JOC Article

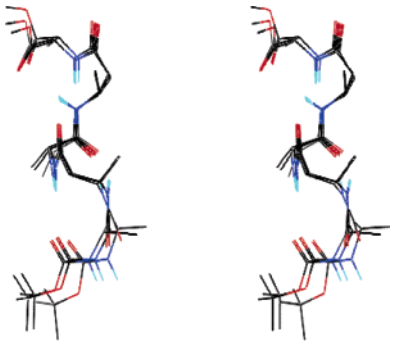
11/9-Mixed Helices in the α/β-Peptides Derived from Alternating α- and β-Amino Acids with Proteinogenic Side Chains*

Gannoju Srinivasulu,† Singarapu Kiran Kumar,† Gangavaram V. M. Sharma,*,‡ and Ajit C. Kunwar*,†

Centre for Nuclear Magnetic Resonance and D-211, Discovery Laboratory, Organic Chemistry Division III, Indian Institute of Chemical Technology, Hyderabad 500 007, India

esmvee@iict.res.in; kunwar@iict.res.in

Received June 23, 2006



α/β-Hybrid peptides are prepared from amino acids with proteinogenic side chains on the basis of the concept of "alternating chirality", involving D-Phe and a β^3-hVal. Through the extensive NMR, CD, and MD studies, robust left-handed 11/9-mixed helices were identified in these peptides in $CDCl_3$ solutions, wherein the 11/9-mixed helix was observed even in a small peptide with three residues.

Introduction

Foldamers[1] derived from alternating α- and β-amino acid residues adopt a variety of helical structures.[2−5] Such hybrid peptides containing constrained cyclic β-amino acids, reported by Gellman et al. and Reiser et al., display 11-, 13-, and 14/15-helices,[2−4] whereas those derived from acyclic C-linked carbo-β^3-amino acid [(*S*)-Caa] reported[5] by us have shown robust 9/11- (11/9)-mixed helices. The demonstration of antimicrobial activity[6] and protein surface recognition[7] by α/β-peptides has generated a lot of research interest, creating an imminent need to find new secondary structural elements for such exploration in this class of peptides. It has been suggested that preorganization[4] of β-residues is essential for the helix stability, whereas the β-branched side chains in α-residues lower the propensity of helix formation. In β-peptides, enhanced helical stability has been very well documented for β-residues with branched side chains.[8] On the basis of the very robust design of mixed helices from Caa's with "alternating chirality"[9] as well as of the above considerations on the helical stability, in the present communication, we report the synthesis of peptides **1**−**5** (Figure 1) and the identification of new robust left-handed 11/

* IICT Communication No. 051005.
† Centre for Nuclear Magnetic Resonance.
‡ D-211, Discovery Laboratory.

(1) (a) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071−13072. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.
(2) Hayen, A.; Schmitt, M. A.; Nagassa, N.; Thomson, K. A.; Gellman, S. H. *Angew. Chem.* **2004**, *116*, 511−516; *Angew. Chem., Int. Ed.* **2004**, *43*, 505−510.
(3) De Pol, S.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. *Angew. Chem.* **2004**, *116*, 517−520; *Angew. Chem., Int. Ed.* **2004**, *43*, 511−514.
(4) Schmitt, M. A.; Choi, S. H.; Guzei, A. I.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 13130−13131.
(5) Sharma, G. V. M.; Nagendar, P.; Jayaprakash, P.; Radha Krishna, P.; Ramakrishna, K. V. S.; Kunwar, A. C. *Angew. Chem.* **2005**, *117*, 6028−6032; *Angew. Chem., Int. Ed.* **2005**, *44*, 5878−5882.
(6) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2004**, *126*, 6848−6849.
(7) Sadowsky, J. D.; Schmitt, M. A.; Lee, H.-S.; Naoki, U.; Wang, S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966−11968.
(8) (a) Raguse, T. L.; Lai, J. R.; Gellman, S. H. *Helv. Chim. Acta* **2002**, *85*, 4154−4164. (b) Hart, S. A.; Bahadoor, A. B. F.; Mathews, E. E.; Qiu, X. J.; Schepartz, A. *J. Am. Chem. Soc.* **2003**, *125*, 4022−4023.
(9) (a) Sharma, G. V. M.; Ravinder Reddy, K.; Radha Krishna, P.; Ravi Sankar, A.; Narsimulu, K.; Kiran Kumar, S.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *J. Am. Chem. Soc.* **2003**, *125*, 13670−13671. (b) Sharma, G. V. M.; Ravinder Reddy, K.; Radha Krishna, P.; Ravi Sankar, A.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *Angew. Chem.* **2004**, *116*, 4051−4055; *Angew. Chem., Int. Ed.* **2004**, *43*, 3961−3965.

10.1021/jo0612980 CCC: $33.50
Published on Web 09/27/2006

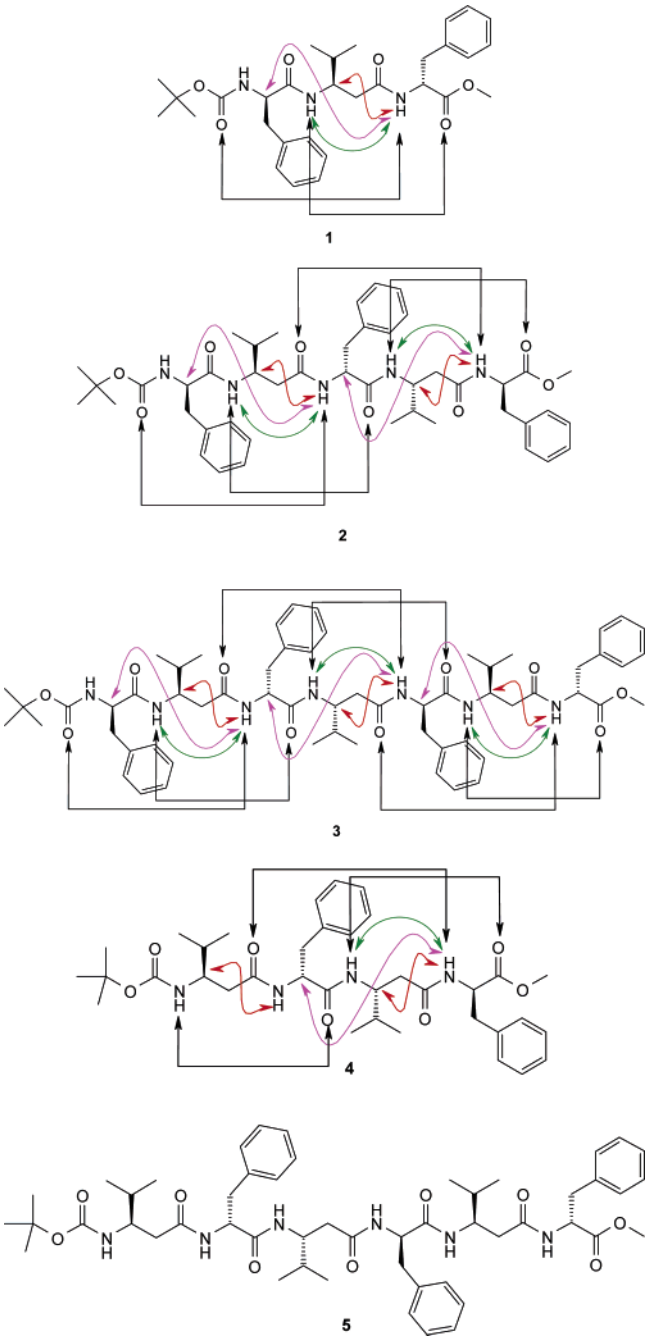


FIGURE 1. Structures of peptides **1**−**5**; H-bonds (solid lines) and diagnostic nOe's (curved lines) in **1**−**4**.

9-mixed helices in the peptides containing alternating α-D-Phe and β^3-hVal, from incisive NMR, CD, and molecular dynamics (MD) studies.

Hofmann et al.[10] have carried out a detailed theoretical study of α/β-peptides with a dimeric repetitive pattern. They have shown that the most favored structure in apolar solvents is a mixed helix with a nine-membered H-bond in the forward direction and an 11-membered H-bond in the backward direction of the peptide chain. They further distinguish between the two possibilities of such helices. A structure where an α-amide proton participates in a nine-membered (9-mr) H-bond and a

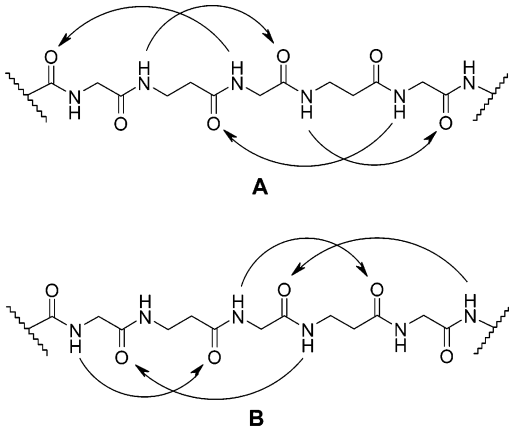


FIGURE 2. Hydrogen-bonding pattern in (A) 11/9- and (B) 9/11-mixed helices in α/β-hybrid peptides.

β-amide proton is involved in an 11-membered (11-mr) H-bond is referred to as a 9/11-helix (Figure 2). On the other hand, when the α-amide proton makes an 11-mr pseudoring and the β-amide proton participates in a 9-mr ring, it is called an 11/9-helix (Figure 2). However, in our earlier studies,[5] the mixed 9/11- and 11/9-helices have been differentiated depending upon whether the first H-bonded pseudoring was 9-mr or 11-mr, respectively. On the basis of the new convention,[10] these mixed helices correspond to the family of the 11/9-helix.[5]

Results and Discussions

1. Synthesis of Peptides 1−5. The α/β-peptides **1**−**5** (Scheme 1) have been synthesized in the solution phase from Boc-β^3-hVal-OMe **6** and Boc-D-Phe-OH **7** by the standard peptide coupling method (EDCI/HOBt). First, **6** was converted into the corresponding amine salt **8** on exposure to CF_3COOH in CH_2Cl_2 for 3 h and then coupled with acid **7** in the presence of EDCI/HOBt and Et_3N in CH_2Cl_2 for 12 h to afford the dipeptide **9**. The ester group in **9** was saponified with LiOH in THF/MeOH/H_2O (2:1:1) to give acid **10** (99%), which on coupling with H-D-Phe-OMe **11** under the above conditions gave tripeptide **1** (61.3%). Unmasking of Boc protection in **1** with CF_3COOH and coupling of the resulting salt **12** with acid **10** afforded pentapeptide **2** (93.6%). Similarly, treatment of **2** with CF_3COOH gave the amine salt **13**, which on further coupling with acid **10** furnished the heptapeptide **3** (38.8%).

Likewise, the peptides **4** and **5** (Scheme 1), having β^3-hVal at the N-terminus, were prepared adopting the above procedure using the amine salts **12** and **13**. Accordingly, **6** was saponified to acid **14** and coupled independently with **12** and **13** to afford the tetrapeptide **4** and hexapeptide **5**, respectively.

2. Conformational Analysis of Peptides 1−5. The ^{1}H NMR spectrum[11] of peptide **1** in $CDCl_3$ shows the signatures of a stable secondary structure. Though none of the amide protons has a chemical shift (δNH) >7 ppm, a small change of 0.54 ppm in the NH(2) chemical shift ($\Delta\delta$NH), during the solvent titration studies,[12] confirms its participation in H-bonding. For the β-hVal residue, $^3J_{NH-C\beta H} = 9.8$ Hz and $^3J_{C\alpha H-C\beta H} < 5$ Hz are consistent with the values of dihedral angles ϕ (C(O)−N−Cβ−Cα) ∼ 100° and θ (N−Cβ−Cα−C(O)) ∼ ± 60°,

(10) Baldauf, C.; Gunther, R.; Hofmann, H. *Biopolymers* (*Pept. Sci.*) **2006**, *84*, 408−413.

(11) See Supporting Information.

(12) Solvent titration studies were carried out by sequentially adding up to 33% of DMSO-d_6 to $CDCl_3$ solutions of the peptides.

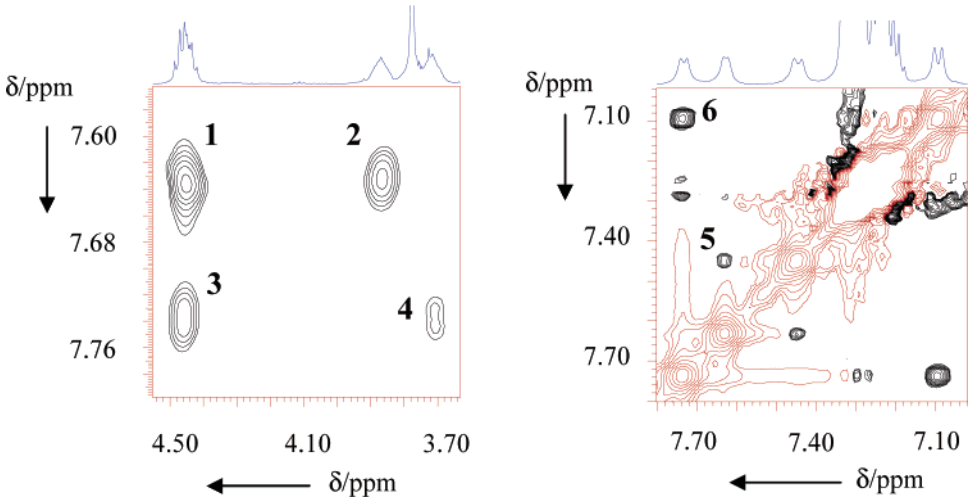
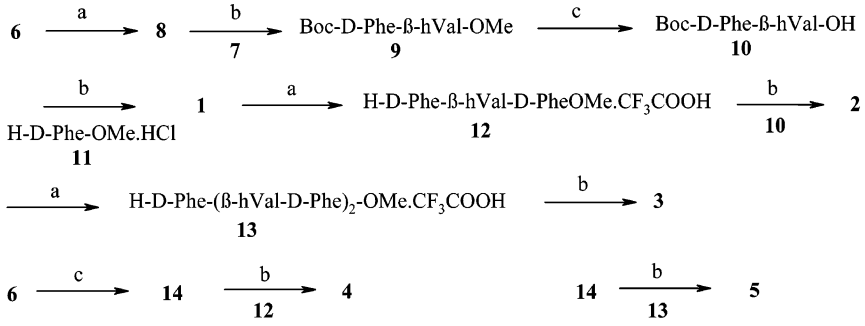


FIGURE 3. Characteristic nOe's in the ROESY spectrum of **2**: (1) CαH(1)/NH(3), (2) Cβ H(2)/NH(3), (3) CαH(3)/NH(5), (4) Cβ H(4)/NH(5), (5) NH(2)/NH(3), (6) NH(4)/NH(5).

SCHEME 1. Synthesis of Peptides 1−5



a) CF_3COOH, CH_2Cl_2 (1:1), 3 h; b) HOBt, EDCI, Et_3N, CH_2Cl_2, 0 °C, 12 h; c) LiOH, THF : MeOH : H_2O (2:1:1), 3 h

respectively. The unequivocal assignments for $C\alpha H_{(pro-S)}$ and $C\alpha H_{(pro-R)}$, based on strong NH(3)/$C\alpha H_{(pro-S)}$(2) and more intense $C\alpha H_{(pro-S)}$(2)/NH(2) compared to $C\alpha H_{(pro-R)}$(2)/NH(2) nOe's, support a value of θ of about −60°, an important signature for a left-handed helix. From the conformationally diagnostic nOe's, CαH(1)/NH(3), Cβ H(2)/NH(3), and NH(2)/NH(3), nucleation of an 11/9-helix is evident. $^3J_{NH-C\alpha H}$ values of 7.5 and 8.5 Hz for D-Phe(1) and D-Phe(3), respectively, are not very distinctive and reflect the influence of fraying in the termini. However, in light of the expected value of C(O)−N−Cα−C(O) (ϕ) ∼ 70° for the α-residues in 11/9-helices,[5] the couplings might even correspond to a predominant single conformation. The data presented above, along with the CD spectrum (discussed in latter sections), show a significant propensity of an unprecedented left-handed 11/9-helix in **1**. To have more definitive evidence for the underlying structures in this family, peptides **2** and **3** were investigated.

For **2**, the ^{1}H NMR spectrum showing four amide protons, except NH(1), at δ > 7 ppm and the solvent titration studies ($\Delta\delta$NH < 0.31 ppm)[12] support their participation in H-bonding. For β-hVal(2) and β-hVal(4), $^3J_{C\alpha H-C\beta H}$ < 4.7 Hz and $^3J_{NH-C\beta H}$ = 10.0 Hz are in conformity with $\theta \sim \pm 60°$ and $\phi \sim 100°$, respectively. In light of our deductions of $\phi \sim 70°$, for the α-residues in the 11/9-helix,[5] we tentatively believe that, for D-Phe-(1) and D-Phe(3), the values of $^3J_{NH-C\alpha H}$ = 7.0 and 6.7 Hz, respectively, are arising due to a predominant population with $|\phi| \sim 70°$. This observation was also supported by the low-temperature studies at 263 K, resulting in an enhanced population of the lowest-energy conformers, which showed values of $^3J_{NH-C\alpha H}$ = 6.1 and 5.6 Hz for D-Phe(1) and D-Phe(3), respectively. $C\alpha H_{(pro-S)}$ and $C\alpha H_{(pro-R)}$ protons were assigned as discussed for **1**, and a value of about −60° for θ was deduced. In the ROESY spectrum (Figure 3), the distinctive nOe's for an 11/9-helix, CαH(1)/NH(3) and CαH(3)/NH(5), consistent with an 11-mr H-bond between Boc CO−NH(3) and NH(5)−CO(2) and NH(2)/NH(3) and NH(4)/NH(5) supporting 9-mr H-bonds between NH(2)−CO(3) and NH(4)−CO(5) were observed. The NMR data provide compelling evidence for a left-handed 11/9/11/9-H-bonded pattern for **2**.

In a detailed study of **2** in CD_3OD and CD_3OH, weak signatures of the 11/9-helix are noticed. $^3J_{NH-C\alpha H}$ and $^3J_{NH-C\beta H}$ are similar to those in $CDCl_3$ solution. The disappearance time of the NH(2) and NH(4) resonances is about 8 h (Figure 4), suggesting some of the molecular population is involved in H-bonding. The theoretical calculations of Hofmann et al.[10] also showed that the mixed helices are more stable in apolar solvents.

Despite the poor solubility in $CDCl_3$, propagation of an 11/9-helix in peptide **3** was unmistakably noticed. The ^{1}H NMR spectrum showed NH(2)−NH(7) at δ > 7 ppm as well as a very small $\Delta\delta$NH of <0.26 ppm in the solvent titration studies[12] confirming their involvement in H-bonding. Line broadening precluded determination of some of the $^3J_{NH-C\alpha H}$ values at 303 K; however, studies at 243 K showed $^3J_{NH-C\alpha H}$ = 6.7, 5.8, and 6.4 Hz for D-Phe(1), D-Phe(3), and D-Phe(5), respectively, corresponding to a value of $\phi \sim 70°$. Similarly, for β-hVal residues, $^3J_{NH-C\beta H}$ > 9.8 Hz and $^3J_{C\alpha H-C\beta H}$ < 5.0 Hz, along

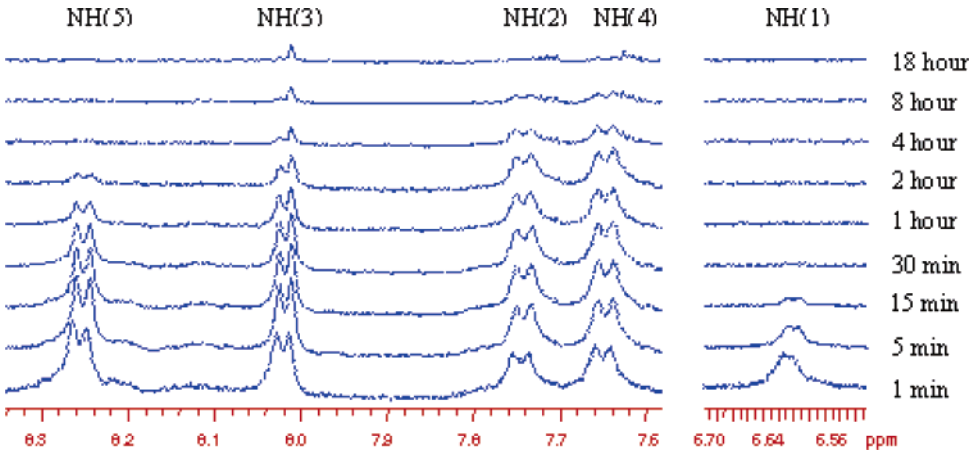


FIGURE 4. H/D exchange of amide protons for **2** in CD_3OD (500 MHz) at 303 K.

with the unequivocal assignment for $C\alpha H_{(pro-S)}$ and $C\alpha H_{(pro-R)}$ protons, imply that $\phi \sim 100°$ and $\theta \sim -60°$. The nOe correlations, $C\alpha H(1)/NH(3)$, $C\alpha H(3)/NH(5)$, $C\alpha H(5)/NH(7)$, NH(2)/NH(3), NH(4)/NH(5), and NH(6)/NH(7), in addition to the information on H-bonding and the dihedral angles, confirmed 11-mr H-bonds between NH(3)−Boc CO, NH(5)−CO(2), and NH(7)−CO(4) and 9-mr H-bonds between NH(2)−CO(3), NH-(4)−CO(5), and NH(6)−CO(7). These observations amply support a left-handed 11/9-helix with an 11/9/11/9/11/9-H-bonded configuration in **3**.

Like our earlier studies on α/β-peptides with β-Caa,[5] **4** and **5** with the β-hVal residue at the N-terminus are also expected to exist as 11/9-helices. The ^{1}H NMR spectrum of **4** showed NH(3) and NH(4) at $\delta > 7$ ppm, and solvent titration studies confirmed their participation in H-bonding.[12] Further, the characteristic long-range nOe's, $C\alpha H(2)/NH(4)$, NH(1)/NH(2), and NH(3)/NH(4), as well as the couplings, providing information on some of the backbone dihedral angles, and the CD spectrum yield strong evidence for the presence of a unique left-handed helix with a 9/11/9-H-bonded pattern. For peptide **5**, poor solubility did not permit determination of detailed structural information. However, CD spectra (discussed below) showed characteristic signatures of a left-handed mixed 11/9-helix.

For the β-hVal residue, especially in **2** and **3**, $^3J_{C\beta H-C\gamma H}$ was found to be ~10 Hz, suggesting the preponderance of a single conformer with CβH and CγH being antiperiplanar to each other. Further support for this comes from the nOe's between CγH of β-hVal(i) and $C\alpha H_{(pro-S)}$ of β-hVal(i+2) in **2** and **3**. On the other hand, it was intriguing that only the D-Phe residue at the C-terminus shows $^3J_{C\alpha H-C\beta H_{(pro-R)}} > 10$ Hz and $^3J_{C\alpha H-C\beta H_{(pro-S)}} < 5$ Hz indicating a large population of a single conformer about the $C_\alpha-C_\beta$ bond, which is more conspicuous as the length of the peptide increases. It was observed that the most populated conformer, with $\chi_1(N-C\alpha-C\beta-C\gamma) \sim 180°$, had populations of about 80% for **2** and of 90% for **3**.[13]

The CD studies for **1**, **2**, and **4** were undertaken in 50 μM MeOH solutions (Figure 5), whereas for **3** and **5**, because of poor solubility, the spectra were obtained in saturated solutions.[11] The spectra show two very distinct minima at 196 and 220 nm, with no isodichroic point. The stronger peak at about 198 nm resembles those observed in 10/12-[9,14] as well as in 11/9-mixed helices[5] at about 200 nm, whereas the weaker peak corresponds

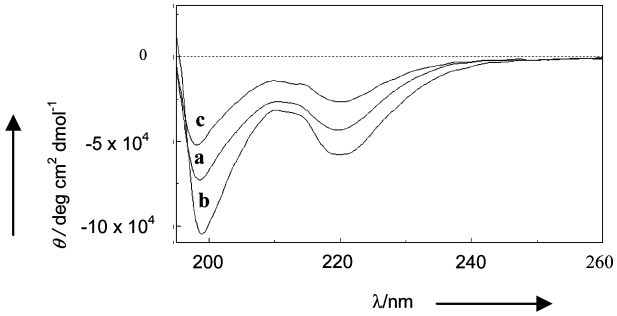


FIGURE 5. CD spectra of peptides (a) **1**, (b) **2**, and (c) **4** in CH_3OH.

to the broad peak at about 225 nm in an 11/9-helix.[5] The negative intensities of the peaks very clearly indicate the change in the handedness in these helices compared to those reported earlier.

The MD calculations for **1**−**4** were carried out using the quantitative restraints obtained from the ROESY spectra using the volume integrals and a two-spin approximation. Figure 6 depicts a superposition of the 25 lowest-energy structures for **2**. For the sake of clarity, the side chains have been replaced with a methyl group after the MD calculations. Maximum violations for the NMR-derived constraints for **2** and **3** are 0.18 and 0.20 Å, respectively. Average pairwise backbone and heavy atom root-mean-square deviations are 0.19 and 0.41 Å for **2** and 0.17 and 0.42 Å for **3**, respectively. The average values of backbone dihedral angles were obtained by excluding the first and the last residues. In **2** the ϕ, θ, and $C\beta-C\alpha-C(O)-N$ (ψ) for the β-residues are $-93 \pm 8°$, $-58 \pm 1°$, and $85 \pm 1°$, respectively, whereas for the α-residue, $\phi = 72 \pm 3°$ and $C\alpha-C(O)-N-C\alpha$ (ψ) $= -141 \pm 2°$. The corresponding values for **3** are $-94 \pm 4°$, $-60 \pm 2°$, $86 \pm 2°$, $76 \pm 2°$, and $-142 \pm 5°$, respectively.[11] These values are very similar to those reported for mixed 11/9-helices in α/β-peptides derived from a C-linked carbo-β^3-amino acid.[5]

Conclusions

We have found that alternating sequences of β^3-hVal and D-Phe provide stable and robust mixed 11/9-helices in peptides

(13) Demarco, A.; Llinas, M.; Wuthrich, K.; *Biopolymers* **1978**, *17*, 617−636.

(14) (a) Seebach, D.; Gademann, K.; Schreiber, J. V.; Mathews, J. L.; Hintermann, T.; Jaun, B.; Ober, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1997**, *80*, 2033−2037. (b) Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Mathews, J. L.; Schreiber, J. V.; Ober, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1998**, *81*, 932−982.

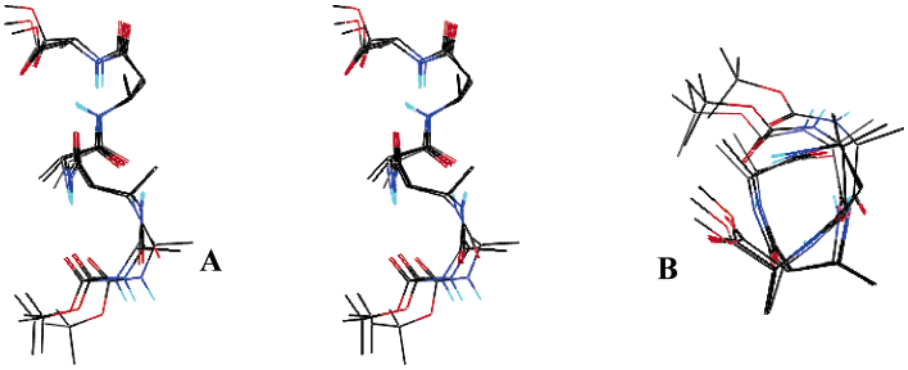


FIGURE 6. Superimposed MD structures for **2**: (A) stereoview, (B) top view from the C-terminal end.

1−**5**. Despite the markedly inherent lower propensity for helix formation in proteinogenic amino acids,[4,15] even peptides with as few as only three residues display the signatures of a helix. The hindrance in the studies due to the poor solubility of these hydrophobic peptides, during both synthesis and NMR studies, may be alleviated by incorporating amino acids with charged side chains, which may additionally endow this class of molecules with other desirable properties. Controlled folding of biomolecules into well-defined compact structures allows precise orientation and placement of the functional groups to enable them to carry out desired functions. Extending the conformational space for the family of mixed helices by using α- and β-amino acids with proteinogenic side chains opens up novel options and enhances the variety of secondary structural elements available for bioevaluation and obtaining higher-order assemblies in synthetic peptides and proteins.

Experimental Section

Boc-D-Phe-β-hVal-OMe (9). A solution of **6** (0.88 g, 3.6 mmol) and TFA (5 mL) in CH_2Cl_2 (5 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and resulting H-β-hVal-OMe·CF_3COOH (**8**) was dried under high vacuum and used without further purification.

A solution of Boc-D-Phe-OH **7** (0.95 g, 3.6 mmol), HOBt (0.58 g, 4.3 mmol), and EDCI (0.8 g, 4.3 mmol) in CH_2Cl_2 (10 mL) at 0 °C was stirred under a N_2 atmosphere for 30 min and treated sequentially with the above TFA salt **8** and Et_3N (1.5 mL, 10.8 mmol) and stirred for 12 h. Solvent was evaporated, and the residue was dissolved in $CHCl_3$ (10 mL). The organic layer was washed with aq NH_4Cl solution (2 × 10 mL), $NaHCO_3$ solution (2 × 10 mL), and brine (10 mL), dried (Na_2SO_4), and evaporated. The residue obtained was purified by column chromatography (silica gel, 10% ethyl acetate, and pet ether) to afford dipeptide **9** (1.12 g, 79.8%) as a white solid: mp 122−125 °C; IR (KBr) 3477 (w), 3340 (m), 3306 (m), 2952 (m), 1732 (m), 1687 (m), 1653 (s), 1519 (s), 1441 (m), 1368 (m), 1290 (m), 1241 (m), 1165 (m), 1046 (w), 1019 (w), 865 (w), 766 (s), 695 (m), 664 (m), 626 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−716 (m, Ar-5H), 6.27 (d, J = 9.5 Hz, 1H), 4.97 (br s, 1H), 4.31 (q, J = 7.3 Hz, 1H), 4.03 (m, 1H), 3.64 (s, 3H), 3.06 (d, J = 6.9 Hz, 2H), 2.45 (d, J = 6.0 Hz, 2H), 1.72 (m, 1H), 1.41 (s, 9H), 0.79 (d, J = 6.8 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 170.7, 155.3, 136.7, 129.2, 128.6, 126.8, 80.1, 56.1, 51.7, 51.4, 38.2, 36.3, 31.2, 28.2, 19.1, 18.4; FAB-MS 393 ($[M + 1]^+$).

Boc-D-Phe-β-hVal-D-Phe-OMe (1): A solution of ester **9** (0.40 g, 1.02 mmol) in a mixture of THF/MeOH:H_2O (2:1:1) was treated with LiOH (0.10 g, 2.5 mmol) at 0 °C and stirred for 3 h at room temperature. Solvent was evaporated, and the residue was diluted with H_2O (10 mL) and washed with ether (2 × 10 mL). The aq layer was neutralized with citric acid at 0 °C and extracted with ether (2 × 10 mL). The organic layer was dried (Na_2SO_4) and evaporated to give Boc-D-Phe-β-hVal-OH (**10**), which was used as such for a further coupling reaction.

As described for the synthesis of **9**, a mixture of **10** (0.38 g, 1.02 mmol), HOBt (0.16 g, 1.22 mmol), and EDCI (0.23 g, 1.22 mmol) in CH_2Cl_2 (8 mL) was stirred at 0 °C for 30 min and treated with H-D-Phe-OMe·HCl (**11**; 0.28 g, 1.3 mmol) and Et_3N (0.4 mL, 3.9 mmol) under a nitrogen atmosphere for 12 h. Workup and purification of the residue by column chromatography (silica gel, 25% ethyl acetate, and pet ether) afforded **1** (0.35 g, 61.3%) as a white solid: mp 178−180 °C; IR (KBr) 3283 (m), 2954 (m), 1744 (m), 1688 (m), 1656 (m), 1648 (m), 1641 (m), 1539 (m), 1455 (w), 1366 (w), 1219 (m), 1170 (w), 771 (s), 698 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42−7.15 (m, 10H, Ar−H), 7.0 (d, J = 8.5 Hz, 1H, NH-3), 6.86 (d, J = 9.8 Hz, 1H, NH-2), 5.08 (d, J = 7.5 Hz, 1H, NH-1), 4.84 (ddd, J = 5.0, 9.6, 8.5 Hz, 1H, CαH-3), 4.26 (q, J = 7.5 Hz, 1H, CαH-1), 3.75 (s, 3H, OMe), 3.20 (dd, J = 5.0, 14.2 Hz, 1H, $C\beta H_{(pro-S)}$-3), 3.08 (dd, J = 7.5, 13.6 Hz, 1H, $C\beta H_{(pro-S)}$-1), 3.01 (dd, J = 9.6, 14.2 Hz, 1H, $C\beta H_{(pro-R)}$-3), 2.96 (dd, J = 7.5, 13.6 Hz, 1H, $C\beta H_{(pro-R)}$-1), 2.38 (dd, J = 4.5, 14.0 Hz, 1H, $C\alpha H_{(pro-R)}$-1), 2.30 (dd, J = 4.5, 14.0 Hz, 1H, $C\alpha H_{(pro-S)}$-1), 1.58 (m, 1H, Cγ H-2), 1.39 (s, 9H, Boc), 0.87 (d, J = 6.6 Hz, 3H, δCH_3), 0.62 (d, J = 6.6 Hz, 3H, $\delta' CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 171.3, 170.8, 155.9, 136.7, 129.3, 129.0, 128.6, 128.5, 126.9, 80.2, 56.7, 53.6, 52.7, 52.4, 38.6, 38.1, 37.2, 30.4, 29.7, 28.2, 19.7, 19.2; FAB-MS 545 ($[M + Li]^+$).

Boc-D-Phe-β-hVal-D-Phe-β-hVal-D-Phe-OMe (2): A solution of **1** (0.18 g, 0.33 mmol) and TFA (5 mL) in CH_2Cl_2 (5 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure, and the resulting H-D-Phe-β-hVal-D-Phe-OMe·CF_3COOH (**13**) was dried under high vacuum and used without further purification.

A solution of **10** (0.12 g, 0.32 mmol), HOBt (0.05 g, 0.37 mmol), and EDCI (0.75 g, 0.37 mmol) in CH_2Cl_2 (8 mL) was stirred at 0 °C for 30 min and treated with the above H-D-Phe-β-hVal-D-Phe-OMe·CF_3COOH (**12**) and Et_3N (0.04 mL, 10 mmol) and stirred for 12 h. Solvent was removed, and the resultant solid was washed with pet ether and a 1:1 mixture of pet ether and EtOAc to give **2** (0.24 g, 93.6%) as a white solid: mp 206−210 °C; IR (KBr) 3301 (s), 3065 (m), 2961 (s), 1756 (s), 1691 (s), 1653 (s), 1541 (s), 1460 (s), 1383 (s), 1169 (s), 1045 (m), 990 (m), 855 (w), 748 (s), 699 (s), 493 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42−7.10 (m, 15H, Ar−H), 7.74 (d, J = 8.5 Hz, 1H, NH-5), 7.63 (d, J = 6.72 Hz, 1H, NH-3), 7.45 (d, J = 10.0 Hz, 1H, NH-2), 7.09 (d, J = 10.0 Hz, 1H, NH-4), 5.20 (d, J = 7.0 Hz, 1H, NH-1), 4.79 (ddd, J = 10.9, 8.5, 4.0 Hz, 1H, CαH-5), 4.46 (q, J = 6.7 Hz, 1H, CαH-3), 4.44 (q, J = 7.0 Hz, 1H, CαH-1), 3.87 (tt, J = 10.0, 4.5 Hz, 1H, Cβ H-1), 3.77 (s, 3H, OCH_3), 3.18 (dd, J = 13.8, 4.0 Hz, 1H, Cβ′H-5), 3.12 (dd, J = 13.4, 7.0 Hz, 1H, Cβ′H-1), 3.07 (dd, J = 12.9, 6.7

(15) Leplae, P. R.; Fisk, J. D.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 6820−6821.

Hz, 1H, Cβ'H-3), 3.04 (dd, J = 12.9, 6.7 Hz, 1H, CβH-3), 2.98 (dd, J = 13.4, 7.0 Hz, 1H, CβH-1), 2.97 (dd, J = 13.8, 10.9 Hz, 1H, CβH-5), 2.42 (dd, J = 13.0, 4.5 Hz, 1H, CαH$_{(pro-R)}$-2), 2.40 (dd, J = 13.0, 4.5 Hz, 1H, CαH$_{(pro-S)}$-2), 2.35 (dd, J = 12.5, 3.4 Hz, 1H, CαH$_{(pro-R)}$-4), 2.17 (dd, J = 12.5, 4.7 Hz, 1H, CαH$_{(pro-S)}$-4), 1.57 (m, 1H, CγH-2), 1.52 (m, 1H, CγH-4), 1.38 (s, 9H, Boc), 0.95 (d, J = 6.7 Hz, 3H, CδMe-2), 0.93 (d, J = 6.7 Hz, 3H, CδMe-4), 0.64 (d, J = 6.7 Hz, 3H, Cδ'Me-2), 0.51 (d, J = 6.7 Hz, 3H, Cδ'Me-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.6, 172.6, 172.3, 171.8, 171.0, 156.2, 137.3, 137.0, 136.6, 129.4, 129.2, 129.1, 128.58, 128.3, 126.9, 126.7, 80.2, 57.4, 56.7, 54.3, 53.7, 53.1, 52.5, 38.6, 38.4, 38.0, 37.1, 36.5, 30.1, 29.7, 28.3, 20.0, 19.8, 19.7, 19.5; ES$^+$ MS: 801 ([M + 1]$^+$).

Boc-D-Phe-β-hVal-D-Phe-β-hVal-D-Phe-β-hVal-D-Phe-OMe (3): A solution of **2** (0.13 g, 0.16 mmol) and TFA (5 mL) in CH_2Cl_2 (5 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure, and the resulting H-D-Phe-β-hVal-D-Phe-β-hVal-D-Phe-OMe·CF_3COOH (**13**) was dried under high vacuum and used without further purification.

A solution of **10** (0.05 g, 0.15 mmol), HOBt (0.025 g, 0.18 mmol), and EDCI (0.035 g, 0.18 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min and treated with the above TFA salt **13** and Et_3N (0.05 mL, 0.8 mmol) and then stirred for 12 h under a nitrogen atmosphere. Solvent was removed, and the resultant solid was washed with pet ether and a 1:1 mixture of pet ether and EtOAc to give **3** (0.06 g, 38.8%) as a white solid: mp > 250 °C; IR (KBr) 3281 (s), 3066 (m), 3031 (m), 2963 (s), 1722 (s), 1644 (s), 1550 (s), 1454 (m), 1368 (m), 1292 (m), 1242 (m), 1172 (m), 1119 (m), 1050 (w), 960 (w), 914 (w), 742 (m), 698 (s), 621 (w); ^{1}H NMR (750 MHz, $CDCl_3$, 5 °C) δ 8.21 (d, J = 6.4 Hz, 1H, NH-5), 7.89 (d, J = 10.1 Hz, 1H, NH-4), 7.86 (d, J = 8.4 Hz, 1H, NH-7), 7.66 (d, J = 5.8 Hz, 1H, NH-3), 7.65 (d, J = 9.8 Hz, 1H, NH-2), 7.33 (d, J = 10.0 Hz, 1H, NH-6), 7.0−7.4 (m, 20H, Ar−H), 5.14 (d, J = 6.7 Hz, 1H, NH-1), 4.72 (ddd, J = 12.0, 8.4, 3.6 Hz, 1H, CαH-1), 4.66 (dq, J = 7.6, 5.8 Hz, 1H, CαH-3), 4.36 (ddd, J = 9.0, 6.4, 5.4 Hz, 1H, CαH-5), 3.81 (m, 1H, CβH-2), 3.75 (s, 3H, OMe), 3.71 (m, 1H, CβH-4), 3.68 (m, 1H, CβH-6), 3.11 (dd, J = 14.0, 3.6 Hz, 1H, CβH$_{(pro-S)}$-7), 3.08 (dd, J = 13.7, 6.7 Hz, 1H, CβH$_{(pro-S)}$-1), 3.05 (m, 2H, CβH-3), 3.04 (dd, J = 13.0, 9.0 Hz, 1H, CβH$_{(pro-S)}$-5), 2.93 (dd, J = 13.7, 6.7 Hz, 1H, CβH$_{(pro-R)}$-1), 2.92 (dd, J = 13.0, 6.4 Hz, 1H, CβH$_{(pro-R)}$-5), 2.87 (dd, J = 14.0, 12.0 Hz, 1H, CβH$_{(pro-R)}$-7), 2.39 (dd, J = 13.0, 4.5 Hz, 1H, CαH$_{(pro-R)}$-2), 2.35 (m, 2H, CαH$_{(pro-R)}$-4 and CαH$_{(pro-S)}$-2), 2.31 (dd, J = 12.6, 3.1 Hz, CαH$_{(pro-R)}$-6), 2.20 (m, 1H, CαH$_{(pro-S)}$-4), 2.09 (dd, J = 12.6, 5.0 Hz, 1H, CαH$_{(pro-S)}$-6), 1.56 (m, 1H, CγH-6), 1.55 (m, 1H, CγH-2), 1.49 (m, 1H, CγH-4), 1.31 (s, 9H, Boc), 0.94 (d, J = 6.7 Hz, 1H, Cδ' Me-6), 0.93 (d, J = 6.4 Hz, 1H, Cδ' Me-4), 0.92 (d, J = 6.5 Hz, 1H, Cδ' Me-2), 0.61 (d, J = 6.7 Hz, 1H, Cδ' Me-6), 0.60 (d, J = 6.5 Hz, 1H, Cδ' Me-2), 0.47 (d, J = 6.4 Hz, 1H, Cδ' Me-4); HRMS-MS ($C_{60}H_{81}N_7O_{10}Na$) 1082.5942 (calcd), 1082.5906 (obsd).

Boc-β-hVal-D-Phe-β-hVal-D-Phe-OMe (4): A solution of **14** (0.07 g, 0.46 mmol), HOBt (0.07 g, 0.55 mmol), and EDCI (0.1 g, 0.55 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min and treated with TFA salt **12** (0.03 g, 0.55 mmol) and Et_3N (0.08 mL, 1.65 mmol) and stirred for 12 h under a nitrogen atmosphere. Solvent was removed, and the resultant solid was washed with pet ether and a 1:1 mixture of pet ether and EtOAc to give **3** (0.055 g, 29.6%) as a white solid: mp 250−254 °C; IR (KBr) 3308 (s), 3064 (m), 2962 (s), 1755 (s), 1691 (s), 1646 (s), 1536 (s), 1454 (m), 1368 (m), 1247 (m), 1173 (s), 1047 (m), 960 (w), 749 (m), 699 (s), 587 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, J = 7.9 Hz, 1H, NH-4), 7.07 (d, J = 9.2 Hz, 1H, NH-3), 6.79 (d, J = 6.5 Hz, 1H, NH-2), 5.05 (d, J = 9.2 Hz, 1H, NH-1), 4.75 (ddd, J = 9.4, 7.9, 5.0 Hz, 1H, CαH-4), 4.51 (q, J = 6.5 Hz, 1H, CαH-2), 3.80 (m, 1H, CβH-3), 3.74 (s, 3H, OMe), 3.64 (dddd, J = 10.0, 7.7, 6.4, 4.4 Hz, 1H, CβH-1), 3.14 (dd, J = 13.5, 5.0 Hz, 1H, CβH$_{(pro-S)}$-4), 3.13 (dd, J = 13.5, 6.5 Hz, 1H, CβH$_{(pro-S)}$-2), 2.99 (dd, J = 13.5, 6.5 Hz, 1H, CβH$_{(pro-R)}$-2), 2.97 (dd, J = 13.5, 9.4 Hz, 1H, CβH$_{(pro-R)}$-4), 2.39 (dd, J = 14.5, 7.7 Hz, 1H, CαH$_{(pro-R)}$-1), 2.36 (m, 2H, CαH$_{(pro-R)}$-3 and CαH$_{(pro-S)}$-3), 2.33 (dd, J = 14.5, 4.4 Hz, 1H, CαH$_{(pro-S)}$-1), 1.67 (m, 1H, CγH-1), 1.59 (m, 1H, CγH-3), 0.88 (d, J = 6.6 Hz, 3H, CδH-Me-3), 0.87 (d, J = 6.6 Hz, 6H, CδH-Me-1 and CδH-Me′-1), 0.65 (d, J = 6.6 Hz, 3H, CδH-Me′-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 171.6, 171.2, 171.0, 157. 0, 136.8, 129.3, 129.1, 128.6, 126.9, 79.5, 55.5, 54.0, 52.6, 52.4, 39.6, 38.8, 37.5, 36.9, 31.9, 30.6, 28.4, 19.7, 19.3, 18.4; FAB MS 653 [M + 1]$^+$.

Boc-β-hVal-D-Phe-β-hVal-D-Phe-β-hVal-D-Phe-OMe (5): A solution of **14** (0.01 g, 0.045 mmol), HOBt (0.008 g, 0.055 mmol), and EDCI (0.01 g, 0.055 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min and treated with TFA salt (**13**) of **2** (0.03 g, 0.04 mmol) and Et_3N (pH adjusted to basic) and then stirred under a nitrogen atmosphere for 12 h. Solvent was removed, and the resultant solid was washed with pet ether and a 1:1 mixture of pet ether and EtOAc to give **5** (0.036 g, 90.7%) as a white solid: mp > 250 °C; IR (KBr) 3294 (s), 3064 (w), 3031 (w), 2961 (m), 2873 (w), 1745 (m), 1690 (s), 1645 (s), 1538 (s), 1455 (m), 1368 (m), 1247 (m), 1134 (s), 748 (m), 699 (m), 638 (m), 616 (m); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 6.5 Hz, 1H), 7.61 (d, J = 9.8 Hz, 1H), 7.41−7.13 (m, 15H, Ar−H), 5.10 (d, J = 9.8 Hz, 1H), 4.77 (m, 1H), 4.69 (q, J = 7.1 Hz, 1H), 4.37 (m, 1H), 3.86 (m, 1H), 3.78 (s, 3H, OMe), 3.68 (m, 2H), 3.30−2.87 (m, 6H), 2.5−2.15 (m, 6H), 1.44 (s, 9H, Boc), 0.96 (d, J = 6.2 Hz, 3H), 0.93 (d, J = 6.2 Hz, 3H), 0.85 (d, J = 6.6 Hz, 6H), 0.70 (d, J = 6.2 Hz, 3H), 0.57 (d, J = 6.2 Hz, 3H); FAB MS 914 [M + 1]$^+$.

Acknowledgment. S.K.K. is thankful to CSIR, New Delhi, for financial support. A.C.K. is thankful to M. Goerlach and R. Ramachandran for 750 MHz NMR and to INSA and DFG for financial support.

Supporting Information Available: NMR details, solvent titration plots, and distance constraints used in MD calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0612980