Peptide Structures

Left-Handed Helical Twists in "Mixed β-Peptides" Derived From Alternating C-Linked Carbo-β^3-Amino Acids and β-hGly Units**

*Gangavaram V. M. Sharma,**
Kondreddi Ravinder Reddy, Palakodety Radha Krishna, Ampapathi Ravi Sankar, Pagadala Jayaprakash, Bulusu Jagannadh, and Ajit C. Kunwar**

Synthetic oligomers of β-amino acids, that is, β-peptides,[1] are amongst the most studied class of molecules in foldamer chemistry. Changes in their substitution pattern generate a variety of interesting structural features in these molecules,[2–5] which have thus begun to prove immensely useful in bioactive peptide mimicry.[6] Amongst the various peptide secondary structures, mixed helices are unique to β-peptides. These structures contain intertwined 12 and 10-membered H-bonded rings and are unprecedented in α-proteins. Seebach et al.[4a] were the first to demonstrate the existence of right-handed 12/10 mixed helical structures in β-peptides with alternating β^2 and β^3 residues. Kessler and co-workers[4b] have reported such structures in mixed peptides containing constrained f-sugar amino acid and β-hGly repeats. We recently reported[7] the formation of both 10/12 and 12/10 right-handed helices by β-peptides derived from C-linked carbo-β^3-amino acids (Caa) with "alternating chirality." Although a variety of helical structures have been discovered in β-peptides, no reports have been published in this active area of research that describe left-handed mixed helices. Several natural proteins[8] have been found to contain small fragments of left-handed helices, most of which occur in or near an active or binding site and are thus likely to be of functional importance. Herein, we report the design, synthesis, and structural study of "mixed β-peptides" with right-handed and novel left-handed mixed (10/12 and 12/10) helical structures. These peptides are derived from alternating epimeric C-linked carbo-β^3-amino acids[9] (**1** or **2**; Scheme 1) and β-hGly units.

Our previous study revealed that Caa **1** occupies an energetically unfavorable position in the carbo-β-peptides **3** and **4**,[7] although robust mixed helical structures were formed by these peptides. We envisaged a new design intended to provide more conformational freedom and relieve steric strain[10,11] through the inclusion of alternating Caa (**1** or **2**) and β-hGly residues. Mixed β-peptides **5**–**12** (**5**/**6** and **7**/**8** with Caa **2** and **1** at the N terminus, respectively; **9**/**10** and **11**/**12** with β-hGly at the N terminus) were prepared by conventional procedures (1-ethyl-3(3-dimethylaminopropyl)carbodiimide, 1-hydroxy-1*H*-benzotriazole) and differences in the conformational behavior of the various peptides were studied. We anticipated that Caas **1** and **2** would define the conformational behavior of β-hGly in **5**–**12** (Scheme 1).

Structural studies on these peptides were carried out by using NMR and CD spectroscopic techniques. The ^{1}H NMR spectra of **5** and **6** in $CDCl_3$ show well-resolved backbone proton signals, as well as low-field NH resonances (NH(2)-NH(3) for **5**; NH(2)-NH(5) for **6**), which indicate the involvement of these NH groups in H bonding. This bonding was further confirmed by solvent titration studies.[12] The coupling constants $^3J_{C\alpha H/C\beta H}$ of the peptides (>10 Hz or <5 Hz) clearly demonstrate the predominance of a single conformation about the Cα–Cβ bond (single θ value), except in the N-terminal residue. The $^3J_{C\alpha H/C\beta H}$ values, along with various CαH/CβH and strong sequential NOE correlations (between the NH group of β-hGly or Caa **2** and the preceding $C\alpha H_{(pro\text{-}R)}$ or $C\alpha H_{(pro\text{-}S)}$ atom, respectively), confirm a θ value of around 60° for these peptides. The ROESY spectrum of **5** revealed the presence of long-range NOE correlations (CβH(1)/NH(3), $C\beta H(1)/C\alpha H_{(pro\text{-}R)}(3)$) unique to the signature of a 12-membered ring connected by an H bond involving *tert*-butoxycarbonyl (Boc)-CO and NH(3). A weak NH(2)/NH(3) NOE was observed, which supports the existence of a 10-membered ring with an H bond between NH(2) and CO(3), and confirms that **5** has a right-handed 12/10 helical structure. Similarly, the strong long-range backbone NOE interactions CβH(1)/NH(3), $C\beta H(1)/C\alpha H_{(pro\text{-}R)}(3)$, CβH(3)/NH(5), and $C\beta H(3)/C\alpha H_{(pro\text{-}R)}(5)$, and weak NH(2)/NH(3) and NH(4)/NH(5) NOE correlations observed in the ROSEY spectrum of **6** (Figure 1 A) provide ample evidence of a propagated right-handed 12/10/12/10 helical structure for this peptide. The coupling constants of both **5** and **6** ($^3J_{NH/C\beta H} > 8.9$ Hz for Caa **2**, $^3J_{NH/C\beta H} > 8.9$ Hz and < 3.3 Hz for β-hGly except in the C-terminal residue) correspond to $|\phi| \approx 100°$, which is consistent with a mixed helical structure. The first Caa residue has a $^3J_{C\beta H/C4H}$ value of 6.8 Hz, which indicates averaging of the conformation about CβH–Cβ–C4–C4H (χ^1). The other Caa residues have coupling constants $^3J_{C\beta H/C4H} > 9.5$ Hz; this value implies the preponderance of a structure with $\chi^1 \approx 180°$. The presence of several conforma-

[*] Dr. G. V. M. Sharma, K. R. Reddy, P. R. Krishna
D-211, Discovery Laboratory
Organic Chemistry Division III
Indian Institute of Chemical Technology
Hyderabad 500 007 (India)
Fax: (+91) 40-2716-0387 or 2716-0757
E-mail: esmvee@iict.res.in

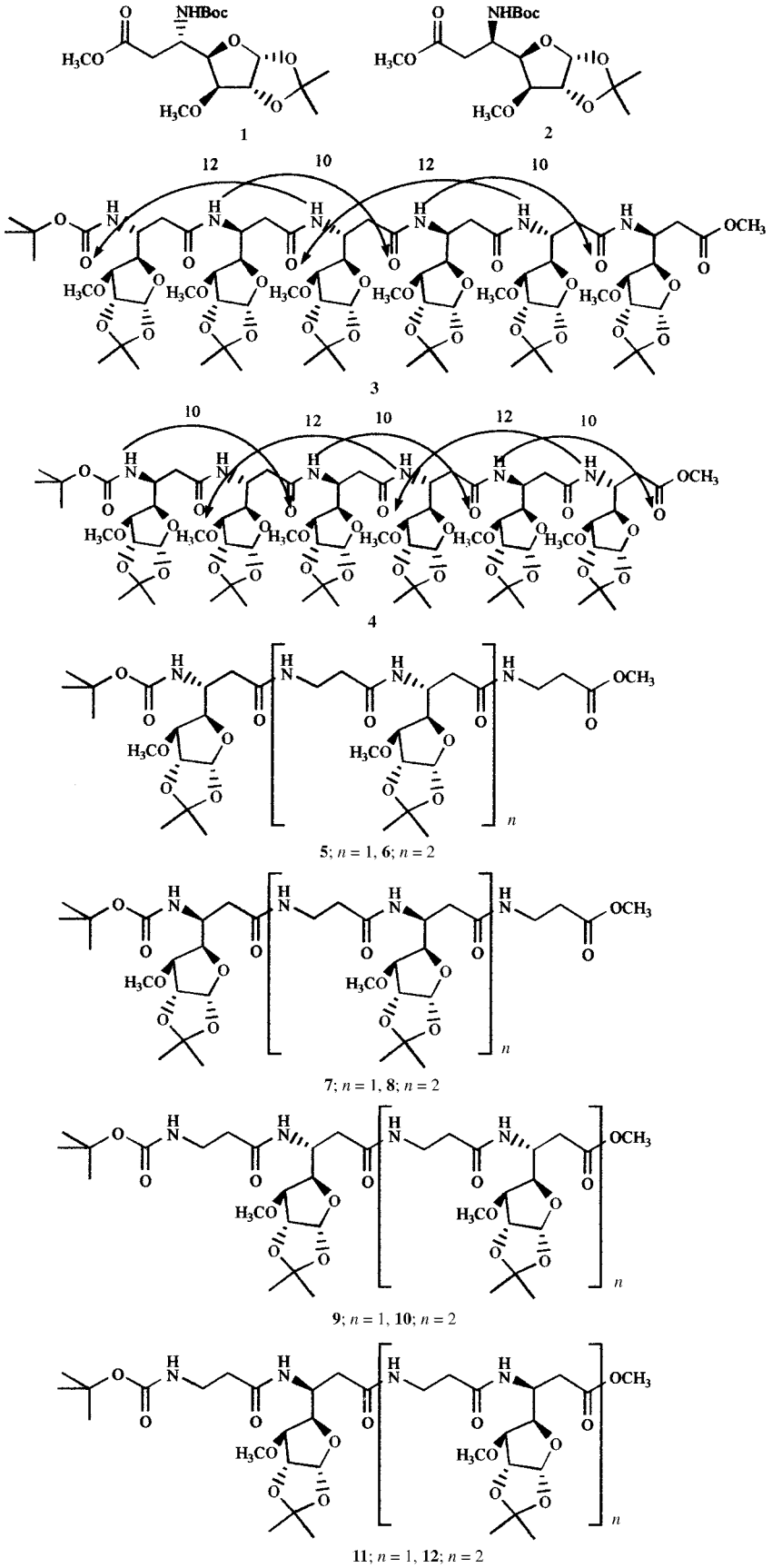
P. Jayaprakash, Dr. B. Jagannadh
Molecular Modelling Unit
Indian Institute of Chemical Technology
Hyderabad 500 007 (India)
Fax: (+91) 40-2716-0512
E-mail: jagan@iict.res.in

A. R. Sankar, Dr. A. C. Kunwar
Centre for Nuclear Magnetic Resonance
Indian Institute of Chemical Technology
Hyderabad 500 007 (India)
Fax: (+91) 40-2719-3108
E-mail: kunwar@iict.res.in

[**] Indian Institute of Chemical Technology Communication No. 031115. K.R.R. and A.R.S. are thankful to the Council of Scientific and Industrial Research, New Delhi, for financial support.

Supporting information for this article (details of NMR experiments, solvent titration plots, and the distance constraints used in MD calculations) is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200353467 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 1. Structures of monomers **1** and **2**, carbo-β-peptides **3** and **4** (with H bonding indicated by arrows), and mixed β-peptides **5**–**12**.

tions as a result of fraying at the C-terminal residue is reflected by an averaging of the coupling constants.

The CD spectra (Figure 2) of **5** and **6** in methanol (0.1 mM), have maxima at about 203 nM,[4a] which is a very similar value to that observed for **3**[7] and corresponds to the signature of a mixed helical conformation. In spite of the insertion of β-hGly[10] and the resulting increase in conformational freedom, mixed helices were the intrinsically favored structures of **5** and **6**. In both peptides, the amide group of Caa **2** participates in an H bond connecting a 12-membered ring, whilst that of the β-hGly residue is effectively induced to participate in an H bond in a 10-membered ring; the result is a robust right-handed 12/10 helix.

^{1}H NMR and CD studies on **7** and **8** gave interesting results. The appearance of NH(2) and NH(3) signals at $\delta > 7$ ppm in the NMR spectrum of **7**, as well as the presence of weak NOE correlations (for example, CβH(1)/NH(3) and CβH(1)/CαH$_{(pro\text{-}S)}$(3)), suggests that the NH(3) proton of **7** participates in an H bond that results in formation of a 12-membered ring and indicates the nucleation of a structure. Definitive information about this structure could not be obtained because of averaging of the coupling constants owing to several conformations. In contrast, peptide **8** has a well-defined structure. The observation of low-field amide δ values and the results of solvent titration studies[12] confirmed that H bonding occurs from NH(2) to NH(5) in **8**. The $^3J_{C\alpha H/C\beta H}$ values (> 10 Hz or < 5 Hz) for residues (1)–(5) indicate the predominance of a single conformer (single θ value). The presence of CβH(1)/NH(3), CβH(1)/CαH$_{(pro\text{-}S)}$(3), CβH(3)/NH(5), and CβH(3)/CαH$_{(pro\text{-}S)}$(5) NOE correlations (Figure 1B) suggests the existence of 12-membered rings involving H bonds from NH(3) to CO(Boc) and NH(5) to CO(2), whilst weak NH(2)/NH(3) and NH(4)/NH(5) NOE correlations indicate the presence of 10-membered rings with H bonds from NH(2) to CO(3) and NH(4) to CO(5). These results imply a 12/10/12/10 helical structure for **8**. In contrast to the situation in previously studied peptides,[7] the amide protons of the Caa **1** residues in **8** participate in H bonds to form 12-membered instead of 10-membered rings. Moreover, NOE interactions CβH(i) and CαH$_{(pro\text{-}S)}$($i+2$) ($i = 1$ and 3) were detected for **8**. These NOE correlations verify the existence of a 12-membered ring but differ from those observed for right-handed mixed helices, which show CβH(i)/CαH$_{(pro\text{-}R)}$($i+2$) NOE correlations. The strong sequential NOE correlations observed for **8** between the NH proton of the β-hGly residue and the preceding CαH$_{(pro\text{-}S)}$ proton, and between the NH group of Caa **1** and the preceding

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

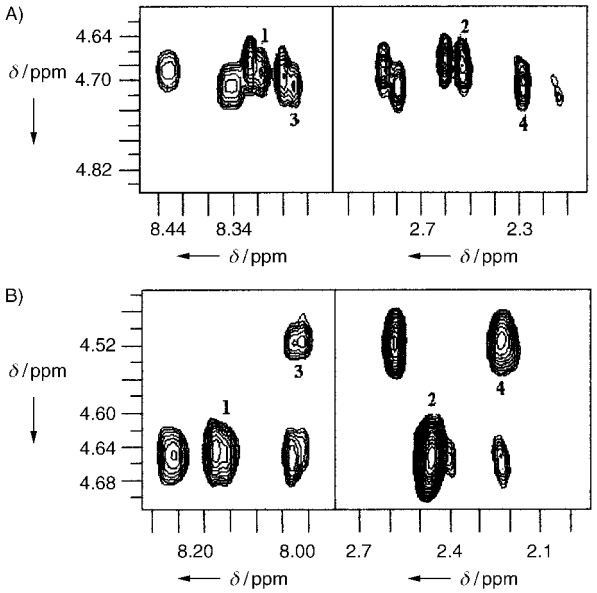


Figure 1. A) ROESY spectrum of **6**. The NOE correlations CβH(3)/NH(5), CβH(3)/CαH$_{(pro\text{-}R)}$(5), CβH(1)/NH(3), and CβH(1)/CαH$_{(pro\text{-}R)}$(3) are marked 1, 2, 3, and 4, respectively. B) ROESY spectrum of **8**. The NOE correlations CβH(3)/NH(5), CβH(3)/CαH$_{(pro\text{-}S)}$(5), CβH(1)/NH(3), and CβH(1)/CαH$_{(pro\text{-}S)}$(3) are marked 1, 2, 3, and 4, respectively.

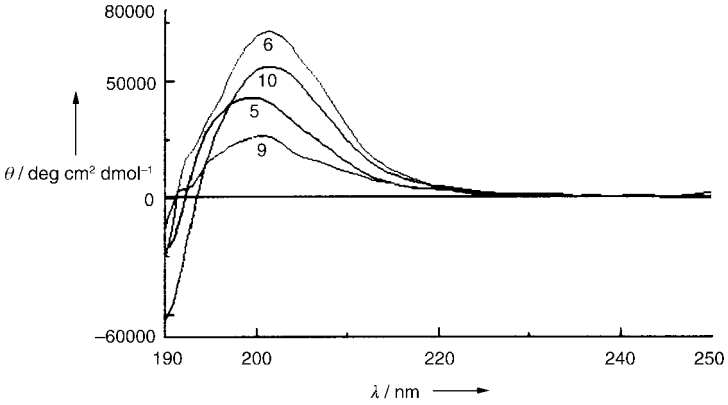


Figure 2. CD spectra of **5**, **6**, **9**, and **10**.

CαH$_{(pro\text{-}R)}$ proton are in agreement with the well-defined $^3J_{C\alpha/C\beta H}$ values recorded and imply a predominant conformation with $\theta \approx -60°$ for all these residues. Comparison of this result with the θ value of around $+60°$ determined for peptides **3**–**6** unequivocally demonstrates the occurrence of a "switch" in the sense of the helical twist in **8** relative to that in **3**–**6**. This twist leads to the formation of an unprecedented left-handed 12/10/12/10 helix. The $^3J_{NH/C\beta H}$ values ($^3J_{NH/C\beta H} >$ 9.6 Hz for Caa **1**; $^3J_{NH\text{-}C\beta H} > 9.0$ Hz and < 2.1 Hz for β-hGly) of **8** correspond to $|\phi| \approx 100°$, which is consistent with a mixed helical structure. A $^3J_{C\beta H/C4H}$ value of 6.4 Hz for the first Caa residue indicated an averaging about χ^1. The other Caa residues had large coupling constants, $^3J_{C\beta H/C4H} \approx 9$ Hz, which implies the preponderance of a structure with $\chi^1 \approx 180°$. The left-handed helical twist in **8** was further confirmed by the CD spectrum of the peptide. A minimum was observed at 205 nm (Figure 3) and the molar ellipticity of about $-12\,000$ deg cm^2 dmol^{-1} per residue is lower than the values recorded for **5** and **6**.

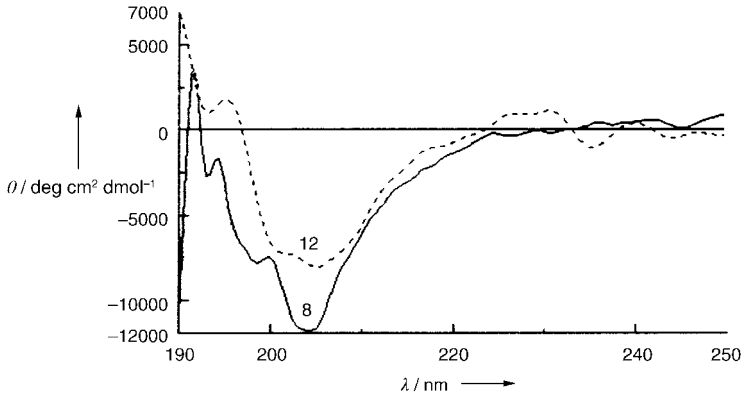


Figure 3. CD spectra of **8** and **12**.

The switch in handedness observed in **8** can be rationalized by applying energy considerations, as shown by the Newman projections about Cα–Cβ presented in Figure 4. An *anti*-periplanar arrangement of the amide carbonyl group and the sugar moiety ($\theta = 60°$) in Caa **2** (Figure 4a) is energetically favored and leads to a right-handed helix. Under similar conditions, the amide carbonyl group and sugar moiety of "epimeric" Caa **1** are in an energetically less favored (−) *syn*-clinical arrangement, as indicated in Figure 4b. However, Figure 4c shows that these groups adopt a favorable *anti*-periplanar low-energy conformation in a left-handed helical structure with $\theta = -60°$. The results of theoretical studies[10,11] are consistent with these observations. The structure depicted in Figure 4b leads to a sterically less favored axial arrangement of the Cβ–Cγ bond connecting the sugar moiety to the backbone in **1**, whereas the left-handed helical structure in Figure 4c places the Cβ–Cγ bond in a more favorable lateral arrangement with respect to the helix axis.

The study described above revealed the helical structures of mixed β-peptides **5**–**8** and clearly shows that β-hGly participates in an H bond to form a 10-membered ring under the influence of Caas **1** and **2**. By analogy, we expected the mixed β-peptides **9**–**12**, in which N-terminal β-hGly units alternate with **2** or **1**, to generate right and left-handed 10/12 helices, respectively.

The results of studies on **9** and **10** were found to be in agreement with results we reported previously for 10/12 helices.[7] Low-field amide proton resonances (NH(1), NH(3), NH(4)) and solvent titration studies[12] confirmed the presence of H bonds in **9**. The specific NOE values showed the predominance of a single conformation with $\theta \approx 60°$ for all the β-hGly and Caa **1** residues. The CβH(2)/NH(4) and CβH(2)/CαH$_{(pro\text{-}R)}$(4) NOE correlations and weak NH(1)/NH(2) and NH(3)/NH(4) NOE correlations present in the

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

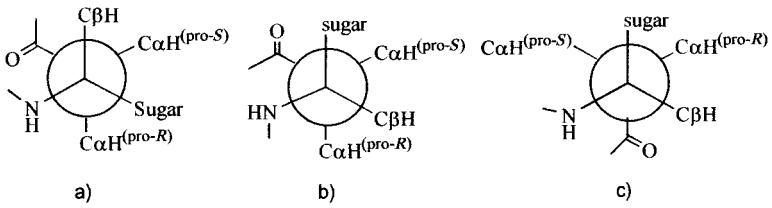


Figure 4. Newman projections about three bond axes illustrating the stereochemistries of **1** and **2** in left-handed and right-handed mixed helical arrangements.

ROESY spectrum of **9** form the distinctive signature of a right-handed 10/12/10 helix. Similarly, all the amide protons in hexapeptide **10**, except those in the second residue, participate in H bonding. Characteristic NOE correlations and coupling constants indicated an extended right-handed 10/12/10/12/10 helix conformation for **10**.

The structure of **11** could not be determined conclusively because the NOE correlations are too weak, but the signatures of helix nucleation were observed. The appearance of five amide proton signals (not NH(2)) at low field in the spectrum of **12**, along with the results of solvent titration studies,[12] confirmed the participation of these protons in H bonding. The $^{3}J_{C\alpha H/C\beta H}$ values (>10 Hz or <5 Hz) for residues (2)–(6) and the presence of strong characteristic sequential NOEs similar to those observed for **8** indicated a θ value of around $-60°$ and a single predominant conformation for all the residues. Distinctive backbone NOEs (CβH(2)/NH(4), CβH(2)/C$\alpha H_{(pro\text{-}S)}$(4), CβH(4)/NH(6), CβH(4)/C$\alpha H_{(pro\text{-}S)}$(6); weak signals: NH(1)/NH(2), NH(3)/NH(4), and NH(5)/NH(6)) confirmed an unprecedented left-handed 10/12/10/12/10 helix conformation for **12**. The CD spectra of **9**, **10** (Figure 2), and **12** (Figure 3) confirm the above-mentioned results. The incorporation of β-hGly at the N terminus resulted in the successful synthesis of peptides **9**/**10** and **12** with right and left-handed 10/12 helical structures, respectively.

NOE-restrained[13] molecular dynamics (MD) calculations for **5**, **6**, **8**–**10**, and **12** gave results that are in agreement with the structures discussed above. A large number of long-range NOE constraints were derived from the volume integrals of the ROESY spectra by using the two-spin approximation and these values were used quantitatively. Figure 5 depicts a superposition of the 10 lowest-energy structures calculated for **6** and **8**, which have right and left-handed helical structures, respectively. The backbone is well-defined, especially for the middle residues, and there is fraying at the C terminus. The calculations converge well; the maximum violations are 0.32 and 0.31 Å for **6** and **8**, and the average pair-wise heavy-atom and backbone root-mean-square deviations are 0.48 and 0.46 Å for **6** and 0.46 and 0.30 Å for **8**.

In conclusion, we have shown that the "chirality" of "epimeric" Caas **1** and **2** residues in mixed β-peptides derived from alternating β-Caa and β-hGly units induces β-hGly to participate in H bonding, which results in novel helices. In addition, the β-hGly residues provided the requisite conformational freedom for a facile "switch" in handedness leading to the formation of unprecedented left-handed 10/12 and 12/10 helices. The design and synthesis of peptides that form left-handed helices provides an opportunity to understand the active role of these secondary structures in biological functions and to create novel tertiary structures with far-reaching implications for peptide chemistry. Efficient "design control" leading to the successful formation of left and right-handed mixed helices should open up new routes towards the development of artificial tertiary structures through incorporation of the various above-mentioned secondary structural elements into new complex structures with useful functions.

Figure 5. MD structures. A) Side view of **6**; B) side view of **8**. Hydrogen atoms are omitted and sugar moieties are replaced by methyl groups after calculations.

Received: December 5, 2003
Revised: April 20, 2004 [Z53467]

Keywords: β-peptides · amino acids · helical structures · hydrogen bonds · peptides

[1] a) D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015–2022; b) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180; c) K. Gademann, T. Hintermann, J. V. Schreiber, *Curr. Med. Chem.* **1999**, *6*, 905–925; d) W. F. DeGrado, J. P. Schneider, Y. Hamuro, *J. Pept. Res.* **1999**, *54*, 206–217; e) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232.

[2] a) D. Seebach, M. Overhand, F. N. M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913–941; b) D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 13071–13072.

[3] D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi, Jr., S. H. Gellman, *Nature* **1997**, *387*, 381–384.

[4] a) D. Seebach, K. Gademann, J. V. Schreiber, J. L. Matthews, T. Hintermann, B. Jaun, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1997**, *80*, 2033–2038; b) S. A. W. Gruner, V. Truffault, G. Voll, E. Locardi, M. Stockle, H. Kessler, *Chem. Eur. J.* **2002**, *8*, 4366–4376.

[5] a) T. D. W. Claridge, J. M. Goodman, A. Moreno, D. Angus, S. F. Barker, C. Taillefumier, M. P. Watterson, G. W. J. Fleet, *Tetrahedron Lett.* **2001**, *42*, 4251–4255; b) K. Gademann, A. Hane,

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

M. Rueping, B. Jaun, D. Seebach, *Angew. Chem.* **2003**, *115*, 1573 – 1575; *Angew. Chem. Int. Ed.* **2003**, *42*, 1534 – 1537.
[6] J. A. Patch, A. E. Barron, *Curr. Opin. Chem. Biol.* **2002**, *6*, 872 – 877.
[7] G. V. M. Sharma, K. Ravinder Reddy, P. Radha Krishna, A. Ravi Sankar, K. Narsimulu, S. Kiran Kumar, P. Jayaprakash, B. Jagannadh, A. C. Kunwar, *J. Am. Chem. Soc.* **2003**, *125*, 13670 – 13671.
[8] a) A. A. Adzhubei, M. J. E. Sternberg, *J. Mol. Biol.* **1993**, *229*, 472 – 493; b) P. K. Vlasov, G. T. Kilosanidze, D. L. Ukrainskii, A. V. Kuzmin, V. G. Tumanyan, N. G. Esipova, *Biophysics*, **2001**, *3*, 546 – 550; c) F. Jourdan, S. Lazzaroni, B. L. Mendez, P. Lo Cantore, M. de Julio, P. Amodeo, N. S. Iacobellis, A. Evidente, A. Motta, *Proteins* **2003**, *52*, 534 – 543.
[9] G. V. M. Sharma, V. Goverdhan Reddy, A. Subhash Chander, K. Ravinder Reddy, *Tetrahedron: Asymmetry* **2002**, *13*, 21 – 24.
[10] D. Seebach, P. E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 2043 – 2066.
[11] a) Y.-D. Wu, D.-P. Wang, *J. Am. Chem. Soc.* **1999**, *121*, 9352 – 9362; b) Y.-D. Wu, J.-Q. Lin, Y.-L. Zhao, *Helv. Chim. Acta* **2002**, *85*, 3144 – 3160; c) R. Gunther, H.-J. Hoffman, K. Kuczera, *J. Phys. Chem. B* **2001**, *105*, 5559 – 5567.
[12] Solvent titration was carried out by adding [D_6]dimethylsulfoxide (DMSO) stepwise to $CDCl_3$ solutions of the peptides to give a final [D_6]DMSO level of 33%.
[13] For details, see the Supporting Information.

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim