An Artificial Antiparallel β-Sheet Containing a New Peptidomimetic Template

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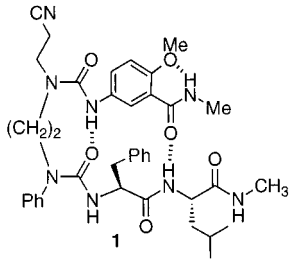
During the past few years, we have been developing compounds that mimic the structures and hydrogen-bonding patterns of protein β-sheets (artificial β-sheets).[1] In 1996, we reported studies of artificial β-sheet **1**.[2] This



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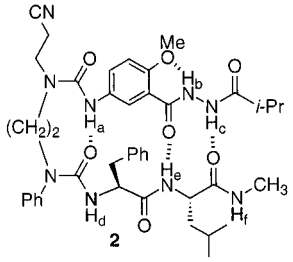

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compound comprises a 5-amino-2-methoxybenzamide template that is linked to a phenylalanylleucine dipeptide by a hydrogen-bonded 1,2-diaminoethane diurea turn unit. The 5-amino-2-methoxybenzamide template forms only one hydrogen bond to the dipeptide and is too short to hydrogen bond to the carbonyl group of the leucine residue.[3] Here, we report artificial β-sheet **2**, which contains a 5-amino-2-methoxybenzoic hydrazide template that is of sufficient length to form two hydrogen bonds to the attached dipeptide.

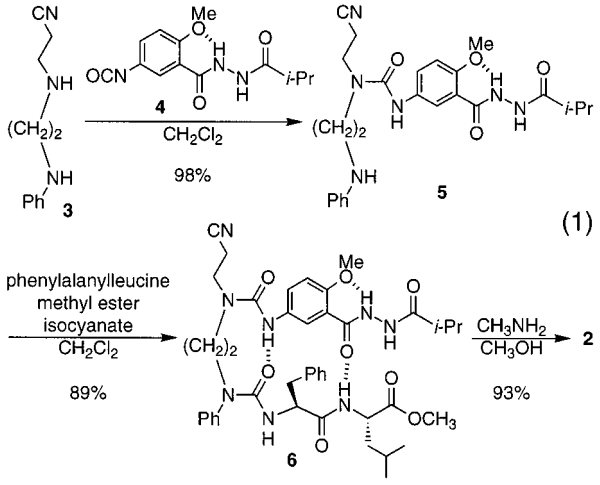
Artificial β-sheet **2** was synthesized efficiently and in high yield from diamine **3**[4] (eq 1). Reaction of diamine **3** with isocyanate **4**[5] afforded urea **5**. Treatment of urea **5** with phenylalanylleucine methyl ester isocyanate[6] generated diurea **6**. Aminolysis with methylamine converted the methyl ester group of **6** to a methylamide group, affording artificial β-sheet **2** in 81% overall yield. Artificial β-sheet **2** was also prepared by solid-phase synthesis on Merrifield resin in 60% overall yield.[5]

^{1}H NMR spectroscopic studies establish that **2** is intramolecularly hydrogen bonded and that it adopts an antiparallel β-sheet conformation in $CDCl_3$ solution. ^{1}H NMR chemical shift studies indicate that the appropriate NH groups are hydrogen bonded. Thus, H_a, H_c, and H_e in **2** are shifted downfield by 3.62, 1.67, and 1.92 ppm relative to the corresponding protons in controls **7** and **8**

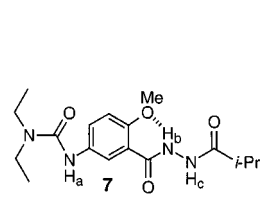
Table 1. ^{1}H NMR Chemical Shifts of the NH Protons of 2, and 7-9[a]

	H_a	H_b	H_c	H_d	H_e	H_f
2	9.93	10.97	10.45	4.82	8.29	5.79
7	6.31	10.85	8.78			
8				4.43	6.37	6.71
9					5.82	6.00

[a] Spectra were recorded at 295 K in 1.0 mM $CDCl_3$ solution.



(Table 1).[7] In contrast, protons H_b and H_d exhibit little (0.12 and 0.39 ppm) downfield shifting. Proton H_f of **2** is shifted *upfield* by 0.92 ppm relative to that of dipeptide **8**, because **8** adopts a β-turn conformation.[2,8] In this conformation, the methylamide NH group is hydrogen bonded to the urea carbonyl group and is shifted downfield. For this reason, monopeptide **9** serves as a better control for H_f.



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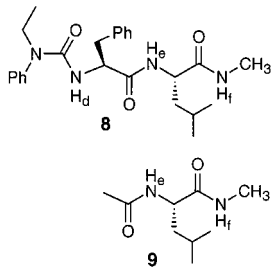

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^{1}H NMR nuclear Overhauser effect (NOE) studies provide compelling evidence that **2** adopts a β-sheet conformation. Because the molecular weight of **2** is moderately high (784), its NOEs are small. For this reason, we performed these studies in the rotating frame, using the transverse-ROESY (Tr-ROESY) method.[9] A one-dimensional spectrum was first recorded, and 32 distinct resonances were identified and designated 1−32 in order of increasing chemical shift.[10] The resonances were then assigned by a combination of one-dimensional and two-dimensional (PFG COSY and Tr-ROESY) methods. These assignments are shown in Figure 1. Separate

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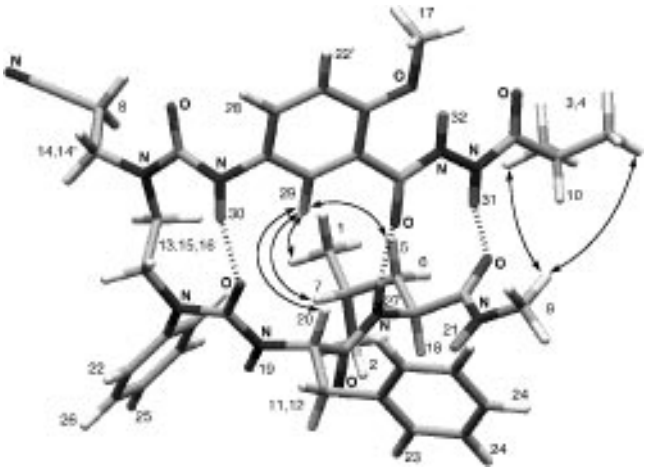

Figure 1. Model of artificial β-sheet **2** illustrating interstrand ROEs. The model was generated using MacroModel V5.5 with the AMBER* force field. The starting geometry (before minimization) was chosen to reflect the preferred (anti) conformation of the 1,2-diaminoethane diurea backbone.[4,12] The starting conformation of the leucine side-chain was chosen to reflect measured coupling constants and NOEs; that of the phenylalanine is largely arbitrary.

regions of some resonances (14 and 14′, 22 and 22′) were identified through the two-dimensional NMR studies.

The Tr-ROESY studies show long-range ROEs between the upper (peptidomimetic) and lower (peptide) strands that are indicative of antiparallel β-sheet structure.[11] Most notably, the proton at the 6-position of the aromatic ring (29) exhibits ROEs to the phenylalanine α-proton (20) and the leucine side-chain (1, 5, and 7). These ROEs are shown as arrows in Figure 1. Also noteworthy are ROEs between the leucine methylamide methyl group (9) and the isobutyryl methyl groups of the hydrazide (3 and 4). One weak cross-peak between the phenylalanine NH proton (19) and the proton at the 6-position of the aromatic ring (29), which is not consistent with the model shown in Figure 1, may be an artifact resulting from coupling between 19 and 20.[9b] ROEs between the upper urea proton (30) and the 1,2-diaminoethane backbone protons (13, 15, and 16) provide evidence that the 1,2-diaminoethane diurea forms a turn structure. The presence of moderate-strong ROEs between 30 and at least three of the backbone protons suggests that the 1,2-diaminoethane diurea turn comprises multiple conformations (e.g., two diastereomeric anti conformers of the 1,2-diaminoethane backbone).[4,12]

Strong ROEs between the α and NH protons of adjacent residues (20 and 27, 18 and 21), and large (8.4 and 9.2 Hz) $^3J_{HN\alpha}$ coupling constants, provide evidence for a β-strand conformation in the phenylalanylleucine peptide strand.[13] Weak-moderate ROEs between the phenylalanine and leucine NH groups (19 and 27), and between the leucine NH and leucine methylamide NH groups (27 and 21), suggest that non-β-strand conformers may also be present.

In conclusion, these studies show that the 5-amino-2-methoxybenzoic hydrazide template forms a hydrogen-bonded antiparallel β-sheet with the phenylalanylleucine dipeptide in artificial β-sheet **2**. Artificial β-sheet **2** is similar in structure to systems developed by Kemp et al.[14] and by Michne and Schroeder.[15] These systems also contain templates that mimic peptide β-strands; however, the templates are tetracyclic and bicyclic aromatic molecules. The simplicity of the 5-amino-2-methoxybenzoic hydrazide template renders it an attractive alternative to these polycyclic aromatic templates.

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Supporting Information Available: Synthetic procedures and one- and two-dimensional ^{1}H NMR spectra of artificial β-sheet **2** (24 pages).

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(10) ^{1}H NMR of **2** (25 mM in $CDCl_3$, 298 K): 0.66 (d, J = 6.4 Hz, 3 H, *1*), 0.79 (d, J = 6.3 Hz, 3 H, *2*), 1.24 (d, J = 6.9 Hz, 3 H, *3*), 1.27 (d, J = 6.9 Hz, 3 H, *4*), 1.39 (ddd, J = 13.3, 9.3, 5.4 Hz, 1 H, *5*), 1.48 (ddd, J = 13.8, 8.0, 6.2 Hz, 1 H, *6*), 1.56−1.48 (m, 1 H, *7*), 2.68−2.65 (m, 2 H, *8*), 2.68 (d, J = 4.8 Hz, 3 H, *9*), 2.81 (septet, J = 6.9 Hz, 1 H, *10*), 2.85 (dd ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 8.7 Hz, 1 H, *11*), 3.00 (dd ABX pattern, J_{AB} = 13.8 Hz, J_{BX} = 7.5 Hz, 1 H, *12*), 3.48−3.41 (m, 1 H, *13*), 3.56−3.45 (m, 2 H, *14* and *14′*), 3.69−3.61 (m, 2 H, *15*), 3.92−3.86 (m, 1 H, *16*), 4.05 (s, 3 H, *17*), 4.44 (td, J = 9.2, 5.7 Hz, 1 H, *18*), 4.82 (d, J = 8.4 Hz, 1 H, *19*), 4.94 (q, J = 8.2 Hz, 1 H, *20*), 5.80 (br q, J = 4.7 Hz, 1 H, *21*), 6.96−6.94 (m, 2 H, *22*), 6.96 (d, J = 9.0 Hz, 1 H, *22′*), 7.15 (appar d, J = 7.0 Hz, 2 H, *23*), 7.29−7.23 (m, 3 H, *24*), 7.36 (appar t, J = 7.2 Hz, 1 H, *25*), 7.40 (appar t, J = 7.1 Hz, 2 H, *26*), 8.28 (d, J = 9.2 Hz, 1 H, *27*), 8.39 (dd, J = 9.0, 2.8 Hz, 1 H, *28*), 8.62 (d, J = 2.8 Hz, 1 H, *29*), 9.92 (s, 1 H, *30*), 10.44 (d, J = 7.0 Hz, 1 H, *31*), 10.97 (d, J = 7.5 Hz, 1 H, *32*).

(11) ^{1}H NMR Tr-ROESY cross-peaks for **2** (25 mM in $CDCl_3$, 298 K). (Cross-peaks in the F_1 dimension are tabulated for each resonance in the F_2 dimension.) *1*: 5 (m), 6 (s), 7 (s), 18 (m), 27 (w), 29 (w). *2*: 5 (m), 6 (m), 7 (s), 18 (s), 27 (w). *3*: 9 (m), 10 (s). *4*: 9 (m), 10 (s). *5*: 1 (s), 2 (m), 6 (s), 7 (m), 18 (m), 27 (m), 29 (w). *6*: 1 (s), 2 (s), 5 (s), 18 (m), 27 (m). *7*: 1 (s), 2 (s), 5 (m), 18 (m), 27 (m), 29 (w). *8*: 14 (s), 14′(s). *9*: 3 (m), 4 (m), 21 (s). *10*: 3 (s), 4 (s), 31 (m). *11*: 12 (s), 19 (s), 20 (s), 23 (m). *12*: 11 (s), 19 (m), 20 (s), 23 (m). *13*: 15 (s), 30 (m). *14*: 8 (s), 14′ (s). *14′*: 8 (s), 14 (s). *15*: 13 (s), 16 (s), 22 (m), 30 (s). *16*: 15 (s), 22 (w), 30 (m). *17*: 22′ (s). *18*: 1 (m), 2 (s), 5 (m), 6 (m), 7 (m), 21 (s), 27 (m). *19*: 11 (s), 12 (m), 22 (m), 23 (m), 27 (w), 29 (w). *20*: 11 (s), 12 (s), 23 (s), 27 (s), 29 (m). *21*: 9 (s), 18 (s), 27 (w). *22*: 19 (s), 26 (s). *22′*: 17 (s), 28 (s). *23*: 11 (s), 12 (s), 19 (m), 20 (s), 24 (s). *24*: 23 (s). *26*: 22 (s). *27*: 5 (m), 6 (m), 7 (m), 18 (m), 19 (m), 20 (s), 21 (w). *28*: 22′ (s), 30 (w). *29*: 1 (w), 5 (w), 7 (w), 20 (s), 30 (s). *30*: 13 (m), 15 (s), 16 (m), 28 (w), 29 (s). *31*: 10 (m). *32*: 17 (m).

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