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Quaternary Centres as a Tool for Modulating Foldamer Conformation

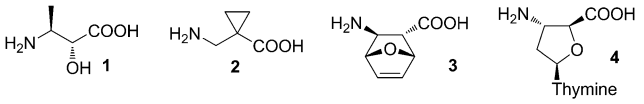
Roberta Galeazzi,[a] Gianluca Martelli,*[a] Andrea Mazzanti,[b] Mario Orena,*[a] and Samuele Rinaldi[a]

Dedicated to Professor Giuliana Cardillo

In recent years, oligomers of unnatural peptidic residues,[1–3] mainly built up with β-amino acids[4–5] or their hybrids with natural α-amino acids,[6] have emerged as versatile structural templates (foldamers) that exhibit predictable and well-defined secondary structures, such as helices, turns and strands, and offer a variety of possibilities for orienting functional side-chains. When a linear α-polypeptide folds into well-ordered and compact secondary structures,[7–11] the preferred backbone conformation arises in part from minimization of Newman and Pitzer strain, as well as pseudoallylic A(1,3) strain, which restricts the ϕ and χ torsion angle values accessible to proteinogenic amino acid residues.[12] In addition, proteinogenic α-amino acids display intrinsic and distinct propensities for helices and sheets and can be selected accordingly to stabilise a given fold,[13–15] but a higher stabilisation can be achieved by further restricting the available conformational space of amino acids in the sequence.[5]

Thus, in α-peptides, Thorpe–Ingold effects (C(α)-tetrasubstitution) have been used extensively to impose such a restriction on ϕ and χ angles[16] and Aib (α-aminoisobutyric acid) is a very strong promoter of helical (3_{10} and α-helices) and β-turn structures.[5,11] On the other hand, β-peptides mostly occur in the 12- or 14-helical conformation, but oligomers consisting of $\beta^{2,3}$-amino acids of *unlike* configuration or of geminally disubstituted amino acids cannot fit in any of the two folds. In fact, (2*R*,3*S*)-α-hydroxy $\beta^{2,3}$-amino acid **1** gives a foldamer, which in polar solvent, displays a helical conformation based on repetitive 8-membered H bonded rings resulting from 1←3 H bond interactions ($C{=}O_i{\cdots}H{-}N_{i+2}$).[17] A remarkably similar C_8-based conformation has also been reported for oligomers consisting of 1-aminomethyl cyclopropane carboxylic acid residues (**2**, Scheme 1).[18]

Moreover, the β-peptide consisting of *trans*-oxabornene-β-amino acid **3** adopts a C_8-based helix conformation;[19] this causes the cyclohexyl ring bridging and unsaturation to impose angular constraints that translate the robust 3_{14}-helix sustained by *trans*-ACHC-$\beta^{2,3}$ amino acid units into a new folding pattern.[1,20,21] Eventually, homo-oligomers derived from nucleoside β-amino acids **4**, in which strain probably arises from 1,3-*cis*-disubstitution at the oxygen bridge, display a similar conformational pattern.[22] However, all these results do not question the proposal formulated by Gellman that 1←3 H bonding between nearest-neighbour amide groups in β-peptides is not favoured, but rather suggest that extra interactions or specific angular constraints can overcome this general feature.[23]



Scheme 1. β-Amino acids leading to foldamers displaying an 8-helix conformation.

Since the change from 12- or 14-helix into a 8-helix conformation for a β-foldamer seems to arise from conformational restrictions, we investigated whether introducing a quaternary centre in (3*R*,4*S*,1′*S*)-4-amino-1-(1′-(4-methoxyphenyl)ethyl)-5-oxopyrrolidine-3-carboxylic acid ((3*S*,4*R*,1′*S*)AMOPC; **5a**)[24] could also give rise to a conformational change. In fact, in connection with our interest towards applications, such as peptidomimetics,[25] antimicrobial agents[26] and components in nanostructured materials,[27] we had already prepared a foldamer starting from **5b**, the diprotected form of **5a**, the secondary structure of which was determined to be a 12-helix by means of ^{1}H NMR spectroscopy data and supported by molecular dynamics (MD) simulations.[28]

Directed towards the preparation of an amphiphilic foldamer, we envisaged the more rigid 8-helix conformation can allow a better separation between the substituents at the lactam nitrogen on the two sides of the helix. In fact, owing to the easy removal of the 4-methoxyphenylethyl group, the N-1 of the pyrrolidin-2-one ring could be appropriately functionalised with polar chains. Thus, stereoselective alkylation of **5b** led to the corresponding derivative **6b**, the fully protected (3*R*,4*S*,1′*S*)-4-amino-1-(1′-(4-methoxyphenyl)ethyl)-3-methyl-5-oxopyrrolidine-3-carboxylic acid ((3*R*,4*S*,1′*S*)-AMMOPC; **6a**, Scheme 2),[29] and this unit was employed in an attempt to modify the foldamer conformation to intro-

[a] Dr. R. Galeazzi, Dr. G. Martelli, Prof. M. Orena, Dr. S. Rinaldi
Di.S.V.A. Chemistry, Polytechnic University of Marche
Via Brecce Bianche, I-60131 Ancona (Italy)
Fax: (+39) 071-2204714
E-mail: gmartell@univpm.it
m.orena@univpm.it

[b] Prof. A. Mazzanti
Department of Organic Chemistry "A. Mangini"
University of Bologna, Via Risorgimento, I-40126 Bologna (Italy)

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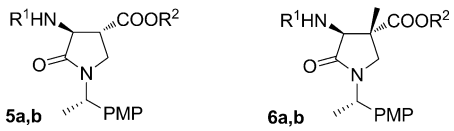
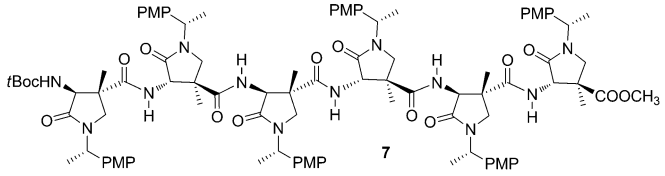


a. $R^1 = R^2 = H$; **b.** $R^1 = t$Boc, $R^2 = CH_3$

Scheme 2. Structures of (3*S*,4*R*,1′*S*)-AMOPC (**5a**), (3*R*,4*S*,1′*S*)-AMMOPC (**6a**) and of their derivatives, **5b** and **6b**.

duce a quaternary centre in the monomer. Thus, by using standard homogeneous peptide synthesis, starting from **6b** the corresponding hexamer **7** was obtained and its conformation was first assigned by means of NMR analysis and then confirmed by MD simulations.



First, the occurrence of intramolecular C=O···H–N hydrogen bonds in the foldamer leading to an 8-helix was confirmed by investigation of the $[D_6]$DMSO dependence of NH proton chemical shifts.[30] This solvent is a strong hydrogen-bond acceptor and if it is bound to a free NH proton, it is expected to dramatically move its chemical shift downfield. The results for the $[D_6]$DMSO/$CDCl_3$ titrations of the NH protons of hexamer **7** are reported in Figure 1. The oligomers are not aggregated under the experimental conditions since even on changing the concentration the spectra remained identical (see the Supporting Information). The outcome of these titrations was in perfect agreement with results arising from ROESY spectral data, as the NH protons of **7** are nearly insensitive to DMSO; this confirms that this hexamer is able to form a H bond-driven secondary structure. Then, ROESY spectral data were used in order to assign non-adjacent NOE interactions, and the correlation types observed were consistent with a population of secondary structures identified as an 8-helix (see the Supporting Information).

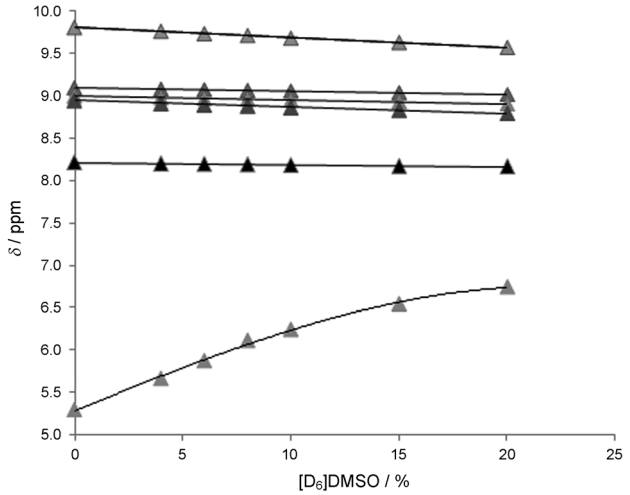


Figure 1. Variation of NH proton chemical shifts (ppm) of hexamer **7** as a function of increasing percentage of $[D_6]$DMSO added to the $CDCl_3$ solution (v/v; concentration 5 mM). From top to bottom: NH-Res2, NH-Res3, NH-Res4, NH-Res5, NH-Res6, NH-Res1.

In order to definitely confirm the real strong preference for this conformational structure, totally unconstrained MD simulations were carried out by using the protocol described in the Supporting Information. The cluster analysis (RMS = 0.39) pointed out the existence of 72 conformers grouped into three clusters, all showing the same 8-helix backbone arrangement, whereas the main differences concern the orientation of the side-chains. The lowest energy conformer with its 8-helix structure and the most populated cluster including the lowest energy conformer (number of members 70) are reported in Figures 2 and 3, respectively.

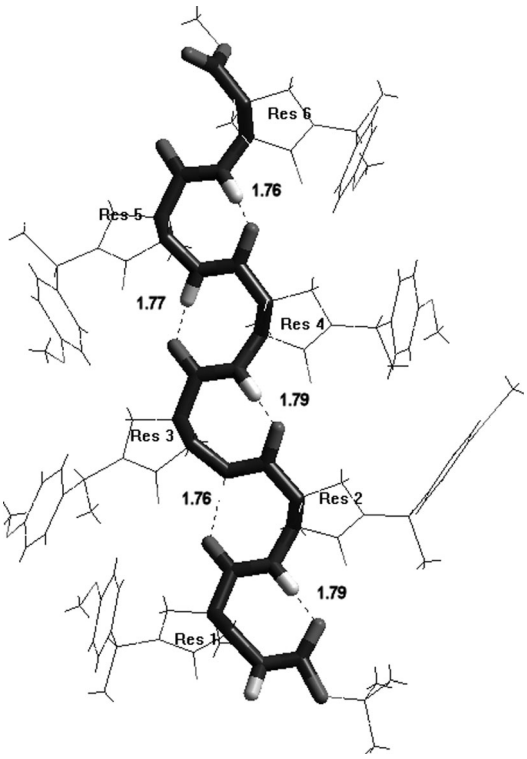


Figure 2. Minimum energy structure for hexamer **7** showing the hydrogen bonds implicated in the 8-helix conformation.

These findings were confirmed by high temperature quenched molecular dynamics (QMD) simulations by using ROESY distance restrained QMD simulations. In fact, a total agreement was observed between these two independently obtained results and the NOE interatomic distances. Thus, as observed from the ^{1}H NMR spectroscopy data and

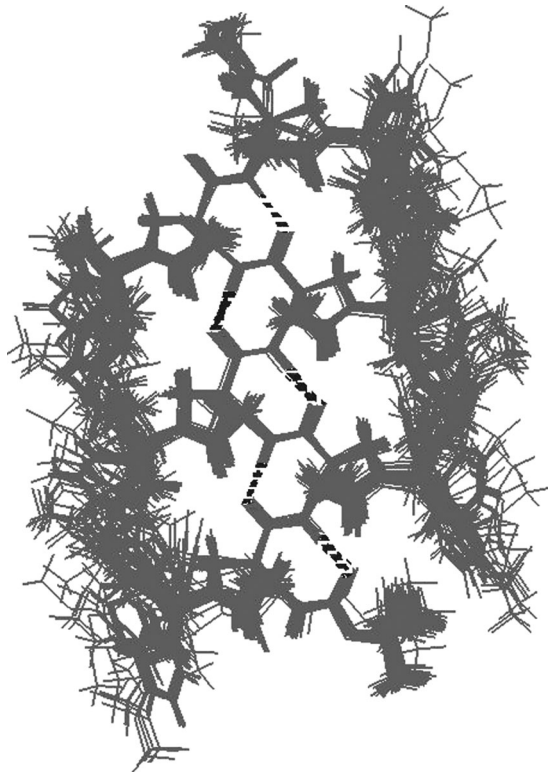


Figure 3. Most populated cluster for compound **7**.

MD simulations, foldamer **7** displays an 8-helical secondary structure in $CDCl_3$ solution.

Having assigned the conformation of **7**, using MD simulations we started a preliminary computational analysis directed to ascertain whether introduction of (3*S*,4*R*,1′*S*)-AMMOPC (**6a**) within a structure built up with (3*S*,4*R*,1′*S*)-AMOPC (**5a**) could give rise to significant conformational changes. From the MD simulations carried out without any experimental restraints, the introduction of a sole **6a** unit at any position of the hexamer built up with **5a** led only to minor effects. Random conformational changes perturbed the population distribution of the 12-helix structure, which was locally distorted, with the position most prone to give conformational changes being unit 3. On the other hand, when monomers **5a** and **6a** were alternated, the conformation changed dramatically from 12- to 8-helix, which was

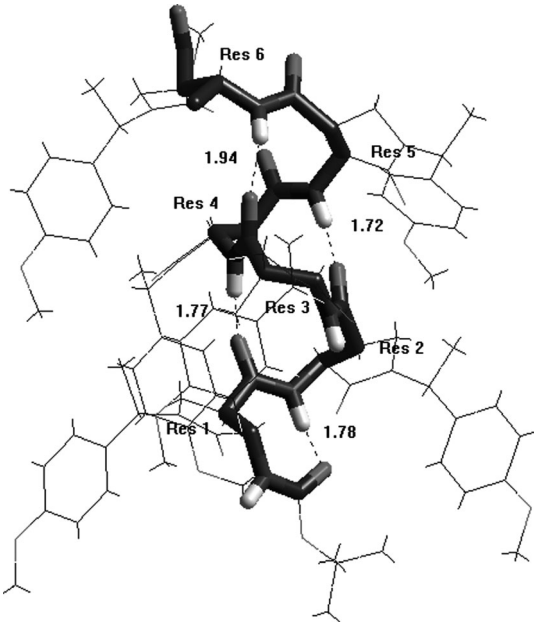
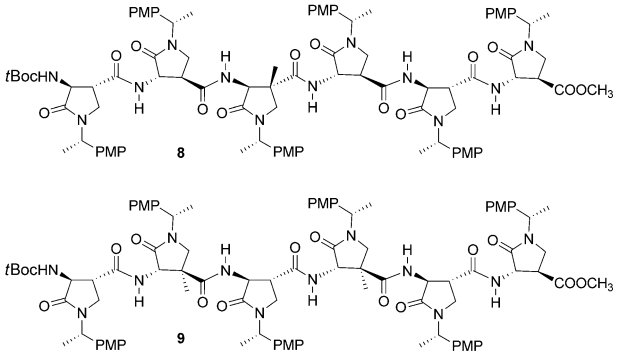


Figure 4. Minimum energy structure for hexamer **8** showing the hydrogen bonds implicated in the 8-helix→12-helix conformation.



Scheme 3. Structures of the hexamers **8** and **9** showing the insertion of (3*R*,4*S*,1′*S*)-AMMOPC (**6a**).

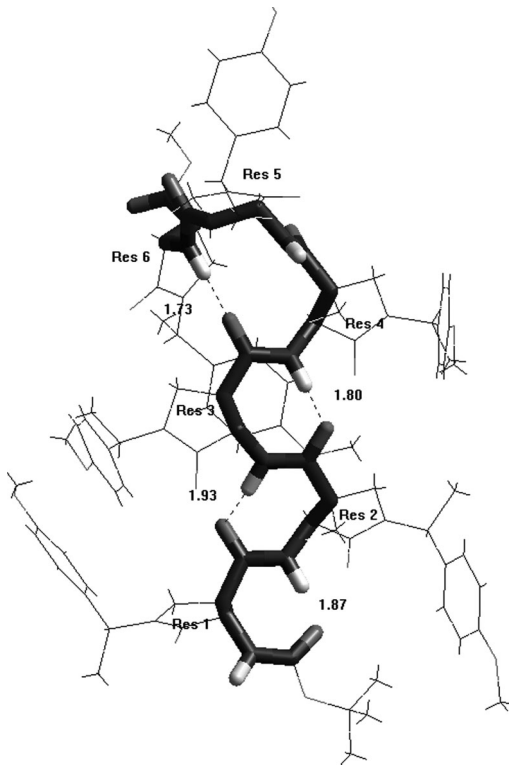


Figure 5. Minimum energy structure for hexamer **9** showing the hydrogen bonds implicated in the mixed 8-helix→12-helix conformation.

most stable when the double substitution occurred at positions 2 and 4.

With the aim to confirm these results, following a standard homogeneous phase synthesis (see the Supporting Information) the mixed hexamers **8** and **9** were prepared, in which **6a** was introduced at positions 3 and (2,4), respectively, and the remaining building blocks were **5a** (Scheme 3). From both NMR spectroscopy data and MD simulations, it was found that **6a** is able to modify the 12-helix of the hexamer built up from **5a**, and the new conformation was assigned as a mixed 8-helix–12-helix. In fact, for compound **8** we observed a large conformational variability and only a locally induced 8-helix folding within 12-helix structural elements (see the Supporting Information for further details; Figure 4). On the contrary, cluster analysis of compound **9** carried out with NOE restricted MD simulations pointed out the existence of 22 conformers, arranged into 13 clusters, and the minimum energy structures alternate 8-helix and 12-helix conformations (Figure 5).

For a better insight, a comparison between the backbones of hexamers **7**–**9** is reported in Figure 6. From these results, **6a** could be a useful tool to generate local changes of the conformationally preferred 12-helix in a β-foldamer, and this behaviour was ascribed to the presence of the quaternary carbon centre.

In summary, we have found that the hexamer of (3*S*,4*R*,1′*S*)-AMMOPC (**6a**) in $CDCl_3$ folds around short-range hydrogen bonds leading to eight-membered rings (8-helix conformation). In addition, insertion of **6a** into the β-peptide built up from (3*S*,4*R*,1′*S*)-AMOPC (**5a**), which shows a clear preference for longer-range hydrogen bonding interactions that lead to a stabilised 12-helix, changed its conformation from 12- to 8-helix. This behaviour might enhance the understanding of the principles behind the design of functionally folded architectures in large synthetic structures. To this end, synthesis of a series of β-amino acids tethered on a γ-lactam containing a quaternary centre is underway, and results arising from their insertion into β-foldamers will be reported in due course.

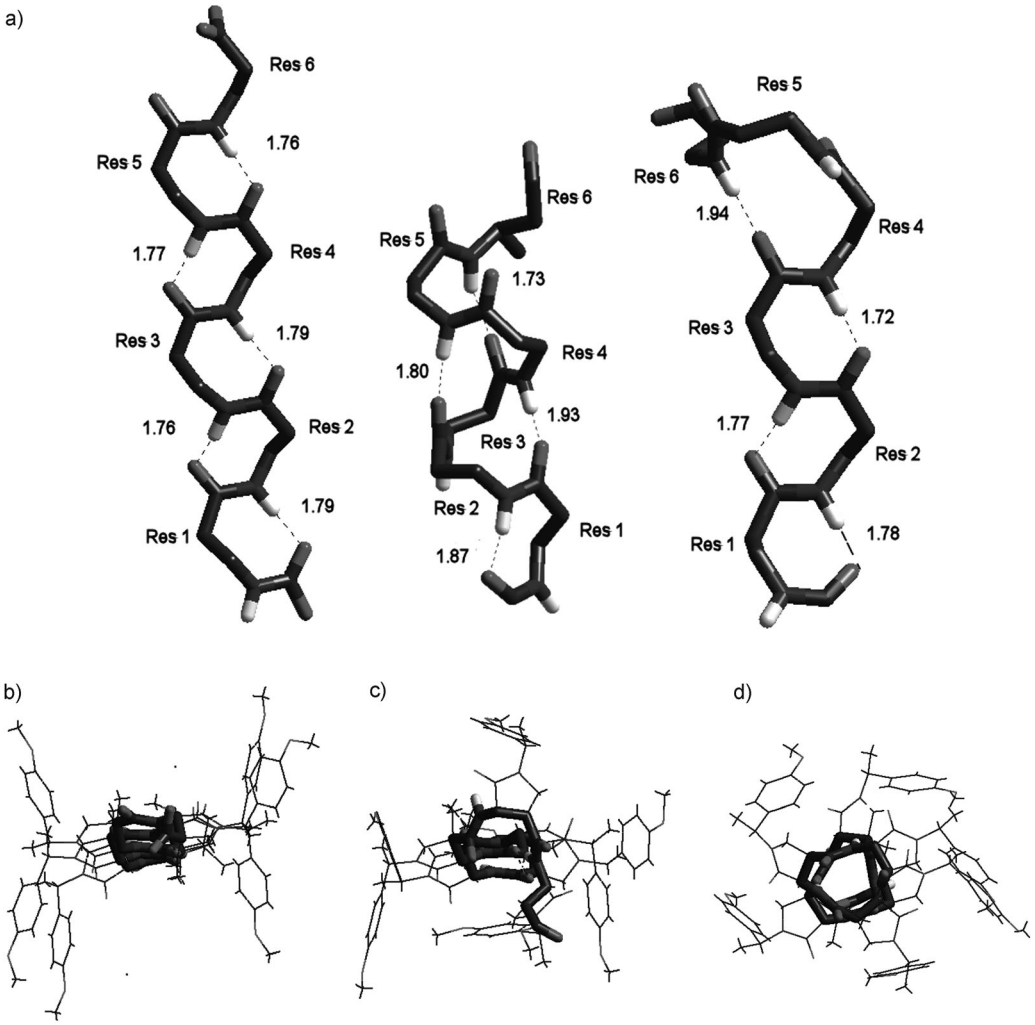


Figure 6. Comparison of lateral and perpendicular views of backbones of foldamers **7**, **8** and **9** (from left to right).

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Keywords: conformation analysis · foldamers · helical structures · lactams · quaternary carbon

[1] C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252–262, and references therein.
[2] D. Seebach, A. K. Beck, D. J. Bierbaum, *Chem. Biodiversity* **2004**, *1*, 1111–1239, and references therein.
[3] R. S. Roy, I. L. Karle, S. Raghothama, P. Balaram, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16478–16482.
[4] S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180.
[5] R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232.
[6] W. S. Horne, S. H. Gellman, *Acc. Chem. Res.* **2008**, *41*, 1399–1408.
[7] W. F. DeGrado, C. M. Summa, V. Pavone, F. Nastri, A. Lombardi, *Annu. Rev. Biochem.* **1999**, *68*, 779–819.
[8] M. J. I. Andrews, A. B. Tabor, *Tetrahedron* **1999**, *55*, 11711–11743.
[9] E. Lacroix, T. Kortemme, M. Lopez de La Paz, L. Serrano, *Curr. Opin. Struct. Biol.* **1999**, *9*, 487–493.
[10] S. H. Gellman, *Curr. Opin. Chem. Biol.* **1998**, *2*, 717–725.
[11] G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Protein Chem.* **1985**, *37*, 1–110.
[12] G. Quinkert, E. Egert, C. Griesinger, *Macromolecular and Supramolecular Chemistry in Aspects of Organic Chemistry, Structure*, (Eds.: G. Quinkert, E. Egert, C. Griesinger), Helvetica Chimica Acta, Basel, **1996**.
[13] A. Chakrabartty, R. L. Baldwin, *Adv. Protein Chem.* **1995**, *46*, 141–176.
[14] A. G. Street, S. L. Mayo, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9074–9076.
[15] D. L. Minor, Jr., P. S. Kim, *Nature* **1994**, *367*, 660–663.
[16] C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396–419.
[17] K. Gademann, A. Häne, M. Rueping, B. Jaun, D. Seebach, *Angew. Chem.* **2003**, *115*, 1573–1575; *Angew. Chem. Int. Ed.* **2003**, *42*, 1534–1537.
[18] a) S. Abele, P. Seiler, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1559–1571; b) S. Abele, D. Seebach, *Eur. J. Org. Chem.* **2000**, 1–15.
[19] R. J. Doerksen, B. Chen, J. Yuan, J. D. Winkler, M. L. Klein, *Chem. Commun.* **2003**, 2534–2535.
[20] D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 6206–6212.
[21] J. J. Barchi, Jr., X. Huang, D. H. Apella, L. A. Christianson, S. R. Durell, S. Gellman, *J. Am. Chem. Soc.* **2000**, *122*, 2711–2718.
[22] R. Threlfall, A. Davies, N. M. Howarth, J. Fisher, R. Cosstick, *Chem. Commun.* **2008**, 585–587.
[23] G. P. Dado, S. H. Gellman, *J. Am. Chem. Soc.* **1994**, *116*, 1054–1062.
[24] R. Galeazzi, G. Martelli, M. Orena, S. Rinaldi, P. Sabatino, *Tetrahedron* **2005**, *61*, 5465–5473.
[25] a) T. K. Chakraborty, S. Ghosh, S. Jayaprakassh, J. A. R. P. Sharma, V. Ravikanth, P. V. Diwan, N. Nagaraj, A. C. Kunwar, *J. Org. Chem.* **2000**, *65*, 6441–6457; b) J. D. Sadowsky, W. D. Fairlie, E. B. Hadley, H. S. Lee, N. Umezawaq, Z. Nikolovska-Coleska, S. M. Wang, D. C. S. Huang, Y. Tomita, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 139–154.
[26] a) R. F. Epand, L. Raguse, S. H. Gellman, *Biochemistry* **2004**, *43*, 9527–9535; b) P. I. Arvidsson, N. S. Ryder, H. M. Weiss, G. Gross, O. Kretz, R. Woessner, D. Seebach, *ChemBioChem* **2003**, *4*, 1345–1347.
[27] T. A. Martinek, A. Hetényi, L. Fülop, I. M. Mándity, G. K. Tóth, I. Dékány, F. Fülop, *Angew. Chem.* **2006**, *118*, 2456–2460; *Angew. Chem. Int. Ed.* **2006**, *45*, 2396–2400.
[28] I. Menegazzo, A. Fries, S. Mammi, R. Galeazzi, G. Martelli, M. Orena, S. Rinaldi, *Chem. Commun.* **2006**, 4915–4917.
[29] E. Crucianelli, R. Galeazzi, G. Martelli, M. Orena, S. Rinaldi, P. Sabatino, *Tetrahedron* **2010**, *66*, 400–405.
[30] a) K. D. Kopple, M. Ohnishi, A. Go, *Biochemistry* **1969**, *8*, 4087–4095; b) L. Belvisi, A. Bernardi, L. Manzoni, D. Potenza, C. Scolastico, *Eur. J. Org. Chem.* **2000**, 2563–2569; c) M. M. Fernández, A. Diez, M. Rubiralta, E. Montenegro, N. Casamitjana, *J. Org. Chem.* **2002**, *67*, 7587–7599; d) A. Trabocchi, E. G. Occhiato, D. Potenza, A. Guarna, *J. Org. Chem.* **2002**, *67*, 7483–7492.

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