Foldamers

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De Novo Design and Synthesis of Helix–Turn–Helix Structure from Short and Robust Mixed Helices Derived from C-Linked Carbo-β-Amino Acids**

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Peptides and proteins are molecular devices that adopt specific compact folded and organized structures for performing diverse functions in 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 development of nonnatural peptides[1–4] with compact and specific conformations is of considerable interest to understand the folding and stabilization and for designing new materials with specific biological functions. In spite of rapid progress made in the design and understanding of foldamers[2a] (mostly from β-amino acids) with a variety of secondary structures, generating distinct tertiary structures from them has so far remained a serious challenge. The helix–turn–helix (HTH) motif,[5] 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 by using β- or γ-amino acids have so far not been successful. Herein, we present the first report on the de novo design of helix–turn and helix–turn–helix motifs in hepta- and undecapeptides, respectively, derived from C-linked carbo-β-amino acids (Caas) and a "turn motif" consisting of β-hGly-D-Pro-Gly-β-hGly (the common name of β-hGly is β-alanine[4]).

We have recently shown that the oligomers of Caas,[6] derived from D-xylose ($Caas_{(x)}$), adopt novel and robust right- as well as left-handed 12/10 helices[7,8] (the 12/10 helices consist of alternating 12- and 10-membered H-bonded pseudo rings[4]), whereas heterogeneous backbone peptides from L-Ala and $Caa_{(x)}$ resulted in unprecedented 11/9 helices.[9] "Alternating chirality"[7,10] of the epimeric $Caa_{(x)}$ (*S* and *R* conformations at C_β) was efficiently used as the design control to successfully identify the mixed helical patterns in as short as tri- and tetrapeptides. Herein, the tetrapeptide β-hGly-D-Pro-Gly-β-hGly is envisioned as a "turn motif",[11,12] which plays a crucial role as the strategic conformation nucleator. Flanking D-Pro-Gly on either side with two units of β-hGly in the tetrapeptide is aimed to provide the necessary continuity for the mixed helices and to provide conformational freedom for accommodating the helical structural elements. The turn motif is linked with tri- and tetrapeptides with mixed helical structures at the N and C termini, respectively, to furnish the HTH structure.

Peptides **1**–**5** (Scheme 1) were prepared by conventional peptide synthesis methods.[13] Alternating sequences of "epimeric" (*R*)- and (*S*)-$Caa_{(l)}$ (from D-mannose) were used in the synthesis of **1** and **2**. Peptide **3** was first condensed with tripeptide **1** to result in a heptapeptide **4**, which upon modification at the C terminus by coupling with **2** gave the undecapeptide **5**. The structural information of these peptides was obtained from extensive NMR spectroscopic, molecular dynamics (MD), and CD studies.[13]

NMR spectroscopic studies on **1**–**5** were carried out at 303 K in 5–10 mM solutions in $CDCl_3$. Peptides **1** and **2**, like our earlier peptides derived from $Caa_{(x)}$,[7] showed the evidence for 12/10-mixed helical structures (see the Supporting Information). The robustness of **1** and **2** is similar to that observed for the corresponding tripeptide and tetrapeptide prepared from $Caa_{(x)}$.[7]

For the turn motif **3**, the ^{1}H NMR spectrum showed an ≈1:24 *cis*/*trans* isomer ratio. The NOE correlations, between the $C_\delta H(2)$ and $C_\alpha H(1)$ protons helped in characterizing the major isomer as the *trans* isomer, whereas the *cis* isomer shows distinctive $C_\alpha H(1)/C_\alpha H(2)$ NOE interactions (for the minor isomer, their small populations did not permit us to obtain structural information[13]). In the major isomer, NH(4) resonated at 7.09 ppm and displayed a small shift in the solvent titration studies, implying its participation in H bonding. The $C_\alpha H(2)/NH(4)$ NOE correlation additionally confirmed the β turn around Pro-Gly. The NOE correlation $C_\alpha H(2)/NH(3)$ was stronger than the $C_\alpha H(2)/NH(4)$, $C_\alpha H_{Pro\text{-}(R)}(3)/NH(4)$, and $C_\alpha H_{(S)\text{-}Pro}(3)/NH(4)$ contacts, suggesting the presence of a type II′ β turn.

In the ^{1}H NMR spectrum of heptapeptide **4**, *cis*/*trans* isomers about the imide bond were observed in an approximate 1:9 ratio. The *trans*-imide bond preceding D-Pro was confirmed with the help of NOE values between $C_\delta H(5)$ and both $C_\alpha H(4)$ protons. Only the major isomer was studied in detail. Wide dispersion of the backbone proton resonances, for amide as well as $C_\alpha H$, suggests that the two structural elements do not destabilize the proposed secondary structure. NH(2), NH(3), and NH(7), in addition to their low-field shifts, display small $\Delta\delta$ values of about −0.01, 0.26, and 0.66 ppm, respectively, in solvent titration studies[14] confirming their participation in intramolecular H bonding. The

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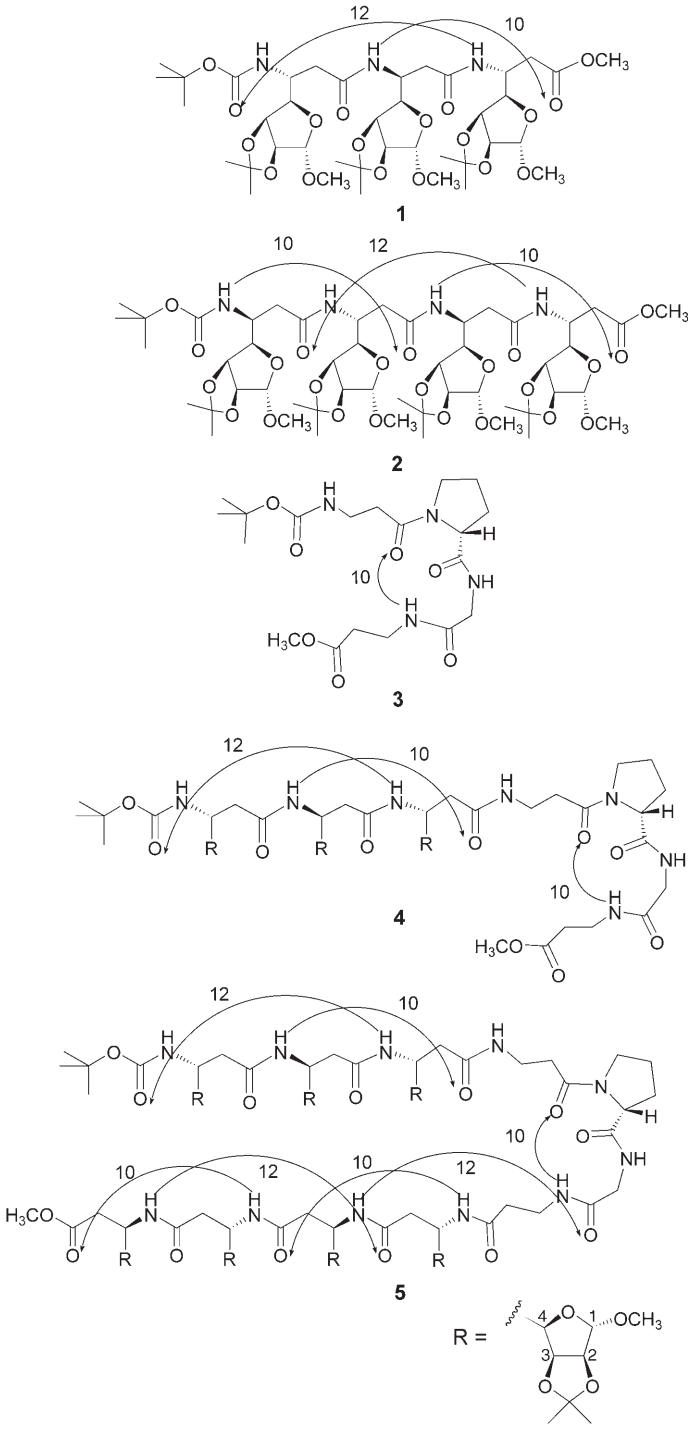

Scheme 1. Structures of peptides **1**–**5** with H-bond interactions shown by arrows.

coupling constants for the first three residues are very similar to that for **1**. Additionally, NOE cross-correlations like $C_\beta H(1)/NH(3)$ and $C_\beta H(1)/C_\alpha H_{Pro\text{-}(R)}(3)$ provide sufficient support for a 12/10-mixed helix involving the first three residues. The presence of $C_\alpha H(5)/NH(6)$ NOE correlations, which are stronger than the $C_\alpha H(5)/NH(7)$, $C_\alpha H_{Pro\text{-}(R)}(6)/NH(7)$, and $C_\alpha H_{Pro\text{-}(S)}(6)/NH(7)$ NOE correlations, in conjunction with the participation of NH(7) in H bonding, show that the last four residues organize into a type II′ β turn. The β-hGly(4) residue has $^3J_{NH\text{-}C_\beta H} = 4.3$ Hz and 8.0 Hz, which corresponds to $|\phi| \approx 120°$. From correlation experiments,[13] it was inferred that the $C_\beta H$ proton (at $\delta = 3.74$ ppm and with large coupling with NH), which is antiperiplanar to the amide proton, has a small coupling with both the $C_\alpha H$ protons, whereas the other $C_\beta H$ proton (at $\delta = 3.32$ ppm) has large coupling with one of the $C_\alpha H$ protons (at $\delta = 2.62$ ppm), therefore suggesting $\theta \approx 60°$. Further support for these observations came from the much smaller intensity of the NOE correlation between $C_\beta H$ ($\delta = 3.32$ ppm) and $C_\alpha H$ ($\delta = 2.62$ ppm) when compared with other $C_\beta H/C_\alpha H$ cross-peaks. These data permitted us to stereospecifically assign the $C_\alpha H$ and $C_\beta H$ protons and further show the $\phi \approx 120°$ and $\theta \approx 60°$ for the fourth residue,[13] which corresponds to further extending of the 12/10 helix in to the turn. These results thus provide generous support for the absence of destabilization of the two structural motifs in **4** when joined by an amide bond. The presence of a super secondary structure of the helix–turn family in heptapeptide **4** is noteworthy and provides credence to our desire to generate a helix–turn–helix tertiary structure de novo.

The ^{1}H NMR spectrum shows that undecapeptide **5** has *cis* and *trans* isomers in an ≈1:6 ratio. For the major isomer, the *trans*-imide bond preceding D-Pro was confirmed by the NOE contacts $C_\alpha H(4)/C_\delta H(5)$, whereas the *cis* isomer shows $C_\alpha H(4)/C_\alpha H(5)$ NOE values. The spectrum displays a large number of amide protons with $\delta > 7$ ppm. Solvent titration studies showed small changes in δNH for eight of the amide protons, confirming their participation in intramolecular H bonding. Typical signatures of a right-handed 12/10-mixed helix discussed for the first three residues of **1** and **4** are distinctly present in the couplings and the NOE data, permitting full characterization of a 12/10 helix for the first three residues in **5**. Similarly, the couplings as well as the characteristic long-range NOE values, $C_\beta H(9)/NH(11)$, $C_\beta H(9)/C_\alpha H_{Pro\text{-}(R)}(11)$, NH(8)/NH(9), and NH(10)/NH(11) provide compelling evidence for the 10/12/10 helical structures arising from the H bonding between NH(8)–CO(9), NH(11)–CO(8), and NH(10)–CO(11) for the last four residues. This validates the use of the tetrapeptide **2** as a helical motif at the C terminus. In addition, observation of $C_\beta H_{Pro\text{-}(R)}(7)/NH(9)$, $C_\beta H_{Pro\text{-}(R)}(7)/C_\alpha H_{Pro\text{-}(R)}(9)$, and involvement of NH(9) in H bonding suggests the presence of an additional 12-membered NH(9)–CO(6) H bond, resulting in an extended 12/10/12/10 helix. Thus, it is evident that the turn-inducing tetrapeptide **3** does not destabilize the robust helices both at the C and the N termini. With these two helical elements firmly in place, it was necessary to look for the distinctive features for a turn involving D-Pro-Gly residues. The NOE cross-correlations (Figure 1), $C_\alpha H(5)/NH(7)$ and NH(6)/NH(7), and involvement of the NH(7) in H bonding provide adequate support for the induction of a β turn. Furthermore, $C_\alpha H(5)/NH(6)$ NOE values, which are stronger than the $C_\alpha H(5)/NH(7)$, $C_\alpha H_{Pro\text{-}(R)}(6)/NH(7)$, and $C_\alpha H_{Pro\text{-}(S)}(6)/NH(7)$, suggests that β-hGly-D-Pro-Gly-β-hGly participates in a type II′ β turn. The fourth residue has $^3J_{NH\text{-}C_\beta H} = 4.0$ and 8.3 Hz, whereas the corresponding values for the seventh residue are 3.8 and 9.3 Hz. Like in **4** for β-hGly(4), the

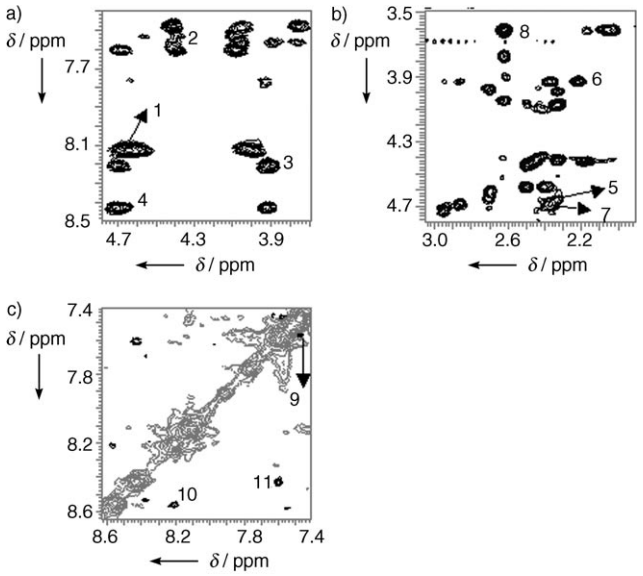

Figure 1. ROESY spectrum of **5**. a) The NOE interactions $C_\beta H(i)/NH(i+2)$ ($i=1$, 7, and 9) are marked as 1, 3, and 4, respectively. $C_\alpha H(5)/NH(7)$ is marked as 2; b) $C_\beta H(i)/C_\alpha H_{Pro\text{-}(R)}(i+2)$ ($i=1$, 7, and 9) are marked as 5, 6, and 7, respectively, and $C_\alpha H(4)/C_\delta H(5)$ (*trans* geometry of D-Pro) is marked as 8; c) $NH(i)/NH(i+1)$ ($i=6$, 8, 10) are marked as 9, 10, and 11, respectively.

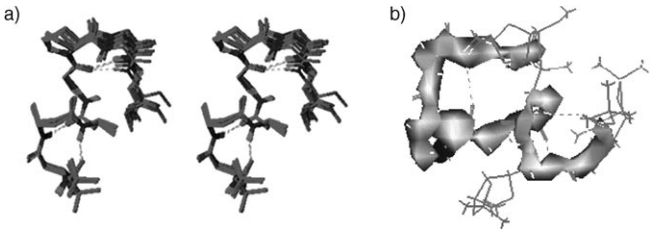

Figure 2. MD structures of **4**. a) Stereoview of 15 superimposed minimum-energy structures (sugar moieties replaced with a methyl groups after the calculations for clarity); b) backbone has been shown as a ribbon for a better display.

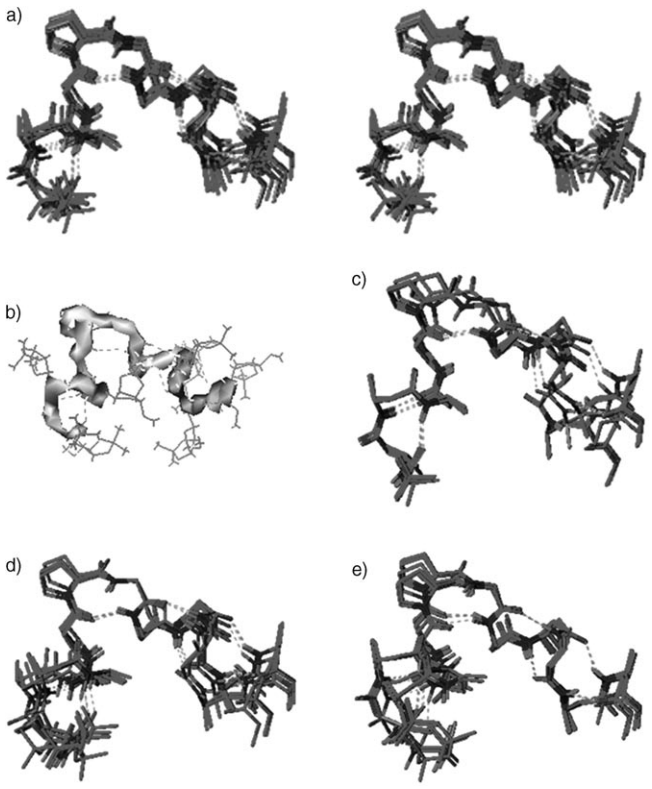

Figure 3. MD structure of undecapeptide **5.** a) Stereoview of 15 superimposed minimum-energy structures (sugar moieties replaced with a methyl groups after the calculations for clarity); b) backbone has been shown as a ribbon for a better display; c) view highlighting helix at the N terminus (only residues 1–4 superimposed); d) view highlighting the turn (only residues 4–7 superimposed); e) view highlighting helix at the C terminus (only residues 7–11 superimposed).

information on $^3J_{C_\alpha H\text{-}C_\beta H}$ and the NOE correlations between the $C_\alpha H$ and $C_\beta H$ permitted us to make stereospecific assignments of $C_\alpha H$ and $C_\beta H$, which in turn showed the predominance of structures with $\phi \approx 120°$ and $\theta \approx 60°$. Similarly for β-hGly(7), ϕ was deduced to be $\approx -120°$ and $\theta \approx 60°$.[13] Observation of strong $C_\alpha H_{Pro\text{-}(R)}(7)/NH(8)$ NOE correlations further support $\psi \approx 120°$. The flanking of D-Pro-Gly with β-hGly generates backbone conformational space, which further extends the two helices into the turn and imparts desired robustness to the structure. All the data presented above provides unequivocal evidence of a HTH tertiary structure in **5**, which contains only 11 amino acid residues.

Except for the first residue, for all Caa residues in **4** and **5**, the $^3J_{C_\beta H\text{-}C_4H} > 9$ Hz implies $\chi 1(C_\beta H\text{-}C_\beta\text{-}C_4\text{-}C_4H) \approx 180°$. However, like earlier observations,[7] the first residue in **1**, **4**, and **5** and the second residue in **2** have $^3J_{C_\beta H\text{-}C_4H} \approx 6$ Hz, suggesting predominance of structures with $|\chi 1| \approx 60°$. The sugar ring couplings of $^3J_{C_1H\text{-}C_2H} \approx 0$ Hz, $^3J_{C_2H\text{-}C_3H} \approx 5.8$ Hz, and $^3J_{C_3H\text{-}C_4H} \approx 3.2$ Hz are in conformity with the $^2T_3$ sugar pucker for the furanose ring.

Fifteen superimposed structures of **4** and **5**, obtained from restrained MD studies are shown in Figures 2 and 3, respectively (the backbones are also represented in ribbon form for better visualization). For peptide **4** (Figure 2), the 12/10 helix as well as the type II′ β turn, characterized from the NMR spectroscopic data, are very clearly visible. The backbone and the heavy-atom root mean square deviation (RMSD) are 0.75 Å and 0.81 Å, respectively. Various views highlighting the two helices and the turn in **5** are presented in Figure 3. The 12/10-mixed helices as well as the type II′ β turn are very well elaborated in these structures. The backbone and the heavy-atom RMSD are 0.45 Å and 0.64 Å, respectively. The premise of using the individual structural elements appears appropriate and resulted in the desired tertiary structure. The two helices, tethered through a turn, are almost orthogonal to each other, with an angle of about 80°, and resemble the ubiquitous protein structural element in DNA-binding motif common to transcription factors and the EF hand of a calcium binding protein.[5,15]

The CD spectra for **1**, **2**, **4**, and **5**[13] correspond to a typical 10/12-mixed helix,[7] with a maximum at 198 nm, whereas that of **3**, on the other hand, showed weak signatures of a turn. The

characteristics of a turn are not distinctly deciphered in the spectra of **4** and **5**.

In conclusion, the helix–turn–helix motif has been generated de novo with a small peptide containing 11 amino acid residues. The robust individual secondary structures of the oligomers obtained from $Caa_{(l)}$ and the novel turn motif β-hGly-D-Pro-Gly-β-hGly are conserved, resulting in a very well defined HTH tertiary structure with the helices almost orthogonal to each other. These investigations demonstrate that the challenges of assembling higher-order molecular assemblies by using unnatural amino acids can be achieved based on such basic structural units. Availability of the large registry of helical scaffolds in the foldamer domain from the unnatural amino acids would thus permit the creation of a variety of new materials with diverse tertiary structures to understand their implications in biology.

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