Amino Acids and Peptides. XLVI. Introduction of Carboxyl Function into Pyrazinone by Using Newly Developed Procedure for Pyrazinone Ring Formation: Observation of Immediate Decarboxylation[1,2]

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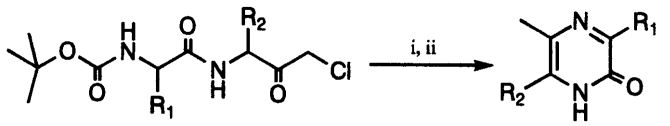
The carboxyl function was easily introduced into a pyrazinone ring by means of a newly developed procedure for pyrazinone ring formation from Asp- or Glu-containing dipeptidyl chloromethyl ketones: during the reaction, rapid decarboxylation from a carboxymethyl group at position 3 of 2(1*H*)-pyrazinone occurred due to the low electron density at position 3 of 2 (1*H*)-pyrazinone.

Key words synthesis; 2(1*H*)-pyrazinone; carboxyl function; decarboxylation; dipeptidyl chloromethyl ketone

Recently, rational design of agonists and antagonists based on the structure of natural peptide hormones has become possible. We are interested in the introduction of pyrazinone derivatives into peptide hormones to obtain super agonists or antagonists by changing the length and restricting the conformation of peptides.[3,4] To introduce pyrazinone derivatives into peptides, amino and/or carboxyl function(s) should be introduced into the pyrazinone ring. This paper deals with the introduction of a carboxyl function into the pyrazinone ring, the observation of rapid decarboxylation from the carboxymethyl group at position 3 of 2(1*H*)-pyrazinone, and studies on the electron density at positions 3 and 6 of 2(1*H*)-pyrazinone.

Previously, we found that 2(1*H*)-pyrazinone derivatives can be easily prepared from dipeptidyl chloromethyl ketones under slightly acidic conditions.[5-7] To introduce a carboxyl function into the pyrazinone ring, Asp or Glu was selected as a constituent amino acid of dipeptidyl chloromethyl ketones. As shown in Chart 1, several Boc-dipeptidyl chloromethyl ketones which contain Asp or Glu residue were prepared. After removal of the Boc group with HCl–dioxane, the dipeptidyl chloromethyl ketone hydrochlorides in MeOH were refluxed for 2 h to give the desired 2(1*H*)-pyrazinone derivatives which contain the protected carboxyl function at position 3 or 6 of 2(1*H*)-pyrazinone (**8**, **11**). However, HCl·H–Asp(OBzl)–Phe–CH_2Cl was unexpectedly converted to a mixture of 3-benzyloxycarbonylmethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (**2**) and 3-methoxycarbonylmethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (**3**) in a ratio of 6:1. Studies on this reaction by measuring the reaction products by HPLC as a function of time indicated that the above ester interchange occurred after pyrazinone ring formation, because the peak of compound **3** was not observed in Fig. 1a, but appeared in Fig. 1b. To find a solvent that would suppress this ester interchange, the cyclization reaction was carried out in various solvents. The reaction rate was studied by measuring the reaction products (**2**, **3**) formed from HCl·H–Asp(OBzl)–Phe–CH_2Cl by HPLC as a function of time. These results are summarized in Fig. 2. The reaction in MeOH gave **2** in low yield owing to the occurrence of ester interchange, but the reaction in other solvents gave **2** in high yield. Therefore, we decided to employ MeCN as a solvent for the cyclization reaction because of the ease of work-up (low boiling point compared with those of other solvents) (Chart 2). To obtain the free carboxyl function-containing pyrazinone derivatives, the benzyl group was removed from **2** by hydrogenation over a Pd catalyst. However, 3,5-dimethyl-6-benzyl-2(1*H*)-pyrazinone (**4**)[7] instead of 3-carboxymethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (**5**) was obtained in this hydrogenation reaction (Chart 2). The reaction rate was studied by measuring the reaction products by HPLC as a function of time. In the reaction mixture, compound **5** was not detected by HPLC (Fig. 3), indicating that decarboxylation occurred immediately after the removal of the benzyl group. To examine whether the above phenomenon is observed at position 6 of pyrazinone, 3-benzyl-5-methyl-6-benzyloxycarbonylmethyl-2(1*H*)-pyrazinone (**8**) was hydrogenated over a Pd catalyst, affording 3-benzyl-5-methyl-6-carboxymethyl-2(1*H*)-pyrazinone (**9**) (Chart 3a) in high yield (83.5%). From these results, it can be deduced that the electron



1. R_1= CH_2CO_2Bzl; R_2=Bzl
7. R_1= Bzl; R_2= CH_2CO_2Bzl
10. R_1= $(CH_2)_2CO_2Bzl$; R_2=Bzl

2*. R_1= CH_2CO_2Bzl; R_2=Bzl
3*. R_1= CH_2CO_2Me; R_2=Bzl
8. R_1= Bzl; R_2= CH_2CO_2Bzl
11. R_1= $(CH_2)_2CO_2Bzl$; R_2=Bzl

Reagents and conditions: i, HCl–dioxane; ii, reflux in MeOH, 2 h.
* Compound **2**, **3** were derived from **1** in a ratio of 6:1

Chart 1

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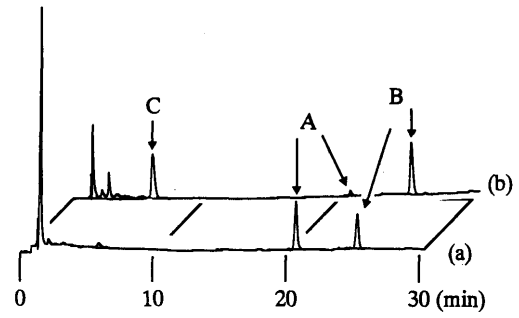


Fig. 1. HPLC Profiles of Cyclization Reaction Mixture in MeOH

(a) 30 min, (b) 360 min. A, H–Asp(OBzl)–Phe–CH_2Cl; B, compound **2**; C, compound **3**. Conditions: see experimental section.

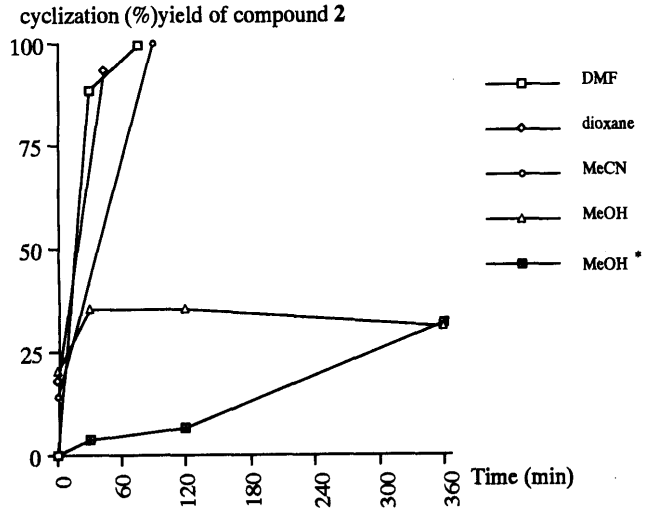
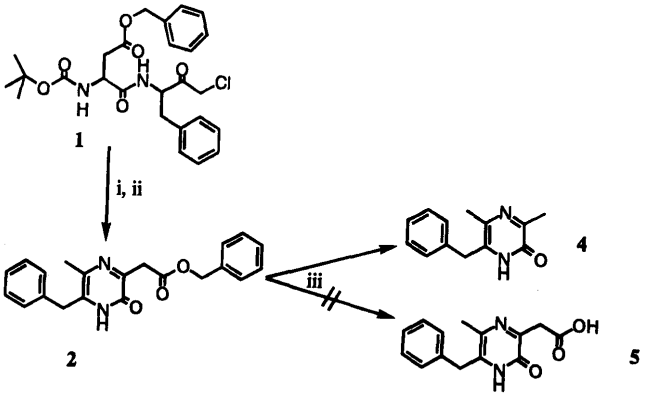


Fig. 2. Comparison of Different Solvents for Cyclization Reaction

* Yield of compound **3**.



Reagents and conditions: i, HCl–dioxane; ii, MeCN, 55 °C, 1 h; iii, Pd/H_2

Chart 2

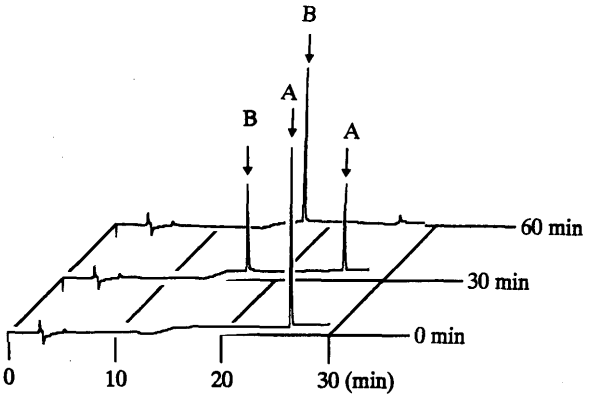
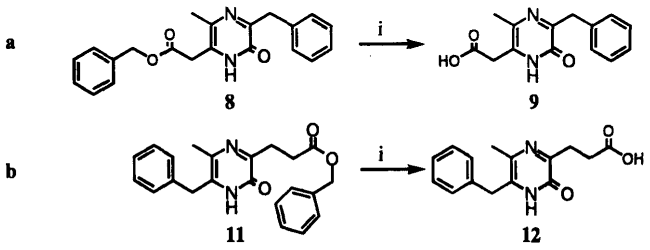


Fig. 3. HPLC Profiles of Pd-Catalyzed Reduction Reaction Mixture

A, compound **2**; B, compound **4**. Conditions: see experimental section.



Reagent: i, Pd/H_2

Chart 3

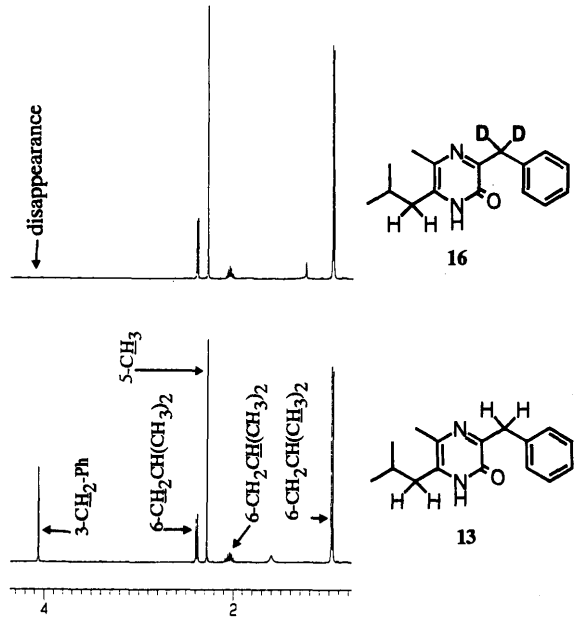


Fig. 4. ^{1}H-NMR Spectra of Compound **13** (Lower) and Compound **16** (Upper, after Deuteration)

density is lower at position 3 than at position 6 of 2(1*H*)-pyrazinone. Next, 3-benzyloxycarbonylethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (**11**) was hydrogenated over a Pd catalyst to afford 3-carboxyethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (**12**) (Chart 3b). Since the free carboxyl group of the carboxyethyl moiety at position 3 of 2(1*H*)-pyrazinone was obtained, the methylene group of the ethyl moiety has a sufficient shielding effect on the electron-withdrawing property of pyrazinone to prevent decarboxylation.

Next, to confirm the low electron density at position 3 of 2(1*H*)-pyrazinone, the acidity of the methylene function of the 3-benzyl or isobutyl moiety at position 3 of 2(1*H*)-pyrazinone was studied in comparison with that of position 6 by measuring the deuteration rate of the methylene proton(s) of pyrazinone derivatives. After dissolution of the pyrazinone derivatives (**13**—**15**) in a deuterated solvent mixture (D_2O–DCl–CD_3OD), the ^{1}H-NMR spectra were measured. In compound **13**,[5] the active methylene group of the 3-benzyl moiety was completely deuterated, but the methylene group of the 6-isobutyl moiety was not (Fig. 4, compound **16**). Next,

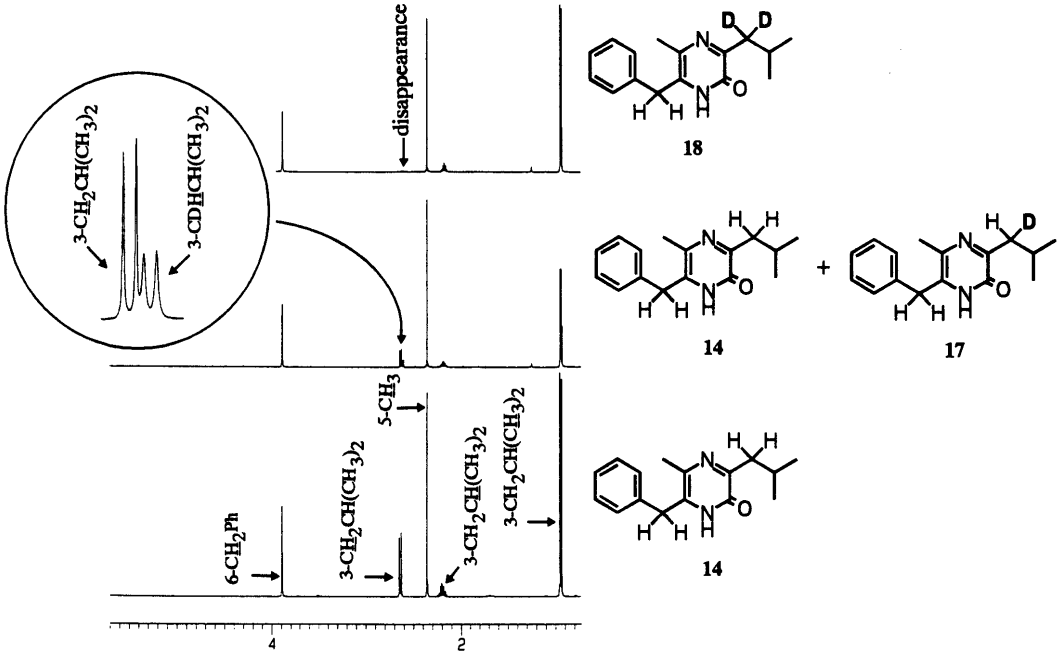
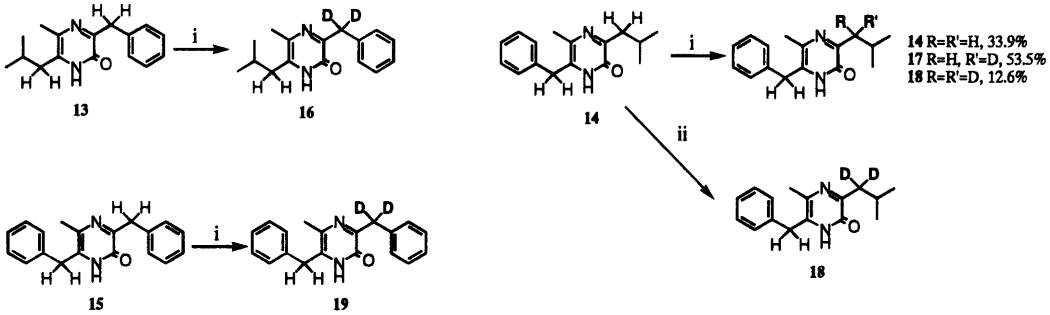


Fig. 5. ^{1}H-NMR Spectra of Compound **14** (Lower), Compound **14** and **17** (Middle, Deuteration for 0.5 h) and Compound **18** (Upper, Deuteration for 2 h)



Conditions: i, reflux in DCl–D_2O–CD_3OD for 0.5 h; ii, reflux in DCl–D_2O–CD_3OD for 2 h

Chart 4

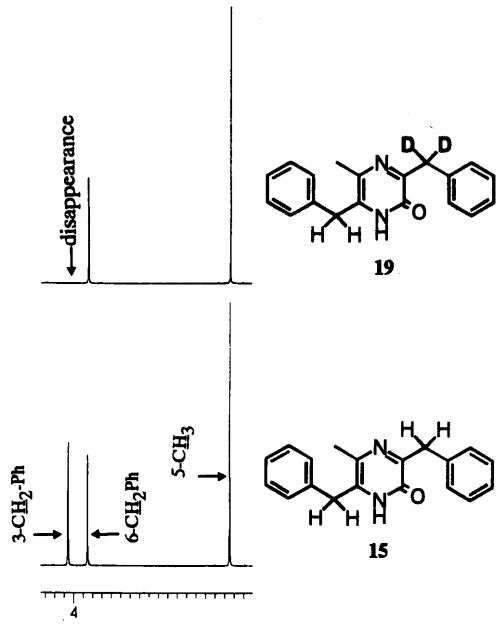


Fig. 6. ^{1}H-NMR Spectra of Compound **15** (Lower) and Compound **19** (Upper, after Deuteration)

in compound **14**,[7] besides the parent molecule **14**, two compounds, **17** and **18**, were observed in a ratio of 33.9 : 53.5 : 12.6, from calculation based on the methylene signal of the isobutyl moiety [3-C$\underline{H}_2$CH$(CH_3)_2$, 2.64 ppm; 3-CD$\underline{H}$CH$(CH_3)_2$, 2.62 ppm] (Fig. 5, compounds **14** and **17**). However, on treatment of **14** for 2 h under the same conditions, the methylene group of the 3-isobutyl moiety was completely deuterated (Fig. 5, compound **18**). Finally, in compound **15**,[8] selective deuteration occurred to give compound **19**. This structure was confirmed by comparing the ^{1}H-NMR spectrum of compound **19** with that of the parent compound **15** (Fig. 6). These results (summarized in Chart 4) show that the electron density is lower at position 3 than at position 6 of 2(1*H*)-pyrazinone.

In conclusion, carboxyl function-containing 2(1*H*)-pyrazinone derivatives were easily synthesized by our newly developed synthetic procedure under mild conditions. We found that ester interchange and decarboxylation occurred at position 3 of the pyrazinone ring, and it was confirmed by measuring the deuteration rate of the active methylene proton(s) that these phenomena were a consequence of the strong electron-withdrawing effect of

the nitrogen atom at position 4 and the carbonyl function at position 2 of 2(1*H*)-pyrazinone. Carboxyl function-containing 2(1*H*)-pyrazinone derivatives might be useful as building blocks for the synthesis of various peptide mimetics.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.) and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H (400, 500 MHz)- and ^{13}C (100 MHz)-NMR spectra were recorded on either a Bruker AM400 or ARX500 spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard (δ-value). The *J* values are given in Hz. The ^{13}C signals were assigned with the aid of distortionless enchancement by polarization transfer (DEPT) experiments, and multiplicities are indicated by prim (primary), sec (secondary), tert (tertiary) or quart (quaternary). For HPLC analysis, the following conditions were employed: (A), solvent: eluent A, 0.05% TFA/H_2O; eluent B, 0.05% TFA/MeCN; 80% A for 5 min, to 20% A for 20 min, and 20% A for 5 min; column: Cosmosil-C18 (4.6 × 250 mm), flow rate: 1 ml/min; (B), solvent: eluent A, 0.05% TFA/H_2O; eluent B, 0.05% TFA/MeCN; 75% A for 5 min, and to 50% A for 25 min; column: Waters Nova-Pak C18 (3.9 × 150 mm), flow rate: 1 ml/min; the retention time is reported as t_R (A) or t_R (B). On TLC (Kiesegel G, Merck), Rf^1, Rf^2, Rf^3, Rf^4 and Rf^5 values refer to the systems of $CHCl_3$–MeOH (20 : 1), BuOH–AcOH–H_2O (4 : 1 : 5), AcOEt–hexane (1 : 2), AcOEt–hexane (2 : 1) and $CHCl_3$–AcOEt–MeOH (30 : 19 : 1), respectively.

Boc-Asp(OBzl)-Phe-CH_2Cl (1) A solution of the mixed anhydride [prepared from Boc–Asp(OBzl)–OH (1.9 g, 6.38 mmol), Et_3N (0.98 ml, 7.02 mmol) and isobutyl chloroformate (0.83 ml, 6.38 mmol) as usual] in tetrahydrofuran (THF) (50 ml) was added to an ice-cold solution of HCl·H–Phe–CH_2Cl [prepared from Boc–Phe–CH_2Cl[9] (1.9 g, 6.38 mmol) and 7.2 N HCl/dioxane (8.9 ml, 63.8 mmol) as usual] in DMF, (50 ml) containing Et_3N (0.98 ml, 7.02 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 2.4 g (74.8%), mp 160 °C, $[\alpha]_D^{25}$ −7.4° (*c* = 1.0, $CHCl_3$), Rf^5 0.51. *Anal.* Calcd for $C_{26}H_{31}ClN_2O_6$: C, 62.1; H, 6.21; N, 5.57. Found: C, 62.0; H, 6.33; N, 5.43.

3-Benzyloxycarbonylmethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (2) and 3-Methoxycarbonylmethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (3) HCl·H–Asp(OBzl)–Phe–CH_2Cl [prepared from Boc–Asp(OBzl)–Phe–CH_2Cl (**1**, 1.5 g, 2.98 mmol) and 7.2 N HCl/dioxane (4.1 ml, 29.8 mmol) as usual] was dissolved in MeOH (100 ml), and the mixture was refluxed for 4 h. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and evaporated. Ether was added to the residue to afford crystals, which were collected by filtration and separated by flash column-chromatography (AcOEt : hexane = 2 : 1), yield **2**: 520 mg (50.1%), **3**: 91 mg (11.2%), mp **2**: 174—176 °C, **3**: 131—132 °C, Rf^4 **2**: 0.86, **3**: 0.69, t_R (A) **2**: 26.48 min, t_R (B) **2**: 25.24 min, **3**: 6.00 min. **2**: ^{1}H-NMR ($CDCl_3$ 400 MHz) δ: 13.1 (1H, br s, NH), 7.31—7.21 (10H, m, 3-$CH_2CO_2CH_2$-Ph + 6CH_2-Ph), 5.12 (2H, s, 3-$CH_2CO_2CH_2$-Ph), 3.84 (2H, s, 6-CH_2-Ph), 3.83 (2H, s, 3-$CH_2CO_2CH_2$-Ph), 2.33 (3H, s, 5-CH_3), ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: 169.7 (*quart*, 3-$CH_2CO_2CH_2$-Ph), 157.4 (*quart*, C-2), 149.6 (*quart*, C-3), 136.0 (*quart*, 6-C-1″), 135.8 (*quart*, 3-C-1′), 134.8 (*quart*, C-6), 130.2 (*quart*, C-5), 128.9 (*tert*, 6-C-3″, 5″), 128.7 (*tert*, 6-C-2″, 6″ + 3-C-3′, 5′), 128.1 (*tert*, 3-C-4′), 128.0 (*tert*, 3-C-2′, 6′), 127.2 (*tert*, 6-C-4″), 66.6 (*sec*, 3-$CH_2CO_2CH_2$-Ph), 39.2 (*sec*, 3-$CH_2CO_2CH_2$-Ph), 36.2 (*sec*, 6-CH_2-Ph), 18.7 (*prim*, 5-CH_3). *Anal.* Calcd for $C_{21}H_{20}N_2O_3$: C, 72.4; H, 5.79; N, 8.04. Found: C, 72.1; H, 5.65; N, 7.82. **3**: ^{1}H-NMR ($CDCl_3$ 400 MHz) δ: 13.0 (1H, br s, NH), 7.33—7.22 (5H, m, 6-CH_2-Ph), 3.89 (2H, s, 6-CH_2-Ph), 3.78 (3H, s, 3-$CH_2CO_2CH_3$), 3.66 (2H, s, 3-$CH_2CO_2CH_3$), 2.35 (3H, s, 5-CH_3). ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: 169.7 (*quart*, 3-$CH_2CO_2CH_3$), 157.1 (*quart*, C-2), 149.7 (*quart*, C-3), 135.9 (*quart*, 6-C-1′), 134.7 (*quart*, C-6), 130.2 (*quart*, C-5), 129.0 (*tert*, 6-C-3′, 5′), 128.9 (*tert*, 6-C-2′, 6′), 127.2 (*tert*, 6-C-4′), 52.1 (*prim*, 3-$CH_2CO_2CH_3$), 39.1 (*sec*, 3-$CH_2CO_2CH_3$), 36.3 (*sec*, 6-CH_2-Ph), 18.8 (*prim*, 5-CH_3). *Anal.* Calcd for $C_{15}H_{16}N_2O_3$: C, 66.2; H, 5.92; N, 10.3. Found: C, 66.1; H, 5.98; N, 10.1.

3,5-Dimethyl-6-benzyl-2(1*H*)-pyrazinone[7] (4) The title compound was prepared from 3-benzyloxycarbonylmethyl-5-methyl-6-benzyl-2-(1*H*)-pyrazinone (**2**, 600 mg, 1.7 mmol) by hydrogenation in dioxane (100 ml) over a Pd catalyst. After removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from acetone, yield 280 mg (77.0%), mp 153—155 °C (154—155 °C, lit.[7]), Rf^5 0.28. ^{1}H-NMR ($CDCl_3$ 400 MHz) δ: 11.3 (1H, br s, NH), 7.34—7.22 (5H, m, CH_2-Ph), 3.89 (2H, s, 6-CH_2-Ph), 2.41 (3H, s, 3-CH_3), 2.34 (3H, s, 5-CH_3). ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: 157.0 (*quart*, C-2), 154.4 (*quart*, C-3), 135.8 (*quart*, C-1′), 132.1 (*quart*, C-6), 129.12 (*quart*, C-5), 129.10 (*tert*, C-3′, 5′), 128.8 (*tert*, C-2′, 6′), 127.4 (*tert*, C-4′), 36.2 (*sec*, 6-CH_2-Ph), 19.9 (*prim*, 5-CH_3), 18.7 (*prim*, 5-CH_3). *Anal.* Calcd for $C_{13}H_{14}N_2O$: C, 72.9; H, 6.59; N, 13.1. Found: C, 73.2; H, 6.54; N, 13.1.

General Procedure for Examination of Cyclization Reaction in Various Solvents HCl·H_2N–Asp(OBzl)–Phe–CH_2Cl (0.03 mmol) was dissolved in DMF (15 ml), dioxane (15 ml), MeCN (15 ml) or MeOH (15 ml). Each reaction mixture was stirred at room temperature. An aliquot (0.05 ml) was taken periodically and diluted with MeOH (0.2 ml), and 0.01 ml of the diluted solution was analyzed by HPLC. The peak areas corresponding to compounds **2** and **3** were plotted as a function of time.

3-Benzyloxycarbonylmethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (2) HCl·H–Asp(OBzl)–Phe–CH_2Cl [prepared from Boc–Asp(OBzl)–Phe–CH_2Cl (2.0 g, 4.0 mmol) and 7.2 N HCl/dioxane (5.6 ml, 40.0 mmol) as usual] was dissolved in MeCN (125 ml). The reaction mixture was stirred at 60 °C for 2 h. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and evaporated. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 1.1 g (78.9%).

Boc-Asp(OBzl)-CH_2Cl (6) Diazomethane [prepared from *p*-toluenesulfonyl-*N*-methyl-*N*-nitrosoamide (30.4 g, 0.14 mol)] in ether (200 ml) was added to a mixed anhydride [prepared from Boc–Asp(OBzl)–OH (15.4 g, 47.4 mmol), NMM (6.2 ml, 56.8 mmol) and isobutyl chloroformate (6.2 ml, 47.4 mmol) as usual] in THF (200 ml) at −15 °C and the reaction mixture was stirred at 4 °C overnight. Then 8.3 N HCl/dioxane (11.3 ml, 94.2 mmol) was added to the reaction mixture at 0 °C, and the resulting solution was stirred at 0 °C for 3 h. After neutralization of the solution with Et_3N and removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over $MgSO_4$ and evaporated down. Hexane was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 11.4 g (67.6%), mp 72—74 °C, $[\alpha]_D^{25}$ −43.6° (*c* = 1.0, $CHCl_3$), Rf^3 0.63. *Anal.* Calcd for $C_{17}H_{22}ClNO_5$: C, 57.4; H, 6.23; N, 3.94. Found: C, 57.2; H, 6.11; N, 3.70.

Boc-Phe-Asp(OBzl)-CH_2Cl (7) A solution of the mixed anhydride [prepared from Boc–Phe–OH (1.4 g, 5.4 mmol), Et_3N (0.83 ml, 5.9 mmol) and isobutyl chloroformate (0.71 ml, 5.4 mmol) as usual] in THF (80 ml) was added to an ice-cold solution of HCl·H–Asp(OBzl)–CH_2Cl [prepared from **6** (1.92 g, 5.4 mmol) and 4.9 N HCl/dioxane (5.5 ml, 27 mmol) as usual] in DMF (80 ml) containing Et_3N (0.83 ml, 5.9 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 2.12 g (78.1%), mp 143—145 °C, $[\alpha]_D^{25}$ −41.6° (*c* = 1.0, MeOH), Rf^3 0.44. *Anal.* Calcd for $C_{26}H_{31}ClN_2O_6$: C, 62.1; H, 6.21; N, 5.57. Found: C, 61.9; H, 6.09; N, 5.37.

3-Benzyl-5-methyl-6-benzyloxycarbonylmethyl-2(1*H*)-pyrazinone (8) HCl·H–Phe–Asp(OBzl)–CH_2Cl [prepared from **7** (3.3 g, 6.56 mmol) and 8.6 N HCl/dioxane (7.6 ml, 65.6 mmol) as usual] was dissolved in MeCN (100 ml). The reaction mixture was stirred at 60 °C for 2 h. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and evaporated. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 1.8 g (78.8%), mp 136.5—138 °C, Rf^1 0.48, t_R (A) 26.51 min. ^{1}H-NMR ($CDCl_3$ 400 MHz) δ: 13.0 (1H, br s, NH), 7.36—7.14 (10H, m, 3-CH_2-Ph + 6-$CH_2CO_2CH_2$-Ph), 5.14 (2H, s, 6-$CH_2CO_2CH_2$-Ph), 4.03 (2H, s, 3-CH_2-Ph), 3.56 (2H, s, 6-CH_2CO_2CH2-Ph), 2.23 (3H, s, 5-CH_3), ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: 168.2 (*quart*, 6-$CH_2CO_2CH_2$-Ph), 157.0 (*quart*, C-2), 155.9 (*quart*, C-3), 137.7 (*quart*, 3-C-1′), 135.2 (*quart*, 6-C-1″), 131.3 (*quart*, C-5), 129.4

(*tert*, 6-C-2″, 6″), 128.6 (*tert*, 3-C-3′, 5′), 128.5 (*tert*, 3-C-4′), 128.26 (*tert*, 3-C-2′, 6′), 128.23 (*tert*, 6-C-3″, 5″), 127.6 (*quart*, C-6), 126.4 (*tert*, 6-C-4″), 67.4 (*sec*, 6-$CH_2CO_2\underline{C}H_2$-Ph), 39.4 (*sec*, 3-$\underline{C}H_2$-Ph), 35.7 (*sec*, 6-$\underline{C}H_2CO_2CH_2$-Ph), 18.6 (*prim*, 5-CH_3). *Anal.* Calcd for $C_{21}H_{20}N_2O_3$: C, 72.4; H, 5.79; N, 8.04. Found: C, 72.2; H, 5.84; N, 7.99.

3-Benzyl-5-methyl-6-carboxymethyl-2(1*H*)-pyrazinone (9) The title compound was prepared from **8** (1.6 g, 4.59 mmol) by catalytic hydrogenation in dioxane (50 ml). After removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 990 mg (83.5%), mp 135—140 °C, Rf^2 0.83, t_R (A) 16.65 min. ^{1}H-NMR ($CDCl_3+CD_3OD$ 400 MHz) δ: 7.32—7.12 (5H, m, 3-CH_2-$\underline{Ph}$), 4.03 (2H, s, 3-$C\underline{H}_2$-Ph), 3.50 (2H, s, 6-$C\underline{H}_2CO_2H$), 2.25 (3H, s, 5-CH_3), ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: 170.2 (*quart*, 3-$CH_2\underline{C}O_2H$), 157.0 (*quart*, C-2), 155.2 (*quart*, C-3), 137.7 (*quart*, 3-C-1′), 130.7 (*quart*, C-5), 129.4 (*tert*, 3-C-2′, 6′), 128.2 (*tert*, 3-C-3′, 5′), 127.9 (*quart*, C-6), 126.0 (*tert*, 6-C-4′), 38.5 (*sec*, 3-$C\underline{H}_2$-Ph), 35.1 (*sec*, 6-$\underline{C}H_2CO_2H$), 17.6 (*prim*, 5-CH_3). *Anal.* Calcd for $C_{14}H_{14}N_2O_3\cdot H_2O$: C, 64.7; H, 5.50; N, 10.8. Found: C, 64.6; H, 5.66; N, 10.7.

Boc–Glu(OBzl)–Phe–CH_2Cl (10) A solution of the mixed anhydride [prepared from Boc–Glu(OBzl)–OH (2.26 g, 6.7 mmol), Et_3N (1.03 ml, 7.4 mmol) and isobutyl chloroformate (0.88 ml, 6.7 mmol) as usual] in THF (80 ml) was added to an ice-cold solution of HCl·H–Phe–CH_2Cl [prepared from Boc-Phe-CH_2Cl (2.0 g, 6.7 mmol) and 7.2 N HCl/dioxane (9.3 ml, 67.0 mmol) as usual] in DMF (80 ml) containing Et_3N (1.03 ml, 7.4 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 3.0 g (86.6%), mp 129—130.5 °C, $[\alpha]_D^{25}$ −15.4° (c=1.0, $CHCl_3$), Rf^3 0.33. *Anal.* Calcd for $C_{27}H_{33}ClN_2O_6$: C, 62.7; H, 6.43; N, 5.42. Found: C, 62.7; H, 6.46; N, 5.40.

3-Benzyloxycarbonylethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (11) HCl·H–Glu(OBzl)–Phe–CH_2Cl [prepared from **10** (1.0 g, 1.9 mmol) and 7.2 N HCl/dioxane (2.7 ml, 19.0 mmol) as usual] was dissolved in MeCN (65 ml), and the mixture was stirred at 60 °C for 2 h. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and evaporated. Ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 547 mg (79.4%), 108—109 °C, Rf^4 0.56, t_R (A) 27.58 min. ^{1}H-NMR ($CDCl_3$ 400 MHz) δ: 12.8 (1H, br s, NH), 7.32—7.21 (10H, m, 3-$CH_2CH_2CO_2CH_2$-$\underline{Ph}$+6-CH_2-$\underline{Ph}$), 5.13 (2H, s, 3-$CH_2CH_2CO_2C\underline{H}_2$-Ph), 3.86 (2H, s, 6-$C\underline{H}_2$-Ph), 3.10 (2H, d, J=7.0 Hz, 3-$C\underline{H}_2CH_2CO_2CH_2$-Ph), 2.80 (2H, d, J=7.0 Hz, 3-$CH_2C\underline{H}_2CO_2CH_2$-Ph), 2.26 (3H, s, 5-$CH_3$), ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: 173.0 (*quart*, 3-$CH_2CH_2\underline{C}O_2CH_2$-Ph), 157.4 (*quart*, C-2), 154.2 (*quart*, C-3), 136.3 (*quart*, 6-C-1″), 136.2 (*quart*, 3-C-1′), 132.9 (*quart*, C-6), 129.7 (*quart*, C-5), 128.9 (*tert*, 6-C-3″, 5″), 128.7 (*tert*, 6-C-2″, 6″), 128.5 (*tert*, 3-C-3′, 5′), 128.12 (*tert*, 3-C-2′, 6′), 128.07 (*tert*, 3-C-4′), 127.1 (*tert*, 6-C-4″), 66.1 (*sec*, 3-$CH_2CH_2CO_2\underline{C}H_2$-Ph), 36.1 (*sec*, 6-$\underline{C}H_2$-Ph), 30.5 (*sec*, 3-$CH_2\underline{C}H_2CO_2CH_2$-Ph), 27.5 (*sec*, 3-$\underline{C}H_2CH_2CO_2CH_2$-Ph), 18.8 (*prim*, 5-$CH_3$). *Anal.* Calcd for $C_{22}H_{22}N_2O_3$: C, 72.9; H, 6.12; N, 7.73. Found: C, 72.6; H, 6.25; N, 7.57.

3-Carboxyethyl-5-methyl-6-benzyl-2(1*H*)-pyrazinone (12) The title compound was prepared from **11** (180 mg, 0.5 mmol) by catalytic hydrogenation in MeCN (60 ml). After removal of Pd and the solvent, ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from acetone, yield 85 mg (62.4%), mp 192—196 °C, t_R (A) 18.32 min. ^{1}H-NMR ($CDCl_3+CD_3OD$ 400 MHz) δ: 7.42—7.16 (5H, m, 6-CH_2-$\underline{Ph}$), 3.87 (2H, s, 6-$C\underline{H}_2$-Ph), 3.06 (2H, d, J=7.0 Hz, 3-$C\underline{H}_2CH_2CO_2H$), 2.75 (2H, d, J=7.0 Hz, 3-$CH_2CH_2\underline{C}O_2H$), 2.28 (3H, s, 5-$CH_3$), ^{13}C-NMR ($CDCl_3+CD_3OD$ 100 MHz) δ: 175.3 (*quart*, 3-$CH_2CH_2\underline{C}O_2H$), 156.1 (*quart*, C-2), 154.3 (*quart*, C-3), 135.8 (*quart*, 6-C-1′), 132.8 (*quart*, C-6), 129.7 (*quart*, C-5), 128.6 (*tert*, 6-C-3′, 5′), 128.0 (*tert*, 6-C-2′, 6′), 126.8 (*tert*, 6-C-4′), 35.6 (*sec*, 6-$\underline{C}H_2$-Ph), 30.2 (*sec*, 3-$CH_2\underline{C}H_2CO_2H$), 27.2 (*sec*, 3-$\underline{C}H_2CH_2CO_2H$), 18.0 (*prim*, 5-$CH_3$). *Anal.* Calcd for $C_{14}H_{14}N_2O_3\cdot H_2O$: C, 64.7; H, 5.50; N, 10.8. Found: C, 64.6; H, 5.66; N, 10.7.

General Procedure for Deuteration of 2(1*H*)-Pyrazinone Derivatives A 2(1*H*)-pyrazinone derivative (**13**—**15**, 20 mg) was dissolved in deuterated solvent mixture (DCl: D_2O: CD_3OD=1:1:1, 3 ml), and the mixture was refluxed for 0.5 or 2 h. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and evaporated. Ether was added to the residue to afford crystals, which were collected by filtration, yield 20 mg (100%).

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References and Notes

1) Part XLV: Okada Y., Wang J., Yamamoto T., Mu Y., Yokoi T., *J. Chem. Soc. Perkin Trans. 1.*, **1996**, 2139—2143.
2) The customary L indication for amino acid residues is omitted. Standard abbreviations for the amino acids, peptides and their derivatives are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Other abbreviations used are: Boc, *tert*-butyloxycarbonyl; Et_3N, triethylamine; NMM, *N*-methylmorpholine; DMF, dimethylformamide; AcOEt, ethyl acetate; THF, tetrahydrofuran; BuOH, butanol; Bzl, benzyl.
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6) Okada Y., Taguchi H., Nishiyama Y., Yokoi T., *Tetrahedron Lett.*, **35**, 1231—1234 (1994).
7) Taguchi H., Yokoi T., Tsukatani M., Okada Y., *Tetrahedron*, **51**, 7361—7372 (1995).
8) mp 174—175 °C, δ_H 13.1 (1H, br s, NH), 7.38—7.14 (10H, m, 3-CH_2-$\underline{Ph}$, 6-CH_2-$\underline{Ph}$), 4.06 (2H, s, 3-$C\underline{H}_2$-Ph), 3.85 (2H, s, 6-$C\underline{H}_2$-Ph), 2.34 (3H, s, 5-CH_3); δ_C: 157.3 (*quart*, C-2), 155.0 (*quart*, C-3), 137.9 (*quart*, 6-C1″), 136.3 (*quart*, 3-C1′), 133.6 (*quart*, C-6), 129.9 (*quart*, C-5), 129.3 (*tert*, 3-C2′, 6′), 129.0 (*tert*, 6-C3″, 5″), 128.8 (*tert*, 6-C2″, 6″), 128.3 (*tert*, 3-C3′, 5′), 127.1 (*tert*, 6-C4″), 126.3 (*tert*, 3-C4′), 39.4 (*sec*, 3-CH_2-Ph), 36.1 (*sec*, 6-CH_2-Ph), 18.9 (*prim*, 5-CH_3). *Anal.* Calcd for $C_{19}H_{18}N_2O$: C, 64.8; H, 6.57; N, 6.30. Found: C, 64.7; H, 6.52; N, 6.31. *m/z*: 290 (M^+). Experimental details will be described elsewhere.
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