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A New Artificial β-Sheet That Dimerizes through Parallel β-Sheet Interactions

Sergiy Levin and James S. Nowick*

Department of Chemistry, University of California, Irvine, Irvine, California 92697-2025

jsnowick@uci.edu

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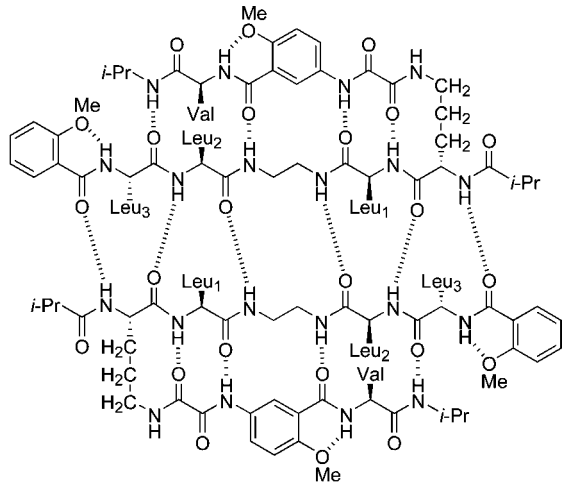
ABSTRACT



This paper introduces a chemical model of a β-sheet that dimerizes through parallel β-sheet interactions in $CDCl_3$ solution. The model consists of two *C*-terminally linked dipeptides connected to a molecular template. ^{1}H NMR studies establish the β-sheet folding and dimerization of the model system. This system corroborates that linking two peptide strands and blocking one edge of the assembly creates soluble, easy-to-study systems that participate in the types of interactions that occur widely in peptide and protein aggregates.

Intermolecular parallel β-sheet interactions occur widely in peptide and protein aggregation and are important in Alzheimer's and a variety of other neurodegenerative disorders.[1] These noncovalent interactions are difficult to study because they often occur in an uncontrolled fashion and lead to insoluble aggregates. We recently reported the first well-defined chemical model system with which to study intermolecular parallel β-sheet formation.[2] Here, we report a second chemical model system that forms well-defined dimers through parallel β-sheet formation.[3]

In our first system, we achieved dimer formation by *N*-terminally linking two short peptides with a succinic acid unit and blocking one of the exposed hydrogen-bonding edges with a molecular template. In the new system, we

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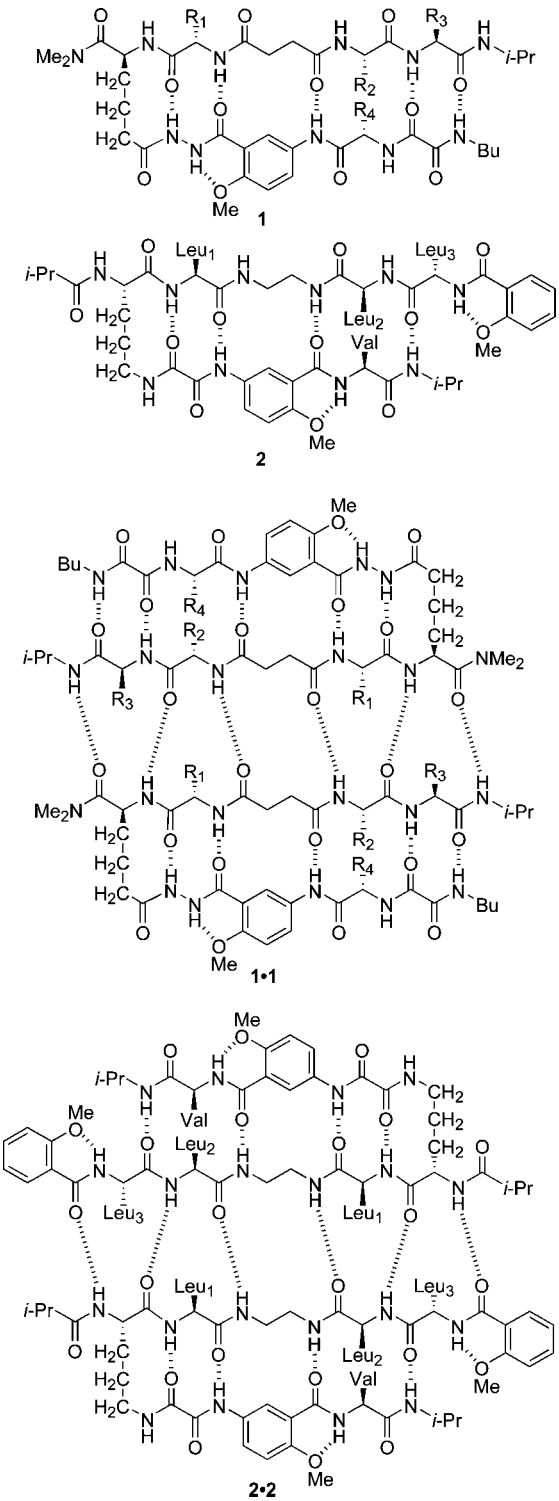
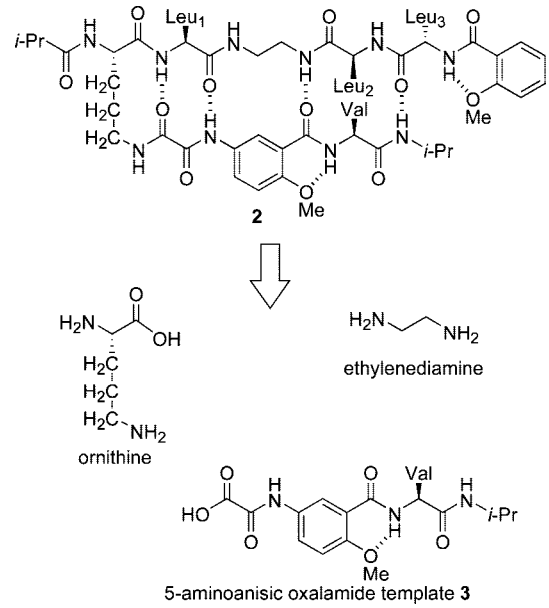


Figure 1. Artificial β-sheets **1** and **2** and corresponding parallel β-sheet dimers **1·1** and **2·2**.

achieve dimer formation by *C*-terminally linking two short peptides with an ethylenediamine unit and blocking one of the exposed hydrogen-bonding edges. Figure 1 illustrates the structure of the two systems, artificial β-sheets **1** and **2**, respectively, and the corresponding hydrogen-bonded dimers **1·1** and **2·2**.

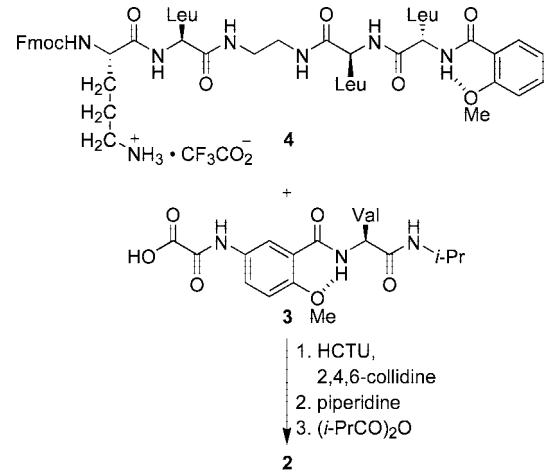
Scheme 1. Building Blocks of Artificial β-Sheet **2**



Artificial β-sheet **2** consists of two *C*-terminally linked dipeptide strands that are blocked along one edge by a hydrogen-bonding template. The dipeptide strands are linked with ethylenediamine, and the template comprises 5-aminoanisic acid oxalamide and valine. The template is connected to one of the dipeptide strands by a turn unit composed of δ-linked ornithine.[4] Scheme 1 illustrates these components. The exposed edge of the linked dipeptide strands of **2** presents six alternating hydrogen-bond donor and acceptor groups that participate in the formation of the hydrogen-bonded dimer. We incorporated the nonpolar amino acids leucine and valine in the peptide strands and template to allow studies in chloroform, a solvent that supports hydrogen bonding.

Artificial β-sheet **2** was assembled from 5-aminoanisic oxalamide template **3** and ethylenediamine-linked peptide **4**

Scheme 2. Assembly of Artificial β-Sheet **2**



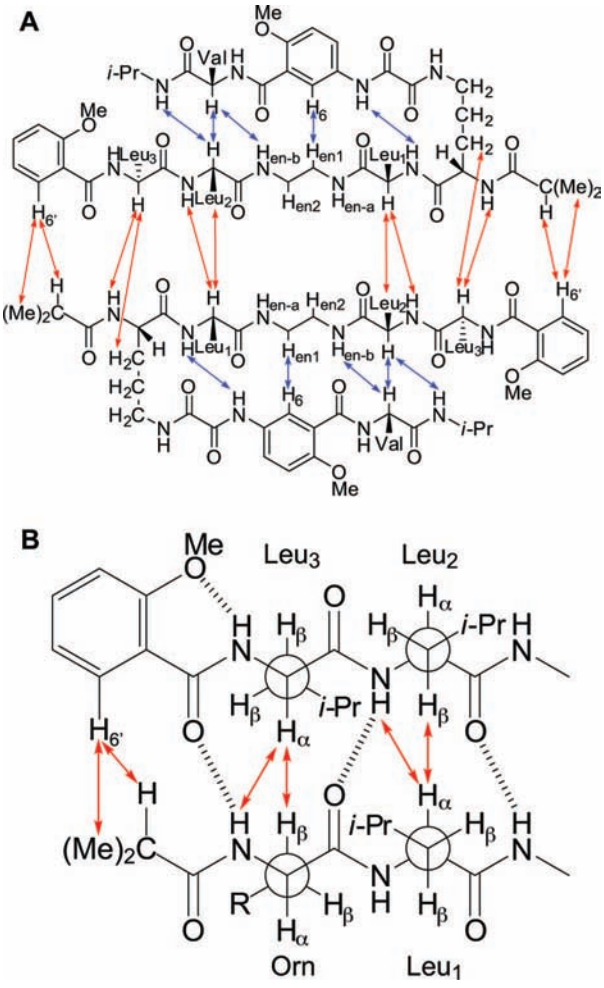


Figure 2. Key NOEs observed in artificial β-sheet **2**. A: Red arrows represent intermolecular NOEs; blue arrows represent intramolecular NOEs. B: Newman projection with intramolecular NOEs (red arrows).

using standard solution-phase peptide synthesis techniques (Scheme 2). Peptide-coupling reactions were performed with HCTU and 2,4,6-collidine or DIPEA.[5] The 5-aminoanisic oxalamide template was prepared from Fmoc-protected 5-aminoanisic acid.[6] Ethylenediamine-linked peptide **4** was prepared from Boc-protected ethylenediamine ($BocNHCH_2CH_2NH_2$) by standard solution-phase Boc- and Cbz-based peptide synthesis procedures.

^{1}H NMR studies establish the folding and dimerization of artificial β-sheet **2** in $CDCl_3$ solution. The proton resonances of artificial β-sheet **2**, although broadened at ambient temperature, are sufficiently sharp at 268 K and 800 MHz to be assigned definitively by COSY, TOCSY, and NOESY techniques. Consistent with a hydrogen-bonded β-sheet structure, the amide NH resonances are shifted downfield

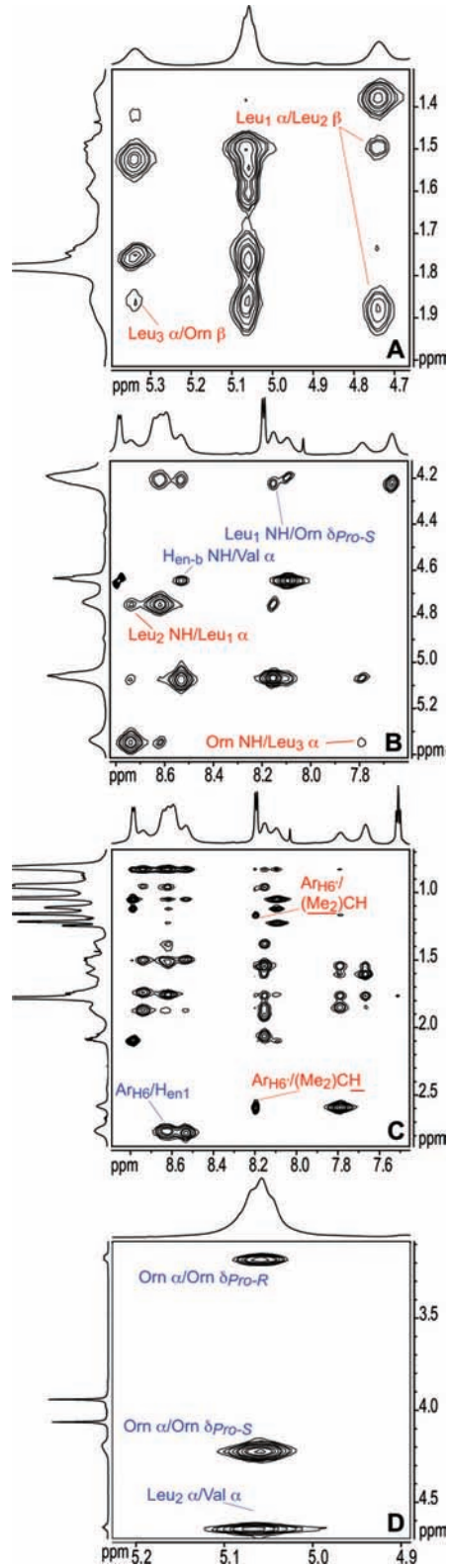


Figure 3. Selected NOEs in artificial β-sheet **2**. 800 MHz NOESY spectrum recorded at 4 mM and 268 K in $CDCl_3$ with a 400-ms mixing time. Intramolecular NOEs are shown in blue; intermolecular NOEs are shown in red.

compared to non-hydrogen-bonded amide resonances and the amino acid α-proton resonances are shifted downfield

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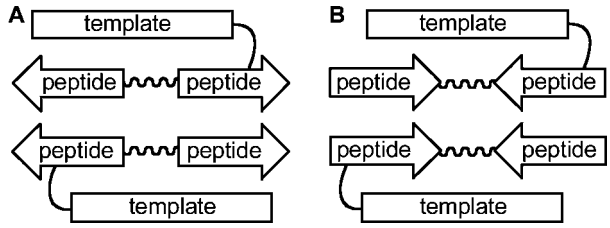

Figure 4. Schematic representations of parallel β-sheet dimers **1**·**1** (A) and **2**·**2** (B).

compared to random coil values.[7] The ornithine δ-protons show significant magnetic anisotropy (1.06 ppm) and strong NOE signals with the ornithine α-proton, reflecting a turn conformation of the ornithine side chain.[4]

The 800 MHz NOESY studies definitively establish the formation of a well-defined and folded hydrogen-bonded dimer in $CDCl_3$ solution. Artificial β-sheet **2** shows a rich array of intramolecular NOEs characteristic of antiparallel β-sheet folding and intermolecular NOEs characteristic of parallel β-sheet dimerization.[8] Figure 2 illustrates these NOEs. Figure 2A shows the overall structure of the dimer, while Figure 2B represents half of the 2-fold symmetrical parallel β-sheet dimerization interface as a Newman projection. Intramolecular NOEs associated with folding are represented by blue arrows, while intermolecular NOEs associated with dimerization are represented by red arrows.

Figure 3 shows key regions of the NOESY spectrum. Noteworthy NOEs associated with antiparallel β-sheet folding include strong NOEs between Leu_2 α and Val α (Figure 3D) and H_6 of the template and H_{en1} of the linker (Figure 3C). Noteworthy NOEs associated with parallel β-sheet dimerization include NOEs between Leu_1 α and Leu_2 β and between Leu_3 α and Orn β (Figure 3A). Additional noteworthy NOEs associated with parallel β-sheet dimerization include NOEs between Leu_2 NH and Leu_1 α and between Orn NH and Leu_3 α (Figure 3B).

The present system is complementary to the original system in that the peptide strands in **2** are *C*-terminally linked, while those in **1** are *N*-terminally linked. Thus, this system offers a new topology for dimer formation and provides a distinct new class of dimers. This new system also demonstrates the generality of the approach to achieving dimerization through parallel β-sheet interactions by linking two peptide strands and blocking one of the exposed edges with a molecular template. Figure 4 provides cartoons that show the structural relationship between the dimers of these two systems.

In future studies, we will use systems such as these to explore noncovalent interactions among parallel β-sheets. These types of systems are ideally suited to such studies, because they do not form uncontrolled aggregates, like simple peptides. We further anticipate applying the design principles established through these studies to create systems that participate in parallel β-sheet interactions in aqueous solution and using these systems to antagonize peptide and protein aggregation.

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Supporting Information Available: Synthetic procedures and NMR spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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