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Studies on intramolecular hydrogen bonding between the pyridine nitrogen and the amide hydrogen of the peptide: synthesis and conformational analysis of tripeptides containing novel amino acids with a pyridine ring

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Abstract: For the first time tripeptides, Z-AA_1-Xaa-AA_3-OMe (AA_1 and AA_3 = Gly or Aib, Xaa=2Pmg and 2Pyg) were prepared containing α-methyl-α-(2-pyridyl)glycine (2Pmg) and α-(2-pyridyl)glycine (2Pyg) by solid-phase Ugi reaction. These results clearly indicate that for the preparation of tripeptides containing an amino acid with a pyridine ring, the solid-phase Ugi reaction is very useful.

NMR analysis clarified that 2Pmg-containing tripeptides adopt a unique conformation with an intramolecular hydrogen bond between 2Pmg-NH and the pyridine nitrogen. However, in the case of Z-Gly-2Pyg-Gly-OMe, the intramolecular hydrogen bonding between 2Pyg-NH and the pyridine nitrogen was not observed, whereas Z-Aib-2Pyg-Aib-OMe adopts a unique conformation with an intramolecular hydrogen bond between 2Pyg-NH and a pyridine nitrogen. Conformational analysis of the tripeptides, Z-AA_1-Xaa-AA_3-OMe (AA_1, AA_3 = Gly or Aib, Xaa = α,α-di(2-pyridyl)glycine (2Dpy), α-phenyl-α-(2-pyridyl)glycine (2Ppg), 2Pmg and 2Pyg), clarified that when an α,α-disubstituted glycine with a 2-pyridyl group at an α-carbon atom is introduced into any peptide, an intramolecular hydrogen bond between a pyridine nitrogen and an amide proton is formed and conformational mobility of the peptide backbone is restricted.

Keywords: α-methyl-α-(2-pyridyl)glycine (2Pmg); α-(2-pyridyl) glycine (2Pyg); Ugi reaction; solid-phase reaction; intramolecular hydrogen bonding

INTRODUCTION

There has been increasing interest in the incorporation of α,α-disubstituted glycine (DSG) into peptides, because of their particular role restricting the conformational mobility of peptide backbones [1–5]. Synthesis of the DSG-containing peptides presents challenging problems since steric hindrance associated with the quaternary α-carbon atom of the DSG gives rise to difficulty in the conventional synthesis of their peptides. The four-component condensation (Ugi reaction) [6,7] is relatively insensitive to steric hindrance and constitutes a convenient method for the synthesis of DSGs, such as α,α-dibenzyl glycine, α,α-diethyl glycine, α,α-di-*n*-propyl glycine, α,α-di-*n*-butyl glycine and α,α-diisopropyl glycine [8,9], and the peptide containing them [10]. A variety of fully protected tripeptides containing an extremely crowded DSG, α,α-diphenylglycine (Dph), were synthesized previously by the modified Ugi reaction using diphenylmethanimine as a key compound [11–14]. Furthermore, in connection with Dph which has two phenyl groups at C^α, the synthesis and conformation were reported recently of peptides containing a novel DSG, α,α-di(2-pyridy)glycine (2Dpy) which has two 2-pyridyl groups at C^α and α-phenyl-α-(2-pyridy)glycine (2Ppg) which has one 2-pyridyl group at C^α by the modified Ugi reaction (Figure 1) [15–17]. 2Dpy and 2Ppg are expected not only to provide a similar conformational constraint to Dph, but also to adopt conformations containing intramolecular hydrogen bonding in which pyridine nitrogens participate. In fact, ^{1}H NMR analysis of 2Dpy-containing tripeptides (Z-AA_1-2Dpy-AA_3-OMe (**1**): AA_1, AA_3 = Gly, Aib) indicates that **1a** adopts a novel conformation stabilized by two intramolecular hydrogen bonds between NH of 2Dpy and N of a pyridine ring and between NH of AA_3 (Gly_3) and N of another pyridine ring [15]. Both **1b** and **2** adopt a β-turn conformation stabilized by two intramolecular hydrogen bonds between NH of 2Dpy and N of a pyridine ring and between NH of AA_3 and C=O of the Z group [15,16]. The same conformation was clarified for **1b** by x-ray crystal structural analysis [15,17]. No report has yet described such intramolecular hydrogen bonds in peptide chemistry. It was also found that **1b** is able to self-assemble in the presence of Cu(II) ion, by the coordination of the metal ion to pyridine nitrogens [16].

Although the Ugi reaction provides an excellent method for the construction of DSG with aryl or heteroaryl groups, yields of DSG-containing tripeptides with

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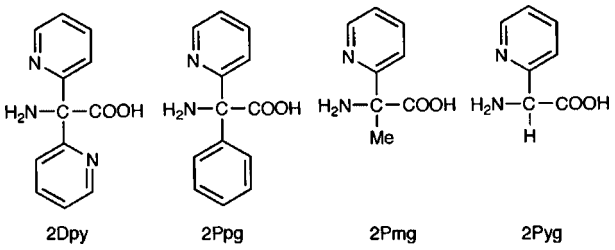


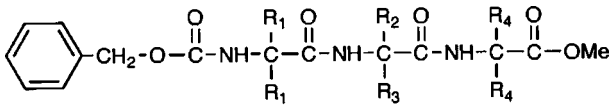
Figure 1 Structure of DSGs with 2-pyridyl groups and the related amino acids.

a pyridine ring were very low with the exception of those containing 2Ppg. The reaction mixture colorized very darkly and many by-products were produced. Therefore, purification of the desired Ugi product was very troublesome. This may be due to the steric crowding in the reaction intermediate and the reactivity of the pyridine ring itself [15].

Solid-phase synthesis is a powerful tool for organic synthesis and combinatorial chemistry [18–20]. Multicomponent condensation reactions such as the Ugi reaction have also been developed in the area of solid-phase synthesis [21–24]. Many by-products which occur from several side reactions may be removable from the resin only by washing with excess solvents.

Recently, a solid-phase synthesis was reported of the tripeptides containing the very crowded DSGs, such as 2Dpy and Dph [25]. Although many by-products were also produced, in these cases the purification of **1** could be performed easily.

Now, we report a solid-phase synthesis of the tripeptides containing α-methyl-α-(2-pyridyl)glycine (2Pmg) and the corresponding tripeptides containing α-(2-pyridyl)glycine (2Pyg) by the Ugi reaction (**3** and **4** in Scheme 1) in order to assess the participation of



1a: R_1=R_4=H, R_2=R_3=2-pyridyl; (Z-Gly-2Dpy-Gly-OMe)

1b: R_1=R_4=Me, R_2=R_3=2-pyridyl; (Z-Aib-2Dpy-Aib-OMe)

2a: R_1=R_4=H, R_2=2-pyridyl, R_3=phenyl; (Z-Gly-2Ppg-Gly-OMe)

2b: R_1=R_4=Me, R_2=2-pyridyl, R_3=phenyl; (Z-Aib-2Ppg-Aib-OMe)

3a: R_1=R_4=H, R_2=2-pyridyl, R_3=Me; (Z-Gly-2Pmg-Gly-OMe)

3b: R_1=R_4=Me, R_2=2-pyridyl, R_3=Me; (Z-Aib-2Pmg-Aib-OMe)

4a: R_1=R_4=H, R_2=2-pyridyl, R_3=H; (Z-Gly-2Pyg-Gly-OMe)

4b: R_1=R_4=Me, R_2=2-pyridyl, R_3=H; (Z-Aib-2Pyg-Aib-OMe)

5a: R_1=R_4=H, R_2=phenyl, R_3=Me; (Z-Gly-Phg(αMe)-Gly-OMe)

5b: R_1=R_4=Me, R_2=phenyl, R_3=Me; (Z-Aib-Phg(αMe)-Aib-OMe)

6a: R_1=R_4=H, R_2=phenyl, R_3=H; (Z-Gly-Phg-Gly-OMe)

6b: R_1=R_4=Me, R_2=phenyl, R_3=H; (Z-Aib-Phg-Aib-OMe)

Scheme 1

the nitrogen of the pyridine ring bound to C^{α} in an intramolecular hydrogen bonding with an amide hydrogen of the peptide bond.

MATERIALS AND METHODS

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 500 MHz instrument at 499.85 MHz and 125.68 MHz, respectively. ^{1}H NMR spectra were also recorded on a Varian Unity 300 MHz instrument at 299.85 MHz. Tetramethylsilane was used as an internal standard. Assignments of signals for the peptides were made by COSY and gHMBC correlations. 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. Fmoc-Rink amide resin was obtained from Watanabe Chemical Industries, Ltd.

General Procedure for the Solid-phase Ugi Reaction

Fmoc protected Rink amide resin (300 mg, 0.19 mmol, 0.62 mmol/g, 100–200 mesh) was put in the solid-phase reaction vessel and the resin was treated with 20% piperidine/DMF (3 ml, 2 × 15 min). Then, the solvent was drained and the resin was washed with DMF (3 × 3 ml), MeOH (3 × 3 ml) and DCM (3 × 3 ml), successively. The resin was dried under vacuum for 30 min. To the reaction vessel was added Z-Gly or Z-Aib (1.05 mmol), 2-acetylpyridine or pyridine-2-carboxyaldehyde (1.05 mmol) and DCM or DCM/MeOH (4 : 1 v/v) at room temperature. After shaking for 2 h, methyl isocyanoacetate or methyl 2-isocyano-2-methylpropanoate (1.05 mmol) was added to the mixture. The reaction mixture was shaken for 2 days. The reaction mixture was drained and the resin was then washed with DMF (3 × 3 ml), MeOH (3 × 3 ml) and DCM (3 × 3 ml), successively. The resin was dried under vacuum for 1 day. The products were cleaved off from the resin with 10% TFA/DCM (2 × 5 ml, 5 min) and finally washed with DCM (3 × 10 ml). The combined solutions were concentrated under reduced pressure, and the residue was dried *in vacuo*. The residue was purified by preparative thin-layer chromatography (silica gel, 9% MeOH in $CHCl_3$).

Z-Gly-2Pmg-Gly-OMe (3a). Yield 28.3%, oil; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.85 (3H, s, 2Pmg-CH_3), 3.55 (3H, s, OCH_3), 3.99 (2H, dd, J = 5.5 and 12.0 Hz, Gly_1-CH_2), 4.02 (2H, dd, J = 6.5 and 11.5 Hz, Gly_3-CH_2), 5.00 (2H, dd, J = 12.5 Hz, Z-CH_2), 5.50 (1H, br, Gly_1-NH), 7.21–7.34 (6H, m, ϕH, Py-5H), 7.36 (1H, br, Gly_3-NH), 7.61 (1H, d, J = 8.0 Hz, Py-3H), 7.71 (1H, t, J = 8.0 Hz, Py-4H), 8.36 (1H, d, J = 5.0 Hz, Py-6H), 9.08 (1H, s, 2Pmg-NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 26.7 (2Pmg-βC), 41.9 (Gly_1-αC), 45.0 (Gly_3-αC), 52.1 (OCH_3), 61.3 (Z-CH_2), 66.6 (2Pmg-αC), 120.7 (Py-C5), 122.6 (Py-C3), 127.7 (ϕ-oC), 128.1 (ϕ-pC), 128.5 (ϕ-mC), 136.1 (ϕ-*ipso*C), 137.1 (Py-C4), 147.3 (Py-C6), 155.5 (Z–C=O), 158.6 (Py-C2), 170.5 (2Pmg C=O), 172.0 (Gly_1 C=O), 174.8 (Gly_3 C=O).

MALDI-TOF (m/z): Calcd. for $C_{25}H_{32}N_4O_6$ $M+H^+$ 429.1774, $M+Na^+$ 451.1594; Found: $M+H^+$ 429.1770, $M+Na^+$ 451.1590.

Z-Aib-2Pmg-Aib-OMe (3b). Yield 26.9%, mp 146°–148 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.28 and 1.49 (6H, s × 2,

Aib_3-CH_3), 1.55 and 1.59 (6H, s × 2, Aib_1-CH_3), 1.85 (3H, s, 2Pmg-CH_3), 3.55 (3H, s, OCH_3), 5.00 (2H, dd, $J = 12.5$ Hz, Z-CH_2), 5.36 (1H, s, Aib_1-NH), 7.21–7.33 (6H, m, ϕH, Py-5H), 7.36 (1H, s, Aib_3-NH), 7.61 (1H, d, $J = 8.0$ Hz, Py-3H), 7.71 (1H, t, $J = 8.0$ Hz, Py-4H), 8.36 (1H, d, $J = 5.0$ Hz, Py-6H), 9.16 (1H, s, 2Pmg-NH). ^{13}C NMR (125 MHz, $CDCl_3$) $\delta = 23.6$ (Aib_3-βC), 25.2 (Aib_1-βC), 26.7 (2Pmg-βC), 51.8 (OCH_3), 56.1 (Aib_3-αC), 57.3 (Aib_1-αC), 62.1 (Z-CH_2), 66.8 (2Pmg-αC), 120.7 (Py-C5), 122.6 (Py-C3), 127.7 (ϕ-*o*C), 128.1 (ϕ-*p*C), 128.5 (ϕ-*m*C), 136.1 (ϕ-*ipso*C), 137.1 (Py-C4), 147.3 (Py-C6), 155.5 (Z-C=O), 158.6 (Py-C2), 170.5 (2Pmg C=O), 172.0 (Aib_1 C=O), 174.8 (Aib_3 C=O).

MALDI-TOF (*m*/*z*): Calcd. for $C_{25}H_{32}N_4O_6$ $M + H^+$ 485.2400, $M + Na^+$ 507.2220; Found: $M + H^+$ 485.2397, $M + Na^+$ 507.2215.

Z-Gly-2Pyg-Gly-OMe (4a). Yield 47.0%, oil; ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 3.69$ (3H, s, OCH_3), 3.99 (2H, dd, $J = 6.5$ and 12.0 Hz, Gly_1-CH_2), 4.03 (2H, dd, $J = 6.0$ and 11.0 Hz, Gly_3-CH_2), 5.15 (2H, dd, $J = 12.0$ Hz, Z-CH_2), 5.50 (1H, br, Gly_1-NH), 5.61 (1H, d, $J = 6.0$ Hz, 2Pyg-αH), 7.22–7.36 (6H, m, ϕH, Py-5H), 7.41 (1H, d, $J = 8.0$ Hz, Py-3H), 7.81 (1H, t, $J = 8.0$ Hz, Py-4H), 7.69 (1H, br, Gly_3-NH), 7.88 (1H, d, 2Pyg-NH), 8.51 (1H, d, $J = 5.0$ Hz, Py-6H). ^{13}C NMR (125 MHz, $CDCl_3$) $\delta = 40.5$ (Gly_1-αC), 43.2 (Gly_3-αC), 51.6 (OCH_3), 58.7 (2Pyg-αC), 61.5 (Z-CH_2), 122.5 (Py-C5), 123.9 (Py-C3), 127.7 (ϕ-*o*C), 128.1 (ϕ-*p*C), 128.5 (ϕ-*m*C), 136.4 (ϕ-*ipso*C), 136.6 (Py-C4), 147.6 (Py-C6), 156.6 (Z-C=O), 158.1 (Py-C2), 169.8 (2Pyg C=O), 171.9 (Gly_1 C=O), 174.7 (Gly_3 C=O).

MALDI-TOF (*m*/*z*): Calcd. for $C_{20}H_{22}N_4O_6$ $M + H^+$ 415.1618, $M + Na^+$ 437.1437; Found: $M + H^+$ 415.1610, $M + Na^+$ 437.1432.

Z-Aib-2Pyg-Aib-OMe (4b). Yield 45.2%, oil; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.42$ and 1.49 (6H, s × 2, Aib_3-CH_3), 1.55 and 1.61 (6H, s × 2, Aib_1-CH_3), 3.55 (3H, s, –OCH_3), 5.14 (2H, dd, $J = 12.5$ Hz, Z-CH_2), 5.32 (1H, s, Aib_1-NH), 5.41 (1H, d, $J = 6.0$ Hz, 2Pyg-αH), 7.22–7.36 (6H, m, ϕH, Py-5H), 7.42 (1H, d, $J = 8.5$ Hz, Py-3H), 7.61 (1H, t, $J = 8.5$ Hz, Py-4H), 7.69 (1H, s, Aib_3-NH), 8.21 (1H, d, $J = 6.0$ Hz, 2Pyg-NH), 8.51 (1H, d, $J = 5.0$ Hz, Py-6H). ^{13}C NMR (125 MHz, $CDCl_3$) $\delta = 23.7$ (Aib_3-βC), 25.3 (Aib_1-βC), 51.5 (OCH_3), 56.0 (Aib_3-αC), 57.4 (Aib_1-αC), 58.8 (2Pyg-αC), 62.3 (Z-CH_2), 120.5 (Py-C5), 122.4 (Py-C3), 127.7 (ϕ-*o*C), 128.0 (ϕ-*p*C), 128.5 (ϕ-*m*C), 136.2 (ϕ-*ipso*C), 137.3 (Py-C4), 147.1 (Py-C6), 155.3 (Z-C=O), 158.6 (Py-C2), 169.9 (2Pyg C=O), 172.2 (Aib_1 C=O), 174.7 (Aib_3 C=O).

MALDI-TOF (*m*/*z*): Calcd. for $C_{24}H_{30}N_4O_6$ $M + H^+$ 471.2244, $M + Na^+$ 493.2063; Found: $M + H^+$ 471.2243, $M + Na^+$ 493.2060.

General Procedure for Tripeptides containing α-Methyl-α-phenylglycine (Phg(αMe)) and α-Phenylglycine (Phg)

The peptides used were prepared by the usual Z strategy in the solution method. The coupling reactions were performed according to the carbodiimide-HOBt method. The removal of the protecting groups of dipeptides was attained by the HBr/AcOH method.

Z-Gly-DL-Phg(αMe)-Gly-OMe (5a). mp 121°–123 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.83$ (3H, s, Phg(αMe)-CH_3), 3.70 (3H, s, OCH_3), 3.82 (2H, d, $J = 5.8$ Hz, Gly_1-CH_2), 4.02 (2H, d, $J = 6.2$ Hz, Gly_3-CH_2), 5.02 (2H, s, Z-CH_2), 5.11 (1H, br, Gly_1-NH), 6.42 (1H, br, Gly_3-NH), 7.21–7.34 (10H, m, Z-ϕH, (Phg(αMe)-ϕH), 7.35 (1H, s, Phg(αMe)-NH).

MALDI-TOF (*m*/*z*): Calcd. for $C_{22}H_{25}N_3O_6$ $M + H^+$ 428.1822, $M + Na^+$ 450.1641; Found: $M + H^+$ 428.1805, $M + Na^+$ 450.1651.

Z-Aib-DL-Phg(αMe)-Aib-OMe (5b). mp 135°–136 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.28$ and 1.35 (6H, s × 2, Aib_3-CH_3), 1.49 and 1.56 (6H, s × 2, Aib_1-CH_3), 1.83 (3H, s, Phg(αMe)-CH_3), 3.56 (3H, s, –OCH_3), 5.00 (2H, s, Z-CH_2), 5.10 (1H, s, Aib_1–NH), 6.62 (1H, s, Aib_3–NH), 7.23–7.34 (10H, m, Z-ϕH, Phg(αMe)-ϕH, 7.35 (1H, s, Phg(αMe)-NH).

MALDI-TOF (*m*/*z*): Calcd. for $C_{26}H_{33}N_3O_6$ $M + H^+$ 484.2448, $M + Na^+$ 506.2267; Found: $M + H^+$ 484.2446, $M + Na^+$ 506.2268.

Z-Gly-D-Phg-Gly-OMe (6a). mp 99°–101 °C, $[\alpha]_D^{25}$ –4.3° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 3.59$ (3H, s, –OCH_3), 3.71 (2H, d, $J = 6.0$ Hz, Gly_1-CH_2), 3.84 (2H, d, $J = 5.7$ Hz, Gly_3-CH_2), 5.02 (2H, s, Z-CH_2), 5.11 (1H, br, Gly_1-NH), 5.41 (1H, d, $J = 8.1$ Hz, Phg-αH), 6.45 (1H, br, Gly_3-NH), 7.01 (1H, d, $J = 8.1$ Hz Phg-NH), 7.21–7.34 (10H, m, Z-ϕH, Phg(αMe)-ϕH).

MALDI-TOF (*m*/*z*): Calcd. for $C_{21}H_{23}N_3O_6$ $M + H^+$ 414.1665, $M + Na^+$ 436.1485; Found: $M + H^+$ 414.1652, $M + Na^+$ 436.1484.

Z-Aib-D-Phg-Aib-OMe (6b). mp 128°–129 °C, $[\alpha]_D^{25}$ –10.3° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.46$ and 1.50 (6H, s × 2, Aib_3-CH_3), 1.52 and 1.54 (6H, s × 2, Aib_1-CH_3), 3.59 (3H, s, –OCH_3), 5.00 (2H, s, Z-CH_2), 5.10 (1H, s, Aib_1-NH), 5.39 (1H, d, $J = 8.1$ Hz, Phg-αH), 6.99 (1H, br, Aib_3-NH), 7.12 (1H, d, $J = 8.1$ Hz, Phg-NH), 7.23–7.34 (10H, m, Z-ϕH, Phg(αMe)-ϕH).

MALDI-TOF (*m*/*z*): Calcd. for $C_{25}H_{31}N_3O_6$ $M + H^+$ 470.2291, $M + Na^+$ 492.2107; Found: $M + H^+$ 470.2278, $M + Na^+$ 492.2111.

RESULTS AND DISCUSSION

Synthesis of 2Pmg- and 2Pyg-containing Tripeptides using the Solid-phase Ugi Reaction

Synthesis of the amino acids with a pyridine ring [26–28] or a bipyridyl group [29–35] has been reported. However, synthesis of the peptide containing an α-(2-pyridyl)glycine derivative has scarcely been reported [36,37], because the α-(2-pyridyl)glycine derivative decarboxylates very easily in an acidic medium [37]. Previously, we succeeded in the synthesis of tripeptides containing an N-benzyl-2Pmg residue and an N-benzyl-2Pyg residue by the Ugi reaction [38]. However, no linear tripeptide esters without the N-benzyl group were obtained because the selective removal of an N-benzyl group by catalytic hydrogenolysis was tried but was not successful [38]. Therefore, no 2Pmg- or 2Pyg-containing tripeptide was obtained by the solution method. If a resin with an amino group is utilized as an amine component, the resin part may be removed selectively from the peptide backbone under

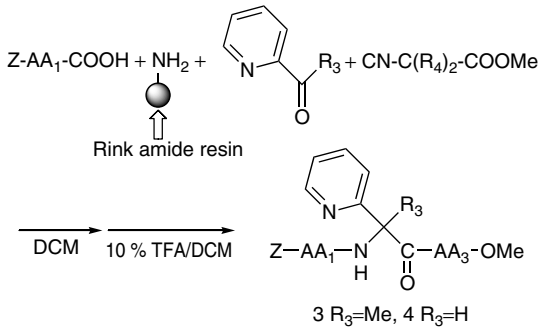


Figure 2 Synthetic route of **3** and **4**.

Table 1 Synthesis of 2Pmg- and 2Pyg-containing Tripeptides (**3** and **4**) in DCM

	AA_1	$R_3(AA_2)$	$R_4(AA_3)$	Reac. time (d)	Yield (%)
4a	Gly	H (2Pyg)	H (Gly)	1	35.1
4b	Aib	H	Me(Aib)	1	25.2
4a	Gly	H	H (Gly)	2	47.1
4b	Aib	H	Me(Aib)	2	45.2
3a	Gly	Me (2Pmg)	H (Gly)	1	Trace
3b	Aib	Me	Me(Aib)	1	Trace
3a	Gly	Me	H (Gly)	2	4.5
3b	Aib	Me	Me(Aib)	2	3.1

mild conditions. In the Ugi reaction by the solid-phase method, Rink amide resin has been often used as an amine component [39,40]. Rink resin was also used as an amine component in order to synthesize 2Pmg and 2Pyg-containing tripeptides (**3** and **4)** by the solid-phase Ugi reaction (Figure 2). The results are summarized in Table 1. The peptides (**4a** and **4b**) were obtained in 47% and 45% yields in DCM for 2 days, respectively. However, the yield of **3** by the solid-phase reaction in DCM was very low.

To improve the yield of **3** by the solid-phase Ugi reaction, the effects of several solvents on the preparation of **3** were examined as shown in Table 2. The yields of **3** were scarcely improved when DMF, *N*-methylpyrrolidone (NMP) and 2,2,2-trifluoroethanol (TFE) were employed as the solvent (entry 2–4 and 11–13). However, the addition of TFE, MeOH and NMP into DCM remarkably improved the yields of **3** (entry 5–9, 14–18). Further, the effects of the ratio of solvents on the yield of **3** were examined. The best results in the synthesis of **3** were obtained in DCM–MeOH (4 : 1) (entry 8, 17). Therefore, the control of the solvent polarity seems to be an important factor when the synthesis of DSG-containing tripeptides with a pyridine ring is performed by the solid-phase method [26]. The solid-phase Ugi reaction is very useful for the synthesis of tripeptides containing an amino acid, particularly α,α-disubstituted glycine, with a pyridine ring.

Table 2 Survey of Conditions for the Solid-phase Ugi Reaction of **3**[a]

Entry	Product	AA_1	$R_4(AA_3)$	Solvent	Yield (%)
1	**3a**	Gly	H (Gly)	DCM	4.5
2	**3a**	Gly	H (Gly)	NMP	9.9
3	**3a**	Gly	H (Gly)	DMF	7.5
4	**3a**	Gly	H (Gly)	TFE	2.5
5	**3a**	Gly	H (Gly)	DCM-TFE (5 : 1, v/v)	23.5
6	**3a**	Gly	H (Gly)	DCM-TFE (4 : 1, v/v)	27.9
7	**3a**	Gly	H (Gly)	DCM-MeOH (5 : 1, v/v)	22.8
8	**3a**	Gly	H (Gly)	DCM-MeOH (4 : 1, v/v)	28.3
9	**3a**	Gly	H (Gly)	DCM-NMP (4 : 1, v/v)	19.5
10	**3b**	Aib	Me(Aib)	DCM	3.1
11	**3b**	Aib	Me(Aib)	NMP	10.2
12	**3b**	Aib	Me(Aib)	DMF	9.7
13	**3b**	Aib	Me(Aib)	TFE	2.1
14	**3b**	Aib	Me(Aib)	DCM-TFE (5 : 1, v/v)	20.3
15	**3b**	Aib	Me(Aib)	DCM-TFE (4 : 1, v/v)	26.3
16	**3b**	Aib	Me(Aib)	DCM-MeOH (5 : 1, v/v)	19.5
17	**3b**	Aib	Me(Aib)	DCM-MeOH (4 : 1, v/v)	26.9
18	**3b**	Aib	Me(Aib)	DCM-NMP (4 : 1, v/v)	16.7

[a] Reaction time: 2 days.

NMR Analysis of 2Pmg- and 2Pyg-containing Tripeptides (3 and 4)

Comparison of the ^{1}H-NMR spectra of 2Pmg-containing tripeptides (**3**) in $CDCl_3$ with those of the corresponding Phg(αMe)-containing tripeptides (**5**) showed that the chemical shifts of both 2Pmg-NH and AA_3-NH of **3** were in markedly lower fields than those of Phg(αMe)-NH and AA_3-NH of **5**, respectively (Table 3). The chemical shift difference between AA_3-NH of **3** and **5** was similar to that of 2Pmg-NH.

Similarly, the chemical shifts of both 2Pyg-NH and AA_3-NH of 2Pyg-containing tripeptides (**4**) were in markedly lower fields than those of Phg-NH and AA_3–NH of the corresponding Phg-containing tripeptides (**6**), respectively (Table 3). The chemical shift difference of AA_3-NH (1.33 ppm) between **4a** and **6a** was larger than that of Xaa-NH (0.72 ppm), whereas the chemical shift difference of AA_3-NH (0.76 ppm) between **4b** and **6b** was smaller than that of Xaa-NH (1.29 ppm).

Tables 4 and 5 summarize NH chemical shifts and the temperature dependence of **3** and **4** in $CDCl_3$ and

Table 3 ^{1}H NMR Chemical Shifts of NH Protons of 2Pmg- and 2Pyg-containing Tripeptides (**3** and **4**) and Chemical Shift Difference from those of the Corresponding Phg(αMe)- and Phg-containing Tripeptides (**5** and **6**) in $CDCl_3$, respectively

	AA_1	Xaa	AA_3	Chemical shift (δ)/ppm		
				(Δδ/ppm)		
				AA_1-NH	Xaa-NH	AA_3-NH
3a	Gly	2Pmg	Gly	5.60	8.62	7.42
5a	Gly	Phg(αMe)	Gly	5.23	7.56	6.13
				(0.37)[a]	(1.06)[a]	(1.29)[a]
3b	Aib	2Pmg	Aib	5.47	9.16	7.34
5b	Aib	Phg(αMe)	Aib	5.36	7.55	6.13
				(0.11)[a]	(1.61)[a]	(1.21)[a]
4a	Gly	2Pyg	Gly	5.50	7.88	7.69
6a	Gly	Phg	Gly	5.40	7.16	6.36
				(0.10)[b]	(0.72)[b]	(1.33)[b]
4b	Aib	2Pyg	Aib	5.32	8.27	7.74
6b	Aib	Phg	Aib	5.40	6.98	6.98
				(−0.08)[b]	(1.29)[b]	(0.76)[b]

[a] δ(2Pmg-peptide) −δ(Phg(αMe)-peptide).
[b] δ(2Pyg-peptide) −δ(Phg-peptide).

Table 4 Solvent Effect on NH Chemical Shifts of **3** and **4**[a]

	Solvent	δ (ppm)		
		AA_1	Xaa	AA_3
3a	$CDCl_3$	5.60	8.62	7.42
	DMSO-d_6	7.35	8.54	8.36
3b	$CDCl_3$	5.47	9.16	7.34
	DMSO-d_6	7.36	9.02	8.27
4a	$CDCl_3$	5.50	7.88	7.69
	DMSO-d_6	7.54	8.51	8.71
4b	$CDCl_3$	5.32	8.27	7.74
	DMSO-d_6	7.51	8.13	8.94

[a] [peptide] = 1 mM.

DMSO-d_6, respectively. The chemical shifts of 2Pmg-NH of **3a** and **3b** were approximately equal in both solvents, whereas those of both AA_1-NH and AA_3-NH of **3a** and **3b** were rather different between both solvents (Table 4). Furthermore, very little dependence of the chemical shifts of 2Pmg-NH of **3a** and **3b** on temperature was observed in DMSO-d_6 (Table 5). Therefore, the hydrogen bonding of 2Pmg-NH of **3** may be not intermolecular with the solvent but intramolecular with the pyridine nitrogen. Similarly, the chemical shifts of AA_1-NH and AA_3-NH of both **4a** and **4b** were rather different between $CDCl_3$ and DMSO-d_6, and the chemical shift of 2Pyg-NH of **4b** was approximately equal in both solvents. However,

Table 5 Temperature Dependence of NH Chemical Shifts of **3** and **4**[a]

	Solvent	$(-d\delta/dT)/10^{-3}K^{-1}$		
		AA_1	Xaa	AA_3
3a	$CDCl_3$	1.6	3.5	1.6
	DMSO-d_6	3.2	0.6	3.7
3b	$CDCl_3$	1.7	3.9	1.3
	DMSO-d_6	2.9	0.2	3.1
4a	$CDCl_3$	1.7	1.8	1.5
	DMSO-d_6	4.0	2.6	3.8
4b	$CDCl_3$	1.2	2.9	1.1
	DMSO-d_6	3.6	1.0	2.6

[a] [peptide] = 1 mM, temperature 298–328 K.

the chemical shift of 2Pyg-NH of **4a** was different between both solvents and the change from $CDCl_3$ to DMSO-d_6 caused a lower-field shift, in contrast with **3a, 3b** and **4b**. The temperature dependence of the chemical shift of 2Pyg-NH of **4b** showed a similar tendency to that of 2Pmg-NH of **3**, i.e. 2Pyg-NH showed much smaller $-d\delta/dT$ in DMSO-d_6 than in $CDCl_3$.

On the other hand, 2Pyg-NH of **4a** showed larger $-d\delta/dT$ in DMSO-d_6 than in $CDCl_3$, and further, the temperature dependence of the chemical shift of 2Pyg-NH of **4a** in DMSO-d_6 was larger than those of **3a, 3b** and **4b**. These results suggest that the pyridine nitrogen of 2Pyg in **4b** forms an intramolecular hydrogen bond with its own NH, whereas the 2Pyg-NH in **4a** hardly participates in intramolecular hydrogen bonding with the pyridine nitrogen even in $CDCl_3$.

The solvent dependence of NH chemical shifts of **3** and **4**, observed by adding increasing amounts of strong hydrogen-bonding acceptor solvent DMSO-d_6 to the $CDCl_3$ solution, showed that the chemical shifts of both AA_1-NH and AA_3-NH in **3** and **4**, and 2Pyg-NH in **4a** were sensitive to the addition of DMSO-d_6 (Figure 3).

On the contrary, 2Pmg-NH in **3** and 2Pyg-NH in **4b** are insensitive to the addition of DMSO-d_6 and thus display a behavior characteristic of protons shielded from the solvent. Although the chemical shift of 2Pyg-NH in $CDCl_3$ of **4b** (8.27 ppm) is in higher-field than those of 2Dpy-NH and 2Ppg-NH, Yu and coworkers reported that the chemical shifts of two carbamoyl protons of 2,6-bis(carbamoylpeptide)pyridine were observed at ca. 8.5 ppm in $CDCl_3$ and the compound adopted a conformation based on an intramolecular hydrogen bond between two carbamoyl protons and a pyridine nitrogen [41]. The chemical shift of 2Pyg-NH in $CDCl_3$ of **4a** (7.88 ppm) is in much higher-field and is similar to that of Gly_3-NH of **4a**.

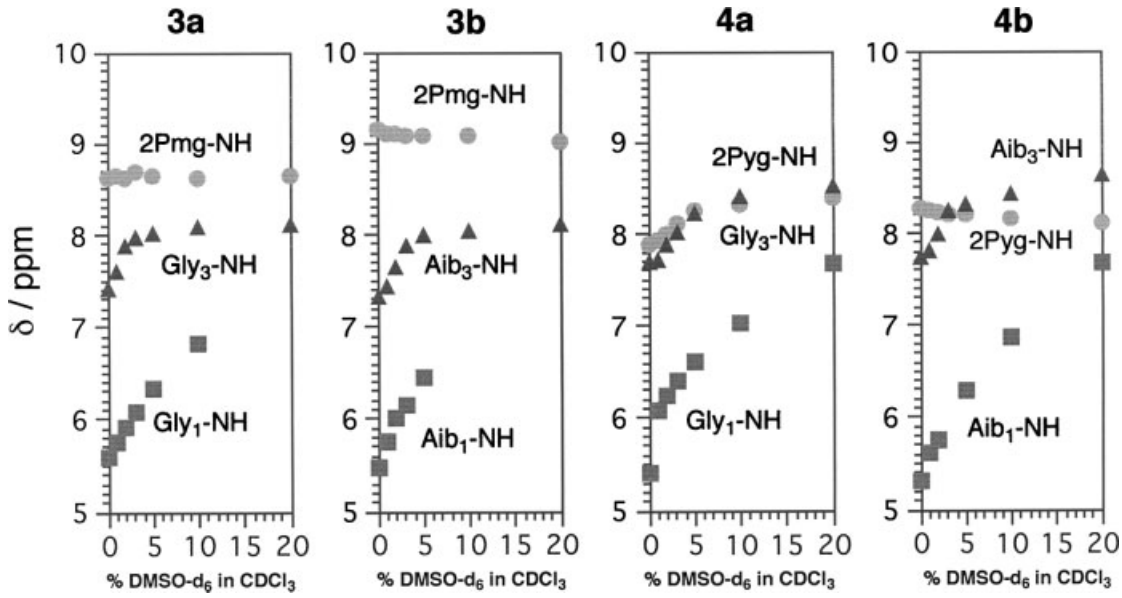


Figure 3 Plots of NH chemical shifts of (a) Z-Gly_1-2Pmg-Gly_3-OMe (**3a**), (b) Z-Aib_1-2Pmg-Aib_3-OMe (**3b**), (c) Z-Gly_1-2Pyg-Gly_3-OMe (**4a**) and (d) Z-Aib_1-2Pyg-Aib_3-OMe (**4b**) *vs* increasing percentages (v/v) of DMSO-d_6 in $CDCl_3$.

These results clearly indicate that 2Pmg-containing tripeptides (**3a** and **3b**) and **4b** adopt a unique conformation with an intramolecular hydrogen bond between the pyridine nitrogen and its own NH group (Figure 4), but **4a** does not. All data of chemical shifts, solvent effect (Table 4), temperature dependence (Table 5) and solvent titration (Figure 3) reveal that every AA_3-NH of **3a, 3b, 4a** and **4b** does not participate in an intramolecular hydrogen bonding. Therefore, both **3** and **4** do not adopt a β-turn structure that 2Ppg-containing tripeptides (**2**) adopt [17].

The NH chemical shifts of **1**–**4** are shown in Table 6. The remarkable lower-field shifts of 2Dpy-NH and 2Ppg-NH were attributed to the hydrogen bonding and the ring-current effect associated with a pyridine ring [15,16]. The chemical shifts of 2Pmg-NH of **3** in $CDCl_3$ are in higher field than those of 2Dpy-NH and 2Ppg-NH of the corresponding 2Dpy- and 2Ppg-containing tripeptides (**1** and **2**). Further, the chemical shifts of 2Pyg-NH of 2Pyg-containing tripeptides (**4**) are in considerably higher field than those of 2Dpy-NH of **1** and 2Ppg-NH of **2**. The chemical shift of Xaa-NH of **1**–**4** except **4a** decrease in the order of 2Dpy-NH>2Ppg-NH>2Pmg-NH>2Pyg-NH. This may reflect the stability of hydrogen bonding and the ring-current effect associated with a pyridine ring.

Table 6 ^{1}H NMR Chemical Shifts of NH Protons of **1**–**4**[a] in $CDCl_3$

	XAA	δ		
		AA_1	Xaa	AA_3
1a	2Dpy	5.63	9.45	10.41
2a	2Ppg	5.51	9.28	8.61
3a	2Pmg	5.60	8.62	7.42
4a	2Pyg	5.50	7.88	7.69
1b	2Dpy	5.42	9.70	9.07
2b	2Ppg	5.42	9.62	8.54
3b	2Pmg	5.50	9.16	7.34
4b	2Pyg	5.32	8.27	7.74

[a] [peptide]: 1 mM.

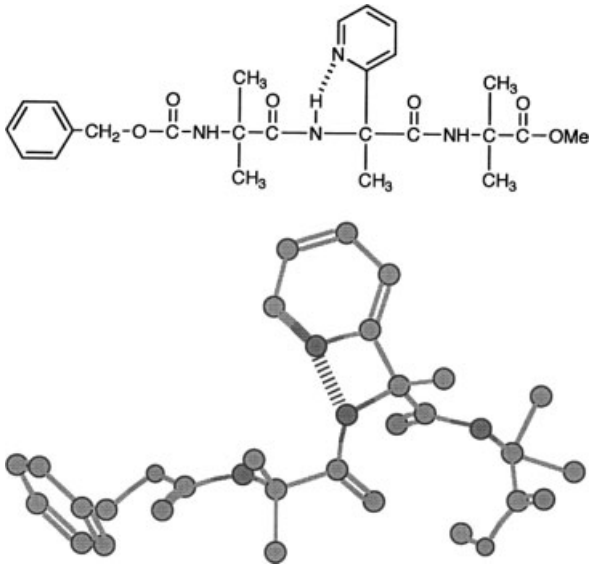


Figure 4 Possible conformation of Z-Aib-2Pmg-Aib-OMe (**3b**).

CONCLUSION

In conclusion, tripeptides containing α-methyl-α-(2-pyridyl)-
glycine (2Pmg) and α-(2-pyridyl)glycine (2Pyg) have been prepared for the first time by the solid-phase Ugi reaction. This approach was demonstrated to be effective in the construction of peptides containing DSGs with a hetero-aryl group. Furthermore, it was clarified by conformational analysis of tripeptides **1**–**4** that when an α-amino acid, particularly α,α-disubstituted glycine, with a 2-pyridyl group at the α-carbon atom is introduced into any peptide, an intramolecular hydrogen bond between a pyridine nitrogen and an amide proton may be formed and the conformational mobility of the peptide backbone may be restricted. Various α,α-disubstituted glycines with a 2-pyridyl group will provide promising building-blocks applicable to *de novo* design of functional peptides.

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