Helical Structures

DOI: 10.1002/anie.201306404

Structural Characterization of α/β-Peptides having Alternating Residues: X-ray Structures of the 11/9-Helix from Crystals of Racemic Mixtures**

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The development of proteinlike structures of oligomers which contain unnatural amino acids (peptidic foldamers) is an active area of research.[1] A variety of distinct conformations of peptidic foldamers that are analogous to protein secondary structures have been discovered to date. Helical structures of peptidic foldamers are particularly interesting because diverse helical conformations with unconventional hydrogen-bonding interactions are available.[1f–h] Two prominent helices, the α-helix and the 3_{10}-helix, in natural proteins arise from $(i,i+4)$ and $(i,i+3)$ C=O···H–N hydrogen bonding, respectively.[2] A number of helical structures in peptidic foldamers arise from a single type of C=O···H–N hydrogen bond, thus resulting in a macrodipole along the helical axis. In contrast, there are unique helical structures of peptidic foldamers and they feature the alternation of two hydrogen-bonding types with opposite directions along the helical axis (mixed helices).[3–6] Mixed helices give rise to relatively small macrodipoles because of the alternation of hydrogen-bonding directions. Since Seebach and co-workers reported the β-peptide 12/10-helix that arises from alternating $(i,i-1)$ and $(i,i+3)$ hydrogen bonds,[3k] a number of mixed helices have been reported for diverse unnatural peptide backbones, such as β-peptides,[3] α/β-peptides,[4] and α/γ-peptides.[5] The 11/9-helix, one of mixed helices for α/β-peptides, contains two types of intramolecular hydrogen bonds: an 11-atom ring hydrogen bond between the C=O of β-residue(i) and the H-N of α-residue(i+3), and a 9-atom ring hydrogen bond between the C=O of α-residue(i) and the H-N of β-residue($i-1$).[4a,b,7] Among the α/β-peptide helices with alternating residue types, the 11/9-helix has been predicted to be most stable, by theoretical calculations, both in vacuum and in solution.[7] A couple of 11/9-helical α/β-peptide backbones have been characterized by two-dimensional NMR experiments.[4a,b] However, the application of this unique helical peptide backbone is limited because detailed structural information for the 11/9-helix, which is derived from high-resolution structures, is not available to date. There are crystal structures of αβα-tripeptides that display 11- and 9-atom ring hydrogen bonds.[8] It is still insufficient to derive structural parameters for the 11/9-helix from these trimer structures because one helical turn is defined by at least four points.[9] It is thus desirable to obtain the crystal structures of 11/9-helical α/β-peptides containing four residues or more. Herein we report crystal structures of the α/β-peptide 11/9-helix with multiple helical turns, which allowed us to derive the structural parameters for the 11/9-helix. We hypothesized that the conformational preference of *cis*-2-aminocyclohexanecarboxylic acid (*cis*-ACHC) is amenable to the 11/9-helix in light of the local conformations of *cis*-ACHC in the crystal state, as reported previously.[10] In addition, oligomers of *cis*-ACHCs with alternating chiralities display 12/10-helical conformations in solution,[3d] and are homologous to the 11/9-helix in α/β-peptides. We chose (1*R*,2*S*)-2-aminocyclohexanecarboxylic acid and D-alanine to prepare a series of α/β-peptide oligomers. NOESY or ROESY spectra for the α/β-peptides **1**–**3** are consistent with the characteristic NOEs for the 11/9-helix, that is, α-residue $C_\alpha H(i)$–α-residue NH(i+2) and β-residue NH(i)–α-residue NH(i+1) as reported by Sharma and co-workers (Figure 1).[4a,b] All but one of these characteristic NOEs were observed for **1**–**3**. Only the NOE between the β-residue NH(2) and α-residue NH(3) was not observed for the trimer **1**.

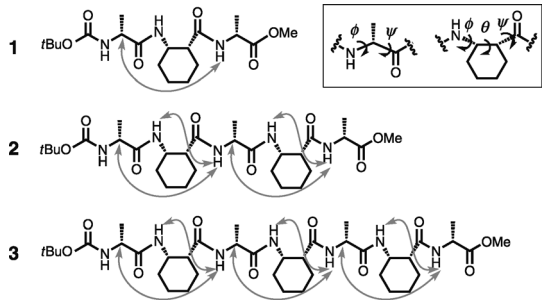

Figure 1. Structures of α/β-peptides. Arrows indicate characteristic NOEs for the 11/9-helix observed in $CDCl_3$.[4b]

Far-UV CD spectra of **1**–**3** were acquired in methanol (see Figure S1 in the Supporting Information). The α/β-peptide trimer **1** does not show any CD signature for a specific folding, presumably because of weak folding propensity in polar solvent. The pentamer **2** and heptamer **3** have CD signatures

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[**] This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (2011-0015106), the Yonsei University Research Fund of 2011, and TJ Science Fellowship of POSCO TJ Park Foundation. We thank Prof. Moon-Gun Choi for helpful discussions about X-ray crystallography.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201303404.

which are very similar to those for the 11/9-helix: negative maxima at 200 nm and 220 nm.[4a,b,11]

The trimer **1** crystallized with two symmetry-independent conformations in the solid state, conformations designated as folded and unfolded (Figure 2).[16] The folded conformation forms two intramolecular hydrogen bonds which correspond to those for the 11/9-helix: a 11-atom ring hydrogen bond as in

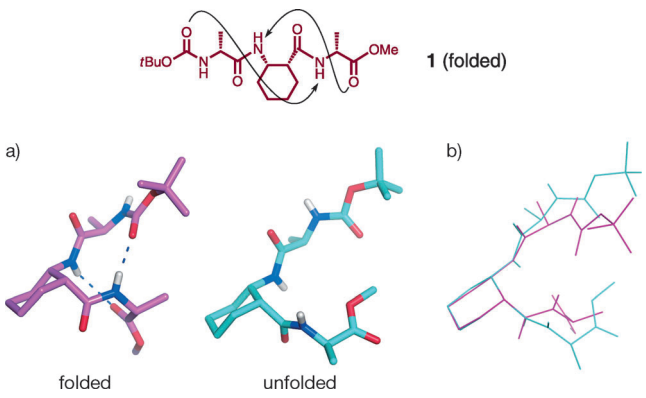

Figure 2. Crystal structures of the α/β-peptide trimer **1**: a) two symmetry-independent conformations; b) overlay of the two conformations. Arrows and dotted lines indicate hydrogen bonds.

the C=O(*i*)···H–N(*i*+3) type interaction between the N-terminal C=O and the NH of Ala(3), and a 9-atom ring hydrogen bond as in the C=O(*i*)···H–N(*i*−1) type interaction between the C=O of Ala(3) and the NH of *cis*-ACHC(2). In contrast, the unfolded conformation contains no intramolecular hydrogen bonds, but forms intermolecular hydrogen bonds with the other unfolded neighbors. The local conformations of *cis*-ACHC in the two structures of **1** are very similar, with axial C=O and the equatorial NH groups, but relatively flexible D-Ala residues cause different overall conformations (Figure 2b).

Several attempts to crystallize longer α/β-peptides **2** and **3** were not successful. We chose to grow crystals from racemic mixtures afterward. We were inspired by the racemic protein crystallography, which has been used to characterize a number of protein structures and secondary structures of peptide oligomers.[12] To prepare racemic mixtures, we synthesized enantiomers of **2** and **3** with (1*S*,2*R*)-2-aminocyclohexanecarboxylic acid and L-alanine. In addition, the α/β-peptide pentamer **4**, with a C-terminal benzyl ester group, and its enantiomer *ent*-**4** were prepared. The racemate of pentamers (*rac*-**4**) and heptamers (*rac*-**3**) crystallized in centrosymmetric space groups.[12c,16] Figure 3a shows the crystal structure of *rac*-**4** in the space group $P2_1/c$. Each of the two enantiomers (**4** and *ent*-**4**) adopts two symmetry-independent conformations, which are very similar and fully folded 11/9-helical conformations having two sets of 11- and 9-atom ring hydrogen bonds (Figure 3b and c). The C=O and the NH groups of Ala(1) in **4** do not participate in intramolecular hydrogen bonding, but form two intermolecular hydrogen bonds with the NH and the C=O groups, respectively, of Ala(1) in *ent*-**4**. Figure 4a shows the crystal packing of *rac*-**3** in the space group $P\bar{1}$. The NH and the C=O groups of

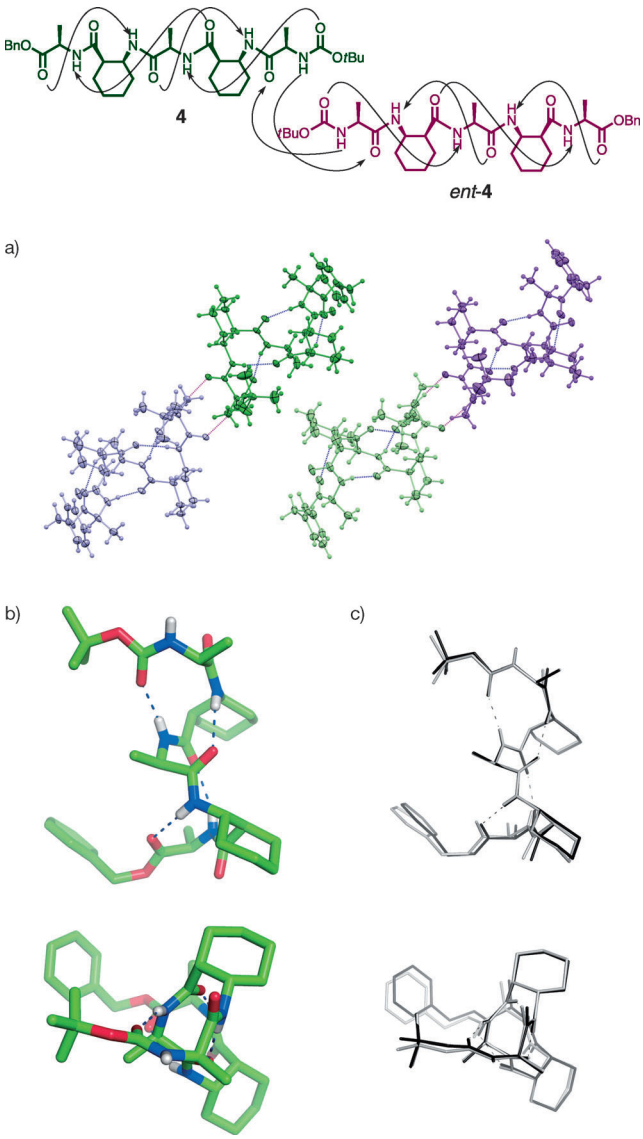

Figure 3. Crystal structure of the α/β-peptide pentamer *rac*-**4**: a) molecular drawing of crystal packing with 50% probability level (green: **4**; blue/lilac: ent-**4**); b) 11/9-helical structure of **4** viewed perpendicular to the helical axis (top) and along the helical axis (bottom); c) overlay of two symmetry-independent conformations of **4**. Arrows and dotted lines indicate hydrogen bonds. Cocrystallized solvent molecules are omitted.

Ala(1) in **3** interact with the C=O and the NH groups, respectively, of Ala(1) in *ent*-**3** through intermolecular hydrogen bonds, which are analogous to those for **4** and *ent*-**4**. The α/β-peptide heptamer **3** adopts a left-handed, fully folded 11/9-helical conformation with three consecutive sets of alternating 11- and 9-atom ring hydrogen bonds (Figure 4b). The average distance between the α-residue $C_\alpha H(i)$ and α-residue NH(*i*+2) is 3.3 Å, and the average distance between the β-residue NH(*i*) and α-residue NH(*i*+1) is 3.1 Å. Both of the average distances are within a 5 Å NOE detection range, and are consistent with the characteristic NOEs for the 11/9-helix observed in solution (Figure 1). The superposition of the three 11/9-helical backbone structures shows little deviation from one another (Figure 4c). In addition, the average

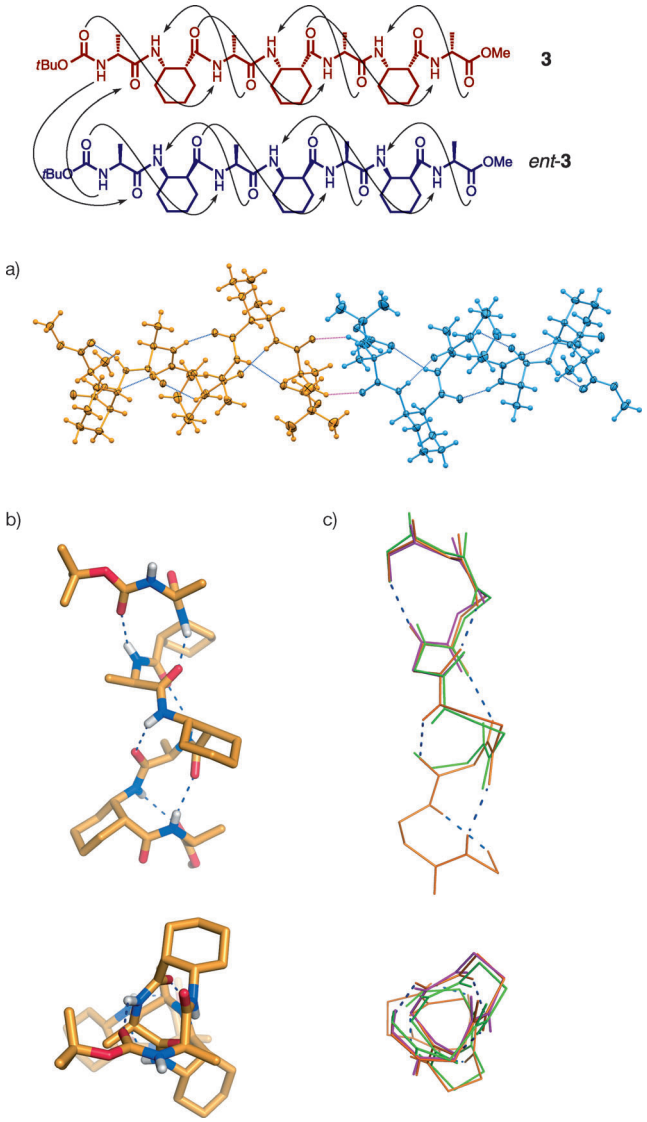

Figure 4. Crystal structure of the α/β-peptide heptamer *rac*-**3**: a) molecular drawing of crystal packing with 50% probability level; b) 11/9-helical structure of **3** viewed perpendicular to the helical axis (top) and along the helical axis (bottom); c) overlay of three 11/9-helical backbone structures (pink: **1**; green: **4**; gold: **3**). Arrows and dotted lines indicate hydrogen bonds.

backbone torsion angles for the 11/9-helix in the crystal state are very similar to those predicted by theoretical calculations (Table 1).[7] These results suggest that the 11/9-helical crystal structures in this study are canonical helices with little structural distortion. The *cis*-ACHC residue is preorganized to have opposite signs for the ϕ and the ψ angles, and may be a prerequisite for mixed helices. In contrast, β-residues that promote the 11-helix or the 14/15-helix for α/β-peptides adopt the same sign for the ϕ and the ψ angles in the crystal state.[15]

Table 1: Average backbone torsion angles for the 11/9-helix.

Residue	Torsion angle[a]	Crystal structures[b]	Theoretical model[c]
α-residue	ϕ	61	60
	ψ	−151	−150
β-residue	ϕ	−87	−77
	θ	−52	−60
	ψ	97	100

[a] Figure 1. [b] α-residue = D-Ala, β-residue = (1*R*,2*S*)-ACHC. [c] Reference [7].

The helical parameters of the 11/9-helix were derived from the crystal structures of **3** and **4** (Table 2).[9] It is noteworthy that the 11/9-helical backbone appears to be analogous to the 3_{10}-helix, even though the two helices arise from different types of hydrogen bonds. The number of residues per turn and the radius of the 11- and 9-helix are 3.0 and 2.0 Å, respectively, both of which are similar to those of the 3_{10}-helix.[2,13] The rise per residue for the 11/9-helix (2.1 Å) is slightly longer than that for the 3_{10}-helix (1.8 Å in globular proteins and 1.9 Å in model peptides).

Table 2: Helical parameters.

Helix type	11/9-Helix (α/β-peptides)	3_{10}-Helix[a] (globular proteins)	3_{10}-Helix[b] (model α-peptides)
n[c]	3.0	3.2	3.2
p [Å][d]	6.1	5.8	6.3
d [Å][e]	2.1	1.8	1.9
r [Å][f]	2.0	2.0	–

[a] Reference [13]. [b] Reference [2]. [c] Number of residues per turn. [d] Pitch (rise per turn). [e] Rise per residue. [f] Radius of the helix.

The α/β-peptide 11/9-helix with alternating (*1R,2S*)-*cis*-ACHC and D-alanine in this study are complementary to extended conformations adopted by oligomers with alternating (*1R,2S*)-*cis*-ACHC and L-alanine.[10a] These examples are in good agreement with the stereochemical patterning approach by Fülöp and co-workers,[14] thus suggesting that secondary structures of α/β-peptides can be controlled by the stereochemistry of the *cis*-ACHC residue in conjunction with L-α-residues.

In conclusion, we used the racemates of α/β-peptide oligomers to provide the centrosymmetric crystal structures of the 11/9-helix, which features unconventional types of hydrogen bonds and a small helix macrodipole. The helical parameters that are derived from these atomic-resolution structures should enable the precise disposition of side-chain groups along the 11/9-helical backbone to facilitate the function-based foldamer design.

Received: July 23, 2013
Revised: September 5, 2013
Published online: October 2, 2013

Keywords: amino acids · foldamers · helical structures · hydrogen bonds · peptides

[1] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180; b) T. A. Martinek, F. Fulop, *Chem. Soc. Rev.* **2012**, *41*, 687–702; c) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232; d) L. K. A. Pilsl, O. Reiser, *Amino Acids* **2011**, *41*, 709–718; e) W. S. Horne, S. H. Gellman, *Acc. Chem. Res.* **2008**, *41*, 1399–1408; f) G. Guichard, I. Huc, *Chem. Commun.* **2011**,

47, 5933 – 5941; g) A. Roy, P. Prabhakaran, P. K. Baruah, G. J. Sanjayan, *Chem. Commun.* **2011**, *47*, 11593 – 11611; h) D. Seebach, D. F. Hook, A. Glättli, *Biopolymers* **2006**, *84*, 23 – 37.

[2] M. Crisma, F. Formaggio, A. Moretto, C. Toniolo, *Biopolymers* **2006**, *84*, 3 – 12.

[3] a) C. Baldauf, R. Gunther, H. J. Hofmann, *Angew. Chem.* **2004**, *116*, 1621 – 1624; *Angew. Chem. Int. Ed.* **2004**, *43*, 1594 – 1597; b) É. Szolnoki, A. Hetényi, I. M. Mándity, F. Fülöp, T. A. Martinek, *Eur. J. Org. Chem.* **2013**, 3555 – 3559; c) G. V. M. Sharma, N. Y. Reddy, R. Ravi, B. Sreenivas, G. Sridhar, D. Chatterjee, A. C. Kunwar, H. J. Hofmann, *Org. Biomol. Chem.* **2012**, *10*, 9191 – 9203; d) I. M. Mándity, L. Fülöp, E. Vass, G. K. Tóth, T. A. Martinek, F. Fülöp, *Org. Lett.* **2010**, *12*, 5584 – 5587; e) G. V. A. Sharma, V. Manohar, S. K. Dutta, V. Subash, A. C. Kunwar, *J. Org. Chem.* **2008**, *73*, 3689 – 3698; f) T. A. Martinek, I. M. Mándity, L. Fülöp, G. K. Tóth, E. Vass, M. Hollósi, E. Forró, F. Fülöp, *J. Am. Chem. Soc.* **2006**, *128*, 13539 – 13544; g) G. V. M. Sharma, K. R. Reddy, P. R. Krishna, A. R. Sankar, P. Jayaprakash, B. Jagannadh, A. C. Kunwar, *Angew. Chem.* **2004**, *116*, 4051 – 4055; *Angew. Chem. Int. Ed.* **2004**, *43*, 3961 – 3965; h) G. V. M. Sharma, K. R. Reddy, P. R. Krishna, A. R. Sankar, K. Narsimulu, S. K. Kumar, P. Jayaprakash, B. Jagannadh, A. C. Kunwar, *J. Am. Chem. Soc.* **2003**, *125*, 13670 – 13671; i) S. A. W. Gruner, V. Truffault, G. Voll, E. Locardi, M. Stöckle, H. Kessler, *Chem. Eur. J.* **2002**, *8*, 4365 – 4376; j) D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. L. Matthews, J. V. Schreiber, *Helv. Chim. Acta* **1998**, *81*, 932 – 982; k) 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.

[4] a) G. Srinivasulu, S. K. Kumar, G. V. M. Sharma, A. C. Kunwar, *J. Org. Chem.* **2006**, *71*, 8395 – 8400; b) G. V. M. Sharma, P. Nagendar, P. Jayaprakash, P. R. Krishna, K. V. S. Ramakrishna, A. C. Kunwar, *Angew. Chem.* **2005**, *117*, 6028 – 6032; *Angew. Chem. Int. Ed.* **2005**, *44*, 5878 – 5882; c) G. V. M. Sharma, T. Sridhar, P. P. Reddy, A. C. Kunwar, *Eur. J. Org. Chem.* **2013**, 3543 – 3554; d) L. Berlicki, L. Pilsl, E. Weber, I. M. Mandity, C. Cabrele, T. A. Martinek, F. Fulop, O. Reiser, *Angew. Chem.* **2012**, *124*, 2251 – 2255; *Angew. Chem. Int. Ed.* **2012**, *51*, 2208 – 2212; e) G. V. M. Sharma, K. S. Reddy, S. J. Basha, K. R. Reddy, A. V. S. Sarma, *Org. Biomol. Chem.* **2011**, *9*, 8102 – 8111; f) G. Angelici, G. Luppi, B. Kaptein, Q. B. Broxterman, H. J. Hofmann, C. Tomasini, *Eur. J. Org. Chem.* **2007**, 2713 – 2721.

[5] a) P. G. Vasudev, S. Chatterjee, K. Ananda, N. Shamala, P. Balaram, *Angew. Chem.* **2008**, *120*, 6530 – 6532; *Angew. Chem. Int. Ed.* **2008**, *47*, 6430 – 6432; b) G. V. M. Sharma, V. B. Jadhav, K. V. S. Ramakrishna, P. Jayaprakash, K. Narsimulu, V. Subash, A. C. Kunwar, *J. Am. Chem. Soc.* **2006**, *128*, 14657 – 14668; c) L. Guo, W. Zhang, I. A. Guzei, L. C. Spencer, S. H. Gellman, *Org. Lett.* **2012**, *14*, 2582 – 2585.

[6] G. V. M. Sharma, B. S. Babu, D. Chatterjee, K. V. S. Ramakrishna, A. C. Kunwar, P. Schramm, H. J. Hofmann, *J. Org. Chem.* **2009**, *74*, 6703 – 6713.

[7] C. Baldauf, R. Gunther, H. J. Hofmann, *Biopolymers* **2006**, *84*, 408 – 413.

[8] a) M. Tanaka, M. Oba, T. Ichiki, H. Suemune, *Chem. Pharm. Bull.* **2001**, *49*, 1178 – 1181; b) C. J. Saavedra, A. Boto, R. Hernandez, J. I. Miranda, J. M. Aizpurua, *J. Org. Chem.* **2012**, *77*, 5907 – 5913; c) C. Saavedra, R. Hernandez, A. Boto, E. Alvarez, *J. Org. Chem.* **2009**, *74*, 4655 – 4665; d) C. André, B. Legrand, C. Deng, C. Didierjean, G. Pickaert, J. Martinez, M. C. Averlant-Petit, M. Amblard, M. Calmes, *Org. Lett.* **2012**, *14*, 960 – 963.

[9] a) H. Sugeta, T. Miyazawa, *Biopolymers* **1967**, *5*, 673 – 679; b) P. C. Kahn, *Comput. Chem.* **1989**, *13*, 185 – 189.

[10] a) S. H. Choi, M. Ivancic, I. A. Guzei, S. H. Gellman, *Eur. J. Org. Chem.* **2013**, 3464 – 3469; b) N. V. Nagy, S. Van Doorslaer, T. Szabo-Planka, S. Van Rompaey, A. Hamza, F. Fulop, G. K. Toth, A. Rockenbauer, *Inorg. Chem.* **2012**, *51*, 1386 – 1399; c) L. Fábián, A. Kálmán, G. Argay, G. Bernáth, Z. C. Gyarmati, *Cryst. Growth Des.* **2005**, *5*, 773 – 782.

[11] The folding propensity of **2** and **3** might be weaker in polar solvent. ROESY spectra for **2** and **3** in CD_3OH show NOEs indicative of the 11/9-helix with lower intensities than in $CDCl_3$ (see the Supporting Information).

[12] a) M. Avital-Shmilovici, K. Mandal, Z. P. Gates, N. B. Phillips, M. A. Weiss, S. B. H. Kent, *J. Am. Chem. Soc.* **2013**, *135*, 3173 – 3185; b) M. Kudo, V. Maurizot, B. Kauffmann, A. Tanatani, I. Huc, *J. Am. Chem. Soc.* **2013**, *135*, 9628 – 9631; c) T. O. Yeates, S. B. Kent, *Annu. Rev. Biophys.* **2012**, *41*, 41 – 61; d) D. E. Mortenson, K. A. Satyshur, I. A. Guzei, K. T. Forest, S. H. Gellman, *J. Am. Chem. Soc.* **2012**, *134*, 2473 – 2476; e) B. L. Pentelute, K. Mandal, Z. P. Gates, M. R. Sawaya, T. O. Yeates, S. B. H. Kent, *Chem. Commun.* **2010**, *46*, 8174 – 8176; f) J. R. Banigan, K. Mandal, M. R. Sawaya, V. Thammavongsa, A. P. A. Hendrickx, O. Schneewind, T. O. Yeates, S. B. H. Kent, *Protein Sci.* **2010**, *19*, 1840 – 1849; g) K. Mandal, B. Pentelute, V. Tereshko, V. Thammavongsa, O. Schneewind, A. Kossiakoff, *Protein Sci.* **2009**, *18*, 1146 – 1154; h) B. L. Pentelute, Z. P. Gates, V. Tereshko, J. L. Dashnau, J. M. Vanderkooi, A. A. Kossiakoff, S. B. H. Kent, *J. Am. Chem. Soc.* **2008**, *130*, 9695 – 9701; i) S. K. Sia, P. S. Kim, *Biochemistry* **2001**, *40*, 8981 – 8989; j) W. R. Patterson, D. H. Anderson, W. F. DeGrado, D. Cascio, D. Eisenberg, *Protein Sci.* **1999**, *8*, 1410 – 1422; k) C. Toniolo, C. Peggion, M. Crisma, F. Formaggio, X. Shui, D. S. Eggleston, *Nat. Struct. Biol.* **1994**, *1*, 908 – 914.

[13] I. K. McDonald, J. M. Thornton, *J. Mol. Biol.* **1994**, *238*, 777 – 793.

[14] I. M. Mándity, E. Wéber, T. A. Martinek, G. Olajos, G. K. Tóth, E. Vass, F. Fülöp, *Angew. Chem.* **2009**, *121*, 2205 – 2209; *Angew. Chem. Int. Ed.* **2009**, *48*, 2171 – 2175.

[15] S. H. Choi, I. A. Guzei, L. C. Spencer, S. H. Gellman, *J. Am. Chem. Soc.* **2008**, *130*, 6544 – 6550.

[16] CCDC 947861 (**1**), 946117 (*rac*-**4**), and 946279 (*rac*-**3**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

