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Metal Ion-binding Ability of Tetrapeptides Containing α-Aminoisobutyric Acid

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Abstract: α-Aminoisobutyric acid (Aib), one of the $C^{\alpha,\alpha}$-disubstituted glycines, is a sterically hindered amino acid that acts as a conformational constraint in peptides. However, studies for the application of the ability of Aib to control conformation are quite few. The paper focuses on the molecular recognition ability of acyclic oligopeptides containing Aib. Liquid–liquid extraction of nine kinds of metal ions from aqueous layers to nonpolar organic layers with acyclic tetrapeptides, X-Trp-Xaa_2-Gly-Xaa_4-NH-Ar (X = H or $C_6H_5CH_2OCO$ (Z), Xaa_2 = Aib or Gly, Xaa_4 = Leu or Ala, Ar = phenyl or 3,5-dimethylphenyl) was examined using picrate as the anion of ion pairs. The extraction behaviour of the metal ions with the tetrapeptides was investigated in the pH range from 3 to 9. In the case of basic pH regions, Cu(II) and Ag(I) were effectively extracted with Trp-Aib-Gly-Leu-NH-Ar. Pd(II) was specifically extracted with Trp-Aib-Gly-Leu-NH-Ar in acidic pH regions. The extraction percent (%E) of the peptide host, which has a 3,5-dimethylphenyl group, was even larger than that of the host, which has a phenyl group. Moreover, Pd(II) was extracted with a peptide host which has Leu and a 3,5-dimethylphenyl group in the absence of picrate as the anion of ion pairs. The free α-amino group, the turn conformation and the hydrophobicity of peptide molecules were important factors for the extraction of the metals.

Keywords: α-aminoisobutyric acid; β-turn structure; liquid–liquid extraction; metal ion-binding

INTRODUCTION

Metalloenzymes such as carboxypeptidase, catalase and nitrogenase form the coordination site and the recognition site for metal ions by means of the construction of structures such as a helix and a sheet [1,2]. Those structures are stabilized by multiple non-covalent intramolecular forces, e.g. hydrogen bonding, ionic interaction, hydrophobic interaction and steric hindrance. Designs of oligopeptides that have recognition ability were usually based on conformational control due to many amino acid residues. α-Aminoisobutyric acid (Aib), one of the $C^{\alpha,\alpha}$-disubstituted glycines, is a strong helix-promoting residue [3]. Previously, it was reported that the peptide, Dnp-Val-Aib-Gly-Leu-pNA, prefers the β-turn structure due to the steric effect of the methyl groups of Aib, the intramolecular hydrogen bonds, and the stacking of 2,4-dinitrophenyl (Dnp) and *p*-nitroanilino (pNA) groups [4, 5]. As an application of the ability of Aib to control conformation, the quaternary ammonium-binding ability of Aib-containing tetrapeptides, Trp-Aib-Gly-Leu-NH-Ar (Ar = phenyl or 3,5-dimethylphenyl) was reported recently [6]. Although studies of various ligands such as crown ether [7,8], thio crown ether [9,10], cyclophane [11], amino acids [12] and peptides [13–18] have been reported, studies on the design of the ligand of the peptide containing $C^{\alpha,\alpha}$-disubstituted glycine are scarce.

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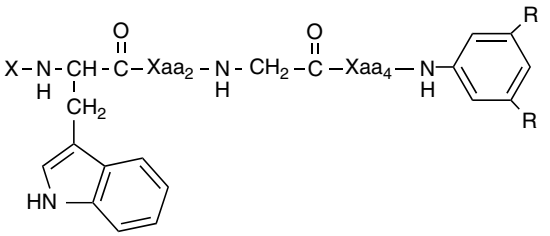
The metal ion-binding ability is reported of Aib-containing tetrapeptides, X-Trp-Xaa_2-Gly-Xaa_4-NH-Ar (**1**–**7**), the structures of which are shown in Scheme 1. These peptides have an amino group, amide bonds and π-basic aromatic groups, all of which are expected to act as binding sites for the metal ions. In order to examine the effect of the free amino group on the extraction of metal ions, peptide (**7**) which has no free amino group was also analysed.

MATERIALS AND METHODS

^{1}H NMR spectra were recorded on a Varian Unity 300 MHz instrument at 299.94 MHz. Tetramethylsilane was used as the internal standard. Assignments of signals for the peptides were made by COSY and gHMBC correlations. Specific rotations were measured on a Jasco DIP 1000 digital polarimeter. MALDI-TOF mass spectra were recorded on a PerSeptive Biosystems Voyager DE PRO Biospectrometry Workstation, where α-cyano-4-hydroxycinnamic acid was used as a matrix reagent. The quantitative analysis of metal ions was carried out by a Hitachi Z-5310 atomic absorption spectrophotometer. Circular dichroism (CD) spectra of peptides were obtained by using a Jasco J-600 spectropolarimeter with 0.1 cm pathlength quartz cell at 25 °C. Spectrograde 1,2-dichloroethane was used. The values are expressed in terms of $[\theta]_T$, the total molar ellipticity (deg cm^2 dmol^{-1}).



1 X=H, Xaa_2=Aib, Xaa_4=Leu, R=H

2 X=H, Xaa_2=Aib, Xaa_4=Leu, R=CH_3

3 X=H, Xaa_2=Aib, Xaa_4=Ala, R=H

4 X=H, Xaa_2=Aib, Xaa_4=Ala, R=CH_3

5 X=H, Xaa_2=Gly, Xaa_4=Leu, R=H

6 X=H, Xaa_2=Gly, Xaa_4=Leu, R=CH_3

7 X=Z, Xaa_2=Aib, Xaa_4=Leu, R=H

Scheme 1

Peptide Synthesis

The peptides used as hosts were prepared by the usual Z strategy in the liquid phase. The preparation of various peptides, Trp-Aib-Gly-Leu-NH-C_6H_5 (**1**), Trp-Aib-Gly-Leu-NH-$C_6H_3(CH_3)_2$ (**2**) and Z-Trp-Aib-Gly-Leu-NH-C_6H_5 (**7**) were described in our previous report [6]. The coupling reactions were performed according to the carbodiimide-HOBt method. The removal of protecting groups of di-, tri- and tetrapeptides was attained by the HBr/AcOH method or catalytic hydrogenation. Aniline, 3,5-dimethylaniline, picric acid and all metal ions were obtained commercially.

General Procedure for Z-Ala-NH-Ar

To a solution of Z-Ala (50.0 mmol), aniline (52.0 mmol) and HOBt (50 mmol) in chloroform (50 cm^3) and MeCN (50 cm^3), EDC·HCl (52 mmol) was added slowly at 0 °C. The solution was stirred for 24 h at room temperature and was then dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (70 cm^3). The solution was washed with 1 M HCl (3 × 50 cm^3), 1 M $NaHCO_3$ (3 × 50 cm^3) and saturated NaCl (50 cm^3) and dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was recrystallized from ethyl acetate–hexane.

Z-Ala-NH-Ph. Yield: 65.7%, mp 162°–163 °C, $[\alpha]_D^{25}$ −29.3° (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.46 (3H, d, J = 6.9 Hz, Ala-βCH_3), 4.38 (1H, q, J = 6.6 Hz, Ala-αCH), 5.14 (2H, dd, J = 12.0 and 15.6 Hz, Z-CH_2), 5.33 (1H, d, J = 5.6 Hz, Ala-NH) 7.09 (1H, t, J = 7.5 Hz, Ph(anilide)-4H), 7.28 (2H, t, J = 7.5 Hz, Ph(anilide)-3H), 7.32 (5H, m, Ph(Z)), 7.47 (2H, d, J = 7.5 Hz, Ph(anilide)-2H), 8.18 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 321.12. ($C_9H_{13}N_2O + H)^+$ requires 321.13].

Z-Ala-NH-C$_6$H$_3$(3,5-Me$_2$). Yield 76.1%, mp 186°–187 °C, $[\alpha]_D^{25}$ + 5.02° (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.45 (3H, d, J = 6.9 Hz, Ala-βCH_3), 4.41 (1H, br, Ala-αCH), 5.13 (2H, dd, J = 12.0 and 15.6 Hz, Z-CH_2), 5.50 (1H, d, J = 5.7 Hz, Ala-NH) 6.74 (1H, s, xylyl-4H), 7.13 (2H, s, xylyl-2H), 7.34 (5H, m, Ph(Z)), 9.79 (1H, s, xylidide-NH).

m/z (MALDI-TOF MS) [Found: 349.15. ($C_9H_{13}N_2O + H)^+$ requires 349.15].

General Procedure for Ala-NH-Ar·HBr

Z-Ala-NH-Ar (16.8 mmol) was added to a solution of 25% hydrogen bromide in acetic acid (16.8 g) and allowed to stand for 1.5 h at room temperature. Precipitation occurred with the addition of ether (100 cm^3). It was filtered and then washed with ether. A white amorphous solid was obtained.

Ala-NH-Ph·HBr. Yield 96.7%, $[\alpha]_D^{25} + 5.02^\circ$ (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d_6), δ (ppm) = 1.43 (3H, d, $J = 7.2$ Hz, Ala-βCH_3), 4.03 (1H, br, Ala-αCH), 7.07–7.13 (1H, m, Ph(anilide)-4H), 7.31–7.37 (2H, m, Ph(anilide)-3H), 7.58–7.62 (2H, m, Ph(anilide)-2H), 8.17 (3H, br, Ala-NH_3^+), 10.45 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 165.10. ($C_9H_{13}N_2O + H)^+$ requires 165.10].

Ala-NH-C$_6$H$_3$(3,5-Me$_2$)·HBr. Yield 96.7%, $[\alpha]_D^{25} + 4.16^\circ$ (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d_6) δ (ppm) = 1.43 (3H, d, $J = 7.2$ Hz, Ala-βCH_3), 2.23 (6H, s, xylyl-CH_3) 4.02 (1H, br, Ala-αCH), 6.74 (1H, s, xylyl-4H), 7.22 (2H, s, xylyl-2H), 8.17 (3H, br, Ala-NH_3^+), 10.32 (1H, s, xylidide-NH).

m/z (MALDI-TOF MS) [Found: 193.13. ($C_9H_{13}N_2O + H)^+$ requires 193.13].

General Procedure for Z-Gly-Leu-NH-Ar

To a solution of Z-Gly (5.87 mmol), Leu-NH-Ar (5.87 mmol) and HOBt (5.87 mmol) in DMF (100 cm^3), EDC·HCl (5.89 mmol) was added slowly at 0 °C. The solution was stirred for 24 h at room temperature and was then dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (60 cm^3). The solution was washed with 1 M HCl (3×40 cm^3), 1 M $NaHCO_3$ (3×40 cm^3), and saturated NaCl (30 cm^3), and dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was recrystallized from ethyl acetate–hexane.

Z-Gly-Leu-NH-Ph. Yield 83.9%, mp 138°–139 °C, $[\alpha]_D^{25} - 54.6^\circ$ (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.46 (3H, dd, $J = 6.9$ and 7.8 Hz, Leu-δCH_3), 1.57–1.82 (3H, m, Leu-βCH_2 and Leu-γCH), 3.88 (2H, $J = 5.4$ Hz, Gly-αCH_2), 4.62 (1H, q, $J = 7.8$ Hz, Leu-αCH), 5.10 (2H, s, Z-CH_2), 5.66 (1H, d, $J = 5.4$ Hz, Gly-NH), 6.89 (1H, d, $J = 7.8$ Hz, Leu-NH), 7.09 (1H, t, $J = 7.5$ Hz, Ph(anilide)-4H), 7.24–7.32 (7H, m, Ph(anilide)-3H and Ph(Z)), 7.52 (2H, d, $J = 7.5$ Hz, Ph(anilide)-2H), 8.62 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 420.19. ($C_{22}H_{27}N_3O_4 + Na)^+$ requires 420.19].

Z-Gly-Leu-NH-C$_6$H$_3$(3,5-Me$_2$). Yield 73.5%, mp 162°–163 °C, $[\alpha]_D^{25} - 54.9^\circ$ (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.46 (3H, dd, $J = 6.9$ and 7.8 Hz, Leu-δCH_3), 1.58–1.72 (3H, m, Leu-βCH_2 and Leu-γCH), 2.23 (6H, s, xylyl-CH_3), 3.88 (2H, $J = 5.4$ Hz, Gly-αCH_2), 4.69 (1H, q, $J = 7.8$ Hz, Leu-αCH), 5.11 (2H, s, Z-CH_2), 5.56 (1H, d, $J = 5.4$ Hz, Gly-NH), 6.73 (1H, s, xylyl-4H), 6.85 (1H, d, $J = 7.8$ Hz, Leu NH), 7.16 (2H, s, xylyl-2H), 7.32 (5H, m, Ph(Z)), 8.18 (1H, s, xylydide-NH).

m/z (MALDI-TOF MS) [Found: 448.22. ($C_{24}H_{31}N_3O_4 + Na)^+$ requires 448.22].

Gly-Leu-NH-Ph. Hydrogen was bubbled through a solution of Z-Gly-Leu-NH-Ph (1.27 g, 3.20 mmol) in THF (20 cm^3) in the presence of 5% palladium on activated carbon (320 mg) for 1.5 h at room temperature. The catalyst was filtered off and the filtrate was dried under reduced pressure. A white amorphous solid was obtained.

Yield 87.2%, mp 152°–153 °C, $[\alpha]_D^{25} + 19.1^\circ$ (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 0.97 (3H, dd, $J = 6.9$ and 7.8 Hz, Leu-δCH_3), 1.43 (2H, br s, Gly-NH_2), 1.60–1.89 (3H, m, Leu-βCH_2 and Leu-γCH), 3.49 (2H, s, Gly-αCH_2), 4.65 (1H, q, $J = 7.8$ Hz, Leu-αCH), 7.07 (1H, t, $J = 7.5$ Hz, Ph(anilide)-4H), 7.28 (1H, t, Ph(anilide)-3H), 7.53 (2H, d, $J = 7.5$ Hz, Ph(anilide)-2H), 7.78 (1H, d, $J = 7.8$ Hz, Leu-NH), 8.18 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 264.17. ($C_{14}H_{21}N_3O_2 + H)^+$ requires 264.18].

Gly-Leu-NH-C$_6$H$_3$(3,5-Me$_2$)·HBr. The same procedure as described above was performed with HBr/AcOH.

Yield 68.3%, $[\alpha]_D^{25} + 21.2^\circ$ (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d_6), δ (ppm) = 1.46 (3H, dd, $J = 6.9$ and 7.8 Hz, Leu-δCH_3), 1.57–1.82 (3H, m, Leu-βCH_2 and Leu-γCH), 2.23 (6H, s, xylyl-CH_3), 3.69 (2H, s, Gly-αCH_2), 4.02 (1H, br, Leu-αCH), 6.74 (1H, s, xylyl-4H), 7.22 (2H, s, xylyl-2H), 8.17 (3H, br, Gly-NH_3^+), 10.32 (1H, s, xylidide-NH)

m/z (MALDI-TOF MS) [Found: 292.20. ($C_{16}H_{25}N_3O_2 + H)^+$ requires 292.20].

General Procedure for Z-Xaa$_2$-Gly-Xaa$_4$-NH-Ar

To a solution of Z-Aib (1.90 mmol), Gly-Leu-NH-Ar (1.91 mmol) and HOBt (1.90 mmol) in chloroform

(20 cm^3), EDC·HCl (2.28 mmol) was added slowly at 0 °C. The solution was stirred for 24 h at room temperature and was then dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (60 cm^3). The solution was washed with 1 M HCl (3 × 40 cm^3), 1 M $NaHCO_3$ (3 × 40 cm^3), and saturated NaCl (30 cm^3), and dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was recrystallized from ethyl acetate.

Z-Aib-Gly-Leu-NH-Ph. Yield 77.0%, mp 161°–162 °C, $[\alpha]_D^{25}$ − 2.88° (*c* 1.0, MeOH);

^{1}H NMR ($CDCl_3$), δ (ppm) = 0.93 (3H, dd, J = 6.9 and 7.8Hz, Leu-δCH_3), 1.50 (6H, s, Aib-βCH_3), 1.67–1.79 (3H, m, Leu-βCH_2 and Leu-γCH), 3.93 (2H, ddd, J = 5.4, 6.6 and 17.7 Hz, Gly-αCH_2), 4.62 (1H, br, Leu-αCH), 4.96 (2H, dd, J = 9.9 and 12.0 Hz, Z-CH_2), 5.70 (1H, s, Aib-NH), 7.06 (1H, t, J = 7.5 Hz, Ph(anilide)-4H), 7.17 (1H, t, J = 5.4 Hz, Gly-NH), 7.28–7.32 (7H, m, Ph(anilide)-3H and Ph(Z)), 7.73 (1H, d, J = 7.8 Hz, Leu-NH), 7.80 (2H, d, J = 7.5 Hz, Ph(anilide)-2H), 8.66 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 505.25. ($C_{26}H_{34}N_4O_5 + Na)^+$ requires 505.24].

Z-Gly-Gly-Leu-NH-Ph. Yield 56.0%, mp 143–144 °C, $[\alpha]_D^{25}$ − 24.6° (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 0.97 (3H, dd, J = 6.9 and 7.8 Hz, Leu-δCH_3), 1.53–1.78 (3H, m, Leu-βCH_2 and Leu-γCH), 3.98–4.05 (4H, m, 2-Gly-αCH_2 and 3-Gly-αCH_2), 4.38 (1H, q, J = 7.8 Hz, Leu-αCH), 5.14 (2H, s, Z-CH_2), 6.04 (1H, d, J = 5.4 Hz, 2-Gly-NH), 7.09 (1H, t, J = 7.5 Hz, Ph(anilide)-4H), 7.28–7.32 (7H, m, Ph(anilide)-3H and Ph(Z)), 7.43 (1H, d, J = 7.8 Hz, Leu-NH), 7.56 (2H, d, J = 7.5 Hz, Ph(anilide)-2H), 7.66 (1H, d, J = 5.4 Hz, 3-Gly-NH), 8.18 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 436.46. ($C_{22}H_{27}N_3O_5 + Na)^+$ requires 436.46].

Z-Gly-Gly-Leu-NH-C_6H_3(3,5-Me_2). Yield 87.0%, mp 127°–128 °C, $[\alpha]_D^{25}$ − 56.5° (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.46 (3H, dd, J = 6.9 and 7.8 Hz, Leu-δCH_3), 1.57–1.82 (3H, m, Leu-βCH_2 and Leu-γCH), 2.23 (6H, s, xylyl-CH_3), 3.88–3.99 (4H, m, 2-Gly-αCH_2 and 3-Gly-αCH_2), 4.69 (1H, q, J = 7.8 Hz, Leu-αCH), 5.11 (2H, s, Z-CH_2), 5.56 (1H, d, J = 5.4 Hz, Gly-NH), 6.73 (1H, s, xylyl-4H), 6.85 (1H, d, J = 7.8 Hz, Leu-NH), 7.16 (2H, t, J = 7.5Hz, xylyl-2H), 7.32 (5H, m, Ph(Z)), 7.65 (1H, d, J = 5.4 Hz, 3-Gly-NH), 8.18 (1H, s, xylydide-NH).

m/z (MALDI-TOF MS) [Found: 448.22. ($C_{24}H_{31}N_3O_5 + Na)^+$ requires 448.22].

General Procedure for Xaa_2-Gly-Xaa_4-NH-Ar

Removal of Z groups of Z-Aib-Gly-Leu-NH-Ph was carried out with the same procedure (H_2/Pd) as described above.

Aib-Gly-Leu-NH-Ph. Yield 89.3%, $[\alpha]_D^{25}$ − 69.7° (*c* 1.0, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) 0.97 (3H, dd, J = 3.1 and 6.8 Hz, Leu-δCH_3), 1.36 (6H, s, Aib-βCH_3), 1.43 (2H, br, Aib-NH_2), 1.58–1.77 (3H, m, Leu-βCH_2 and Leu-γCH), 3.95 (2H, d, J = 5.7 Hz, Gly-αCH_2), 4.60 (1H, q, J = 7.8 Hz, Leu-αCH), 7.07 (1H, t, J = 7.5 Hz, Ph(anilide)-4H), 7.28 (1H, t, Ph(anilide)-3H), 7.53 (2H, d, J = 7.5 Hz, Ph(anilide)-2H), 7.78 (1H, d, J = 7.8 Hz, Leu-NH), 8.53 (1H, br, Gly-NH), 8.73 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 371.20. ($C_{18}H_{28}N_4O_3 + Na)^+$ requires 371.20].

Gly-Gly-Leu-NH-Ph. Removal of Z groups of Z-Gly-Gly-Leu-NH-Ph was carried out with the same procedure (H_2/Pd) as described above.

Yield 98.4%, $[\alpha]_D^{25}$ + 21.2° (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d_6), δ(ppm) = 0.87 (6H, dd, J = 6.9 and 7.8Hz, Leu-δCH_3), 1.49–1.60 (3H, m, Leu-βCH_2 and Leu-γCH), 1.43 (2H, br, Gly-NH_2), 3.59 (2H, br, 3-Gly-αCH_2), 3.89 (2H, br, 2-Gly-αCH_2), 4.02 (1H, br, Leu-αCH), 7.07 (1H, t, J = 7.5 Hz, Ph(anilide)-4H), 7.28 (1H, t, J = 7.5 Hz, Ph(anilide)-3H), 7.53 (2H, d, J = 7.5 Hz, Ph(anilide)-2H), 7.78 (1H, d, J = 7.8 Hz, Leu NH), 8.53 (1H, br, Gly-NH), 8.73 (1H, s, anilide-NH).

m/z (MALDI-TOF MS) [Found: 337.39. ($C_{16}H_{22}N_4O_4 + H)^+$ requires 337.39].

Gly-Gly-Leu-NH-C_6H_3(3,5-Me_2)·HBr. Removal of Z groups of Z-Gly-Gly-Leu-NH-C_6H_3(3,5-Me_2) was carried out with the same procedure (HBr/AcOH) as described above.

Yield 68.3%, $[\alpha]_D^{25}$ + 21.2° (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d_6) δ (ppm) = 0.87 (3H, dd, J = 6.9 and 7.8 Hz, Leu-δCH_3), 1.57–1.82 (3H, m, Leu-βCH_2 and Leu-γCH), 2.23 (6H, s, xylyl-CH_3), 3.69 (2H, s, Gly-αCH_2), 4.02 (1H, br, Leu-αCH), 6.74 (1H, s, xylyl-4H), 7.22 (2H, s, xylyl-2H), 8.17 (3H, br, Gly-NH_3^+), 10.32 (1H, s, xylidide-NH).

m/z (MALDI-TOF MS) [Found: 365.22. ($C_{18}H_{28}N_4O_4 + H)^+$ requires 365.22].

General Procedure for Z-Trp-Gly-Gly-Leu-NH-Ar

To a solution of Z-Trp (3.61 mmol), Gly-Gly-Leu-NH-Ar (3.61 mmol), and HOBt (3.61 mmol) in DMF

(20 cm^3), EDC·HCl (4.33 mmol) was added slowly at 0 °C. The solution was stirred for 24 h at room temperature and was then dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (60 cm^3). The solution was washed with 1 M HCl (3 × 40 cm^3), 1 M $NaHCO_3$ (3 × 40 cm^3), and saturated NaCl (30 cm^3), and dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was recrystallized from ethyl acetate–hexane.

Z-Trp-Gly-Gly-Leu-NH-Ph. Yield 77.6%, mp 124°–125 °C, $[\alpha]_D^{25}$ − 27.7° (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d_6), δ (ppm) = 0.86 (6H, dd, J = 6.9 and 7.8Hz, Leu-δCH_3), 1.54–1.61 (3H, m, Leu-βCH_2 and Leu-γCH), 3.05 (2H, ddd, J = 6.6, 7.2 and 14.7 Hz, Trp- βCH_2), 3.75–3.79 (4H, m, 2-Gly-αCH_2 and 3-Gly-αCH_2), 4.41 (1H, q, J = 7.2 Hz, Trp-αCH), 4.60 (1H, apparent q, J = 7.2Hz, Leu-αCH), 4.92 (2H, s, Z-CH_2), 7.04 (1H, d, J = 1.5 Hz, indolyl-2H), 7.02–7.09 (3H, m, indolyl-5H, Ph(anilide)-4H, Trp-NH), 7.11 (1H, t, J = 8.1 Hz, indoly-6H), 7.17–7.32 (8H, m, Ph(Z), Ph(anilide)-3H and 2-Gly-NH), 7.35 (1H, J = 8.1 Hz, indolyl-7H), 7.44 (1H, d, J = 7.2 Hz, Leu-NH), 7.63 (1H, d, J = 7.8 Hz, indolyl-4H), 7.74 (2H, d, J = 7.8 Hz Ph(anilide)-2H), 8.34 (1H, br, 3-Gly-NH), 9.90 (1H, s, anilide-NH), 10.7 (1H, br, indolyl-NH).

m/z (MALDI-TOF MS) [Found: 679.29. ($C_{35}H_{40}N_6O_7 + Na)^+$ requires 679.29].

Z-Trp-Gly-Gly-Leu-NH-C$_6$H$_3$(3,5-Me$_2$). Yield 73.4%, mp 127°–128 °C, $[\alpha]_D^{25}$ − 56.5° (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d_6), δ (ppm) = 0.85 (6H, dd, J = 6.9 and 7.8 Hz, Leu-δCH_3), 1.54–1.61 (3H, m, Leu-βCH_2 and Leu-γCH), 3.05 (2H, ddd, J = 6.6, 7.2 and 14.7 Hz, Trp-βCH_2), 3.75–3.79 (4H, m, 2-Gly-αCH_2 and 3-Gly-αCH_2), 4.41 (1H, q, J = 7.2 Hz, Trp-αCH), 4.60 (1H, apparent q, J = 7.2 Hz, Leu-αCH), 4.92 (2H, s, Z-CH_2), 6.73 (1H, s, xylyl-4H), 7.04 (1H, d, J = 1.5 Hz, indolyl-2H), 7.02–7.06 (2H, m, indolyl-5H, Trp-NH), 7.11 (1H, t, J = 8.1 Hz, indoly-6H), 7.16 (2H, t, J = 7.5 Hz, xylyl-2H), 7.21–7.32 (8H, m, Ph(Z), and 2-Gly-NH), 7.35 (1H, J = 8.1Hz, indolyl-7H), 7.44 (1H, d, J = 7.2 Hz, Leu-NH), 7.63 (1H, d, J = 7.8 Hz, indolyl-4H), 8.34 (1H, br, 3-Gly-NH), 9.78 (1H, s, anilide-NH), 10.6 (1H, br, indolyl-NH).

m/z (MALDI-TOFMS) [Found: 707.31. ($C_{37}H_{44}N_6O_7 + Na)^+$ requires 707.31].

General Procedure for Z-Trp-Aib-Gly-Ala-NH-Ar

To a solution of Z-Trp-Aib-Gly (10.0 mmol), Ala-NH-Ph (10.0 mmol), HOBt (10.0 mmol), and TEA (10 mmol) in chloroform (20 cm^3) and MeCN (10 cm^3), EDC·HCl (12.2 mmol) was added slowly at 0 °C. The solution was stirred for 24 h at room temperature and was then dried under reduced pressure. The resulting residue was dissolved in ethyl acetate (60 cm^3). The solution was washed with 1 M HCl (3 × 40 cm^3), 1 M $NaHCO_3$ (3 × 40 cm^3), and saturated NaCl (30 cm^3), and dried over anhydrous sodium sulfate. The crude product obtained by evaporation of the solution was purified by chromatography on silica gel with 9% MeOH–chloroform.

Z-Trp-Aib-Gly-Ala-NH-Ph. Yield 58.3%, $[\alpha]_D^{25}$ − 20.2° (*c* 1.0, chloroform).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.24 and 1.36 (6H, s × 2, Aib-βCH_3), 1.58 (3H, d, J = 7.2 Hz, Ala-βCH_3), 3,20 (2H, ddd, J = 6.6, 7.2 and 14.7 Hz, Trp-βCH_2), 3.54 (2H, ddd, J = 5.1, 5.4 and 17.7 Hz, Gly-αCH_2), 4.41 (1H, q, J = 7.2 Hz, Trp-αCH), 4.60 (1H, apparent q, J = 7.2 Hz, Ala-αCH), 5.02 (2H, s, Z-CH_2), 5.62 (1H, d, J = 5.7 Hz Trp NH), 6.57 (1H, br, Gly-NH), 6.72 (1H, s, Aib-NH), 7.04 (1H, d, J = 1.5 Hz, indolyl-2H), 7.02–7.07 (2H, m, indolyl-5H, Ph(anilide)-4H), 7.11 (1H, t, J = 8.1 Hz, indoly-6H), 7.17–7.32 (7H, m, Ph(Z) and Ph(anilide)-3H), 7.35 (1H, J = 8.1 Hz, indolyl-7H), 7.59 (1H, d, J = 7.2 Hz, Ala-NH), 7.63 (1H, d, J = 7.8 Hz, indolyl-4H), 7.74 (2H, d, J = 7.8 Hz Ph(anilide)-2H), 8.31 (1H, s, anilide-NH), 8.65 (1H, br, indolyl-NH).

m/z (MALDI-TOF MS) [Found: 649.27. ($C_{34}H_{38}N_6O_6 + Na)^+$ requires 649.27].

Z-Trp-Aib-Gly-Ala-NH-C$_6$H$_3$(3,5-Me$_2$). Yield 58.2%, $[\alpha]_D^{25}$ − 20.3° (*c* 1.0, chloroform).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.25 and 1.35 (6H, s × 2, Aib-βCH_3), 1.58 (3H, d, J = 7.2 Hz, Ala-βCH_3), 2.23 (6H, s, xylyl-CH_3) 3.20 (2H, ddd, J = 6.6, 7.2 and 14.7 Hz, Trp-βCH_2), 3.54 (2H, ddd, J = 5.1, 5.4 and 17.7Hz, Gly-αCH_2), 4.40 (1H, q, J = 7.2 Hz, Trp-αCH), 4.59 (1H, apparent q, J = 7.5 Hz, Ala-αCH), 5.02 (2H, dd, J = 11.4 and 12.3 Hz, Z-CH_2), 5.62 (1H, d, J = 5.7 Hz Trp-NH), 6.57 (1H, br, Gly-NH), 6.72 (1H, s, Aib-NH), 6.71 (1H, s, xylyl-4H), 7.05 (1H, d, J = 1.5 Hz, indolyl-2H), 7.11 (1H, t, J = 7.2 Hz, indolyl-5H), 7.11–7.32 (7H, m, indoly-6H and Ph(Z)), 7.34 (2H, s, xylyl-2H), 7.36 (1H, J = 8.1 Hz, indolyl-7H), 7.53 (1H, d, J = 7.2 Hz, Ala NH), 7.63 (1H, d, J = 7.5 Hz, indolyl-4H), 8.30 (1H, s, xylydide-NH), 8.57 (1H, br, indolyl-NH).

m/z (MALDI-TOF MS) [Found: 677.30. ($C_{36}H_{42}N_6O_6 + Na)^+$ requires 677.30].

General Procedure for Trp-Xaa$_2$-Gly-Xaa$_4$-NH-Ar

Hydrogen was bubbled through a solution of Z-Trp-Xaa$_2$-Gly-Xaa$_4$-NH-Ar (2.5 mmol) in MeOH (20 cm^3) in the presence of 5% palladium on activated carbon (200 mg) for 1 h at room temperature. The catalyst was filtered off and the filtrate was dried under reduced pressure. The crude products were purified by flash column chromatography (chloroform/MeOH/NH_4OH 95 : 15 : 0.1).

Trp-Aib-Gly-Ala-NH-Ph (3). Yield 71.1%, mp 132°–133 °C, $[\alpha]_D^{25} + 13.0°$ (*c* 0.5, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.41 and 1.45 (6H, s × 2, Aib-βCH_3), 1.56 (3H, d, J = 7.5 Hz, Ala-βCH_3), 3.06 (2H, ddd, J = 5.4, 7.8 and 14.4 Hz, Trp-βCH_2), 3.67–3.71 (3H, m, Gly-αCH_2 and Trp-αCH), 4.60 (1H, apparent q, J = 7.6 Hz, Ala-α CH), 6.57 (1H, t, J = 4.5 Hz, Gly NH), 6.92 (1H, d, J = 2.1 Hz, indolyl-2H), 7.03–7.13 (2H, m, indolyl-5H, Ph(anilide)-4H), 7.11 (1H, t, J = 6.9 Hz, indoly-6H), 7.23–7.31 (3H, m, Ph(anilide)-3H and indolyl-7H), 7.49 (1H, d, J = 8.1 Hz, indolyl-4H), 7.62(1H, s, Aib-NH), 7.76 (2H, d, J = 7.8 Hz Ph(anilide)-2H), 7.79 (1H, d, J = 7.5 Hz, Ala-NH), 8.14 (1H, s, anilide-NH), 8.73 (1H, br, indolyl-NH).

m/z (MALDI-TOF MS) [Found: 493.25. ($C_{26}H_{36}N_6O_4 + H)^+$ requires 493.25].

Trp-Aib-Gly-Ala-NH-C$_6$H$_3$(3,5-Me$_2$) (4). Yield 73.1%, $[\alpha]_D^{25} - 6.16°$ (*c* 0.5, MeOH).

^{1}H NMR ($CDCl_3$), δ (ppm) = 1.40 and 1.44 (6H, s, Aib-βCH_3), 1.54 (3H, d, J = 6.9 Hz, Ala-βCH_3), 1.95 (2H, br, Trp-NH_2), 2.25 (6H, s, xylyl-CH_3) 3.07 (2H, ddd, J = 5.4, 7.8 and 14.4 Hz, Trp-βCH_2), 3.60–3.79 (3H, m, Gly-αCH_2 and Trp-αCH), 4.58 (1H, apparent q, J = 7.5 Hz, Ala-α CH), 6.57 (1H, br, Gly-NH), 6.72 (1H, s, xylyl-4H), 7.06 (1H, d, J = 2.1 Hz, indolyl-2H), 7.06 (1H, t, J = 7.9 Hz, indolyl-5H), 7.16 (1H, t, J = 7.9 Hz, indolyl-6H), 7.28 (1H, d, J = 7.9 Hz, indolyl-7H), 7.37 (2H, s, xylyl-2H), 7.51 (1H, d, J = 7.8 Hz, indolyl-4H), 7.65 (1H, s, Aib-NH), 7.77 (1H, d, J = 7.5 Hz, Ala NH), 8.25 (1H, s, xylydide-NH), 8.68 (1H, s, indolyl-NH).

m/z (MALDI-TOF MS) [Found: 521.28. ($C_{28}H_{36}N_6O_4 + H)^+$ requires 521.28].

Trp-Gly-Gly-Leu-NH-Ph (5). Yield 75.3%, mp 136°–137 °C, $[\alpha]_D^{25} - 49.6°$ (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d$_6$), δ (ppm) = 0.86 (6H, dd, J = 6.9 and 7.8 Hz, Leu-δCH_3), 1.54–1.65 (3H, m, Leu-βCH_2 and Leu-γCH), 1.87 (2H, br, Trp-NH_2) 2.98 (2H, ddd, J = 5.1, 7.6 and 13.8 Hz, Trp-βCH_2), 3.49 (1H, q, J = 5.1 Hz, Trp-αCH), 3.75–3.79 (4H, m, 2-Gly-αCH_2 and 3-Gly-αCH_2), 4.42 (1H, apparent q, J = 7.2 Hz, Leu-αCH), 7.04 (1H, d, J = 1.5 Hz, indolyl-2H), 7.02–7.09 (2H, m, indolyl-5H, Ph(anilide)-4H), 7.21 (1H, t, J = 8.1 Hz, indoly-6H), 7.32 (2H, t, J = 7.8 Hz Ph(anilide)-3H), 7.35 (1H, J = 8.1 Hz, indolyl-7H), 7.63 (1H, d, J = 7.8 Hz, indolyl-4H), 7.74 (2H, d, J = 7.8 Hz Ph(anilide)-2H), 8.08 (1H, d, J = 8.1 Hz, Leu-NH), 8.34 (1H, t, J = 5.7 Hz 3-Gly-NH), 8.34 (1H, br, 2-Gly-NH), 9.85 (1H, s, anilide-NH), 10.8 (1H, br, indolyl-NH).

m/z (MALDI-TOF MS) [Found: 523.27. ($C_{27}H_{38}N_6O_5 + H)^+$ requires 551.30].

Trp-Gly-Gly-Leu-NH-C$_6$H$_3$(3,5-Me$_2$) (6). Yield 72.7 %, $[\alpha]_D^{25} - 49.6°$ (*c* 1.0, MeOH).

^{1}H NMR (DMSO-d$_6$), δ (ppm) = 0.86 (6H, dd, J = 6.9 and 7.8 Hz, Leu-δCH_3), 1.54–1.65 (3H, m, Leu-βCH_2 and Leu-γCH), 1.82 (2H, br s, Trp-NH_2), 2.25 (6H, s, xylyl-CH_3) 2.96 (2H, ddd, J = 5.4, 7.1 and 14.1 Hz, Trp-βCH_2), 3.50 (1H, q, J = 5.1 Hz, Trp-αCH), 3.76–3.77 (4H, m, 2-Gly-αCH_2 and, 3-Gly-αCH_2), 4.40 (1H, apparent q, J = 7.2 Hz, Leu-αCH), 6.72 (1H, s, xylyl-4H), 7.06 (1H, d, J = 2.1 Hz, indolyl-2H), 7.06 (1H, t, J = 7.9 Hz, indolyl-5H), 7.16 (1H, t, J = 7.9 Hz, indolyl-6H), 7.28 (1H, d, J = 7.9 Hz, indolyl-7H), 7.37 (2H, s, xylyl-2H), 7.51 (1H, d, J = 7.8 Hz, indolyl-4H), 8.02 (1H, d, J = 8.1 Hz, Leu-NH), 8.31 (1H, t, J = 5.7 Hz 3-Gly-NH), 8.36 (1H, br, 2-Gly-NH), 9.85 (1H, s, xylydide-NH), 10.8 (1H, br, indolyl-NH).

m/z (MALDI-TOF MS) [Found: 551.30. ($C_{29}H_{38}N_6O_5 + H)^+$ requires 551.30].

Liquid–Liquid Extraction of the Metal Ions

An aliquot (10 ml) of an aqueous solution containing a metal ion (5×10^{-5} M), picrate ion (1×10^{-3} M), and buffer (acetate-sodium acetate in pH 3–5, borate-sodium borate in pH 6–8, and sodium hydroxide, pH 9; 1×10^{-3} M) was inserted into a 50 ml glass cylindrical tube with a glass stopper. The ionic strength was kept at 0.1 with sodium sulfate. After the addition of 10 ml of a peptide solution in 1,2-dichloroethane (5×10^{-4} M), the mixture was shaken for 30 min at 180 strokes/min at 25° ± 0.1 °C. After the mixture was centrifuged for 5 min, the pH of the aqueous phase was measured and the metal concentration was measured by atomic absorption spectrometry using the resonance line for the metal. The metal concentration in the organic phase was determined as follows: 5 ml of the 1,2-dichloroethane phase was allowed to evaporate. The residue was dissolved in acetic acid (5 ml), and then

the metal ion in this solution was determined by atomic absorption spectrometry.

RESULTS AND DISCUSSION

Conformation of Peptides

Conformational analysis of the peptides in solution was performed by ^{1}H NMR spectroscopy. The effects on NH chemical shifts of the tetrapeptides by temperature and solvents are shown in Tables 1 and 2, respectively. Very little dependence of chemical shifts of Xaa_4-NH of **1**–**4** and **7** on temperature was observed in DMSO-d_6. However, in the cases of peptides (**5** and **6**) where Xaa_2 is Gly, a large dependence of chemical shifts of Leu-NH on temperature was observed in DMSO-d_6. The hydrogen bonding of Xaa_4-NH of **1**–**4** and **7** was not to the solvent but to the carbonyl oxygen in the same molecule. The hydrogen bonding of Xaa_4-NH of both **5** and **6** was to the solvent. On the other hand, a relatively large dependence of Xaa_4-NH in $CDCl_3$ demonstrated the existence of a hydrogen bond even in a non-polar solvent. Furthermore, the chemical shifts of Xaa_4-NH of **1**–**4** and **7** were approximately equal in both solvents. The chemical shifts of Xaa_4-NH of both **5** and **6** were rather different between both solvents. Therefore, both **5** and **6** probably adopt a random structure.

The dependence of NH chemical shifts on solvents is shown in Figure 1. Xaa_4-NH of **1** showed only a small change in chemical shifts in up to 5 : 1 mixtures of the $CDCl_3$-DMSO-d_6. Xaa_4-NH of **2**–**4** and **7** exhibited the same behaviour. Further, analysis of the shielded NH groups of **1** and **3** was carried out by using free radical (TEMPO) induced line broadening of NH resonances [19]. Figure 2 illustrates the behaviour of the NH resonances of **1** and **3** in addition of TEMPO. Broadening of line width for both Xaa_4-NH of **1** and **3** was not observed in addition to TEMPO. These results show that NH of Xaa_4 participates in the intramolecular hydrogen bonding. Although the line width of the signal for Aib-NH of both **1** and **3** exhibited only little change in addition to TEMPO, the result may suggest that TEMPO hardly approaches Aib-NH because of the steric hindrance by two methyl groups of Aib. Aib-NH and Xaa_4-NH of **2**, **4** and **7** also exhibited the same behaviour. These results show that Xaa_4-NH participated in the intramolecular hydrogen-bonding, and probably stabilized β-turn structure, as did the peptides previously reported [4,5].

Table 1 Temperature Dependence of NH Chemical Shifts of **1**–**7**[a]

		$(-d\delta/dT)/10^{-3}K^{-1}$					
Peptide	Solvent	Indole	Trp	Xaa_2	Gly	Xaa_4	NH-Ar
1	$CDCl_3$	1.6	—	1.7	1.6	3.0	1.6
	DMSO-d_6	1.8	—	2.7	2.5	0.8	2.6
2	$CDCl_3$	1.6	—	1.8	1.5	2.8	1.8
	DMSO-d_6	1.6	—	2.8	2.6	0.7	2.6
3	$CDCl_3$	1.6	—	2.2	1.3	—[b]	1.5
	DMSO-d_6	1.8	—	3.9	2.8	0.8	2.9
4	$CDCl_3$	2.5	—	2.2	2.0	3.5	1.4
	DMSO-d_6	1.8	—	3.8	2.5	0.9	2.4
5	$CDCl_3$	1.1	—	2.1	2.4	2.6	2.5
	DMSO-d_6	2.0	—	2.1	3.4	2.8	2.5
6	$CDCl_3$	2.5	—	2.2	2.2	2.7	2.4
	DMSO-d_6	2.2	—	2.3	3.1	2.5	2.5
7	$CDCl_3$	1.1	0.7	1.6	—[d]	1.7	1.0
	DMSO-d_6	1.7	—[c]	4.3	2.8	0.9	1.8

[a] [Peptide]: 0.5 mM, temperature: 298–328 K.
[b] Ala-NH of **3** is not observed due to overlap with 2-H of anilide.
[c] Trp-NH of **7** is not observed due to overlap with 4-H of anilide.

Table 2 Solvent Effect on NH Chemical Shifts of **1**–**7** at 298 K[a]

		δ					
Peptide	Solvent	Indole	Trp	Xaa_2	Gly	Xaa_4	NH-Ar
1	$CDCl_3$	8.14	—	7.62	6.35	7.77	8.69
	DMSO-d_6	10.82	—	8.39	8.19	7.79	9.54
2	$CDCl_3$	8.12	—	7.62	6.42	7.79	8.34
	DMSO-d_6	10.84	—	8.50	8.17	7.81	9.36
3	$CDCl_3$	8.72	—	7.62	6.20	7.77	8.04
	DMSO-d_6	10.86	—	8.38	8.13	7.93	9.55
4	$CDCl_3$	8.63	—	7.63	6.52	7.74	8.14
	DMSO-d_6	10.85	—	8.43	8.11	7.89	9.40
5	$CDCl_3$	8.41	—	7.85	6.90	7.56	8.51
	DMSO-d_6	10.83	—	8.31	8.20	8.12	9.82
6	$CDCl_3$	8.42	—	7.88	7.97	7.56	8.56
	DMSO-d_6	10.84	—	8.36	8.19	8.20	9.92
7	$CDCl_3$	8.71	5.39	6.19	6.93	7.51	8.16
	DMSO-d_6	10.82	—[b]	8.42	7.89	7.79	9.42

[a] [peptide]: 1.0 mM.
[b] Trp-NH of **7** is not observed due to overlap with 4-H of anilide.

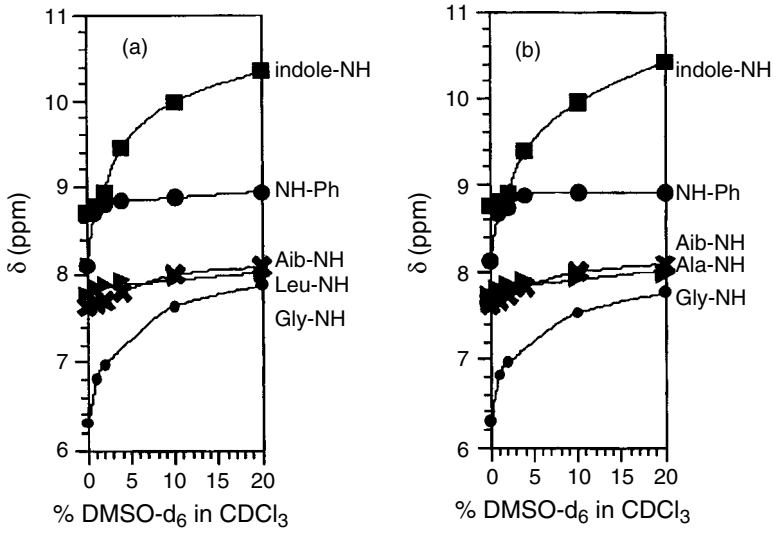


Figure 1 Solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures at 298.5 K: (a) **1**, 1 mM; (b) **3**, 1 mM.

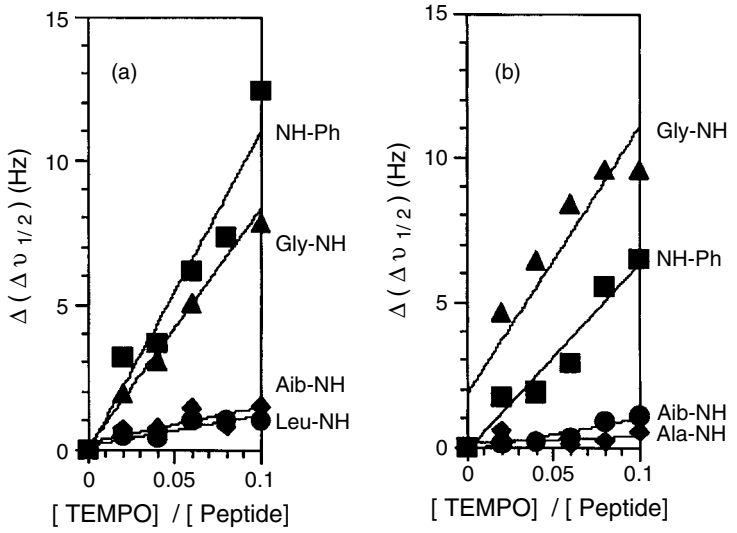


Figure 2 Plot of the line width of the NH protons vs increasing percentages of TEMPO ([TEMPO]/[Peptide]) in $CDCl_3$ at 298.5 K: (a) **1**, 5 mM; (b) **3**, 5 mM.

The conformation of peptides from the corresponding CD spectra, shown in Figure 3, were examined. The CD spectra of **1** and **2** in 1,2-dichloroethane (5×10^{-5} M) are characterized by a minimum at 207 nm. The spectra could not be recorded below 205 nm because of the absorbance of this solvent in the far UV region. Balaram *et al.* reported that the CD spectrum of a β-turn model tetrapeptide showed a minimum at 207 nm in an apolar solvent [20]. Therefore, peptides **1** and **2** probably adopt β-turn structures. Peptides **3**, **4** and **7** may also adopt β-turn structures stabilized by an intramolecular hydrogen bond, in which the NH of Xaa_4 participates.

Extraction of Metals

The liquid–liquid extraction of various metal ions with peptides **1** and **7** was examined. The following metal ions were examined: Na(I), K(I), Mg(II), Co(II), Ni(II), Zn(II), Cu(II), Fe(III), Cd(II), Ag(I) and Pd(II). The results of the extraction of metal ions with **1** and **7** into 1,2-dichloroethane in the presence of picrate as a counter anion are shown in Table 3. Cu (II)

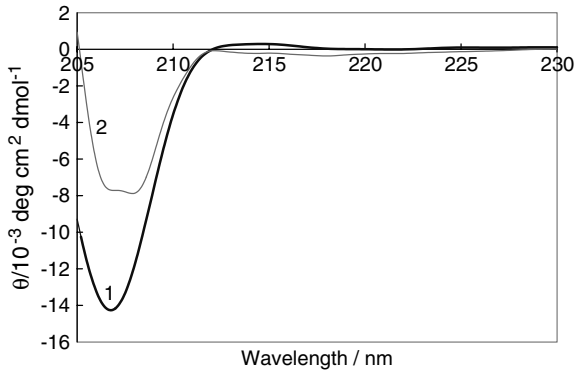


Figure 3 CD spectra of **1** and **2** in 1,2-dichloroethane at 298 K.

and Ag(I) were effectively extracted with **1** from the aqueous layer into 1,2-dichloroethane. Pd(II) was poorly extracted with **1** at pH 7. All metal ions, however, were not extracted with **7** at all. The results clearly indicate that an amino group of peptide is needed for the extraction.

The plot of the extraction percent (%E) of Cu(II), Ag(I) and Pd(II) with **1** in the presence and absence of picrate vs pH are shown in Figure 4. Cu (II), Ag(I) and Pd(II) were optimally extracted with **1** from the aqueous layer into 1,2-dichloroethane at pH 7.3, 7.5 and 4.0, respectively. The decreases of %E in the extraction of Cu(II) and Ag(I) with **1** below pH 7 may be attributed to the protonation of the picrate anion. The %E in the extraction of Cu(II) and Ag(I) with **1** in the absence of picrate was much lower than that in the presence of picrate. In the case of pH 4, picric acid exists both in the organic phase and in the aqueous phase (ca. 50% each), whereas in the case of pH 7.5, picric acid scarcely exists in the organic phase. The picrate anion in the aqueous phase easily forms the ion pair with Cu(II) and Ag(I), and the ion pairs are extracted with the peptides into the organic phase. This fact suggests that the formation of ion pairs with picrate is very important for the extraction of Cu(II) and Ag(I). On the other hand, Pd(II) was effectively extracted with **1** in the acidic region, but scarcely extracted in the basic region. This may indicate that the Pd(II) ion forms complex anions with a hydroxide ion in the basic region, so that it is not extracted into the organic layer.

Table 3 Extraction Percent of Various Metal Ions with **1** and **7** into 1,2-Dichloroethane at pH 7

Metal ion	**1** %E	**7** %E
Na(I)	0	0
K(I)	0	0
Mg(II)	0	0
Co(II)	0	0
Ni(II)	0	0
Zn(II)	0	0
Cu(II)	65	0
Fe(III)	0	0
Cd(II)	0	0
Ag(I)	79	0
Pd(II)	5.1	0

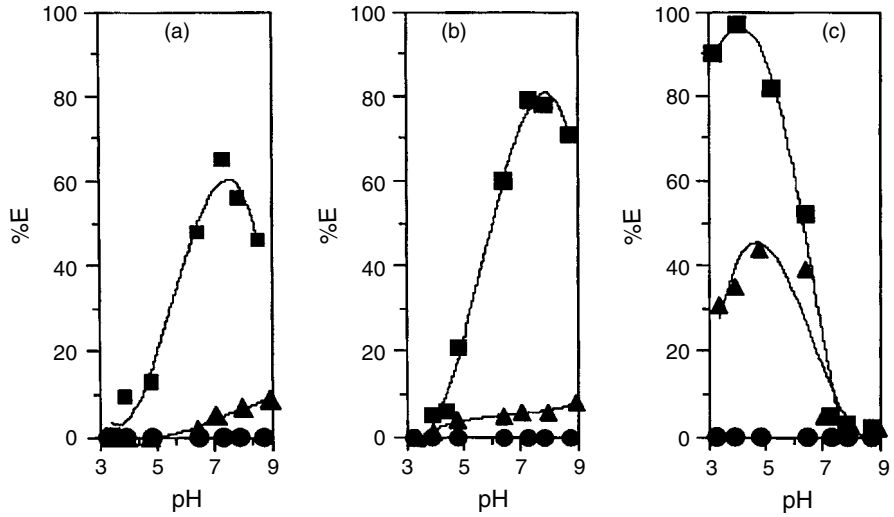


Figure 4 Plot of %E of the metal ion vs pH with **1** in the presence (■) and the absence (▲) of picrate and with **7** (●) for Cu(II) (a), Ag(I) (b) and Pd(II) (c).

Na(I), K(I), Mg(II), Co(II), Ni(II), Zn(II), Fe(III) and Cd(II) were not extracted with **1** in the pH range 3–9. The remarkable difference in extraction between Cd(II) and Ag(I), both of which are classed as Pearson's soft acids, may be speculated to be attributed to the difference in the extent of softness. Yamada and Tanaka reported that the following general trend is evident: soft acids have large a values, a constant parameter in the equation for the complex formation constants, (greater than 1.5), whereas hard acids have small a values (smaller than 1) and the first transition metal ions have intermediate values [21]. According to them, the a values of Cd(II) and Ag(I) are 1.66 and 3.60, respectively, and therefore, the softness of Cd(II) may be much less than that of Ag(I).

Next, based on the above data, an attempt was made to extract Cu(II), Ag(I) and Pd(II) with **2**–**6** in the pH range 3–9. Leu-NH-Ph, Gly-Leu-NH-Ph and Aib-Gly-Leu-NH-Ph were also examined as reference compounds. Table 4 shows the %E of the optimal pH. The %E for the extraction of Cu(II), Ag(I) and Pd(II) with **1** and **2** (Xaa_2 = Aib) were greater than those with **5**, **6** (Xaa_2 = Gly), Leu-NH-Ph, Gly-Leu-NH-Ph and Aib-Gly-Leu-NH-Ph. A turn structure of the peptide, which is well formed due to the steric hindrance of Aib, seems to be important for the extraction of metal ions.

As shown in Table 4, the %E of metal ions with **2** and **4** (Ar = 3,5-dimethylphenyl group) were much higher than those with **1** and **3** (Ar = phenyl group) for all of metal ions, respectively. This shows that hydrophobicity of aromatic moiety is an important factor for extraction. Moreover, the %E of metal ions with **4** (Xaa_4 = Ala) was lower than those with **2** (Xaa_4 = Leu) in the case of every metal. The other peptides exhibited similar behaviour. Leu and a 3,5-dimethyl group are more hydrophobic than Ala and a phenyl group, respectively. These results clearly indicate that the hydrophobicity of the peptide molecule is an important factor for the extraction of metal ions.

Table 4 Extraction Percent of Cu(II), Ag(I) and Pd(II) of Various Peptides into 1,2-Dichloroethane at Optimal pH

Peptide	Cu(II)		Ag(I)		Pd(II)	
	pH	%E	pH	%E	pH	%E
1	7.3	65	7.5	79	4.0	97
1[a]	7.1	9.2	7.4	6.2	4.5	44
2	5.9	75	7.3	92	4.9	98
2[a]	5.9	9.5	8.9	17	4.7	91
3	7.2	5.2	7.2	22	4.2	16
4	6.5	37	7.3	25	4.7	66
5	7.1	25	7.6	35	3.9	19
6	7.3	29	7.5	41	4.5	45
Leu-NH-Ph	7.3	13	7.3	2.2	4.1	10
Gly-Leu-NH-Ph	7.2	7.6	7.3	0.2	4.3	17
Aib-Gly-Leu-NH-Ph	7.1	10	7.3	0	4.2	45

[a] In the absence of picrate.

The plot of %E vs pH in the extraction of Cu(II), Ag(I) and Pd(II) with **2** in the presence and absence of picrate is shown in Figure 5. The %E of Cu

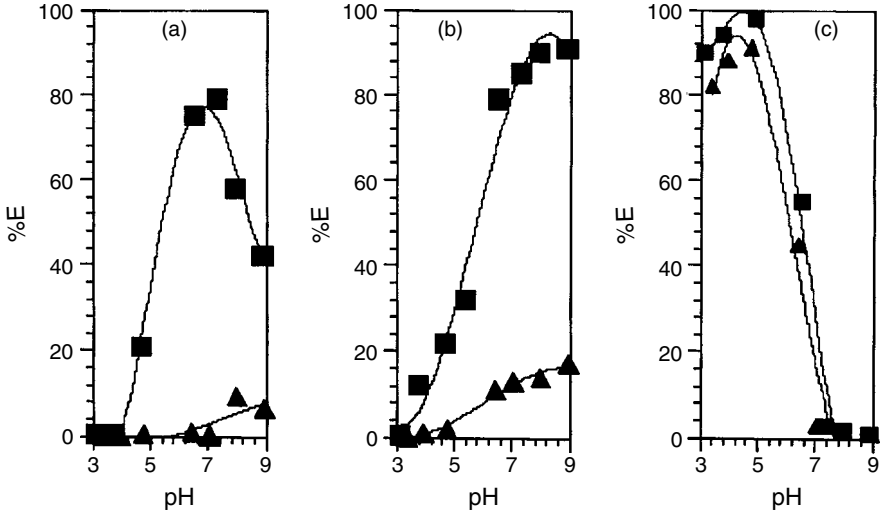


Figure 5 Plot of %E of the metal ion vs pH with **1** in the presence (■) and the absence (▲) of picrate of Cu(II) (a), Ag(I) (b) and Pd(II) (c).

(II), Ag (I) and Pd (II) with **2** were higher than those with **1**. Moreover, Pd (II) was extracted with **2** even in the absence of picrate as the counter anion. This result may suggest that the SO_4^{2-} ion in the sodium sulfate solution or the AcO^- ion in the buffer solution are used as the counter anion. These results indicate that **2** is specifically bound to Pd (II) in the acidic regions. The free α-amino group, the turn conformation and the hydrophobicity of peptides molecule were important factors for metal ions to be effectively extracted from the aqueous layer to the nonpolar organic layer.

In conclusion, a new model of Aib-containing tetrapeptide, Trp-Aib-Gly-Leu-NH-$C_6H_3(CH_3)_2$ (**2**), which binds to Pd(II) was established. The introduction of Aib into small peptides as a conformational constraint represents a powerful approach for the design of novel peptide-based ligands.

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REFERENCES

1. William NL, Norbert S. Recent advances in zinc enzymology. *Chem. Rev.* 1996; **96**: 2375–2450.
2. Robert RE. Structure–function relationships of alternative nitrogenases. *Chem. Rev.* 1996; **96**: 3013–3065.
3. Venkatraman J, Shankaramma C, Balaram P. Design of folded peptides. *Chem. Rev.* 2001; **101**: 3131–3152.
4. Yamada T, Nakao M, Miyazawa T, Kuwata S, Sugiura M, In Y, Ishida T. Conformational difference between diastereomers of Dnp-pNA derivatives of Aib-containing tetrapeptides. In *Peptide Chemistry 1991*, Suzuki A (ed.). Protein Research Foundation: Osaka, 1992; 71–76.
5. Yamada T, Nakao M, Miyazawa T, Kuwata S, Sugiura M, In Y, Ishida T. Conformational difference between diastereomers of Dnp-Val-Aib-Gly-Leu-pNA studied by x-ray crystal analyses. *Biopolymers* 1993; **33**: 813–822.
6. Yanagihara R, Katoh M, Hanyu M, Miyazawa T, Yamada T. Recognition of quaternary ammonium salts with tetrapeptides containing α-aminoisobutyric acid as a conformational constraint. *J. Chem. Soc. Perkin Trans. 2* 2000; 551–556.
7. Pererson CJ. Cyclic polyethers and their complexes with metal salts. *J. Am. Chem. Soc.* 1967; **89**: 2495–2498.
8. Nakatsuji Y, Muraoka M, Kajiya H, Zhng W, Kida T, Ikeda I. Synthesis and complexing ability of a C-pivot type of double-armed 15-crown-5 ethers toward alkali metal cations. *Bull. Chem. Soc. Jpn* 2002; **75**: 1765–1770.
9. Chayama K, Sekido E. Liquid–liquid extraction of metal ions by cyclic and acyclic tetrathio ethers. *Anal. Sci.* 1987; **3**: 535–541.
10. Desper MJ, Gellman HS, Wolf ER Jr, Cooper RS. Enhanced nickel(II) chelation by *gem*-dimethyl-substituted macrocyclic tetrathioethers. *J. Am. Chem. Soc.* 1991; **113**: 8663–8671.
11. Kang CH, Hanson WA, Eaton B, Boekelheide V. [2$_6$](1,2,4,5)Cyclophane(deltaphane) and related compounds. Simultaneous π-electron interaction among three benzene rings. *J. Am. Chem. Soc.* 1985; **107**: 1979–1985.
12. Odani A, Yamauchi O. Ternary α-amino acid-palladium(II) complexes with ligand–ligand hydrogen bonding. *Bull. Chem. Soc. Jpn* 1981; **54**: 3773–3779.
13. Sigel H, Martin BR. Coordinating properties of the amide bond. Stability and structure of metal ion complexes of peptides and related ligands. *Chem. Rev.* 1982; **82**: 385–426.
14. Luo X, Huang W, Mei Y, Zhou S, Zhu L. Interraction of palladium(II) complexes with sulfur-containing peptides studies by electrospray mass spectrometry. *Inorg. Chem.* 1999; **38**: 1474–1480.
15. Jacobs AS, Margerum WD. Solution properties of bis(dipeptide)nickelate(III) complexes and kinetics of their decomposition in acid. *Inorg. Chem.* 1984; **23**: 1195–1201.
16. Kim S-H, Martin BR. Noncovalent ligand–metal and ligand–ligand interactions in tridentate (dipeptide)palladium(II) complexes. *J. Am. Chem. Soc.* 1984; **106**: 1707–1712.
17. McDonald RM, Fredericks CF, Margerum WD. Characterization of copper(III)-tetrapeptide complexes with histidine as the third residue. *Inorg. Chem.* 1997; **36**: 3119–3124.
18. Rossi P, Felluga F, Tecilla P, Formaggio F, Crisma M, Toniolo C, Scrimin P. An azacrown-functionalized peptide as a metal ion based catalyst for the cleavage of a RNA-model substrate. *Biopolymers (Peptide Sci.)* 2000; **55**: 496–501.
19. Kopple DK, Schamper JT. Proton magnetic resonance line broadening produced by association with a nitroxide radical in studies of amide and peptide conformation. *J. Am. Chem. Soc.* 1972; **94**: 3644–3646.
20. Crisma M, Fasman GD, Balaram H, Balaram P. Peptide models for β-turns: A circular dichroism study. *Int. J. Peptide Protein Res.* 1984; **23**: 411–419.
21. Yamada S, Tanaka M. Softness of some metal ions. *J. Inorg. Nucl. Chem.* 1975; **37**: 587–589.

