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Participation of aromatic side chains in diketopiperazine ensembles

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ABSTRACT

This study probes the beneficial role of aromatic side chains in peptide self-assembly by choosing four diketopiperazine model systems variably composed of glycine, proline, phenylalanine, and tryptophan residues.

The self-assembly process is governed by synergistic participation of multiple non-covalent interactions.[1] Of the various possibilities, hydrophobic aromatic interactions are known to be the crucial determinants of stability of many bioinspired self-assembled systems. The significance of these interactions is reflected in nucleic acid structure stabilization, ligand-receptor interaction, and protein structure stabilization.[2] The aromatic interactions represent a combination of non-covalent contacts which include electrostatic, hydrophobic, and van der Waals' interactions.[3]

In this connection, formation of peptide and protein fibers and aggregates by invoking favorable hydrophobic interactions is an area of contemporary interest.[4] Within the context of protein/peptide nanotechnology, aromatic side chains support self-assembled structures from short peptides[5] and remarkably, even aromatic dipeptides afford ordered nanostructures where the aromatic interactions are implicated in providing the order and directionality needed for the self-assembly process.[6,7]

Diketopiperazines (DKPs) are interesting model systems for studying the self-assembly process as they afford a variety of structures including entangled and elongated aggregates such as rods, ribbons, helices, and tubules.[8] It is possible that these structures may be modulated by varying the substituents on the DKP ring. This study involves the synthesis of four synthetic DKPs: *cyclo*-(Gly-Gly) **1**, *cyclo*-(Trp-Pro) **2**, *cyclo*-(Trp-Trp) **3**, and *cyclo*-(Phe-Phe) **4** (Fig. 1), and an investigation of the gross morphology of self-assembled structures on different surfaces.[9]

Interestingly, atomic force microscopy (AFM) investigation of the four samples (**1**–**4**; 0.3 mM in 50% aqueous methanol) afforded diverse morphologies when studied on highly ordered pyrolytic graphite (HOPG) surface. A fresh sample of **1** displayed a cross-linked mesh-like network with a pore-size of 50–250 nm (Fig. 2a). In contrast, compound **2** lacked an ordered structure, while **3** displayed the formation of fibers and **4** exhibited a tape-like morphology (Fig. 2b–d). DKPs **3** and **4** afforded similar morphology on a more hydrophilic mica surface where we observed fibrous networks and closely packed fibers leading to filamentous aggregates (Fig. 2e and f).

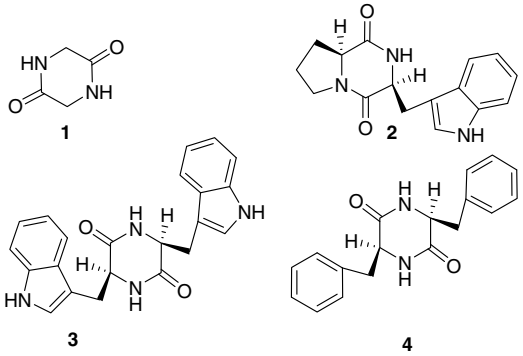

Figure 1. Molecular structures of: (**1**) *cyclo*-(Gly-Gly); (**2**) *cyclo*-(Trp-Pro); (**3**) *cyclo*-(Trp-Trp), and (**4**) *cyclo*-(Phe-Phe).

Such dichotomy in morphologies provided us with the impetus to probe the reasons responsible for such effects. In fact, the effect of surface characteristics on the growth of peptide nanofilaments has been reported recently.[10]

It appears that the mesh-like structure for unsubstituted DKP **1** results from a discrete molecular cross-linked network derived from a hydrogen bonding-mediated assembly. DKP **2** has a lopsided structure which contains a puckered proline ring and an aromatic indole skeleton. It has been proposed that **2** exists in two different conformations,[9c] in which the diketopiperazine ring exists in a typical boat conformation. DKP **3** possesses two aromatic groups suggesting that π–π stacking is a dominant factor behind self-organization. Such a possibility is further confirmed in the solid state structure of **3** which exhibits aromatic interactions (van der Waals, hydrophobic, and electrostatic forces) between the indole rings. Akin to nanotubular structures formed by Phe-Phe, DKP **4** also reveals the formation of tubular structures, possibly due to favorable aromatic interactions between the two phenyl rings, which is further aided by the hydrophobic nature of the HOPG surface.

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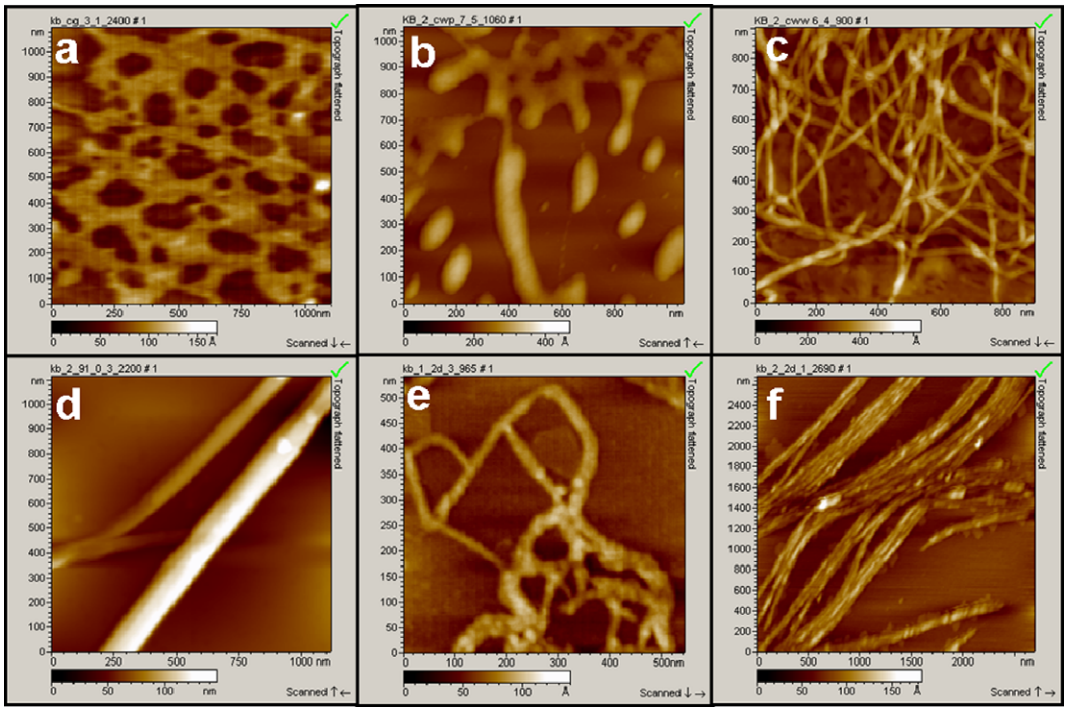

Figure 2. AFM micrographs of fresh samples in 50% aqueous methanol of the self-assembled morphologies of compounds (a) **1**, (b) **2**, (c) **3**, and (d) **4** on HOPG surfaces. Micrographs of (e) **3** and (f) **4** on mica surfaces.

Encouraged by these results, we decided to further confirm the structure of peptide aggregates by scanning electron microscopy (SEM). Interestingly, SEM micrographs confirmed the AFM observations (Fig. 3a and b). A fresh solution of sample **1** in 50% aqueous methanol demonstrated very dense sheet-like fibers (data not shown).

Disordered and unsystematic aggregates of ~1 μm diameter were obtained for a fresh solution of compound **2** (data not shown). A fresh solution of compound **3** afforded fibrillar morphology, with diameter of 100–300 nm (Fig. 3a).

On the other hand, a solution of compound **4** in 50% aqueous methanol showed bundled, straight fibers having diameters of 0.4–1 μm (Fig. 3b). Aging of the solutions of samples **3** and **4** for 2 days at ambient temperature resulted in mature fiber formation (Fig. S1; Supplementary data) confirming a time-dependent aggregation and fibrillation event. A course fibrillar network was observed after aging sample **3** for 2 days (Fig. S1a and c; Supplementary data). However, the aged sample of **4**, showed bundled fiber formation which build up from the packing of small fibers (Fig. S1b and d; Supplementary data).

We further decided to determine the effect of solvent composition on the ultrastructures of the DKP ensembles formed in **3** and **4**.

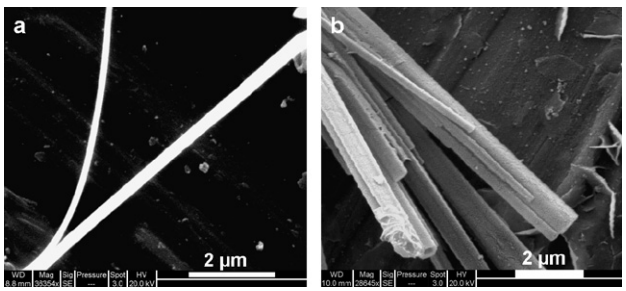

Figure 3. SEM micrographs of fresh samples of self-assembled morphologies of compounds (a) **3** and (b) **4** in 50% aqueous methanol.

Fluorinated solvents are well-known for stabilizing peptide and protein structures.[11] The fiber cross-sections of fresh **3** and **4** in 50% aqueous methanol were ~30 and ~80 nm, respectively, whereas in 50% aqueous 2,2,2-trifluoroethanol (TFE), the fiber cross-section increased up to ~500 and 200 nm for **3** and **4**, respectively, suggesting that the fluorinated solvent induces coalescence in the aggregated ensembles. Interestingly, the structures observed in 50% aqueous methanol were also retained in this solvent giving rise to fiber-like aggregates on the HOPG surface (Fig. 4), suggesting that the overall morphologies displayed by **3** and **4** remain unaffected by a change in solvent composition.

FT-IR measurements were used to probe significant structural features responsible for the self-assembly process.[12] The deconvoluted FT-IR spectra of the amide I and II regions of dipeptides **1**, **2**, **3**, and **4**, are shown in Figure 5. The amide I region of compound **1** contains three absorptions at 1619, 1678, and 1511 cm^{-1}. The bands at 1678 and 1619 cm^{-1} are usually assigned to anti-parallel β-sheets,[12a] while the band at 1511 cm^{-1} is representative of the

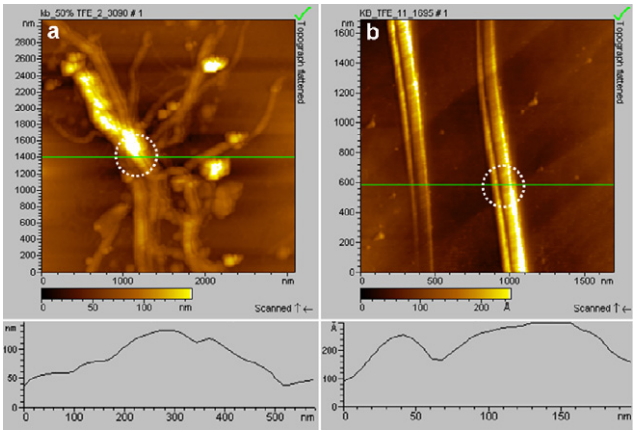

Figure 4. AFM micrographs of samples (a) **3** and (b) **4** in 50% aqueous TFE.

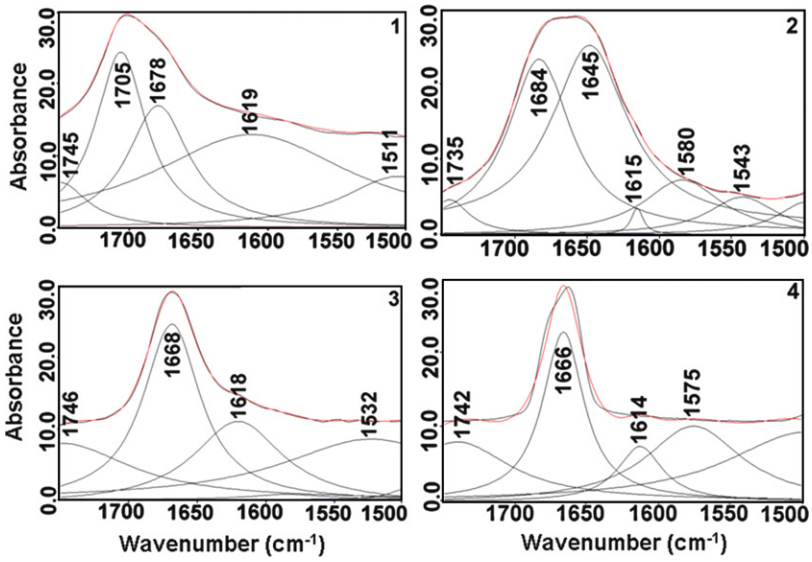

Figure 5. FT-IR deconvolution spectra. Amide I and II regions of compounds **1**, **2**, **3**, and **4**.

presence of a β-sheet conformation. The bands at 1734 cm^{-1} are assigned to C=O stretching vibrations of the free or non-hydrogen bonded groups, while that at 1705 cm^{-1} is due to the bifurcated hydrogen bond or laterally hydrogen-bonded groups.

The amide I region of compound **2** exhibited bands at 1684 and 1615 cm^{-1} due to a β-sheet conformation with the measured components at 1645 cm^{-1} showing some structural randomness in structures. Such structures were further confirmed by a band at 1543 cm^{-1} in the amide II region which could tentatively be assigned to an unordered component. The bands at 1746 cm^{-1} were assigned to C=O stretching vibrations of free or non-hydrogen bonded groups. However, the presence of a band at 1668 cm^{-1} indicates the occurrence of β-turns together with a β-sheet conformation, as represented by a band at 1618 cm^{-1}. Furthermore, the presence of small amount of β conformation was also confirmed by a band at 1532 cm^{-1} for compound **3**. Compound **4** displayed a band at 1742 cm^{-1} due to the C=O stretching vibrations of free or non-hydrogen bonded groups, while a band at 1666 cm^{-1} may be attributed to β-turns with the participation of β-sheet conformations.[12a,13a–d]

DKP supramolecular structures are governed primarily through amide functionalities where they serve as interacting links via hydrogen bonding interactions to reveal numerous structures such as capsules, spheres, channels, helices, ribbons or tapes, rods, sheets or layers, and tubes.[13e–h] The structural implications for the four DKPs **1–4**, on the basis of FT-IR spectra, are summarized in Table 1.

The self-assembled structures were further evaluated to ascertain their thermal stability, with the help of thermogravimetric

Table 1
Structural implications of DKPs **1–4**

		DKP			
DKP		*cyclo*-(Gly-Gly) 1	*cyclo*-(Trp-Pro) 2	*cyclo*-(Trp-Trp) 3	*cyclo*-(Phe-Phe) 4
Frequency of the amide I band in cm^{-1}					
Deconvoluted bands	1678, 1619	1684, 1645, 1615	1668, 1618	1666, 1614	
Secondary structures	β-Sheets	Random structures with β-sheets	β-Turn and β-sheets	β-Turn and β-sheets	

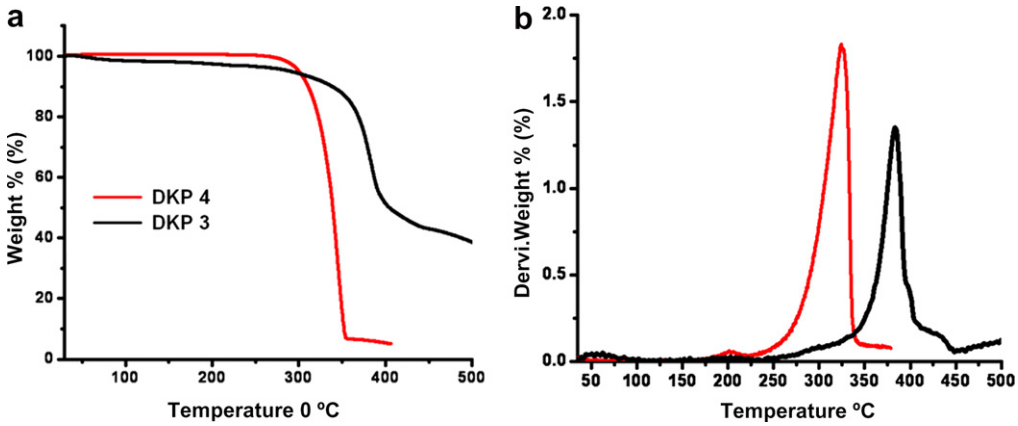

Figure 6. TGA thermograms of (a) DKP **3** (black trace) and DKP **4** fiber (red trace); (b) derivative plots suggesting the higher stability of DKP **3** compared to DKP **4**.

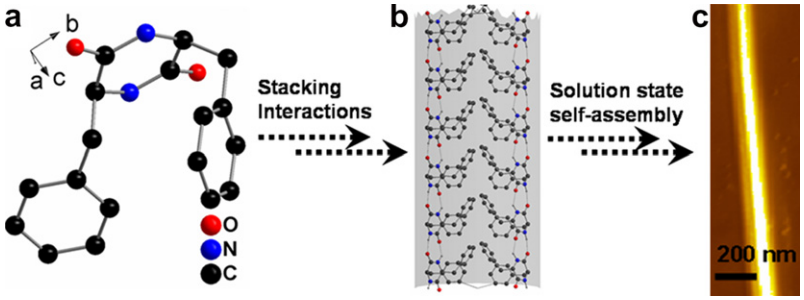

Figure 7. Proposed model for the fiber formation by compound **4** in solution state. (a) A single molecule of *cyclo*-(Phe-Phe), **4**, (b) crystal packing extending along the '*a*' axis, and (c) an AFM micrograph of **4**.

analysis (TGA), by monitoring the loss of weight as a function of increasing temperature for compounds **3** and **4**. Such studies have been reported previously for peptide-based soft structures in order to ascertain their thermal stability.[14] The TGA thermograms of **3** and **4** showed ~2–10% weight-loss going from room temperature to ~300 °C (Fig. 6a). A major decrease in weight was observed above 300 °C for both **3** and **4** suggesting rugged thermal stability of these DKPs perhaps due to the presence of aromatic substituents (Fig. 6). Figure 6b shows major peaks at 326 and 383 °C for the *cyclo*-(Phe-Phe) and *cyclo*-(Trp-Trp) nanotubes, respectively.

Finally, we tried to correlate the solution state self-assembly structures with the available solid state structure. As an example, fiber formation by compound **4** is hypothesized on the basis of its solid state structure.[9d] It is possible that the growth of fibers in **4** invokes π-stacking and hydrogen bonding interactions between planar diketopiperazine rings resulting in a tubular fiber-like morphology as observed by atomic force microscopy (Fig. 7).

In conclusion, this study gives an insight into possible contributions of aromatic amino acid side chains in DKP self-assembly and allows us to propose that beneficial aromatic interactions might dictate formation and stability of diketopiperazine ensembles. It is expected that careful use of such interactions will help us to understand their role as a stabilizing feature in aggregation and as an aid in de novo design of self-assembled structures from peptide-based molecular frameworks.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.156.

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