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Nanozipper formation in the solid state from a self-assembling tripeptide with a single tryptophan residue

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Abstract—The self-assembly of a terminally protected tripeptide Boc-γ-Abu(1)-Ala(2)-Trp(3)-OMe (γ-Abu = γ-aminobutyric acid) **1** results in the formation of a nanostructured supramolecular zipper through various non-covalent interactions in the crystal in which the indole side-chain of the Trp(3) residue plays a key role via N–H···π interactions.

Various supramolecular structures including helices, sheets and ladders can be obtained through the molecular self-assembly of the corresponding molecular building blocks using various non-covalent interactions[1] and nanostructured material can be obtained from these supramolecular structures using the 'bottom-up' approach.[2] However, the supramolecular nanozipper created from a synthetic tripeptide reported herein represents a new type of nanostructure. A recent study includes the utilization of a biologically important leucine zipper motif[3] attached to a core dendrimer for constructing monodisperse and supramolecular assemblies spanning the range of a nanometer to a micrometer.[4] There are several examples of non-peptide based supramolecular zipper formation in the literature. Hunter and co-workers have demonstrated the formation of double-stranded zipper complexes from an assembly of isophthalic acid and a bisaniline derivative through hydrogen bonding interactions in solution.[5] In de novo designed synthetic proteins, alanine zipper[6] and isoleucine zipper[7] structures are found and they exhibit native-like structural properties. Liu and co-workers have engineered a 'Trp-Zipper' protein containing tryptophan residues at the first and fourth positions of the heptad repeat of an α–helical coiled-coil protein. This engineered Trp-Zipper protein forms a stable α-helical pentamer at physiological pH in aqueous medium.[8] However, to the best of our knowledge, there is no example of a small self-assembling peptide (tripeptide) containing a single Trp residue that forms a nanozipper in the solid state. Here, we demonstrate the formation of a supramolecular zipper by self-aggregation of terminally protected linear tripeptide Boc-γ-Abu(1)-Ala(2)-Trp(3)-OMe (γ-Abu = γ-aminobutyric acid) **1** through various non-covalent interactions.

The tripeptide subunit Boc-γ-Abu(1)-Ala(2)-Trp(3)-OMe **1** contains a conformationally flexible γ-aminobutyric acid residue at the N-terminus. γ-Aminobutyric acid is a naturally occurring amino acid found in the mammalian brain[9] where it is enzymatically produced and acts as a neurotransmitter.[10] In our previous work, we have demonstrated that contiguously located γ-Abu and Aib (Aib: α-aminoisobutyric acid) residues provide a folded turn structure whereas, adjacently positioned γ-Abu and Ala residues in a sequence do not form the folded structure, instead forming an extended backbone conformation.[11] Thus, we used the sequence Boc-γ-Abu-Ala-Trp-OMe to observe mainly the role of the Trp-residue in crystal engineering and the γ-Abu-Ala portion to provide an extended backbone structure. The peptide was synthesized by conventional solution phase methodology.[12] The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC–HOBt). The final compound was fully characterized by ^{1}H NMR spectroscopy

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and mass spectrometry. A colourless monoclinic crystal, suitable for single crystal X-ray diffraction study was obtained from methanol–water solution by slow evaporation. The molecular conformation of the peptide **1** in the crystal state is depicted in Figure 1. From X-ray crystallography,[13] it was evident that **1** has an intramolecular N–H···N hydrogen bond[14] (N11–H···N8, H···A 2.43 Å, D···A 2.79(1) Å and D-H···A 106°), in which the tryptophan NH is the donor and the Ala(2) nitrogen is an acceptor (Table 1). Most of the torsional angles, ϕ and ψ values (except $\phi 1$ −113.1(7) and $\psi 1$ 162.1(7)°) of the constituent amino acids residues fall within the helical region of the Ramachandran plot (Table 2). The peptide backbone, containing this five-membered intramolecular N–H···N hydrogen bonded turn structure, then self-assembles through three intermolecular N–H···O hydrogen bonds along the axis parallel to the crystallographic *a* axis to form a columnar supramolecular sheet structure, in which peptide molecules are arranged in a parallel fashion (Fig. 2). Of the three intermolecular hydrogen bonds, two N–H···O hydrogen bonds (N3–H3···O71 and N8–H8···O10) are stronger (N···O 2.97(1), 2.97(1) Å) whereas the N11–H11··· O124 bond is comparatively weak (N···O 3.52(1) Å). These intermolecular hydrogen bonds are formed by exploiting the hydrogen bond capabilities of the γ-Abu(1), Ala(2) and Trp(3) carbonyl oxygen atoms with the γ-Abu(1), Ala(2) and the Trp(3) amide NH groups, respectively. The hydrogen bonding parameters of peptide **1** are listed in Table 1. The individual β-sheet columns are regularly stacked together with an adjacent column through van der Waals' interactions and intermolecular N–H···π hydrogen bonds[15] to form a supramolecular nanozipper along the crystallographic *a* axis (Fig. 3). The internal width of the zipper is about 9.2 Å (0.92 nm), the total width of this zipper (including the widths of both of the arms of the zipper) is 2.378 nm (23.78 Å) and the average length of each tooth of the zipper is 6.2 Å (0.62 nm) (Fig. 3). From Figure 3, it is clear that the indole functional group of the Trp(3) residue plays a key role in zipper formation and acts as an interlocking agent between the two columns of the

Table 1. Intramolecular and intermolecular hydrogen bonding parameters of peptide **1** in the crystal state

D-H···A	H···A/Å	D···A/Å	D-H···A/°
N11–H11···N8	2.43	2.79(1)	106
N3–H3···O71[a]	2.18	2.97(1)	152
N8–H8···O10[a]	2.11	2.97(1)	176
N11–H11···O124[a]	2.71	3.52(1)	156
N21–H21··· C15[b]	2.78	3.54(1)	150
N21–H21···C16[b]	2.63	3.48(1)	176
N21–H21···C17[b]	2.89	3.65(1)	148

[a] Symmetry element $x-1, y, z$.
[b] Symmetry element $3-x, 0.5+y, -1+z$.

Table 2. Selected torsional angles (°) of peptide **1**

Torsional angles	γ-Abu(1)[a]	Ala(2)	Trp(3)
ϕ	−113.1(7)	−73.1(6)	−80.3(7)
ψ	162.1(7)	−29.4(7)	−51.6(8)
ω	−176.0(5)	−166.9(6)	−174.8(5)
θ_1	−53.8(10)	—	—
θ_2	−165.8(7)	—	—

[a] The torsion angles for rotation about the bonds of the peptide backbone: (ϕ,ψ,ω); torsions in the main chain in the N-terminal γ-Abu residue about C^{α}–C^{β} and C^{β}–C^{γ} are termed as θ_2 and θ_1, respectively.

supramolecular zipper. Figure 4 shows that N–H···π hydrogen bonds (Table 1) are formed involving the indole NH of a column with the phenyl ring of Trp(3) of the parallel column along the crystallographic *c* axis. There are close contacts from this N–H to three adjacent carbon atoms in the phenyl ring with H···C distances of 2.63, 2.78 and 2.89 Å and N–H···C angles of 176°, 150° and 148°, Trp residues in proteins are most frequently involved in N–H···π interactions.[16] In this study, N–H···π hydrogen bonds have given additional strength to the formation and stability of this nanozipper system. Liu and co-workers, have shown that the Trp Zipper pentamer forms a specific knobs-into-holes packing of hepted repeats of α-helical coiled-coil protein and in this Trp-Zipper system the main stabilizing interactions are

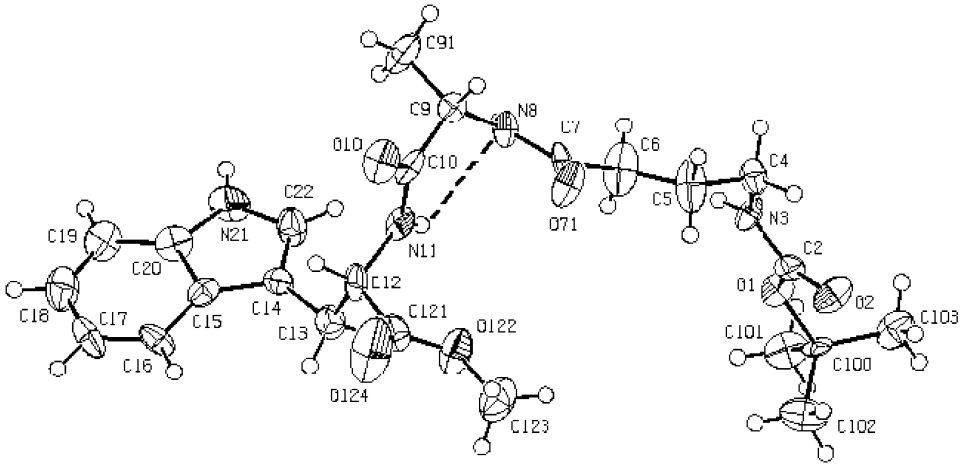

Figure 1. ORTEP diagram with atomic numbering scheme of the peptide **1**. Thermal ellipsoids are shown at 30% probability level. Intramolecular N–H···N hydrogen bonds are shown as dotted lines.

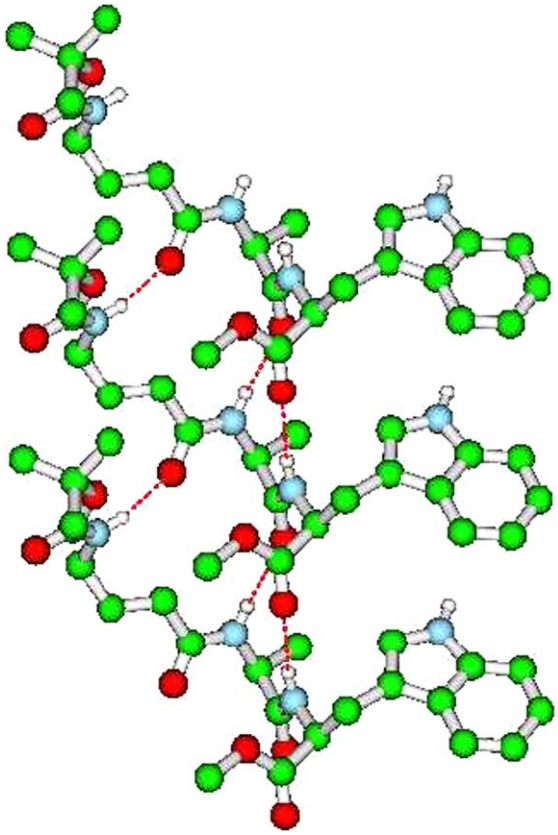

Figure 2. The packing diagram of peptide **1** showing the intermolecular hydrogen-bonded supramolecular columnar sheet structure along the crystallographic *a* axis. Intermolecular N–H···O hydrogen bonds are shown as dotted lines.

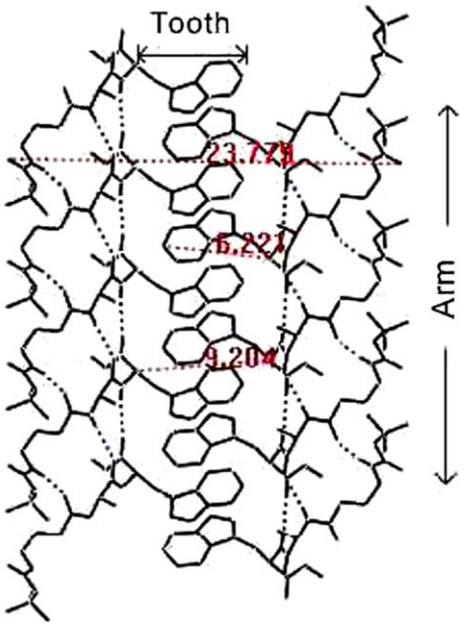

Figure 3. The higher order self-assembly of peptide Boc-γ-Abu-Ala-Trp-OMe (peptide **1**) representing the supramolecular nanozipper structure along crystallographic *c* axis. The important sections of the nanozipper are marked. The width of each tooth of this zipper is 6.22 Å (0.622 nm), the internal width is 9.2 Å (0.92 nm) and the total width (including the widths of both the arms of the zipper on which the teeth are anchored) is 23.78 Å (2.378 nm). The black dotted lines indicate hydrogen bonds.

the C–H···π and N–H···π interactions involving Trp residues.[8] Short synthetic peptides generally self-aggregate in low polarity solvents such as C_6H_6 and $CHCl_3$.[17] However, the peptide **1** does not aggregate in a low polarity deuteriated solvent like $CDCl_3$ (Supplementary data, Fig. 4) and the solvent perturbation experiment shows the solvent shielding nature of the Trp(3)-NH indicating its involvement in intramolecular hydrogen bonding (Supplementary data, Fig. 5a and Fig. 5b).

This report clearly demonstrates the formation of a nanostructured supramolecular zipper in the solid state from a self-assembling tripeptide with a single Trp residue and this Trp residue plays a vital role in nanostructured zipper formation using the N–H···π interaction. To the best of our knowledge this nanozipper is a new nanostructure and the building block of this structure is a tripeptide containing only natural amino acids. The formation of this self-assembling short peptide-based nanozipper may be important for the design and construction of new, bioinspired nano-materials in the future.

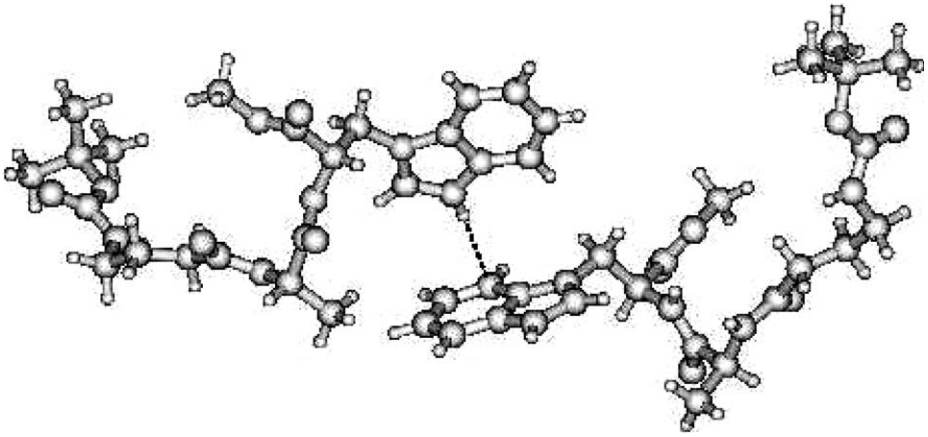

Figure 4. The N–H···π hydrogen bonds between the indole NH of Trp(3) of a molecule peptide **1** with the phenyl ring of Trp(3) of another molecule along the crystallographic *c* axis.

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Supplementary data

The spectral data and crystallographic data for peptide **1** are included in the supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.02.070.

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12. The peptide Boc-γ-Abu(1)-Ala(2)-Trp(3)-OMe ($C_{24}H_{34}N_4O_6$) was synthesized by conventional solution phase methodology (Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer: New York, 1984, pp 1–282). Coupling of Boc-γ-Abu-OH with H-Ala-OMe was followed by saponification yielding the dipeptide acid Boc-γ-Abu-Ala-OH, which was further coupled to H-Trp-OMe using *N*,*N*′-dicyclohexylcarbodiimde (DCC) and 1-hydroxybenzotriazole mediated condensation. The final compound was purified on a silica gel column (100–200 mesh size) using ethyl acetate and toluene mixture (3:1) as eluent. Yield = 2.24 g (4.73 mmol, 86%); δ_H (300 MHz, $CDCl_3$): 8.77 (1H, s); 7.53–7.36 (2H, m); 7.12 (3H, m); 6.69 (1H, d, $J = 8.3$ Hz); 6.09 (1H, d, $J = 7.5$ Hz); 4.87 (1H, m); 4.76 (1H, br); 4.45 (1H, m); 3.71 (3H, s); 3.30 (2H, m); 3.04 (2H, m); 2.09 (2H, m); 1.92 (2H, m); 1.46 (9H, s); 1.30 (3H, d, $J = 6.9$ Hz); IR (KBr): 3397, 3354, 3314, 1724, 1654, 1523 cm^{-1}; $[\alpha]_D^{20}$ −13.4 (*c* 0.55, CH_3OH). Anal. Calcd for $C_{24}H_{34}N_4O_6$ (474) C, 60.76; H, 7.17; N, 11.81. Found: C, 60.52; H, 7.28; N, 11.76. Mass spectral data $(M+H)^+ = 475.4$, $M_{calcd} = 474$.
13. Single crystal X-ray data for peptide **1**: $C_{24}H_{34}N_4O_6$, $M = 474.55$, monoclinic, $a = 5.903(7)$, $b = 8.451(9)$, $c = 25.29(3)$ Å, $\beta = 93.891(10)$, $V = 1259$ Å^3, $T = 293$ K, space group $P2_1$, $Z = 2$, dm = 1.252 Mg m^{-3}. Intensity data were collected with Mo Kα radiation using the MAR research Image Plate System. The crystal was positioned at 70 mm from the Image Plate. One hundred frames were measured at 2° intervals with a counting time of 2 min to give 4292 independent reflections. Data analysis was carried out with the XDS program.[18] The structure was solved using direct methods with the Shelx86 program.[19] The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The structure was refined on F^2 using Shelxl.[20] The final *R* values were *R*1 0.0994 and *wR*2 0.1870 for 3308 data with $I > 2\sigma(I)$. The largest peak and hole in the final difference Fourier were −0.26 and 0.24 e Å^{-3}. The data have been deposited at the Cambridge Crystallographic Data Centre with reference number CCDC 281551.
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