H, m), 5.20 (2H, s), 7.41 (5H, s), 9.00 (2H, s), 9.37 (1H, d, J=6 Hz). *Anal.* Calcd for $C_{32}H_{49}N_3O_8 \cdot 1/2H_2O$: C, 62.72; H, 8.22; N, 6.86. Found: C, 62.61; H, 8.29; N, 6.59.

L-Lactoyl(OAc)-L-Ala-γ-D-Glu(α-OBzl)-(L)-Boc-(D)*meso*-A_2pm(D)-NHNHBoc-(L)-Gly (18a)——A solution of **16a** (844 mg, 2 mmol) and *N*-methylmorpholine (202 mg, 2 mmol) in CH_2Cl_2 (8.5 ml) was cooled to −15 °C and isobutyl chloroformate (273 mg, 2 mmol) was added dropwise with stirring at −15——10 °C. After stirring for 30 min at the same temperature, a solution of the silyl ester of **17** [prepared from **17** (922 mg, 2 mmol) and bis(trimethylsilyl)acetamide (1.42 g, 7 mmol) in a mixture of CH_2Cl_2 (10 ml) and DMF (2 ml) by stirring for 15 min at room temperature] was added dropwise and the whole was stirred at −10 °C for 2 h and at 0 °C for 1 h. After concentration of the reaction mixture to about 5 ml, the concentrate was poured into AcOEt (100 ml) and washed with 3% HCl and H_2O. Drying over $MgSO_4$ and evaporation gave an amorphous solid, which was crystallized from AcOEt–isopropylether to give 1.32 g (77%) of **18a**: mp 139—141 °C. $[\alpha]_D$ −5.7° (c=0.5, MeOH). IR (Nujol): 3270, 1740 (sh), 1730, 1670, 1645 cm^{-1}. NMR ($CDCl_3$) δ: 1.3—1.95 (30H, m), 2.16 (3H, s), 2.1—2.45 (4H, m), 3.96 (2H, s), 4.3—4.6 (4H, m), 5.06 (1H, m), 5.24 (2H, s), 7.37 (5H, s). *Anal.* Calcd for $C_{39}H_{59}N_7O_{15}$: C, 53.45; H, 6.96; N, 11.48. Found: C, 53.38; H, 6.90; N, 11.62.

D-Lactoyl(OAc)-D-Ala-γ-D-Glu(α-OBzl)-(L)-Boc-(D)*meso*-A_2pm(D)-NHNHBoc-(L)-Gly (18b)——The DCHA salt of **16b** (510 mg, 0.83 mmol) was suspended in AcOEt (20 ml). Then 2% HCl (10 ml) was added and the mixture was stirred for 15 min at room temperature. The resulting precipitate was filtered off, and the organic layer was separated and washed with brine. Drying over $MgSO_4$ and evaporation gave **16b** as an amorphous solid (307 mg, 88%), which was coupled to **17** in the same manner as described for the preparation of **18a**, in 81% yield: mp 100—106 °C. $[\alpha]_D$ 32.3° (c=0.2, MeOH). IR (Nujol): 3280, 1740, 1650 cm^{-1}; NMR ($CDCl_3$) δ: 1.15—2.00 (30H, m), 2.04 (3H, s), 2.1—2.5 (4H, m), 3.85 (2H, s), 4.0—4.6 (4H, m), 4.99 (1H, q, J=6 Hz), 5.10 (2H, s), 7.27 (5H, s). *Anal.* Calcd for $C_{39}H_{59}N_7O_{15}$: C, 53.45; H, 6.96; N, 11.48. Found: C, 53.52; H, 6.90; N, 11.71.

D-Lactoyl(OAc)-L-Ala-γ-L-Glu(α-OBzl)-(L)-Boc-(D)*meso*-A_2pm(D)-NHNHBoc-(L)-Gly (18c)——Compound **18c** was prepared from the DCHA salt **16c** in the same manner as described for the preparation of **18a** and **18b**, in 90% yield: mp 127—129 °C. $[\alpha]_D$ −19.3° (c=0.5, MeOH). IR (Nujol): 3300, 1740 (sh), 1730, 1670, 1650, 1640 cm^{-1}. NMR ($CDCl_3$) δ: 1.30—2.05 (30H, m), 2.2—2.6 (4H, m), 2.13 (3H, s), 3.95 (2H, s), 4.10—4.65 (4H, m), 5.05 (1H, q, J=7 Hz), 5.19 (2H, s), 7.38 (5H, s). *Anal.* Calcd for $C_{39}H_{59}N_7O_{15}$: C, 53.45; H, 6.96; N, 11.48. Found: C, 53.31; H, 7.12; N, 11.27.

L-Lactoyl-L-Ala-γ-D-Glu(α-OH)-(L)*meso*-A_2pm(L)-Gly (4)——A solution of **18a** (1.0 g, 1.17 mmol) in AcOH (20 ml) was hydrogenated over 10% Pd–C (0.2 g) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was pulverized with ether to give 850 mg of **19a**. A 825 mg (1.08 mmol) sample of **19a** was dissolved in 50% aqueous MeOH (20 ml) and the solution was adjusted to pH 9 with 5% aqueous K_2CO_3. The stirring was continued for 13 h at pH 9 at room temperature. After evaporation of the MeOH, the resulting aqueous solution was acidified to pH 3 with 3% HCl and applied to a column of Diaion HP-20. Elution with MeOH–H_2O (1 : 1) and evaporation of the eluate gave 600 mg of **20a**.

A 600 mg (0.74 mmol) sample of **20a** was dissolved in trifluoroacetic acid (3 ml) and stirred at room temperature for 30 min. After evaporation of trifluoroacetic acid, the residue was pulverized with ether and the resulting powder was dissolved in H_2O (10 ml). This solution was cooled to 0 °C and acidified to pH 1 with 0.1 N H_2SO_4. A solution of $NaIO_4$ (430 mg, 2 mmol) in H_2O (5 ml) was added with stirring and the mixture was stirred for 1 h at the same temperature. After decomposition of the excess oxidant with $NaHSO_3$, the reaction mixture was brought to pH 2.5 with saturated $NaHCO_3$ and concentrated to about 3 ml. The concentrate was applied to a column of Diaion HP-20 (120 ml) and eluted with H_2O. The eluate was concentrated and lyophilized to give 260 mg (44% from **18a**) of **4**: mp 170—174 °C (dec.). $[\alpha]_D$ −33.2° (c=0.25, H_2O). *Rf*, 0.18 (A), 0.33 (B). HPLC t_R, 14.55 min. IR (Nujol): 3270, 1720, 1620 cm^{-1}. NMR (D_2O) δ: 1.38 (3H, d, J=7 Hz), 1.45 (3H, d, J=7 Hz), 3.89 (1H, t, J=7 Hz), 4.01 (2H, s), 4.20—4.55 (4H, m). Amino acid anal., Ala 1.00, Glu 0.98, Gly 1.02, A_2pm 1.04. *Anal.* Calcd for $C_{20}H_{33}N_5O_{11}\cdot 2H_2O$: C, 43.24; H, 6.71; N, 12.61. Found: C, 43.10; H, 6.89; N, 12.50.

D-Lactoyl-D-Ala-γ-D-Glu(α-OH)-(L)*meso*-A_2pm(L)-Gly (5)——Compound **5** was prepared from **18b** in 52% total yield in a manner similar to that used for the preparation of **4**: mp 103—106 °C. $[\alpha]_D$ 11.6° (c=0.25, H_2O). *Rf*, 0.18 (A), 0.34 (B). HPLC t_R, 9.43 min. IR (Nujol): 3280, 1720, 1640 (br) cm^{-1}. NMR (D_2O) δ: 1.36 (3H, d, J=7 Hz), 1.42 (3H, d, J=7 Hz), 3.91 (1H, t, J=6 Hz), 3.99 (2H, s), 4.14—4.50 (4H, m). Amino acid anal., Ala 1.00, Glu 1.04, Gly 0.97, A_2pm 1.04. *Anal.* Calcd for $C_{20}H_{33}N_5O_{11}\cdot H_2O$: C, 44.69; H, 6.56; N, 13.03. Found: C, 44.43; H, 6.58; N, 12.95.

D-Lactoyl-L-Ala-γ-L-Glu(α-OH)-(L)*meso*-A_2pm(L)-Gly (6)——Compound **6** was prepared from **18c** in 50% total yield in a manner similar to that used for the preparation of **4**: mp 115—118 °C (dec.). $[\alpha]_D$ −34.7° (c=0.15, H_2O). *Rf*, 0.18 (A), 0.34 (B). HPLC t_R, 8.05 min. IR (Nujol): 3280, 1720, 1640 cm^{-1}. NMR (D_2O) δ: 1.35 (3H, d, J=7 Hz), 1.42 (3H, d, J=7 Hz), 3.85 (1H, t, J=7 Hz), 3.98 (2H, s), 4.13—4.50 (4H, m). Amino acid anal., Ala 1.00, Glu 1.02, Gly 1.01, A_2pm 1.05. *Anal.* Calcd for $C_{20}H_{33}N_5O_{11}\cdot H_2O$: C, 44.69; H, 6.56; N, 13.03. Found: C, 44.51; H, 6.68; N, 12.90.

Acknowledgement The authors are grateful to Drs. J. Mori, H. Senoh, and Y. Mine for the biological assays. Thanks are also due to Messrs. Y. Miyazaki and T. Nakamura for their technical assistance.

References and Notes

1) Review: for example, E. Lederer, *J. Med. Chem.*, **23**, 819 (1980).
2) *a*) K. Hemmi, H. Takeno, S. Okada, O. Nakaguchi, Y. Kitaura, and M. Hashimoto, *J. Am. Chem. Soc.*, **103**, 7026 (1981); *b*) K. Hemmi, M. Aratani, H. Takeno, S. Okada, Y. Miyazaki, O. Nakaguchi, Y. Kitaura, and M. Hashimoto, *J. Antibiot.*, **35**, 1300 (1982).
3) R. Wade, S. M. Birnbaum, M. Winitz, R. J. Koegel, and J. P. Greenstein, *J. Am. Chem. Soc.*, **79**, 648 (1957).
4) It is known[5] that at a 1 : 1 ratio of ligand to metal the order of cupric ion binding is Gly-Gly > Gly. In our case, the copper complex of the dipeptide moiety in compound **9** might also be more stable than that of α-amino moiety, and consequently carbobenzyloxylation would occur at the latter moiety.
5) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1, John Wiley and Sons, Inc., New York, 1961.
6) E. Work, S. M. Birnbaum, M. Winitz, and J. P. Greenstein, *J. Am. Chem. Soc.*, **77**, 1916 (1955).
7) K. Koga, S. Yamada, M. Yoh, and T. Mizoguchi, *Carbohydr. Res.*, **36**, C9 (1974).
8) *a*) Y. Kitaura, O. Nakaguchi, H. Takeno, S. Okada, S. Yonishi, K. Hemmi, J. Mori, H. Senoh, Y. Mine, and M. Hashimoto, *J. Med. Chem.*, **25**, 335 (1982); *b*) H. Takeno, S. Okada, S. Yonishi, K. Hemmi, O. Nakaguchi, Y. Kitaura, and M. Hashimoto, *Chem. Pharm. Bull.*, **32**, 2932 (1984).
9) The usual symbols of peptide chemistry are used in the experimental section. For the designation of substituents on diaminopimelic acid, we used the abbreviations advocated by Bricas *et al.*[11]
10) G. Biozzi, B. Benacerraf, and B. N. Halpern, *Br. J. Exp. Pathol.*, **34**, 441 (1953).
11) E. Bricas, C. Nicot, and E. Lederer, *Bull. Soc. Chim. Biol.*, **44**, 1115 (1962).