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Orthanilic acid-promoted reverse turn formation in peptides†‡

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Orthanilic acid (2-aminobenzenesulfonic acid, ^{S}Ant), an aromatic β-amino acid, has been shown to be highly useful in inducing a folded conformation in peptides. When incorporated into peptide sequences (*Xaa*-^{S}Ant-*Yaa*), this rigid aromatic β-amino acid strongly imparts a reverse-turn conformation to the peptide backbone, featuring robust 11-membered-ring hydrogen-bonding.

β-Amino acids, the next higher homologues of α-amino acids, provide considerable insight into designing synthetic peptides with improved proteolytic and structural stability.[1] Since β-amino acids are stable against proteolytic degradation, they are considered to be one of the key structural entities in drug design as well.[1a] The oligomers derived from β-amino acids form stable secondary structures such as helices,[2,3] sheets,[4] *etc.*, offering avenues for the structural exploration of diversely functionalized novel β-amino acid building blocks and further investigation of their conformational preferences.

Recent years have witnessed the development of diversely functionalized β-amino acids with varying degrees of conformational freedom on the backbone, with respect to the parent β-alanine – the only naturally existing β-amino acid among the β-amino acid family. Amongst the huge repertoire of β-amino acids, 2-aminocyclopropanecarboxylic acid (ACC),[5] 2-aminocyclopentanecarboxylic acid (ACPC),[6] 2-aminocyclohexanecarboxylic acid (ACHC),[7] and 2-aminobenzoic acid (anthranilic acid, Ant)[8] (Fig. 1) attain special status owing to their backbone rigidity coupled with considerable scope for backbone functionalization.[9] It is noteworthy that the secondary structures

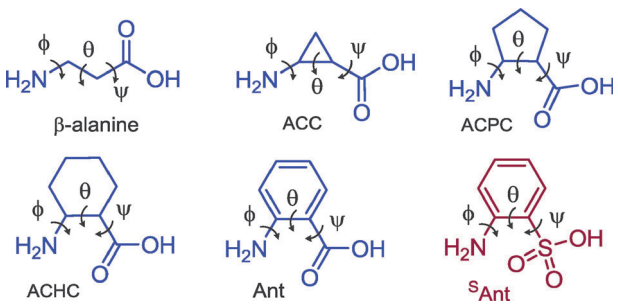

Fig. 1 Selected examples of β-amino acid building blocks.

attainable from some of these amino acid building blocks have found application in developing ligands for intervening protein–protein interactions.[10] Among the various plausible intracatenary hydrogen-bonded rings formed from β-amino acids, 11-membered hydrogen bonded ring is unusual and is considered as an alternative to Pauling's α-helix (13-membered hydrogen bonded ring)[11a] and also as the backbone extended analogue of the typical β-turn (10-membered hydrogen bonded ring).[11]

Herein, we demonstrate orthanilic acid (2-aminobenzenesulfonic, ^{S}Ant) as a unique aromatic β-amino acid with a very high propensity to induce a β-turn-like conformation in peptides, displaying robust 11-membered-ring intramolecular H-bonding interaction. With its amino and sulfonic acid groups attached to the rigid aromatic framework, ^{S}Ant is characterized by well-defined torsional angles and constraints. It is noteworthy that sulfonic acid groups have a tetrahedral geometry[12] which is in stark contrast to the that of planar carboxyl groups usually found in amino acids. Thus, we envisioned that introduction of the rigid ^{S}Ant residue into peptide sequences would not only cause rigidification of the peptide backbone but also might have a dramatic outcome in their conformation. Our efforts were also emboldened by the general observation that sulfonamides as a class of compounds are ushering into prominence owing to their potential use in the development of drug candidates to treat different ailments.[13] Using several peptide sequences (*Xaa*-^{S}Ant-*Yaa*), we could unequivocally demonstrate the utility of ^{S}Ant in

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† Dedicated to Prof. K. N. Ganesh on the occasion of his 60th birthday.

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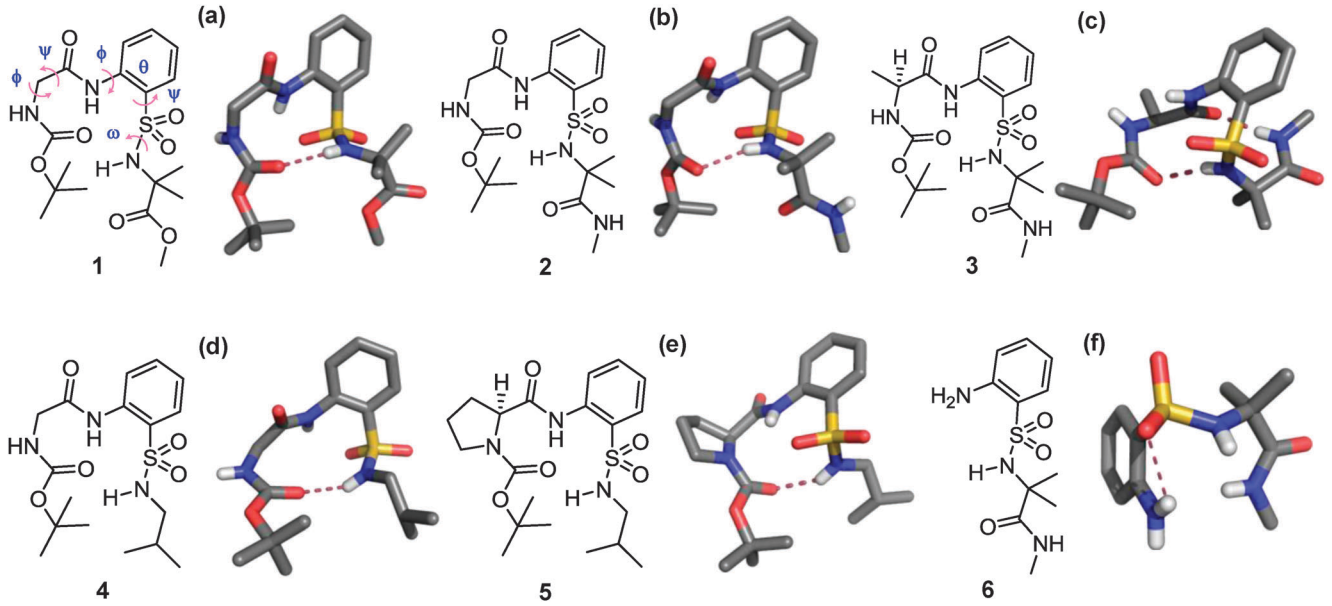

Fig. 2 Molecular structures (left) and their corresponding crystal structures (right) of peptides **1–6**. Hydrogens, other than the ones involved hydrogen bonding, have been omitted for clarity.

strongly promoting the reverse-turn conformation featuring 11-membered robust intramolecular H-bonding, as clearly evident from the solid and solution-state conformation of the peptides. The robustness of the C-11 H-bonding has been tested by modulating N- and C-termini of the peptides (**1–6**, Fig. 2). The N-terminus modification has been achieved by altering the residues ranging from the conformationally flexible glycine (Gly) to conformationally restricted proline (Pro). Similarly, the C-terminus has also been altered in an effort to understand the role of amino acid residues at this position.

All the designed peptides **1–5** commonly display two types of intramolecular H-bonding: one type involving 6-membered hydrogen bonding (C6 H-bonding) and another type involving strong 11-membered hydrogen bonding (C11 H-bonding) (Fig. 2). Whereas the C6 H-bonding is an *intra-residual* interaction, formed within ^{S}Ant itself, the C11 H-bonding is an *inter-residual* strong H-bonding interaction, formed of four residues between the C═O of the *i*th residue and the sulfonamide NH of the (*i* + 3)rd residue, in the backward direction (4 ← 1), as seen in typical β-turns. The hydrogen-bonding distances [*d*(H···A)] of the C11 reverse turns in **1–5** were found to be ranging from 2.08 Å (**1**) up to 2.25 Å (**5**), suggesting that the C11 H-bonded network is robust (Table 2). The hydrogen-bonding angle [Δ(D–H···A)] varies from 137° (**4**) to 169° (**1**). The dihedral angle descriptors of ^{S}Ant (Table 1), with its twisted conformation, are informative. Of particular note is the characteristic sulfonamide torsion (*ω*) of ^{S}Ant observed in **1–5** which is in the range of 63.9(2)° (**5**) to 93.5(3)° (**1**). It is noteworthy that in majority of the sulfonamide crystal structures reported in the literature,[14] the sulfonamide torsion *ω* has been found to be ∼90° (±30), which is in stark contrast to the *ω* of planar carboxamides (∼180° (±30)). The restriction of *ω* to ∼90°, obviously, imparts ^{S}Ant its twisted conformation which is translated into the peptide backbone.

Table 1 Backbone torsional angles observed in crystals **1–6**

	Gly/Ala/Pro$^{(i+1)}$			^{S}Ant$^{(i+2)}$		
No.	*ϕ* (°)	*ψ* (°)	*ϕ* (°)	*θ* (°)	*ψ* (°)	*ω* (°)
1	79.9(3)	11.1(4)	149.2(3)	−5.0(3)	−68.8(2)	−93.5(3)
2	86.7(2)	9.0(2)	134.9(2)	−5.9(2)	−62.7(2)	−90.7(1)
3	−75.7(4)	−24.6(4)	−102.5(4)	−0.2(4)	58.0(3)	91.4(3)
4	−55.9(7)	139.8(4)	128.6(5)	−4.8(6)	−67.7(5)	−71.2(4)
5	−85.6(2)	−6.8(3)	−151.9(2)	5.4(3)	69.9(2)	63.9(2)
6	—	—	—	0.7(3)	61.5(2)	77.2(2)

Table 2 Hydrogen-bonding parameters observed in crystals **1–5**[a]

	Hydrogen bonding parameters (C11 H-bonding)		
Compound no.	H···A (Å)	D···A (Å)	D–H···A (degree)
1	2.20	3.044(3)	169
2	2.17	2.992(2)	160
3	2.08	2.914(4)	162
4	2.20	2.887(6)	137
5	2.25	3.017(3)	148

[a] *Note*: For further details, see ESI (S57–S70).

Finally, in order to understand the role of ^{S}Ant in folding, we made analog **6** which is devoid of the N-terminus arm essential for the C-11 H-bonding. The crystal structure of **6** clearly reveals a folded conformation even in the absence of any *inter-residual* H-bonding which clearly vindicates the pivotal role of ^{S}Ant with its twisted conformation in inducing folding (Fig. 2f). As in all other cases, the usual *intra-residual* C-6 H-bonding of ^{S}Ant was observed in **6**. The torsional angles and H-bonding parameters evidently provide insight into the conformational rigidity imparted by ^{S}Ant, leading to the reverse turn conformation of the peptides. It is noteworthy that **3** displays one more C11 H-bonding between the C═O of the (*i* + 1) residue and NH of

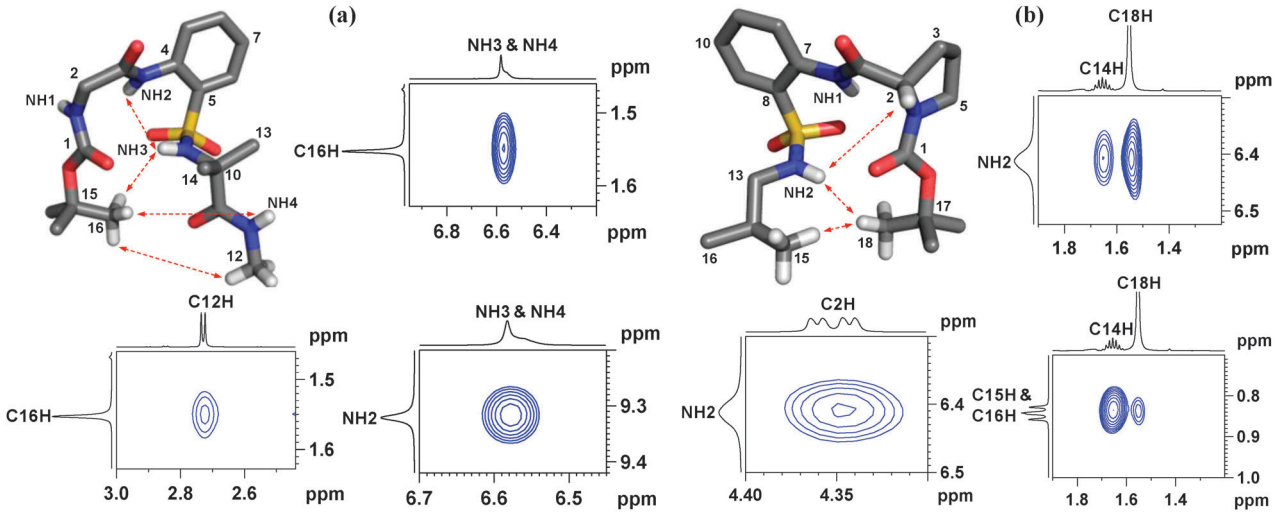

Fig. 3 Selected 2D nOe excerpts of **2** (a) and **5** (b) displaying C-11 membered hydrogen bonds ($CDCl_3$, 400 MHz and 500 MHz, respectively).

the $(i + 5)$ residue along with the regular reverse turn, described earlier. This intriguing conformational feature singles out **3** among others in the series, raising its prospects of being used for the development of foldamers with perfect back-to-back hydrogen bonding.[2]

The results of solution-state NMR studies firmly suggest that the solid-state conformation is prevalent in the solution state as well. The characteristic inter-residual nOe interactions supportive of the folded conformation are the diagnostic long range *inter-residual* dipolar couplings between the N- and C-termini groups. Some of the selected nOes (Fig. 3a) that support the reverse-turn conformation of **2** are ^{t}Boc (C16H) *vs.* NH3 and NH4, C16H *vs.* C12H and NH2 *vs.* NH3. Similarly, some of the selected nOes observed in **5** (Fig. 3b), which support its folded conformation are NH2 *vs.* C2H, NH2 *vs.* ^{t}Boc (C18H) and C15H *vs.* C18H. Compounds **3** and **4** also display characteristic *inter-residual* nOes supporting their folded conformation (ESI,‡ pages S42–S51). The negligible chemical shift [$\Delta\delta$ (NH) $<$ 0.33 ppm (ESI,‡ pages S29–S32)] observed in the DMSO-d_6 titration experiments strongly support the intramolecular nature of the amide protons involved in the 11-membered hydrogen bonding in compounds **2–5**. The results of variable temperature studies of compounds **2–5** [temperature coefficients $(\Delta\delta/\Delta T) \sim 3.8$ ppb) (ESI,‡ pages S33–S36)] further support the strong intramolecular H-bonding pattern observed in these peptides.

In conclusion, we could demonstrate that orthanilic acid (2-aminobenzenesulfonic acid, ^{S}Ant) is a strong reverse-turn inducer when incorporated into peptide sequences (*Xaa*-^{S}Ant-*Yaa*). All the designed peptides containing ^{S}Ant displayed 11-membered-ring hydrogen bonding, formed in the backward direction of the sequence as seen in native four-residue β-turns, clearly vindicating the significance of the conformational rigidity offered by ^{S}Ant to efficiently promote folding. The results of diverse structural perturbations carried out in-and-around the turn segment suggest that this reverse turn conformation is robust. Comparison of the crystal structures[15] clearly revealed that it is the typical torsional constraint of orthanilic acid (^{S}Ant) that is primarily responsible for inducing folding in peptides.

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