ACCOUNTS
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Exploring β-Sheet Structure and Interactions with Chemical Model Systems

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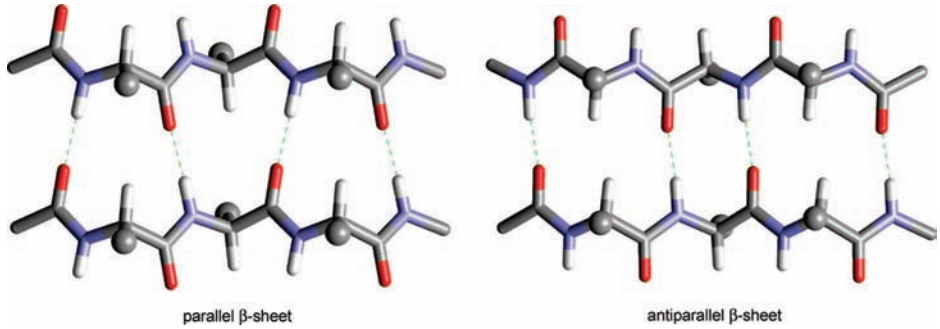

What I cannot create, I do not understand.—Richard P. Feynman

β-Sheets consist of extended polypeptide strands (β-strands) connected by a network of hydrogen bonds and occur widely in proteins. Although the importance of β-sheets in the folded structures of proteins has long been recognized, there is a growing recognition of the importance of intermolecular interactions among β-sheets. Intermolecular interactions between the hydrogen-bonding edges of β-sheets constitute a fundamental form of biomolecular recognition (like DNA base pairing) and are involved protein quaternary structure, protein−protein interactions, and peptide and protein aggregation. The importance of β-sheet interactions in biological processes makes them potential targets for intervention in diseases such as AIDS, cancer, and Alzheimer's disease.

This Account describes my research group's use of chemical model systems to study the structure and interactions of β-sheets. Chemical model systems provide an excellent vehicle with which to explore β-sheets, because they are smaller, simpler, and easier to manipulate than proteins. Synthetic chemical models also provide the opportunity to control or modulate natural systems or to develop other useful applications and may eventually lead to new drugs with which to treat diseases.

In our "artificial β-sheets", molecular template and turn units are combined with peptides to mimic the structures of parallel and antiparallel β-sheets. The templates and turn units form folded, hydrogen-bonded structures with the peptide groups and help prevent the formation of complex, ill-defined aggregates. Templates that duplicate the hydrogen-bonding pattern of one edge of a peptide β-strand while blocking the other edge have proven particularly valuable in preventing aggregate formation and in promoting the formation of simple monomeric and dimeric structures.

Artificial β-sheets that present exposed hydrogen-bonding edges can form well-defined hydrogen-bonded dimers. Dimerization occurs readily in chloroform solutions but requires additional hydrophobic interactions to occur in aqueous solution. Interactions among the side chains, as well as hydrogen bonding among the main chains, are important in dimer formation. NMR studies of artificial β-sheets have elucidated the importance of hydrogen-bonding complementarity, size complementarity, and chiral complementarity in these interactions. These pairing preferences demonstrate sequence selectivity in the molecular recognition between β-sheets.

These studies help illustrate the importance of *intermolecular* edge-to-edge interactions between β-sheets in peptides and proteins. Ultimately, these model systems may lead to new ways of controlling β-sheet interactions and treating diseases in which they are involved.

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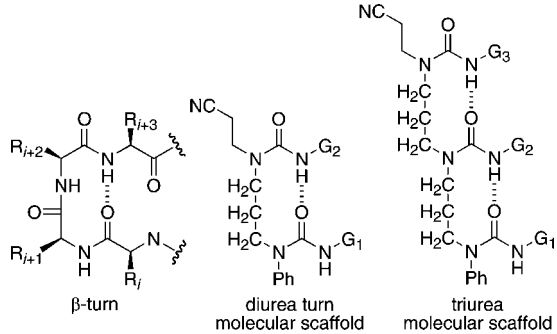
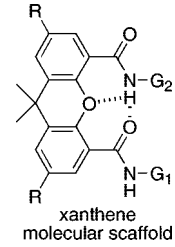
Introduction

During the past 17 years, my co-workers and I have studied *β*-sheet structure and interactions by developing peptide-based chemical model systems in which synthetic building blocks of our own design help induce *β*-sheet folding, structure, and interactions. The studies began with the development of chemical models of protein *β*-sheets in which molecular templates induce *β*-sheet structure in attached peptide strands in chloroform solution. The serendipitous discovery of well-defined dimerization interactions among these structures lead to an appreciation of the importance of molecular recognition involving *β*-sheet interactions among peptides and proteins. A desire to more closely mimic biological systems, in which solvation plays a key role, lead to systems that fold and interact in water. Ongoing efforts are focused on developing *β*-sheet systems that inhibit peptide aggregation and bind proteins through *β*-sheet interactions.

Our initial efforts began with the development of turn structures that loosely resemble the amide-based *β*-turn structures prevalent in peptides and proteins.[1] Inspired in part by peptides and proteins and in part by DNA, we developed a urea-based turn structure, which we termed a "molecular scaffold". Like a *β*-turn, the molecular scaffold allows two groups (G_1 and G_2) to be held in proximity. Unlike peptides and proteins, the urea-based turn can be concatenated to form triurea molecular scaffolds and larger organized structures. We initially focused on structures in which the urea groups are connected by trimethylene chains (-$CH_2CH_2CH_2$-), because they form hydrogen-bonded ten-membered rings like those found in *β*-turns. We subsequently explored structures in which the urea groups are connected by dimethylene chains (-CH_2CH_2-) and found that these structures form hydrogen-bonded nine-membered rings that are more stable than those of the larger homologues.[2,3]

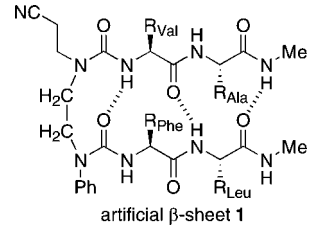


Several key influences set the stage for this work. There had been considerable interest in mimicking peptide and protein turn structures, with the goal of duplicating the structural and functional properties of proteins or creating peptide and protein analogues with improved pharmacological properties.[4] Gellman and co-workers had published a series of fascinating papers on the formation of turn structures in simple amide derivatives.[5] (This work served as the starting point for the important area that Gellman subsequently termed "foldamers".[6]) I had previously developed a xanthene-based molecular template while a postdoctoral scholar and had become fascinated by the ability of intramolecular hydrogen bonding to control structure and reactivity in this system.[7] I had also become fascinated with the observation of Shudo and co-workers that secondary amides with both alkyl and aryl groups on the amide nitrogen atom prefer a conformation in which the aryl group is *trans* to the carbonyl group,[8] and I envisioned using this observation to control the conformation of our oligourea molecular scaffolds.



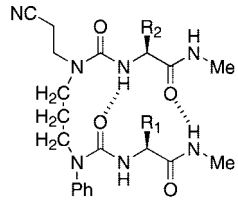
Artificial *β*-Sheets

The resemblance of our urea-based turns to *β*-turns inspired us to combine our molecular scaffolds with amino acids to form structures that we termed "artificial *β*-sheets".[9−11] In our first foray into this area, we constructed an artificial *β*-sheet (**1**) composed of two dipeptides attached to a diurea molecular scaffold with a dimethylene chain. ^{1}H NMR spectroscopic studies showed that the molecule adopts a hydrogen-bonded *β*-sheet-like structure in chloroform ($CDCl_3$) solution and that the phenyl group on the diurea scaffold helps control the relative orientation of the two dipeptide strands. Unlike naturally occurring *β*-turns, which form antiparallel *β*-sheets, the urea-based turn structure of **1** forms a parallel *β*-sheet.



The formation of hydrogen-bonded *β*-sheets with well-defined structures proved challenging, even in noncompetitive solvents such as $CDCl_3$, which facilitate hydrogen bonding. Artificial *β*-sheet **1** teeters on an equilibrium between folded and unfolded states. The equilibrium readily permits the eval-

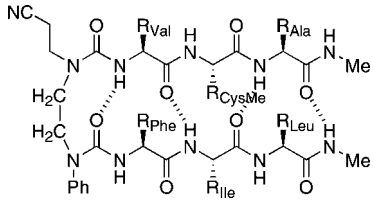
uation of the propensities of different amino acids to adopt *β*-sheet structures. We subsequently used a related system, artificial *β*-sheets **2**, to evaluate the propensities of glycine, alanine, valine, and leucine to adopt *β*-sheet structures.[12] The



artificial β-sheets **2**
R_1, R_2 = H (Gly), Me (Ala), *i*-Pr (Val), *i*-Bu (Leu)

propensities of these amino acids to adopt *β*-sheet structures was widely known from their statistical occurrence in protein secondary structures (commonly referred to as the "Chou−Fasman parameters")[13] and from empirical studies.[14] Our studies showed that the propensities of valine and leucine to adopt *β*-sheet structure is greater than that of alanine, which in turn is greater than that of glycine (V, L > A > G). These results largely match the established propensities (V > L > A > G) and demonstrate that even relatively simple model systems can provide information relevant to the folding and interactions of larger proteins.

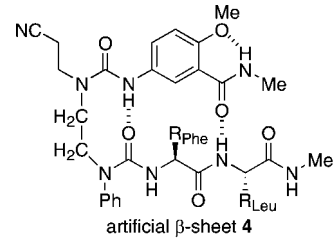
Inspired by artificial *β*-sheet **1**, my co-workers and I contemplated constructing larger artificial *β*-sheets with longer peptide strands and more peptide strands. We synthesized artificial *β*-sheet **3**, which comprises two tripeptides, but did not seriously pursue its characterization because of concerns about the potential for incomplete folding and aggregation.[15]



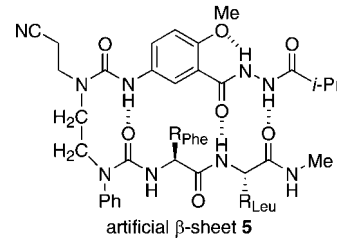
artificial β-sheet **3**

Instead, we pursued additional tactics to enhance the folding of our artificial *β*-sheets and reduce uncontrolled intermolecular interactions.

To enhance folding and reduce uncontrolled intermolecular interactions, we introduced a second template that mimics the hydrogen-bonding pattern of one edge of a peptide in a *β*-strand conformation.[16,17] The template consists of a 5-amino-2-methoxybenzoic acid amide or hydrazide and offers the same pattern of hydrogen-bonding groups as one edge of a peptide *β*-strand. We termed the template a "*β*-strand mimic." Artificial *β*-sheets **4** and **5** illustrate the structures of the *β*-strand mimic and show how it hydrogen bonds to the adjacent peptide strand. In contrast to artificial *β*-sheets



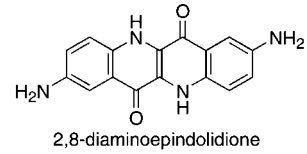
artificial β-sheet **4**



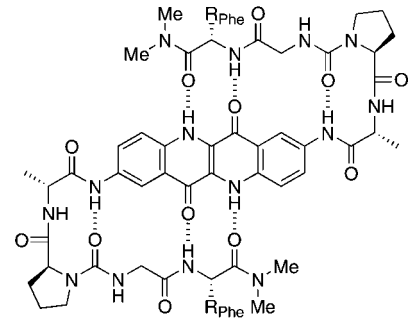
artificial β-sheet **5**

1 and **2**, these structures are largely or wholly folded in $CDCl_3$ solution. Also, in contrast to the earlier artificial *β*-sheets, these compounds mimic the hydrogen-bonding pattern of *antiparallel* *β*-sheets.

Part of the inspiration for the *β*-strand mimic came from the 2,8-diaminoepindolidione template developed by Kemp and co-workers.[4a] This template provides the same pattern of hydrogen-bonding groups as one edge of a peptide in a *β*-strand conformation and induces a 2-fold symmetrical *β*-sheet structure when combined with suitable peptides to form artificial *β*-sheet **6**. The effectiveness of the template in



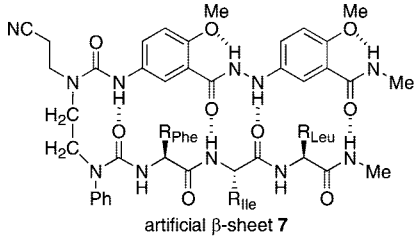
2,8-diaminoepindolidione



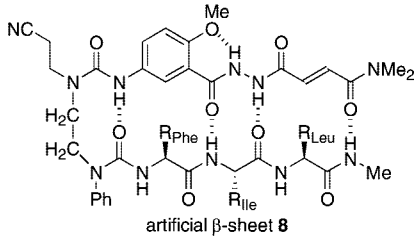
2,8-diaminoepindolidione-templated β-sheet **6**

inducing folding demonstrated the benefits of a preorganized hydrogen-bonding template. In artificial *β*-sheets **4** and **5** and in subsequent systems, we have used 2-methoxybenzoic acid amide and hydrazide derivatives to create preorganization through intramolecular hydrogen bonding.

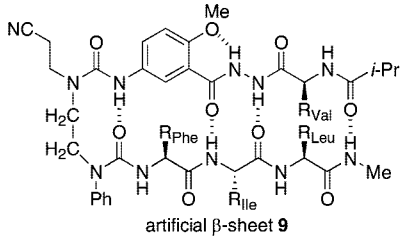
Our dual-template strategy permitted the preparation of larger artificial *β*-sheets. We explored ways of extending the template by synthesizing and studying artificial *β*-sheets **7**−**9**.[18,19] We extended the dual-template strategy to prepare



artificial β-sheet **7**

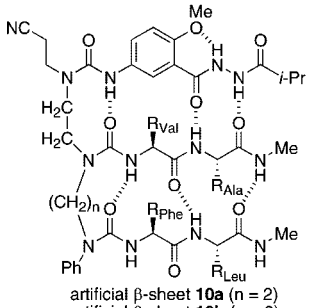


artificial β-sheet **8**

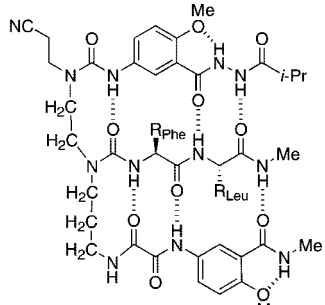


artificial β-sheet **9**

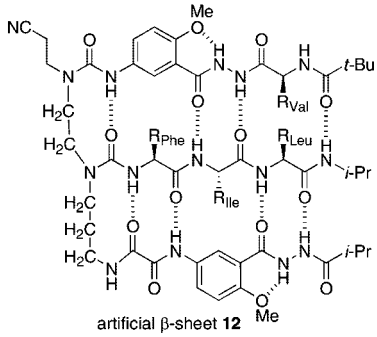
a variety of three-stranded artificial *β*-sheets, including **10a**, **10b**, **11**, and **12**.[20−22] All of these artificial *β*-sheets exhibit robust folding in $CDCl_3$ solution.



artificial β-sheet **10a** (n = 2)
artificial β-sheet **10b** (n = 3)



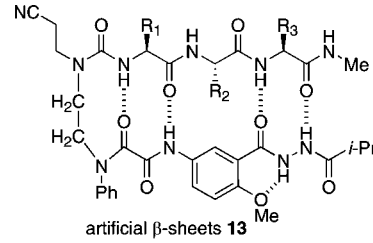
artificial β-sheet **11**



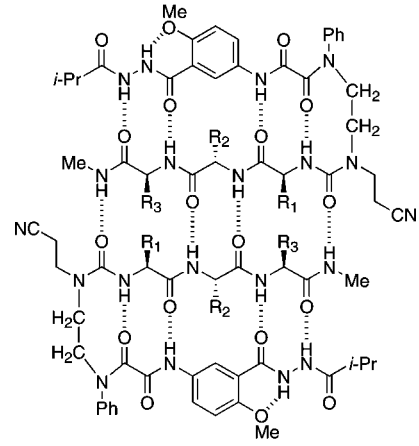
artificial β-sheet **12**

Artificial *β*-Sheets That Dimerize

Relatively little intermolecular interaction occurs among artificial *β*-sheets **1**, **2**, **4**, **5**, and **7**−**12**, because most of the hydrogen-bond-donor groups are satisfied by intramolecular hydrogen bonding or blocked by the phenyl group of the molecular scaffold. In preparing and studying artificial *β*-sheets **13**, we observed the formation of the well-defined hydrogen-bonded dimers **13·13**.[23] This observation brought to our attention the importance of edge-to-edge interactions among *β*-sheets.



artificial β-sheets **13**
13a $R_1 = R_{Phe}$, $R_2 = R_{Ile}$, $R_3 = R_{Leu}$
13b $R_1 = R_{p\text{-nitroPhe}}$, $R_2 = R_{Val}$, $R_3 = R_{Met}$



artificial β-sheet dimers **13•13**

In artificial *β*-sheets **13**, the peptide strand presents an exposed edge with two hydrogen-bond-donor groups and two hydrogen-bond-acceptor groups available to form four intermolecular hydrogen bonds. In $CDCl_3$ solution at millimolar concentrations, **13** forms well-defined hydrogen-bonded dimers through interaction of this exposed edge. Artificial *β*-sheet **13a** dimerizes with an association constant of 600 M^{-1}, while artificial *β*-sheet **13b** dimerizes with an association constant of 90 M^{-1}. This interesting difference between the two homologues suggests that interactions among the side chains, as well as hydrogen bonding among the main chains, are important in molecular recognition between *β*-sheets.

This mode of edge-to-edge interaction among *β*-sheets constitutes a fundamental form of biomolecular recognition.[24] It occurs widely in protein quaternary structure, mediates protein−protein interactions, and is involved in peptide and protein aggregation. The structure of the interleukin 8 dimer illus-

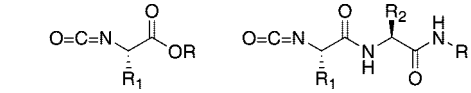
trates this mode of interaction in an actual protein.[25] In collaboration with bioinformatics researchers, we have developed a database of intermolecular β-sheet interactions among

β-sheet dimer of interleukin 8 (PDB ID 1il8)

proteins, which we have called the "Interchain β-Sheet (ICBS) Database."[26,27]

Amino Acids That Induce β-Sheet Folding and Dimerization

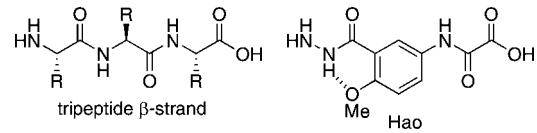
The syntheses of all of the artificial β-sheets described above required specialized techniques and methodology because the peptide strands are attached to the molecular scaffold by urea linkages. To synthesize these structures, we developed methods for the preparation of peptide isocyanates (a hitherto unreported class of compounds) and improved methods for the synthesis of amino acid ester isocyanates.[28−30] The isocyan-



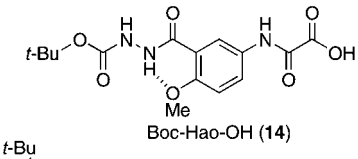
amino acid ester isocyanate peptide isocyanate

ates were used primarily in solution-phase syntheses of the artificial β-sheets; additional methods were developed for the solid-phase syntheses.[31]

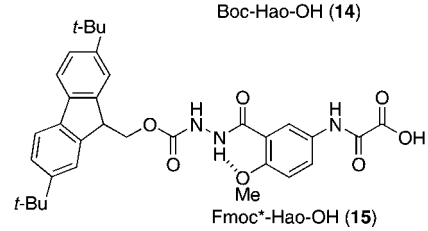
A major advance in the design and synthesis of artificial β-sheets came through the introduction of an unnatural amino acid "Hao" based on the β-strand mimic of **13**.[32] The amino acid is composed of hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid, hence the name Hao, and mimics the hydrogen-bonding edge of a tripeptide in a β-strand conformation. Because Hao is an amino acid, it can be incorporated into peptides through standard solid-phase peptide synthesis techniques. This feature makes Hao-containing peptides faster



tripeptide β-strand Hao



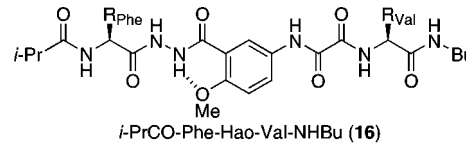
Boc-Hao-OH (**14**)



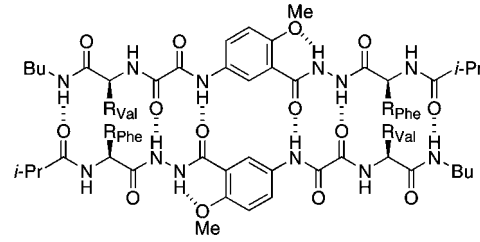
Fmoc*-Hao-OH (**15**)

and easier to prepare than other artificial β-sheets. We developed both a Boc-protected version of Hao (**14**) and a version protected by the 2,7-di-*tert*-butyl analogue of the Fmoc protecting group (Fmoc*), which we created to address the poor solubility of Fmoc derivatives in organic solvents.[33] We have used the latter version, Fmoc*-Hao-OH (**15**), extensively in solid-phase synthesis of Hao-containing peptides.

The amino acid Hao promotes β-sheet folding and interactions among peptides that contain it. The peptide *i*-PrCO-Phe-Hao-Val-NHBu (**16**) forms the β-sheet dimer **16·16** in $CDCl_3$ solution.[32] The association constant (ca. 10^6 M^{-1}) is far



i-PrCO-Phe-Hao-Val-NHBu (**16**)

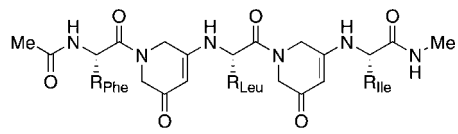


i-PrCO-Phe-Hao-Val-NHBu dimer **16•16**

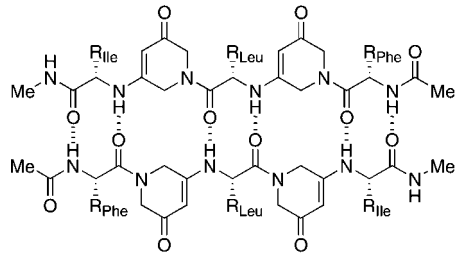
greater than that of simple peptides or of artificial β-sheets **13**, reflecting the preorganization of the Hao amino acid and the energetic contributions of *six* intermolecular hydrogen bonds in a noncompetitive organic solvent. Addition of methanol (CD_3OD) weakens the dimer by competitively hydrogen bonding to the monomer; in 10% CD_3OD−$CDCl_3$, the dimerization constant drops to 900 M^{-1}.

Bartlett and co-workers have subsequently reported a clever system based on 1,2-dihydro-3(6*H*)-pyridinone units, which they termed "@-tides", that also induces β-sheet folding and interactions among peptides.[34] The @-tide Ac-Phe-

Ach-Leu-Ach-Ile-NHMe (**17**), for example, forms hydrogen bonded *β*-sheet dimer **17·17** in $CDCl_3$ solution. Bartlett and



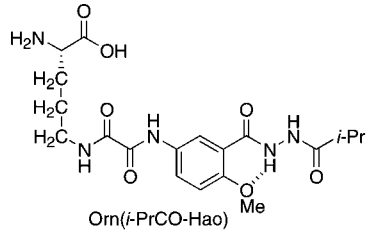
Ac-Phe-Ach-Leu-Ach-Ile-NHMe (**17**)



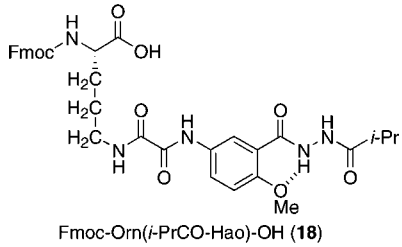
Ac-Phe-Ach-Leu-Ach-Ile-NHMe dimer **17•17**

co-workers have used the @-tide peptidomimetic system to study amino acid side-chain interactions within *β*-sheets and to bind protein PDZ domains through *β*-sheet interactions.[35]

The amino acid Hao lead us to think about studying *β*-sheet folding and interactions in purely peptidic systems and allowed us to rapidly design and synthesize molecules that participate in these interactions. Another major advance came through the combination of the *β*-strand mimic Hao with another amino acid, ornithine.[36] By linking Hao to the *δ*-amino group of ornithine to create Orn(*i*-PrCO-Hao), we created a hybrid amino acid consisting of both a *β*-strand mimic and a turn unit. The *δ*-linked ornithine in this hybrid structure is akin to the molecular scaffold in our artificial *β*-sheets or a *β*-turn in a peptide or protein. The Orn(*i*-PrCO-Hao) amino acid can be incorporated into peptides through standard solid-phase peptide synthesis techniques using Fmoc-Orn(*i*-PrCO-Hao)-OH (**18**).

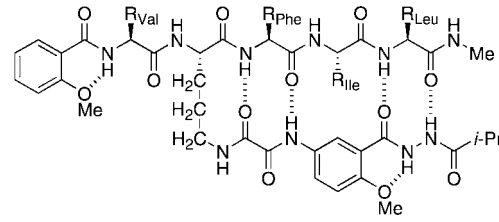


Orn(*i*-PrCO-Hao)

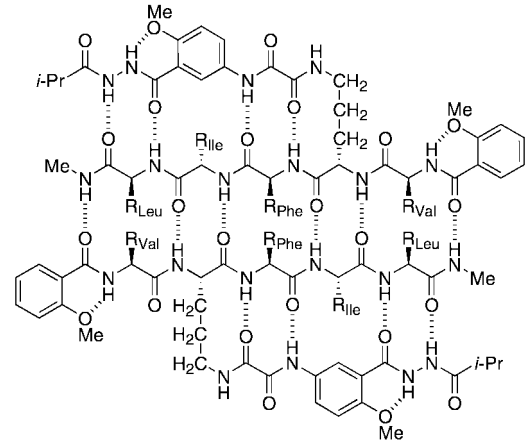


Fmoc-Orn(*i*-PrCO-Hao)-OH (**18**)

Peptides containing Orn(*i*-PrCO-Hao) fold and dimerize through *β*-sheet interactions. The peptide *o*-anisoyl-Val-Orn(*i*-PrCO-Hao)-Phe-Ile-Leu-NHMe (**19**), for example, forms the well-defined *β*-sheet dimer **19·19** in $CDCl_3$ solution.[36] In this



o-anisoyl-Val-Orn(*i*-PrCO-Hao)-Phe-Ile-Leu-NHMe (**19**)



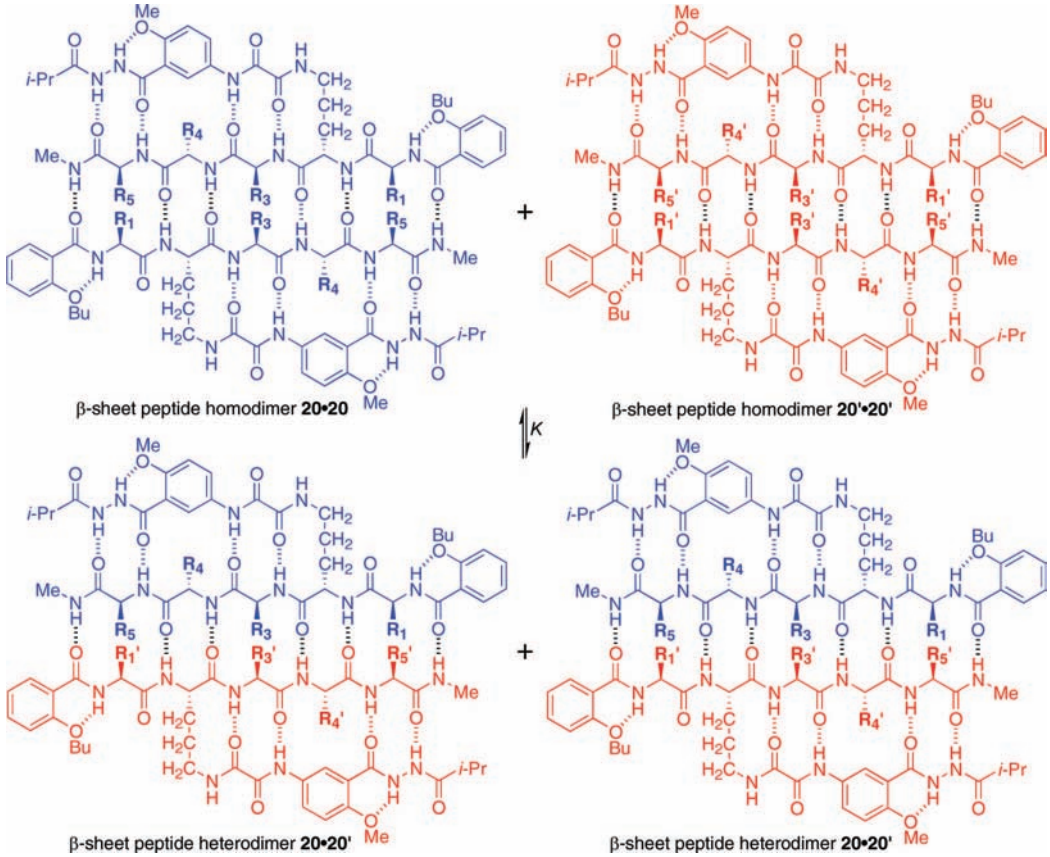
o-anisoyl-Val-Orn(*i*-PrCO-Hao)-Phe-Ile-Leu-NHMe dimer **19•19**

molecule, the *i*-PrCO-Hao unit acts as a template to enforce *β*-sheet structure in the phenylalanine, isoleucine, and leucine amino acids of the adjacent pentapeptide strand. The *i*-PrCO-Hao unit helps block one edge of the pentapeptide strand and prevent the formation of higher oligomers while preorganizing the other edge to participate in *β*-sheet dimer formation.

Studies of Artificial *β*-Sheets That Dimerize

We have used the robust dimers that form among Orn(*i*-PrCO-Hao)-containing peptides to explore molecular recognition that occurs between the edges of *β*-sheets. The homodimers of Orn(*i*-PrCO-Hao)-containing peptides equilibrate slowly on the NMR time scale, particularly at subambient temperatures. Mixing two different homodimers (shown as **20·20** and **20′·20′**, Scheme 1) generates an equilibrium mixture of the homodimers and the heterodimer (**20·20′**), which give distinct ^{1}H NMR resonances associated with the hydrazide and anilide groups. Quantification of the three species by integration or curve fitting permits the determination of the equilibrium constant (*K*). The equilibrium interchanges the pairs of side chains that interact at the interfaces of the *β*-sheets: in homodimer **20·20**, R_1 and R_5 interact; in homodimer **20′·20′**, R_1′ and R_5′ interact; and in heterodimer **20·20′**, R_1

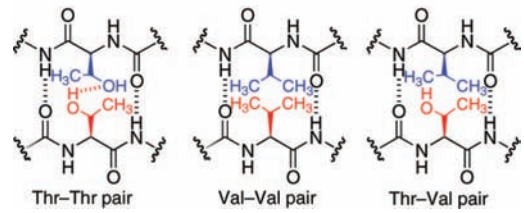
SCHEME 1



and R_5' and R_1' and R_5 interact. The position of the equilibrium reflects the difference in interaction energy among the side chains.

We have used this system to measure the interaction differences among various amino acid side chains.[37] For example, we have used Orn(*i*-PrCO-Hao)-containing peptides with valine and threonine groups at the R_1 and R_5 positions (**20a**−**d**) to study the interactions among valine and threonine.[38] The studies demonstrated substantial preference for Thr−Thr and Val−Val pairing over Val−Thr pairing in 10% CD_3SOCD_3 in $CDCl_3$ solution. Experiments in which the four peptides were mixed in all six possible binary combinations demonstrated that a Thr−Thr pair and a Val−Val pair are 0.6 kcal/mol more stable than two Thr−Val pairs. The two mixing experiments that resulted in different side-chain interactions (**20a** with **20b** and **20c** with **20d**) showed strong preferences for hetero- and homodimer formation. The four experiments that resulted in no different side-chain interactions (**20a** with **20c**, **20a** with **20d**, **20b** with **20c**, and **20a** with **20d**) served as controls and showed no significant preferences. Analogous experiments with serine and valine showed similar but slightly smaller self-pairing preferences

20a $R_1 = R_{Thr}$, $R_5 = R_{Val}$
20b $R_1 = R_{Val}$, $R_5 = R_{Thr}$
20c $R_1 = R_{Thr}$, $R_5 = R_{Thr}$
20d $R_1 = R_{Val}$, $R_5 = R_{Val}$

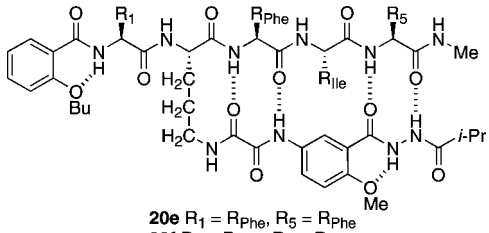


and demonstrated that a Ser−Ser pair and a Val−Val pair are 0.4 kcal/mol more stable than two Ser−Val pairs.[39]

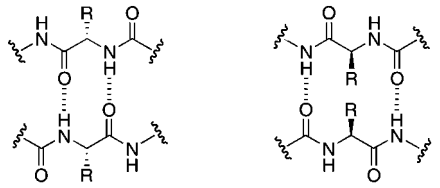
These findings are significant, because they demonstrate that pairing among β-sheets is sequence selective. These experiments suggest that hydrogen bonding among the threonine or serine side chains may play an important role in the observed selectivity. Threonine and serine are self-complementary and can hydrogen bond together. Interestingly, the

interaction is not selective in pure $CDCl_3$, suggesting that the CD_3SOCD_3 may mediate the hydrogen-bonding interaction.[40] In subsequent studies, we have found preferences among the hydrophobic amino acids alanine, valine, leucine, and isoleucine that suggest size and shape complementarity of the amino acid side chains are also important in sequence selectivity.[39]

Interactions among aromatic amino acids are generally thought to be important in protein folding and interaction.[41] To test whether these interactions are important in molecular recognition between *β*-sheets, we studied the pairing preferences of artificial *β*-sheets containing phenylalanine and cyclohexylalanine groups at the R_1 and R_5 positions (**20e**−**h**).[42] We were surprised to find no preference for phenylalanine pairing with itself in $CDCl_3$ solution, particularly in light of the established importance of interactions among aromatic amino acids in the folding of *β*-hairpin peptides.[43] Statistical studies of Phe−Phe pairings within protein *β*-sheets shed light on this interesting finding: Phe−Phe pairings occur frequently in the *hydrogen-bonded* cross-strand pairs of antiparallel *β*-sheets but not in the *non-hydrogen-bonded* cross-strand pairs.[37b,d] Examination of *non-hydrogen-bonded* cross-strand Phe−Phe pairs at the interface between antiparallel *β*-sheets in the ICBS database showed little significant contact between the aromatic rings. Collectively, these studies suggest that Phe−Phe interactions within the non-hydrogen-bonded cross-strand pairs do not appear to mediate interactions between antiparallel *β*-sheets. Although contact between aromatic rings is favorable when geometry permits, the energetic penalty of achieving such contact apparently offsets the energetic benefit in proteins and in this model system.



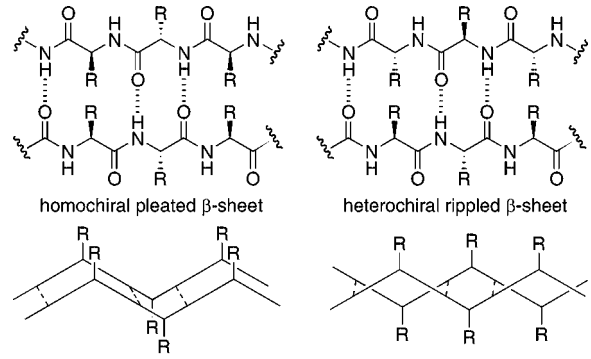
20e $R_1 = R_{Phe}$, $R_5 = R_{Phe}$
20f $R_1 = R_{Cha}$, $R_5 = R_{Cha}$
20g $R_1 = R_{Phe}$, $R_5 = R_{Cha}$
20h $R_1 = R_{Cha}$, $R_5 = R_{Phe}$

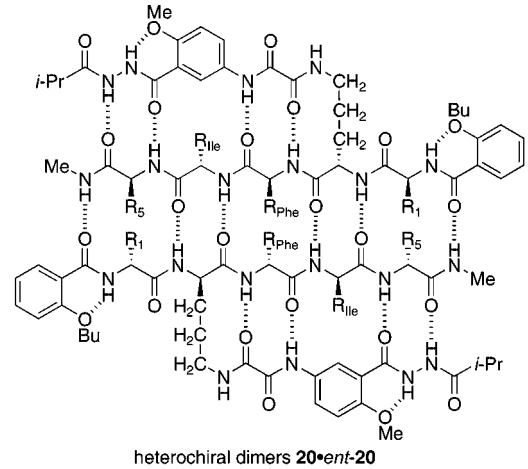


hydrogen-bonded cross-strand pair

non-hydrogen-bonded cross-strand pair

Chemical model systems permit the testing of ideas and relationships that cannot readily be studied with naturally occurring peptides and proteins. In 1953, Pauling and Corey speculated on the *rippled-sheet* structure that might form when alternate strands of L- and D-polypeptides hydrogen bond together to form *β*-sheets.[44] In contrast to the *pleated-sheet* structure of naturally occurring peptides and proteins, which are composed wholly of *homochiral* strands (L-polypeptides),[45] the side chains of the adjacent polypeptide strands of the hypothetical *heterochiral* structures point in opposite directions. To test the relative stability of homochiral and heterochiral *β*-sheets, we mixed all-L-polypeptide homochiral dimers **20**·**20** and all-D-polypeptide homochiral dimers *ent*-**20**·*ent*-**20** in $CDCl_3$ solution and quantified the formation of heterochiral dimers **20**·*ent*-**20** by ^1H NMR spectroscopy.[46] Homochiral dimer formation was favored strongly, by 3.1−4.2 kcal/mol, in the three systems that we studied (**20d**,**i**,**j** and *ent*-**20d**,**i**,**j**). These results clearly demonstrate that homochiral pleated *β*-sheets are far more stable than heterochiral rippled *β*-sheets. The greater stability of the homochiral *β*-sheets might result from favorable nonbonded contacts between side chains of



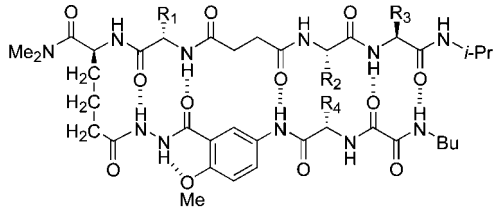


heterochiral dimers **20**•*ent*-**20**

20d, *ent*-**20d** $R_1 = R_{Val}$, $R_5 = R_{Val}$
20i, *ent*-**20i** $R_1 = R_{Leu}$, $R_5 = R_{Leu}$
20j, *ent*-**20j** $R_1 = R_{Val}$, $R_5 = R_{Ala}$

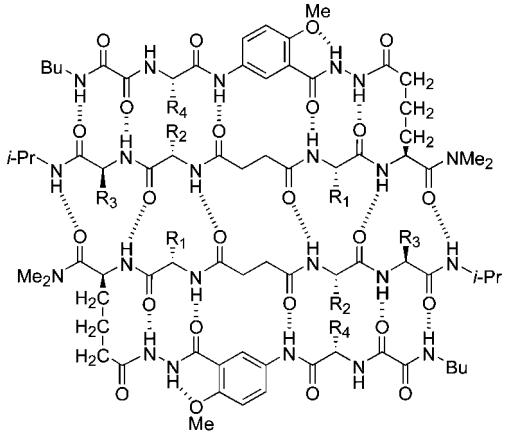
the homochiral β-strands, the complementary twisting of homochiral β-strands, or some other factor.

All of the dimers described thus far involve the formation of antiparallel β-sheets. Parallel β-sheet formation, although less prevalent in protein−protein interaction, is widely involved in peptide and protein aggregation.[47] The aggregation of peptides and proteins has emerged as the key molecular process in many devastating neurodegenerative diseases, such as Alzheimer's, Huntington's, and the prion diseases, as well as a variety of other diseases. To study this important process, we have developed artificial β-sheets that dimerize through parallel β-sheet interactions.[48] Artificial β-sheets **21** consist of two dipeptides that are N-terminally linked with succinic acid and combined with suitable turn and template units. The artificial β-sheets form the well-defined dimers **21·21** in



artificial β-sheets **21**

21a $R_1 = R_{Leu}$, $R_2 = R_{Leu}$, $R_3 = R_{Ile}$, $R_4 = R_{Val}$
21b $R_1 = R_{Leu}$, $R_2 = R_{Leu}$, $R_3 = R_{Leu}$, $R_4 = R_{Val}$
21c $R_1 = R_{Leu}$, $R_2 = R_{Ala}$, $R_3 = R_{Leu}$, $R_4 = R_{Ala}$
21d $R_1 = R_{Leu}$, $R_2 = R_{Leu}$, $R_3 = R_{Leu}$, $R_4 = R_{Ala}$
21e $R_1 = R_{Phe}$, $R_2 = R_{Leu}$, $R_3 = R_{Ile}$, $R_4 = R_{Ala}$
21f $R_1 = R_{Leu}$, $R_2 = R_{Phe}$, $R_3 = R_{Leu}$, $R_4 = R_{Ala}$



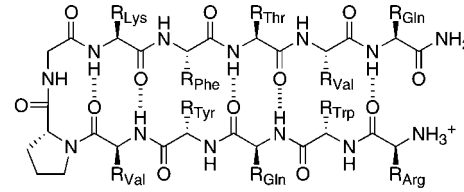
parallel β-sheet dimers **21•21**

$CDCl_3$ solution in which the two dipeptides interact by parallel β-sheet formation. In ongoing studies, we are using artificial β-sheets **21** to investigate interactions among amino acid side chains in parallel β-sheets.

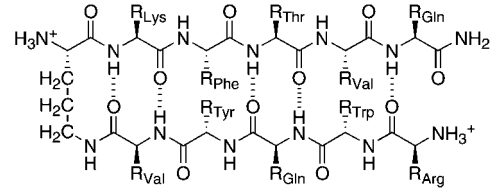
Artificial β-Sheets That Fold and Dimerize in Water

Studying the folding and interactions of peptides and proteins in water is important, because water is ubiquitous in natural biological systems. Most small peptides do not adopt well-defined folded structures in aqueous solution. Hydrogen bonding plays a smaller role in stabilizing folded structures in water, because water hydrogen bonds to peptide amide groups. Hydrophobic interactions are important in folding in water and occur extensively in larger proteins. In smaller peptides, it is difficult to form the types of extensive hydrophobic structures that are generally needed for stable folding. Small peptide β-hairpins, which constitute minimal β-sheet structures, have been created through the judicious choice of turn and side-chain groups.[49] Most of these structures teeter on the brink of unfolding and do not tolerate substantial changes in amino acid sequence.

To evaluate the suitability of our δ-linked ornithine turn unit to create β-sheet structures that fold in water, we compared the δ-linked ornithine turn to β-turns in a water-soluble β-hairpin peptide.[50] Gellman and co-workers had shown that Arg-Trp-Gln-Tyr-Val-D-Pro-Gly-Lys-Phe-Thr-Val-Gln-NH_2 (**22**) folds into a well-defined β-hairpin structure.[51] We compared this peptide to analogue Arg-Trp-Gln-Tyr-Val-$^{\delta}$Orn-Lys-Phe-Thr-Val-Gln-NH_2 (**23**), in which the D-Pro-Gly turn was replaced with δ-linked ornithine ($^{\delta}$Orn) and found both peptides to exhibit comparable degrees of β-hairpin folding. $^{\delta}$Orn-peptide **23** showed superior folding to the homologue with a turn unit based on Asn-Gly, which is also known to form β-turns and support β-hairpin formation. Peptides in which the δ-linked ornithine turn structure was replaced with δ-aminovaleric acid, δ-linked D-ornithine, or ε-linked lysine showed little or no β-hairpin folding. Collectively, these studies established that the δ-linked ornithine turn is well suited to creating β-sheet structures that fold in water.



Arg-Trp-Gln-Tyr-Val-D-Pro-Gly-Lys-Phe-Thr-Val-Gln-NH_2 (TFA salt, **22**)

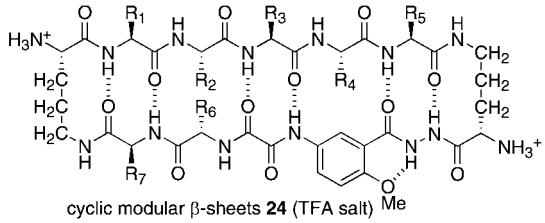


Arg-Trp-Gln-Tyr-Val-$^{\delta}$Orn-Lys-Phe-Thr-Val-Gln-NH_2 (TFA salt, **23**)

A limitation of existing β-hairpin structures is that specific amino acid sequences are required to achieve β-sheet folding. We envisioned that β-sheets that fold in water without requiring specific amino acid sequences would be particularly

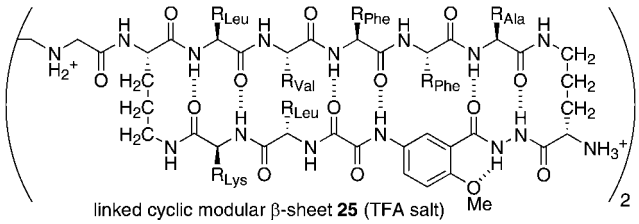
well suited to studying and controlling the types of intermolecular interactions between *β*-sheets that occur among proteins and in peptide and protein aggregation. Such preorganized *β*-sheets would allow us to explore the effects of different amino acid side chains and sequences upon intermolecular interactions without having to account for the differing propensities of different amino acids to adopt *β*-sheet structures.

To create *β*-sheets that do not require specific amino acid sequences to fold in water, we developed macrocyclic structures **24**, which comprise two *δ*-linked ornithine turns, the Hao amino acid *β*-strand mimic, a pentapeptide strand, and two additional amino acids.[52] We termed the 42-membered ring



cyclic modular β-sheets **24** (TFA salt)

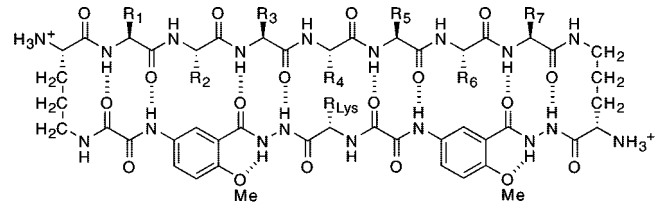
24a R_1=R_{Lys}, R_2=R_{Leu}, R_3=R_{Val}, R_4=R_{Phe}, R_5=R_{Phe}, R_6=R_{Val}, R_7=R_{Glu}
24b R_1=R_{Leu}, R_2=R_{Val}, R_3=R_{Phe}, R_4=R_{Phe}, R_5=R_{Ala}, R_6=R_{Leu}, R_7=R_{Lys}
24c R_1=R_{Ala}, R_2=R_{Ile}, R_3=R_{Ile}, R_4=R_{Gly}, R_5=R_{Leu}, R_6=R_{Leu}, R_7=R_{Lys}
24d R_1=R_{Ala}, R_2=R_{Ile}, R_3=R_{Ile}, R_4=R_{Gly}, R_5=R_{Leu}, R_6=R_{Tyr}, R_7=R_{Lys}
24e R_1=R_{Ala}, R_2=R_{Ile}, R_3=R_{Ile}, R_4=R_{Ala}, R_5=R_{Leu}, R_6=R_{Leu}, R_7=R_{Lys}
24f R_1=R_{Ala}, R_2=R_{Ile}, R_3=R_{Ile}, R_4=R_{Phe}, R_5=R_{Leu}, R_6=R_{Leu}, R_7=R_{Lys}
24g R_1=R_{Ser}, R_2=R_{Leu}, R_3=R_{Ser}, R_4=R_{Val}, R_5=R_{Thr}, R_6=R_{Ala}, R_7=R_{Thr}
24h R_1=R_{Ser}, R_2=R_{Leu}, R_3=R_{Ser}, R_4=R_{Val}, R_5=R_{Thr}, R_6=R_{Tyr}, R_7=R_{Thr}



linked cyclic modular β-sheet **25** (TFA salt)

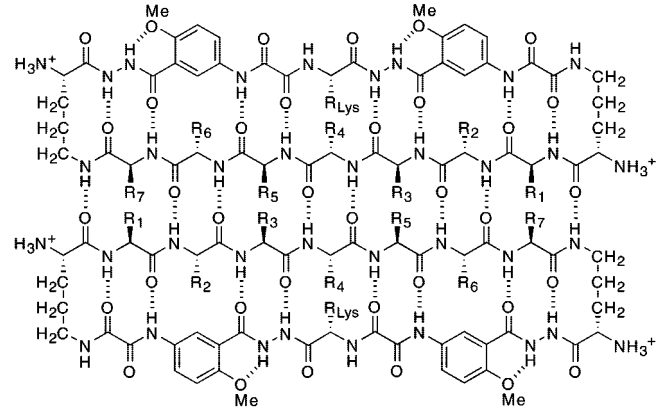
macrocycles "cyclic modular *β*-sheets". Cyclic modular *β*-sheets **24** tolerate a variety of peptide sequences while generally maintaining well folded or at least moderately folded *β*-sheet structures. The $^{\delta}$Orn turn and Hao template units help maintain a *β*-sheet conformation in the pentapeptide strand and block one edge of the pentapeptide while leaving the other edge available to participate in intermolecular *β*-sheet interactions. The macrocycles can be connected through the *α*-amino groups of the $^{\delta}$Orn turn units to create multivalent *β*-sheet structures with more than one *β*-sheet domain, such as linked cyclic modular *β*-sheet **25**.

We hypothesized that macrocyclic *β*-sheets that present a larger *β*-sheet edge might form dimers in water and tested this hypothesis with macrocyclic *β*-sheets **26**.[53] Macrocyclic *β*-sheets **26** comprise two *δ*-linked ornithine turns, two Hao amino acids, a heptapeptide strand, and one additional amino acid in a

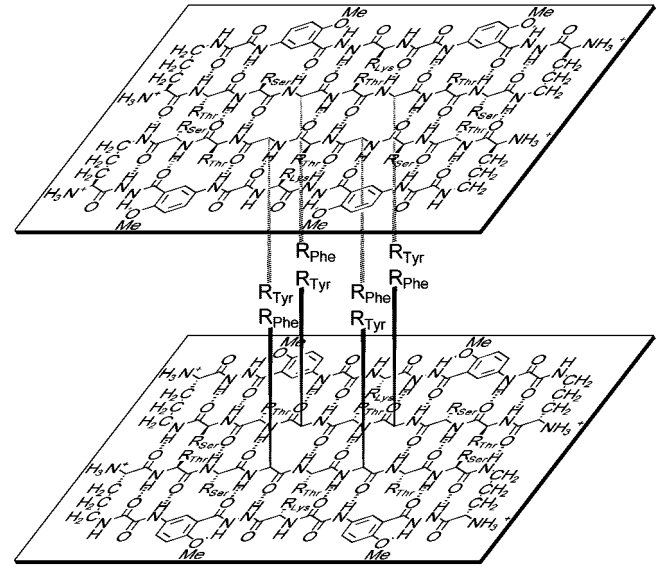


macrocyclic β-sheets **26** (TFA salt)

26a R_1=R_{Thr}, R_2=R_{Ser}, R_3=R_{Phe}, R_4=R_{Thr}, R_5=R_{Tyr}, R_6=R_{Thr}, R_7=R_{Ser}
26b R_1=R_{Thr}, R_2=R_{Ser}, R_3=R_{Phe}, R_4=R_{Glu}, R_5=R_{Tyr}, R_6=R_{Thr}, R_7=R_{Ser}
26c R_1=R_{Thr}, R_2=R_{Ser}, R_3=R_{Phe}, R_4=R_{Leu}, R_5=R_{Tyr}, R_6=R_{Thr}, R_7=R_{Ser}
26d R_1=R_{Thr}, R_2=R_{Ser}, R_3=R_{Phe}, R_4=R_{Tyr}, R_5=R_{Tyr}, R_6=R_{Thr}, R_7=R_{Ser}
26e R_1=R_{Thr}, R_2=R_{Leu}, R_3=R_{Phe}, R_4=R_{Thr}, R_5=R_{Tyr}, R_6=R_{Val}, R_7=R_{Ser}
26f R_1=R_{Thr}, R_2=R_{Tyr}, R_3=R_{Phe}, R_4=R_{Thr}, R_5=R_{Tyr}, R_6=R_{Phe}, R_7=R_{Ser}
26g R_1=R_{Thr}, R_2=R_{Tyr}, R_3=R_{Phe}, R_4=R_{Thr}, R_5=R_{Tyr}, R_6=R_{Tyr}, R_7=R_{Ser}
26h R_1=R_{Glu}, R_2=R_{Ser}, R_3=R_{Phe}, R_4=R_{Thr}, R_5=R_{Tyr}, R_6=R_{Thr}, R_7=R_{Lys}
26i R_1=R_{Thr}, R_2=R_{Ser}, R_3=R_{Leu}, R_4=R_{Thr}, R_5=R_{Val}, R_6=R_{Thr}, R_7=R_{Ser}
26j R_1=R_{Thr}, R_2=R_{Tyr}, R_3=R_{Ile}, R_4=R_{Thr}, R_5=R_{Val}, R_6=R_{Tyr}, R_7=R_{Ser}
26k R_1=R_{Thr}, R_2=R_{Tyr}, R_3=R_{Ile}, R_4=R_{Thr}, R_5=R_{Thr}, R_6=R_{Tyr}, R_7=R_{Ser}
26l R_1=R_{Thr}, R_2=R_{Tyr}, R_3=R_{Ser}, R_4=R_{Thr}, R_5=R_{Val}, R_6=R_{Tyr}, R_7=R_{Ser}
26m R_1=R_{Thr}, R_2=R_{Tyr}, R_3=R_{Ser}, R_4=R_{Thr}, R_5=R_{Thr}, R_6=R_{Tyr}, R_7=R_{Ser}
26n R_1=R_{Phe}, R_2=R_{Tyr}, R_3=R_{Ser}, R_4=R_{Thr}, R_5=R_{Thr}, R_6=R_{Phe}, R_7=R_{Tyr}



macrocyclic β-sheet dimers **26•26** (TFA salt)



macrocyclic β-sheet dimer of dimers **26a•26a–26a•26a** (TFA salt)

54-membered ring. Most of the variants of these 54-membered ring macrocycles that we prepared and studied form well-defined *β*-sheet dimers **26·26** through edge-to-edge interactions in

water at millimolar concentrations. The dimers further self-assemble to form dimers of dimers (tetramers), or in some cases higher oligomers, through hydrophobic face-to-face interactions. Macrocyclic peptide **26a**, for example, forms dimer of dimers **26a·26a**−**26a·26a**, in which the phenylalanine and tyrosine groups at the R_3 and R_5 positions create a hydrophobic core that stabilizes the hydrogen-bonded dimers. This tetramer is reminiscent of a β-sandwich protein, which has a hydrophobic core between two β-sheets.

The free dimers **26·26** are never observed, and those variants of **26** that do not self-assemble to form higher oligomers at millimolar concentrations do not dimerize. The requirement that the dimers further self-associate and that the resulting oligomers form a hydrophobic core suggests that hydrogen bonds alone are not generally sufficient to stabilize β-sheet structure and interactions in water and that additional hydrophobic interactions are necessary. In ongoing investigations, we are studying related macrocyclic β-sheets that bind a small protein through edge-to-edge β-sheet interactions and appropriate additional contacts.

Conclusion

By using a bottom-up approach to create molecules that mimic β-sheets, we have learned about the structure and interactions of β-sheets. Molecules that fold to form β-sheet structures in chloroform solution can be created by combining suitable turn, template, and peptide units. If the molecules present a preorganized β-sheet edge, the molecules form well-defined hydrogen-bonded dimers. These types of edge-to-edge interactions among β-sheets are important in protein quaternary structure, protein−protein interactions, and peptide and protein aggregation. Molecules that fold to form β-sheet structures in water are harder to create but can be formed by combining suitable turn, template, and peptide units to make macrocycles. Macrocyclic β-sheets can interact through edge-to-edge interactions in water if additional face-to-face hydrophobic interactions permit higher oligomer formation. Ongoing studies are focused on the interaction of macrocyclic β-sheets with β-sheet proteins and peptides that aggregate through β-sheet formation. Through these studies, we aim to continue to learn how to control β-sheet interactions in biological systems.

I am deeply indebted to all of my co-workers, named in the references, who have done the experimental research described in these studies. We thank the National Institutes of Health (Grant GM-49076) and the National Science Foundation for financial support.

BIOGRAPHICAL INFORMATION

James S. Nowick was born in 1964 and earned an A.B. degree from Columbia University in 1985 and a Ph.D. from MIT in 1989. After an NSF postdoctoral fellowship at MIT, he began his independent faculty career at UCI in 1991, where is currently a Professor of Chemistry.

FOOTNOTES

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