This thesis set out to explore the conformational properties of short designed peptide sequences, in which transitions between structural states may be anticipated. The use of conformationally constrained residues like α-aminoisobutyric acid (Aib) and D-proline (^{D}Pro) permits the design of model sequences for structural studies. The principle of imposing conformational constraints by multiple substitutions at backbone atoms in aminoacid residues may also be extended to the higher homologs of α-amino acids, namely β and γ residues. The experimental results presented in this thesis also examine the potential of using cross-strand interactions between aromatic residues as a probe of structure in designed peptide β-hairpins.

Chapter 1 provides a very brief introduction to the necessary background on which the experimental studies in this thesis are based.

Chapter 2 describes studies aimed at establishing chain length effects on helix-hairpin conformational distributions in short synthetic sequences, containing centrally positioned Aib-^{D}Ala and Aib-Aib segments.The Aib-^{D}Ala dipeptide segment has a tendency to form both type-I'/III' and type-I/III β-turns. The occurrence of prime turns facilitates the formation of β-hairpin conformations, while type-I/III turns can nucleate helix formation. The octapeptide Boc-Leu-Phe-Val-Aib-^{D}Ala-Leu-Phe-Val-OMe (**1**) has been previously shown to form a β-hairpin in the crystalline state and in solution. The effects of sequence truncation have been examined using the model peptides Boc-Phe-Val-Aib-Xxx-Leu-Phe-NHMe (**2**, **6**), Boc-Val-Aib-Xxx-Leu-NHMe (**3**, **7**) and Boc-Aib-Xxx-NHMe (**4**, **8**), where Xxx = ^{D}Ala, Aib. For peptides with central Aib-Aib segments, Boc-Phe-Val-Aib-Aib-Leu-Phe-NHMe (**6**), Boc-Val-Aib-Aib-Leu-NHMe (**7**) and Boc-Aib-Aib-NHMe (**8**) local helical conformations have been established by NMR studies in both hydrogen

bonding (CD_3OH) and non-hydrogen bonding ($CDCl_3$) solvents. In contrast, the corresponding hexapeptide Boc-Phe-Val-Aib-^{D}Ala-Leu-Phe-Val-NHMe (**2**) favors helical conformations in $CDCl_3$ and β-hairpin conformations in CD_3OH. β-Turn conformations (type-I′/III) stabilized by intramolecular 4→1 hydrogen bonds are observed for the peptide Boc-Aib-^{D}Ala-NHMe (**4**) and Boc-Aib-Aib-NHMe (**8**) in crystals. The tetrapeptide Boc-Val-Aib-Aib-Leu-NHMe (**7**) adopts an incipient 3_{10}-helical conformation stabilized by three 4→1 hydrogen bonds. The peptide Boc-Val-Aib-^{D}Ala-Leu-NHMe (**3**) adopts a novel α-turn conformation, stabilized by three intramolecular hydrogen bonds (two 4→1 and one 5→1). The Aib-^{D}Ala segment adopts a type-I' β-turn conformation. The observation of the NOE Val(1) NH↔$HNCH_3$ (5), in CD_3OH, suggests that the solid state conformation of peptide **3** is maintained in methanol solutions.

Peptide hairpins provide an ideal scaffold for exploring cross-strand interactions between residues on facing antiparallel strands. **Chapter 3** reports studies directed towards probing, aromatic interactions between facing Phe residues, positioned at the non-hydrogen bonding positions in designed octapeptide β-hairpins. The studies described in this Chapter employ ring current shifted aromatic proton resonances as a means of probing aromatic ring orientations. Crystal structures of eight peptide β-hairpins with the sequence Boc-Leu-Phe-Val-Xxx-Yyy-Leu-Phe-Val-OMe revealed that the Phe(2) and Phe(7) aromatic rings are in close spatial proximity, with a centroid-centroid distance (R_{cen}) of 4.4Å to 5.4Å between the two phenyl rings. Proton NMR spectra in chloroform and methanol solutions reveal a significant upfield shift of the Phe(7) $C^{\delta,\delta'}H_2$ protons (6.65 ppm to 7.04 ppm). Specific assignments of the aromatic protons have been carried out in the peptide Boc-Leu-Phe-Val-^{D}Pro-^{L}Pro-Leu-Phe-Val-OMe (**6**). The anticipated ring current shifts have been estimated from the aromatic ring geometries observed in

crystals for all eight peptides. Only one of the $C^{\delta,\delta'}H$ proton lies in the shielding zone, with rapid ring flipping, resulting in averaging between the two extreme chemical shifts. An approximate estimate of the population of conformations which resemble crystal state orientations may be obtained. Key nuclear Overhauser effects (NOEs) between facing Phe sidechains provide support for close similarity between the solid state and solution conformations. Temperature dependence of aromatic ring proton chemical shifts and line widths for peptide **6** (Boc-Leu-Phe-Val-^{D}Pro-^{L}Pro-Leu-Phe-Val-OMe) and the control peptide Boc-Leu-Val-Val-^{D}Pro-Gly-Leu-Phe-Val-OMe establish an enhanced barrier to ring flipping, when the two Phe rings are in proximity. Modeling studies suggest that small, conformational adjustments about the C^{α}-C^{β} (χ^1), and C^{β}-C^{γ} (χ^2) bonds of the Phe residues may be required in order to permit unhindered, uncorrelated flipping of both the Phe rings. The maintenance of specific aromatic ring orientations in organic solvents provides evidence for significant stabilizing interactions.

Earlier studies from this laboratory established that a centrally positioned ^{D}Pro-^{L}Pro-^{D}Ala segment could induce hairpin formation in nonapeptide sequences, facilitated by a *three residue* loop segment. The ^{D}Ala residue at position 6 in the nonapeptide Boc-Leu-Phe-Val-^{D}Pro-^{L}Pro-^{D}Ala-Leu-Phe-Val-OMe has been shown to adopt a left handed helical (α_L) conformation. The studies described in **Chapter 4**, examine the effects of aminoacid replacements at positions 5 and 6. NMR studies on eight nonapeptides, with the general sequence Boc-Leu-Phe-Val-^{D}Pro-Xxx-Yyy-Leu-Phe-Val-OMe are described. In the case of peptides with a central ^{D}Pro-^{L}Pro-Yyy sequence, two kinds of hairpin conformations are formed in solution. These are; i) β-hairpin structures with a central three residue loop, resulting in registered antiparallel tripeptide strands, and ii) a slipped hairpin structure, nucleated by a central ^{D}Pro-^{L}Pro type-II′ β-turn, with residue 6 being incorporated into the

C-terminal strand. The three residue loop β-hairpins are favored for ^{D}Ala(6) and Aib(6), while the ^{L}Ala(6) peptide favors a "slipped" hairpin structure. Replacement of the Pro(5) residue by ^{L}Ala results in a reduced population of three residue hairpins in the nonapeptide with the ^{D}Pro-^{L}Ala-^{D}Ala segment. Replacement of Pro(5) by Aib, abolished hairpin formation. Aromatic proton chemical shifts provide a convenient diagnostic for the presence of three residue loop hairpin conformations in these nonapeptides.

A great deal of current interest has focused on the conformations of peptides incorporating β and γ aminoacid residues. Earlier studies from this laboratory have focused on the conformational properties of the β,β′-disubstituted γ residue gabapentin (1-aminomethylcyclohexane acetic acid). Subsequent work with the related β aminoacid $\beta^{3,3}Ac_6c$ (1-aminocyclohexaneacetic acid) revealed that intramolecularly hydrogen bonded conformations are infrequently observed in short peptides. The studies described in **Chapter 5**, examine the conformational properties for model peptides containing the isomeric β-aminoacid, $\beta^{2,2}Ac_6c$ (1-aminomethylcyclohexane-1-carboxylic acid). The effect of *gem* dialkyl substituents on the backbone conformations of β amino acid residues in peptides, has been investigated using four model peptides, Boc-Xxx-$\beta^{2,2}Ac_6c$-NHMe [Xxx = Leu (**1**), Phe(**2**)] and Boc-Xxx-$\beta^{3,3}Ac_6c$-NHMe [Xxx = Leu (**3**), Phe(**4**)]. Tetrasubstituted carbon atoms restrict the ranges of stereochemically allowed conformations about flanking single bonds. The crystal structure of Boc-Leu-$\beta^{2,2}Ac_6c$-NHMe (**1**) establishes a C_{11} hydrogen bonded turn in the αβ hybrid sequence. The observed torsion angles [α($\phi\approx$ -60°, $\psi\approx$ -30°), β($\phi\approx$ -90°, $\theta\approx$ 60°, $\psi\approx$ -90°)] correspond to a C_{11} helical turn, which is a backbone expanded analog of the type III β-turn in αα sequences. The crystal structure of the peptide Boc-Phe-$\beta^{3,3}Ac_6c$-NHMe (**4**) establishes a

C_{11} hydrogen bonded turn with distinctly different backbone torsion angles [α($\phi\approx$ -60°, $\psi\approx$ 120°), β($\phi\approx$ 60°, $\theta\approx$ 60°, $\psi\approx$ -60°)], which corresponds to a backbone expanded analog of the type II β-turn, observed in $\alpha\alpha$ sequences. In peptide **4**, the two molecules in the asymmetric unit adopt backbone torsion angles of opposite signs. In one of the molecules, the Phe residue adopts an unfavourable backbone conformation, with the energetic penalty being offset by favourable aromatic interactions between proximal molecules in the crystal. NMR studies provide evidence for the maintenance of folded structures in solution, in these $\alpha\beta$ hybrid sequences.

The result presented in this thesis suggests that it should be possible to construct designed synthetic peptides, which can undergo transitions between two distinct and energetically favourable conformational states. The ability to design peptide sequences that can undergo switching between helical and β-hairpin states, or between hairpin structures with variations in connecting loop length may prove valuable in providing further insights into the factors influencing conformational dynamics.