Insertion of Methylene Units into the Turn Segment of Designed β-Hairpin Peptides

S. C. Shankaramma, S. Kumar Singh, Aruna Sathyamurthy, and P. Balaram*

Contribution from the Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, India

Received February 9, 1999

Abstract: The effect of insertion of methylene groups into the turn segment of β-hairpin peptides has been investigated in the model sequence Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe. This sequence is related to the previously well-characterized model β-hairpin octapeptide, Boc-Leu-Val-Val-^{D}Pro-Gly-Leu-Val-Val-OMe. Replacement of Gly by δ-Ava (δ-aminovaleric acid) formally corresponds to expansion of the turn segment from a two-residue loop to a three-residue loop. Backbone proton chemical shifts, vicinal coupling constants, and circular dichroism spectra for the two peptides are virtually indistinguishable. Nuclear Overhauser effects corresponding to short cross-strand interproton distances confirm that the registry of the β-hairpin structure is maintained in the δ-Ava peptide. Restrained molecular dynamics simulations, using experimental constraints, yield two structural families that are consistent with the NOE data. Both families correspond to β-hairpin conformations and differ only in the backbone torsion angles at the δ-Ava residue.

Introduction

The insertion of methylene groups into polypeptide chains is readily accomplished by incorporation of ω-amino acids into peptide sequences.[1] Peptides containing ω-amino acids differ from their α-amino acid counterparts in the number of C-atoms which separate two linked peptide units. Greater stereochemical variability and resistance to proteolytic cleavage is anticipated in ω-peptides, a feature which should make these systems attractive in the design of peptidomimetics.[2] Considerable recent interest in the design of β-amino acid-containing peptides has been stimulated by the observation of novel stereochemical features in model sequences.[1,3] Synthetic and stereochemical interest has also been focused on peptides containing γ- and δ-amino acids.[4] The incorporation of a β-Ala-γ-Abu segment into peptide helices has been demonstrated by crystallographic analysis.[5] In this report, we describe the expansion of the nucleating β-turn segment in a model β-hairpin peptide by insertion of a δ-aminovaleric acid residue ($-NH-(CH_2)_4-CO-$, δ-Ava). β-Hairpin structures in peptides have been shown to be favored in sequences incorporating centrally located ^{D}Pro-Gly segments.[6] The crystal structure of the designed hairpin structure Boc-Leu-Val-Val-^{D}Pro-Gly-Leu-Val-Val-OMe (**I**) has been previously reported.[7] In this study, spectroscopic evidence for a β-hairpin structure in the analogous peptide Boc-Leu-Val-Val-^{D}Pro-δ-Ava-Leu-Val-Val-OMe (**II**) is presented. The incorporation of δ-Ava in place of Gly corresponds to the insertion of three additional backbone atoms into the β-turn segment. The δ-Ava residue may thus be considered as formally homomorphous with a Gly-Gly segment (Figure 1).[8] Peptide **II** may consequently be viewed as a sequence in which a two-residue hairpin turn has been expanded to a "three-residue turn". Recent analyses of hairpins in protein structures reveal the widespread occurrence of three-residue loops connecting the two β-strand segments.[9]

Experimental Procedures

Peptide **II** was synthesized by conventional solution-phase methods,[10] using a fragment condensation strategy involving a final 3 + 5 coupling.

* To whom correspondence should be addressed. Fax: (80) 3348535. Phone: (80) 3341683. E-mail: pb@mbu.iisc.ernet.in.

(1) (a) Seebach, D.; Matthews, J. L. *J. Chem. Soc., Chem. Commun*. **1997**, 2015−2022. (b) Banerjee, A.; Balaram, P. *Curr. Sci*. **1997**, *73*, 1067−1077. (c) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.

(2) (a) Goodman, M.; Ro, S. In *Medicinal Chemistry and Drug Design*; Burger, A., Ed.; John Wiley & Sons: New York, 1994; Vol. I, pp 803−861. (b) Hruby, V. J.; Li, G.; Haskell-Luevano, C.; Shenderovich, M. *Biopolymers* **1997**, *43*, 219−266.

(3) (a) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer H. *Helv. Chim. Acta* **1996**, *79*, 913−941. (b) Daura, X.; Van Gunsteren, W. F.; Rigo, D.; Jaun, B.; Seebach, D. *Chem. Eur. J.* **1997**, *3*, 1410−1417. (c) Hintermann, T.; Gademann, K.; Jaun, B.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 983−1002. (d) Apella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071−13072. (e) Apella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. *Nature* **1997**, *387*, 381−382. (f) Krauthaser, S.; Christianson, L. A.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11719−11720. (g) Wu, Y. D.; Wang, D. P. *J. Am. Chem. Soc.* **1998**, *120*, 13485−13493. (h) Gademann, K.; Jaun, B.; Seebach, D.; Perozzo, R.; Scapozza, L.; Folkers, G. *Helv. Chim. Acta* **1999**, *82*, 1−11. (i) Gung, B. W.; Mackay, J.; Zou, D. *J. Org. Chem.* **1999**, *64*, 700−706.

(4) (a) Snyder, K. R.; Murray, T. F.; DeLander, G. E.; Aldrich, J. V. *J. Med. Chem*. **1993**, *36*, 1100−1103. (b) Seebach, D.; Abele, S.; Sifferlen, T.; Hanggi, M.; Gruner, S.; Seiler, P. *Helv. Chim. Acta* **1998**, *81*, 2218−2243. (c) Hanessian, S.; Yang, H.; Schaum, R. *J. Am. Chem. Soc.* **1996**, *118*, 2507−2508. (d) Hanessian, S.; Luo, X.; Schaun, R.; Michnick, S. *J. Am. Chem. Soc.* **1998**, *120*, 8569−8570. (e) Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074−1078.

(5) Karle, I. L.; Pramanik, A.; Banerjee, A.; Bhattacharjya, S.; Balaram, P. *J. Am. Chem. Soc.* **1997**, *119*, 9087−9095.

(6) (a) Raghothama, S.; Awasthi, S. K.; Balaram, P. *J. Chem. Soc., Perkin Trans. 2* **1998**, 137−143. (b) Schenck, H. L.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4869−4870. (c) Struthers, M. D.; Cheng, R. P.; Imperiali, B. *Science* **1996**, *271*, 342−345. (d) Das, C.; Raghothama, S.; Balaram, P. *J. Am. Chem. Soc.* **1998**, *120*, 5812−5813.

(7) Karle, I. L.; Awasthi, S. K.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8189−8193.

(8) (a) Homomorphous segments are defined as having the same number of backbone atoms between the −NH and C═O groups of the ω-amino acids. (b) Banerjee, A.; Pramanik, A.; Bhattacharjya, S.; Balaram, P. *Biopolymers* **1996**, *39*, 769−777.

(9) Gunasekaran, K.; Ramakrishnan, C.; Balaram, P. *Protein Eng*. **1997**, *10*, 1131−1141.

(10) Awasthi, S. K.; Raghothama, S.; Balaram, P. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2701−2706.

10.1021/ja990412b CCC: $18.00
Published on Web 05/27/1999

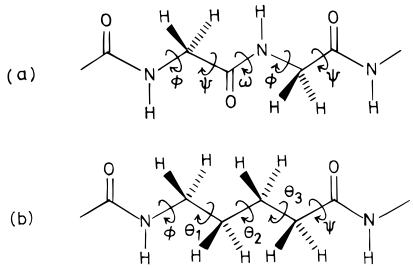

Figure 1. Comparison of homomorphic segments (a) Gly-Gly with (b) δ-Ava.

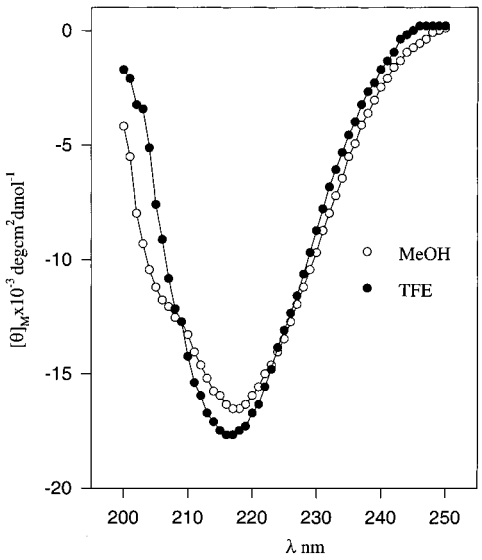

Figure 2. CD spectra of peptide **II** in MeOH and TFE. Peptide concentration, 1.89×10^{-4} mmol. Molar ellipticities ($[\theta]_M$) are expressed as deg cm^2 dmol^{-1}.

The Boc group was used for N-terminal protection, and the C-terminus was protected as a methyl ester. Deprotections were performed using 98% formic acid or saponification, respectively. Couplings were mediated by dicyclohexylcarbodiimide−1-hydroxybenzotriazole (DCC/HOBt). All the intermediates were characterized by ^{1}H NMR (80 MHz) and thin-layer chromatography (TLC) on silica gel and used without further purification. The final peptide was purified by medium-pressure liquid chromatography (MPLC) on a C-18 (40−60 μm) column, followed by high-performance liquid chromatography (HPLC) purification, on a reversed-phase (RP) C-18 (10μ) column using methanol−water gradient elution. The melting point was 162−164 °C. Peptide homogeneity was demonstrated by analytical RP-HPLC (C-18, 5 μm), and the identity of the peptide was confirmed by MALDI mass spectra, M + Na (obsd) = 972.6, M (calcd) = 949.6. The final peptide was characterized by complete assignment of 500-MHz ^{1}H NMR spectra.

All NMR experiments were carried out on a Bruker DRX-500 spectrometer. Peptide concentrations were in the range of 7−8 mM, and the probe temperature was maintained at 298 K. Resonance assignments were done using two-dimensional double-quantum filtered COSY and rotating frame nuclear Overhauser effect (ROESY) experiments. All 2D experiments were recorded in a phase-sensitive mode using the time proportional phase incrementation method. A total of 1024 and 512 data points were used in t_2 and t_1 dimensions, respectively. The resultant data set was zero-filled to finally yield 1K × 1K data points. A shifted square sine bell window was used in both dimensions. Spectral widths were in the region of 4500 Hz. CD spectra were recorded on a JASCO J-500 spectropolarimeter using a 1 mm path length cell.

Results and Discussion

The CD spectrum of peptide **II** (Figure 2) consists of a single negative band at 217 nm in trifluoroethanol and was almost indistinguishable from that of the parent peptide **I.**[11] Both peptides yield identical spectra in methanol, where a shoulder at 208 nm is detectable in addition to the strong negative band at 218 nm. The spectra closely resemble those reported in the literature for a range of β-hairpin peptides.[11−13] Assignments of backbone resonances in 500-MHz ^{1}H NMR spectra of **II** in $CDCl_3$ were readily achieved. The relevant NMR parameters are summarized in Table 1 and compared with the parameters obtained for peptide **I**. Clearly, there is a remarkable correspondence in the C$^\alpha$H and NH chemical shifts of the two peptides. The high $J_{NHC^\alpha H}$ values in peptide **II** are also consistent with extended conformations at the Leu and Val residues. Addition of varying amounts of the strongly hydrogen bonding solvent, dimethyl sulfoxide ($(CD_3)_2SO$), to $CDCl_3$ solutions reveals that NH resonances of Val(2), Val(7), and δ-Ava(5) showed much larger downfield shifts as compared with those of Leu(1), Val(3), Leu(6), and Val(8) (Figure 3). These data suggests that the latter four NH groups may be solvent shielded, a feature consistent with their involvement in cross-strand

Table 1. Comparison of NMR Parameters for Peptides **I** and **II** in $CDCl_3$[a]

	NH		C$^\alpha$H		$J_{NHC^\alpha H}$ (Hz)	
residue	**I**	**II**	**I**	**II**	**I**	**II**
Leu(1)	5.62	5.65	4.09	4.13	8.5	8.3
Val(2)	6.54	6.64	4.74	4.80	9.1	9.1
Val(3)	8.73	8.73	4.54	4.70	9.0	9.1
^{D}Pro(4)			4.33	4.45		
Xxx(5)	6.13	6.39	3.97	3.35		
Leu(6)	7.65	7.46	4.66	4.57	8.0	8.8
Val(7)	6.44	6.76	4.69	4.70	8.6	8.0
Val(8)	8.33	8.22	4.56	4.58	8.0	8.6

[a] Peptide **I**, Xxx = Gly; peptide **II**, Xxx = δ-Ava. Parameters for peptide **I** are from ref 6a.

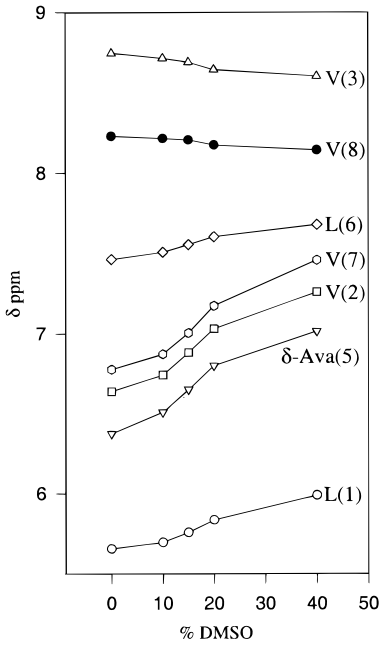

Figure 3. Dependence of NH chemical shifts on the concentration of $(CD_3)_2SO$ (v/v) in $CDCl_3$ in peptide **II**. Assignments are indicated using the one-letter code.

(11) Awasthi, S. K.; Raghothama, S.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 375−381.
(12) Alvarado, M. R.; Blanco, F. J.; Serrano, L. *Nature Struct. Biol.* **1996**, *3*, 604−612.
(13) Nesloney, C. L.; Kelly, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 5836−5845.

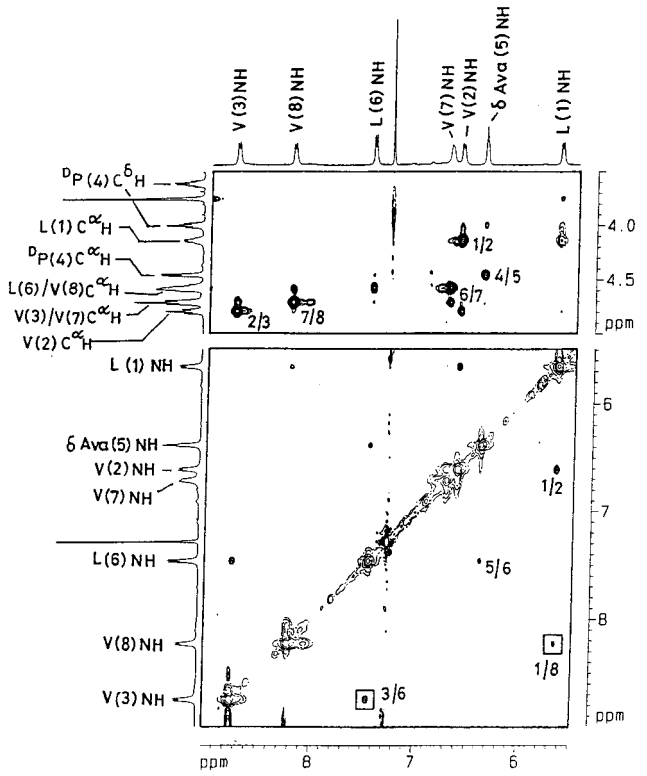

Figure 4. Partial 500-MHz ROESY spectra of peptide **II** in $CDCl_3$. The top panel shows $C^{\alpha}H \leftrightarrow NH$ NOEs, and the lower panel shows $NH \leftrightarrow NH$ NOEs. Assignments are marked on the 1D spectra.

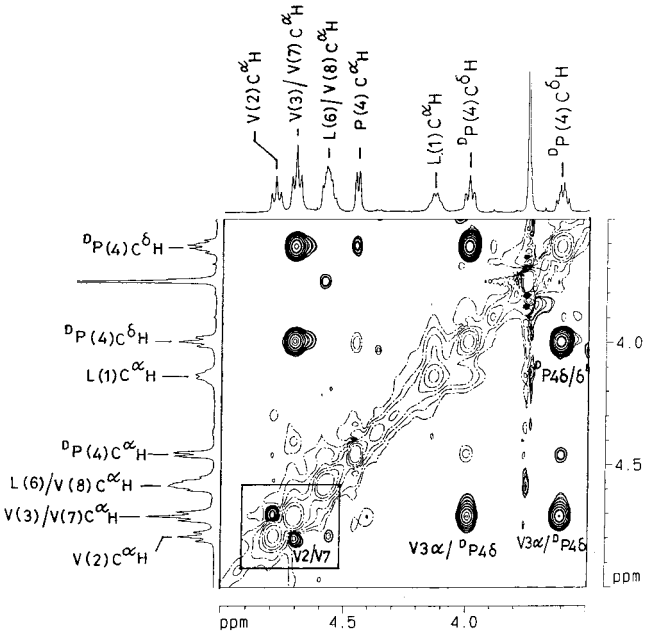

Figure 5. Portion of the 500-MHz ROESY spectrum of peptide **II** in $CDCl_3$, highlighting $C^{\alpha}H \leftrightarrow C^{\alpha}H$ cross-strand NOEs.

hydrogen bonding in a β-hairpin structure. The NOEs between backbone protons are illustrated in Figure 4. The relatively intense interresidue $C_i^{\alpha}H$ and $N_{i+1}H$ ($d_{\alpha N}$) NOEs and the very weak or absent intraresidue $N_iH \leftrightarrow N_{i+1}H$ (d_{NN}) NOEs for the segments 1−3 and 6−8 suggest that the Leu-Val-Val units favor extended conformations. Interestingly, an NOE, albeit weak, is observed between δ-Ava(5) NH and Leu(6) NH (d_{NN} 5/6). While the d_{NN} NOE at the $i + 2$ position of a β-turn is expected in both types I and II turns for a normal α-amino acid residue, in the present case three additional methylene units have been inserted at this position. The observation of this NOE is strongly suggestive of "turn" formation bringing the NH groups of residue 5 and 6 into proximity. The d_{NN} 3−6 and 1−8 connectivity supports extension of the hairpin structure. The relatively weak d_{NN} 1−2 NOE may be indicative of conformational heterogeneity at the N-terminus. Firm support for the β-hairpin conformation is obtained from the observation of a $C^{\alpha}H \leftrightarrow C^{\alpha}H$ cross-strand NOE between Val(2) and Val(7) (Figure 5). The strong Val(3) α -^{D}Pro(4) δ NOE also confirms the trans geometry about the Val(3)-^{D}Pro(4) bond.

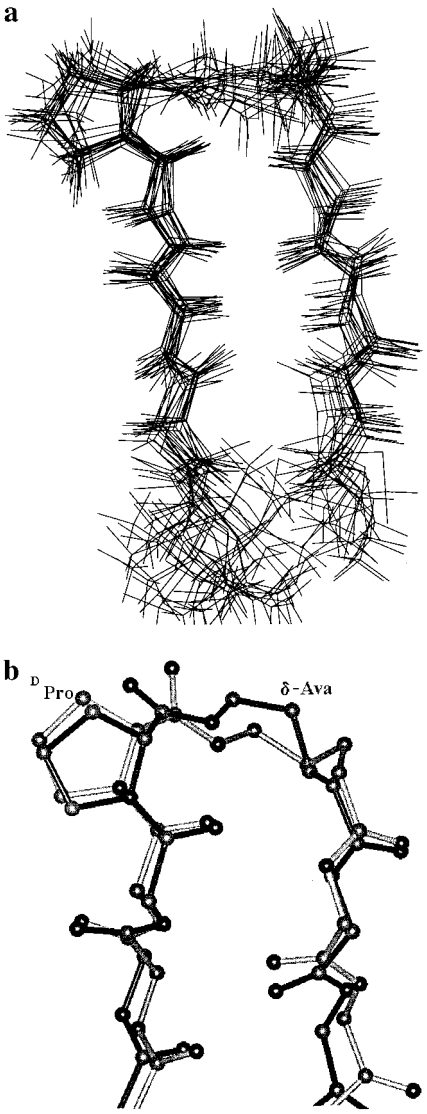

Figure 6. (a) Superposition of 25 structures of one structural family of peptide **II** obtained by molecular dynamics calculations with NOE derived restraints. (b) Superposition of the turn region of two structural families, highlighting the stereochemical differences at the δ-Ava residue. The dark bonds correspond to family 1, while the light bonds correspond to family 2.

The generation of structures consistent with the NMR data was achieved by using starting models for the ^{D}Pro-δ-Ava segment, in which the Val(3) C═O and Leu (6) NH groups were brought within approximately hydrogen bonding distance (N···O, 3.0 Å) and the dihedral angles at the δ-Ava residue were maintained at 180° ± 30° (trans) or ±60° ± 30° (gauche). Restrained molecular dynamics simulations using 15 NOE restraints yielded two distinct families of β-hairpin structures, which were stereochemically acceptable and of comparable

energies (Figure 6). Figure 6a shows the superposition of structures obtained corresponding to one structural family. The two structural families differ only in the conformation of the δ-Ava residue. The relevant portions of the average structures highlighting the stereochemical difference are superposed in Figure 6b. In family 1, the dihedral angles for δ-Ava are $\phi = -158.15°$, $\theta_1 = -67.68°$, $\theta_2 = 97.40°$, $\theta_3 = 69.01°$, $\psi = -112.98°$,while in family 2, the corresponding values are $\phi = 178.88°$, $\theta_1 = 67.50°$, $\theta_2 = -87.52°$, $\theta_3 = 179.40°$, $\psi = -114.03°$.[14] Since all the nuclear Overhauser effect restraints are effectively between the two strand segments and the δ-Ava segment has no major experimental restraints, these two conformational families correspond to two possible closely related minima which have been probed in the course of the molecular dynamics. The two structures differ in a correlated torsional flip about the $C^{\alpha}-C^{\beta}-C^{\gamma}-C^{\delta}$ segment of the δ-Ava residue. It is pertinent to note that concerted flips involving correlated changes in backbone torsion angles have been implicated in interconversions between classical β-turn structures.[15]

Conclusion

The NMR results presented above unambiguously establish that the insertion of δ-Ava at the turn position in a β-hairpin can be accomplished without loss of the antiparallel β-sheet registry. The ability to insert ω-amino acids into canonical secondary structural motifs such as helices,[5] β-turns,[16] and β-hairpins should prove valuable in peptidomimetic design, where it is desirable to maintain overall three-dimensional shape despite covalent modification of the peptide backbone. At first glance, the incorporation of polymethylene units into folded backbone structures may appear surprising; however, it should be noted that the main prerequisite for the generation of a compact fold is the ability of the carbon chain to adopt gauche conformations ($\theta = \pm 60°$). This indeed appears to be an energetically accessible alternative in several theoretical calculations on model systems.[17]

Acknowledgment. S.C.S. and S.K.S. were supported by a Research Associateship and Senior Research Fellowship of the CSIR, respectively. A.S. was a summer trainee supported by the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore. This work was partly funded by the program support grant in Drug and Molecular Design from the Department of Biotechnology. NMR spectra were recorded at the Sophisticated Instruments Facility, Indian Institute of Science, Bangalore.

JA990412B

(14) The nomenclature for the dihedral angles in δ-Ava is as defined in ref 8. See also refs 1b and 5 for a suggestion regarding nomenclature for ω-amino acids in general.

(15) Gunasekaran, K.; Gomathi, L.; Ramakrishnan, C.; Chandrasekhar, J.; Balaram, P. *J. Mol. Biol.* **1998**, *284*, 1505−1516.

(16) (a) Chung, Y. J.; Christianson, L. A.; Stanger, H. E.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 10555−10556. (b) Jones, I. G.; Jones, W.; North, M. *J. Org. Chem.* **1998**, *63*, 1505−1513. (c) Lombardi, A.; Saviano, M.; Nastri, F.; Maglio, O.; Mazzeo, M.; Pedone, C.; Iserniac, C.; Pavone, V. *Biopolymers* **1996**, *38*, 683−691. (d) Mantsch, H. H.; Perczel, A.; Hollosi, M.; Fasman, G. D. *Biopolymers* **1993**, *33*, 201−207.

(17) (a) Aleman, C.; Navarro, E.; Puiggali, J. *J. Org. Chem.* **1995**, *60*, 6135−6140. (b) Navarro, E.; Aleman, C.; Puiggali, J. *J. Am. Chem. Soc.* **1995**, *117*, 7307−7310. (c) Price, D. J.; Roberts, J. D.; Jorgenson, W. L. *J. Am. Chem. Soc.* **1998**, *120*, 9672−9679.