TITLE: Helix packing motif common to the crystal structures of two undecapeptides containing dehydrophenylalanine residues: Implications for the *de novo* design of helical bundle super secondary structural modules.

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RUNNING TITLE: An approach to the design of helical bundles using dehydrophenylalanine residues based on the packing of helices in the solid state.

Abstract:

De novo designed peptide based super secondary structures are expected to provide scaffolds for the incorporation of functional sites as in proteins. Self-association of peptide helices of similar screw sense, mediated by weak interactions has been probed by the crystal structure determination of two closely related peptides:

Ac-Gly^1-Ala^2-ΔPhe^3-Leu^4-Val^5-ΔPhe^6-Leu^7-Val^8-ΔPhe^9-Ala^{10}-Gly^{11}-NH$_2$, I and Ac-Gly^1-Ala^2-ΔPhe^3-Leu^4-Ala^5-ΔPhe^6-Leu^7-Ala^8-ΔPhe^9-Ala^{10}-Gly^{11}-NH$_2$, II. The crystal structures determined to atomic resolution and refined to R-factors 8.12% and 4.01% respectively reveal right-handed 3_{10}-helical conformations for both the peptides. Circular dichroism has also revealed the preferential formation of right-handed 3_{10}-helical conformations for both the molecules. Our aim was to critically analyze the packing of the helices in the solid state with a view to elicit clues for the design of super secondary structural motifs such as two, three and four helical bundles based on helix-helix interactions. An important finding is that a packing motif could be identified common to both the structures, in which a given peptide helix is surrounded by six other helices reminiscent of transmembrane seven helical bundles. The outer helices are oriented either parallel or antiparallel to the central helix. The helices interact laterally through a combination of N-H···O, C-H···O and C-H···π hydrogen bonds. Layers of interacting Leucine residues are seen in both the peptide crystal structures. The packing of the peptide helices in the solid state appears to provide valuable leads for the design of super secondary structural modules such as two, three or four helix bundles by connecting adjacent antiparallel helices through suitable linkers such as tetraglycine segments.

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Introduction:

De novo protein design endeavors to understand the daunting complexity of protein architecture and has the ambitious goal of constructing novel molecules with predetermined structures and functions.[1-4] The success in the *de novo* synthesis of protein mimics, [5-12] relies heavily on the ability to design relatively short peptides that can adopt stable secondary structures, such as a helix. In this connection, non-protein amino acids such as α,β-dehydrophenylalanine (ΔPhe) have been used as conformation-determining residues.[13-21] A recent example from our laboratory includes, a ΔPhe containing decapeptide, showing self-association of opposite-handedness helices.[22] This self-association concept was successfully exploited by us in the design of super secondary structure helix-turn-helix, where the adjacent helices that docked to each other are of opposite screw sense.[23] However, a strategy has to be evolved for the development of discrete folded super secondary structural motif in which the interacting helices are right-handed, for which there has been till date no crystal structure containing ΔPhe residue as a model for pairing and interaction. Here, we report the synthesis and characterization of two closely related undecapeptides namely: Ac-Gly^1-Ala^2-ΔPhe^3-Leu^4-Val^5-ΔPhe^6-Leu^7-Val^8-ΔPhe^9-Ala^{10}-Gly^{11}-NH$_2$, I; and Ac-Gly^1-Ala^2-ΔPhe^3-Leu^4-Ala^5-ΔPhe^6-Leu^7-Ala^8-ΔPhe^9-Ala^{10}-Gly^{11}-NH$_2$, II; that have been envisioned to fold as right-handed 3$_{10}$-helices. Val residues at positions 5 and 8 in peptide I have been replaced by less bulky Ala residues in peptide II, except for which the two sequences are similar. Crystal structure and CD studies have both confirmed the preferential folding of the designed peptides into right-handed 3$_{10}$-helices. Interestingly in both the crystal structures, a given helix is surrounded by six other helices reminiscent of transmembrane seven helical-bundles. The outer helices

have either parallel or antiparallel orientation relative to the central helix. Apart form other interactions the parallel helices interact through C-H…O[24] and C-H…π[25] hydrogen bonds while the antiparallel helices interact through N-H…O and C-H…π hydrogen bonds. Leucine residues occurring in layers is a feature common to both the structures. Here we discuss the molecular and crystal structures of the peptides I and II and the clues that have emerged to realize the successful design of higher order super secondary structural elements such as helical bundles.

Experimental procedures

Synthesis and characterization of peptides

Fmoc-protected amino acids for solid-phase peptide synthesis were obtained from Novabiochem or Bachem. The two undecapeptides (I and II) were synthesized manually at a 0.5 mmol scale. Fmoc-Rinkamide MBHA (Novabiochem) resin (0.5mmol/g) was used to afford carboxyl-terminal primary amides. Couplings were performed by using carbodiimide. **Solution phase methodology was used to introduce the ΔPhe residue in the undecapeptides as a dipeptide block by dehydration of Fmoc-Aa-DL-threo-β-Phenyl Serine (Aa=Valine or Alanine in case of (I) and aa=Alanine in case of (II)) using fused sodium acetate and freshly distilled acetic anhydride as reported earlier.**[26] All reactions were monitored by TLC on precoated silica plates in at least two different solvent systems.[26] All the couplings were followed by a 5 minute reaction with acetic anhydride and HOBT in DMF/DCM to cap any unreacted amines. Fmoc deprotection was performed with piperidine (20% in DMF). After addition of the final residue, the amino terminus was acetyl-capped and the resin was rinsed with DMF/DCM/MeOH then dried. The final peptide deprotection and cleavage from the resin was achieved with 10 ml of

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95:2.5:2.5 TFA: H2O: triisopropylsilane for 2 hours. The resin was filtered and the filtrate concentrated to ca. 1 ml volume after which the crude peptides were precipitated with cold ether. The supernatant was decanted and crude peptides were suspended in water with a minimal amount of acetonitrile then frozen and lyophilized to dryness. The lyophilized crude peptides were dissolved in DMSO and purified by preparative reverse phase HPLC (RP-HPLC) (C5 SUPELCO PREP Column/Shimadzu HPLC/linear gradient 40-60% acetonitrile (0.1%TFA)/H2O (0.1%TFA) over 50 min). Peptide identities were confirmed on a mass spectrometer. (I) ($C_{61}H_{82}N_{12}O_{12}$) calculated 1174 **Daltons,** observed 1197 **Daltons (sodium salt)**; (II) ($C_{57}H_{74}N_{12}O_{12}$) calculated 1118 **Daltons**, observed 1141 **Daltons (sodium salt)**.

The α,β-dehydrophenylalanine residues in both the peptides correspond to the Z-isomer[27] (Δ^Z Phe) and the symbol ΔPhe is used here to represent Δ^Z Phe.

X-ray Crystallography:

The peptide, I; ($C_{61}H_{82}N_{12}O_{12}$, MW = 1197) was crystallized by controlled slow evaporation of the peptide solution in a (1:1 v/v) mixture of acetone-ethanol. Platy crystals were obtained over a period of 15 days. For the suitable crystal, X-ray diffraction data was collected on a Bruker AXS SMART APEX CCD diffractometer equipped with MoKα radiation (λ=0.71073 Å). The structure solution was obtained by using direct methods as employed in Shake and Bake[28] (SnB). The structure was refined to an R-factor of 8.12%. No solvent molecules could be located in the electron density map. The crystallographic details of this peptide are summarized in Table 1.

The peptide, II; ($C_{57}H_{74}N_{12}O_{12}$, MW = 1141) was crystallized by controlled slow evaporation of the peptide solution in a methanol-water mixture. Good platy crystals were obtained over a period of 7 days. X-ray diffraction data was collected on a

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Bruker AXS SMART APEX CCD diffractometer equipped with Mo Kα radiation (λ=0.71073Å). The structure solution was obtained by using direct methods employed in SHELXS[29]. The structure was refined to an R-factor of 4.01%. No solvent molecules could be located in the electron density map. The crystallographic details of this peptide are summarized in Table 1.

Circular Dichroism spectroscopy:

Circular dichroism (CD) measurements were carried out on a JASCO J720 spectropolarimeter with a data processor attached. A 1mm pathlength was used. The spectrum was recorded in various solvents such as acetonitrile (ACN), dichloromethane (DCM), chloroform (CHCl$_3$), methanol (MeOH), trifluoroethanol (TFE) and dimethylsulfoxide (DMSO).

Results and Discussions:

Molecular and Crystal structures of Peptide I and Peptide II:

Molecular conformations:

Atomic resolution structures of peptides I and II are depicted in the Figure 1. As designed, both peptides folded into right-handed 3$_{10}$-helices. In both of these peptide molecules, the segments 2-9 are in 3$_{10}$-helical conformations (Table 2), stabilized by 4→1 hydrogen bonds as shown in Table 3. The average (ϕ, ψ) values for helical stretch in Peptide I and II are (-56°, -20°) and (-57°, -21°) respectively.

In 3$_{10}$-helices, every third residue lies on the same face of the helix. Accordingly in both structures, peptide I and II, three ΔPhe residues (ΔPhe3, ΔPhe6, ΔPhe9), which are placed at two-residue spacer in sequence during design, lie on the same face of

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helix, similarly Leu4, Leu7, and Ala10 lie on the same face of helix. The rest Ala2, Val5, Val8 residues are on the same face of helix in peptide I and Ala2, Ala5, Ala8 residues on the same side of helix in peptide II as is clearly seen in Figure 1. Figure (1b, 1d) also depicts the occurrence of wedges and grooves defined by the main chain and protruding side chain atoms, a characteristic feature of 3_{10}-helices. The least square superposition[30] of the corresponding main chain atoms of peptides I and II results in an R.M.S.D of 0.36Å indicating that both the peptides have very nearly the same backbone conformation.

CD studies

CD spectroscopy can be a very useful tool to establish the screw sense of dehydrophenylalanine containing peptides.[31] The CD spectra displays a couplet of bands that appear as a typical exciton splitting of the dehydrophenylalanine chromophore at 280 nm. This splitting pattern is an indication of two or more ΔPhe residues, generally involved in a system of consecutive beta-turns, i.e. 3_{10}-helix, [32,33] the sign of the couplet dictating the handedness of the helices. For the present undecapeptides, CD studies were carried out in various solvents at room temperature. The spectra display couplet of bands, the most striking feature being the sign of the couplet. A negative CD couplet (- +), characteristic of a right-handed 3_{10}-helix with negative band at 299 nm, a positive band at 268 nm and a crossover point at λ=287 nm is observed for the undecapeptides in various solvents tried. Thus the undecapeptides preferentially form a right-handed 3_{10}-helical conformation. The sign of the couplet does not change with the solvent. Only a variation in the intensity of the couplet is observed which reflects the instability of the peptide conformation in different solvents. Both the peptides, however, show small featureless CD spectra in DMSO, suggesting loss of structure in this chaotropic solvent (CD data not shown).

Crystal packing:

Arrangement of helices:

It is very interesting to note that, despite differences between their sequences, both these peptides have crystallized in same space group $P2_1 2_1 2_1$ and with quite similar cell parameters (Table 1). In the crystal packing of both molecules, peptide I and II, the helices are arranged in both antiparallel and parallel orientations. Figure (2a - d) shows view down the helical axis for the helical arrangement in crystal lattice of Peptide I and II, when they are in antiparallel and parallel orientations.

Further, the helices are arranged laterally in such a way that they are in level with each other and a given helix is surrounded by six other helices, two helices being parallel and four helices being antiparallel to it. Figure (3a - d) shows such packing of helices in a layer. This arrangement has resulted in two kinds of helix interfaces, Leu – Leu and Val – Val in case of Peptide I and Leu – Leu and Ala – Ala in case of peptide II. In (Figure 4a, c) it is clearly seen that helices in central row are having Leucine – Leucine interface with those in upper row in both crystal structures. While helices in central row are having Valine – Valine or Alaine – Alaine interface with those in lower row in peptide I and II respectively. Figure 5e depicts the schematic representation of packing of helices and the naming of peptide chains in both peptides I and II.

Association of Helices:

In both the cases of helical arrangements, the parallel and antiparallel, the helices are associated through wedges into grooves (Figure 2, 3a, c) as opposed to knobs into holes in α-helices in proteins[34]. In Leu – Leu interfaces of both peptides, and Ala – Ala interface in Peptide II, there were no close approaches of methyl moieties. Their

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C…C distance were > 4.0 Å except in the case of $C^{\gamma 1}(Val^8)…C^{\gamma 2}(Val^5)$ = 3.4 Å, in Peptide I at the Val – Val interface.

Hydrogen Bonding Network:

In crystal packing of both the peptides, helices are found to have head-to-tail kind of hydrogen bonds N2···O9', N3···O11' and N12···O1 (Table 3) in a direction parallel to the helical axis.   In addition to other non-bonding interactions (wedges into grooves, helix dipole – dipole interactions), packing of helices along lateral direction are further stabilized by network of classical N-H···O hydrogen bonds N1···O10' (Table 3) and **non-classical** C-H···O and C-H···π weak hydrogen bonds (Table 4). Figure 4 explicitly shows the occurrence of the intermolecular hydrogen bonds. There is a distribution of type and concentration of hydrogen bonds in the packing of the helices. In the crystal structures of both the peptides, parallel helices interact through aromatic side-chain of ΔPhe to main chain C-H…O hydrogen bonds (Figure 4a,b and Table 4), three C-H…O bonds between parallel helices in the case of peptide I and two in the case of Peptide II. In addition to the three C-H…O bonds, parallel helices are having a C-H…π bond at termini in case of peptide I. At Leu – Leu interface, common to both crystal packing the antiparallel helices are engaged in a N-H…O hydrogen bond at termini. In addition, in case of Peptide II a C-H…π bond is found between antiparallel helices (Figure 4b, d, f and Table 4). At Val-Val interface in Peptide I, antiparallel helices are interacting through two C-H…π bonds (Figure 4e and Table 4). While at Ala – Ala interface, in Peptide II, a C-H…π bond is observed between antiparallel helices (Figure 4f and Table 4).

This network of hydrogen bonds in a direction lateral to the helices can be represented schematically as shown in Figure 5. In case of Peptide I each helix is involved in a total of two N-H…O hydrogen bonds, six C-H…O and six C-H…π intermolecular hydrogen bonds. In case of Peptide II each helix is involved in a total of two N-H…O hydrogen bonds, four C-H…O and four C-H…π intermolecular hydrogen bonds. Thus in the crystal structure of these peptides, each helix is acting as donor and acceptor of intermolecular hydrogen bonds.

Presumably, the presence of well defined wedges and grooves in 3_{10}-helices encourage the bringing together of adjacent helices facilitated by wedge into groove association resulting in the occurrence of a multitude of classical as well as non-classical interactions at the helix interfaces. The overall arrangement of helices in the crystal structure of these two peptides may be best described as a hexagonal close packing of regular cylinders where the cylinders are in flush with each other.

A careful study and a proper understanding of interactions between helices and the pattern of their association is important, since it has been reported recently that both metal binding and catalytic activity could be successfully incorporated into a hetero oligomeric association of *de novo* designed peptide helices as tetramers[35].

Discussions:

In the crystal structures of both molecules Peptide I and II, identical helical arrangements are observed. A given helix is surrounded by two parallel and four antiparallel helices. This mode of aggregation of helices via the parallel and antiparallel orientations is similar to that observed in membrane proteins[36] and water-soluble proteins including helical bundles. Parallel and antiparallel and only parallel arrangements of α-helices in crystal polymorphism were observed for Aib containing decapeptides [37,38], where the packing of helices was reportedly dominated by the close

approach of methyl moieties (3.4 – 4.1 Å). But in the present peptide molecules, helical packing seems to be stabilized by concerted action of weak C-H...O, C-H...π and classical N-H...O hydrogen bonds along with other non-bonding interactions and close approach of methyl moieties does not seem to be dominating the helical packing. A comparison of calculated crystal densities (Table 1) of the Peptide I and II (1.22 g/cm^3 each) with those of Aib containing decapeptides[37, 38] (1.161, 1.117, 1.120, 1.126 and 1.143 g/cm^3) suggests that packing of helices in crystal structures of Peptide I and II is more compact. This can presumably be attributed to the presence of hydrogen bond networks, other reason being smaller radius of 3_{10}-helices compared to that of α helices[39] and wedges into groves association of 3_{10}-helices that brings them together. In the recently reported helix-turn-helix structure from our laboratory a tetraglycine segment links adjacent antiparallel helices.[21] The folding of the peptide was realized through a concerted action of C-H…O and N-H…O hydrogen bonds between helices. The self association of helices in the crystal structures of peptides I and II reported here, the occurrence of a common helix packing motif in the two crystal structures and the presence of C-H…O, C-H…π and N-H…O hydrogen bonds between the helices in the lateral direction, points to the possibility of controlled design of covalently linked helical aggregates and the construction of real helical bundles.[40]

Conclusions:

As designed, peptides I and II have folded into right-handed 3_{10}-helical conformation. Despite the differences between their sequences, both **peptide** molecules have identical helical arrangements in the crystal packing; a given helix is surrounded by six other helices, two helices being parallel and four being antiparallel to the central helix. The packing of helices is characterized by a network of hydrogen bonds along

with other non-bonded interactions. These hydrogen bonds include conventional N-H…O and a multitude of weak C-H…O and C-H…π weak hydrogen bonds.

Weak interactions along with other non-bonded interactions that determine the stability of the **supramolecular system** and play an important role in their folding.[41, 42,43] appear to dictate the packing of helices in both the crystal structures. The occurrence of conventional N-H…O hydrogen bond between adjacent antiparallel helices appears to further stabilize the helix association.

The lateral association of right-handed helices observed in the crystal structures of peptides I and II can be exploited for the construction of super secondary structures like helical-hairpin, triple and four-helix bundles. This could be achieved by the covalent insertion of flexible residues (tetraglycine linker segment) between the adjacent helices. For example, the adjacent antiparallel helices A and G (Figures 2, 3) may be connected to form a helical hairpin and the helices A, G and C may be connected sequentially to form a three-helix bundle. There are many examples where the self-association of molecules has led to the design of super secondary structural elements[21, 23, 44]. In these molecules, tetraglycine linker has been used as a connecting segment between adjacent helices resulting in the successful design of peptide based super secondary structural modules.

The crux of our design strategy involves the use of conformation-constraining amino acid residue ΔPhe to induce long- range interactions. As may be seen, the aromatic ring in the ΔPhe residue acts as a donor in C-H…O interactions and as an acceptor in C-H...π interactions in both the crystal structures. Also, the careful positioning of other protein-coded hydrophobic amino acid residues has helped us realize both parallel and antiparallel orientation of the right-handed helices. The association of the

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right-handed 3_{10}-helices through the concerted action of N-H...O, C-H...O and C-H...π interactions has provided possible clues for the construction of helical bundles. There is an advantage in including the ΔPhe residue in the design of super secondary structural elements as it exhibits preferential secondary structural features, i.e., helix inducer, both in solid state and in solution and provides greater stability to the scaffold. The design of higher order super secondary structural elements using ΔPhe is likely to be helpful in realizing stable molecular frameworks for incorporating activity.

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Table 1. Data collection and Refinement parameters for Ac-Gly-Ala-ΔPhe-Leu-Xxx--ΔPhe-Leu-Xxx--ΔPhe-Ala-Gly-NH$_2$. (Xxx=Val I, Xxx=Ala II)

	Peptide I	Peptide II
Empirical Formula	C$_{61}$ H$_{82}$ N$_{12}$ O$_{12}$	C$_{57}$ H$_{74}$ N$_{12}$ O$_{12}$
Molecular weight	1174	1118
Crystal System	Orthorhombic	Orthorhombic
Space Group	P2$_1$2$_1$2$_1$	P2$_1$2$_1$2$_1$
Cell Parameter	a=10.69Å, b=15.37 Å, c=38.95 Å	a=10.73 Å, b=14.56 Å, c=39.08 Å
Cell Volume	V=6400 Å^3	V=6105 Å^3
Z	4	4
Molecules/asymmetric Unit	1	1
Density Calculated	1.22 g cm^{-3}	1.22 g cm^{-3}
μ	9.0 cm^{-1}	9.0 cm^{-1}
Radiation used	Mo (λ=0.71073 Å)	Mo (λ=0.71073 Å)
Resolution	1.00 Å	0.9 Å
Unique reflections	6711	8755
Observed reflections	5383 (|Fo| > 4σ(|Fo|)	7331 (|Fo| > 4σ(|Fo|)
Structure Solution	SnB V2.1	Shelxs
Refinement Procedure	Full-matrix least-squares refinement on Fo^2 using Shelxl (97-2)	Full-matrix least-squares refinement on Fo^2 using Shelxl (97-2)
Number of parameter refined	767	737
Data/Parameter	7.0	9.95
R-factor	8.12%	4.01%
wR2	17.43%	8.73%
GooF (s)	1.119	1.047
Residual electron density	Max. =0.39e/Å^3 Min= -0.24e/Å^3	Max. =0.13e/Å^3 Min. =-0.12e/Å^3

Table 2. Torsion angles (deg) in the peptides, Ac-Gly-Ala-ΔPhe-Leu-Xxx--ΔPhe-Leu-Xxx--ΔPhe-Ala-Gly-NH$_2$. (Xxx=Val I, Xxx=Ala II)

Peptide I

Residue	Phi	Psi	Omega	Chi1	Chi2
1 GLY	-71	168	-179		
2 ALA	-50	-34	179		
3 ΔPHE	-53	-22	176	-3	-17
4 LEU	-59	-16	174	-114	-178
5 VAL	-61	-16	170	-55	
6 ΔPHE	-52	-21	-177	0	-21
7 LEU	-66	-2	162	-66	179
8 VAL	-54	-26	170	-69	
9 ΔPHE	-53	-20	178	-4	-40
10 ALA	-93	3	-178		
11 GLY	-108	-177			

Peptide II.

Residue	Phi	Psi	Omega	Chi1	Chi2
1 GLY	-77	169	-179		
2 ALA	-50	-33	179		
3 ΔPHE	-59	-17	173	-4	-16
4 LEU	-61	-14	171	-69	177
5 ALA	-49	-32	179		
6 ΔPHE	-55	-23	-180	3	-6
7 LEU	-71	-3	162	-61	177
8 ALA	-53	-27	172		
9 ΔPHE	-55	-17	177	-3	-30
10 ALA	-86	0	-180		
11 GLY	-96	178			

Table 3. **Hydrogen bonds in the structures of peptides, Ac-Gly-Ala-ΔPhe-Leu-Xxx--ΔPhe-Leu-Xxx--ΔPhe-Ala-Gly-NH₂. (Xxx=Val I, Xxx=Ala II)**

Peptide I

Type	D (Donor)	A (Acceptor)	D···A (Å)	H···A (Å)	D-H···A (°)	Symmetry code*
Lateral	N1	O10'	2.950	2.09	174	1
Head-to-Tail	N2	O9'	2.771	1.97	155	2
	N3	O11'	3.116	2.19	171	2
3_{10}-helix (4→1)	N4	O1'	2.966	2.11	171	0
	N5	O2'	3.063	2.20	178	0
	N6	O3'	3.033	2.18	169	0
	N7	O4'	2.980	2.13	172	0
	N8	O5'	3.024	2.17	176	0
	N9	O6'	2.904	2.06	167	0
	N10	O7'	2.881	2.06	161	0
	N11	O8'	3.003	2.17	163	0
Head-to-Tail	N12	O1'	2.774	1.92	171	3

Peptide II

Type	D (Donor)	A (Acceptor)	D···A (Å)	H···A (Å)	D-H···A (°)	Symmetry code*
Lateral	N1	O10'	2.879	2.03	169	1
Head-to-Tail	N2	O9'	2.810	1.99	159	2
	N3	O11'	2.947	2.10	170	2
3_{10}-helix (4→1)	N4	O1'	2.958	2.11	169	0
	N5	O2'	2.935	2.09	169	0
	N6	O3'	3.056	2.21	168	0
	N7	O4'	2.917	2.08	165	0
	N8	O5'	2.991	2.14	171	0
	N9	O6'	2.902	2.08	161	0
	N10	O7'	2.892	2.04	169	0
	N11	O8'	2.988	2.16	161	0
Head-to-Tail	N12	O1'	2.885	2.04	169	3

- 0) x, y, z
  1) ½+x, -y+½, -z   2) -x+½+1, -y, z-½   3) –x+½, -y, z+½

Table 4. C-H⋯O/π hydrogen bonds between the helices observed in the structure of peptides, Ac-Gly-Ala-ΔPhe-Leu-Xxx--ΔPhe-Leu-Xxx--ΔPhe-Ala-Gly-NH$_2$. (Xxx=Val I, Xxx=Ala II)

Peptide I

Donor (D)	Acceptor (A)	D⋯A (Å)	H⋯A (Å)	D-H⋯A (°)
CE2_3A	O_1B	3.415	2.64	141
CE2_6A	O_4B	3.384	2.54	151
CE2_9A	O_7B	3.588	2.66	177
CG2_8D	X_3A	3.863	3.05	143
CG2_5D	X_6A	3.868	3.21	128
CA_11B	X_9A	4.102	3.36	135
CG2_8F	X_3C	3.863	3.05	143
CG2_5F	X_6C	3.868	3.21	128
CA_11A	X_9C	4.102	3.36	135
CG2_8B	X_3D	3.863	3.05	143
CG2_5B	X_6D	3.868	3.21	128
CG2_8A	X_3F	3.863	3.05	143
CG2_5A	X_6F	3.868	3.21	128
CA_11D	X_9F	4.102	3.36	135
CA_11E	X_9G	4.102	3.36	135
CG2_8D	X_3A	3.863	3.05	143

Peptide II

Donor (D)	Acceptor (A)	D⋯A (Å)	H⋯A (Å)	D-H⋯A (°)
CE2_3A	O_4B	3.416	2.71	134
CE2_6A	O_1B	3.508	2.80	134
CB_5F	X_6A	3.589	2.65	168
CD2_7G	X_6A	4.112	3.28	146
CB_5D	X_6B	3.589	2.65	168
CD2_7E	X_6B	4.112	3.28	146
CB_5A	X_6D	3.589	2.65	168
CD2_7A	X_6E	4.112	3.28	146
CB_5C	X_6F	3.589	2.65	168
CD2_7C	X_6G	4.112	3.28	146

X= Centroid of aromatic ΔPhe ring
A, B, C, D, E, F and G represent chain ID of molecules in crystal packing (Figure 4).

Legends to the figures:

Figure 1. Molecular conformation in crystals of. Ac-Gly-Ala-ΔPhe-Leu-Val-ΔPhe-Leu-Val-ΔPhe-Ala-Gly-NH$_2$. (a) view perpendicular to the helix axis, (b) view along the helix axis, and in crystals of . Ac-Gly-Ala-ΔPhe-Leu-Ala-ΔPhe-Leu-Ala-ΔPhe-Ala-Gly-NH$_2$. (c) view perpendicular to the helix axis, (d) view along the helix axis. **(e) and (f) Electron density maps (2Fo-Fc) contoured at 2.0 σ levels for the peptides.**

Figure 2. Wedge into groove association of interacting helices in the crystal structure of Ac-Gly-Ala-ΔPhe-Leu-Val-ΔPhe-Leu-Val-ΔPhe-Ala-Gly-NH$_2$, (a) view for antiparallel helices (b) view for parallel helices and in crystals of. Ac-Gly-Ala-ΔPhe-Leu-Ala-ΔPhe-Leu-Ala-ΔPhe-Ala-Gly-NH$_2$ (c) view for antiparallel helices (d) view for parallel helices, Where N- N terminus, C- C terminus. In all the figures, along the helix axis, the label corresponds to the terminus closest to the viewer.

Figure 3. Packing of helices in the crystals of Ac-Gly-Ala-ΔPhe-Leu-Val-ΔPhe-Leu-Val-ΔPhe-Ala-Gly-NH$_2$. (a) view along the helix axis. (b) view perpendicular to the helix axis, and in the crystals of Ac-Gly-Ala-ΔPhe-Leu-Ala-ΔPhe-Leu-Ala-ΔPhe-Ala-Gly-NH$_2$. (c) view along the helix (d) view perpendicular to the helix axis. Note that in both peptide structures, the central helix is surrounded by six other helices; outer helices are either parallel or antiparallel to the central helix. In view (b) and (d) it may be seen that the helices are packed in flush with each other. In e) the peptide chains are named and the packing of helices in both the peptide crystal structures I and II is shown schematically.

Figure 4. a) C-H…O and C-H…π inter helical hydrogen bonds between parallel helices in case of Peptide I, b) C-H…O inter helical hydrogen bonds between parallel helices in case of Peptide II, (c, d) N-H…O hydrogen bond observed between the termini of antiparallel helices in case of Peptide I and II at Leucine-Leucine interface, e) Inter helical C-H…π interaction between antiparallel helices at Leucine-Leucine interface in case of Peptide I and f) inter helical C-H…π hydrogen bond between antiparallel helices at Leucine-Leucine and Alanine-Alanine interfaces in case of Peptide II.

Figure 5. Schematic presentation of the network of weak hydrogen bonds in crystal structure of Ac-Gly-Ala-ΔPhe-Leu-Val-ΔPhe-Leu-Val-ΔPhe-Ala-Gly-NH$_2$. (a) the network of C-H…π hydrogen bonds. (b) the network of C-H…O hydrogen bonds, and in the crystal structure of Ac-Gly-Ala-ΔPhe-Leu-Ala-ΔPhe-Leu-Ala-ΔPhe-Ala-Gly-NH$_2$ (c) the network of C-H…π hydrogen bonds, (d) the network of C-H…O hydrogen bonds. e) Network of N-H…O hydrogen bond observed in both the peptide crystal structures. Arrow points to the acceptor of hydrogen bond and numbers represent the number of hydrogen bonds. Note that A represents central helix in both the peptides. Helices B and C are parallel to A, whereas D, E, F, G are antiparallel to A. The relative orientation of the helices **is** as depicted in figure 3e.
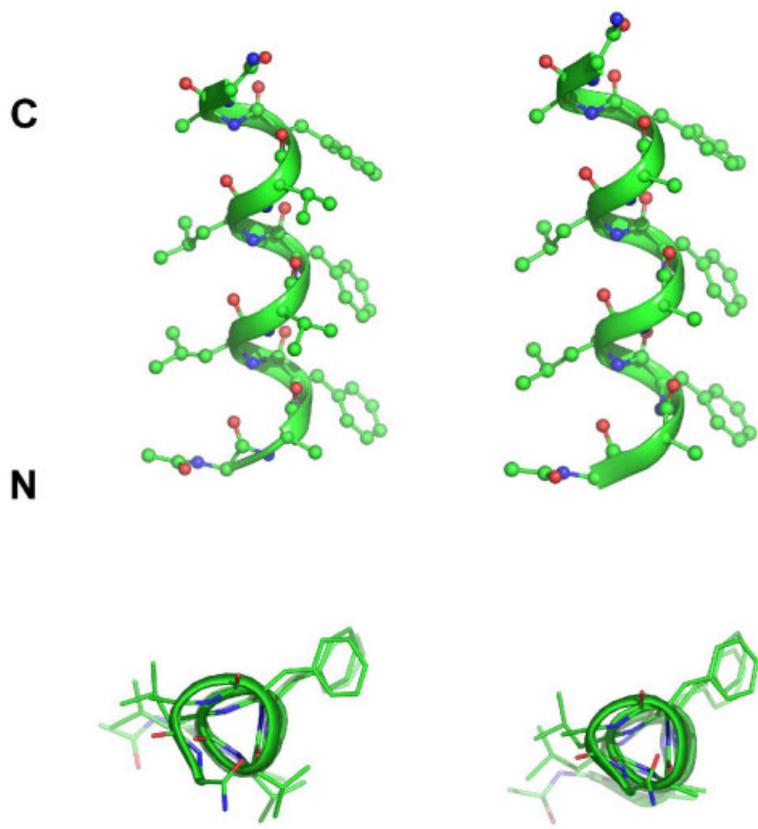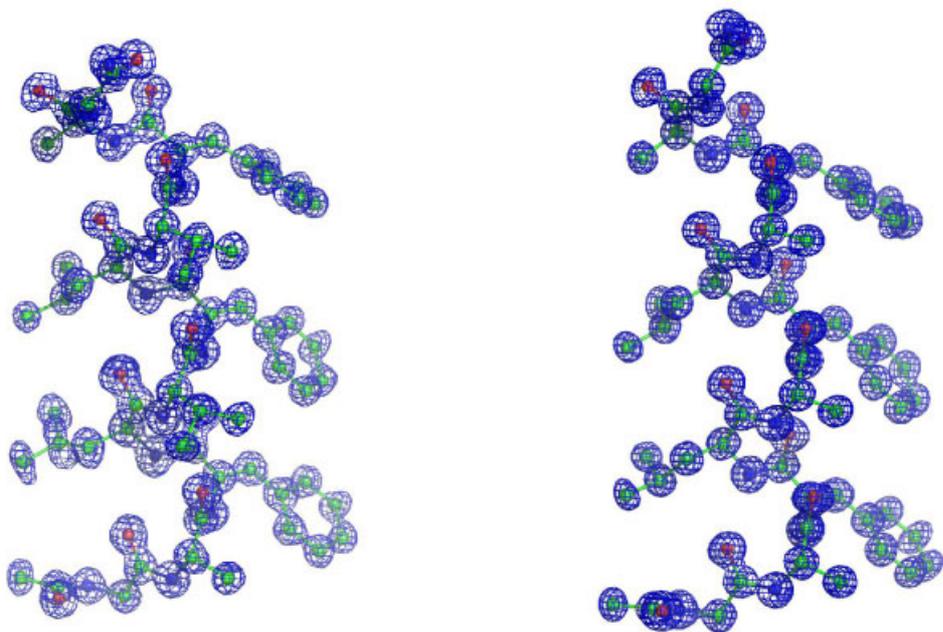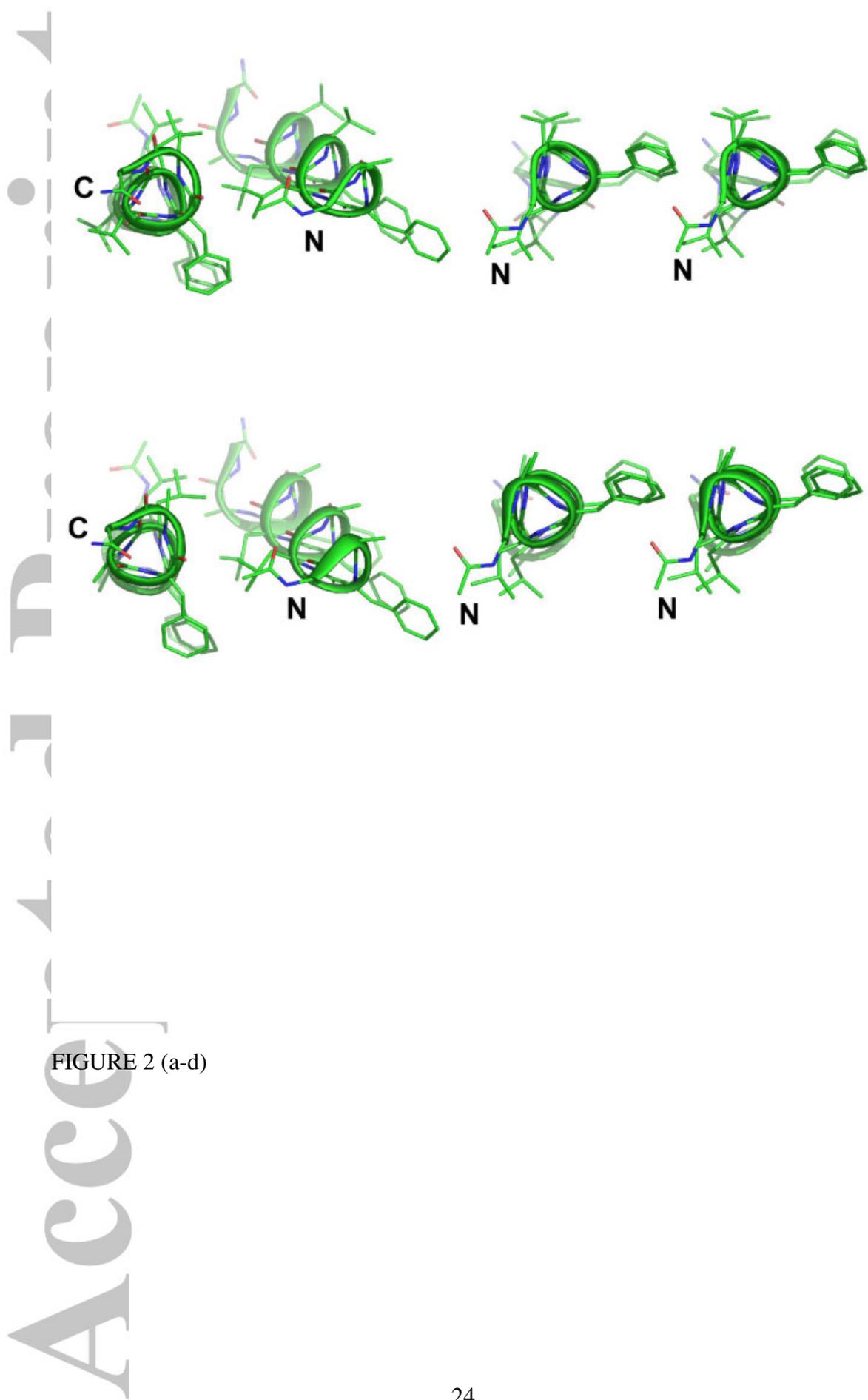
C

N

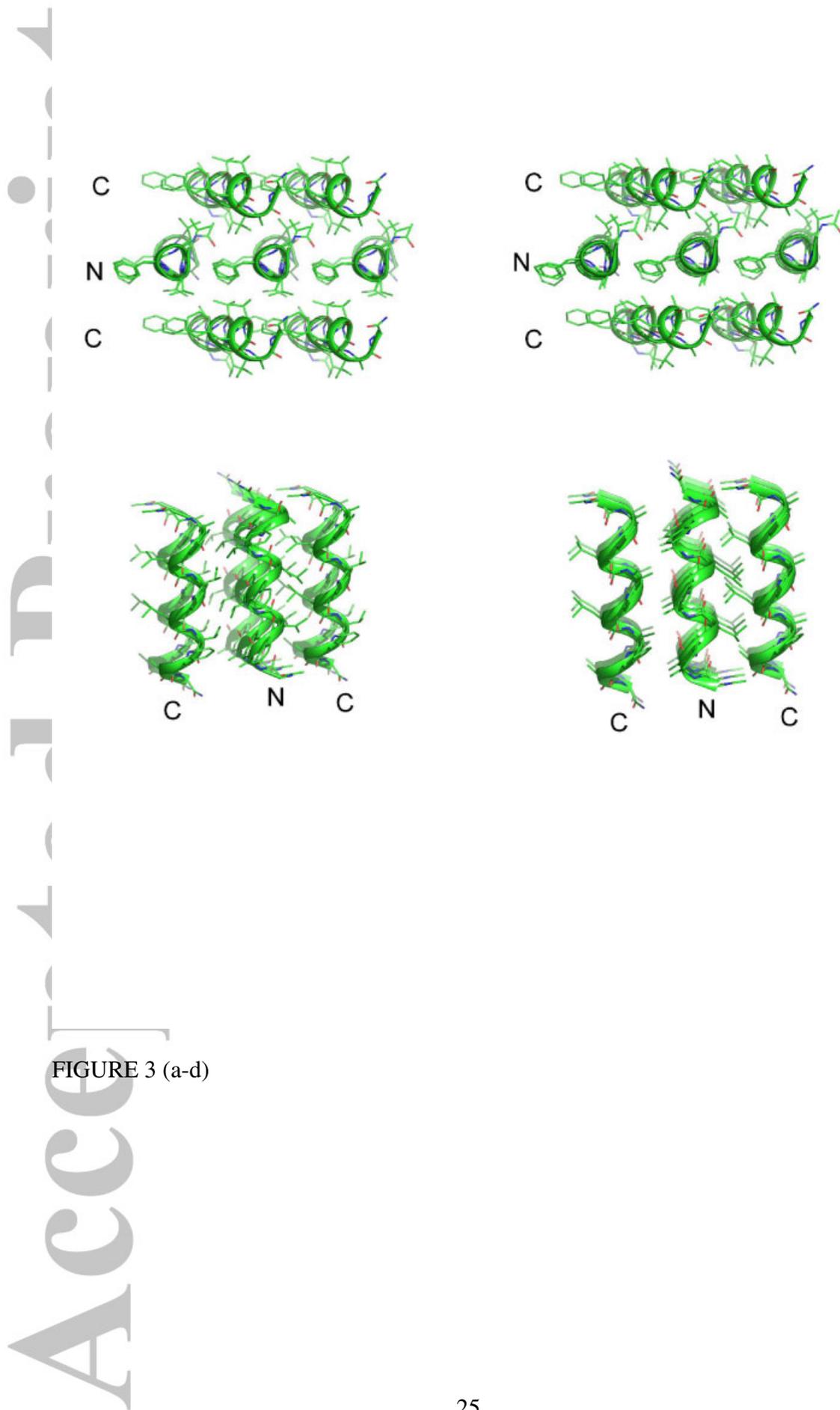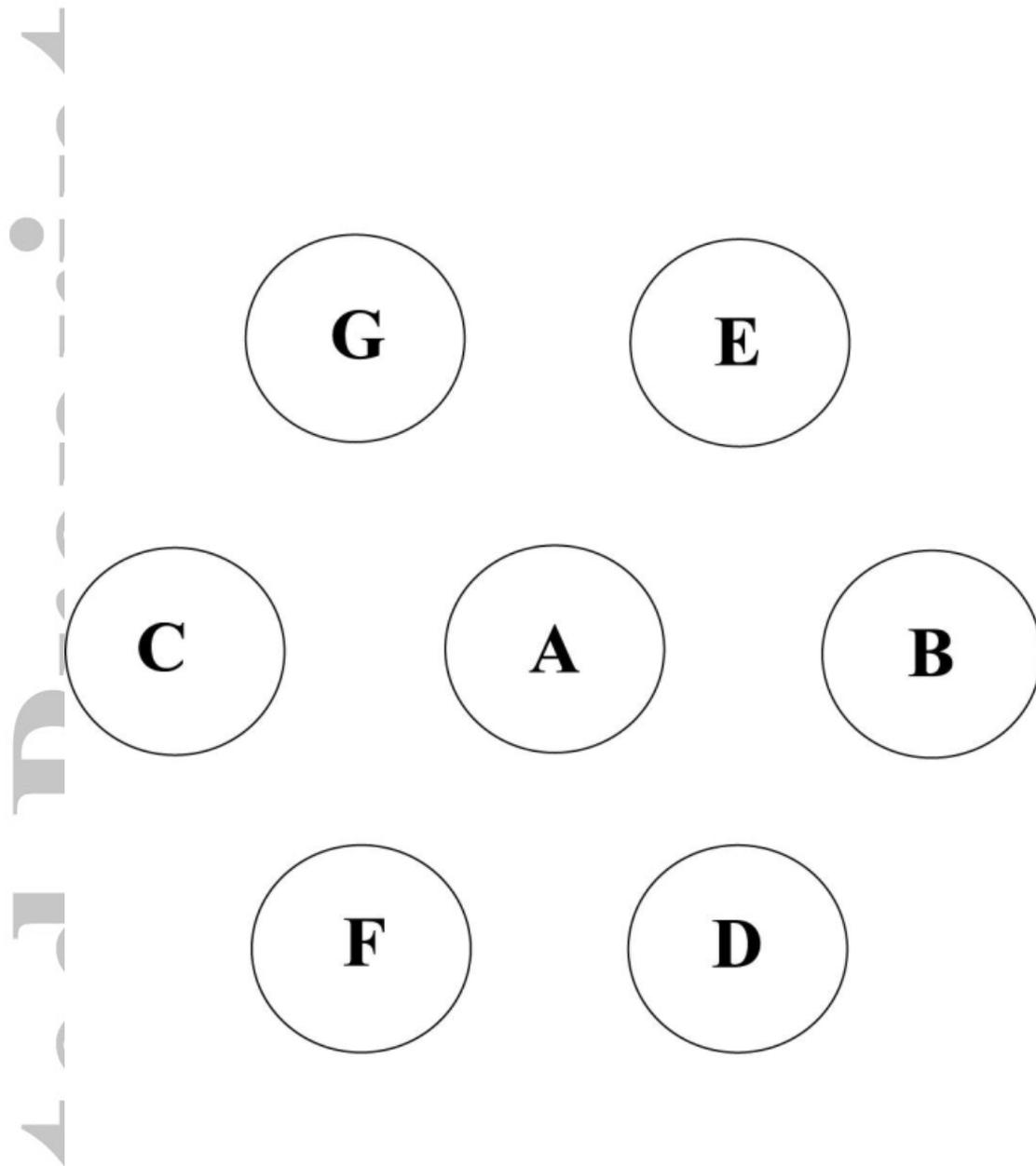FIGURE 1 (a-d)

FIGURE 1 (e-f)

FIGURE 2 (a-d)

FIGURE 3 (a-d)

FIGURE 3 (e)

FIGURE 4 (a-f)
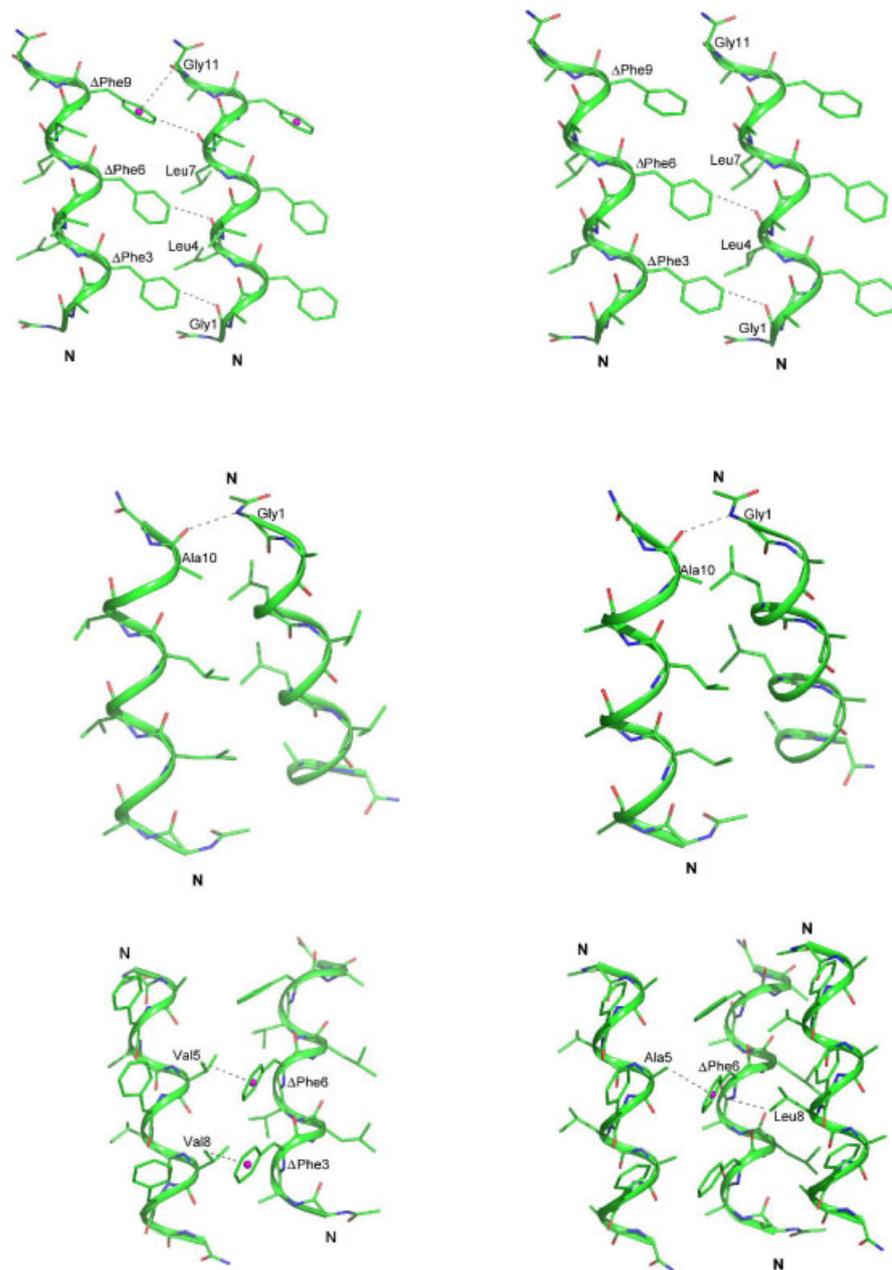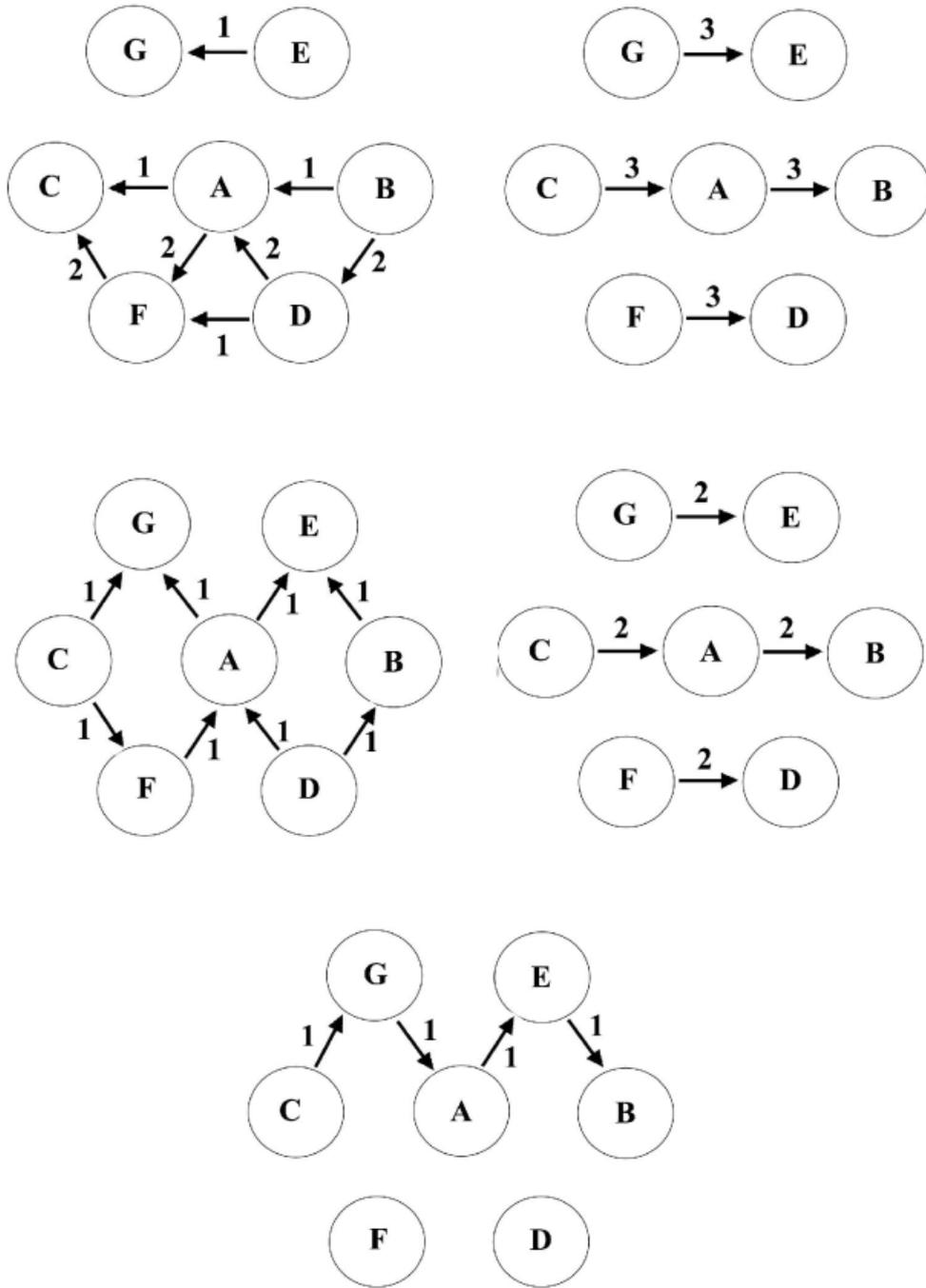
FIGURE 5