General Introduction

Bioorganic chemistry has expanded rapidly over the past three decades, enriching the disciplines of biology and chemistry and providing important insights into the working of biological systems at a molecular level. Peptides and proteins control all biological processes at transcriptional, translational or posttranslational levels. Yet, at the molecular level, our understanding of the relationship between structure and function remains rudimentary. The problems involved in clarifying these issues are somewhat different for peptides and proteins. The dissection of multidomain proteins into small, synthetic, conformationally restricted components is an important step in the design of low molecular weight nonpeptides and modified peptides that mimic the activity of the native proteins. Mimetics of critical functional domains might possess beneficial properties in comparison to the intact proteinaceous species with regards to specificity and therapeutic potential, and are valuable probes for the study of molecular recognition events. Peptide mimetics are powerful tools for the study of molecular recognition and are providing a unique opportunity to dissect and investigate structure-function relationships in peptides and complex proteins.

Peptides are small segments of proteins made up of linear sequences of amino acids joined together by amide bonds having partial double bond character with nearly trans configuration. The biological functions and activities of the proteins or polypeptides depend upon their specific three-dimensional structures. The native three-dimensional conformation of a protein or oligopeptides is maintained by a range of non-covalent interactions such as electrostatic forces, hydrogen bonds, Van der Waals interactions, hydrophobic interactions, \(\pi\)-stacking interactions etc.\(^1\) In some cases covalent disulfide bonds give stability to the three-dimensional structure.\(^1\) Previously, different approaches have been pursued to determine the structure and folding of a protein\(^2\), yet, it is not possible to predict the tertiary folding pattern of a protein simply on the basis of its primary structure. Examination of protein 3-dimensional structure suggests that complex tertiary folds and quaternary association can be deconstructed into a limited number of secondary structural modules such as helices, strands, hairpins and reverse turns.\(^3\)
**Structural Organization in Proteins**

Hierarchical nature of protein structural organization is illustrated in Figure 1. Only a few secondary structures are discussed below, they are relevant to the work embodied in the thesis.

![Diagram](image.png)

**Figure 1.** (a) Primary and secondary structural organization observed in proteins. $R_1$, $R_2$, $R_3$, $R_4$ and $R_5$ refer to various side chains of amino acid residues, (b) some super secondary, tertiary and quaternary structures existing in proteins.
Backbone Conformational Parameters: Tools for Analyzing Protein Secondary Structures

The backbone conformation of a polypeptide chain depends upon its main chain torsion angles (Figure 2). The value of dihedral angle $\mathrm{C}^\alpha$-$\mathrm{N}$-$\mathrm{C}'$-$\mathrm{C}^\alpha$ ($\omega$) is restricted to take an extended conformation having values nearly $180^\circ$ or $0^\circ$ due to the planarity of the peptide bond geometry. Generally the trans conformation ($\omega \sim 180^\circ$) of peptide bond is preferred over the cis conformation ($\omega \sim 0^\circ$) to avoid steric crowding of the bulky side chains present at $\mathrm{C}^\alpha$ carbon atoms of amino acid residues (Figure 2). Hence, it is practically the two other main chain torsion angles, $\mathrm{C}'$-$\mathrm{N}$-$\mathrm{C}^\alpha$-$\mathrm{C}'$ ($\phi$) and $\mathrm{N}$-$\mathrm{C}^\alpha$-$\mathrm{C}'$-$\mathrm{N}$ ($\psi$) (Figure 3) that dictate the backbone conformation of a polypeptide chain. Conformational freedom of the torsion angles, $\phi$ and $\psi$, in a polypeptide chain are restricted by steric hindrance.

![Figure 2. Trans and Cis configurations of peptide bond ($\omega$).](image)

![Figure 3. Two linked peptide units and single headed arrows indicating the backbone torsion angles $\phi$, $\psi$ and $\omega$. The torsion angles for rotation about the amino acid side chains is designated as $\chi$.](image)
The sterically allowed values of $\phi$ and $\psi$ are visualized in a contour diagram, popularly known as $\phi$, $\psi$ diagram or Ramachandran plot (Figure 4).\textsuperscript{5} The diagram exhibits that there are only three small sterically allowed regions in the Ramachandran plot that are physically accessible to a polypeptide chain, and within this region the $\phi$, $\psi$ values produces the right handed $\alpha$-helix, the parallel and antiparallel $\beta$-pleated sheets and the collagen triple helix.\textsuperscript{6,7} The $\phi$, $\psi$ values for the various secondary structures are listed in Table 1. For a particular secondary structure, $\phi$, $\psi$ values at each amino acid residues are same along the polypeptide chain. The secondary structures are stabilized by hydrogen bonding interactions. The Ramachandran plot generated for Gly, L-Ala and D-Ala residues using both normal and relaxed Van der Waals interaction are represented in Figure 5. As all proteinous amino acids excepting Glycine have C$^\beta$ carbon atom, the Alanine map can be considered to be a prototype for allowed conformations for others.

**Figure 4.** Ramachandran plot showing the allowed regions for poly-L-alanine (black region). $\alpha$, $\phi$, $\psi$ values correspond to the right handed $\alpha$-helix; $\beta$ for the antiparallel $\beta$-pleated sheet region; $\beta'$ for the parallel $\beta$-pleated sheet; C for the collagen helix.
Figure 5. The Ramachandran map for (a) Glycine (b) L-Alanine and (c) D-Alanine. The area enclosed within the solid line corresponds to the fully allowed region. The dotted line encloses the partially allowed region.
### Table 1. Parameters for defining regular secondary structures of polypeptide chains

<table>
<thead>
<tr>
<th>Structure type</th>
<th>Residues per turn</th>
<th>Residues per residue (Å)</th>
<th>Torsion angles (°)</th>
<th>Hydrogen bonding</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-helix</td>
<td>3.6</td>
<td>1.5</td>
<td>-57, -47</td>
<td>180</td>
<td>13-membered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5→1)</td>
</tr>
<tr>
<td>3_{10}-helix</td>
<td>3.0</td>
<td>2.0</td>
<td>-60, -30</td>
<td>180</td>
<td>10-membered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(4→1)</td>
</tr>
<tr>
<td>π-helix</td>
<td>4.4</td>
<td>1.15</td>
<td>-57, -70</td>
<td>180</td>
<td>16-membered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6→1)</td>
</tr>
<tr>
<td>Antiparallel β-sheet</td>
<td>2.0</td>
<td>3.4</td>
<td>-139, -135</td>
<td>178</td>
<td>b</td>
</tr>
<tr>
<td>Parallel β-sheet</td>
<td>2.0</td>
<td>3.4</td>
<td>-119, -113</td>
<td>180</td>
<td>b</td>
</tr>
<tr>
<td>Polyproline I-helix</td>
<td>3.33</td>
<td>1.9</td>
<td>-83, 158</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Polyproline II-helix</td>
<td>3.0</td>
<td>3.12</td>
<td>-78, 149</td>
<td>180</td>
<td>5</td>
</tr>
<tr>
<td>Polyglycine II-Helix</td>
<td>3.0</td>
<td>3.1</td>
<td>-80, 150</td>
<td>180</td>
<td>6</td>
</tr>
<tr>
<td>Collagen-helix</td>
<td>3.3</td>
<td>2.9</td>
<td>-60, 140</td>
<td>180</td>
<td>7</td>
</tr>
</tbody>
</table>

*References where these structures were first proposed are listed.

Hydrogen bonds are usually between strands.
Secondary Structures

The regular folding patterns along a polypeptide chain in a protein is known as secondary structures. Important, abundant and recognizable secondary structures found in proteins are reverse turns, helices, β-strands and β-hairpins.

Reverse turns

Presence of a reverse turn changes the direction of a polypeptide chain.\textsuperscript{12} Turns were first identified by Venkatachalam (1968) by model building studies and their occurrence are confirmed in peptides and proteins.\textsuperscript{13} There are three types of turns each containing a hydrogen bond between the C=O of n residue and NH of n + 3 residue and these three types of turns are designated as I, II and III. Each of these three types of turns has a backbone mirror image conformation that is also found in some proteins designated as I', II' and III' even though these conformations are disfavored due to steric hindrance.\textsuperscript{14} Various types of β-turns observed in proteins are shown in Figure 6.
Several descriptions and analyses of various reverse turn structures in proteins and peptides have been reported in the literature. The geometric parameters to form different types of β-turns are listed in Table 2.

γ-turns

Single amino acid chain inverters that can form 3→1 hydrogen bonds are known as γ-turns. In comparison with the β-turn, γ-turns are rare element of proteins. Depending upon the torsion angle, there are two types of γ-turn present in proteins, (a) inverse γ-turn and (b) classical γ-turn (Figure 7). Inverse γ-turns are frequently found in proteins in contrast to classical γ-turns. The characteristic parameters of different γ-turns are listed in Table 3.

Figure 6. Structures of different reverse turns involving L-amino acid residues. Only hydrogen bonded atoms are shown.

Figure 7. Structures of (a) classical γ-turn and (b) inverse γ-turn with a 1→3 intramolecular hydrogen bond. Only hydrogen-bonded hydrogen atoms are shown for clarity.
Table 2. Torsion angles at the two-residue reverse turns\(^a\)

<table>
<thead>
<tr>
<th>Type</th>
<th>Dihedral angles(^b)(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\phi_{+1})</td>
</tr>
<tr>
<td>I</td>
<td>-60</td>
</tr>
<tr>
<td>I'</td>
<td>60</td>
</tr>
<tr>
<td>II</td>
<td>-60</td>
</tr>
<tr>
<td>II'</td>
<td>60</td>
</tr>
<tr>
<td>III</td>
<td>-60</td>
</tr>
<tr>
<td>III'</td>
<td>60</td>
</tr>
<tr>
<td>V</td>
<td>-80</td>
</tr>
<tr>
<td>V'</td>
<td>80</td>
</tr>
<tr>
<td>Via(^c)(cis)</td>
<td>-60</td>
</tr>
<tr>
<td>Vib(^d)(cis)</td>
<td>-120</td>
</tr>
<tr>
<td>VIII</td>
<td>-60</td>
</tr>
</tbody>
</table>

\(^a\) Taken from Zimmerman and Scheraga, 1977.

\(^b\) The dihedral angles for Type I-III' were given by Venkatachalam, 1969. Type IV and VII are not listed here. Type IV has dihedral angles similar to Type I, II and III with two or more angles differing by at least 40°. Type VII has a kink in the peptide chain with \(|\psi_{i+1}|>140^\circ\) and \(|\phi_{i+2}|<60^\circ\) or \(|\psi_{i+1}|<60^\circ\) \(|\phi_{i+2}|>140^\circ\).

\(^c\) From Richardson, 1981.


Table 3. Torsion angle of the single residue turns (\(\gamma\)-turn)

<table>
<thead>
<tr>
<th>Turn type</th>
<th>(\phi)</th>
<th>(\psi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical (\gamma)-turn</td>
<td>75.0</td>
<td>-64.0</td>
</tr>
<tr>
<td>Inverse (\gamma)-turn</td>
<td>-79.0</td>
<td>69.0</td>
</tr>
</tbody>
</table>

---

\(\chi\)
**Helices**

Helices are one of the most abundant (26%) secondary structures observed in proteins. Helices are very well studied structural elements among the protein structural elements. Various types of helices proposed in the literature are illustrated in Figure 8.

![Figure 8](image)

Figure 8. A perspective view of ideal (a) $3_{10}$- (b) $\alpha$- and (c) $\pi$ helical structures. 4→1 hydrogen bonding in the $3_{10}$ helix, 5→1 hydrogen bonding in the $\alpha$-helix and 6→1 hydrogen bonding in the $\pi$ helix are observed. The broken lines indicate hydrogen bonds.

The $\alpha$-helix and $3_{10}$-helix are frequently observed in proteins. Detailed analysis on helix geometry has been reported in the literature. Characteristic geometric parameters of different helices are listed in Table 4. The criteria used for identifying different helices in proteins are:

(a) hydrogen bonding pattern,

(b) torsion angles (\(\phi\), \(\psi\)) and

(c) C'' position.
Table 4. Characteristic parameters of different helices

<table>
<thead>
<tr>
<th>Helix</th>
<th>Torsion angles (°)</th>
<th>Pitch (Å) per turn</th>
<th>Residues</th>
<th>Radius (Å)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>φ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-helices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pauling et al. (1951)b</td>
<td>-48</td>
<td>-57</td>
<td>5.5</td>
<td>3.65</td>
<td>2.3</td>
</tr>
<tr>
<td>Perutz (1951)c</td>
<td>-67</td>
<td>-44</td>
<td>5.2</td>
<td>3.67</td>
<td>2.4</td>
</tr>
<tr>
<td>Arnott and Wonacottd (1966)</td>
<td>-57</td>
<td>-47</td>
<td>5.4</td>
<td>3.54</td>
<td>2.3</td>
</tr>
<tr>
<td>Barlow and Thornton (1988)e</td>
<td>-62</td>
<td>-41</td>
<td>5.4</td>
<td>3.54</td>
<td>2.3</td>
</tr>
<tr>
<td>Toniolo and Benedetti f (1991)</td>
<td>-63</td>
<td>-42</td>
<td>5.67</td>
<td>3.63</td>
<td>-</td>
</tr>
<tr>
<td>3_{10}-helices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pauling et al. (1951)b</td>
<td>-74</td>
<td>-4</td>
<td>6.0</td>
<td>3.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Perutz (1951)c</td>
<td>-49</td>
<td>-26</td>
<td>5.8</td>
<td>3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Arnott and Wonacottd (1966)</td>
<td>-54</td>
<td>-28</td>
<td>5.8</td>
<td>3.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Barlow and Thornton (1988)e</td>
<td>-71</td>
<td>-18</td>
<td>5.4</td>
<td>3.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Toniolo and Benedetti f (1991)</td>
<td>-57</td>
<td>-30</td>
<td>6.29</td>
<td>3.24</td>
<td>-</td>
</tr>
<tr>
<td>Vainshtein et al., (1986)</td>
<td>-57</td>
<td>-70</td>
<td>10</td>
<td>4.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Adapted from Barlow and Thornton, 1988<sup>19c</sup>; b from model building; c average values for haemoglobin; d from fiber diffraction data; e average values obtained from protein data analysis; f average values obtained from peptide crystal structures.

α-helix

The α-helix was discovered through model building in 1951 by Linus Pauling at the California Institute of Technology.<sup>27</sup> He predicted that it is the energetically stable structure in protein on the basis of accurate geometrical parameters obtained from crystallographic analysis of small molecules. α-Helices have 3.6 residue per turn with 5→1 hydrogen bonds between C=O of residue n and NH of residue n + 4 and positioned at the allowed region in the bottom left quadrant of the Ramachandran plot. An α-helix
can adopt a right-handed (φ, -57; ψ, -47) or a left-handed (φ, 57; ψ, 47) arrangement depending upon the screw direction of the peptide chain. A left-handed α-helix is not allowed for L-amino acids due to the close approach of the side chains and the C=O groups. Some important features of the α-helices have been mentioned such as length distribution, conformation at the termini, geometry and bending, residue preferences at the termini, helix signals, spatial preferences of ion pairs, water insertion, hydrophobic moments, amphipathicity in helices and helix dipole.

3₁₀-helix

A 3₁₀-helix is energetically unfavorable and rare element compared to α-helix in proteins. It is characterized by 3.0 peptide units per turn with 4→1 hydrogen bonds between C=O of residue n and NH of residue n + 3 and positioned at the edge of an allowed region in Ramachandran map. This is why 3₁₀-helix is occasionally observed in proteins, and in short fragments that are frequently distorted from ideal 3₁₀ conformations. The 3₁₀-helix most often occurs as a single turn transition between the end of an α-helix and the next portion of a polypeptide chain. However, this 3₁₀-helix commonly observed in synthetic peptides with α-aminoisobutyric acids (Aib) residues.

π-helix

Apart from the abundant α-helix and relatively less abundant 3₁₀-helix, extremely rare π-helices are also observed in proteins. It is mainly characterized by 4.4 peptide unit per turn with 6→1 hydrogen bonds and is wider and shorter than an α-helix. A shorter stretch of π-helix has been found in Catalase.

β-strands

After helix, the second major structural element that is ubiquitous (20-28%) in globular proteins is the β-strand. The β-strand are usually 5 to 10 residues long and are in almost fully extended conformation with φ, ψ angles close to -120°, 140° within the broad structurally allowed region in the upper left quadrant of the Ramachandran plot. The
extended nature of the polypeptide chain causes to positioning the side chains of consecutive residues in opposite directions. There are no intrastrand hydrogen bonds and Van der Waals interactions in comparison to helix between atoms of neighboring residues. Several such adjacent \(\beta\)-strands associate to form either parallel or antiparallel \(\beta\)-sheets with \(C^\alpha\) atoms successively a little above and below the plane of the \(\beta\)-sheets. In \(\beta\)-pleated sheets, however, the hydrogen bonding occurs between neighbouring polypeptide chains rather than within one as in \(\alpha\)-helices. There are two types of \(\beta\)-pleated sheets:

(a) The amino acids in the aligned \(\beta\)-strands can run in the same direction to form the parallel \(\beta\)-pleated sheets (Figure 9a)

(b) The amino acid residues in successive strands can run through in opposite direction to produce the antiparallel \(\beta\)-pleated sheets (Figure 9b).

The antiparallel \(\beta\)-sheet is thought to be intrinsically more stable than a parallel \(\beta\)-sheet structure due to more optimal orientation of the inter-strand hydrogen bonds and minimum peptide bond dipole. The peptide chain within a \(\beta\)-pleated sheet is fully extended maintaining a distance of 0.35 nm between the neighboring \(C^\alpha\) atoms. \(\beta\)-sheets are slightly curved and sometimes assemble to form a \(\beta\)-barrel. \(^{31}\) Analysis of various features of the \(\beta\)-strands so far includes the number of residues in a strand and no. of strands in a sheet,\(^{41}\) preferences towards parallel and antiparallel association,\(^{42}\) preferences of residue “in register” in forming \(\beta\)-strands,\(^{43}\) salt bridge stabilization between residues at position \(i\) and \(i + 2\),\(^{44}\) bulges in \(\beta\)-strands, packing and higher ordered structures.\(^{45}\)
Supramolecular structures through molecular self-assembly

Several self-assembling peptide systems have been developed, ranging from models for studying protein folding and protein conformational diseases, to molecular materials that produce peptide nanofibers, peptide scaffolds, peptide surfactants and...
peptide ink. These self-assembling peptide systems represent a significant advance in molecular design and engineering for diverse technological innovations.

(a) The amyloid hypothesis and the implications of self-assembled peptides in Amyloid fibril formation

Specific folding of proteins enable them to perform many biochemical functions critical to life. However, the misfolding and aggregation of proteins has become a topic of current interest as mounting evidence indicate that the aggregation of normally soluble proteins or protein fragments leads to the formation of insoluble amyloid deposits which is a key factor for the development of many neurodegenerative diseases including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, prion protein encephalopathies and other prevalent disease like Type II diabetes. Environment and mutations that destabilize the native structures of protein facilitate the formation of partially unfolded intermediates and it has been demonstrated that this is the possible cause for aggregation and amyloid fibril formation of these proteins.

Thus, it is of fundamental interest to understand how so many different protein sequences can adopt this aggregated fibrillar structure and it is of medical interest to understand and control disease related amyloid diseases, detailed knowledge about fibrillogenesis i.e. the putative conformational requirements, the pathway(s) of fibril formation detection of intermediate species and the interaction between intermediates is required. Unfortunately, amyloid fibrils are non-crystalline and insoluble and therefore they are not amenable to X-ray crystallography and solution NMR, the classic tool of structural biology.

Previously, amyloid fibrils have been widely studied by different electron microscopic techniques (SEM, TEM, STEM), solid state nuclear magnetic resonance and X-ray fiber diffraction studies, which show that although the sequences and native structures of amyloid proteins are vastly different, the morphology and properties of fibrils share some common features. The high resolution X-ray diffraction patterns indicate that the amyloid fibrils are composed of helical array of β-sheets, in which the hydrogen bonding direction is parallel and the β-strands are perpendicular to the fiber
axis (Figure 10).\textsuperscript{56} However, fiber diffraction data cannot determine the chemical structural details that are needed to understand fibrillogenesis thoroughly.

\textbf{Figure 10.} Cross $\beta$-model of amyloid fibrils. The $\beta$-sheets are aggregated step by step from A$\rightarrow$E to obtain mature fibrils. In the fibrils the extended $\beta$-sheets are organized such that the sheet is parallel to the long axis of the fiber, with the constituent $\beta$-strands perpendicular to this axis.

Which part of the sequence form the $\beta$-strands and which specific amino acid residues are crucial for self-aggregations in amyloid formation are not found out from fiber diffraction data analysis of amyloid fibrils. These informations have now been deduced for Alzheimer's $\beta$-amyloid fibril using a variety of solid state NMR experiments.\textsuperscript{55} Besides the structural similarity, the physiological properties of all amyloid fibrils are similar. They bind with physiological dyes like Congo-red and thioflavin-T.\textsuperscript{57}
However the mechanism of fibrillogenesis pathways is not well understood. Amyloid fibrillogenesis has been reported to proceed through the formation of monomeric\textsuperscript{58} even through dimeric to tetrameric to hexameric amyloidogenic intermediates.\textsuperscript{59} Aβ fibrillogenesis also include intermediates like soluble spherical aggregates\textsuperscript{60} and protofibrils.\textsuperscript{61}

Some reports have suggested that not only β-sheets but also helices have a significant role in amyloid fibril formation.\textsuperscript{62} Arvinte \textit{et al.} have established that significant amounts of secondary structures other than β-sheets present in amyloid fibrils formed by human calcitonin where the α-helices are stacked along the fibril axis.\textsuperscript{63} Muñoz and coworkers have introduced a new family of pathological aggregates formed by the intermolecular packing of α-helices in Alzheimer’s disease associated Tau-protein.\textsuperscript{64}

Many aspects of fibrillogenesis process are still elusive due to the insolubility and noncrystallinity of the amyloidogenic sequences. So, it is necessary to design and construct the supramolecular β-sheet model and supramolecular helical assemblage which can easily be studied using the high resolution NMR spectroscopy in solution and X-ray crystallography for better understanding of molecular self-aggregation mechanism and amyloid-like fibril formation.
(b) Self-assembling peptides as structural building motifs

Molecular self-assembly is ubiquitous in Nature and has recently emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials and engineering. Many self-assembling systems have been developed, and they represent a significant advance in the molecular engineering of simple molecular building blocks useful for wide range of applications.

The objective of the present thesis is to develop synthetic peptides that will mimic secondary structural elements of proteins such as β-turns, β-hairpins, β-sheets, helices etc. Various coded amino acids along with non-coded amino acids, both conformationally restricted (α-Amino isobutyric acid, Aib) as well as flexible (β-Alanine, γ-Amino butyric acid) and template molecules such as m-amino benzoic acid and m-nitroaniline etc., have been utilized in the design to stabilize the secondary structural elements. The other aspect of the thesis work is to study the self-assembly mechanism of synthetic peptides in the solid state. The formation of various supramolecular architectures such as fibrils, amyloid-like fibrils, non-twisted ribbon-like structures etc. from self-assembly of small synthetic peptides will be investigated.

α-Amino isobutyric acid (Aib) β-Alanine (β-Ala) γ-Amino butyric acid (γ-Abu)

m-Aminobenzoic acid (m-ABA) m-Nitroaniline (m-NA)
References


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40. Vainshtein, B. K.; Melik-Adamyam, W. R.; Baryming, V. V.; Grebenko, A. I.; Borisob, V. V. J. Mol. Biol. 1986, 188, 49. (catalase)


xxxii
Materials and Methods

This section provides a detailed description of all spectroscopic measurements and many microscopic studies used in this work. The respective chapters describe the specific synthetic procedures pertaining to the sequences studied in this thesis.

Source of Chemicals

All amino acids, tertiary butyl carbazate, dicyclohexyl carbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) were obtained from Sigma Chemical Company; Aldrich, U. S. A.; E-Merck, Germany; Lancaster, England, SRL and Spectrochem, India. Deuterated solvents for NMR studies, CDCI₃, (CD₃)₂SO were supplied by Sigma Chemical Company, U. S. A. Silica gel for TLC as well as for column chromatography (100-200 mesh) was supplied by Spectrochem, India. All other chemicals used were obtained from local manufactures like Ranbaxy, SD Fine Chemicals Pvt. Ltd., Bengal Chemical and Pharmaceutical Ltd., SRL, India, E-Merck India etc.

Purification of Solvents and Reagents

The solvents used during the course of synthesis were purified as follows: ethyl acetate, chloroform and petroleum ether (60-80°C) were distilled and used. Dichloromethane were passed through basic alumina before use. Methanol was fractionally distilled and used. Absolute methanol was prepared from distilled methanol using magnesium and iodine. Dimethylformamide (DMF) was fractionally distilled using condenser packed with fenske helices under reduced pressure over ninhydrin (3g/lit).

Spectroscopic Measurements

FT-IR spectroscopy

IR spectra were examined in Perkin Elmer – 782 model spectrophotometer. The solid-state FT-IR measurements were performed using the KBr disk technique.
NMR experiments

All \(^1\)H NMR and \(^{13}\)C NMR studies were recorded on a Bruker Avance 300 model spectrometer operating at 300, 75 MHz respectively. The peptide concentrations were in the range 1-10 mM in CDCl\(_3\) for \(^1\)H NMR and 30 – 40 mM in CDCl\(_3\) for \(^{13}\)C NMR. Solvent titration experiments were carried out at a concentration of 10mM in CDCl\(_3\) with gradual addition of \(d_7\)-DMSO. The 2D Experiment was carried out in CDCl\(_3\) on a Bruker DRX 500 MHz equipped with a 5mm broad band inverse probe head. Resonance assignment of the hexapeptide was done using double-quantum filtered COSY and ROESY methods at 300K.

Circular Dichroism spectroscopy

Solvent methanol, water and acetonitrile (1.5 mM as final concentration) were used for obtaining the spectra of peptides. Far- UV CD measurements were recorded at 25°C with a 0.5 sec averaging time, a scan speed of 50 nm/min, using a JASCO spectropolarimeter (J 720 model) equipped with a 0.1 cm pathlength cuvette. The measurements were taken at 0.2 nm wavelength intervals, 2.0 nm spectral bandwidth and five sequential scans were recorded for each sample.

Mass Spectrometry

Mass spectra of few compounds were recorded on a HEWLETT PACKARD Series 1100MSD and Micromass Qtof Micro YA263 mass spectrometers by positive mode electrospray ionization.

Field Emission scanning electron microscopic study

Morphology of the peptides was investigated using field emission scanning electron microscope (FESEM). For the study, fibrous materials (slowly grown from different solvents) were dried and gold coated. The micrograph was taken in a FESEM apparatus (JEOL JSM - 6700F).