A Triply Templated Artificial β-Sheet

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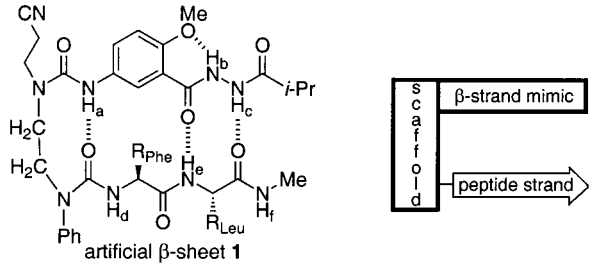
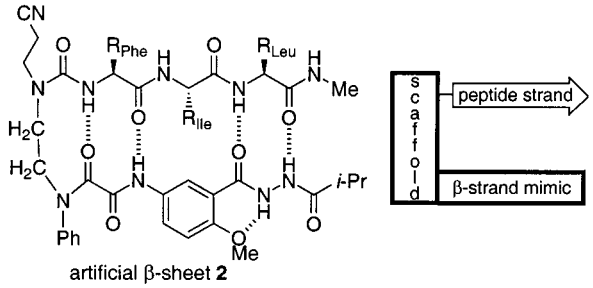
Abstract: This paper describes the design, synthesis, and structural evaluation of a compound (**4**) comprising three molecular templates and a peptide strand that mimics a three-stranded protein β-sheet. Two of the templates mimic the hydrogen-bonding functionality of peptide β-strands and serve as the top and bottom strands by embracing the peptide strand, which is located in the middle of the sheet. The remaining template holds the three strands next to each other. The synthesis of artificial β-sheet **4** begins with the bottom template and involves the sequential addition of the middle and top strands. ^{1}H NMR chemical shift and NOE studies establish that this compound folds to adopt a hydrogen-bonded β-sheetlike structure in $CDCl_3$ solution. Chemical shift studies indicate that triply stranded artificial β-sheet **4** is more tightly folded than its smaller doubly stranded homologue, artificial β-sheet **1**.

Over the past few years, our laboratory has sought to gain insight into β-sheet structure and interactions and develop useful peptidomimetic building blocks by designing, synthesizing, and studying molecules that mimic protein β-sheets.[1] In these molecules, which we have termed *artificial β-sheets*, molecular templates are combined with peptide groups to form the characteristic conformations and hydrogen-bonding patterns of protein β-sheets. Our efforts have complemented and built upon the pioneering use of templates to stabilize β-sheet structure in attached peptides by the research groups of Feigel,[2] Kemp,[3] and Kelly[4] and the ongoing efforts of groups such as Gellman[5] and Seebach,[6] who create folded proteinlike structures in unnatural peptides without using molecular templates. Collectively, these studies are beginning to reveal how molecules that mimic the structure and function of proteins can be designed and synthesized.

One of our ongoing concerns has been the development of larger and more complex designed molecules that begin to rival the complex folding of proteins. We have previously reported artificial β-sheets with β-strand mimics either along the upper edge or along the lower edge. Artificial β-sheets **1**[7] and **2**[8] illustrate two of these structures. In artificial β-sheet **1**, a 5-amino-2-methoxybenzoic hydrazide β-strand mimic and a dipeptide strand are attached by urea linking groups to the upper and lower nitrogen atoms of a 1,2-diaminoethane group. The two urea groups hydrogen bond together to form a turnlike





"molecular scaffold". In artificial β-sheet **2**, the 5-amino-2-methoxybenzoic hydrazide β-strand mimic resides on the lower edge, rather than the upper, and the urea linking group has been replaced by an oxalic acid group to achieve an appropriate hydrogen-bonding pattern. To accommodate the oxalic acid linking group, the peptide is elongated to a tripeptide.

In the present study, we sought to combine the elements of these two doubly templated structures to form a triply templated artificial β-sheet. Initially, we envisioned artificial β-sheet **3** as embodying the combination of the types of structures represented by artificial β-sheets **1** and **2**. In this structure, the diamine has been extended to a triamine and the upper β-strand mimic is extended by the addition of an amino acid to match a tripeptide in length.[9] Difficulties in synthesizing this compound and concerns about its folded structure prompted us to design and synthesize artificial β-sheet **4**.[10] Artificial β-sheet **4** contains

(1) Nowick, J. S. *Acc. Chem. Res.* **1999**, *32*, 287−296.
(2) (a) Wagner, G.; Feigel M. *Tetrahedron* **1993**, *49*, 10831−10842. (b) Brandmeier V.; Sauer, W. H. B.; Feigel M. *Helv. Chim. Acta* **1994**, *77*, 70−85.
(3) (a) Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* **1988**, *29*, 5077−5080. (b) Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* **1988**, *29*, 5081−5082. (c) Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* **1990**, *55*, 4650−4657.
(4) (a) Diaz, H.; Tsang, K. Y.; Choo, D.; Espina, J. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 3790−3791. (b) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988−4005.
(5) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.
(6) Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015−2022.
(7) Smith, E. M.; Holmes, D. L.; Shaka, A. J.; Nowick, J. S. *J. Org. Chem.* **1997**, *62*, 7906−7907.
(8) Nowick, J. S.; Tsai, J. H.; Bui, Q.-C. D.; Maitra, S. *J. Am. Chem. Soc.* **1999**, *121*, 8409−8410.
(9) Tsai, J. H.; Waldman, A. S.; Nowick, J. S. *Bioorg. Med. Chem.* **1999**, *7*, 29−38.

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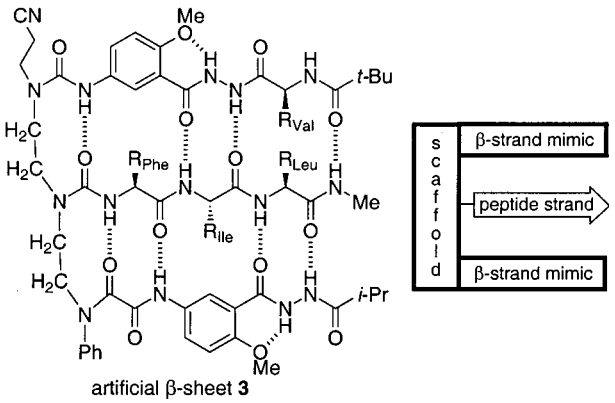
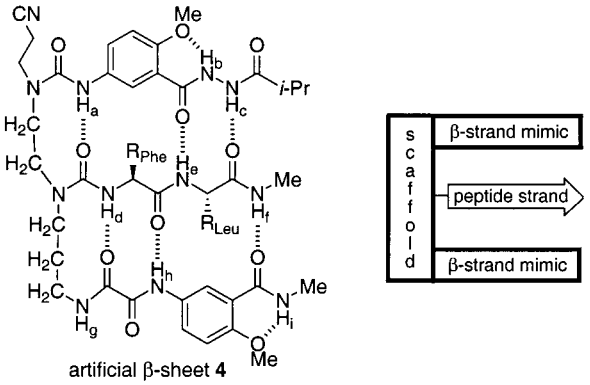


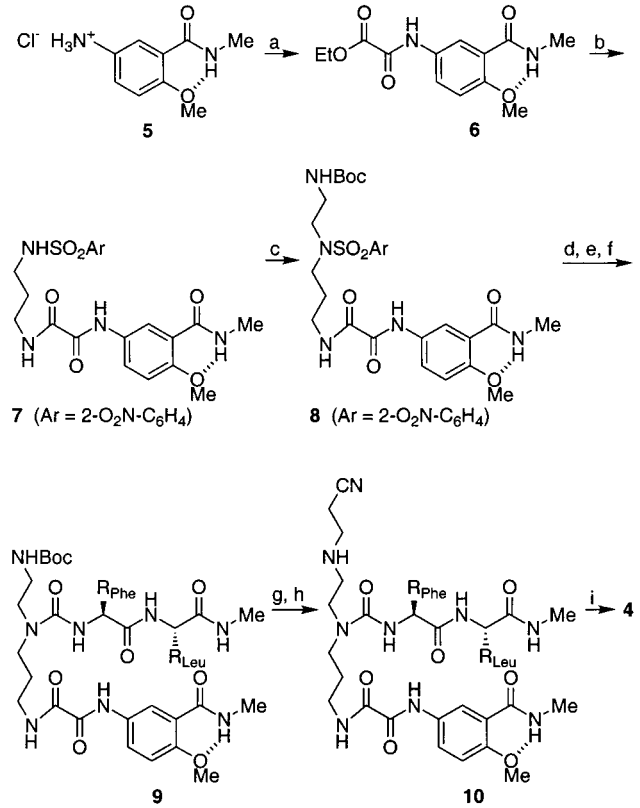
artificial β-sheet **3**



artificial β-sheet **4**

a dipeptide strand and a truncated lower β-strand mimic and is smaller and easier to synthesize than **3**. To allow the lower β-strand mimic to better align with the peptide strand, artificial β-sheet **4** incorporates a longer 1,3-diaminopropane group in place of the lower 1,2-diaminoethane portion of the molecular scaffold of **3**.[11] To mitigate synthetic difficulties associated with the formation of the amide bond of the tertiary anilide group and concerns about its conformational heterogeneity, it contains a hydrogen in place of the phenyl group in its lower left-hand corner.[12,13] In this paper, we report the evaluation of this triply templated design through synthetic and ^{1}H NMR spectroscopic structural studies of artificial β-sheet **4**.

Scheme 1[a]



[a] Reagents: (a) EtO_2CCOCl, Et_3N/CH_2Cl_2 (91%). (b) 2-O_2N-$C_6H_4SO_2NH(CH_2)_3NH_2/CH_2Cl_2$ (44%). (c) $BocNH(CH_2)_2Br$, Cs_2CO_3/DMF (52%). (d) PhSH, K_2CO_3/DMF (98%). (e) phenyalanylleucine methyl ester isocyanate, Et_3N/CH_2Cl_2 (82%). (f) CH_3NH_2/CH_3OH (86%). (g) (i) TFA/CH_2Cl_2; (ii) saturated aqueous K_2CO_3 (60%). (h) CH_2═$CHCN/CH_3OH$:$CHCl_3$ 5:2 (81%). (i) 5-OCN-2-MeO-$C_6H_3CONHNHCOi$-Pr/CH_2Cl_2 (73%).

Results

Artificial β-sheet **4** was synthesized starting with the bottom β-strand mimic (5-amino-2-methoxy-*N*-methylbenzamide) by successively building up the triamine backbone and then adding the middle peptide strand and the upper β-strand mimic (Scheme 1). 5-Amino-2-methoxy-*N*-methylbenzamide hydrochloride (**5**)[14] was coupled with ethyl oxalyl chloride ($EtO_2COCOCl$) in the presence of triethylamine to form the corresponding ethyl oxamate (**6**). Oxamate esters are especially reactive and can be coupled with simple amines by simply mixing or by mixing and warming; thus, oxamate **6** was coupled with *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide[15] to give sulfonamide **7** by mixing the two compounds in CH_2Cl_2 solution for 2 days at room temperature.[16] The triamine backbone was then generated by alkylation of the sulfonamide group with *tert*-butyl *N*-(2-bromoethyl)carbamate[17] to give tertiary sulfonamide **8**.[18] Removal of the sulfonyl group by treatment with benzenethiol and K_2CO_3,[18] followed by reaction of the resulting secondary amine[19] with phenylalanylleucine methyl ester isocyanate[20] and aminolysis of the ester group with methylamine, introduced the

(10) Preliminary studies suggest that artificial β-sheet **3** forms a well-defined β-sheet structure in chloroform solution. For details, see: Tsai, J. H. Ph.D. Dissertation, University of California, Irvine, 2000.

(11) Previous studies by our research group have shown that both the 1,2-diaminoethane group and the 1,3-diaminopropane group form turn structures, but that the 1,2-diaminoethane turn is more stable and structurally well defined. See: (a) Nowick, J. S.; Powell, N. A.; Martinez, E. J.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 3763−3765. (b) Nowick, J. S.; Abdi, M.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Noronha, G.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 89−99. (c) Nowick, J. S.; Mahrus, S.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 1066−1072.

(12) Simple oxalamides bearing phenyl and alkyl groups on one nitrogen [RNHCOCON(Ph)R′ (R′ = alkyl)] exhibit two conformers in the ^{1}H NMR spectrum at ambient temperatures. The observation of two conformers is associated with the partial double-bond character of the CO−N(Ph)R′ bond. Ureas bearing phenyl and alkyl groups on one nitrogen [RNHCON(Ph)R′ (R′ = alkyl)] preferentially adopt a conformer in which the carbonyl oxygen is cis to the alkyl group (R′) and trans to the phenyl group. The relatively rapid rotation about the CO−N(Ph)R′ bond in these compounds precludes the observation of discreet conformers by ^{1}H NMR spectroscopy at ambient temperatures.

(13) X-ray crystallographic and molecular modeling studies suggest that the replacement of the tertiary amide group with a secondary group also changes the preferred geometry of the oxalamide group from twisted (ca. 125° N−C−C−N torsion angle) to linear (ca. 180° N−C−C−N torsion angle). This change in geometry makes the oxalamide group more complementary to a peptide β-strand.

(14) Holmes, D. L.; Smith, E. M.; Nowick, J. S. *J. Am. Chem. Soc.* **1997**, *119*, 7665−7669.

(15) Hidai, Y.; Kan, T.; Fukuyama, T. *Tetrahedron Lett.* **1999**, *40*, 4711−4714.

(16) The low yield of this step (44%) may result from incomplete reaction; longer reaction times, higher concentrations, or heating may give better yields.

(17) Beylin, V. G.; Goel, O. P. *Org. Prep. Proc. Int.* **1987**, *19*, 78−80.

(18) Fukuyama, T.; Jow, C.-K.; Cheung. M. *Tetrahedron Lett.* **1995**, *36*, 6373−6374.

Table 1. ^{1}H NMR Chemical Shifts of NH Protons of Artificial β-Sheets and Controls[a]

	H_a	H_b	H_c	H_d	H_e	H_f	H_g	H_h	H_i
artificial β-sheet **1**	9.93	10.97	10.45	4.82	8.29	5.79			
artificial β-sheet **4**	10.27	11.21	11.58	6.69	8.69	8.55	7.70	10.76	8.11
control **11**[7]	6.31	10.85	8.78						
control **12**				4.63	6.28	6.71			
control **13**[7]					5.82	6.00			
control **14**							7.49	9.27	7.82

[a] Spectra were recorded in 1 mM $CDCl_3$ solution at 298 K.

peptide strand and afforded urea **9**. Removal of the Boc protecting group and alkylation of the resulting primary amine with acrylonitrile[21] generated secondary amine **10**. Coupling of secondary amine **10** with the isocyanate 5-OCN-2-MeO-C_6H_3-CONHNHCO-*i*-Pr[14] introduced the upper β-strand mimic by way of a urea linkage and completed the synthesis of artificial β-sheet **4**.

^{1}H NMR chemical shift studies indicate that artificial β-sheet **4** is intramolecularly hydrogen bonded in $CDCl_3$ solution and provide a pattern of data consistent with a folded β-sheet structure. In $CDCl_3$ solution, NH protons that are hydrogen bonded typically appear about 2 ppm downfield of similar types of NH protons that are not hydrogen bonded. Non-hydrogen-bonded peptide amide protons typically appear at about 6 ppm, for example, while hydrogen-bonded peptide amide protons typically appear at about 8 ppm. Comparison of the chemical shifts of the NH protons of artificial β-sheet **4** to those of suitable controls in dilute $CDCl_3$ solution elucidates the hydrogen-bonding states of the protons. For these studies, compound **11**[7] serves as a control for the upper β-strand mimic of **4**, compounds **12** and **13**[7] serve as controls for the peptide strand, and compound **14** serves as a control for the lower β-strand mimic.

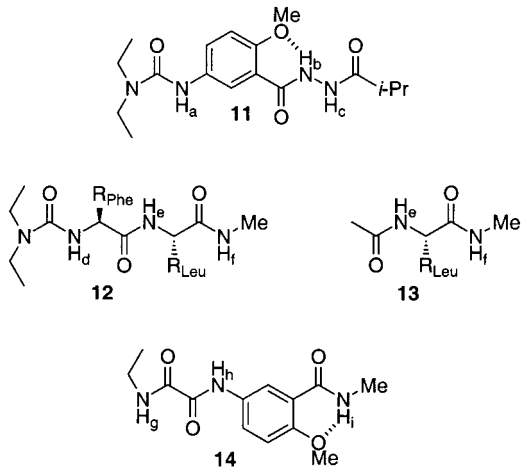


Table 1 summarizes the chemical shifts of the NH groups of these compounds.[22] Protons H_a and H_c of the upper β-strand mimic of artificial β-sheet **4** appear 3.96 and 2.80 ppm downfield of those of control **11**,[7] indicating that the protons are hydrogen bonded in **4**. Proton H_b appears at similar positions in both **4** and **11**, reflecting that it is intramolecularly hydrogen bonded to the adjacent methoxy group in both compounds.[23] Protons H_d and H_e of the peptide strand of **4** appear 2.06 and 2.41 ppm downfield of control **12**,[24] indicating that these protons are hydrogen bonded in **4**. Proton H_f of **4** appears 2.55 ppm downfield of that of control **13**,[7] indicating it is also hydrogen bonded.[25] Proton H_h of artificial β-sheet **4** appears 1.49 ppm downfield of that of control **14**,[26] indicating it is hydrogen bonded in **4**. Protons H_g and H_i are comparable in chemical shift in both **4** and **14**, reflecting that they have similar hydrogen-bonding states in both compounds.[27] Thus, the pattern and magnitudes of the chemical shifts of NH protons H_a-H_i support a model in which **4** is largely or wholly folded into a triply stranded β-sheetlike structure.

^{1}H NMR NOE studies provide compelling evidence that artificial β-sheet **4** folds into a well-defined β-sheet structure in $CDCl_3$ solution.[28] Because **4** is of intermediate size (MW = 999), these studies were performed in the rotating frame using the transverse-ROESY (Tr-ROESY) method.[29,30] These studies reveal one prominent interstrand NOE between the proton at the 6-position of the upper β-strand mimic and the α-proton of the phenylalanine residue and a second prominent interstrand NOE between the proton at the 6-position of the lower β-strand mimic and the α-proton of the leucine residue. (Figure 1 illustrates these and other key interstrand NOEs graphically.) Other key interstrand NOEs, which provide additional evidence for a folded β-sheet structure, occur between the isobutyryl methyl protons of the upper β-strand mimic and the methylamide methyl group of the middle peptide strand, the urea proton (H_d) of the middle peptide strand and the anilide proton (H_h) of the lower β-strand mimic, the leucine methyl groups (δ-protons)

(19) It is not clear whether the free secondary amine or an indeterminate salt or adduct is isolated after treatment of sulfonamide **8** with PhSH and K_2CO_3. The ^{1}H NMR and mass spectrum are consistent with the amine, but do not preclude a salt or unstable adduct (such as that which might form with CO_2). Consistent with a salt or unstable adduct, the solubility of this material in organic solvents is unexpectedly low, its melting point is unexpectedly high, and it requires the addition of triethylamine to react with phenylalanylleucine methyl ester isocyanate. The IR spectrum suggests the presence of an amine salt.

(20) Nowick, J. S.; Holmes, D. L.; Noronha, G.; Smith, E. M.; Nguyen, T. M.; Huang, S.-L. *J. Org. Chem.* **1996**, *61*, 3929−3934.

(21) (a) Bergeron, R. J.; Burton, P. S.; McGovern, K. A.; Kline, S. J.; *Synthesis* **1981**, 732−733. (b) Jasys, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Rosnack, K. J.; Saccomano, N. A.; Stroh, J. G.; Volkmann, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 6696−6704.

(22) The ^{1}H NMR spectra of these compounds were recorded at 1 mM in $CDCl_3$ solution at 298 K. Studies of the NH chemical shifts as a function of concentration indicate no significant intermolecular association at this concentration.

(23) The slight downfield shifting of H_b in **4** relative to **11** ($\Delta\delta$ = 0.36 ppm) may reflect polarization of the amide group in **4** by hydrogen bonding of the carbonyl.

(24) Control **12** was prepared by coupling of phenylalanylleucine methyl ester isocyanate with diethylamine in CH_2Cl_2 to give Et_2NCO-Phe-Leu-OMe, followed by aminolysis with methylamine in CH_3OH.

(25) The chemical shift of H_f of control **12** is 0.71 ppm downfield of that of control **13**. This downfield shifting may reflect that **12** can adopt a β-turnlike conformation in which H_f is intramolecularly hydrogen bonded to the urea carbonyl group. For this reason, **13** is likely a better control for the chemical shift of H_f.

(26) Control **14** was prepared by aminolysis of oxamate ester **6** with ethylamine in CH_3OH.

(27) The slight downfield shifting of H_g and H_i in **4** relative to **14** ($\Delta\delta$ = 0.21 and 0.29 ppm, respectively) may reflect polarization of these amide groups in **4** by hydrogen bonding of the carbonyl groups.

(28) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986; pp 125−129.

(29) (a) Hwang, T. L.; Shaka, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 3157−3159. (b) Hwang, T. L.; Shaka, A. J. *J. Magn. Reson. Ser. B* **1993**, *102*, 155−165.

(30) The studies were performed at 10 mM with a mixing time of 350 ms and were run at 312 K to minimize overlap of key resonances.

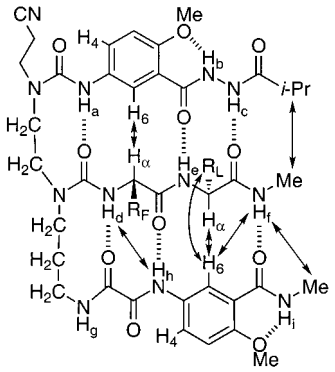


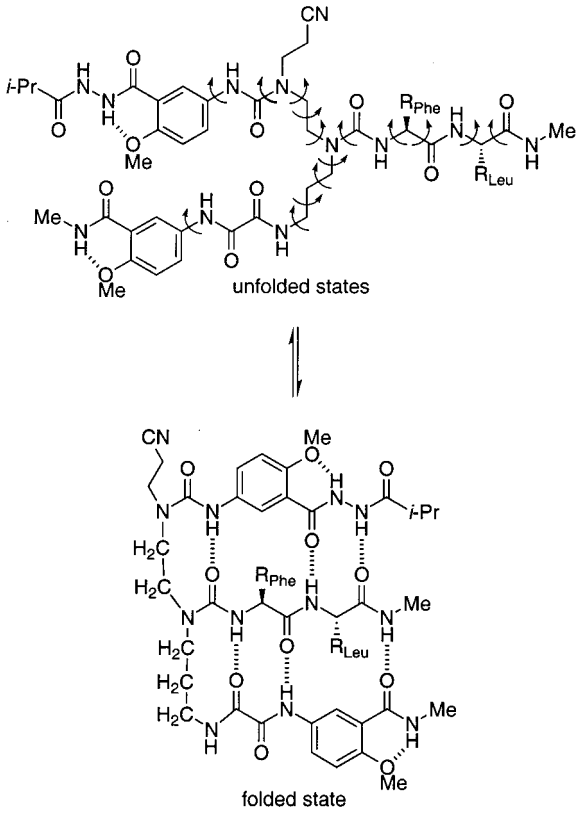
Figure 1. Key interstrand NOEs in artificial β-sheet **4**. Studies were performed in 10 mM $CDCl_3$ solution at 312 K. Interstrand NOEs are represented by arrows.

and the proton at the 6-position of the lower β-strand mimic, the methylamide proton (H_f) of the middle peptide strand and the proton at the 6-position of the lower β-strand mimic, and the methylamide proton (H_f) of the middle peptide strand and the methylamide methyl group of the lower β-strand mimic.

Additional evidence for a β-sheetlike conformation of **4** comes from the intrastrand NOEs of the peptide and peptidomimetic strands and the coupling constants of the peptide strand.[28] The peptide strand shows relatively strong interresidue NOEs between the NH and α-protons and relatively weak intraresidue NOEs between the NH and α-protons. Similarly, the upper β-strand mimic shows a relatively strong NOE between the NH proton H_a and the proton at its 6-position and only a very weak NOE between this NH proton and the proton at its 4-position; the lower β-strand mimic shows a relatively strong NOE between the NH proton H_h and the proton at its 6-position and only a weak NOE between this NH proton and the proton at its 4-position. The $^3J_{HN\alpha}$ coupling constants of the peptide strand are 8.9 Hz (Phe) and 9.3 Hz (Leu).[31] Collectively, these NOE and coupling constant data indicate that the component peptide and strand mimics adopt β-strandlike conformations.

^{1}H NMR studies indicate that the β-sheetlike structure into which **4** folds in the noncompetitive solvent $CDCl_3$ is lost in the competitive solvent CD_3OD. Tr-ROESY studies in this solvent show no NOEs between the protons at the 6-positions of the aromatic rings of the β-strand mimics and the α-protons of the phenylalanine and leucine residues.[32] Additional evidence for the loss of structure in this solvent comes from the chemical shifts of the phenylalanine and leucine α-protons. In $CDCl_3$ solution, these protons appear at 5.45 and 4.97 ppm, respectively. These values are substantially (>0.5 ppm) downfield of the typical random-coil values of Phe and Leu in proteins (4.66 and 4.17 ppm, respectively)[33] and in control **12** (4.46 and 4.38 ppm, respectively). This downfield shifting should reflect the anisotropic effects of the adjacent aromatic rings of the upper and lower β-strand mimics, as well as the general propensity of the α-protons of β-sheets to be shifted downfield.[33] In CD_3OD solution, the α-protons of the phenylalanine and leucine residues show little downfield shifting, appearing at 4.75 and 4.41 ppm, respectively. These studies reflect the importance of hydrogen bonding in the folding of artificial β-sheet **4** and the ability of competitive solvents to interrupt this folding.

Scheme 2



Discussion

That artificial β-sheet **4** adopts a folded β-sheetlike conformation is noteworthy, considering all of the other conformations that this large and complex molecule can adopt. The molecule could adopt unfolded states, in which the β-strand mimics and peptide strand are not hydrogen bonded (Scheme 2). It could also adopt misfolded states with different hydrogen-bonding patterns, such as the pairing of the β-strand mimics to the exclusion of the peptide strand. Partially folded and aggregated states are also possible. Despite the plethora of potential states, all of the data described above are consistent with the folded state and suggest that this state predominates.

To fold properly, artificial β-sheet **4** must form six hydrogen bonds and restrict rotation about fifteen bonds (shown with arrows in Scheme 2). Previous experience has shown us that in chloroform solution each hydrogen bond is able to offset up to roughly four degrees of rotational freedom. In doubly stranded artificial β-sheets **1** and **2**, rotation about three bonds must be significantly constrained for each hydrogen bond formed: Artificial β-sheet **1** has nine bonds about which substantial rotation must be constrained to achieve its three hydrogen bonds; artificial β-sheet **2** has twelve for its four hydrogen bonds. That the ratio of restricted rotations to hydrogen bonds is smaller for **4** than for **1** (2.5:1 vs 3:1) suggests that **4** could be better folded than **1**.

Comparison of the chemical shifts of the NH groups of **4** to those of **1** reveals this to be the case. Notably, the chemical shift of upper strand hydrazide proton H_c of triply stranded artificial β-sheet **4** appears 1.13 ppm downfield of that of doubly stranded artificial β-sheet **1**. This dramatic difference may result mainly from a greater hydrogen-bonded population of **4**, stronger

(31) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986; pp 166−168.

(32) NOEs involving the NH groups were not observed in CD_3OD, because the NH protons are lost by exchange with deuterium in this solvent.

(33) (a) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *J. Mol. Biol.* **1991**, *222*, 311−333. (b) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *Biochemistry* **1992**, *31*, 1647−1651.

hydrogen bonds in **4**, or both.[34] The other NH groups that are hydrogen bonded in both **4** and **1** (H_a, H_b, and H_e) are also shifted downfield in **4**, but the downfield shifting is considerably less pronounced (0.24−0.40 ppm). These smaller differences may reflect the polarization of these hydrogen-bonding groups associated with their participation in hydrogen-bonded networks, strengthening of the hydrogen bonds, or anisotropic effects associated with small conformational differences between the two molecules and are less significant than the dramatic downfield shifting of H_c. Collectively, these shift data suggest that the bottom β-strand mimic of **4** contributes cooperatively to its folding and helps reduce fraying of the interface between the upper β-strand mimic and the middle peptide strand.[35]

Conclusion

The studies described herein establish that three molecular templates can be combined with a peptide strand to form a compound that folds to resemble a three-stranded protein β-sheet in chloroform solution. This triply templated artificial β-sheet (**4**) is more tightly folded than its smaller doubly stranded homologue (**1**). These findings lend further support to our modular approach to the creation of proteinlike structures and bode well for the creation of even larger and more complex molecules that begin to rival the complex folding of proteins.

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Supporting Information Available: Synthetic procedures and ^{1}H NMR, TOCSY, and Tr-ROESY spectra of artificial β-sheet **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(34) (a) Mackay, J. P.; Gerhard, U.; Beauregard, D. A.; Westwell, M. S.; Searle, M. S.; Williams, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 4581−4590. (b) Williams, D. H.; Maguire, A. J.; Tsuzuki, W.; Westwell, M. S. *Science* **1998**, *280*, 711−714.

(35) (a) Sharman, G. J.; Searle, M. S. *Chem. Commun.* **1997**, 1955−1956. (b) Schenck, H. L.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4869−4870. (c) Das, C.; Raghothama, S.; Balaram, P. *J. Am. Chem. Soc.* **1998**, *120*, 5812−5813. (d) Kortemme, T.; Ramírez-Alvarado, M.; Serrano, L. *Science* **1998**, *281*, 253−256. (e) Griffiths-Jones, S. R.; Searle, M. S. *J. Am. Chem. Soc.* **2000**, *122*, 8350−8356.