Syntheses of Several Dihydrochalcone-Amino Acid and -Peptide Conjugates

Shintaro KAMIYA,* Sachiko ESAKI,** Naoko SHIBA*
and Takeshi HOSHI***

*Laboratory of Organic Chemistry, **Laboratory of Food Chemistry
and ***Laboratory of Physiology,
School of Food and Nutritional Sciences, University of Shizuoka,
Yada 395, Shizuoka 422, Japan

Received December 12, 1988

In order to find out some specific blockers of the membrane transport of amino acids and small peptides in animal small intestine and kidney, the following dihydrochalcone (DHC)-amino acid and -peptide conjugates were synthesized. [*N*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yloxy]acetyl]glycyl-glycine, *N*^6-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yloxy]acetyl]lysine, *O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]homoserine, [*O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]homoseryl]glycine, *N*-glycyl-*O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]homoserine, and [*O*-[(4,4',6'-trihydroxy-DHC)-2'-yl]homoseryl]glycine.

Among the naturally occurring dihydrochalcone (DHC) derivatives, phlorizin¹⁾ is produced in most species of the genus *Malus* and has the structure of 2'-(β -D-glucopyranosyloxy)-4,4',6'-trihydroxy-DHC (**1**). Compound **1** is known as a powerful inhibitor²⁾ of Na⁺-dependent D-glucose uptake in the small intestine and kidney of animals. Its inhibition constant (*K*_i) is usually 1000 times lower than the *K*_m value of D-glucose, and the inhibition is purely competitive.³⁾ Phloretin (**2**), the aglycone of **1**, is much less potent than **1** in its ability to block Na⁺-dependent D-glucose transport, and the mode of its action is non-competitive. It has generally been considered that the sugar carrier has two separate binding sites, the "sugar" and "phenol" sites, and that phlorizin binds strongly to the carrier by interacting with the two sites.⁴⁾

At the present time, the active transport of such organic solutes as glucose, amino acids and peptides across the intestinal and renal epithelia is known to be mediated by respective carriers (protein) located in the brush border membranes. The cation dependence of these carriers is different in each. D-Glucose carrier is Na⁺-dependent in the presence of Na⁺;

however, it becomes H⁺-dependent when Na⁺ is absent.⁵⁾ Phlorizin competitively inhibits both Na⁺-dependent and H⁺-dependent D-glucose transport. The carrier for neutral amino acids is completely Na⁺-dependent,⁶⁾ and that for small peptides (di- and tripeptides) is entirely dependent on the H⁺ gradient.^{6,7)} Phloretin was found to partially inhibit both Na⁺-dependent amino acid and H⁺-dependent peptide transport, as it does for the Na⁺ (H⁺)-dependent sugar transport (Hoshi, unpublished observations).

From an analogy with the biological function of **1**, it may be expected that, among the phloretin-amino acid or peptide conjugates, there must be some which possess the properties of interacting with amino acid and peptide carriers, respectively, to inactivate their transport action. If such blockers can be found, they will be powerful tools for an extensive elucidation of the transport mechanisms and the significance of amino acid and small peptide carriers in the small intestine.

For the foregoing purpose, the present study was designed to synthesize the titled compounds.

The catalytic hydrogenation of 5-hydroxy-

4. R = H
 6. R = β -D-glucosyl
 7. R = $\text{CH}_2\text{CO}_2\text{C}_2\text{H}_5$
 8. R = $\text{CH}_2\text{CO}_2\text{H}$

chloride by a method similar to that already described to afford [*N*-benzyloxycarbonyl-*O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]-homoseryl]glycine ethyl ester (**22**). After alkaline hydrolysis, **22** gave [*N*-benzyloxycarbonyl-*O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]-homoseryl]glycine (**23**), which was subsequently deprotected with hydrogen to yield **21** in a 43.9% overall yield based on **22**.

Hydrogenolysis of **16** in the presence of an equimolar amount of hydrochloric acid in dioxane afforded *O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]homoserine methyl ester hydrochloride (**24**) in an 81.5% yield. The treatment of **24** with *N*-benzyloxycarbonylglycine in DMF using DEPC as a catalyst resulted in the formation of *N*-[(benzyloxycarbonyl)glycyl]-*O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]homoserine methyl ester (**25**) in a 67.9% yield. Alkaline hydrolysis of **25** proceeded smoothly to yield *N*-[(benzyloxycarbonyl)glycyl]-*O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]homoserine (**26**) in a quantitative yield. Hydrogenolytic deprotection of **26** gave *N*-glycyl-*O*-[(6'-hydroxy-4,4'-dimethoxy-DHC)-2'-yl]homoserine (**27**) in a 69.9% yield.

To prepare [*O*-[(4,4',6'-trihydroxy-DHC)-2'-yl]homoseryl]glycine hydrochloride (**28**), direct benzylation of **2** with three molar equivalents of benzyl bromide in the presence of potassium carbonate in DMF was examined. However, the desired 4,4',6'-tribenzyloxy-2'-hydroxy-DHC (**29**) was obtained in a poor yield, together with many unidentified by-products. As the yield of **29** was not improved by varying the conditions, we finally adopted the benzylation of 4',5,7-trihydroxyflavanone (naringenin, **30**) with three molar equivalents of benzyl bromide as already described, and 4,4',6'-tribenzyloxy-2'-hydroxychalcone (**31**) could be obtained in a good yield.

Treatment of **31** with **15** in acetone in the presence of potassium carbonate for 48 hr under reflux, following by chromatographic purification on a silica gel column, yielded *N*-benzyloxycarbonyl-*O*-[(4,4',6'-tribenzyloxychalcone)-2'-yl]homoserine methyl ester (**32**), which was subsequently hydrolyzed with sodium hydroxide in aqueous acetone to give *N*-benzyloxycarbonyl-*O*-[(4,4',6'-tribenzyloxychalcone)-2'-yl]homoserine (**33**). Coupling of **33** with **19** in the presence of DEPC and

triethylamine in DMF resulted in the formation of [*N*-benzyloxycarbonyl-*O*-[(4,4',6'-tribenzyloxychalcone)-2'-yl]homoseryl]glycine benzyl ester (**34**, 85.8%), which was hydrogenolyzed to give **28** in an 87% yield.

The results of a biological investigation of the synthetic compounds (**11**, **14**, **21**, **27** and **28**) will be reported by one of the authors (Hoshi) elsewhere.

Experimental

Thin-layer chromatography (TLC) was performed on Silica Gel G (Merck), with detection by charring with sulfuric acid, or by spraying with 2% ferric chloride in ethanol or with 1% ninhydrin in butanol. Column chromatography was carried out on Kieselgel G 60, and optical rotations were measured with a Horiba SEPA-200 digital polarimeter. NMR spectra (internal tetramethylsilane) were recorded with a Hitachi R-90 H spectrometer, and UV spectra were measured with a Hitachi double-wave-length (557) spectrophotometer.

2',6'-Dihydroxy-4,4'-dimethoxydihydrochalcone (4). To a solution of **3** (3 g) in ethyl acetate (130 ml) were added 10% Pd-C (2 g) and triethylamine (1.5 ml), before the mixture was stirred under hydrogen for 4 hr at room temperature. After filtration, the filtrate was evaporated and a residue was crystallized from chloroform-ethanol to give **4** (2.6 g, 86.1%) as colorless needles, mp 142°C; *R*_f 0.65 (chloroform-ethyl acetate, 8:1). In the absence of triethylamine, the reduction was completed after 72 hr. *Anal.* Found: C, 67.51; H, 5.86. Calcd. for C₁₇H₁₈O₅: C, 67.55; H, 5.96%. NMR δ_C (CDCl₃): 29.20 (CH₂ β); 45.36 (CH₂ α); 54.88, 55.23 (each OCH₃); 93.17 (C-3', C-5'); 104.54 (C-1'); 113.65 (C-2, 6); 133.27 (C-1); 157.36 (C-4); 163.86 (C-2', 6'); 165.36 (C-4'); 204.48 (C=O). δ_H (CDCl₃): 1.60 (1H, s, 2'-OH); 2.70~3.03 (2H, m, CH₂); 3.16~3.40 (2H, m, CH₂); 3.70 (6H, s, 2 OCH₃); 5.79 (2H, s, H-3', 5'); 6.66 (2H, d, *J*=7 Hz, H-3, 5); 7.00 (2H, d, *J*=7 Hz, H-2, 6); 9.60 (1H, br. s, 6'-OH).

2'-(β -D-Glucopyranosyloxy)-6'-hydroxy-4,4'-dimethoxydihydrochalcone (6). To a mixture of **4** (285 mg) and **5** (342 mg) in dry quinoline (30 ml) was added freshly prepared silver carbonate (275 mg) and 4A molecular sieves (1 g), before the mixture was stirred overnight at room temperature with all light excluded. The reaction mixture was centrifuged and the supernatant was poured into ice-cooled 10% acetic acid with stirring. After standing overnight in a refrigerator, the precipitate was collected by filtration, and dissolved in pyridine. The solution was again poured into 10% acetic acid, and the mixture was treated as just described. The well-dried precipitate was dissolved in 0.1 N methanolic sodium methoxide (50 ml),

and the solution was left overnight at room temperature. After neutralizing with Amberlite IR-120 resin (H⁺), the filtered solution was evaporated, and the residual syrup was chromatographed on a silica gel column, using ethyl acetate saturated with water as the eluent. The fractions containing only **6** were collected and evaporated to a syrup (430 mg), which was crystallized from 50% ethanol as needles. Yield, 170 mg (36.0%); mp 163°C (50% ethanol); $[\alpha]_D$ -46° (*c*=0.5, ethanol); *R*_f 0.35 (ethyl acetate sat. with water). *Anal.* Found: C, 59.27; H, 6.00. Calcd. for C₂₃H₂₈O₁₀: C, 59.48; H, 6.03%. Calcd. for [M]_D (methyl α -D-glucoside, +30900)+[M]_D (**4**, 0): +30900. Calcd. for [M]_D (methyl β -D-glucoside, -6600)+[M]_D (**4**, 0): -6600. Found for [M]_D: -21334. The glycosidic linkage of **6** was, therefore, of β -configuration. Acetate, mp 78°C. *Anal.* Found: C, 58.26; H, 5.61. Calcd. for C₃₃H₃₈O₁₅: C, 58.75; H, 5.64%. NMR δ_H (CDCl₃): 1.93, 1.96, 2.00, 2.05, 2.08 (each s, 3H, OAc); 2.70~3.10 (1H, m, CH₂-CH₂); 3.70 (6H, s, 2OCH₃); 4.05~4.20 (2H, m, sugar CH₂); 4.80~5.10 (4H, sugar H-2~5); 5.16 (1H, d, *J*=8 Hz, sugar H-1); 6.30 (1H, d, *J*=2 Hz, H-3'); 6.40 (1H, d, *J*=2 Hz, H-5'); 6.66 (2H, d, *J*=8 Hz, H-3, 5); 7.03 (2H, d, *J*=8 Hz, H-2, 6).

2'-(Ethoxycarbonyl)methoxy-6'-hydroxy-4,4'-dimethoxydihydrochalcone (7). To a solution of **4** (3.02 g) in DMF (30 ml) were added ethyl monochloroacetate (6 ml) and anhydrous potassium carbonate (1.4 g), before the mixture was stirred for 72 hr at room temperature. The reaction mixture was poured into ice-cooled 1% hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with water twice and dried (MgSO₄). After filtration, the filtrate was evaporated and the residue was crystallized from benzene to yield **7** (2.23 g, 54.5%) as needles, mp 117°C. *Anal.* Found: C, 64.75; H, 6.21. Calcd. for C₂₁H₂₄O₇: C, 64.94; H, 6.19%. NMR δ_C (CDCl₃): 14.10 (CH₃); 29.60 (CH₂ β); 45.82 (CH₂ α); 55.22, 55.51 (each OCH₃); 61.53, 65.51 (each CH₂); 91.41 (C-3'); 94.49 (C-5'); 105.89 (C-1'); 113.76 (C-3', 5'); 129.32 (C-2, 6); 133.70 (C-1); 159.79 (C-4); 160.43 (C-6'); 165.53 (C-2'); 167.48 (CO); 167.63 (C-4'); 204 (CO). δ_H (CDCl₃): 1.23 (3H, t, *J*=7 Hz, CH₃); 2.95 (2H, t, *J*=6 Hz, CH₂); 3.43 (2H, t, *J*=6 Hz, CH₂); 3.73 (6H, s, 2 OCH₃); 4.13 (2H, q, *J*=7 Hz, CH₂); 4.66 (2H, s, -OCH₂O-); 5.70 (1H, d, *J*=2 Hz, H-5'); 6.03 (1H, d, *J*=2 Hz, H-3'); 6.73 (2H, d, *J*=8 Hz, H-3, 5); 7.13 (2H, d, *J*=8 Hz, H-2, 6).

2'-(Carboxy)methoxy-6'-hydroxy-4,4'-dimethoxydihydrochalcone (8). A suspension of **7** (7 g) in 5% potassium hydroxide (300 ml) was stirred at room temperature until a clear solution was obtained (after about 6 hr). The solution was acidified with 10% hydrochloric acid, before the white precipitate was collected by filtration and recrystallized from methanol to afford **8** (5.36 g, 82.6%) as needles, mp 173°C. *Anal.* Found: C, 43.70; H, 3.82. Calcd. for C₁₉H₂₀O₇: C, 43.85; H, 3.85%. NMR δ_C (DMSO-*d*₆): 29.09 (CH₂ β); 45.11 (CH₂ α); 54.89, 55.64 (each OCH₃);

65.35 (CH₂); 91.62 (C-3'); 94.29 (C-5'); 105.70 (C-1'); 113.60 (C-3, 5); 129.18 (C-2, 6); 133.18 (C-1); 157.36 (C-4); 160.57 (C-6); 165.34, 165.83 (C-2', 4'); 169.34 (CO); 204.65 (CO). δ_{H} (CDCl₃): 2.80 (2H, t, $J=6$ Hz, CH₂); 3.33 (2H, t, $J=6$ Hz, CH₂); 3.61, 3.70 (each 3H, s, OCH₃); 4.68 (2H, s, OCH₂); 5.95 (2H, d, $J=1.5$ Hz, H-3', 5'); 6.66 (2H, d, $J=8$ Hz, H-3, 5); 7.00 (2H, d, $J=8$ Hz, H-2, 6).

[*N*-[(6'-Hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yloxy]acetyl]glycyl]glycine ethyl ester (**10**). To a mixture of **8** (1.44 g) and **9** (0.8 g) in DMF (20 ml) were added DEPC (0.72 ml) and triethylamine (1.12 ml), before the solution was stirred for 1 hr at 0°C and then allowed to stand overnight at room temperature. The reaction mixture was poured into ice-cooled water with stirring, and the precipitate was collected, washed with water and recrystallized from methanol to give **10** (1.5 g, 75.0%) as needles, mp 165~167°C. *Anal.* Found: C, 59.33; H, 6.02, N, 5.74. Calcd. for C₂₅H₃₀O₆N₂: C, 59.76; H, 5.98, N, 5.58%. NMR δ_{C} (DMSO-*d*₆): 13.99 (CH₃); 28.94 (CH₂ β); 45.20 (CH₂ α); 54.89, 55.60 (each OCH₃); 60.83 (CH₂); 67.46 (CH₂); 91.62 (C-3'); 94.39 (C-5'); 106.01 (C-1'); 113.59 (C-3, 5); 129.17 (C-2, 6); 133.12 (C-1); 157.33 (C-4); 160.67 (C-6'); 165.16 (C-2'); 165.31 (C-4'); 167.19, 168.67, 169.54, 204.52 (each CO). δ_{H} (DMSO-*d*₆): 1.16 (3H, t, $J=6$ Hz, CH₃); 2.63~2.93 (4H, m, -CH₂-CH₂); 3.36, 3.70 (each 3H, s, OCH₃); 4.00 (2H, $J=6$ Hz, CH₂); 4.60 (2H, s, OCH₂CO); 5.95 (2H, d, $J=1.5$ Hz, H-3', 5'); 6.66 (2H, d, $J=8$ Hz, H-3, 5); 7.00 (2H, d, $J=8$ Hz, H-2, 6); 8.00~8.27 (2H, m, 2NH); 13.2 (1H, s, OH).

[*N*-[(6'-Hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yloxy]acetyl]glycyl]glycine (**11**). To a solution of **10** (1.4 g) in DMF (32 ml) was added 1 N sodium hydroxide solution (7 ml), before the mixture was stirred for 1.5 hr at room temperature. After the reaction was complete, the mixture was acidified with 4% hydrochloric acid. The precipitate was collected and recrystallized from methanol or ethyl acetate to give **11** (almost quantitative) as needles, mp 180~181°C. *Anal.* Found: C, 58.06; H, 5.46; N, 5.78. Calcd. for C₂₃H₂₆N₂O₉: C, 58.23; H, 5.49; N, 5.91%. *Rf* 0.56 (chloroform-methanol-water, 7:5:1). NMR δ_{C} (DMSO-*d*₆): 29.05 (CH₂ β); 40.71, 41.65 (each CH₂); 45.33 (CH₂ α); 54.97, 55.67 (each OCH₃); 67.56 (C-3); 91.71 (C-3'); 94.50 (C-5'); 106.04 (C-1'); 113.70 (C-3, 5); 129.27 (C-2, 6); 133.24 (C-9); 157.44 (C-4); 160.81 (C-6'); 165.29 (C-2'); 165.56 (C-4'); 167.31, 168.82, 171.06, 204.62 (each CO). δ_{H} (DMSO-*d*₆): 2.90 (2H, t, $J=6$ Hz, CH₂); 3.40 (2H, t, $J=6$ Hz, CH₂); 3.70, 3.80 (each 3H, OCH₃); 4.70 (2H, s, -OCH₂CO); 3.50~4.00 (4H, m, 2NH-CH₂-CO); 6.03 (2H, d, $J=2$ Hz, H-3', 5'); 6.76 (2H, d, $J=8$ Hz, H-3, 5); 7.13 (2H, d, $J=8$ Hz, H-2, 6); 8.23 (2H, t, $J=4$ Hz, 2NH).

N^ε-Benzyloxycarbonyl-*N*^ε-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yloxy]acetyl-L-lysine benzyl ester (**13**). To a mixture of **8** (1.31 g) and **12** (1.93 g) in DMF (25 ml), DEPC (0.62 ml) and triethylamine (1 ml) were added,

before the mixture was allowed to stand for 1 hr at 0°C and then overnight at room temperature. After the reaction was complete, the solution was poured into ice-cooled water with stirring, and the precipitate was extracted with ethyl acetate. The organic layer was washed successively with 4% hydrochloric acid, 4% sodium hydrogencarbonate solution and water, and then dried (MgSO₄) and evaporated to a residue, which was recrystallized from ethanol to give **13** (1.25 g, 48.3%) as needles, mp 139~141°C. *Rf* 0.50 (chloroform-ethyl acetate, 2:1). *Anal.* Found: C, 67.42; H, 6.18; N, 3.98. Calcd. for C₄₀H₄₄N₂O₁₀: C, 67.42; H, 6.18; N, 3.93%. NMR δ_{C} (DMSO-*d*₆): 22.81, 28.45 (each CH₂); 28.99 (CH₂ β); 30.30, 38.33 (each CH₂); 45.27 (CH₂ α); 53.98 (CH), 54.88, 55.58 (each OCH₃); 65.48, 65.84 (each PhCH₂); 67.67 (CH), 91.63 (C-3'); 94.26 (C-5'); 106.00 (C-1'); 113.57 (C-3, 5); 127.40, 127.66, 127.92, 128.24 (each PhCH₂-2~6); 128.30 (C-2, 6), 133.07 (C-1), 135.85, 136.78 (each PhCH₂-1); 156.08 (CO); 157.33 (C-4'); 160.61 (C-6'); 165.11, 165.34 (C-2', 4'); 166.55, 172.18, 204.51 (each CO). δ_{H} (CDCl₃): 0.80~1.85 (6H, m, 3CH₂); 2.66~3.50 (m, 6H, 3CH₂); 3.70, 3.73 (each 3H, s, OCH₃); 5.03, 5.10 (each 2H, s, PhCH₂); 6.78 (1H, NH); 6.08 (2H, br. s, H-3', 5'); 6.75 (2H, d, $J=8$ Hz, H-3, 5); 7.10 (2H, d, $J=8$ Hz, H-2, 6); 7.33 (10H, s, 2PhCH₂).

N^ε-[(6'-Hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yloxy]acetyl-L-lysine (**14**). A mixture of **13** (500 mg) and 10% Pd-C (500 mg) in acetic acid (15 ml) was stirred under a hydrogen atmosphere for 7.5 hr at room temperature. The catalyst was filtered off through a celite bed, and the filtrate was evaporated to give **14** as long needles (263 mg, 70.3%), mp 173~175°C, $[\alpha]_{\text{D}}^{+10}$ ($c=1$, acetic acid). *Rf* 0.82 (ethanol-acetic acid-water, 3:2:1). The ninhydrin test was positive. *Anal.* Found: C, 56.29; H, 6.17; N, 5.13. Calcd. for C₂₅H₃₂N₂O₈·2½H₂O: C, 56.28; H, 6.94; N, 5.25%. NMR δ_{C} (CD₃COOD): 16.00, 19.95, 20.66, 20.10 (each CH₂, CH₂ β); 30.00 (CH₂); 36.65 (CH₂ α); 46.00, 46.30 (each OCH₃); 58.50 (CH₂ α , CH); 83.50 (C-3'); 86.00 (C-5'); 105.00 (C-3, 5, 1'); 124.60 (C-1); 148.50 (C-4); 151.70 (C-6'); 157.00 (C-4', 2'); 158.00, 168.50, 197.50 (each CO). δ_{H} (CD₃COOD): 1.05~2.10 (m, lysine CH₂); 2.70~3.39 (m, CH₂-CH₂, lysine CH₂); 3.60, 3.64 (each 3H, s, OCH₃); 4.50 (2H, s, OCH₂CO); 5.76 (1H, s, H-3'); 5.94 (1H, s, H-5'); 6.57 (2H, d, $J=8$ Hz, H-3, 5); 6.90 (2H, d, $J=6$ Hz, H-2, 6).

N-Benzyloxycarbonyl-*O*-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine methyl ester (**16**). A mixture of **4** (4.05 g), **15** (4.41 g) and anhydrous potassium carbonate (2.04 g) in DMF (70 ml) was stirred for 72 hr at room temperature. The reaction mixture was poured into ice-cooled water (600 ml) with stirring, acidified with 10% hydrochloric acid, and then extracted with ethyl acetate. The organic layer was washed with water, dried (MgSO₄) and evaporated. The residue was crystallized from methanol to give **16** as needles (4.5 g, 60.9%), mp 115~116°C

(methanol). *R_f* 0.52 (chloroform–ethyl acetate, 10:1). *Anal.* Found: C, 65.30; H, 6.01; N, 2.52. Calcd. for C₃₀H₃₃NO₉: C, 65.33; H, 5.99; N, 2.54%. NMR δ_c (CDCl₃): 28.77 (CH₂ β); 30.09 (CH₂); 45.15 (CH₂ α); 50.91 (CH₂); 51.89 (CH₃); 54.82, 55.53 (each OCH₃); 65.15 (CH); 65.59 (CH₂); 91.25 (C-5'); 93.91 (C-3'); 105.55 (C-1'); 113.54 (C-3, 5); 127.54, 127.69, 128.18, 129.02 (PhCH₂-2~6); 132.95 (C-1); 136.72 (PhCH₂-1); 156.05 (CO); 157.29 (C-4); 161.32 (C-6'); 165.43, 165.80 (C-2', 4'); 172.12, 204.10 (each CO). δ_H (CDCl₃): 1.85~2.60 (2H, m, -OCH₂CH₂-); 2.70~3.43 (4H, m, Ar-CH₂-CH₂CO); 3.60 (3H, s, COCH₃); 3.70, 3.75 (each 3H, s, OCH₃); 4.00 (2H, t, *J*=6 Hz, OCH₂CH₂); 4.43 (1H, d, *J*=6 Hz, C-CH-N); 5.03 (2H, s, PhCH₂); 5.33 (1H, d, *J*=6 Hz, -NH); 5.80 (1H, d, *J*=2 Hz, H-3'); 6.00 (1H, d, *J*=2 Hz, H-5'); 6.70 (2H, d, *J*=8 Hz, H-3, 5); 7.06 (2H, d, *J*=8 Hz, H-2, 6); 7.25 (5H, s, PhCH₂).

N-Benzyloxycarbonyl-*O*-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine (**17**). To a suspension of **16** (4 g) in 4% sodium hydroxide solution (100 ml) was added acetone (about 100 ml) until a clear solution was obtained. After standing for 2 hr at room temperature, the reaction mixture was neutralized with 4% hydrochloric acid with ice-cooling. To this, acetone (80 ml) was added until the syrup deposited was completely dissolved, and then the solution was acidified with 4% hydrochloric acid. After standing for a while, the crystals deposited were collected by filtration and recrystallized from ethanol to yield 3.6 g (92.3%) of **17** as needles, mp 142~143°C. *Anal.* Found: C, 64.68; H, 5.77; N, 2.63. Calcd. for C₂₉H₃₁NO₉: C, 64.80; H, 5.77; N, 2.61%. NMR δ_c (DMSO-*d*₆): 28.67 (CH₂ β); 30.16 (CH₂); 44.86 (CH₂ α); 50.86 (CH₂); 54.82, 55.53 (each OCH₃); 65.41 (CH₂, CH); 91.23 (C-5'); 93.85 (C-3'); 105.64 (C-1'); 113.55 (C-3, 5); 127.49, 127.60, 128.13, 129.98 (C-2, 6; PhCH₂-2~6); 132.89 (C-1); 136.78 (PhCH₂-1); 156.04 (CO); 157.24 (C-4); 161.28 (C-6'); 165.35, 165.60 (C-2', 4'); 173.17, 204.13 (each CO). δ_H (DMSO-*d*₆): 1.66~2.60 (2H, m, OCH₂CH₂); 2.63~3.50 (4H, m, PhCH₂CH₂CO); 3.63, 3.73 (each 3H, s, OCH₃); 3.86~4.32 (3H, m, OCH₂CH₂, CHNH); 4.96 (2H, s, PhCH₂); 6.00 (2H, s, H-3', 5'); 6.70 (2H, d, *J*=8 Hz, H-3, 5); 7.06 (2H, d, *J*=8 Hz, H-2, 6); 7.23 (5H, s, PhCH₂); 7.63 (1H, d, *J*=8 Hz, NH).

O-[(6'-Hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine (**18**). A mixture of **17** (1 g) and 10% Pd-C (500 mg) in tetrahydrofuran (20 ml) containing acetic acid (2 ml) was stirred under hydrogen for 6 hr, and then treated as described for the preparation of **14** from **13** to afford **18** as needles (680 mg, 90.7%), mp 189~191°C. *Anal.* Found: C, 62.77; H, 5.98; N, 3.65. Calcd. for C₂₁H₂₅NO₇: C, 62.53; H, 6.20; N, 3.47%.

Compound **18** (419 mg) was dissolved by heating in 70 ml of 1N hydrochloric acid. After being cooled to ambient temperature, the crystallization solution was filtered, and the crystals were allowed to air dry to yield

370 mg (80.5%) of the hydrochloride salt (**18'**) as long needles, mp 185°C (sint. 180°C).

[*N*-Benzyloxycarbonyl-*O*-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine]glycine benzyl ester (**20**). To a mixture of **17** (2.42 g) and **19** (1.52 g) in DMF (25 ml) were added DEPC (0.72 ml) and triethylamine (1.26 ml), before the mixture was stood for 1 hr at 0°C and then overnight at room temperature. Ice-cooled water was added to the reaction mixture, and the white viscous oil deposited was extracted with a large quantity of ethyl acetate. The organic layer was treated as described for the preparation of **13** from **8**, and the final crystalline residue was recrystallized from ethanol to give **20** as needles (2.56 g, 82.6%), mp 150~153°C. *Anal.* Found: C, 64.50; H, 5.30; N, 5.20. Calcd. for C₂₉H₃₀N₂O₉: C, 64.27; H, 5.45; N, 5.09%. NMR δ_c (DMSO-*d*₆): 28.77 (CH₂ β); 31.03, 41.35 (each CH₂); 44.90 (CH₂ α); 51.80 (CH₂); 54.83, 55.56 (each OCH₃); 65.24 (CH); 65.48, 65.79 (each CH₂); 91.24 (C-3'); 93.80 (C-5'); 105.72 (C-1'); 113.56 (C-3, 5); 126.70~128.26 (2PhCH₂-2~6); 129.05 (C-2, 6); 132.98 (C-1); 135.78, 136.75 (2PhCH₂-1); 155.81 (CO); 157.24 (C-4); 161.35 (C-6'); 165.37 (C-2'); 165.54 (C-4'); 169.45, 171.73, 204.31 (each CO). δ_H (CDCl₃): 1.80~4.33 (13H, m, -OCH₂CH₂CH(NH)CONHCH₂, PhCH₂CH₂-CO); 3.63, 3.80 (each 3H, s, OCH₃); 5.00, 5.02 (each 2H, s, PhCH₂); 6.03 (2H, s, H-3', 5'); 6.73 (2H, d, *J*=8 Hz, H-3, 5); 7.06 (2H, d, *J*=8 Hz, H-2, 6); 7.30 (10H, s, 2PhCH₂).

[*O*-[(6'-Hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine]glycine (**21**). A mixture of benzyl ester (**20**, 1 g) and 10% Pd-C (600 mg) in warm acetic acid (35 ml, 50°C) was stirred under hydrogen for 2 hr at 50°C and then for 3 hr at room temperature. The reaction mixture was treated in the same way as described for the preparation of **14** from **13** to give a syrup, which was crystallized from ethanol to give **21** (660 mg, 98.5%) as needles, mp 114~118°C. *Anal.* Found: C, 59.50; H, 6.38; N, 5.86. Calcd. for C₂₃H₂₈N₂O₈: C, 60.00; H, 6.09; N, 6.09%. NMR δ_c (CD₃CO₂D): 30.44 (CH₂ β); 31.60, 42.30 (each CH₂); 46.50 (CH₂ α); 52.30 (CH₂); 55.57, 56.10 (each OCH₃); 65.44 (CH); 92.95 (C-3'); 95.13 (C-5'); 106.89 (C-1'); 114.78 (C-3, 5); 130.22 (C-2, 6); 134.60 (C-1); 158.84 (C-4); 162.50 (C-6'); 167.09 (C-2'); 167.63 (C-4'); 170.36, 177.73, 205.91 (each CO). δ_H (CD₃CO₂D): 2.64~3.44 (4H, m, PhCH₂CH₂CO); 2.12~4.46 (m, -OCH₂CH₂CH-CONHCH₂); 3.66, 3.70 (each 3H, OCH₃); 6.00 (2H, m, H-3', 5'); 6.66 (2H, d, *J*=8 Hz, H-3, 5); 7.00 (2H, d, *J*=8 Hz, H-2, 6).

N-Benzyloxycarbonyl-*O*-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine]glycine ethyl ester (**22**). To a mixture of **17** (1.61 g) and glycine ethyl ester hydrochloride (0.41 g) in DMF (15 ml) were added DEPC (0.48 g) and triethylamine (0.84 ml), before the mixture was treated in the same way as that described for the preparation of **20**. The final syrup was crystallized from

methanol to give **22** as plates (1.82 g, 97.9%), mp 143°C (methanol). *R_f* 0.16 (chloroform-ethyl acetate, 10:1). *Anal.* Found: C, 63.58; H, 6.03; N, 4.48. Calcd. for $C_{33}H_{38}N_2O_{10}$: C, 63.66; H, 6.11; N, 4.50%. NMR δ_c ($CDCl_3$): 13.97 (CH_3); 28.84 ($CH_2 \beta$); 31.14, 40.81 (each CH_2); 44.98 ($CH_2 \alpha$); 51.80 (CH_2); 54.83, 55.50 (each OCH_3); 60.42 (CH_2); 65.57 (CH, CH_2); 91.27 (C-3'); 93.86 (C-5'); 105.72 (C-1'); 113.59 (C-3, 5); 127.57, 127.67, 127.85, 128.21 ($PhCH_2$ -2~6); 129.09 (C-2, 6); 133.04 (C-1); 136.81 ($PhCH_2$ -1); 155.87 (C=O); 157.32 (C-4); 161.46 (C-6'); 165.47 (C-2'); 165.75 (C-4'); 169.54, 171.74, 204.36 (each CO). δ_H ($CDCl_3$): 1.16 (3H, t, $J=6$ Hz, CH_3); 4.06 (2H, q, $J=6$ Hz, CH_2); 1.70~4.50 (m, $OCH_2CH_2CHCONHCH_2$); 3.75, 3.80 (6H, each 3H, s, $2OCH_3$); 5.00 (2H, s, $PhCH_2$); 6.03 (2H, s, H-3', 5'); 6.73 (2H, d, $J=8$ Hz, H-3, 5); 7.10 (2H, d, $J=8$ Hz, H-2, 6); 7.26 (5H, s, $PhCH_2$); 7.60 (1H, d, $J=6$ Hz, NH); 8.33 (1H, t, $J=6$ Hz, NH); 13.52 (1H, s, 6'-OH).

[*N*-Benzyloxycarbonyl]-[O-(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine methyl ester (23). To a solution of **22** (1 g) in DMF (10 ml) was added 1 N sodium hydroxide solution (2 ml), and the solution was allowed to stand overnight at room temperature. The solution was poured into ice-cooled 1% hydrochloric acid and extracted with ethyl acetate. The extract was washed with water, dried over $MgSO_4$ and evaporated. The residual syrup was crystallized from ethanol-ether to give **23** (515 mg, 53.9%), mp 157~159°C. *Anal.* Found: C, 61.92; H, 5.76; N, 4.67. Calcd. for $C_{31}H_{34}N_2O_{12}$: C, 62.63; H, 5.72; N, 4.71%. NMR δ_c ($DMSO-d_6$): 28.79 ($CH_2 \beta$); 31.12, 41.36 (each CH_2); 44.93 ($CH_2 \alpha$); 51.75 (CH_2); 54.85, 55.58 (each OCH_3); 65.50 (CH_2 , CH); 91.28 (C-3'); 93.82 (C-5'); 105.72 (C-1'); 113.57 (C-3, 5); 127.52, 127.64 ($PhCH_2$ -2~6); 128.19 (C-2, 6); 133.00 (C-1); 136.78 ($PhCH_2$ -1); 155.81 (CO); 157.26 (C-4); 161.39 (C-6'); 165.38, 165.56 (C-2', 4'); 170.91, 171.46, 204.36 (each CO). δ_H ($DMSO-d_6$): 1.70~4.46 (m, $OCH_2CH_2CHCONHCH_2$); 2.66~3.43 (4H, m, $PhCH_2CH_2CO$); 3.70, 3.80 (each 3H, s, OCH_3); 5.00 (2H, s, $PhCH_2$); 6.05 (2H, s, H-3', 5'); 6.73 (2H, d, $J=8$ Hz, H-3, 5); 7.10 (2H, d, $J=8$ Hz, H-2, 6); 7.30 (5H, s, $PhCH_2$); 7.60 (1H, d, $J=8$ Hz, NH); 8.20 (1H, br. s, NH); 13.40 (1H, 6'-OH).

Hydrogenolysis of 23. A mixture of **23** (500 mg) and 10% Pd-C (400 mg) in dioxane (15 ml) was stirred under hydrogen for 5 hr. The usual work-up gave **21** in an almost quantitative yield.

O-[(6'-Hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine methyl ester hydrochloride (**24**). To a solution of **16** (553 mg) in dioxane (10 ml) were added 4.6 N hydrochloric acid in dioxane (0.25 ml) and 10% Pd-C (260 mg), before the mixture was stirred under hydrogen for 6 hr at room temperature. To this, warm acetic acid was added to dissolve the reaction product deposited, and the mixture was filtered. The filtrate was evaporated, and

the crystalline residue was washed with ether to yield **24** as plates (370 mg, 81.5%), mp 183~184°C. *Anal.* Found: C, 58.23; H, 6.07; N, 3.00. Calcd. for $C_{22}H_{27}O_7N \cdot HCl$: C, 58.28; H, 6.18; N, 3.09%. NMR δ_c ($CDCl_3$): 28.82 ($CH_2 \beta$); 29.41 (CH_2); 45.16 ($CH_2 \alpha$); 49.43 (CH_3); 52.80 (CH_2); 54.93, 55.69 (each OCH_3); 64.61 (CH); 91.57 (C-3'); 94.11 (C-5'); 106.04 (C-1'); 113.64 (C-3, 5); 129.11 (C-2, 6); 133.04 (C-1); 157.33 (C-4); 160.83 (C-6'); 165.02 (C-2'); 165.16 (C-4'); 169.25, 204.11 (each CO). δ_H ($CDCl_3$): 2.10~4.42 (m, OCH_2CH_2CH , $PhCH_2CH_2CO$, $CHNH$, $PhOCH_2$); 3.62, 3.65, 3.76 (each 3H, s, $2OCH_3$ and CO_2CH_3); 6.02 (2H, s, H-3', 5'); 6.70 (2H, d, $J=8$ Hz, H-3, 5); 7.06 (2H, d, $J=8$ Hz, H-2, 6); 8.83 (br. s, 3H, NH_3^+); 13.32 (1H, s, OH).

N-[(Benzyloxycarbonyl)glycyl]-O-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine methyl ester (**25**). Compound **24** (1.81 g) was added to DMF (20 ml), and the suspension was stirred until a clear solution was obtained. After a while, long needles (**24'**) were deposited from the solution. After filtration, **24'** was washed with ether. This compound was an adduct of **24** and DMF, mp 179~180°C. *Anal.* Found: C, 56.93; H, 6.63; N, 5.33. Calcd. for $C_{25}H_{34}O_8N_2 \cdot HCl$: C, 57.03; H, 6.65; N, 5.32%. NMR δ_H ($DMSO-d_6$): 2.00~4.40 (m, OCH_2CH_2 , $PhCH_2CH_2CO$, $CH(NH)CO$); 2.73, 2.86 (each 3H, s, CH_3); 3.63, 3.66, 3.76 (each 3H, s, OCH_3); 6.03 (2H, s, H-3', 5'); 6.73 (2H, d, $J=8$ Hz, H-3, 5); 7.06 (2H, d, $J=8$ Hz, H-2, 6); 7.83 (1H, s, CHO); 8.73 (3H, br. s, NH_3^+). Compound **24'** was returned to the mother liquid, to which *N*-benzyloxycarbonylglycine (803 mg), triethylamine (1.2 ml) and DEPC (0.64 ml) were added, before the clear solution was stood for 1 hr at 0°C and then overnight at room temperature. The reaction mixture was worked up as described for the preparation of **13** from **8**, and the final crystalline residue was recrystallized from ethanol to give **26** as needles (1.65 g, 67.9%), mp 124°C. *Anal.* Found: C, 63.01; H, 5.88; N, 4.89. Calcd. for $C_{32}H_{36}N_2O_{10}$: C, 63.16; H, 5.92; N, 4.61%. NMR δ_c ($DMSO-d_6$): 28.80 ($CH_2 \beta$); 30.30, 43.38 (each CH_2); 45.11 ($CH_2 \alpha$); 48.85 (CH_2); 51.92 (CH_2); 54.88, 55.58 (each OCH_3); 65.09 (CH_2); 65.44 (CH); 91.39 (C-3'); 93.80 (C-5'); 105.61 (C-1'); 113.56 (C-3, 5); 127.57, 127.64 ($PhCH_2$ -2~6); 128.21 (C-2, 6); 132.97 (C-1); 136.89 ($PhCH_2$ -1); 156.34 (CO); 157.29 (C-4); 161.25 (C-6'); 165.37 (C-2'); 165.61 (C-4'); 169.31; 171.72; 204.18 (each CO). δ_H ($DMSO-d_6$): 1.90~4.20 (m, $OCH_2CH_2CH(NH)$, $NHCH_2CO$); 3.50, 3.66, 4.03 (each 3H, s, OCH_3); 4.90 (2H, s, $PhCH_2$); 5.96 (2H, s, H-3, 5); 6.70 (2H, d, $J=8$ Hz, H-3', 5'); 7.06 (2H, d, $J=8$ Hz, H-2, 6); 7.23 (2H, s, $PhCH_2$); 8.23 (1H, d, $J=8$ Hz, NH); 13.40 (1H, s, OH).

N-[(Benzyloxycarbonyl)glycyl]-O-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine (**26**). To a solution of **25** (1.25 g) in DMF (16 ml) was added a 1 N sodium hydroxide solution (2.6 ml), and the mixture was allowed to stand overnight at 23°C. The reaction mixture

was worked up in the same manner as described for the preparation of **23** from **22**. The final residue was crystallized from ethanol to give **26** in a quantitative yield, mp 156°C (ethanol). *Anal.* Found: C, 62.49; H, 5.74; N, 4.74. Calcd. for $C_{31}H_{34}N_2O_{10}$: C, 62.63; H, 5.72; N, 4.71%. NMR δ_c (DMSO- d_6): 30.30 (CH₂ β); 32.04, 44.92 (each CH₂); 46.82 (CH₂ α); 50.70 (CH₂); 55.54, 56.01 (each OCH₃); 66.00 (CH); 68.32 (CH₂); 92.72 (C-3'); 94.73 (C-5'); 106.68 (C-1'); 114.75 (C-3, 5); 128.79, 129.00, 129.35, 130.21 (PhCH₂-2~6); 134.66 (C-1); 137.21 (PhCH₂-1); 158.75 (CO, C-4); 162.84 (C-6'); 167.04 (C-2'); 167.89 (C-4'); 172.82, 176.23, 205.88 (each CO). δ_H (CD₃CO₂D): 2.13~2.65 (2H, m, OCH₂CH₂); 2.65~3.53 (4H, m, COCH₂CH₂Ph); 3.70~4.30 (OCH₂CH₂, NHCH₂CO); 3.66, 3.69 (each 3H, s, OCH₃); 4.60~4.90 (1H, br. s, CHNH); 5.03 (2H, s, PhCH₂); 5.90, 6.00 (each s, d, J = 1.5 Hz, H-3', 5'); 6.70 (2H, d, J = 8 Hz, H-3, 5); 7.10 (2H, d, J = 8 Hz, H-2, 6); 7.21 (5H, s, PhCH₂).

N-Glycyl-O-[(6'-hydroxy-4,4'-dimethoxydihydrochalcone)-2'-yl]homoserine (27). A mixture of **26** (710 mg) and 10% Pd-C (480 mg) in acetic acid (20 ml) was stirred under hydrogen for 6 hr at room temperature. The reaction mixture was worked up as usual to give a syrup, which was crystallized from aqueous ethanol to give **27** as needles (407 mg, 69.9%), mp 166~168°C, decomposed above 200°C. *Anal.* Found: C, 56.46; H, 5.98; N, 5.84%. Calcd. for $C_{23}H_{28}N_2O_8 \cdot 1\frac{1}{2}H_2O$: C, 56.67; H, 6.36; N, 5.75%. NMR δ_c (CD₃CO₂D): 30.33 (CH₂ β); 32.07, 42.11 (each CH₂); 46.81 (CH₂ α); 51.17 (CH₂); 55.57, 56.03 (each OCH₃); 65.94 (CH); 92.73 (C-3'); 94.78 (C-5'); 106.71 (C-1'); 114.79 (C-3, 5); 130.24 (C-2, 6); 134.66 (C-1); 158.83 (C-4'); 162.40 (C-6'); 162.83 (C-2'); 167.10, 168.29 (C-2', CO); 178.14, 205.85 (each CO). δ_H (CD₃CO₂D): 2.10~2.63 (2H, m, OCH₂C₂); 2.92, 3.40 (each 2H, t, J = 6 Hz, COCH₂CH₂Ph); 3.63, 3.76 (each 3H, 2OCH₃); 3.90~4.30 (4H, m, OCH₂CH₂, NHCH₂CO); 4.66~4.90 (1H, m, CHNH); 5.96, 6.10 (each 1H, d, J = 2 Hz, H-3', 5'); 6.76 (2H, d, J = 8 Hz, H-3, 5); 7.13 (2H, d, J = 8 Hz, H-2, 6).

4,4',6'-Tribenzyloxy-2'-hydroxydihydrochalcone (29). To a solution of **2** (3.97 g) in DMF (110 ml) were added benzyl bromide (5.2 ml) and anhydrous potassium carbonate (5.94 g), before the mixture was stirred for 72 hr at room temperature. The reaction mixture was poured into ice-cooled 1% hydrochloric acid with stirring, and the reddish brown syrup deposited was extracted with ethyl acetate. The extract was washed with water, dried over MgSO₄ and then evaporated. The residual syrup was chromatographed on a silica gel column, eluting with benzene-ethyl acetate (9:1), to give **29**, which was crystallized from ethanol (1 g, 14.7%), mp 105~106°C. *Anal.* Found: C, 79.20; H, 5.93. Calcd. for $C_{36}H_{32}O_5$: C, 79.41; H, 5.88%. *Rf* 0.90 (benzene-ethyl acetate, 9:1). NMR δ_c (CDCl₃): 29.28 (CH₂ β); 45.74 (CH₂ α); 69.94, 70.11, 71.16 (each PhCH₂); 92.23 (C-3'); 94.89 (C-5'); 105.95 (C-1'); 114.64 (C-3, 5); 127.26~128.60 (PhCH₂-2, 6); 130.00 (C-

2, 6); 133.55 (C-1); 135.27, 135.81, 137.24 (PhCH₂-1); 156.80 (C-4); 161.74 (C-6'); 164.78 (C-2'); 167.51 (C-4'); 204.40 (CO). δ_H (CDCl₃): 2.66~2.96, 2.96~3.36 (each 2H, m, COCH₂CH₂Ph); 4.93 (6H, s, 3PhCH₂); 5.96, 6.06 (each 1H, d, J = 1.5 Hz, H-3', 5'); 6.70 (4H, s, H-2, 6); 7.20, 7.26 (10H, each s, 2Ph); 13.86 (1H, s, OH).

Further elution of the column gave a mixture of dibenzyloxy-2'-hydroxydihydrochalcones, which could not be separated, as well as other unidentified by-products.

4,4',6'-Tribenzyloxy-2'-hydroxychalcone (4,4',6'-tribenzyloxynaringenin chalcone, 31). A mixture of **30** (2.72 g), benzyl bromide (3.6 ml) and anhydrous potassium carbonate (4.2 g) in dry DMF (70 ml) was stirred for 72 hr at room temperature. The reaction mixture was poured into ice-cooled 1% hydrochloric acid (500 ml), and a yellow precipitate (5.5 g) was collected by filtration, washed with water and dried in air. The well-dried sample was dissolved in benzene and chromatographed on a silica gel column, eluting with benzene-hexane (7:3) to give **31**, which was recrystallized from chloroform-ethanol as yellow plates. Yield, 2.68 g (56.1%), mp 140°C. *Rf* 0.61 (benzene). *Anal.* Found: C, 79.02; H, 5.53. Calcd. for $C_{36}H_{30}O_5$: C, 79.70; H, 5.54%. NMR δ_c (CDCl₃): 70.03, 70.23, 71.37 (each CH₂); 92.43 (C-3'); 95.11 (C-5'); 106.33 (C-1'); 114.98 (C-3, 5); 125.23 (CH α); 127.37~128.85 (3PhCH₂-2~6); 130.07 (C-1); 135.46, 135.90, 136.54 (3PhCH₂-1); 142.61 (CH β); 160.22 (C-6'); 161.61 (C-4); 165.00 (C-2'); 168.73 (C-4'); 194.22 (CO). δ_H (CDCl₃): 5.00 (6H, s, 3PhCH₂); 6.10 (2H, s, H-3', 5'); 6.65 (2H, d, J = 8 Hz, H-3, 5); 6.93 (2H, d, J = 8 Hz, H-2, 6); 7.33 (15H, s, 3PhCH₂); 7.66 (2H, s, H α , β); 14.60 (1H, s, OH).

N-Benzyloxycarbonyl-O-[(4,4',6'-tribenzyloxychalcone)-2'-yl]homoserine methyl ester (32). A mixture of **31** (5.33 g), **15** (3.26 g) and anhydrous potassium carbonate (1.4 g) in dry acetone (100 ml) was refluxed for 48 hr. After filtration, the filtrate was evaporated and the residue was chromatographed on a silica gel column, eluting with benzene-ethyl acetate (15:1) to afford **32** as a syrup (5.7 g, 73.2%), which was contaminated with a minor amount of impurities. NMR δ_c (CDCl₃): 31.25, 51.97 (each CH₂); 52.29 (CH₂); 65.52 (CH); 66.67, 70.02, 70.28, 70.46 (each PhCH₂); 93.36, 93.70 (C-3', 5'); 113.27 (C-1'); 115.10 (C-3, 5); 126.96~130.02 (CH α , 4PhCH₂-2~6); 136.39, 136.46, 136.55 (4PhCH₂-1); 143.85 (CH β); 157.80, 157.90 (C-4, 6'); 160.43 (C-2'); 161.32 (C-4'); 171.92, 193.22 (each CO).

N-Benzyloxycarbonyl-O-[(4,4',6'-tribenzyloxychalcone)-2'-yl]homoserine (33). To a suspension of crude **32** (5.7 g) in 4% sodium hydroxide solution (20 ml) was added acetone until a clear solution was obtained. After standing for 2 hr at room temperature, the solution was neutralized with acetic acid and evaporated. The residue was dissolved in a small quantity of DMF and poured into ice-cooled 1% hydrochloric acid, and the precipitate was extracted with

ethyl acetate. The extract was washed with brine, dried over MgSO_4 , and evaporated. The residue was crystallized from ethanol to give **33** as needles (2.94 g, 52.5%), mp 123.5°C. *Anal.* Found: C, 73.93; H, 5.56; N, 1.73. Calcd. for $\text{C}_{48}\text{H}_{43}\text{NO}_9$: C, 74.13; H, 5.53; N, 1.80%. NMR δ_{C} (CDCl_3): 31.20, 51.91 (each CH_2); 65.00 (CH); 66.83, 70.28, 70.44, 70.58 (4PhCH_2); 93.07, 93.54 ($\text{C}-3',5'$); 112.47 ($\text{C}-1'$); 115.09 ($\text{C}-3,5$); 126.94 ($\text{CH } \alpha$); 127.35~130.19 ($4\text{PhCH}_2-2\sim6$); 136.28 (4PhCH_2-1); 144.73 ($\text{CH } \beta$); 156.10 ($\text{C}-6'$); 157.84, 158.00 ($\text{C}-4$, CONH); 160.53 ($\text{C}-2'$); 161.59 ($\text{C}-4'$); 174.10, 194.24 (each CO). δ_{H} (CDCl_3): 2.13 (2H, br. s, OCH_2CH_2); 3.70~4.00 (2H, m, OH_2CH_2); 4.20~4.50 (1H, m, CHNH); 4.83, 4.86, 4.90, 4.93 (each 2H, s, PhCH_2); 5.83 (1H, d, $J=6$ Hz, NH); 6.08 (2H, d, $J=3$ Hz, H-3',5'); 6.70 (2H, d, $J=8$ Hz, H-3,5); 6.75 (2H, d, $J=8$ Hz, H-2,6); 7.03, 7.13, 7.20 (22H, each s, $\text{CH}=\text{CH}$, 4PhCH_2).

[*N*-Benzyloxycarbonyl-*O*-[$(4,4',6'$ -tribenzyloxychalcone)-2'-yl]homoseryl]glycine benzyl ester (**34**). A mixture of **33** (2.94 g), **19** (1.3 g), triethylamine (1.1 ml) and DEPC (0.65 ml) in DMF (24 ml) was stirred for 1 hr at 0°C and then overnight at room temperature. The reaction mixture was worked-up as described before, and the final syrup was crystallized from chloroform-ethanol to give **34** as needles (3 g, 85.8%), mp 118°C (chloroform-ethanol). *Anal.* Found: C, 73.78; H, 5.61; N, 2.96. Calcd. for $\text{C}_{57}\text{H}_{52}\text{N}_2\text{O}_{10}$: C, 74.03; H, 5.63; N, 3.03%. NMR δ_{C} (CDCl_3): 32.23, 41.30, 52.93 (each CH_2); 65.64 (CH); 66.69, 70.26, 70.41 (5PhCH_2); 93.12, 93.44 ($\text{C}-3',5'$); 112.61 ($\text{C}-1'$); 115.09 ($\text{C}-3,5$); 126.78 ($\text{CH } \alpha$); 126.95~130.16 ($5\text{PhCH}_2-2\sim6$); 136.17~136.46 (5PhCH_2-1); 144.01 ($\text{CH } \beta$); 155.90 ($\text{C}-6'$); 158.08 ($\text{C}-4$, CONH); 160.49 ($\text{C}-2'$); 161.65 ($\text{C}-4'$); 168.99 (CONH); 171.68, 193.74 (each CO). δ_{H} (CDCl_3): 1.80~2.50 (2H, m, OCH_2CH_2); 3.76~4.50 (5H, m, OCH_2CH_2 , $\text{CH}(\text{NH})$, NHCH_2CO); 4.80~5.40 (10H, m, 5PhCH_2); 6.20 (2H, s, H-3',5'); 6.80 (2H, d, $J=9$ Hz, H-3,5); 7.00 (2H, d, $J=9$ Hz, H-2,6); 7.00~8.20 (27H, m, 5PhCH_2 , $\text{CH}=\text{CH}$). UV. λ_{max} (nm): 330.

[*O*-[$(4,4',6'$ -Trihydroxydihydrochalcone)-2'-yl]homoseryl]glycine hydrochloride (**28**). A mixture of **34** (924 mg) and 10% Pd-C (900 mg) in dioxane (70 ml) containing conc. hydrochloric acid (0.5 ml) was stirred under hy-

drogen for 48 hr at room temperature. The reaction mixture was worked up as usual to give a very hygroscopic powder (**28**; 420 mg, 87.3%). The ninhydrin test was positive. NMR δ_{C} ($\text{DMSO}-d_6$): 29.03 ($\text{CH}_2 \beta$); 30.33, 44.99 (each CH_2); 49.65 ($\text{CH}_2 \alpha$); 62.77 (CH_2); 64.31 (CH); 92.08 ($\text{C}-3'$); 95.91 ($\text{C}-5'$); 104.79 ($\text{C}-1'$); 115.01 ($\text{C}-3,5$); 128.95 ($\text{C}-2,6$); 131.21 ($\text{C}-1$); 155.24 ($\text{C}-4$); 161.50 ($\text{C}-6'$); 164.50 ($\text{C}-2'$); 165.40 ($\text{C}-4'$); 168.24, 170.44, 203.87 (each CO). δ_{H} (CDCl_3): 5.93 (2H, s, H-3',5'); 6.60 (2H, d, $J=9$ Hz, H-3,5); 6.90 (2H, d, $J=9$ Hz, H-2,6); 8.85 (3H, br. s, NH_3^+); 13.21 (1H, s, COOH). UV. λ_{max} (nm): 283, 222.

In the absence of hydrochloric acid or in the presence of acetic acid instead of hydrochloric acid, the reaction products showed negative to the ninhydrin test. In the ^1H NMR spectrum, the signals of the NH_3^+ and COOH groups were not recognized. It is thought that the diketopiperazine ring was perhaps formed in **28**.

References

- 1) de L. Koninck, *Liebigs Ann. Chem.*, **15**, 75, 258 (1835); J. S. Challica and A. H. Williams, *Phytochemistry*, **9**, 1261 (1970).
- 2) D. F. Diedrich, *Ark. Biochem. Biophys.*, **117**, 248 (1966); D. F. Diedrich, *Biochim. Biophys. Acta*, **71**, 688 (1966).
- 3) E. Brot-Laroche and F. Alvarado, in "Intestinal Transport," ed. by M. Gilles-Baillien and R. Gilles, Springer, Berlin, 1983, pp. 147~169.
- 4) F. Alvarado, *Biochim. Biophys. Acta*, **135**, 483 (1967).
- 5) T. Hoshi, N. Takuwa, M. Abe and A. Tajima, *Biochim. Biophys. Acta*, **861**, 483 (1986).
- 6) M. Abe, T. Hoshi and A. Tajima, *J. Physiol. (London)*, **394**, 481 (1987).
- 7) T. Hoshi, *Jpn. J. Physiol.*, **35**, 179 (1985).
- 8) Y. Asahina and M. Inubuse, *Yakugaku Zasshi*, **48**, 868 (1928). E. Wollenweber and K. Egger, *Ztsch. Pflanzenphysiol.*, **65**, 427 (1971).
- 9) W. Klyne, *Biochem. J.*, **47**, XLI (1950).
- 10) S. Takuma, Y. Hamada and T. Shioiri, *Chem. Pharm. Bull.*, **38**, 3147 (1982).
- 11) G. D. DuBois, G. A. Crosby, J. F. Lee, R. A. Stephenson and P. C. Wang, *J. Agric. Food Chem.*, **29**, 1261 (1981).