The Propensities of Amino Acids To Form Parallel β-Sheets

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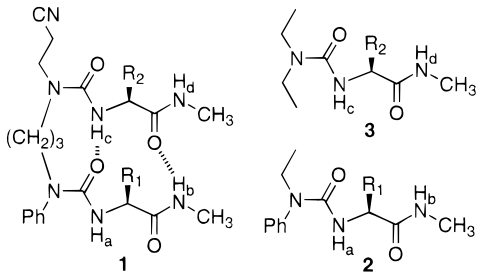
Abstract: This paper reports synthetic and structural studies of a 16-membered combinatorial library of chemical models for parallel β-sheets (**1**) and two four-membered libraries of controls (**2** and **3**). The libraries contain glycine, alanine, valine, and leucine residues, and the structural studies use ^{1}H NMR and IR spectroscopy to measure the relative degrees of intramolecular hydrogen bonding of the parallel β-sheet models in chloroform solution. These studies indicate that leucine and valine are relatively good at forming parallel β-sheets, alanine is moderate, and glycine is poor (L, V > A > G).

In 1993 and 1994, three teams of researchers reported studies of the propensities of different amino acids to form antiparallel β-sheets.[1−3] These studies involved systematically varying amino acids within small β-sheet containing proteins and quantifying the effects of the mutations upon the thermodynamic stabilities of the proteins. To our knowledge, no similar study of the propensities of different amino acids to form parallel β-sheets has been reported.[4]

In this paper, we report initial findings of the propensities of four different amino acids (glycine, alanine, valine, and leucine) to form parallel β-sheets. In contrast to the earlier studies of antiparallel β-sheets, the current studies use a small chemical model system, which we term an *artificial* β-sheet.[5,6] Also in contrast to the previous studies, the current studies use chloroform as a solvent, rather than water, because β-sheets often occur in the hydrophobic core of proteins, which is much less polar than water.[7]

To study the propensities of different amino acids to form parallel β-sheets, we have prepared a combinatorial library of artificial β-sheets **1**. These artificial β-sheets consist of two amino acid groups attached to a diamine backbone by way of two urea groups. The urea groups form a hydrogen-bonded turn structure,[8] which juxtaposes two amino acids and orients them to promote the formation of a parallel β-sheet.[9] 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 have prepared four-membered libraries **2** and **3**, which mimic the top and bottom groups of **1** and also contain glycine, alanine, valine, and leucine residues.



Results

The combinatorial libraries were prepared by multiple parallel solid-phase synthesis upon Merrifield resin, as shown in eq 1.[10,11] Boc-protected glycine, alanine, valine, and leucine resins were deprotected by treatment with trifluoroacetic acid (TFA), treated with triethylamine (TEA) to liberate the free amino group, and coupled with the carbamoyl chloride PhN(COCl)-$CH_2CH_2CH_2$N(Boc)CH_2CH_2CN (**4**). The Boc protective group was removed by treatment with TFA, and the amino group was liberated by treatment with TEA and coupled with alanine, valine, or leucine methyl ester isocyanate, or with glycine ethyl ester isocyanate.[12] Cleavage from the resin by aminolysis with methylamine, followed by chromatographic purification, afforded pure artificial β-sheets **1**. Controls **2** and **3** were prepared by treatment of deprotected glycine, alanine, valine, and leucine Merrifield resins with either *N*-ethyl-*N*-phenylcarbamoyl chloride[13] or *N*,*N*-diethylcarbamoyl chloride, followed by aminolysis with methylamine and chromatographic purification.

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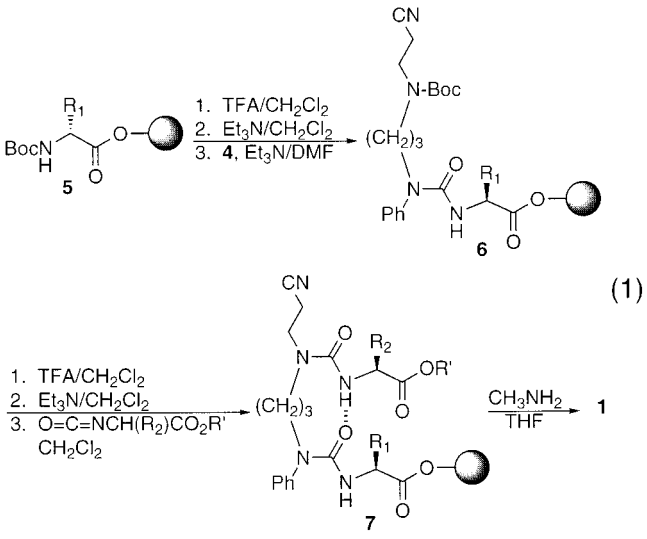
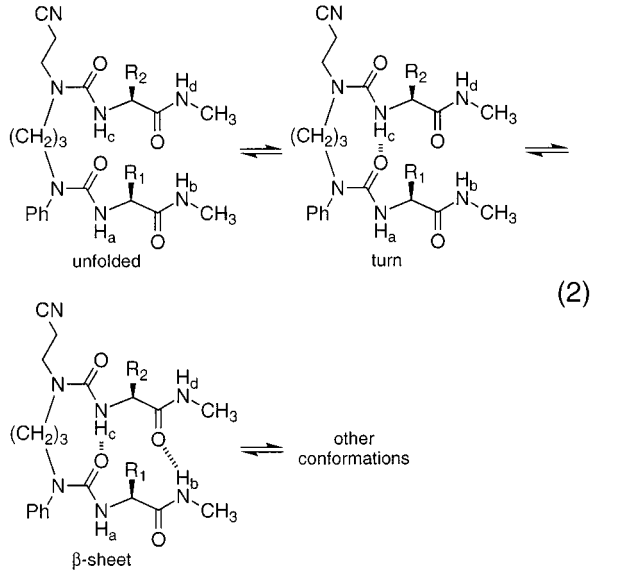


Previous studies in our laboratory have established that the diurea backbone forms a hydrogen-bonded turn structure,[8] and that this turn induces attached peptide groups to form a parallel β-sheet structure.[9] The artificial β-sheet is not fully hydrogen bonded, however, and participates in a rapid equilibrium involving several conformations, as illustrated in eq 2. For the



purposes of subsequent discussion, we will refer to these as the *unfolded*, *turn*, and β-sheet conformations. The unfolded conformation lacks hydrogen bonds; the turn conformation contains a hydrogen bond between the urea groups; the β-sheet conformation comprises a minimal parallel β-sheet with two hydrogen bonds. Any other conformations, if present, involve other hydrogen-bonding patterns (e.g., hydrogen bonding of H_d to the oxygen atom of the bottom urea or amide group).

The spectroscopic properties of the artificial β-sheets reflect the position of the conformational equilibrium. In $CDCl_3$ solution, the ^{1}H NMR resonances of hydrogen-bonded amide and urea NH groups appear downfield of the resonances of similar NH groups that are not hydrogen bonded. Upon hydrogen bonding, NH groups typically shift downfield by 2−3 ppm; downfield shifting of 1 ppm corresponds to a proton spending roughly equal time in the hydrogen-bonded and non-hydrogen-bonded states.[8,9] In the infrared spectrum, the hydrogen-bonded NH stretching vibrations appear as a broad band at ca. 3325 cm^{-1}, while the non-hydrogen-bonded NH stretching vibrations appear as several narrower overlapping bands at 3420−3450 cm^{-1}. The magnitude of the hydrogen-bonded NH band also reflects the position of the conformational equilibrium and provides independent corroboration of the ^{1}H NMR chemical shift data. In contrast to the ^{1}H NMR data, the IR data reflect the total degree of hydrogen bonding, rather than the hydrogen bonding of individual NH groups.

Table 1. Spectroscopic Properties of the NH Groups of **1**−**3**

	^{1}H NMR (ppm)				
compd[a]	H_a[b]	H_b[b]	H_c[b]	H_d[b]	IR absorbance[c,d]
1GG	4.90	6.27	6.60	6.60	0.0587
1AG	4.78	6.45	6.75	6.58	0.0737
1VG	4.80	6.42	6.85	6.60	0.0823
1LG	4.66	6.60	6.86	6.60	0.0833
1GA	4.88	6.34	6.25	6.40	0.0643
1AA	4.78	6.50	6.54	6.66	0.0873
1VA	4.83	6.72	6.72	6.60	0.1003
1LA	4.67	6.87	6.71	6.53	0.1050
1GV	4.88	6.46	5.98	6.15	0.0553
1AV	4.72	6.74	6.26	6.19	0.0750
1VV	4.77	6.78	6.41	6.26	0.0903
1LV	4.65	7.04	6.51	6.25	0.1033
1GL	4.89	6.38	6.12	6.35	0.0623
1AL	4.78	6.73	6.47	6.45	0.0893
1VL	4.84	6.90	6.71	6.59	0.1183
1LL	4.68	7.03	6.70	6.49	0.1119
2G	4.72	6.30			
2A	4.50	6.39			
2V	4.55	6.08			
2L	4.38	6.33			
3G			5.03	6.36	
3A			4.80	6.41	
3V			4.90	6.04	
3L			4.67	6.29	

[a] The letters in **1**−**3** refer to amino acids. In **1**, the letters correspond respectively to bottom (R_1) and top (R_2) amino acids. [b] ^{1}H NMR spectra were recorded at 5 mM in $CDCl_3$ solution at 295 K. [c] Intensity of the hydrogen-bonded NH stretching band at ca. 3325 cm^{-1}. [d] IR spectra were recorded at 5 mM in $CHCl_3$ solution at 295 K. Spectra were recorded with use of a 1.0 mm path length CaF_2 cell against a $CHCl_3$ reference and are baseline corrected. Absorbances are the averages of three independent runs.

The ^{1}H NMR and IR spectra of artificial β-sheets **1** and controls **2** and **3** were recorded at 5 mM in $CDCl_3$ and $CHCl_3$ solution, respectively (Table 1). At this concentration, these compounds undergo negligible self-association, and the recorded spectra reflect the properties of the unaggregated compounds.[14] The ^{1}H NMR spectral resonances of artificial β-sheets **1** were assigned to H_a−H_d by a combination of decoupling and NOE studies.[15]

The chemical shift data in Table 1 support the model in eq 2, in which proton H_c is largely hydrogen bonded, proton H_b is partially hydrogen bonded, and protons H_a and H_d of artificial β-sheets **1** are largely not hydrogen bonded. The ^{1}H NMR resonances of H_c in **1** are substantially downfield of those of

(14) ^{1}H NMR dilution titration studies indicate that artificial β-sheet **1LL** self-associates with a dimerization constant of about 3 M^{-1}; related compounds that we have studied previously exhibit similar dimerization constants.

(15) In artificial β-sheet **1**, NH resonances were assigned to H_a−H_d as follows: The α-protons of glycine, alanine, valine, and leucine exhibit distinct coupling patterns (ABX pattern, quintet, triplet or doublet of doublets, and quartet or triplet of doublets, respectively). H_a and H_c were assigned by decoupling experiments. H_b and H_d were assigned by ^{1}H NMR NOE experiments. In **1GG**, **1AA**, **1VV**, and **1LL**, a combination of NOE and decoupling experiments were required to distinguish H_a from H_c and H_b from H_d.

the corresponding controls. In **1GG**, **1AG**, **1VG**, and **1LG** H_c appears 1.57−1.83 ppm downfield of control **3G**; in **1GA**, **1AA**, **1VA**, and **1LA** H_c appears 1.45−1.92 ppm downfield of control **3A**; in **1GV**, **1AV**, **1VV**, and **1LV** H_c appears 1.08−1.61 ppm downfield of control **3V**; in **1GL**, **1AL**, **1VL**, and **1LL** H_c appears 1.45−2.04 ppm downfield of control **3L**. (The letters in **1**−**3** refer to amino acids. In **1**, the letters correspond respectively to bottom (R_1) and top (R_2) amino acids.) As discussed above, the large downfield shifting of H_c (1.1−2.0 ppm) reflects that it is largely hydrogen bonded; the variability in downfield shifting reflects that the position of the equilibrium differs among the members of the library.

Although the ^{1}H NMR resonances of H_b in **1** generally appear downfield of those of the corresponding controls, the degree of downfield shifting is less. In **1GG**, **1GA**, **1GV**, and **1GL** H_b appears −0.03−0.16 ppm downfield of control **2G**; in **1AG**, **1AA**, **1AV**, and **1AL** H_b appears 0.06−0.35 ppm downfield of control **2A**; in **1VG**, **1VA**, **1VV**, and **1VL** H_b appears 0.34−0.82 ppm downfield of control **2V**; in **1LG**, **1LA**, **1LV**, and **1LL** H_b appears 0.27−0.71 ppm downfield of control **2L**. The smaller degree of downfield shifting (≤0.8 ppm) indicates a lesser degree of hydrogen bonding; the variability reflects differences among the members of the library.

The chemical shifts of H_a and H_d in **1** are generally more similar to those of the corresponding controls, and the variability in downfield shifting is less than that of H_b and H_c. In **1GG**, **1GA**, **1GV**, and **1GL** H_a appears 0.16−0.18 ppm downfield of control **2G**; in **1AG**, **1AA**, **1AV**, and **1AL** H_a appears 0.22−0.28 ppm downfield of control **2A**; in **1VG**, **1VA**, **1VV**, and **1VL** H_a appears 0.22−0.29 ppm downfield of control **2V**; in **1LG**, **1LA**, **1LV**, and **1LL** H_a appears 0.27−0.30 ppm downfield of control **2L**. In **1GG**, **1AG**, **1VG**, and **1LG** H_d appears 0.22−0.24 ppm downfield of control **3G**; in **1GA**, **1AA**, **1VA**, and **1LA** H_d appears −0.01−0.25 ppm downfield of control **3A**; in **1GV**, **1AV**, **1VV**, and **1LV** H_d appears 0.11−0.22 ppm downfield of control **3V**; in **1GL**, **1AL**, **1VL**, and **1LL** H_d appears 0.06−0.30 ppm downfield of control **3L**.

The lesser variability and lesser downfield shifting (≤0.3 ppm) of H_a and H_d reflect that these protons are largely spectators in the equilibrium outlined in eq 2. The small degree of downfield shifting that does occur may result from the participation of the corresponding carbonyl groups as hydrogen bond donors: when the bottom urea group hydrogen bonds to H_c, electron density is pulled away from H_a, shifting H_a downfield; when the top amide group hydrogen bonds to H_b, electron density is pulled away from H_d, shifting H_d downfield.[16] Alternatively, the downfield shifting of H_a and H_d may reflect the involvement, to a limited extent, of other conformations in the equilibrium outlined in eq 2.

The downfield shifting of H_b reflects the β-sheet forming propensities of the different amino acids. Figure 1 provides a three-dimensional representation of these data. Because these data comprise a 4 × 4 matrix, there are *eight* series with which to make comparisons. These eight series consist of the four rows and four columns of Figure 1 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. These eight series indicate that leucine and valine are relatively good at forming parallel β-sheets, alanine is moderate, and glycine is poor (L, V > A > G). In addition, the bottom amino acid appears to have more effect upon parallel β-sheet formation than the top amino acid in this model system.

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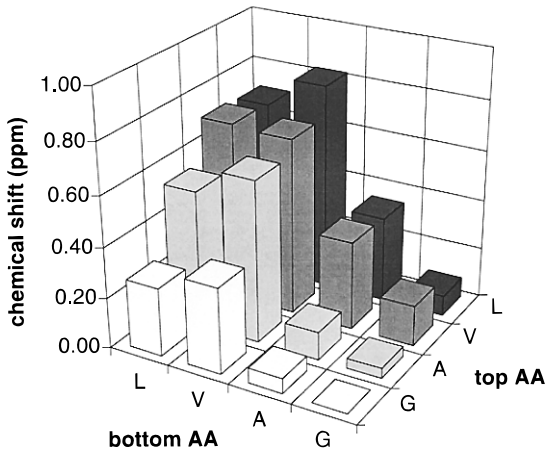


Figure 1. ^{1}H NMR chemical shift differences between H_b of artificial β-sheets **1** and controls **2**. (Downfield shifting of this proton reflects the population of the β-sheet conformation.)

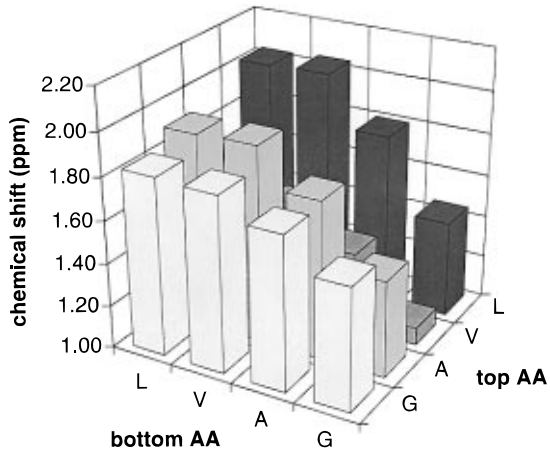


Figure 2. ^{1}H NMR chemical shift differences between H_c of artificial β-sheets **1** and controls **3**. (Downfield shifting of this proton reflects the overall populations of the turn and β-sheet conformations.)

The downfield shifting of H_c reflects the overall population of the turn and β-sheet conformations (Figure 2). The amino acids exhibit the same trend as they do for sheet formation (L, V > A > G), when the bottom amino acid is varied and the top amino acid is held fixed (the rows in Figure 2). However, valine proves distinctly poorer than leucine, alanine, or glycine at stabilizing the turn conformation, when the top amino acid is varied and the bottom amino acid is held fixed (the columns in Figure 2). Although H_a and H_d also exhibit some downfield shifting, the degree of downfield shifting is small (≤0.30 ppm) and the data exhibit no significant trends.

The IR data (Table 1) exhibit trends similar to those of the ^{1}H NMR data, providing independent corroboration of the NMR studies described above. The intensities of the hydrogen-bonded NH stretching band in the IR spectra of **1** reflect the overall degree of hydrogen bonding in these compounds. Figure 3 illustrates these data graphically. Because the IR data reflect the total degree of hydrogen bonding, rather than the hydrogen bonding of individual NH groups, each IR datum is best compared to the *sum* of the NMR downfield shifting of H_b and H_c for the same compound. Figure 4 shows the sum of the NMR downfield shifting of H_b and H_c. The similarities between Figures 3 and 4 are remarkable, and there is clearly a strong correlation between the IR and NMR data. This correlation lends further support to the use of the ^{1}H NMR chemical shift differences as a measure of the degree of hydrogen bonding in artificial β-sheets **1**.

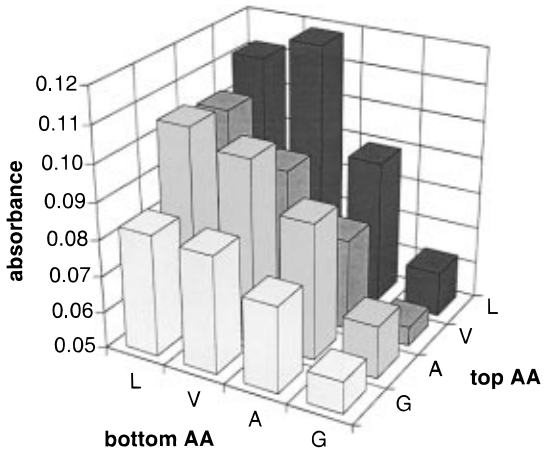


Figure 3. Intensity of the hydrogen-bonded NH stretching band (ca. 3325 cm^{-1}) in the IR spectra of artificial β-sheets **1**. (The intensity of this band reflects the overall degree of hydrogen bonding in **1**.)

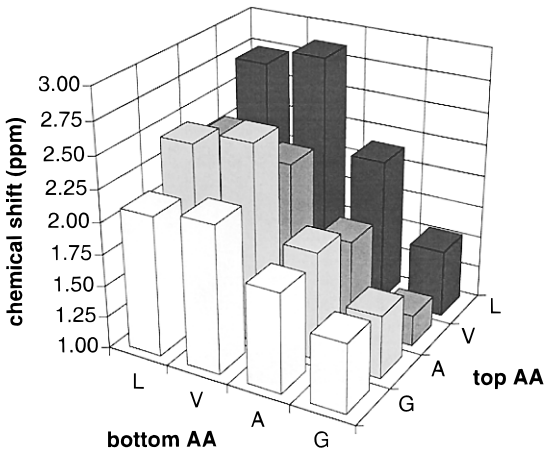


Figure 4. Sum of the ^{1}H NMR chemical shift differences between H_b of artificial β-sheets **1** and controls **2** and between H_c of artificial β-sheets **1** and controls **3**. (This is the sum of data from Figures 1 and 2 and reflects the overall degree of hydrogen bonding of H_b and H_c.)

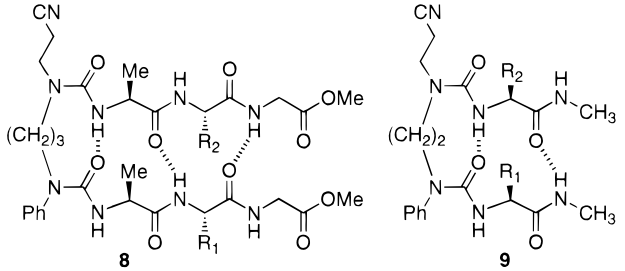
Discussion

The observed propensities of the different amino acids to form parallel β-sheets (L, V > A > G) largely parallels findings from earlier studies. Chou and Fasman reported the conformational parameter P_β for the β-sheet forming propensities of all 20 amino acids based upon their frequency of occurrence in β-sheets in 15 proteins.[17] Values for P_β of 1.65, 1.22, 0.97, and 0.81 were obtained for valine, leucine, alanine, and glycine. Kemp and co-workers measured the β-sheet forming propensities of valine, alanine, glycine, and phenylalanine in an antiparallel β-sheet model system and found the following ordering of β-sheet forming propensities in this system: F, V > A > G.[5] The studies that were described at the beginning of this paper were performed by Kim and Berg, Minor and Kim, and Smith, Withka, and Regan. Kim and Berg measured the antiparallel β-sheet propensities using a zinc-finger peptide and obtained free energies favoring β-sheet formation by 0.53, 0.48, 0.35, and 0 kcal/mol for valine, leucine, alanine, and glycine.[1] Minor and Kim measured the β-sheet propensities using the IgG-binding domain from protein G and obtained values of 0.82, 0.51, 0.00, and −1.2 kcal/mol for these residues in the middle of an antiparallel β-sheet and values of 0.17, −0.24, 0.00, and −0.85 when valine, leucine, alanine, and glycine were on the edge of a β-sheet.[2] The authors note that the context in which a residue occurs may affect its propensity to form β-sheets. Smith, Withka, and Regan performed similar studies of residues in the middle of an antiparallel β-sheet and obtained values of 0.94, 0.45, 0.00, and −1.21.kcal/mol.[3] In a subsequent study, Smith and Regan examined the energetics of side-chain interactions between pairs of amino acids in antiparallel β-sheets.[18] Blasie and Berg recently reported a similar study of electrostatic interactions across the antiparallel β-sheet of the zinc finger system.[19]

Our results differ from most of the studies described above, in that our studies show no significant differences in the β-sheet forming propensities of leucine and valine. Although this difference may reflect differing properties of parallel and antiparallel β-sheets, we believe that it does not. Even though the ^{1}H NMR data allow the degree of hydrogen bonding of H_b and H_c to be determined independently, it is likely that the hydrogen bonding of these two NH groups is cooperative. For this reason, the ^{1}H NMR data for H_b do not reflect exclusively the propensities of the four amino acids to form parallel β-sheets. Since a valine residue at the top of **1** disfavors the turn conformation, it may also destabilize the β-sheet conformation. If the data in the third row of Figure 1 are neglected for this reason, and comparisons are only made along the rows and not along the columns, then the remaining data (the first, second, and fourth rows) suggest that valine favors β-sheet formation over leucine. In short, the experiments described in this paper cannot determine with certainty whether leucine and valine are comparable at forming parallel β-sheets, or whether valine is better than leucine.

In an ideal model system in which to study β-sheet formation, the property being measured would depend exclusively upon the inherent β-sheet forming propensities of the amino acids. As the studies of Minor and Kim have shown, it is difficult to separate the inherent β-sheet forming propensities of the amino acids from the effects of the context in which the amino acids occur.[2] In other words, many different factors may contribute to effects that involve small (≤1 kcal/mol) differences in energy.

With the goal of developing an optimal model system, we had initially considered model systems other than **1**. We prepared and studied several derivatives of the general structure **8**, with the anticipation that the alanine and glycine residues at the ends of the tripeptide strands would isolate the middle (R_1 and R_2) residues from the turn portion of the molecules and that chemical shift differences would better reflect the β-sheet forming propensities of these residues. We decided not to use



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

this system, however, because of its greater potential for conformational heterogeneity, greater difficulty of synthesis, and greater complexity in the ^{1}H NMR spectrum. We also considered model system **9**, which differs from **1** in that a two-carbon chain, instead of a three-carbon chain, links the two urea groups. Previous studies in our laboratory have established that

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diureas linked by a two-carbon tether favor a turn conformation more strongly than diureas linked by a three-carbon tether.[8b] We anticipated that model system **9** would equilibrate between the turn and β-sheet conformations and that there would be little involvement of the unfolded conformation and of other conformations. The diurea turn structure with the two-carbon tether is very conformationally constrained, however, and it does not allow the two peptides to align in a fashion that is completely parallel.[8b,c] We chose not to use this model system, because we were concerned that the β-sheet forming propensities that were determined would reflect the ability of the amino acids to tolerate this conformationally-constrained turn, as well as the inherent β-sheet forming propensities of the amino acids. We finally chose to use system **1**, because it is simpler than **8** and less conformationally constrained than **9**.

Conclusion

In summary, the studies described in this paper establish that a combinatorial library of chemical models for parallel β-sheets can be synthesized and used to study the parallel β-sheet forming propensities of different amino acids. The trends in parallel β-sheet forming propensities that we have observed (L, V > A > G) are largely similar to the trends in antiparallel β-sheet forming propensities that other researchers have measured using small antiparallel β-sheet proteins.[1−3] One advantage of the model system is that it is particularly well-suited to the study of *parallel* β-sheet structure. In proteins, turn structures can create antiparallel β-sheets, but not parallel β-sheets. Typically a much longer segment of polypeptide (e.g., an α-helix) is require to link the β-strand segments of a parallel β-sheet. In the present system, the diurea turn unit permits the juxtaposition of two peptide groups in a parallel fashion, without requiring long polypeptide segments.

Another advantage of the present system is that it is a combinatorial library. As such, this system allows multiple comparisons to be performed. In the present 4 × 4 library, it is possible to make eight comparisons, indicating the β-sheet forming propensities of a given residue. In a 20 × 20 library comprising all of the amino acids, it would be possible to make 40 comparisons for a given residue. The greater number of comparisons afforded by combinatorial libraries should better allow trends to be distinguished from anomalies, context effects to be identified, and specific pairwise interactions to be studied.

One disadvantage of the present library is that it was prepared by multiple parallel synthesis, a labor-intensive process. The preparation, purification, and characterization of the 16-membered library and eight controls required several weeks of effort. The work to prepare a 20 × 20 library would be prohibitive. More sophisticated combinatorial methods, such as single-bead techniques or the synthesis and analysis of mixtures, will be required to allow the preparation and study of larger libraries. We are currently considering such approaches for future studies.

Experimental Section

General Methods. Merrifield resin (1% divinylbenzene crosslinked polystyrene) bearing Boc-protected amino acids was obtained from Peptides International or Advanced Chemtech. A 3:1 (v/v) mixture of dichloromethane and trifluoroacetic acid containing 1 mg/mL of indole was used to remove Boc protective groups. The solution was prepared at least 48 h before use. Solid-phase syntheses were performed with use of a 30- or 500-mL reaction tube with a sintered glass frit and stopcock at one end and a stoppered ground glass joint at the other. Solid-phase reaction mixtures were shaken with a mechanical shaker. The reaction vessel was drained by using nitrogen gas at ca. 5 psi to expel the solvent. The resin was washed by adding solvent, shaking for ca. 1 min, and draining the reaction vessel.

General Procedure for the Preparation of Artificial β-Sheets 1. A 500-mL solid-phase reaction vessel was charged with 10 g of Boc-glycine, Boc-alanine, Boc-valine, or Boc-leucine Merrifield resin (0.76−1.27 mmol/gm). The resin was washed with three 100-mL portions of CH_2Cl_2 and 100 mL of CH_2Cl_2:CF_3CO_2H:indole solution and then shaken with 100 mL of CH_2Cl_2:CF_3CO_2H:indole solution for 30 min. The solution was drained, and the resin was washed with six 100-mL portions of CH_2Cl_2, two 100-mL portions of CH_2Cl_2:Et_3N (10:1, v/v), six 100-mL portions of CH_2Cl_2, and 100 mL of DMF. The resin was then shaken with 3.0 equiv of $PhN(COCl)CH_2CH_2CH_2N(Boc)CH_2CH_2CN$ (**4**), 7.5 mL of Et_3N, and 90 mL of DMF for 24 h. The solution was drained, and the resin was washed alternately with 100 mL of CH_2Cl_2 and 100 mL of methanol three times. After the final methanol wash, the resin was dried under a stream of N_2 and then in vacuo to afford 11−12 g of white resin.

A 30-mL solid-phase reaction vessel was charged with 1.0 g of the resin. The resin was washed with three 15-mL portions of CH_2Cl_2 and 15 mL of CH_2Cl_2:CF_3CO_2H:indole solution, and was then shaken with 15 mL of CH_2Cl_2:CF_3CO_2H:indole solution for 30 min. The solution was drained, and the resin was washed with six 15-mL portions of CH_2Cl_2, two 15-mL portions of CH_2Cl_2:Et_3N (10:1, v/v), and six 15-mL portions of CH_2Cl_2. The resin was then shaken with 3.0 equiv of alanine, valine, or leucine methyl ester isocyanate, or with glycine ethyl ester isocyanate, in 15 mL of CH_2Cl_2 for 2 h.[12] The solution was drained, and the resin was washed alternately with 15 mL of CH_2Cl_2 and 15 mL of methanol three times, and then with 15 mL of THF. The resin was then shaken with 15−20 mL of a 6 M solution of CH_3NH_2 in THF for 24 h.[20] [**Caution**: *Pressure develops in the reaction vessel.*] The solution was collected, the resin was washed with three 20-mL portions of THF, and the combined CH_3NH_2 solution and THF washes were concentrated by rotary evaporation. The CH_3NH_2 treatment and THF washing of the resin was repeated, and the combined residues from both treatments were concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel to afford a colorless glassy solid or white foam. If further purification was needed, preparative HPLC was performed with use of a C_{18} reverse phase column.

General Procedure for the Preparation of Controls 2 and 3. A 30-mL solid-phase reaction vessel was charged with 1.0 g of Boc-glycine, Boc-alanine, Boc-valine, or Boc-leucine Merrifield resin (0.76−1.27 mmol/gm). The resin was washed with three 15-mL portions of CH_2Cl_2 and 15 mL of CH_2Cl_2:CF_3CO_2H:indole solution and then shaken with 15 mL of CH_2Cl_2:CF_3CO_2H:indole solution for 30 min. The solution was drained, and the resin was washed with six 15-mL portions of CH_2Cl_2, two 15-mL portions of CH_2Cl_2:Et_3N (10:1, v/v), six 15-mL portions of CH_2Cl_2, and 15 mL of DMF. The resin was then shaken with 3.0 equiv of $PhN(COCl)Et$[13] or Et_2NCOCl, 0.64−1.06 mL (6.0 equiv) of Et_3N, and 15 mL of DMF for 16−24 h. The solution was drained, and the resin was washed alternately with 15 mL of CH_2Cl_2 and 15 mL of methanol three times and then with 15 mL of THF. The resin was then shaken with 15−20 mL of a 6 M solution of CH_3NH_2 in THF for 20 h. [**Caution**: *Pressure develops in the reaction vessel.*] The solution was collected, the resin was washed with three 20-mL portions of THF, and the combined CH_3NH_2 solution and THF washes were concentrated by rotary evaporation. The CH_3NH_2 treatment and THF washing of the resin was repeated, and the combined residues from both treatments were concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel.

$PhN(COCl)CH_2CH_2CH_2N(Boc)CH_2CH_2CN$ (4). A solution of $PhNHCH_2CH_2CH_2NHCH_2CH_2CN$[8b] (10.16 g, 49.96 mmol) and di-*tert*-butyl dicarbonate (10.96 g, 50.22 mmol) in 150 mL of methanol was stirred for 20 h and then concentrated by rotary evaporation and dried under vacuum to yield 14.92 g (98%) of $PhNHCH_2CH_2CH_2N(Boc)CH_2CH_2CN$ as a viscous pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (t, J = 7.7 Hz, 2 H), 6.70 (t, J = 6.8 Hz, 1 H), 6.61 (d, J = 7.0

(20) Twenty-four hours is not sufficient for the aminolysis to go to completion in the preparation of **1GV**, **1AV**, **1VV**, and **1LV**. In the preparation of these compounds, derivatives in which the top (valine) methyl ester groups are not aminolyzed are also isolated.

Hz, 2 H), 3.47 (t, $J = 6.5$ Hz, 2 H), 3.41 (t, $J = 6.8$ Hz, 2 H), 3.15 (q, $J = 6.3$ Hz, 2 H), 2.70−2.50 (m, 2 H), 1.93−1.75 (m, 2 H), 1.48 (s, 9 H).

A 1.93 M solution of phosgene in toluene (4.0 mL, 7.7 mmol) was added to a rapidly stirred, ice-cooled solution of $PhNHCH_2CH_2CH_2N(Boc)CH_2CH_2CN$ (0.98 g, 3.2 mmol) in 40 mL of methylene chloride and 40 mL of saturated aqueous $NaHCO_3$ solution. After 10 min, the phases were separated, the organic phase was extracted with three 20-mL portions of methylene chloride, and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated by rotary evaporation to afford 1.20 g (101%) of $PhN(COCl)CH_2CH_2CH_2N(Boc)CH_2CH_2CN$ as a colorless oil: IR ($CDCl_3$) 1724, 1691 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.54−7.36 (m, 2 H), 7.32−7.12 (m, 3 H), 3.73 (t, $J = 6.6$ Hz, 2 H), 3.47−3.28 (m, 2 H), 3.32 (t, $J = 7.1$ Hz, 2 H), 2.68−2.47 (m, 2 H), 1.88 (quintet, $J = 7.2$ Hz, 2 H), 1.45 (s, 9 H).

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Supporting Information Available: Spectroscopic and analytical data for **1**−**3** and ^1H NMR spectra of **1**−**4** (37 pages). See any current masthead page for ordering and Internet access instructions.

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