Foldamer Structure

Parallel Sheet Secondary Structure in β-Peptides**

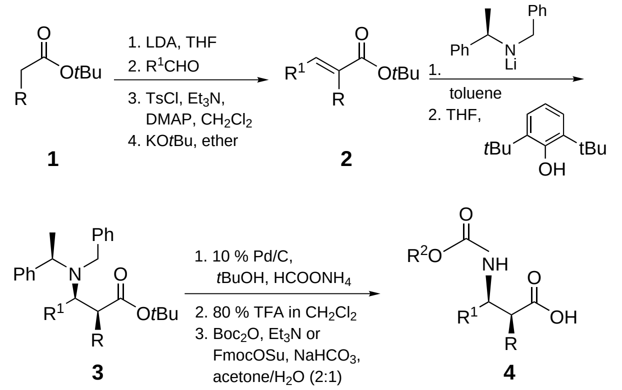
*Joseph M. Langenhan, Ilia A. Guzei, and Samuel H. Gellman**

Non-natural oligomers that adopt discrete conformations ("foldamers") are the focus of an extensive research effort.[1] β-Peptide foldamers[2] have been particularly interesting because several distinct secondary structures (for example, different types of helices, two kinds of reverse turns, and antiparallel sheet) can be induced by using appropriately substituted β-amino acid residues. Here we extend the secondary structure list by documenting parallel sheet secondary structure between α,β-*syn*-dialkyl β-amino acid residues, linked with a diamine connector, both in solution and in the solid state.[3]

A hairpin (that is, a strand–loop–strand motif) is the smallest possible increment of autonomous sheet secondary structure. The hairpin architecture has been widely employed to generate β-sheet model systems among conventional peptides[4] and to explore sheet secondary structure involving non-natural units, which include systems containing strand mimics,[5] nonpeptidic loops,[6] and α/β-amino acid combinations.[7] In proteins, two classes of secondary structure display long-range order, helices and sheets. Development of autonomously folding β sheets proved to be more difficult than creating autonomously folding α helices because noncovalent contacts within a helix occur between residues that are a fixed and short distance apart along the backbone, while noncovalent juxtapositions in sheet secondary structure do not involve fixed sequential relationships. Success in β-sheet design requires identification of appropriate strategies for linking the strand-forming segments.[4] Manipulation of the linker has allowed both antiparallel and parallel juxtapositions of β-strand segments among conventional peptides. The resulting model systems are invaluable tools for probing the forces that control β-sheet stability in natural peptides and proteins,[4] and for evaluating fundamental noncovalent interactions.[8] Small hairpin molecules should be useful for identifying the parameters that influence sheet secondary structure in unnatural foldamers, as has already been demonstrated for antiparallel β-peptide sheet.[3b,9]

Parallel hairpins[5c,f,6b,6c,10] require a reverse-turn unit that can link together two strand segments via their C or N termini; segments constructed from only α-, β-, or other amino acids cannot fulfill this need because they have only one N and one C terminus. The prolyl-(1,1-dimethyl)-1,2-diaminoethyl (Pro-DADME) unit has recently been identified as an effective reverse turn for both α- and γ-peptide strand segments.[11,12] Here we explore the Pro-DADME unit as a promoter of parallel sheet formation between β-peptide strands.

We have previously shown that antiparallel β-peptide sheet secondary structure is promoted by α,β-*syn*-dialkyl β-amino acid residues, which favor an *anti* torsion angle about the $NC_\beta{-}C_\alpha C({=}O)$ bond.[9a,13] These residues form antiparallel hairpins in organic solvents when connected by either β-peptide[3b,9b–9d] or non-β-peptide linkers.[9a] Our initial studies utilized α,β-*syn*-dialkyl β-amino acids that had been synthesized from L-aspartic acid;[14] however, this methodology did not provide ready access to diverse side chains. Dialkyl β-amino acids have also been obtained by α-alkylation of β^3-substituted β-amino methyl esters, but this process results in mixtures of diastereomers, which are sometimes inseparable by chromatography.[15] We are now able to synthesize α,β-*syn*-dialkyl β-amino acids with high enantiomeric excesses by a route pioneered by Davies et al. (Scheme 1).[16] *E*-α,β-unsaturated *tert*-butyl esters **2** result from an aldol reaction between *tert*-butyl esters **1** and the appropriate aldehydes, followed by tosylation and subsequent potassium *tert*-butoxide-mediated elimination. These unsaturated esters undergo conjugate addition with lithium (*R*)-(+)-*N*-benzyl-*N*-α-methylbenzylamide under the reaction conditions developed by Davies and co-workers. The resultant amino esters **3** are then fully deprotected; the amino groups are reprotected with Boc or Fmoc groups, for solution or solid-phase synthesis, respectively. This general scheme can afford a variety of amino acids that contain β-alkyl groups and α-alkyl or α-aryl groups.



Scheme 1. Synthesis of α,β-*syn*-disubstituted β-amino acids. LDA = lithium diisopropylamide; DMAP = 4-dimethylamino pyridine; TFA = trifluroacetic acid; Boc = *t*-butyloxycarbonyl; FmocOSu = 9-fluorenylmethyloxycarbonyl-*N*-hydroxysuccinimide

With appropriate reverse-turn and strand building blocks in hand, we synthesized hairpin **5**, in which two *N*-acetylated

---

[*] Prof. S. H. Gellman, J. M. Langenhan, Dr. I. A. Guzei+
Department of Chemistry
University of Wisconsin
1101 University Ave., Madison, WI 53706 (USA)
Fax: (+1) 608-265-4534
E-mail: gellman@chem.wisc.edu

[+] Correspondence regarding X-ray diffraction analysis should be addressed to I.A.G. (E-mail: iguzei@chem.wisc.edu).

[**] This research was supported by the U.S. National Institutes of Health (GM 56414). J.M.L. was supported in part by a National Science Foundation Predoctoral Fellowship. The NMR spectrometer was purchased in part with a U.S. National Institutes of Health grant (NIH 1 S10 RR13866-01).

DOI: 10.1002/ange.200350932

α,β-*syn*-dialkyl β-amino acid strand residues are connected by a D-Pro-DADME linker. Molecule **5** adopts a parallel hairpin conformation in the solid state (Figure 1; top).[17] Within the crystal, the hairpins are packed in a parallel fashion into columns (Figure 1; middle). There is a single intermolecular hydrogen bond between each hairpin molecule within these columns. The columns pack together in the manner shown in the lower section of Figure 1. Within each individual hairpin, the O(1)···H–N(5) and O(2)···H–N(4) separations (2.1 Å and 2.2 Å, respectively) strongly suggest the presence of two hydrogen bonds between the two β-peptide strands (Figure 1; top). This result demonstrates that the desired parallel sheet folding pattern is accessible to **5**, but, as with any crystal structure of an inherently flexible molecule, these solid-state data do not provide insight on the propensity for parallel-sheet formation.

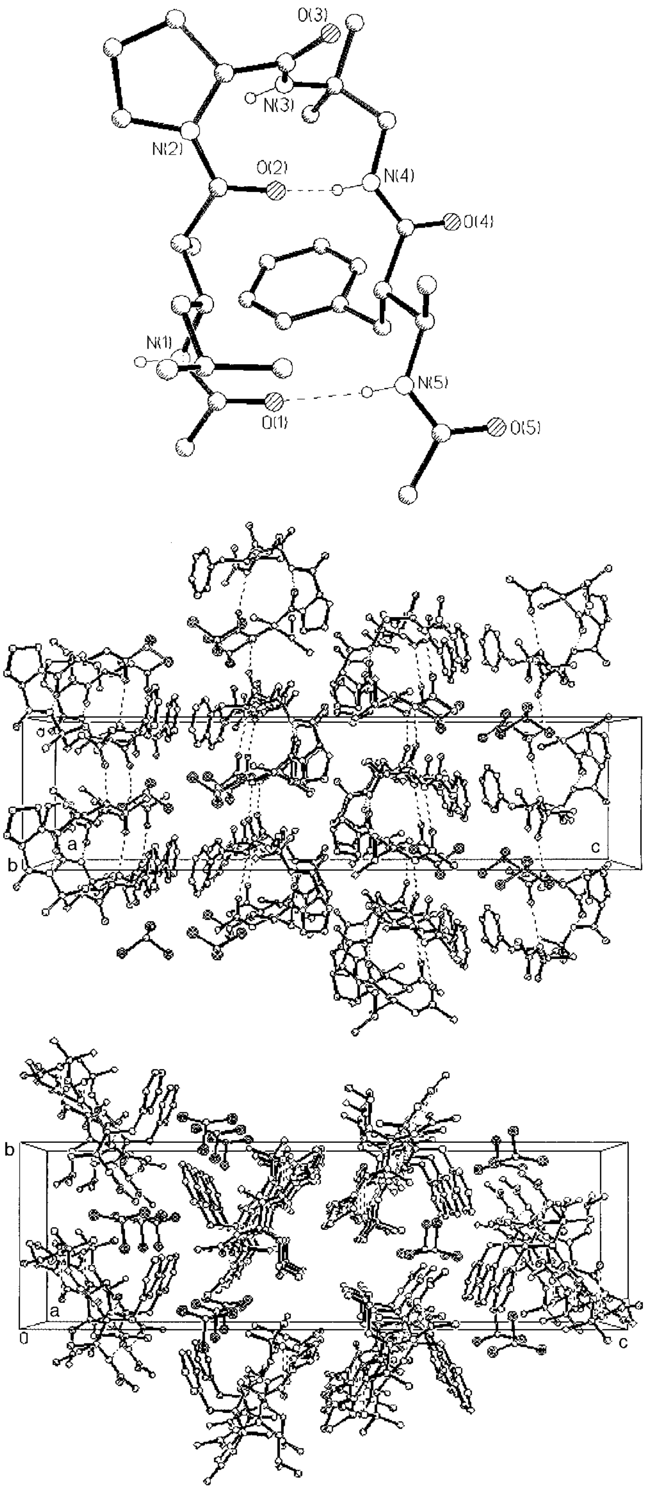
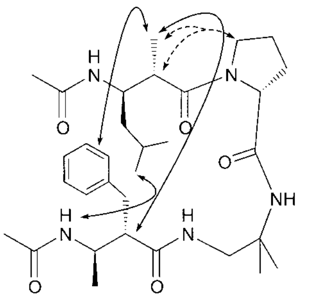


Figure 1. Top: The solid-state conformation of **5**. Hydrogen bonds are shown as dashed lines; middle: crystal packing diagram viewed along the *b* axis; bottom: crystal packing diagram viewed along the *a* axis.

To gain qualitative insight on the favorability of the hairpin conformation observed for **5** in the solid state, we examined this molecule by two-dimensional NMR spectroscopy in d_3-methanol at 4 °C. The chemical shifts for amide protons did not change between 0.05 mM and 5 mM, which is an indication that no aggregation of **5** occurs within this concentration range. All further experiments were performed at 5 mM. Because methanol is a protic solvent, a folding pattern stabilized exclusively by two internal hydrogen bonds should not be highly populated in this solvent. Detection of the hairpin conformation of **5** in methanol would suggest that other factors in addition to the hydrogen bonds preorganize **5** for parallel-sheet formation. Other secondary structures (helices[18] and antiparallel sheets[3b,9d]) have been detected among short β-peptides in methanol.

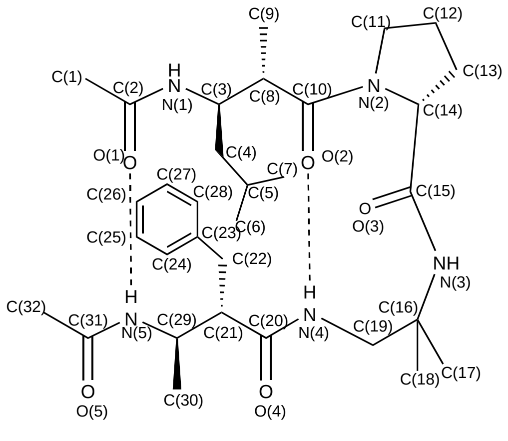
Two sets of proton resonances were observed for **5** in methanol, in a 93:7 ratio. We attribute this doubling of resonances to slow rotation about the tertiary amide C–N bond.[19] The proton resonances of the major conformer were fully assigned using COSY,[20] TOCSY,[21] and NOESY[22] measurements. Relatively strong NOEs were observed between the δ protons of the proline group and both the α proton and the α-methyl group of the adjacent residue (Scheme 2). Analogous NOEs involving the α proton of the proline group were not observed. These data establish the rotational state about the tertiary amide bond of the major rotamer as the *Z* configuration (as depicted in Scheme 2). This rotational state is consistent with the desired hairpin conformation. Most importantly, three interstrand NOEs were observed (Scheme 2). Each of these NOEs is consistent with the hairpin conformation observed in the solid state (Table 1); no NOEs inconsistent with the parallel hairpin structure were observed.



5

Scheme 2. NOEs observed for **5** in d_3-methanol that define the observed tertiary amide rotamer (dashed) and interstrand NOEs that establish a hairpin conformation in solution (solid).

Table 1: Solid-state separations between protons of **5** for which NOEs are observed in solution (**5** or **6**)[a].



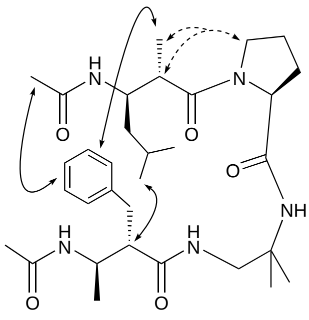
5

^{1}H atom	^{1}H atom	Separation [Å]
C(11)–H	C(8)–H	2.2
C(11)–H	C(9)–H	2.6
C(21)–H	C(9)–H	2.6
N(5)–H	C(6)–H	2.6
C(28)–H	C(9)–H	2.8
C(21)–H	C(6)–H	3.8
C(28)–H	C(1)–H	3.1

[a] For atoms that possess more than one proton, the smallest separation is reported. Protons on the phenyl ring (from C(24)–H to C(28)–H) could not be fully assigned. The separations reported involving the phenyl group are the shortest separations observed.

We and others have demonstrated that the α-peptide hairpin structure is dramatically influenced by the stereochemistry of the reverse-turn segment.[23] D-Pro-Gly and D-Pro-DADME reverse-turn segments induce antiparallel or parallel hairpin conformations, respectively; however, the enantiomeric L-Pro-Gly and L-Pro-DADME segments disallow folding. The stereochemistry of reverse-turn segments exerts a more subtle influence on antiparallel hairpin structure among β-peptides.[9c] For example, both an (*R*)-nipecotic acid-(*S*)-nipecotic acid segment and the enantiomeric (*S*)-nipecotic acid-(*R*)-nipecotic acid segment promote hairpin structure, but the *S*/*R* segment results in a larger population of a minor rotamer relative to the *R*/*S* segment (22% versus 6%), for α,β-syn-dialkyl β-amino acid strand residues with the absolute configuration of those used in the present study. Inspection of conventional Corey–Pauling–Koltun (CPK) models suggests that the possible minor rotamers do not allow hairpin folding. Therefore, this difference in the rotamer ratio indicates that the *R*/*S* reverse turn is slightly more effective than the *S*/*R* turn at promoting antiparallel sheet interactions between these strand residues.

We examined **6**, a diastereomer of **5** containing an L-Pro-DADME linker, to ascertain the influence exerted by the prolyl stereocenter on parallel hairpin formation in β-peptides. Molecule **6** displayed a 74:26 rotamer ratio in d_3-methanol at 5 mM, a concentration at which no aggregation occurs (4 °C). The proton resonances of the major rotamer were fully assigned using two-dimensional NMR spectroscopy. Strong NOEs between the δ protons of the proline group (but not the α protons) and both the α proton and the α-methyl group of the adjacent residue established a *Z* configuration about the tertiary amide in the major rotamer, as shown in Scheme 3. Several interstrand NOEs were observed (Scheme 3), which suggests the presence of a significant population of the hairpin conformation, with parallel-sheet interactions between the β-amino acid residues. No NOEs inconsistent with the desired hairpin conformation were observed (Table 1).



6

Scheme 3. NOEs observed for **6** in d_3-methanol that define the observed tertiary amide rotamer (dashed) and interstrand NOEs that establish a hairpin conformation in solution (solid).

The main difference between the D-Pro-DADME- and L-Pro-DADME-containing parallel hairpins (**5** and **6**, respectively) is the larger population of a minor rotamer in **6** (26%) relative to **5** (7%). This difference suggests that the L-Pro-DADME reverse turn of **6** is slightly less compatible with parallel-sheet interactions between α,β-*syn*-dialkyl β-amino acid residues (of the absolute configuration used in our studies) than is the D-Pro-DADME turn of **5**, because CPK modeling indicates that parallel sheet interactions are not possible if the tertiary amide configuration is *E*.

It is interesting that β-peptide hairpins, whether parallel or antiparallel, are less strongly influenced by reverse-turn stereochemistry than their α-peptide counterparts. For α-peptides, reverse-turn stereochemistry is important because the twist of the turn segment must be compatible with the twist preferred by the strand segments. β-Peptide strands may exhibit a significantly less pronounced twist than their α-peptide counterparts,[24] and therefore the local twist of the reverse-turn segment may be less influential in β-peptide sheet than for α-peptide sheet. Our demonstration of minimal systems for creating parallel sheet among β-peptides will enable a complete characterization of the factors that control this secondary structure.

Received: January 14, 2003 [Z50932]

Keywords: foldamers · NMR spectroscopy · peptides · secondary structure · structure elucidation

[1] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173; b) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893.

[2] a) D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, *21*, 2015; b) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219.

[3] Intermolecular parallel-sheet structure has previously been observed in the solid state for two three-residue β-peptides. One of these peptides has a high intrinsic preference for helix formation in solution, which highlights the fact that solid-state data fail to provide insight on the intrinsic folding propensity of solvated molecules. See a) D. Seebach, M. Overhand, F. N. M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913; b) D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem.* **1999**, *111*, 1700; *Angew. Chem. Int. Ed.* **1999**, *38*, 1595.

[4] a) S. H. Gellman, *Curr. Opin. Chem. Biol.* **1998**, *2*, 717; b) L. Serrano, *Adv. Protein Chem.* **2000**, *53*, 49; c) M. S. Searle, *J. Chem. Soc. Perkin Trans. 2* **2001**, 1011.

[5] a) J. M. Langenhan, J. D. Fisk, S. H. Gellman, *Org. Lett.* **2001**, *3*, 2559; b) D. S. Kemp, B. R. Bowen, *Tetrahedron Lett.* **1988**, *29*, 5081; c) J. S. Nowick, *Acc. Chem. Res.* **1999**, *32*, 287; d) J. S. Nowick, D. M. Chung, K. Maitra, S. Maitra, K. D. Stigers, Y. Sun, *J. Am. Chem. Soc.* **2000**, *122*, 7654; correction: J. S. Nowick, D. M. Chung, K. Maitra, S. Maitra, K. D. Stigers, Y. Sun, *J. Am. Chem. Soc.* **2001**, *123*, 1545; e) J. S. Nowick, J. M. Cary, J. H. Tsai, *J. Am. Chem. Soc.* **2001**, *123*, 5176; f) J. S. Nowick, E. M. Smith, J. W. Ziller, A. J. Shaka, *Tetrahedron* **2002**, *58*, 727; g) J. S. Nowick, K. S. Lam, T. V. Khasanova, W. E. Kemnitzer, S. Maitra, H. T. Mee, R. Lui, *J. Am. Chem. Soc.* **2002**, *124*, 4972.

[6] a) J. P. Schneider, J. W. Kelly, *Chem. Rev.* **1995**, *95*, 2169; b) D. Ranganathan, V. Haridas, S. Kurur, A. Thomas, K. P. Madhusudanan, R. Nagaraj, A. C. Kunwar, A. V. S. Sarma, I. L. Karle, *J. Am. Chem. Soc.* **1998**, *120*, 8448; c) I. G. Jones, W. Jones, M. North, *J. Org. Chem.* **1998**, *63*, 1505; d) R. R. Gardner, G.-B. Liang, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 1806; e) L. Belvisi, C. Gennari, A. Madder, A. Mielgo, D. Potenza, C. Scolastico, *Eur. J. Org. Chem.* **2000**, 695; f) B. Aguilera, G. Siegal, H. S. Overkleeft, N. J. Meeuwenoord, F. P. J. T. Rutjes, J. C. M. van Hest, H. E. Schoemaker, G. A. van der Marel, J. H. van Boom, M. Overhand, *Eur. J. Org. Chem.* **2001**, 1541.

[7] a) S. Hanessian, H. Yang, *Tetrahedron Lett.* **1997**, *38*, 3155; b) B. R. Huck, J. D. Fisk, S. H. Gellman, *Org. Lett.* **2000**, *2*, 2607; c) I. L. Karle, H. N. Gopi, P. Balaram, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3716; d) H. N. Gopi, R. S. Roy, S. R. Raghothama, I. L. Karle, P. Balaram, *Helv. Chim. Acta* **2002**, *85*, 3313; e) I. L. Karle, H. N. Gopi, P. Balaram, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5160.

[8] C. D. Tatko, M. L. Waters, *J. Am. Chem. Soc.* **2002**, *124*, 9372.

[9] a) S. Krauthauser, L. A. Christianson, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719; b) Y. J. Chung, L. A. Christianson, H. E. Stanger, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 10555; c) Y. J. Chung, B. R. Huck, L. A. Christianson, H. E. Stanger, S. Krauthauser, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **2000**, *122*, 3995; d) X. Daura, K. Gademann, H. Schafer, B. Jaun, D. Seebach, W. F. van Gunsteren, *J. Am. Chem. Soc.* **2001**, *123*, 2393.

[10] a) D. S. Kemp, D. E. Blanchard, C. C. Muendel in *Peptides—Chemistry and Biology* (Eds.: J. Smith, J. River), ESCOM, Leiden, **1992**, p. 319; b) G. Wagner, M. Feigel, *Tetrahedron* **1993**, *49*, 10831; c) J. S. Nowick, S. Insaf, *J. Am. Chem. Soc.* **1997**, *119*, 10903; d) M. L. Skar, J. S. Svendsen, *Tetrahedron* **1997**, *53*, 17425; e) P. Chitnumsub, W. R. Fiori, H. A. Lashuel, H. Diaz, J. W. Kelly, *Bioorg. Med. Chem.* **1999**, *7*, 39; f) J. D. Fisk, S. H. Gellman, *J. Am. Chem. Soc.* **2001**, *123*, 343.

[11] J. D. Fisk, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **2000**, *122*, 5443.

[12] 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.

[13] Y.-L. Zhao, Y.-D. Wu, *J. Am. Chem. Soc.* **2002**, *124*, 1570.

[14] a) C. W. Jefford, J. McNulty, *Helv. Chim. Acta* **1994**, *77*, 2142; b) C. W. Jefford, J. Wang, *Tetrahedron Lett.* **1993**, *34*, 1111.

[15] D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. L. Matthews, J. V. Schreiber, *Helv. Chim. Acta* **1998**, *81*, 932.

[16] S. G. Davies, O. Ichihara, I. A. S. Walters, *J. Chem. Soc. Perkin Trans. 1* **1994**, 1141.

[17] Crystal data for $C_{32}H_{51}N_5O_5{\cdot}CHCl_3$: crystal size $0.43\times0.32\times0.26\,mm^3$, orthorhombic, $P2_12_12_1$, $a=9.1688(13)$, $b=11.3311(15)$, $c=36.444(5)$ Å, $V=3786.3(9)$ Å^3, $\rho_{calcd}=1.237\,Mg\,m^{-3}$, $2\theta_{max}=53.0°$, $Mo_{K\alpha}$ radiation ($\lambda=0.71073$ Å), ω scans, $T=100(1)$ K, 30860 independent and 7713 unique reflections. The data were corrected for Lorentz and polarization effects; the empirical absorption correction was performed with SADABS V2.03 (Bruker-AXS, 2001), $\mu=0.286\,mm^{-1}$, Tmax/min$^{-1}=0.93/0.89$. The structure was solved and refined usimg SHELXTL V6.10 (Bruker-AXS, 2000). All non-hydrogen atoms except C(15) and C(23) were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients. The C(23) phenyl ring was refined with an idealized geometry. There is also one molecule of solvate chloroform in the asymmetric unit. The final least-squares refinement of 390 parameters against 7713 data (with 264 constraints) resulted in residuals R (based on F^2 for $I\geq2\sigma$) and wR (based on F^2 for all data) of 0.1111 and 0.2936, respectively. The slightly high R value results from the necessity of recording data from a weakly diffracting crystal. The final difference Fourier map was featureless. CCDC-200849 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

[18] a) D. Seebach, M. Overhand, F. N. M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913; b) D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 13071; c) D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. L. Huang, J. J. Barchi, S. H. Gellman, *Nature* **1997**, *387*, 381.

[19] The difference in resonance ratios observed in *d*-chloroform versus d_3-methanol confirmed that resonance doubling is attributable to rotamers.

[20] W. P. Aue, E. Bartholdi, R. R. Ernst, *J. Chem. Phys.* **1976**, *64*, 2229.

[21] A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *65*, 355.

[22] S. Macura, R. R. Ernst, *Mol. Phys.* **1980**, *41*, 95.

[23] a) T. S. Haque, J. C. Little, S. H. Gellman, *J. Am. Chem. Soc.* **1994**, *116*, 4105; b) S. K. Awasthi, S. Raghothama, P. Balaram, *Biochem. Biophys. Res. Commun.* **1995**, *216*, 375; c) I. L. Karle, S. K. Awasthi, P. Balaram, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 8189; d) T. S. Haque, J. C. Little, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 6975; e) T. S. Haque, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 2303; f) H. E. Stanger, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 4236; g) S. R. Raghothama, S. K. Awasthi, P. Balaram, *J. Chem. Soc. Perkin Trans. 2* **1998**, 137.

[24] J.-Q. Lin, S.-W. Luo, Y.-D. Wu, *J. Comput. Chem.* **2002**, *23*, 1551.