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The Design of Water Soluble β-Sheet Structure Based On a Nucleation Strategy

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Abstract. This manuscript demonstrates that incorporation of the amino acid residue 4-(2-aminoethyl)-6-dibenzofuranpropionic acid **1** is necessary but not sufficient to stabilize the β-sheet structure of a heptapeptide in water. A sequence which facilitates intrastrand hydrophobic interactions is also important.

Introduction. β-sheet secondary structure is as common as α-helical structure in proteins, but unlike the latter, β-sheets are not well understood due to the lack of well-defined β-sheets amenable to detailed biophysical evaluation.[1] Conformational investigations on high molecular weight polypeptides composed of a single type of amino acid residue and analogous studies on sequential polypeptides have contributed significantly to our understanding of β-sheet structure in aqueous solutions.[2] However, a significant limitation of employing polymers for the examination of β-sheet structure is that self-association occurs, even for high molecular weight samples capable of forming intramolecular β-sheet structure.[2f-i] Usually a mixture of intramolecularly folded and linear polypeptides self-associate to form large heterogeneous β-sheets (Figure 1). Additional structural complications arise from the orientation of the neighboring chains which can be either parallel or antiparallel.[2] Furthermore, two different kinds of self association are possible, lateral association (assisted by hydrogen bonding) and stacking of laterally associated β-sheets (mediated through hydrophobic interactions). The ultimate difficulty arises when these large associated β-sheets become insoluble and precipitate from solution.

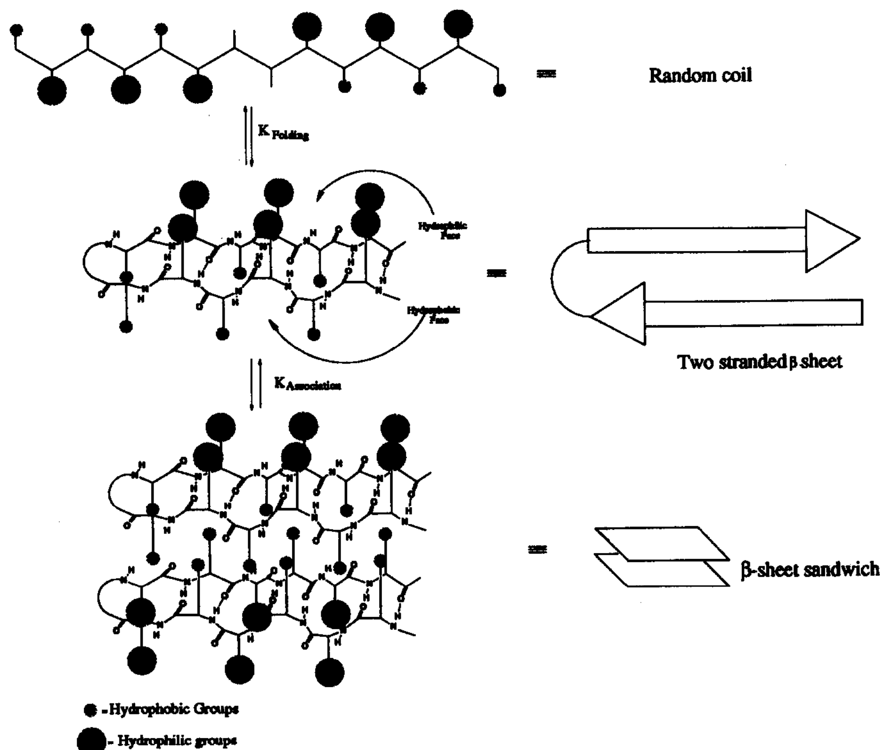
The use of small hydrophobic oligopeptides or amphiphilic oligopeptides as models for β-sheet structure has generally resulted in the formation of heterogeneous β-sheets which self-associate and precipitate.[3] Only a few exceptions have been noted.[3b] In most cases the handling and purification of these peptides has proven to be very difficult.[4] Our approach for creating well defined β-sheets centers around facilitating rapid intramolecular chain folding which affords a well defined β-sheet and thus avoids many of the problems discussed above. Fast intramolecular folding allows the rational design of β-sheet structures that do not self-associate or only associate to create dimers or small oligomers (Figure 1A). Controlling self-association is accomplished by the proper choice of peptide sequence, which will be discussed later. The pivotal component in our peptides is an unnatural amino acid which directs the folding of a polypeptide chain into a β-sheet secondary structure.[5] In order to discuss the design of this residue it is important to briefly outline what is known about β-sheet folding.

The equilibrium between the random coil state and the folded β-sheet state is controlled by the $\Delta G_{folding}$ which is entropically based. The enthalpy changes associated with β-sheet folding are negligible.[6]

$$\Delta G_{folding} = \Delta H_{water} + \Delta H_{chain} - T\,(\Delta S_{water} + \Delta S_{chain}) \qquad \text{(Equation 1)}$$

Polypeptides fold when the ΔS_{water} term is greater in magnitude than the ΔS_{chain} term (Equation 1). In the denatured state the S_{water} term is unfavorable relative to the folded state due to the ordered hydration shells required to solvate the hydrophobic side chains. As the peptide chain folds, the S_{water} term increases due to the ordered water liberated from the interacting hydrophobic groups. Releasing ordered water overcomes the unfavorable entropy term associated with restricting the conformation of the chain which occurs during secondary structure formation.

A. Ideally, Intramolecular Folding Preceeds Self-Association Affording a Homogenous β-Sheet Structure



B. Often Folding and Self-Association Have Comparable Rates Which Leads to a Heterogeneous β-Sheet

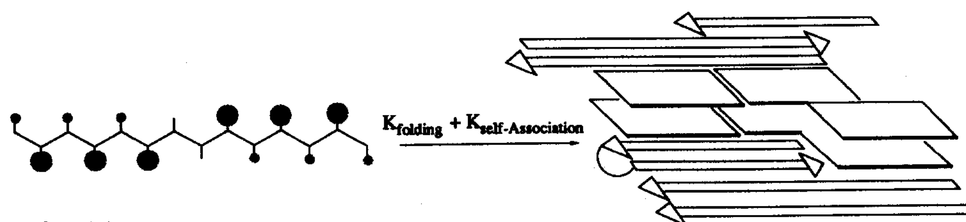


FIGURE 1.

The mechanism(s) by which β-sheets form is still not understood owing to the lack of a good model system to test theoretical predictions.[7] It is established that the initiation (nucleation) of an α-helix from a random coil conformation is the slowest and energetically most costly step in helix folding. Contrarily, subsequent growth of the helix is rapid and thermodynamically favored. Nucleation is thermodynamically unfavorable because of the entropic penalty associated with fixing the dihedral angles in the three residues intervening between residues i and i+4, which must be H-bonded in order to form a core structure which facilitates helix propagation. Growth of the helix from such a nucleus is favored because of the increase in the effective concentration of H-bonding donors and acceptors in the residue proximal to the nucleus and because the dihedral angles in only one residue need to be restricted in order to add that residue to the helix. Recent computational papers suggest that β-sheets could also form from a nucleus of a partial β-sheet.[7] For this reason we aim to synthesize a molecule that can act as a β-sheet nucleus.

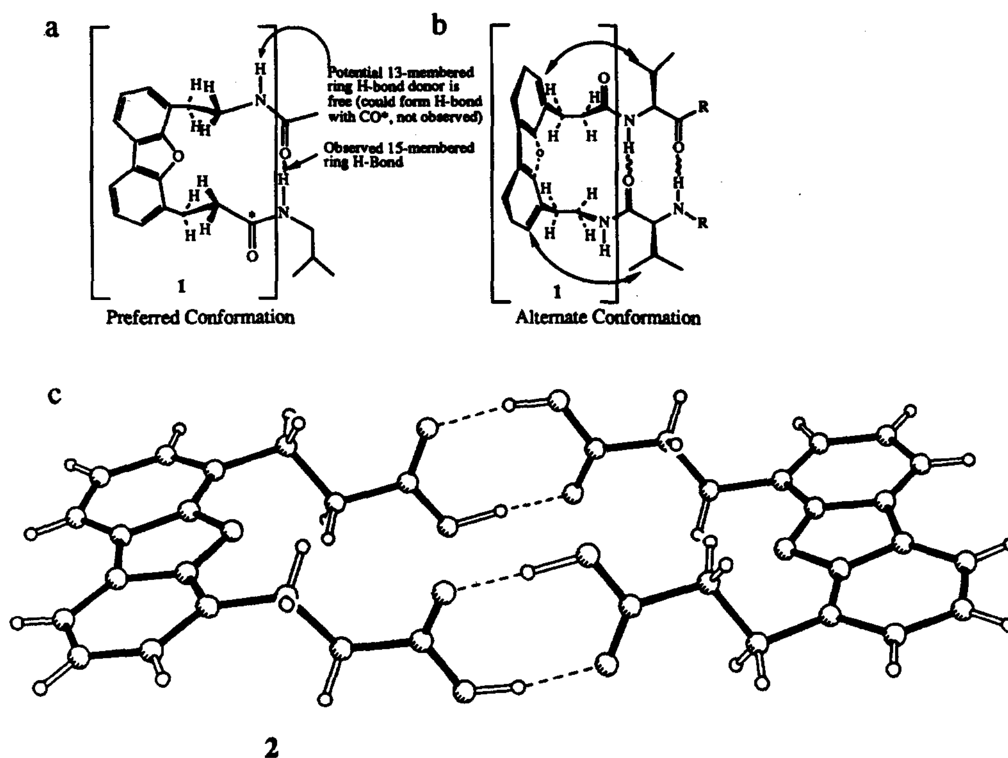


Figure 2. (a) Low energy conformer of **1**. (b) Alternate conformer of **1** which is potentially important in aqueous solution owing to the hydrophobic interactions between the dibenzofuran skeleton and the side chains of the amino acids proximal to **1** (indicated by the arrows). (c) Ball and stick representation of the solid state structure of the diacid **2** (4,6-dibenzofuran-dipropionic acid) (see Table 1 for atomic coordinates)

In principle, small linear polypeptides can adopt a β-sheet conformation in aqueous solution by forming a reverse turn (β-turn) that facilitates the hydrophobic interactions and hydrogen bonding interactions which are the hallmark of antiparallel β-sheet structure.[8] Despite the fact that some small peptides can adopt a β-turn conformation in aqueous solution, the incorporation of these sequences into peptides having a propensity for the formation of antiparallel β-sheet structure has not resulted in well defined β-sheet structure in our hands. However, the incorporation of a conformationally restricted β-sheet nucleator has proven effective for the creation of water soluble β-sheets.

Results and Discussion. An X-ray crystallographic study combined with computational and CPK model building suggested that a structurally simple and effective β-turn mimetic could result from functionalizing the aromatic skeleton of dibenzofuran.[9] The distance and geometry between C4 and C6 (4.9 Å) is very close to the distance between the strands of an antiparallel β-sheet (4.85 Å).[9] We chose to incorporate aminoethyl and carboxyethyl functional groups at positions 4 and 6, respectively, to create 4-(2-aminoethyl)-6-dibenzofuran-propionic acid **1** (Figure 2).[10] Studies on the conformation of ethyl benzene, phenethylamine and other related derivatives in a variety of solvents have shown that rotation around the aryl-alkyl carbon-carbon bond is restricted by 1.2 - 4.8 Kcal / mol (calculated values).[11] Moreover, the low energy conformer is that which positions the aliphatic carbon-carbon bond perpendicular to the plane of the aromatic ring which is desirable for two reasons.[11] First, the perpendicular conformation facilitates an intramolecular hydrogen bond of the type needed to stabilize β-sheet structure formation over alternative conformations in aqueous solution, Figure 2a. Secondly, the hydrogen bonded structure appears to be free of significant torsional and non-bonded strain which is essential if the hydrogen bond is to contribute towards stabilizing the β-sheet conformation in 55M water.[5a] Evidence supporting the perpendicular conformation as the low energy conformer is exhibited by the X-ray crystal structure of the intermolecularly hydrogen bonded 4,6-dibenzofurandipropionic acid **2**, which is a synthetic precursor to the putative nucleator 4-(2-aminoethyl)-6-dibenzofuranpropionic acid **1** (Figure 2c). It is important to note that the desired conformation is obtained even in cases where intramolecular interactions are not observed.

Molecular dynamics / molecular mechanics studies reported previously suggest that another conformation, which is just slightly higher in energy than that considered in Figure 2a, may also be worthy of consideration (Figure 2b).[5a] This conformer also exhibits a perpendicular structure that allows intramolecular hydrogen bonding, but differs in that it permits hydrophobic interactions between the dibenzofuran skeleton and the side chains of the hydrophobic amino acids flanking the dibenzofuran nucleus. We suggest that these interactions may be important for nucleating β-sheet structure in aqueous solution. A recent NMR study on a peptide incorporating **1** supports the existance of this hydrophobic cluster.[15a]

We have recently reported ^{1}H NMR and FT-IR data as evidence in favor of one of two possible intramolecularly hydrogen bonded structures in simple diamide derivatives of **1** in non-polar solvents.[5a] The 15-membered ring intramolecular hydrogen bond shown in Figure 2 is observed whereas the alternative 13-membered hydrogen bond is not detected. The presence of intramolecular amide-amide hydrogen bonding can be readily determined by analyzing the temperature dependence of the amide proton NMR chemical shift and

the amide N-H IR stretch region (3200-3500 cm^{-1}).[12] These techniques have been extensively employed for determining a hydrogen bonded conformation in several polyamide model systems.[12]

In nonhydrogen bonding solvents, a large temperature dependence ($\Delta\delta / \Delta T = 0.01$ ppm / K) indicates that the amide N-H is hydrogen bonded. On the other hand, a small temperature dependence ($\Delta\delta / \Delta T = 0.003$ ppm / K) usually means that the amide N-H is not forming a hydrogen bond. Alternatively, a small temperature coefficient is also consistent with the N-H being locked in a rigid hydrogen bonded conformation. These two possibilities can be easily distinguished employing FT-IR and sometimes just by considering the N-H chemical shift. A resonance at low field (δ 7-8 ppm, in CD_2Cl_2) is indicative of an amide that is strongly bonded, while a nonbonded amide N-H appears at a higher field (δ 5.5-6 ppm). In both cases, the observed chemical shift is a weighted average of those conformational states populated.

Examination of the more complex tetraamide **3** was carried out in order to demonstrate that the putative dibenzofuran nucleator could support a multiply hydrogen bonded antiparallel β-sheet-like structure. Proton NMR reveals three amide signals in CD_2Cl_2. The upfield signal δ 6.2 (25°C) exhibits a moderate temperature dependence (0.008 ppm / °K) consistent with an NH that is solvent exposed. The two remaining amide NH's appear to be strongly hydrogen bonded as evidenced by their downfield chemical shifts. The relatively small temperature dependencies (0.005 ppm / °K) displayed by these downfield protons suggest that the amide groups are in a rigid hydrogen bonded conformation (Figure 3).[12] Since a small temperature coefficient for an amide proton is also consistent with a non-hydrogen bonded state or a mixture of non-hydrogen bonded and hydrogen bonded states having similar enthalpies, it is essential to examine these peptides by FT-IR which has a much faster time scale to ensure that these amide NH's are hydrogen bonded.[12c]

Typically, a hydrogen bonded N-H displays a broad IR absorption between 3275 - 3350 cm^{-1} (in CH_2Cl_2) whereas a free amide N-H absorbs energy between 3400 - 3500 cm^{-1}. In the case of tetraamide **3**, the hydrogen bonded N-H stretch appears to be shifted to a much lower frequency (3305 cm^{-1}) when compared to the simple amides previously examined,[5a] suggesting that the hydrogen bonds are stronger in **3** (Figure 3). These data clearly suggest that **1** facilitates the formation of a multiply hydrogen bonded conformation, which is required for β-sheet formation. The ability of amides composed of **1** to undergo intramolecular hydrogen bonding in non-polar solvents at room temperature is required, but is not sufficient to predict their efficacy as nucleators in aqueous solutions.[5a]

The ability of **3** to form a predominantly hydrogen bonded conformation in CH_2Cl_2 provided encouragement to test the efficacy of **1** as a β-sheet nucleator in peptides that are water soluble. The peptide sequences examined here are based on the β-sheet region of the cyclic peptide gramicidin S (Figure 4).[13] Gramicidin S is an amphiphilic peptide that is unique in that it is monomeric in aqueous solution.[13c] An acyclic derivative of gramicidin S was obtained by eliminating one of the D-Phe-Pro reverse turns and replacing the other with the dibenzofuran-based amino acid residue **1**. We reasoned that the positive charges in each strand, as well as the positive charge at the amino terminus would prevent self-association due to electrostatic repulsions. In addition, the positive charges should prove to be important for solubility purposes. Consideration was given to the possibility that this peptide may simply be too small to adopt a stable intramolecular β-sheet fold. At best,

we expected this sequence to exhibit a partial β-sheet structure since it has been predicted, and in some secondary structures established, that the N-and C-terminal residues are conformationally ill-defined.[14]

Several acyclic derivatives of gramicidin S were prepared using solid phase peptide synthesis methodology. The benzhydryl amine resin was employed to afford C-terminal amides, creating sequences void of negative charge in order to avoid potential favorable electrostatic interactions which could lead to unwanted self-association. Several acyclic gramicidin S derivatives were prepared in order to examine the influence of the various residues on the β-sheet structural stability.

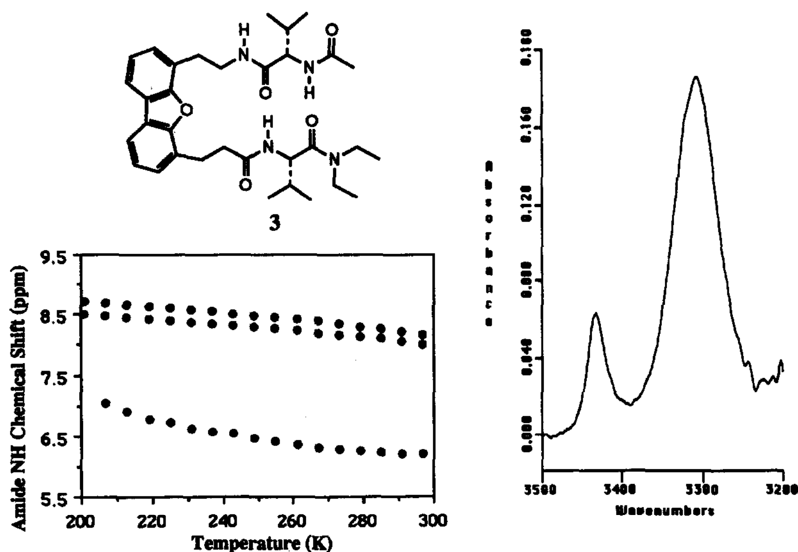
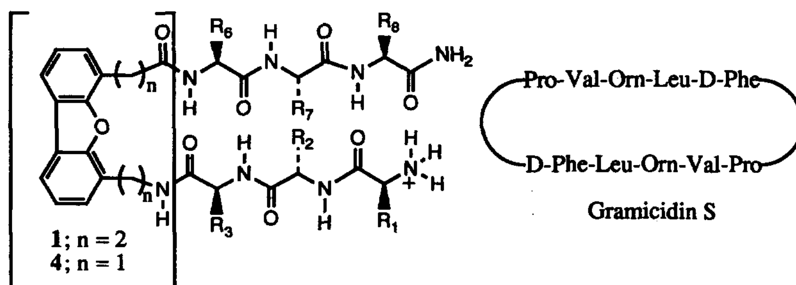


Figure 3. Temperature dependence of the amide proton NMR chemical shifts of a 1.5mM solution of peptide **3** in CD_2Cl_2 at 400 MHz (left panel) and the FT-IR spectrum of the N-H region of peptide **3** in 1.5mM CD_2Cl_2 at 298 °K. All spectra were recorded on a Galaxy 6021 spectrometer. Baseline corrections were applied.

We have recently completed spectroscopic studies on peptide **5** and related analogs by NMR and / or circular dichroism, which provide evidence that **1** stabilizes the β-sheet fold in aqueous solution.[15a] Briefly, the CD spectrum exhibited by peptide **5** is consistent with a peptide that is partly β-sheet and partly random coil (Figure 5).[15a] It is remarkable that such a short sequence exhibits a partial β-sheet structure due to the preference of the terminal residues to adopt a coil conformation.[7a, 14] Several control peptides were prepared in order to ensure that the precedented β-sheet CD signal at 213 nm originated from the β-sheet structure and not from an electronic transition within the aromatic chromophore.[15a,b] Evidence that β-sheet structure is responsible for the CD minimum centered at 213 nm derives from the CD spectra of analogs of peptide **5** that have a constant hydrophobic core, which includes **1** and the flanking amino acid residues, and variable β-strand sequences. That these peptides exhibit decidely different amounts of β-sheet structure and have a constant

hydrophobic core is strong evidence that that conformation of the strands contribute to the majority of the intensity at 214nm (data not shown).[15c] Furthermore, the intensity of the random coil component at 197nm decreases in every case where the intensity of the 214 nm signal increases. In all cases these peptides exhibit similar near-UV CD spectra indicating that the aromatic contribution to the 214 nm band, if any, should be constant since the hydrophobic cluster seems to be populated in all peptides. Taken together the CD data combined with the NMR data suggest that the 214 nm band does report on the amount of β-sheet structure present.[15b]

Having established that **1** is required for sheet formation in these acyclic gramicidin S derivatives, it is important to investigate the role that the non-turn residues in the sequence play in stabilizing the β-sheet structure. It was expected that the sequence of the β-strands would also play a significant role in stabilizing the β-sheet fold due to hydrophobic interactions between the side chains of residues flanking **1** and the dibenzofuran skeleton. Also, residues far apart in primary sequence but close in space (e.g. R_3 and R_6) may also interact with one another to stabilize or destabilize the structure, Figure 4 . Hydrophobic interactions appear to contribute much more to the stability of protein structure than does



PEPTIDE	R1	R2	R3	R4		R5	R6	R7	R8
Peptide **5**	Val	Lys	Leu	-	**1**	-	Val	Lys	Leu-NH_2
Peptide **6**	Ala	Lys	Ala	-	**1**	-	Ala	Lys	Ala-NH_2
Peptide **7**	Val	Orn	Leu	-	**1**	-	Val	Orn	Leu-NH_2
Peptide **8**	Leu	Orn	Val	-	**1**	-	Val	Orn	Leu-NH_2
Peptide **9**	Val	Orn	Leu	Gly		Gly	Val	Orn	Leu-NH_2

Figure 4

hydrogen bonding.[6,13d] The latter seems to be important from the standpoint of stabilizing one fold over an alternative fold.[16] To examine individual residue contributions, peptides **5** - **9** were prepared on the benzhydryl amine solid support using solid phase methodology.[17] The importance of intrastrand hydrophobic interactions

was examined by replacing the Val and Leu residues with Ala residues to afford peptide **6**. The circular dichroism spectra of peptide **6** is consistent with a random coil conformation implying that the hydrophobic interactions in addition to the presence of **1** is necessary for β-sheet formation (Figures 3 and 5).[18]

In our initial studies we replaced Orn with Lys because the latter is common in proteins.[15a] Peptide **7** was prepared to examine the effect, if any, that this substitution has on the formation of β-sheet structure in the acyclic gramicidin S analogs. The CD analysis of peptide **7** reveals that the spectrum of peptides **5** and **7** are virtually identical, hence the Lys for Orn substitution has no detectable effect on the development of β-sheet structure. This is expected since residues R_2 and R_7 are not expected to interact due to their like charge.

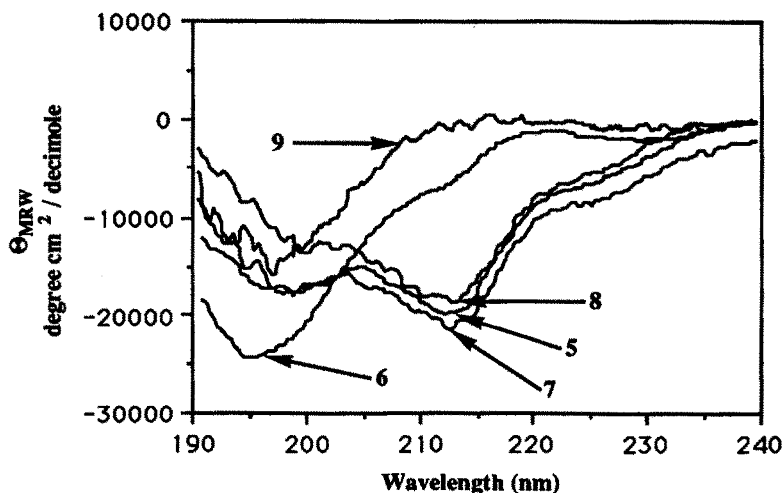


Figure 5. Circular dichroism spectra of peptides **5** - **9** (0.2 mM) at pH 2.9 (10 mM phosphate buffer).

To verify the importance of hydrophobic interactions, peptide **8** was prepared which has the same amino acid composition as peptide **7**, but differs with respect to sequence. Peptide **8** was designed to probe the general importance of hydrophobic interactions between residues R_3, R_6 and the dibenzofuran skeleton. The CD spectra of peptides **7** and **8** are nearly identical, indicating that specific hydrophobic interactions are not required, rather, a general dependence on the extent of the interacting hydrophobic surface area seems to be critical.

Replacing residue **1** with -Gly-Gly- in peptide **7** affords peptide **9** which was prepared because of our concern that the previously reported controls to examine the propensity of the sequence in the non-turn region to adopt a β-sheet structure. It is conceivable that D-Phe-Pro and the dibenzofuran analog 4-(aminomethyl)-6-dibenzofuran ethanoic acid **4** could actually be destabilizing the desired β-sheet conformation.[15a] In order to be confident that the strand sequences by themselves were not responsible for the β-sheet structure observed in peptide **7** and by analogy in peptide **5**, -Gly-Gly- was incorporated in place of **1**. Since dipeptide -Gly-Gly- can sample the largest range of conformational space available to any dipeptide, peptide **9** seemed to be the ideal

control to ensure that the strand sequences were not solely responsible for the observed β-sheet structure in peptide **7** and by analogy in peptide **5**. A CD study of peptide **9** identifies its conformation as a random coil, further supporting the hypothesis that both a good nucleator (**1**) and a carefully chosen sequence capable of intrachain hydrophobic interactions are required for β-sheet formation.

Summary. This manuscript demonstrates that the dibenzofuran nucleator **1** is necessary but not sufficient to stabilize β-sheet structure in a heptapeptide. The experiments described within suggest that intrastrand hydrophobic interactions are required in addition to the presence of **1** for β-sheet nucleation.

Refined X-ray Coordinates:

Table 1. Atomic coordinates ($x10^4$) and equivalent isotropic displacement parameters ($Å^2$ x 10^3)

X	Y	Z	U (eq)[a,b]
7747 (2)	338 (10)	7623 (2)	38 (2)
6452 (3)	2751 (13)	4689 (3)	62 (2)
5801 (3)	6205 (12)	4923 (3)	77 (3)
5369 (3)	-4910 (12)	6504 (3)	54 (2)
4751 (2)	-1135 (10)	6654 (2)	49 (2)
8890 (3)	2567 (15)	8509 (3)	37 (3)
9506 (3)	4395 (15)	8701 (4)	40 (3)
9557 (4)	5878 (16)	8090 (4)	48 (3)
8995 (3)	5220 (16)	7293 (4)	44 (3)
8367 (3)	3662 (15)	7077 (4)	39 (3)
8334 (3)	2264 (14)	7706 (3)	37 (3)
7758 (3)	3174 (16)	6216 (3)	48 (3)
7015 (4)	4880 (20)	5981 (4)	68 (4)
6404 (4)	4473 (19)	5131 (4)	52 (3)
8633 (3)	630 (14)	8958 (3)	35 (3)
8914 (3)	-171 (16)	9758 (3)	42 (3)
8502 (4)	-2174 (17)	9951 (4)	47 (3)
7805 (3)	-3330 (15)	9371 (3)	40 (3)
7504 (3)	-2572 (14)	8569 (3)	35 (3)
7942 (3)	-584 (14)	8405 (3)	34 (3)
6758 (3)	-3827 (14)	7933 (3)	37 (3)
6082 (3)	-1711 (15)	7583 (3)	44 (3)
5375 (4)	-2750 (16)	6866 (4)	38 (3)

H-atomic coordinates ($x10^4$)

Atom	X	Y	Z	U
H2A	9893	4629	9247	80
H3A	9985	7166	8218	80
H4A	9040	6599	6883	80
H7A	7608	1242	6146	80
H7B	7987	3603	5863	80
H8A	7167	6810	6053	80
H8B	6788	4451	6337	80
H11A	9381	677	10162	80
H12A	8701	-2757	10498	80
H13A	7529	-4694	9527	80
H16A	6596	-5355	8157	80
H16B	6861	-4544	7509	80
H17A	6276	-59	7440	80
H17B	5926	-1211	7989	80
H105	4286	-1834	6047	50
H102	5350	5952	4383	50

(a) equivalent isotopic U defined as one third of the trace of the orthogonalized U_{ij} tensor. (b) Estimated standard deviations are given in parenthesis.

Experimental. Routine NMR spectra were obtained on a Varian XL-200E or XL 400 spectrometer and the chemical shifts are reported in ppm from tetramethylsilane in terms of singlets (s), doublets (d), triplets (t), quartets (q), or multiplets (m). Melting points were obtained on a Mel-Temp apparatus in open capillary tubes

and are uncorrected. High resolution mass determinations were carried out on a VG-70S double focusing high resolution mass spectrometer. Details regarding the variable temperature NMR and IR studies can be found in reference 5a. The synthesis of **1** and **4** is described in references 10 and 15a, respectively.

Synthesis of tetraamide **3**. A 25 ml round bottomed flask was charged with 1.03g (6 mmol) of valine diethylamide and 1.1 g (2 mmol) of 4-(N-tert-butyloxycarbonyl-2-aminoethyl-)-6-dibenzofuran-pentafluorophenylpropionate **10** in 25 ml of CH_2Cl_2. Triethylamine 0.3 g (3 mmol) was then added. After overnight stirring, the mixture was diluted with CH_2Cl_2 (50 ml) and washed with 1 M citric acid (3 x 30 ml) and 5 % K_2CO_3 (3 x 30 ml). The organic layer was dried and concentrated to afford 1.04 g of an oily solid. This solid was treated with 100 ml of 25 % TFA in CH_2Cl_2 for 45 min. The excess TFA was removed under vacuum followed by treatment with 10 g of amberlyst A-21 resin to afford 0.86 g (96 % yield) of 4-(-2-aminoethyl-)-6-dibenzofuran valinyl propionamide **11**.

A 25 ml round bottomed flask was charged with **11** and 8 mmol of Boc-valine-pentafluorophenyl ester as well as 15 ml of CH_2Cl_2 and 1 ml of TEA. After overnight stirring, 2 ml of N,N-dimethylethylenediamine was added in order to convert the remaining valine active ester into a water soluble side product. After 1 h the reaction mixture was diluted with CH_2Cl_2 (25 ml) and washed with 1 M citric acid (3 x 40 ml), 5 % $KHCO_3$ (2 x 30 ml) and saturated NaCl (35 ml). The organic layer was dried and concentrated to afford 1.23 g of a brown oil. The crude oil was treated with TFA and neutralized using 12 g of amberlyst A-21. After acetylation with excess acetic anhydride / DIEA, the resulting solid was recrystallized from THF / cyclohexane to afford 0.25 g (21 % yield) of pure **40**: IR (1.5 mM in CH_2Cl_2, cm^{-1}) 3432, 3307, 1657, 1627, 1526; ^1H NMR (400 MHz, DMSO-d_6) δ 8.19 (t, J = 5.7 Hz, 1 H, $ArCH_2CH_2N\underline{H}$) 8.11 (d, J = 9.2 Hz, 1 H, $ArCH_2CH_2CON\underline{H}$) 7.93 (dd, J = 6.0 Hz, 1.6 Hz, 1 H, Ar-1 or 9 $\underline{H}$) 7.91 (dd, J = 6.2 Hz, 1.3 Hz, 1 H, Ar-1 or 9 $\underline{H}$) 7.82 (d, J = 8.9 Hz, 1 H, $\underline{H}N\alpha CHCOCH_3$) 7.27 (m, 4 H,Ar-2,3,7,8 H) 4.45 (t, J = 9.2 Hz, 1 H, $\underline{H}N\alpha CHCON(CH_2CH_3)_2$) 4.06 (dd, J = 8.9 Hz, 7.1 Hz, 1 H, $HN\alpha C\underline{H}COCH_3$) 3.74 (m, J = 6.6 Hz, 1 H, $ArCH_2C\underline{H}_2NH$) 3.42 (m, 1 H, $ArCH_2C\underline{H}_2NH$) 3.40 (m, 3 H, $CON(C\underline{H}_2CH_3)_2$) 3.20 (m, 2 H, $ArC\underline{H}_2CH_2CO$) 3.05 (m, 2 H, $ArC\underline{H}_2CH_2NH$) 3.03 (m, 1 H, $CON(C\underline{H}_2CH_3)_2$) 2.81 (m, J = 7.0 H, 1 H, $ArCH_2C\underline{H}_2CO$) 2.64 (m, J = 7.4 Hz, 1 H, $ArCH_2C\underline{H}_2CO$) 1.84 (s, 3 H, $HNCOC\underline{H}_3$) 1.83 (m, 1 H, $C\underline{H}(CH_3)_2$) 1.78 (m, J = 6.8 Hz, 1 H, $C\underline{H}(CH_3)_2$) 1.10 (t, J = 7.12 Hz, 3 H, $N(CH_2C\underline{H}_3)_2$) 0.97 (t, J = 7.12 Hz, 3 H, $N(CH_2C\underline{H}_3)_2$) 0.75 (d, J = 6.8 Hz, $CH(C\underline{H}_3)_2$) 0.68 (d, J = 7.0 Hz, 3 H, $CH(C\underline{H}_3)_2$) 0.65 (dd, J = 5.82 Hz, 0.97 Hz, 6 H, $CH(C\underline{H}_3)_2$); MS m / z (M^+) calcd 578.3468, obsd 578.3486.

Peptide Synthesis. Solid phase peptide synthesis was carried out employing the benzhydrylamine solid support[17] (Advanced Chemtech) with a loading of 0.66 meq / g. Dichloromethane (DCM), isopropanol (IPA), and dimethylformamide (DMF) were reagent grade solvents. DMF was stored over molecular sieves 4A. Boc-amino acids were obtained from Advanced ChemTech. Trifluoroacetic acid was purchased from PCR Fluorochemicals and used as a 25-50 % solution in CH_2Cl_2 containing 1 % thioanisole (Aldrich). Diisopropylethylamine (Aldrich) was distilled from ninhydrin prior to use. (Benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium (Bop) hexafluorophospate reagent was purchased from Richelieu Biotechnologies Inc. and was handled in a fume hood (**Beware**: HMPA source).

The peptide synthesis protocol was a combination of the procedures reported by Kaiser and Castro.[20] Peptides **5** through **9** were synthesized on a 0.6 mmol scale. Each coupling step was monitored by the ninhydrin test. The first amino acid was loaded onto the resin employing 1.6 eq of diisopropylcarbodiimide-activated ester for 24 h in CH_2Cl_2. The resin was washed with DMF (2 x 1 min), CH_2Cl_2 (1 x 1min), IPA (1 x 1 min), CH_2Cl_2 (2 x 1 min), IPA (1 x 1 min), and CH_2Cl_2 (4 x 1 min). The following cycle was used for each coupling; TFA prewash (25 -50 % TFA x 1 min), TFA deprotection (25 -50 % TFA x 50 min), and the following washes: CH_2Cl_2 (2 x 1 min), IPA (1 x 1 min), CH_2Cl_2 (2 x 1 min), IPA (1 x 1 min), CH_2Cl_2 (4 x 1 min), preneutralize (12 % DIEA x 1 min), neutralize (12 % DIEA x 9 min), couple (3 eq of amino acid, 3 eq of Bop, 4 eq of DIEA in CH_2Cl_2 containing 10 - 15 % DMF for 2 - 6 h), and perform the following washes: DMF (2 x 1 min), CH_2Cl_2 (1 x 1 min), IPA (1 x 1 min), CH_2Cl_2 (2 x 1 min), IPA (1 x 1 min), and CH_2Cl_2 (4 x 1 min). The coupling of **1** to the growing peptide was performed employing 3 eq of 4-(N-tert-butyloxycarbonyl-2-aminoethyl-)-6-dibenzofuran pentafluorophenylpropionate **10** and 3 eq of DIEA overnight. At the end of each peptide synthesis, the Boc N-terminal protecting group was removed with TFA. The resin-bound peptide was then subjected to HF cleavage which deprotects the side-chains and liberates the peptide from the resin.[21]

The samples were purified by preparative HPLC on a dual pump system equipped with Altex 110A pumps and a 420 gradient programmer. The column employed was a Waters RCM Delta Pak C_{18} (15 μm, 300 Å, 25 x 100 mm) attached to a variable wavelength detector model Knauer 86 set at 254 nm. Solvent A was composed of 95 % water, 5 % acetonitrile (Fisher, Optima grade), and 0.2 % TFA. Solvent B was composed of 5 % water, 95 % acetonitrile, and 0.2 % TFA. The yields of purified peptides, based on initial resin loading, were in the range of 10 - 40 %. All peptides were characterized by laser desorption time of flight mass spectrometry.[21]

Purification of peptide 5. After HF cleaveage, the crude peptide was dissolved in a minimum amount of 50 mM acetate buffer (pH 4.0) and purified using a 35% - 50% linear gradient in acetonitrile over twenty minutes. MS m / z (M^+ + 1) calcd 964.3, obsd 964.1.

Purification of peptide 6. After HF cleaveage, the crude peptide was dissolved in the minimum amount of 50 mM acetate buffer (pH 4.0) and purified using a 20% - 45% linear gradient in acetonitrile over twenty minutes. MS m / z (M^+ + 1) calcd 824.0, obsd 824.0.

Purification of peptide 7. After HF cleaveage, the crude peptide was dissolved in a minimum amount of 50 mM acetate buffer (pH 4.0) and purified using a 0% - 55% linear gradient in acetonitrile over twenty two minutes. MS m / z (M^+ + 1) calcd 936.2, obsd 935.6.

Purification of peptide 8. After HF cleaveage, the crude peptide was dissolved in the minimum amount of water pH = 6 and purified using a 35% - 50% linear gradient in acetonitrile over twenty minutes. MS m / z (M^+ + 1) calcd 936.2 , obsd 935.2.

Purification of peptide 9. After HF cleaveage, the crude peptide was dissolved in a minimum amount of 50 mM acetate buffer (pH 4.0) and purified using a 0% - 55% linear gradient in acetonitrile over twenty minutes. MS m / z (M^+ + 1) calcd 784.0, obsd 784.7.

Circular dichroism studies. CD spectra were collected on a Jasco J-600 spectropolarimeter. The samples were prepared as stock solutions (1 to 5 mM) in the appropriate buffer and diluted to the final

concentration. The solutions were degassed by sonication under vacuum for 30 sec. Spectra were obtained at a scan speed of 50 nm / min, a time constant of 0.5 sec and a band width of 1 nm at 25°C and were reported in mean residue ellipticity.[15a]

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