Artificial β-Sheets

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1 Introduction

In the early 1950s, Pauling and coworkers published an ingenious series of papers in which they used wooden molecular models in conjunction with X-ray crystallography to elucidate the regular conformational patterns exhibited by peptides and proteins.[1] These patterns have become known as *secondary structures* and include α-helices, β-turns, and β-strands.[2–5] In a β-strand, the peptide main-chain adopts an extended conformation with a pleated shape (Fig. 1). The amino acid side-chains (shown as methyl groups in Fig. 1) project above and below the faces of the β-strand, while the amide NH and C=O groups stud its edges.

Isolated β-strands rarely occur in proteins, and the edges of β-strands are generally hydrogen bonded to each other to form β-sheets. The relative orientations of the β-strands may be parallel, antiparallel, or mixed. Parallel β-sheets are characterized by a series of twelve-membered hydrogen-bonded rings, while antiparallel β-sheets are characterized by an alternating series of ten- and fourteen-membered hydrogen-bonded rings (Figs. 2 and 3).

β-Sheets are important to the structure and biological activity of many peptides and proteins. Silk is composed predominantly of β-sheets, and most other proteins contain β-sheets as key structural elements. β-Sheets are involved in processes as diverse as electron transfer, protein dimerization, and substrate recognition by proteolytic enzymes. The deposition of an insoluble polypeptide with

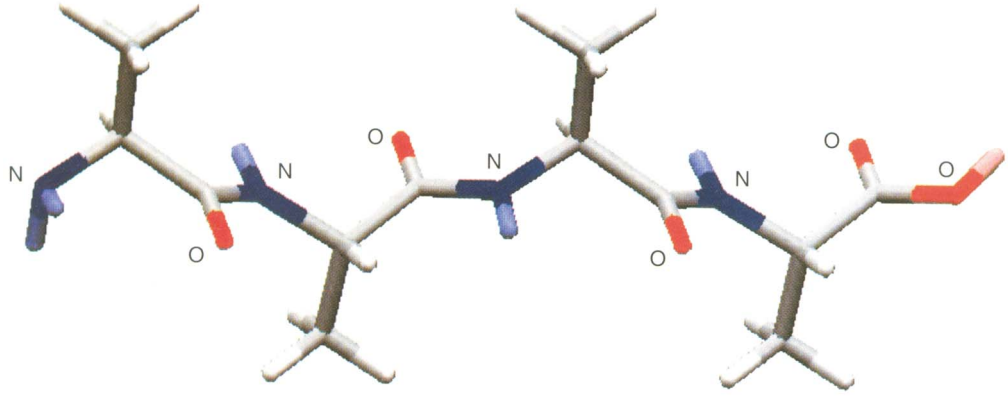
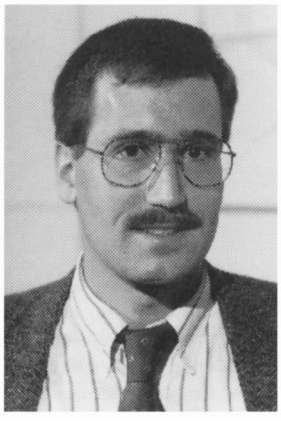
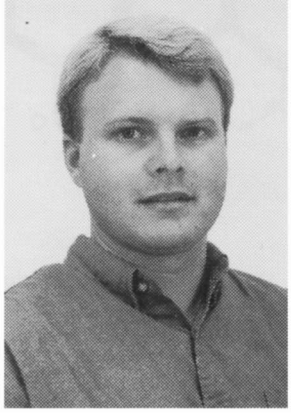


Figure 1 A pentapeptide in a β-strand conformation

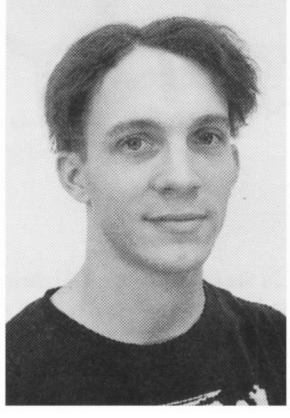
James S. Nowick was born in Scarsdale, New York (USA) in 1964 and received his AB degree from Columbia University in 1985. He was a National Science Foundation (NSF) graduate fellow at the Massachusetts Institute of Technology (MIT) under the supervision of Professor Rick L. Danheiser. After earning his PhD in 1990, he continued at MIT as an NSF postdoctoral fellow in the laboratories of Professor Julius Rebek. In 1991, he began his independent career as an Assistant Professor at the University of California, Irvine (UCI) and was promoted to Associate Professor in 1996. Professor Nowick's honours and awards include a Camille and Henry Dreyfus Foundation award for distinguished newly appointed faculty (1991), an American Cancer Society Junior Faculty Research Award (1992), a National Science Foundation Young Investigator Award (1992), an Arnold and Mabel Beckham Foundation Young Investigator Award (1994), a UCI Award for Outstanding Faculty Contributions to Undergraduate Research (1995), a Presidential Faculty Fellow Award (1995), and a Camille Dreyfus Teacher-Scholar Award (1996).



Eric M. Smith was born in Cincinnati, Ohio (USA) in 1969. He earned BS degrees in both chemistry and biological sciences from UCI. He is currently working towards a PhD in organic chemistry under the direction of Professor Nowick. Eric plans to finish his degree in 1997 and begin a career in the pharmaceutical industry.



Mason Pairish was born in Orange, California (USA) in 1970. He earned a BS degree in chemistry in 1992 from the University of California at Riverside and a MS degree at UCI in 1996 under the direction of Professor Nowick. He is currently employed at Roche BioSciences in Palo Alto, California



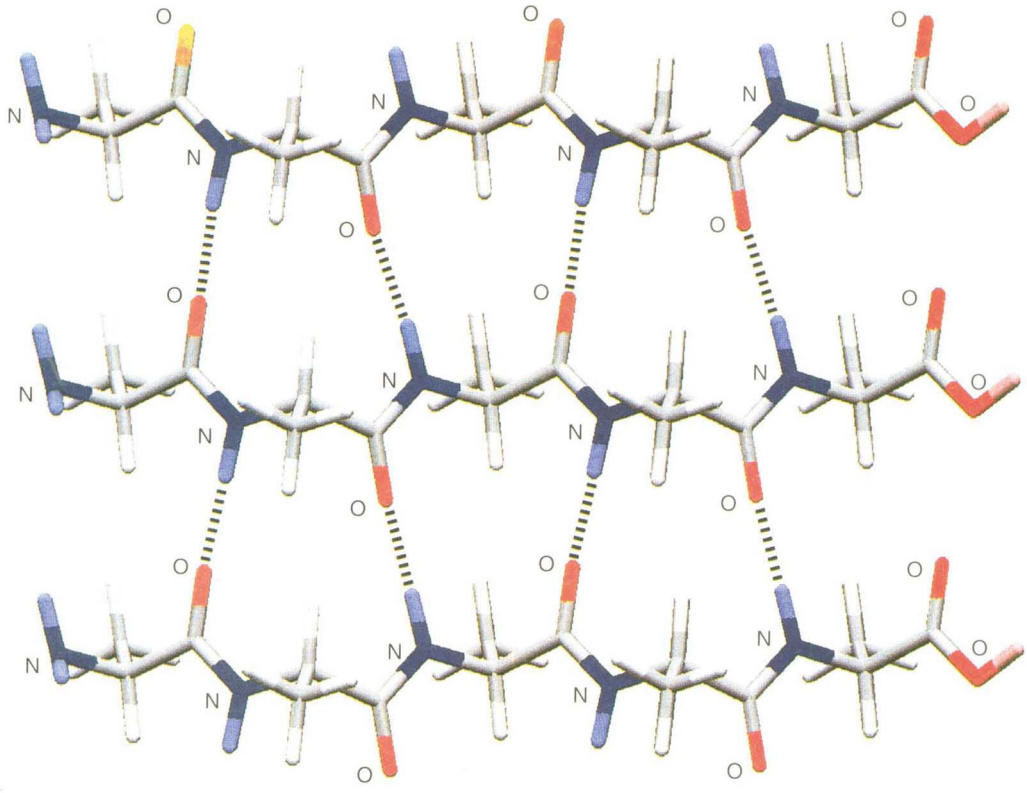


Figure 2 A parallel β-sheet

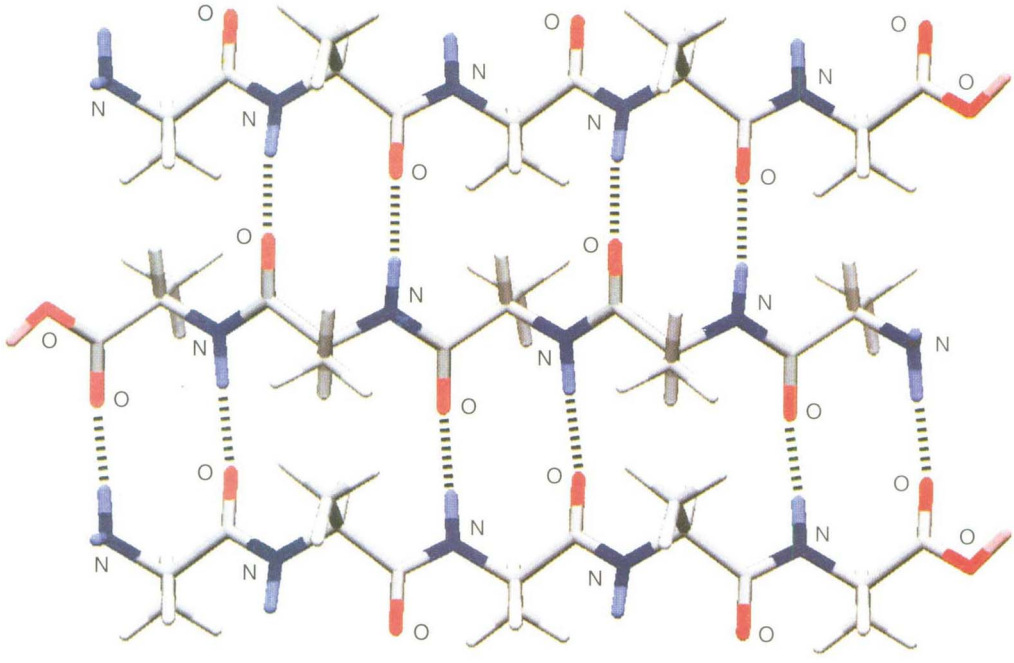


Figure 3 An antiparallel β-sheet

β-sheet structure plays a key role in the progression of Alzheimer's disease. A conformational change involving the conversion of α-helices into β-sheets is involved in scrapie and other prion diseases.

Almost half a century after Pauling's pioneering studies, β-sheets are still not well understood. Although individual amino acids are known to exhibit slight preferences for forming β-sheets, α-helices, or β-turns, and various algorithms for the prediction of protein structure have been developed, the folding pattern of a protein cannot generally be predicted from its sequence of amino acids. An improved understanding of β-sheet structure would help solve the *protein folding problem* and would facilitate the rational design of new drugs. A related problem, the *de novo design* of artificial proteins that form well-defined three-dimensional structures, offers the promise of creating useful molecular receptors and catalysts. Thus

far, there has been good progress in the *de novo* design of α-helical proteins and more limited progress in the *de novo* design of β-sheet proteins

During the past two decades, the development of small molecules that mimic the structures of peptides and proteins has attracted considerable interest, and the field of *peptidomimetic chemistry* has emerged [6–17] Although initial efforts in peptidomimetic chemistry focused upon the development of enzyme inhibitors and peptide hormone analogues, this field now encompasses both the creation of pharmacologically useful analogues of biologically active peptides and the development of compounds that mimic protein structures Current objectives include developing new drugs, gaining an enhanced understanding of protein folding, and creating catalysts and new materials with useful properties

Within the past decade, several research groups have synthesized and studied compounds that mimic the structures and hydrogen-bonding patterns of β-sheets In these compounds, rigid molecular templates stabilize β-sheet structure in attached peptides This review seeks to summarize these studies and explain the growing interest in *artificial β-sheets* These studies involve a bottom-up approach to protein structure and are complementary to top-down approaches, such as the *de novo* design of Richardson and Erickson and the *template assembled synthetic protein* (TASP) approach of Mutter A comprehensive treatment of these areas is beyond the scope of this review Also omitted from this review are β strand mimics, β-turn mimics, and other related templates that have not been used to form hydrogen-bonded β-sheets The reader is directed to references 6–17 for further reading on these areas

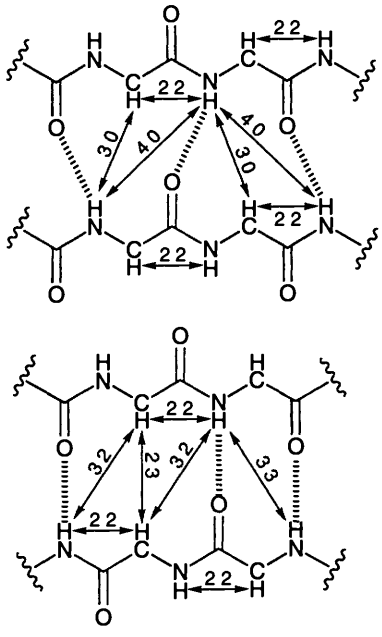


Figure 4 Short interproton distances (Å) in parallel (top) and antiparallel (bottom) β sheets that give rise to characteristic NOEs (ref 18)

2 Techniques for Structural Studies

Studies of artificial β-sheets involve the design and synthesis of molecular templates, the synthesis of compounds in which the templates are linked to peptides, and the structural evaluation of these molecules The literature associated with structural studies of peptides, proteins, and peptidomimetic compounds is sufficiently confusing that data from structural studies are sometimes overinterpreted or misinterpreted To provide a better understanding of the means by which artificial β sheets are studied, this section critically reviews the most important techniques used to elucidate the structure of peptidomimetic compounds, peptides and proteins

X-ray crystallography is perhaps the most powerful technique for studying molecular structure If a compound can be coaxed to generate suitable crystals, X-ray crystallography can rapidly generate an accurate three-dimensional picture of the molecule That this technique generates a unique structure is both an advantage and a limitation X-ray crystallography is unable to identify the range of conformations that may be present in solution, and crystal-packing forces may affect the structure of a molecule in the solid state

NMR spectroscopy offers a wealth of information on the structure of small proteins, peptides and peptidomimetic compounds [18–20] The nuclear Overhauser effect (NOE) can provide either qualitative or quantitative information about the proximity of protons (or other nuclei) Both one-dimensional and two-dimensional (NOESY and ROESY) experiments can be performed with a standard NMR spectrometer, and NOEs between protons separated by distances ranging from less than 2 to more than 4 Å may be detected Since the magnitudes of NOEs decrease as a function of the sixth power of internuclear separation, protons separated by 2 Å generally give very strong NOEs, while protons separated by 4 Å produce much weaker NOEs that are not always observed

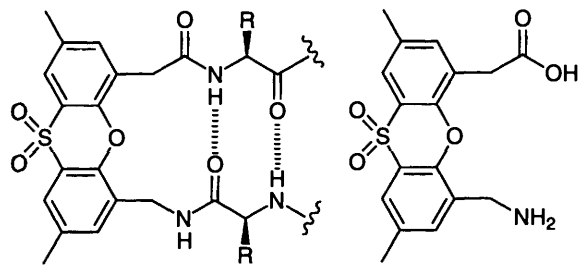
Different secondary structures give characteristic patterns of NOEs Fig 4 illustrates typical interresidue ^{1}H NOEs involving the main-chains of parallel and antiparallel β-sheets Characteristic interproton distances are shown in angstroms Patterns of long-range NOEs involving the α and NH protons of adjacent peptide strands can establish participation in parallel or antiparallel β-sheet structure, while *strong* NOEs between the α and NH protons of sequential residues are consistent with a β-strand conformation The latter NOEs must be interpreted with caution, however, since unstructured peptides also give NOEs between sequential α and NH protons Interstrand NOEs involving the side-chains can provide additional evidence for β-sheet structures In combination with molecular modelling techniques, NOE and other NMR studies can provide structural information that rivals X-ray crystallography in the detail provided

Different secondary structures have different patterns of hydrogen bonding, and the NH resonances in the ^{1}H NMR spectrum often reflect these patterns Protons that are hydrogen bonded intramolecularly or to the solvent appear downfield of protons that are not hydrogen bonded In solvents that do not form strong hydrogen bonds (*e g* $CDCl_3$), hydrogen-bonded peptide amide protons appear at *ca* 8 ppm while non-hydrogen-bonded peptide amide protons appear at *ca* 6 ppm Other types of NH protons, such as those of ureas and aromatic amides, exhibit different characteristic chemical shifts In solvents that are strong hydrogen-bond acceptors (*e g* CD_3SOCD_3 and D_2O), amide protons do not show such pronounced differences in chemical shift

The temperature dependence of the chemical shift of peptide NH groups can also provide evidence for intramolecular hydrogen bonding In CD_3SOCD_3 solution, peptide amide protons that are intramolecularly hydrogen-bonded generally exhibit a small temperature dependence ($-\Delta\delta/\Delta T \leq 2 - 3 \times 10^{-3}$ ppm K^{-1}), while peptide amide protons that are not intramolecularly hydrogen bonded generally exhibit a large temperature dependence ($-\Delta\delta/\Delta T \geq 4 - 5 \times 10^{-3}$ ppm K^{-1}) The situation is more complicated in $CDCl_3$ solution In this solvent, protons that are either not hydrogen bonded or are locked in a hydrogen-bonded conformation exhibit a small temperature dependence in chemical shift, while protons that participate in an equilibrium between a hydrogen bonded state and a non hydrogen bonded state exhibit a large temperature dependence

^{1}H NMR coupling constants provide information about the conformation of the peptide main chain The vicinal coupling constant between the NH and $C_\alpha H$ groups of a peptide ($^3J_{HN\alpha}$) reflects the dihedral angle between these two protons, and hence the main chain ϕ angle Coupling constants greater than 7 Hz are consistent with β sheet structure, while coupling constants less than 6 Hz are consistent with α helical structure Because random coil conformations typically have coupling constants of 7–8 Hz, coupling constants in this range should not be regarded as proof of β-sheet structure Many other NMR phenomena, including heteronuclear coupling constants, rates of exchange of NH protons, ^{13}C relaxation times, and magnetic anisotropy of aromatic rings, can provide additional insight into the structure of peptides, proteins and peptidomimetic compounds

Circular dichroism (CD) spectroscopy has also been widely used to study proteins peptides, and peptidomimetic compounds.[4,5] α-Helices and β-sheets give characteristic CD spectra that are distinct from each other and from those of unstructured (random coil) conformations: α-helices exhibit maxima in the CD spectra at 191 nm and minima at 208 and 222 nm; β-sheets exhibit maxima at 195 and minima at 217 nm; and random coil conformations exhibit minima at 197 and weak maxima at 217 nm. The percentage of each component in a peptide or protein may be estimated by fitting a linear combination of these characteristic spectra to its CD spectrum. In contrast to X-ray crystallography and ^{1}H NMR spectroscopy, CD spectroscopy provides information on the overall structure of a peptide or protein without elucidating structural detail. Furthermore, this technique is not compatible with organic solvents that absorb UV light near 200 nm (*e.g.* chloroform and dimethylsulfoxide), and chromophores in peptidomimetic templates may make unpredictable contributions to CD spectra.



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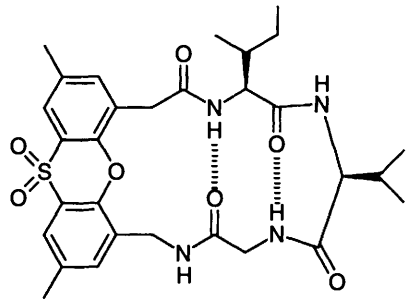

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3 Approaches to Artificial β-Sheets

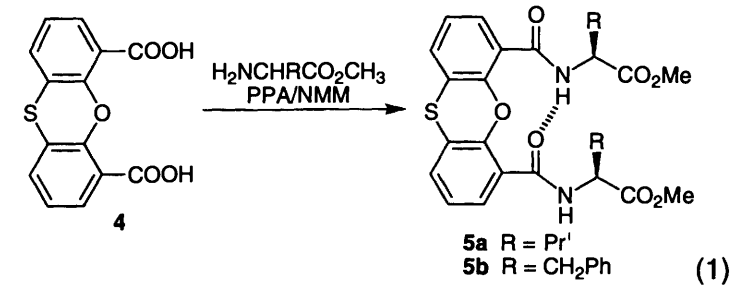
During the past decade, Feigel, Kemp, Kelly, Nowick, and a number of other researchers have designed, synthesized and studied artificial β-sheets. This section summarizes these studies.

3.1 Feigel's Artificial β-Sheets

In 1986, Feigel voiced the concept of using a rigid aromatic template to induce antiparallel β-sheet formation between two attached peptide strands (**1**).[21] Feigel implemented this concept by preparing amino acid **2** and coupling it with the tripeptide Ile-Val-Gly to form cyclopeptide **3**. ^{1}H NMR NOE studies and variable-temperature chemical shift studies of the NH groups in CD_3SOCD_3 indicate that **3** adopts a hydrogen-bonded β-sheet conformation similar to that found in certain cyclic peptides. These studies show that nonpeptide templates can be combined with peptides to form compounds that mimic β-sheets.

In subsequent studies, Feigel and coworkers prepared a variety of cyclopeptides containing pairs of templates. Artificial parallel β-sheets **7** were synthesized by coupling phenoxathiin-4,6-dicarboxylic acid (**4**) with the methyl esters of valine and phenylalanine, followed by coupling of the resulting diesters (**5**) with diamine **6** using the azide method (eqn. 1).[22] The two peptide strands of **7** display two sets of resonances in the ^{1}H NMR spectrum at low temperatures, indicating that they experience two different environments. ^{1}H NMR ROESY studies of **7a** in $CDCl_3$ solution at −61 °C show crosspeaks consistent with a parallel β-sheet structure. In conjunction with ROESY and coupling constant data, molecular modelling studies indicate that **7a** adopts a parallel β-sheet conformation. Fig. 5 provides a model of this structure and illustrates important NOEs.

Artificial antiparallel β-sheets **9** were prepared by coupling suitably protected versions of template **8** and tripeptides, coupling of the resulting tetrapeptides to form octapeptides, and macrocyclization of the octapeptides using the azide method.[23] The biphenyl templates add elements of atropisomeric chirality to macrocycles **9**, causing these compounds to exist as *R,R*-, *S,S*-, and *R,S*-diastereomers. Macrocycle **9b** crystallizes as one of the two possible C_2 symmetric diastereomeric forms, but equilibrates in solution below 0 °C to form a 1.0:0.7:0.2 mixture of the C_2, C_1 and other C_2 isomeric forms. Molecular dynamics calculations and ^{1}H NMR ROESY, coupling constant, and temperature-dependent chemical shift studies in CD_3SOCD_3 solution suggest that the *R,R*-diastereomer predominates and adopts the antiparallel β-sheet conformation shown in Fig. 6. Additional support for this model is provided by the alanine methyl resonance, which appears at 0.34 ppm in the ^{1}H NMR spectrum. The unusual upfield shift of this resonance is consistent with a model in which the methyl group sits over the face of one of the biphenyl rings, as shown in Fig. 6.



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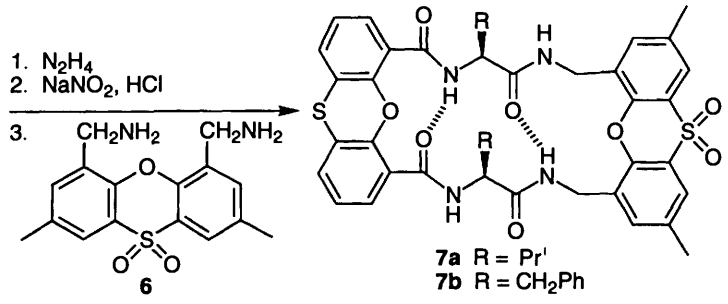
5a R = Pr^i
5b R = CH_2Ph (1)



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7a R = Pr^i
7b R = CH_2Ph

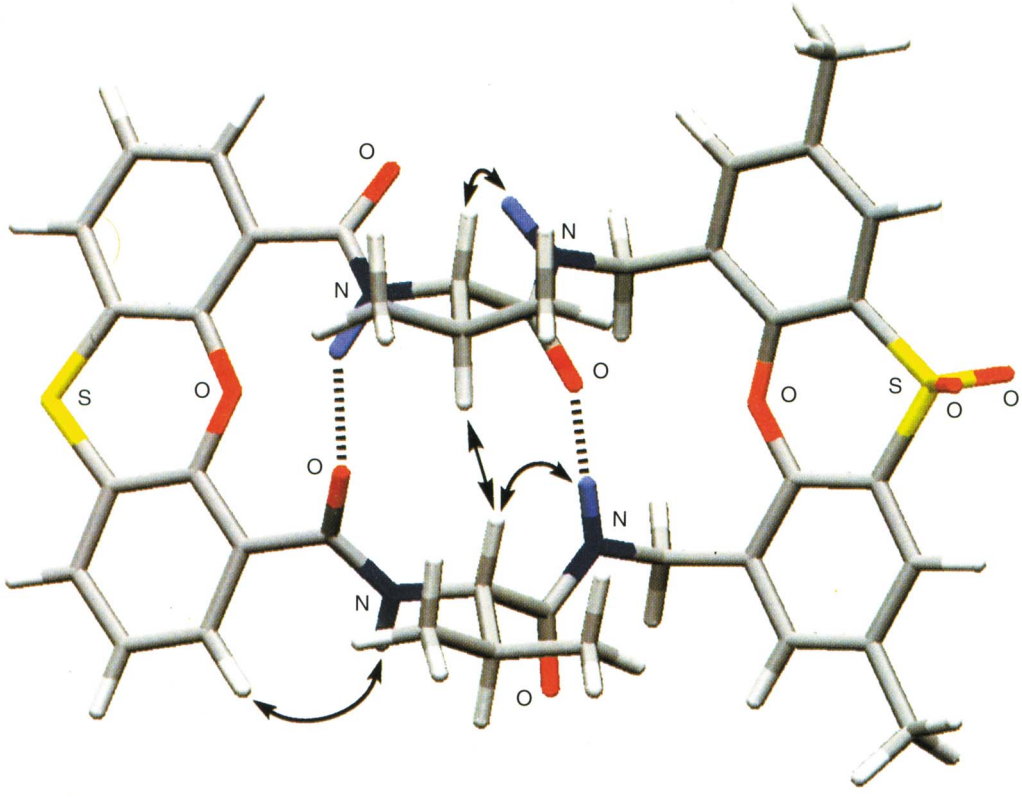
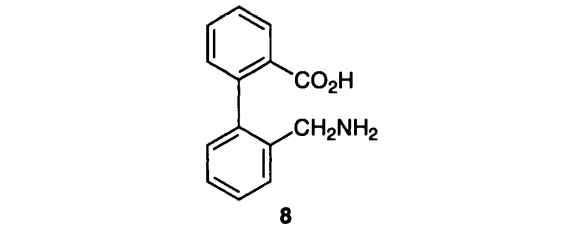


Figure 5 Model of artificial β-sheet **7a** in a minimum energy conformation (local minimum) as calculated using MACROMODEL V5.0 with the AMBER* force field. Sequential and long-range NOEs are shown with arrows.



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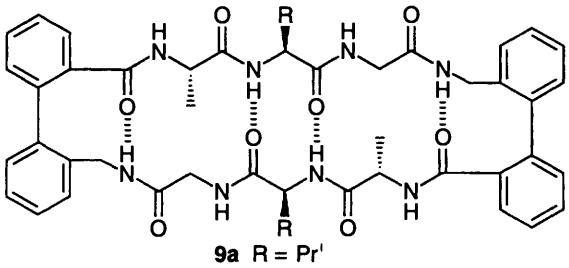


9a R = Pr^{i}
9b R = CH_2Ph

3.2 Kemp's Artificial β-Sheets

Two years after Feigel's initial publication, Kemp and coworkers reported artificial β-sheets featuring a tetracyclic β-strand mimic that duplicates the hydrogen-bonding functionality of one edge of a peptide in a β-strand conformation.[6,24–26] The antiparallel version of the artificial β-sheet (**12**) comprises an epindolidione β-strand mimic connected by Pro-D-Ala β-turns and urea linking groups to dipeptide β-strands. The compound is C_2 symmetric and may be thought of as a pair of two-stranded antiparallel β-sheets sharing a common β-strand mimic.

Artificial β-sheet **12** was prepared as shown in eqn. (2). 2,8-Diaminoepindolidione (**10**) was coupled with Boc-protected D-alanine and proline residues to form **11**. Removal of the Boc protective groups, followed by reaction of the proline residues with an amino acid ester isocyanate ($O{=}C{=}NCHR^3CO_2Et$) to form the urea linker, cleavage of the ethyl ester, and coupling with the last amino acid group afforded **12**. A variety of derivatives of **12** containing different amino acid residues R^3 and R^4 were prepared using this procedure. Derivatives in which the D-Ala and Pro turn region was varied were also synthesized.

X-Ray crystallography reveals that the glycylphenylalanine version of **12** (R^3 = H, R^4 = CH_2Ph) adopts a hydrogen-bonded antiparallel β-sheet conformation in the solid state (Fig. 7). ^{1}H NMR studies indicate that derivatives of **12** containing suitable amino acid groups also adopt an antiparallel β-sheet structure in CD_3SOCD_3 solution. Thus, the glycylphenylalanine version of **12** exhibits NOEs, coupling constants, and variable-temperature chemical shifts of the NH groups consistent with a hydrogen-bonded β-sheet structure.

The ^{1}H NMR chemical shifts of the epindolidione H-3 and NH groups vary among artificial β-sheets **12** comprising different amino acids. The chemical shifts of these groups reflect the degree of β-sheet structure and allow the β-sheet forming propensities of these amino acids to be determined. Derivatives in which residue 4 varies from Gly to Ala to Phe to Val exhibit increasing β-sheet structure. This trend parallels the empirically observed frequencies of these amino acids in protein β-sheets and provides independent corroboration that phenylalanine and valine are good at forming β-sheets.

Artificial parallel β-sheets **13** were prepared by omitting the urea linking groups and connecting two tetrapeptides to the 2,8-diaminoepindolidione template.[27] The valylvaline version of **13** (R^3 = Pr^{i}, R^4 = Pr^{i}) shows NOEs, coupling constants, and chemical shifts consistent with the hydrogen-bonded β-sheet structure shown in Fig. 8. Bulkier amino acids better stabilize the parallel sheets, and the alanylalanine version of **13** (R^3 = Me, R^4 = Me) exhibits little or no β-sheet structure. A water-soluble homologue (**14**) was also prepared. ^{1}H NMR ROESY studies indicate that this compound adopts a β-sheet conformation in aqueous solution.

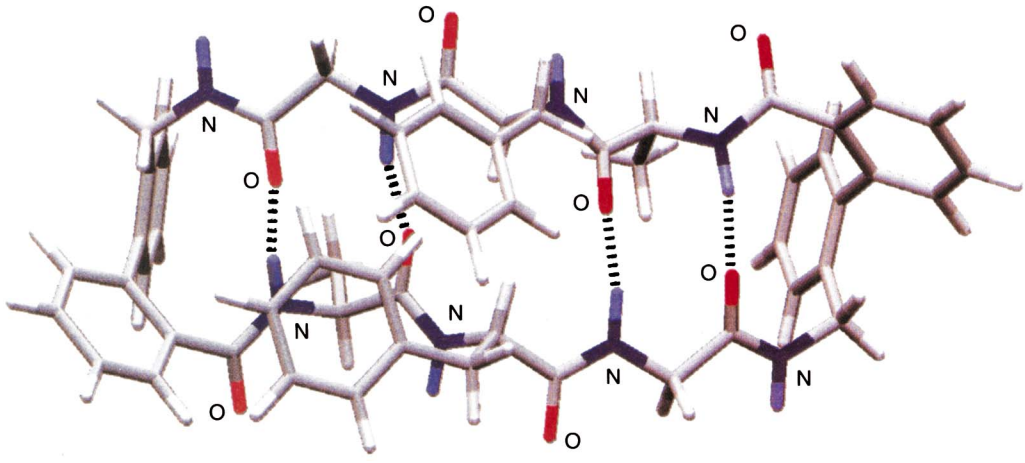
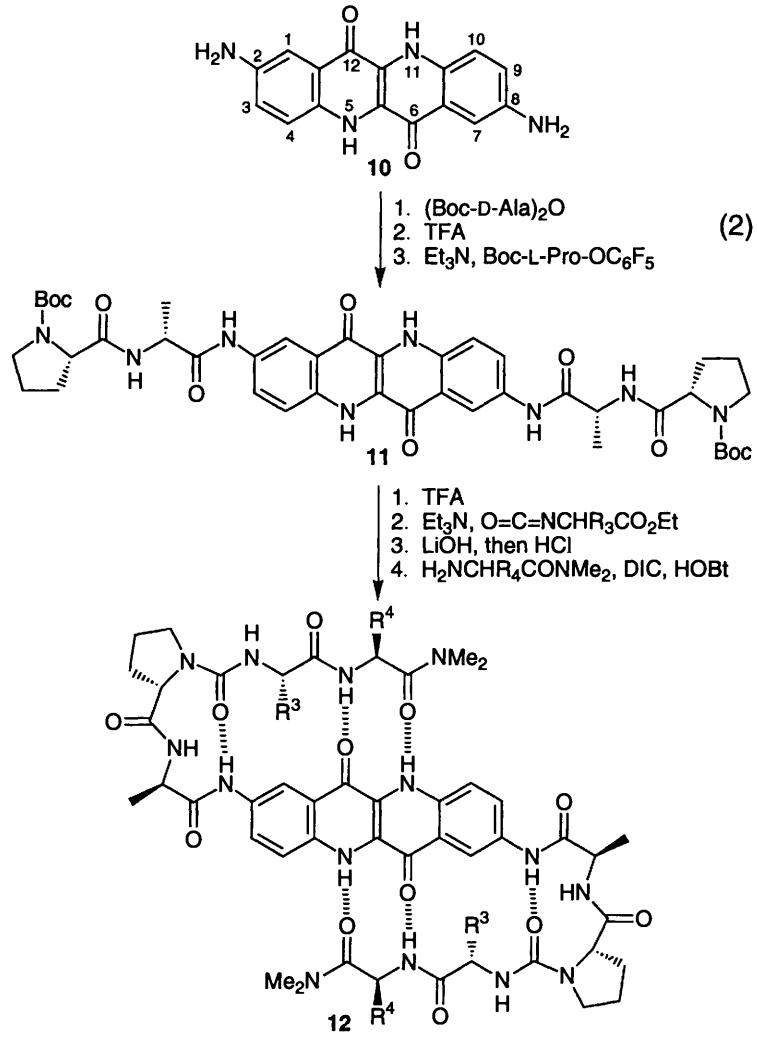


Figure 6 Model of artificial β-sheet **9b** in a minimum energy conformation (local minimum) as calculated using MACROMODEL V5.0 with the AMBER* force field.



3.3 Kelly's Artificial β-Sheets

Beginning in 1991, Kelly and coworkers have published an approach to artificial antiparallel β-sheets that adopt folded structures in aqueous solution.[17,28] Like Feigel, Kelly uses aromatic templates to enforce proximity between two attached peptide strands. However, Kelly's artificial β-sheets are acyclic and are stabilized by hydrophobic effects. Most of Kelly's templates are designed to fold onto the side-chains of the flanking peptide strands, thus forming a hydrophobic cluster and stabilizing a β-sheet structure.

Dibenzofuran template **15** was incorporated into heptapeptides **16** by standard solid-phase synthetic techniques.[29] These compounds were studied by CD and NMR spectroscopy in aqueous solution. Peptide **16a** exhibits minima at both 197 and 214 nm in the CD spectrum, suggesting the presence of both β-sheet and random coil structure. In the ^{1}H NMR spectrum, the R^3 leucine and R^6 valine methyl groups of **16a** appear upfield at 0.36—0.64 ppm and exhibit NOEs to the dibenzofuran rings, indicating that these groups form a hydrophobic cluster. Fig. 9 illustrates these

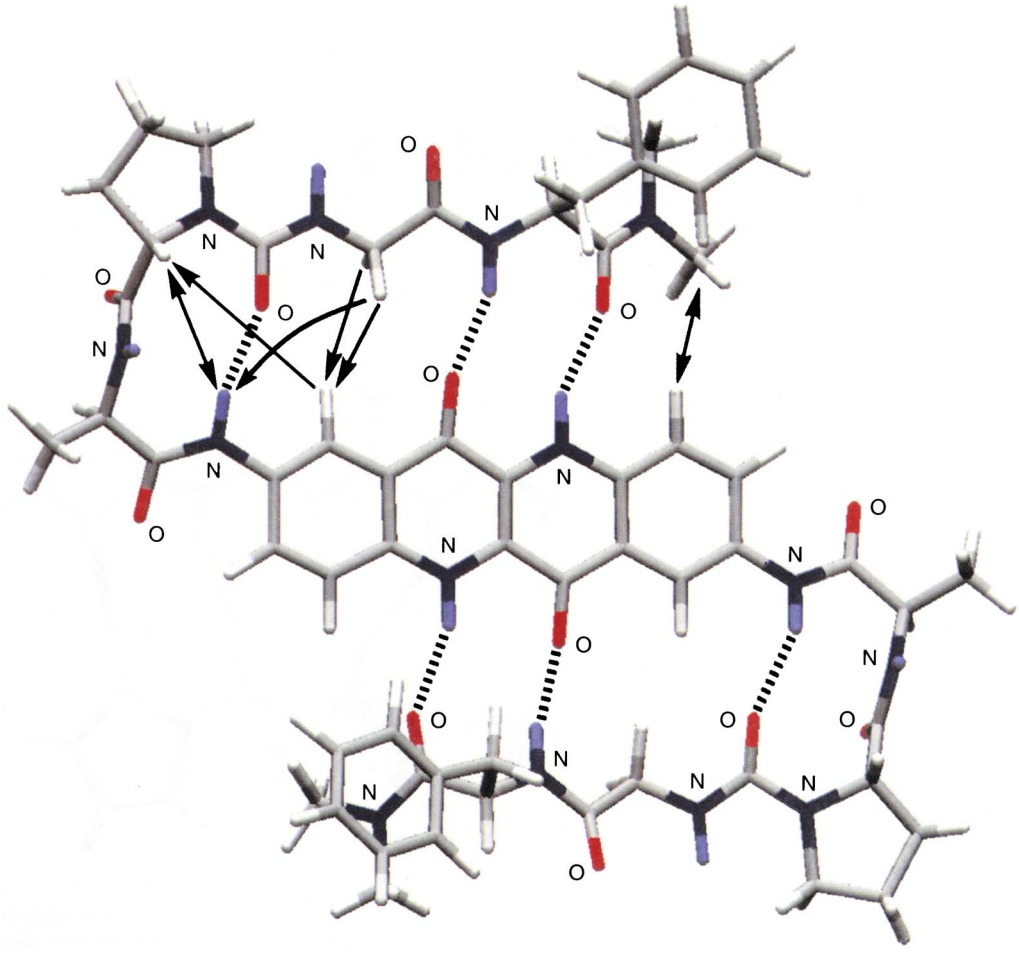
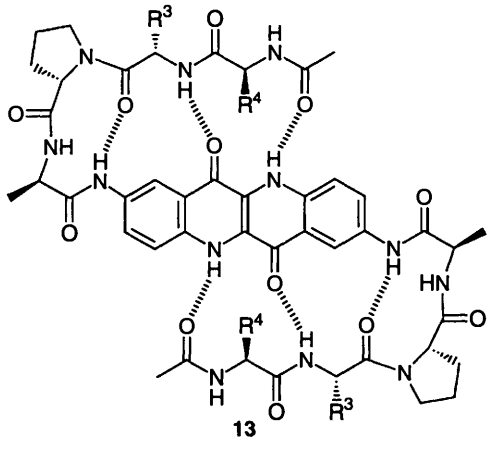


Figure 7 X-Ray crystallographic structure of the glycylphenyalanine version of artificial antiparallel β-sheet **12** (R^3 = H, R^4 = CH_2Ph). Coordinates were obtained from D. E. Blanchard, Thesis, MIT, 1992. Medium- and long-range NOEs observed in CD_3SOCD_3 solution are shown with arrows.



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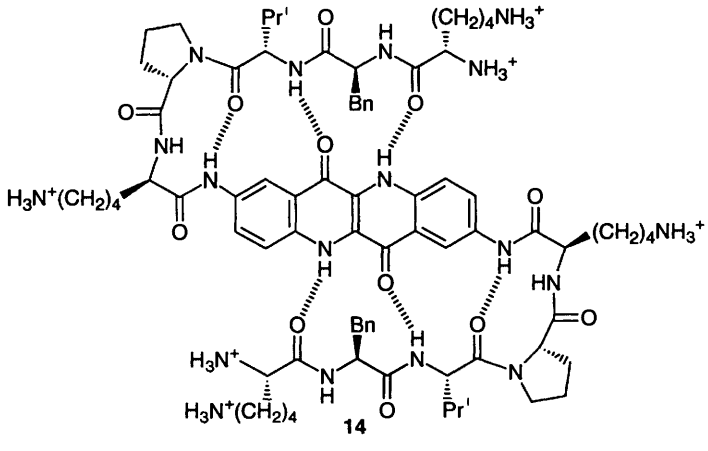

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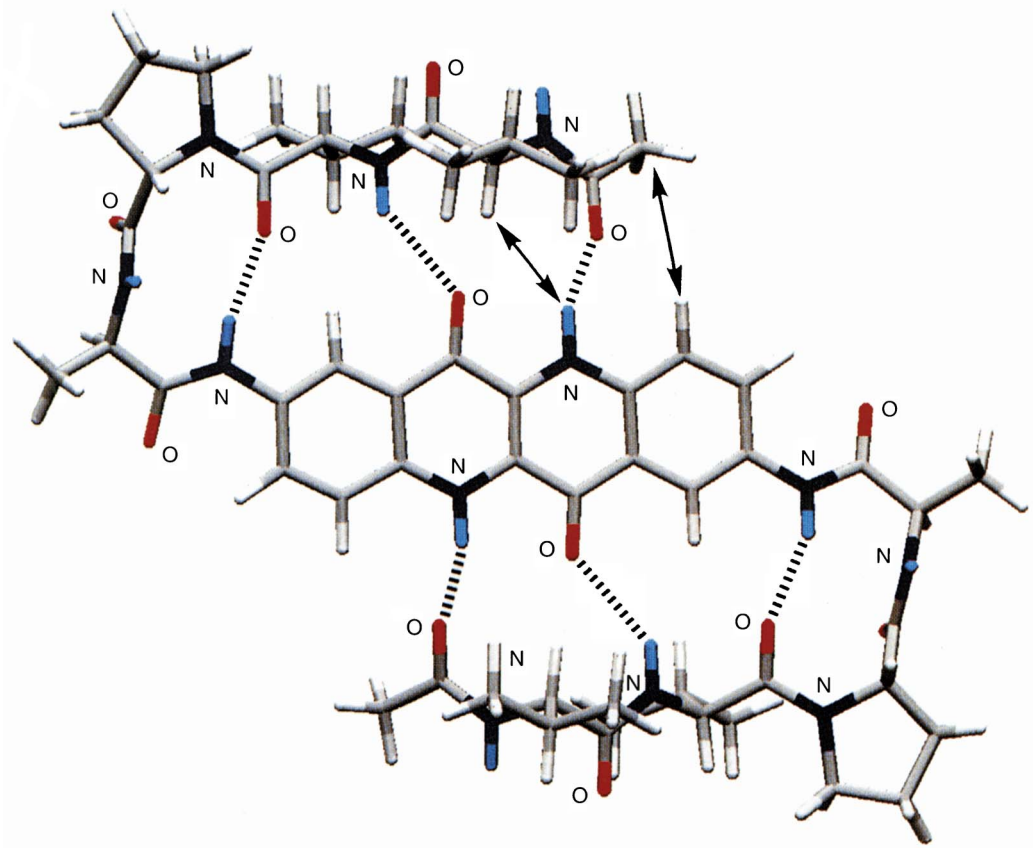
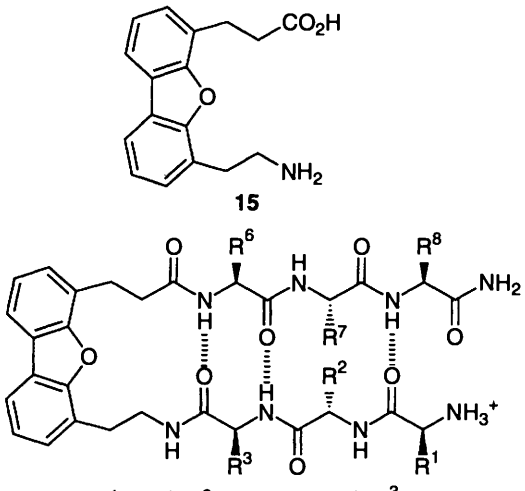


Figure 8 Crysta[illegible]graphically based model of the valylvaline version of artificial parallel β-sheet **13** (R^3 = Pr^i, R^4 = Pr^i). In building the model, crystallographic coord[illegible] for the β-turn units and epindolidione template were used (see Fig. 7), the acetylvalylvaline peptides were added, and a minimum energy conformation was calculated using MACROMODEL V5.0 with the AMBER* force field. The geometry of the epindolidione template and β-turn units were held fixed during minimization. Long-range NOEs observed by ^{1}H NMR studies in CD_3SOCD_3 solution are shown with arrows.



15

16a R^1 = Pr^i, R^2 = $(CH_2)_4NH_3^+$, R^3 = Bu^i
R^6 = Pr^i, R^7 = $(CH_2)_4NH_3^+$, R^8 = Bu^i
16b R^1 = Pr^i, R^2 = $(CH_2)_4NH_3^+$, R^3 = Me
R^6 = Me, R^7 = $(CH_2)_4NH_3^+$, R^8 = Bu^i
16c R^1 = Pr^i, R^2 = Bu^i, R^3 = $(CH_2)_4NH_3^+$
R^6 = $(CH_2)_4NH_3^+$, R^7 = Pr^i, R^8 = Bu^i

and other key NOEs and provides a model of this artificial β-sheet.

By varying the sequences of heptapeptides **16**, Kelly and coworkers established that hydrophobic cluster formation is required for β-sheet formation in these compounds. When the Leu and Val residues at the R^3 and R^6 positions of **16a** are replaced with less hydrophobic Ala groups (**16b**), or the sequence is permuted to introduce hydrophilic Lys groups at these positions (**16c**), the hydrophobic cluster cannot form and peptides **16** adopt random coil conformations. Random coil conformations also form when template **15** is replaced with templates that cannot form hydrophobic clusters or with a dipeptide sequence designed to favour a reverse turn.

Equilibrium ultracentrifugation studies indicate that heptapeptide **16a** does not aggregate in aqueous solution. This finding is significant, because β-sheets often aggregate in aqueous solution, and aggregation may stabilize β-sheet structure. Tridecapeptide homologues of **16** comprising two amphiphilic hexapeptide strands attached to template **15** exhibit a greater degree of β-sheet structure than **16a**. These molecules aggregate, however, and hydrophobic interactions between the faces of the β-sheets may contribute to the greater degree of β-sheet structure.

Recently, Kelly and coworkers reported that templates **17**—**19** induce β-sheet structure when attached to amphiphilic peptides.[30] Templates **17** and **18** bind Cu^{II}, and peptides containing **17** require Cu^{II} for β-sheet formation. Templates **18** and **19** participate in hydrophobic cluster formation with the side-chains of the attached peptide strands, and hydrophobic cluster formation plays a key role in β-sheet formation involving these compounds. With one exception, all the β-sheet forming peptides containing templates **17**—**19** that were studied form high molecular mass aggregates, and the effects of the template and of aggregation could not be analysed separately.

In contrast, peptide **20** forms monomeric β-sheets in aqueous

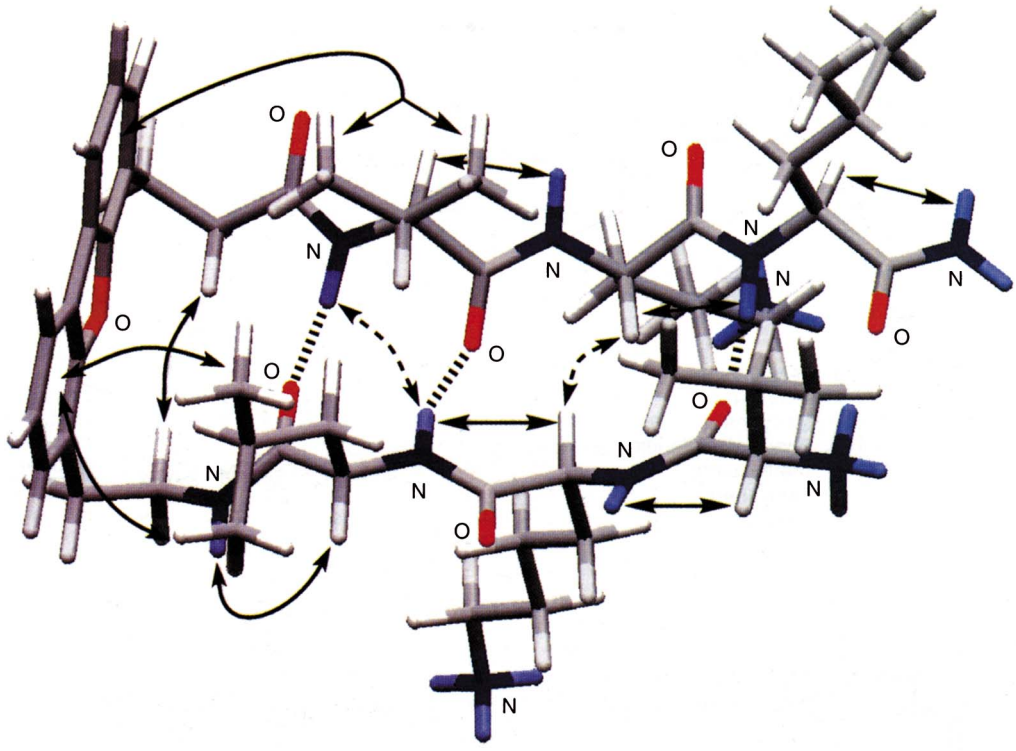
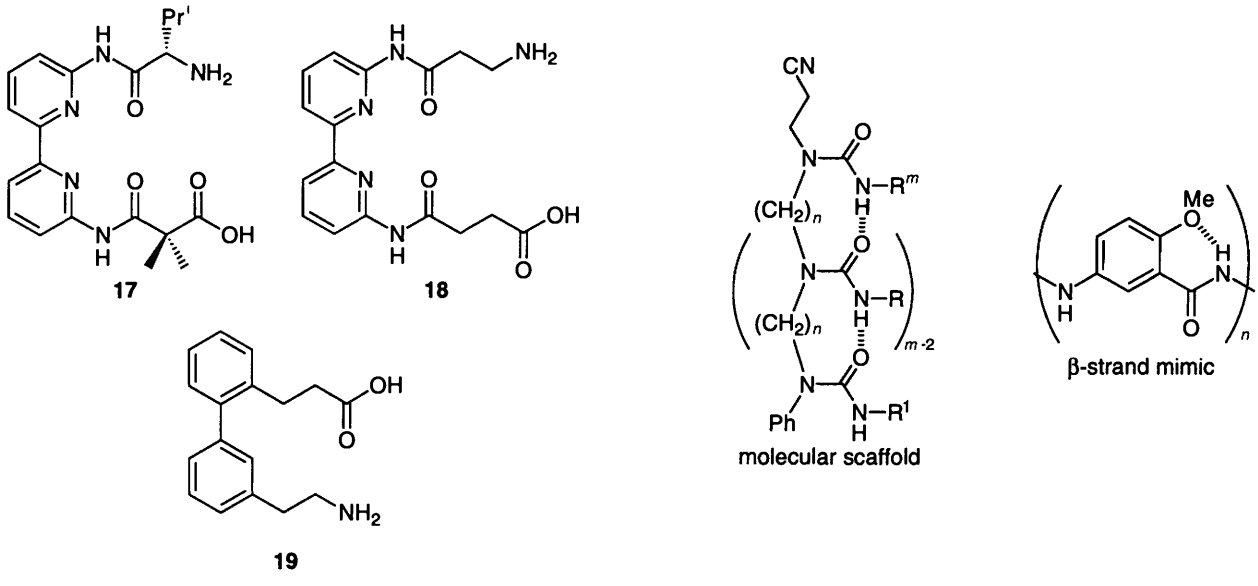
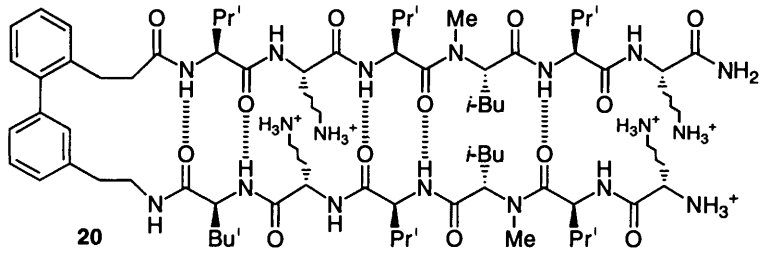
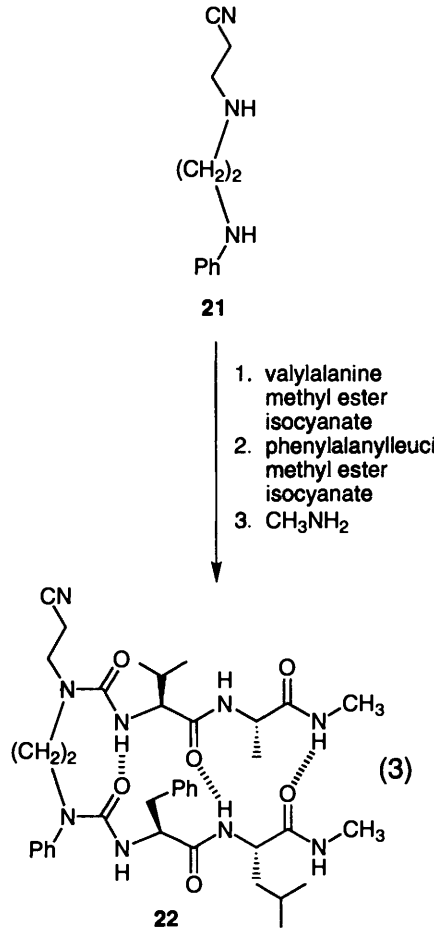
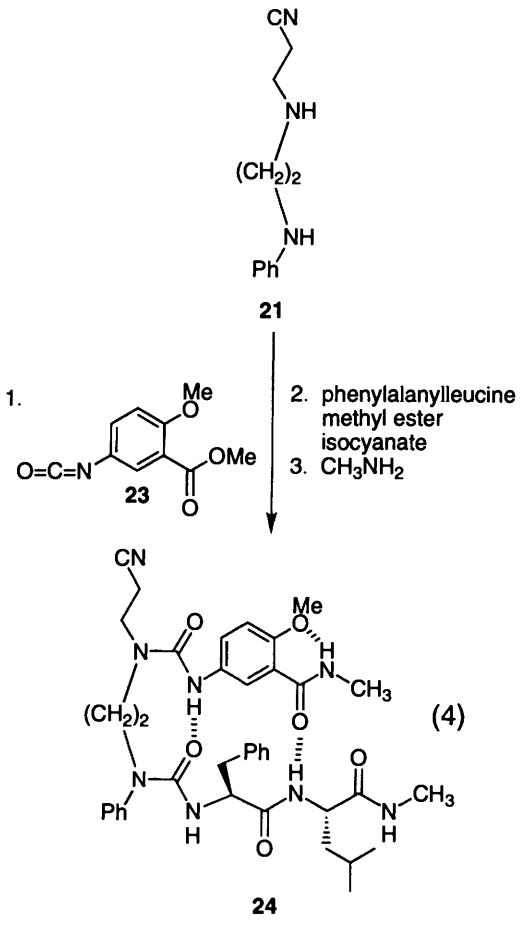


Figure 9 Model of artificial β-sheet **16a** in a minimum energy conformation (local minimum) as calculated using MACROMODEL V5.0 with the AMBER* force field. Sequential, medium-range, and long-range NOEs are shown with arrows. Dashed arrows represent crosspeaks observed by NOESY but not uniquely assigned.









solution. In this compound, template **19** enforces proximity between two peptide strands containing *N*-methylated amino acids. The template is designed to form a hydrophobic cluster with the side-chains of the adjacent valine and leucine residues while the *N*-methyl groups block the exposed edges of the peptide strands and prevent aggregation. CD spectroscopy shows minima at 198 and 223 nm, suggesting that **20** exhibits both β-sheet and random coil structure. Deuterium exchange studies indicate that the amino acids on the inner edges of the peptide strands are hydrogen bonded, and NOESY studies provide evidence for the hydrophobic cluster. Of concern, however, is that interstrand NOEs characteristic of an antiparallel β-sheet are absent. These experiments suggest that **20** is sheetlike near the template, but is frayed at the ends. Collectively, Kelly's studies indicate that hydrophobic cluster formation and intermolecular hydrophobic interactions can play important roles in stabilizing β-sheets in aqueous solution.

3.4 Nowick's Artificial β-Sheets

In 1992, we began publishing a series of papers aimed at creating larger and more complex artificial sheets, in which the templates are not limited in length and the attached peptide strands are not limited in number.[31] To achieve this goal, we developed two complementary templates, an oligourea *molecular scaffold* and a β-strand mimic. The oligourea molecular scaffold is designed to hold two or more peptide or peptidomimetic strands in proximity, and the β-strand mimic is designed to attach to the oligourea template, rigidify the artificial β-sheet, and help prevent intermolecular association. Over the past two years, we have combined these building blocks with peptide strands to create four different artificial β-sheets of increasing size and complexity.

We first synthesized and studied a small artificial parallel β-sheet by combining a diurea scaffold with two peptide strands.[32] Sequential reaction of diamine **21** with valylalanine methyl ester isocyanate and phenylalanylleucyl methyl ester isocyanate, followed by aminolysis of the methyl ester groups with methylamine, afforded artificial β-sheet **22** (eqn. 3). ^{1}H NMR NOE and chemical shift studies establish that **22** adopts a hydrogen-bonded parallel β-sheet conformation in $CDCl_3$ solution. Fig. 10 provides a model of this conformation and illustrates key NOEs. Comparison of the chemical shifts of the NH groups with those of appropriate controls indicates that **22** exists as a rapidly-equilibrating mixture of conformers, which comprises approximately 50% of the β-sheet conformer. This study establishes that the oligourea molecular scaffold can induce β-sheet formation between attached peptide strands.

To evaluate the concept of using both the molecular scaffold and a β-strand mimic, we prepared a small artificial antiparallel β-sheet (**24**) in which the upper dipeptide strand of **22** was replaced with a 5-amino-2-methoxybenzamide β-strand mimic.[33] The synthesis of **24** is analogous to that of **22** and is outlined in eqn. (4). ^{1}H NMR chemical shift studies indicate that the NH groups of the leucine and the β-strand mimic are hydrogen bonded in $CDCl_3$ solution. One of the leucine methyl groups appears upfield, at 0.44 ppm, suggesting that it is near the face of the aromatic ring of the β-strand mimic. NOE studies confirm that these two groups are next to each other and show an extensive network of NOEs between the phenylalanylleucine peptide strand and the 5-amino-2-methoxybenzamide β-strand mimic. A model consistent with these NOEs is shown in Fig. 11. This study establishes the feasibility of using two complementary templates to induce β-sheet formation. Artificial β-sheet **24** appears to be more conformationally well-ordered than artificial β-sheet **22**, suggesting that two templates are better than one at inducing β-sheet structure.

We envisioned extending the 5-amino-2-methoxybenzamide β-strand mimic by connecting a series of these units end-to-end. Coupling of a 5-amino-2-methoxybenzoic acid unit and a 5-hydrazino-2-methoxybenzamide unit doubled the length of the β-strand mimic and allowed the generation of artificial antiparallel β-sheet **30** (unpublished results). Eqn. (5) illustrates the synthesis of this compound. Amine **25** was coupled with phenylalanylisoleucylleucine methyl ester isocyanate and the methyl ester group was aminolysed with methylamine to form urea **26**. The Boc

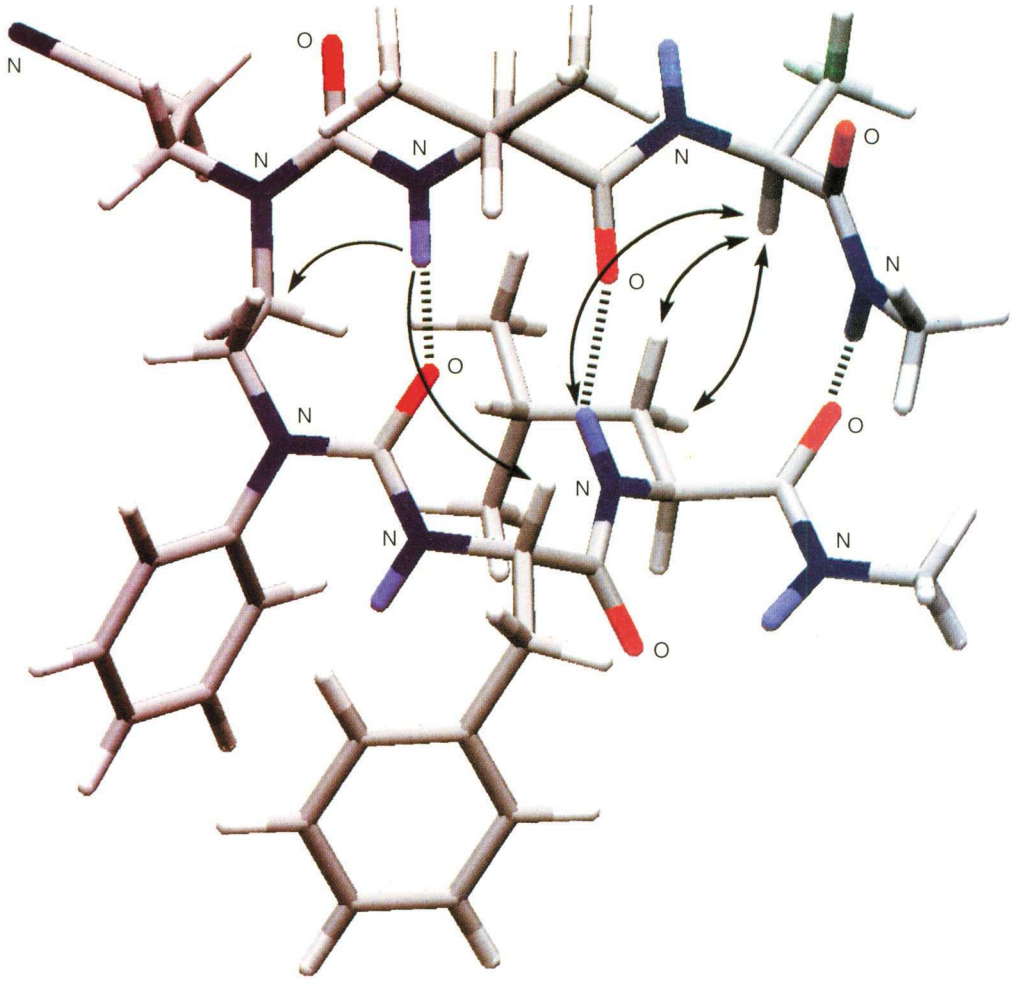


Figure 10 Model of artificial β-sheet **22** in a minimum energy conformation (local minimum) as calculated using MACROMODEL V5.0 with the AMBER* force field. Medium- and long-range NOEs are shown with arrows.

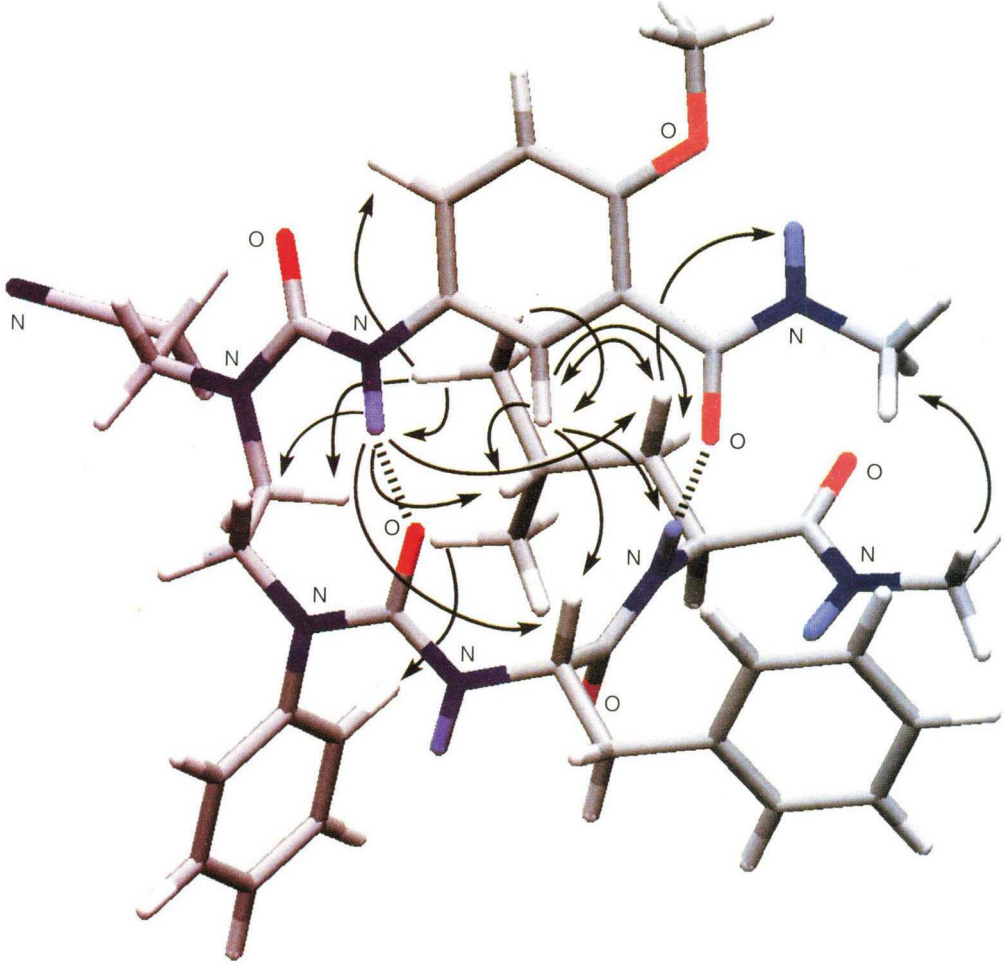
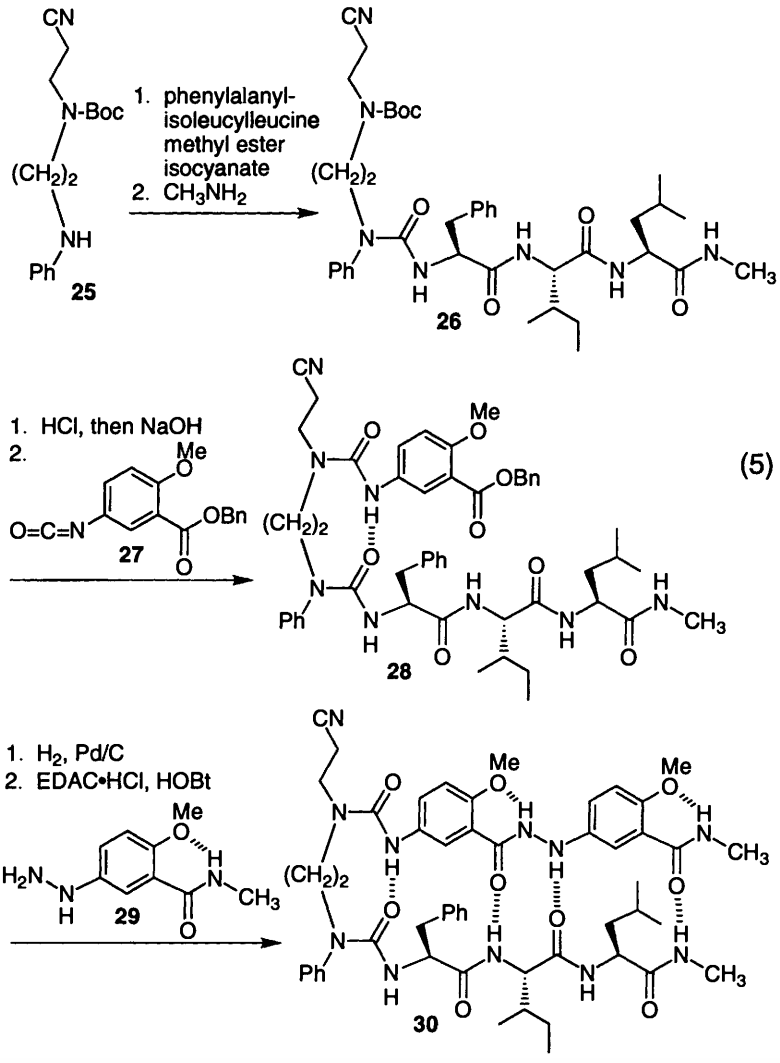


Figure 11 Model of artificial β-sheet **24** in a minimum energy conformation (local minimum) as calculated using MACROMODEL V5.0 with the AMBER*



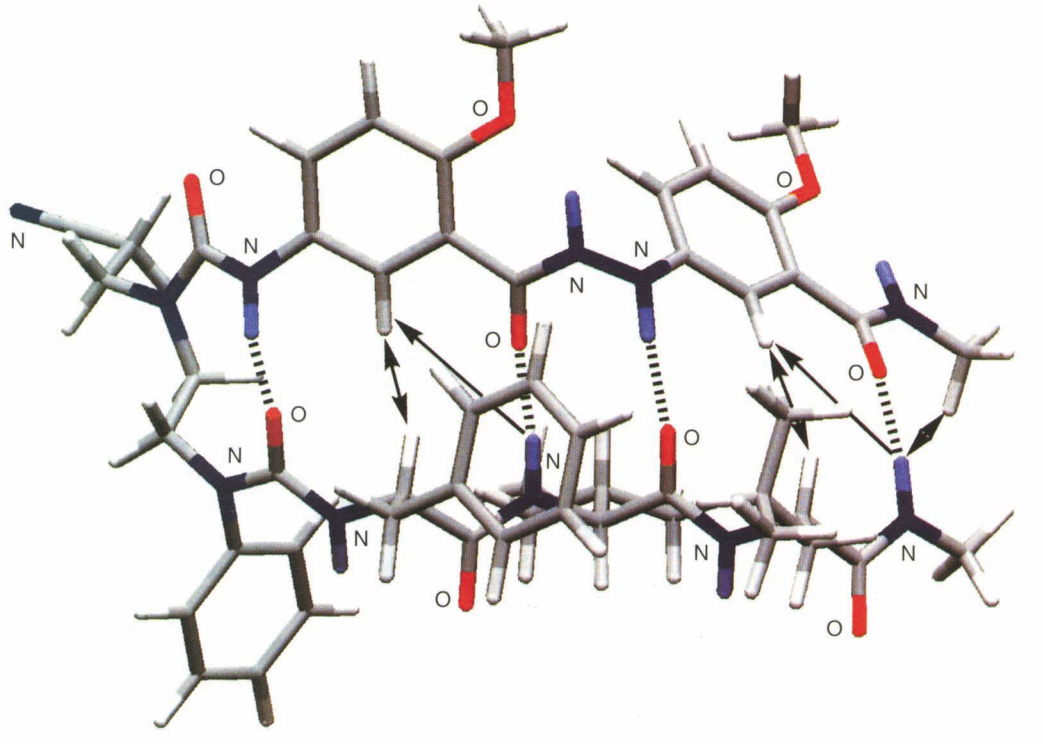


Figure 12 Model of artificial β-sheet **30** in a minimum energy conformation (local minimum) as calculated using MACROMODEL V5.0 with the AMBER* force field. Adequate parameters for the C-N-N-C torsion angle of the hydrazide group were not available, and this torsion was constrained to the crystallographically observed value of 80° during minimization. Long-range NOEs are shown with arrows.

protective group of **26** was removed with HCl, and the resulting amino group was coupled with isocyanate **27** to form diurea **28** Removal of the benzyl ester protective group by hydrogenolysis, followed by coupling with 5-hydrazino-2-methoxybenzamide **29**, generated artificial β-sheet **30**

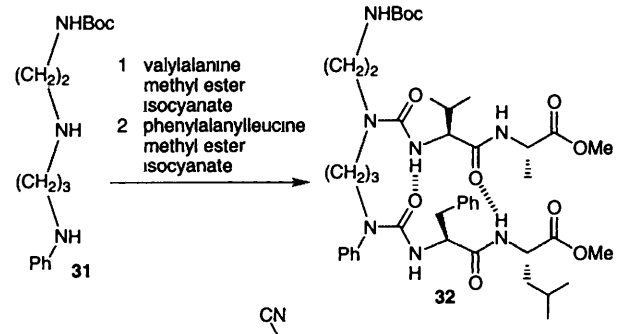
^{1}H NMR chemical shift studies establish that the tripeptide strand and the β-strand mimic are hydrogen bonded in $CDCl_3$ solution, and NOE studies show contacts between these two groups that are appropriate for an antiparallel β-sheet Fig 12 provides a model of **30** and illustrates these NOEs These synthetic and structural studies establish the feasibility of extending the β-strand mimic Presumably, it should be possible to use oligomers of 5-hydrazino-2-methoxybenzoic acid as extended β-strand mimics in artificial β-sheets containing longer peptide strands

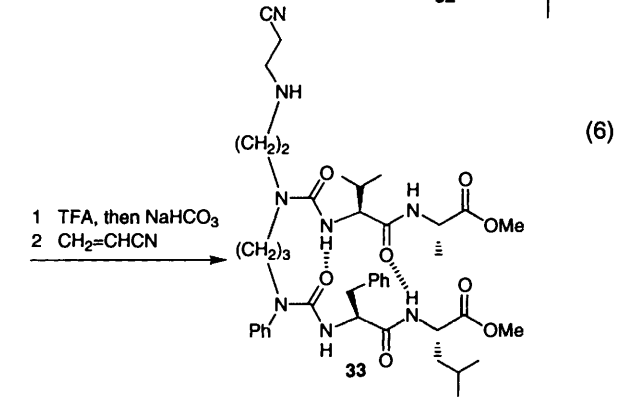
We also envisioned extending the oligourea molecular scaffold to form artificial β-sheets comprising more than two peptide or peptidomimetic strands Three-stranded artificial β-sheet **35**, containing a β-strand mimic and exhibiting both parallel and antiparallel hydrogen-bonding patterns, was prepared as shown in eqn (6) (unpublished results) Reaction of diamine **31** with valylalanine methyl ester isocyanate and phenylalanylleucine methyl ester isocyanate gave diurea **32** Removal of the Boc protective group, followed by conjugate addition of the resulting primary amino group to acrylonitrile, afforded secondary amine **33** Aminolysis of the methyl ester groups with methylamine and reaction of the sec ondary amino group with isocyanate **34** generated artificial β-sheet **35**

Comparison of the ^{1}H NMR chemical shifts of the NH groups of **35** to those of suitable controls indicates that this compound adopts a hydrogen-bonded β-sheet conformation in $CDCl_3$ solution ^{1}H NMR NOESY studies show interstrand NOEs that further support a β-sheet structure A model of artificial β-sheet **35** illustrating the interstrand NOEs is shown in Fig 13 These studies indicate that our dual-template approach is not limited to doubly-stranded artifi cial β-sheets The success of artificial β-sheets **22**, **24**, **30** and **35** is very encouraging and suggests that the preparation of even larger artificial β-sheets may be possible using this strategy

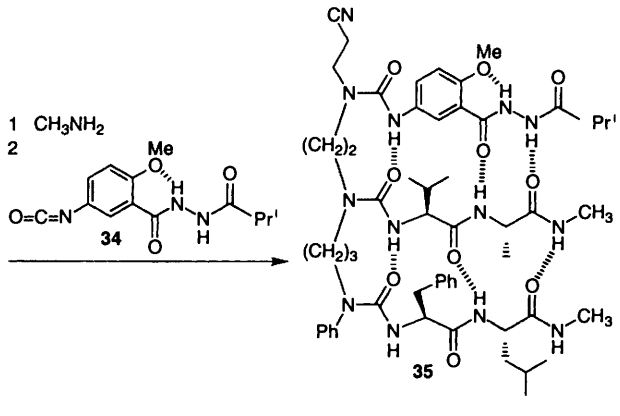
3.5 Other Artificial β-Sheets

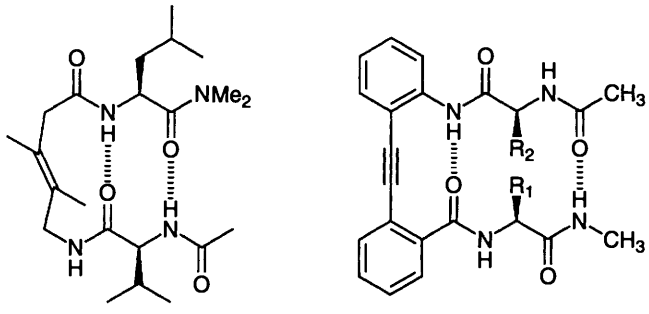
A number of other artificial β-sheets have been reported recently Gellman and coworkers reported that a tetrasubstituted *trans*-alkene template induces attached peptide strands to form a β-hairpin in CD_2Cl_2 solution (**36**) [34] Kemp and Li described a diphenylacetylene template that induces β-sheet formation between two attached peptide strands in a variety of organic solvents (**37**) [35] Ogawa and coworkers reported that a $Ru(bpy)_3^{2+}$ complex linked to two valyl-valine dipeptides forms parallel β-sheets **38** in aqueous solution [36] For a β-sheet to form, one of the linking amide groups must adopt an unstable *cis*-amide conformation, however, and we do not believe that the data presented provide sufficient evidence for this structure With the goal of developing biologically active mimics of the cell adhesion protein ICAM-1, Michne and Schroeder prepared artificial β-sheets **39** [37] These compounds contain an analogue of Kemp's epindolidione β-strand mimic and adopt hydrogen-bonded antiparallel sheet structures in CD_3SOCD_3 solution

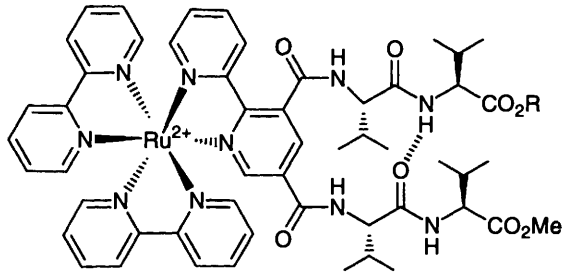




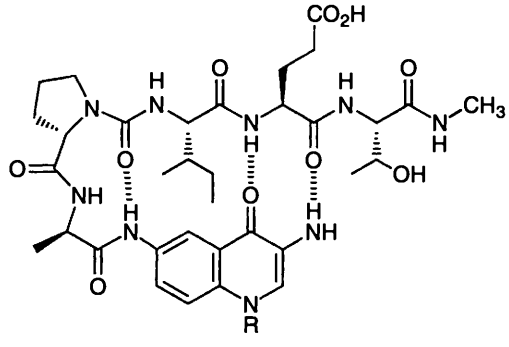
(6)







38a R = Me
38b R = $Co(NH_3)_5^{3+}$



39a R = H
39b R = $CH_2CH{=}CH_2$

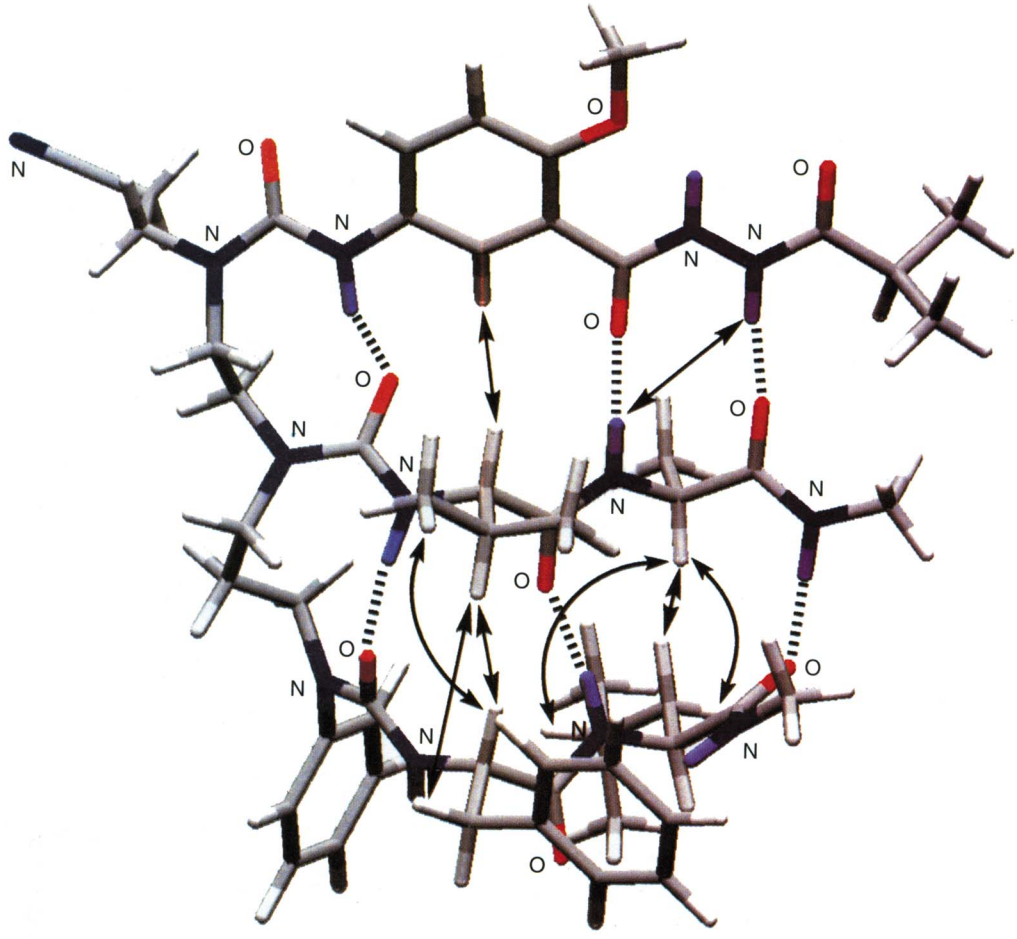


Figure 13 Model of artificial β-sheet **35** in a minimum energy conformation (local minimum) as calculated using MACROMODEL V5.0 with the AMBER* force field. Long-range NOEs are shown with arrows.

4 Conclusions and Future Directions

The studies described in the preceding section establish that templates can help induce β-sheet structure in attached peptides. Kemp's studies provide independent confirmation of the sheet forming propensities of several amino acids. Further reports of the energetic contributions of different amino acids to β-sheet formation are anticipated from the Kemp laboratories. Kelly's studies establish that hydrophobic interactions can play a major role in β-sheet structure and lend support to the hypothesis that hydrophobic cluster formation is important to the formation of β-sheets during protein folding. Ongoing investigations in the Kelly laboratories are focused upon the role of β-sheet self-assembly in β-amyloid formation and Alzheimer's disease and the application of β-sheet self-assembly to the creation of new materials.

We have developed two complementary templates that allow the creation of larger and more complex artificial β-sheets. We are currently studying three-stranded β-sheets and we are beginning to prepare a four-stranded artificial β-sheet containing these templates. We are also in the process of developing additional templates and linkers to create artificial β-sheets with new topologies. Thus far, we have focused upon developing structures that fold in chloroform solution, because the interior of proteins resembles an organic solvent and β-sheets are generally found in the interior of proteins. However, we are also beginning to study artificial β-sheets in aqueous solution, because proteins fold in water. Our studies have laid the groundwork to allow us to apply our artificial sheets to various problems. To determine how interactions between amino acids affect β-sheet stability, we are preparing a combinatorial library in which different amino acids are juxtaposed. With the goal of developing drugs to treat Alzheimer's disease, we are preparing β-strand mimics and artificial β-sheets designed to inhibit β-amyloid self-assembly. In the future, we will prepare artificial β-sheets that are missing peptide strands with the goal of creating molecular receptors and catalysts that bind substrates using a β-sheet motif.

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