This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Reversal of H-bonding Direction by N-Sulfonylation: A Case Study With a Synthetic Reverse-Turn Peptide Motif‡


Received (in XXX, XXX) Xth XXXXXXXX 20XX, Accepted Xth XXXXXXXX 20XX
DOI: 10.1039/b000000x

This communication depicts an intriguing example of hydrogen-bonding reversal upon introduction of sulfonyl linkage at the N-terminus of a synthetic reverse-turn peptide motif. The ready availability of two sulfonyl oxygen atoms, as hydrogen-bonding acceptors, combined with the inherent twisted conformation of sulfonylides are seen to act as switches that engage/disengage the hydrogen-bond at the sticky ends/termini.

Introduction

Hydrogen-bonding, one of the prominent non-covalent interactions, plays a significant role in rigidifying the conformation of biopolymers – especially peptides and proteins. The highly directional nature of H-bonding is the primary cause for the precise folding phenomenon in various protein secondary structures like reverse turns, helices, β-sheets, etc. Reverse turns are the smallest secondary structural units with wide range of functions in biological systems and their mimics are one of the most preferred motifs for the development of peptide derived drugs. Introduction of sulfonylamide functionality into peptides provides a solution for the limited usage of proteolytically unstable peptide drugs. Moreover, the modified peptido-
sulfonylamide linkages can act as an interesting structural motifs for peptidomimetics due to their immense bio-active properties. The key feature of sulfonylides, an efficient surrogate of carboxamide linkage, is the presence of two oxygen atoms that enhances the hydrogen-bonding possibilities. In addition, the limited rotation barrier about the S-N bond, along with the dihedral disparity of ~90° (compared to 180° of carboxamides) renders twists to peptide chains.

In this regard, the use of orthanilic acid has earned special attention due to its intrinsic feature of inducing reverse turn formation as well as structural rigidification in peptides. Our earlier studies on orthanilic acid ($^5$Ant) and $^3$proline (Pro)-derived dipeptides led to the discovery of reverse turn motifs featuring strong C-9 or C-11 inter-residual H-bonding in the sequence order of β(1) and α(2), respectively. In earlier investigations, it was found that when $^5$Ant was conjoined with Pro - a conformationally constrained amino acid, it preserved the pseudo-β-turn assembly, by assuming the highly deviated C-S-N-C dihedral angle (α) = 163° (α′ ~ 160° for carboxamide analogues). This instance demonstrated that the fundamental torsional preference of $^5$Ant (sulfonylamide bond) could be dramatically modulated by the influence of adjoining residues. Extending the torsional restraints by addition of another Pro unit to the N-terminus of the $^5$Ant-Pro turn motif also retained the similar conformational features, as seen in peptide 1.

Fig. 1: Molecular structures of $\text{Xaa}^5\text{Ant-Yaa}$ turn motifs with the observed hydrogen-bonding patterns. Note: The H-bonding sites and their orientation have been highlighted.

Intriguingly, substitution of the N-terminus carboxamide in 1 by sulfonylamide, as in 2-4, resulted in dramatic conformational change which could be clearly seen from their crystal structures (Fig. 2). Sulfonylamides 2 and 3 feature a terminal C-14 hydrogen-bonding network with d(N-H-O) = 2.44Å. The hydrogen-bonding interactions were seen to be involving oxygen of SO of the i-th residue and the NH of the (i + 4)th residue, in the backward direction (5→1), akin to typical α-turns (5 residues in C-13 turn). And in 4, wherein the C-terminal Pro was replaced with constraint amino acid 2-aminoisobutyric acid (Aib), a weak C-7 hydrogen-bonding is also observed, in addition to a C-11 hydrogen-bonding.
Results and Discussion

Synthesis

Compounds 1-7, required for the present study, were synthesized using multi-step synthetic strategies, as depicted in schemes 1-3 (ESI, page S3-S5).

Conformational Analysis

Solid-state structural studies

From the previous studies, it was noted that both the dihedral constraints ‘ω’ of sulfonamide bond and ‘θ’ of S\textsuperscript{Ant} play crucial role in the formation and stabilization of S\textsuperscript{Ant}-Pro C-9 hydrogen-bonding.\textsuperscript{5} Torsion angle ‘ω’ in tripeptide 1 also adopted the unusual value of 152° as it featured the presence of S\textsuperscript{Ant}-Pro C-9 turn with hydrogen-bonding distance [d(N-H···O): 2.2Å] (Table 1, vide infra).

It was noted that the S\textsuperscript{Ant}-Pro motif, which was devoid of a pseudo β-turn (C-9 turn) in 2 and 3, showed the C-S-N-C dihedral angle ‘ω’ close to a value of 66°, which is of a typical sulfonamide bond. The three-dimensional architecture of the tripeptide with characteristic C-14 hydrogen-bonding was well evidenced from solid-state single crystal X-ray diffraction studies of 2 and 3. Thus, the sulfonamide-to-carboxamide modification at the N-termini completely abolished the C-9 conformation observed in 1, indicating the significance of the twisted sulfonamide bond in bringing the hydrogen-bonding sites into proximity and thereby, altering the hydrogen-bonding patterns as observed in the carboxamide analog 1.

In order to explore the role of torsional requisites causing the formation of this 5-residue turn featuring a planar aromatic residue, we carried out further structural alterations within the tripeptide sequence, including the replacement of terminal pro residues with another constrained amino acid 2-aminoisobutyric acid (Aib) - an unnatural α-aminoacid with unique preferences of dihedral angles to induce folding in peptides.\textsuperscript{7} The significance of the dihedral angle constraints and their structural implications were thereby expounded. Substitution effects within the turn motif resulted in deleterious effect on the C-14 hydrogen-bonding pattern, when the N- and C-terminal prolines were swapped with Aib. This modification also revealed the involvement of an additional ‘NH’ as a hydrogen-bond donor unlike to that of proline. In the tripeptide 4 (Fig 2), the Aib at the N-terminus showed the involvement of Aib ‘NH’ in an 11-membered hydrogen-bonding with proline ‘SO’ in the backward direction (4←1) [d(N-H···O): 2.3Å]. The turn system also featured a weak 7-membered H-bonding between the terminal sulfonyl ‘O’ and S\textsuperscript{Ant} ‘NH’, in the backward direction (3←1) [d(N-H···O): 3.0Å], similar to typical β-turns (3 residue C-7 turn).\textsuperscript{9}

Table 1. Table showing the crystal data analysis of peptides 1-7

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>αXaa</th>
<th>αS\textsuperscript{Ant}</th>
<th>αYaa</th>
<th>C-S-N-C dihedral angle for sulfonamide bond</th>
<th>Type of inter-residual H-bonding\textsuperscript{a}</th>
<th>Torsion Angle C=O–H-N (deg.)</th>
<th>H-bonding Distance d(NH–O) (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-67.00</td>
<td>146.81</td>
<td>-127.75</td>
<td>168.11</td>
<td>C-9</td>
<td>155.17</td>
<td>2.216</td>
</tr>
<tr>
<td>2</td>
<td>-86.86</td>
<td>-31.16</td>
<td>-178.38</td>
<td>-1.01</td>
<td>C-14</td>
<td>-91.78</td>
<td>66.55</td>
</tr>
<tr>
<td>3</td>
<td>-118.80</td>
<td>11.82</td>
<td>167.80</td>
<td>1.25</td>
<td>C-14</td>
<td>-58.74</td>
<td>58.78</td>
</tr>
<tr>
<td>4</td>
<td>-107.89</td>
<td>6.11</td>
<td>-154.77</td>
<td>0.51</td>
<td>Weak C-7 &amp; C-11</td>
<td>-127.91</td>
<td>3.080</td>
</tr>
<tr>
<td>5</td>
<td>-67.21</td>
<td>-45.70</td>
<td>160.28</td>
<td>-9.41</td>
<td>-97.78</td>
<td>-10.20</td>
<td>3.292</td>
</tr>
<tr>
<td>6</td>
<td>- -</td>
<td>-158.56</td>
<td>-0.69</td>
<td>86.66</td>
<td>-163.12</td>
<td>-0.83</td>
<td>-161.98</td>
</tr>
<tr>
<td>7</td>
<td>- -</td>
<td>148.56</td>
<td>6.17</td>
<td>-68.56</td>
<td>120.93</td>
<td>-</td>
<td>2.132</td>
</tr>
</tbody>
</table>

\textsuperscript{a}inter-residual hydrogen-bonding.
The substitution of N-terminus Pro with Aib abolished the C-9 hydrogen-bonding too, a prominent feature of β-Ant-Pro turn systems and the resultant crystal structure, instead, showed the C-6 hydrogen-bonding of 3Ant in 5 (Fig. 2). The absence of 9-membered hydrogen-bonding was due to the presence of dihedral constraints, as discussed earlier. Replacement of L-Pro of β-Ant-Pro turn motif with D-Pro and NMe-Aib resulted in the persistence of C-9 hydrogen-bonding networks in 6 (Fig. 2) and 7 (ESI S72). In those cases, the ‘ω’ remain 163° and 155° for 6 and 7, respectively. This experimentation suggested that the N-terminal constraints of Pro and NMe-Aib residue have major roles in maintaining the large value for C-S-N-C dihedral angle ‘ω’ for sulfonamide bond.10 The absence of β-Ant-Pro 9-membered hydrogen-bonding in 2-4 is mainly due to the dihedral constraints offered by N-terminal Pro/Aib residues.

The conformational changes that occur upon backbone modification, governed by their Φ and ψ values, are summarized in table-1 (vide supra). From the table, it is apparent that the sulfonamide group has provided the prolific use of its acceptor sulfonyl oxygens for hydrogen-bonding interactions, although weak, and induce folding owing to its inherent twisted geometry.

Solution-state NMR studies

The solution-state behavior of 1 was also evaluated from 2D NOESY studies (ESI S52-S53), which supported the presence of C-9 H-bonding pattern. NMR DMSO-d6 titration (δNH1 = 0.08 ppm & δNH2 = 0.33 ppm) and variable temperature experiments (δNH1 = 0.07 ppm; Δδ/ΔT = -1.27 ppmK-1 & δNH2 = 0.23 ppm; Δδ/ΔT = -4.18 ppmK-1) in CDCl3 (ESI S61 & S64), validated the strength of the hydrogen-bonding interaction.

Also, the solution-state NMR 2D NOESY studies of 3 confirmed its C-14 hydrogen-bonded arrangement through the diagnostic NOEs like NH2 vs C1H, C1H vs C18H, C23H vs C22H & NH1 vs C15H (Fig. 3 and ESI S49-S50). The nature of hydrogen-bonding interactions (intra vs inter) were studied by DMSO-d6 titration (Fig. 4B) and variable temperature (Fig. 4C) studies in CDCl3, where both the NHs showed negligible chemical shift (δNH1 ≤ 0.13 ppm; ESI, S60 and S64), suggestive of robust intramolecular C-14 hydrogen-bonding.

Thus, the sulfonamide-to-carboxamide modification at the N-termini completely abolished the C-9 conformation observed in 1, indicating the significance of the twisted sulfonamide bond in bringing the hydrogen-bonding sites into proximity and thereby, altering the hydrogen-bonding patterns as observed in the carboxamide analog 1.

![PyMol rendered crystal structure](A), stacked plots of NMR DMSO-d6 titration studies (B) and variable temperature studies (C) of 3 (400 MHz, 5 mmol, CDCl3).

In order to explore the role of torsional requisites causing the formation of this 5-residue turn featuring a planar aromatic residue, we carried out further structural alterations within the tripeptide sequence, including the replacement of terminal pro residues with another constrained amino acid 2-aminoisobutyric acid (Aib) - an unnatural α-aminoacid with unique preferences of dihedral angles to induce folding in peptides.2 The significance of the dihedral angle constraints and their structural implications were thereby expounded. Substitution effects within the turn motif resulted in deleterious effect on the C-14-hydrogen-bonding pattern, when the N- and C-terminal prolines were swapped with Aib. This modification also revealed the involvement of an additional ‘NH’ as a hydrogen-bond donor unlike to that of proline. In the tripeptide 4 (Fig 2), the Aib at the N-terminus showed the involvement of Aib ‘NH’ in an 11-membered hydrogen-bonding with proline ‘SO’ in the backward direction (4v-1) [d(N-H-O): 2.3Å]. The turn system also featured a weak 7-membered H-bonding between the terminal sulfonyl ‘O’ and 5Ant ‘NH’, in the backward direction (3v-1) [d(N-H-O): 3.0Å], similar to typical γ-turns (3 residue C-7 turn).9 This was clearly revealed

![Figure showing the selected 2D NOESY extracts of 3.](C)
in their solution-state 2D NMR studies as well (ESI S58–S59), solvent titration (ESI S63) and variable temperature studies (ESI S66). The solid-state behaviour of 5 was well reflected in the solution-state 2D NOESY studies (ESI S55–S56). The presence of intra-molecular hydrogen-bonding was vindicated from the negligible chemical shifts observed from DMSO-d6, titration studies (δNHH = 0.33ppm, δNH2 = 0.1 ppm & δNHS = 0.23ppm) and variable temperature NMR studies carried out in CDCl3 (δNHH = 0.33ppm; Δδ/ΔT = -6.6ppbK-1, δNH2 = 0.07ppm; Δδ/ΔT = -1.27ppbK-1, δNHS = 0.21ppm; Δδ/ΔT = -3.82ppbK-1) (ESI S62 & S67).

Conclusions

In summary, the work presented herein illustrates the reversal of hydrogen-bonding orientation at the termini, when a N-carboxyamide moiety is swapped with N-sulfonamide. The three-residue folded peptides mesyl-Pro-3-Ant-Pro 2 and 3 exhibited an unusual C-14 membered hydrogen-bonding pattern, unlike that of Piv-Pro-3-Ant-Pro 1 that adopts C-9 turn. The mutation of Pro to Aib at both termini validated the essentiality of Pro unit in the formation of C-14 hydrogen-bond. The different folding patterns are dependent on the terminal aminoaic residues (i and i+2) and presence of sulfonamide bond between the residues as evidenced from single crystal analyses and NMR studies. This work illustrates the implications of the conformational features of peptides when the N-terminus is swapped with sulfonamide group with its distinctive geometrical and hydrogen-bonding preferences - starkingly different when compared to its group with its distinctive geometrical and hydrogen-bonding preferences - starkingly different when compared to its

Acknowledgement

KNV, RVN & ASK thank CSIR (New Delhi) for research fellowships. This work was funded by CSIR Biodiversity network programme (BSC-0120).

Experimental procedures

Crystal X-ray Crystallographic Studies:

Crystallographic Data for the compounds 1, 2, 3, 4 and 5 were collected on SMART APEX-II CCD using Mo-Kα radiation (λ = 0.7107 Å) to a maximum θ range of 25.00°. Crystal to detector distance 5.00 cm, 512 x 512 pixels / frame, Oscillation / frame -0.5°, maximum detector swing angle = –30.0°, beam center = (260.2, 252.5), in plane spot width = 1.24, SAINT integration with different exposure time per frame and SADABS correction applied. All the structures were solved by direct methods using SHELXTL. All the data were corrected for Lorentzian, polarisation and absorption effects. SHELX-97 (Shelxtl) was used for structure solution and full matrix least squares refinement on F2. Hydrogen atoms were included in the refinement as per the riding model.

Crystal data for 1

Single crystals of 1 were grown by slow evaporation of the solution mixture of ethyl acetate and pet. ether. Colorless block crystal of size 0.49 x 0.42 x 0.31 mm3, was used for data collection, Temperature = 296(2) K, Wave length = 0.71073 Å

Quadrant data acquisition, F(000) = 1256, θ range = 1.76° to 28.29°, completeness to θ is 100 %. Goodness-of-fit on F2 = 1.067, C27H35BrN2O2S, M = 605.54. Crystals belong to Orthorhombic, space group P212121, a = 6.7186(2), b = 15.8096(5), c = 26.5176(7) Å, α = β = γ = 90°, V = 2816.66(14) Å3, Z = 4, De = 1.428 g/cc, µ (Mo–Kα) = 1.577 mm-1, 6952 total reflections, 4854 unique reflections, R value 0.0698, wR2 = 0.1124.

Crystal data for 2

Single crystals of 2 were grown by slow evaporation of the solution mixture of ethylacetate and pet.ether. Colorless needle crystal of size 0.64 x 0.19 x 0.13 mm3, was used for data collection, Temperature = 297(2) K, Wave length = 0.71073 Å

Quadrant data acquisition, Total scans = 4, F(000) = 1064, θ range = 2.19° to 25.49°, completeness to θ of 24.99 % is 100 %. Goodness-of-fit on F2 = 1.018, C12H21BrN2O2S2, M = 500.63.

Crystals belong to Monoclinic, space group C2, a = 18.6244(11), b = 8.4267(4), c = 16.0611(8) Å, α = 90, β = 93.118(5), γ = 90, V = 2516.92(2) Å3, Z = 4, Dc = 1.321 g/cc, µ (Mo–Kα) = 0.254 mm-1, 4429 total reflections, 4052 unique [I>2σ(I)], R value 0.0430, wR2 = 0.1171.

Crystal data for 3

Single crystals of 3 were grown by slow evaporation of the solution mixture of ethylacetate and pet.ether. Colorless needle crystal of size 0.65 x 0.33 x 0.29 mm3, was used for data collection, Temperature = 296(2) K, Wave length = 0.71073 Å

Quadrant data acquisition, F(000) = 616, θ range = 2.36° to 30.42°, completeness to θ is 95 %. Goodness-of-fit on F2 = 0.993, C12H21BrN2O2S2, M = 599.53. Crystals belong to Monoclinic, space group P21, a = 8.1060(12), b = 10.5928(16), c = 14.9832(2) Å, α = 90, β = 96.2738(8), γ = 90, V = 1278.83(3) Å3, Z = 2, Dc = 1.557 g/cc, µ (Mo–Kα) = 1.817 mm-1, 7389 total reflections, 5331 unique reflections, R value 0.0355, wR2 = 0.0875.

Crystal data for 4

Single crystals of 4 were grown by slow evaporation of the solution mixture of ethylacetate and pet.ether. Colorless block crystal of size 0.50 x 0.45 x 0.35 mm3, was used for data collection, Temperature = 296(2) K, Wave length = 0.71073 Å

Quadrant data acquisition, F(000) = 944, θ range = 2.53° to 28.10°, completeness to θ is 100 %. Goodness-of-fit on F2 = 1.082, C11H20N2O2S2, M = 446.54. Crystals belong to Orthorhombic, space group P212121, a = 9.5896(2), b = 14.7665(3), c = 15.8096(5), α = 90, β = 93.118(5), γ = 90, V = 26.5176(7) Å3, Z = 4, Dc = 1.428 g/cc, µ (Mo–Kα) = 1.577 mm-1, 9302 total reflections, 7203 unique reflections, R value 0.0346, wR2 = 0.0908.

Crystal data for 5

Single crystals of 5 were grown by slow evaporation of the solution mixture of ethylacetate and pet.ether. Colorless plate crystal of size 0.47 x 0.31 x 0.05 mm3, was used for data collection, Temperature = 297(2) K, Wave length = 0.71073 Å

Quadrant data acquisition, F(000) = 472, θ range = 1.65° to 30°, completeness to θ is 87 %. Goodness-of-fit on F2 = 1.027, C12H20N2O2S2, M = 446.54. Crystals belong to Monoclinic, space group P21, a = 7.7425(4), b = 10.0108(6), c = 13.8284(8) Å, α = 90, β = 90, γ = 90, V = 1766.54(14) Å3, Z = 2, Dc = 1.415 g/cc, µ (Mo–Kα) = 0.296 mm-1, 5303 total reflections, 4805 unique reflections, R value 0.0317, wR2 = 0.0819.
Crystal data for 6

Single crystals of 6 were grown by slow evaporation of the solution mixture of Dichloromethane and methanol. Colorless needle type crystal of approximate size 0.45 x 0.23 x 0.19 mm³, was used for data collection, Temperature = 297(2) K, Wave length = 0.71073 Å, Quadrant data acquisition, Total scans = 4, F(000) = 952, θ range = 2.56° to 28.31°, Goodness-of-fit on F² = 0.988, C₂H₅BrN₂O₂S, M = 466.35. Crystals belong to Orthorhombic, space group P2₁2₁2₁, a = 7.1146(3) Å, b = 8.0387(3) Å, c = 15.0865(6) Å, V = 800.21(6) Å³, α = 90.323(2), β = 90.054(2), γ = 109.252(2), Dc = 1.363 g/cc, µ (Mo–Kα) = 0.227 Å⁻¹. The C-S-N-C dihedral angle ‘φ’ for sulfonamide bond is ≤140°, the C=O bond is 1.249 Å, C-N bond is 1.358 Å, O-H bond is 0.924 Å.

Crystals data for 7

Single crystals of 7 were grown by slow evaporation of the solution of methanol. Colorless block crystal of approximate size 0.66 x 0.27 x 0.14 mm³, was used for data collection, Temperature = 297(2) K, Wave length = 0.227 Å, Quadrant data acquisition, F(000) = 348, θ range = 2.56° to 28.31°, Goodness-of-fit on F² = 0.71073 Å, Quadrant data acquisition, Total scans = 4, F(000) = 952, θ range = 2.56° to 28.31°, Goodness-of-fit on F² = 0.988, C₂H₅BrN₂O₂S, M = 466.35. Crystals belong to Orthorhombic, space group P2₁2₁2₁, a = 9.2942(10) Å, b = 13.9119(13) Å, c = 15.3096(17) Å, α = β = γ = 90°, V = 1979.5 (4) Å³, Z = 4, Dc = 1.565 g/cc, µ (Mo–Kα) = 0.71073 mm⁻¹, total reflections = 4865, 3065 unique reflections, R value 0.0432, wR = 0.0926.

Notes and references


2) CCDC no: of 1-7 are CCDC 1027031-1027037, respectively.