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Two New β-Strand Mimics

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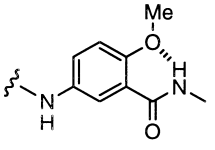
Abstract—In a previous report, Nowick and co-workers described β-strand mimic **A**, which duplicates the structure and hydrogen-bonding pattern of one edge of a tetrapeptide in a β-strand conformation (Nowick, J. S.; Pairish, M.; Lee, I. Q.; Holmes, D. L.; Ziller, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 5413). β-Strand mimic **A** is composed of a 5-amino-2-methoxybenzoic acid unit linked to a 5-hydrazino-2-methoxybenzamide unit by means of an acylhydrazine group. This paper introduces two related β-strand mimics (**B** and **C**) and reports their comparison to β-strand mimic **A**. β-Strand mimic **B** is composed of a 5-amino-2-methoxybenzoic acid unit linked by a diacylhydrazine group to a fumaramide unit; β-strand mimic **C** is composed of a 5-amino-2-methoxybenzoic acid unit linked by a diacylhydrazine group to a peptide. β-Strand mimics **A–C** were connected to tripeptide (Phe-Ile-Leu) groups by means of 1,2-diaminoethane diurea turn units to form artificial β-sheets **1–3**. ^{1}H NMR studies, involving ROESY, chemical shift, coupling constant, and variable temperature experiments, reveal that **1–3** adopt hydrogen-bonded antiparallel β-sheet conformations and establish that all three templates are viable β-strand mimics.

Introduction

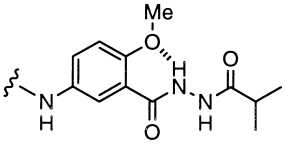
β-Sheet formation plays a critical role in many biological processes associated with diseases and normal functions. β-Sheet interactions between proteins have been shown or hypothesized to be involved in cell signaling and oncogene expression associated with the binding of Ras and Rap by the serine/threonine kinase Raf,[1] the clustering of membrane ion channels by PDZ domains,[2] the binding of lymphocyte function-associated antigen-1 (LFA-1) by the intercellular adhesion molecule-1 (ICAM-1),[3] and the interaction between the CD4 receptor and the HIV viral protein gp120.[4,5] To cleave peptides, proteolytic enzymes, such as HIV-1 protease and renin, form β-sheet-like networks of hydrogen bonds with their peptide substrates.[6–8] HIV-1 protease dimerizes through four-interdigitating β-strands.[9] The *met* repressor, a protein involved in gene regulation, also functions as a β-sheet dimer; in this case, the β-sheet that forms is directly involved in binding the major groove of DNA.[10] Many proteins aggregate to form insoluble β-sheet structures that are associated with Alzheimer's disease,[11–13] Creutzfeld–Jacob disease and other prion diseases,[14] and progressive neurodegenerative disorders that are associated with trinucleotide (CAG) repeats.[15–17]

An intriguing possibility for the development of new therapeutic strategies for treating diseases associated with β-sheet formation involves chemical decoys that modulate β-sheet formation.[1–17] These decoys must mimic aspects of β-sheet structure, such as the placement of the amino acid side-chains or the alternating array of hydrogen-bond donors and acceptors provided by one edge of a peptide in a β-strand conformation. Leading examples of β-strand mimics have been developed by Kemp and co-workers,[18–20] Smith, Hirschmann, and co-workers,[6–8] Pallai, Rebek, and co-workers,[4,5] Schrader and Kirsten,[21,22] and our own research group.[23–25] These β-strand mimics have the potential to be used as inhibitors and antagonists of processes involving β-sheet formation.

In 1996, we introduced 5-amino-2-methoxybenzamide as a β-strand mimic.[23] This mimic duplicates the hydrogen-bonding functionality of one edge of a dipeptide. To duplicate a tripeptide, we extended the β-strand mimic with a diacylhydrazine group to form a 5-amino-2-methoxybenzoic hydrazide.[25]



5-amino-2-methoxybenzamide



5-amino-2-methoxybenzoic hydrazide

Key words: Amino acids and derivs; mimetics; peptides and polypeptides; solid phase synthesis.

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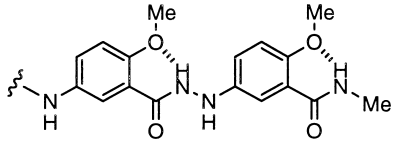
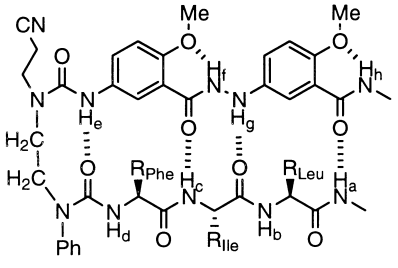
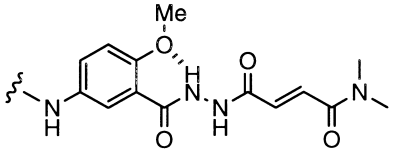
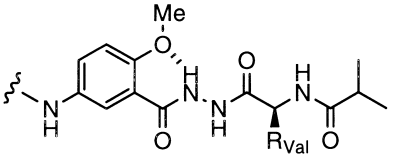
We also introduced β-strand mimic **A**, which contains a 5-amino-2-methoxybenzoic acid unit coupled to a 5-hydrazino-2-methoxybenzamide unit by means of an acylhydrazine linkage.[24] This β-strand mimic duplicates the hydrogen bonding functionality of one edge of a tetrapeptide in a β-strand conformation. To evaluate the effectiveness of this mimic, we synthesized artificial β-sheet **1**, which contains β-strand mimic **A** linked to a peptide strand by means of a 1,2-diaminoethane diurea turn unit.[26,27] ^{1}H NMR chemical shift and NOE studies revealed that artificial β-sheet **1** adopts a hydrogen-bonded β-sheet structure in $CDCl_3$ solution.[24] The chemical shift studies showed that the NH groups are hydrogen bonded, and the NOE studies showed that the β-strand mimic is proximal to the peptide strand. Although many NOEs characteristic of β-sheet structure were present, we did not observe an NOE between the aniline-type NH proton (H_g) and the Ile NH proton (H_c). The absence of this NOE suggested that these NH groups were further apart than the 3.1 Å separation that is characteristic of β-sheets. We attributed the absence of the H_c-H_g NOE to the propensity of the acylhydrazine group of the β-strand mimic to adopt a twisted geometry (C–N–N–C dihedral angle $\approx 90°$).[24]





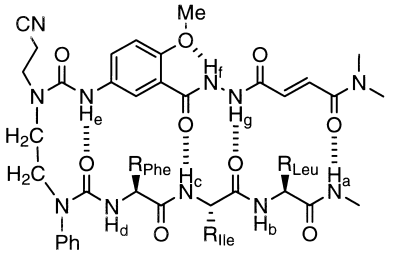
Although β-strand mimic **A** is an effective template in artificial β-sheet **1**, our concern over its twist prompted us to investigate alternative β-strand mimics. In this paper, we report two alternative β-strand mimics (**B** and **C**) and compare them to β-strand mimic **A**. β-Strand mimic **B** contains a diacylhydrazine group in place of the monoacylhydrazine group of β-strand mimic **A** and a fumaramide group in place of the 5-hydrazino-2-methoxybenzamide group. β-Strand mimic **C** also contains a diacylhydrazine group and contains a peptide in place of the 5-hydrazino-2-methoxybenzamide group. X-ray crystallographic and theoretical data suggest that the diacylhydrazine group should better be able to adopt a linear geometry (C–N–N–C dihedral angle $\approx$ 180°), allowing it to better mimic a peptide β-strand.[28,29] The diacylhydrazine group also contains an amide NH group, which should be a better hydrogen-bond donor than the aniline NH group of β-strand mimic **A**. With these considerations in mind, we set out to compare β-strand mimics **B** and **C** to β-strand mimic **A**. This paper describes this comparison.

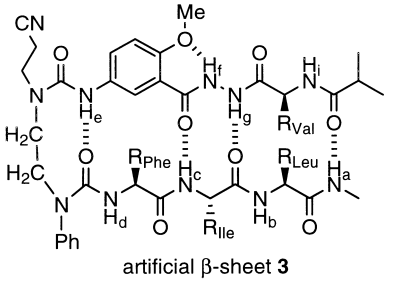




Results and Discussion

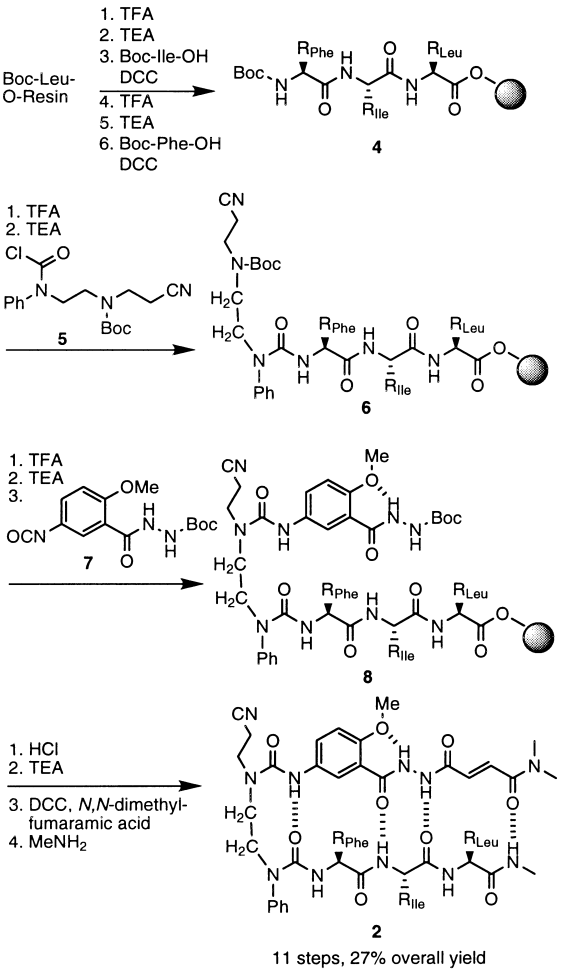
To evaluate these β-strand mimics, we synthesized and studied artificial β-sheets **1–3**, which incorporate β-strand mimics **A–C**. ^{1}H NMR studies involving ROESY, chemical shift, coupling constant, and variable temperature experiments in $CDCl_3$ solution reveal that **1–3** form hydrogen-bonded antiparallel β-sheet structures.



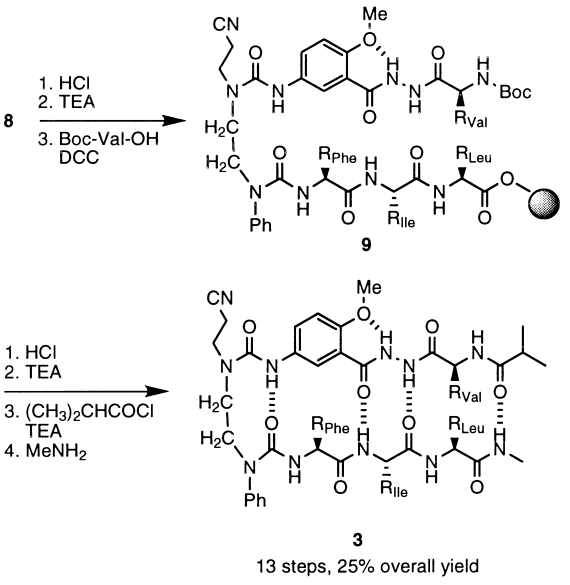


Synthesis of artificial β-sheets 1–3

Artificial β-sheets **2** and **3** were synthesized by solid-phase methods in a fashion analogous to that which we had previously reported for the solid-phase synthesis of artificial β-sheet **1**.[30] Scheme 1 illustrates the preparation of **2**. Resin-bound tripeptide **4** was constructed on Merrifield resin by standard solid-phase peptide synthesis methods.[31] The Boc protective group of **4** was removed by treatment with TFA and the free amino group was then liberated by treatment with TEA. The



Scheme 1.



Scheme 2.

resulting amine was coupled with carbamoyl chloride **5** to form urea **6**. The Boc protective group was removed from urea **6** by treatment with TFA. The free amino group was liberated by treatment with TEA and coupled with isocyanate **7** to form diurea **8**. The Boc protective group of **8** was removed with gaseous HCl,[32] and the resulting hydrazine was coupled to *N*,*N*-dimethylfumaramic acid (*trans*-$Me_2NCOCH{=}CHCO_2H$) with DCC to afford the resin-bound β-sheet. Aminolytic cleavage from the resin followed by chromatographic purification afforded artificial β-sheet **2** in 27% overall yield.

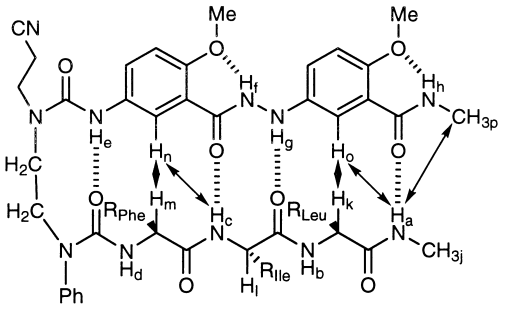
Artificial β-sheet **3** was synthesized in a similar fashion to artificial β-sheet **2**, in 25% overall yield. Scheme 2 illustrates its preparation from diurea **8**. The Boc protective group of **8** was removed by treatment with gaseous HCl and the free hydrazino group was then liberated by treatment with TEA. The resulting hydrazine was coupled to Boc-valine with DCC to give **9**. The Boc protective group of **9** was removed by treatment with gaseous HCl and the resulting hydrochloride salt was treated with TEA and coupled with isobutyryl chloride to afford the resin-bound β-sheet. Artificial β-sheet **3** was then liberated from the resin by aminolysis with methylamine.

^{1}H NMR ROESY studies of artificial β-sheets 1–3

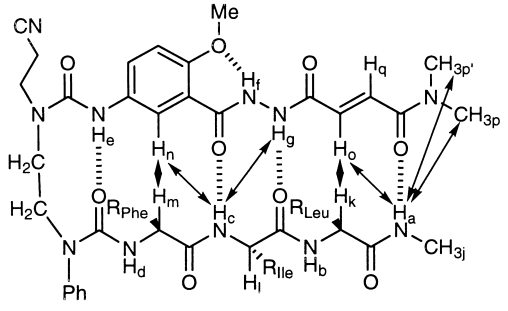
^{1}H NMR nuclear Overhauser effect studies indicate that artificial β-sheets **1–3** adopt antiparallel β-sheet conformations in $CDCl_3$ solution. These studies were performed in the rotating frame using the transverse-ROESY (Tr-ROESY) method,[33,34] and reveal 12–14 interstrand ROEs between the β-strand mimics and the adjacent main-chains and side-chains of the peptide strands in each of the artificial β-sheets.

Artificial β-sheets **1–3** each exhibit networks of interstrand ROEs between their β-strand mimics and their tripeptide groups (Fig. 1). All the artificial β-sheets exhibit interstrand ROEs between aromatic ring protons H_n and the Phe α-protons (H_m) and between aromatic ring protons H_n and the Ile amide protons (H_c). Analogous ROEs between β-strand mimic protons H_o and the Leu α-protons (H_k) and between β-strand mimic protons H_o and methylamide protons H_a are present. Artificial β-sheets **1–3** also exhibit ROEs between methylamide protons H_a and β-strand mimic protons H_p (or H_p and $H_{p'}$). Artificial β-sheet **3** shows ROEs between H_p and $H_{p'}$ and the terminal methyl group of the peptide H_j. Artificial β-sheets **2** and **3** exhibit ROEs between hydrazine protons H_g and the Ile amide protons (H_c); the analogous ROE was not detected in artificial β-sheet **1**. This difference between artificial β-sheets **2** and **3** and artificial β-sheet **1** is consistent with a model in which the diacylhydrazine group of the former better adopts a linear geometry than the monoacylhydrazine group of the latter.

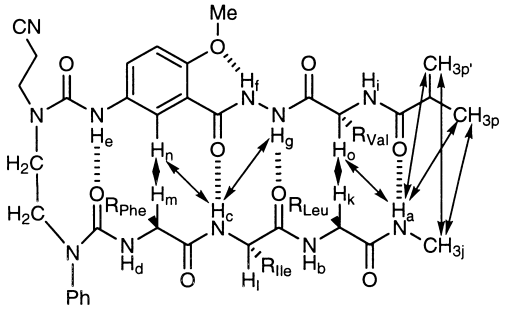
The artificial β-sheets also exhibit interstrand ROEs between the β-strand mimics and the side-chains of the peptide strands (not shown in Figure 1). These include ROEs between aromatic ring protons H_n and the Phe and Ile side-chains, β-strand mimic protons H_o and H_p and the Leu side-chains, and hydrazine protons H_g and the Ile side-chains.



artificial β-sheet **1**



artificial β-sheet **2**



artificial β-sheet **3**

Figure 1. Important ROEs between the β-strand mimics and peptide main chains in artificial β-sheets **1–3**.

Interresidue ROEs within the peptide strands of **1–3** provide further evidence for β-sheet structure. In each of these compounds the interresidue ROEs between the α-protons of one peptide residue and the NH protons of the adjacent residue are strong; these ROEs are much stronger than the intraresidue ROEs between the α-protons and the NH protons of each residue. These ROEs indicate that the peptides adopt β-strand conformations.

Several weak (or very weak) ROEs are not wholly consistent with β-sheet structure. These include ROEs between H_a and H_b, H_b and H_c, H_c and H_d, H_d and H_n, and H_f and H_o. Molecular modeling indicates that the distances between these protons should be 4.0–4.5 Å; the intensity of these ROEs should be only a few percent of those of the strongest ROEs. These ROEs may reflect deviation from ideal β-sheet structure or conformational mobility, or may result from TOCSY artifacts, which can arise if the 180° pulse of the Tr-ROESY experiments is not well calibrated. Artificial β-sheet **2** exhibits a number of unexpected ROEs involving β-strand mimic protons H_o and H_q (H_g–H_q, H_k–H_q, H_o–H_p, and H_o–$H_{p'}$). These ROEs may reflect that H_o and H_q are strongly coupled and that their respective resonances do not wholly correspond to the individual protons.

^{1}H NMR coupling constant studies of artificial β-sheets 1–3

The ^{1}H NMR coupling constants between the NH and α-protons reflect the conformation of a peptide; coupling constants of less than 6 Hz are consistent with α-helical structure while coupling constants of greater than 7 Hz are consistent with β-strand structure.[35] Consistent with a β-strand conformation, the Phe-Ile-Leu tripeptide strands of artificial β-sheets **1–3** have coupling constants of 7.2–9.5 Hz. The coupling constant for H_b in **1** is slightly smaller than in **2** and **3** (Table 1). This (minor) difference may indicate that the peptide strand in **1** adapts to the twist in the adjacent β-strand mimic by adopting a distorted β-strand conformation.

^{1}H NMR chemical shift studies of artificial β-sheets 1–3

The ^{1}H NMR chemical shifts of the NH and α-protons of the peptide and peptidomimetic groups of **1–3** also indicate that these compounds form β-sheet structures. In $CDCl_3$, and in other non-competitive solvents, protons that are hydrogen bonded typically appear several ppm downfield of similar protons that are not hydrogen bonded. Non-hydrogen-bonded peptide amide protons generally appear at about 6 ppm, while hydrogen-bonded peptide amide protons typically appear at about 8 ppm.[36] In compounds **1–3**, H_a and H_c appear at 8.02–8.62 ppm, while H_b appears at 6.07–6.40 ppm (Table 2). These chemical shifts are consistent with a β-sheet conformation in which H_a and H_c lie on the hydrogen-bonded edge of the peptide, and H_b lies on the non-hydrogen-bonded edge.

Protons H_d cannot be directly compared to protons H_a–H_c, because protons H_d are part of a peptide urea. The chemical shifts of H_d in **1–3** (4.80–4.88 ppm) are typical of what we have previously observed for similar non-hydrogen-bonded ureas.[26] Protons H_e are part of an aromatic urea and appear at 9.95–10.10 ppm; these shifts are consistent with values we have previously seen for related hydrogen-bonded urea protons.[23–25] Protons H_g differ among compounds **1–3**: in **1**, it is part of an *N*-acyl-phenylhydrazine group, while in **2** and **3** it is part of a diacylhydrazine group. For this reason, the chemical shift values for the two different groups cannot be compared to each other. They are, however, consistent with those that we have previously observed for similar hydrogen-bonded protons.[24,25]

The ^{1}H NMR chemical shifts of the α-protons of the peptide strands in **1–3** provide further evidence that these compounds form β-sheet structures. In β-sheets, the α-protons of each amino acid residue typically appear several tenths of a ppm downfield of the same residue in a random coil.[37,38] In random coils, Phe, Ile, and Leu exhibit chemical shifts of 4.66 ± 0.10,

Table 1. ^{1}H NMR coupling constants (Hz) of the NH protons of **1–3**[a]

	H_b(Leu)	H_c(Ile)	H_d(Phe)
β-sheet **1**	7.2	9.1	8.5
β-sheet **2**	9.1	9.4	9.1
β-sheet **3**	9.5	9.1	8.3

[a]Spectra were recorded in 1 mM in $CDCl_3$ solution.

Table 2. ^{1}H NMR chemical shifts of the NH protons of **1–3**[a]

	H_a	H_b	H_c	H_d	H_e	H_f	H_g	H_h	H_i
β-sheet **1**	8.02	6.07	8.14	4.80	9.95	9.55	8.45	8.10	—
β-sheet **2**	8.16	6.40	8.62	4.88	10.10	11.28	12.04	—	—
β-sheet **3**	8.03	6.29	8.38	4.88	10.02	10.85	11.56	—	6.29

[a]Spectra were recorded in 1 mM in $CDCl_3$ solution.

3.95 ± 0.10, and 4.17 ± 0.10 ppm, respectively.[37,38] The α-protons of artificial β-sheets **1–3** all appear downfield of these values (Table 3); the Phe α-protons appear at 5.00–5.35 ppm, the Ile α-protons appear at 4.14–4.31 ppm, and the Leu α-protons appear at 4.41–4.83 ppm. Caution must be exercised in comparing these values to the random coil values, however, since the α-proton of Phe is next to a urea group, and since the chemical shifts of the artificial β-sheets were measured in $CDCl_3$, while those of the random coils were measured in water.

Variable-temperature ^{1}H NMR studies of artificial β-sheets 1–3

The temperature dependence of the chemical shifts of amide protons reflects their state of hydrogen bonding. Peptide amide protons that are not hydrogen bonded or are locked in a hydrogen-bonded conformation display small temperature dependencies (−2 to −3 ppb/K) in chloroform solution, while peptide amide protons that equilibrate between hydrogen-bonded and non-hydrogen-bonded states display larger temperature dependencies (−4 to −8 ppb/K).[39,40] In artificial β-sheet **1**, all protons exhibit small temperature dependencies of chemical shifts (−0.5 to −3.3 ppb/K), with the exception of methylamide proton H_a, which exhibits a temperature dependence of −8.5 ppb/K (Table 4). These data are consistent with a model in which artificial β-sheet **1** adopts a hydrogen-bonded β-sheet conformation, but in which the hydrogen bond to the leucine methylamide NH proton 'frays' upon warming. In artificial β-sheets **2** and **3**, the temperature dependence of H_a is less (−5.3 to −5.5 ppb/K), suggesting that less fraying occurs and that these β-sheets are more stable. In these two β-sheets, H_g exhibits a large temperature dependence (−5.8 to −6.3 ppb/K), possibly indicating a greater degree of fraying of its hydrogen bond. Protons H_b in **2** and H_i in **3** also exhibit a larger temperature dependence (−4.0 to −4.1 ppb/K). Although these data may reflect the involvement of other conformations or equilibria, we hesitate to place too much emphasis on the interpretation of these data: much of what is known about temperature dependence of NH shifts has been established for peptide amide NH groups, but **1–3** contain not only peptide amide NH groups but also urea, amine, acylhydrazine, and aromatic amide NH groups.

Table 3. ^{1}H NMR chemical shifts of α-CH protons of **1–3**[a]

	H_k(Leu)	H_l(Ile)	H_m(Phe)
β-sheet **1**	4.41	4.14	5.00
β-sheet **2**	4.83	4.31	5.35
β-sheet **3**	4.83	4.18	5.07

[a]Spectra were recorded in 1 mM in $CDCl_3$ solution.

Table 4. Temperature dependence of the ^{1}H NMR chemical shifts (ppb/K) of the NH protons of **1–3**[a]

	H_a	H_b	H_c	H_d	H_e	H_f	H_g	H_h	H_i
β-sheet **1**	−8.5	−2.7[b]	−2.1	−0.5	−1.5	−2.2	−3.3	−3.1	—
β-sheet **2**	−5.3	−4.0	−3.3	0.1	−1.2	−3.0	−5.8	—	—
β-sheet **3**	−5.5	−1.4	−1.2	−0.5	−1.6	−1.5	−6.3	—	−4.1

[a]Spectra were recorded in 1 mM in $CDCl_3$ solution at 10 °C intervals from −30 °C to 30 °C.
[b]Temperature-dependence of the NH shift data exhibited poor linear correlation.

Conclusions

These synthetic and ^{1}H NMR spectroscopic studies indicate that β-strand mimics **A–C** can template β-sheet structure in adjacent peptide strands and establish that β-strand mimics **B** and **C** are viable alternatives to β-strand mimic **A**. It is difficult to conclude whether **B** and **C** are better templates than **A**, since all three do an admirable job. A few slight differences exist between artificial β-sheets **2** and **3** and artificial β-sheet **1** (additional ROEs, slight differences in coupling constants, slightly greater downfield shifting of H_a, H_c, H_k, H_l, and H_m, a slightly smaller temperature dependence of H_a), which suggest that **B** and **C** are slightly better than **A**. These differences may reflect the greater propensity of the diacylhydrazine groups of **B** and **C** to adopt linear geometries and the better hydrogen-bonding abilities of the amide-type protons H_g of these groups.

Experimental

General. Commercially available reagents and solvents were used without further purification. Phosgene was obtained from Fluka as a 20% (1.93 M) solution in toluene. Merrifield resin (1% divinylbenzene cross-linked polystyrene) bearing Boc-protected amino acids was obtained from Advanced ChemTech. Each solid-phase synthesis was performed using a 50- or 100-mL reaction vessel with a sintered-glass frit and a stopcock at one end and a stoppered 14/20-ground joint at the other end. The reaction vessels were shaken with an air-driven, 270° rotary shaker. The resin was washed by adding solvent, shaking for 1 min, and draining the reaction vessel. The reaction vessels were drained using nitrogen gas at ca. 5 psi to expel the solvent. Either a 3/1 (v/v) mixture of dichloromethane (CH_2Cl_2) and trifluoroacetic acid (TFA) containing 1 mg/mL of indole or HCl gas was used to remove Boc protective groups. A 9/1 (v/v) mixture of CH_2Cl_2 and triethylamine (TEA)

was used to convert the resulting trifluoroacetic acid salts to the free amines.

Artificial β-Sheet 2. A 100-mL solid-phase reaction vessel was charged with 8.00 g of Boc-Leu Merrifield resin (0.50 mmol/gm, 4.0 mmol). The resin was washed with CH_2Cl_2 (3×80 mL) and CH_2Cl_2/TFA/indole solution (1×80 mL) and shaken with CH_2Cl_2/TFA/indole solution (80 mL) for 30 min. The resin was drained and washed with CH_2Cl_2 (6×80 mL), CH_2Cl_2/TEA solution (2×80 mL), and CH_2Cl_2 (6×80 mL). The resin was shaken with a mixture of *N*-Boc-Ile-OH (2.5 g, 10 mmol), 1.0 M solution of DCC in CH_2Cl_2 (10 mL, 10 mmol), and CH_2Cl_2 (70 mL) for 2 h. The resulting white suspension was filtered, and the residue was washed alternately (3×) with CH_2Cl_2 (80 mL) and MeOH (80 mL) to give the resin-bound dipeptide as white beads.

The beads were washed with CH_2Cl_2 (3×80 mL) and CH_2Cl_2/TFA/indole solution (1×80 mL) and shaken with CH_2Cl_2/TFA/indole solution (80 mL) for 30 min. The resin was washed with CH_2Cl_2 (6×80 mL), CH_2Cl_2/TEA solution (2×80 mL), and CH_2Cl_2 (6×80 mL). The resulting amine was shaken with a mixture of *N*-Boc-Phe-OH (2.7 g, 10 mmol), 1.0 M solution of DCC in CH_2Cl_2 (10 mL, 10 mmol), and CH_2Cl_2 (70 mL) for 2 h. The resulting white suspension was filtered, and the residue was washed alternately (3×) with CH_2Cl_2 (80 mL) and MeOH (80 mL) to give resin-bound tripeptide **4** as white beads.

Resin **4** was washed with CH_2Cl_2 (3×80 mL) and CH_2Cl_2/TFA/indole solution (1×80 mL) and shaken with CH_2Cl_2/TFA/indole solution (80 mL) for 30 min. The resin was washed with CH_2Cl_2 (6×80 mL), CH_2Cl_2/TEA solution (2×80 mL), CH_2Cl_2 (6×80 mL), and DMF (3×80 mL). The resulting amine was shaken with a mixture of DMF (80 mL), TEA (0.50 mL, 6.8 mmol), and carbamoyl chloride **5** (2.3 g, 6.6 mmol) for 24 h. The resulting white suspension was filtered, and the residue was washed alternately (3×) with CH_2Cl_2 (80 mL) and MeOH (80 mL) to afford resin **6** as white beads.

Resin **6** was washed with CH_2Cl_2 (3×80 mL) and CH_2Cl_2/TFA/indole solution (1×80 mL) and shaken with CH_2Cl_2/TFA/indole solution (80 mL) for 30 min. The resin was drained and washed with CH_2Cl_2 (6×80 mL), CH_2Cl_2/TEA solution (2×80 mL), and CH_2Cl_2 (6×80 mL). The resulting amine was shaken with a solution of isocyanate **7** (2.0 g, 6.5 mmol) in CH_2Cl_2 (80 mL) for 2 h. The resulting suspension was filtered, and the residue was washed alternately (3×) with CH_2Cl_2 (80 mL) and MeOH (80 mL) to afford 10.5 g of resin **8** as yellow beads.

A 50-mL reaction vessel was charged with 0.50 g (dry weight) of resin **8** (0.38 mmol/gm, 0.19 mmol), and the resin was washed with CH_2Cl_2 (3×20 mL). The vessel was charged with CH_2Cl_2 (20 mL) and HCl gas was bubbled into the reaction vessel through a pipette for 10 min. The solution was drained, and the resin was washed with CH_2Cl_2 (2×20 mL), CH_2Cl_2/TEA (2×20 mL), and CH_2Cl_2 (2×20 mL). The resulting amine was shaken with a mixture of fumaric acid **9** (0.20 g, 1.4 mmol), DCC (0.20 g, 9.7 mmol), and CH_2Cl_2 (20 mL) for 2 h. The resin was washed with CH_2Cl_2 (3×20 mL) and shaken with 40% (w/w) methylamine in MeOH (25 mL) for 3 h. The solution was collected and concentrated by rotary evaporation. The residue was purified by chromatography on silica gel (*i*-PrOH/CH_2Cl_2, 7/93) followed by reverse-phase HPLC (C_{18} column, CH_3CN/H_2O, 60/40) to afford 49 mg (27%) of **2** as a white solid: IR ($CHCl_3$) 3417, 3315, 2251, 1655, 1626, 1597 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 12.08 (d, J = 8.5 Hz, 1H), 11.30 (d, J = 8.5 Hz, 1H), 10.11 (s, 1H), 8.86 (br s, 1H), 8.64 (d, J = 9.4 Hz, 1H), 8.38 (dd, J = 8.9, 2.8 Hz, 1H), 8.19 (br q, J = 4.6 Hz, 1H), 7.63 (d, AB pattern, J = 14.9 Hz, 1H), 7.51 (d, AB pattern, J = 14.9 Hz, 1H), 7.39–7.32 (m, 3H), 7.20–7.16 (m, 3H), 7.10–7.08 (m, 2H), 6.98 (d, J = 9.0 Hz, 1H), 6.88 (d, J = 7.4 Hz, 2H), 6.57 (d, J = 9.0 Hz, 1H), 5.35 (td, J = 9.4, 5.6 Hz, 1H), 4.93 (d, J = 9.0 Hz, 1H), 4.83 (q, J = 7.6 Hz, 1H), 4.31 (t, J = 8.6 Hz, 1H), 4.09 (s, 3H), 3.95–3.82 (m, 1H), 3.63–3.51 (m, 1H), 3.48 (t, J = 6.3 Hz, 2H), 3.42–3.32 (m, 2H), 3.23 (s, 3H), 3.07 (s, 3H), 3.01 (dd, J = 14.1, 5.4 Hz, 1H), 2.81–2.75 (m, 1H), 2.77 (d, J = 4.6 Hz, 3H), 2.72–2.59 (m, 2H), 1.74–1.66 (m, 1H), 1.65–1.43 (m, 4H), 1.15–1.04 (m, 1H), 0.91 (d, J = 5.6 Hz, 3H), 0.90 (d, J = 5.5 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H), 0.75 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 171.5, 170.8, 165.2, 158.6, 158.3, 157.1, 155.6, 152.3, 140.7, 136.9, 135.0, 133.1, 130.3, 129.3, 128.6, 128.3, 128.2, 127.5, 126.7, 123.3, 122.1, 118.7, 118.1, 111.5, 57.7, 56.3, 56.0, 51.5, 51.1, 48.8, 45.3, 43.0, 39.4, 38.1, 37.5, 35.9, 26.0, 25.1, 24.8, 22.7, 22.5, 17.6, 15.2, 11.2; HRMS (FAB) m/e for $C_{49}H_{66}N_{11}O_9$ $(M+H)^+$ calcd 952.5045, found 952.5045. Anal. calcd for $C_{49}H_{66}N_{11}O_9$: C, 61.81; H, 6.88; N, 16.18. Found: C, 61.70; H, 7.09; N, 15.99.

Artificial β-Sheet 3. A 50-mL reaction vessel was charged with 0.43 g (dry weight) of resin **8** (0.52 mmol/g, 0.22 mmol), and the resin was washed with dry CH_2Cl_2 (3×20 mL). The vessel was charged with CH_2Cl_2 (20 mL) and HCl gas was bubbled into the reaction vessel through a pipette for 10 min. The solution was drained, and the resin was washed with CH_2Cl_2 (2×20 mL), CH_2Cl_2/TEA solution (2×20 mL), and CH_2Cl_2 (2×20 mL). The resulting amine was shaken with a mixture of DCC (0.20 g, 1.0 mmol), Boc-Val-OH (0.20 g, 0.92 mmol), and CH_2Cl_2 (20 mL) for 2 h. The resulting suspension was filtered and the residue was washed alternately (3×) with CH_2Cl_2 (20 mL) and MeOH (20 mL) to afford resin **9** as yellow beads.

Resin **9** was washed with CH_2Cl_2 (3×20 mL). The vessel was charged with CH_2Cl_2 (20 mL) and HCl gas was bubbled into the reaction vessel through a pipette for 10 min. The solution was drained, and the resin was washed with CH_2Cl_2 (2×20 mL), CH_2Cl_2/TEA solution (2×20 mL), and CH_2Cl_2 (2×20 mL). The resin was shaken with a mixture of isobutyryl chloride (0.50 mL, 7.2 mmol), TEA (0.50 mL, 7.2 mmol), and CH_2Cl_2 (20 mL) for 30 min. The resin was washed with CH_2Cl_2 (3×20 mL) and shaken with 40% (w/w) methylamine in MeOH (25 mL) for 3 h. The solution was collected and concentrated by rotary evaporation. The residue was

purified by chromatography on silica gel (*i*-PrOH/CH_2Cl_2, 7/93) followed by reverse-phase HPLC (C_{18} column, CH_3CN/H_2O, 70/30) to afford 55 mg (25%) of **3** as a white solid: IR ($CHCl_3$) 3421, 3309, 2251, 1657, 1632, 1599 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.61 (d, $J=7.0$ Hz, 1H), 10.84 (d, $J=7.5$ Hz, 1H), 10.04 (s, 1H), 8.77 (d, $J=2.8$ Hz, 1H), 8.40 (d, $J=9.8$ Hz, 1H), 8.38 (dd, $J=8.6$, 2.4 Hz, 1H), 8.07 (br q, J = 4.6 Hz, 1H), 7.39–7.26 (m, 3H), 7.24–7.21 (m, 3H), 7.14 (d, $J=$ 7.0 Hz, 2H), 6.96–6.93 (m, 3H), 6.51 (d, $J=9.0$ Hz, 1H), 6.34 (d, $J=9.3$ Hz, 1H), 5.07 (td, $J=8.9$, 6.6 Hz, 1H), 4.96–4.92 (m, 2H), 4.83 (dt, $J=9.1$, 7.3 Hz, 1H), 4.18 (t, $J=9.2$ Hz, 1H), 4.04 (s, 3H), 3.90–3.75 (m, 1H), 3.68–3.66 (m, 2H), 3.56–3.51 (m, 1H), 3.48–3.42 (m, 2H), 2.99 (dd, ABX pattern, $J_{AB}=14.0$ Hz, $J_{AX}=6.4$ Hz, 1H), 2.84 (dd, ABX pattern, $J_{AB}=14.0$ Hz, $J_{AX}=9.4$ Hz, 1H), 2.76 (d, $J=4.5$ Hz, 3H), 2.71–2.60 (m, 2H), 2.44 (septet, $J=6.9$ Hz, 1H), 2.19–2.02 (m, 1H), 1.68–1.49 (m, 3H), 1.43–1.36 (m, 2H), 1.20 (d, $J=6.9$ Hz, 3H), 1.14 (d, $J=6.8$ Hz, 3H), 1.06 (d, $J=6.7$ Hz, 3H), 1.02 (d, $J=6.8$ Hz, 3H), 0.95 (d, $J=6.1$ Hz, 6H), 0.97–0.90 (m, 1H), 0.67 (d, $J=6.6$ Hz, 3H), 0.51 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 172.4, 171.6, 170.8, 166.1, 159.7, 157.4, 155.5, 152.2, 141.0, 136.8, 135.0, 130.3, 129.2, 128.4, 128.0, 127.4, 126.7, 123.2, 122.1, 118.7, 118.2, 111.4, 57.8, 56.3, 56.2, 55.5, 51.3, 50.8, 48.5, 45.3, 42.3, 38.6, 38.0, 35.6, 32.6, 26.1, 24.8, 24.6, 23.0, 22.6, 20.1, 19.2, 19.0, 18.4, 17.6, 15.2, 10.8; HRMS (FAB) *m/e* for $C_{52}H_{74}N_{11}O_9$ $(M+H)^+$ calcd 996.5671, found 996.5659. Anal. calcd for $C_{52}H_{73}N_{11}O_9$: C, 62.69; H, 7.39; N, 15.47. Found: C, 62.50; H, 7.46; N, 15.06.

Carbamoyl chloride 5. An ice-cooled, biphasic mixture of $PhNH(CH_2)_2N(Boc)$-$(CH_2)_2CN$[24] (0.050 g, 0.17 mmol), 15 mL of CH_2Cl_2, and 15 mL of satd aq $NaHCO_3$ was rapidly stirred while a 1.93 M phosgene in toluene (0.5 mL, 0.97 mmol) was added as a single portion. [CAUTION: PHOSGENE VAPOR IS HIGHLY TOXIC—USE HOOD.] The reaction mixture was stirred for 30 min, the organic phase was collected, and the aqueous phase was extracted with CH_2Cl_2 (2×15 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 0.061 g (100%) of **5** as a thin film: IR (neat) 2249, 1738, 1697; ^1H NMR (500 MHz, $CDCl_3$, 50 °C) δ 7.45–7.33 (m, 5H), 3.87 (br s, 2H), 3.58 (t, $J=6.7$ Hz, 2H), 3.46 (br s, 2H), 2.59 (t, $J=6.5$ Hz, 2H), 1.40 (br s, 9H).

Isocyanate 7. A solution of 2-methoxy-5-nitrobenzoic acid[24] (15.0 g, 76.1 mmol), *t*-butyl carbazate, and 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide hydrochloride (16.1 g, 83.7 mmol) in THF was stirred for 8 h. The reaction mixture was diluted with 500 mL of water, and a yellow precipitate formed. The precipitate was isolated by filtration and recrystallized ($MeOH/H_2O$, 1/1) to afford 20.5 g (87%) of 5-NO_2-2-MeO-C_6H_3-CONHNHBoc as a white solid: IR ($CHCl_3$) 3410, 1741, 1678 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.40 (br s, 1H), 9.10 (d, $J=2.8$ Hz, 1H), 8.37 (dd, $J=9.6$, 3.0 Hz, 1H), 7.11 (d, $J=9.4$ Hz, 1H), 6.96 (br s, 1H), 4.13 (s, 3H), 1.51 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.0, 161.6, 155.1, 141.7, 128.6, 128.3, 120.2, 111.9, 81.9, 57.2, 28.1; HRMS (CI) *m/e* for $C_{13}H_{18}N_3O_6$ $(M+H)^+$, calcd 312.1195, found 312.1193. Anal. calcd for $C_{13}H_{17}N_3O_6$: C, 50.16; H, 5.50; N, 13.50. Found: C, 49.96; H, 5.69; N, 13.38.

A suspension of 5-NO_2-2-MeO-C_6H_3-CONHNHBoc (0.300 g, 0.964 mmol) and 10% palladium on activated carbon (0.100 g) in MeOH was stirred under hydrogen gas for two hours. The suspension was filtered through Celite, the filtrate was concentrated by rotary evaporation, and the residue was purified by column chromatography on silica gel (*i*-PrOH/CH_2Cl_2, 1/9) to afford 0.265 g (98%) of 5-NH_2-2-MeO-C_6H_3-CONHNHBoc as a white solid: IR (KBr) 3356, 3248, 1736, 1657, 1618 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.67 (br s, 1H), 7.50 (d, $J=2.9$ Hz, 1H), 7.36 (br s, 1H), 6.78 (dd, $J=8.6$, 2.7 Hz, 1H), 6.74 (d, $J=9.0$ Hz, 1H), 3.84 (s, 3H), 3.63 (br s, 2H), 1.47 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2, 155.1, 150.7, 140.5, 120.0, 119.6, 118.3, 112.8, 81.5, 56.5, 28.1; HRMS (CI) *m/e* for $C_{13}H_{19}N_3O_4$ (M^+), calcd 281.1375, found 281.1370. Anal. calcd for $C_{13}H_{19}N_3O_4$: C, 55.51; H, 6.81; N, 14.94. Found: C, 55.18; H, 6.70; N, 14.80.

An ice-cooled, biphasic mixture of 5-NH_2-2-MeO-C_6H_3-CONHNHBoc (0.150 g, 0.533 mmol), 40 mL of CH_2Cl_2, and 40 mL of satd aq $NaHCO_3$ was rapidly stirred while a 1.93 M phosgene in toluene (1.0 mL, 1.9 mmol) was added as a single portion. [CAUTION: PHOSGENE VAPOR IS HIGHLY TOXIC—USE HOOD.] The reaction mixture was stirred for 30 min, the organic phase was collected, and the aqueous phase was extracted with CH_2Cl_2 (2×15 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 0.17 g (102%) of **7** as a white solid: IR (neat) 3367, 2270, 1738, 1659 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.53 (br s, 1H), 7.97 (d, $J=2.8$ Hz, 1H), 7.19 (dd, $J=8.8$, 2.8 Hz, 1H), 6.94 (d, $J=8.8$ Hz, 1H), 6.93 (br s, 1H), 4.00 (s, 3H), 1.51 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.9, 155.1, 155.0, 128.9, 128.2, 126.8, 124.4, 120.2, 112.4, 81.5, 56.4, 28.0; HRMS (CI) *m/e* for $C_{14}H_{17}N_3O_5$ (M^+), calcd 307.1168, found 307.1164. Anal. calcd for $C_{14}H_{17}N_3O_5$: C, 54.72; H, 5.58; N, 13.67. Found: C, 55.06; H, 5.90; N, 13.33.

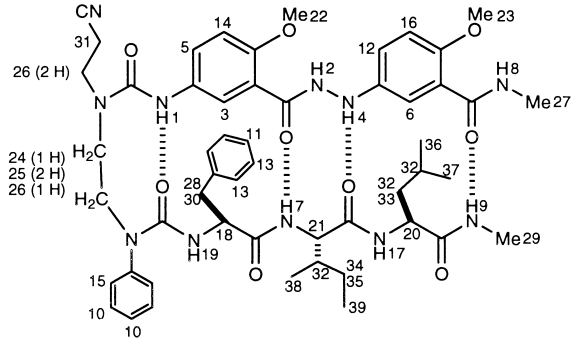
***N,N*-Dimethylfumaramic acid.** A solution of fumaric acid monoethyl ester (5.10 g, 35.4 mmol), 40% (w/w) aq Me_2NH (1.5 mL, 33 mmol), and 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide hydrochloride (7.50 g, 39.1 mmol) in THF was stirred for 8 h and then concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel (EtOAc/hexanes, 3/1) to afford 2.3 g (45%) of ethyl *N,N*-dimethylfumaramate (*trans*-$Me_2NCOCH=CHCO_2Et$) as a colorless oil: IR (neat) 1722, 1659, 1630 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, $J=15.5$ Hz, 1H), 6.79 (d, $J=15.0$ Hz, 1H), 4.26 (q, $J=7.0$ Hz, 2H), 3.14 (s, 3H), 3.05 (s, 3H), 1.32 (t, $J=7.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 164.7, 133.6, 131.1, 61.0, 37.4, 35.7, 14.0; HRMS (CI) *m/e* for $C_8H_{14}NO_3$ $(M+H)^+$, calcd 172.0973, found 172.0971. Anal. calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 55.84; H, 7.67; N, 7.97.

A mixture of ethyl *N,N*-dimethylfumaramate (1.80 g, 10.5 mmol), THF (100 mL), and 150 mL of 0.5 M

NaOH was stirred at 0°C for 45 min. The aqueous layer was washed with CH_2Cl_2 (3×100 mL) and slowly acidified to pH 2 with concentrated HCl while cooling with an ice bath. The resulting aqueous solution was concentrated to dryness. The solid residue was extracted with CH_2Cl_2, and the resulting suspension was filtered and concentrated to dryness to afford 1.39 g (92%) of *N*,*N*-dimethylfumaramic acid as a white solid: IR ($CHCl_3$) 2939, 2765, 2449, 1703, 1649, 1628 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.00 (br s, 1H), 7.48 (d, AB pattern, J=15.3 Hz, 1H), 6.80 (d, AB pattern, J=15.0 Hz, 1H), 3.15 (s, 3H), 3.07 (s, 3H); ^{13}C NMR (125 MHz, CD_3SOCD_3) δ 166.6, 164.1, 134.3, 130.8, 37.0, 35.2; HRMS (CI) *m*/*e* for $C_6H_{10}N_3O$ $(M+H)^+$ calcd 144.0660, found 144.0661. Anal. calcd for $C_6H_9N_3O$: C, 50.35; H, 6.34; N, 9.79. Found: C, 49.95; H, 6.26; N, 9.43.

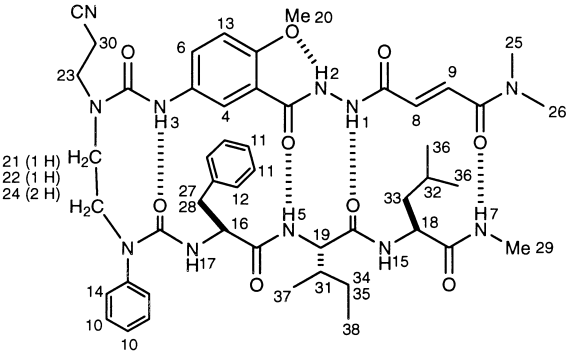
^1H NMR Tr-ROESY experiments. Tr-ROESY studies of artificial β-sheets **1–3** were performed on a Bruker DRX500 spectrometer using 10 mM samples in $CDCl_3$ that were degassed by five freeze–pump–thaw cycles on a high-vacuum line (<0.001 mm Hg) and sealed under vacuum. ROESY spectra were recorded at 30 °C with a mixing time of 300 ms using the Tr-ROESY pulse sequence of Hwang and Shaka.[33,34] A spin locking field strength of 5 kHz was employed to collect 2048 points in the f_2 dimension and 1024 points in the f_1 dimension, a relaxation delay of 1 s was used, and 16 transients were collected for each t_1 value. The data were processed using Bruker XWINNMR software. A sine-squared window function shifted by 60° was applied in both dimensions, and forward linear prediction of 1024 points was performed in the f_1 dimension to give a final matrix of 1024 by 1024 real points. An automatic baseline correction was applied in both dimensions after Fourier transforming the data. The ROESY cross-peaks were tabulated as follows: The proton resonances for each of the three artificial β-sheets were numbered in order of decreasing chemical shifts, and the resonances were assigned by means of the ROESY and COSY experiments. (These assignments are shown on the diagrams that follow.) The cross-peaks in the f_1 dimension were tabulated for each resonance in the f_2 dimension and were characterized as strong (s), medium (m), or weak (w) on the basis of their relative peak heights (45–100%, s; 15–45%, m; 3–15%, w).

^1H NMR Tr-ROESY cross-peaks for artificial β-sheet 1.



1: 3 (s), 5 (w), 24 (m), 25 (m), 26 (m). 2: 3 (w), 4 (w), 6 (w), 12 (m), 22 (m). 3: 1 (s), 2 (w), 7 (m), 13 (w), 18 (m), 19 (w), 34 (w), 35 (w), 39 (w). 4: 2 (w), 6 (m). 5: 1 (w), 14 (s). 6: 2 (w), 4 (m), 9 (s), 12 (w), 20 (s), 36 (w). 7: 3 (m), 13 (w), 17 (w), 18 (s), 19 (w), 21 (w), 34 (w), 35 (w), 38 (w). 8: 23 (w), 27 (s). 9: 6 (m), 17 (w), 20 (s), 27 (w), 29 (m), 36 (w). 10: 15 (s). 11: 13 (s). 12: 2 (s), 6 (w), 16 (s). 13: 3 (w), 7 (w), 11 (s), 18 (m), 19 (m), 28 (m), 30 (m). 14: 5 (s), 22 (s). 15: 10 (s), 19 (m), 24 (w), 25 (w), 26 (w). 16: 12 (s), 23 (s). 17: 7 (w), 9 (w), 20 (m), 21 (s), 32 (m), 33 (m), 38 (w). 18: 3 (m), 7 (s), 13 (m), 19 (s), 28 (m), 30 (m). 19: 3 (w), 7 (w), 13 (w), 15 (m), 18 (s), 28 (m), 30 (m). 20: 6 (m), 9 (s), 17 (m), 32 (m), 33 (m), 36 (m), 37 (m). 21: 7 (m), 17 (s), 32 (m), 34 (w), 35 (w), 38 (s), 39 (m). 22: 2 (m), 14 (s). 23: 8 (m), 16 (s). 24: 1 (m), 15 (w), 25 (s), 26 (w). 25: 1 (m), 15 (w), 24 (s), 26 (m). 26: 1 (m), 15 (w), 24 (w), 25 (m), 31 (m). 27: 8 (s), 9 (w), 36 (m). 28: 13 (m), 18 (m), 19 (m), 30 (s). 29: 9 (s). 30: 13 (m), 18 (w), 19 (m), 28 (s). 31: 26 (m). 32: 17 (m), 20 (m), 21 (m), 33 (s), 34 (m), 36 (s), 38 (s), 39 (m). 33: 17 (m), 20 (w), 32 (s), 36 (m), 37 (m). 34: 3 (w), 7 (w), 21 (w), 32 (m), 35 (s), 39 (m). 35: 3 (w), 7 (w), 21 (w), 34 (s), 38 (m), 39 (m). 36: 6 (w), 9 (w), 20 (m), 27(w), 32 (s), 33 (s). 37: 20 (m), 33 (m). 38: 7 (w), 17 (w), 21 (s), 32 (s), 35 (m), 39 (s). 39: 3 (w), 21 (m), 32 (m), 34 (m), 35 (s), 38 (s).

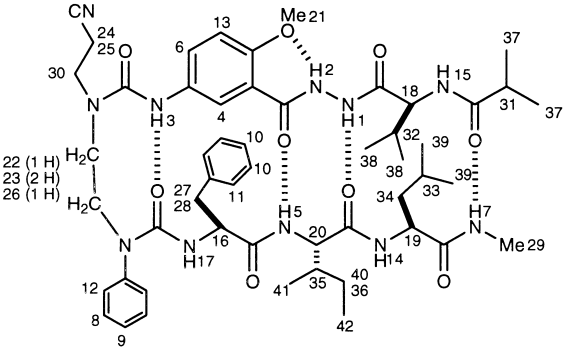
^1H NMR Tr-ROESY cross-peaks for artificial β-sheet 2.



1: 2 (m), 5 (w), 8 (m), 9 (w), 31 (w), 37 (w). 2: 1 (m), 20 (m). 3: 4 (s), 6 (w), 21 (m), 22 (w), 24 (s). 4: 3 (s), 5 (m), 6 (w), 12 (w), 16 (m), 37 (w). 5: 1 (w), 4 (m), 16 (s), 17 (w), 19 (m), 27 (w), 31 (m), 34 (w), 35 (w), 37 (m). 6: 3 (w), 4 (w), 13 (s). 7: 8 (m), 18 (s), 26 (m), 29 (s), 32 (w), 36 (w). 8: 1(s), 7 (m), 9 (s), 18 (s), 25 (m), 26 (w), 36 (m). 9: 1 (w), 8 (s), 18 (m), 25 (s), 26 (w). 10: 14 (s). 11: 12 (s). 12: 4 (w), 11 (s), 16 (s), 17 (m), 27 (m), 28 (s), 36 (w). 13: 6 (s), 20 (s). 14: 10 (s), 17 (m), 21 (w), 24 (m). 15: 18 (m), 19 (s), 32 (w), 33 (w), 37 (w). 16: 4 (m), 5 (s), 12 (m), 17 (m), 27 (m), 28 (m). 17: 5 (w), 12 (w), 14 (m), 16 (m), 27 (w), 28 (m). 18: 7 (s), 8 (m), 9 (w), 15 (m), 32 (m), 33 (m), 36 (s). 19: 5 (m), 15 (s), 31 (m), 34 (m), 35 (w), 37 (s), 38 (m). 20: 2 (m), 13 (s). 21: 3 (m), 14 (w), 22 (m). 22: 3 (w), 21 (m). 23: 30 (s). 24: 3 (s), 14 (w). 25: 8 (m), 9 (s). 26: 7 (m), 8 (w), 9 (m), 36 (m). 27: 5 (w), 12 (m), 16 (m), 17 (w), 28 (s). 28: 12 (m), 16 (m), 17(m), 27 (s). 29: 7 (s). 30: 23 (s). 31: 1 (w), 5 (m), 19 (m), 34 (m), 35 (m), 37 (s), 38 (m). 32: 7 (w), 15 (m), 18 (m), 36 (s). 33: 15 (w), 18 (m), 36 (s). 34: 5 (w), 19 (m), 31 (m), 35 (s), 37 (m), 38 (s). 35:

5 (w), 19 (w), 31 (m), 34 (s), 37 (m), 38 (m). 36: 7 (m), 8 (m), 12 (m), 18 (s), 26 (m), 32 (s), 33 (s). 37: 1 (w), 5 (m), 15 (w), 19 (m), 31 (s), 34 (m), 35 (s). 38: 19 (m), 31 (m), 34 (s). 35 (s).

^{1}H NMR Tr-ROESY cross-peaks for artificial β-sheet 3.



1: 2 (m), 5 (m), 18 (s), 32 (w), 35 (w), 38 (w), 41 (w). 2: 1 (m), 21 (m). 3: 4 (s), 22 (m), 23 (s), 26 (w). 4: 3 (s), 5 (m), 11 (w), 16 (m), 36 (w), 42 (w). 5: 1 (w), 4 (m), 11 (w), 16 (s), 17 (w), 20 (m), 35 (m), 41 (w). 6: 13 (s). 7: 18 (w), 19 (s), 29 (s), 37 (w), 39 (w). 8: 12 (s). 9: 12 (s). 10: 11 (s). 11: 4 (w), 5 (w), 10 (s), 16 (m), 17 (m), 27 (m), 28 (m). 12: 8 (s), 9 (s), 17 (m), 23 (m). 13: 6 (s), 21 (s). 14: 19 (m), 20 (m), 33 (w), 34 (w). 15: 18 (m), 31 (m), 32 (w), 37 (w), 38 (m). 16: 4 (m), 5 (s), 11 (m), 17 (s), 27 (m), 28 (m). 17: 5 (w), 11 (w), 12 (m), 16 (s), 27 (m), 28 (m). 18: 1 (s), 7 (w), 15 (m), 19 (s), 32 (m), 38 (m), 39 (m). 19: 7 (s), 14 (m), 18 (s), 33 (m), 34 (m), 38 (w), 39 (s). 20: 5 (m), 14 (s), 35 (m), 40 (w), 41 (m), 42 (w). 21: 2 (m), 13 (s). 22: 3 (m). 23: 3 (s), 12 (w), 26 (m). 24: 25 (s), 30 (m). 25: 24 (s), 30 (m). 26: 3 (w), 23 (m). 27: 11 (m), 16 (m), 17 (w), 28 (s). 28: 11 (m), 16 (m), 17 (m), 27 (s). 29: 7 (s), 37 (m). 30: 24 (m), 25 (m). 31: 15 (s), 37 (s). 32: 1 (w), 15 (w), 18 (m), 38 (s). 33: 14 (w), 19 (m), 39 (s). 34: 14 (w), 19 (m), 39 (s). 35: 1 (w), 5 (m), 20 (m), 41 (s), 42 (m). 36: 4 (w), 40 (s). 37: 7 (w), 15 (w), 29 (m), 31 (s). 38: 1 (w), 15 (m), 18 (m), 19 (w), 32 (s). 39: 7 (w), 18 (w), 19 (s), 33 (s), 34 (s), 42 (m). 40: 20 (m), 36 (s). 41: 1 (w), 5 (w), 20 (m), 35 (s), 42 (s). 42: 4 (w), 20 (m), 35 (s), 39 (s), 41 (s).

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