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Hydrogen bonded antiparallel β-strand motifs promoted by 2,6-bis(carbamoylpeptide)pyridine

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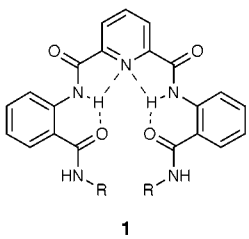
A series of peptide dimers of 2,6-carbamoylpyridine have been isolated and their solid-state structures evaluated for the formation of strands motifs and lamellar architecture.

Many fibrous proteins found in nature (*e.g.* silks) have a significant amount of β-sheet structure which leads to lamellar architectures in the solid state.[1,2] The desirable physical properties associated with these proteins, such as high tensile strength, are proposed to result, in part, from their β-sheet structures.[1,2] Most attempts to duplicate these structural properties in synthetic systems have been hindered by difficulties in controlling secondary and tertiary peptide structures.[3] One useful approach to organize peptide structure is to incorporate a rigid template into the peptide backbone.[4,5] Our laboratory,[6] and that of Hamilton,[7] have been exploring the use of 2,6-bis[(2-carbamoylphenyl)carbamoyl]pyridine templates (**1**) to nucleate synthetic helices. Compounds with **1** have an inherent twist that results from the two appended phenyl rings being disposed on opposite faces of the planar 2,6-bis(carbamoyl)pyridine unit. We have found that replacing the phenyl groups with peptides would yield more planar compounds containing chiral clefts (**2**). In this design, direct intramolecular interactions between appended peptides are not possible; however intermolecular hydrogen bonding can occur to form supramolecular β-strands with antiparallel arrangements of peptides. We report herein our structural findings for a small family of peptide dimers.

Four dipeptides were synthesized that include all possible combinations of alanine and valine **2**(X,Y). Valine and alanine were chosen for their differing propensities to form β-sheets in proteins as shown by statistical[8] and thermodynamic studies.[9] Each pendant peptide arm was synthesized using standard DCC coupling protocol for Boc-protected amino acids.[10] Species **2**(X,Y) were obtained by treating THF solutions of the deprotected peptides with 2,6-bis(chlorocarbonyl)pyridine. All new compounds gave satisfactory FTIR, ^{1}H and ^{13}C NMR, FAB-MS, and elemental data.



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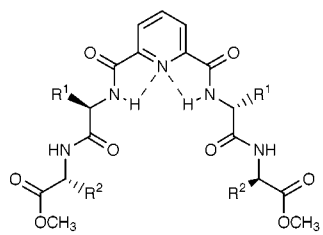
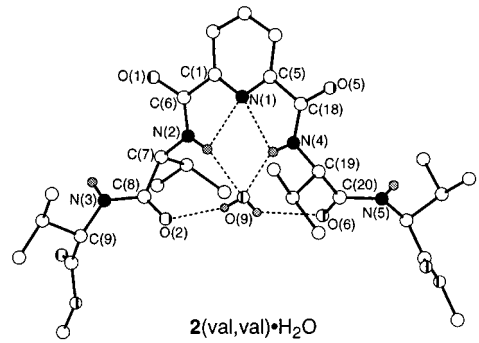


2(ala,ala): $R^1 = R^2 = CH_3$
2(val,val): $R^1 = R^2 = CH(CH_3)_2$
2(ala,val): $R^1 = CH_3$, $R^2 = CH(CH_3)_2$
2(val,ala): $R^1 = -CH(CH_3)_2$, $R^2 = CH_3$

NMR data in $CDCl_3$ obtained for the four peptide dimers suggest that the compounds are C_2 symmetric in solution. The chemical shift for the 2,6-carbamoylpyridine protons are observed at *ca.* 8.5 ppm which is indicative of intramolecular hydrogen bonding (*i.e.* $N–H_{amide}\cdots N_{py}$).[11] Moreover, the chemical shifts of these protons are sensitive to the presence of water. For **2**(val,ala), the resonance for these proton shifts downfield by 0.20 ppm with the addition of 1 equiv. of H_2O. Furthermore, the observed temperature dependence of the 2,6-carbamoyl protons of **2**(val,ala) in the presence of water ($\Delta\delta/\Delta T = -0.012$ ppm K^{-1}) is significantly greater than that under anhydrous conditions ($\Delta\delta/\Delta T = -0.0017$ ppm K^{-1}). Variable temperature ^{1}H NMR data also show that the chemical shift of the water present in **2**(val,ala) has a large temperature dependence of ($\Delta\delta/\Delta T = -0.0345$ ppm K^{-1}. Taken together these NMR data suggest the **2**(val,ala)·H_2O adduct forms *via* multiple hydrogen bonds.[12]

The solid state structures of **2**(val,ala)·H_2O and **2**(val,val)·H_2O were examined by single-crystal X-ray diffraction methods and agree with the structures suggested from solution studies. Both compounds crystallize in non-centrosymmetric space groups: **2**(val,ala)·H_2O in the tetragonal space group $P4_12_12_1$† and **2**(val,val)·H_2O in the orthorhombic space group $P2_12_12_1$.‡ The compounds have similar 'V'-shaped molecular structures with a guest water molecule residing in the chiral cleft formed by the two appended peptides [see Fig. 1 for **2**(val,val)·H_2O]. Species **2**(val,ala)·H_2O has exact C_2-symmetry where the axis bisects the pyridine ring and coincides with N(1) and O(9) of the water molecule. N(2)–H(2) of the carbamoyl moieties are involved in H-bonding between the pyridyl nitrogen [N(2)···N(1), 2.724(7) Å]. The guest water molecule also H-bonds to the internal valine carbonyl oxygens of each peptide as indicated by the heavy atom O(9)···O(2) distance of 3.097(7) Å. Compound **2**(val,val)·H_2O is not C_2-symmetric in the crystalline state; however, it also interacts with its guest water molecule through four H-bonding interactions: (i) O(9) H-bonds to carbamoyl N–H groups with N(2)···O(9) and N(4)···O(9) distances of 3.054(16) and 2.931(16) Å; and (ii) the water hydrogens H-bond to the appended peptides through the



2(val,val)•H_2O

Fig. 1 Molecular structure of **2**(val,val)·H_2O. Selected distances (Å) and dihedral angles (°): N(2)–O(9) = 3.053(16); N(4)–O(9) = 2.928(15); C(30)–O(2) = 3.206(15); O(9)–O(2) = 2.830(16); O(9)–O(6) = 2.697(16); N(2)–C(7)–C(8)–N(3) = 136.3; N(4)–C(19)–C(20)–N(5) = 124.2; C(6)–N(2)–C(7)–C(8) = −130.9; C(18)–N(4)–C(19)–C(20) = −113.5.

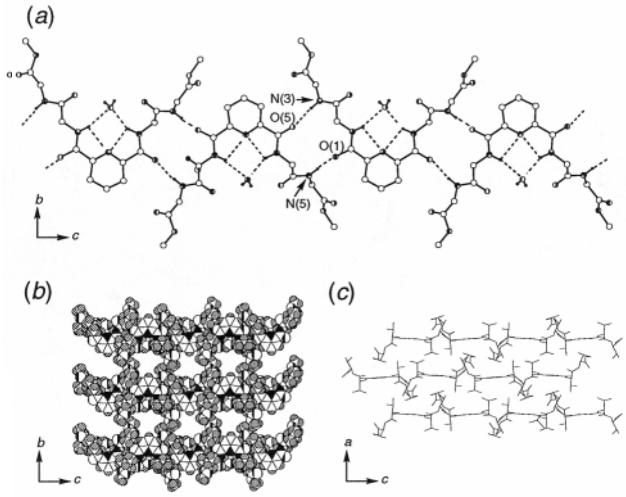


Fig. 2 Crystal lattice architecture in **2**(val,ala)·H_2O. (*a*) intermolecular hydrogen bonding network formed within antiparallel β-sheet (viewed along crystallographic *a*-axis, isopropyl groups removed for clarity), (*b*) lamella structure viewed in the *a*,*b* plane and (*c*) *a*,*c* plane. Chloroform solvent molecules removed for clarity.

internal valine carbonyl oxygens where heavy atom O(9)···O(2) and O(9)···O(6) distances are 2.830(15) and 2.698(15) Å.[13]

Although similar in molecular structure, **2**(val,val)·H_2O and **2**(val,ala)·H_2O have significantly different lattice architectures. Strand motifs are the dominant structural element found in the lattice of **2**(val,val)·H_2O. Individual strands are formed by an antiparallel arrangement of molecules connected by a network of intermolecular H-bonds which runs parallel to the crystallographic *c*-axis. Molecules within a strand have four intermolecular H-bonds, two bonds to each of its two nearest neighbors at distances of N(3)···O(5) = 2.860(16) and O(1)···N(5) = 2.970(18)Å [Fig. 2(*a*)]. The nearly coplanar positioning of the N(3)–H and C(6)–O(1) vectors (RMS planar deviation 0.064 Å) offers a favorable geometric arrangement for the formation of strands.

The strand motif in **2**(val,val)·H_2O resembles antiparallel β-strands (sheets) observed in proteins. The strands in this synthetic system have dihedral angles that are similar to the ψ (136°) and ϕ (−139°) angles found in protein antiparallel β-sheets.[14] The ψ angles for the internal valine in the molecular structure of **2**(val,val) are 136.2 and 124.2°, while those for ϕ are −130.9 and −113.5° (see legend to Fig. 1). These strand motifs assemble to produce sheets that lie in the crystallographic *b*,*c*-plane [Fig. 2(*b*)]. Note that each layer has an open framework which is formed by the interactions of peptides in neighboring strands. In **2**(val,val)·H_2O, the resultant open spaces within a layer are partially occupied by chloroform molecules. The arrangement of sheets within this crystal lattice is a lamellar structure [Fig. 2(*c*)].

In contrast to **2**(val,val)·H_2O, **2**(val,ala)·H_2O associates with neighboring molecules to form a three-dimensional network of hydrogen bonds which has neither strands nor a lamellar structure. Moreover, each molecule in the lattice of **2**(val,ala)·H_2O is involved in only two intermolecular H-bonds.[15] A possible explanation for the differences between the lattice architecture of **2**(val,ala)·H_2O and **2**(val,val)·H_2O is the magnitude of the molecular dihedral angles ψ and ϕ. In **2**(val,ala)·H_2O, the angles are ψ = −133.6° and ϕ = 118.6°, approximately 180° different than found for **2**(val,val)·H_2O (*vide supra*).

The β-strand formation observed in **2**(val,val)·H_2O and absent in **2**(val,ala)·H_2O is consistent with the results found in proteins, where valine and alanine confer different propensities for sheet formation. Moreover, β-sheets are often found in the interior of proteins where side chains engage in hydrophobic interactions with other units of secondary structure.[16] These interactions lead to alternating positioning of side chains that results in a 'tongue and groove' structure as observed in the lattice of **2**(val,val)·H_2O [Fig. 2(*c*)]. However, in **2**(val,ala)·H_2O the side chains do not permit the same long-range interactions, therefore an alternative lattice structure is adopted. The small size of **2**(val,val)·H_2O in comparison to proteins makes it particularly notable that antiparallel strand formation and lamellar structure is observed. Extension of this design to other peptide systems is currently under investigation.

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Notes and references

† *Crystal data* for **2**(val,ala)·H_2O: $C_{25}H_{37}N_5O_8·H_2$), crystal habit, blocks, space group $P4_12_12_1$, a = 12.535(3), c = 18.258(8) Å, V = 2868.9(19) Å^3, T = 231 K, Z = 4; μ = 0.98 cm^{-1}, 3535 reflections collected, 1649 independent reflections [$I > 2\sigma(I)$]. $R(F)$ = 0.0514, $R(wF^2)$ = 0.1024 with a GOF(F^2) = 0.94.

‡ *Crystal data* for **2**(val,val)·H_2O·$CHCl_3$: $C_{29}H_{45}N_5O_8·H_2O·1/2CHCl_3$, crystal habit, blocks, space group $P2_12_12_1$, a = 13,407(1), b = 14.817(2), c = 20.314(4) Å, V = 4035.5(8) Å^3, T = 298(2) K, Z = 4, μ = 1.76 cm^{-1}, 5123 reflections collected, 2303 independent reflections [$I > 2\sigma(I)$]. $R(F)$ = 0.0971, $R(wF^2)$ = 0.2597 with a GOF(F^2) = 1.175. CCDC 182/1285.

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