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Studies on Structure–Activity Relationships of FK-156, an Immunostimulating Peptide, and Related Compounds. II.[1] Synthesis of N^2-(γ-D-Glutamyl)-2(L), 2′(D)-diaminopimelic Acid as the Minimal Essential Structure of FK-156[2]

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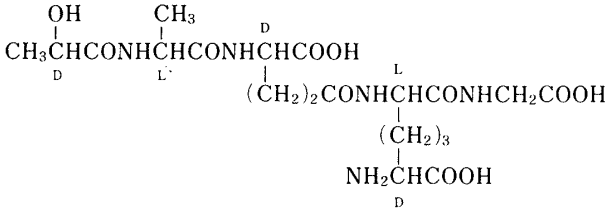
Five partial structures, **3**—**7**, of FK-156 (**1**), an immunostimulating acyl peptide, have been prepared. The compounds were evaluated for biological activities, and it was found that N^2-(γ-D-glutamyl)-2(L),2′(D)-diaminopimelic acid (**3**) represents the minimal active structure unit essential for the immunostimulating property of FK-156 (**1**). Its caprylyl derivative **8** and stearoyl derivative **9** were found to be capable of increasing resistance to bacterial infection as efficiently as **1**. Moreover, compound **9** showed a potent tumor-suppressive activity lacking in **1**.

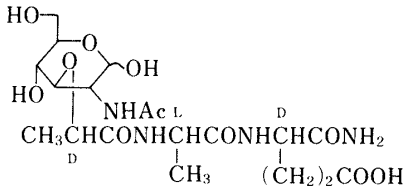
Keywords——immunostimulant; peptide synthesis; FK-156; tumor-suppressive activity; structure–activity relationship

In recent years considerable attention has been focused on the bacterial cell-wall peptidoglycans because of their strong immunostimulating activity. Since the discovery in 1974 that muramyl dipeptide (MDP) is the minimal essential unit of the peptidoglycans, many compounds related to MDP have been prepared.[3] Very recently it has been found that FK-156 (**1**), isolated as a metabolite of *Streptomyces olivaceogriseus*, possesses an activity similar to that of MDP. The structure of **1** is unique, as compared with that of MDP (**2**), in that FK-156 lacks the glucosamine residue at the O-terminal of the D-lactoyl side chain and instead carries the *meso*-2,2′-diaminopimelylglycine residue at γ-C-terminal of D-glutamic acid. It is particularly interesting that FK-156 presents a wide range of immunological activities despite the lack of the muramyl moiety, which had, until recently, been considered to be essential for the biological activity of compounds of the MDP series.[5] It was therefore considered that the 2,2′-diaminopimelylglycine moiety in **1**, like the muramyl moiety in **2**, might play an important role in the unique biological activity.

As part of a program of research on this natural product, we were interested in studying the structure–activity relationships of this series and also in preparing simpler active



FK-156 (**1**)



MDP (**2**)

Fig. 1

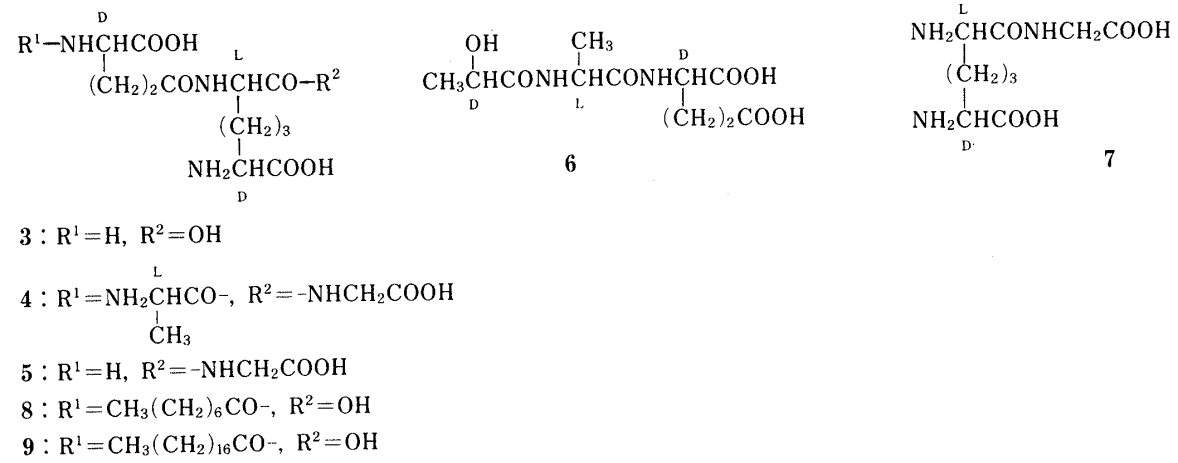


Fig. 2

molecules containing the important component, 2,2′-diaminopimelic acid. Herein we report the syntheses and immunological activities of the partial structures **3**—**7**. N^2-(γ-D-Glutamyl)-*meso*-2(L), 2′(D)-diaminopimelic acid (**3**) was shown to be the minimal structural unit capable of eliciting the biological effects characteristic of FK-156. Its caprylyl derivative (**8**) proved to be a potential broad-spectrum immunostimulant as efficient as **1**, and further, the stearoyl derivative (**9**) showed a potent tumor-suppressive activity that was not present in the original compound (**1**).

Fragment **3** was prepared from **10** and **11** as outlined in Chart 1. α-Benzyl *N*-carbobenzyloxy-D-glutamate (**10**)[6] was preactivated with isobutylchloroformate and coupled to the silyl ester of **11**,[4b] prepared by treatment of **11** with bis(trimethylsilyl)acetamide, giving the condensation product **12** in 89% yield. For removal of the protecting groups in **12**, alkaline hydrolysis was first carried out to yield **13**, which in turn was treated with trifluoroacetic acid and oxidized with sodium metaperiodate to give **14**. Catalytic hydrogenation of **14** over palladium charcoal afforded **3** in 52% total yield from **12**. It is conceivable that alkaline hydrolysis of the γ-glutamyl peptides might also yield the α-rearrangement by-product, as in the case of the rearrangement of glutamine methyl ester to isoglutamine.[7] In the preparations of **3** and other glutamic acid-containing compounds, however, such by-products, even if coproduced, would most probably be removed during the purification by Diaion HP-20 column chromatography.

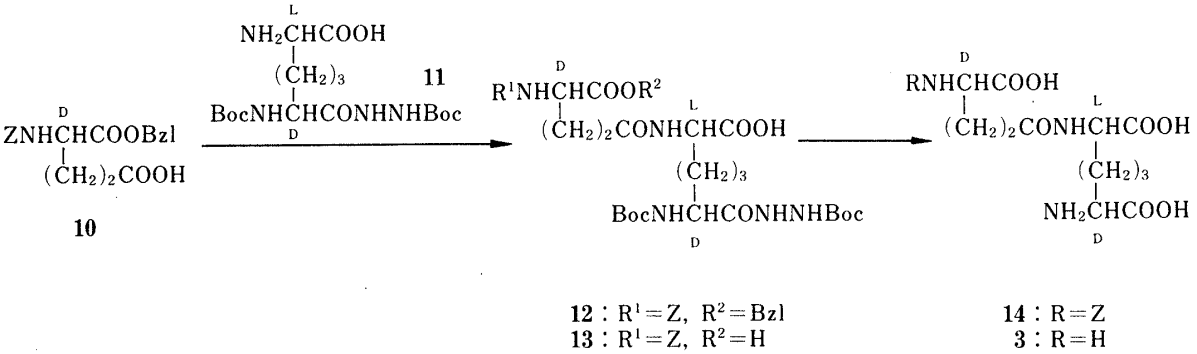


Chart 1

The lactic acid-free derivative **4** and the lactoylalanine-free derivative **5** were prepared as outlined in Chart 2. The fragment **15**[8] was converted *in situ* to a mixed anhydride with isobutyl chloroformate and allowed to react with the silyl ester of **16**,[4b] resulting in an 82% yield of the

condensation product **17**. The protecting groups of **17** were removed in the same way as described for the deprotection of **12** to **3**. Thus, **17** was successively subjected to alkaline hydrolysis, trifluoroacetic acid treatment, oxidation with sodium metaperiodate and catalytic hydrogenation to provide **4** in 43% yield.

Compound **5** was prepared in a similar manner. Thus, **10** was coupled with the silyl ester of **16** by the mixed anhydride method, giving **18** in 90% yield. Deprotection of **18** by alkaline hydrolysis, treatment with trifluoroacetic acid, oxidation with sodium metaperiodate and catalytic hydrogenation gave **5** in 44% yield.

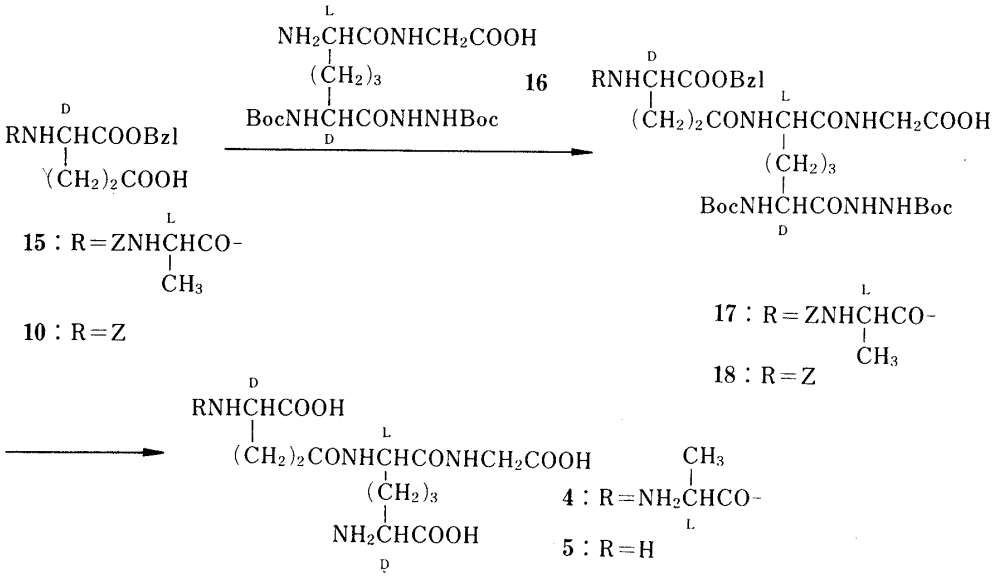


Chart 2

D-Lactoyl-L-alanyl-D-glutamic acid (**6**) and *N*-[2(L),2′(D)-diaminopimel-1-yl]glycine (**7**) were prepared as outlined in Chart 3. The protected D-lactoyl dipeptide **19**, which had been prepared in the total synthesis of **1**,[4b] was deprotected by alkaline hydrolysis to give **6** in 53% yield. The protected diaminopimelyl-glycine (**20**), which had also been prepared in the synthesis of **1**, was treated with trifluoroacetic acid and then oxidized with sodium metaperiodate to give **21** in 76% yield. Subsequent hydrogenation of **21** over palladium charcoal gave **7** in 87% yield.

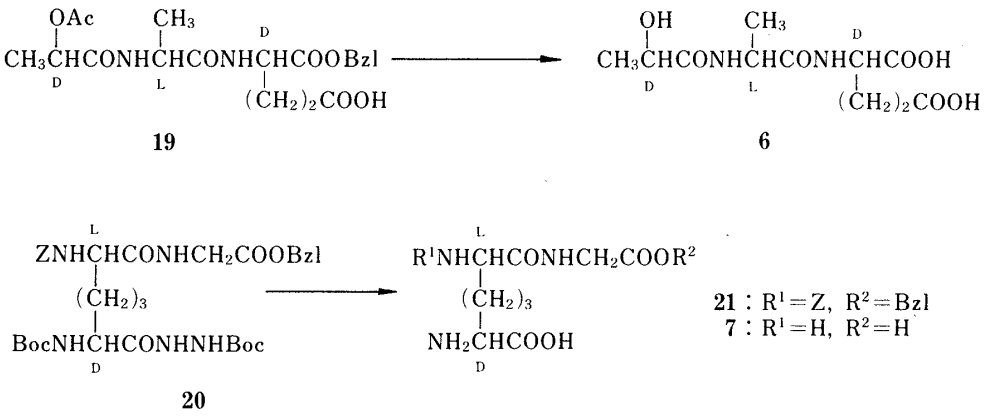


Chart 3

We also synthesized acyl derivatives of **3**, because **3** was found to be the minimal active fragment as described below. Thus, the caprylyl and stearoyl derivatives of **3** were prepared as

outlined in Chart 4. Acylation of α-benzyl D-glutamate with caprylyl chloride gave α-benzyl *N*-caprylyl-D-glutamate (**26**) in 83% yield; this product was converted to the *N*-hydroxysuccinimide ester and coupled to **11** to give **28** in 67% yield. Removal of the protecting groups in **28** by hydrogenolysis, treatment with trifluoroacetic acid and oxidation with sodium metaperiodate gave **8** in 78% yield.

The procedure described above was found to be inapplicable for the preparation of the stearoyl derivative **9**, because of the sparing solubilities of the intermediates in the deprotection processes. This problem was solved by removal of the hydrazide protecting group of *meso*-2,2′-diaminopimelic acid in an early step before introduction of the fatty acid. Thus, **22**[4b] was treated with trifluoroacetic acid and oxidized with sodium metaperiodate to afford **23** in 76% yield. Reprotection of **23** with the *tert*-butyloxycarbonyl group gave **24** in quantitative yield. Subsequent hydrogenolysis of **24** gave **25** in 81% yield. α-Benzyl *N*-stearoyl-D-glutamate (**27**), prepared from α-benzyl D-glutamate by acylation with stearoyl chloride, was derived to the *N*-hydroxysuccinimide ester, which was coupled to **25** to give **29** in 89% yield. Compound **29** was deprotected by hydrogenolysis and trifluoroacetic acid treatment to produce **9** in 89% yield.

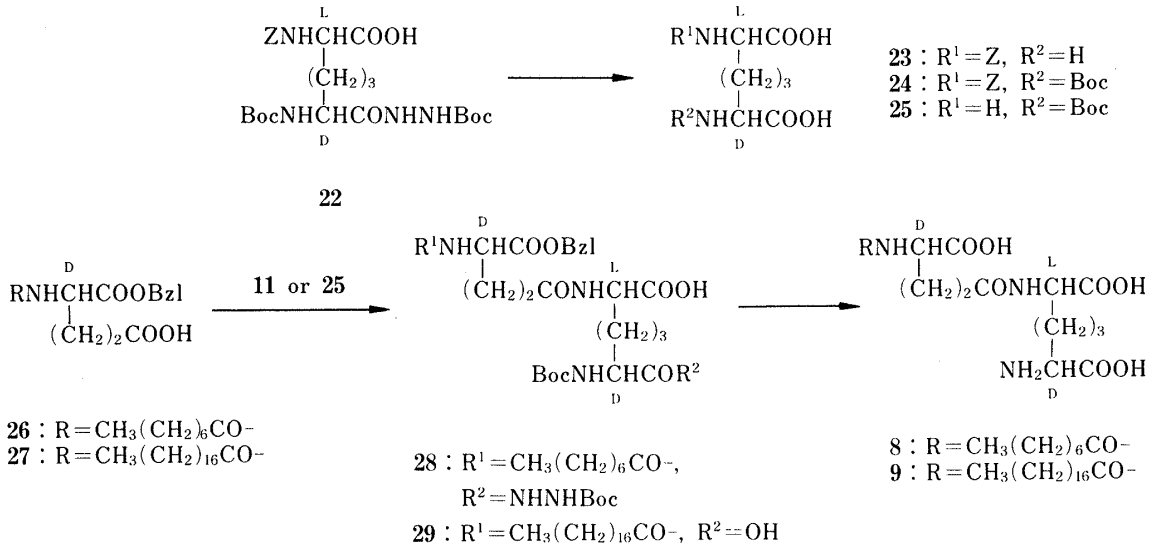


Chart 4

The compounds **3**—**9** thus prepared were tested for phagocytic activity in DDY mice (carbon clearance assay), induction of delayed-type hypersensitivity to egg albumin in guinea pigs and protective effect against *E. coli* infection in mice. Increases in the rate of carbon clearance were observed with **3** at doses of 10 mg/kg and **4** and **5** at doses of 100 mg/kg. Compounds **6** and **7**, on the other hand, were inactive (see Table I).

As shown in Table II, three compounds **3**, **4**, and **5** were found to stimulate the delayed-type hypersensitivity reaction, though they were somewhat less potent than **1**, while **6** and **7** were inactive.

The results of protective effect against *E. coli* infection are summarized in Table III. Compound **4** was about as active as **1**, while **3** was slightly less active than **1**. Compounds **5** and **6** were less potent than **3** and **4**, and again **7** showed no activity.

In summary, compounds **3**, **4**, and **5**, which contain the N^2-(γ-D-glutamyl)-*meso*-2(L), 2′(D)-diaminopimelyl moiety, were active in the bioassays described above, while **6** and **7** were entirely inactive. This suggests that **3** is the minimal active structure essential for eliciting the biological effects so far examined.

Compounds **8** and **9**, both acyl derivatives of the minimal unit **3**, showed carbon

TABLE I. Effects of Compounds **1**—**9** on Carbon Clearance in DDY Mice (Male)[a]

Compd.	Dose, mg/kg	K (mean ± S.E.)	Stimulation index (k_t/k_c)
Controls		0.020 ± 0.003	1.0
1	0.1	0.029 ± 0.001	1.5
	1	0.038 ± 0.007[b]	1.9
3	1	0.021 ± 0.005	1.1
	10	0.058 ± 0.010[c]	2.9
4	1	0.016 ± 0.003	0.8
	100	0.042 ± 0.003[b]	2.1
5	1	0.030 ± 0.003	1.5
	100	0.035 ± 0.003[b]	1.8
6	1	0.023 ± 0.004	1.2
	100	0.029 ± 0.004	1.5
7	1	0.019 ± 0.002	1.0
	100	0.026 ± 0.007	1.3
8	0.1	0.032 ± 0.009	1.6
	1	0.048 ± 0.009[c]	2.4
9	0.1	0.032 ± 0.006	1.6
	1	0.048 ± 0.005[c]	2.4

a) Clearance from the blood was measured according to the method described by Biozzi *et al.*[9] Compounds were administered to mice (five animals in each series) *s.c.* 24 h before injecting a colloidal carbon suspension (170 mg/ml) at a dose of 1 ml/100 g by the same route (*b*) = $p < 0.005$ and *c*) = $p < 0.01$ as compared to the controls).

TABLE II. Induction of Delayed-Type Hypersensitivity to Egg Albumin in Hartley Guinea Pigs (Male)[a]

Compd.	Dose, μg/site	Skin response (mean ± S.E.)
Controls		0
1	1	8.2 ± 1.7[c]
	10	10.0 ± 1.3[c]
3	1	3.3 ± 1.4[b]
	10	6.9 ± 2.0[c]
4	1	1.8 ± 0.7
	10	8.9 ± 0.9[c]
5	1	2.7 ± 0.7[b]
	10	N.T.
6	1	0
	10	1.6 ± 1.0
7	1	0
	10	0
8	1	8.3 ± 1.5[c]
	10	3.7 ± 1.6
9	1	1.7 ± 1.1
	10	3.1 ± 1.3[b]

a) Guinea pigs (five animals in each series) were immunized in both hind footpads with 1 mg of egg albumin plus test compounds in Freund's incomplete adjuvant. After 2 weeks, 5 μg of egg albumin was given as a challenge, and skin reaction (diameter of induration, mm) was measured after 48 h (*b*) = $p < 0.005$ and *c*) = $p < 0.01$ as compared to the controls).

clearance assay activities of the same order as or rather superior to that of **1**. In the delayed-type hypersensitivity assay, **8** was shown to be as active as **1** (Table II). Compound **9**, on the other hand, did not affect the adjuvant potency as markedly in this assay system. It was

TABLE III. Protective Effect against *Escherichia coli* Infection in ICR Mice (Male)[a]

Compd.	Dose, mg/kg	Survival[b]
Controls		1/10
1	0.1	6/10
	1	7/10
3	0.1	4/10
	1	4/10
4	0.1	8/10
	1	5/10
5	0.1	N.T.
	1	3/10
6	0.1	N.T.
	1	3/10
7	0.1	N.T.
	1	0/10
8	0.1	7/10
	1	6/10
9	0.1	7/10
	1	8/10

a) Compounds were administered to mice *i.p.* on the 4th day before challenge with *E. coli* 22 (7.2×10^7) by the same route. Results were obtained on the 3rd day after the bacterial challenge.
b) Number of survivors/number of mice tested.

TABLE IV. Suppressing Effect[a] on Meth-A Fibrosarcoma in BALB/c Mice (Female)[b]

Compd.	Dose, μg/site	Suppression[c]
Controls		0/10
1	100	0/10
9	100	9/9

a) Reference 12.
b) A mixture of Meth-A (1×10^5 cells) and compounds dissolved (in the case of **1**) or suspended (in the case of **9**) in a 0.5% solution of methylcellulose in saline was inoculated intradermally into mice. Results were obtained on the 28th day after the tumor inoculation.
c) Number of tumor-free mice/number of mice tested.

noteworthy, however, that compound **9** exhibitied a potent tumor-suppressive activity (Table IV). In fact, when Meth-A fibrosarcoma in BALB/C mice was used, **9** was highly effective in suppressing the tumor growth, while the original compound **1** was entirely inactive. In the assay of protective effect against bacterial infection, compounds **8** and **9** were both as active as **1**.

The present work shows that compound **3**, N^2-(γ-D-glutamyl)-*meso*-2(L),2′(D)-diaminopimelic acid, represents the minimal active structural unit essential for the characteristic biological response of **1**. Its acyl derivatives **8** and **9** were found to be capable of increasing resistance to bacterial infection as efficiently as **1**. Moreover, compound **9** proved to possess tumor-suppression activity lacking in **1**.

Experimental[10]

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 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 (3:2); AcOEt–AcOH (2:1). The spots were detected by visualization with ultraviolet (UV) light or by spraying 0.5% ninhydrin solution in EtOH.

Z-γ-D-Glu(α-OBzl)-(L)-Boc-(D)*meso*-A_2pm(D)-NHNHBoc (12)——A solution of **10** (743 mg, 2 mmol) and *N*-methylmorpholine (202 mg, 2 mmol) in CH_2Cl_2 (15 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 25 min at the same temperature, a solution of the silyl ester of **11** [prepared from **11** (809 mg, 2 mmol) and bis(trimethylsilyl)acetamide (BSA) (1.46 g, 7.2 mmol) in a mixture of CH_2Cl_2 (8 ml) and dimethylformamide (DMF) (2 ml) by stirring for 30 min at room temperature] was added dropwise and the whole was stirred at −10 °C for 1 h and at 0 °C for 30 min. After concentration of the reaction mixture, AcOEt (80 ml) was added to the residue and the solution was washed with 4% HCl and H_2O. Drying over $MgSO_4$ and evaporation gave 1.35 g (89%) of **12** as an amorphous solid: mp 70—73 °C. $[\alpha]_D$ 23.2° ($c=0.55$, MeOH). IR (Nujol): 3280, 1700 (br), 1685 cm^{-1}. NMR (CD_3OD) δ: 1.25—2.56 (10H, m), 1.45 (18H, s), 3.89—4.50 (3H, m), 5.09 (2H, s), 5.16 (2H, s), 7.31 (10H, s). *Anal.* Calcd for $C_{37}H_{51}N_5O_{12}$: C, 58.64; H, 6.78; N, 9.24. Found: C, 58.78; H, 7.02; N, 9.34.

γ-D-Glu(α-OH)-(L)*meso*-A_2pm (3)——A solution of **12** (400 mg, 0.53 mmol) in a mixture of MeOH (3 ml) and H_2O (3 ml) was cooled to 0 °C and 1 N NaOH (1.21 ml) was added dropwise with stirring. After being stirred for 1 h at the same temperature, the reaction mixture was concentrated to about 2 ml and poured into a mixture of H_2O (10 ml) and ether (5 ml). The aqueous layer was acidified with 3% HCl and extracted with AcOEt (20 ml). The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation gave 327 mg of **13** as an amorphous powder.

Compound **13** (320 mg, 0.48 mmol) was dissolved in trifluoroacetic acid (3 ml) and stirred at room temperature for 30 min. After evaporation of the trifluoroacetic acid, the residue was pulverized with ether and the resulting powder was dissolved in H_2O (8 ml). This solution was cooled to 0 °C and acidified to pH 1 with 0.1 N H_2SO_4. A solution of $NaIO_4$ (256 mg, 1.2 mmol) in H_2O (3 ml) was added and the mixture was stirred for 1 h at 0 °C. After decomposition of the excess oxidant with $NaHSO_3$, the reaction mixture was brought to pH 2 with saturated $NaHCO_3$ solution and concentrated to about 2 ml. The concentrate was applied to a column of Diaion HP-20 and eluted with 70% aqueous MeOH. The eluate was concentrated to give **14** (168 mg) as an amorphous powder, which was dissolved in MeOH (5 ml) and hydrogenated over 10% Pd–C (40 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was dissolved in H_2O (2 ml) and lyophilized to give 86 mg (52% from **12**) of **3** as an amorphous powder: $[\alpha]_D$ −17.0° ($c=0.2$, H_2O). *Rf*, 0.13 (A), 0.64 (B). IR (Nujol): 3500—2500 (br), 1710 (sh), 1620 cm^{-1}. NMR (D_2O) δ: 1.20—2.60 (10H, m), 3.60—3.92 (2H, m), 4.0—4.2 (1H, m). Amino acid anal. Glu 1.00, A_2pm 1.03. *Anal.* Calcd for $C_{12}H_{21}N_3O_7 \cdot 3/2H_2O$: C, 41.60; H, 6.98; N, 12.13. Found: C, 41.30; H, 6.84; N, 12.27.

Z-L-Ala-γ-D-Glu(α-OBzl)-(L)-Boc-(D)*meso*-A_2pm(D)-NHNHBoc-(L)-Gly (17)——A solution of **15** (2.21 g, 5 mmol) and *N*-methylmorpholine (505 mg, 5 mmol) in CH_2Cl_2 (22 ml) was cooled to −15 °C and isobutyl chloroformate (682 mg, 5 mmol) was added dropwise with stirring at −16——12 °C. After stirring for 30 min, the silyl ester of **16** [prepared from **16** (2.48 g, 5 mmol) and BSA (2.54 g, 12.5 mmol) in a mixture of CH_2Cl_2 (20 ml) and DMF (4 ml) by stirring for 15 min at room temperature] was added dropwise and the mixture was stirred at −20——10 °C for 2 h and at 0 °C for 1 h. The reaction mixture was evaporated and the residue was poured into AcOEt (150 ml). This solution was washed with 3% HCl (50 ml) and H_2O (100 ml), dried over $MgSO_4$ and evaporated to afford 3.71 g (82%) of **17** as white crystals: mp 145—149 °C. $[\alpha]_D$ 3.4° ($c=0.4$, MeOH). IR (Nujol): 3300, 1720, 1670 (br) cm^{-1}. NMR ($CDCl_3$–CD_3OD) δ: 1.20—2.42 (31H, m), 3.94 (2H, s), 5.12 (2H, s), 5.16 (2H, s), 7.31 (10H, s). *Anal.* Calcd for $C_{42}H_{59}N_7O_{14} \cdot H_2O$: C, 55.80; H, 6.80; N, 10.85. Found: C, 56.01; H, 6.58; N, 10.62.

L-Ala-γ-D-Glu(α-OH)-(L)*meso*-A_2pm(L)-Gly (4)——A solution of **17** (1.0 g, 1.1 mmol) in a mixture of MeOH (10 ml) and H_2O (7 ml) was cooled to 0 °C and 1 N NaOH (2.8 ml) was added with stirring. The mixture was stirred for 3 h at room temperature and evaporated *in vacuo*. H_2O (10 ml) and ether (8 ml) were added to the residue. The aqueous layer was acidified with 3% HCl and extracted with AcOEt (25 ml). The organic layer was washed with brine and dried over $MgSO_4$. Evaporation gave 700 mg (80%) of the hydrolyzed compound as a white powder.

This compound (600 mg, 0.75 mmol) was dissolved in trifluoroacetic acid (4 ml) and stirred for 30 min at room temperature. Trifluoroacetic acid was evaporated off *in vacuo* and the residue was dissolved in H_2O (10 ml). The solution was cooled to 0 °C and acidified to pH 1 with 0.1 N H_2SO_4. A solution of $NaIO_4$ (401 mg, 1.87 mmol) in H_2O (3 ml) was added with stirring and the mixture was stirred for 1 h at 0 °C. The excess oxidant was decomposed with $NaHSO_3$ and the reaction mixture was brought to pH 2 with saturated $NaHCO_3$ solution. The solution was applied to a column of Diaion HP-20 and eluted with 50% aqueous MeOH. The eluate was concentrated and lyophilized to give a white powder, which was dissolved in a mixture of H_2O (10 ml) and MeOH (10 ml). The solution was hydrogenated over 10% Pd–C (50 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was dissolved in H_2O (5 ml). Lyophilization gave 197 mg (43% from **17**) of **4**: mp 187 °C. $[\alpha]_D$ −10.6° ($c=0.4$, MeOH). *Rf*, 0.38 (B). IR (Nujol): 3250 (br), 1630 cm^{-1}. NMR (D_2O) δ: 1.55 (3H, d, $J=7$ Hz), 1.2—2.8 (10H, m), 3.6—4.5 (4H, m), 3.85 (2H, s). Amino acid anal. Ala 1.00, Glu 1.03, Gly 0.98, A_2pm

1.01. *Anal.* Calcd for $C_{17}H_{29}N_5O_9 \cdot 5/2H_2O$: C, 41.46; H, 6.96; N, 14.22. Found: C, 41.24; H, 6.97; N, 14.25.

Z-γ-D-Glu(α-OBzl)-(L)-Boc(D)*meso*-A_2pm(D)-NHNHBoc-(L)-Gly (18)——A solution of **10** (1.49 g, 4 mmol) and *N*-methylmorpholine (404 mg, 4 mmol) in CH_2Cl_2 (20 ml) was cooled to −15 °C and isobutyl chloroformate (546 mg, 4 mmol) was added dropwise with stirring at −15—−13 °C. After stirring for 30 min at the same temperature, the silyl ester of **16** [prepared from **16** (1.84 g, 4 mmol) and BSA (2.85 g, 14 mmol) in a mixture of CH_2Cl_2 (20 ml) and DMF (4 ml) by stirring for 20 min at room temperature] was added dropwise and the whole was stirred at −30—−10 °C for 2 h and at 0 °C for 30 min. The reaction mixture was evaporated and the residue was poured into AcOEt (40 ml). The solution was washed with 3% HCl (20 ml) and H_2O (20 ml), dried over $MgSO_4$ and evaporated to give 3.0 g (90%) of **18** as a white powder: mp 92—97 °C. $[\alpha]_D$ 11.9° ($c=1.1$, MeOH). IR (Nujol): 3300, 1735, 1700, 1640 cm^{-1}. NMR (CD_3OD) δ: 1.40 (18H, s), 3.93 (2H, s), 3.80—4.53 (3H, m), 5.04 (2H, s), 5.12 (2H, s), 7.27 (10H, s). *Anal.* Calcd for $C_{39}H_{54}N_6O_{13} \cdot H_2O$: C, 56.24; H, 6.78; N, 10.09. Found: C, 56.36; H, 6.64, N, 9.88.

γ-D-Glu(α-OH)-(L)*meso*-A_2pm(L)-Gly (5)——A solution of **18** (2.5 g, 3 mmol) in a mixture of MeOH (20 ml) and H_2O (15 ml) was treated with 1 N NaOH (7.7 ml) at room temperature. After being stirred for 1 h at the same temperature, the mixture was concentrated *in vacuo*. H_2O (15 ml) and ether (10 ml) were added to the residue. The aqueous layer was acidified with 3% HCl and extracted with AcOEt (40 ml). The extract was washed with H_2O and dried over $MgSO_4$. Evaporation gave 1.97 g (86%) of the hydrolyzed product as a white powder, which was used without further purification in the next reaction. Thus, this compound (1.50 g, 2.1 mmol) was dissolved in trifuloroacetic acid (6 ml) and the solution was stirred for 1 h at room temperature. Trifluoroacetic acid was evaporated *in vacuo* and the residue was dissolved in H_2O (10 ml). The solution was cooled to 0 °C and acidified to pH 1 with 0.1 N H_2SO_4. A solution of $NaIO_4$ (1.11 g, 5.2 mmol) in H_2O (10 ml) was added with stirring and the mixture was stirred for 1 h at 0 °C. The excess oxidant was decomposed with $NaHSO_3$ and the reaction mixture was brought to pH 2 with saturated $NaHCO_3$ solution. The solution was applied to a column of Diaion HP-20 and eluted with 50% aqueous MeOH. The eluate was concentrated and lyophilized to give a white powder, which was dissolved in a mixture of H_2O (25 ml) and MeOH (25 ml) and 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 H_2O (10 ml). Lyophilization gave 422 mg (44% from **18**) of **5**: mp 174 °C. $[\alpha]_D$ −26.0° ($c=0.3$, H_2O). *Rf*, 0.10 (A), 0.50 (B). IR (Nujol): 3300 (br), 1630 (br) cm^{-1}. NMR (D_2O) δ: 1.2—2.8 (10H, m), 3.65—3.95 (4H, m), 4.2—4.6 (1H, m). Amino acid anal. Glu 1.00, Gly 0.97, A_2pm 1.02. *Anal.* Calcd for $C_{14}H_{24}N_4O_8 \cdot H_2O$: C, 42.64; H, 6.65; N, 14.21. Found: C, 42.38; H, 6.72, N, 14.01.

D-Lactoyl-L-Ala-D-Glu (6)——A solution of **19** (1.09 g, 2.6 mmol) in a mixture of H_2O (5 ml) and MeOH (10 ml) was cooled to 0 °C and 1 N NaOH (9 ml) was added. After being stirred for 3 h at 0 °C, the reaction mixture was evaporated. The residue was dissolved in H_2O (3 ml) and the solution was applied to a charcoal column and eluted with 50% aqueous EtOH. The eluate was concentrated and lyophilized to give 410 mg (53%) of **6** as an amorphous powder: $[\alpha]_D$ −13.1° ($c=0.4$, H_2O). *Rf* 0.30 (C). NMR (D_2O) δ: 1.34 (3H, d, $J=7$ Hz), 1.40 (3H, d, $J=7$ Hz), 1.80—2.70 (4H, m), 4.10—4.80 (3H, m). Amino acid anal. Ala 1.00, Glu 1.02. *Anal.* Calcd for $C_{11}H_{18}N_2O_7 \cdot 1/2H_2O$: C, 44.14; H, 6.40; N, 9.36. Found: C, 43.95; H, 6.56; N, 9.08.

Z-(L)*meso*-A_2pm(L)-GlyOBzl (21)——A sample (1.37 g, 2 mmol) of **20** was dissolved in trifluoroacetic acid (10 ml) and the solution was stirred for 30 min at room temperature. After evaporation of trifluoroacetic acid, the residue was pulverized with ether and the resulting powder was dissolved in H_2O (20 ml). The solution was cooled to 0 °C and acidified to pH 1 with 0.1 N H_2SO_4. A solution of $NaIO_4$ (1.07 g, 5 mmol) in H_2O (10 ml) was added with stirring and the mixture was stirred for 1 h at 0 °C. The excess oxidant was decomposed with $NaHSO_3$ and the reaction mixture was brought to pH 4 with saturated $NaHCO_3$ solution. The resulting precipitate was collected by filtration, washed with H_2O and dried under reduced pressure to give 740 mg (76%) of **21**: mp 162—167 °C. $[\alpha]_D$ −16.1° ($c=0.6$, MeOH). IR (Nujol): 3280, 1720, 1690, 1650 cm^{-1}. NMR (CD_3OD) δ: 1.28—2.16 (6H, m), 3.47—3.77 (1H, m), 3.96 (2H, s), 4.17 (1H, m), 5.05 (2H, s), 5.12 (2H, s), 7.30 (10H, s). *Anal.* Calcd for $C_{24}H_{29}N_3O_7 \cdot H_2O$: C, 58.89; H, 6.38; N, 8.58. Found: C, 58.60; H, 6.18; N, 8.61.

***meso*-A_2pm(L)-Gly (7)**——A solution of **21** (980 mg, 2 mmol) in AcOH (10 ml) was hydrogenated over 10% Pd–C (200 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated. The residue was dissolved in H_2O (4 ml) and lyophilized to give 490 mg (87%) of **7** as a white powder: mp 141—145 °C. $[\alpha]_D$ 37.9° ($c=0.4$, H_2O). *Rf*, 0.14 (A). IR (Nujol): 3200 (sh), 2800—2300, 1660 cm^{-1}. NMR (D_2O) δ: 1.27—2.30 (6H, m), 3.76—4.27 (4H, m). Amino acid anal. Gly 1.00, A_2pm 1.03. *Anal.* Calcd for $C_9H_{17}N_3O_5 \cdot 2H_2O$: C, 38.16; H, 7.47; N, 14.83. Found: C, 37.87; H, 7.40; N, 14.85.

***N*-Caprylyl-D-Glu(OH)OBzl (26)**——Caprylyl chloride (3.17 g, 20 mmol) was added dropwise to a solution of α-benzyl D-glutamate (4.74 g, 20 mmol) in a mixture of CH_2Cl_2 (50 ml) and BSA (4.10 g, 20 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was evaporated to give an oily residue, to which H_2O (50 ml) was added. This mixture was extracted with AcOEt (100 ml). The extract was washed with H_2O (50 ml), dried over $MgSO_4$ and evaporated to give a crystalline residue (7.10 g), which was recrystallized from isopropyl ether to give 6.0 g (83%) of **26**: mp 79—80 °C. $[\alpha]_D$ −2.6° ($c=0.2$, $CHCl_3$). IR (Nujol): 3300, 3200 (sh), 1740, 1620 cm^{-1}. NMR ($CDCl_3$) δ: 0.87 (3H, t, $J=7$ Hz), 1.0—2.6 (16H, m), 4.5—5.0 (1H, m), 5.20 (2H, s), 6.35 (1H, d, $J=7$ Hz), 7.40 (10H, s), 9.90 (1H, s). *Anal.* Calcd for $C_{20}H_{29}NO_5$: C, 66.10; H, 8.04; N, 3.85. Found: C, 65.82; H, 8.07; N, 3.93.

***N*-Caprylyl-γ-D-Glu(α-OBzl)-(L)-Boc-(D)*meso*-A_2pm(D)-NHNHBoc (28)**——A solution of **26** (3.63 g, 10 mmol) in tetrahydrofuran (THF, 50 ml) was cooled to 0 °C, then *N*-hydroxysuccinimide (1.15 g, 10 mmol) and dicyclohexylcarbodiimide (2.06 g, 10 mmol) were added. The mixture was stirred for 12 h at 0 °C, then the resulting precipitate was filtered off and the filtrate was evaporated to give a crystalline residue, which was recrystallized from isopropyl ether–isopropanol to give 3.65 g (79%) of the *N*-hydroxysuccinimide ester of **26** (mp 83—85 °C). This active ester (2.30 g, 5 mmol) was added at room temperature to a solution of **11** (2.02 g, 5 mmol) in a mixture of dioxane (40 ml), H_2O (40 ml) and triethylamine (1.01 g, 10 mmol). The reaction mixture was stirred for 18 h at room temperature. Dioxan was evaporated off *in vacuo* and the residue was brought to pH 3 with 1 N H_2SO_4 and extracted with AcOEt (100 ml). The extract was washed with H_2O, dried over $MgSO_4$ and evaporated to give 3.20 g (85%, 67% from **26**) of **28** as white crystals: mp 145—147 °C. $[\alpha]_D$ 17.5 ($c=0.2$, $CHCl_3$). IR (Nujol): 3300, 1740 (sh), 1720, 1680, 1650, 1640 cm^{-1}. NMR ($CDCl_3$) δ: 0.7—2.5 (43H, m), 4.0—4.8 (3H, m), 5.20 (2H, s), 5.5—5.8 (1H, m), 6.3—6.6 (2H, m), 6.8—7.1 (2H, m), 7.35 (5H, s), 9.10 (1H, s). *Anal.* Calcd for $C_{37}H_{59}N_5O_{11}$: C, 59.26; H, 7.93; N, 9.34. Found: C, 59.01; H, 7.81; N, 9.10.

***N*-Caprylyl-γ-D-Glu(α-OH)-(L)*meso*-A_2pm (8)**——A solution of **28** (1.50 g, 2 mmol) in AcOH (40 ml) was hydrogenated over 10% Pd–C (300 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was dissolved in trifluoroacetic acid (15 ml). After stirring for 30 min at room temperature, trifluoroacetic acid was evaporated off *in vacuo*. The residual oil was dissolved in a mixture of H_2O (35 ml) and 1 N H_2SO_4 (3 ml). A solution of $NaIO_4$ (1.02 g, 5 mmol) in H_2O (10 ml) was added at 0 °C and the mixture was stirred for 1 h at the same temperature. After decomposition of excess oxidant with $NaHSO_3$, the reaction mixture was applied to a column of Diaion HP-20 and eluted with 50% aqueous MeOH. The eluate was concentrated *in vacuo* to give 710 mg (78%) of **8** as white crystals: mp 182—184 °C. $[\alpha]_D$ −9.7° ($c=0.2$, H_2O). *Rf*, 0.37 (A), 0.34 (B). IR (Nujol): 3500, 3350, 1720, 1660, 1640, 1620 cm^{-1}. NMR (D_2O) δ: 0.68—2.60 (25H, m), 3.80 (1H, t, $J=7$ Hz), 4.16—4.50 (2H, m). Amino acid anal. Glu 1.00, A_2pm 1.11. *Anal.* Calcd for $C_{20}H_{35}N_3O_8 \cdot 1/2H_2O$: C, 52.85; H, 7.98; N, 9.25. Found: C, 52.65; H, 7.88; N, 9.25.

Z-(L)*meso*-A_2pm (23)——A sample (1.0 g, 1.86 mmol) of **22** was dissolved in trifluoroacetic acid (5 ml) and the solution was stirred for 30 min at room temperature. After evaporation of the trifluoroacetic acid, the residue was pulverized with ether and the resulting powder was dissolved in H_2O (20 ml). This solution was cooled to 0 °C and acidified to pH 1 with 0.1 N H_2SO_4. A solution of $NaIO_4$ (995 mg, 4.65 mmol) in H_2O (8 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 applied to a column of Diaion HP-20 and eluted with 50% aqueous MeOH. The eluate was evaporated *in vacuo* to give 470 mg (76%) of **23** as white crystals. An analytical sample was recrystallized from H_2O. mp 245 °C. $[\alpha]_D$ −12.7° ($c=0.2$, AcOH). *Rf*, 0.53 (A). IR (Nujol): 3480, 3350, 3200, 1690 cm^{-1}. NMR ($NaHCO_3$–D_2O) δ: 1.25—2.15 (6H, m), 3.60—4.15 (2H, m), 5.17 (2H, s), 7.45 (5H, s). *Anal.* Calcd for $C_{15}H_{20}N_2O_6 \cdot 1/2H_2O$: C, 54.05; H, 6.35; N, 8.40; Found: C, 53.77; H, 6.23; N, 8.59.

Z-(L)-Boc-(D)*meso*-A_2pm (24)——Triethylamine (253 mg, 2.5 mmol) was added to a solution of **23** (333 mg, 1 mmol) in a mixture of H_2O (7 ml) and dioxane (7 ml). Next, Boc-ON (295 mg, 1.2 mmol) was added at 0 °C, and the reaction mixture was stirred for 20 h at room temperature. Dioxan was evaporated off and H_2O was added to the residue. The mixture was washed with ether, acidified with 3% HCl and extracted with AcOEt (30 ml). The extract was washed with brine, dried over $MgSO_4$ and concentrated to give 423 mg (100%) of **24** as an amorphous powder: mp 49 °C. $[\alpha]_D$ −3.2° ($c=0.24$, MeOH). IR (Nujol): 3300, 1750—1650 (br) cm^{-1}. NMR (DMSO-d_6) δ: 1.2—2.0 (15H, m), 3.63—4.16 (2H, m), 5.06 (2H, s), 6.93 (1H, d, $J=8$ Hz), 7.33 (5H, s). *Anal.* Calcd for $C_{20}H_{28}N_2O_8$: C, 56.59; H, 6.65; N, 6.60. Found: C, 56.34; H, 6.73; N, 6.50.

Boc-(D)*meso*-A_2pm (25)——A solution of **24** (850 mg, 2 mmol) in AcOH (10 ml) was hydrogenated over 10% Pd–C (150 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was dissolved in H_2O (4 ml). This solution was applied to a column of Diaion HP-20 and eluted with 50% aqueous MeOH. The eluate was concentrated to give 470 mg (81%) of **25** as white crystals: mp > 250 °C. $[\alpha]_D$ 21.5° ($c=0.2$, AcOH). *Rf*, 0.38 (A). IR (Nujol): 3400, 1710, 1640 cm^{-1}. NMR (D_2O) δ: 1.16—2.16 (15H, m), 3.6—4.2 (2H, m). *Anal.* Calcd for $C_{12}H_{22}N_2O_6$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.53; H, 7.81; N, 9.72.

***N*-Stearoyl-D-Glu(OH)OBzl (27)**——Stearoyl chloride (6.04 g, 20 mmol) was added to a solution of α-benzyl D-glutamate (4.74 g, 20 mmol) in a mixture of CH_2Cl_2 (50 ml) and BSA (4.10 g, 20 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was evaporated *in vacuo*. H_2O (80 ml) was added to the residue and the mixture was extracted with AcOEt (100 ml). The extract was washed with H_2O, dried over $MgSO_4$ and evaporated to give a crystalline residue (9.60 g), which was recrystallized from isopropyl ether to give 8.97 g (89%) of **27**: mp 80—81 °C. $[\alpha]_D$ −4.3° ($c=0.2$, $CHCl_3$). IR (Nujol): 3300, 1750, 1710, 1650 cm^{-1}. NMR ($CDCl_3$) δ: 0.87 (3H, m), 1.0—2.7 (34H, m), 4.5—5.0 (1H, m), 5.17 (2H, s), 6.33 (1H, d, $J=8$ Hz), 7.33 (5H, s), 10.30 (1H, s). *Anal.* Calcd for $C_{30}H_{49}NO_5$: C, 71.53; H, 9.81, N, 2.78. Found: C, 71.24, H, 9.94; N, 2.89.

***N*-Stearoyl-γ-D-Glu(α-OBzl)-(L)-Boc-(D)*meso*-A_2pm (29)**——A solution of **27** (5.03 g, 10 mmol) in THF (50 ml) was cooled to 0 °C. *N*-Hydroxysuccinimide (1.15 g, 10 mmol) and dicyclohexylcarbodiimide (2.06 g, 10 mmol) were added to the solution. After stirring for 18 h at 0 °C, the resulting precipitate was filtered off and filtrate was

evaporated to give a crystalline residue, which was recrystallized from a mixture of isopropyl ether and isopropyl alcohol to give 5.70 g (95%) of the *N*-hydroxysuccinimide ester of **27** (mp 90—92 °C). This active ester (1.20 g, 2 mmol) was added to a solution of **25** (580 mg, 2 mmol) in a mixture of CH_2Cl_2 (50 ml), MeOH (10 ml) and triethylamine (404 mg, 4 mmol). The reaction mixture was stirred for 18 h at room temperature, then evaporated *in vacuo*. H_2O (20 ml) was added to the pasty residue, then the mixture was acidified with 1 N H_2SO_4 and extracted with AcOEt (50 ml). The extract was washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent gave 1.46 g (94%, 89% from **27**) of **29**: mp 125—127 °C. $[\alpha]_D$ −3.7° (c=0.2, $CHCl_3$). IR (Nujol): 3300, 1750—1700 (br), 1650 (br) cm^{-1}. NMR (DMSO-d_6) δ: 0.86 (3H, m), 1.0—2.4 (51H, m), 4.0—4.5 (3H, m), 5.13 (2H, s), 6.90 (1H, m), 7.36 (5H, s), 7.7—8.3 (2H, m). *Anal.* Calcd for $C_{42}H_{69}N_3O_{10}$: C, 65.00; H, 8.96; N, 5.42. Found: C, 64.87; H, 9.01; N, 5.45.

***N*-Stearoyl-γ-D-Glu(α-OH)-(L)*meso*-A_2pm (9)**——A solution of **29** (775 mg, 1 mmol) in AcOH (21 ml) was hydrogenated over 10% Pd–C (150 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated and the residue was dissolved in trifluoroacetic acid (10 ml). After stirring for 30 min at room temperature, trifluoroacetic acid was evaporated off *in vacuo*. H_2O (20 ml) was added to the residue, and the resulting precipitate was collected by filtration, washed with H_2O (50 ml) and dried over P_2O_5 under reduced pressure to give 545 mg (89%) of **9**: mp 194—198 °C. $[\alpha]_D$ −7.9° (c=0.4, AcOH). *Rf*, 0.39 (A), 0.40 (B). IR (Nujol): 3250 (sh), 1720, 1640 cm^{-1}. NMR (NaOD–D_2O) δ: 0.8—2.8 (45H, m), 3.12—3.40 (1H, m), 4.00—4.32 (2H, m). Amino acid anal. Glu 1.00, A_2pm 1.09. *Anal.* Calcd for $C_{30}H_{55}N_3O_8 \cdot 3/2H_2O$: C, 58.80; H, 9.54; N, 6.86. Found: C, 58.72; H, 9.70; N, 6.68.

Hydrochloride of 9[12]——A sample of **9** (306 mg, 0.5 mmol) was dissolved in AcOH (20 ml) and hydrogen chloride gas was bubbled into the cooled and stirred solution during a period of 10 min. The mixture was evaporated and the crystalline residue was washed with ether to give 295 mg (92%) of the hydrochloride of **9**: mp 125 °C (dec.). *Anal.* Calcd for $C_{30}H_{56}ClN_3O_8 \cdot H_2O$: C, 56.27; H, 9.13; N, 6.56; Cl, 5.54. Found: C, 55.99; H, 9.07; N, 6.61; Cl, 5.27.

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