ARTICLE

OBC
www.rsc.org/obc

A novel intramolecular hydrogen bonding between a side-chain pyridine ring and an amide hydrogen of the peptide backbone in tripeptides containing the new amino acid, α,α-di(2-pyridyl)glycine

Takashi Yamada,* Tomoyuki Ichino, Masayuki Hanyu, Daisuke Ninomiya, Ryoji Yanagihara, Toshifumi Miyazawa and Takashi Murashima

Department of Chemistry, Faculty of Science and Engineering, Konan University, Kobe, Japan. E-mail: yamada@konan-u.ac.jp; Fax: +81-78-435-2539; Tel: +81-78-435-2503

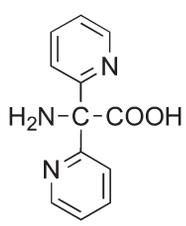
Received 30th April 2004, Accepted 18th June 2004
First published as an Advance Article on the web 27th July 2004

Four tripeptides (Z-AA_1-2Dpy-AA_3-OMe; AA_1, AA_3 = Gly, Aib) containing a novel amino acid, α,α-di(2-pyridyl)glycine (2Dpy), were synthesized by the modified Ugi reaction. NMR analysis clearly indicated that the 2Dpy-containing tripeptides except the peptide in which AA_1, AA_3 = Aib, adopt a unique conformation with two intramolecular hydrogen bonds between 2Dpy-NH and a pyridine nitrogen and between AA_3-NH and another pyridine nitrogen. This conformation has so far not been reported. On the other hand, the peptide Z-Aib-2Dpy-Aib-OMe probably adopts a β-turn structure which is stabilized by two intramolecular hydrogen bonds between 2Dpy-NH and a pyridine nitrogen and between AA_3-NH and the C=O of the Z group.

Introduction

α,α-Disubstituted amino acids provide an excellent tool for the construction of conformationally rigid peptides due to the steric hindrance associated with the quaternary α-carbon atom.[1–6] It may shown that peptides, even small oligopeptides, rich in α,α-disubstituted glycines, adopt a β-turn structure, 3_{10}/α-helical structures or planar, fully-extended (C_5) conformations.[7–10] This makes them attractive building blocks for the construction of supramolecular devices with peptides as the framework. Recently, novel α,α-disubstituted glycines which have additional functions are of growing interest: 4-aminopiperidine-4-carboxylic acid (Api) with a piperidine ring,[11] 2,2,6,6-tetramethylpiperidine-*N*-oxyl-4-amino-4-carboxylic acid (TOAC) with a piperidine-*N*-oxide ring,[12–16] 9-aminofluorene-9-carboxylic acid (Afc) with a fluorene ring,[6,17–19] 9-amino-4,5-diazafluorene-9-carboxylic acid (Daf) with a 4,5-diazafluorene ring,[20–24] conformationally constrained glycosylated amino acids,[25–27] 6-amino-1,11-(20-crown-6)-6,7-dihydro-5*H*-dibenzo-[*a*,*c*]cycloheptene-6-carboxylic acid ([20-C-6]-Bip),[28] 3-thymine-1-(ᵗbutoxycarbonyl)aminocyclopentane-1-carboxylic acid,[29] *etc.*, were reported.

We recently synthesized a variety of fully protected tripeptides containing a bulky α,α-disubstituted glycine, α,α-diphenylglycine (Dph), by the modified Ugi reaction,[30,31] and could clarify that the Ugi reaction[32] is very useful and potent for the synthesis of sterically hindered peptides containing α,α-disubstituted glycines. A systematic structural analysis of the Dph-containing tripeptides by X-ray analysis revealed that the Dph residue adopts a folded conformation in the 3_{10}-helical region if the following residue adopts a folded conformation,[5] although most of the Dph-containing peptides adopt the C_5-extended conformation.[33–36] In connection with Dph which has two phenyl groups at C^α, we here report the synthesis and conformation of peptides containing a novel α,α-disubstituted glycine, α,α-di(2-pyridy)glycine (2Dpy) which has two 2-pyridyl groups at C^α (Scheme 1). 2Dpy will be expected not only to provide a similar conformational constraint to Dph, but also to adopt conformations containing hydrogen bonding in which pyridine nitrogens participate. Recently, various compounds containing 2-pyridinecarboxylic acid or 2,6-pyridinedicarboxylic acid have been reported to adopt conformations based on an intramolecular hydrogen bond between a pyridine nitrogen and an amide proton.[37–43] In this paper, we focus on the participation of each nitrogen of the two pyridine rings bound to C^α in intramolecular hydrogen bonding with the amide hydrogen of a peptide bond.



Scheme 1 Chemical structure of α,α-di(2-pyridyl)glycine (2Dpy).

Results and discussion

Synthesis of 2Dpy-containing tripeptides (5a–5b)

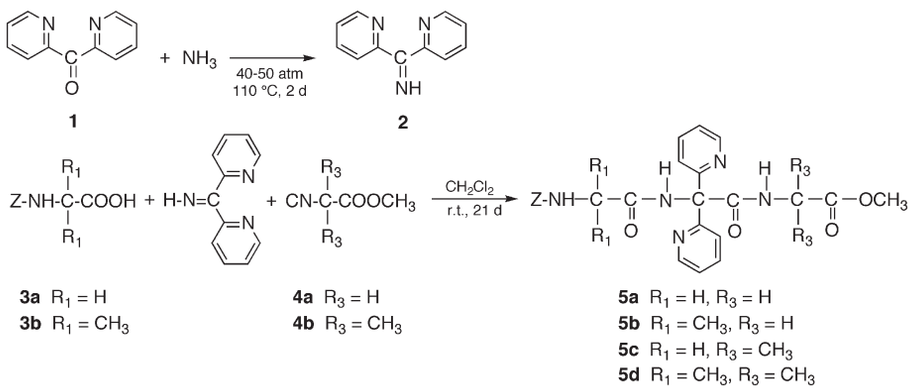
Four tripeptides containing 2Dpy [Z-AA_1-2Dpy-AA_3-OMe (**5a**–**5d**); AA_1, AA_3 = Gly, Aib (α-aminobutyric acid)] could be synthesized by the modified Ugi reaction (Scheme 2). Di(2-pyridyl)methanimine (**2**), which is a key compound in this reaction, was smoothly prepared from di(2-pyridyl)ketone (**1**) and liquid ammonia according to the Verardo method.[44] Condensation of Z-Gly-OH (**3a**) or Z-Aib-OH (**3b**), Schiff's base (**2**) and isocyanides (**4a** or **4b**) which were derived from Gly or Aib, respectively, was carried out in CH_2Cl_2 for 3 weeks. The reaction mixture colorized very darkly and many by-products were produced. Therefore, purification of the desired Ugi product was very troublesome. Yields of these tripeptides (**5a**–**5d**) were low. This may be due to the steric crowding in the reaction intermediate and the reactivity of the pyridine ring itself. All four tripeptides (**5a**–**5d**) were obtained as crystalline compounds after chromatographic purification.

NMR analysis of 2Dpy-containing tripeptides (5a–5d)

In order to reveal the conformational difference caused by the presence of a nitrogen of a pyridine ring, the ^{1}H-NMR spectra in $CDCl_3$ of **5a**–**5d** were compared with those of the corresponding Dph-containing tripeptides (**6a**–**6d**).[31] Fig. 1 shows a comparison of the ^{1}H-NMR spectra in $CDCl_3$ of Z-Gly_1-2Dpy-Gly_3-OMe (**5a**) and Z-Gly_1-Dph-Gly_3-OMe (**6a**). The chemical shifts of NH protons of *C*-terminal Gly (Gly_3) and 2Dpy of **5a** are at markedly lower-field than those of Gly_3 and Dph of **6a**, though the chemical shift of Gly_1-NH scarcely differs between **5a** and **6a**. This is also the case in the other peptides (**5b**–**5d**), as seen in Table 1. This means that such NH protons may participate in an intramolecular hydrogen bonding.

Although both 2Dpy-NH and AA_3-NH of **5** have relatively similar chemical shifts (mostly 9–10 ppm), the chemical shift

DOI: 10.1039/b406545j



Scheme 2 Synthetic route for the preparation of 2Dpy-containing tripeptides (**5a**–**5d**).

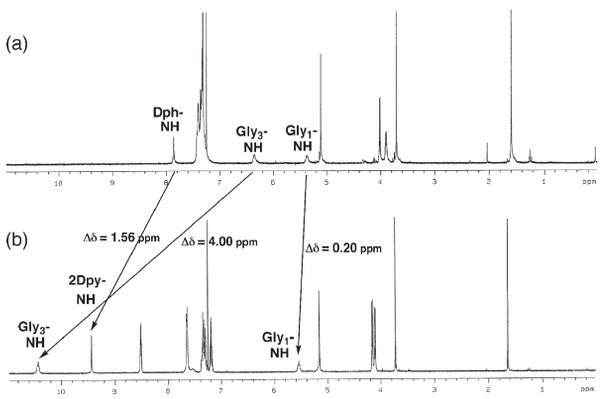


Fig. 1 Comparison of ^{1}H NMR spectra of Z-Gly$_1$-Dph-Gly$_3$-OMe (**6a**) and Z-Gly$_1$-2Dpy-Gly$_3$-OMe (**5a**) in $CDCl_3$.

difference between AA_3-NH of **5** and that of **6** is much larger than the corresponding difference between 2Dpy-NH of **5** and Dph-NH of **6**; more specifically, in the cases of **5a**, **5b** and **5c**, the former differences are 4.00, 3.01 and 3.53 ppm, whereas the latter are 1.57, 1.85 and 1.49 ppm, respectively.

The solvent dependence of NH chemical shifts of **5**, observed by adding increasing amounts of the strong hydrogen-bonding acceptor solvent DMSO-d_6 to the $CDCl_3$ solution, showed that the chemical shift of only AA_1-NH among the three amide hydrogens is sensitive to the addition of DMSO-d_6 (Fig. 2). By contrast, both 2Dpy-NH and AA_3-NH are insensitive to the addition and display a behavior characteristic of protons shielded from the solvent. Therefore, these two amide protons probably participate in an intramolecular hydrogen bonding. On the other hand, in Dph-containing peptides (**6**) only Dph-NH is insensitive to the addition of DMSO-d_6 (Fig. 3). From the previous results,[5,33–36] it is reasonable to conclude that

Table 1 ^{1}H NMR chemical shifts of NH protons of 2Dpy-containing tripeptides (**5a**–**5d**) and chemical shift differences from those of the corresponding Dph-containing tripeptides (**6a**–**6d**)[31] in $CDCl_3$

			Chemical shift (δ)/ppm ($\Delta\delta^a$/ppm)		
	AA_1	AA_3	AA_1-NH	2Dpy-NH	AA_3-NH
5a	Gly	Gly	5.70	9.46	10.41
6a			5.43	7.89	6.41
			(0.27)	(1.57)	(4.00)
5b	Aib	Gly	5.51	9.72	9.83
6b			5.43	7.87	6.82
			(0.08)	(1.85)	(3.01)
5c	Gly	Aib	5.67	9.39	9.95
6c			5.39	7.90	6.42
			(0.26)	(1.49)	(3.53)
5d	Aib	Aib	5.42	9.70	9.07
6d			5.42	7.87	6.85
			(0.00)	(1.83)	(2.22)

$^a\delta$(2Dpy-peptide) − δ(Dph-peptide).[31]

tripeptides (**6**) adopt the C_5-conformation with an intramolecular hydrogen bond between Dph-NH and Dph-C=O in $CDCl_3$.

These results clearly indicate that the 2Dpy-containing tripeptides (**5a**–**5c**) adopt a unique conformation with two intramolecular hydrogen bonds between 2Dpy-NH and a pyridine nitrogen and between AA_3-NH and another pyridine nitrogen (Fig. 4). The remarkable lower-field shift of both NH proton resonances of 2Dpy and AA_3 can be attributed to the hydrogen bonding and the ring-current effect of the pyridine rings. Huc and coworkers[42] reported that the hydrazides derived from 2-pyridinecarboxylic acids have strongly deshielded NH protons (at 9.54 or 9.88 ppm) in $CDCl_3$ and adopt conformations based on an intramolecular hydrogen bond between

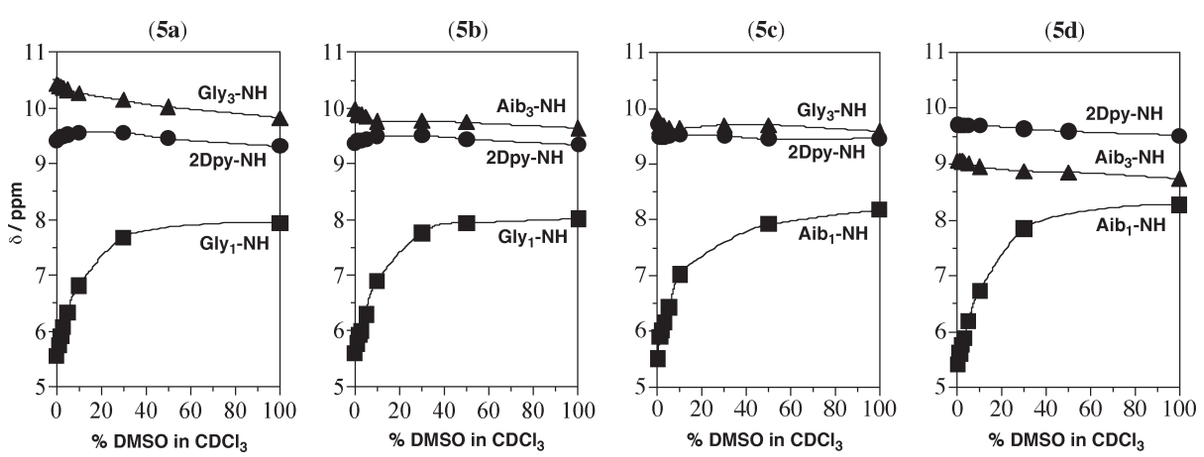


Fig. 2 Plots of NH chemical shifts of Z-Gly$_1$-2Dpy-Gly$_3$-OMe (**5a**), Z-Aib$_1$-2Dpy-Gly$_3$-OMe (**5b**), Z-Gly$_1$-2Dpy-Aib$_3$-OMe (**5c**) and Z-Aib$_1$-2Dpy-Aib$_3$-OMe (**5d**) *vs.* increasing percentages (v/v) of DMSO-d_6 in $CDCl_3$.

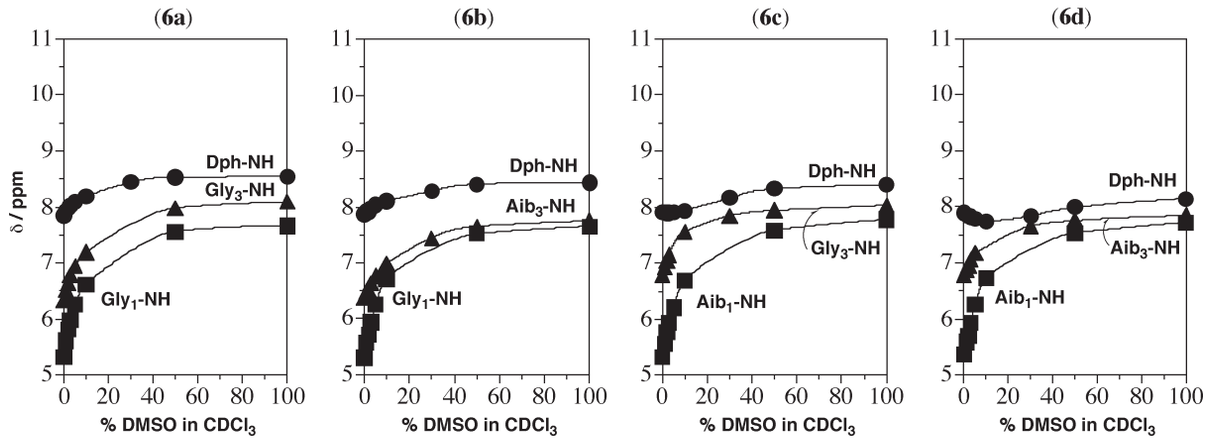


Fig. 3 Plots of NH chemical shifts of Z-Gly$_1$-Dph-Gly$_3$-OMe (**6a**), Z-Aib$_1$-Dph-Gly$_3$-OMe (**6b**), Z-Gly$_1$-Dph-Aib$_3$-OMe (**6c**) and Z-Aib$_1$-Dph-Aib$_3$-OMe (**6d**) *vs.* increasing percentages (v/v) of DMSO-d_6 in $CDCl_3$.

a hydrazide proton and the pyridine nitrogen. Most of the chemical shifts of 2Dpy-NH and AA$_3$-NH in **5a**–**5d** are at 9.4~10.0 ppm and are well consistent with Huc's results. The chemical shift difference between 2Dpy-NH of **5** and Dph-NH of **6** ($\Delta\delta$ 1.85~1.49 ppm) is much less than that in the case of AA$_3$-NH ($\Delta\delta$ 4.00~3.01 ppm), except for **5d**–**6d** ($\Delta\delta$ 2.22 ppm). This should probably be attributed to the fact that the Dph-NH has already been significantly deshielded (δ 7.8~7.9 ppm) by forming an intramolecular hydrogen bond in the C_5-conformation in $CDCl_3$. Thus, the chemical shift difference between 2Dpy-NH of **5** and Dph-NH of **6** may be attributed to an additional ring-current effect in the former.

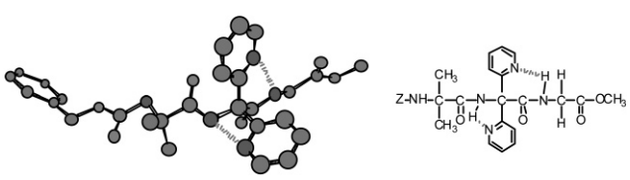


Fig. 4 Possible conformation of Z-Aib$_1$-2Dpy-Gly$_3$-OMe (**5b**).

The peptide Z-Aib$_1$-2Dpy-Aib$_3$-OMe (**5d**) is relatively different from the other peptides (**5a**–**5c**), both in chemical shifts and in solvent effects. The chemical shift of Aib$_3$-NH of **5d** (δ 9.07 ppm) is at much higher field than those of AA$_3$-NH of **5a**–**5c** (δ 10.41–9.83 ppm), while the chemical shifts of 2Dpy-NH of **5a**–**5d** are almost the same (δ 9.37~9.72 ppm). Moreover, the chemical shift of Aib$_3$-NH of **5d** (δ 9.07 ppm) is also at much higher field than that of 2Dpy-NH (δ 9.70 ppm) in the same peptide, but it is not sensitive to the addition of DMSO-d_6, as in the case of 2Dpy-NH (Fig. 2d). Therefore, Aib$_3$-NH of **5d** seems to take part in an intramolecular hydrogen bonding but not with a pyridine nitrogen, because a ring-current effect is not observed. The peptide (**5d**) probably adopts a β-turn structure which is stabilized by two intramolecular hydrogen bonds between 2Dpy-NH and a pyridine nitrogen and between the Aib$_3$-NH and the C=O of the Z group (Fig. 5). This conformation of **5d** may be due to the presence of the strong helix-promoting residue Aib before and behind the 2Dpy residue. The same conformation was also found in the crystal structure of **5d**, which has been reported by us in another paper.[45]

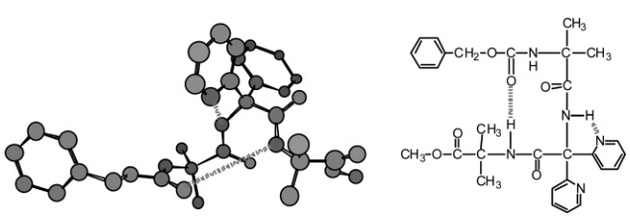


Fig. 5 Possible conformation of Z-Aib$_1$-2Dpy-Aib$_3$-OMe (**5d**).

Conclusion

The results described in this paper clearly show that a novel α,α-disubstituted glycine, 2Dpy, provides a promising conformationally-constrained building block, generating a unique rigid structure stabilized by two intramolecular hydrogen bonds associated with pyridine nitrogens.

Although several amino acids having a pyridine ring in their side chain have been prepared, there are only few reports on peptides, except those containing a bipyridyl group, and those in which an amino acid with a pyridyl group is contained: one is the RNase S-peptide 1–14 analog with histidine replaced by β-(2-, 3- or 4-pyridyl)alanine[46,47] and another is the Leu-enkephaline analog with tyrosine replaced by α-(5-hydroxy-2-pyridyl)glycine.[48] No report has yet described such intramolecular hydrogen bonding between a pyridine nitrogen in a side chain and an amide hydrogen on the peptide backbone.

Experimental

Most of the reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd. and Tokyo Kasei Kogyo Co. and used without further purification. Di(2-pyridyl)ketone was purchased from Aldrich Chemical Co. Methyl isocyanoacetate[49] and methyl 2-isocyano-2-methylpropionate[31] were prepared by the literature methods. Products were isolated by preparative TLC, on silica gel (Wakogel B-5F, Wako Pure Chemical Industries, Ltd.), by flash column chromatography on silica gel (Wakogel FC-40) or by open column chromatography (Wakogel C-300). Reactions using liquid ammonia were carried out in a stainless steel portable reactor (TVS-1), which was purchased from Taiatsu Techno Co. Melting points were measured on a Yamato melting point apparatus (MP-21) and are uncorrected. IR spectra were run on a Nicolet FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian UNITY 300 spectrometer at 299.94 MHz and 75.4 MHz, respectively, TMS or the solvent being used as internal standard. Assignments of signals for the peptides were carried out by COSY and gHMBC correlations. ^{1}H NMR and ^{13}C NMR spectra of the peptide **5d** were also recorded on Varian UNITY 500 spectrometer at 500 MHz and 125.7 MHz, and assignments of signals were made by DEPT, HMQC and HMBC techniques. MALDI-TOF mass spectra were recorded on a PerSeptive Biosystems Voyager DE PRO Biospectrometry Workstation, where α-cyano-4-hydroxycinnamic acid was used as the matrix reagent.

Preparation of di(2-pyridyl)methanimine (2)

A mixture of di(2-pyridyl)ketone (**1**) 22.1 g (120 mmol), ammonium chloride 7.06 g (132 mmol), dry THF (90 cm^3) and liquid NH_3 (130 cm^3) was heated in a sealed stainless reaction vessel (300 cm^3) at 110 °C by using an oil bath. The pressure in the vessel became *ca.* 50 atm. After 48 h, the vessel was cooled down and ammonia gas was removed. Yellow oil was filtered and concentrated

under reduced pressure. The residue was distilled under reduced pressure, affording a colorless oil (18.0 g, 81.8%), which solidified in the freezer (mp < 0 °C). Bp 121 °C/1.0 mmHg; IR (neat): ν_{max}/cm^{-1} 1641 (C=N); δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.40 (2H, br, Py-5H), 7.75 (2H, br-s, Py-4H), 7.88 (1H, br-t, Py-3H), 8.12 (1H, d, J = 7.2 Hz, Py-3H), 8.70, 8.75 (2H, br-s, Py-6H), 11.61 (1H, s, C=NH).

General procedure for synthesis of the Z-AA$_1$-2Dpy-AA$_3$-OMe (5) tripeptides

A solution of Z-AA$_1$-OH (6.06 mmol), di(2-pyridyl)methanimine (**2**) (6.0 mmol) and methyl isocyanoacetate or methyl 2-isocyano-2-methylpropionate (5.66 mmol) in CH_2Cl_2 (5 cm^3) was stirred at room temperature for 3 weeks. The solvent was removed under vacuum, and the residue was dissolved in $CHCl_3$ (50 cm^3). This solution was washed with 1 M HCl (20 cm^3 × 4), H_2O (20 cm^3), 1 M $NaHCO_3$ (20 cm^3 × 4) and NaCl saturated H_2O (20 cm^3 × 2) successively, and dried over Na_2SO_4. The solvent was evaporated and the crude product was purified by preparative thin-layer chromatography (PTLC) and/or by recrystallization from EtOAc.

Z-Gly-2Dpy-Gly-OMe (5a). Yield 7.2%; mp 155.5–156 °C; δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.72 (3H, s, OCH_3), 4.07 (2H, d, J = 5.7 Hz, Gly$_1$-CH_2), 4.15 (2H, d, J = 5.1 Hz, Gly$_3$-CH_2), 5.16 (2H, s, Z-CH_2), 5.70 (1H, br-t, Gly$_1$-NH), 7.18 (2H, dd, J = 3.0 Hz, J = 5.1 Hz, Py-5H), 7.25–7.4 (5H, m, ϕH), 7.65 (4H, m, Py-4H, Py-3H), 8.50 (2H, dd, J = 4.8 Hz, Py-6H), 9.46 (1H, s, 2Dpy-NH), 10.41 (1H, br-t, Gly$_3$-NH); *m*/*z* (MALDI-TOF) Found M + H$^+$ 492.1855, M + Na$^+$ 514.1463 (Calcd. M + H$^+$ 492.1883, M + Na$^+$ 514.1702).

Z-Aib-2Dpy-Gly-OMe (5b). Yield 8.4%; mp 149–151 °C; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.61 (6H, s, Aib-CH_3), 3.68 (3H, s, OCH_3), 4.10 (2H, d, J = 5.7 Hz, Gly-CH_2), 5.13 (2H, s, Z-CH_2), 5.51 (1H, s, Aib-NH), 7.18 (2H, ddd, $J_{4,5}$ = 7.5 Hz, $J_{5,6}$ = 4.8 Hz, $J_{3,5}$ = 1.5 Hz, Py-5H), 7.2–7.4 (5H, m, ϕH), 7.64 (2H, ddd, $J_{3,4}$ = 7.8 Hz, $J_{4,5}$ = 7.5 Hz, $J_{4,6}$ = 1.5 Hz, Py-4H), 7.75 (2H, dd, $J_{3,4}$ = 7.8 Hz, $J_{3,5}$ = 1.5 Hz, Py-3H), 8.47 (2H, dd, $J_{5,6}$ = 4.8 Hz, $J_{4,6}$ = 1.5 Hz, Py-6H), 9.72 (1H, s, 2Dpy-NH), 9.83 (1H, br, Gly-NH); *m*/*z* (MALDI-TOF) Found M + H$^+$ 520.2160, M + Na$^+$ 542.1956 (Calcd. M + H$^+$ 520.2196, M + Na$^+$ 542.2015).

Z-Gly-2Dpy-Aib-OMe (5c). Yield 9.3%; mp 104.5–106.5 °C; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.55 (3H, s, Aib-CH_3), 3.59 (3H, s, OCH_3), 4.03 (2H, d, J = 5.7 Hz, Gly-CH_2), 5.16 (2H, s, Z-CH_2), 5.67 (1H, br, Gly-NH), 7.18 (2H, qr, J = 4.5 Hz, Py-5H), 7.2–7.4 (m, 5H, ϕH), 7.64 (2H, m, Py-4H), 7.65 (2H, m, Py-3H), 8.48 (2H, d, J = 4.8 Hz, Py-6H), 9.39 (1H, s, 2Dpy-NH), 9.95 (1H, br, Aib-NH). *m*/*z* (MALDI-TOF) Found M + H$^+$ 520.2184, M + Na$^+$ 542.2042 (Calcd. M + H$^+$ 520.2196, M + Na$^+$ 542.2015).

Z-Aib-2Dpy-Aib-OMe (5d). Yield 12.8%. mp 165–166 °C. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.50 (6H, s, Aib$_3$-CH_3), 1.55 (6H, s, Aib$_1$-CH_3), 3.55 (3H, s, OCH_3), 5.14 (2H, s, Z-CH_2), 5.42 (1H, s, Aib$_1$-NH), 7.16 (2H, dd, J = 7.5 Hz, J = 5.0 Hz, Py-5H), 7.26–7.29 (3H, m, ϕ*m*H, *p*H), 7.35 (2H, m, ϕ*o*H), 7.63 (2H, t, J = 7.5 Hz, Py-4H), 7.75 (2H, d, J = 7.5 Hz, Py-3H), 8.43 (2H, d, J = 5.0 Hz, Py-6H), 9.07 (1H, br-s, Aib$_3$-NH), 9.70 (1H, s, 2Dpy-NH). *m*/*z* (MALDI-TOF); Found M + H$^+$ 548.2468, M + Na$^+$ 570.2298 (Calcd. M + H$^+$ 548.2509, M + Na$^+$ 570.2328).

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by the Hirao Taro Foundation of the Konan University Association for Academic Research.

References

1 C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone and C. Pedone, *Biopolymers*, 1983, **22**, 205–215.
2 V. Barone, F. Lelj, A. Bavoso, B. Di Blasio, P. Grimaldi, V. Pavone and C. Pedone, *Biopolymers*, 1985, **24**, 1759–1767.
3 C. Toniolo and E. Benedetti, *ISI Atlas Sci.: Biochem.*, 1988, **1**, 225–230.
4 B. Di Blasio, V. Pavone, A. Lombardi, C. Pedone and E. Benedetti, *Biopolymers*, 1993, **33**, 1037–1049.
5 V. Pavone, A. Lombardi, M. Saviano, F. Nastri, L. Zaccaro, O. Maglio, C. Pedone, Y. Omote, Y. Yamanaka and T. Yamada, *J. Pept. Sci.*, 1998, **4**, 21–32.
6 A. Lombardi, G. De Simone, S. Galdiero, F. Nastri, L. Di Costanzo, K. Makihira, T. Yamada and V. Pavone, *Biopolymers*, 2002, **53**, 150–160.
7 C. Toniolo, *Biopolymers*, 1989, **28**, 247–257.
8 I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747–6756.
9 C. Toniolo and E. Benedetti, *Macromolecules*, 1991, **24**, 4004–4009.
10 V. Pavone, A. Lombardi, F. Nastri, M. Saviano, B. Di Blasio, F. Fraternali, C. Pedone and T. Yamada, *J. Chem. Soc., Perkin Trans. 2*, 1992, 971–977.
11 C. L. Wysong, T. S. Yokum, M. L. McLaughlin and R. P. Hammer, *CHEMTECH*, 1997, 26–33.
12 R. Marchetto, S. Schreier and C. R. Nakaie, *J. Am. Chem. Soc.*, 1993, **115**, 11042–11043.
13 C. Toniolo, E. Valente, F. Formaggio, M. Crisma, G. Pilloni, C. Corvaja, A. Toffoletti, G. V. Martinez, M. P. Hanson, G. L. Millhauser, C. George and J. L. Flippen-Anderson, *J. Pept. Sci.*, 1995, **1**, 45–57.
14 M. L. Smythe, C. R. Nakaie and G. R. Marshall, *J. Am. Chem. Soc.*, 1995, **117**, 10555–10562.
15 P. Hanson, G. V. Martinez, G. Millhauser, F. Formaggio, M. Crisma, C. Toniolo and C. Vita, *J. Am. Chem. Soc.*, 1996, **118**, 271–272.
16 C. Toniolo, M. Crisma and F. Formaggio, *Biopolymers*, 1998, **47**, 153–158.
17 T. Yamada, K. Makihira, S. Suzuki, K. Yoneda, R. Yanagihara and T. Miyazawa, in *Peptides Science—Present and Future*, ed. Y. Shimonishi, Kluwer Academic Publishers, Dordrecht, 1999, pp. 300–302; T. Yamada, K. Makihira, S. Suzuki, R. Yanagihara and T. Miyazawa, in *Peptides 1998*, eds. S. Bajusz and F. Hudecz, Akadémiai Kiadó, Budapest, 1999, pp. 404–405; T. Yamada, K. Makihira, R. Yanagihara, T. Miyazawa, V. Pavone, A. Lombardi and G. De Simone, in *Peptides Science 1998*, ed. M. Kondo, Protein Research Foundation, Osaka, 1999, pp. 353–356.
18 J. Savrda, J.-P. Mazaleyrat, M. Wakselman, F. Formaggio, M. Crisma and C. Toniolo, *J. Pept. Sci.*, 1999, **5**, 61–74.
19 M. Crisma, F. Formaggio, S. Mezzato, C. Toniolo, J. Savrda, J.-P. Mazaleyrat and M. Wakselman, *Lett. Pept. Sci.*, 2000, **7**, 123–131.
20 T. Sheradsky, G. Salemnick and Z. Nir, *Tetrahedron*, 1972, **28**, 3833–3843.
21 M. T. DuPriest, R. E. Conrow and D. Kuzmich, *Tetrahedron Lett.*, 1990, **31**, 1959–1962.
22 J.-P. Mazaleyrat, M. Wakselman, F. Formaggio, M. Crisma and C. Toniolo, *Tetrahedron Lett.*, 1999, **40**, 6245–6248.
23 J.-P. Mazaleyrat, K. Wright, M. Wakselman, F. Formaggio, M. Crisma and C. Toniolo, *Eur. J. Org. Chem.*, 2001, 1821–1829.
24 C. Peggion, M. Crisma, F. Formaggio, C. Toniolo, K. Wright, M. Wakselman and J.-P. Mazaleyrat, *Biopolymers*, 2002, **63**, 314–324.
25 J. W. Lane and R. L. Halcomb, *Tetrahedron*, 2001, **57**, 6531–6538.
26 A. Avenoza, J. M. Peregrina and E. S. Martín, *Tetrahedron Lett.*, 2003, **44**, 6413–6416.
27 J. W. Lane and R. L. Halcomb, *J. Org. Chem.*, 2003, **68**, 1348–1357.
28 J.-P. Mazaleyrat, Y. Goubard, M.-V. Azzini, M. Wakselman, C. Peggion, F. Formaggio and C. Toniolo, *Eur. J. Org. Chem.*, 2002, 1232–1247.
29 N. M. Howarth, L. P. G. Wakelin and D. M. Walker, *Tetrahedron Lett.*, 2003, **44**, 695–698.
30 T. Yamada, T. Yanagi, Y. Omote, T. Miyazawa, S. Kuwata, M. Sugiura and K. Matsumoto, *J. Chem. Soc., Chem. Commun.*, 1990, 1640–1641.
31 T. Yamada, Y. Omote, Y. Yamanaka, T. Miyazawa and S. Kuwata, *Synthesis*, 1998, 991–998.
32 G. Gokel, P. Hoffmann, H. Kleimann, H. Klusacek, G. Ludke, D. Marquaerding and I. Ugi, in *Isonitrile Chemistry*, ed. I. Ugi, Academic Press, New York and London, 1971, p. 201; A. Dömling and I. Ugi, *Angew. Chem., Int. Ed.*, 2000, **39**, 3168–3210.
33 V. Pavone, A. Lombardi, M. Saviano, B. Di Blasio, F. Nastri, R. Fattorusso, L. Zaccaro, O. Maglio, T. Yamada, Y. Omote and S. Kuwata, *Biopolymers*, 1994, **34**, 1595–1604.
34 V. Pavone, A. Lombardi, M. Saviano, F. Nastri, O. Maglio, Y. Omote, Y. Yamanaka and T. Yamada, *Biopolymers*, 2000, **53**, 161–168.
35 M. Crisma, G. Valle, G. M. Bonora, E. De Menego, C. Toniolo, F. Lelj, V. Barone and F. Fraternali, *Biopolymers*, 1990, **30**, 1–11.
36 M. Crisma, G. Valle, G. M. Bonora, C. Toniolo, F. Lelj, V. Barone, F. Fraternali, P. M. Hardy and H. L. S. Maia, *Biopolymers*, 1991, **31**, 637–641.
37 Y. Hamuro, S. J. Geib and A. D. Hamilton, *J. Am. Chem. Soc.*, 1997, **119**, 10587–10593.

38 Q. Yu, T. E. Baroni, L. Liable-Sands, G. P. A. Yap, A. L. Rheibgol and A. S. Borovik, *Chem. Commun.*, 1999, 1467–1468.
39 V. Berl, I. Huc, R. G. Khoury, M. J. Krische and J.-M. Lehn, *Nature*, 2000, 720–723.
40 D. Ranganathan, S. Kurur, A. C. Kunwar, A. V. S. Sarma, M. Vairamani and I. L. Karle, *J. Pept. Res.*, 2000, **56**, 416–426.
41 I. Huc, V. Maurizot, H. Goritzka and J.-M. Léger, *Chem. Commun.*, 2002, 578–579.
42 J. Garric, J.-M. Léger, A. Grelard, M. Ohkita and I. Huc, *Tetrahedron Lett.*, 2003, **44**, 1421–1424.
43 I. Odriozola, N. Kyritsakas and J.-M. Lehn, *Chem. Commun.*, 2004, 62–63.
44 G. Verardo and A. G. Giumanini, *Synth. Commun.*, 1988, **18**, 1501–1511.
45 L. De Costanzo, S. Geremia, L. Randaccio, T. Ichino, R. Yanagihara, T. Yamada, D. Marasco, A. Lombardi and V. Pavone, *Dalton Trans.*, 2003, 787–792.
46 C. Hoes, J. Raap, W. Bloemhoff and K. E. T. Kerling, *Recl. J. R. Neth. Chem. Soc.*, 1980, **3**, 99–104.
47 C. Hoes, K. E. T. Kerling and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, 1983, **102**, 140–147.
48 C. Herdeis and R. Gebhard, *Arch. Pharm. (Weinheim)*, 1987, **320**, 546–553.
49 G. Skorna and I. Ugi, *Angew. Chem., Int. Ed. Engl.*, 1977, **16**, 259.