Sudhanand Prasad
Department of Chemistry
Banaras Hindu University
Varanasi 221 005, India
Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012, India

R. Balaji Rao
Department of Chemistry
Banaras Hindu University
Varanasi 221 005, India

P. Balaram
Molecular Biophysics Unit
Indian Institute of Science
Bangalore 560 012, India

Contrasting Solution Conformations of Peptides Containing α,α-Dialkylated Residues with Linear and Cyclic Side Chains

The conformational properties of α,α-dialkylated amino acid residues possessing acyclic (diethylglycine, Deg; di-n-propylglycine, Dpg; di-n-butylglycine, Dbg) and cyclic (1-amino-cycloalkane-1-carboxylic acid, Ac_nc) side chains have been compared in solution. The five peptides studied by nmr and CD spectroscopy are Boc-Ala-Xxx-Ala-OMe, where Xxx = Deg (I), Dpg (II), Dbg (III), Ac_6c (IV), and Ac_7c (V). Delineation of solvent-shielded NH groups have been achieved by solvent and temperature dependence of NH chemical shifts in $CDCl_3$ and $(CD_3)_2SO$ and by paramagnetic radical induced line broadening in peptide III. In the Dxg peptides the order of solvent exposure of NH groups is $Ala(1) > Ala(3) > Dxg(2)$, whereas in the Ac_nc peptides the order of solvent exposure of NH groups is $Ala(1) > Ac_nc(2) > Ala(3)$. The nmr results suggest that Ac_nc peptides adopt folded β-turn conformations with $Ala(1)$ and $Ac_nc(2)$ occupying $i + 1$ and $i + 2$ positions. In contrast, the Dxg peptides favor extended C_5 conformations. The conformational differences in the two series are clearly borne out in CD studies. The solution conformations of peptides I–III are distinctly different from the β-turn structure observed in crystals. Low temperature nmr spectra recorded immediately after dissolution of crystals of peptide II provide evidence for a structural transition. Introduction of an additional hydrogen-bonding function in Boc-Ala-Dpg-Ala-NHMe (VI) results in a stabilization of a consecutive β-turn or incipient 3_{10}-helix in solution. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

α,α-Dialkylated amino acid residues have acquired considerable importance as a means of introducing backbone conformation constraints in synthetic peptides.[1,2] The prototype residue α-aminoisobutyric acid (Aib) is a strong helix-promoting residue,[3,4] a property predicted by early conformational energy calculations.[5] Recent work on peptides containing 1-aminocycloalkane-1-carboxylic

CH₃   CH₃

$(CH_2)_n$ $(CH_2)_n$ H_2C————$(CH_2)_n$

C   C

H_2N COOH H_2N COOH

n = 1 , Deg   n = 4 , Ac₆c

n = 2 , Dpg   n = 5 , Ac₇c
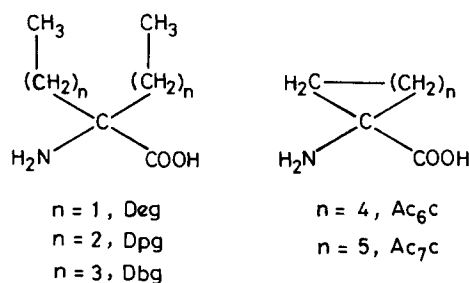
n = 3 , Dbg

FIGURE 1 Structures of α,α-dialkylated amino acids with linear and cycloalkyl sidechains.

acid (Ac$_n$c, where n is the number of carbon atoms in the cycloalkane ring; Figure 1) establishes that these residues also favor helical conformations,[6-10] with $\phi \sim \pm 60°$, $\psi \sim \pm 30°$. In contrast, α,α-di-n-propylglycine (Dpg) (Figure 1) appears to be capable of stabilizing fully extended C_5 conformations ($\phi \sim \psi \sim 180°$) in short homooligopeptides.[11-13] However, di-n-propylglycyl and di-n-butylglycyl (Dbg) residues have also been shown to be incorporated into helical/β-turn conformations in the crystal structures of peptides ranging in length from three to ten residues.[14,15] The crystallographic studies described in the preceding report establish that Dpg/Dbg residues in protected peptides of the type Boc-Ala-Xxx-Ala-OMe, where Xxx = Dpg or Dbg adopt conformations in the $3_{10}/\alpha$-helical of ϕ,ψ space.[16]

We describe in this paper conformational analysis of the peptides Boc-Ala-Xxx-Ala-OMe, where Xxx = Deg (I), Dpg (II), and Dbg (III), and compare their behavior with that observed for the corresponding cycloalkane side chain containing residues Ac₆c (IV) and Ac₇c (V). The effect of providing an additional intramolecular hydrogen bond donor on peptide conformation has been examined by studying the peptide Boc-Ala-Dpg-Ala-NHMe (VI). The results establish that Ac$_n$c residues stabilize folded β-turn conformations, whereas residues with linear alkyl side chains (Dxg) appear to favor fully extended conformations in solution. There is a clear and unambiguous difference between conformations observed for Dxg containing tripeptides in solution and the crystalline state conformations described in the preceding paper.

EXPERIMENTAL PROCEDURES

Synthesis of Amino Acids

α,α-Dialkylated amino acids were synthesized from the appropriate ketones. The ketones were first converted

into their corresponding hydantoins, which on hydrolysis with 60% H_2SO_4 gave the respective amino acids (Table I).

General Method for the Synthesis of 5,5'-Disubstituted Hydantoins[17]

In a 1-L round-bottomed flask, 0.1 mol of ketone was dissolved in 300 mL of 50% aqueous methanol, to which 0.3 mol of KCN and 0.5 mol of (NH_4)₂CO_3 were added. The mixture was refluxed for 4-6 h on a water bath. The solution was then concentrated to approximately half its volume and chilled in an ice bath. Hydantoins generally precipitated. In case no precipitate appeared, the solution was acidified with 2N HCl. The precipitate thus obtained was filtered and washed several times with ice cold water to remove traces of cyanide (the washings were tested with FeSO₄ solution until a negative Prussian blue test was obtained). The solid was further dried and recrystallized from aqueous alcoholic solution. Yields, melting points, and characteristic ir bands are summarized in Table I.

Hydrolysis of Hydantoins

In a 250 mL round-bottomed flask, 0.05 mol of 5,5'-disubstituted hydantoin was dissolved in 60% H_2SO_4 ($\sim$ 45 mL) and refluxed at 150-160°C for about 24-50 h on an oil bath. (Table I). The reaction mixture was cooled to room temperature and diluted with water (150 mL). The diluted solution was filtered to remove charred particles. The clear solution was chilled in ice cold water, neutralized with ammonia solution, until alkaline. In most cases a precipitate was obtained directly, whereas in some cases the precipitate did not appear immediately. In such cases the solution was concentrated to about half its volume. On cooling, crystals were obtained. The second and third crop could be obtained by further concentrating the mother liquor. The precipitate thus obtained was washed several times with ice cold water and recrystallized several times from water or aqueous alcoholic solution. Amino acids were characterized by their ir spectra, positive ninhydrin reaction and characterization of amino and carboxy terminal protected derivatives.[18]

Synthesis of Peptides

The peptides Boc-Ala-Xxx-Ala-OMe were synthesized by conventional solution phase procedures. The t-butyloxycarbonyl and methyl ester group were used for amino and carboxyl protections and dicyclohexylcarbodiimide (DCC) or DCC 1-hydroxybenzotriazole (HOBT) as coupling agents. Methyl ester hydrochlorides of Ala, Ac₆c, and Ac₇c were prepared by the thionyl chloride-methanol procedure.[19] The esterification of Dxg amino

Table I **Synthetic Yields of Hydantoins[a] and Amino Acids[b]**

Ketones	Hydantoins (Yield %)	mp (°C)[c]	Hydrolysis Reflux Times (h)	Amino Acids	Crude (Yield %)
3-Pentanone	5,5'-Diethyl hydantoin (85)	165–166	36–38	Diethylglycine (Deg)	(70)
4-Heptanone	5,5'-Di-*n*-propyl hydantoin (84)	198–200	46–48	Di-*n*-propylglycine (Dpg)	(73)
5-Nonanone	5,5'-Di-*n*-butyl hydantoin (86)	160–161	48–50	Di-*n*-butylglycine (Dbg)	(80)
Cyclohexanone	5,5'-Spirocyclohexane hydantoin (84)	210	24–26	1-Aminocyclohexane-1-carboxylic acid (Ac$_6$c)	(84)
Cycloheptanone	5,5'-Spirocycloheptane hydantoin (86)	15	26–28	1-Aminocycloheptane-1-carboxylic acid (Ac$_7$c)	(86)

[a] Characteristic carbonyl stretching bands (cm^{-1}) in the ir spectra of the hydantoins are 1710–1740 and 1760–1780.

[b] Characteristic ir bands (cm^{-1}) of amino acids are 1610–1640 (-COO$^-$ groups) and 30603090 (-NH$_3^+$) were observed.

[c] Melting points are uncorrected.

acids was effected by passing dry HCl gas (until saturation) into solutions of amino acids in dry methanol, followed by storage at ~ 10°C for 3 days and then refluxing for 6 h.[18] All the intermediates obtained were checked for purity by thin layer chromatography (tlc) on silica gel and characterized by ¹H-nmr (80 MHz). All the final peptides were purified by high performance liquid chromatography on a Lichrrosorb RP C-18 column using MeOH/H₂O gradients.[18]

Boc-Ala-Deg-Ala-OMe **(I)**. *Boc-Ala-Deg-OMe (1)*. Boc-Ala-OH (0.95 g, 5 mmol) was dissolved in dimethylformamide (DMF; 3 mL). 0.73 g (5 mmol) of Deg-OMe obtained from its hydrochloride was added followed by DCC (1.0 g, 5 mmol) and HOBT (0.67 g). The reaction mixture was stirred at room temperature for 3 days. The precipitated dicyclohexylurea (DCU) was filtered and diluted with ethyl acetate (80 mL). The organic layer was washed with excess of water, 1N HCl (3 × 30 mL), 1*M* Na₂CO₃ solution (3 × 30 mL) and again with water. The solvent was then dried over anhydrous Na₂SO₄ and evaporated in vacuo, giving a light yellow gum. Yield: 0.735 g (46%).

¹H-nmr (CDCl₃, δ) 0.77, 6H, t (Deg C$^\gamma$H₃s); 1.27, 1.36, 1.77, 2.09, 2.40, 7H (Deg C$^\beta$H₂s, AlaC$^\beta$H₃); 1.45, 9H, s (Boc CH₃s); 3.76, 3H, s (— COOCH₃); 4.18, 1H, m (AlaC$^\alpha$H); 5.72, 1H, d (Ala NH) 7.14, 1H, s (Deg NH).

Boc-Ala-Deg-OH (2). 0.73 g (2.3 mmol) of **1** was dissolved in methanol (10 mL) and 4N NaOH (3 mL) was added. The reaction mixture was stirred at room temperature for 2 days. The progress of the reaction was monitored by tlc. After completion of the reaction, methanol was evaporated. The residue obtained was diluted with

water and washed with diethyl ether. The aqueous layer was cooled in ice and neutralized by 2N HCl and extracted with ethyl acetate. The solvent was evaporated in vacuo to give a white solid. Yield: 0.45 g (50%); mp = 146–148°C.

Boc-Ala-Deg-Ala-OMe (I). 0.31 g (1 mmol) of **2** was dissolved in DMF (4 mL). Ala-OMe, obtained from its hydrochloride (0.21 g, 2 mmol), was added followed by DCC (0.2 g, 1 mmol) and HOBT (0.14 g). The reaction mixture was stirred at room temperature for 5 days. The work up of the reaction was done as in case of **1**. Yield: 0.3 g (77%), mp = 136–138°C.

¹H-nmr (CDCl₃, δ) 0.81, 6H, t (Deg C$^\gamma$H₃s); 1.40, 1.45, 1.90, 2.54, 10H, m (Deg C$^\beta$H₂s, Ala C$^\beta$H₂s); 1.5, 9H, s (Boc CH₃s); 3.84, 3H, s(— COOCH₃) 4.16, 4.18, 2H, m(Ala C$^\alpha$Hs); 5.11, 1H, d [Ala(1)NH]; 6.59, 1H, d [Ala(3)NH]; 7.18, 1H, s (Deg NH).

Boc-Ala-Dpg-Ala-OMe **(II)**. *Boc-Ala-Dpg-OMe (3)*. 0.57 g (3 mmol) of Boc-Ala-OH was coupled to Dpg-OMe (0.4 g, 3 mmol) using 0.6 g (3 mmol) of DCC and HOBT (0.41 g) as described in case of **1**. The peptide was obtained as a gum. Yield: 0.4 g (41%).

¹H-nmr (CDCl₃, δ): 0.8, 1.16, 1.20, 1.34, 2.01, 2.3, 2.4, 17H (Dpg —CH₂, —CH₃ protons, Ala C$^\beta$H₃s); 1.38, 9H, s (BocCH₃s); 3.67, 3H, s (— COOCH₃); 4.0, 1H, m (Ala C$^\alpha$H); 5.28, 1H, d (Ala NH); 6.95, 1H, s (Dpg NH).

Boc-Ala-Dpg-OH (4). 0.4 g of **4** was saponified in MeOH (10 mL) using 4N NaOH as described for **2**. Peptide **4** was obtained as a white solid. Yield: 0.35 g (91%) mp = 162–164°C.

Boc-Ala-Dpg-Ala-OMe (II). 0.3 g of (1.06 mmol) of **4** was coupled to Ala-OMe (2 mmol) as in the case of I.

^{1}H-nmr (CDCl$_3$, δ) 0.8, 1.16, 1.20, 1.34, 1.42, 1.61, 2.01, 2.3, 2.4, 20H (Dpg CH$_2$, CH$_3$ protons, Ala C$^\beta$H$_3$); 1.47, 9H, s (Boc CH$_3$s); 3.73, 3H, s (— COOCH$_3$); 4.05, 4.50, 2H, m (AlaC$^\alpha$Hs); 4.95, 1H, d [Ala(1)NH]; 6.41, 1H, d [Ala(3)NH]; 7.05, 1H, s (Dpg NH).

Boc-Ala-Dpg-Ala-NHMe (VI). 0.3 g of peptide II was taken in dry methanol and methylamine gas was passed into the solution until saturation. It was then kept in at −20°C for three days after which the solvent was evaporated to give a white solid. Yield = 0.14 g (46%). mp = 157–158°C.

^{1}H-nmr (CDCl$_3$, δ) 0.9, 1.32, 1.37, 1.6, 2.0, 2.1, 20H (Dpg CH$_2$, CH$_3$ protons, Ala C$^\beta$H$_3$); 1.45, 9H, s (Boc CH$_3$s); 2.75, 3H d (NH-CH$_3$ protons) 4.0, 4.45, 2H m (Ala C$^\alpha$Hs) 4.95, 1H, d [Ala(3) NH]; 6.45, 1H, s (Dpg NH) 7.05, 1H, d [Ala(3) NH] 7.0, 1H, m (NH-CH$_3$).

Boc-Ala-Dbg-Ala-OMe (III). *Boc-Ala-Dbg-OMe (5)*. 0.8 g (4.2 mmol) of Boc-Ala-OH was coupled to Dbg-OMe (0.85 g, 4.2 mmol) using DCC (0.84 g) and HOBT (0.67 g) as described in the case of **2**. The peptide **5** was obtained as a gum. Yield: 0.4 g (25%).

^{1}H-nmr (CDCl$_3$, δ) 0.8, 0.93, 1.1, 1.26, 2.05, 2.3, 2.4, 18H, m (Dbg CH$_2$, CH$_3$ protons); 1.36, 3H, d (Ala C$^\beta$H$_3$s); 1.42, 9H, s (Boc CH$_3$s); 3.7, 3H, s (— COOCH$_3$); 4.06, 1H, m (AlaC$^\alpha$H); 5.06, 1H, d (Ala NH) 6.9, 1H, s (Dbg NH).

Boc-Ala-Dbg-OH (6). 0.4 g of **5** was saponified in methanol (15 mL) using 4N NaOH as described in case of **2**. The peptide **6** was obtained as a solid. Yield: 0.3 g (83%). mp = 118–120°C.

Boc-Ala-Dbg-Ala-OMe (III). 0.3 g (0.03 mmol) of **6** was dissolved in DMF (5 mL) and coupled to Ala-OMe obtained from its hydrochloride (0.28 g, 2 mmol) using DCC (0.2 g) and HOBT (0.14 g) as described in the case of I. The peptide was obtained as a white solid. Yield: 0.25 g (5%) mp = 128–130°C.

^{1}H-nmr (CDCl$_3$, δ) 0.85, 0.9, 1.15, 1.30, 1.35, 1.4, 2.1,

2.25, 2.4 24H (Dbg CH$_2$, CH$_3$ protons, Ala CH$_3$s); 1.5, 9H, s (Boc-CH$_3$s); 3.73, 3H, (COOCH$_3$); 4.05 δ, 1H, m [Ala(1)C$^\alpha$H]; 4.52, 1H, m [Ala(3)C$^\alpha$H]; 4.95, 1H, d [Ala(1)NH]; 6.4, 1H, d [Ala(3)NH]; 7.02, 1H, s (Dbg NH).

Boc-Ala-Ac$_6$c-Ala-OMe (IV). *Boc-Ala-Ac$_6$c-OMe (7)*. 0.95 g (5 mmol) of Boc-Ala-OH was dissolved in CH$_2$Cl$_2$ (20 mL and cooled in an ice bath: Ac$_6$c-OMe obtained from 1.38 g (7 mmol) of its hydrochloride was added followed by DCC (1.0 g, 5 mmol). The reaction mixture was stirred overnight at room temperature, and precipitated DCU was filtered. The CH$_2$Cl$_2$ was evaporated and the solid was dissolved in ethyl acetate. The organic solution was washed with excess of water, 1 M Na$_2$CO$_3$ (3 × 40 mL) 1N HCl (3 × 40 mL) and water. The organic layer was dried over anhydrous Na$_2$SO$_4$ and evaporated in vacuo. Peptide **7** was obtained as a gum. Yield: 1.4 g (85%).

^{1}H-nmr (CDCl$_3$, δ) 1.38, 3H, d (Ala C$^\beta$H$_3$s); 1.5, 9H, s (Boc CH$_3$s); 1.68, 2.05, 10H, m (Ac$_6$c ring —CH$_2$— protons); 3.71, 3H, s (— COOCH$_3$) 4.18 δ, 1H, m (Ala C$^\alpha$H); 5.23, d (Ala NH); 6.73, 1H, s (Ac$_6$c NH).

Boc-Ala-Ac$_6$c-OH (8). 1.4 g (4.26 mmol) of **7** was saponified in MeOH (20 mL) using 4N NaOH described in case of **2**. Peptide **8** was obtained as a gum. Yield: 1.2 g (90%).

Boc-Ala-Ac$_6$c-Ala-OMe (IV). 1.2 g (3.8 mmol) of **8** was dissolved in DMF (6 mL) and coupled to Ala-OMe (1.12 g, 8 mmol) using DCC (0.8 g) and HOBT (0.6 g) as described in the case of I. Peptide IV was obtained as a white solid. Yield: 0.85 g (56%) mp = 176–178°C.

^{1}H-nmr (CDCl$_3$, δ) 1.35, 1.37, 6H (Ala C$^\beta$H$_3$); 1.45, 9H, s (Boc CH$_3$s); 1.49, 1.68, 2.09, 10H (Ac$_6$c ring —CH$_2$— protons); 3.66, 3H, S(— COOCH$_3$); 4.05, 4.45, 2H, m (Ala C$^\alpha$Hs); 4.96, 1H, d [Ala(1)NH]; 6.40, 1H, s (Ac$_6$c NH), 7.25, 1H d [Ala(3)NH].

Boc-Ala-Ac$_7$c-Ala-OMe (V). *Boc-Ala-Ac$_7$c-OMe (9)*. 0.95 g (5 mmol) of Boc-Ala-OH was coupled to Ac$_7$c-OMe, obtained from 1.25 g (6 mmol) of its hydrochloride in CH$_2$CL$_2$ (10 mL) using DCC (1.0 g, 5 mmol) as in the case of **8**. Peptide **9** was obtained as a gum. Yield 1.31 g (76%).

^{1}H-nmr (CDCl$_3$, δ) 1.3, 3H, d (Ala C$^\beta$H$_3$); 1.51, 9H, s (Boc CH$_3$s); 1.67, 2.08, 12H (Ac$_7$c ring —CH$_2$— protons); 3.78, 3H, s (— COOCH$_3$); 4.16, 1H, m (Ala C$^\alpha$H); 5.07, 1H, d (Ala NH) 6.5, 1H, s (Ac$_7$c NH).

Boc-Ala-Ac$_7$c-OH (10). 1.31 g (3.8 mmol) of **9** was saponified using MeOH (10 mL) and 4N NaOH (5 mL) as described for **2**. Peptide **10** was obtained as a gum. Yield: 1.0 g (80%).

Boc-Ala-Ac$_7$c-Ala-OMe (V). The amount of 0.7 g (2.13 mmol) of **10** was coupled to Ala-OMe obtained from its hydrochloride (0.56 g, 4 mmol) in DMF using DCC (0.5 g) and HOBT (0.33 g) as described for I. Peptide V was obtained as a white solid. Yield: 0.62 g (70%); mp = 173–175°C.

^{1}H-nmr (CDCl$_3$, δ) 1.35, 1.39, d (Ala C$^\beta$H$_3$s); 1.45, 9H, s (Boc CH$_3$s); 1.49, 1.56, 2.09, 12H (Ac$_7$c ring —CH$_2$— protons); 3.71, 3H, s (—COOCH$_3$); 4.06, 4.50, 2H, m (Ala C$^\alpha$H$_3$); 5.01, 1H, d [Ala(1)NH]; 6.47, 1H, s (Ac$_7$c NH); 7.17, 1H, d [Ala(3)NH].

NMR Measurements

The NMR studies were carried out on a Varian FT-80 nmr spectrometer. Difference one-dimensional (1D) nuclear Overhauser effect (NOE) spectra were recorded on a Bruker WH 270 FT-nmr spectrometer at the Sophisticated Instruments Facility, I.I.Sc. as described earlier.[20]

CD Measurements

All CD spectra were recorded on a JASCO J-500 spectropolarimeter. A cell of path length 1 mm was used. CD band intensities are expressed as molar ellipticities.

RESULTS AND DISCUSSION

NMR Studies

Peptide conformations were probed in solution using the solvent dependence of NH chemical shifts in CDCl$_3$/(CD$_3$)$_2$SO mixtures, free radical induced line broadening of NH resonances in CDCl$_3$, and temperature coefficients of NH resonances in (CD$_3$)$_2$SO.[21,22] In addition, selective 1D difference NOE experiments were also employed.[20] Assignment of NH resonances in all the cases was straightforward since the two Ala residues appear as doublets that are easily distinguished by the high field appearance of the Ala(1) NH group, which is part of urethane function. The Ac$_n$c and Dxg reso-

nances appear as singlets in all cases. Table II summarises the nmr parameters for NH resonances in peptides I–V. Figure 2 shows representative results of solvent perturbation and free radical induced line broadening of NH resonances in peptide Boc-Ala-Dbg-AlaOMe (III). It can be clearly seen that the order of solvent exposure of NH groups is Ala(1)NH > Ala(3) NH > Dbg NH. A similar order is also obtained by examining temperature coefficients ($d\delta/dT$) in (CD$_3$)$_2$SO (Table II). While the Dbg NH has a very low value of 2×10^{-3} ppm/K, the Ala(1) NH and Ala(3) NH groups have values of 8×10^{-3} ppm/K and 5×10^{-3} ppm/K, respectively. An examination of Table II reveals that in the Boc-Ala-Dxg-Ala-OMe peptides in all three cases the order of NH group solvent exposure is Ala(1) > Ala(3) > Dxg. In sharp contrast, in Boc-Ala-Ac$_n$c-Ala-OMe peptides the order of solvent exposure is Ala(1) > Ac$_n$c (2) > Ala(3). In peptides I–III the Dxg NH resonances are clearly shielded from solvent, with characteristically low values of $\Delta\delta$ (0.45 ppm) and $d\delta/dT$ (1.8–2 ppm/K). In peptides IV and V, the Ala(3) resonance is solvent shielded with $\Delta\delta = 0.12$–0.58 ppm and $d\delta/dT$ values in (CD$_3$)$_2$SO of 1.5–1.9 ppm/K. The solvent-shielded nature of Ala(3) NH in peptides Boc-Ala-Ac$_n$c-Ala-OMe (IV, V) clearly supports a significant population of β-turn conformations involving this group in intramolecular hydrogen bonding with the Boc-CO group (Figure 3a). These observations are consistent with the known tendency of Ac$_n$c residues to favor β-turn conformations in short peptides. Indeed, incorporation of Ac$_n$c residues in different peptide sequences such as Boc-Pro-Ac$_6$c-Ala-OMe,[7] Boc-(Ac$_6$c)$_3$-OMe,[7] and Boc-Pro-Ac$_7$c-Ala-OMe[8] have been shown to induce β-turn conformations.

The solvent-exposed nature of Ala(3)NH in the Dxg residue containing peptides (I–III) clearly rules out the existence of a significant population of β-turn conformations in solution. The solvent-shielded nature of the Dxg NH group may be rationalized by invoking two distinct conformations:

1. C$_7$ (γ) turn conformations[23] centered at Ala(1) involving the Dxg NH in an intramolecular (3 → 1) hydrogen bond with the Boc-CO group;
2. C$_5$ conformations[24] at Dxg in which a fully extended backbone places the Dxg NH group in close proximity to the Dxg CO group (Figure 3b).

Table II NMR Parameters for NH Resonances in Peptides Boc-Ala-Xxx-Ala-OMe[a]

Boc-Ala-Xxx-Ala-OMe	Chemical Shift (δ ppm) CDCl₃			Chemical Shift (δ ppm) (CD₃)₂ SO			Δδ NH (ppm) (CD₃)₂ SO-CDCl₃			$d\delta/dT \times 10^{-3}$ (ppm /K) (CD₃)₂SO			$J_{\text{NHC}^{\alpha}\text{H}}$ (Hz)[b] CDCl₃		$J_{\text{NHC}^{\alpha}\text{H}}$ (Hz)[b] (CD₃)₂SO	
Xxx	Ala (1)	Xxx	Ala (3)	Ala (1)	Xxx	Ala (3)	Ala (1)	Xxx	Ala (3)	Ala (1)	Xxx	Ala (3)	Ala (1)	Ala (3)	Ala (1)	Ala (3)
Deg	5.11	7.18	6.59	7.51	7.62	8.37	2.4	0.44	1.78	7.4	1.8	6.1	6.8	7.8	6.8	7.8
Dpg	4.95	7.05	6.41	7.2	7.5	8.16	2.25	0.45	1.75	6.2	1.8	4.4	6.4	6.8	7.2	6.8
Db	4.95	7.02	6.4	7.25	7.47	8.15	2.3	0.45	1.75	8.0	2.0	5.0	6.8	7.6	7.0	7.4
Ac₆c	4.96	6.40	7.25	6.86	7.5	7.37	1.9	1.10	0.12	5.3	4.1	1.9	6.0	6.0	6.4	7.0
Ac₇c	5.01	6.47	7.17	7.04	7.86	7.75	2.03	1.39	0.58	4.6	4.5	1.5	5.6	6.8	6.0	6.8

[a] Peptide concentration ~ 10 mM.
[b] Error in J values ± 0.5 Hz.

C_5 conformations at Dxg residues would appear to be a much better conformational choice, since ample precedence for such structures exists in crystal structures of short Dxg containing peptides.[12,13] There does not appear to be any compelling reason for Dxg residues to stabilize C_7 conformations at the preceding residue in the sequence. Indeed, C_7 conformations have almost never been observed in crystal structures of short acyclic peptides. It is noteworthy that stabilization of C_7 conformations at a preceding residue has been invoked in a study of acyclic dehydroalanine peptides on the basis of limited spectral evidence.[25]

Figure 4 shows the results of a difference NOE experiment carried out on the peptide Boc-Ala-Dbg-Ala-OMe (III). Irradiation of three NH resonances and Ala(3) C^{α}H reveals only one strong interresidue NOE between Ala(1) C^{α}H and Dbg NH. This is consistent with an extended conformation at Ala(1). The ψ values of +60° to 180° result in interproton distances $d_{\alpha N} < 2.5$ Å.[26]

CD Studies

Figures 5 and 6 illustrates the CD spectra obtained for peptides IV, in trifluoroethanol. Table III sum-
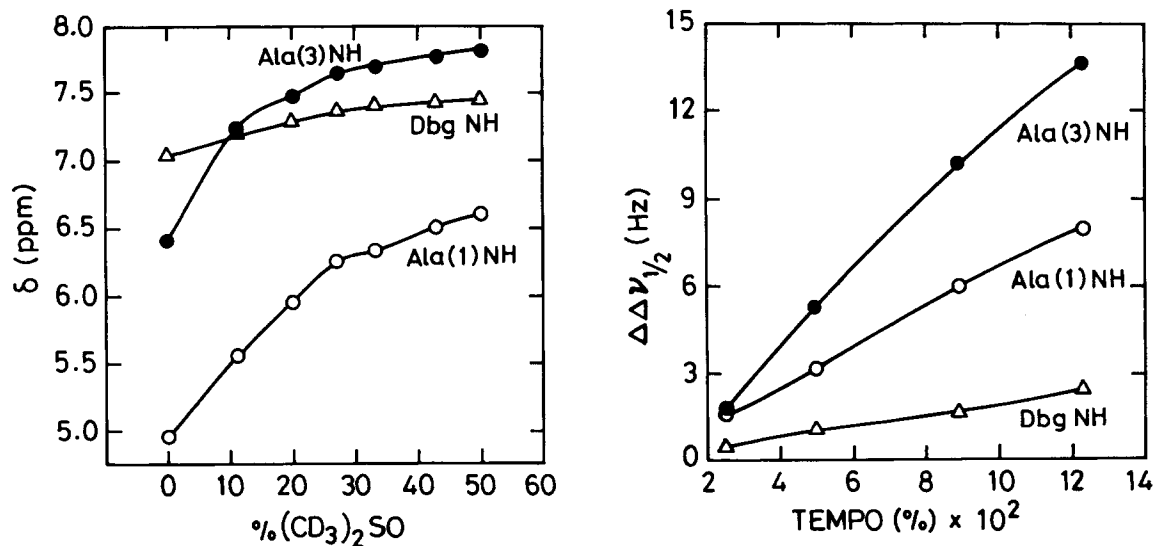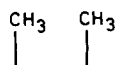

FIGURE 2 Left: Nmr solvent titration curves for NH protons in Boc-Ala-Dbg-Ala-OMe (III) in CDCl₃ — (CD₃)₂SO mixtures. Right: Free radical induced line broadening for NH protons in III as a function of TEMPO concentration in CDCl₃.

(b)

CH₃   CH₃

that the CD spectra of Ac$_n$c peptides are very similar, as are the spectra of the Dxg series. The spectra
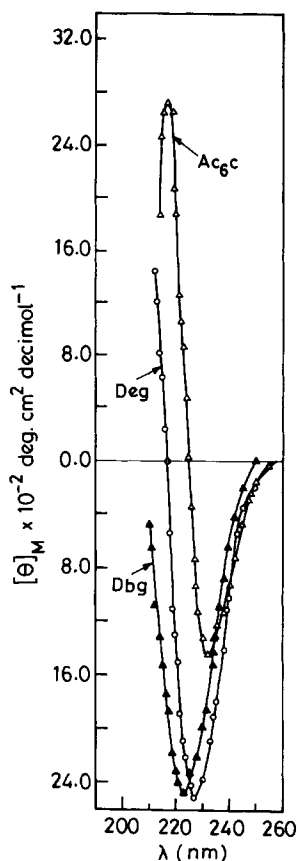
FIGURE 6 CD spectra of Boc-Ala-Ac$_6$c-Ala-OMe (IV) and Boc-Ala-Dxg-Ala-OMe (Deg, I; Dpg, II) in tri-fluoroethanol.

Conformation of Dxg Residues

The spectroscopic studies described above clearly indicate that the Dxg containing tripeptides do not adopt folded β-turn conformations in solution. This is in marked contrast to the results described in the preceding paper, where type II β-turn conformations have been established for Boc-Ala-Dpg-Ala-OMe and Boc-Ala-Dbg-Ala-OMe, with the Dxg residue occupying the *i* + 2 position, having φ,ψ values lying in the α-helical region of φ,ψ space (Dpg, φ = 66.2°, ψ = 19.3°; Dbg, φ = 66.5°, ψ = 21.1°).[16] While dramatic differences between solution and solid state conformations can be expected in principle, experimental evidence in support of such differences is often not available. In the case of Aib and Ac$_n$c residues, very good agreement between solid state and solution conformations have generally been observed.[3,7,8] One noteworthy example of differences in solution and solid state conformations at an Aib residue has been established for a cyclic peptide disulfide.[30]

In order to obtain direct evidence for a conformational transition on dissolution of crystals, an attempt was made to record 400 MHz 1D nmr spectra at low temperature (233 K), immediately after addition of peptide to a precooled $CDCl_3$ solution. Under these conditions it may be possible to observe conformational species that cannot be detected at ambient temperatures, because of relatively low barriers to conformational interconversions. Indeed, conformational transitions in a peptide containing a Pro-Pro sequence have been

Table III   CD Parameters for Peptides Boc-Ala-Xxx-Ala-OMe^a

Peptides	TFE		Methanol		TMP	
	λ (nm)	$[\theta]_M \times 10^2$	λ (nm)	$[\theta]_M \times 10^2$	λ (nm)	$[\theta]_M \times 10^2$
Boc-Ala-Deg-Ala-OMe (I)	227	−25.15	228	−22.08	229	−12.5
	212	+14.31				
Boc-Ala-Dpg-Ala-OMe (II)	225	−20.9	226	−20.6		
	210	+6.83				
Boc-Ala-Dbg-Ala-OMe (III)	224	−24.86	227	−22.87	226	−10.56
Boc-Ala-Ac$_6$c-Ala-OMe (IV)	233	−14.57	234	−10.37		
	217	+27.1	213	+30.5	206	+16.9
Boc-Ala-Ac$_7$c-Ala-OMe (V)	233	−8.24			239	−1.65
	212	+32.75	212	+28.2	209	+15.04

^{b} Peptide concentration 2 m*M*. Band intensities are expressed as $[\theta]_M$ deg cm^2 decimol^{-1}. TFE, 2,2,2-trifluoroethanol; TMP, tri-methylphosphate.

monitored by low temperature dissolution of single crystals.[31] Figure 7 shows the partial nmr spectra of Boc-Ala-Dpg-Ala-OMe recorded immediately after dissolution of a crystalline peptide sample at 233 K. The peaks marked with arrows correspond to resonances that appear in the low temperature experiment, which broaden and disappear on heating. The four observed resonances correspond to Dpg NH, Ala(3) NH, Ala(1) NH, and Ala(1) $C^{\alpha}H$ of a minor conformation, which is in slow exchange on the nmr time scale, with a major conformational species already established as a fully extended form. A definitive conformational assignment of the minor species is not possible on the basis of available data at low temperature. However, the chemical shifts of the NH groups of the central residue in Boc-Ala-Xxx-Ala-OMe peptides (where Xxx = $Ac_{n}c$, Dxg) provide a diagnostic of the conformational state of the Xxx residue.

Figure 8 shows a correlation diagram for the chemical shift of NH resonances of peptide I–V in $CDCl_3$ and $(CD_3)_2SO$. It is clearly seen that when the Xxx residue occurs in an extended conformation, the Xxx NH resonance appears between 7.02 δ and 7.18 δ in $CDCl_3$ and 7.47δ and 7.62δ in $(CD_3)_2SO$ (peptides I–III). In contrast, when the central residue occurs in the $i + 2$ position of a β-turn as in peptides IV and V, the Xxx NH reso-
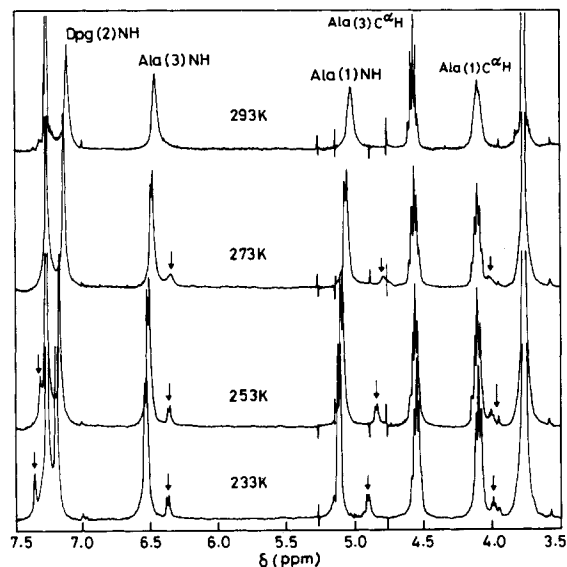

FIGURE 7 Partial 400 MHz ^{1}H-nmr spectra obtained by dissolving a crystalline sample of Boc-Ala-Dpg-Ala-OMe (II) at low temperatures (233 K) in $CDCl_3$. The lowermost trace was recorded immediately after dissolution. Upper spectra were recorded at the temperatures indicated against the traces. Arrows indicate minor resonances detectable at low temperatures.
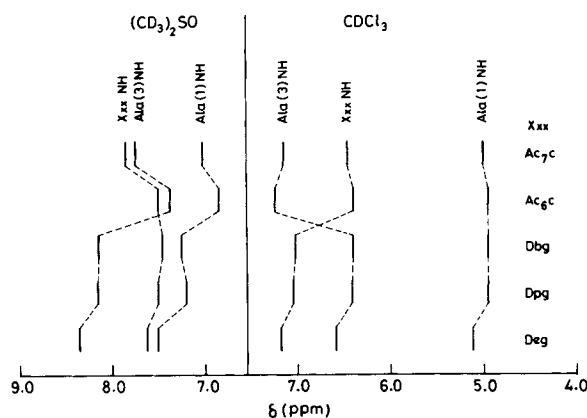

FIGURE 8 Correlation diagram for NH resonances in peptides I–V in $CDCl_3$ and $(CD_3)_2SO$.

nance occurs between 6.40δ and 6.47δ in $CDCl_3$ and 7.5δ in $(CD_3)_2SO$. Inspection of the NH chemical shifts in the minor conformation detected at low temperatures suggest that the observed species may not correspond to the β-turn conformation detected in crystals.[16] The possibility that this minor species corresponds to an intermediate in the conformational transition between the folded form observed in crystals and extended form observed in solution cannot be excluded.

Solution Conformation of Boc-Ala-Dpg-Ala-NHMe (VI)

The temperature coefficient $(d\delta/dT \times 10^{-3})$ values in $(CD_3)_2SO$ for the NH resonances of peptide VI are Ala(1) 5.0, Dpg(2) 4.0, Ala(3) 2.5, and NHMe 2.5. Solvent perturbation experiment using $CDCl_3(CD_3)_2SO$ mixtures yielded the following $\Delta\delta$ values of NH chemical shifts; Ala(1) 2.1, Dpg(2) 1.1, Ala(3) 0.5, and NHMe 0.45. These results clearly suggest that the Ala(3)NH and methylamide NH groups are solvent shielded and presumably intramolecularly hydrogen bonded. As discussed in the preceding paper, peptide VI adopts a consecutive β-turn or incipient 3_{10}-helical structure in the crystalline state.[16] The nmr results in solution are consistent with the retention of this conformation in solution. It is noteworthy that in peptide II, which differs from peptide VI only at the C-terminus with a methyl ester group replacing methyl amide function, the solution and solid state conformations are distinctly different.

These results emphasize the fact that both heli-

cal and fully extended conformations are energetically readily accessible for Dxg residues. Subtle changes in the molecular environments in crystals or solutions can determine the nature of the conformation that is populated. Increasing the intramolecular hydrogen-bonding capacity as in the case of peptide VI appears to tilt the balance toward folded, potentially helical structures. Indeed, several crystal structure determinations of long peptides containing Dxg residues (Ref. 15 and unpublished results) have invariably resulted in the characterization of helical conformations.

The use of Dxg residues in the design of peptides of specific backbone conformations will be facilitated by further investigations, which define the conditions under which helical and extended conformations can be exclusively stabilized. Studies presently underway in our laboratories focus on the conformation of Dxg residues inserted into sequences with very low helix propensities.

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