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Supramolecular double helix from capped γ-peptide†

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The single crystal X-ray diffraction study of capped γ-peptide reveal that the peptide adopts helical conformation which self-assemble to form a supramolecular parallel double helical structure using intermolecular hydrogen bonding as well as π–π stacking interactions in the solid state.

Nature achieves a wide range of double helical structures and functions like information storage, transcription and formation of ion channels from relatively small number of building block such as four nucleobases and twenty amino acids.[1] Chemical synthesis of biopolymer mimics has advantage to play with the wide range of building blocks.[2] Over the past few years, considerable progress has been achieved in the design, synthesis and characterization of artificial oligomers that can wind around one another based on the intrinsic nature of the foldamers.[3] Plenty of intertwined supramolecular duplexes from the abiotic backbone that are assembled by H-bond donor/acceptor sites are known, although most of the reported double helices are DNA analogues and derivatives containing regular base-pairs.[4] A small number of studies are also representative of non-DNA-based hydrogen bonded double helices.[5] Yashima and colleagues have designed and synthesized an amidinium–carboxylate salt bridge *m*-terphenyl derivative by direct double helix-to-double helix transformations using the Pt(II) acetylide complexes as the surrogate linkers and removing the Pt(II) linkers by treatment with iodine, where the supramolecular double helical structure can be stabilized by intermolecular salt bridge formation.[6] Aromatic interactions based on alternating stacking of electron rich 1,5-dialkoxy-naphthalene and electron poor 1,4,5,8-naphthalene-tetracarboxylic diimide systems to stabilize the double helical structure were introduced by Gabriel and Iverson.[7] The principle of π–π and CH–π interactions also exists between oligoresorcinols that self-assemble into double helices in water.[8] Synthetic double-stranded molecules that are held together by noncovalent interactions also have potential application.[9] For example, hydrogen-bonded duplexes obtained from linear oligoamide strands have been used as a template for cross-olefin metathesis.[10] Huc and coworkers have designed helical molecular tapes of aromatic oligoamides[11] to slowly wind around rod-like guests and then to rapidly slide along them, where the winding process requires helix unfolding and refolding, as well as a strict match between helix length and anchor points on the rods.[12]

We have reported that the interstrand side chains–side chains interactions can significantly increase the hybridization constant of the pyridine derived oligoamide foldamer.[13,14] Moreover, the hybridization of pyridine carboxyamide oligomers enhanced dramatically with increasing oligoamide length and terminal Boc protection.[15] Intriguing the information from previous reports we have designed foldamers **1** and **2** incorporating *m*-amino benzoic acid and capped with Boc and *N*,*N*′-dicyclohexylurea. In the solid state the peptide **1** forms an antiparallel hydrogen bonded dimer and peptide **2** dimerizes as a parallel double helix through intermolecular hydrogen bonding and π–π stacking interactions.

The γ-peptides **1** and **2** containing *m*-amino benzoic acid and capped with Boc and *N*,*N*′-dicyclohexylurea have been synthesized by conventional solution-phase methodology, purified, characterized, and studied (Fig. 1). The assumption was that *m*-aminobenzoic acid should impart a helical propensity in the γ-peptides[16] and *N*,*N*′-dicyclohexylurea should enhance intermolecular hydrogen bonding interaction as seen in the other urea derivatives.

In order to understand conformational features of the capped γ-peptides in solution, NMR studies were performed. The concentration dependent ^{1}H NMR study in $CDCl_3$ exhibits significant downfield shift of the amide protons with increasing concentration (Fig. S1, ESI†), which suggest that

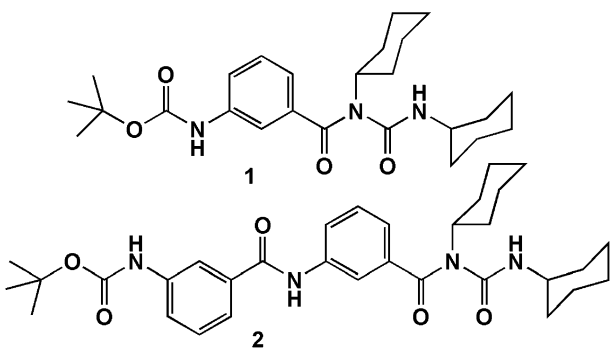

Fig. 1 The schematic presentation of the reported γ-peptides **1** and **2**.

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† Electronic supplementary information (ESI) available: Synthesis and characterization of peptides, ^{1}H NMR, ^{13}C NMR, solid state FTIR spectra, Table S1, Fig. S1–S19. CCDC 832274 and 832275. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c1cc15570a

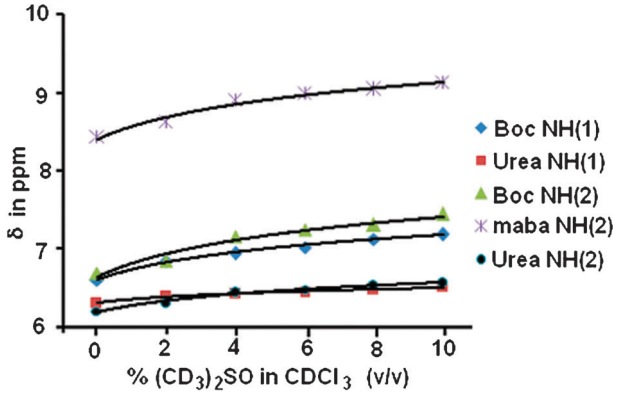

Fig. 2 Plot of solvent dependence of NH chemical shifts of peptides **1** and **2** at varying concentrations of $(CD_3)_2SO$ in $CDCl_3$ solutions.

the NH protons are hydrogen bonded. The upfield shifts of amide protons upon heating of peptide **2** in $CDCl_3$ (Fig. S2, ESI†) suggest that some hydrogen bonds are broken upon increasing temperature. To determine whether the hydrogen bonds are intramolecular or intermolecular, solvent titration experiments have been performed. The effects of adding a hydrogen bond accepting solvent like $(CD_3)_2SO$ to $CDCl_3$ solutions of peptides **1** and **2** are represented in Fig. 2. Generally, addition of small amounts of $(CD_3)_2SO$ in $CDCl_3$ brings about monotonic downfield shifts of exposed NH groups in peptides, leaving solvent-shielded NH groups largely unaffected.[17] Fig. 2 shows that all NHs are solvent exposed as it is evident from their significant chemical shift upon the addition of $(CD_3)_2SO$ in $CDCl_3$ solutions. For both the peptides, urea NH exhibits minimum chemical shift ($\Delta\delta$ 0.20 for peptide **1** and 0.36 for peptide **2**) even at higher percentages of $(CD_3)_2SO$. However, the Boc NH shows maximum chemical shift ($\Delta\delta$ 0.58 for peptide **1** and 0.76 for peptide **2**). Table S1 (ESI†) illustrates $\Delta\delta$ values of all NHs for peptides **1** and **2**. The Maba(2)NH of peptide **2** is also solvent exposed ($\Delta\delta$ 0.70). This demonstrates that peptides **1** and **2** cannot form any intramolecular hydrogen bonded structure in solution.[17] Moreover, the NOESY spectrum at 50 mM concentration exhibits NOE intensities which are responsible for intermolecular interaction between aromatic protons (Fig. S3, ESI†). Other solution phase techniques such as circular dichroism, UV-vis and fluorescence spectroscopy were performed. CD spectrums of peptides **1** and **2** in $CHCl_3$ (Fig. S4, ESI†) have positive bands at 212 nm and 226 nm and a negative band at 220 nm.

The solid state conformations of peptides **1** and **2** have been studied by X-ray crystallography. Colorless triclinic crystals of peptide **1** and colorless orthorhombic crystals of peptide **2** suitable for X-ray diffraction studies were obtained from their chloroform–hexane solutions by slow evaporation.‡ In the solid state, peptide **1** crystallizes with two molecules in the asymmetric unit (Fig. S5a, ESI†). This is a centrosymmetric structure. The two molecules in the asymmetric unit are actually very similar except the orientations of the cyclohexyl groups attached to N2/N5, and when these are removed, a least squares fit is close to perfect (Fig. S5b, ESI†). From the crystal structure of peptide **1**, it is evident that there are two intermolecular hydrogen bonds (N3–H3···O4, 2.12 Å, 2.97(7) Å, 172° and N6–H6···O8, 2.05 Å, 2.89(7) Å, 167°) between urea

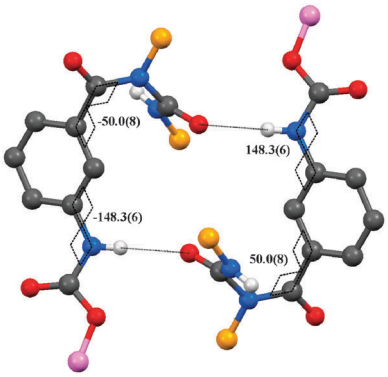

Fig. 3 Crystal structure of intermolecular hydrogen bonded (dotted lines) antiparallel dimers obtained from peptide **1**. Cyclohexyl groups here appear as orange spheres and *t*-butyl groups as violet spheres. Non-active H atoms have been omitted for clarity. Some torsion angles are included.

C═O and *m*-aminobenzoic acid N–H leading to anti-parallel dimers (Fig. 3). These dimers are in turn connected to neighboring dimers through the N1–H1···O7 (2.14 Å, 2.96(6) Å, 160°) and N4–H4···O3 (2.12 Å, 2.97(7) Å, 175°) hydrogen bonds into two-dimensional layers (Fig. S6, ESI†), viewed here along the *a* axis.

However, one molecule of peptide **2** crystallizes with one molecule of chloroform in the asymmetric unit (Fig. S7, ESI†). The torsion angle around the *m*-aminobenzoic acid residues ($\phi1 = -31.3(3)°$, $\psi1 = -28.0(2)°$, $\phi2 = -154.7(16)°$, $\psi2 = 142.4(17)°$) appears to play a critical role in dictating the overall structural features. For peptide **2**, in higher order assembly, a parallel double helix has been observed. Contacts between the main chains of the two strands consist of edge-to-face aromatic stacking and four interstrand N–H···O hydrogen bonds. The duplex is stabilized through intermolecular hydrogen bonding interactions (N1–H1···O3, 2.12(2) Å, 2.92(2) Å, 153(2)°, $1 - x$, y, $1/2 - z$ and N3–H3···O2, 2.35(2) Å, 3.18(2) Å, 163(2)°, $1 - x$, y, $1/2 - z$) between two strands (Fig. 4).

Thus the urea and Boc capping have significant contribution in the duplex stabilization. Unlike other oligoamide double helical foldamers, there is only a π–π interaction between the

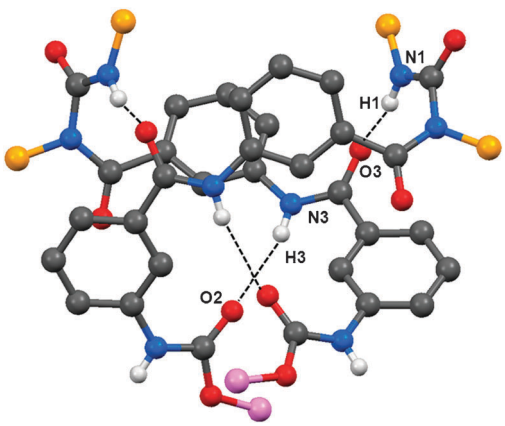

Fig. 4 The inter strand hydrogen bonded double helix of peptide **2**. Hydrogen bonds are shown as dotted lines. Cyclohexyl groups here appear as orange spheres and *t*-butyl groups as violet spheres. Non-active H atoms have been omitted for clarity.

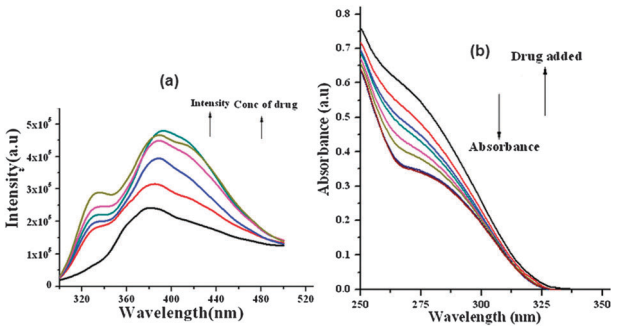

Fig. 5 (a) Fluorescence spectra and (b) UV-vis spectra of peptide **2** with different concentrations of sulfamethoxazole added.

strands. Maba(1) of strand A stacks over Maba(2) of strand B (shortest C–C distance is 3.54 Å) and reciprocally.[18] The two molecules related by proper two-fold rotation symmetry generate a dimer. There is no solvent molecule inside the double helix channel. In higher order packing each double helical structure forms an intermolecular hydrogen bond (N4–H4···O5, 2.01(2) Å, 2.83(2) Å, 161(2)°, $-1/2 + x$, $1/2 + y$, $1/2 - z$) with four other double helices (Fig. S8, ESI†) where $CHCl_3$ molecules are simply filling voids in the crystal lattice.

Moreover, the NMR chemical shift of NH protons with addition of sulfamethoxazole exhibits that peptide **2** interacts with a potent bacteriostatic antibiotic sulfamethoxazole (Fig. S9, ESI†). For further investigation, UV-vis and fluorescence experiments were performed as it is a very sensitive technique to study the changes in microenvironment (Fig. 5). Initially the fluorescence intensity at 375 nm increases with increasing drug concentration. But, after a certain point, a red shift at 395 nm has been observed, which indicates a strong interaction between drug molecules and peptide **2**. The fluorescence of sulfamethoxazole interferes with that of peptide **2**. X-Ray quality crystal of the complex is not obtained.

In summary, we have shown that the ability of helical foldamers to form double helices is not restricted to the aromatic oligoamides but also applies to urea and Boc capped conformationally constrained peptides. The parallel double helix is stabilized by multiple intermolecular hydrogen bonds as well as π–π stacking interaction. The capped γ-peptides can be considered as a new molecular scaffold for supramolecular double helix formation in the solid state. The results presented here may foster new studies for the design of capped γ-peptides leading to cross-hybridization and sequence-selective recognition.

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Notes and references

‡ Crystallographic data: Peptide **1**: $C_{25}H_{37}N_3O_4$, M_w = 443.58, triclinic, space group $P1$, a = 12.4834(8), b = 13.8403(9), c = 14.9746(10) Å, α = 91.506(4)°, β = 95.452(4)°, γ = 90.632(4)°, V = 2574.4(3) Å^3, Z = 4, d_c = 1.145 Mg m^{-3}, T = 296 K, R_1 = 0.0541 and wR_2 = 0.1700 for 2678 data with $I > 2\sigma(I)$. Peptide **2**: $C_{32}H_{42}N_4O_5$, M_w = 562.70, orthorhombic, space group $Pbcn$, a = 19.0609(13), b = 19.6825(14), c = 18.9838(13) Å, V = 7122.1(9) Å^3, Z = 8, d_c = 1.050 Mg m^{-3}, T = 100 K, R_1 = 0.0540 and wR_2 = 0.1398 for 5064 data with $I > 2\sigma(I)$. Intensity data were collected with MoKα radiation for peptide **1** at 296 K and MoKα radiation for peptide **2** at 100 K using a Bruker APEX-2 CCD diffractometer. Data were processed using the Bruker SAINT package and the structure solution and refinement procedures were performed using SHELX97.[19] For peptide **1** non-hydrogen atoms were refined with anisotropic thermal parameters. For peptide **2**, the non-hydrogen atoms were refined with isotropic thermal parameters due to closely spaced Cl atoms in the solvent molecule. The PLATON/SQUEEZE program[20] was used on the raw data to generate a new dataset that removed the scattering contribution of disordered solvent molecule.

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