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Chemical Models of Protein β-Sheets

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The 1997 Nobel Prizes highlighted the importance of proteins in virtually all of life's processes. The Nobel Prize in Chemistry was awarded to Paul Boyer and John Walker "for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)" and to Jens Skou "for the first discovery of an ion-transporting enzyme, Na^+,K^+-ATPase".[1] Stanley Prusiner received the Nobel Prize in Medicine "for his discovery of prions (proteinaceous infectious particles)—a new biological principle of infection".[2]

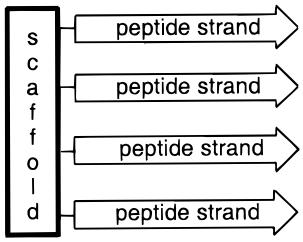
Proteins derive their unique functions as enzymes, receptors, infectious agents, etc. because of their ability to fold into well-defined three-dimensional structures. Protein folding is not well understood. A protein's three-dimensional structure cannot yet be predicted with confidence from its sequence of amino acids, and the design of functional proteins from scratch remains challenging. The folded structures of proteins are composed of simpler structural elements, such as α-helices and β-sheets. With the goals of learning about protein folding, developing molecular receptors and catalysts, and discovering new drugs, chemists have begun to design and synthesize compounds that fold to mimic these structural elements.[3] This Account describes our efforts in this area. Our approach to creating chemical models of protein β-sheets relies upon molecular templates to induce β-sheet structure in attached peptide strands. The first half of the paper describes the syntheses of these compounds; the second half describes structural studies.

Design of Oligourea Molecular Scaffolds

In 1991, my students and I became interested in designing, synthesizing, and studying compounds that mimic the structures and hydrogen-bonding patterns of protein β-sheets. Through these studies, we aim to gain insight into factors affecting β-sheet structure and stability and to create compounds that may eventually lead to new treatments for Alzheimer's disease and for other diseases in which β-sheet formation plays a key role.

We conceived of structures in which molecular templates induce β-sheet formation in attached peptide strands, and we termed these structures *artificial β-sheets*. Initially, we envisioned artificial β-sheets in which a sort of *molecular scaffold* could be constructed to hold a number of peptide strands in proximity. Chart 1 illustrates this notion. To translate this cartoon into an actual molecule, we required a suitable molecular scaffold and a means of attaching peptide strands.

Chart 1



artificial β-sheet

Existing molecular scaffolds did not appear suitable. Mutter and co-workers had developed protein-like molecules ("template-assembled synthetic proteins") in which polypeptide templates hold peptide strands in proximity.[4] These templates permit the creation of α-helical structures but have proven less successful at creating β-sheets. Other studies by Feigel[5] and by Kelly[6] have used fused tricyclic molecules as templates to hold two peptide strands in proximity and create β-sheetlike structures. Although these tricyclic templates are tractable and easy to synthesize, larger fused polycyclic templates capable of holding three or more peptide strands in proximity would likely be difficult to synthesize and have poor solubility properties.

With the goal of creating larger artificial β-sheets, we set out to develop acyclic molecular scaffolds that would be easy to synthesize and would allow several peptide strands to be attached. We postulated that rigid scaffolds would better nucleate β-sheets with well-defined structures, and we decided to use intramolecular hydrogen bonding to rigidify the scaffold. Since ureas are good at hydrogen bonding, we decided to examine oligomeric urea derivatives. Initially, we designed, synthesized, and studied di- and triureas of the general structures **1** and **2** as scaffolds capable of holding two or three peptide strands in proximity. We have begun to prepare tetraureas **3**, and we now anticipate being able to prepare higher oligomers, such as **4**.

Each of these oligoureas incorporates a phenyl group at the "bottom" of the scaffold and a cyanoethyl group at the "top". The phenyl group provides conformational control. In ureas and amides in which one of the nitrogen atoms bears both a phenyl group and an alkyl group, there

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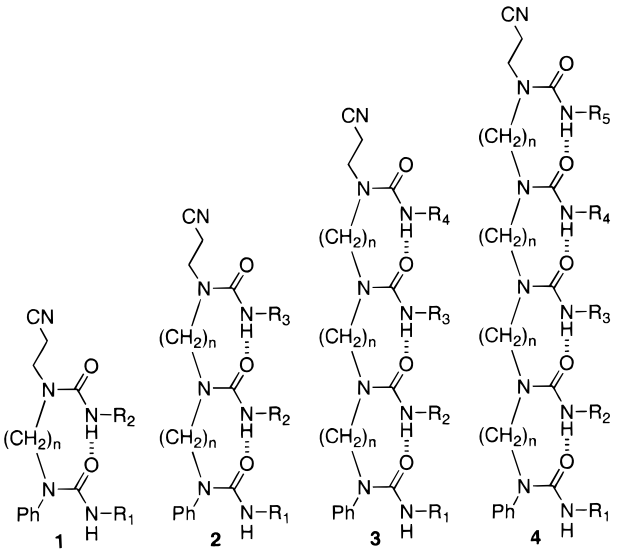
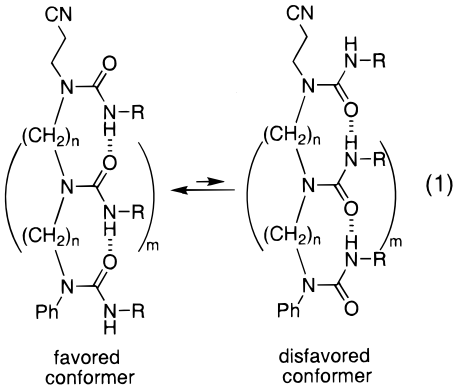


is a strong conformational bias for the phenyl group to be s-trans to the carbonyl group.[7] This bias makes the "lower" carbonyl group point "upward" (eq 1). Intra-



(1)

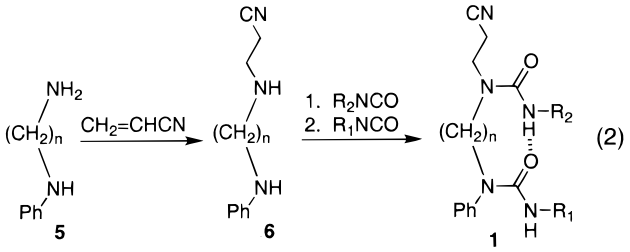
molecular hydrogen bonding aligns the "upper" carbonyl groups in the same direction. This conformational control is important, because it distinguishes the upper and lower edges of the attached peptide strands and promotes conformational homogeneity of artificial β-sheets containing the oligourea scaffold.

The scaffold must be "capped" by some sort of alkyl group, to make the top urea group point upward. The capping is best accomplished during the synthesis of the oligourea scaffold, by alkylating the primary amino group at the top of its polyamine backbone. The cyanoethyl group provides a convenient cap, because it is readily introduced by reaction of a primary amine with acrylonitrile. Recently, we discovered that the cyanoethyl group also helps stabilize the hydrogen-bonded conformation of the scaffold and that the cyanoethyl group, like the phenyl group, plays a key role in rigidifying the molecular scaffold.[8]

To determine the optimal length of the methylene chain linking the urea groups, we prepared a number of simple diurea derivatives with two- and three-carbon linkers (**1**, $n = 2$, 3) and studied them by IR and NMR spectroscopy in chloroform solution.[9] These studies revealed that diureas containing the two-carbon linker are largely or wholly hydrogen bonded, while diureas containing the three-carbon linker are partially hydrogen bonded. In the infrared spectra, the former compounds show only NH stretching bands associated with the hydrogen-bonded state, while the latter compounds show bands associated with both hydrogen-bonded and non-hydrogen-bonded conformers. In the ^{1}H NMR spectra, NH resonances of the upper urea groups of the two-carbon compounds appear 2−3 ppm downfield of those of suitable controls, while those of the three-carbon compounds are downfield shifted to a lesser extent. These studies indicate that both the two- and three-carbon linkers are suitable for forming rigidified scaffolds but that the two-carbon linker is especially well suited.

Synthesis of Oligourea Molecular Scaffolds

We have developed syntheses of di-, tri-, and higher oligourea molecular scaffolds that allow the regioselective attachment of different substituents at the various urea groups. The synthesis of diurea derivatives **1** is simple, efficient, and high yielding (eq 2).[9b] Reaction of *N*-phenyl-

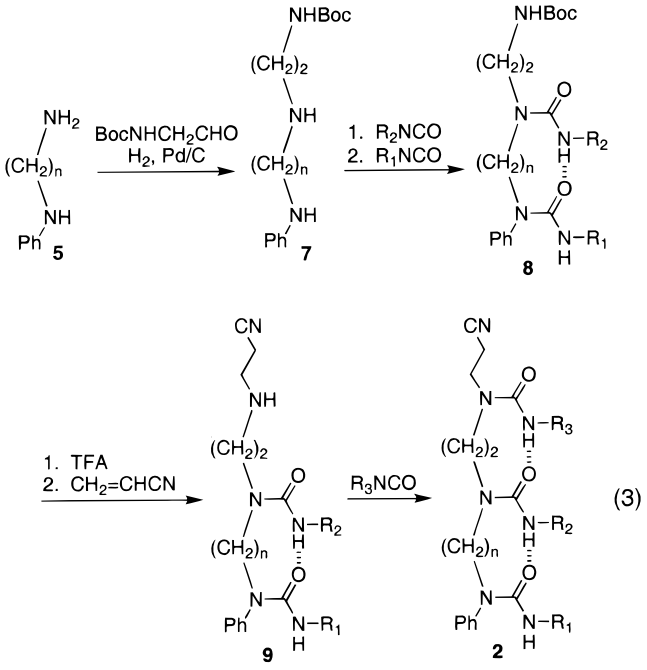


(2)

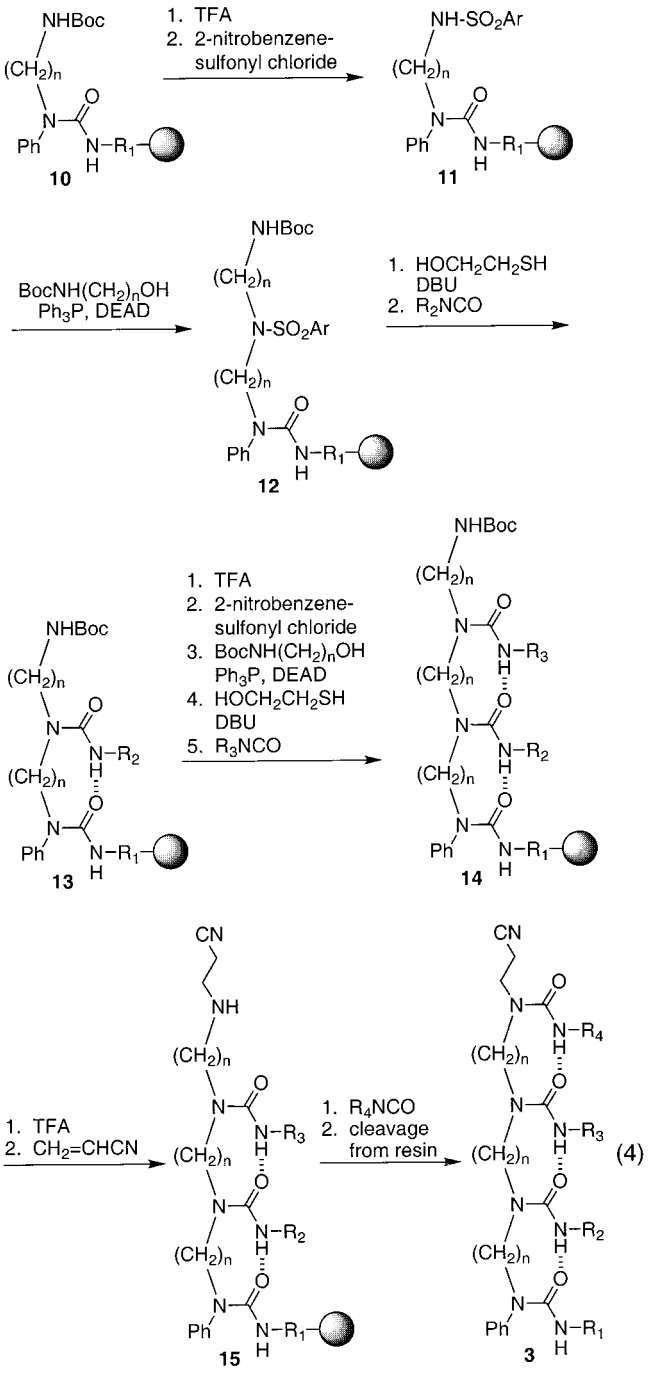
1,2-ethanediamine (**5**, $n = 2$) or *N*-phenyl-1,3-propanediamine (**5**, $n = 3$) with acrylonitrile affords aminonitrile **6** in good yield. The addition occurs exclusively at the aliphatic amino group of **5**, which is considerably more basic and nucleophilic than the aromatic amino group. The aliphatic and aromatic amino groups of **6** also differ substantially in nucleophilicity. When 1 equiv of an isocyanate (R_2NCO) is added, it reacts exclusively with the aliphatic nitrogen; when a second 1 equiv of a different isocyanate (R_1NCO) is added, it reacts at the aromatic nitrogen, generating diurea **1**.

By conversion of diamine **5** to Boc-protected triamine **7**, the homologous triurea derivatives **2** can be prepared (eq 3).[10] Reductive alkylation of **5** with Boc-glycinal (*t*-BuO_2CNHCH_2CHO) affords **7**. Treatment of **7** with isocyanates R_2NCO and R_1NCO yields diurea **8**. Removal of the Boc protective group, followed by reaction with acrylonitrile, affords aminonitrile **9**. Reaction of **9** with isocyanate R_3NCO generates triurea **2**.

Solid-phase synthesis provides an attractive alternative to solution-phase synthesis, because excess reagents and reaction byproducts can be washed away, while the desired molecule remains tethered to a solid support. The intermediates need not be isolated, purified, and characterized, allowing the reactions to be carried out in rapid succession. For these reasons, we have adapted the syntheses of diureas **1** and triureas **2** to the solid phase.[11] Recently, we developed an iterative method of synthesiz-



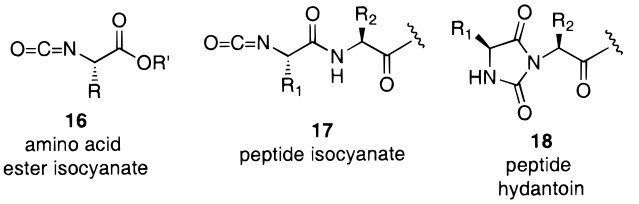
ing larger oligoureas on the solid support (eq 4).[12] This method is based upon Fukuyama's sulfonamide alkylation, in which an amine is converted to the corresponding nitrobenzenesulfonamide, the sulfonamide is alkylated, and the nitrobenzenesulfonyl group is removed by treatment with a thiol.[13] The iterative synthesis begins with urea **10**. Urea **10** is first converted to 2-nitrobenzenesulfonamide derivative **11** by removal of its Boc protective group and reaction of the amino group that is liberated with 2-nitrobenzenesulfonyl chloride. Alkylation of **11** with a Boc-protected amino alcohol under Mitsunobu conditions generates sulfonamide **12**. Removal of the sulfonamide group, followed by reaction with isocyanate R_2NCO, forms diurea **13**. Repetition of this series of five steps yields triurea **14**. Cleavage of the Boc protective group of **14**, followed by alkylation with acrylonitrile, affords amine **15**. Reaction of **15** with isocyanate R_4NCO, followed by cleavage from the resin, liberates tetraurea **3**.



Peptide Isocyanates

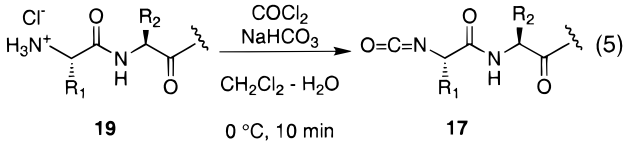
As the preceding equations have shown, the reaction of amines with isocyanates provides a convenient and efficient method of preparing ureas. To prepare oligoureas bearing peptides, we required isocyanate derivatives of peptides. Although isocyanate derivatives of amino acids (**16**) had been known since 1950, peptide isocyanates (**17**) had not been reported.[14] One reported attempt to prepare a peptide isocyanate resulted in the formation of a peptide hydantoin (**18**).[14] For this reason, we were concerned that peptide isocyanates might not be stable and might spontaneously cyclize to the corresponding hydantoins.

When we began our studies, the best method for preparing amino acid ester isocyanates involved the reaction of amino acid ester hydrochlorides with gaseous phosgene.[14] This method has the disadvantages that it requires high temperatures and liberates hydrogen chloride as a byproduct and that gaseous phosgene is extremely hazardous. We improved the method by adding either pyridine or aqueous sodium bicarbonate as a base



and by using either triphosgene or a solution of phosgene in toluene, instead of gaseous phosgene.[15,16] Under these conditions, the reaction proceeds rapidly at room tem-

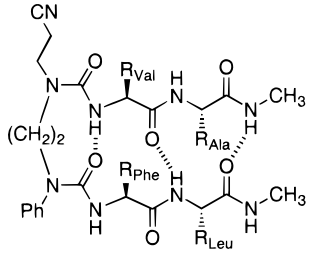
perature or at 0 °C. The mildness of these reaction conditions permits the preparation of peptide isocyanates (eq 5).



The peptide isocyanates allow the introduction of peptide strands during the synthesis of artificial β-sheets. Because isocyanates react cleanly with nucleophiles, such as amines and alcohols, these compounds are also attractive synthetic building blocks for the creation of biologically relevant molecules. For this reason, we anticipate that peptide isocyanates will find many applications in combinatorial chemistry and drug discovery efforts.

A Parallel Artificial β-Sheet

With convenient procedures for the preparation of oligoureas and peptide isocyanates in hand, we set out to test the idea of using these building blocks to create artificial β-sheets. We began these endeavors by synthesizing and studying artificial β-sheet **20**, in which a diurea

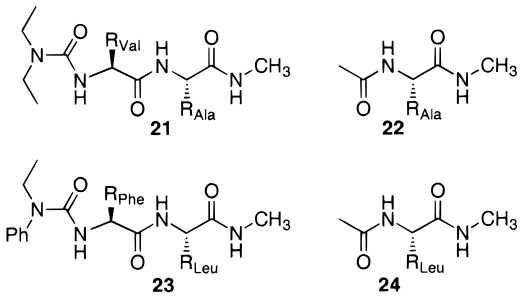


artificial β-sheet **20**

molecular scaffold juxtaposes two dipeptide strands.[17] We chose to incorporate phenylalanine, leucine, valine, and alanine residues into the dipeptides, because these nonpolar amino acids lack hydrogen-bonding side-chain functionality, which might complicate the structure of the β-sheet.

^{1}H NMR studies reveal that **20** adopts a β-sheet conformation in $CDCl_3$ solution. In this solvent, the chemical shift of NH protons is a good indicator of their hydrogen-bonding states; hydrogen-bonded NH protons generally appear 2−3 ppm downfield of similar NH protons that are not hydrogen bonded. Comparison of the chemical shifts of the NH groups of **20** to those of controls **21**−**24** indicates that the valine NH, leucine NH, and alanine methylamide NH groups are hydrogen bonded. The valine NH group is shifted downfield by 1.9 ppm, indicating that it is largely hydrogen bonded; the leucine NH and alanine methylamide NH are shifted downfield by 1.0 and 0.9 ppm, suggesting that these groups are partially hydrogen bonded.

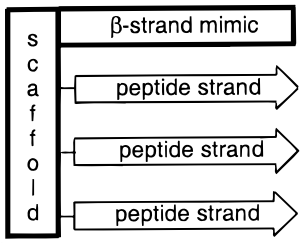
Nuclear Overhauser effect (NOE) experiments indicate that the upper and lower dipeptide strands are next to each other. Thus, the alanine α-proton exhibits NOEs with the leucine NH and side-chain protons, and the valine NH proton exhibits an NOE with the phenylalanine α-proton. Figure 1a provides a model of artificial β-sheet **20** and illustrates these interstrand NOEs. Although the NOE and chemical shift data establish that **20** adopts a β-sheetlike structure, the chemical shift data also suggest that the structure frays and is fragile.



Artificial β-Sheets Containing β-Strand Mimics

We decided to see whether we could create artificial β-sheets that were sturdier and less fragile, by incorporating a second template that is complementary to the oligourea molecular scaffold. This template is designed to mimic the geometry and hydrogen-bonding functionality of one edge of a peptide in a β-strand conformation and is more rigid than a peptide. The molecular scaffold and the β-strand mimic work in conjunction to create a sort of corner bracket, which should better induce β-sheet formation in attached peptide strands. Chart 2 illustrates this idea.

Chart 2



artificial β-sheet containing a β-strand mimic

Kemp and co-workers had previously developed a β-strand mimic that is complementary in length and hydrogen-bonding functionality to a dipeptide and had established that this template can help induce β-sheet formation in an attached peptide strand.[18] Kemp's β-strand mimic is a fused tetracyclic molecule that is difficult to synthesize and incorporate into β-sheetlike structures because it is insoluble in common solvents. We did not envision this template as being suitable for use in our structures because it is insoluble and because we wanted to be able to prepare larger homologues that are complementary in length to longer peptides.

For this reason, we set out to develop a class of β-strand mimics that could be prepared in varying lengths and was not based on a fused polycyclic system. Molecular modeling and X-ray crystallographic studies suggested that

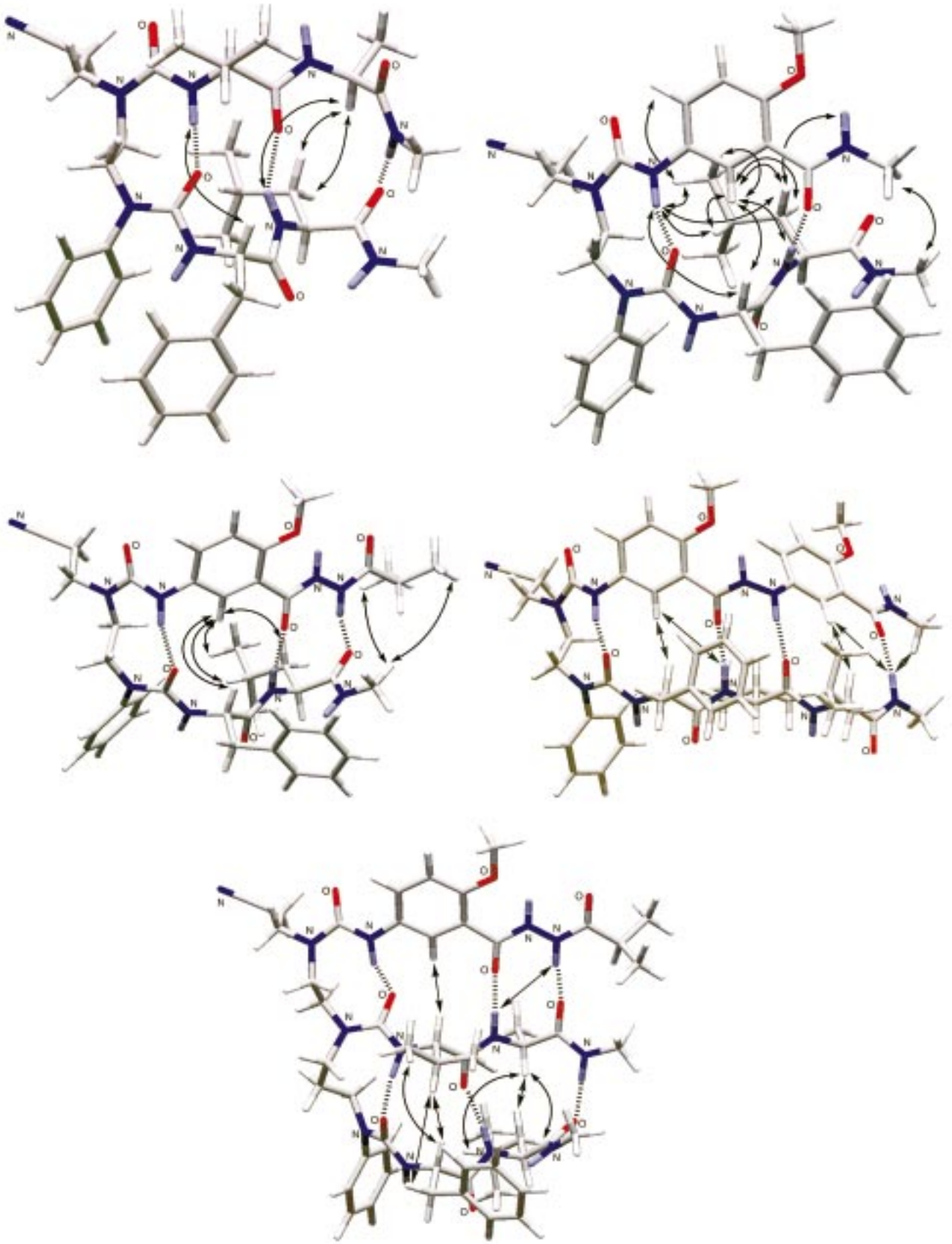
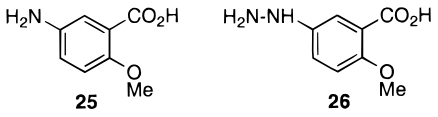


FIGURE 1. Models of artificial β-sheets illustrating important interstrand NOEs (arrows): (a, top left) **20**; (b, top right) **27**; (c, middle left) **28**; (d, middle right) **29**; (e, bottom) **30b**.

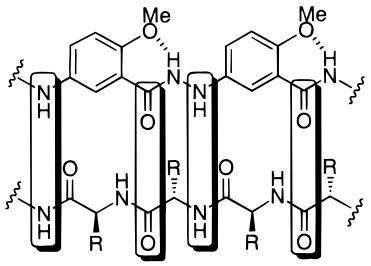
derivatives of 5-amino-2-methoxybenzoic acid (**25**) and 5-hydrazino-2-methoxybenzoic acid (**26**) would be suitable. Amide or hydrazide derivatives of these compounds provide an alternating array of hydrogen-bond donors and



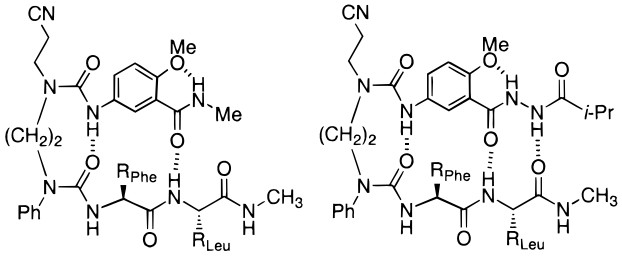
25 **26**

acceptors similar to those of one edge of a peptide in a β-strand conformation. Chart 3 illustrates this relationship.

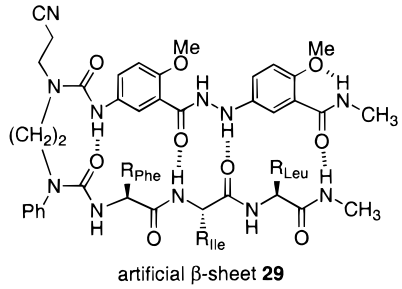
Chart 3



We prepared artificial β-sheets **27**−**29** to test these β-strand mimics and to evaluate the idea of using two templates in conjunction with peptide strands to create



artificial β-sheet **27** artificial β-sheet **28**



artificial β-sheet **29**

β-sheet structures.[19−21] Artificial β-sheet **27** is an analogue of **20**, in which the upper peptide strand has been replaced with a 5-amino-2-methoxybenzamide β-strand mimic that is complementary in length to a monopeptide. In **28**, the β-strand mimic has been elongated by replacement of the amide group with a hydrazide group, making it complementary in length to a dipeptide. In artificial β-sheet **29**, the β-strand mimic has been elongated by coupling a 5-hydrazino-2-methoxybenzamide unit to the 5-amino-2-methoxybenzoic acid unit. This β-strand mimic is complementary in length to a tripeptide; the lower peptide strand has, in turn, been extended by addition of a third amino acid residue.

^{1}H NMR chemical shift studies indicate that **27**−**29** form stable hydrogen-bonded structures in chloroform solution.[19−21] In these compounds, all but one of the hydrogen-bonded NH groups appear about 2 ppm downfield of controls that are not hydrogen bonded. In $CDCl_3$ solution, non-hydrogen-bonded peptide amide protons typically appear at about 6 ppm, while hydrogen-bonded peptide amide protons typically appear at about 8 ppm. Thus, the leucine NH protons of **27** and **28** appear at 8.2 and 8.3 ppm, respectively, and the isoleucine NH proton of **29** appears at 8.1 ppm. In contrast, the leucine methylamide NH protons of **27** and **28** appear at 5.5 and 5.8 ppm, respectively, and the leucine NH proton of **29** appears at 6.1 ppm. In each of these compounds, the urea NH group of the β-strand mimic is shifted exceptionally far downfield, appearing 3.6−3.7 ppm downfield of controls that are not hydrogen bonded.

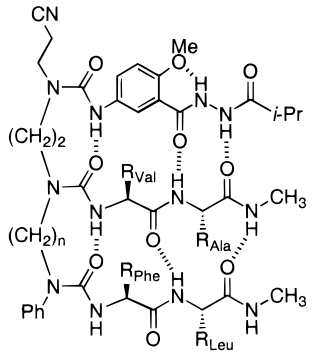
These ^{1}H NMR chemical shift studies indicate that artificial β-sheets **27**−**29** are more stable and well-structured than artificial β-sheet **20**. The large downfield shifting of the hydrogen-bonded NH groups in **27**−**29** suggests that these NH groups are largely or wholly hydrogen bonded. In contrast, the leucine NH and alanine methylamide NH groups of **20** are shifted downfield by only about 1 ppm, suggesting that these groups are partially hydrogen bonded.

Nuclear Overhauser effect experiments indicate that **27**−**29** adopt β-sheetlike structures. Each of these compounds exhibits a network of NOEs between the β-strand mimic and the peptide strand that is consistent with a β-sheet structure. The protons at the 6-positions of the aromatic rings of the β-strand mimics provide a particularly rich array of NOEs because they are right next to the adjacent peptide strands. Parts b−d of Figure 1 provide models of these molecules that illustrate the observed interstrand and other long-range NOEs. Collectively, the NOE and chemical shift data indicate that the oligourea molecular scaffold and the β-strand mimic work in conjunction to induce β-sheet formation in the attached peptide strands.

Three-Stranded Artificial β-Sheets

To further test the effectiveness of the dual-template strategy, we prepared three-stranded analogues of artificial β-sheet **20** that incorporate a β-strand mimic as the top strand.[12,22] X-ray crystallographic studies of triurea derivatives of diethylenetriamine (**2**, $n = 2$) had shown that the oligourea molecular scaffold has a distinct curvature when the urea groups are linked by two-carbon chains.[9b] This curvature raised concerns that the triurea derivatives containing two-carbon linkers might not permit the proper alignment of the peptide strands of a three-stranded artificial β-sheet. For this reason, we prepared two variants of the three-stranded artificial β-sheet, **30a** and **30b**. In **30a**, two-carbon linkers connect both the bottom and middle urea groups and the middle and top urea groups; in **30b**, a three-carbon linker connects the bottom and middle urea groups and a two-carbon linker connects the middle and top urea groups.

^{1}H NMR chemical shift studies indicate that artificial β-sheets **30** are hydrogen bonded in chloroform solution. In these compounds, most of the hydrogen-bonded NH groups appear about 2 ppm downfield of suitable controls.



artificial β-sheet **30a** (n = 2)
artificial β-sheet **30b** (n = 3)

As in the case of artificial β-sheets **27**−**29**, the urea NH groups of the β-strand mimics of **30** are shifted exceptionally far downfield, appearing 3.7−4.1 ppm downfield of controls that are not hydrogen bonded.

These studies suggest that artificial β-sheets **30** are more robust than artificial β-sheet **20**. In artificial β-sheet **20**, the leucine NH and alanine methylamide NH resonances appear 1.0 and 0.9 ppm downfield of the controls. In **30a**, these resonances are 1.8 and 1.9 ppm downfield, and in **30b**, they are 1.9 and 1.4 ppm downfield. The greater downfield shifting of these resonances establishes that the β-strand mimic helps further stabilize β-sheet structure in these compounds.

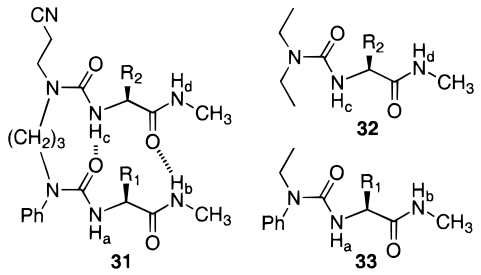
NOE studies further establish that **30a** and **30b** adopt β-sheet conformations. Both of these compounds exhibit NOEs between the β-strand mimic and middle peptide strand and between the middle and lower peptide strands. Figure 1e provides a model of **30b** and illustrates the NOEs detected using the NOESY technique. Because the molecular weights of artificial β-sheets **30** are just over 1000, these NOEs could not conveniently be measured at room temperature, and the NOESY experiments were performed at −15 °C. We have also performed these studies at room temperature in the rotating frame using the transverse-ROESY (Tr-ROESY) method, with largely similar results.[23]

Propensities of Amino Acids To Form Parallel β-Sheets

Having developed a practical approach to creating β-sheet structures, we decided to apply our approach to an important problem—understanding protein folding. The folding of proteins is so fundamental to their function that it has been termed the "second genetic code". Unlike the "first" genetic code, the protein folding code remains largely uncracked.

One approach to understanding protein folding is to determine the propensities of different amino acids to form various secondary structures.[24] Recently, several teams of researchers measured the β-sheet-forming propensities of amino acids by systematically varying amino acids within small β-sheet-containing proteins and quantifying the effects of these mutations upon the thermodynamic stabilities of these proteins.[25−27] These studies have focused upon the propensities of amino acids to form antiparallel or mixed β-sheets; similar studies of the propensities of amino acids to form parallel β-sheets were lacking.

Since our oligourea scaffold is ideally suited to creating parallel β-sheets (e.g., **20**), we decided to use our system to determine the propensities of amino acids to form parallel β-sheets.[28] To do this, we prepared a combinatorial library of artificial β-sheets **31**, in which two amino

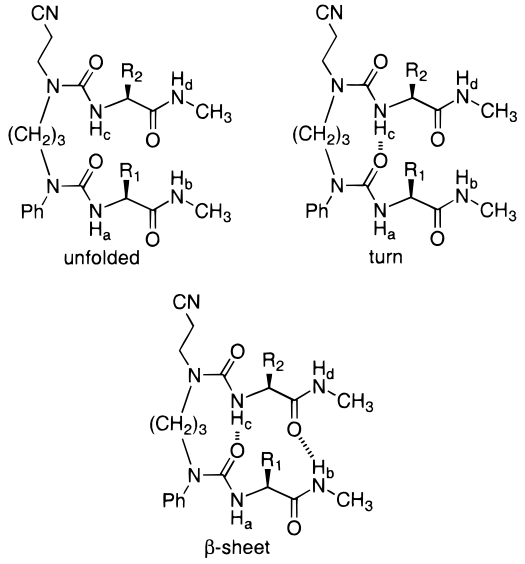


R_1, R_2 = H (Gly), Me (Ala), *i*-Pr (Val), *i*-Bu (Leu)

acids are attached to a diurea molecular scaffold to form a minimal artificial parallel β-sheet. The library comprises 16 compounds, with glycine, alanine, valine, and leucine residues in the top and bottom halves of the β-sheets. As controls, we prepared two four-membered libraries, **32** and **33**, which mimic the top and bottom halves of **31**.

Artificial β-sheets **31** adopt both β-sheetlike and non-β-sheetlike conformations, which are in rapid equilibrium (Chart 4). The β-sheet-forming propensities of the com-

Chart 4



ponent amino acids determine the position of this equilibrium, and the ^{1}H NMR chemical shifts of the NH groups in **31** reflect the position of the equilibrium. When **31** adopts a β-sheet conformation, the methylamide NH of the lower amino acid (H_b) is hydrogen bonded. Thus, the degree of downfield shifting of H_b in **31**, relative to that of control **33**, reflects the degree of β-sheet structure.

The degree of downfield shifting of H_b for all 16 compounds **31** is presented graphically in Figure 2. One of the advantages of using a combinatorial library for these

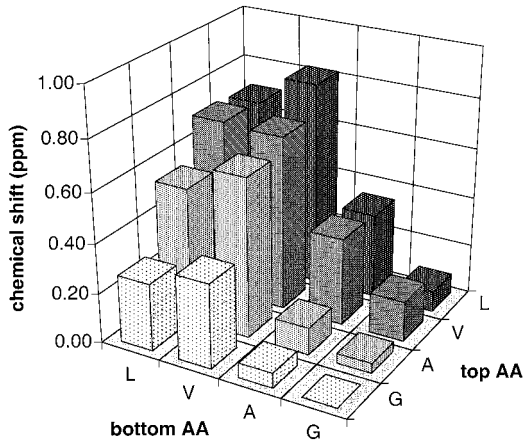


FIGURE 2. ^{1}H NMR chemical shift differences between H_b of artificial β-sheets **31** and controls **33**.

studies is that redundant data are generated and anomalies are minimized. Thus, Figure 2 provides *eight* series with which to make comparisons. These eight series consist of the four rows and four columns of the figure and involve four sets of compounds in which the top amino acid is held fixed and the bottom amino acid is varied and four sets of compounds in which the bottom amino acid is held fixed and the top amino acid is varied. From the four rows and four columns in the figure, it is evident that leucine and valine are relatively good at forming parallel β-sheets, alanine is moderate, and glycine is poor (L, V > A > G). This trend largely parallels the statistical propensities of amino acids to form β-sheets (P_β), which were first described by Chou and Fasman (P_β = 1.65, 1.22, 0.97, and 0.81 for valine, leucine, alanine, and glycine).[24]

Folding of Artificial β-Sheets in Competitive Solvents

Until recently, we have focused upon developing artificial β-sheets that fold in chloroform solution because β-sheets often occur in the hydrophobic core of proteins, which is much less polar than water.[29] However, we have now begun to study artificial β-sheets in aqueous solution, since proteins fold in water. Water and other highly polar solvents are challenging because they can hydrogen-bond to amide and urea groups and compete with the intramolecular hydrogen bonds that help stabilize the β-sheet structure. To determine the effect of competitive solvents, we performed NOE studies on artificial β-sheet **27** in chloroform, methanol, 50% aqueous methanol, and dimethyl sulfoxide solutions using the Tr-ROESY method.[23,30] Figure 3 illustrates key NOEs detected through these studies. In chloroform, there are many interstrand NOEs between the β-strand mimic and the dipeptide; in methanol and aqueous methanol, there are fewer interstrand NOEs; and in dimethyl sulfoxide there are none. In dimethyl sulfoxide, many short-range NOEs involving the urea groups are inconsistent with β-sheet structure; in aqueous methanol and in methanol, these inconsistent NOEs are fewer and weaker; and in chloroform, they are absent. These studies indicate that artificial β-sheet **27** is

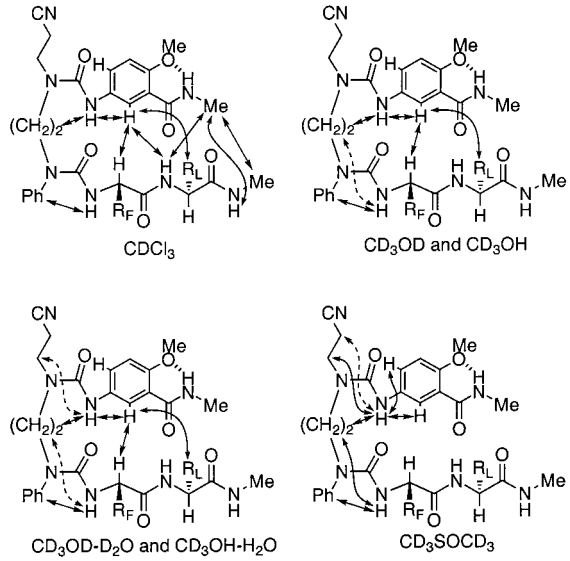


FIGURE 3. Key NOEs detected by Tr-ROESY studies of artificial β-sheet **27** in chloroform, methanol, aqueous methanol, and dimethyl sulfoxide. Interstrand NOEs are shown with arrows between the upper and lower halves of the molecule. Short-range NOEs involving the urea groups are shown with arrows to these groups; dotted arrows represent short-range NOEs that are relatively weak.

tightly folded into a β-sheet in chloroform, partially folded into a β-sheet in methanol and aqueous methanol, and unfolded in dimethyl sulfoxide.

Conclusions and Future Directions

The studies described above have established that our approach to creating β-sheet structures works. The oligourea molecular scaffold holds multiple peptide and peptidomimetic strands in proximity and induces β-sheet formation. The β-strand mimic helps reinforce and rigidify the β-sheets. The solution-phase and solid-phase syntheses are practical and permit the artificial β-sheets to be easily prepared. The peptide isocyanates provide an efficient means of introducing the peptide strands into the artificial β-sheets. The ^{1}H NMR chemical shift and NOE studies indicate that the artificial β-sheets fold properly in chloroform solution.

One of the directions in which we are now heading is to build larger artificial β-sheets. For many years, chemists and biochemists have dreamed of creating artificial proteins that can mimic the well-defined structures and diverse functions of naturally occurring proteins. The artificial β-sheets that we have developed thus far are smaller than most naturally occurring β-sheets. In the future, we will prepare larger artificial β-sheets containing more peptide strands and longer peptide strands; we are currently using the iterative method of synthesizing larger oligoureas (eq 4) to prepare four-stranded artificial β-sheets.[12] We are also developing longer β-strand mimics and new β-strand mimics that may have better synthetic and structural properties than the ones that we are currently using.[31] Eventually, we wish to develop molecular receptors and catalysts based upon these β-sheet

structures. Another exciting area that we wish to explore involves modulating β-sheet interactions among proteins and peptides. β-Sheet formation plays a critical role in many diseases, including AIDS, Alzheimer's disease, and prion diseases. Researchers have begun to explore therapeutic strategies aimed at blocking β-sheet formation and have reported approaches in which peptide derivatives block the dimerization of HIV-1 protease[32] and the self-assembly of β-amyloid.[33] We believe that our artificial β-sheets and β-strand mimics are ideally suited to blocking β-sheet interactions among peptides and proteins, and we are now beginning to study their effect upon β-amyloid aggregation and other processes involving β-sheet interactions.

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