The conformational properties of $\alpha,\alpha$-dialkylated amino acid residues possessing acyclic (diethylglycine, Deg; di-$n$-propylglycine, Dpg; di-$n$-butylglycine, Dbg) and cyclic (1-$\alpha$-amino-cycloalkane-1-carboxylic acid, Ac$_6$C) 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$_6$C (IV), and Ac$_{10}$C (V). Delineation of solvent-shielded NH groups have been achieved by solvent and temperature dependence of NH chemical shifts in CDCl$_3$ and (CD$_3$)$_2$SO and by paramagnetic radical induced line broadening in peptide III. In the Dpg peptides the order of solvent exposure of NH groups is Ala(1) > Ala(3) > Dpg(2), whereas in the Ac$_6$C peptides the order of solvent exposure of NH groups is Ala(1) > Ac$_6$C(2) > Ala(3). The nmr results suggest that Ac$_6$C peptides adopt folded $\beta$-turn conformations with Ala(1) and Ac$_6$C(2) occupying $i+1$ and $i+2$ positions. In contrast, the Dpg peptides favor extended C$_\beta$ 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 $\beta$-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 $\beta$-turn or incipient $3_1\alpha$-helix in solution. ©1995 John Wiley & Sons, Inc.

INTRODUCTION

$\alpha,\alpha$-Dialkylated amino acid residues have acquired considerable importance as a means of introducing backbone conformation constraints in synthetic peptides.$^{1,2}$ The prototype residue $\alpha$-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-$\alpha$-aminocycloalkane-1-carboxylic acid (Ac$_n$C) side chains has shown that these residues can adopt folded $\beta$-turn conformations.$^{6,8}$ The conformational stability of such peptides is dependent on the nature of the $\alpha$-aminoalkyl side chain and the conformational constraints imposed by the backbone. $^{9,10}$

Received March 23, 1994; Accepted May 6, 1994
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Contrasting Solution Conformations of Peptides Containing $\alpha,\alpha$-Dialkylated Residues with Linear and Cyclic Side Chains
acid (Ac, 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 \( \phi \sim \pm 60^\circ \), \( \psi \sim \pm 30^\circ \). In contrast, \( \alpha,\alpha \)-di-\( n \)-propylglycine (Dpg) (Figure 1) appears to be capable of stabilizing fully extended C\(_3\) conformations (\( \phi \sim \psi \sim 180^\circ \)) in short homooligopeptides.\(^11\)\(^-\)\(^13\)

However, di-\( n \)-propylglycyl and di-\( n \)-butylglycyl (Dbg) residues have also been shown to be incorporated into helical/\( \beta \)-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 \( \phi,\psi \) 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 Ac,Ac (IV) and Ac,Ac (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,Ac residues stabilize folded \( \beta \)-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**

\( \alpha,\alpha \)-Dialkylated amino acids were synthesized from the appropriate ketones. The ketones were first converted into their corresponding hydantoins, which on hydrolysis with 60\% \( \text{H}_2\text{SO}_4 \) 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 \((\text{NH}_4)_2\text{CO}_3\) 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 \( \text{FeSO}_4 \) 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\% \( \text{H}_2\text{SO}_4 \) (\~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 \( \beta \)-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, Ac,Ac, and Ac,Ac were prepared by the thionyl chloride-methanol procedure.\(^19\) The esterification of Dxg amino
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. 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 Lichrosorb RP C-18 column using MeOH/H2O gradients.

**Boc-Ala-Deg-OH (1).** Boc-Ala-Deg-OH (0.95 g, 5 mmol) was dissolved in dimethylformamide (DMF; 3 mL). 0.73 g (5 mmol) of Deg-OH 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, 1 N 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 C7H3s); 1.27, 1.36, 1.77, 2.09, 2.40, 7H (Deg C9H18, AlaC9H18); 1.45, 9H, s (Boc CH3); 3.76, 3H, s (− COOCH3); 4.18, 1H, m (Ala CH3); 5.72, 1H, d (Ala NH) 7.14, 1H, s (Deg NH).

**Boc-Ala-Deg-Ala-OMe (2).** Boc-Ala-Deg-Ala-OMe (0.31 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 (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%).

<table>
<thead>
<tr>
<th>Ketones</th>
<th>Hydantoins (Yield %)</th>
<th>mp (°C)</th>
<th>Hydrolysis Reflux Times (h)</th>
<th>Amino Acids</th>
<th>Crude (Yield %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Pentanone</td>
<td>5,5'-Diethyl hydantoin (85)</td>
<td>165–166</td>
<td>36–38</td>
<td>Diethylglycine (Deg)</td>
<td>(70)</td>
</tr>
<tr>
<td>(84)</td>
<td></td>
<td>198–200</td>
<td>46–48</td>
<td>Di- n-propylglycine (Dpg)</td>
<td>(73)</td>
</tr>
<tr>
<td>5-Nonanone</td>
<td>5,5'-Di-n-butyl hydantoin (86)</td>
<td>160–161</td>
<td>48–50</td>
<td>Di-n-butylglycine (Dbg)</td>
<td>(80)</td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>5,5'-Spirocyclohexane hydantoin (84)</td>
<td>210</td>
<td>24–26</td>
<td>1-Aminocyclohexane-1-carboxylic acid (Ac-c)</td>
<td>(84)</td>
</tr>
<tr>
<td>Cycloheptanone</td>
<td>5,5'-Spirocycloheptane hydantoin (86)</td>
<td>15</td>
<td>26–28</td>
<td>1-Aminocycloheptane-1-carboxylic acid (Ac-c)</td>
<td>(86)</td>
</tr>
</tbody>
</table>

* Characteristic carbonyl stretching bands (cm⁻¹) in the ir spectra of the hydantoins are 1710–1740 and 1760–1780.

* Characteristic ir bands (cm⁻¹) of amino acids are 1610–1640 (−COO− groups) and 3060–3090 (−NH2) were observed. Melting points are uncorrected.
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.

\[ ^1H \text{-nmr (CDCl}_3, \delta) 0.8, 1.16, 1.2, 1.34, 1.42, 1.61, 2.0, 2.3, 2.4, 2.0H (Dpg CH2, CH3 protons, Ala C\text{H}_3); 1.47, 9H, s (Boc CH3); 3.73, 3H, s (COOCH3); 4.05, 4.50, 2H, m (AlaCH3); 4.95, 1H, d [Ala(3)NH]; 6.45, 1H, s (DpgNH). \]

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: 0.85 g (85%).

\[ ^1H \text{-nmr (CDCl}_3, \delta) 0.85, 0.9, 1.15, 1.3, 1.35, 1.4, 2.1, 2.25, 2.4 2H (Dpg CH2, CH3 protons, Ala CH3); 1.5, 9H, s (Boc-CH3); 3.73, 3H, (COOCH3); 4.05 \delta, 1H, m [Ala(1)C\text{H}_3]; 4.52, 1H, m [Ala(3)C\text{H}_3]; 4.95, 1H, d [Ala(1)NH]; 6.4, 1H, d [Ala(3)NH]; 7.02, 1H, s (DpgNH). \]

Boc-Ala-Dpg-Ala-OMe (IV). Boc-Ala-Ac\text{c}-OMe (7). 0.95 g (5 mmol) of Boc-Ala-OMe was dissolved in CH2Cl2 (20 mL) and cooled in an ice bath; Ac\text{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 DUC was filtered. The CH2Cl2 was evaporated and the solid was dissolved in ethyl acetate. The organic solution was washed with excess of water, 1M Na2CO3 (3 × 40 mL) 1N HCl (3 × 40 mL) and water. The organic layer was dried over anhydrous Na2SO4 and evaporated in vacuo. Peptide 7 was obtained as a gum. Yield: 1.4 g (85%).

\[ ^1H \text{-nmr (CDCl}_3, \delta) 1.38, 3H, d (Ala C\text{H}_3); 1.5, 9H, s (Boc CH3); 1.68, 2.05, 10H, m (Ac\text{c} ring — CH2 — protons); 3.71, 3H, s (COOCH3) 4.18, 1H, m (Ala C\text{H}_3); 5.23, d (Ala NH); 6.73, 1H, s (Ac\text{c}NH). \]

Boc-Ala-Ac\text{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\text{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.

\[ ^1H \text{-nmr (CDCl}_3, \delta) 1.35, 1.37, 6H (Ala C\text{H}_3); 1.45, 9H, s (Boc CH3); 1.49, 1.68, 2.09, 10H (Ac\text{c} ring — CH2 — protons); 3.66, 3H, s (COOCH3); 4.05, 4.45, 2H, m (Ac\text{c}NH); 4.96, 1H, d [Ala(1)NH]; 6.40, 1H, s (Ac\text{c}NH), 7.25, 1H, d [Ala(3)NH]. \]
**Boc-Ala-Ac,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,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 1. Peptide V was obtained as a white solid. Yield: 0.62 g (70%); mp = 173–175°C.

**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 CDCl₃/(CD₃)₂SO mixtures, free radical induced line broadening of NH resonances in CDCl₃, and temperature coefficients of NH resonances in (CD₃)₂SO.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,c and Dxg resonances 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 (dδ/dT) in (CD₃)₂SO (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-Dbg-AlaOMe peptides in all three cases the order of NH group solvent exposure is Ala(1) > Ala(3) > Dsg. In sharp contrast, in Boc-Ala-Ac,c-Ala-OMe peptides the order of solvent exposure is Ala(1) > Ac,c (2) > Ala(3). In peptides I–III the Dsg NH resonances are clearly shielded from solvent, with characteristically low values of Δδ (0.45 ppm) and dδ/dT (1.8–2 ppm/K). In peptides IV and V, the Ala(3) resonance is solvent shielded with Δδ = 0.12–0.58 ppm and dδ/dT values in (CD₃)₂SO of 1.5–1.9 ppm/K. The solvent-shielded nature of Ala(3) NH in peptides Boc-Ala-Ac,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,c residues to favor β-turn conformations in short peptides. Indeed, incorporation of Ac,c residues in different peptide sequences such as Boc-Pro-Ac,c-Ala-OMe,7 Boc-(Ac,c) dword,7 and Boc-Pro-Ac,c-Ala-OMe⁸ have been shown to induce β-turn conformations.

The solvent-exposed nature of Ala(3) NH in the Dsg 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 Dsg NH group may be rationalized by invoking two distinct conformations:

1. C₇ (γ) turn conformations centered at Ala(1) involving the Dsg NH in an intramolecular (3 → 1) hydrogen bond with the Boc-CO group;

2. C₅ conformations at Dsg in which a fully extended backbone places the Dsg NH group in close proximity to the Dsg CO group (Figure 3b).
Table II  NMR Parameters for NH Resonances in Peptides Boc-Ala-Xxx-Ala-OMe

<table>
<thead>
<tr>
<th>Boc-Ala-Xxx-Ala-OMe</th>
<th>Chemical Shift (δ ppm) CDCl₃</th>
<th>Chemical Shift (δ ppm) (CD₃)₂SO</th>
<th>Δδ NH (ppm) CDCl₃</th>
<th>db/dT × 10⁻³ (ppm K⁻¹) CDCl₃</th>
<th>J₈NH⁻¹H (Hz)</th>
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</thead>
<tbody>
<tr>
<td>Xxx</td>
<td>Ala (1) XXX (3)</td>
<td>Ala (1) XXX (3)</td>
<td>Ala (1) XXX (3)</td>
<td>Ala (1) XXX (3)</td>
<td>Ala (1) XXX (3)</td>
</tr>
<tr>
<td>Deg</td>
<td>5.11 7.18 6.59</td>
<td>7.51 7.62 8.37</td>
<td>2.4 0.44 1.78</td>
<td>7.4 1.8 6.1</td>
<td>6.8 7.8 6.8</td>
</tr>
<tr>
<td>Dpg</td>
<td>4.95 7.05 6.41</td>
<td>7.2 7.5 8.16</td>
<td>2.25 0.45 1.75</td>
<td>6.2 1.8 4.4</td>
<td>6.4 6.8 7.2</td>
</tr>
<tr>
<td>Db</td>
<td>4.95 7.02 6.4</td>
<td>7.25 7.47 8.15</td>
<td>2.3 0.45 1.75</td>
<td>8.0 2.0 5.0</td>
<td>6.8 7.6 7.0</td>
</tr>
<tr>
<td>Ac⁻c</td>
<td>4.96 6.40 7.25</td>
<td>6.86 7.5 7.37</td>
<td>1.9 1.10 0.12</td>
<td>5.3 4.1 1.9</td>
<td>6.0 6.0 6.4</td>
</tr>
<tr>
<td>Ac⁻c</td>
<td>5.01 6.47 7.17</td>
<td>7.04 7.86 7.75</td>
<td>2.03 1.39 0.58</td>
<td>4.6 4.5 1.5</td>
<td>5.6 6.8 6.0</td>
</tr>
</tbody>
</table>

a  Peptide concentration ~ 10 mM.

b  Error in J values ± 0.5 Hz.

C₅ conformations at Dsg residues would appear to be a much better conformational choice, since ample precedence for such structures exists in crystal structures of short Dsg containing peptides. There does not appear to be any compelling reason for Dsg residues to stabilize C₇ conformations at the preceding residue in the sequence. Indeed, C₇ conformations have almost never been observed in crystal structures of short acyclic peptides. It is noteworthy that stabilization of C₇ conformations at a preceding residue has been invoked in a study of acyclic dehydroalanine peptides on the basis of limited spectral evidence.

Figure 4 shows the results of a difference NOE experiment carried out on the peptide Boc-Ala-Dsg-Ala-OMe (III). Irradiation of three NH resonances and Ala(3) C“H reveals only one strong interresidue NOE between Ala(1) C“H and Dsg NH. This is consistent with an extended conformation at Ala(1). The ψ values of +60° to 180° result in interproton distances dₖN < 2.5 Å.

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-Dsg-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.
marizes the ellipticity values obtained in different solvents. A comparison of the CD spectra of Boc-Ala-Ac₅C-Ala-OMe and Boc-Ala-Dpg-Ala-OMe (Figure 5) clearly establishes dramatic differences between the backbone conformations of the two peptides. It may be noted that the two peptides possess the same number of carbon atoms in the residue 2 side chain, with the sole difference that a cyclic side chain is present in Ac₅C, whereas a linear side chain is present in Dpg. Figure 6 establishes that the CD spectra of Ac₅C peptides are very similar, as are the spectra of the Dpg series. The spectra of Ac₅C peptides IV and V show the presence of a weak negative band at 233–234 nm and a more intense positive band at 215 nm, which may be suggestive of type II β-turn conformations, having Ala(i) and Ac₅C as the i + 1 and i + 2 residues, respectively. The possibility of CD spectra arising from contributions of both type I and type II β-turn structures must also be considered.²⁷,²⁸

The Dpg peptides show only a single negative band at 224–227 nm. This feature may be representative of fully extended conformations. It may be noteworthy that spectra obtained for polypeptides adopting classical β-sheet conformations show a single negative band.²⁹

Taken together, the nmr and CD studies unequivocally establish differences in the conformational properties of α,ω-dialkylated residues with linear (Dpg) and cycloalkyl (Ac₅C) side chains, in short model peptides in solution.
Conformation of Dsg Residues

The spectroscopic studies described above clearly indicate that the Dsg containing tripeptides do not adopt folded \( \beta \)-turn conformations in solution. This is in marked contrast to the results described in the preceding paper, where type II \( \beta \)-turn conformations have been established for Boc-Ala-Dpg-Ala-OMe and Boc-Ala-Dbg-Ala-OMe, with the Dsg residue occupying the \( i+2 \) position, having \( \phi, \psi \) values lying in the \( \alpha \)-helical region of \( \phi, \psi \) space (Dpg, \( \phi = 66.2^\circ, \psi = 19.3^\circ \); Dbg, \( \phi = 66.5^\circ, \psi = 21.1^\circ \)).\(^{16}\) 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\(_6\)c residues, very good agreement between solid state and solution conformations have generally been observed.\(^{33,8}\) 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

<table>
<thead>
<tr>
<th>Peptides</th>
<th>TFE</th>
<th>Methanol</th>
<th>TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \lambda ) (nm)</td>
<td>([\theta]_M \times 10^2)</td>
<td>( \lambda ) (nm)</td>
</tr>
<tr>
<td>Boc-Ala-Deg-Ala-OMe (I)</td>
<td>227</td>
<td>-25.15</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>212</td>
<td>+14.31</td>
<td></td>
</tr>
<tr>
<td>Boc-Ala-Dpg-Ala-OMe (II)</td>
<td>225</td>
<td>-20.9</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>+6.83</td>
<td></td>
</tr>
<tr>
<td>Boc-Ala-Dbg-Ala-OMe (III)</td>
<td>224</td>
<td>-24.86</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td>233</td>
<td>-14.57</td>
<td>234</td>
</tr>
<tr>
<td>Boc-Ala-Ac(_6)c-Ala-OMe (IV)</td>
<td>217</td>
<td>+27.1</td>
<td>213</td>
</tr>
<tr>
<td></td>
<td>233</td>
<td>-8.24</td>
<td>239</td>
</tr>
<tr>
<td>Boc-Ala-Ac(_6)c-Ala-OMe (V)</td>
<td>212</td>
<td>+32.75</td>
<td>212</td>
</tr>
</tbody>
</table>

* Peptide concentration 2 mM. Band intensities are expressed as \([\theta]_M\) deg cm\(^2\) decimol\(^{-1}\). TFE, 2,2,2-trifluoroethanol; TMP, trimethylphosphate.
monitored by low temperature dissolution of single crystals. 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, c, Dpg) 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\(_3\))\(_2\)SO. It is clearly seen that when the Xxx residue occurs in an extended conformation, the Xxx NH resonance appears between 7.02 \(\delta\) and 7.18 \(\delta\) in CDCl\(_3\) and 7.47\(\delta\) and 7.62\(\delta\) in (CD\(_3\))\(_2\)SO (peptides I-III). In contrast, when the central residue occurs in the \(i+2\) position of a \(\beta\)-turn as in peptides IV and V, the Xxx NH resonance occurs between 6.40\(\delta\) and 6.47\(\delta\) in CDCl\(_3\) and 7.55\(\delta\) in (CD\(_3\))\(_2\)SO. Inspection of the NH chemical shifts in the minor conformation detected at low temperatures suggest that the observed species may not correspond to the \(\beta\)-turn conformation detected in crystals. 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\(_3\))\(_2\)SO 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\(_3\))\(_2\)SO 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 \(\beta\)-turn or incipient 3\(_{10}\)-helical structure in the crystalline state. 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 D$_x$g 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 D$_x$g residues (Ref. 15 and unpublished results) have invariably resulted in the characterization of helical conformations.

The use of D$_x$g 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 D$_x$g residues inserted into sequences with very low helix propensities.

This research was supported by grants from the Department of Science and Technology, Government of India.

REFERENCES