Molecular Scaffolds. 3. An Artificial Parallel β-Sheet

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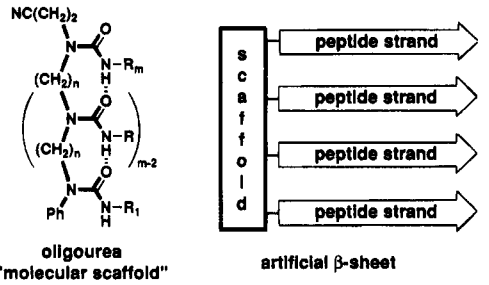
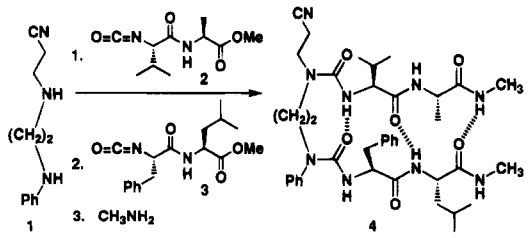
Within the past few years, there has been an explosion of interest in peptidomimetic chemistry.[1] This area of research offers fertile ground for the development of pharmacologically useful analogs of biologically active peptides and can provide fundamental insights into the factors affecting protein structure and stability. Although a number of models for two-stranded antiparallel β-sheets have been developed, progress toward artificial parallel β-sheets has been limited, and there are no models for β-sheets containing well-characterized hydrogen-bonded networks of three or more peptide β-strands.[2]

We recently embarked upon a program of research aimed at creating artificial β-sheets in which an oligourea "molecular scaffold" organizes multiple peptide strands into hydrogen-bonded β-sheets.[3] The following diagrams illustrate the structure of the oligourea molecular scaffold and its role in an artificial parallel β-sheet. In this paper, we report our initial efforts toward this goal—the creation of a two-stranded parallel β-sheet, in which two dipeptide strands are attached to a diurea molecular scaffold.



Artificial parallel β-sheet **4** was synthesized by sequential treatment of diamine **1** with peptide isocyanates[4] **2** and **3**, followed by aminolysis of the methyl ester groups with methylamine (eq 1). Diamine **1** reacts with peptide



(1)

isocyanates in a highly regioselective fashion because the aliphatic and aromatic amino groups differ substantially in reactivity.[3b]

Proton magnetic resonance nuclear Overhauser effect (NOE) studies reveal proximity between the Val-Ala and Phe-Leu peptide strands of **4** in chloroform solution. NOEs were identified by two-dimensional (NOESY) experiments and corroborated and quantified by one-dimensional experiments in which the following protons were irradiated: Val NH, Val α-CH, Ala α-CH, Ala NHC*H*$_3$, Phe NH and α-CH (coincident), Leu α-CH, Leu *pro-R* β-CH$_2$, Leu *pro-S* β-CH$_2$, and Leu NHC*H*$_3$. Table 1 provides data from the four one-dimensional experiments in which significant NOEs between peptide strands were observed.[5] The following data are noteworthy: irradiation of the valine NH enhances the phenylalanine α-CH resonance; irradiation of the alanine α-CH enhances the leucine β-CH$_2$ (*pro-S*) resonance; irradiation of the leucine β-CH$_2$ (*pro-S*) enhances the leucine β-CH$_2$ (*pro-R*) resonance; irradiation of the leucine β-CH$_2$ (*pro-R*) enhances the leucine γ-CH resonance.

Table 1. Percentage NOE Observed upon Irradiation of Selected Protons in 4[a]

protons enhanced	irrad. of Val NH	irrad. of Ala α-CH	irrad. of Leu β-CH$_2$ (*pro-R*)[b]	irrad. of Leu β-CH$_2$ (*pro-S*)[b]
NCH$_2$CH$_2$N	0.9, 1.2, 1.5, 3.0			
Val α-CH	1.0			
Val β-CH	1.9			
Val CH$_3$'s	0.3, 0.6			
Ala NH	0.6	1.0		
Ala α-CH			1.2	0.5
Ala CH$_3$		1.4		
Ala NHCH$_3$		3.1		
Phe α-CH	0.4		0.3	0.2
Leu NH		0.6	2.6	0.7
Leu α-CH			3.4	3.7
Leu β-CH$_2$ (*pro-R*)[b]		0.6		7.1
Leu β-CH$_2$ (*pro-S*)[b]		0.2	6.7	
Leu γ-CH			1.4	1.9
Leu CH$_3$'s			0.4, 0.8	0.7, 0.4

[a] One-dimensional NOE experiment performed at 21 °C and 500 MHz with 2 s irradiation of a freeze–pump–thaw degassed 25 mM sample in $CDCl_3$ solution. [b] The *pro-R* and *pro-S* assignments of the Leu β-CH$_2$ resonances were made on the basis of the model shown in Figure 1.[6]

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(4) We will describe the synthesis of peptide isocyanates, a hitherto unreported class of compounds, in a subsequent paper. For a related synthesis of amino acid ester isocyanates, see: Nowick, J. S.; Powell, N. A.; Nguyen, T. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 7364.

(5) The observed nuclear Overhauser effects, although small, are significant. Measurement of spectral subtraction errors of the various methyl resonances suggests that NOEs of 0.2% or greater can be interpreted to be real, rather than spectroscopic artifacts, under the experimental conditions that were employed. We attribute the small magnitude of the observed NOEs to the molecular weight of **4** (734). Consistent with this interpretation, the NOE between the diastereotopic leucine β-CH$_2$ protons is only 7%, and negative NOEs occur at −35 °C.

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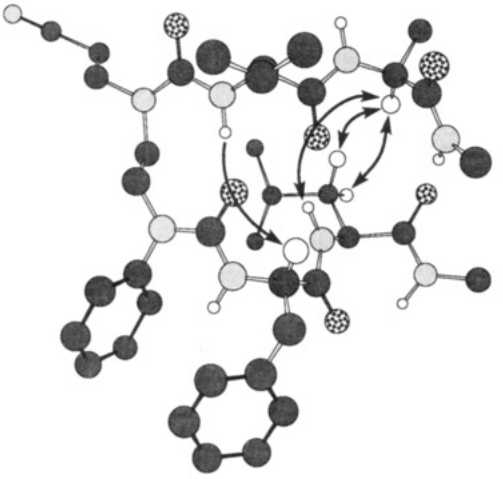


Figure 1. Model of parallel β-sheet conformation of **4**. NOEs observed between the peptide strands are shown with arrows. The model is a minimum energy conformer (local minimum) as calculated using MacroModel V4.5 with the AMBER*[7] force field. For clarity, only selected hydrogen atoms are shown.

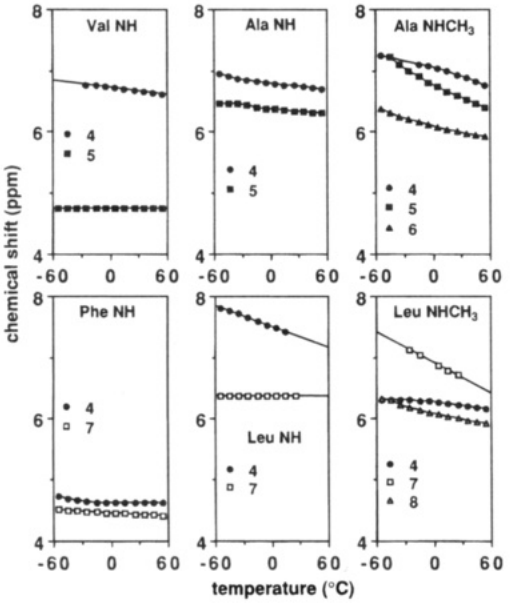
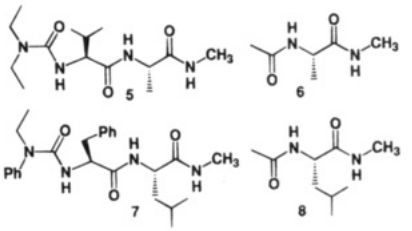


Figure 2. ^{1}H NMR chemical shifts of the NH groups of **4**–**8** as a function of temperature (1.0 mM, $CDCl_3$).

hances the leucine NH and β-CH_2 resonances; irradiation of each of the leucine β-CH_2 protons enhances the alanine α-CH resonance.

These data are consistent with a parallel β-sheet conformation in which the 1,2-diaminoethane diurea group forms a hydrogen-bonded nine-membered ring U-turn,[3b] the two urea groups juxtapose the Val-Ala and Phe-Leu peptide strands, and the peptide strands hydrogen bond to each other. A model of this conformation is shown in Figure 1.[6]

Comparison of the ^{1}H NMR chemical shifts of the amide and urea protons of **4** to those of controls **5**–**8** (Figure 2) provides further evidence for hydrogen-bonded parallel β-sheet structure. In the ^{1}H NMR spectra the



valine NH, leucine NH, and alanine methylamide NH resonances of **4** are substantially downfield of the valine NH of **5**, the leucine NH of **7**, and the alanine methylamide NH of **6** (1.9, 1.0, and 0.9 ppm, respectively, at 25 °C). In contrast, the phenylalanine NH, the alanine NH, and the leucine methylamide NH resonances of **4** are only slightly downfield of the phenylalanine NH of **7**, the alanine NH of **5**, and the leucine methylamide NH of **8** (0.2, 0.4, and 0.2 ppm, respectively, at 25 °C).[8] This pattern of downfield shifting is consistent with the hydrogen-bonded parallel β-sheet conformation shown in Figure 1.[9]

^{1}H NMR titration studies, in which $CDCl_3$ solutions of controls **5**–**8** were titrated with $DCON(CD_3)_2$ and 1:1 binding isotherms were fitted to the observed chemical shifts, indicate that non-hydrogen-bonded amide NH groups shift downfield by ca. 2 ppm at 25 °C upon hydrogen bonding to another amide group. From this number and the observed downfield shifts of the leucine NH of **4** (1.0 ppm relative to **7**) and the alanine methylamide NH of **4** (0.9 ppm relative to **6**), we estimate **4** to be roughly 50% in a parallel β-sheet conformation at 25 °C.

In summary, the NOE and chemical shift data described herein indicate that compound **4** adopts a parallel β-sheet conformation in chloroform solution. Ongoing studies in this laboratory are aimed at synthesizing and studying artificial β-sheets containing longer peptide strands and more peptide strands.

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Supporting Information Available: Synthetic procedures, ^{1}H NMR, difference NOE, and COSY spectra of **4** (13 pages).

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(6) In this model, the rotameric orientation of the Leu side chain was chosen to reflect the relative magnitudes of the NOEs from the Leu *pro-R* and *pro-S* β-protons to the Leu NH (2.6% and 0.7%, respectively) and the ^{1}H NMR coupling constants ($J_{\alpha,\beta pro\text{-}R}$ = 9.6 Hz, $J_{\alpha,\beta pro\text{-}S}$ = 5.5 Hz, $J_{\gamma,\beta pro\text{-}R}$ = 5.6 Hz, $J_{\gamma,\beta pro\text{-}S}$ = 8.5 Hz).

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(8) The slight downfield shift of the alanine NH resonance of **4** (0.4 ppm relative to **5**) suggests the existence of a minor conformer in which this NH group acts as a hydrogen-bond donor.

(9) The methylamide NH resonances of **5** and **7** appear substantially downfield of methylamide NH resonances of **6** and **8** (0.6 and 0.7 ppm, respectively, at 25 °C). These downfield shifts suggest that **5** and **7** can adopt β-turn conformations in which the methylamide NH groups are hydrogen bonded to the urea carbonyl groups. Although a similar conformation of the "upper" peptide strand of **4** could account for the downfield shifting of the alanine methylamide NH group, this conformation would not account for the downfield shifting of the leucine NH group or the NOEs between the peptide strands.