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Peptide Turns Through Just 'One Atom'! Sulfamide Group Nucleates Folding and Stabilizes New Supramolecular Topologies in Short Peptides

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Peptide segments with centrally placed sulfamide groups showed remarkable tendency to adopt turn conformation and exhibited supramolecular topologies like 'helical stacks' and 'hairpin sheets' through highly co-ordinated array of strong and weak hydrogen bonds.

Reverse turns, stabilized by intramolecular hydrogen bonds are integral part of many functional proteins.[1] Apart from reversing the chain direction to stabilize higher order structures like β-trefoil superfolds,[2] such turns also appear as part of the recognition domains in hormones, antigens and receptors.[3] This has inspired the scientific community to develop artificial turn mimics based on peptidic- or non-peptidic central scaffolds with the objective of developing competitive inhibitors of macromolecular recognition events relevant to various disease conditions.[4] Such designs were based on either turn-inducing segments from α-amino acids such as AsnGly,[5] DProXxx[6] or rationally designed synthetic frameworks with rings or conformationally locked bicyclic skeletons (eg. benzodiazepines, indolizinoindole, functionalized dibenzofurans)[7] Although structurally different, covalent- or non-covalent restriction of conformation enable them to orient the peptide chains to the same direction as done by residues at i+1 and i+2 positions in natural systems.

What is the simplest turn-inducing segment one can have? While looking for such scaffolds, we came across sulfamide class of compounds which contain NH-SO_2-NH group substituted symmetrically/unsymmetrically at the ends, or existing as part of a cyclic structure.[8] Bing Gong *et al*. have elegantly shown the self-assembly profiles of a number of simple sulfamides.[9] We however noticed that the ability of this moiety to induce and stabilize specific conformations in peptides remains to be explored, and if successful, could lead to new systems with applications in both medicine and material science. We envisioned that rotational restriction brought about by electron delocalization across N-S-N bonds, coupled with mutual exclusion of groups adjacent to nitrogen atoms to avoid steric crowding, could make it useful as a turn inducer in peptides. As a first step to explore this possibility, we have synthesized a series of symmetric sulfamido peptides (Figure 1), and have systematically studied their conformation and self- assembly profiles. They can be considered as inducers of parallel β-sheet like structures based on the directionality of peptide chains. A number of reports highlighting the ability of rationally designed diamine- or diacid spacers to facilitate formation of such structural motifs are available, and are equally important as those used in the design of antiparallel sheets.[10] Our investigations in this direction involving peptides **1a-d** showed that the sulfamide group not only facilitates folding but also promotes unique supramolecular topologies like helical stacks and hairpin sheets through a combination of inter- and intramolecular secondary interactions (Figure 1b). These findings are elaborated below.

Preparation of sulfamides **1a-1d** typically involves reaction of appropriate amino acid methyl ester with sulfuryl chloride to get a diester intermediate, hydrolysis, and subsequent coupling with the second amino acid component under EDCI condition.

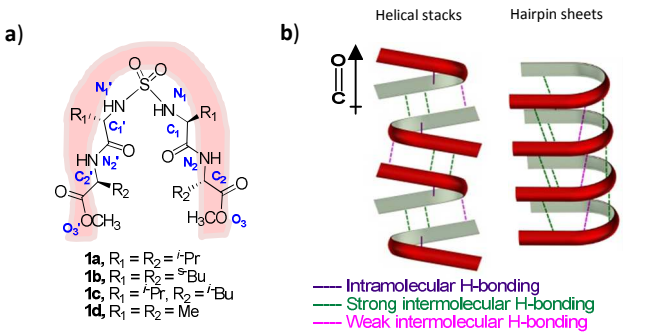
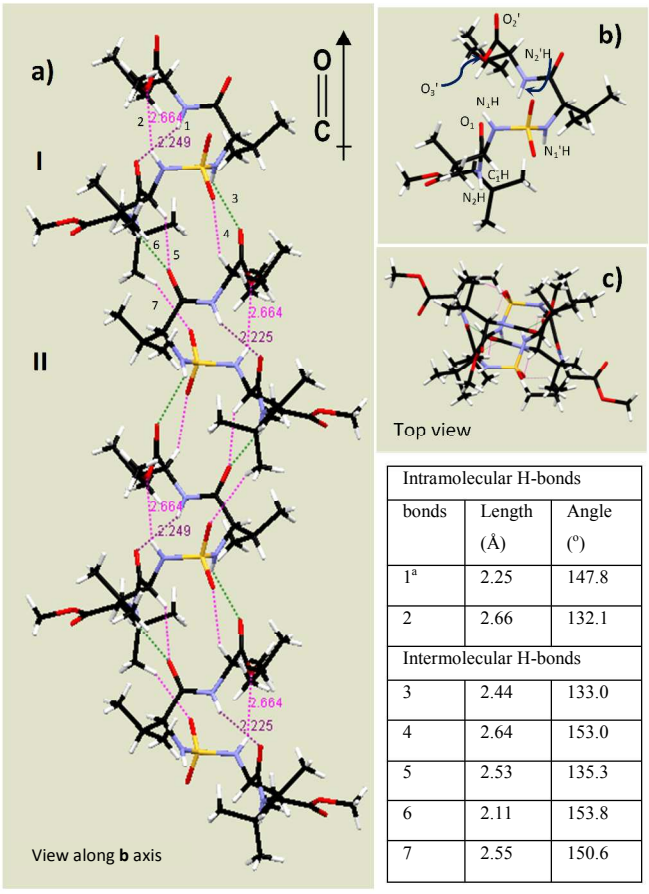


Fig. 1. a) Structures of sulfamido peptides **1a-1d**; b) Cartoon representation of helical stacks and hairpin sheets.

Their synthetic- and characterization details are presented in the supporting material. Due to symmetry, their ^{1}H NMR spectra had

peaks corresponding to only one half of the molecule. HRMS data however could unambiguously confirm their molecular integrities.

These peptides have different combinations of hydrophobic groups at 1,1' and 2,2' positions. They were chosen to understand the effect of side-chain chirality and branching on the overall conformation. To get an insight into these, X-ray quality crystals were secured by slow evaporation of their solutions in methanol and diffraction analyses were carried out. All, except **1d** gave orthorhombic unit cells in $P2_12_12_1$ space group. One of the astonishing features in the crystal structure of the di-Val derivative **1a** was the presence of a 10-memberd NH···O=C hydrogen bonding involving N_2'H and O_1 (bond 1, Figure 2a), akin to that seen in natural systems. Though short, with an N-N distance of ~2.65 Å, orientation of sulfamide NHs to opposite faces, and outward dispositioning of adjacent isopropyl groups, essentially created a turn in the backbone. The two arms were slightly twisted with respect to the N-S-N plane which allowed the molecules to form perfect helical stacks (Figure 2a). Another attraction was the presence of a rare array of weak- and strong intermolecular hydrogen bonds which aligned the amide groups vertically and the side chains horizontally to give a cylindrical appearance to the overall assembly. A closer examination of the lattice revealed that this supramolecular helix is in fact formed by alternate arrangement of conformers (I and II) related by pseudo two-fold screw operation.



Intramolecular H-bonds		
bonds	Length (Å)	Angle (°)
1[a]	2.25	147.8
2	2.66	132.1
Intermolecular H-bonds		
3	2.44	133.0
4	2.64	153.0
5	2.53	135.3
6	2.11	153.8
7	2.55	150.6

Fig. 2. a) Helical stacks from peptide **1a**; b) isomorph I with atom labelling; c) view along c axis; [a] For isomorph II, the N_2'H ··· O_1 bond length is 2.23 Å and the angle is 148°.

Orientation of amide C=O and NH bonds parallel to the helix-axis facilitated intermolecular hydrogen bondings between O_1' and O_2' of one arm with N_2H and N_1'H of another molecule on the top as shown (bonds 6 and 3 respectively, Figure 2a). This is reminiscent of the arrangement of amide C=O and NH groups in natural peptide-helices which is responsible for the net dipole.

As evident from Figure 2a, the central sulfonyl group of each molecule bridged those on either side through weak C-H···O interactions, making the hydrogen bonding network continuous (bonds 4 and 7). The weak intramolecular hydrogen bonding with N_1H and O_3' (bond 2), and bifurcated interaction between C_1H and O_1' (bond 5) were other contributing factors for the overall assembly. The side chains and carboxylate moieties were oriented radially outward with respect to the central hydrogen bonded helical core as shown in Figure 2c. Such supramolecular topology through self-organization of simpler units, which individually are conformationally restricted through intramolecular hydrogen bonding, is very rare; despite having a discontinuous backbone, the overall hydrogen bonding network is similar to that in peptide helices, which is also remarkable.

The fact that the observed assembly profile arises out of a preference became clear when we looked at the crystal structures of other members in this series, namely **1b** and **1c**. The former is based solely of isoleucine and hence possess sec-butyl group at each of 1,1' and 2,2' positions, whereas the latter has Valine at 1,1'- and Leucine at 2,2' positions. The 10-membered intramolecular hydrogen bonding and helical-stacking through secondary interactions involving properly aligned amide C=O and NH groups were present in these cases as well (Figures 3a and 3b).

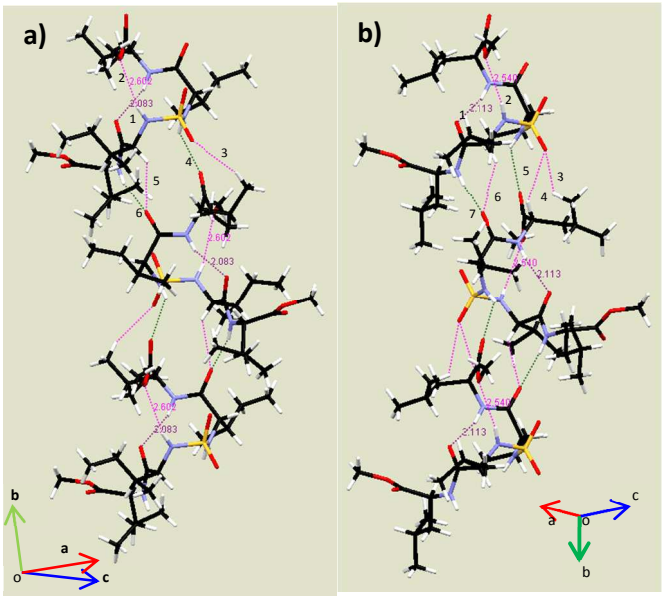


Fig. 3. a) Helical stacking in **1b**, and that in **1c** (b); atoms are labelled in the same manner as that in **1a**.

In the case of di-Ileu derivative **1b** (Figure 3a), the intramolecular hydrogen bonding between N_2'H···O_1 (2.08 Å, ∢ 158.1) was found to rigidify the turn (bond 1). As in the case of **1a**, the bend conformation in this case was also supported by a weak

$N_1H\cdots O_3'_{(sp3)}$ hydrogen bonding (bond 2; 2.60 Å, ∢ 134.7). The N_2H and $N_1'H$ atoms at the same time maintained interactions with O_1' and O_2' of the molecule underneath (bond 6, $N_2H\cdots O_1'$ = 2.03 Å, ∢ 163.63; bond 4, $N_1'H\cdots O_2'$ = 2.3 Å, ∢ 133.47). Presence of additional weak $C_1H\cdots O_1'$ (bond 5) and $C_{2\gamma}'$-H…O_1=S (bond 3, bifurcated) interactions involving backbone and side chain elements gave continuity to the bonding network as was seen in **1a** ($C_{2\gamma}'H$ represents the hydrogen atom attached to the CH_3 group of the second Ileu residue).

A high degree of consistency in bonding network was also observed in **1c** (Figure 3b). Apart from the 10-membered hydrogen bonding between $N_2'H\cdots O_1$ (bond 1; 2.11 Å, ∢ 156.95), the weak intramolecular $N_1H\cdots O_3'_{(sp3)}$ hydrogen bonding (bond 2; 2.54 Å, ∢ 132.38) and the intermolecular $N_2H\cdots O_1'$ & $N_1'H\cdots O_2'$ interactions involving adjacent molecules (bond 7, $N_2H\cdots O_1'$, 2.02 Å, ∢ 170.3; bond 5, $N_1'H\cdots O_2'$, 2.33 Å, ∢ 132.8) were found contributing to the assembly as shown in Figure 3b. In addition, the bifurcated weak interaction of O_1' with C_1H of the molecule on the top (bond 6; $C_1H\cdots O_1'$, 2.61 Å, ∢ 134.4) and similar CH···O interaction between sulfonyl oxygen with $C_2'H$ (bond 4) and $C_{2\delta}'H$ (bond 3) of the molecule underneath ($C_2'H\cdots O_1$=S, 2.65 Å, ∢ 137.9; $C_{2\delta}'H\cdots O_1$=S, 2.7 Å, ∢ 122.4) were part of the closed array of intermolecular secondary interactions responsible for the supramolecular helix formed here.

Hydrogen bonded helical arrays in **1a-c** in fact arise due to a twist in the peptide turn. β-branching in amino acid residues at 1,1' positions is likely responsible for this conformational bias which became obvious when the structure of **1d** was analyzed. It gave triclinic crystals under P_1 space group. Having alanine with methyl groups at 1,1' positions, this molecule could assume a perfect 'U'-shape without affecting the orientation of sulfamide NHs with respect to the backbone (Figure 4). Surprisingly, there was no intramolecular hydrogen bonding to stabilize the turn which underscores the ability of sulfamide group to direct and stabilize a bend conformation by itself. At a supramolecular level, this led to an extended assembly which can be called as 'hairpin sheet' to indicate both the conformation of the building block and the backbone alignment. A view of the arrangement along c axis is shown in Figure 4. This arrangement enabled O_1 of each molecule to have bifurcated hydrogen bonding with N_2H (bond 3, 2.12 Å, ∢ 172.87) and C_1H (bond 2, 2.59 Å, ∢ 148.57) of another one in front. The N_1H and N_2H' of each molecule at the same time had hydrogen bondings with S=O (bond 1; 2.07 Å,∢ 153.5) and O_1' (bond 4; 2.08 Å, ∢ 176.15) respectively. The weak interaction between $C_{2\beta}'H$ with the sp^3 oxygen atom of the ester O_3' (bond 5; 2.56 Å, ∢, 156.1) was also consistent and continuous throughout the hairpin sheet.

As most of the donor-acceptor sites in **1a-c** were utilized for intermolecular interactions leading to one-dimensional assembly, the packing of resulting helical arrays/hairpin sheets in their lattices had stabilizations mainly through clustering of hydrophobic side chains; in **1b-d**, the sulfonyl oxygen also had weak CH···O type interaction with molecules on adjacent column (supporting information). Interestingly, the close packing of supramolecular helices in **1b** and **1c** with clustered alkyl chains seemed analogous to the arrangement in Leucine zippers where the proper placement of side chains are responsible for the super secondary structure.

While deriving conclusions from solid-state structures of organic molecules with rotatable bonds, it is important to understand that the conformational preference, if any, is an outcome of interplay between many competing factors. Entropic considerations, solvation effects, along with inter- and intramolecular secondary interaction possibilities play specific roles in the selection of molecular conformation and the overall lattice. With due consideration of these, analysis of the structures of **1a-d** reveal the following. β-branching in amino acid residues at 1,1' positions in general induce a twist in the turn which, at a supramolecular level, enable the molecules to form helical stacks following 2_1 or pseudo two fold screw (in **1a**). The continuous and closed array of weak and strong

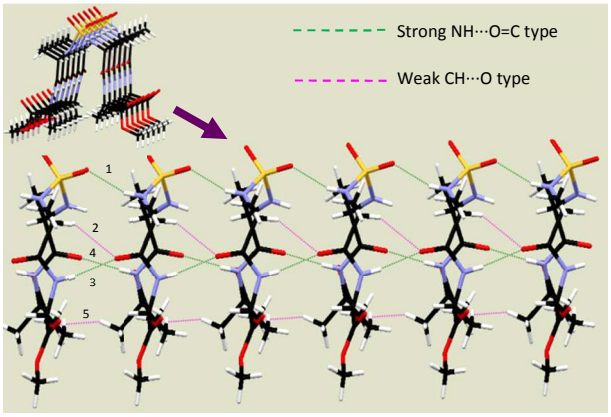


Fig. 4. Assembly of **1d** in the crystal lattice.

hydrogen bondings between molecules make the assembly appear like a helix with the amide C=O and N-H groups parallel to the helix axis, and side chains perpendicular to it. This is comparable to the orientation of these groups in natural peptide helices. Apart from demonstrating the first example of peptide turn through just one atom (sulfur of the sulfamide group, as NHs are part of the amino acid residues) supported additionally by 10-membered intramolecular hydrogen bonding, this report brings the unique manifestation of cylindrical arrays of weak and strong hydrogen bondings, making the observations relevant to the domains of crystal engineering and peptide science alike.[11] The pseudo two-fold screw axis seen in **1a** having two molecules in the asymmetric unit is also notable. The isomorphism in this case seems to result from the tendency of the system to accommodate maximum number of inter- and intramolecular secondary interactions in the given lattice. Availability of large number of intermolecular interactions for extended assembly could be a driving force for the selection of 'U'-shape in **1d**. This is also supported by outward orientation of relatively less bulky methyl groups at 1,1'-positions. Nevertheless, its preference for a perfect β-turn like structure without the involvement of intramolecular hydrogen bond is unique.

Helical columnar assemblies from functionalized ureidotriazines reported by Meijer and co-workers;[12] supramolecular helices and sheets from pseudopeptides having cores based on malonic acid-, 1,1-cyclopropane dicarboxylic acid or 1,1-cyclobutane dicarboxylic acid;[13] and helical assemblies from: i) calixarenes substituted with but-3-yn-2-ol groups,[14] ii) 8-oxoinosine- and 8-oxoguanosine derivatives,[15] and iii) amphiphilic pyrene

derivative with BocTrp counter ions,[16] are some of the notable examples from literature showing the power of self-assembly in the development of higher order supramolecular architectures. With the shortest spacer inducing the turn conformation and cylindrical arrays of weak and strong secondary interactions stabilizing the helical/hairpin sheet structures, the lattices of **1a-d** are new additions to this field of science. Since there is high consistency in the pattern of secondary interactions which form the backbone of the assembly, this class of compounds also offer promise in the design of new mesomorphic functional materials.

Conclusions

A group of sulfamido-peptides was synthesized and studied as part of our pursuit towards simplest structural units capable of inducing and stabilizing hairpin conformation. With a sulfonyl group bridging their N-terminus, these peptides not only adopted a perfect bend conformation with 10-membered intramolecular hydrogen bonding, but also showed remarkable tendency to self-organize into specific supramolecular topologies through directed array of weak and strong interactions. Helical stacks with most of the amide C=O and NHs oriented towards opposite poles, and the side chains distributed radially outward, was reminiscent of the arrangement of these groups in natural peptide helices. This was consistently seen in all systems having β-branched amino acid residues at 1,1' positions. When there were no sterically demanding groups at these positions as in the case of alanine derivative **1d**, the perfect U-shape allowed the molecules to give an extended hairpin sheet-like structure.

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Notes and references

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Electronic Supplementary Information (ESI) available: Experimental details, NMR spectra, mass analysis and crystallographic summaries. CCDC 980490, 980491, 980493 and 1009176 corresponding to compounds **1a**, **1b**, **1c** and **1d** respectively. See DOI: 10.1039/c000000x/

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Graphical Abstract:

Peptide Turns Through Just 'One Atom'! Sulfamide Group Nucleates Folding and Stabilizes New Supramolecular Topologies in Short Peptides

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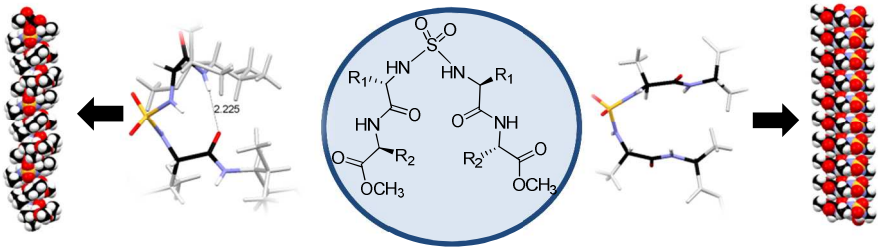

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