An Artificial β-Sheet Comprising a Molecular Scaffold, a β-Strand Mimic, and a Peptide Strand[1]

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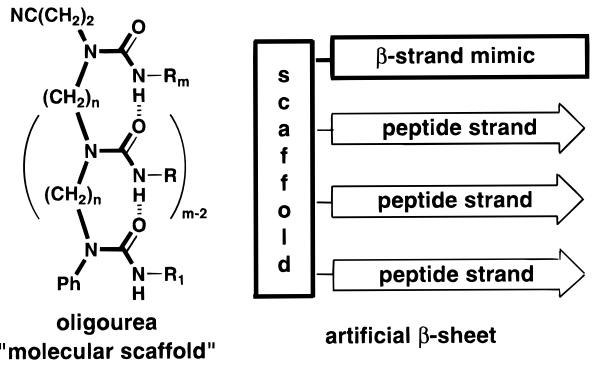
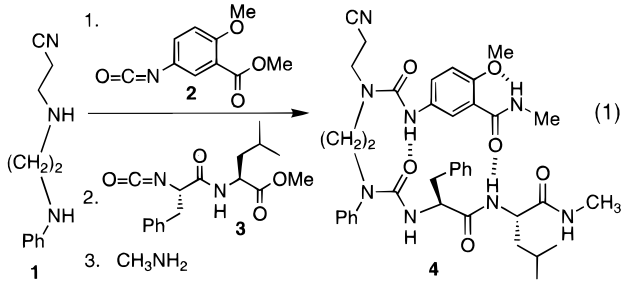
β-Sheets are a widespread, yet poorly understood, element of protein structure. A number of research groups are investigating β-sheet structure and stability by preparing and studying artificial β-sheets comprising molecular templates and peptide groups.[2] With the goal of synthesizing and studying artificial β-sheets of greater size and complexity than have been prepared previously, we are developing oligourea "molecular scaffolds" that hold multiple peptide strands in proximity and induce β-sheet formation.[3] We recently reported an artificial parallel β-sheet in which two dipeptide amides were attached to a diurea molecular scaffold.[3c] We are now extending this strategy by introducing a β-strand mimic as a second structural template, designed to confer greater order and solubility upon these structures. The following cartoon illustrates the roles of the

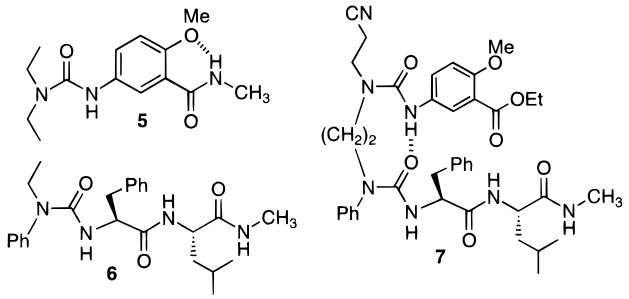


two templates and the peptide strands in these structures. In this paper, we report the combination of a β-strand mimic, a diurea scaffold, and one dipeptide amide to form a small artificial β-sheet.

Derivatives of 5-amino-2-methoxybenzamide were envisioned as conformationally-constrained compounds providing the same array of hydrogen-bonding groups as one edge of a peptide β-strand.[2b] To evaluate the ability of these derivatives to serve as β-strand mimics, we combined a 5-amino-2-methoxybenzamide unit with a 1,2-diaminoethane diurea molecular scaffold and a dipeptide to create artificial β-sheet **4**. This compound was prepared by sequential treatment of diamine **1** with isocyanates **2** and **3**, followed by aminolysis of the methyl ester groups with methylamine (eq 1).



Compound **4** exhibits exceptional downfield shifting of the "upper" urea NH and leucine NH resonances in the ^{1}H NMR spectrum.[4] In a 1.0 mM solution in $CDCl_3$ (294 K), these resonances appear at 10.00 and 8.20 ppm, respectively, while the corresponding resonances of control compounds **5** and **6**



appear at 6.31 and 6.37 ppm. The downfield shifting of these resonances (by 3.69 and 1.83 ppm, respectively) suggests that **4** adopts a pattern of hydrogen bonding similar to that of an antiparallel β-sheet.[5]

The magnitude of these shifts indicates that compound **4** is largely or wholly hydrogen bonded in chloroform solution. We have previously established that urea groups of related compounds shift downfield by about 2.5−2.8 ppm upon hydrogen bonding.[3a,b] The unusually large downfield shifting of the "upper" urea group of **4** (3.69 ppm relative to that of **5**) may be attributed to anisotropic effects associated with changes in the torsion angle about the bond between the urea group and the

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(4) ^{1}H NMR (500 MHz, $CDCl_3$) for compound **4** (assignments by COSY in italics, using numbering scheme shown in Figure 1): δ 0.48 (d, J = 6.2 Hz, 3 H, *1*), 0.79 (d, J = 6.2 Hz, 3 H, *2*), 1.13 (td, J = 12.3, 3.2 Hz, 1 H, *3*), 1.48−1.55 (m, 1 H, *4*), 1.57 (td, J = 11.8, 3.6 Hz, 1 H, *5*), 2.48 (d, J = 4.7 Hz, 3 H, *6*), 2.61−2.72 (m, 2 H, *7*), 2.90 (dd, ABX pattern, J_{AB} = 12.7 Hz, J_{BX} = 10.5 Hz, 1 H, *8*), 2.91 (d, J = 4.7 Hz, 3 H, *9*), 2.96 (dd, ABX pattern, J_{AB} = 13.1 Hz, J_{AX} = 6.1 Hz, 1 H, *10*), 3.48 (dt, J = 14.1, 6.3 Hz, 1 H, *11*), 3.56 (dt, J = 14.0, 6.2 Hz, 1 H, *12*), 3.62 (appar ddd, J = 14.6, 9.2, 4.3 Hz, 1 H, *13*), 3.71 (appar ddd, J = 15.2, 9.8, 5.3 Hz, 1 H, *14*), 3.76 (appar ddd, J = 15.1, 10.0, 5.1 Hz, 1 H, *15*), 3.86 (appar ddd, J = 13.6, 9.5, 4.9 Hz, 1 H, *16*), 3.95 (s, 3 H, *17*), 4.38 (ddd, J = 11.8, 8.9, 3.4 Hz, 1 H, *18*), 4.94 (td, J = 9.2, 6.2 Hz, 1 H, *19*), 5.00 (d, J = 8.6 Hz, 1 H, *20*), 5.51 (appar q, J = 4.3 Hz, 1 H, *21*), 6.92 (d, J = 9.1 Hz, 1 H, *22*), 7.14 (d, J = 7.7 Hz, 2 H, *23*), 7.27−7.31 (m, 5 H, *24*), 7.38 (t, J = 7.4 Hz, 1 H, *25*), 7.45 (t, J = 7.6 Hz, 2 H, *26*), 8.07 (appar q, J = 4.6 Hz, 1 H, *27*), 8.13 (d, J = 8.9 Hz, 1 H, *28*), 8.36 (dd, J = 8.9, 2.8 Hz, 1 H, *29*), 8.51 (d, J = 3.0 Hz, 1 H, *30*), 9.94 (s, 1 H, *31*).

(5) Other smaller chemical shift differences are also observed. Compound **4**: leucine methylamide NH (21), 5.45 ppm; phenylalanine NH (20), 5.02 ppm; "upper" methylamide NH (27), 8.11 ppm. Compound **5**: methylamide NH, 7.92 ppm. Compound **6**: phenylalanine NH, 4.44 ppm; leucine methylamide NH, 6.73 ppm. We attribute the difference in chemical shifts of the leucine methylamide groups to anisotropic effects of the aromatic rings of **4** and to the propensity of **6** to adopt a hydrogen-bonded β-turn conformation, in which the methylamide NH is hydrogen bonded to the urea carbonyl group.

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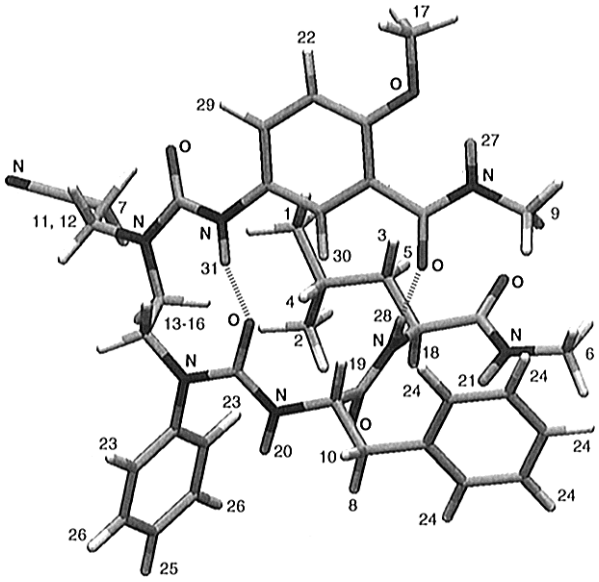

Figure 1. Model of **4** in a minimum energy conformation (local minimum) as calculated using MacroModel V4.5 with the AMBER* force field. To generate the starting geometry (prior to minimization), X-ray crystallographic coordinates of a related diurea (compound **2c** in ref 3b) were used, the dipeptide group was introduced in a β-strand conformation, and χ_1 and χ_2 angles were chosen to reflect observed coupling constants and NOEs (footnotes 4 and 7).

"upper" aromatic ring. The chemical shift of the leucine NH group (8.20 ppm) is also consistent with a hydrogen-bonded structure; the leucine NH group of **6** appears at 8.02 ppm in dimethylformamide-d_7, a good hydrogen-bond acceptor.

The ^{1}H NMR chemical shifts of **7** support this analysis. In this compound, the "upper" methylamide group of **4** has been replaced with an ethyl ester group, a weaker hydrogen-bond acceptor. The "upper" urea NH and leucine NH resonances appear at 9.51 and 6.97 ppm, respectively (1.0 mM solution in $CDCl_3$, 295 K). The large downfield shift of the urea group (3.20 ppm relative to that of **5**) indicates that this group is largely or wholly hydrogen bonded, while the small downfield shift of the leucine NH group (0.60 ppm relative to that of **6**) suggests that this group spends only a fraction of its time in a hydrogen-bonded conformation.

The chemical shifts of the leucine methyl resonances of **4** provide additional insight into the structure of the artificial β-sheet. One of the leucine methyl resonances appears unusually upfield at 0.44 ppm, while the other leucine methyl resonance appears at 0.79 ppm and those of **6** appear at 0.89 and 0.91 ppm, respectively. The upfield shift of this methyl group suggests that it is over the face of an aromatic ring. Figure 1 provides a model of **4** consistent with the chemical shifts of the methyl and NH groups.

^{1}H NMR DPFGSE NOE (double-pulsed field gradient spin echo NOE) experiments[6] shed light on the chemical shift of the upfield methyl group and support the model put forth in Figure 1.[7] Key results are summarized as follows: Irradiation of this methyl group (numbered 1 in Figure 1) enhances the "upper" urea NH resonance (31 in Figure 1), "upper" aromatic ring resonances 29 and 30, and 1,2-diaminoethane backbone resonance 13. Irradiation of the other leucine methyl group (2) enhances phenyl resonances 23 and 1,2-diaminoethane backbone resonance 13. Irradiation of leucine β-proton 3 enhances "upper" aromatic ring resonance 30 and the "upper" methylamide NH resonance (27). Irradiation of the leucine methylamide methyl (6) enhances the "upper" methylamide methyl (9). Irradiation of the "upper" aromatic ring proton 30 enhances the leucine β- and γ-resonances (3−5), the leucine NH (28), and the phenylalanine α-resonance (19). Irradiation of the "upper" urea NH (31) gives a large enhancement of the "upper" aromatic ring resonance 30, a much smaller enhancement of "upper" aromatic ring resonance 29, enhancements of leucine β-resonance 3 and γ-resonance (4), the phenylalanine α-resonance (19), and the 1,2-diaminoethane backbone resonances (13−16). The NOEs to all four backbone protons (13−16) are relatively large, suggesting that the backbone is conformationally mobile, allowing all the backbone protons to spend time in the vicinity of the "upper" urea NH. A number of small NOEs are inconsistent with the model in Figure 1 (e.g., $H_{21} \rightarrow H_{30}$ and $H_{24} \rightarrow H_{30}$), suggesting that other portions of the molecule (e.g., Leu ψ and Phe χ_1) may also be conformationally mobile and providing a reminder that no single structure can provide a complete picture of a conformationally dynamic molecule.

In summary, the ^{1}H NMR studies described herein indicate that the diurea molecular scaffold and 5-amino-2-methoxybenzamide β-strand mimic act in conjunction to stabilize a β-strand conformation in a dipeptide strand, thus forming a small artificial β-sheet. Ongoing efforts in this laboratory are aimed at synthesizing and studying larger artificial β-sheets comprising more peptide strands, longer peptide strands, and larger β-strand mimics.

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(7) DPFGSE NOE experiments were performed using 0.5 s mixing times. NOEs are reported in parentheses as percentage enhancement relative to area of the irradiated peak in the DPFSGE NOE spectrum and are normalized for the number of protons involved. (These values are *not* steady-state NOEs and only indicate the relative enhancements of resonances within a given experiment.) NOEs of 0.2% or greater are reported. Percentage enhancements of resonances 4 and 5 are approximate because these resonances overlap. Percentage enhancements of resonances 8 and 10 are approximate because these resonances overlap. Irradiation of methyl group 1 enhances resonances 2 (1.1), 3 (2.5), 4 (2.7), 5 (4.4), 13 (0.2), 18 (0.4), 29 (0.2), 30 (0.4), and 31 (0.3). Irradiation of methyl group 2 enhances resonances 1 (1.2), 4 (2.5), 5 (4.5), 13 (0.2), 18 (5.0), and 23 (0.3). Irradiation of proton 3 enhances resonances 1 (0.8), 4 (6.2), 5 (18.1), 18 (0.9), 21 (0.3), 27 (0.3), 28 (2.4), and 30 (0.5). Irradiation of methyl group 6 enhances resonances 9 (0.3), 18 (0.2), 21 (4.7), and 24 (0.4). Irradiation of proton 18 enhances resonances 2 (1.1), 3 (0.5), 4 (0.9), 5 (2.5), 21 (0.9), and 28 (1.3). Irradiation of proton 21 enhances resonances 3 (0.2), 6 (1.7), 9 (0.3), 18 (1.3), 24 (0.3), 28 (3.1), and 30 (0.2). Irradiation of protons 23 enhances resonances 13 (0.4), 14 (0.5), 15 (0.8), 16 (0.7), 19 (0.6), 20 (1.5), 25 (0.5), and 26 (3.2). Irradiation of protons 24 enhances resonances 6 (0.3), 8 (1.7), 10 (2.7), 19 (1.8), 20 (1.0), 21 (0.7), 28 (0.3), and 30 (0.2). Irradiation of proton 30 enhances resonances 3 (0.3), 4 (0.9), 5 (0.3), 19 (1.6), 20 (0.2), 28 (1.9), and 31 (5.2). Irradiation of proton 31 enhances resonances 3 (0.2), 4 (0.7), 13 (5.5), 14 (5.0), 15 (3.7), 16 (3.1), 19 (0.3), 29 (0.8), and 30 (9.9).