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Cyclic Modular β-Sheets

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Abstract: The development of peptide β-hairpins is problematic, because folding depends on the amino acid sequence and changes to the sequence can significantly decrease folding. Robust β-hairpins that can tolerate such changes are attractive tools for studying interactions involving protein β-sheets and developing inhibitors of these interactions. This paper introduces a new class of peptide models of protein β-sheets that addresses the problem of separating folding from the sequence. These model β-sheets are macrocyclic peptides that fold in water to present a pentapeptide β-strand along one edge; the other edge contains the tripeptide β-strand mimic Hao [*JACS* **2000**, *122*, 7654] and two additional amino acids. The pentapeptide and Hao-containing peptide strands are connected by two δ-linked ornithine ($^{\delta}$Orn) turns [*JACS* **2003**, *125*, 876]. Each $^{\delta}$Orn turn contains a free α-amino group that permits the linking of individual modules to form divalent β-sheets. These "cyclic modular β-sheets" are synthesized by standard solid-phase peptide synthesis of a linear precursor followed by solution-phase cyclization. Eight cyclic modular β-sheets **1a**−**1h** containing sequences based on β-amyloid and macrophage inflammatory protein 2 were synthesized and characterized by ^{1}H NMR. Linked cyclic modular β-sheet **2**, which contains two modules of **1b**, was also synthesized and characterized. ^{1}H NMR studies show downfield α-proton chemical shifts, $^{\delta}$Orn δ-proton magnetic anisotropy, and NOE cross-peaks that establish all compounds but **1c** and **1g** to be moderately or well folded into a conformation that resembles a β-sheet. Pulsed-field gradient NMR diffusion experiments show little or no self-association at low (≤2 mM) concentrations. Changes to the residues in the Hao-containing strands of **1c** and **1g** improve folding and show that folding of the structures can be enhanced without altering the sequence of the pentapeptide strand. Well-folded cyclic modular β-sheets **1a**, **1b**, and **1f** each have a phenylalanine directly across from Hao, suggesting that cyclic modular β-sheets containing aromatic residues across from Hao are better folded.

Introduction

Synthetic compounds that imitate the hydrogen-bonding edges and side-chain faces of protein β-sheets are valuable tools for studying and regulating protein−protein and protein−DNA interactions.[1] Peptides that fold into β-hairpins are especially suited for such studies, because these β-sheet models permit the use of actual amino acid sequences from protein β-sheets. Several reports of β-hairpins that bind DNA or proteins have been published in recent years.[2] Waters and co-workers developed a divalent β-hairpin that binds single-stranded DNA with an affinity similar to that of proteins.[2c] Wells and co-workers and Robinson and co-workers developed β-hairpins that bind the Fc domain of human immunoglobulin G and inhibit the binding of immunoglobulin G to protein A.[2a,d] Robinson and co-workers also developed a β-hairpin inhibitor of the p53−HDM2 protein−protein interaction.[2b]

The development of peptide β-hairpins has historically been difficult, but groundbreaking work in the 1990s led to reports of peptide β-hairpins that were either isolated from protein sequences or designed de novo.[3,4] Subsequent studies have revealed the importance of cross-strand hydrophobic and aromatic−aromatic side-chain interactions and of good β-turn sequences for the folding of β-hairpins.[5,6] In spite of this progress, the development of β-hairpins remains problematic, because β-hairpin folding is intimately linked to the amino acid sequence of the peptide strands and because well-folded structures often form intractable aggregates.[5b] Only certain

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10.1021/ja0667965 CCC: $37.00

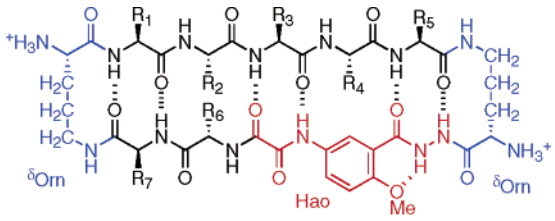
sequences give folded structures, and changes in the sequence of a folded β-hairpin can disrupt folding by disrupting key side-chain−side-chain interactions. In studies with well-folded β-hairpins, Gellman and co-workers carried out carefully selected mutations to show the dependence of β-hairpin folding on interstrand hydrophobic side-chain−side-chain interactions.[6b,c] Cochran and co-workers[6a] and Serrano and co-workers[4c] have reported similar sequence dependences. To probe the effects of mutations within a β-hairpin on *intermolecular* interactions, it is important to be able to change the sequence of the β-strands while maintaining a folded structure and avoiding aggregation. Robust models of protein β-sheets, which tolerate a variety of sequences, are needed.

Robust models of protein β-sheets can be created by combining peptides with peptidomimetic templates that nucleate and reinforce a β-sheet structure.[5a] One class of templates is the turn mimics, which nucleate parallel or antiparallel β-sheet structures in attached peptide chains. Examples of turn mimics include structures such as the dibenzofuran-based amino acid developed by Kelly and co-workers,[7] the β-peptide reverse-turn mimic developed by Gellman and co-workers,[8] and the oligourea "molecular scaffolds" developed by Nowick and co-workers.[9]

Another class of templates is the β-strand mimics, which duplicate the pattern of hydrogen-bond donors and acceptors of one edge of a peptide β-strand. These templates can serve as frameworks to nucleate and reinforce the β-strand conformation of attached polypeptide strands. A pioneering example of such a template is the 2,8-diaminoepindolidione β-strand mimic developed by Kemp and co-workers in the late 1980s.[10] More recently developed examples of β-strand mimics, such as the Hao amino acid, invented by Nowick and co-workers,[11] and the @-unit, invented by Bartlett and co-workers,[12] are easily incorporated into peptides and nucleate the formation of well-folded β-hairpin-like structures in organic solvents. Other templates such as the hydrogen-bonded duplex developed by Gong and co-workers[13] and the pyrrolinone-based peptidomimetics developed by Hirschmann and co-workers[14] were not designed for use in β-hairpins but do mimic certain aspects of β-sheets.

Although the existing β-sheet templates have allowed the creation of many novel β-sheet structures, a robust model of protein β-sheets that folds in water is still lacking. Most of the existing templates require either organic solvents or sequence-dependent interactions with neighboring amino acids to nucleate a β-sheet structure. There is considerable room for improvement in developing β-sheet structures that fold in aqueous solution and tolerate a greater variety of different amino acid sequences.

In this paper, we address these issues with a new class of cyclic models of protein β-sheets, **1**. These compounds contain two of our previously reported peptidomimetic templates and tolerate a variety of different amino acid sequences. Many of these compounds fold into β-sheets and do not aggregate or self-associate significantly at low-millimolar concentrations. These individual β-sheets are readily linked to form multivalent structures with more than one β-sheet domain.



cyclic modular β-sheets **1**

These "cyclic modular β-sheets" are 42-membered rings that contain a pentapeptide in the "upper" strand, the amino acid Hao[11] and two α-amino acids in the "lower" strand, and two δ-linked ornithine β-turn mimics.[15] Hao is a relatively rigid tripeptide β-strand mimic that serves as a template for the folding of the upper strand and blocks the lower strand to minimize edge-to-edge aggregation.[11,16] The two α-amino acids in the lower strand permit tuning the folding and solubility of the cyclic modular β-sheets without changing the upper strand. The two δ-linked ornithine ($^{\delta}$Orn) residues form hairpin turns and have free α-amino groups that serve as sites for linking the cyclic modular β-sheets.[15,17] We developed a *cyclic* β-sheet model, because cyclization is known to confer conformational stability to synthetic peptide β-hairpins[6e,18] and to natural peptide β-hairpins such as gramicidin S.[19,20] This paper describes the synthesis and NMR structural studies of eight cyclic modular β-sheets and one linked cyclic modular β-sheet.

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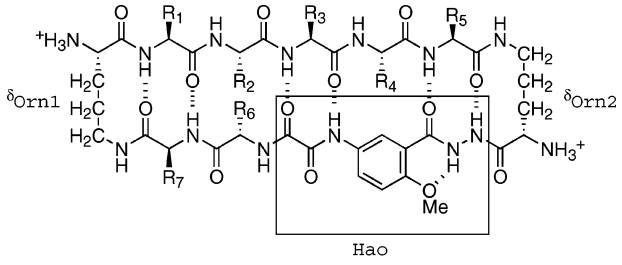
Chart 1



	δOrn1- 1 - 2 - 3 - 4 - 5 -	δOrn2-Hao- 6 - 7
1a (TFA salt)	Lys-Leu-Val-Phe-Phe	Val-Glu
1b (TFA salt)	Leu-Val-Phe-Phe-Ala	Leu-Lys
1c (TFA salt)	Ala-Ile-Ile-Gly-Leu	Leu-Lys
1d (TFA salt)	Ala-Ile-Ile-Gly-Leu	Tyr-Lys
1e (TFA salt)	Ala-Ile-Ile-Ala-Leu	Leu-Lys
1f (TFA salt)	Ala-Ile-Ile-Phe-Leu	Leu-Lys
1g (TFA salt)	Ser-Leu-Ser-Val-Thr	Ala-Thr
1h (TFA salt)	Ser-Leu-Ser-Val-Thr	Tyr-Thr

Results

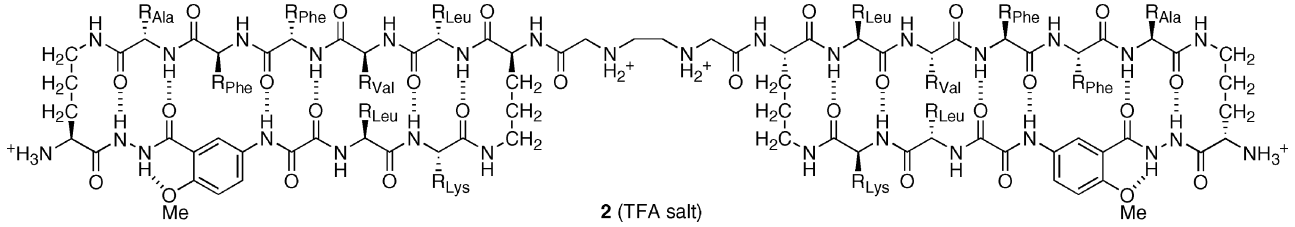
1. Design. Pentapeptide sequences based on β-amyloid (Aβ)[21] and macrophage inflammatory protein 2 (MIP-2)[22] were incorporated into the upper strands of cyclic modular β-sheets **1a**−**1h** (Chart 1). The upper strands of **1a** and **1b** each present opposite edges of a hydrophobic sequence that plays a key role in the fibrilization of Aβ: **1a** contains Aβ_{16-20} (KLVFF) and **1b** contains Aβ_{17-21} (LVFFA).[23] Selection of the two residues for the lower strands of **1a** and **1b** was guided by observations of cross-strand β-sheet pairings in various proteins.[24,25] The initial selection of Leu-Val for positions 6 and 7 of **1b** resulted in poor solubility in water; the sequence was subsequently changed to Leu-Lys to improve the solubility.

The upper strands of **1c** and **1d** both contain a hydrophobic sequence from an alternate region of Aβ, Aβ_{30-34} (AIIGL). The Leu-Lys sequence that was suitable for the lower strand of **1b** was also included in the lower strand of **1c** but was replaced with Tyr-Lys in **1d** to improve folding. The upper strands of **1e** and **1f** contain the pentapeptide sequences AIIAL and AIIFL, respectively, which are modified versions of the Aβ_{30-34} sequence. Cyclic modular β-sheets **1e** and **1f** also contain Leu-Lys in the lower strands.

The upper strands of cyclic modular β-sheets **1g** and **1h** contain a relatively hydrophilic sequence from the β-sheet dimerization interface of MIP-2 (SLSVT). The lower strand of **1g** contains Ala-Thr, which in MIP-2 are the cross-strand neighbors of the first two residues of the pentapeptide sequence. The Ala-Thr sequence in the lower strand of **1g** was replaced with Tyr-Thr in **1h** to improve folding.

We prepared linked cyclic modular β-sheet **2** to demonstrate the potential of this system to generate multivalent β-sheet structures. Linked cyclic modular β-sheet **2** contains two modules derived from **1b**. The two $^{\delta}$Orn β-turn mimics provide two possible linkage sites on each cyclic modular β-sheet. The two modules of linked cyclic modular β-sheet **2** are connected at the "left" $^{\delta}$Orn ($^{\delta}$Orn1) by an *N*,*N*′-ethylenediaminediacetic acid linker ($HO_2CCH_2NHCH_2CH_2NHCH_2CO_2H$). An initial version of this linked cyclic modular β-sheet, which contained a 3,6-dioxaoctanedioic acid linker ($HO_2CCH_2OCH_2CH_2OCH_2CO_2H$), exhibited poor solubility in water. The *N*,*N*′-ethylenediaminediacetic acid linker was then chosen to solve this problem.

2. Synthesis.[26] The cyclic modular β-sheets are conveniently prepared by standard Fmoc solid-phase synthesis of the corresponding linear peptides on 2-chlorotrityl chloride resin, followed by solution-phase cyclization.[27] The initial step of the synthesis involves loading commercially available Boc−Orn-(Fmoc)−OH onto 2-chlorotrityl chloride resin (Scheme 1). Amino acids are added to the growing peptide by removing the terminal Fmoc group with piperidine and then adding a solution of the Fmoc-protected amino acid and the coupling reagents HCTU[28] and *N*,*N*-diisopropylethylamine (DIEA). Most



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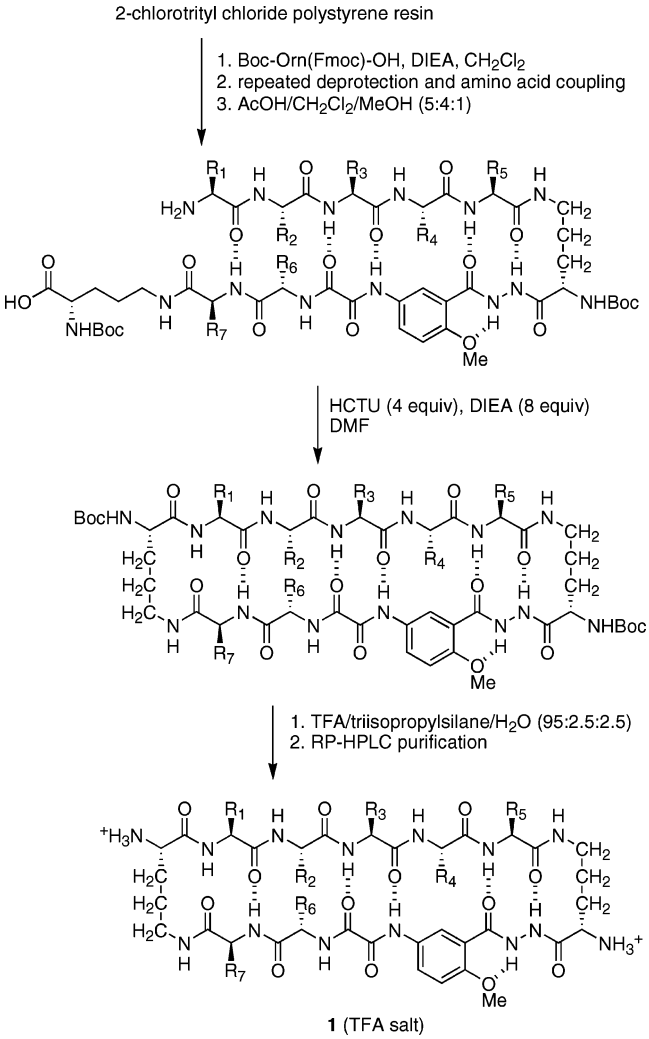
Scheme 1



coupling reactions are completed within 30 min. The coupling of Fmoc*−Hao−OH[11a,29] is sluggish and is incomplete even after several hours when HCTU and DIEA are used as the coupling reagents. The reaction proceeds cleanly in 4−6 h, however, when 2,4,6-collidine is used in place of DIEA.[30]

After the coupling of the final amino acid, the terminal Fmoc group is removed and the linear protected peptide is cleaved from the resin by treatment with 50% acetic acid and 10% methanol in methylene chloride. Any residual acetic acid is removed by repeated rotary evaporation with hexanes, which forms an azeotrope with acetic acid. This important step eliminates the potential of capping the linear protected peptide with acetic acid instead of cyclization. Analytical HPLC typically shows that the linear protected peptide is relatively clean; the HPLC trace of the linear protected peptide intermediate in the synthesis of **1g** is representative (Figure 1a).

The cyclization reaction occurs cleanly with HCTU and DIEA in dilute (ca. 0.5 mM) DMF solution. Since the *C*-terminus of the protected linear peptide contains an α-amino acid carbamate (BocNH−CHR−COOH), rather than an α-amino acid amide (RCONH−CHR−COOH), epimerization during this reaction is not expected. Treatment of the cyclic intermediate with trifluoroacetic acid (TFA) removes the acid-labile protecting groups (Figure 1b), and purification of the deprotected peptide by reversed-phase HPLC affords cyclic modular β-sheet **1** as

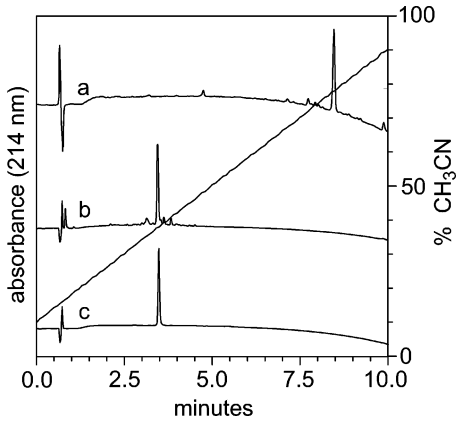


Figure 1. Analytical HPLC traces of (a) the linear protected peptide intermediate in the synthesis of **1g**, (b) unpurified cyclic modular β-sheet **1g**, and (c) purified cyclic modular β-sheet **1g**. Conditions: Alltech Alltima Rocket C18 column (53 mm × 4.5 mm), gradient of 10−90% CH_3CN in H_2O with 0.1% TFA.

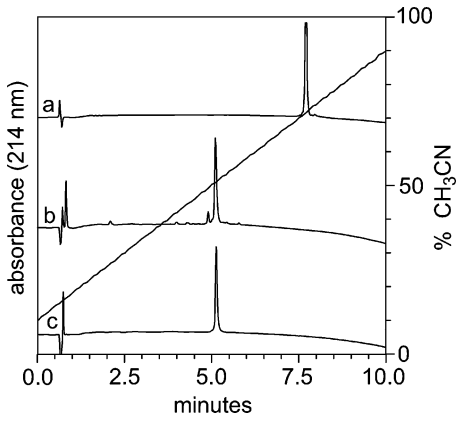


Figure 2. Analytical HPLC traces of **(**a) purified intermediate **4**, (b) unpurified linked cyclic modular β-sheet **2**, and (c) purified linked cyclic modular β-sheet **2**. Conditions: Alltech Alltima Rocket C18 column (53 mm × 4.5 mm), gradient of 10−90% CH_3CN in H_2O with 0.1% TFA.

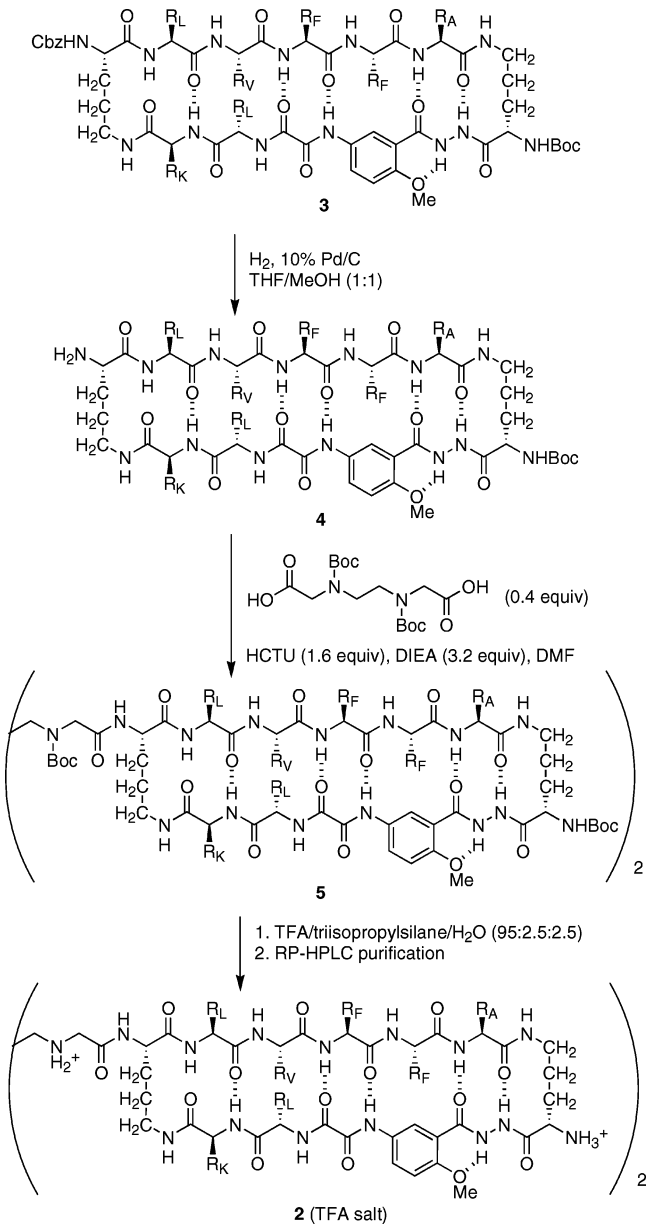
the TFA salt (Figure 1c). A 15−25% yield, based on the loading of the resin with Boc−Orn(Fmoc)−OH, is typically obtained; a 0.1 mmol scale synthesis typically affords 20−30 mg of the purified cyclic modular β-sheet.

Linked cyclic modular β-sheets are prepared by connecting individual modules at one of the $^{\delta}$Orn ($^{\delta}$Orn1 or $^{\delta}$Orn2) α-amino groups with a dicarboxylic acid linker. The two $^{\delta}$Orn residues in each cyclic modular β-sheet must be differentiated, and this differentiation can be accomplished with the Cbz protecting group. Commercially available Cbz−Orn(Fmoc)−OH is used instead of Boc−Orn(Fmoc)−OH at the turn position that serves as the linkage site. The Cbz protecting group is removed by catalytic hydrogenation, which leaves the Boc protecting group on the other $^{\delta}$Orn α-amino group intact.

The synthesis of linked cyclic modular β-sheet **2** begins with the synthesis of fully protected cyclic peptide **3** (Scheme 2). Catalytic hydrogenation of **3** with palladium on carbon selectively removes the Cbz protecting group. The resulting Boc-protected intermediate **4** is purified by reversed-phase HPLC (Figure 2a). Coupling of **4** to the Boc-protected *N*,*N*′-ethylenediaminediacetic acid linker[31] in a 1.0:0.4 molar ratio, with HCTU and DIEA, yields linked intermediate **5** as a relatively clean

(31) McMurry, T. J.; Brechbiel, M.; Kumar, K.; Gansow, O. A. *Bioconjugate Chem.* **1992**, *3*, 108−117.

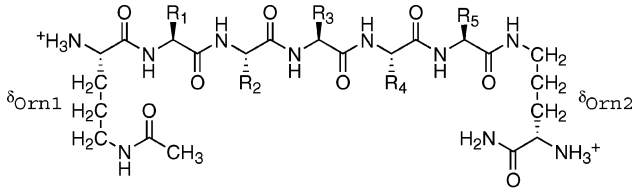
Scheme 2



product. Treatment of **5** with TFA removes the acid-labile protecting groups (Figure 2b). Purification by reversed-phase HPLC affords linked cyclic modular β-sheet **2** as the TFA salt. The synthesis of **2** from 2-chlorotrityl chloride resin and Cbz−Orn(Fmoc)−OH proceeded in 9% overall yield; a 0.2 mmol scale synthesis afforded 24 mg of **2**.

3. ^{1}H NMR Structural Studies. ^{1}H NMR studies (1D, 2D TOCSY, and 2D ROESY) of cyclic modular β-sheets **1** and linked cyclic modular β-sheet **2** in aqueous solutions (D_2O and H_2O/D_2O mixtures) establish the β-sheet folding and reveal the extent of folding.[32] Analysis of the α-proton chemical shifts,

Chart 2



Ac-$^{\delta}$Orn1- 1 - 2 - 3 - 4 - 5 -$^{\delta}$Orn2-NH_2

6a (TFA salt)	Lys-Leu-Val-Phe-Phe
6b (TFA salt)	Leu-Val-Phe-Phe-Ala
6c (TFA salt)	Ala-Ile-Ile-Gly-Leu
6e (TFA salt)	Ala-Ile-Ile-Ala-Leu
6f (TFA salt)	Ala-Ile-Ile-Phe-Leu
6g (TFA salt)	Ser-Leu-Ser-Val-Thr

Table 1. α-Proton Chemical Shifts (ppm) of the Cyclic Modular β-Sheets and Linear Controls[a]

	position						
	1	2	3	4	5	6	7
1a	4.67	4.32	4.26	4.78	4.60	4.16	4.60
1b	4.65	4.41	4.98	4.60	4.37	4.83	4.54
1c	4.42	4.14	4.09	3.94	4.32	4.43	4.25
1d	4.47	4.18	4.27	3.93	4.36	4.63	4.27
1e	4.45	4.11	4.28	4.35	4.12	4.43	4.29
1f	4.55	4.08	4.38	4.74	4.42	4.38	4.49
1g	4.53	4.38	4.53	4.26	4.26	4.58	4.26
1h	4.59	4.06	4.64	4.40	4.27	4.70	4.22
2	4.61	4.47	5.04	4.61	4.42	4.92	4.58
6a	4.33	4.35	4.06	4.59	4.40	–	–
6b	4.41	4.04	4.57	4.52	4.07	–	–
6c	4.39	4.13	4.16	3.93	4.24	–	–
6e	4.38	4.12	4.13	4.31	4.21	–	–
6f	4.38	4.06	4.14	4.62	4.21	–	–
6g	4.54	4.41	4.47	4.22	4.19	–	–

[a] 500 or 600 MHz data were collected on 2 mM solutions in D_2O at 6 °C. Data for **1a**−**1f** and **6a**−**6f** were collected in a buffer of 50 mM CD_3COOD and 50 mM CD_3COONa; data for **1g**, **1h**, **2**, and **6g** were collected in pure D_2O.[32]

$^{\delta}$Orn δ-proton magnetic anisotropy, and NOE cross-peaks establishes well-folded β-sheet structures for **1a**, **1b**, **1f**, and **2**; moderately folded β-sheet structures for **1d**, **1e**, and **1h**; and poorly folded β-sheet structures for **1c** and **1g**.

The ^{1}H NMR spectra of cyclic modular β-sheets **1a**−**1h** show peaks that are well dispersed and sharp (line widths ca. 2−4 Hz); the ^{1}H NMR spectrum of linked cyclic modular β-sheet **2** shows peaks that are also well dispersed but slightly broader (line width ca. 5 Hz).[33] The spectrum of **1a** in D_2O, shown in Figure 3, is representative of the spectra of **1b**−**1h**. Peaks corresponding to a minor (4%) species are present in the spectrum of **1a**, suggesting the occurrence of an alternate conformation. Minor peaks also occur in the spectra of **1b**−**1h** and **2**. The minor peaks are most intense in **1d** and **1f** (10%). ROESY experiments on **1f** at 50 °C show chemical exchange cross-peaks (EXSY) that confirm that the minor peaks originate from slow exchange with an alternate conformation.[34]

a. α-Proton Chemical Shifts. The chemical shifts of the α-protons of peptides in β-sheets are generally downfield of those in unstructured "random coil" environments.[35] Linear

(32) ^{1}H NMR studies in H_2O/D_2O mixtures (typically 9:1) with water suppression permit observation of amide resonances, which are not observed in D_2O. All resonances were assigned by TOCSY and ROESY studies. ^{1}H NMR studies were conducted at 5 or 6 °C to minimize overlap of the HOD resonance with the α-proton resonances. All cyclic modular β-sheets except **1g**, **1h**, and **2** were studied in a buffer of 50 mM CD_3COOD and 50 mM CD_3COONa. Cyclic modular β-sheets **1g**, **1h**, and **2** showed signs of extensive aggregation (highly viscous solutions and very broad ^{1}H NMR resonances) in the buffered solutions; these compounds were therefore studied without buffer.

(33) The ^{1}H NMR spectra of the cyclic modular β-sheets were referenced to the *cationic* internal standard DSA, which is less prone to association with cationic peptides than the popular *anionic* internal standard DSS. Nowick, J. S.; Khakshoor, O.; Hashemzadeh, M.; Brower, J. O. *Org. Lett.* **2003**, *5*, 3511−3513.

(34) This alternate conformation may involve a *cis*-amide linkage between the Hao hydrazide group and $^{\delta}$Orn2.

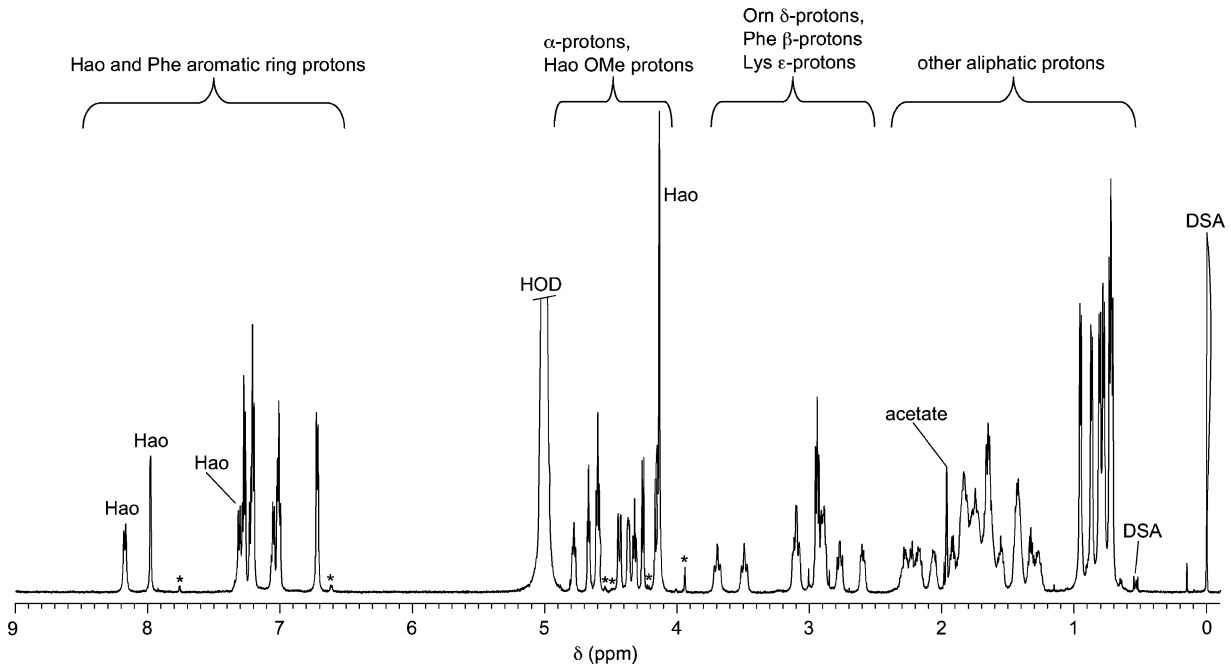


Figure 3. 600 MHz ^{1}H NMR spectrum of a 2 mM solution of **1a** in D_2O with 50 mM CD_3CO_2D and 50 mM CD_3CO_2Na at 6 °C.[33] Peaks marked with an asterisk (*) likely correspond to an alternate conformation.

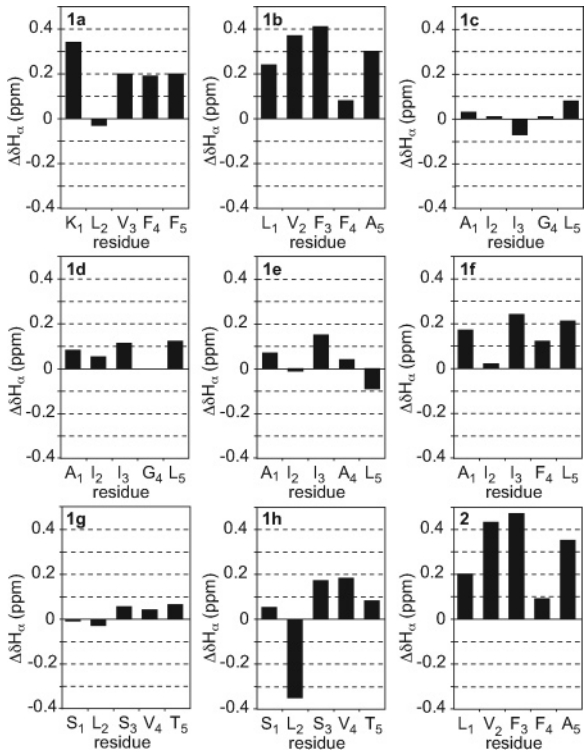


Figure 4. $\Delta\delta H_\alpha$ [δH_α(cyclic modular β-sheet) − δH_α(linear control)] values for the upper strands of the cyclic modular β-sheets.

control peptides **6a**−**6g** serve as references for the random coil conformations of the cyclic modular β-sheets (Chart 2).[36] Each of these linear control peptides contains two $^{\delta}$Orn residues and one of the six different pentapeptide sequences of **1a**−**1h**. ROESY studies of the control peptides show no long-range NOE cross-peaks, suggesting that these peptides are largely unstructured. Table 1 lists the α-proton chemical shifts of cyclic modular β-sheets **1a**−**1h**, linked cyclic modular β-sheet **2**, and control peptides **6a**−**6g**.

Comparison of the chemical shifts of the α-protons of peptides **1** and **2** to those of linear controls **6** reflects the folding of the cyclic modular β-sheets. Figure 4 illustrates the $\Delta\delta H_\alpha$ values for the pentapeptide strands of **1a**−**1h** and **2**, where $\Delta\delta H_\alpha$ corresponds to the difference between the chemical shifts of the α-protons of the cyclic modular β-sheets and the linear controls. Positive $\Delta\delta H_\alpha$ values provide evidence that the cyclic modular β-sheets are folded. The $\Delta\delta H_\alpha$ values of four or five of the α-protons in the upper strands of **1a**, **1b**, **1d**, **1f**, and **1h** are positive, suggesting at least partial β-sheet folding. The $\Delta\delta H_\alpha$ values are largest for **1a**, **1b**, and **1f**, suggesting that these cyclic modular β-sheets are the best folded in the series. Linked cyclic modular β-sheet **2**, which contains two modules derived from **1b**, shows $\Delta\delta H_\alpha$ values that are only slightly different than those of **1b**, suggesting that the folding of each module of **2** is similar to that of **1b**.

The $\Delta\delta H_\alpha$ values are smaller for **1d** and **1h**, suggesting that these cyclic modular β-sheets are not as strongly folded as **1a**, **1b**, and **1f**. A strongly negative (−0.35 ppm) $\Delta\delta H_\alpha$ value for residue 2 of **1h** may result from the magnetic anisotropy of the tyrosine aromatic side chain at position 6, the cross-strand neighbor of residue 2. The $\Delta\delta H_\alpha$ values of at least two α-protons of **1c**, **1e**, and **1g** are essentially zero or slightly negative, and most of the other $\Delta\delta H_\alpha$ values are only slightly positive. These data suggest that **1c**, **1e**, and **1g** are poorly folded.

b. $^{\delta}$Orn δ-Proton Magnetic Anisotropy. The ^{1}H NMR spectra of **1a**−**1f** and **1h** show magnetic anisotropy between the diastereotopic *pro-R* δ-proton ($H_{\delta R}$) and *pro-S* δ-proton ($H_{\delta S}$) resonances of both $^{\delta}$Orn residues (Figures 5 and 6). The

(35) (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.

(36) Random coil reference peptides such as these help to account for the effects of specific neighboring residues on α-proton chemical shifts. For other examples of reference peptides for the unfolded state of a peptide β-hairpin, see ref 6b and Butterfield, S. M.; Waters, M. L. *J. Am. Chem. Soc.* **2003**, *125*, 9580−9581.

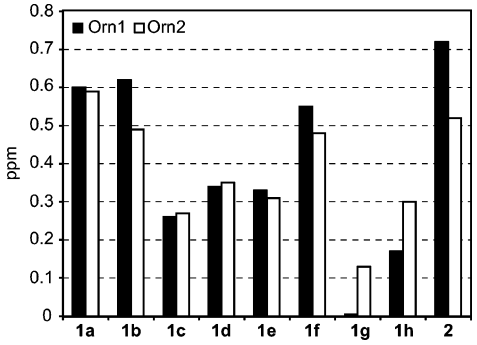


Figure 5. Magnetic anisotropy between the diastereotopic δ-proton resonances of each $^{\delta}$Orn residue in the cyclic modular β-sheets.

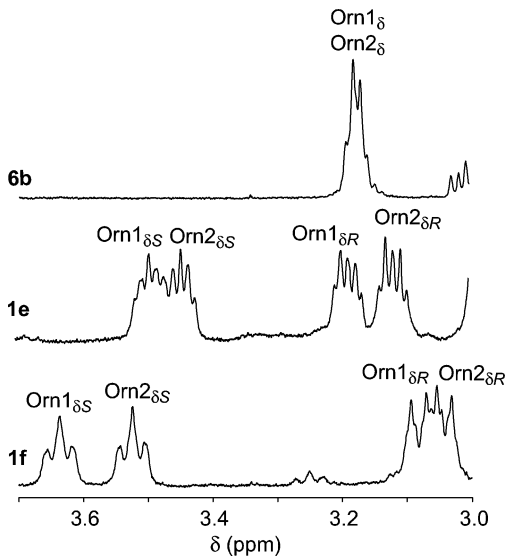


Figure 6. Expansions of the 600 MHz ^{1}H NMR spectra of 2 mM solutions of **6b**, **1e**, and **1f** in D_2O at 6 °C showing the $^{\delta}$Orn δ-proton resonances.[33] Assignments of the diastereotopic δ-proton resonances of **1e** are tentative and are based on an analogy with **1f**.

magnitude of the magnetic anisotropy (0.17−0.62 ppm) suggests the $^{\delta}$Orn residues to be moderately or well folded and provides a measure of the folded populations. In contrast, the δ-protons of control peptides **6a**−**6f** show ≤0.01 ppm magnetic anisotropy, and those of **6g** show 0.01 and 0.05 ppm magnetic anisotropy (Figure 6). Previous and ongoing studies in our laboratories strongly suggest that roughly 0.6 ppm is the maximum magnetic anisotropy of $^{\delta}$Orn δ-protons in aqueous solution when the $^{\delta}$Orn has an unacylated α-amino group.[37] These studies involve three different systems: a cyclic β-sheet peptide related to those studied by Gellman,[15] cyclic modular β-sheets **1a**−**1h** and other cyclic modular β-sheets in this family, and a family of larger macrocyclic model β-sheets.[38]

The ^{1}H NMR spectra of cyclic modular β-sheets **1a**, **1b**, and **1f** show large $^{\delta}$Orn δ-proton magnetic anisotropy (0.48−0.62 ppm), demonstrating these turns to be well folded (Figure 5). The spectrum of linked cyclic modular β-sheet **2** shows 0.72 ppm

(37) A different class of β-hairpin mimics that contain a $^{\delta}$Orn turn unit that is acylated at the $^{\delta}$Orn α-amino group shows $^{\delta}$Orn δ-proton magnetic anisotropy of up to 1.3 ppm in chloroform solution (ref 11b). The larger separation of the $^{\delta}$Orn δ-proton resonances is partially an effect of the anisotropy of the $^{\delta}$Orn α-amido carbonyl group on the $^{\delta}$Orn *pro-S* δ-proton. The large anisotropy may also reflect the formation of a more highly ordered structure through stronger hydrogen bonding in a noncompetitive organic solvent.

(38) Khakshoor, O.; Demeler, B.; Nowick, J. S. Submitted to *J. Am. Chem. Soc.*

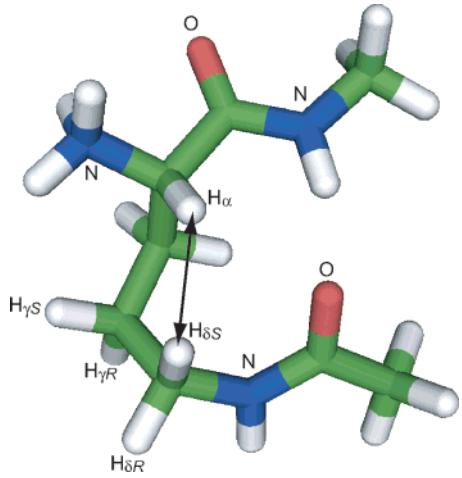


Figure 7. Model of a $^{\delta}$Orn β-turn mimic (Ac−$^{\delta}$Orn−NHMe, global minimum: MacroModel v7.0; AMBER* force field; GB/SA H_2O solvent model).[15] An arrow indicates the NOE between the $H_{\delta S}$ and H_{α}.

δ-proton magnetic anisotropy for $^{\delta}$Orn1 and 0.52 ppm δ-proton magnetic anisotropy for $^{\delta}$Orn2, demonstrating that the turns in **2** are also well folded. The exceptionally large value for linked $^{\delta}$Orn1 in **2** (0.72 ppm) likely arises from the magnetic anisotropy of the carbonyl group of the amide linker that is attached to $^{\delta}$Orn1. The spectra of **1c**, **1d**, **1e**, and **1h** show smaller $^{\delta}$Orn δ-proton magnetic anisotropy (0.17−0.35 ppm), demonstrating these turns to be moderately folded. The spectrum of **1g** shows essentially no δ-proton anisotropy in $^{\delta}$Orn1 (≤0.01 ppm), demonstrating this turn to be unfolded, and 0.13 ppm anisotropy in $^{\delta}$Orn2, demonstrating this turn to be slightly folded.

The ^{1}H NMR spectra of the cyclic modular β-sheets with well-folded $^{\delta}$Orn turns show characteristic coupling patterns of the $^{\delta}$Orn δ-proton resonances that are consistent with the model that our research group has previously proposed for the $^{\delta}$Orn β-turn mimic (Figure 7).[15] The ^{1}H NMR spectrum of cyclic modular β-sheet **1f** clearly illustrates these coupling patterns (Figure 6). Each $^{\delta}$Orn of **1f** gives two diastereotopic δ-proton resonances that are separated by ca. 0.5 ppm. The downfield resonance ($H_{\delta S}$) appears as a broad triplet, with a coupling constant of ca. 13 Hz, while the upfield resonance ($H_{\delta R}$) appears as a broad doublet with a coupling constant of 13 or 14 Hz. The triplet of $H_{\delta S}$ results from two strong couplings, geminal coupling with $H_{\delta R}$ and vicinal coupling with $H_{\gamma R}$, which is in an anti orientation. Coupling with $H_{\gamma S}$, which is in a gauche orientation, is sufficiently weak that it only contributes to broadening of the triplet. The doublet of $H_{\delta R}$ results from one strong coupling—geminal coupling with the $H_{\delta S}$. Coupling with the γ-protons, which are both gauche, is sufficiently weak that it only contributes to broadening or slight further splitting of the doublet.

In contrast to the differentiated $^{\delta}$Orn δ-proton coupling patterns of the well-folded $^{\delta}$Orn turns, moderately and poorly folded $^{\delta}$Orn turns give coupling patterns that suggest an ensemble of different conformations. The spectrum of **1e** illustrates these coupling patterns and shows a doublet of triplets for each of the diastereotopic H_{δ} resonances of each $^{\delta}$Orn residue (Figure 6). These coupling patterns show each δ-proton to have a strong (ca. 13 Hz) coupling with the geminal H_{δ} and a weaker (ca. 6 Hz) coupling with each vicinal H_{γ}.

c. NOE Cross-Peaks. NOEs between peptide strands are a hallmark of β-sheets and reflect proximity between residues that

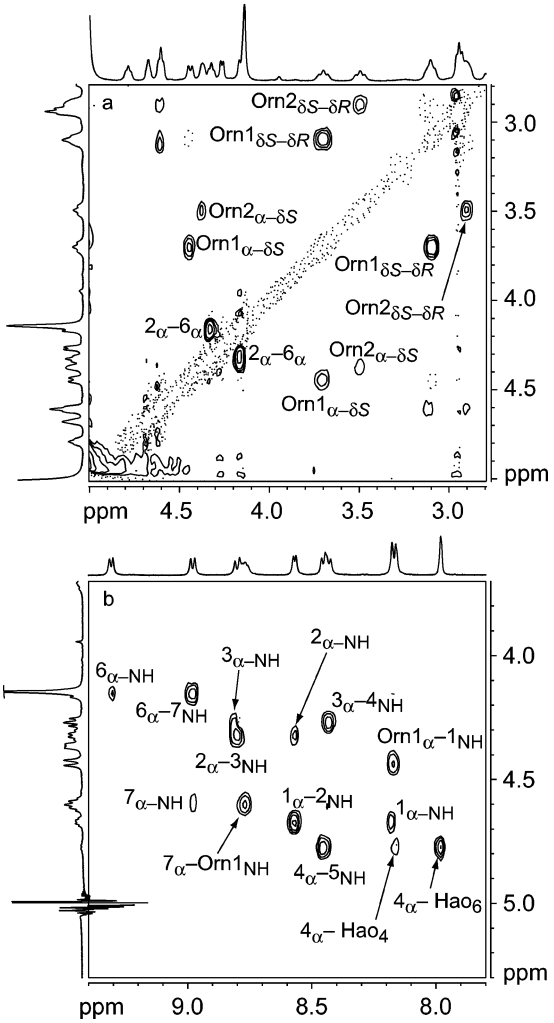


Figure 8. Selected expansions of the ROESY spectra of **1a**: (a) 2 mM in D_2O with 50 mM CD_3CO_2D and 50 mM CD_3CO_2Na at 500 MHz and 6 °C with a 200-ms spin lock time; (b) 2 mM in 9:1 H_2O/D_2O with 50 mM CD_3CO_2D and 50 mM CD_3CO_2Na at 600 MHz and 6 °C with a 200-ms spin lock time and gradient water suppression.

are otherwise remote in the unfolded peptide. The ROESY spectra of **1a** at 500 and 600 MHz show NOE cross-peaks that demonstrate this cyclic modular β-sheet to be folded into a β-sheet-like conformation (Figure 8). Most importantly, these spectra show strong long-range NOEs between the α-protons of the leucine at position 2 and the valine at position 6 ($2_\alpha-6_\alpha$) and between the α-proton of the phenylalanine at position 4 and aromatic proton 6 of Hao ($4_\alpha-Hao_6$). These NOEs are associated with the characteristic short interstrand $H_\alpha-H_\alpha$ contacts in the non-hydrogen-bonded pairs within β-sheets and are illustrated in Figure 9. The ROESY spectra of **1a** also show strong NOEs between the α-proton of each $^{\delta}$Orn and the corresponding *pro-S* δ-proton ($^{\delta}Orn_{\alpha-\delta S}$), demonstrating the folding of the $^{\delta}$Orn turns (Figure 8). These NOEs agree with the model of a $^{\delta}$Orn β-turn mimic (Figure 7), in which only the $H_{\delta S}$ is near the H_α, and are also illustrated in Figure 9.

The 600 MHz ROESY spectra of **1b**, **1f**, and **1h** also show strong $2_\alpha-6_\alpha$, $4_\alpha-Hao_6$, and $^{\delta}Orn_{\alpha-\delta S}$ NOEs, suggesting these cyclic modular β-sheets to also be folded into β-sheet-like conformations. The spectrum of linked compound **2** also shows these NOEs, suggesting both modules to be folded. The spectra of **1d** and **1e** show strong $2_\alpha-6_\alpha$ and $^{\delta}Orn_{\alpha-\delta S}$ NOEs but

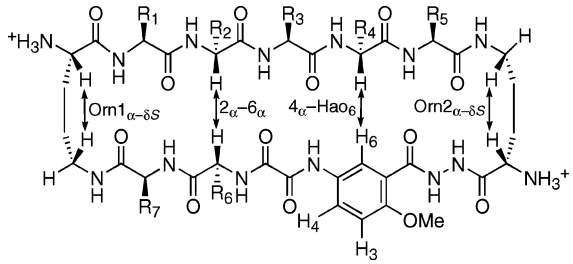


Figure 9. Key NOEs shown in the ROESY spectra of cyclic modular β-sheets **1a**, **1b**, **1d**, **1e**, **1f**, and **1h** in D_2O.

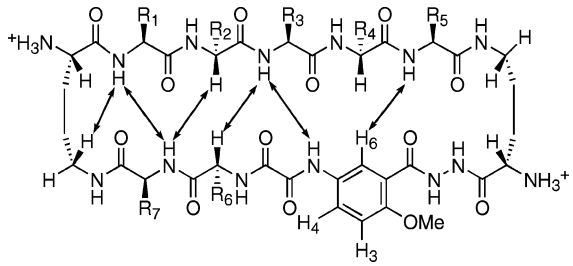


Figure 10. Interstrand NH−NH and H_α−NH NOEs in the 800 MHz NOESY spectra of **1a** and/or **1b** in 9:1 H_2O/D_2O. The hydrazide NH groups do not show up under these conditions and do not give NOEs.

Table 2. $^{\delta}$Orn Turn and Interstrand NOEs in the Cyclic Modular β-Sheets[a]

	$2_\alpha-6_\alpha$	$4_\alpha-Hao_6$	$^{\delta}Orn1_{\alpha-\delta S}$	$^{\delta}Orn1_{\alpha-\delta R}$[b]	$^{\delta}Orn2_{\alpha-\delta S}$	$^{\delta}Orn2_{\alpha-\delta R}$[b]
1a	strong	strong	strong	−	strong	−
1b	strong	strong	strong	−	strong	−
1c	weak	−	weak	weak	weak	weak
1d	strong	weak	strong	−	strong	−
1e	strong	weak	strong	weak	strong	weak
1f	strong	strong	strong	weak	strong	weak
1g	−	weak	−	−	weak	−
1h	strong	strong	strong	−	strong	−
2	strong	strong	strong	−	strong	−

[a] A dash (−) indicates that no NOE cross-peak was detected. [b] NOEs suggesting alternate turn conformations.

relatively weak $4_\alpha-Hao_6$ NOEs, suggesting that these cyclic modular β-sheets are not as strongly folded as **1a**, **1b**, **1f**, and **1h**. The spectrum of **1c** does not show a $4_\alpha-Hao_6$ NOE and only shows weak $2_\alpha-6_\alpha$ and $^{\delta}Orn_{\alpha-\delta S}$ NOEs. The spectrum of **1g** does not show $2_\alpha-6_\alpha$ or $^{\delta}Orn1_{\alpha-\delta S}$ NOEs and only shows weak $4_\alpha-Hao_6$ and $^{\delta}Orn2_{\alpha-\delta S}$ NOEs. The absence of key NOEs in each of these compounds suggests that **1c** and **1g** are poorly folded. Table 2 summarizes these key NOEs for cyclic modular β-sheets **1a**−**1h** and **2**.

800 MHz NOESY spectra of **1a** and **1b** show interstrand NH−NH and H_α−NH NOEs that further demonstrate a β-sheet structure for these compounds. Figure 10 summarizes these data. These NOEs were largely not seen in the 500 or 600 MHz ROESY spectra of **1a**, **1b**, or any of the other cyclic modular β-sheets. The weakness or absence of these NOE cross-peaks may reflect the lower sensitivity of the 500 or 600 MHz ROESY experiments and the longer distances associated with NH−NH and H_α−NH NOEs in β-sheets (ca. 3.2 or 3.3 Å vs ca. 2.3 Å for interstrand $H_\alpha-H_\alpha$[39]).

Several of the cyclic modular β-sheets show NOEs that suggest a rapid equilibrium with alternate conformations. For example, a weak $4_\alpha-Hao_4$ NOE occurs in the 600 MHz ROESY

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

Table 3. Diffusion Coefficients of the Cyclic Modular β-Sheets[a]

	MW	D (cm^2/s)
gramicidin S	1140	12.9 ($\pm$0.5) $\times$ 10^{-7}
1a	1327	10.7 ($\pm$0.3) $\times$ 10^{-7}
1b	1283	11.9 ($\pm$0.7) $\times$ 10^{-7}
1c	1172	12.9 ($\pm$0.8) $\times$ 10^{-7}
1d	1222	12.0 ($\pm$0.2) $\times$ 10^{-7}
1e	1186	12.2 ($\pm$0.3) $\times$ 10^{-7}
1f	1263	11.4 ($\pm$0.3) $\times$ 10^{-7}
1g	1123	12.8 ($\pm$0.3) $\times$ 10^{-7}
1h	1214	12.4 ($\pm$0.5) $\times$ 10^{-7}
2	2705	7.2 ($\pm$0.1) $\times$ 10^{-7}

[a] The diffusion coefficients were measured by 800 MHz PFG NMR diffusion experiments at 6 °C in D_2O.[41] The concentrations of **1a**−**1h** and **2** were 2 mM; the concentration of gramicidin S was 1.1 mM. Data for **1a**−**1f** were collected in a buffer of 50 mM CD_3COOD and 50 mM CD_3COONa; data for gramicidin S, **1g**, **1h**, and **2** were collected in pure D_2O. Each value represents an average measured for multiple resonances. Uncertainties ($\pm$values) are the standard deviations of these measurements.

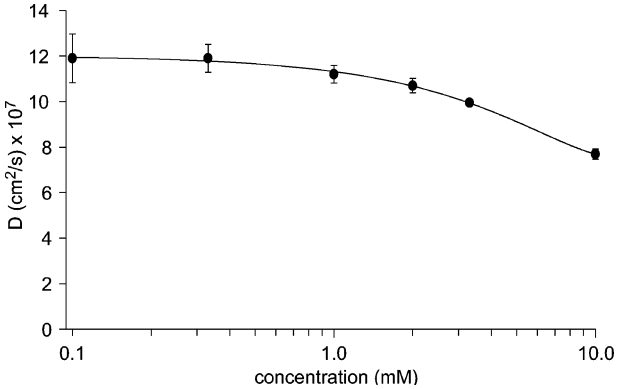


Figure 11. Diffusion coefficient of **1a** as a function of concentration. The diffusion coefficient was measured by 800 MHz PFG NMR diffusion experiments at 6 °C in D_2O in a buffer of 50 mM CD_3COOD and 50 mM CD_3COONa.[41] Each value represents an average measured for three resonances. Error bars are the standard deviations of these measurements.

Table 4. Folding of Cyclic Modular β-Sheets **1**

1a	cyclo($^{\delta}$Orn-K-L-V-F-F-Hao-V-E-$^{\delta}$Orn)	well folded
1b	cyclo($^{\delta}$Orn-L-V-F-F-A-Hao-L-K-$^{\delta}$Orn)	well folded
1c	cyclo($^{\delta}$Orn-A-I-I-G-L-Hao-L-K-$^{\delta}$Orn)	poorly folded
1d	cyclo($^{\delta}$Orn-A-I-I-G-L-Hao-Y-K-$^{\delta}$Orn)	moderately folded
1e	cyclo($^{\delta}$Orn-A-I-I-A-L-Hao-L-K-$^{\delta}$Orn)	moderately folded
1f	cyclo($^{\delta}$Orn-A-I-I-F-L-Hao-L-K-$^{\delta}$Orn)	well folded
1g	cyclo($^{\delta}$Orn-S-L-S-V-T-Hao-A-T-$^{\delta}$Orn)	poorly folded
1h	cyclo($^{\delta}$Orn-S-L-S-V-T-Hao-Y-T-$^{\delta}$Orn)	moderately folded

spectrum of **1a**, suggesting an alternate rotamer involving Hao (Figure 8b). Weak $^{\delta}Orn_{\alpha-\delta R}$ cross-peaks occur in the 600 MHz ROESY spectra of **1c**, **1e**, and **1f**, suggesting alternate turn conformations (Table 2). A few weak NOE cross-peaks occur in the 800 MHz NOESY spectrum of **1a**, also suggesting alternate conformations.

While the $^{\delta}$Orn turn and *inter*strand NOE cross-peaks demonstrate folding of the cyclic modular β-sheets, *intra*strand NOE cross-peaks are consistent with extended (β-strand) conformations of the peptide strands. The 600 MHz ROESY spectrum of **1a** in 9:1 H_2O/D_2O shows strong interresidue H_α−NH(i,i + 1) NOE cross-peaks and weak intraresidue H_α−NH-(i,i) NOE cross-peaks (Figure 8b). It should be noted that this pattern of strong interresidue NOEs and weak intraresidue NOEs does not rigorously distinguish β-sheet structures from non-β-sheet structures. This pattern occurs in all of the cyclic modular β-sheets and in control peptides **6**.

4. PFG NMR Diffusion Studies. Pulsed-field gradient (PFG) NMR diffusion studies provide insight into the molecular weights and association states of molecules in the same sample and under the same conditions as NMR structural studies.[40] PFG NMR diffusion studies are based on the attenuation of the NMR signal that occurs when opposing z-axis magnetic field gradient pulses are applied with an intervening delay. This attenuation results from molecular diffusion and permits the calculation of the diffusion coefficient (D). PFG NMR diffusion studies of cyclic modular β-sheets **1a**−**1h** show little or no self-association at the 2 mM concentration used for structural studies.[41]

The diffusion coefficients of cyclic modular β-sheets **1a**−**1h** and **2** were measured at 2.0 mM (Table 3), and that of **1a** was also measured at 0.1, 0.33, 1.0, 3.3, and 10 mM to assess the effects of concentration (Figure 11). The diffusion coefficient of **1a** shows little or no decrease from 0.1 mM to 1.0 mM (from 11.9 ($\pm$1.1) $\times$ 10^{-7} cm^2/s to 11.2 ($\pm$0.4) $\times$ 10^{-7} cm^2/s). It decreases slightly at 2.0 mM (10.7 ($\pm$0.3) $\times$ 10^{-7} cm^2/s), more at 3.3 mM (10.0 ($\pm$0.1) $\times$ 10^{-7} cm^2/s), and substantially at 10 mM (7.7 ($\pm$0.2) $\times$ 10^{-7} cm^2/s). These changes suggest significant self-association at 10 mM, slight self-association at 2.0 mM, and no significant self-association at lower concentrations. Consistent with these trends, the ^{1}H NMR peak widths of **1a** do not increase significantly from 0.1 mM to 2.0 mM but increase significantly from 2.0 mM to 10 mM. The ^{1}H NMR chemical shifts do not change significantly from 0.1 mM to 10 mM, suggesting that folding does not depend on self-association.

The diffusion coefficients of **1a**−**1h** at 2.0 mM range from 10.7 ($\pm$0.3) $\times$ 10^{-7} cm^2/s to 12.9 ($\pm$0.8) $\times$ 10^{-7} cm^2/s and, as expected, generally increase with decreasing molecular weight (Table 3). These values are comparable to that of gramicidin S (12.9 ($\pm$0.5) $\times$ 10^{-7} cm^2/s), a monomeric cyclic peptide with a molecular weight close to that of the cyclic modular β-sheets, which was used as a reference. The similarities of the diffusion coefficients of **1b**−**1h** to those of gramicidin S and **1a** suggest little or no self-association of these compounds at 2 mM.

The diffusion coefficient of linked cyclic modular β-sheet **2** is 7.2 ($\pm$0.1) $\times$ 10^{-7} cm^2/s at 2.0 mM (Table 3). This value is substantially lower than that of the monovalent homologue **1b**, as expected with the larger size of **2**.[42]

Discussion

Collectively, the ^{1}H NMR structural studies show that **1a**, **1b**, **1f**, and **2** adopt well-folded β-sheet conformations; **1d**, **1e**, and **1h** adopt moderately folded β-sheet conformations; and **1c**

(40) (a) Gibbs, S. J.; Johnson, C. S., Jr. *J. Magn. Reson.* **1991**, *93*, 395−402. (b) Altieri, A. S.; Hinton, D. P.; Byrd, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 7566−7567. (c) Yao, S.; Howlett, G. J.; Norton, R. S. *J. Biomol. NMR* **2000**, *16*, 109−119. (d) Cohen, Y.; Avram, L.; Frish, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 520−554.

(41) The sLED pulse sequence was used in the PFG NMR diffusion studies of the cyclic modular β-sheets (ref 40b).

(42) The diffusion coefficient of **2** is slightly lower than would be expected for a simple dimer of **1b** and may reflect some minor additional self-association. For discussions of the relationship between diffusion coefficients and molecular weight, see: (a) Teller, D. C.; Swanson, E.; DeHaen, C. *Methods Enzymol.* **1979**, *61*, 103−124. (b) Polson, A. *J. Phys. Colloid Chem.* **1950**, *54*, 649−652.

and **1g** adopt poorly folded β-sheet conformations (Table 4). The well-folded cyclic modular β-sheets show large $\Delta\delta H_\alpha$ values for four or five α-protons in the pentapeptide strand, $^{\delta}$Orn δ-proton magnetic anisotropy of at least 0.45 ppm, and strong key NOE cross-peaks (Figure 9). The moderately folded cyclic modular β-sheets also demonstrate a β-sheet structure but have smaller $\Delta\delta H_\alpha$ values, more moderate $^{\delta}$Orn δ-proton magnetic anisotropy, and weaker NOE cross-peaks, which suggests that the population of the β-sheet structure is lower. The poorly folded cyclic modular β-sheets show two or more negative or essentially zero $\Delta\delta H_\alpha$ values, moderate or small $^{\delta}$Orn δ-proton magnetic anisotropy, and only three or fewer of the four key NOEs in Figure 9. Although portions of the poorly folded cyclic modular β-sheets may be folded, the population of the β-sheet structure is low and the folding is incomplete.

The spectra of divalent cyclic modular β-sheet **2**, which contains two modules derived from **1b**, show that the folding of the modules is comparable to that of **1b**. This result is significant, because it shows that acylation of a $^{\delta}$Orn turn residue does not disrupt the folding of a cyclic modular β-sheet. This finding is similar to that of Waters and Cooper, who showed that modification of the side chain of an asparagine turn residue caused little change in the folding of a β-hairpin peptide.[43] We have now prepared several other linked cyclic modular β-sheets that are linked at either of the two $^{\delta}$Orn residues. In all cases the folding of the linked compounds is comparable to that of the monovalent homologues.

Varying the two α-amino acids in the lower strand can enhance the folding of a cyclic modular β-sheet without altering the sequence of the upper pentapeptide strand. The development of homologues of **1c** and **1g** with enhanced folding demonstrates how tuning the lower strand can improve β-sheet folding. Cyclic modular β-sheet **1g** contains sequences from MIP-2 in the upper and lower strands. After ^{1}H NMR studies of **1g** showed poor folding, we attempted to increase the folding by incorporating residues into the lower strand that would allow for greater cross-strand hydrophobic interactions. Our initial change to **1g** of replacing the alanine of position 6 with valine resulted in slightly improved β-sheet folding. Our second change of incorporating tyrosine into position 6 resulted in greater improvement in folding and gave moderately folded cyclic modular β-sheet **1h**. Insight from the design of **1h** led to improvements to poorly folded **1c**. Replacement of the leucine at position 6 of **1c** with tyrosine resulted in moderately folded **1d**.

Tuning of the lower strand was also necessary to prepare a cyclic modular β-sheet related to the huntingtin protein associated with Huntington's disease and containing five glutamine residues in the upper strand.[44,45] We initially selected two arginine residues for the lower strand to enhance solubility. ^{1}H NMR studies on this compound showed essentially no $^{\delta}$Orn δ-proton magnetic anisotropy (≤0.01 ppm) and no detectable NOE cross-peaks, thus indicating the compound to be completely without any β-sheet-like structure. Attempts at improving the folding by replacing the arginines in the lower strand with hydrophobic residues eventually resulted in an analogue that is moderately folded.

While some of our initial cyclic modular β-sheet designs have resulted in poorly folded structures, some others resulted in structures with poor water solubility. This is a potential problem for cyclic modular β-sheets that contain hydrophobic pentapeptide sequences, such as those from β-amyloid. This problem is illustrated by the homologue of **1b** that we first prepared, which contained Val-Leu in the lower strand. The compound had poor solubility (<0.5 mM) in water, and no studies of the structure of this compound were attempted. Replacement of the valine in the lower strand with lysine substantially increased the solubility.

^{1}H NMR studies of **1a**, **1b**, and **1f** suggest that pentapeptide sequences that allow cross-strand aromatic−aromatic interactions involving the Hao aromatic ring result in the best-folded cyclic modular β-sheets.[46,47] The three monovalent cyclic modular β-sheets in this paper that are well folded each contain at least one phenylalanine residue across from Hao. The phenylalanine side chains at position 4 of the well-folded cyclic modular β-sheets **1a**, **1b**, and **1f** can participate in aromatic−aromatic interactions with the Hao aromatic ring, and the spectra of these compounds suggest that such interactions do occur. The spectra of **1a** show *ortho*-proton resonances of the side chain of the phenylalanine at position 4 that are strongly upfield by ca. 0.5 ppm of those in control **6a**. The spectra of **1b** and **1f** also show ca. 0.5 ppm upfield shifting of the *ortho*-proton resonances of the phenylalanine at position 4 relative to the respective controls. ROESY studies of **1a**, **1b**, and **1f** show NOE cross-peaks between the phenylalanine aromatic rings and the Hao aromatic ring, further suggesting cross-strand interactions.[48]

Cyclic modular β-sheets **1c**, **1e**, and **1f** demonstrate the positive effect that a phenylalanine at position 4 can have on folding. These compounds are identical except for the residues at position 4: **1c** contains glycine, **1e** contains alanine, and **1f** contains phenylalanine. Compound **1c** is poorly folded, **1e** is moderately folded, and **1f** is well folded.

Conclusions

Combination of the Hao amino acid β-strand mimic with two $^{\delta}$Orn β-turn mimics and α-amino acids results in cyclic modular β-sheets that present pentapeptide β-strands along one edge. The linear peptide precursors are quickly and easily synthesized by standard Fmoc solid-phase chemistry, cyclization is clean and efficient, and the resulting cyclic modular β-sheets are easily purified. The α-amino groups of the $^{\delta}$Orn residues provide attractive sites for linking individual cyclic modular β-sheets. Several cyclic modular β-sheets have been prepared that show clear evidence (α-proton chemical shifts, $^{\delta}$Orn δ-proton magnetic anisotropy, and NOE cross-peaks) of being folded into a β-sheet-like conformation. A linked cyclic modular β-sheet also shows evidence of being folded. PFG NMR diffusion studies demonstrate that little or no self-association occurs in the cyclic modular β-sheets at low millimolar (≤2 mM) concentrations.

The potential for improving the folding of cyclic modular β-sheets by tuning the lower strand is a powerful feature that expands the number of sequences that can be placed in the top

(43) Cooper, W. J.; Waters, M. L. *Org. Lett.* **2005**, *7*, 3825−3828.
(44) Castellanos, E.; Nowick, J. S. Unpublished results.
(45) Macdonald, M. E., et al. *Cell* **1993**, *72*, 971−983.
(46) Other studies have demonstrated the importance of aromatic−aromatic interactions in β-hairpin stability. For examples, see ref 6a, d, and k.
(47) A study by Nowick and coworkers suggests that aromatic interactions are not important in *inter*molecular β-sheet interactions. Chung, D. M.; Dou, Y.; Baldi, P.; Nowick, J. S. *J. Am. Chem. Soc.* **2005**, *127*, 9998−9999.
(48) The interaction between the phenylalanine residue at position 4 and Hao is similar to the "aromatic rescue of glycine in β-sheets" reported by Merkel and Regan: Merkel, J. S.; Regan, L. *Fold. Des.* **1998**, *3*, 449−455.

strand. The modularity of the cyclic modular β-sheets is another powerful feature. Monovalent cyclic modular β-sheets can be thought of as discrete building blocks for the synthesis of divalent structures with two separate β-sheet domains. Such divalent structures have the potential to be ligands for biomolecular assemblies that present multiple binding sites (e.g., β-amyloid or huntingtin aggregates). Preparation of multivalent cyclic modular β-sheets with more than two β-sheet domains should also be possible. Both monovalent and multivalent cyclic modular β-sheets are potential tools with which to mimic β-sheet interactions involving proteins and to inhibit those interactions.

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Supporting Information Available: Experimental procedures and NMR spectra, mass spectra, and HPLC traces for the cyclic modular β-sheets; complete ref 45. This material is available free of charge via the Internet at http://pubs.acs.org.

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