Studies on the Mechanism of 1,2-Dihydropyrazin-2-one Ring Formation from Dipeptidyl Chloromethyl Ketone and Its Chemical Properties: Immediate Deamination during Catalytic Hydrogenation

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1,2-Dihydropyrazin-2-one derivatives, which have two aminoalkyl groups at the positions 3 and 6, were found to be efficient tools for the construction of potent, selective and long-acting opioid mimetics. During the course of preparation, we found that the catalytic hydrogenation of 3,6-bis(benzyloxycarbonylaminomethyl)-5-methyl-1,2-dihydropyrazin-2-one to remove the benzyloxycarbonyl groups resulted in a side reaction. By MS and NMR studies and by preparation of additional 1,2-dihydropyrazin-2-one derivatives, the structure of the by-product was identified as 3-aminomethyl-5,6-dimethyl-1,2-dihydropyrazin-2-one. Preparation of additional compounds substituted with deuterium provided us with sufficient information to confirm the structure of the product and to support a cyclization mechanism in its formation.

Key words 1,2-dihydropyrazin-2-one; deuterium substitution; cyclization mechanism; catalytic hydrogenation; deamination

1,2-Dihydropyrazin-2-one derivatives were conveniently synthesized from dipeptidyl chloromethyl ketones or methyl ketones.[1,2] According to this novel procedure, various functional groups could be introduced into positions 3 and 6 of 1,2-dihydropyrazin-2-one by using appropriate amino acids (Chart 2).[1—4] It had been assumed that the formation of the 1,2-dihydropyrazin-2-one ring from dipeptidyl chloromethyl ketones proceeded according to the mechanism shown in Chart 1.

We previously reported that various opioid mimetics containing a series of 1,2-dihydropyrazin-2-one derivatives exhibited broad binding activity to μ- and δ-opioid receptors whose affinities ranged from nanomolar to micromolar[3—5]; although the binding activity and selectivity were lower than endomorphin-1 and -2, which are endogenous mammalian opioid ligands, but considerably greater than the endorphins or β-endorphin. To develop more potent, selective and long-acting opioid ligands, a series of 1,2-dihydropyrazin-2-one derivatives (Fig. 1, **1**—**4**), which possess two Z-protected aminoalkyl moieties as the side chains, were synthesized. Next, we attempted to convert these protected compounds (**1**—**4**) into compounds (**5**—**8**). With the exception of **4**, compounds **1**—**3** were converted to **5**—**7**, respectively, by catalytic hydrogenation over a Pd catalyst. However, catalytic hydrogenation of compound **4** produced an unexpected compound instead of **8**. A preliminary report revealed that a special deamination from 6-Z-aminomethyl moiety on 1,2-dihydropyrazin-2-one occurred.[6] This paper will describe the further details of the above side reaction and confirmation of the cyclization mechanism.

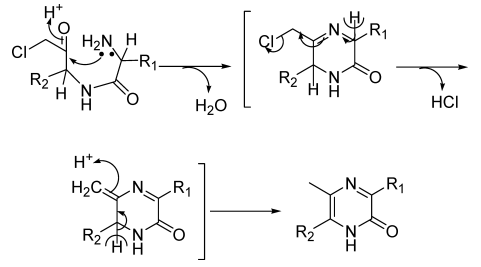
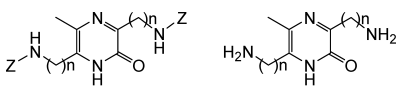


Chart 1. Ring Formation Mechanism from Dipeptidyl Chloromethyl Ketones

Results and Discussion

Studies on Hydrogenation Reaction of 1—4 Peptides and peptide mimetics were synthesized by a liquid phase method. N^{α}-*tert*-Butyloxycarbonyl(Boc)-Lys(Z)-OH and N^{α}-Boc-Orn(Z)-OH were prepared by generally known procedures.[7] N^{α}-Boc-Gln-OH and N^{α}-Boc-Asn-OH were treated with bis(trifluoroacetoxy)-iodobenzene (BTIB)[8—11] in DMF–water in the presence of pyridine to transform the amide of side chain into amine.[8] The amino function was protected with benzyloxycarbonyl (Z) group by treating with 4-(benzyloxycarbonyloxy)phenyl dimethylsulfonium (Z-DSP) under basic condition to give N^{α}-Boc-Dab(Z)-OH (Dab: 2,4-diaminobutylic acid) or N^{α}-Boc-Dap(Z)-OH (Dap: 2,3-diaminopropionic acid), respectively. The carboxyl moiety of these compounds [N^{α}-Boc-Xaa(Z)-OH; Xaa=Lys, Orn, Dab or Dap] was converted to the corresponding chloromethyl ketone by treating with diazomethane, followed by HCl–dioxane to give N^{α}-Boc-Xaa(Z)-CH_2Cl [Xaa=Lys, Orn, Dab or Dap, respectively]. The Boc group of N^{α}-Boc-Xaa(Z)-CH_2Cl was removed with HCl-dioxane and the resulting amine coupled with N^{α}-Boc-Xaa(Z)-OH by a mixed anhydride method to give N^{α}-Boc-Xaa(Z)-Xaa(Z)-CH_2Cl (Chart 2). The Boc



1 : n=4
2 : n=3
3 : n=2
4 : n=1

5 : n=4
6 : n=3
7 : n=2
8 : n=1

Fig. 1. Structures of 3,6-Bis(Z-aminoalkyl)-5-methyl-1,2-dihydropyrazin-2-ones (**1**—**4**) and Deprotected Compounds (**5**—**8**)

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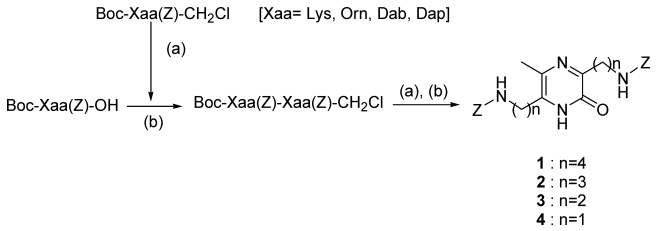
group of the dipeptidyl chloromethyl ketone was removed by HCl–dioxane and the resulting H-Xaa(Z)-Xaa(Z)-CH_2Cl hydrochloride salt was treated under reflux conditions in either acetonitrile (CH_3CN) or methanol (MeOH) to form the 1,2-dihydropyrazin-2-one derivatives (**1**—**4**).

The Z-protected derivatives (**1**—**4**) were hydrogenated over a Pd catalyst in 50% acetic acid (AcOH) in order to remove the Z groups (Chart 3). Although the catalytic hydrogenation of **1**—**3** yielded the corresponding desired products **5**—**7**, respectively, hydrogenation of **4** produced an unexpected compound (**9a** or **9b**) instead of the anticipated compound **8**. The hydrogenolysis reaction for these compounds was investigated as a function of time (10, 20, 30, 60 min) by use of mass spectrometry (MS). In the MS study, the following result was obtained: hydrogenation of **1**—**3** yielded the desired compounds **5**—**7**, respectively, at the early reaction times and essentially no starting materials were detected after 60 min. In contrast, hydrogenation of **4** produced both the desired product **8** and an unexpected compound after 10 min; however, after 60 min all of **4** was transformed into the unexpected product (**9a** or **9b**), which had a lower molecular weight (153 Da) than that of **8** (168 Da). This product was purified by reversed-phase HPLC and identified by one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopy. MS and NMR studies indicated that the unexpected product possessed only one aminomethyl moiety and two methyl groups. These results could be explained by deamination of the amino moiety attached to 1,2-dihydropyrazin-2-one ring at either position 3 or 6 to produce either **9a** or **9b** seen in Chart 3.



Reagents and conditions: (a) HCl/dioxane; (b) IBCF, NMM or Et_3N, DMF/THF; (c) reflux in CH_3CN or MeOH.

Chart 2. Synthetic Scheme for the Z-Protected 1,2-Dihydropyrazin-2-one Derivatives

Two Z-protected 1,2-dihydropyrazin-2-one derivatives **13** and **14** were prepared from **10** and **11**, respectively (Chart 4; I), and hydrogenated to determine from which position the amino group was removed (Chart 4; II and III). Both hydrogenolysis reactions were also studied as stated above, and MS and NMR spectroscopy were utilized to analyze the reaction at 10, 20 and 30 min and 1, 2, 4 and 6 h, in order to identify the final product at each step. Only **9b** was obtained after 6 h in the case of the hydrogenation of **13** (Chart 4; II). On the other hand, by hydrogenation of **14**, a compound which had a molecular weight of 138 Da (**15**), even smaller than the deprotected form (153 Da; **9a**) appeared by 15 min: compound **14** was totally transformed to **15** after 6 h (Chart 4; III). An NMR study indicated that the product produced after 6 h possessed three methyl groups on the 1,2-dihydropyrazin-2-one. Thus, these results confirmed that the deamination from the side chain attached to 1,2-dihydropyrazin-2-one ring occurred specifically at position 6 of the ring. It can be deduced that the property of C–N bond of aminomethyl moiety at position 6 of the ring is similar to that of benzylic or allylic C–N bond, while the property of C–N bond of aminomethyl moiety at the positon 3 of the ring is quite different from that of benzylic C–N bond.[12—18] Furthermore, this deamination could occur much more easily than in the case of benzylamine under atmospheric pressure at room temperature.

Application of Deuteration for Identification of Structure and Confirmation of Ring Formation Mechanism. (i) Identification of Chemical Shifts of Methyl Moieties on 1,2-Dihydropyrazin-2-one On our studies for the preparation of a series of 1,2-dihydropyrazin-2-one derivatives, the structures of each compound were identified by NMR in pyridine-d_5. The NMR signals of the product (Chart 3, **9b**) generated during catalytic hydrogenation of **4** were consistent with those of the authentic compound (Chart 4; II, **9b**). The chemical shifts of the deaminated product (Chart 4; III, **15**) were also consistent with those of synthetic **15** in Chart 4; I. Thus, we identified the chemically generated products by MS and 1D- and 2D-NMR; however, the individual chemical sifts of methyl group in compounds which possessed two or three methyl groups, in particular those com-

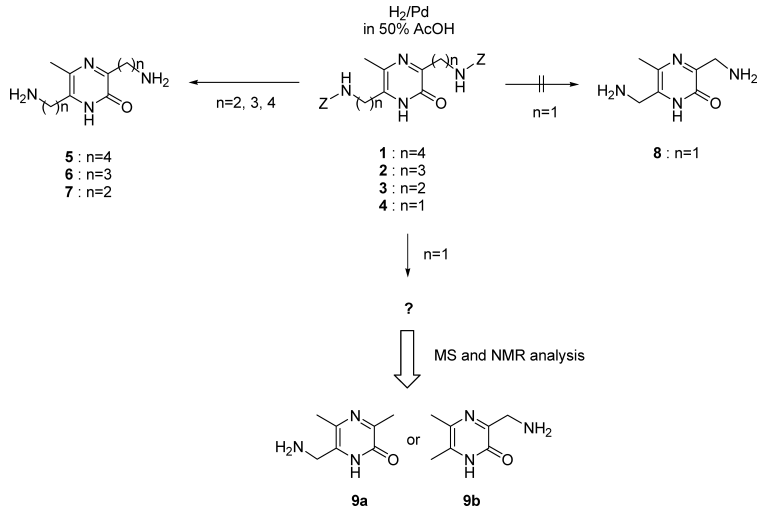
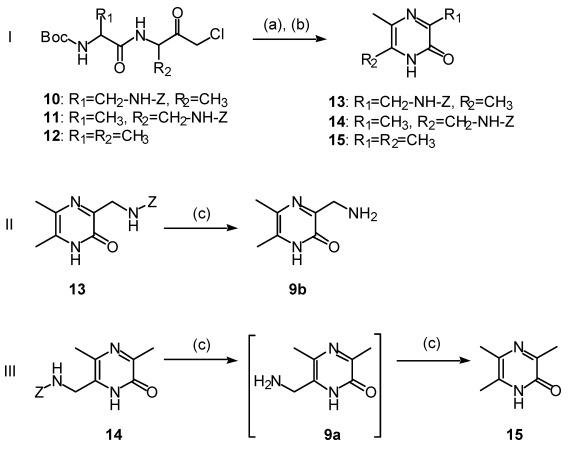


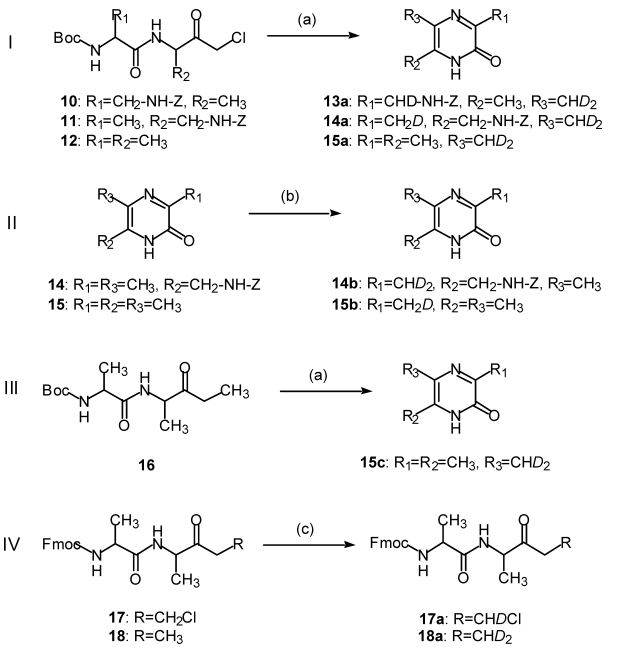
Chart 3. Deprotection of the Z Groups and Side Reaction by the Catalytic Hydrogenation in 50% AcOH

pounds possessing two methyl groups at both positions 5 and 6 on the ring, could not be assigned by utilization of 2D-NMR. It was assumed that specific deuteration of a proton at the side chain functional groups attached to the ring would provide us with clues to the assignment of each chemical shift. Thus, dipeptidyl chloromethyl ketone and 1,2-dihydropyrazin-2-one derivatives (Chart 4; I, **10**—**15**) were employed for the synthesis of deuterated compounds (Chart 5; I, II). One deuterium atom should be introduced into the methyl moiety at position 5 of the ring if the ring was formed in a deuterium solvent according to the hypothesized mechanism shown in Chart 1. In addition, the protons at position 3 associated with the highly acidic side chain function attached to the ring should easily be substituted with deuterium atoms if treated in the same solvent.[2] Chart 5 summarizes the preparation of various compounds substituted with deuterium. Boc-protected dipeptidyl chloromethyl ketones (Chart 5; I, **10**—**12**) were dissolved in mixed deuterium solvent [deuterium oxide : methanol-d_4 : deuterium chloride, 1 : 1 : 1 (v/v/v)], and stirred for 30 min at 65 °C, and for 1 h at 25 °C to give deuterated **13a**, **14a**, and **15a**, respectively, whose structures were established by ^{1}H-NMR in pyridine-d_5. The integral values for non-substituted compounds and the corresponding deuterated compounds are summarized in Table 1. Peak intensity and integral values of the respective signals of deuterated moiety decreased. In the case of **13a**, **14a**, and **15a** (Chart 5; I), if the introduction of a few deuterium atoms into the methyl moiety with a highly acidic group at position 3[2] was ignored, only one signal, that of methyl groups, brought about a change and the integral values decreased to approximate one [integral values (ppm): 1.29 (2.20), 0.86 (2.45) and 1.29 (2.24), respectively], which is in contrast to the three integral values of non-substituted compounds. Therefore, the variable methyl signal was identified with the methyl moiety at position 5. For the discrimination of the chemical shifts of the methyl moiety between position 3 and 5 of the ring, the following experiments were performed: the 1,2-dihydropyrazin-2-one derivatives (Chart 5; II, **14**, **15**) were dissolve in the deuterium solvent, reacted under reflux conditions for 2 h, and yielded the deuterated **14b** and **15b**, under which conditions deuterium should be introduced into the methyl moiety at position 3 on the ring. As shown in Table 1, the peak intensity and integral values decreased [0.32 (2.59 ppm) and 2.29 (2.60 ppm), respectively], and therefore it was concluded that this decreased peak height established that the methyl group was at position 3. Thus, it was revealed that the order of chemical shift of three methyl groups on 1,2-dihyropyrazin-2-one is that of po-



Reagents and conditions: (a) HCl/dioxane; (b) reflux in MeOH; (c) H_2/Pd in 50% AcOH.

Chart 4. Synthesis of Authentic Standards



Reagents and conditions: (a) 65 °C for 30 min then 25 °C for 1h in DCl/D_2O/CD_3OD (v/v/v = 1/1/1); (b) 65 °C for 2 h in DCl/D_2O/CD_3OD (v/v/v = 1/1/1); (c) 65 °C for 30 min in DCl/DMSO-d_6.

Chart 5. Deuteration on the Side Chains of 1,2-Dihydropyrazin-2-one and on the C-Terminal Moiety of Linear Peptide

Table 1. The Extent of Peak Intensity Decreased by Deuteration in ^{1}H-NMR Studies

Entries A	Entries B	Integral values: Entries A/Entries B (chemical shifts: ppm)		
13	13a	3.00/1.29 (2.20)	3.00/3.00 (2.13)	
14	14a	2.95/2.25 (2.59)	2.98/0.86 (2.45)	
	14b	2.95/0.32 (2.59)	2.98/2.98 (2.45)	
15	15a	2.98/2.89 (2.60)	2.98/1.29 (2.24)	3.00/3.00 (2.15)
	15b	2.98/2.29 (2.60)	2.98/3.02 (2.24)	3.00/3.00 (2.15)
	15c	2.98/2.95 (2.60)	2.98/1.40 (2.24)	3.00/3.00 (2.15)
17	17a		2.09/1.23 (4.57)	
18	18a		3.01/0.74 (2.05)	

a) Entry A and B indicates the non-deuterated compounds and the corresponding deuterium substituted compounds, respectively. *b*) These values were indicated respective integral values for entries A (left) and entries B (right) on chemical shifts in parenthesis.

sitions 3, 5 and 6 from lower field to higher field. This information could facilitate the assignment of NMR signals of 1,2-dihydropyrazin-2-one derivatives.

(ii) Studies on the Ring Formation Mechanism With the aid of these NMR experiments, we investigated the details of the reaction mechanism to form 1,2-dihydropyrazin-2-one ring from dipeptidyl chloromethyl ketone. Chemical shifts for an individual methyl moiety were determined by deuteration. However, the varied methyl-proton signal at position 5 of deuterated **13a**, **14a**, and **15a** (Chart 5; I), obtained by cyclization in mixed deuterium solvent, indicated that only one methyl proton (1.29, 0.86, 1.29, respectively) remained, although we expected that the compound should contain two methyl protons if the formation of the ring occurred according to the mechanism shown in Chart 1. On the contrary, we previously reported that the formation of 1,2-dihydropyrazin-2-one derivatives from dipeptidyl methyl ketone derivatives would not occur *via* olefin intermediates as in the case of the formation from dipeptidyl chloromethyl ketone, but would occur oxidatively.[19] Namely, we expected that the cyclization from dipeptidyl methyl ketone in deuterium-solvent might normally produce **15** (Chart 5; II) without substitution by a deuterium atom. In order to further investigate this mechanism, another dipeptidyl methyl ketone, Boc-Ala-Ala-Me (Chart 5; III, **16**), was prepared and cyclized in the deuterated solvent and the extent of deuteration on the methyl moiety at position 5 was studied. Although a similar cyclization reaction from **16** was performed, the result indicated that there was no difference between the product **15c** formed from a dipeptidyl methyl ketone and the former product (Chart 5; I, **15a**) obtained from a dipeptidyl chloromethyl ketone. As for **15c**, more than one deuterium atom was introduced into the methyl moiety at position 5 and the integral value at 2.24 ppm was 1.40 (Table 1). These observations suggested two possibilities: (1) the actual mechanism of ring formation was different from the hypothetical mechanism (Chart 1); or (2) the introduction of one deuterium atom into dipeptidyl chloromethyl ketone molecule occurred at the step prior to cyclization.

These hypotheses were examined by the following studies. 9-Fluorenylmethyloxycarbonyl (Fmoc)-protected derivatives, Fmoc-Ala-Ala-CH_2Cl (Chart 5; IV, **17**) and Fmoc-Ala-Ala-Me (Chart 5; IV, **18**) were prepared and treated in the deuterium solvent, which released deuterium cations. These Fmoc derivatives were dissolved in dimethylsulfoxide-d_6 and deuterium chloride was added to the solution. At the same time, the solution was heated to 65 °C for 30 min to yield the **17a** and **18a** containing deuterium, respectively. Each compound in the solution was analyzed for the extent of deuteration by ^{1}H-NMR (Table 1). A 30-min exposure of **17** to deuterium chloride yielded **17a**, in which the chloromethyl group contained approximately one deuterium atom [1.23 (4.57 ppm)]. In a similar approach for **18**, approximately two deuterium atoms were introduced into the C-terminal methyl group to produce **18a** [0.74 (2.05 ppm)].

These results demonstrated that one deuterium atom was introduced into the chloromethyl group, followed by formation of 1,2-dihydropyrazin-2-one derivatives *via* an olefin intermediate followed by the addition of another deuterium atom. Therefore, the final products would contain two deuterium atoms in a methyl function at position 5. The experiment using Fmoc-Ala-Ala-Me revealed that the introduction of two deuterium atoms occurred at the first step followed by oxidative ring formation with the final cyclic compound having two deuterium atoms in the methyl group at position 5.

Conclusions

The convenient synthetic procedure of 1,2-dihydropyrazin-2-one derivatives from dipeptidyl chloromethyl ketone was developed and the resulting nonpeptide derivatives are valuable in the preparation of peptidomimetic compounds.[1—4] In order to synthesize more potent, selective and long-acting opioid ligands, the preparation of 1,2-dihydropyrazin-2-one derivatives (Fig. 1, **5**—**8**) was attempted. However, catalytic hydrogenation of **4** did not yield **8** with two free amines and produced an unexpected by-product. That substance was determined to be deaminated **9b** with a molecular weight of 153 Da; the amino function of the side chain at position 6 of the pyrazinone ring was specifically removed due to the similar properties of the C–N bond of aminomethyl moiety at position 6 of the ring to that of benzylic or allylic C–N bond. Additional deuteration studies based on reaction mechanisms and character of the 1,2-dyhydropyrazin-2-one derivatives led to the determination of the structures of all the compounds. This deamination occurred much more easily under gentle conditions, such as under atmospheric pressure at room temperature, compared with other *N*-benzyl compounds.[12,13,18] The introduction of deuterium into C-terminal chloromethyl or methyl moiety occurred before ring formation reaction in the deutration reaction of **17** and **18** supporting the previous hypothesis on cyclization mechanisms and confirming that the 1,2-dihydropyrazin-2-one ring smoothly formed from a dipeptidyl chloromethyl ketone *via* an olefin intermediate (Chart 1).

Experimental

General Melting points were determined on a Yanagimoto micro-melting point apparatus and were uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-1000 (Japan Spectroscopic Co.). ^{1}H- (400 MHz) and ^{13}C- (100 MHz) nuclear magnetic resonance (NMR) spectral data were recorded on a Bruker DPX-400 spectrometer. Chemical shift values are expressed as ppm, referenced to tetramethylsilane at 0.00 ppm as an internal standard (δ-values). The J values are given in Hz. Attribution of ^{13}C signals were made also with the aid of distortionless enhancement by polarization transfer (DEPT) experiments and two-dimensional experiments, and multiplicities are indicated by p (primary), s (secondary), t (tertiary) or q (quaternary). Mass spectra of the compounds were taken on a KRATOS-MALDI IV mass spectrometer using TOF techniques.

The following conditions were employed for HPLC analysis and for semi-preparative HPLC: multi solvent delivery system, Waters model 600 E; solvent, 0.05% TFA in water for solvent A and 0.05% TFA in acetonitrile (CH_3CN) for solvent B; column, Cosmosil C18-ARII (4.6×250 mm), [Cosmosil C18-ARII (20×250 mm) for semi-preparative HPLC]; flow rate, 1 ml/min. Thin-layer chromatography (TLC) was carried out on Silica gel 60 F254 (Merck Japan, Ltd) and compounds were visualized by ultraviolet at either 254 nm or 365 nm when an aromatic (benzene or 1,2-dihydropyrazin-2-one) ring was present, by ninhydrin spray, or ninhydrin plus 25% HBr/acetic acid (AcOH) spray when free amino or Boc-, Z-protected amino groups were present. Rf^1, Rf^2, Rf^3, Rf^4, Rf^5 and Rf^6 values refer to the solvent systems of *n*-hexane/ethyl acetate (AcOEt) (1 : 1), *n*-hexane/AcOEt (6 : 5), $CHCl_3$/MeOH/AcOH (90 : 8 : 2), *n*-butanol (*n*-BuOH)/AcOH/pyridine/water (4 : 1 : 1 : 2), *n*-BuOH/AcOH/water (4 : 1 : 5, upper phase) and *n*-BuOH/AcOH/pyridine/water (1 : 1 : 1 : 1), respectively.

General Procedure for Synthesis of Boc-Xaa(Z)-CH_2Cl [Xaa=Dap, Dab, Orn] Diazomethane [prepared from *p*-toluensulfonyl-*N*-methyl-*N*-nitrosoamide (NMTA: 40.9 mmol), KOH (40.9 mmol) and ethanol (EtOH: 40.5 mmol)] in ether (60 ml) was added to a solution of mixed anhydride, which was prepared from Boc-Xaa(Z)-OH (13.6 mmol) [Xaa=Dap, Dab,

Orn; Boc-Dap(Z)-OH or Boc-Dab(Z)-OH was prepared from Boc-Asn-OH or Boc-Gln-OH (1.0 eq), BTIB[8—11] (1.5 eq), pyridine (2.0 eq) and Z-DSP (1.3 eq)], triethylamine (Et_3N: 16.4 mmol) and isobutyl chloroformate (IBCF: 16.4 mmol) in tetrahydrofuran (THF: 50 ml) at −15 °C. The reaction mixture was stirred at 5 °C overnight. Then 6.9 N HCl/dioxane (34.1 mmol) was added to the reaction mixture at −15 °C, and the resulting solution was stirred for 30 min. The solution was diluted with ice-cold water and extracted with AcOEt. The AcOEt phase was washed with water, 5% aqueous $NaHCO_3$ and saturated aqueous NaCl solution, then dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was removed *in vacuo* and petroleum ether was added to the residue to afford crystals, which were collected by filtration. If necessary, recrystallization from ethanol was performed.

Boc-Dap(Z)-CH_2Cl Yield: 2.90 g (75.9%), mp 94—97 °C, Rf^1 0.84, $[\alpha]_D^{25}$ +50.9° (c=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.37—7.31 (m, 5H, Ar–H), 5.69 (d, 1H, J=6.0 Hz, α-NH), 5.22 (br, 1H, β-NH), 5.07 (s, 2H, –CH_2–Ph), 4.59 (br, 1H, α-CH), 4.42 and 4.30 (AB-q, 2H, J=15.6 Hz, –CH_2Cl), 3.70 (br, 1H, β-CH_A), 3.59 (m, 1H, β-CH_B), 1.44 (9H, s, *tert*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 200.3 (q, $COCH_2Cl$), 157.1 and 155.5 (q, carbonyl), 136.9 (q, phenyl), 128.6, 128.3 and 128.1 (t, phenyl), 80.7 (q, *tert*-butyl), 67.3 (s, –CH_2–Ph), 58.2 (t, α-CH), 46.5 (s, –CH_2Cl), 41.9 (s, β-CH_2), 28.3 (p, *tert*-butyl). *Anal.* Calcd for $C_{17}H_{23}ClN_2O_5$: C, 55.1; H, 6.25; N, 7.55. Found: C, 55.3; H, 6.31; N, 7.55.

Boc-Dab(Z)-CH_2Cl Yield: 1.20 g (58.0%), mp 88—95 °C, Rf^1 0.76, $[\alpha]_D^{25}$ −8.77° (c=0.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.31 (5H, m, Ar–H), 5.45 (br, 2H, α-NH and γ-NH), 5.10 (s, 2H, –CH_2–Ph), 4.57 (br, 1H, α-CH), 4.23 (s, 2H, –CH_2Cl), 3.47 (br, 1H, γ-CH_A), 3.11 (br, 1H, γ-CH_B), 2.09 (m, 1H, β-CH_A), 1.67 (m, 1H, β-CH_B), 1.44 (9H, s, *tert*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 202.1 (q, $COCH_2Cl$), 159.1 and 157.7 (q, carbonyl), 136.5 (q, phenyl), 128.6—128.3 (t, phenyl), 80.7 (q, *tert*-butyl), 66.9 (s, –CH_2–Ph), 55.0 (t, α-CH), 46.5 (s, –CH_2Cl), 37.1 (s, γ-CH_2), 31.7 (s, γ-CH_2), 28.4 (p, *tert*-butyl). *Anal.* Calcd for $C_{18}H_{25}ClN_2O_5\cdot 0.3H_2O$: C, 55.3; H, 6.59; N, 7.16. Found: C, 55.1; H, 6.47; N, 7.08.

Boc-Orn(Z)-CH_2Cl Yield: 4.80 g (88.9%), mp 69—73 °C, Rf^1 0.69, $[\alpha]_D^{25}$ −25.5° (c=0.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.30 (m, 5H, Ar–H), 5.18 (br, 1H, α-NH), 5.09 (s, 2H, –CH_2–Ph), 4.94 (br, 1H, δ-NH), 4.51 (br, 1H, α-CH), 4.24 (s, 2H, –CH_2Cl), 3.23 (m, 2H, δ-CH_2), 1.87 (br, 1H, β-CH_A), 1.62—1.48 (m, 3H, β-CH_B and γ-CH_2), 1.44 (9H, s, *tert*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 201.6 (q, $COCH_2Cl$), 156.6 and 155.6 (q, carbonyl), 136.5 (q, phenyl), 128.6—128.1 (t, phenyl), 80.5 (q, *tert*-butyl), 66.8 (s, –CH_2–Ph), 57.0 (t, α-CH), 46.5 (s, –CH_2Cl), 40.3 (s, δ-CH_2), 28.5 (s, β-CH_2), 28.3 (p, *tert*-butyl), 26.1 (s, γ-CH_2). *Anal.* Calcd for $C_{19}H_{27}ClN_2O_5$: C, 57.3; H, 6.80; N, 7.20. Found: C, 57.2; H, 6.82; N, 7.02.

Boc-Ala-Me Boc-Ala-chloromethyl ketone (CMK)[20] (1.00 g, 4.51 mmol) dissolved in 20 ml of MeOH was hydrogenated over Pd-black catalyst and the pH of the reaction mixture was kept at 7 to 8 by Et_3N. The catalyst was removed by filtration after 4 h, and the solution was evaporated *in vacuo*. The resulting residue was extracted with AcOEt, which was washed by 10% citric acid, 5% $NaHCO_3$ and saturated aqueous NaCl solution, then dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was removed to obtain the desired product as an oil. Yield: 806 mg (95.5%). ^{1}H-NMR ($CDCl_3$) δ: 5.24 (br, 1H, α-NH), 4.3 (br quint, 1H, J=6.9 Hz, α-CH), 2.03 (s, 3H, $COCH_3$), 1.44 (9H, s, *tert*-butyl), 1.34 (d, 3H, J=7.2 Hz, methyl of Ala), ^{13}C-NMR ($CDCl_3$) δ: 207.3 (q, $COCH_3$), 155.2 (q, carbonyl of *tert*-butyloxycarbonyl), 79.8 (q, *tert*-butyl), 55.7 (t, α-CH), 28.4 (p, *tert*-butyl), 26.4 (p, $COCH_3$), 17.7 (p, methyl of Ala). *Anal.* Calcd for $C_9H_{17}NO_3\cdot 0.1H_2O$: C, 57.2; H, 9.17; N, 7.41. Found: C, 57.1; H, 8.97; N, 7.29.

General Procedure for Synthesis of Boc-Xaa(Z)-Xaa(Z)-CH_2Cl [Xaa=Dap, Dab, Orn], Boc-Dap(Z)-Ala-CH_2Cl, Boc-Ala-Dap(Z)-CH_2Cl and Boc-Ala-Ala-CH_2Cl To a solution of a mixed anhydride [prepared from Boc-Xaa(Z)-OH or Boc-Ala-OH (3.37 mmol), Et_3N (3.37 mmol) and IBCF (3.37 mmol) in THF (50 ml)], dimethylformamide (DMF: 50 ml) solution containing H-Xaa(Z)-CH_2Cl or H-Ala-CH_2Cl [prepared from Boc-Xaa(Z)-CH_2Cl (4.04 mmol) or Boc-Ala-CH_2Cl and 7.2 N HCl/dioxane (36.4 mmol)] and Et_3N (4.04 mmol) was added at 0 °C. The reaction mixture was stirred at same temperature for 1 h and at room temperature overnight. Then the solvent was removed *in vacuo* and the residue was extracted with AcOEt, which was washed with 10% citric acid, 5% aqueous $NaHCO_3$ and saturated aqueous NaCl, then dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was evaporated down and the residue was precipitated from ether, then the crystals were colleted by filtration. If necessary, the resulting crude products were purified by silica gel chromatography [mobile phase: AcOEt/*n*-hexane=1 : 1 (v/v) for the purification of Boc-Dab(Z)-Dab(Z)-CH_2Cl, AcOEt/*n*-hexane=6 : 5 (v/v) in the case of Boc-Ala-Dap(Z)-CH_2Cl and Boc-Ala-Ala-CH_2Cl].

Boc-Dap(Z)-Dap(Z)-CH_2Cl Yield: 1.33 g (66.8%), mp 175—178 °C, Rf^1 0.56, $[\alpha]_D^{25}$ −51.8° (c=0.2, DMF). ^{1}H-NMR (DMSO-d_6 containing 1% pyridine-d_5) δ: 8.54 (d, 1H, J=7.1 Hz, α-NH), 7.37—7.29 (m, 10H, Ar–H), 7.23 (br, 1H, β-NH), 7.14 (br, 1H, β-NH), 6.90 (d, 1H, J=6.7 Hz, α-NH), 5.03 (s, 2H, –CH_2–Ph), 5.02 (s, 2H, –CH_2–Ph), 4.60 and 4.53 (AB-q, 2H, J=16.8 Hz, –CH_2Cl), 4.40 (br, 1H, α-CH), 4.03 (br, 1H, α-CH), 3.51 (m, 1H, β-CH_A), 3.40—3.24 (m, 3H, β-CH_B and β-CH_2), 1.38 (9H, s, *tert*-butyl), ^{13}C-NMR (DMSO-d_6 containing 1% pyridine-d_5) δ: 199.2 (q, $COCH_2Cl$), 170.9, 156.33, 156.26 and 155.3 (q, carbonyl), 136.9 and 136.8 (q, phenyl), 128.3—127.6 (t, phenyl), 78.6 (q, *tert*-butyl), 65.5 (s, –CH_2-Ph), 56.7 and 54.9 (t, α-CH), 47.9 (s, –CH_2Cl), 41.61 and 40.1 (s, β-CH_2), 28.0 (p, *tert*-butyl). *Anal.* Calcd for $C_{28}H_{35}ClN_4O_8$: C, 56.9; H, 5.97; N, 9.48. Found: C, 56.8; H, 6.08; N, 9.56.

Boc-Dab(Z)-Dab(Z)-CH_2Cl Yield: 580 mg (36.3%), mp 58—68 °C, Rf^1 0.28, $[\alpha]_D^{25}$ −34.6° (c=1.0, DMF). ^{1}H-NMR ($CDCl_3$) δ: 7.37—7.29 (m, 10H, Ar-H), 7.23 (br, 1H, γ-NH), 6.89 (br, 1H, α-NH), 5.40 (br, 2H, γ-NH and α-NH), 5.16—5.08 (m, 4H, –CH_2–Ph), 4.73 (br, 1H, α-CH), 4.30—4.09 (m, 2H, –CH_ACl and α-CH), 3.73 (m, 1H, γ-CH_A), 3.63 (d, 1H, J=10.8 Hz, –CH_BCl), 3.52 (br, 1H, γ-$CH_{A'}$), 3.28 (m, 1H, γ-CH_B), 3.11 (br, 1H, γ-$CH_{B'}$), 2.15 (m, 1H, β-CH_A), 1.93 (m, 1H, β-$CH_{A'}$), 1.82—1.77 (m, 2H, β-CH_B and β-$CH_{B'}$), 1.42 (9H, s, *tert*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 199.0 (q, $COCH_2Cl$), 172.0, 156.9 and 154.5 (q, carbonyl), 136.3 and 136.0 (q, phenyl), 128.7—127.8 (t, phenyl), 80.3 (q, *tert*-butyl), 67.3 and 67.1 (s, –CH_2–Ph), 52.6 and 52.2 (t, α-CH), 45.5 (s, γ-CH_2), 44.8 (s, –CH_2Cl), 37.9 (s, γ-CH_2), 33.9 (s, β-CH_2), 28.0 (p, *tert*-butyl), 27.9 (s, β-CH_2). *Anal.* Calcd for $C_{30}H_{39}ClN_4O_8\cdot 0.2n$-Hexane: C, 58.9; H, 6.58; N, 8.81. Found: C, 58.9; H, 6.70; N, 8.80.

Boc-Orn(Z)-Orn(Z)-CH_2Cl Yield: 1.39 g (43.0%), mp 110—111 °C, Rf^1 0.69, $[\alpha]_D^{25}$ −36.5° (c=0.25, DMF). ^{1}H-NMR ($CDCl_3$) δ: 7.32—7.29 (m, 11H, Ar–H and α-NH), 5.30 (br, 1H, α-NH), 5.22 (br, 2H, 2×δ-NH), 5.06 (s, 4H, –CH_2–Ph), 4.69 (br, 1H, α-CH), 4.27 (br, 1H, α-CH), 4.21 (s, 2H, –CH_2Cl), 3.33 (br, 1H, δ-CH_A), 3.17 (br, 3H, δ-CH_B and δ-CH_2), 1.85—1.78 (m, 2H, β-CH_2), 1.55 (br, 6H, β-CH_2 and 2×$\gamma$$CH_2$), 1.42 (9H, s, *tert*-butyl), ^{13}C-NMR ($CDCl_3$) δ: 200.8 (q, $COCH_2Cl$), 172.9, 157.1, 156.8 and 155.9 (q, carbonyl), 136.5 (q, phenyl), 128.5—127.8 (t, phenyl), 80.1 (q, *tert*-butyl), 66.8 (s, –CH_2–Ph), 56.0 and 53.1 (t, α-CH), 46.6 (s, –CH_2Cl), 40.2 and 39.7 (s, δ-CH_2), 29.8 (s, β-CH_2), 28.4 (p, *tert*-butyl), 28.3 (s, β-CH_2), 26.2 (s, γ-CH_2). *Anal.* Calcd for $C_{32}H_{43}ClN_4O_8$: C, 59.4; H, 6.70; N, 8.66. Found: C, 59.7; H, 6.65; N, 8.83.

Boc-Ala-Ala-CH_2Cl (12) Yield: 603 mg (45.6%), mp 125—130 °C, Rf^2 0.45, $[\alpha]_D^{21}$ −56.2° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.39 (br, 1H, α-NH), 5.04 (d, 1H, J=7.1 Hz, α-NH), 4.76 (quint, 1H, J=7.1 Hz, α-CH), 4.29 and 4.25 (AB-q, 2H, J=15.8 Hz, –CH_2Cl), 4.17 (br, 1H, α-CH), 1.45 (9H, s, *tert*-butyl), 1.40 (d, 3H, J=7.2 Hz, methyl of Ala), 1.36 (d, 3H, J=7.1 Hz, methyl of Ala), ^{13}C-NMR ($CDCl_3$) δ: 201.2 (q, $COCH_2Cl$), 172.8, and 155.5 (q, carbonyl), 80.5 (q, *tert*-butyl), 52.0 (t, α-CH), 50.0 (t, α-CH), 46.1 (s, –CH_2Cl), 28.3 (p, *tert*-butyl), 18.0 (p, methyl of Ala), 17.1 (p, methyl of Ala). *Anal.* Calcd for $C_{12}H_{21}ClN_2O_4$: C, 49.2; H, 7.23; N, 9.57. Found: C, 49.3; H, 6.99; N, 9.31.

Boc-Dap(Z)-Ala-CH_2Cl (10) Yield: 1.15 g (57.6%), mp 182—183 °C, Rf^1 0.60, Rf^3 0.28, $[\alpha]_D^{21}$ −54.4° (c=1.0, DMF). ^{1}H-NMR (DMSO-d_6) δ: 8.43 (d, 1H, J=7.0 Hz, α-NH of Ala), 7.38—7.27 (m, 5H, Ar–H), 7.21 (br, 1H, β-NH of Dap), 6.86 (d, 1H, J=7.2 Hz, α-NH of Dap), 5.02 (s, 2H, –CH_2–Ph), 4.58 and 4.51 (AB-q, 2H, J=16.9 Hz, –CH_2Cl), 4.34 (p, 1H, J=7.0 Hz, α-CH of Ala), 4.02 (br-q, 1H, J=6.7 Hz, α-CH of Dap), 3.29 (t, 2H, J=5.8 Hz, β-CH_2 of Dap), 1.38 (9H, s, *tert*-butyl), 1.20 (d, 3H, J=7.1 Hz, methyl of Ala), ^{13}C-NMR (DMSO-d_6) δ: 200.9 (q, $COCH_2Cl$), 170.4, 156.2 and 155.2 (q, carbonyl), 136.9 (q, phenyl), 128.2, 127.7 and 127.6 (t, phenyl), 78.4 (q, *tert*-butyl), 65.4 (s, –CH_2–Ph), 54.6 (t, α-CH of Dap), 52.3 (t, α-CH of Ala), 47.5 (s, –CH_2Cl), 41.8 (s, β-CH_2 of Dap), 28.0 (p, *tert*-butyl), 15.6 (p, methyl of Ala). *Anal.* Calcd for $C_{20}H_{28}ClN_3O_6$: C, 54.4; H, 6.39; N, 9.51. Found: C, 54.2; H, 6.38; N, 9.44.

Boc-Ala-Dab(Z)-CH_2Cl (11) Yield: 374 mg (56.0%), mp 114—118 °C, Rf^2 0.40, $[\alpha]_D^{21}$ +53.4° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.28 (m, 5H, Ar–H), 7.25 (br, 1H, α-NH of Dap), 5.64 (br, 1H, β-NH of Dap), 5.06 (s, 2H, –CH_2–Ph), 5.03 (br, 1H, α-NH of Ala), 4.85 (m, 1H, α-CH of Dap), 4.45 and 4.29 (AB-q, 2H, J=15.7 Hz, –CH_2Cl), 4.07 (br quint, 1H, J=6.9 Hz, α-CH of Ala), 3.71 (m, 2H, β-CH_2 of Dap), 1.42 (9H, s, *tert*-butyl), 1.32 (d, 3H, J=7.1 Hz, methyl of Ala), ^{13}C-NMR ($CDCl_3$) δ: 199.6 (q, $COCH_2Cl$), 173.3, 157.4 and 155.7 (q, carbonyl), 136.2 (q, phenyl), 128.5, 128.2 and 128.0 (t, phenyl), 80.6 (q, *tert*-butyl), 67.1 (s, –CH_2–Ph),

57.3 (t, α-CH of Dap), 50.7 (t, α-CH of Ala), 46.4 (s, –CH_2Cl), 41.1 (s, β-CH_2 of Dap), 28.3 (p, *tert*-butyl), 17.6 (p, methyl of Ala). *Anal.* Calcd for $C_{20}H_{28}ClN_3O_6$: C, 54.4; H, 6.39; N, 9.51. Found: C, 54.7; H, 6.27; N, 9.21.

Preparation of Boc-Ala-Ala-Me (16) Boc-Ala-Ala-CMK (550 mg, 1.88 mmol) dissolved in 25 ml of MeOH was hydrogenated over a Pd-black catalyst and the pH of the reaction mixture was kept at 7 to 8 by Et_3N. The catalyst was removed by filtration after 4 h and the filtrate was concentrated *in vacuo*. The resulting residue was extracted with AcOEt, which was washed by 10% citric acid, 5% $NaHCO_3$ and saturated aqueous NaCl solution, then dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was removed to obtain the oily residue. Cold *n*-hexane was added to the residue to yield the crystals. Yield: 355 mg (73.1%), mp 123—125 °C, Rf^3 0.67, $[\alpha]_D^{21}$ −7.37° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 6.88 (d, 1H, J=5.9 Hz, α-NH), 5.06 (d, 1H, α-NH), 4.55 (quint, 1H, J=7.1 Hz, α-CH), 4.18 (br, 1H, α-CH), 2.21 (s, 3H, $COCH_3$), 1.45 (9H, s, *tert*-butyl), 1.37 (d, 3H, J=7.2 Hz, methyl of Ala), 1.36 (d, 3H, J=7.1 Hz, methyl of Ala), ^{13}C-NMR ($CDCl_3$) δ: 206.5 (q, $COCH_3$), 172.3 and 155.4 (q, carbonyl), 80.1 (q, *tert*-butyl), 54.6 (t, α-CH), 50.2 (t, α-CH), 28.4 (p, *tert*-butyl), 26.5 (p, $COCH_3$), 18.5 (p, methyl of Ala), 17.3 (p, methyl of Ala). *Anal.* Calcd for $C_{12}H_{22}N_2O_4\cdot 0.1H_2O$: C, 55.4; H, 8.57; N, 10.8. Found: C, 55.5; H, 8.65; N, 10.8.

Preparation of Fmoc-Ala-Ala-CH_2Cl (17) To a solution of a mixed anhydride [prepared from Fmoc-Ala-OH (773 mg, 2.48 mmol), Et_3N (379 μl, 2.71 mmol) and IBCF (337.4 μl, 2.59 mmol) in THF 70 ml], DMF (70 ml) solution containing H-Ala-CH_2Cl hydrochloride salt [prepared from Boc-Ala-CH_2Cl (500 mg, 2.26 mmol) and 8.0 N HCl/dioxane (2.80 ml, 22.6 mmol)] and Et_3N (379 μl, 2.71 mmol) was added at −15 °C. The reaction mixture was stirred at same temperature for 1 h and at room temperature for additional 4 h. Then the solvent was removed *in vacuo* and the residue was extracted with AcOEt, which was washed with 10% citric acid, 5% $NaHCO_3$, 5% Na_2CO_3 and saturated aqueous NaCl, then dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was removed and ether was added to the residue to yield crystals, which were colleted by filtration. Yield: 592 mg (63.2%), mp 198—200 °C, Rf^3 0.54, $[\alpha]_D^{21}$ −46.7° (c=1.0, DMF). ^{1}H-NMR (DMSO-d_6) δ: 8.39 (d, 1H, J=6.6 Hz, α-NH), 7.89 (d, 2H, J=6.6 Hz, C4-H and C5-H of Fmoc), 7.74 (d, 1H, J=6.7 Hz, C1-H of Fmoc), 7.72 (d, 1H, J=6.5 Hz, C8-H of Fmoc), 7.55 (d, 1H, J=7.2 Hz, α-NH), 7.42 (br t, 2H, J=7.2 Hz, C3-H and C6-H of Fmoc), 7.33 (t, 2H, J=7.4 Hz, C2-H and C7-H of Fmoc), 4.60 and 4.55 (AB-q, 2H, J=16.6 Hz, –CH_2Cl), 4.37 (quint, 1H, J=6.9 Hz, α-CH), 4.25 (m, 3H, –CH_2O– and CH– of Fmoc), 4.05 (quint, 1H, J=7.2 Hz, α-CH), 1.24 (d, 3H, J=7.2 Hz, methyl of Ala), 1.23 (d, 3H, J=7.1 Hz, methyl of Ala), ^{13}C-NMR (DMSO-d_6) δ: 201.1 (q, $COCH_2Cl$), 172.8 and 155.7 (q, carbonyl), 143.8 (q, C-8a of Fmoc), 143.7 (q, C-9a of Fmoc), 140.6 (q, C-4a and C-5a of Fmoc), 127.5 (t, C-3 and C-6 of Fmoc), 127.0 (t, C-2 and C-7 of Fmoc), 125.2 (t, C-1 and C-8 of Fmoc), 120.0 (t, C-4 and C-5 of Fmoc), 65.5 (s, –CH_2O– of Fmoc), 52.1 (t, α-CH), 49.7 (t, α-CH), 47.3 (s, –CH_2Cl), 46.6 (t, CH– of Fmoc), 17.7 (p, methyl of Ala), 15.5 (p, methyl of Ala). *Anal.* Calcd for $C_{22}H_{23}ClN_2O_4$: C, 63.7; H, 5.59; N, 6.75. Found: C, 63.7; H, 5.70; N, 6.65.

Preparation of Fmoc-Ala-Ala-Me (18) To a solution of a mixed anhydride [prepared from Fmoc-Ala-OH (1.12 g, 3.58 mmol), Et_3N (0.620 ml, 4.38 mmol) and IBCF (0.490 ml, 3.78 mmol) in THF 60 ml], DMF (30 ml) solution containing H-Ala-Me hydrochloride salt [prepared from Boc-Ala-Me (0.750 g, 3.98 mmol) and 8.0 N HCl/dioxane (2.50 ml, 19.9 mmol)] and Et_3N (0.670 ml, 4.78 mmol) was added at −15 °C. The reaction mixture was stirred at same temperature for 1 h and at room temperature for additional 4 h. Then the solvent was removed *in vacuo* and the residue was extracted with AcOEt, the AcOEt phase was washed with 10% citric acid, 5% $NaHCO_3$, 5% Na_2CO_3 and saturated aqueous NaCl, then dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was removed and ether was added to the residue to give crystals, which were colleted by filtration. Yield: 429 mg (31.5%), mp 192—194 °C, Rf^3 0.78, $[\alpha]_D^{21}$ −28.3° (c=1.0, DMF). ^{1}H-NMR (DMSO-d_6) δ: 8.23 (d, 1H, J=6.8 Hz, α-NH), 7.89 (d, 2H, J=6.6 Hz, C4-H and C5-H of Fmoc), 7.74 (d, 1H, J=6.5 Hz, C1-H of Fmoc), 7.72 (d, 1H, J=6.3 Hz, C8-H of Fmoc), 7.51 (d, 1H, J=7.5 Hz, α-NH), 7.42 (t, 2H, J=7.3 Hz, C3-H and C6-H of Fmoc), 7.33 (br t, 2H, J=7.3 Hz, C2-H and C7-H of Fmoc), 4.32—4.15 (m, 4H, –CH_2O–, CH– of Fmoc and α-CH), 4.08 (quint, 1H, J=7.3 Hz, α-CH), 2.05 (s, 3H, $COCH_3$), 1.25 (d, 3H, J=7.2 Hz, methyl of Ala), 1.18 (d, 3H, J=7.2 Hz, methyl of Ala), ^{13}C-NMR (DMSO-d_6) δ: 207.6 (q, $COCH_3$), 172.4 and 155.6 (q, carbonyl), 143.8 (q, C-8a of Fmoc), 143.7 (q, C-9a of Fmoc), 140.6 (q, C-4a and C-5a of Fmoc), 127.5 (t, C-3 and C-6 of Fmoc), 127.0 (t, C-2 and C-7 of Fmoc), 125.2 (t, C-1 and C-8 of Fmoc), 120.0 (t, C-4 and C-5 of Fmoc), 65.5 (s, –CH_2O– of Fmoc), 54.1 (t, α-CH), 49.7 (t, α-CH), 46.6 (t, CH– of Fmoc), 25.7 (p, $COCH_3$), 17.9 (p, methyl of Ala), 15.6 (p, methyl of Ala). *Anal.* Calcd for $C_{22}H_{24}N_2O_4$: C, 69.5; H, 6.36; N, 7.36. Found: C, 69.4; H, 6.41; N, 7.33.

General Procedure of 1,2-Dihydropyrazin-2-one Derivatives (2, 4, 13, 14) Monohydrochloride salt of dipeptidyl chromethyl ketones [prepared from Boc-protected dipeptidyl chromethyl ketones (1.11 mmol) and 6.3 N HCl/dioxane (45.1 mmol)] was dissolved in distilled MeOH or CH_3CN. The solution was stirred at 45—65 °C for 1—4 h. After the solvent was evaporated down, the residue was dissolved in $CHCl_3$, which was washed with 10% citric acid and saturated aqueous NaCl. The $CHCl_3$ phase was dried over Na_2SO_4 and after removal of Na_2SO_4, the solvent was evaporated. The resulting residue was precipitated from ether and a precipitate was collected by filtration. If necessary, the crude product was recrystallized from MeOH or EtOH.

3,6-*Bis*(3′-*Z*-aminopropyl)-5-methyl-1,2-dihydropyrazin-2-one (2) Yield: 636 mg (52.3%), mp 133—135 °C, Rf^3 0.56. ^{1}H-NMR ($CDCl_3$) δ: 7.32—7.28 (m, 10H, Ar–H), 6.11 (br, 1H, –NH-Z), 5.57 (br, 1H, –NH–Z), 5.08 (s, 2H, –CH_2–Ph), 5.06 (s, 2H, –CH_2–Ph), 3.20—3.14 (m, 4H, 2× –CH_2–CH_2–CH_2–NH–Z), 2.78 (t, 2H, J=7.0 Hz, –CH_2–CH_2–CH_2–NH–Z), 2.53 (t, 2H, J=7.0 Hz, –CH_2–CH_2–CH_2–NH–Z), 2.53 (s, 3H, 5-methyl), 1.79 (br, 4H, 2× –CH_2–CH_2–CH_2–NH–Z), ^{13}C-NMR ($CDCl_3$) δ: 157.7, 156.8, 156.5, 155.3, 136.8, 136.6, 134.4 and 130.6 (q), 128.5—127.7 (t, phenyl), 66.8 (s, –CH_2–Ph), 66.5 (s, –CH_2–Ph), 40.4 (s, –CH_2–CH_2–CH_2–NH–Z), 39.4 (s, –CH_2–CH_2–CH_2–NH–Z), 29.5 (s, –CH_2–CH_2–CH_2–NH–Z), 28.5 (s, –CH_2–CH_2–CH_2–NH–Z), 27.0 (s, –CH_2–CH_2–CH_2–NH–Z), 26.7 (s, –CH_2–CH_2–CH_2–NH–Z), 18.4 (p, 5-methyl). MS *m/z*: 493.6 (Calcd $[M+H]^+$: 493.6), *Anal.* Calcd for $C_{27}H_{32}N_4O_5\cdot 0.3H_2O$: C, 65.1; H, 6.55; N, 11.3. Found: C, 65.2; H, 6.59; N, 11.3.

3,6-*Bis*(Z-aminomethyl)-5-methyl-1,2-dihydropyrazin-2-one (4) Yield: 278 mg (57.5%), mp 190—193 °C, Rf^3 0.56. ^{1}H-NMR (pyridine-d_5) δ: 8.49 (br, 1H, –NH–Z), 8.03 (br, 1H, –NH–Z), 7.48—7.23 (m, 10H, Ar–H), 5.34 (s, 2H, –CH_2–Ph), 5.32 (s, 2H, –CH_2–Ph), 4.93 (d, 2H, J=4.8 Hz, –CH_2–NH–Z), 4.56 (d, 2H, J=4.8 Hz, –CH_2–NH–Z), 2.42 (s, 3H, 5-CH_3), ^{13}C-NMR (pyridine-d_5) δ: 157.4, 156.5, 138.1 and 137.8 (q), 128.8—128.1 (t, phenyl), 66.7 (s, –CH_2–Ph), 66.5 (s, –CH_2–Ph), 42.8 (s, –CH_2–NH–Z), 42.5 (s, –CH_2–NH–Z), 19.1 (p, 5-CH_3). MS *m/z*: 437.7 (Calcd $[M+H]^+$: 437.5), *Anal.* Calcd for $C_{23}H_{24}N_4O_5\cdot 0.5H_2O$: C, 62.0; H, 5.66; N, 12.6. Found: C, 62.3; H, 5.47; N, 12.6.

3-(Z-Aminomethyl)-5,6-dimethyl-1,2-dihydropyrazin-2-one (13) Yield: 87.5 mg (37.2%), mp 199—202 °C, Rf^3 0.52. ^{1}H-NMR (pyridine-d_5) δ: 7.93 (br, 1H, –NH–Z), 7.49—7.28 (m, 5H, Ar–H), 5.35 (s, 2H, –CH_2–Ph), 4.95 (d, 2H, J=4.5 Hz, –CH_2–NH–Z), 2.20 (s, 3H, 5-CH_3), 2.18 (s, 3H, 6-CH_3), ^{13}C-NMR (pyridine-d_5) δ: 157.3, 156.3 and 138.1 (q), 128.8, 128.3 and 128.1 (t, phenyl), 66.4 (s, –CH_2–Ph), 43.1 (s, –CH_2–NH–Z), 18.8 (p, 5-CH_3), 16.9 (p, 6-CH_3). MS *m/z*: 288.3 (Calcd $[M+H]^+$: 288.3), *Anal.* Calcd for $C_{15}H_{17}N_3O_3\cdot 0.5H_2O$: C, 60.8; H, 6.12; N, 14.2. Found: C, 61.1; H, 5.89; N, 14.1.

6-(Z-Aminomethyl)-3,5-dimethyl-1,2-dihydropyrazin-2-one (14) Yield: 136 mg (47.2%), mp 171—173 °C, Rf^3 0.56. ^{1}H-NMR (pyridine-d_5) δ: 8.48 (br, 1H, –NH–Z), 7.47—7.25 (m, 5H, Ar–H), 5.33 (s, 2H, –CH_2–Ph), 4.58 (d, 2H, J=4.5 Hz, –CH_2–NH–Z), 2.59 (s, 3H, 3-CH_3), 2.45 (s, 3H, 5-CH_3), ^{13}C-NMR (pyridine-d_5) δ: 157.4, 157.2 and 137.8 (q), 128.8, 128.34 and 128.25 (t, phenyl), 66.7 (s, –CH_2–Ph), 42.6 (s, –CH_2–NH–Z), 19.8 (p, 3-CH_3), 19.1 (p, 5-CH_3). MS *m/z*: 288.3 (Calcd $[M+H]^+$: 288.4), *Anal.* Calcd for $C_{15}H_{17}N_3O_3\cdot 0.3H_2O$: C, 61.4; H, 6.09; N, 14.3. Found: C, 61.6; H, 5.81; N, 14.0.

Preparation of 3,6-*Bis*(2′-*Z*-aminoethyl)-5-methyl-1,2-dihydropyrazin-2-one (3) Boc-Dab(Z)-Dab(Z)-CH_2Cl (200 mg, 0.32 mmol) was dissolved in 3.0 N HCl (4 ml) in THF (20 ml), the solution was refluxed for 1 h. After removal of THF, the residue was dissolved in $CHCl_3$, which was washed with 10% citric acid and saturated aqueous NaCl, and dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was evaporated down. The resulting crude material was recrystallized from EtOH. Yield: 81.4 mg (54.8%), mp 183—185 °C, Rf^3 0.57. ^{1}H-NMR (pyridine-d_5) δ: 8.34 (br, 1H, –NH–Z), 8.04 (br, 1H, –NH–Z), 7.45—7.24 (m, 10H, Ar–H), 5.32 (s, 2H, –CH_2–Ph), 5.29 (s, 2H, –CH_2–Ph), 3.99 (q, 2H, J=6.5 Hz, –CH_2–CH_2–NH–Z), 3.72 (q, 2H, J=6.5 Hz, –CH_2–CH_2–NH–Z), 3.32 (t, 2H, J=6.9 Hz, –CH_2–CH_2–NH–Z), 2.92 (t, 2H, J=6.9 Hz, –CH_2–CH_2–NH–Z), 2.33 (s, 3H, 5-CH_3), ^{13}C-NMR (pyridine-d_5) δ: 157.34, 157.28, 157.2, 138.2 and 138.0 (q), 128.8—128.1 (t, phenyl), 66.3 (s, –CH_2–Ph), 66.2 (s, –CH_2–Ph), 40.2 (s, –CH_2–CH_2–NH–Z), 39.5 (s, –CH_2–CH_2–NH–Z), 33.6 (s, –CH_2–CH_2–NH–Z), 32.5 (s, –CH_2–CH_2–NH–Z), 19.1 (p, 5-CH_3). MS *m/z*: 464.9 (Calcd $[M+H]^+$: 464.5), *Anal.* Calcd for $C_{25}H_{28}N_4O_5$: C, 64.6; H, 6.08; N, 12.1. Found: C, 64.4; H, 5.87; N, 12.0.

Preparation of 3,5,6-Trimethyl-1,2-dihydropyrazin-2-one (15) Boc-

Ala-Ala-CH_2Cl (250 mg, 0.85 mmol) was dissolved in MeOH, water and 35% HCl (3 ml, v/v/v=1 : 1 : 1), the solution was stirred at 60 °C for 1 h. After the solvent was evaporated down, the residue was dissolved in $CHCl_3$, which was washed with small amount of 10% citric acid, 5% $NaHCO_3$ and saturated aqueous NaCl. The $CHCl_3$ phase was dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was evaporated to give crystals, which were collected. Yield: 68.1 mg (57.7%), mp 207—210 °C, Rf^3 0.63. ^{1}H-NMR (pyridine-d_5) δ: 2.60 (br s, 3H, 3-C$\underline{H}_3$), 2.24 (s, 3H, 5-C$\underline{H}_3$), 2.15 (s, 3H, 6-C$\underline{H}_3$), ^{13}C-NMR (pyridine-d_5) δ: 20.1 (p, 3-$\underline{C}H_3$), 18.9 (p, 5-$\underline{C}H_3$), 16.8 (p, 6-$\underline{C}H_3$). MS *m/z*: 139.3 (Calcd $[M+H]^+$: 139.2), *Anal.* Calcd for $C_7H_{10}N_2O \cdot 0.1H_2O$: C, 60.1; H, 7.28; N, 20.0. Found: C, 60.4; H, 7.18; N, 20.3.

Mass Studies on Catalytic Hydrogenation of 1,2-Dihydropyrazin-2-one Derivatives (1—4) Four 1,2-dihydropyrazin-2-one derivatives (15 mg of each **1**—**3**, of which **1** was prepared according to the previous procedure,[4] and 7.6 mg of **4**, respectively) was dissolved in 50% AcOH (3.0 ml or 2.0 ml in the case of compound **4**), and H_2 gas was passed through the solution in the presence of a small amount of Pd-black catalyst. As a function of time, an aliquot of the reaction mixture at 10, 20, 30 and 60 min was withdrawn and analyzed by MS.

NMR Studies on the Unexpected Products Obtained during Catalytic Hydrogenation In the MS studies of **4**, the reaction mixture was separated from catalyst after 60 min, the solvent was removed and the resulting residues dissolved in water; the compound was purified by reverse-phase HPLC. The following conditions for HPLC purification were employed: 95% solvent A and 5% solvent B (see, "General Procedure") at a flow rate of 1 ml/min, and an analytical column. The main homogenous fractions that possessed a strong extinction at 365 nm was collected, the solvent was removed and the residue was lyophilized from water to give 3.9 mg (84% as mono-TFA salt) of brown amorphous powder. Rf^6 0.51. ^{1}H-NMR (pyridine-d_5) δ: 4.87 (s, 2H, –C$\underline{H}_2$–NH_2), 2.23 (s, 3H, 5-C$\underline{H}_3$), 2.15 (s, 3H, 6-C$\underline{H}_3$), ^{13}C-NMR (pyridine-d_5) δ: 40.3 (s, –$\underline{C}H_2$–NH_2), 18.6 (p, 5-$\underline{C}H_3$), 17.0 (p, 6-$\underline{C}H_3$). MS *m/z*: 154.1 (Calcd $[M+H]^+$: 154.2).

Mass Studies on Catalytic Hydrogenation of 1,2-Dihydropyrazin-2-one Derivatives (13, 14) The compounds **13** and **14** (5 mg, 17.4 μmol) were dissolved in 50% AcOH (2.0 ml), and hydrogenated as above. An aliquot of the reaction mixture (10 μl) was taken as a function of time (10, 20, 30 and 60 min, 2, 4 and 6 h), diluted with water (90 μl) and the diluted solution was analyzed by MS.

NMR Studies on the Products Obtained by Catalytic Hydrogenation of Compounds 13 and 14 To the 50% AcOH (5.0 ml) solution of **13** and **14** (50 mg, 0.174 mmol), H_2 gas was poured in the presence of a small amount of Pd-black catalyst. After 6 h the catalyst was removed by filtration and the filtrate was evaporated down *in vacuo*. The residue was dissolved in water, and purified by RP-HPLC, which was as follows: 95% A for 30 min and to 50% for 15 min (for product **9b**), 95% A for 5 min and to 55% for 40 min (for product **15**), and a semi-preparative column was employed in both cases. Characteristic data of these products was depicted as follows:

Product **9b**: Yield 13.2 mg (55.0%), Rf^6 0.52. ^{1}H-NMR (pyridine-d_5) δ: 4.87 (s, 2H, –C$\underline{H}_2$–NH_2), 2.21 (s, 3H, C$\underline{H}_3$), 2.16 (s, 3H, C$\underline{H}_3$), ^{13}C-NMR (pyridine-d_5) δ: 40.3 (s, –$\underline{C}H_2$–NH_2), 18.6 (p, $\underline{C}H_3$), 16.9 (p, $\underline{C}H_3$). MS *m/z*: 154.2 (Calcd $[M+H]^+$: 154.2).

Product **15**: Yield 17.8 mg (74.0%), Rf^5 0.63. ^{1}H-NMR (pyridine-d_5) δ: 2.60 (s, 3H, C$\underline{H}_3$), 2.24 (s, 3H, C$\underline{H}_3$), 2.15 (s, 3H, C$\underline{H}_3$), ^{13}C-NMR (pyridine-d_5) δ: 20.1 (p, $\underline{C}H_3$), 18.9 (p, $\underline{C}H_3$), 16.8 (p, $\underline{C}H_3$). MS *m/z*: 139.4 (Calcd $[M+H]^+$: 139.2).

Preparation of Deuterated Compounds. Introduction of Deuterium into Position 5 Compounds **10**, **11** and **12** (0.905, 0.226 and 0.342 mmol, respectively) were dissolved in total 2.25 ml deuterium chloride, deuterium oxide and methanol-d_4 (v/v/v=1 : 1 : 1). The solution was stirred at 65 °C for 30 min and at 25 °C for additional 1 h. The reaction mixture was evaporated *in vacuo*, and the residue was extracted with $CHCl_3$. The organic phase was washed with water or 10% citric acid and saturated aqueous NaCl, and dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was removed to yield the corresponding deuterated products **13a**, **14a** and **15a**. The yields were 71.4, 41.4 and 53.6%, respectively. The *Rf* values were same as those of non-deuterium substituted form.

Introduction of Deuterium into Position 3 Compounds **14** and **15** (54.6 μmol, 0.290 mmol, respectively) were dissolved in total 2.25 ml deuterium chloride, deuterium oxide and methanol-d_4 (v/v/v=1 : 1 : 1). The solution was stirred at 65 °C for 2 h. The solvent was removed by evaporation and the residue was extracted with $CHCl_3$. The organic phase was washed with water or small amount of 5% Na_2CO_3 and saturated aqueous NaCl, and dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was evaporated down to yield the corresponding deuterated products **14b**, **15b**. The yields were 95.5 and 63.8%, respectively. The *Rf* values were same as those of non-substituted form.

Cyclization from Boc-Ala-Ala-Me (16) in Deuterated Solvents Compound **16** was dissolved in total 2.25 ml deuterium chloride, deuterium oxide and methanol-d_4 (v/v/v=1 : 1 : 1). The solution was stirred at 65 °C for 30 min and 25 °C for additional 1 h. After removal of solvent, the residue was extracted with $CHCl_3$. The organic phase was washed with small amount of 5% Na_2CO_3 and saturated aqueous NaCl, and dried over Na_2SO_4. After removal of Na_2SO_4, the solvent was removed to yield product **15c**. The yield was 15.6% and the *Rf* value was same as that of non-substituted form.

Introduction of Deuterium into Fmoc-Derivatives (17, 18) Fmoc-derivatives **17** and **18** (50 μmol) dissolved in 0.75 ml of DMSO-d_6 were heated at 65 °C, then 35% deuterium chloride (20.8 μl, 0.198 mmol) was added into the solution. The solution was stored for 30 min to yield the deuterium compounds **17a** and **18a**. The extent of deuterium content was immediately analyzed by ^{1}H-NMR.

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