

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

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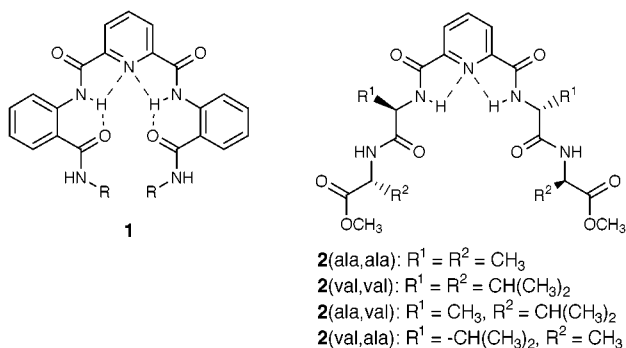
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Received (in Columbia, MO, USA) 5th January 1999, Accepted 3rd June 1999

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 statistical⁸ 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, ¹H and ¹³C NMR, FAB-MS, and elemental data.



NMR data in CDCl₃ obtained for the four peptide dimers suggest that the compounds are C₂ 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}...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 H₂O. 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 ¹H 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)**·H₂O adduct forms *via* multiple hydrogen bonds.¹²

The solid state structures of **2(val,ala)**·H₂O and **2(val, val)**·H₂O 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)**·H₂O in the tetragonal space group P4₁2₁2₁[†] and **2(val,val)**·H₂O in the orthorhombic space group P2₁2₁2₁.[‡] 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)**·H₂O]. Species **2(val,ala)**·H₂O has exact C₂-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)**·H₂O is not C₂-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

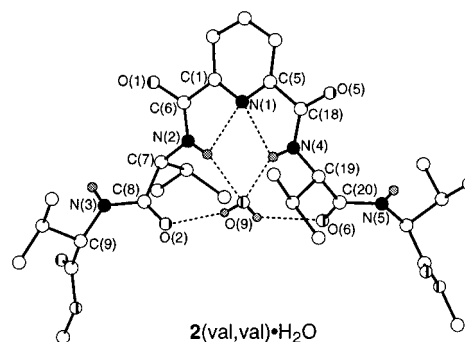


Fig. 1 Molecular structure of **2(val,val)**·H₂O. Selected distances (Å) and dihedral angles (°): N(2)–O(9) = 3.053(16); N(4)–O(9) = 2.928(15); C(30)–O(2) = 3.206(15); O(9)–O(2) = 2.830(16); O(9)–O(6) = 2.697(16); N(2)–C(7)–C(8)–N(3) = 136.3; N(4)–C(19)–C(20)–N(5) = 124.2; C(6)–N(2)–C(7)–C(8) = -130.9; C(18)–N(4)–C(19)–C(20) = -113.5.

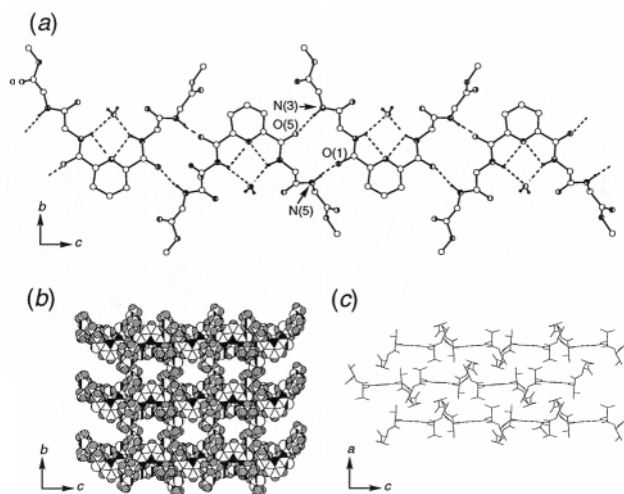


Fig. 2 Crystal lattice architecture in $2(\text{val,ala})\cdot\text{H}_2\text{O}$. (a) intermolecular hydrogen bonding network formed within antiparallel β -sheet (viewed along crystallographic a -axis, isopropyl groups removed for clarity), (b) lamella structure viewed in the a,b plane and (c) a,c plane. Chloroform solvent molecules removed for clarity.

internal valine carbonyl oxygens where heavy atom $\text{O}(9)\cdots\text{O}(2)$ and $\text{O}(9)\cdots\text{O}(6)$ distances are 2.830(15) and 2.698(15) Å.¹³

Although similar in molecular structure, $2(\text{val,val})\cdot\text{H}_2\text{O}$ and $2(\text{val,ala})\cdot\text{H}_2\text{O}$ have significantly different lattice architectures. Strand motifs are the dominant structural element found in the lattice of $2(\text{val,val})\cdot\text{H}_2\text{O}$. 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 $\text{N}(3)\cdots\text{O}(5) = 2.860(16)$ and $\text{O}(1)\cdots\text{N}(5) = 2.970(18)$ Å [Fig. 2(a)]. The nearly coplanar positioning of the $\text{N}(3)\text{--H}$ and $\text{C}(6)\text{--O}(1)$ vectors (RMS planar deviation 0.064 Å) offers a favorable geometric arrangement for the formation of strands.

The strand motif in $2(\text{val,val})\cdot\text{H}_2\text{O}$ 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 $2(\text{val,val})$ 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 $2(\text{val,val})\cdot\text{H}_2\text{O}$, 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 $2(\text{val,val})\cdot\text{H}_2\text{O}$, $2(\text{val,ala})\cdot\text{H}_2\text{O}$ 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 $2(\text{val,ala})\cdot\text{H}_2\text{O}$ is involved in only two intermolecular H-bonds.¹⁵ A possible explanation for the differences between the lattice architecture of $2(\text{val,ala})\cdot\text{H}_2\text{O}$ and $2(\text{val,val})\cdot\text{H}_2\text{O}$ is the magnitude of the molecular dihedral angles ψ and ϕ . In $2(\text{val,ala})\cdot\text{H}_2\text{O}$, the angles are $\psi = -133.6^\circ$ and $\phi = 118.6^\circ$, approximately 180° different than found for $2(\text{val,val})\cdot\text{H}_2\text{O}$ (*vide supra*).

The β -strand formation observed in $2(\text{val,val})\cdot\text{H}_2\text{O}$ and absent in $2(\text{val,ala})\cdot\text{H}_2\text{O}$ 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 $2(\text{val,val})\cdot\text{H}_2\text{O}$ [Fig. 2(c)]. However, in $2(\text{val,ala})\cdot\text{H}_2\text{O}$ the side chains do not permit the same long-range interactions, therefore an alternative lattice structure is adopted. The small size of $2(\text{val,val})\cdot\text{H}_2\text{O}$ 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.

We thank the NIH for financial support.

Notes and references

† Crystal data for $2(\text{val,ala})\cdot\text{H}_2\text{O}$: $\text{C}_{25}\text{H}_{37}\text{N}_5\text{O}_8\cdot\text{H}_2$, crystal habit, blocks, space group $P4_12_12_1$, $a = 12.535(3)$, $c = 18.258(8)$ Å, $V = 2868.9(19)$ Å³, $T = 231$ K, $Z = 4$; $\mu = 0.98$ cm⁻¹, 3535 reflections collected, 1649 independent reflections [$I > 2\sigma(I)$]. $R(F) = 0.0514$, $R(wF^2) = 0.1024$ with a $\text{GOF}(F^2) = 0.94$.

‡ Crystal data for $2(\text{val,val})\cdot\text{H}_2\text{O}\cdot\text{CHCl}_3$: $\text{C}_{29}\text{H}_{45}\text{N}_5\text{O}_8\cdot\text{H}_2\text{O}\cdot 1/2\text{CHCl}_3$, crystal habit, blocks, space group $P2_12_12_1$, $a = 13.407(1)$, $b = 14.817(2)$, $c = 20.314(4)$ Å, $V = 4035.5(8)$ Å³, $T = 298(2)$ K, $Z = 4$, $\mu = 1.76$ cm⁻¹, 5123 reflections collected, 2303 independent reflections [$I > 2\sigma(I)$]. $R(F) = 0.0971$, $R(wF^2) = 0.2597$ with a $\text{GOF}(F^2) = 1.175$. CCDC 182/1285.

- 1 D. L. Kaplan, S. J. Lombardi, W. S. Muller and S. A. Fossey, in *Biomaterials*, ed. D. Byrom, Macmillan, UK, 1991, p. 1; *Silk Polymers*, ed. D. L. Kaplan, W. W. Adams, B. Farmer and C. Viney, *ACS Symp. Ser.* 544, ACS, Washington, DC, 1994; R. V. Lewis, *Acc. Chem. Res.*, 1992, **25**, 392.
- 2 A. H. Simmons, C. A. Michal and L. Jelinski, *Science*, 1996, **271**, 84; D. A. Tirrell, *Science*, 1996, **271**, 39.
- 3 Notable exceptions: M. T. Krechi, E. D. T. Adkins, A. J. Waddon, M. J. Fournier, T. L. Mason and D. A. Tirrell, *Science*, 1996, **265**, 1427; D. Choo, J. P. Schneider, N. R. Graciani and J. W. Kelly, *Macromolecules*, 1996, **29**, 355.
- 4 Recent reviews: J. P. Schneider and J. W. Kelly, *Chem. Rev.*, 1995, **95**, 2169; G. Tushsherer and M. Mutter, *Pure Appl. Chem.*, 1996, **68**, 2153; S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173.
- 5 Selected examples: D. S. Kemp and B. R. Bowen, *Tetrahedron Lett.*, 1988, **29**, 5077; A. B. Smith III, M. C. Guzman, P. A. Sprengeler, T. P. Keenan, R. C. Holcomb, J. L. Wood, P. J. Carroll and R. Hirschmann, *J. Am. Chem. Soc.*, 1994, **37**, 215; J. P. Schneider and J. W. Kelly, *J. Am. Chem. Soc.*, 1995, **117**, 2533; J. S. Nowick, S. Mahrus, E. M. Smith and J. W. Ziller, *J. Am. Chem. Soc.*, 1996, **118**, 1066.
- 6 T. Kawamoto, O. Prakash, A. L. Rheingold, B. A. Ostrander, A. L. Rheingold and A. S. Borovik, *Inorg. Chem.*, 1995, **34**, 4294; T. Kawamoto, B. S. Hammes, B. Haggerty, G. P. A. Yap, A. L. Rheingold and A. S. Borovik, *J. Am. Chem. Soc.*, 1996, **118**, 285; T. Kawamoto, B. S. Hammes, R. Ostrander, A. L. Rheingold and A. S. Borovik, *Inorg. Chem.*, 1998, **37**, 3424; Q. Yu, T. E. Baroni, L. Liable-Sands, A. L. Rheingold and A. S. Borovik, *Tetrahedron Lett.*, 1998, **39**, 6831.
- 7 Y. Hamuro, S. J. Geib and A. H. Hamilton, *Angew. Chem., Int., Ed. Engl.*, 1994, **33**, 446; Y. Hamuro, S. J. Geib and A. H. Hamilton, *J. Am. Chem. Soc.*, 1996, **118**, 7529; Y. Hamuro, S. J. Geib and A. H. Hamilton, *J. Am. Chem. Soc.*, 1997, **119**, 10 587.
- 8 P. Y. Chou and G. D. Fasman, *Biochemistry*, 1973, **12**, 211.
- 9 C. H. Kim and J. M. Berg, *Nature*, 1993, **362**, 267; D. L. Minor Jr. and P. S. Kim, *Nature*, 1994, **367**, 660; C. K. Smith, J. M. Withka and L. Regan, *Biochemistry*, 1994, **33**, 5510; D. L. Minor Jr. and P. S. Kim, *Nature*, 1994, **371**, 264.
- 10 M. Bodansky and A. Bodansky, *The Practice of Peptide Synthesis*, Springer-Verlag, New York, 1994.
- 11 The chemical shift of related protons in 2,6-carbamoylbenzene are at ~ 7.0 ppm.
- 12 See A. B. Kudryavtsev and W. Linert, *Physico-Chemical Applications of NMR*, World Scientific Publishing, Singapore, 1996, pp. 165–178.
- 13 An intermolecular C–H \cdots O hydrogen bond is also observed between $2(\text{val,val})$ and the CHCl_3 solvate [$\text{C}(30)\cdots\text{O}(2)$ distance is 3.206(15) Å]. Typical C–H \cdots O hydrogen bond distances are ~ 3.4 Å: H.-B. Bürgi and J. D. Dunitz, *Structure Correlation*, VCH, New York, 1994.
- 14 T. E. Creighton, *Proteins-Structures and Molecular Principles*, Freeman, New York, 1984, pp. 221–225.
- 15 The intermolecular H-bonds between molecules in the lattice of $2(\text{val,ala})\cdot\text{H}_2\text{O}$ are at distances of 2.954(7) and 3.307(6) Å for the $\text{N}(3)\text{--H}\cdots\text{O}(2)$ and $\text{C}(3)\text{--H}\cdots\text{O}(3)$ interactions.
- 16 For the influence of hydrophobic interactions in stabilizing sheet structures in proteins and synthetic systems see ref. 4 and B. Hazes and W. G. J. Hol, *Proteins: Struct. Funct. Genet.*, 1992, **12**, 278; Y. K. Tsang, H. Diaz, N. Graciani and J. W. Kelly, *J. Am. Chem. Soc.*, 1996, **116**, 3988; D. Neri, M. Billeter, G. Wider and K. Wüthrich, *Science*, 1992, **257**, 1559.