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ARTICLE

Formation of Supramolecular Single and Double Helix-like Structures by Designed Tripeptides

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The conformation and self-assembly of an N- and C- protected tripeptide, Boc-Gly-L-Phg-D-Phe-OMe (**1**, Phg: Phenylglycine) and Boc-Gly-L-Phg-D-Phg-OMe (**2**) have been investigated. The effect of the insertion of two non-coded amino acid residues, L-Phg/D-Phe and L-Phg/D-Phg, consecutively on the structure of two tripepetides has been investigated. The single crystal X-ray diffraction analysis of **1** and **2** suggested that both peptides adopted anti-parallel β-sheet structure but they further self-assembled to form supramolecular single helix and double helix-like architectures, respectively, by various non-covalent interactions in the crystalline state. To the best of our knowledge, this is the first crystallographic report where alternating D/L unnatural amino acid containing small tripeptide exhibited double helix-like architecture. The conformation of these peptides was examined by 2D NMR, solvent dependent NMR titration, and CD spectroscopic studies in solution. Peptide **1** and **2** self-aggregated to form a bunch of flower-petals-like and flower-like architecture, respectively, in an acetonitrile-water medium under field emission scanning electron microscope (FESEM).

Introduction

Formation of various secondary structures, e.g., α-helix, and β-sheet has an essential role in biological system.[1-3] Therefore *de novo* design of helix forming peptide moieties can be useful for biomedical and material chemistry applications. However, design and development of single helical or double helical assembly through peptide or peptidomimetic is a challenging task.[4,5]

With this inspiration, Banerjee and co-workers reported the helical assembly of Aib (α-aminoisobutyric acid) containing tripeptides[6] and the double helical assembly of N-terminal β-alanine containing dipeptides.[7] Haldar *et al.* reported the formation of supramolecular double helix from N-terminal L-tyrosine and central Aib (α-aminoisobutyric acid) containing tripeptides[8] as well as from Boc and N,N'-dicyclohexylurea caped γ-peptide.[9] Dutt Konar *et al.* described the double helical structures of model Aib containing tripeptides[10] and pyridine carboxamide containing tyrosine analog pseudopeptides.[11] Görbitz developed double helical structures from a series of dipeptides belonging to Val-Ala class.[12] Gopi *et al.* reported α/γ[4]-hybrid peptide helices[13] and artificial β-double helices from achiral γ-peptides.[14] Luis and co-works reported the formation of columnar helical assemblies from Val and Phe containing pseudopeptides.[15] Recently, Gazit *et al.* reported a most aggregation-prone tripeptide, Pro-Phe-Phe self-assembled to form a rigid helical-like sheet structure which was confirmed by SC-XRD experiment.[16]

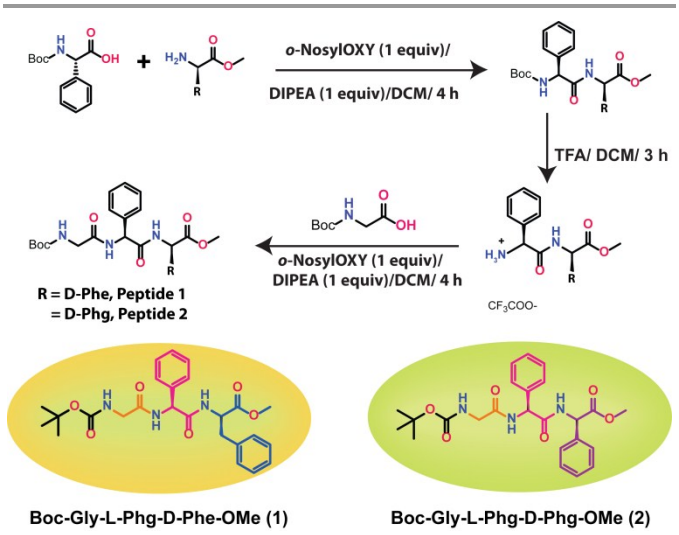

Fig. 1 Peptide synthesis in solution and chemical structures of tripeptides **1** and **2**

Phenylglycine (Phg) is a non-proteinogenic amino acid, which is very similar to Phenylalanine (Phe, proteinogenic) but one methylene (-CH_2-) group less in the side chain than that of Phe. Interestingly, the steric and electronic effect of the phenyl ring in Phg influences the conformation, reactivity, and physical properties of the peptides.[17] Phg containing peptides are used as an antibiotic, e.g., virginiamycin S,[17] streptogramin B[17,18] and pristinamycin I.[17,18] Therefore, to develop new drugs and biologically active materials, Phg is useful. Furthermore, long polypeptides of regularly alternating D/L amino acids usually form channel or cavity through the formation of β-helix, e.g.,

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Gramicidin A, Feglymycin, and Ramoplanin. They are used as antibiotic.[19-21]

Keeping in mind the advantages and structural features of Phg and importance of alternating D/L amino acids in a sequence,[17-21] we designed and synthesized both N- and C-protected tripeptides, Boc-Gly-L-Phg-D-Phe-OMe (**1**) and Boc-Gly-L-Phg-D-Phg-OMe (**2**) (Fig. 1).

Recently, we reported the crystal structures of two tripeptides, Boc-Gly-L-Phe-L-Phe-OMe and Boc-Gly-L-Phg-L-Phe-OMe. They adopted a rare novel open turn and parallel β-sheet structures, respectively.[22] The structural differences observed between above two reported peptides were probably due to the presence of the non-proteinogenic amino acid L-phenylglycine. Therefore in the present study, we kept the N-terminal dipeptide glycylphenylglycine (-Gly-L-Phg-) unaltered but changed the next C-terminal amino acid to D-Phe (**1**) and D-Phg (**2**), respectively, and wanted to observe the effect of the reversion of chirality.

Thus, we investigated the conformation and self-assembly of the above-mentioned alternating D/L peptides in the solid state as well as in a solution. Interestingly, SC-XRD revealed that **1** formed an antiparallel β-sheet structure which further self-assembled through non-covalent interactions to form helix-like architecture in the crystalline state. Whereas, peptide **2** adopted an antiparallel β-sheet structure which further self-associated to form supramolecular double helix-like architecture. To the best of our knowledge, this is the first crystallographic report where alternating D/L unnatural amino acid containing small tripeptide exhibited double helix-like architecture.

At first, peptides were synthesized by using standard conventional coupling method (Fig. 1) in solution[23,24] and purified by column chromatography and then characterized by mass spectrometry and NMR spectroscopy.

The conformation and self-assembly of the synthesized peptides were studied by single crystal X-ray diffraction experiments in the solid state. Colorless block-shaped crystal (Fig. S1, ESI) suitable for X-ray diffraction were matured from the acetonitrile-water medium by slow evaporation at room temperature. The obtained triclinic crystal of **1** and monoclinic crystal of **2** contained space group *P1* and *C2,* respectively. Peptide **1** showed two molecules (**A** in orange and **B** in green color, Fig. 2a) in the asymmetric unit. The conformation of **1** in the crystalline state showed that it had extended backbone conformation. The torsion angles are listed in Table 1. Molecule **A** and **B** of **1** were interconnected through intermolecular H-bond to form an antiparallel β-sheet arrangement along *a* direction (Fig. 2b). Each subunit of **1** was further stabilized by two C-H…π (2.83 and 2.75 Å between aromatic H of L-Phg of **B** and sp^2 C of L-Phg of **A**) and three C-H…O (2.57 Å between βH of D-Phe of **A** and urethane carbonyl oxygen of **B**; 2.70 Å between aromatic H of D-Phe of **B** and urethane carbonyl oxygen of **A**; 2.43 Å between αH of Gly of **B** and amide oxygen of L-Phg of **A**) interactions (Fig. 2c). It was further self-assembled in higher-order packing along crystallographic *a*-direction (Fig. 2d). The structure was formed through the interaction of C-H…π (average CH-π distance 2.83 Å, the aromatic C-H of D-Phe of one particular **B** molecule and aromatic π of D-Phe of **A** molecule, Fig. 2e) and C-H…O (average CH…O distance 2.70 Å, the aromatic C-H of D-Phe of that particular **B** molecule and the C=O of Boc of another **A** molecule). Similarly, two **B** molecules were stabilized by similar interactions with a nearby **A** molecule (Fig. 2f). The backbone orientation of two consecutive **B** molecule of **1** along *b* axis appeared as a helix-like stretch (Fig. 2g). The molecular packing along *b* direction also exhibited a helix-like structure (Fig. 2f).

From Table S1 (ESI), it was observed that the torsion angles of previously reported consecutive L/L amino acids containing tripeptide, Boc-Gly-L-Phg-L-Phe-OMe[22] were different from consecutive D/L amino acid containing tripeptide Boc-Gly-L-Phg-D-Phe-OMe (**1**). As a result, their conformations were also different in the crystalline state, as expected.

On the other hand, peptide **2** exhibited one molecule in the asymmetric unit, and they were interconnected to form antiparallel β-sheet through intermolecular H-bonding along *a* axis (Fig. 3b). Each subunit of **2** was further stabilized by two C-H…π (2.76 and 2.73 Å) and two C-H…O (2.61 and 2.45 Å) interactions (Fig. 3c). Interestingly, it self-assembled to form supramolecular double helix-like architecture with a helical pitch of 18.53 Å in higher-order packing along the *b* axis (Fig. 3d and 3e). This double helix was further stabilized through C-H…π interaction with average distance (CH-π) 2.79 Å and C-H…O (2.40 Å) interactions.

Table 1: The backbone torsion angles (deg) of **1** and **2**

Torsion angles	Boc-Gly-L-Phg-D-Phe-OMe (1)		Boc-Gly-L-Phg-D-Phg-OMe (2)
	Molecule A	Molecule B	
ϕ1	C5-N1-C6-C7= -59.8(4)	C30-N4-C31-C32= 155.7(3)	C5-N1-C6-C7= 172.9(3)
ϕ2	C7-N2-C8-C15= -141.4(3)	C32-N5-C33-C40= -128.7(3)	C7-N2-C8-C15= -138.0(3)
ϕ3	C15-N3-C16-C24= 141.1(3)	C40-N6-C41-C49= 133.6(3)	C15-N3-C16-C23= 142.3(3)
ψ1	N1-C6-C7-N2= 150.1(3)	N4-C31-C32-N5= -143.2(3)	N1-C6-C7-N2= -175.1(3)
ψ2	N2-C8-C15-N3= 112.7(3)	N5-C33-C40-N6= 140.9(3)	N2-C8-C15-N3= 141.3(3)
ψ3	N3-C16-C24-O6= 8.0(5)	N6-C41-C49-O12= -109.7(3)	N3-C16-C23-O25= 18.1(4)
ω1	O1-C5-N1-C6= 165.2(3)	O7-C30-N4-C31= 175(3)	O1-C5-N1-C6= -166.4(3)
ω2	C6-C7-N2-C8= -179.9(3)	C31-C32-N5-C33= -174.4(3)	C6-C7-N2-C8= 179.4(3)
ω3	C8-C15-N3-C16= -175.4(3)	C33-C40-N6-C41= 172(3)	C8-C15-N3-C16= -175.3(3)

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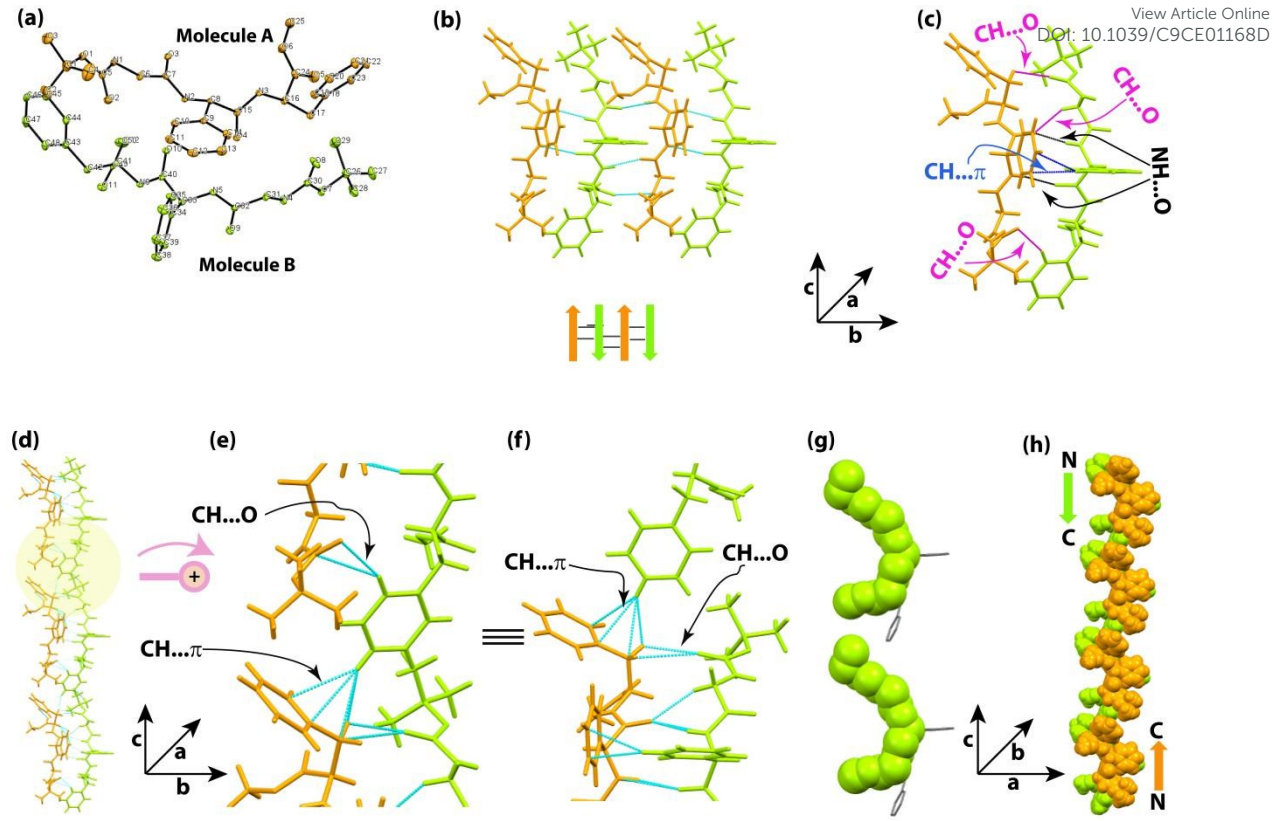

Fig. 2 (a) The ORTEP diagram with ellipsoid of 30% probability, (b) antiparallel β-sheet along *a* axis, (c) non-covalent interactions, (d) higher order assembly along *a* axis, (e) two **A** molecules are held by one **B** molecule, (f) two **B** molecules are held by one **A** molecule, (g) a partial spacefill representation of helical species of **B** molecules, and (f) spacefill representation of helix-like structure in molecular packing along *b* axis, of peptide **1**

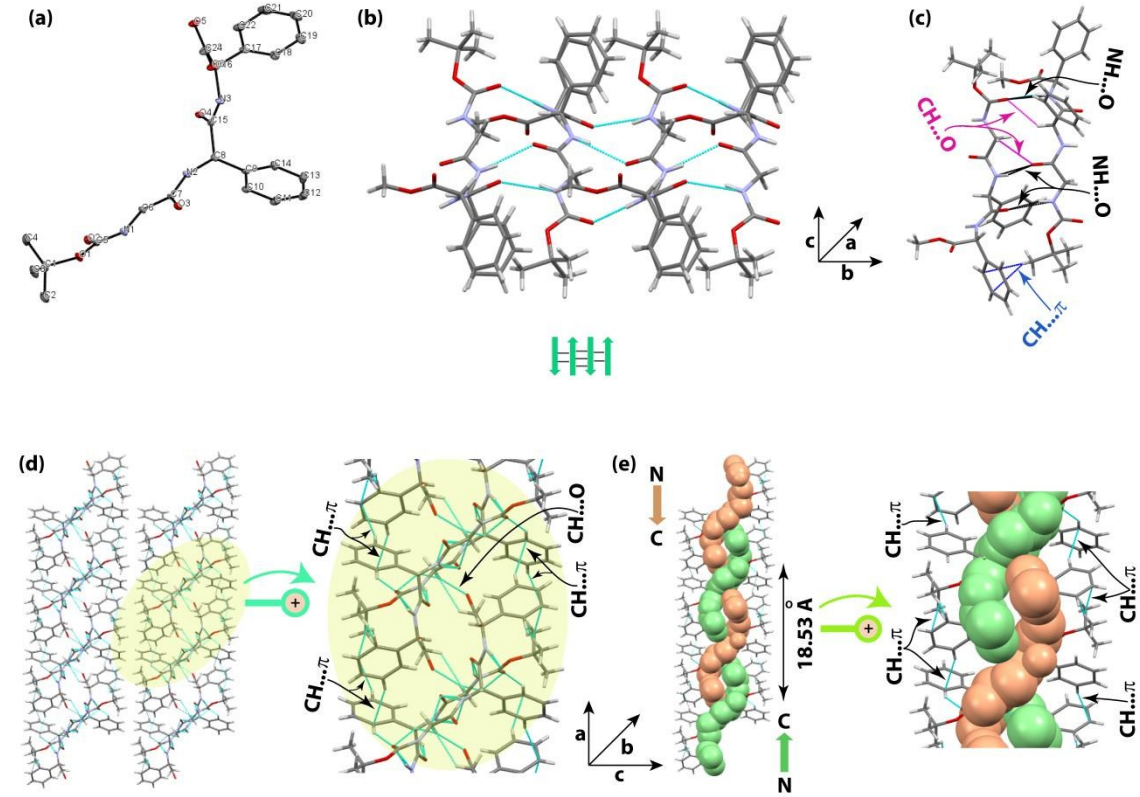

Fig. 3 (a)The ORTEP diagram with ellipsoid of 30% probability, (b) antiparallel β-sheet along *a* axis, (c) non-covalent interactions, (d) double helix-like structure in higher order along the *b* axis, and (e) spacefill representation of double helix-like structure along the *b* axis, of peptide **2**

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Table 2: Crystal Parameters and Refinement Data of **1** and **2**

Parameters	Boc-Gly-L-Phg-D-Phe-OMe (**1**)	Boc-Gly-L-Phg-D-Phg-OMe (**2**)
Formula	$C_{25}H_{31}N_3O_6$	$C_{24}H_{29}N_3O_6$
Fw	469.53	455.50
Crystal system	Triclinic	monoclinic
Space group	*P 1*	*C 2*
a/Å	8.3294(6)	18.5349(8)
b/Å	9.6074(7)	8.8497(3)
c/Å	16.4130(11)	14.3748(5)
α/°	86.861(5)	90
β/°	84.758(5)	91.092(4)
γ/°	74.072(6)	90
V/Å^3	1257.12(16)	2357.45(15)
Z	2	4
D_c/g cm^{-3}	1.240	1.283
μ Mo K_α/mm^{-1}	0.089	0.093
F000	500.0	968.0
T/K	100.00(10)	100.00(10)
θ max.	28.823	28.815
Total no. of reflections	9785	5011
Independent reflections	6889	4110
Observed reflections	6025	3677
Parameters refined	621	302
R_1, I > 2σ(I)	0.0544	0.0546
wR_2, I > 2σ(I)	0.1522	0.1562
GOF (F^2)	0.787	0.817
CCDC No.	1874425	1896847

Two subunits formed a dimer connected by two-fold rotational symmetry. No water molecule or solvent was observed inside the double helix. Interestingly, long chain polypeptides having alternating D/L amino acids usually exhibit β-helix conformation.[25,26] The observed torsion angles of **1** and **2** were quite similar to the β-helix peptides (Table S1). All H-bond distances, bond angles, and crystallographic data are displayed in Table S2 (ESI) and Table 2.

Next, we compared the molecular structures of our previously reported L/L amino acid containing peptides Boc-Gly-L-Phe-L-Phe-OMe (**A**) and Boc-Gly-L-Phg-L-Phe-OMe (**B**)[22] and the newly designed alternating L/D amino acid containing peptides Boc-Gly-L-Phg-D-Phe-OMe (**1**) and Boc-Gly-L-Phg-D-Phg-OMe (**2**). In **A**, phenyl ring is connected through a methylene group, i.e. the ring is not directly attached to Cα of the central L-Phe. As a result, the Cα became flexible and backbone oriented to form an open turn (Fig. 4a) structure.[22] Whereas in **B**, a steric repulsion arises between Cα proton and aromatic *ortho* proton of the central L-Phg, therefore the backbone was extended, and it adopted parallel β-sheet (Fig. 4b) structure.[22]

Peptides **1** and **2** also contained L-Phg at the central position of their sequence. Therefore, similar steric repulsion is present between Cα and *ortho* hydrogens of L-Phg, and it causes backbone extension. Moreover, in the C-terminus, both **1** and **2** contained D-Phe and D-Phg, respectively. The consecutive existence of L and D amino acid forces their side chains to flank in the same direction, which caused more backbone elongation than the L/L amino acid containing peptides. The backbone elongation is more in **2** for the presence of C-terminal D-Phg (the aromatic ring is directly attached to Cα) than **1** (Fig. 4c and 4d).

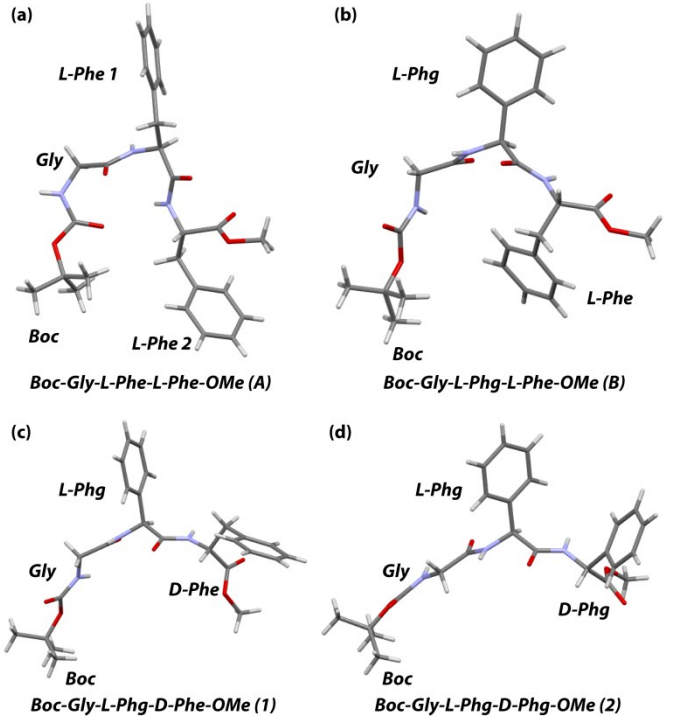

Fig.4 Comparison of the molecular structures between L/L amino acid containing tripeptides (a) Boc-Gly-L-Phe-L-Phe-OMe (b) Boc-Gly-L-Phg-L-Phe-OMe and L/D amino acid containing tripeptides (c) Boc-Gly-L-Phg-D-Phe-OMe (c) Boc-Gly-L-Phg-D-Phg-OMe.

Next, we performed CD experiments to know the conformation of these peptides in solution. After seven days, the solutions (1.5 mM) were diluted to 300 μM (to avoid high voltage in CD), and from them 400 μL of each were taken separately in a quartz cuvette, and CD was measured spanning the wavelength range between 190-260 nm (1 mm path length and 1 nm bandwidth). The CD profile of peptide **1** exhibited a positive Cotton effect at 202 nm and a negative Cotton effect at 194 nm, indicating the presence of a turn-like structure (Fig. 5) in solution.[27,28] Whereas peptide **2** showed a negative Cotton effect at 223 nm and a positive Cotton effect at 198 nm, indicating the presence of a β-sheet conformation.[29]

Then, we performed the solvent dependent NMR titration studies to understand the existence of intra- or inter-molecular H-bond in these peptides. These experiments were carried out by adding d_6-DMSO to the peptide solution in non-polar $CDCl_3$. The obtained solvent titration curve indicated that increasing the percentage of d_6-DMSO in $CDCl_3$ solution (v/v) from 0 to 20 % for **1** and 0 to 12% for **2**, a monotonic

downfield chemical shift of NHs was observed. The net change of chemical shifts (Δδ(NH)) were 0.43 (Gly NH), 0.48 (L-Phg NH), and 0.23 ppm (D-Phe NH) for **1** (ESI, Table S4, and Fig S6), and 0.38 (Gly NH), 0.35 (L-Phg NH), and 0.57 ppm (D-Phg NH) for **2**, respectively (ESI, Table S6, and Fig S14), indicating all NHs were involved in intermolecular H-bonding interactions,[22] which was also supported by the SC-XRD experiment.

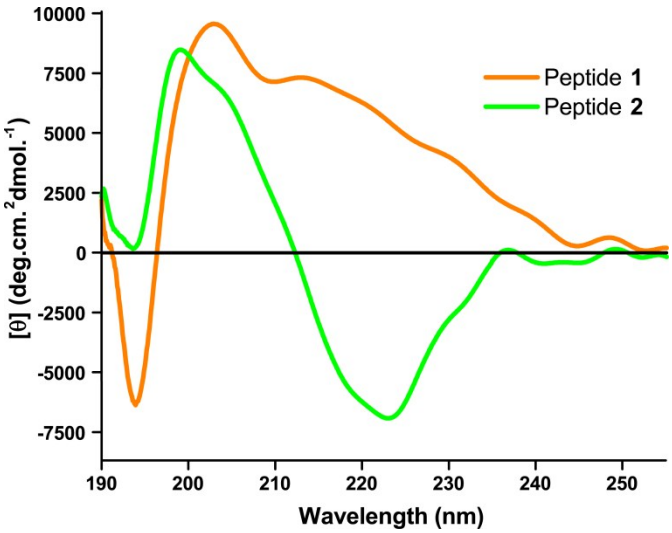

Fig.5 CD spectra of peptide **1** (orange curve) and peptide **2** (green curve) in 30% acetonitrile-water using concentration 300 μM.

Next, the 2D NOESY experiment was performed by using $CDCl_3$ with mixing time 0.60 sec to investigate the existence of inter-residual NOEs in the molecule. The two crucial inter-residual NOE interactions, namely NH Phg ↔ H_α Gly and NH Phe ↔ H_α Phg and others NOEs e.g. H_α Phg ↔ aromatic protons of Phg, NH Phe ↔ *meta* aromatic protons of Phe, NH Phe ↔ aromatic protons of Phg were observed (ESI, Fig. S9), indicating turn like structure present in the solution which was also supported by CD experiment. The results of the CD and NMR experiments corroborated well with the structures observed by SC-XRD. However, the conformation of the peptides usually changes in the solution. On the other hand, no effective NOE interactions were observed for **2** (ESI, Fig. S17).

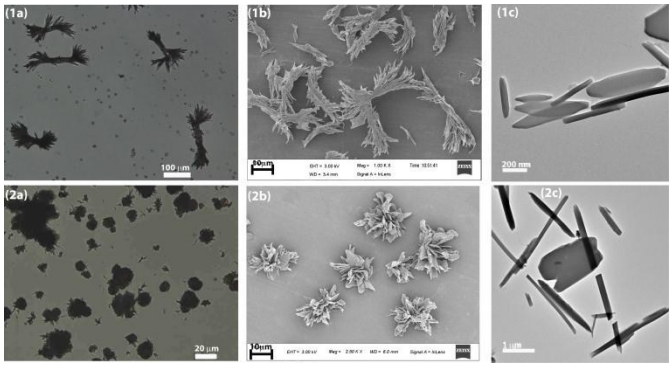

Fig. 6 Optical microscopic, FESEM and TEM images of self-associated tripeptides (1a), (1b) and (1c) of 1 and (2a), (2b) and (2c) of 2, respectively, in 30% acetonitrile-water

Next, to understand the aggregation behavior of these peptides, we checked their morphology. Both, optical microscope and FESEM images indicated that **1** and **2** self-associated in solution to form highly organized a bunch of flower-petals-like (Fig. 6) and a flower-like architecture (Fig. 6), respectively. Then we performed TEM for getting more detail information on the morphology of the self-associated peptides, and rod-like microcrystals of various sizes were observed (Fig. 6). These results indicated that the bunch of flower-like structures which were obtained from FESEM might have been constructed from the self-association of the rod-like microcrystal flakes which were observed under TEM. The rod-like microcrystals, in turn, may have been formed by the accumulation of the single helix and double helix-like structure that was observed by SC-XRD experiments.

Experimental

General Information.

All unprotected and Boc N-protected amino acids, Oxyma and 2-nitrobenzenesulfonyl chloride were obtained from GL Biochem (China). N, N-Diisopropylethylamine (DIPEA), dichloromethane (extra pure grade), acetonitrile (HPLC grade) and trifluoroacetic acid (TFA) were collected from Spectrochem (India). Citric acid, sodium bicarbonate ($NaHCO_3$), ethyl acetate, hexane, and DMSO-d_6 were obtained from Merck (India). $CDCl_3$ was purchased from Sigma Aldrich.

Peptide synthesis

At first, N-terminal t-butyloxycarbonyl (Boc) protected amino acid (1 equiv), coupling reagent, o-NosylOXY (1 equiv), Hünig's base DIPEA (1 equiv) in dichloromethane (DCM) were taken in a 50 mL round-bottom flask (RB) and the reaction mixture stirred with magnetic stirrer bar for 5 min for preactivation. At the same time methyl ester of the next amino acid (1.2 equiv) with DIPEA (1.2 equiv) in DCM was taken in a 25 mL beaker for neutralization. Then, this slightly basic solution was added dropwise to the above reaction vessel and kept stirring for 4-5 h at room temperature. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, the reaction mixture was diluted with DCM and washed with a 10% citric acid solution followed by the saturated $NaHCO_3$ solution 3 times, in each case. Then, the organic layer was dried over anhydrous Na_2SO_4, and the decanted solvent was evaporated under reduce pressure to obtain solid both N- and C- protected dipeptide. Next, this dipeptide was taken in a 50 mL RB and the cleavage cocktail TFA:DCM (90:10) was added onto it. After 3 h reaction, the TFA was evaporated under reduce pressure followed by passing N_2 over it and again neutralized it by adding DIPEA. Then this Boc deprotected dipeptide was coupled with next Boc-N-amino acid, using the procedure as mentioned earlier to get the tripeptide. The desired product was purified by silica gel column chromatography using ethyl acetate-hexane solvent system.

Synthesis of peptide 1

At first, 500 mg (1.992 mmol) of Boc-L-Phg-OH was dissolved in 10 mL DCM, and then 651 mg (1.992 mmol) of *o*-NosylOXY and 257 mg (1.992 mmol) of DIPEA was added to it and kept stirring for 5 min for preactivation. After that, 428 mg (2.390 mmol) of NH_2-D-Phe-OMe was neutralized by 308 mg (2.390 mmol) of DIPEA and added to the above reaction vessel and stirred for 4 h at room temperature. After completion of the reaction, work up the reaction mixture and the obtained solid dipeptide, Boc-L-Phg-D-Phe-OMe was treated with TFA to remove the Boc protecting group. Then, the free amine of H_2N-L-Phg-D-Phe-OMe was coupled with Boc-Gly-OH for 4 h. After purification by column chromatography, we obtained a white solid of Boc-Gly-L-Phg-D-Phe-OMe (**1**). The purity of the peptides was confirmed using analytical HPLC. The isolated peptides were characterized by mass spectrometry as well as 1D [^{1}H] (^{1}H and ^{13}C) and 2D (COSY, TOCSY, and NOESY) NMR spectroscopy (Fig. S2-S9).

White solid; mp 171-173 ^{o}C**.** ^{1}H NMR ($CDCl_3$; 400 MHz) δ 1.43 (9H, s); 2.97-2.95 (2H, d, *J* = 6 Hz); 3.70 (3H, s); 3.81 (2H, s); 4.90-4.85 (1H, q, *J* = 6 Hz, *J* = 8 Hz); 5.24 (1H, brs); 5.50 (1H, brs); 6.55 (1H, brs); 6.68-6.66 (2H, d, *J* = 7.2 Hz), 7.07-7.03 (2H, t, *J* = 7.2 Hz); 7.14-7.11 (1H, t, *J* = 7.2 Hz); 7.33-7.29 (5H, m); 7.41 (1H, brs). ^{13}C NMR ($CDCl_3$; 100 MHz) δ 28.4, 37.8, 44.3, 52.6, 53.3, 57.1, 80.3, 127.1, 127.4, 128.6, 129.2, 129.3, 129.4, 135.4, 137.6, 156.2, 169.2, 169.4, 171.7. HRMS (ESI): calculated $[M+H]^+$ 470.2213, found m/z . 470.4121. HPLC: retention time (t_R) = 11.9 min. Isolated pure product 689 mg (yield: 74% w.r.t. starting meterial Boc-L-Phg).

Synthesis of peptide 2

At first, 500 mg (1.992 mmol) of Boc-L-Phg-OH was dissolved in 10 mL DCM, and then 651 mg (1.992 mmol) of *o*-NosylOXY and 257 mg (1.992 mmol) of DIPEA was added to it and kept stirring for 5 min for preactivation. After that, 394 mg (2.390 mmol) of NH_2-D-Phg-OMe was neutralized by 308 mg (2.390 mmol) of DIPEA and added to the above reaction vessel and stirred for 4 h at room temperature. After completion of the reaction, work up the reaction mixture and the obtained solid dipeptide, Boc-L-Phg-D-Phg-OMe was treated with TFA to remove the Boc protecting group. Then, the free amine of H_2N-L-Phg-D-Phg-OMe was coupled with Boc-Gly-OH for 4 h. After purification by column chromatography, we obtained a white solid of Boc-Gly-L-Phg-D-Phg-OMe (**2**). The purity of the peptides was confirmed using analytical HPLC. The isolated peptides were characterized by mass spectrometry as well as 1D [^{1}H] (^{1}H and ^{13}C) and 2D (COSY, TOCSY, and NOESY) NMR spectroscopy (Fig. S10-S17).

White solid; mp 168-172 ^{o}C**.** ^{1}H NMR ($CDCl_3$; 600 MHz) δ 1.41 (9H, s); 3.70 (3H, s); 3.82 (2H, s); 5.20 (1H, br); 5.52-5.51 (1H, d, *J* = 7.2 Hz); 5.66 (1H, brs); 7.12-7.11 (2H, d, *J* = 6.6 Hz), 7.29-7.23 (10H, m). ^{13}C NMR ($CDCl_3$; 150 MHz) δ 28.4, 44.4, 53.2, 56.8, 80.4, 127.1, 127.6, 128.7, 129.2, 135.8, 137.4, 156.1, 169.1, 171.0. HRMS (ESI): calculated $[M+H]^+$ 456.2136, found m/z . 456.2362. HPLC: retention time (t_R) = 11.8 min. Isolated pure product 650 mg (yield: 71% w.r.t. starting meterial Boc-L-Phg).

High-performance liquid chromatography (HPLC)

The purity of column purified compound was further checked by analytical HPLC, Waters 600E system using a C18 thermo scientific column at a flow rate of 1 mLmin^{-1}, linear gradient of 5-100% CH_3CN over 8 minutes in a total run time of 20 min. Binary solvent system, solvent A (H_2O) and solvent B (CH_3CN) were used. A UV detector, contain dual wavelength at 214 and 254 nm was used.

Mass spectrometry

Mass spectrum of the peptide was recorded from Agilent-Q-TOF 6500 instrument in ESI positive mode and equipped with Mass hunter workstation software.

Nuclear magnetic resonance (NMR) spectroscopy

All NMR spectra were obtained from Bruker Ascend 600 and 400 MHz instrument at 298 K using $CDCl_3$ solvent. 1D [^{1}H] spectra and 2D [^{1}H, ^{1}H] TOCSY (Total Correlation Spectroscopy), 2D [^{1}H, ^{1}H] COSY (Correlated Spectroscopy) were recorded with NS = 16 scans and 2D [^{1}H, ^{1}H] NOESY (Nuclear Overhauser Enhancement Spectroscopy) was recorded with NS = 32 scans. Chemical shifts were referenced to $CDCl_3$ at δ = 7.26 ppm and δ = 77.23 in ^{1}H NMR and ^{13}C NMR, respectively.

Single crystal X-ray diffraction (SC-XRD)

The single crystal XRD experiment was performed on an Oxford SuperNova diffractometer (Agilent Technologies) using MoKα radiation. Data collection was carried out by SMART software. Data refinement and cell reductions were performed by CrysAlisPro 1.171.38.46 (Rigaku OD, 2015).[30] The obtained structures were solved by direct methods implemented in SHELX-2014/7 software.[31]

Sample preparation

2.13 mM of peptide **1** and 2.19 mM of peptide **2** solutions were prepared in an Eppendorf vial (2 mL) by adding 1 mL 30% acetonitrile-water mixture. Then, we prepared 1 mL (1.5 mM) solution (stock solution) from the above solution. This stock solution was incubated for 7 days at 37 ^{o}C. After 7 days, we prepared FESEM and Microscopic slide sample.

Circular dichroism (CD)

CD spectrum was obtained from JASCO J-1500 instrument. After 7 days, the incubated samples (1.5 mM) were diluted to 300 μM and from each sample 400 μL was taken in a CD cuvette, and the CD spectrum were recorded from 190 nm to 260 nm wavelength using 1 mm pathlength and 1 nm bandwidth. The mean residue molar ellipticity was calculated using the following equation:

θ (deg. cm^2 .dmol^{-1}) = Ellipticity (mdeg). 10^6 / Pathlength (mm). [Protein] (μM). N

Optical microscopic images

10 μL of 7 days aged solution was drop casted on a microscopic slide and dried it. The bright field image (40X

magnificence) was captured on Nikon Digital Sight DS-U3 microscope.

Field emission scanning electron microscopy (FESEM)

FESEM samples were prepared by drop casting (10 µL) of 1.5 mM of peptide **1** and **2** on Al-foil and dried them inside a desiccator. FESEM images were captured using a SIGMA-300 (ZEISS) instrument.

Transmission electron microscope (TEM)

TEM images were captured under JEOL (Model: 2100F*)* instrument. The 7 days incubated sample (1.5 mM) was diluted to 100 µM and from this 10 µL of sample was drop-casted on carbon-coated copper grid followed by adding 2% uranyl acetate solution (10 µL) and was allowed to float for 1 min and kept inside desiccator.

Conclusions

In conclusion, we demonstrated the conformation and morphology of both N- and C- protected alternating D/L amino acids containing tripeptides, Boc-Gly-L-Phg-D-Phe-OMe (**1**) and Boc-Gly-L-Phg-D-Phg-OMe (**2**). The SC-XRD analyses suggested that they exhibited anti-parallel β-sheet structures, but **1** self-assembled to form helix-like architecture whereas **2** self-associated to form double helix-like structures. Both supramolecular structures were stabilized by intermolecular H-bond as well as C-H…O and C-H…π interactions. Although there was no intramolecular H-bond in these peptides as suggested by SC-XRD and solvent dependent NMR studies, **1** exhibited turn-like structure in solution suggested by CD and 2D NOESY experiment. They also self-associated to form flower-like structures in 30% acetonitrile-water. The differences in molecular arrangements among these peptides are due to the presence of the methylene group (D-Phe) or absence of methylene group (D-Phg) at the side chain. The obtained results may be helpful for the design of supramolecular single or double helix-like structure using alternating D/L amino acids.

Conflicts of interest

There are no conflicts of interest to declare.

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