Peptide Helices

DOI: 10.1002/ange.200501247

9/11 Mixed Helices in α/β Peptides Derived from C-Linked Carbo-β-Amino Acid and L-Ala Repeats[**]

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Oligomers of nonnatural amino acids, which form a variety of secondary structures, have been studied extensively.[1] The impetus for such efforts has invariably been a desire to understand the conformational behavior and to develop these structures into biomolecules with novel properties. The β peptides, a very important class of foldamers, are derived from β-amino acids and exhibit several novel secondary structures.[2–6] The 10/12-mixed helix, unique to β peptides, was discovered by Seebach et al.[4] in β^2/β^3-dipeptide repeats. Theoretical studies by Hofmann and co-workers[7a] and Wu et al.[7b,c] predicted that 10/12 and 12/10 helices are energetically the most favored secondary structures in β peptides in solution. Subsequently, Kessler and co-workers[8] used a cyclic sugar β-amino acid and β-hGly repeats to obtain such mixed helices. In a later study, we demonstrated the presence of robust right-handed 10/12- and 12/10-mixed helices[9] in heterochiral dipeptide repeats from carbo-β^3-amino acid (Caa) residues with "alternating chirality" at the C^β carbon atom. Further studies on peptides with alternating Caa and β-hGly repeats resulted in right- and left-handed 10/12 and 12/10 helices.[10] Although Hofmann and co-workers[11] predicted the existence of mixed helices in homologous α, β, γ, and δ peptides from theoretical studies, the experimental realization of these structures has so far been limited only to β peptides.[4,8–10] From the above studies, it is amply evident that mixed helices invariably resulted from heterodipeptide repeats. However, the α/β-dipeptide repeats reported by Gellman and co-workers[12] showed the coexistence of an

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[**] IICT Communication No. 050402. P.N. and P.J. are thankful to CSIR, New Delhi, for financial support.

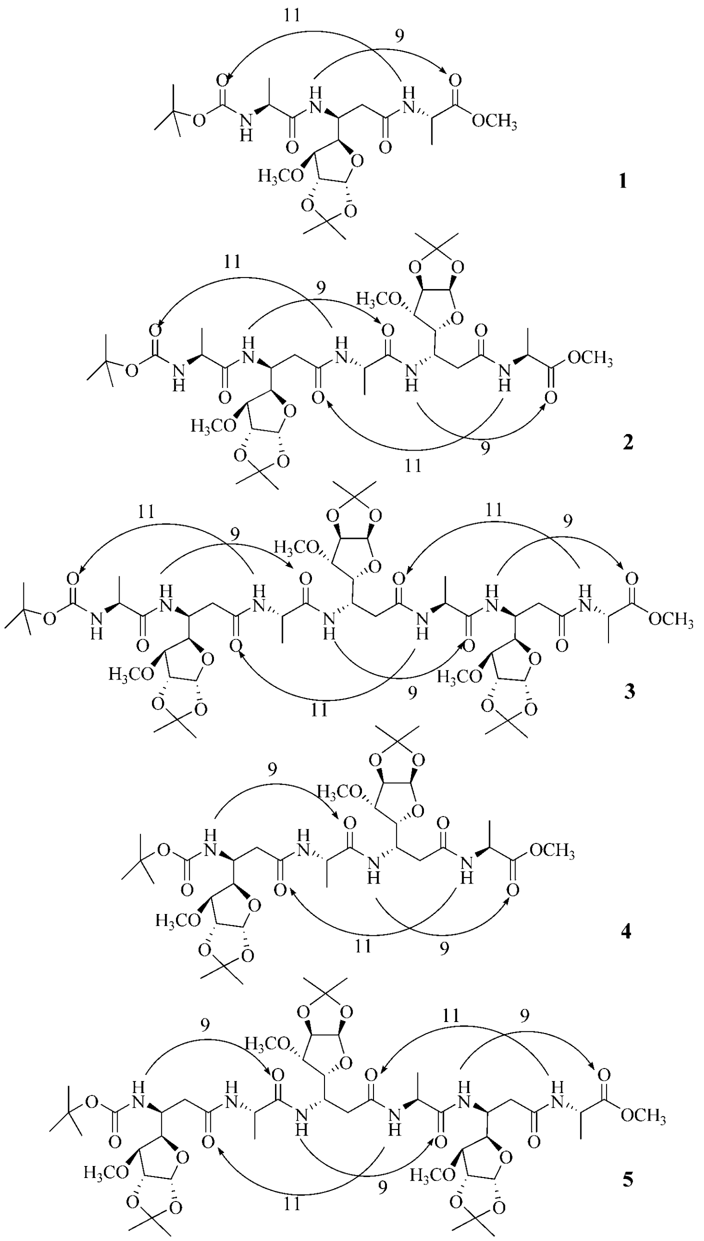
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

11 helix along with a 14/15 helix, whereas Reiser and coworkers[13] observed a 13 helix in the α/β peptides containing alternate *cis*-β-amino cyclopropane carboxylic acid and L-Ala residues.

Based on the above observations on mixed helices and our own studies on Caa-derived β peptides,[9,10] a hybrid α/β-dipeptide motif was envisaged as a "design control" to identify novel secondary structures. Herein, we report the synthesis of α/β-hybrid peptides from the *S* epimer of Caa[14] and L-Ala, as well as the finding of hitherto unknown and unprecedented 9/11 helices in these peptides by extensive NMR spectroscopy, circular dichroism (CD), and molecular dynamics (MD) studies.

The β peptides **1**–**5** (Scheme 1) were prepared by conventional coupling methods, and structural studies were conducted to analyze their conformational details. All of the peptides (100 μM in methanol) displayed novel CD data with maxima at ≈198 nm along with weaker flat maxima at ≈225 nm; this pattern is distinctly different from the CD profile of a 10/12-mixed helix.

The ^{1}H NMR spectrum of tripeptide **1** displayed downfield shift for two amide protons at δNH = 7.16 and 7.58 ppm, a result that suggests their involvement in hydrogen bonding. Solvent titration studies[15] showed very small chemical-shift changes ($\Delta\delta$NH) of 0.213 and 0.422 ppm for NH(2) and NH(3), respectively, thereby confirming their participation in hydrogen bonding. The observed long-range NOE correlations for C$^\alpha$H(1)/NH(3), C$^\beta$H(2)/NH(3), and NH(2)/NH(3) further support a well-defined structure. Furthermore, the coupling constant values of $^3J_{\mathrm{C^\alpha H/C^\beta H}} < 5$ Hz indicate a restricted value of $|\theta| \approx 60°$ for Caa(2). The nondescript $^3J_{\mathrm{NH/C^\alpha H}}$ values of 6.8 and 7.5 Hz for Ala(1) and Ala(3), at this stage, prevent us from deducing the structure for **1**. To obtain concrete information for determining the underlying structure in this family, peptides **2** and **3** were investigated.



Scheme 1. Structures of α/β-hybrid peptides **1**–**5**; hydrogen-bond interactions are indicated by the arrows.

The ^{1}H NMR spectrum of pentapeptide **2** showed that four of the amide protons (except NH(1)) appear at δNH > 7 ppm, which suggests their participation in hydrogen bonding. The same was further confirmed by solvent titration studies ($\Delta\delta$NH < 0.256 ppm).[15] For Caa(2) and Caa(4), small values of $^3J_{\mathrm{NH/C^\alpha H}}$ (< 5.2 Hz) and large values of $^3J_{\mathrm{NH/C^\beta H}}$ (9.3 Hz) are consistent with $|\theta| \approx 60°$ and $|\phi| \approx 100°$, respectively. $^3J_{\mathrm{NH/C^\alpha H}} = 6.1$ and 5.6 Hz for Ala(1) and Ala(3), respectively, values which correspond to $|\phi| \approx 60°$. To derive the handedness of the structures by NMR spectroscopy, it was crucial to distinguish the resonances of the C$^\alpha$H$_{(\text{pro-}S)}$ and C$^\alpha$H$_{(\text{pro-}R)}$ protons. The NOE correlations for C$^\gamma$H(2)/NH(2), C$^\alpha$H$_{(\text{pro-}S)}$(2)/C$^\delta$H(2), C$^\alpha$H$_{(\text{pro-}R)}$(2)/NH(3), C$^\gamma$H(4)/NH(4), C$^\alpha$H$_{(\text{pro-}S)}$(4)/C$^\delta$H(4), and C$^\alpha$H$_{(\text{pro-}R)}$(4)/NH(5) enabled us to assign these resonances unequivocally and allowed us to deduce a value of about +60° for θ. The distinctive signatures of an 11/9 helix were observed in the ROESY spectrum, in which the NOE correlations for C$^\alpha$H(1)/NH(3) and C$^\alpha$H(3)/NH(5) support an 11-membered hydrogen bond between the CO group of *tert*-butoxycarbonyl (Boc) and NH(3), and between NH(5) and CO(2) (Scheme 1). Similarly, the presence of weak but characteristic NOE correlations for NH(2)/NH(3) and NH(4)/NH(5) are consistent with 9-membered hydrogen bonds between NH(2)–CO(3) and NH(4)–CO(5) (Scheme 1). All the distinguishing features discussed above confirm an unprecedented right-handed 11/9/11/9-mixed helix for **2**.

By analogy with the structural information for **2**, the NOE correlations observed in the ROESY spectrum of **1** and the distinct $^3J_{\mathrm{C^\alpha H/C^\beta H}}$ and $^3J_{\mathrm{NH/C^\beta H}}$ values (for Caa) confirm an 11-membered hydrogen bond between the CO group of Boc and NH(3) and a 9-membered hydrogen bond between NH(2)–CO(3), thereby defining an 11/9 helix. The observation of a helix in a molecule as short as a tripeptide is particularly noteworthy.

Propagation of an exceptionally robust 11/9 helix in heptapeptide **3** was confirmed by NMR spectroscopy, which showed a well-resolved spectrum in the amide region, in which NH(2)–NH(6) gave δNH > 7 ppm and small $\Delta\delta$NH values of 0.262 ppm in the solvent titration studies,[15] which confirmed hydrogen bonding. The ${}^3J_{NH/C^{\alpha}H}$ values of 6.1, 5.6, and 5.1 Hz for Ala(1), Ala(3), and Ala(5), respectively, correspond to $|\phi| \approx 60°$. Similarly, for Caa(2), Caa(4), and Caa(6), values of ${}^3J_{NH/C^{\beta}H} > 9.3$ Hz and ${}^3J_{C^{\alpha}H/C^{\beta}H} < 5.6$ Hz imply that $|\phi| \approx 100°$ and $|\theta| \approx 60°$. The assignments of the $C^{\alpha}H_{(pro\text{-}S)}$ and $C^{\alpha}H_{(pro\text{-}R)}$ resonances for the Caa residues were obtained similarly to those for **2**. Additionally, NOE correlations for 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) (Figure 1) as well as the information on hydrogen bonding, confirmed 11-membered hydrogen bonds between NH(3)–CO group of Boc, NH(5)–CO(2), NH(7)–CO(4), and 9-membered hydrogen bonds between NH(2)–CO(3), NH(4)–CO(5), and NH(6)–CO(7) (Scheme 1). The presence of a right-handed 11/9/11/9/11/9 helix and its propagation with the chain length in **3** was evident from all the characteristics described above. The CD profiles of **1**–**3** (Figure 2) showed maxima at around 198 nm and the progressive increase in the molecular ellipticity per residue reflects the enhanced robustness with increasing size of the oligomers.

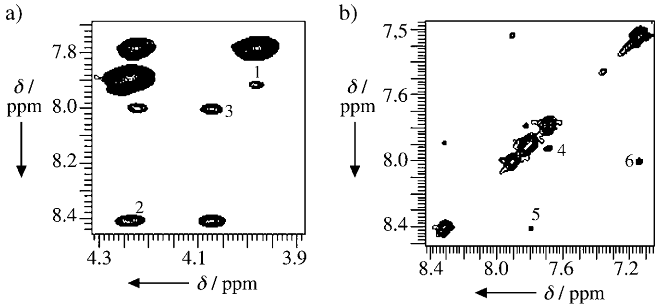


Figure 1. ROESY spectrum of **3**: a) NOE interactions C$^{\alpha}$H(1)/NH(3), C$^{\alpha}$H(3)/NH(5), and C$^{\alpha}$H(5)/NH(7) are marked as 1, 2, and 3, respectively; b) NOE interactions NH(2)/NH(3), NH(4)/NH(5), and NH(6)/NH(7) are marked as 4, 5, and 6, respectively.

Similar to our earlier findings with 12/10-mixed helices,[8,9] two possible helical structures (an 11/9 and a 9/11 helix) were expected in these α/β peptides. Consequently, peptides **4** and **5** were prepared with a Caa monomer at the N terminus to observe the 9/11 helix. The ^{1}H NMR spectrum of **4** showed all the signatures of hydrogen bonding, such as a low-field shift (δNH > 7 ppm) for two amide protons and small changes of the $\Delta\delta$NH values in solvent titration studies. Furthermore, the coupling constants and the characteristic long-range NOE correlations for C$^{\alpha}$H(2)/NH(4), NH(1)/NH(2), and NH(3)/NH(4) provide compelling evidence for the presence of a unique 9/11/9 helix.

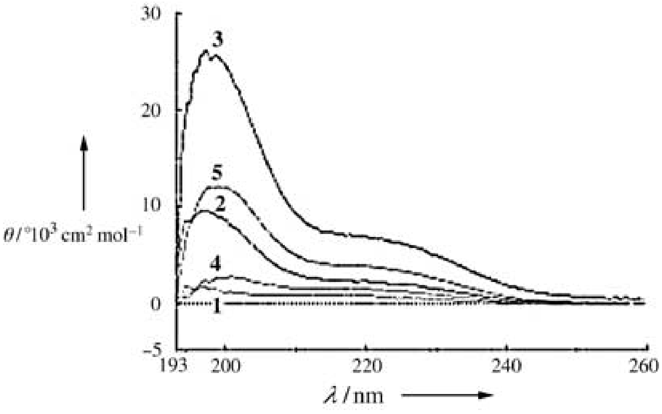


Figure 2. CD profiles of **1**–**5**.

Likewise, a low-field shift (δNH > 7 ppm) for four amide resonances, and the solvent titration studies ($\Delta\delta$H < 0.686 ppm) confirmed the participation of all the amide groups except NH(2) in hydrogen bonding in **5**. The ${}^3J_{NH/C^{\alpha}H}$ coupling constants for Ala(2) and Ala(4) are 4.8 and 5.9 Hz, respectively; these values correspond to $|\phi| \approx 60°$. Furthermore, small ${}^3J_{C^{\alpha}H/C^{\beta}H}$ values for all Caas and distinct NOE correlations for NH(1)/NH(2), NH(3)/NH(4), NH(5)/NH(6), C$^{\alpha}$H(2)/NH(4), and C$^{\alpha}$H(4)/NH(6) support a propagated right-handed 9/11/9/11/9-mixed helix for **5**. The molecular ellipticity per residue for **5** and the NMR spectroscopic data (larger amide chemical shifts) demonstrate that this helix is more robust than that of **4**.

The ${}^3J_{C^{\beta}H/C(4)H}$ values greater than 9 Hz for the Caa residues (except for Caa(1) in **4** and **5**) correspond to χ^1(C$^{\beta}$H–C$^{\beta}$–C(4)–C(4)H) ≈ 180° in all of the oligomers, as observed in oligomers with 10/12-mixed helices in our earlier study.[9] The ${}^3J_{C1H/C2H} \approx 4$ Hz, ${}^3J_{C(2)H/C(3)H} \approx 0$ Hz, and ${}^3J_{C(3)H/C(4)H} \approx 3$ Hz values for the sugar rings are consistent with a $^3T_2$ sugar pucker, which is similar to that observed earlier. Furthermore, the presence of an envelope conformation for the isopropylidine ring is reflected in the strong Me$_{(pro\text{-}R)}$/C(2)H and Me$_{(pro\text{-}R)}$/C(1)H and the weak Me$_{(pro\text{-}S)}$/C(4)H NOE correlations.

The NOE-restrained MD calculations for **1**–**5** resulted in structures that are in excellent agreement with those obtained above. Volume integrals from the ROESY spectra provided quantitative NOE constraints by using the two-spin approximation. Figures 3 and 4 show a superposition of the 25 lowest-energy structures for **3** and **5**, respectively. For the sake of clarity, the sugar moieties were replaced by methyl groups for the structures in Figures 3 and 4 after the MD calculations. The maximum violations for the NMR-derived constraints for **3** and **5** are 0.25 and 0.33 Å, respectively. The average pairwise heavy-atom and backbone root-mean-square deviations are 0.31 and 0.29 Å for **3**, and 0.58 and 0.47 Å for **5**, respectively. The average dihedral angles of the backbones were derived by excluding the first and last residues. In **3**, the ϕ, θ, and ψ values for Caa are 91 ± 7°, 63 ± 3°, and −91 ± 4°, respectively, whereas $\phi = -70 \pm 1°$ and $\psi = 135 \pm 2°$ for L-Ala. The corresponding values for **5** are 86 ± 4°, 66 ± 2°, −91 ± 4°, −70 ± 2°, and 131 ± 2°.

In conclusion, our exploratory study on the hybrid α/β-dipeptide repeats resulted in the unprecedented 9/11-mixed helices (9/11 and 11/9). Furthermore, a robust helical structure (11/9) in the shortest α/β tripeptide (L-Ala–Caaβ^3–L-Ala) is a notable feature of the present design. These peptides, in contrast to the reported α/β-mixed peptides derived from cyclic β-amino acids, show a high propensity for

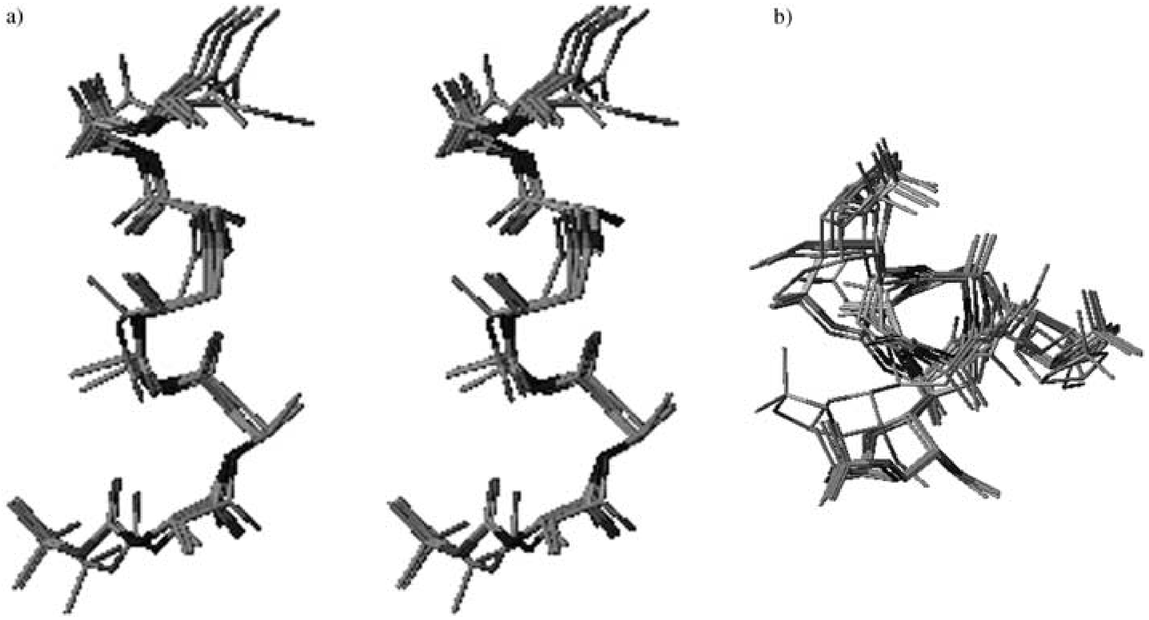


Figure 3. MD structures of **3**: a) stereo view in which sugar moieties were replaced with methyl groups after the calculations for clarity; b) view down the helical axis.

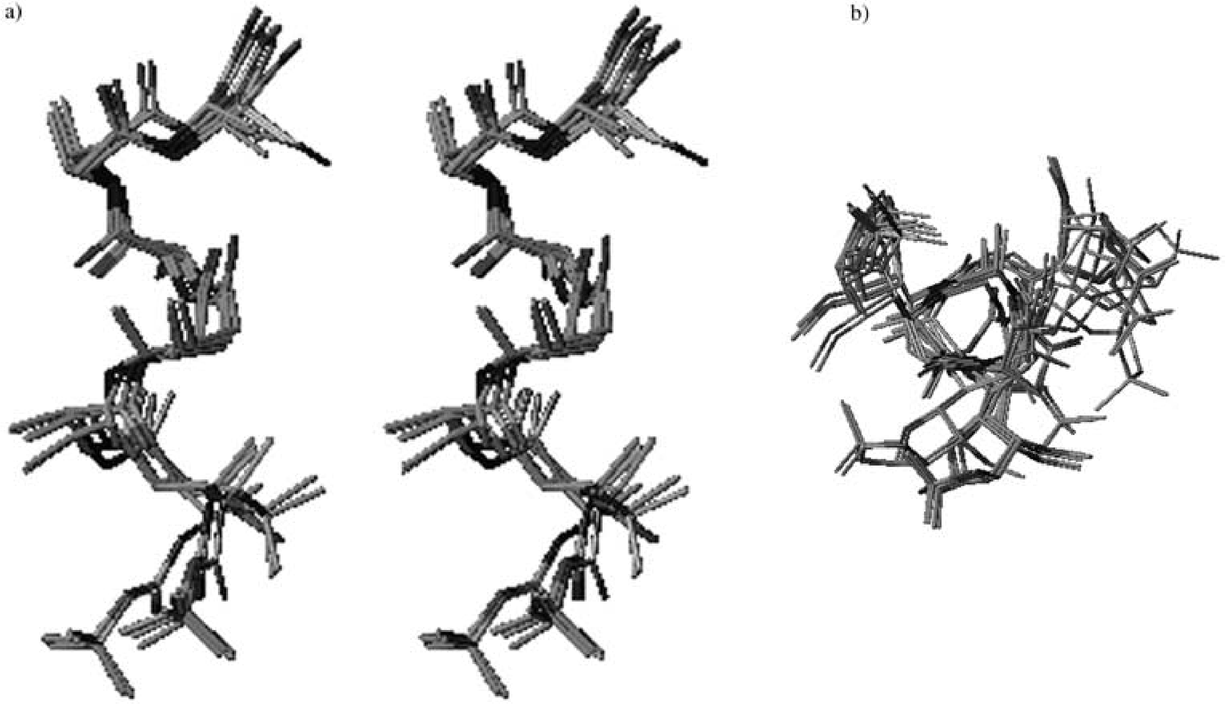


Figure 4. MD structures of **5**: a) stereoview in which sugar moieties were replaced with methyl groups after the calculations for clarity; b) view down the helical axis.

the formation of stable mixed-helical structures. Extrapolation of this study to other α-amino acids will enhance the opportunity of finding not only new secondary structural elements, but also for the introduction of a wide variety of side chains and functionalities to derive desirable properties in this class of peptides.

Received: April 9, 2005
Published online: August 10, 2005

Keywords: amino acids · helical structures · NMR spectroscopy · peptides · structure elucidation

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