

Characteristic Structural Parameters for the γ -Peptide 14-Helix: Importance of Subunit Preorganization**

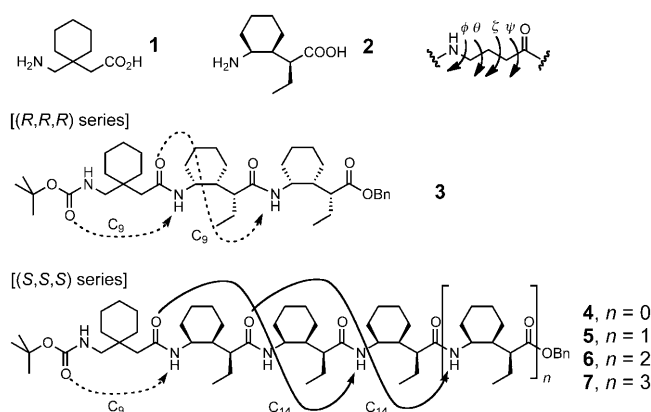
Li Guo, Weicheng Zhang, Andrew G. Reidenbach, Michael W. Giuliano, Ilia A. Guzei, Lara C. Spencer, and Samuel H. Gellman*

Dedicated to Professor Ronald Breslow on the occasion of his 80th birthday

The complexity of structure and function among biopolymers has inspired chemists to extrapolate to non-natural analogues that are intended to emulate the natural archetypes.^[1] β -Amino acid oligomers, for example, can adopt helix, sheet, or reverse-turn conformations comparable to the regular secondary structures found in proteins,^[2] and β -peptides display a wider range of secondary structure variation than do α -peptides.^[3,4] Elucidation of β -peptide folding rules has enabled function-directed design,^[5] which has encouraged exploration of higher amino acids as “foldamer” subunits.^[1] γ -Peptides that form discrete helix, sheet, or reverse-turn secondary structures have been reported,^[6–9] but the pace of γ -peptide exploration lags behind that of β -peptides, in part because of the difficulty of obtaining stereochemically pure building blocks. The γ -peptide 14-helix, defined by 14-atom ring $i, i+3$ C=O...H–N H-bonds, was identified through pioneering efforts of Hanessian et al.^[6] and Seebach et al.^[7] and represents the most thoroughly documented γ -peptide secondary structure. Nevertheless, just one atomic-resolution crystal structure containing this helix has been reported.^[9,10]

Here we report a set of crystal structures that allow the derivation of characteristic parameters for the γ -peptide 14-helix, which was not previously possible. Furthermore, the crystallographic data establish proton NOE patterns that are definitive for 14-helical folding in solution. Use of a conformationally constrained γ -amino acid (**2**, Scheme 1)^[11] was crucial for the generation of these structural data.

We used solution-phase methods to prepare a series of γ -peptides, **3–7** (Scheme 1), which contain a *tert*-butoxycarbonyl (Boc)-protected residue derived from commercially available gabapentin (**1**) at the N termini and cyclically constrained residues derived from γ -amino acid **2** at all other positions. Stepwise synthesis of α -peptides typically proceeds from C terminus to N terminus, because carboxy activation of α -amino acid derivatives in which the backbone nitrogen atom



Scheme 1. Structures of γ -peptides **3–7** (arrows indicate H-bonds in the crystal structures of **3–7**). Bn = benzyl.

is part of an amide group (e.g., at the C terminus of a peptide) often leads to epimerization by transient azalactone formation, while epimerization is suppressed when the amino group is part of a urethane (e.g., Boc). We find a complementary situation with γ -peptides constructed from **2**: the *N*-Boc derivative of **2** is highly prone to γ -lactam formation under standard coupling conditions, and this side reaction is suppressed when the backbone nitrogen atom is part of an amide.^[12] Therefore, γ -peptides **3–7** were constructed by stepwise extension starting from the N terminus. A gabapentin residue was placed at the N terminus because *N*-Boc-gabapentin is not prone to γ -lactam formation during coupling reactions.

γ -Amino acid **2** was selected as the principal component of these oligomers, because protected forms are readily available from butanal and 1-nitrocyclohexene,^[11] and because the ring constraint and stereochemistry are expected to promote a *gauche*⁺, *gauche*⁺ torsion-angle sequence about the C _{α} –C _{β} and C _{β} –C _{γ} bonds, which computational analysis suggests to be conducive to 14-helix formation.^[13] In contrast, the diastereomeric building block with a *trans*-disubstituted cyclohexane ring^[11] is expected to favor a *gauche*⁺, *anti* torsion-angle sequence. The synthetic route that provides **2** enables placement of a wide variety of side chains at the α -position, and it seems likely that the behavior observed with an ethyl group at this position will prove to be representative of other unbranched side chains.

[*] Dr. L. Guo, W. Zhang, A. G. Reidenbach, M. W. Giuliano, Dr. I. A. Guzei, L. C. Spencer, Prof. S. H. Gellman
Department of Chemistry, University of Wisconsin, Madison
Madison, WI 53706 (USA)
Fax: (+1) 608-265-4534
E-mail: gellman@chem.wisc.edu

[**] This research was supported by the NSF (CHE-0848847). NMR spectrometers were purchased with partial support from the NIH and NSF.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201101301>.

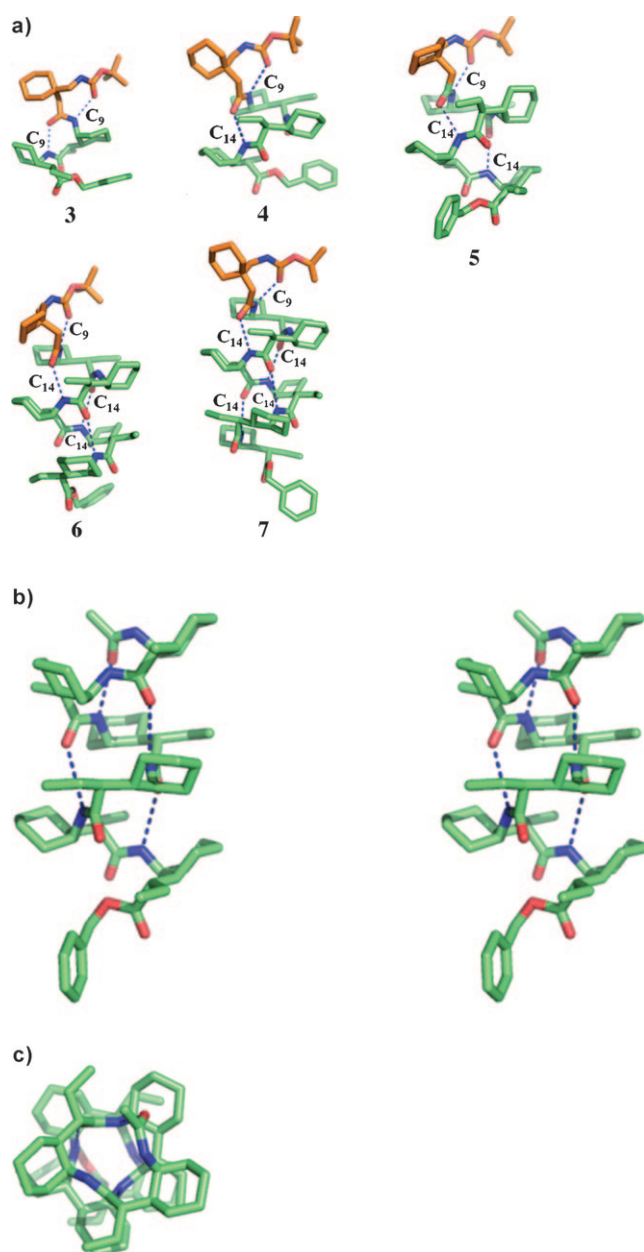


Figure 1. Crystallographic data. a) Crystal structures of **3–7**. b) Stereoview of the 14-helical segment of **7** (N-terminal gabapentin residue not shown). c) View along the helix axis of the 14-helical segment of **7**. Orange: Boc-protected gabapentin residue, green: residues derived from **2**, red: O, blue: N.

In the crystal structures of **4–7** (Figure 1), the segments derived from **2** display the 14-helical conformation. All possible 14-atom-ring H-bonds are formed within each of these segments. This data set permits us to derive robust averages for the backbone torsion angles of a γ -amino acid residue involved in a canonical 14-helix (Table 1). These average values correspond well to relevant torsion angles in the only reported crystal structure of a helical γ -peptide, a tetramer.^[9] Our averages do not include the C-terminal residue from **4–7**. The C terminus itself is an ester in each case, and the lack of an H-bond donor at this position

Table 1: Average torsion angles^[a] from 14-helical segments.

γ -Peptide	Residue	ϕ	θ	ζ	ψ
4–7	gabapentin ^[b]	98.6	−67.2	−76.7	82.5
	γ -AA ^[c]	−154.5	60.2	59.5	−126.8
Seebach et al. ^[9]	γ -AA ^[d]	140.4	−67.1	−54.6	133.6
	γ -AA(4) ^[e]	109.1	174.6	−178.6	51.0

[a] Backbone torsion angles in γ -amino acid residues are defined in Scheme 1. [b] Average backbone torsion angles of gabapentin residues in **4–7**. [c] Average backbone torsion angles of γ -amino acid residues derived from **2** in **4–7**, excluding the C-terminal residue in each case (14 independent γ -amino acid residues from **4–7** were used to generate the torsion-angle averages; see the Supporting Information). [d] Average backbone torsion angles of the first three γ -amino acid residues in the crystal structure of the four-residue γ -peptide reported in Ref. [9]. [e] Torsion angles of the C-terminal residue in the crystal structure of the four-residue γ -peptide reported in Ref. [9].

prevents incorporation of the C-terminal residue into the 14-helical H-bond pattern. Similarly, the C terminus of the previously described tetramer^[9] is an ester, and in this case the C-terminal residue displays torsion angles that deviate dramatically from those required for the 14-helix: the ζ and θ torsion angles (Scheme 1) are *anti* rather than *gauche* for this residue. Among **4–7**, only minor variations in the backbone torsion angle are observed between the C-terminal residue and other residues derived from **2**, a trend that presumably arises because the cyclic constraint of **2** confers strong conformational preorganization, in contrast to the modest conformational preference provided by an acyclic stereochemical control strategy.^[9]

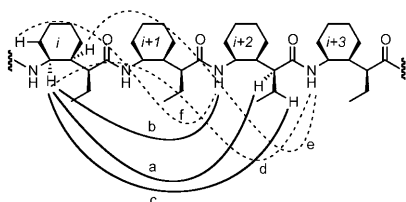
Standard methods for deriving helical parameters such as the number of residues per turn (n), rise per turn (pitch; p), rise per residue (d), and radius (r) require atomic-resolution structures containing segments of four contiguous helical residues.^[14] Hexa- γ -peptide **6** and hepta- γ -peptide **7** provide the first opportunities for such analysis among γ -peptides, with one four-residue segment in **6** and two such segments in **7**. The helical parameters deduced from these three segments are very similar to one another, as shown in Table 2.

Table 2: 14-Helical parameters from structures of **6** and **7**.

Peptide	n	p [Å]	d [Å]	r [Å]
hexamer 6	2.5	5.4	2.1	2.9
	2.6	5.5	2.1	2.9
heptamer 7	2.5	5.5	2.2	2.9
	2.5	5.5	2.2	2.9
average	2.5	5.5	2.1	2.9

The new structural data for segments derived exclusively from **2** allow us to identify H–H distances that are expected to give rise to medium-range nuclear Overhauser effects (NOEs) in the γ -peptide 14-helical conformation. In general, NOEs between protons that are distant from one another in terms of covalent connectivity, specifically, protons that are not from the same residue or from sequentially adjacent residues, provide strong evidence for population of compact conformations in solution. The crystallographic data indicate

- a) $C_{\gamma}H(i)$ to $C_{\alpha}H(i+2)$
 b) $C_{\gamma}H(i)$ to $NH(i+2)$
 c) $C_{\gamma}H(i)$ to $C_{\alpha(\beta')}H(i+2)$
 d) $C_{\gamma}H(i)$ to $NH(i+3)$
 e) $C_{\gamma}H(i)$ to $NH(i+3)$
 f) $C_{\gamma(\beta')}H(i)$ to $NH(i+2)$



Scheme 2. H–H distances in the crystal structures of γ -peptides **4–7**, corresponding to medium-range NOE patterns, expected to be characteristic of γ -peptide 14-helix formation in solution.

six types of H–H juxtapositions, involving protons on the backbone or on the first carbon atom of a side chain, that should give rise to non-sequential NOEs characteristic of the 14-helix (Scheme 2 and Table 3). This analysis reveals that

Table 3: Average H–H distances in crystal structures of **4–7** corresponding to medium-range NOE patterns expected to be characteristic of γ -peptide 14-helix formation in solution.

NOE type	Number of measurements	Distance [Å]
$C_{\gamma}H(i)$ to $C_{\alpha}H(i+2)$	9	2.6 ± 0.4
$C_{\gamma}H(i)$ to $NH(i+2)$	9	2.7 ± 0.1
$C_{\gamma}H(i)$ to $C_{\alpha(\beta')}H(i+2)$	9	3.4 ± 0.4
$C_{\gamma}H(i)$ to $NH(i+3)$	6	4.0 ± 0.1
$C_{\beta}H(i)$ to $NH(i+3)$	6	4.4 ± 0.2
$C_{\gamma(\beta')}H(i)$ to $NH(i+2)$	9	4.0 ± 0.1

$C_{\gamma}H(i)$ – $C_{\alpha}H(i+2)$, $C_{\gamma}H(i)$ – $NH(i+2)$, and $C_{\gamma}H(i)$ – $C_{\alpha(\beta')}H(i+2)$ NOE patterns should be particularly useful, since these H–H distances are between 2.5 and 3.5 Å in the crystal structures. (The designation $C_{\alpha(\beta')}H$ indicates a proton on the first carbon atom (β') of a side chain attached to the backbone α -carbon atom.) NMR spectroscopy data reported by Hanessian et al.^[6] for two γ -peptide hexamers include numerous NOEs of these types, which strongly support 14-helix formation. Our crystallographic data identify three additional H–H distances between 4.0 and 4.5 Å, which might give rise to weak NOEs: $C_{\gamma}H(i)$ – $NH(i+3)$, $C_{\beta}H(i)$ – $NH(i+3)$, and $C_{\gamma(\beta')}H(i)$ – $NH(i+2)$. ($C_{\gamma(\beta')}H$ indicates a proton on the first carbon atom (β') of a side chain attached to the backbone γ -carbon atom.) NMR spectroscopy data reported by Seebach et al.^[7,15] are largely consistent with the data in Table 3, but these studies also revealed NOEs that can now be recognized as inconsistent with the 14-helix. One six-residue γ -peptide displayed a strong $C_{\gamma(\beta')}H(i)$ – $NH(i+3)$ NOE,^[7] but the crystallographic data indicate that this H–H distance is typically (5.3 ± 0.2) Å in the 14-helix (six measurements). Another six-residue γ -peptide displayed a medium-intensity $C_{\gamma}H(i)$ – $C_{\alpha}H(i+3)$ NOE,^[15] but the crystallographic data indicate an H–H distance of (5.9 ± 0.2) Å (six measurements). The non-14-helical NOEs suggest conformational heterogeneity for these γ -peptides in solution.

The gabapentin residue at the N terminus of **3–7** forms a nine-atom-ring H-bond ($C=O(i)$ – $H-N(i+2)$) in each crystal structure. Trimer **3** has a second C_{γ} H-bond, across the central residue, which suggests that this H-bond pattern is energetically reasonable for γ -amino acid residues derived from **2**.

However, no C_{γ} H-bond across a residue derived from **2** is observed in the crystal structures of larger γ -peptides **4–7**, where 14-atom H-bonding becomes possible in the segments composed of **2**. We interpret the dominance of C_{14} H-bonding among **4–7** as strong evidence that the residue derived from **2** has an intrinsic preference for the 14-helical conformation. In contrast, our data indicate that the gabapentin residue has a strong preference for the C_{γ} H-bond: even when the gabapentin residue could participate in a 14-helix, as in **4–7**, this residue consistently forms the shorter-range H-bond. Previously we noted that adoption of compact and specific conformations by unnatural peptidic oligomers requires subunits that disfavor H-bonds between nearest-neighbor backbone amide groups.^[16]

Our conclusions regarding gabapentin conformational preferences are consistent with those suggested by crystal structures of short gabapentin-containing peptides, many of which feature the C_{γ} H-bonding pattern.^[17] This purely local gabapentin folding pattern does not necessarily lead to a regular secondary structure, as illustrated by the crystal structure of a gabapentin tetramer, in which the torsion angles within the C_{γ} rings vary irregularly along the sequence.^[8g] In contrast, NMR spectroscopic analysis of γ -peptide oligomers containing γ^4 -amino acid residues (i.e., γ residues with a side chain at the carbon next to nitrogen) bearing a bulky side chain suggest formation of a regular 9-helix.^[8h] Other γ -peptide conformations containing multiple C_{γ} H-bonded rings have been reported as well.^[8f,i,j]

Atomic-resolution data from a series of 14-helical γ -peptides have enabled us to generate definitive structural parameters for this secondary structure. Furthermore, the crystallographic data identify key backbone proton NOE patterns that should be manifested upon 14-helical folding in solution. These benchmarks are useful both for evaluating previous NMR spectroscopy studies^[6,7,15] and for guiding future conformational explorations. This level of analysis was not previously possible among γ -peptides; the conformational preorganization inherent in γ -amino acid residues derived from **2** presumably contributes to the high propensity of oligomers **3–7** to crystallize and therefore to our success in acquiring multiple 14-helical structures. Atomic-resolution conformational analysis of foldamers containing preorganized β -amino acid residues has provided a foundation for structure-based designs of β - and α/β -peptides with specific functions,^[5,18] and the conformational insights provided here should have comparable value for function-based design of γ -peptides.

Received: February 21, 2011
 Published online: May 12, 2011

Keywords: foldamers · helical structures · NMR spectroscopy · nuclear Overhauser effect · peptides

- [1] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180; b) *Foldamers: Structure, Properties and Applications* (Eds.: S. Hecht, I. Huc), Wiley-VCH, Weinheim, **2007**; c) X. Li, Y.-D. Wu, D. Yang, *Acc. Chem. Res.* **2008**, *41*, 1428–1438; d) B. Gong, *Acc. Chem.*

- Res.* **2008**, *41*, 1376–1386; e) C. E. Schafmeister, Z. Z. Brown, S. Gupta, *Acc. Chem. Res.* **2008**, *41*, 1387–1398.
- [2] a) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232; b) D. Seebach, A. K. Beck, D. J. Bierbaum, *Chem. Biodiversity* **2004**, *1*, 1111–1239; c) D. Seebach, J. Gardiner, *Acc. Chem. Res.* **2008**, *41*, 1366–1375.
- [3] a) D. J. Barlow, J. M. Thornton, *J. Mol. Biol.* **1988**, *201*, 601–619; b) α -Peptide π -helix: R. Chapman, J. L. Kulp, A. Patgiri, N. R. Kallenbach, C. Bracken, P. S. Arora, *Biochemistry* **2008**, *47*, 4189–4195.
- [4] For discussion of the β -peptide 14-, 12-, 10/12- and 10-helices, see Ref. [2a]. For the 14/16-helix, see: M. I. Mándity, E. Weber, 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. For recent developments with 12-helical β -peptides, see: S. Kwon, A. Jeon, S. H. Yoo, I. S. Chung, H.-S. Lee, *Angew. Chem.* **2010**, *122*, 8408–8412; *Angew. Chem. Int. Ed.* **2010**, *49*, 8232–8236; C. Fernandes, S. Faure, E. Pereira, V. Thery, V. Declerck, R. Guillot, D. J. Aitken, *Org. Lett.* **2010**, *12*, 3606–3609.
- [5] a) C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252–262; b) W. C. Pomerantz, V. M. Yuwono, C. L. Pizzey, J. D. Hartgerink, N. L. Abbott, S. H. Gellman, *Angew. Chem.* **2008**, *120*, 1261–1264; *Angew. Chem. Int. Ed.* **2008**, *47*, 1241–1244; c) M. M. Müller, M. A. Windsor, W. C. Pomerantz, S. H. Gellman, D. Hilvert, *Angew. Chem.* **2009**, *121*, 940–943; *Angew. Chem. Int. Ed.* **2009**, *48*, 922–925; d) Y. Imamura, N. Watanabe, N. Umezawa, T. Iwatsubo, N. Kato, T. Tomita, T. Higuchi, *J. Am. Chem. Soc.* **2009**, *131*, 7353–7359; e) M. Hintersteiner, T. Kimmerlin, G. Garavel, T. Schindler, R. Bauer, N. Meisner, J. Seifert, V. Uhl, M. Auer, *ChemBioChem* **2009**, *10*, 994–998; f) A. D. Bautista, J. S. Appelbaum, C. J. Craig, J. Michel, A. Schepartz, *J. Am. Chem. Soc.* **2010**, *132*, 2904–2906.
- [6] S. Hanessian, X. Luo, R. Schaum, S. Michnick, *J. Am. Chem. Soc.* **1998**, *120*, 8569–8570.
- [7] T. Hintermann, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 893–1002.
- [8] a) M. Hagihara, N. J. Anthony, T. J. Stout, J. Clardy, S. L. Schreiber, *J. Am. Chem. Soc.* **1992**, *114*, 6568–6570; b) S. Hanessian, X. Luo, R. Schaum, *Tetrahedron Lett.* **1999**, *40*, 4925–4929; c) M. Brenner, D. Seebach, *Helv. Chim. Acta* **2001**, *84*, 2155–2166; d) M. G. Woll, J. R. Lai, I. A. Guzei, S. J. C. Taylor, M. E. B. Smith, S. H. Gellman, *J. Am. Chem. Soc.* **2001**, *123*, 11077–11078; e) C. R. Jones, M. K. N. Qureshi, F. R. Truscott, S. D. Hsu, A. J. Morrison, M. D. Smith, *Angew. Chem.* **2008**, *120*, 7207–7210; *Angew. Chem. Int. Ed.* **2008**, *47*, 7099–7102; f) J. Farrera-Sinfreu, L. Zaccaro, D. Vidal, X. Salvatella, E. Giralt, M. Pons, F. Albericio, M. Royo, *J. Am. Chem. Soc.* **2004**, *126*, 6048–6057; g) P. G. Vasudev, N. Shamala, K. Ananda, P. Balaran, *Angew. Chem.* **2005**, *117*, 5052–5055; *Angew. Chem. Int. Ed.* **2005**, *44*, 4972–4975; h) G. V. M. Sharma, P. Jayaprakash, K. Narsimulu, A. R. Sankar, K. R. Redder, P. R. Krishna, A. C. Kunwar, *Angew. Chem.* **2006**, *118*, 3010–3013; *Angew. Chem. Int. Ed.* **2006**, *45*, 2944–2947; i) A. A. Edwards, G. J. Sanjayan, S. Hachisu, G. E. Tranter, G. W. J. Fleet, *Tetrahedron* **2006**, *62*, 7718–7725; j) A. Kothari, M. K. N. Qureshi, E. M. Beck, M. D. Smith, *Chem. Commun.* **2007**, 2814–2816.
- [9] D. Seebach, M. Brenner, M. Rueping, B. Schweizer, B. Jaun, *Chem. Commun.* **2001**, 207–208.
- [10] Extensive crystallographic characterization has recently been reported for urea-based oligomers that adopt a helical conformation that is quasi-isostructural with the γ -peptide 14-helix: L. Fischer, P. Claudon, N. Pendem, E. Miclet, C. Didierjean, E. Ennifar, G. Guichard, *Angew. Chem.* **2010**, *122*, 1085–1088; *Angew. Chem. Int. Ed.* **2010**, *49*, 1067–1070. Despite the structural similarity of the oligourea and γ -peptide helices, however, the backbone difference can lead to profound functional differences: P. Claudon, A. Violette, K. Lamour, M. Decossas, S. Fournel, B. Heurtault, J. Godet, Y. Mély, B. Jamart-Grégoire, M.-C. Averlant-Petit, J.-P. Briand, G. Duportail, H. Monteil, G. Guichard, *Angew. Chem.* **2010**, *122*, 343–346; *Angew. Chem. Int. Ed.* **2010**, *49*, 333–336.
- [11] L. Guo, Y. Chi, A. M. Almeida, I. A. Guzei, B. K. Parker, S. H. Gellman, *J. Am. Chem. Soc.* **2009**, *131*, 16018–16020. For related work, see: W. J. Nodes, D. R. Nutt, A. M. Chippindale, A. J. A. Cobb, *J. Am. Chem. Soc.* **2009**, *131*, 16016–16017.
- [12] See the Supporting Information for details.
- [13] C. Baldauf, R. Günther, H.-J. Hofmann, *Helv. Chim. Acta* **2003**, *86*, 2573–2588.
- [14] P. C. Kahn, *Comput. Chem.* **1989**, *13*, 185–189.
- [15] D. Seebach, M. Brenner, M. Rueping, B. Jaun, *Chem. Eur. J.* **2002**, *8*, 573–584.
- [16] G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc.* **1994**, *116*, 1054–1062.
- [17] a) P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaran, *Acc. Chem. Res.* **2009**, *42*, 1628–1639; b) P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaran, *Chem. Rev.* **2011**, *111*, 657–687.
- [18] a) M. Werder, H. Hauser, S. Abele, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1774–1783; b) D. Liu, W. F. DeGrado, *J. Am. Chem. Soc.* **2001**, *123*, 7553–7559; c) T. L. Raguse, E. A. Porter, B. Weisblum, S. H. Gellman, *J. Am. Chem. Soc.* **2002**, *124*, 12774–12785; d) J. A. Kritzer, J. D. Lear, M. E. Hodsdon, A. Schepartz, *J. Am. Chem. Soc.* **2004**, *126*, 9468–9469; e) O. M. Stephens, S. Kim, B. D. Welch, M. E. Hodsdon, M. S. Kay, A. Schepartz, *J. Am. Chem. Soc.* **2005**, *127*, 13126–13127; f) T. B. Potocky, A. K. Menon, S. H. Gellman, *J. Am. Chem. Soc.* **2005**, *127*, 3686–3687; g) W. C. Pomerantz, N. L. Abbott, S. H. Gellman, *J. Am. Chem. Soc.* **2006**, *128*, 8730–8731; h) A. J. Karlsson, W. C. Pomerantz, B. Weisblum, S. H. Gellman, S. P. Palecek, *J. Am. Chem. Soc.* **2006**, *128*, 12630–12631; i) J. D. Sadowsky, W. D. Fairlie, E. B. Hadley, H.-S. Lee, N. Umezawa, Z. Niko-lovska-Coleska, S. Wang, D. C. S. Wang, Y. Tomita, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 139–154; j) J. Smith, P. E. Czabotar, K. J. Peterson-Kaufman, P. M. Colman, S. H. Gellman, W. D. Fairlie, *Angew. Chem.* **2009**, *121*, 4382–4386; *Angew. Chem. Int. Ed.* **2009**, *48*, 4318–4322; k) W. S. Horne, L. M. Johnson, T. J. Ketas, P. J. Klasse, M. Lu, J. P. Moore, S. H. Gellman, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 14751–14756.