ABSTRACT

The thesis entitled "**Design and Structural Investigations of Peptides Containing C-Linked Carbo Amino Acids by NMR Spectroscopy**" consists of four chapters.

Chapter I

This chapter describes the general introduction of nuclear magnetic resonance (NMR) spectroscopy both in one and two dimensions. The overview of the α-, β- and γ-peptides and details of various types of secondary structural features like α-helices, 3_{10}-helices, β-sheets, β-turns, γ-turns and π-turns has been discussed. It also gives a brief overview of the unnatural amino acids, β-peptides and super secondary structures like Hairpins and Helix-Turn-Helix (HTH). The structural parameters, which can be used in the structure elucidation of peptides using NMR spectroscopy, such as scalar couplings, nOe's, H-bonding parameters have been discussed. In addition, the molecular dynamics (MD) simulation study and circular dichroism (CD) spectroscopy studies have also been discussed.

Chapter II

Design and Conformational Studies of Novel C-linked Carbo-β-peptides from Mannose Derived Carbo-β-Amino Acids (β-Caas)

This Chapter has been further divided into two sections:

Section A: De Novo Design and Conformational Studies of Helix-Turn-Helix Structure Derived from C-Linked Carbo-β-Amino Acids

Peptides and proteins are materials and molecular devices, which adopt specific compact folded and organized structures for performing diverse functions in the living systems. The formation of such tertiary and quaternary structures arises from the assembly of stable secondary structures such as helices, sheets and turns. The helix-turn-helix (HTH)[1] motif, a tertiary structure composed of two helices separated by a turn motif, is one of the simplest functional assemblies and has been implicated in various important functions in DNA binding proteins. However, attempts to obtain such a structural motif using β-amino acids has so far remained a serious challenge. Our earlier[2] work on the oligomers derived from carbo-β-amino acids (Caas) generated novel and robust helical structures such as right-handed 10/12- or 12/10-helices. 'Alternating chirality' of the epimeric Caas (*S* and

R) derived from D-xylose, was efficiently used as the design control to successfully identify the mixed helical patterns in as short as tri- and tetrapeptides.

Herein, we present the first report on the '*de novo*' design of HTH motif by the assembly of short peptides with robust helices, with new β-Caas derived from D-mannose, having a D-lyxo-furanoside side chain, utilizing D-Pro-Gly as a turn motif. The main idea behind the preparation of such new Caas with alternating chirality was used in this design to observe the impact of the different furanoside side chains of carbo-β-peptides and their helix forming capability and robustness of the thus derived helices. We have studied the following peptides **1**-**6** (Figure **1**) using NMR, CD and MD studies.

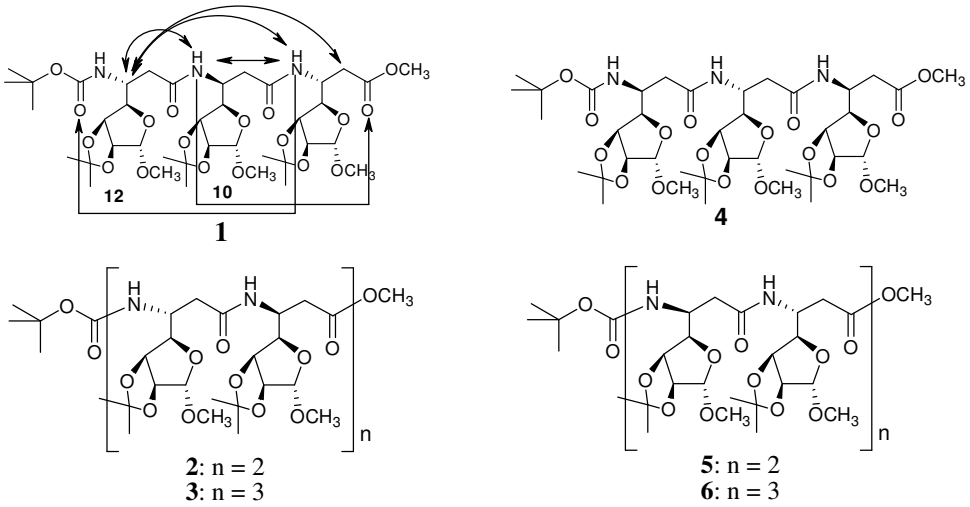


Figure **1**: Schematic representation of chemical structures of peptides **1**-**6**

^{1}H-NMR studies of peptides **1**-**6** fold into mixed 12/10- and 10/12-helices in non-polar solvents like $CDCl_3$. Large dispersion of chemical shifts for the amide and $C_\alpha H$ protons indicated the secondary structure is present. Low field chemical shifts of amide protons (> 7 ppm) suggest that these NHs are participating in intramolecular hydrogen bonding, this was further supported by the solvent titration studies (sequentially adding upto 300 μL DMSO-d_6 to 600 μL $CDCl_3$ solution). The large $^3J_{NH\text{-}C\beta H}$ ~ 9 Hz, supports a *trans* disposition of NH and $C_\beta H$, corresponding to C(O)-N-C_β-C_α (ϕ) ~ ±120°. The $^3J_{C\alpha H\text{-}C\beta H}$ > 10 Hz and < 5Hz very clearly suggest the presence of predominantly a single rotamer with N-C_α-C_β-C(O) (θ) ~ 60°, prerequisite for a helix for each residue in β-peptides.

The distinctive signatures of right-handed 12/10-helix was observed in the ROESY spectrum, wherein the strong intense backbone nOes $C_\beta H$(i)/NH(i+2) and $C_\beta H$(i)/$C_\alpha H_{(pro\text{-}R)}$(i+2) qualify a 12-membered (mr) hydrogen bond between NH(i)-CO(i-3). Whereas

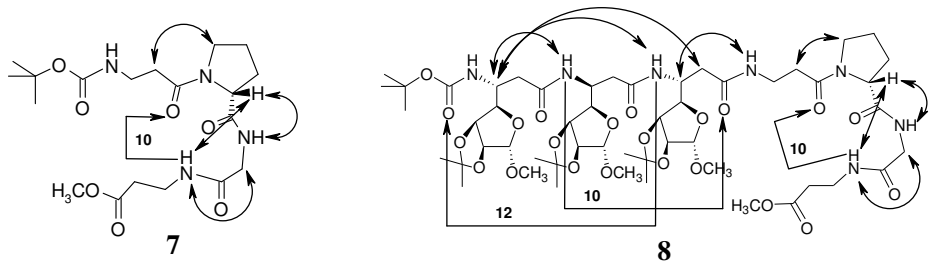
presence of weak NH(i) of "*S*"β^3/NH(i+1) nOe cross correlation supports a 10-mr H-bond between NH(i)-CO(i+1). Thus in **1** characteristic nOes $C_\beta H(1)/NH(3)$, $C_\beta H(1)/C_\alpha H_{(pro\text{-}R)}(3)$ and weak NH(2)/NH(3) nOe, fully characterize a right-handed 12/10-heix. This 12/10 helix appears as robust as those observed for the corresponding tripeptide containing Caas derived from D-xylose[2]. Similar observations were made for tetrapeptide **2** and a 12/10-helix is reinforced and hexapeptide **3** with 12/10/12/10-H-bonding observed. These mixed helices was supported by the nOes like $C_\beta H(1)/NH(3)$, $C_\beta H(1)/C_\alpha H_{(pro\text{-}R)}(3)$ and weak nOe NH(2)/NH(3) in **2** and $C_\beta H(1)/NH(3)$, $C_\beta H(1)/C_\alpha H_{(pro\text{-}R)}(3)$, $C_\beta H(3)/NH(5)$, $C_\beta H(3)/C_\alpha H_{(pro\text{-}R)}(5)$, NH(2)/NH(3) and NH(4)/NH(5) in **3** involving NH(3)-CO(Boc), NH(2)-CO(3) in **2** and NH(3)-CO(Boc), NH(5)-CO(2), NH(2)-CO(3) and NH(4)-CO(5) in **3**.

Unlike those of **1**, NMR and CD spectra of tripeptide **4** showed no helical structure. However, tetrapeptide **5**, hexapeptide **6** very clearly showed wide dispersion of the backbone protons, clearly indicating the presence of a regular secondary structure. In these peptides the distinct signatures for 10/12-helices were observed in the ROESY spectra, where in the strong sequential backbone nOes between $C_\beta H(i)/NH(i+2)$ and $C_\beta H(i)/C_\alpha H_{(pro\text{-}R)}(i+2)$ (i = 2, 4) qualify a 12-mr H bonds between CO(1)-NH(4) in **5**, CO(1)-NH(4), CO(3)-NH(6) in **6**. Weak NH(i)/NH(i+1) (i = 1, 3, 5) suggests 10-mr H-bonding between NH(1)-CO(2), NH(3)-CO(4) in **5**, NH(1)-CO(2), NH(3)-CO(4) NH(5)-CO(6) in **6**. CD Spectra of **1-6** in 100 uM solutions in methanol showed characteristics of a mixed helix with maxima at about 203 nm without an isodichroic point. The increased molecular ellipticity per residue in the CD spectra of **1-3** and **4-6** confirms the stabilization of the helix with the increasing length of the peptide. Further molecular dynamics (MD) study supports, these β-peptides fold into mixed 12/10- and 10/12-helices with a very stable structure even at the terminals reflect the experimental NMR observations.

Having successfully utilized the new Caas with D-lyxose side chain and observed the formation of robust helices in as short as a tri-and tetrapeptides, it was next aimed at the *de novo* design of peptides with a helix-turn-helix (HTH) structure making use of these short helical peptides **1** and **5**.

Thus, in the present study on the *de novo* design and structural studies of HTH, the carbo β-peptides **1** and **5** would be utilized as helical counterparts, while D-Pro-Gly would participate in 'turn motif'. Further, it was proposed to flank the D-Pro-Gly dipeptide with two units of β-hGly on both the sides (C-and N-termini) to form a tetrapeptide β-hGly-D-

Pro-Gly-β-hGly (**7**), wherein, the β-hGly provides required conformational flexibility to the mixed helices that would be interlinked with the turn motif, eventually to form HTH tertiary structure. Accordingly, tetrapeptide **7** and heptapeptide **8** were investigated by NMR and CD studies suggesting it to be a type-II' β-turn around Pro-Gly in **7** and **8** is folding into super secondary structure helix-turn.

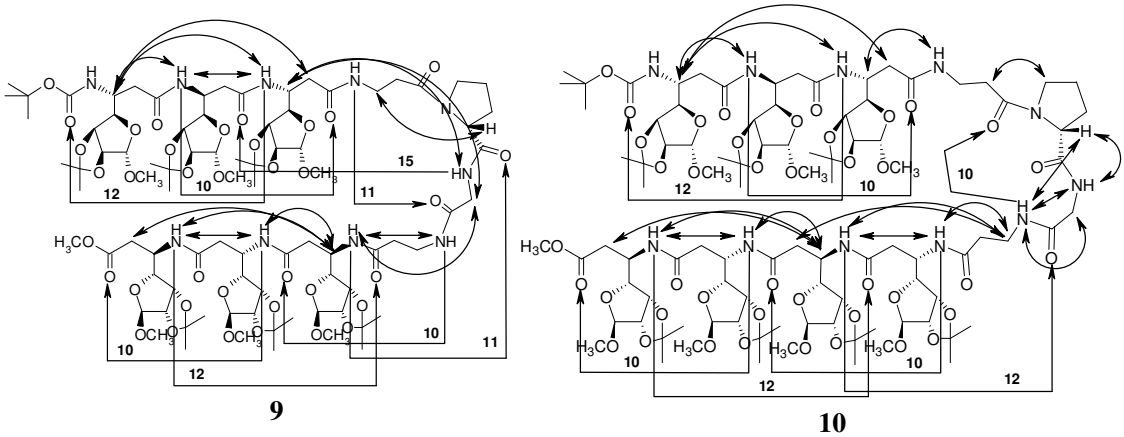


7 **8**

Figure **2**: Characteristic nOes and H-bonding information of **7** and **8**

The presence of a super secondary structure of the helix-turn family in **8** is noteworthy and provides credence to our desire of generating a helix-turn-helix tertiary structure involving a minimal number of amino acid residues, by piecing the three structural motifs together by amide bonds.

Further, the NMR studies of decapeptide **9,** it was amply evident that the 10-mr turn that was observed in **8** is disrupted in the **9**. Instead of the expected HTH structure, the turn motif has shown 11- and 15-mr H-bondings, whereby there was a distraction of 10-mr turn. It was felt that presence of (*R*)-Caa unit of tripeptide at the C-terminus of **9** is causing the damage to the turn. To avoid such fallout and to protect the turn structure, in the new design it was envisioned to have a tetrapeptide **5** at the C-terminus to gave undecapeptide **10**. ^{1}H NMR, CD and MD studies clearly indicated that **10** is folding into super secondary structure helix-turn-helix with 12/10-, type-II' β-turn and 12/10/12/10-helices.



9 **10**

Figure **3**: Characteristic nOes and H-bonding information of **9** and **10**

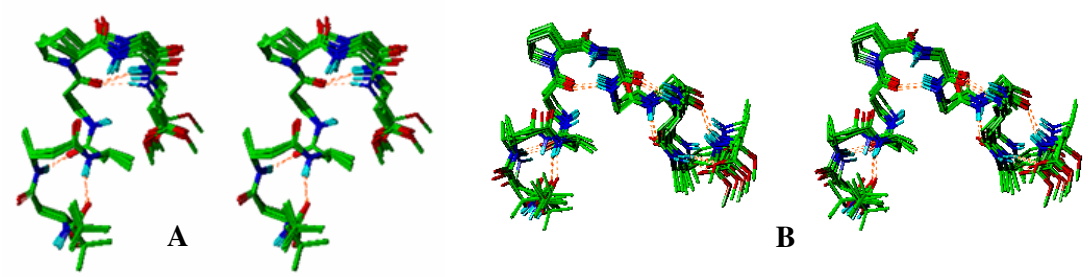


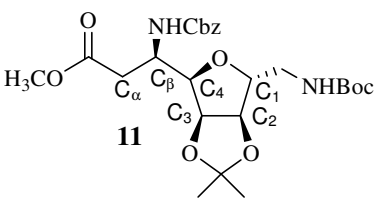
Figure **4**: Stereoview of superimposed 15 minimum energy structures of A) **9** and B) **10**.

The helix-turn-helix motif has been thus generated by a *de novo* design with as small as 11 amino acids residues. The robustness of the oligomers obtained from β-Caa and the classical D-Pro-Gly turn motif retains structure of the individual secondary structure to generate a very well defined tertiary structure with the helices orthogonal to each other.

Section B: Design and Conformational Studies of Peptides Containing Bifunctional C-Linked Carbo-β-Amino Acids (β-Caas) with 10/12-Mixed Helices

The availability of conformationally structured, water soluble β-peptide sequences, together with their known stability and resistance to enzymatic degradation, has led to some early observations regarding the biological activity of this oligomer class. The preceding section described the design and conformational studies of a new class of carbo-β-peptides with robust mixed helical structures such as 10/12- and 12/10-helical patterns in $CDCl_3$ solution with the use of concept of 'alternating chirality' from C-linked carbo-β-amino acids. These peptides, however, are not soluble in water, making them undesirable as the candidates for therapeutic applications. The design principle used by Gellman[3] for water soluble β-peptides with helical structure, had charged hydrophilic residues on one face of the helix and hydrophobic residue on its other face, providing amphiphilicity to the helix.

In the present design, to probe 10/12- and 12/10-helical structures in water, we require water soluble carbo β-animo acid residues with a proper conformational constraint, as a compliment to β-amino acids. Therefore new bifunctional amino acids such as **11** and **12** were designed to help in the water solubility through the additional amine group with the salt formation. We have therefore incorporated these bifunctional residues after every two Caa residues into the corresponding peptides **13**-



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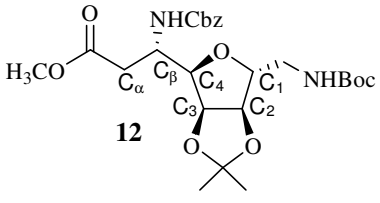

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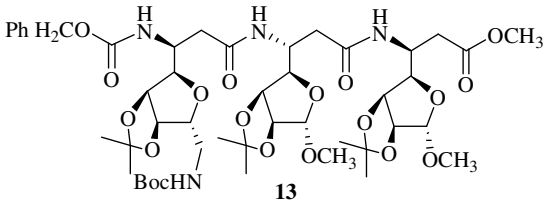
18, yet preserving the concept of alternating chirality, in conformity with the design for the mixed helices. The extensive NMR, MD and CD studies clearly indicated that, carbo-β-peptide **13** has not shown any helical pattern, but **14** and **15** folded in a right-handed 10/12-mixed helical pattern and **16-18** folded into a right-handed 12/10- mixed helical pattern.



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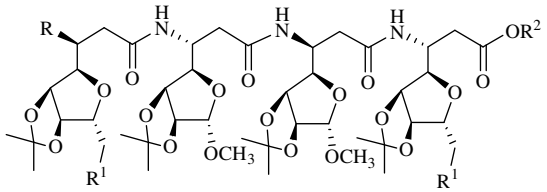
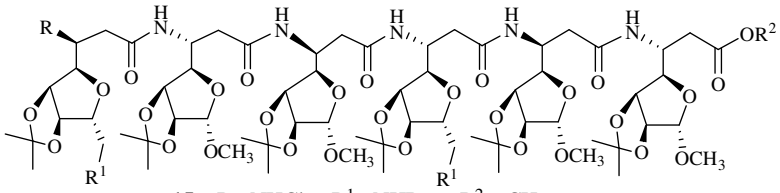
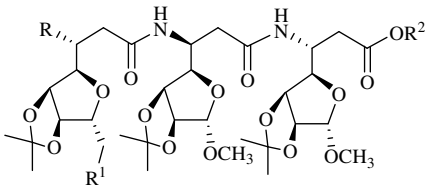


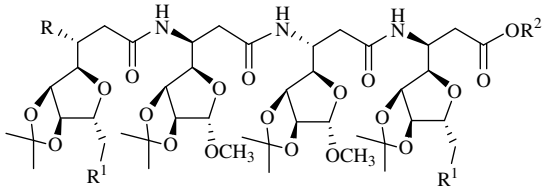
14: R =NHCbz; R^1 = NHBoc; $R^2 = CH_3$
14a: R =NHCbz; $R^1 = NH_2.CF_3COOH$; $R^2 = CH_3$
14b: R=$R^1 = NH_2.CF_3COOH$; R^2 = H



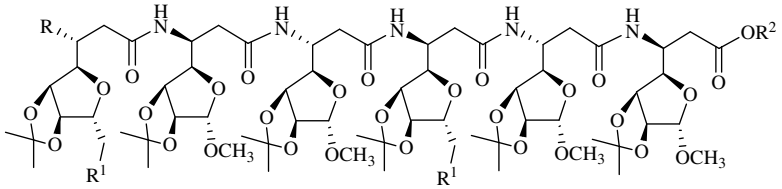
15: R =NHCbz; R^1 =NHBoc; $R^2 = CH_3$
15a: R =NHCbz; $R^1 = NH_2.CF_3COOH$; $R^2 = CH_3$
15b: R = $R^1 = NH_2.CF_3COOH$; R^2 = H



16: R =NHCbz; R^1 = NHBoc; $R^2 = CH_3$
16a: R =NHCbz; $R^1 = NH_2.CF_3COOH$; $R^2 = CH_3$
16b: R = $R^1 = NH_2.CF_3COOH$; R^2 = H



17: R =NHCbz; R^1 = NHBoc; $R^2 = CH_3$
17a: R =NHCbz; $R^1 = NH_2.CF_3COOH$; $R^2 = CH_3$
17b: R = $R^1 = NH_2.CF_3COOH$; R^2 = H



18: R =NHCbz; R^1 = NHBoc; $R^2 = CH_3$
18a: R =NHCbz; $R^1 = NH_2.CF_3COOH$; $R^2 = CH_3$
18b: R =H.CF_3COOH; $R^1 = NH_2.CF_3COOH$; R^2 = H

Figure **5**: Schematic representation of chemical structures of peptides **13**-**18**

NMR studies of **14a**, **15a** and **17a** were in addition also carried out in water (90% H_2O + 10% D_2O) and DMSO-d_6. The spectra in water did not display any distinct signatures of a secondary structure. Most of the NH resonances were grouped in a region of 7.70-8.40 ppm. The large value of the temperature coefficient of the amide proton chemical shifts ruled out their participation in H-bonding. Lastly, was from the absence of medium-range nOes further confirmed, characteristics of such folds. Strong propensity to form intermolecular H-bonds with water molecules might be destabilizing and disrupting the structure. The spectra of these peptides in DMSO-d_6, were, however, broad and lacked the desired resolution, leading to abandoning our efforts for a detailed NMR study. The NMR studies of **15a** were also carried out in CD_3OH, However limited dispersion of amide chemical shift and resonance overlap of $C_\beta H$ and $C_\alpha H$ region are not permitted to analyze. However, found that these peptides, when the charged groups are removed, retain their structure in $CDCl_3$.

The CD spectra of the peptides **14**-**18** (Figure **6**) show distinct signatures of a right-handed 10/12-mixed helix with a maxima at about 203 nm, with very little excursion in the negative molar ellipticity.

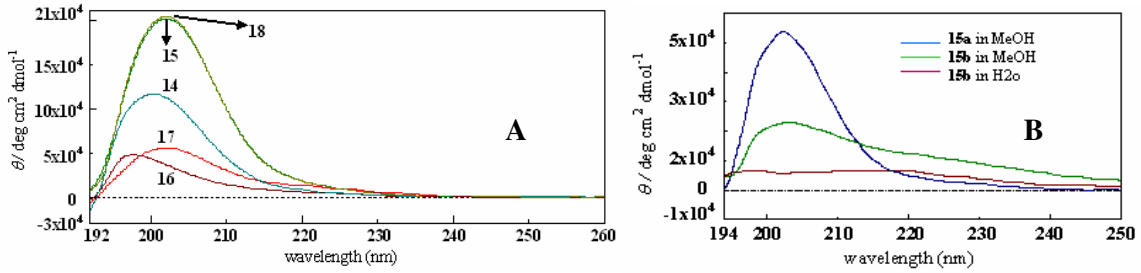


Figure **6**: Circular Dichroism spectra of (**A**) **14**-**18**; (**B**) **15a** and **15b**.

The intensity of this absorption is drastically reduced in aqueous solution (Figure **6B**). This suggests that the 12/10-helical conformation of these peptides is far less stable in water than methanol. The CD spectroscopic analysis shows that peptides **15a** and **15b** are only partially folded in aqueous media. The peptides derived from bifuncational β-Caas, after removing the protecting groups, however, have not shown any helical patterns in aqueous solutions. However, some of these water soluble peptides have shown moderate antibacterial activity.

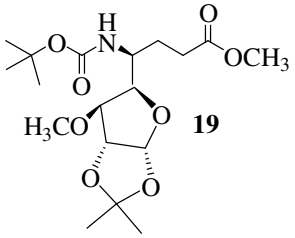
Chapter III

Design and Conformational Studies of γ- and β/γ-Peptides Derived from C-Linked Carbo-β- and γ-Amino Acids

This chapter has been further divided into two sections:

Section A: Left-Handed 9-helix in γ-peptides: Design and Conformational Studies of Oligomers with Dipeptide Repeats of C-linked Carbo-γ^4-Amino Acids and γ-Aminobutyric Acid

The design of non-natural oligomers that form diversified secondary structures is an active area of research. An extensive search of the β-peptide class of foldamers has provided several new secondary structures, while the γ-peptides, despite the possibility of having and traversing a larger conformational space, have received less attention. Based on earlier[4] findings on mixed β-peptides, and Gellman's predictions[5] for γ-aminobutyric acid (GABA) derivatives, about nearest neighbor hydrogen bonds using IR techniques, we envisaged that mixed γ-peptides might adopt novel interesting secondary structures. Hence, the γ-Caas **19** and γ-aminobutyric acid (GABA) **20** were used in this design. We have studied the tetrapeptide **21** and hexapeptide **22**. These peptides have shown left-handed 9-helical structures in $CDCl_3$ confirmed by NMR studies and further supported by the CD and MD studies.



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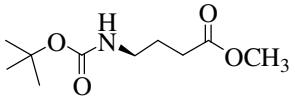

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Wide dispersion of amide protons of peptides **21** and **22** in $CDCl_3$ solution suggesting secondary structure is present. Two amide proton resonances in **21** and five amide proton resonances in **22** appeared at $\delta > 7$ ppm, suggesting their participation in H-bonding. Further, solvent titration studies confirm that all the amides participate in intramolecular hydrogen bonding, with the exception of NH(1). The distinct presence of a left-handed 9-helix with 9-mr hydrogen bond between NH(i)-CO(i-2) is confirmed from ROESY spectra, wherein the long-range nOes like NH(i+1)/$C_\gamma H_{(pro\text{-}S)}$(i) and C_γH(i)/$C_\alpha H_{(pro\text{-}S)}$(i+1) were supported.

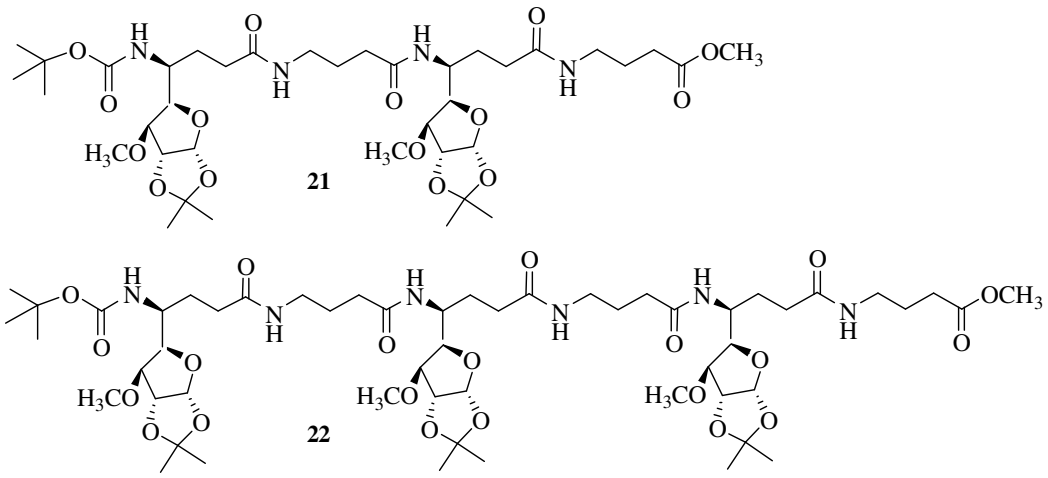


Figure **7**: Schematic representation of chemical structures of peptides **21** and **22**

For the γ-Caas, the $^3J_{NH\text{-}C\gamma H}$ ~ 9.0-9.5 Hz indicated that the NH and $C_\gamma H$ protons are antiperiplanar (*ap*) and imply a value of C(O)-N-C_γ-C_β (ϕ) ~ ±120°. Stereospecific assignments for $C_\beta H$ protons confirmed by the coupling constants and nOe data. The $^3J_{C\beta H\text{-}C\gamma H}$ < 5.0 Hz and > 10.0 Hz support N-C_γ-C_β-C_α (θ_1) ~ -60° or 180°, while a strong nOe, NH(i)/$C_\beta H$(i) (the one *ap* with respect to $C_\gamma H$), confirm θ_1 ~ -60°. For $C_\alpha H$ protons, stereospecific assignments were possible only for $C_\alpha H$(3) protons in **21** and **22**, where one of the protons showed $^3J_{C\alpha H\text{-}C\beta H}$ = 5.0 Hz and = 10.5 Hz, suggesting C_γ-C_β-C_α-C(O) (θ_2) ~ 60° or 180°. The same proton also displayed nOes with NH(3), NH(4) and $C_\gamma H$(2), which is possible only for θ_2 ~ -60° and C_β-C_α-C(O)-N (ψ) ~ ± 100°.

Similarly, for GABA residues, $^3J_{NH\text{-}C\gamma H}$ ~ 5 and 7 Hz, support ϕ ~ 120°. The observation of strong NH(i+1)/$C_\alpha H$(i) nOe correlations suggest that |ψ| may have a value of about 100°. In the ROESY experiments, several long-range nOes such as NH(i+1)/$C_\gamma H$(i), $C_\gamma H$(i)/$C_\alpha H$(i+1), NH(i+1)/$C_\alpha H$(i), NH(i)/$C_\beta H$(i), NH(i)/$C_\alpha H$(i) and $C_\gamma H$(i)/$C_\alpha H$(i) were observed. However, it was difficult to get more definitive structural information, especially for the GABA residues, partly due to fraying at the termini and in part due to spectral overlap. The above data satisfied for ϕ ~ 120°; θ_1 ~ -60°; θ_2 ~ -60° and ψ ~ 100°. This was further supported by the MD studies.

Restrained MD simulations were performed on peptides **21** and **22** using distance constrains, which were derived from the ROSEY experiments to generate a structure for these peptides that is consistent with NMR experimental data. These studies confirmed the presence of a left-handed 9-helical structure. Figure **8** depicts a superimposition with

stereo-view of 15 lowest energy structures for **21** and **22**. For clarity, all the protons have been removed and sugar units were replaced by methyl groups after the MD calculations.

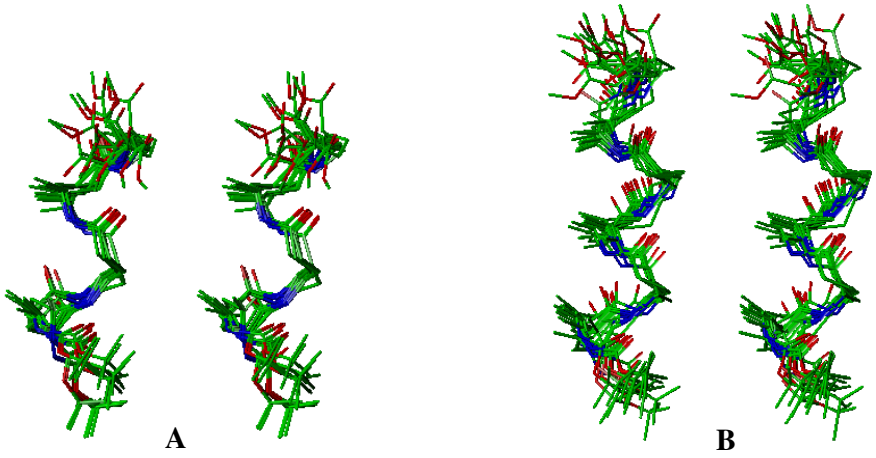


Figure **8**: Stereo-view of superimposed 15 minimum energy structures: (**A**) **21** and (**B**) **22**

This study thus presents the identification of a novel left-handed 9-helix in mixed γ-peptides derived from alternating (*S*)-γ-Caa/GABA residues for the first time, as a new helical structure in the domain of 'foldamers'.

Section B: Design and Conformational Studies of New Class of β/γ- Hybrid Peptides Containing C-Linked Carbo-β-Amino Acids and γ-Amino Acids

Diverse functions carried out by proteins are attributed to their compact three-dimensional structures. Modifications of the peptide backbone with new motifs, while expanding the domain of secondary structural diversity, bring the richness in design with desirable properties in the unnatural oligomers. The thrust to mimic essential features of the proteins has led to the discovery of large variety of secondary structures in the unnatural oligomeric scaffolds derived from β-, γ- and δ-amino acids, falling in the domain of "foldamers". Earlier work[4] on the dipeptide repeats derived from epimeric β-Caas with alternating chirality and β-Caa/β-hGly, repeats to generate very stable and robust right- and left-handed 10/12- (12/10-) helices. It was amply evident that the carbohydrate side chain and the stereochemistry at the amine bearing stereocentre indeed control conformations in the derived peptides. The rigidity differences in the (*S*)- and (*R*)-epimeric β-Caa residues play a crucial role in defining the helical conformations. Based on Hofmann's theoretical calculations[6] and despite having different backbone geometry, we envisaged that hybrid β/γ-peptides might adopt interesting novel secondary structures.

We have designed and studied new class of β/γ-peptides **23**-**25** made from an alternate arrangement consisting of C-linked carbo-β-amino acid (β-Caa) and γ-Caa derived from D-xylose having (*S*)-stereo center in both β-Caa and γ-Caa, have shown to fold into stable an unprecedented left-handed 11/13-helix in solution. The secondary structures of these peptides have been ascertained from detailed NMR studies, while the CD spectroscopy and molecular dynamics investigations provided additional support for the structures derived.

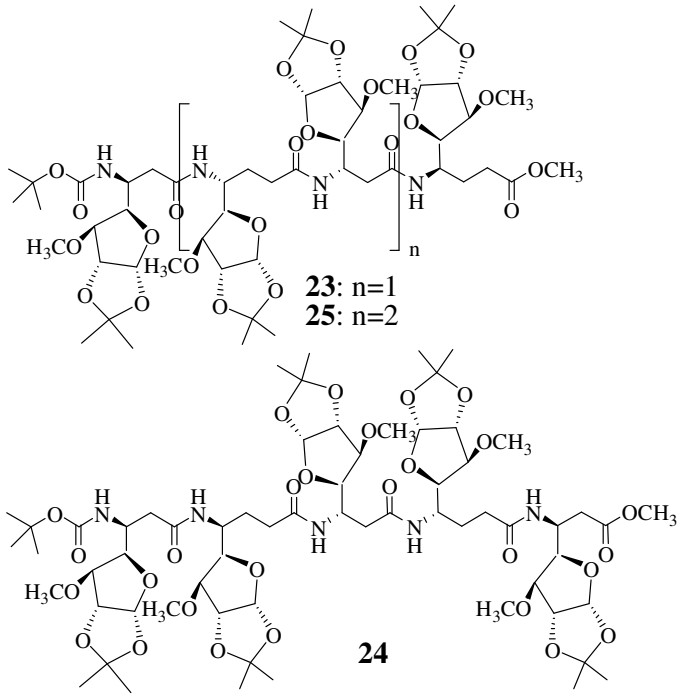


Figure **9**: Schematic representation of chemical structures of peptides **23**-**25**

Here we have given full details of tetrapeptide **23** only. Two amide resonances appear at $\delta > 7$ ppm in $CDCl_3$. Solvent titration studies show that except NH(2) other amide resonances shift by < 0.52 ppm, confirming their participation in hydrogen bonding. For both β- residues, $^3J_{NH\text{-}C\beta H} > 9.3$ Hz and both γ- residues, $^3J_{NH\text{-}C\gamma H} = 8.2$ and 9.6 Hz for the second and fourth residue respectively, suggest preponderance of *ap* arrangement of NH with $C_\beta H$ and $C_\gamma H$, corresponding to a value of ϕ around 120°. Among the β-residues the information on θ_1 for the first residue could be easily derived from small values of both $^3J_{C\alpha H\text{-}C\beta H}$ (~ 5 Hz), which is consistent with θ_1 ~ 60°. This information along with $C_\alpha H(1)$/NH(1) and $C_\alpha H(1)$/NH(2) nOes permits us to assign $C_\alpha H_{(pro\text{-}R)}(1)$ as the one with stronger nOes with NH(1) and NH(2) compared to $C_\alpha H_{(pro\text{-}S)}(1)$. Further, the observation of NH(1)/NH(2) and $C_4H(1)$/NH(2) nOe correlations suggest a ψ value in the vicinity of 0°.

Similar observations of nOes involving the third residue, permit stereospecific assignment of protons at $C_\alpha(3)$ and are consistent with $\theta_1 \sim 60°$, $\psi \sim 0°$. Due to spectral overlap, the detailed coupling pattern could not be deciphered for all $C_\alpha H$ and $C_\beta H$ protons. The stereospecific assignments for some $C_\alpha H$ and $C_\beta H$ protons could be made based on the couplings and nOes. These information suggest the $\theta_1 \sim 60°$, $\theta_2 \sim 60°$ and $\psi \sim -100°$.

Characteristic medium-range nOes like; $C_\gamma H(2)$/NH(4), strongly support mixed helical structure with 13-mr H-bond between NH(4)-CO(1). We also observed the NH(1)/NH(2) and NH(3)/NH(4) nOes, suggesting 11-mr H-bond between NH(1)-CO(2) and NH(3)-CO(4). These considerations provide compelling evidence of the presence of a, hitherto not reported, 11/13-helix, with a 11/13/11-H-bonded arrangement. For the pentapeptide **24** and hexapeptide **25**, NMR data support a structure very similar to that for **23**.

Using the NMR data, restrained MD simulations were performed to further confirm the presence of the 11/13-mixed helix. Figure **10** shows 20 lowest energy superimposed structures of **23** and **24**. The average values of the dihedral angles agree with those for the higher energy $11/13^{III}$-helix reported by Hofmann *et al.*[7] CD spectra for **23-25**, further confirm the presence of an extended 11/13-helical structure.

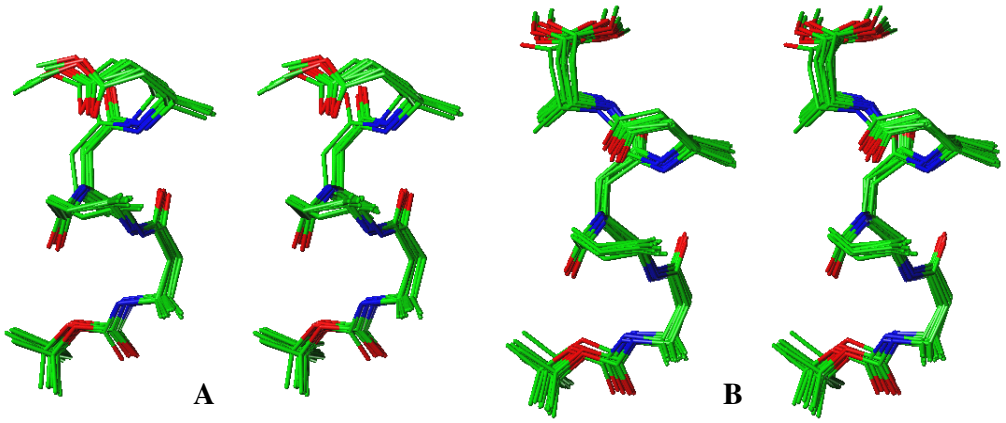


Figure **10**: Stereo-view of (**A**): **23** and (**B**): **24** (sugars are replaced with methyl groups after MD calculations)

Thus, the conformational analyses of β/γ-hybrid peptides **23**-**25** have shown a new class of 11/13-helix for the first time. Further, our new design gives the first experimental proof to Hofmann's recent predictions of the secondary structures in β/γ-peptides.

Chapter IV

Design and Conformational Studies of Unusual Helices and β-Ribbons in Oligomers Derived from C-Linked Carbo α-Amino Acids (α-Caa) and L-Alanine

The α-amino acids are the building blocks of peptides and proteins, which carry out most of the functions in the living cells. The compact secondary structures implicated in these functions are obtained from secondary structural elements like helices, sheets and turns. In this chapter, we have shown that a C-linked carbo α-amino acid (α-Caa), derived from xylose, generates a novel 7-helix containing sequential γ-turns. Such structures contain an additional set of 6-mr intra-residue H-bonds involving the amide proton and the oxygen in the methoxy group of the sugar side chain, and might be crucial to stabilize the unusual helical structure generated by the backbone. Additionally, to accommodate the bulky protected sugar side chains the backbone of the oligomers spans a very narrow conformational space and is severely constrained. To support this view, we have also explored the oligomers with alternate L-Ala/α-Caa, which show an interesting variation in the backbone with a β-bend ribbon, having a 6-mr intra-residue H-bond between backbone NH and sugar ring OMe.

We have studied the α-Caa oligomers **26**-**29** in $CDCl_3$ solution. Well dispersion of NMR spectra and all the amide protons excluding the first NH appear at $\delta > 7$ ppm, suggesting their participation in H-bonding. Solvent titration studies further confirm this information, which exhibit 7-helix containing sequential γ-turns with an additional 6-mr intra-residue H-bonds.

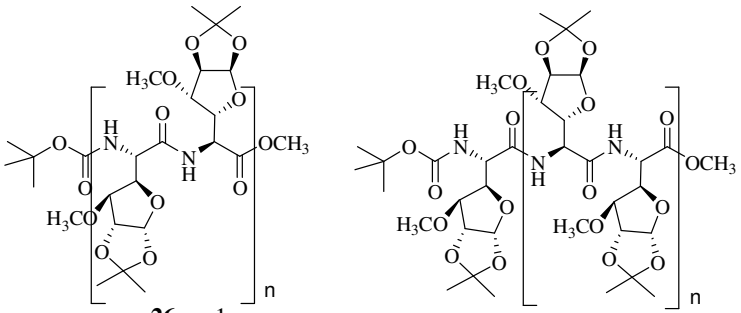


Figure **11**: Schematic representation of chemical structures of peptides **26**-**29**

The observation of $^3J_{NH\text{-}C\alpha H} > 8.2$ Hz, suggests a tendency for an anti-periplanar (*ap*) arrangement of these protons with $\phi \sim \pm 120°$. The distinguishing and distinctive

medium/weak intensity nOe correlations, NH(i)/NH(i+1), C_αH(i)/NH(i+1), NH(i)/C_1H(i+1), OMe(i)/C_1H(i+1) and C_αH(i)/OMe(i+2), right through the peptide chain, characterize the structure completely, specially for the larger oligomers. The absence of two i/i+1 characterstic nOes at the C-termini, is expected to result in significantly ill defined back bone at the C-termini. The nOes involving the sugar protons NH(i)/C_1H(i), show the proximity to the backbone protons. The data also suggests that the sugar moiety is snuggly fitted in the structure without destabilizing the ordered structure.

Molecular Dynamics study fully support the 7-helix formed by these oligomers, stabilized by H-bonds between CO(i) acceptor and NH(i+2) donor. This is first such helix in the domain of the α-peptides. The 6-mr intra-residue H-bond between the amide proton and the methoxy oxygen is present for all the residues. This results in very constrained sugar ring. The $^3J_{C\alpha H\text{-}C4H}$ ~ 6 Hz does seem to represent a very constrained χ of ~ ± 60°.

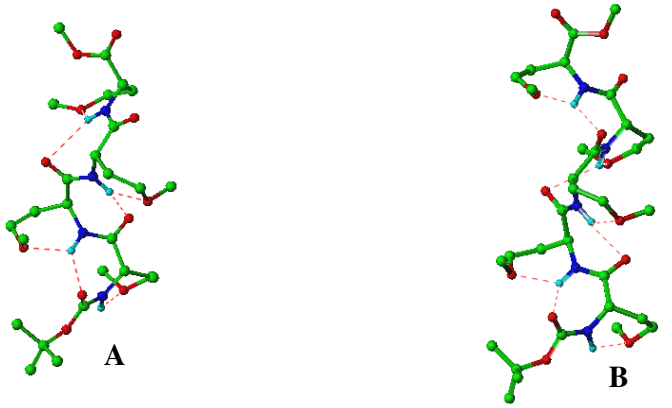
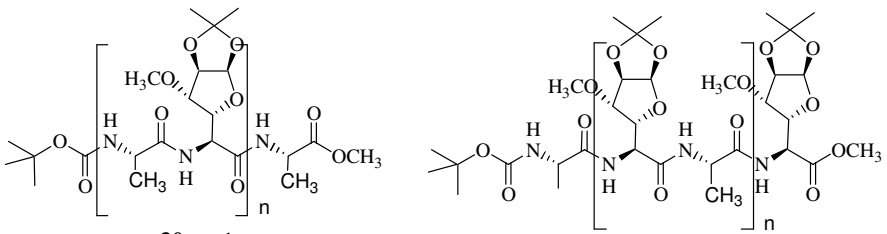


Figure **I2**: Side view with backbone (sugars are removed for clarity) of A) **28** and B) **29**

To verify some of the observations made on α-Caa, we have studied oligomers **30**-**34**, where alternate L-Ala/α-Caa. L-Ala removes the option of the H-bonds between the intra-residue NH with OMe in the sugar ring as well as provides certain amount of conformational freedom in the backbone.



30: n=1
32: n=2
34: n=3

31: n=1
33: n=2

Figure **13**: Schematic representation of chemical structures of peptides **30**-**34**

The tri-peptide **30**, does provide distinctive signatures of a turn structure with involvement of NH(2) and NH(3) in H-bonding. The presence of $C_\alpha H(1)/NH(3)$, NH(1)/NH(2) and NH(2)/NH(3) nOe connectivities as well as $^3J_{NH\text{-}C\alpha H}$ = 5.8, 8.2 and 7.2 Hz for the first, second and third residues respectively provide emphatic support for a type-I β-turn, corresponding a 10-mr H-bond between NH(3)-CO(Boc) and an intra residue 6-mr H-bond in the α-Caa. In **31** we observed that in addition to the type-I β-turn, the NH(4) participates in NH(4)-OMe(4) H-bond. In **32**, excluding NH(1) all amide protons participate in H-bonding. Also the presence of two type-I β-turns, around Ala(1)-α-Caa(2) and Ala(3)-α-Caa(4) is very well supported by the nOe correlations, $C_\alpha H(1)/NH(3)$, NH(1)/NH(2), NH(2)/NH(3), $C_\alpha H(3)/NH(5)$, NH(3)/NH(4) and NH(4)/NH(5). Additionally, unique nOes, $C_\alpha H(2)/NH(5)$ and $C_\alpha H(2)/OMe(4)$, further confirm the presence of the second type-I β-turn, with NH(5)-CO(2) H-bond. The two type-I β-turns are arranged as a β-pleated sheet, very similar to the structure observed in peptides with Pro-Gly repeats. The hexapeptide **33** and heptapeptide **34** behave as expected. In **34** the extension of the β-pleated sheet is evident from the signatures of three type-I β-turns.

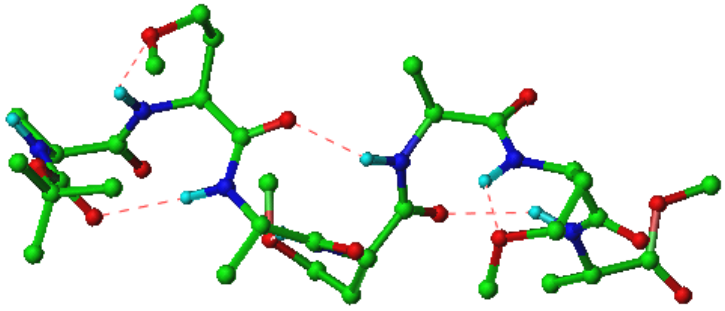


Figure **I4**: Backbone with a β-bend ribbon, shows three type-I β-turns (10-mr) and intra-residue 6-mr H-bond of **34** (Sugars are removed for clarity)

Thus, the observation of such interesting helical structures using the Caa oligomers opens up interesting possibilities. These monomers provide constraints in the conformational space spanned by the backbone and stabilize hitherto not reported helices in α-amino acids. A β-ribbon structure has been noticed in oligomers derived from alternating L-Ala/α-Caa residues, which have probably been stabilized by the intra-molecular 6-mr H-bond between backbone amide proton and the oxygen atom of the OMe group in the sugar side chain. These studies further open up and increase the potential foldamer space exponentially.

References:

[1] a) Harrison, S. C.; Aggarwal, A. C. *Annu. Rev. Biochem*. **1990**, *59*, 993; b) Brennan, R. G.; Maththews, B. W. *J. Biol. Chem*. **1989**, *264*, 1903.

[2] 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.

[3] Raguse, T. L.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *24*, 12774.

[4] Sharma, G. V. M., Ravinder Reddy, K.; Ravi Sankar, A.; Jayaprakash, P.; Radha Krishna, P.; Jagannadh, B.; Kunwar, A. C. *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 3961.

[5] Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054.

[6] (a) Mohle, K.; Günther, R.; Thormann, M.; Sewald, N.; Hofmann, H. J. *Biopolymers.* **1999**, *50*, 167; (b) Baldauf, C.; Günther, R.; Hofmann, H. J. *Angew. Chem. Int. Ed.* **2004**, *43*, 1594.

[7] Baldauf, C.; Günther, R.; Hofmann, H. J. *J. Org. Chem.* **2006**, *71*, 1200.