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

Anna Miyazaki,* Yutaka Fujisawa,* Kimitaka Shiotani,* Yoshio Fujita,* Tingyou Li,b Yuko Tsuda,a,b Toshio Yokoi,a,b Sharon D. Bryant,c Lawrence H. Lazarus,c and Yoshio Okada*a,b

a Faculty of Pharmaceutical Sciences, Kobe Gakuin University; and b High Technology Research Center, Kobe Gakuin University; Nishi-ku, Kobe 651–2180, Japan; and c Medicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences; Research Triangle Park, NC 27709, U.S.A.

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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(benzyloxycarbonylamino)methyl-5-methyl-1,2-dihydropyrazin-2-one to remove the benzyloxy carbonyl 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.1–3) 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 the endorphins although the binding activity and selectivity were lower than the endorphins, but considerably greater than the endorphins.* 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.

Results and Discussion

Studies on Hydrogenation Reaction of 1—4 Peptides and peptide mimetics were synthesized by a liquid phase method. Nα-tert-Butyloxy carbonyl(Boc)-Lys(Z)-OH and Nα-Boc-Orn(Z)-OH were prepared by generally known procedures.3) Nα-Boc-Gln-OH and Nα-Boc-Asn-OH were treated with bis(trifluoroacetoxy)-iodobenzene (BTIB)7–11) in DMF–water in the presence of pyridine to transform the amide of side chain into amine.8) Boc-OH, Orn, Dab or Dap was converted to the corresponding chloromethyl ketone by treating with diazomethane, followed by HCl–dioxane and the resulting amine coupled with benzyl oxycarbonyl(Z) group by treatment with 4-(benzyl oxy carbonyl)phenyl dimethy sulfoxide (Z-DSP) under basic condition to give Nα-Boc-Dab(Z)-OH (Dab: 2,4-diaminobutylic acid) or Nα-Boc-Dap(Z)-OH (Dap: 2,3-diaminopropionic acid), respectively. The carboxyl moiety of these compounds [Nα-Boc-Xaa(Z)-OH; Xaa = Lys, Orn, Dab or Dap] was converted to the corresponding chloromethyl ketone by catalytic hydrogenation over a Pd catalyst. However, catalytic hydrogenation of compound 4 produced an unexpected compound instead of 8. With the exception of 4, compounds 1—3 were converted to 5—7, respectively, by the 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.

Studies on Hydrogenation Reaction of 1—4 Peptides

Fig. 1. Structures of 3,6-Bis(Z-aminooalkyl)-5-methyl-1,2-dihydropyrazin-2-ones (1—4) and Deprotected Compounds (5—8)
group of the dipeptidyl chloromethyl ketone was removed by HCl–dioxane and the resulting H-Xaa(Z)-Xaa(Z)-CH₂Cl hydrochloride salt was treated under reflux conditions in either acetonitrile (CH₃CN) 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.

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 benzyl or allylic C–N bond, while the property of C–N bond of aminomethyl moiety at the position 3 of the ring is quite different from that of benzyl C–N bond. 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-d₅. 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 shifts of methyl group in compounds which possessed two or three methyl groups, in particular those com-
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. 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-d₄ : 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 ¹H-NMR in pyridine-d₅. 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 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-dihydropyrazin-2-one is that of position 5, 3, 6.

### Chart 4. Synthesis of Authentic Standards

Reagents and conditions: (a) HCl/dioxane; (b) reflux in MeOH; (c) H₂/Pd in 50% AcOH.

### 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 ¹H-NMR Studies

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

| 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 deuteriation. 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 deuteron-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 deuteriation 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-Fluorenlymethoxy carbonyl (Fmoc)-protected derivatives, Fmoc-Ala-Ala-CH2Cl (Chart 5; IV, 9) 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-d6 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 deuteriation 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 synthetic convenient 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 deuteriation studies based on reaction mechanisms and character of the 1,2-dihydropyrazin-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 deuteration 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.), \(\lambda = 490(600) \text{ nm (Hz)}\) and \(\lambda = 100 \text{ MHz}\) 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 \(^1\)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 for solvent B; column, Cosmosil C18-ARII (4.6 \times 250 mm), [Cosmosil C18-ARII (4.6 \times 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% F254 (Merck Japan, Ltd) and compounds were visualized by ultraviolet at 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% F254 (Merck Japan, Ltd).

General Procedure for Synthesis of Boc-Xaa(Z)-CH2Cl [Xaa=Dap, Dab, Orn] Diamonostane [prepared from p-toluenesulfonyl-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), BBTr*Cl (1.5 eq), pyridine (2.0 eq) and Z-DSPA (1.3 eq), triethylamine (Et3N; 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 9.8 H2O/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 NaHCO3, and saturated aqueous NaCl solution, then dried over Na2SO4. After removal of Na2SO4, 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)-CH2Cl** Yield: 2.90 g (75.9%), mp 94—97 °C, 1R/*, [α]25 +50.9° (c = 0.5, CHCl3). 1H-NMR (CDCl3) δ: 7.37—7.31 (m, 5H, Ar-H), 5.69 (d, 1H, J = 6.0 Hz, –NH2), 5.22 (br, 1H, β-NH), 5.07 (s, 2H, –CH2–Ph), 4.59 (br, 1H, α-CH), 4.42 and 4.30 (ABq, 2H, J = 15.6 Hz, –CH2–Cl), 3.70 (br, 1H, β-CCH), 3.59 (m, 1H, β-CCH), 1.94 (s, tert-butyl). 13C-NMR (CDCl3) δ: 200.3 (q, COC–C–C–C–CH2), 157.1 and 155.5 (q, carboxyl), 136.9 (q, phenyl), 128.3, 128.1 and 128.1 (t, phenyl), 80.7 (q, tert-butyl), 67.3 (s, –CH2–Ph), 58.2 (t, α-CH), 46.5 (s, –CH2–Cl), 41.9 (s, β-CCH), 28.3 (p, tert-butyl). Anal. Calcd for C24H35ClN2O4: C, 55.9; H, 6.25; N, 5.75. Found: C, 55.3; H, 6.31; N, 5.75.

**Boc-Dap(Z)-CH2Cl** Yield: 1.20 g (58.0%), mp 89—95 °C, 1R/*, [α]25 +5.7° (c = 0.5, CHCl3). 1H-NMR (CDCl3) δ: 7.35—7.31 (m, 5H, Ar-H), 5.45 (br, 2H, α-NH2 and γ-NH), 5.10 (s, 2H, –CH2–Ph), 4.57 (br, 1H, α-CH), 4.23 (2H, –CH2–Cl), 3.47 (br, 1H, γ-CCH), 3.11 (br, 1H, β-CCH), 2.09 (m, 1H, β-CCH), 1.67 (m, 1H, β-CCH), 1.44 (9H, s, tert-butyl). 13C-NMR (CDCl3) δ: 202.1 (q, COC–CH2), 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, –CH2–Ph), 55.0 (p, α-CH), 46.5 (s, –CH2–Cl), 31.7 (p, γ-CCH), 28.4 (p, tert-butyl). Anal. Calcd for C24H35ClN2O4: H2O. C, 55.3; H, 6.59; N, 6.17. Found: C, 55.1; H, 6.47; N, 7.08.

**Boc-Dap(Z)-CH2Cl** Yield: 4.80 g (88.9%), mp 69—73 °C, 1R/*, [α]25 +25.5° (c = 0.2, CHCl3). 1H-NMR (CDCl3) δ: 7.36—7.30 (m, 5H, Ar-H), 5.18 (br, 1H, α-NH2), 5.09 (s, 2H, –CH2–Ph), 4.94 (br, 1H, δ-NH), 4.51 (br, 1H, α-CH), 4.24 (s, 2H, –CH2–Cl), 3.23 (m, 2H, δ-CCH), 1.87 (s, 1H, β-CCH), 1.62—1.48 (m, 3H, β-CCH and γ-CCH), 1.44 (9H, s, tert-butyl). 13C-NMR (CDCl3) δ: 201.6 (q, COC–CH2), 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, –CH2–Ph), 57.0 (t, α-CH), 46.5 (s, –CH2–Cl), 40.3 (s, δ-CCH), 28.5 (s, β-CCH), 28.3 (p, tert-butyl), 26.1 (s, γ-CCH). Anal. Calcd for C24H35ClN2O4: C, 57.3; H, 6.80; N, 7.20. Found: C, 57.2; H, 6.82; N, 7.02.

**Boc-Dap(Z)-CH2Cl** Yield: 603 mg (45.6%), mp 125—130 °C, 1R/*, [α]25 +0.45°, [α]25′ −56.2° (c = 1.0, CHCl3). 1H-NMR (CDCl3) δ: 7.32—7.29 (m, 11H, Ar-H and α-NH), 5.30 (br, 1H, β-NH), 5.22 (2H, –CH2–Cl), 4.96 (s, 2H, –CH2–Ph), 4.74 (br, 1H, α-CH), 4.12 (2H, –CH2–Cl), 3.3 (br, 1H, γ-CCH), 1.82—1.77 (m, 1H, β-CCH), 1.42 (9H, s, tert-butyl). 13C-NMR (CDCl3) δ: 200.8 (q, COC–CH2), 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, –CH2–Ph), 56.0 and 53.1 (t, α-CH), 46.6 (s, –CH2–Cl), 40.2 and 39.7 (s, δ-CCH), 29.8 (s, β-CCH), 28.4 (p, tert-butyl), 28.3 (s, β-CCH). Anal. Calcd for C24H35ClN2O4: C, 59.4; H, 6.70; N, 8.80. Found: C, 59.7; H, 6.65; N, 8.83.

**Boc-Dap(Z)-CH2Cl** Yield: 1.15 g (57.6%), mp 182—183 °C, 1R/*, [α]25′ +0.60°, [α]25 +0.28°, [α]25′ +54.4° (c = 1.0, DMF). 1H-NMR (CDCl3) δ: 7.29—7.25 (m, 10H, Ar-H), 5.15 (br, 1H, α-NH), 5.01 (s, 2H, –CH2–Cl), 4.58 and 4.51 (ABq, 2H, J = 16.9 Hz, –CH2–Ph), 4.34 (p, 1H, β-CCH). Anal. Calcd for C24H35ClN2O4: C, 59.4; H, 6.70; N, 8.80. Found: C, 59.7; H, 6.65; N, 8.83.
57.3 (t, α-CH of Dop), 50.7 (t, α-CH of Ala), 46.4 (s, –CH2Cl), 41.1 (s, β-CH of Dop), 28.3 (p, tert-butyl), 17.6 (p, methyl of Ala). Anal. Calc. for C25H39ClN2O4·0.1H2O: C, 55.4; H, 8.6; N, 10.8. Found: C, 55.4; H, 8.9; N, 10.8.

Preparation of Fmoc-Ala-Ala-CH3 (17) To a solution of a mixed anhydride [prepared from Fmoc-Ala-OH (773 mg, 2.48 mmol), Et3N (577 mg, 5.76 mmol) and DMF (70 ml) solution containing H-Ala-Ala-CH3 hydrochloride salt [prepared from Boc-Ala-CHO, 80.0 μl (0.205 mmol)] and water (1.5 ml)] was stirred 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% NaHCO3, 5% Na2CO3 and saturated aqueous NaCl, then dried over Na2SO4.

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), Et2N (0.620 ml, 4.38 mmol) and DBF (0.490 ml, 3.78 mmol) in THF (60 ml), DMF (30 ml) solution containing H-Ala-Ala-CH3 hydrochloride salt [prepared from Boc-Ala-Ala-Me (0.750 g, 3.98 mmol) and 80.0 μl (0.205 mmol)] and water (1.5 ml)] 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% NaHCO3, 5% Na2CO3 and saturated aqueous NaCl, then dried over Na2SO4.

Preparation of Fmoc-Ala-Ala-OH (19) To a solution of a mixed anhydride [prepared from Fmoc-Ala-OH (1.31 g, 3.85 mmol), Et3N (0.84 ml, 6.00 mmol) and DBF (1.15 ml, 8.70 mmol)] and water (2 ml) was stirred 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% NaHCO3, 5% Na2CO3 and saturated aqueous NaCl, then dried over Na2SO4.

Preparation of Boc-D(Val)-D(Val)-Z(C12H22N2O4·0.1H2O) (20) To a solution of a mixed anhydride [prepared from Boc-protected dipeptidyl chiroleukomethyl ketones (1.11 mmol) and 6.3 n HCl/dioxane (45.1 mmol)] was dissolved in distilled MeOH or CH3CN. The solution was stirred at 45—65 °C for 1—4 h. After the solvent was evaporated, the residue was dissolved in CHCl3, which was washed with 10% citric acid and saturated aqueous NaCl. The CHCl3 phase was dried over Na2SO4 and after removal of Na2SO4, 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.

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Introduction of Deuterium into Fmoc-Derivatives (17, 18)  
Fmoc-derivatives 17 and 18 (50 μmol) dissolved in 0.75 ml of DMSO-d6 were heated at 65 °C, then 35% deuterium chloride (20.8 ml, 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 primarily analyzed by 1H-NMR.

References