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Syntheses and Properties of Tertiary Peptide Bond Containing Polypeptides. 6.[1] Conformational Studies of Oligopeptides Containing the Pro-Pro Sequence by ^{13}C and ^{1}H NMR

Shizuko Isokawa,[†] Tetsuo Asakura,[‡] and Mitsuaki Narita*[†]

Department of Industrial Chemistry and Department of Polymer Engineering, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan.

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ABSTRACT: The conformation of Boc-Leu$_3$-Pro$_2$-Gly-OBzl (1) in Me_2SO-d_6 and $CDCl_3$ has been studied by ^{13}C and ^{1}H NMR spectroscopies. The related oligopeptides Boc-Pro$_2$-Gly-OBzl (2), Boc-Leu-Pro$_2$-Gly-OBzl (3), Boc-Leu$_2$-Pro$_2$-Gly-OBzl (4), Boc-Leu$_3$-Pro-OBzl (5), Boc-Leu$_3$-OBzl (6), and Boc-Leu$_3$-Pro$_2$-Gly-OH (7) have also been studied by NMR spectroscopy. The content of cis isomers about the Pro-Pro bonds was determined from peaks of α carbons of Pro in the ^{13}C NMR spectra and was ca. 15% for the peptides 1, 3, and 4 in Me_2SO-d_6. From the temperature dependencies of the NH chemical shifts in Me_2SO-d_6, the amide protons of 1–6 were shown to be almost fully exposed to the solvent. Only the NH protons of Leu(2) of 1 and 5 and the NH proton of Gly(6) of 1 in the cis configuration about the Pro-Pro bond are slightly shielded from the solvent. The NH chemical shifts of 1 show no concentration dependence, indicating that 1 is not intermolecularly hydrogen bonded among the solutes in Me_2SO-d_6. In the $CDCl_3$ solution of 1, the content of the cis isomer about the Pro-Pro bond was determined as 43% and the NH protons of the Leu$_3$ sequence are intermolecularly hydrogen bonded. In addition, the NH proton of Gly(6) is shielded from the solvent. This is probably due to hydrogen-bond formation between the NH proton of Gly(6) and the carbonyl of Leu(3) (type VI β-turn) in the cis conformation about the Pro-Pro bond in $CDCl_3$.

Introduction

One of the serious problems encountered in achieving smooth couplings in protein syntheses is the decreasing solubility and reactivity of large peptides as the peptide chain length increases.[2,3] The decrease in solubility and reactivity has so far been attributed to the appearance of higher structures of large peptides.[2,3] As a means to solve this solubility problem of large peptides, we have proposed the concept of "peptide segment separation" and have shown that the insertion of tertiary peptide bonds such as X-Pro and X-(Z)Y bonds into central positions of peptide chains could achieve remarkable improvement in solubility.[4,5] X and Y in the above X-Pro and X-(Z)Y bonds are arbitrary amino acids and Z is a suitable protecting group for the X-Y peptide bond. The improvement in solubility of the peptides having tertiary peptide bonds has been attributed to their randomly coiled structure in solution.[6,7]

In order to ascertain the relationship among the conformation, solubility, and reactivity of large peptides, sequential polypeptides, Boc-(Leu$_3$-Pro$_2$-Gly)$_n$-OX (X = H or Bzl, n = 1, 2, 4, 6, 8, 10, and 12) have been prepared previously.[5] These peptides have excellent solubility in polar solvents and their N-deprotected derivatives, H-(Leu$_3$-Pro$_2$-Gly)$_n$-OBzl (n = 2, 4, 6, 8, and 10), have high reactivity to give Boc-(Leu$_3$-Pro$_2$-Gly)$_{n+2}$-OBzl in high yields. The conformational study of these peptides by molar rotation measurement in various polar solvents and CD measurement in methanol also indicated that their predominant structure was that of a random coil in polar solvents.[7]

This paper studies in detail the conformational properties of Boc-Leu$_3$-Pro$_2$-Gly-OBzl (1) and the related peptides by ^{13}C and ^{1}H NMR measurements. In the following paper, we will report on the sequential polypeptides.[8] The information to be obtained from the studies

[†] Department of Industrial Chemistry.
[‡] Department of Polymer Engineering.

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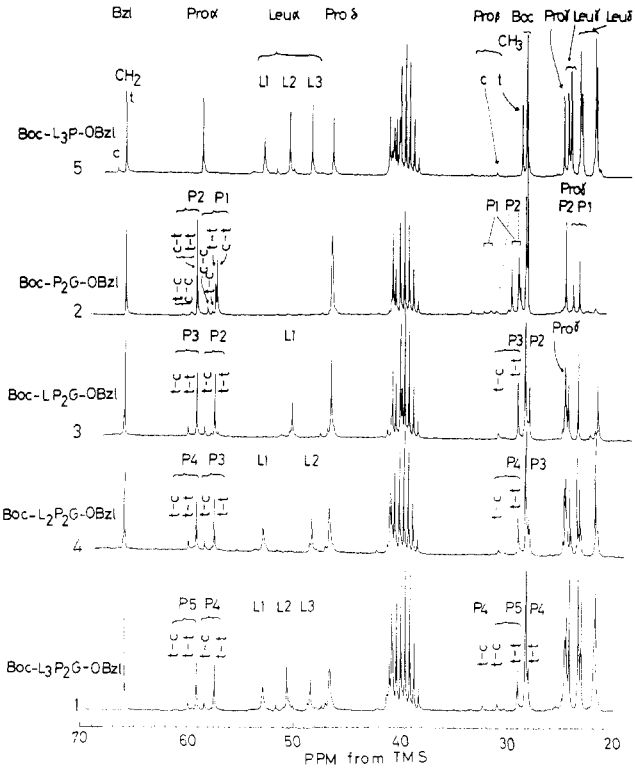

Figure 1. Partial ^{13}C NMR spectra of peptides **1–5** in Me_2SO-d_6 at 50 MHz together with the assignment. Chemical shifts were represented in ppm from Me_4Si.

may be useful in clarifying the local conformation of a large number of natural peptides and proteins containing the Pro-Pro sequence, such as collagen and casein.[9-13]

Experimental Section

Materials. The syntheses and physical properties of Boc-Leu$_3$-Pro$_2$-Gly-OBzl (**1**) Boc-Pro$_2$-Gly-OBzl (**2**), Boc-Leu$_3$-Pro-OBzl (**5**), Boc-Leu$_3$-OBzl (**6**), and Boc-Leu$_3$-Pro$_2$-Gly-OH (**7**) have been reported previously.[4,5] The peptides of Boc-Leu-Pro$_2$-Gly-OBzl (**3**) and Boc-Leu$_2$-Pro$_2$-Gly-OBzl (**4**) were synthesized by the stepwise elongation method as reported previously.[5] The products were purified by reprecipitation from ethyl acetate/*n*-hexane. Peptide **3**: mp 64–66.5 °C. Anal. Found: C, 61.95; H, 7.64; N, 9.69. Calcd for $C_{30}H_{44}O_7N_2 \cdot {}^1/_2H_2O$: C, 61.94; H, 7.80; N, 9.63. Amino acid analysis: Gly, 1.11; Pro, 2.00; Leu, 1.09. Peptide **4**: mp 93–96 °C. Anal. Found: C, 61.49; H, 7.91; N, 9.80. Calcd. for $C_{36}H_{55}O_8N_5 \cdot H_2O$: C, 61.43; H, 8.16; N, 9.95. Amino acid analysis: Gly, 1.05; Pro, 1.83; Leu, 2.00 (The recovery of Phe was 90%.) The amino acid residues of the peptides were numbered from the N terminal of the peptide chain so that, for example, the amide proton of an N-terminal residue is labeled Leu(1) NH and the α proton of the N-terminal Leu is Leu(1) C$^\alpha$H. Tetramethylsilane (Me_4Si) from Merck and Co., Me_2SO-d_6 (99.8%) from CEA, and $CDCl_3$ from Merck and Co. were used.

Measurements. ^{13}C NMR spectra were obtained on a Jeol FX 200 spectrometer at room temperature, with 45° pulse, 16K data points on a spectral width of 10 000 Hz, acquisition time 0.82 s, and with delay time 1.5 s. Up to 16 000 scans were accumulated to improve signal-to-noise ratios for ^{13}C NMR measurements. ^1H NMR spectra were also obtained on a Jeol FX 200 spectrometer at room temperature; 100–200 scans were made for ^1H NMR measurements. Approximately 0.1–0.2 M solutions were prepared for NMR measurements except for the experiments on concentration dependencies of the NH chemical shifts. All variations of chemical shift with temperature change were linear except for the case of the peptide **4**. With regard to the peptide **4**, some deviation from linearity was observed over the temperature range where coalescence of each major and minor NH peak occurred, corresponding to the trans and cis configurations, respectively, about the Boc-Leu and Pro-Pro bonds. Chemical shifts were recorded with Me_4Si as an internal reference.

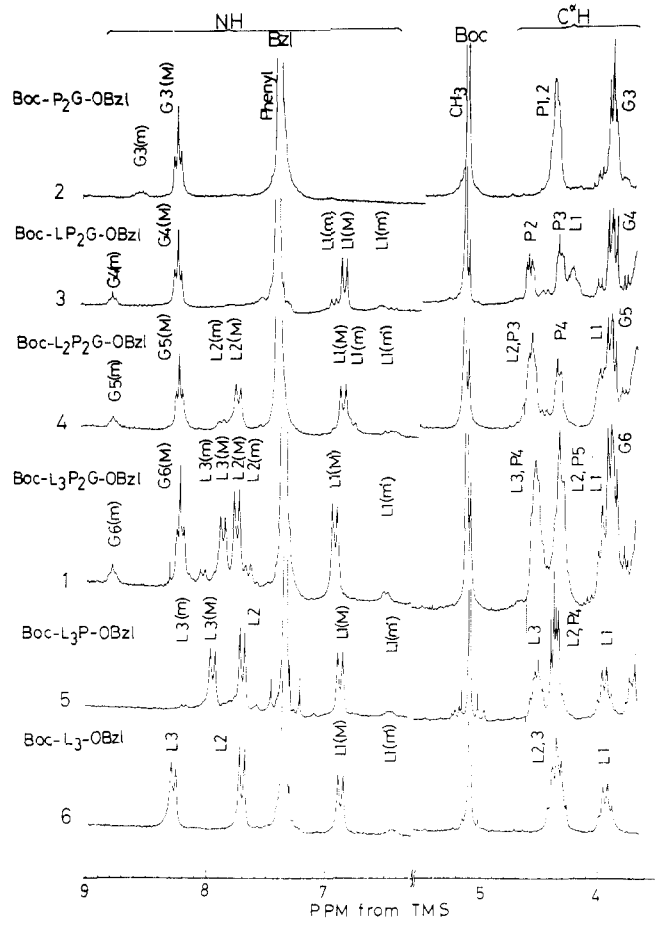

Figure 2. Partial ^1H NMR spectra of peptides **1–6** in Me_2SO-d_6 at 200 MHz together with the assignment. Chemical shifts were represented in ppm from Me_4Si.

Results and Discussion

^{13}C NMR Spectra of 1–5 in Me_2SO-d_6 Solution. Figure 1 shows ^{13}C NMR spectra of **1–5** in the spectral region between 25 and 70 ppm in Me_2SO-d_6. The peaks for carbons of N-terminal Leu were assigned from a comparison of the spectra for **1** and **3–5**. It is known that, because of the steric effect of the Pro residue, the α carbons of the residues preceding Pro resonate upfield by 1.0 ppm compared to the resonance position of the α carbons placed at the other parts in peptide chains.[11] Thus, the peaks for α carbons of Leu and Pro of **1–5** were readily assigned from the chemical shifts. The other peaks were also assigned from ^1H–^{13}C coupling patterns and from comparison of the chemical shifts of these peptides. For example, the ^1H-coupled spectrum of **1** showed doublets for α carbons of Leu and Pro residues, and triplets for β and δ carbons of Pro and methylene of the Bzl group. The assignments were shown in Figure 1, and, except for carbonyl carbons, α carbons of Gly, and β carbons of Leu, all the ^{13}C NMR chemical shifts for **1–5** are listed in Table I. The peak assignment concerning the cis and trans configurations of these peptides will be described later.

^1H NMR Spectra of 1–6 in Me_2SO-d_6 Solution. Figure 2 shows ^1H NMR spectra of **1–6** in the spectral regions between 3.5 and 5.5 ppm and between 6.3 and 9.0 ppm in Me_2SO-d_6 solution. The NMR parameters, including temperature coefficients of the NH protons, are listed in Table II. The high-field resonances in the amide NH region of the spectra of **1** and **3–6** were assigned to the N-terminal Leu(1) NH of the peptides.[14] The low-field triplets in the amide region of the spectra of **1–4** were assigned to the Gly NH protons from the coupling pattern.

Table I
^{13}C Chemical Shifts of Oligopeptides in Me_2SO-d_6

assignment		chem shift, ppm									
		1		2		3		4		5	
Leu	C^α	(1)[a]	52.91				50.13	(1)	57.76	(1)	52.83
			(51.69)[b]				(50.38)				(51.64)
		(2)	50.65							(2)	50.48
			(50.43)								(50.06)
			(50.13)								
		(3)	48.46					(2)	48.19	(3)	48.38
			(48.72)						(48.43)		
	C^γ		24.22				24.08		24.18		24.20
			23.98						23.84		23.94
			23.16								23.89
	C^δ		23.08				23.13		23.18		23.08
			22.94						22.94		23.01
			22.87								22.87
			21.58				21.31		21.43		21.58
			21.47								21.45
Pro	C^α	(4)	57.46	(1)	57.38	(2)	57.36	(3)	57.36		
					57.21[c]						
			(58.43)		(57.72)		(58.31)		(58.31)		
					(58.11)						
		(5)	59.16	(2)	59.09	(3)	59.04	(4)	59.04	(4)	58.58
			(59.99)		*(59.69)*		(59.86)		(59.82)		
	C^β	(4)	27.83	(1)	28.58	(2)	27.73	(3)	27.79		
					29.46						
			(32.21)		(32.13)						
					(31.48)						
		(5)	28.87	(2)	28.83	(3)	28.83	(4)	28.83	(4)	28.51
			(30.80)		(30.99)		(30.70)		(30.70)		(30.94)
					(29.80)						
	C^γ			(1)	23.62						
					23.06						
			24.42	(2)	24.33		24.40		24.37		24.62
			24.35				24.30		24.30		
	C^δ		46.66		46.46		46.49		46.61		46.39
			46.56		46.39				46.49		
Boc	CO		155.24		153.35		155.32		155.12		
					152.91						
	tert C		78.06		78.30		77.79		77.96		77.91
					78.13						
	CH_3		28.10		28.12		28.14		28.07		28.10
					27.93						
benzyl	CH_2		65.90		65.80		65.82		65.80		65.85
			(65.99)				(65.92)		(65.95)		(66.58)
phenyl	C_1		135.78		135.78		135.78		135.76		135.81
			(135.66)				(135.69)		(135.61)		(135.59)
phenyl	C_{2-6}		128.34		128.29		128.32		128.29		128.29
			128.02		127.95		128.00		127.98		127.93
			127.90		127.90		127.90		127.90		127.71

[a] The numbers in parentheses give the position of the amino acid residue from the N terminal. [b] Chemical shifts in parentheses correspond to minor peaks. [c] Italicized chemical shifts are major peaks due to the cis configuration about the Boc-Pro bond (see the text).

From decoupling experiments, the peak centered at 4.55 ppm in the spectrum of **4** was assigned to the $C^\alpha H$ of Leu(2) preceding the Pro residue. The assignment of doublets for Leu(2) NH and Leu(3) NH of **1** was performed from decoupling experiments in the following manner. Irradiation at 4.55 ppm caused collapse of doublets at 7.85 (major, M) and 8.02 (minor, m) ppm. Similarly, irradiation at 4.35 ppm caused collapse of doublets at 7.76 (M) and 7.66 (m) ppm. The peak at 4.55 ppm is attributable to Leu(3) $C^\alpha H$, because the chemical shift coincides with that of the Leu(2) $C^\alpha H$ of **4**. Another peak at 4.35 ppm is attributable to Leu(2) $C^\alpha H$ of **1**. In line with this, the C^α H of Leu existing within the peptide chain in a randomly coiled structure has been reported to resonate at 4.37 ppm in Me_2SO-d_6.[15] Thus, both peaks at 7.85 (M) and 8.02 (m) ppm were assigned to Leu(3) NH and the peaks at 7.76 (M) and 7.66 (m) ppm to Leu(2) NH. The peptide **5** exhibits three doublets corresponding to the three Leu NH protons, and these peaks were assigned from similar decoupling experiments as in the case of **1**. The methylene protons of the Gly residues of **1**–**4** show AB spin-coupling patterns (Figure 2).

Conformation of 1–5 in Me_2SO-d_6 Solution. There are two types of cis–trans equilibrium of peptide **1**: with respect to the Leu-Pro bond and with respect to the Pro-Pro bond. It is well-known that ^{13}C NMR spectroscopy is highly useful to examine the conformation equilibrium between the cis and trans isomers around X-Pro bonds.[11,12,16-18] The energy barrier of the cis–trans interconversion is sufficiently high (18–22 kcal/mol)[19] so that separate peaks can be detected directly in the ^1H and ^{13}C NMR spectra.

Peptide **5** shows only the cis–trans equilibrium about the Leu-Pro bond. (The cis–trans equilibrium about the Boc-Leu bond also exists but differs from the equilibrium under consideration.) In the resonance region of the β carbon of Pro of **5**, two peaks were observed. The chemical shifts of the major (M) and minor (m) peaks were 28.51

Table II
^{1}H NMR Parameters of Peptides in Me_2SO-d_6

peptide	residue	δ_{NH},[a] ppm	cis content, %	$10^3(d\delta_{NH}/dT)$, ppm/°C	$\delta_{C^\alpha H}$ or δ_{CH_2}, ppm	$J_{NH-C^\alpha H}$, Hz
1	Leu(1) M	6.91	15	6.8	3.99	8.0
	Leu(1) m′	6.49		3.7		
	Leu(2) M	7.76		3.3	4.35	8.4
	Leu(2) m	7.66		3.2		8.3
	Leu(3) M	7.85		4.9	4.55	7.6
	Leu(3) m	8.02		5.8		7.6
	Pro(4)				4.55	
	Pro(5)				4.35	
	Gly(6) M	8.21	15	5.0	3.90	5.8
	Gly(6) m	8.78		3.9		5.8
	(benzyl) M				5.11	
	(benzyl) m				5.07	
2	Pro(1)				4.47	
	Pro(2)				4.47	
	Gly(3) M	8.20	12	4.7	3.88	5.9
	Gly(3) m	8.53		4.5		
	(benzyl)				5.12	
3	Leu(1) M	6.76		8.1	4.21	8.1
	Leu(1) m	6.86		8.3		8.0
	Leu(1) m′	6.46	12[b]	7.2		
	Pro(2)				4.55	
	Pro(3)				4.30	
	Gly(4) M	8.18	17	5.0	3.86	5.8
	Gly(4) m	8.73		4.0		6.1
	(benzyl) M				5.09	
	(benzyl) m				5.06	
4	Leu(1) M	6.82	15	4.6	3.97	8.8
	Leu(1) m	6.74		(4.6)		8.6
	Leu(1) m′	6.40	12[b]	7.9		
	Leu(2) M	7.70	15	4.5	4.55	8.3
	Leu(2) m	7.84		5.3		8.1
	Pro(3)				4.55	
	Pro(4)				4.32	
	Gly(5) M	8.19	16	5.3	3.88	5.8
	Gly(5) m	8.75		4.5		
	(benzyl) M				5.10	
	(benzyl) m				5.07	
5	Leu(1) M	7.12		7.2	3.94	8.5
	Leu(1) m′	6.48		5.2		
	Leu(2)	7.71		3.2	4.36	8.3
	Leu(3) M	7.96	10	5.5	4.52	7.8
	Leu(3) m	8.21		5.6		
	Pro(4)				4.36	
	(benzyl)				5.09	
6	Leu(1) M	6.89	12	6.9	3.94	8.7
	Leu(1) m′	6.42				
	Leu(2)	7.71		4.2	4.35	8.5
	Leu(3)	8.28		5.8	4.35	7.3
	(benzyl)				5.09	

[a] The values at 30 °C calculated from temperature dependencies of NH chemical shifts. [b] Calculated from the peak area of m′ divided by the sum of peak areas of M, m, and m′.

and 30.94 ppm, respectively, and the peaks were assigned to the trans and cis configurations about the Leu-Pro bond, respectively.[16] The cis isomer content was determined to be 6% from the relative intensities. The cis content was also obtained as 7% from the two peaks at 65.85 (M) and 66.58 (m) ppm for the methylene carbon of the benzyl ester.

The spectrum of peptide **2** shows two peaks at 57.21 and 57.38 ppm attributable to the α carbon of Pro(1). The spectrum also shows a set of two peaks for each methyl and tertiary carbons of the Boc group with relative intensity similar to that for the α carbon of Pro(1). This fact indicates that these three sets of two peaks are attributable to the cis and trans isomers about the Boc-Pro bonds. Of the three peaks in the spectral region of Pro γ carbons of the peptide **2**, two peaks at 23.06 and 23.62 ppm are attributable to the γ carbon of Pro(1) on the basis of their relative intensities. The position of the two peaks relative to each other shows that the peaks at 23.06 and 23.62 ppm are due to the cis and trans isomers about the Boc-Pro bond, respectively, on the basis of the comparison with the data of the two peaks for the γ carbon of Pro in cis and trans configurations of the Z-Pro sequence of Z-Pro-Pro-OH.[20] The peaks at 29.46 and 28.58 ppm are similarly attributable to the β carbon of Pro(1) in the cis and trans configurations about the Boc-Pro bond on the basis of their relative intensities. The cis content about the Boc-Pro bond was estimated to be 61–64% from the relative intensities of these peaks. The peak at 24.33 ppm was attributed to the γ carbon of Pro(2) by elimination. The chemical shift of the γ carbon of Pro(2) coincides with the value reported previously for the γ carbon of Pro of the trans X-Pro bond.[16] Thus, the major peak at 57.21 ppm for the α carbon of Pro(2) of **2** was due to the trans configuration about the Pro-Pro bond, and the minor peaks in the spectral region of the α carbon of Pro of **2** were due to the cis configuration about the Pro-Pro bond. The major and minor peaks of **2** were assigned by taking into

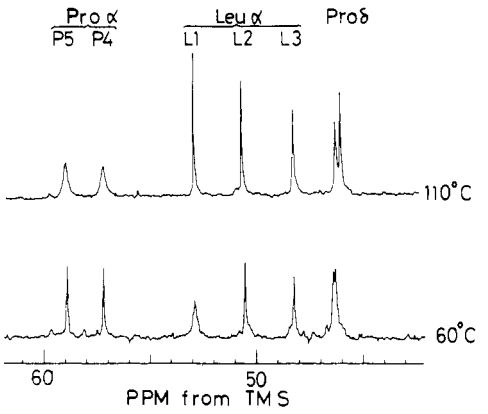

Figure 3. Partial ^{13}C NMR spectra of Boc-Leu_3-Pro_2-Gly-OBzl (1) in Me_2SO-d_6 at 60 and 110 °C.

account their relative intensities as shown in Figure 1. The cis content about the Pro-Pro bond of **2** as estimated from the relative intensities of these peaks was about 7%.

The spectrum of **3** shows a pair of minor peaks at 58.31 and 59.86 ppm, which are assigned to α carbons of Pro(2) and Pro(3) in the cis configuration about the Pro-Pro bond, respectively. Thus, only one minor peak at 30.70 ppm is attributable to the β carbon of Pro(3) in the cis configuration about the Pro-Pro bond, indicating that the Leu-Pro bond is exclusively in the trans configuration. The cis content about the Pro-Pro bond of **3** as estimated from peaks for α carbons of Pro(2) and Pro(3) was about 16%. Similarly, the α carbon resonances of peptides **4** and **1** are attributable to the trans–cis equilibrium about the Pro-Pro bonds. The cis contents about the Pro-Pro bond of **4** and **1** were 16% and 15%, respectively. The cis contents about the Pro-Pro bond of **1**, **3**, and **4** are almost equal to one another and not affected by chain elongation with the Leu residue from the N-terminal of the Pro-Pro sequence. The hexapeptide **7** exhibits a pattern of peaks for α carbons similar to that of **1** and its cis content about the Pro-Pro bond was about 13%.

Figure 3 shows the ^{13}C NMR spectra of **1** at 60 and 110 °C. At 60 °C, some of the minor peaks in the spectral region of α carbons of Leu(1) and Leu(2) residues disappear while the major peaks broaden. This phenomenon is due to rapid exchange between the cis and trans configurations about the Boc-Leu bond. This confirms that these minor peaks are due to the cis configuration rather than to the racemic isomer which might exist in the sample as an impurity. At 110 °C, the minor peaks assigned to α carbons of Pro in the cis configuration about the Pro-Pro bond disappear while the major peaks broaden. The coalescence temperatures of minor and major peaks coincide with those of the NH protons. Namely, the peak for Leu(1) NH (m′) at 6.49 ppm (at 30 °C) of **1** disappeared at about 60 °C (Figure 4). The same tendency was detected for peaks of NH (m′) at about 6.4–6.5 ppm (at 30 °C) of **3–6**. From the ^{13}C NMR observations, the minor peaks at about 6.4–6.5 ppm (at 30 °C) were assigned to Leu(1) NH in the cis configuration about the Boc-Leu bond. The symbol m′ indicates the cis configuration about the Boc-Leu bond.

The activation energy of the interconversion between the trans and cis configurations about a peptide bond can be estimated, as suggested by Shanan-Aditi and Bar-Eli,[21] by using the coalescence temperature and the chemical shift differences of the major and minor peaks. The activation energy of the trans–cis interconversion about the Boc-Leu bond of **1** was determined to be 17.5 kcal/mol. The result is fairly in agreement with that of the barriers

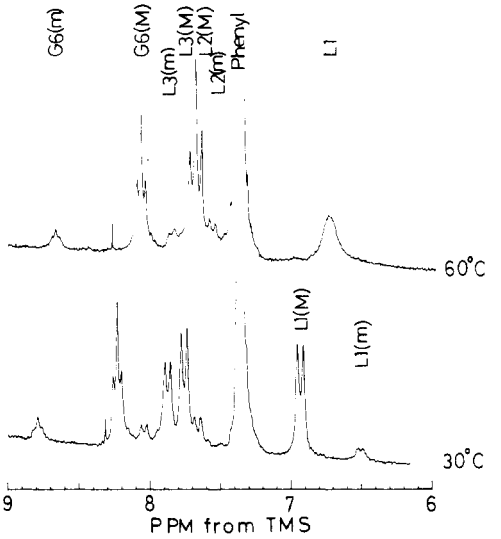

Figure 4. Amide region of ^1H NMR spectra of Boc-Leu_3-Pro_2-Gly-OBzl (**1**) at 30 and 60 °C.

to the cis–trans interconversion about Boc-Pro bonds in several peptides (15.7–19.4 kcal/mol) reported previously.[19]

Next, we discuss the temperature dependence of the cis–trans equilibrium about the Pro-Pro bond. The minor peak, Gly(6) NH (m) of **1**, disappeared at about 110 °C, where the symbol m indicates that the peak is due to the cis configuration about the Pro-Pro bond. Thus, the coalescence temperatures determined from the ^1H and ^{13}C NMR spectra coincide with each other. The activation energy of the trans–cis interconversion about the Pro-Pro bond of **1** was estimated to be 19.3 kcal/mol. The value is also comparable with that obtained for the interconversion about a X-Pro bond (18–22 kcal/mol).[19]

The cis contents obtained from the peak areas of the minor (m) and major (M) peaks are summarized in Table II. As can be seen from the table, they are in good agreement with those obtained from ^{13}C NMR measurements. The minor peaks for the Leu(2) and Leu(3) NH protons of **1** exhibit behaviors similar to the minor peak for the Gly(6) NH proton when the temperature increases. From these observations, it may be concluded that the minor peaks are also due to the cis configuration about the Pro-Pro bond.

The major (M) and minor (m) peaks of Leu(2), Leu(3), and Gly(6) NH and the methylene proton of the benzyl ester of **1** result from the neighboring effect of the trans and cis configurations about the Pro-Pro bond on the wide range of the peptide. As shown in Table II, the minor peaks of NH protons of Leu and Gly, and the methylene protons in the benzyl esters of **3** and **4**, were also detected.

The temperature dependencies of NH chemical shifts of **1–6** are shown in Table II. It has been confirmed that in strongly polar solvents such as Me_2SO the temperature dependence of chemical shifts for NH protons is small when NH protons are shielded from the solvent (2×10^{-3} ppm/°C), while it becomes large when NH protons are exposed to the solvent (6×10^{-3} ppm/°C).[13,14,22,23] The temperature coefficients of most NH chemical shifts of **1–6** are larger than 4×10^{-3} ppm/°C, indicating that the NH protons are almost fully solvated. The chemical shifts of the Leu(2) NH (M and m) of **1** exhibit a small temperature dependence compared to those of Leu(1) and Leu(3) NH of **1**. The small temperature dependence of the Leu(2) NH (M and m) would be explained if it were assumed that the Leu(2) NH is shielded from the solvent due to the large side chains of the neighboring residues or intramolecular hydrogen bonding. The Leu(2) NH chemical shifts of **5**

Table III
Temperature Dependencies of NH Chemical Shifts and Coupling Constants $J_{NH-C^{\alpha}H}$ for Boc-Leu$_3$-Pro$_2$-Gly-OBzl in $CDCl_3$ at Several Concentrations

	residue	concn 0.016 M	0.04 M	0.09 M	0.17 M	0.33 M
$10^3(d\delta_{NH}/dT)$, ppm/°C	Leu(2)	2.5		3.5		7.4
	Leu(3) M					5.7
	Leu(3) m	3.3		5.5		
	Gly(6) M					3.8
	Gly(6) m	2.0		2.6		2.9
$J_{NH-C^{\alpha}H}$, Hz	Leu(2)	7.7	7.7	6.9		
	Leu(3) M				7.6	8.2
	Leu(3) m	8.3	7.7	8.5	6.3	8.5
	Gly(6) M					5.4
	Gly(6) m				5.0	5.8

and 6 also exhibit small temperature dependence, indicating that the small temperature dependence of the Leu(2) NH chemical shift of 1 may be due to shielding of the Leu(2) NH proton from the solvent by the existence of large side chains of the neighboring residues.

The Gly NH (m) chemical shifts of 1, 3, and 4 exhibit smaller temperature dependence than the corresponding values of Gly NH (M) by 1.0×10^{-3} ppm/°C. This indicates that the shielding from the solvent of Gly NH (m) is greater than that of Gly NH (M). The shielding including the case of 2 would be due to a small contribution of turn structures with hydrogen bond between the Gly NH and carbonyl of Leu residues or Boc group preceding the Pro residues on the conformation of 1–4 (type VI β-turn).

The coupling constants $J_{NH-C^{\alpha}H}$ for the peptides 1–6 are listed in Table II. The torsional angle ϕ around the N–C$^{\alpha}$ bond of Leu(3) in the Leu$_3$ sequence of 1, 5, and 6 differs slightly from those of the other two Leu residues.[24] This may be due to a steric effect caused by the presence of large side chains.

The NH chemical shifts of 1 did not exhibit concentration dependence in Me_2SO-d_6 over the concentration range from 0.3 to 0.002 M, indicating that the NH protons of 1 are free from intermolecular hydrogen bonding.[14] This is also supported by the fact that the temperature coefficient of NH chemical shifts was independent of the concentration in Me_2SO-d_6 over the range from 0.3 to 0.016 M. The coupling constants $J_{NH-C^{\alpha}H}$ of 1 were also independent of the concentration, confirming that the conformation of 1 is concentration independent.

^{13}C and ^{1}H NMR Spectra in $CDCl_3$ Solution. The ^{13}C NMR spectrum of 1 in $CDCl_3$ in the spectral region of 45–65 ppm is shown in Figure 5. The ^{1}H NMR spectra in the spectral regions of 6–9 ppm are also shown in Figure 6 at several concentrations. Doublet peaks were observed for the Gly(6) NH, Leu(3) NH, and, although the spectrum of the methylene proton is not shown, the methylene proton of the benzyl ester, which corresponds to the trans and cis configurations about the Pro-Pro bond as mentioned above. The relative intensities of the minor and major peaks of the NH protons are in good agreement with those in the ^{13}C NMR spectrum. Although the data are not shown, the C$^{\alpha}$H peaks centered at 4.72 and 4.47 ppm in $CDCl_3$ were assigned as the Leu(3) preceding the Pro residue and Leu(2), respectively, from solvent titration using the Me_2SO-d_6 and $CDCl_3$ system. The NH peaks for Leu(2) and Leu(3) were assigned from the decoupling experiment.

Conformation of 1 in $CDCl_3$ Solution. The content of the cis isomer about the Pro-Pro bond of 1 was determined as 43% from the relative intensities of the minor and major peaks for α carbons of Pro(4) and Pro(5). The content of the cis isomer of 7 was 38% in $CDCl_3$. The cis content of 1 exhibited no concentration dependence in the range from 0.3 to 0.03 M. The conformations of tripeptides Boc-Pro-Pro-Y-OR (Y = Gly, Ala, Leu, (3,5-Br)-Tyr; R = H or Me) have been studied by ^{13}C NMR and reported to contain 13–40% of the cis isomer about the Pro-Pro bond in $CDCl_3$ as estimated from the intensities of the peaks for β or γ carbons of Pro.[11] Our sample 1 involves relatively high content of the cis isomer in $CDCl_3$.

The concentration dependencies of NH chemical shifts of 1 in $CDCl_3$ are shown in Figure 7. The NH chemical shifts of Leu(1), Leu(2), and Leu(3) exhibit downfield shift with increasing concentration but the chemical shifts of the Gly(6) NH (m) are almost independent of the concentration. These results indicate that the NH protons of Leu(1), Leu(2), and Leu(3) are intermolecularly hydrogen bonded[14] at the site of the Leu$_3$ sequence in $CDCl_3$.

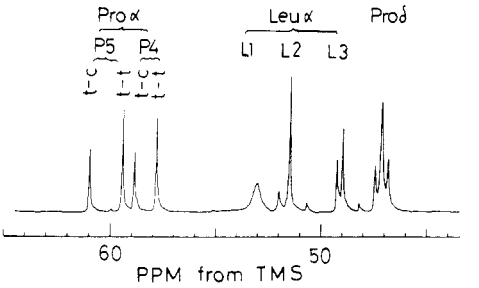

Figure 5. Partial ^{13}C NMR spectrum of Boc-Leu$_3$-Pro$_2$-Gly-OBzl (1) in $CDCl_3$ at 50 MHz.

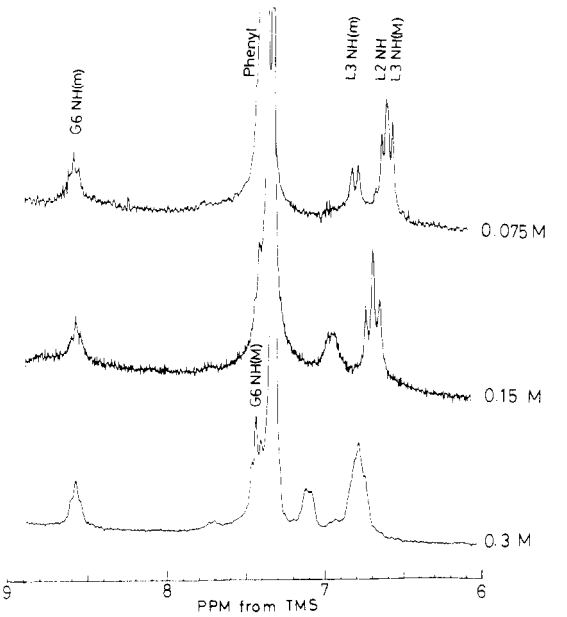

Figure 6. Partial ^{1}H NMR spectra for Boc-Leu$_3$-Pro$_2$-Gly-OBzl in $CDCl_3$ in several concentrations at 200 MHz.

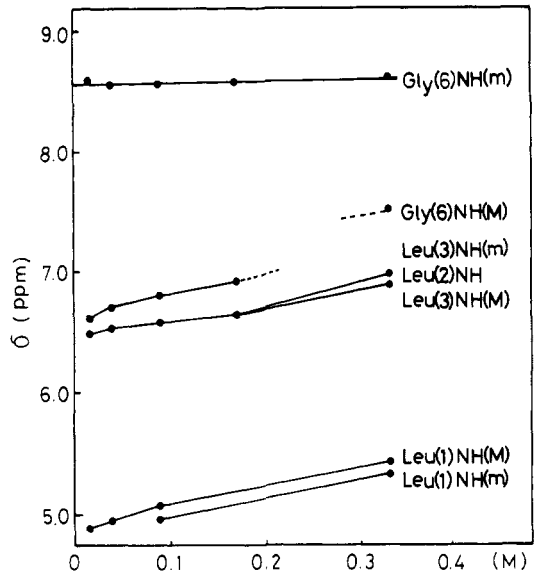

Figure 7. Concentration dependence of the NH chemical shifts of Boc-Leu$_3$-Pro$_2$-Gly-OBzl (1) in $CDCl_3$.

No concentration dependence of the Gly(6) NH (m) indicates that the Gly(6) NH in the cis configuration about the Pro-Pro bond is intramolecularly hydrogen bonded. On the other hand, it may be inferred that the Gly(6) NH in the trans configuration is slightly subject to the intermolecular hydrogen bonding.

The temperature dependencies of the NH chemical shifts of 1 in $CDCl_3$ are shown in Table III at several concentrations. It has been reported that in $CDCl_3$ the temperature coefficient of chemical shifts for NH protons exposed to solvent is small (2.4×10^{-3} ppm/°C) and that for NH protons which are shielded (hydrogen bonded or buried) and remain shielded over the course of temperature variation is also small. For NH protons initially shielded but transferred to an exposed environment in the course of temperature variation, the temperature coefficient has been reported to be significantly larger than 2.4×10^{-3} ppm/°C.[25] Contrary to the observation for Leu(2) and Leu(3) NH, the temperature coefficient as shown in Table III of the NH chemical shifts of the Gly(6) NH (m) is small and independent of the concentration. These results indicate that the Gly(6) NH in the cis configuration is shielded from the solvent. The shielding from the solvent may be attributable to a β-turn structure (type VI) caused by hydrogen bonding of the Gly(6) NH in the cis configuration with the carbonyl group of Leu(3). The possibility of existence of a type VI β-turn structure has also been reported for oligopeptides containing Pro-Pro sequences.[11,12] The small temperature dependence of Gly NH (M) in $CDCl_3$ is also indicative of a large contribution of turn structures in the trans configuration.

The coupling constants, $J_{NH-C^{\alpha}H}$ are also listed in Table III. Each value of $J_{NH-C^{\alpha}H}$ for Leu(2) and Leu(3) is slightly different from the corresponding value in Me_2SO-d_6. The result means that the conformations of the Leu residues in $CDCl_3$ are different from those in Me_2SO-d_6.[24]

From the above, it is concluded that, in Me_2SO, peptide 1 and the related oligopeptides exist as almost fully solvated structures, whereas, in chloroform, aggregation of peptide 1 by intermolecular hydrogen bonding at the site of Leu^3 occurs in spite of the peptide sequence containing the Pro-Pro segment. In nonpolar or slightly polar solvents, intramolecular hydrogen bondings tend to occur in the Leu-Pro$_2$-Gly site. Thus, the polarity of solvents plays a large role in the conformation of peptides.

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