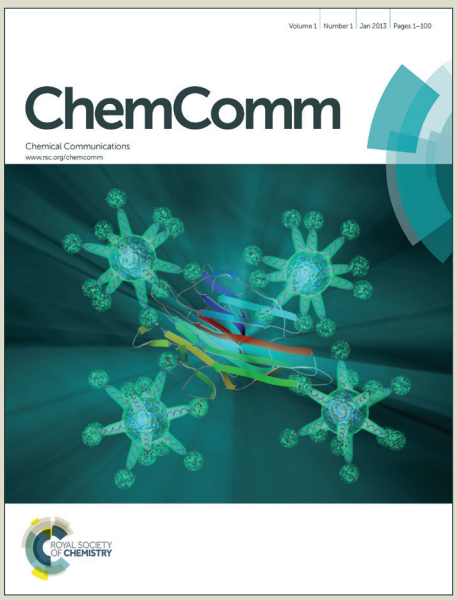
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Conformational modulation of peptide secondary structures using β-aminobenzenesulfonic acid‡

Sangram S. Kale,[a] Shrikant M. Kunjir,[b] Rupesh L. Gawade,[c] Vedavati G. Puranik,[c] P. R. Rajamohanan,[b] Gangadhar J. Sanjayan*[a]

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

This communication describes the influence of β-aminobenzenesulfonic acid (^{S}Ant) on the conformational preferences of hetero foldamers. The designed (Aib-^{S}Ant-Aib)$_n$ and (Aib-^{S}Ant-Pro)$_n$ oligomers display well-defined folded conformation featuring intramolecular mixed hydrogen bonding (7/11) and intra-residual (6/5) H-bonding interactions, respectively.

Modulation and control of secondary structure of peptides and proteins are vital to the *de novo* design of synthetic proteins with intriguing structural architecture and function. Introduction of functionalized homologated amino acid residues (β, γ and δ) in the native peptide leads to conformationally diverse secondary structural architectures.[1] In the past decade, an enormous amount of effort has been expended to diversify the conformational space of peptides using this approach.[1-3] The extent of knowledge generated so far clearly aids the *de novo* design of novel peptide architectures – mimicking any type of secondary structure. Furthermore, unnatural amino acids offer considerable advantage over their natural counterparts, primarily owing to their proteolytic stability.[4]

Among the diverse class of unnatural amino acids, α-aminoisobutyric acid (Aib) has attracted major attention of researchers for modulating secondary structures of polypeptides.[5] The homooligomer of Aib [(-Aib)$_n$] has been shown to display a 3_{10}-helical architecture,[6] though a sequentially inserted Pro analogue [(Aib-Pro)$_n$] exhibits a β-bend ribbon conformation.[7] This conformational disparity clearly suggests that the secondary structural modulation greatly depends on the dihedral angle constraints of amino acids. Herein we demonstrate the utility of β-aminobenzenesulfonic acid in modulating peptide secondary structures. Our primary objective was to investigate the effect of the torsional rigidity of ^{S}Ant on the conformational features of-

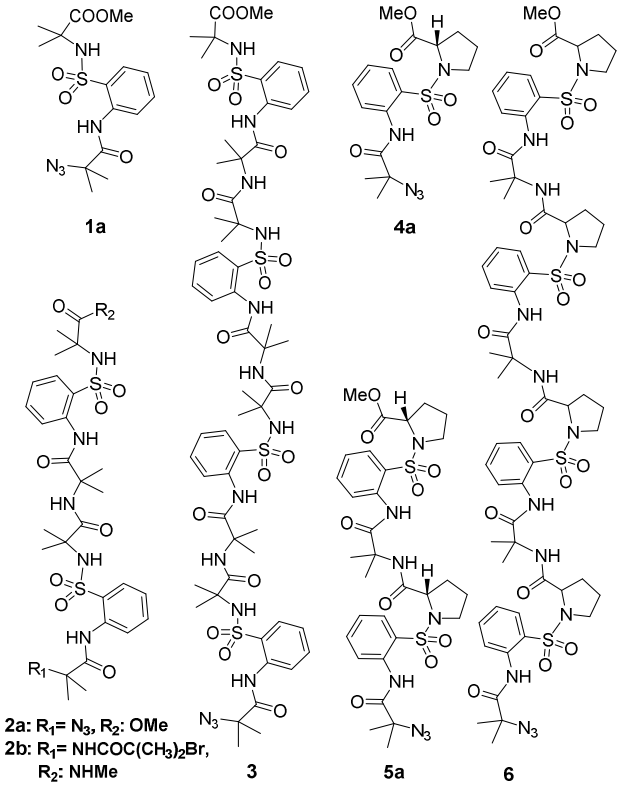


Fig. 1: Molecular structures of oligomers described in this work.

- well studied peptide sequences. In this context, we designed two sets of oligomers having tripeptide building blocks Aib-^{S}Ant-Aib and Aib-^{S}Ant-Pro (Fig. 1) to investigate the extent of conformational modulation in peptides enforced by ^{S}Ant.

Extensive efforts of crystallization trials culminated in the formation of crystals of **2a** and **5b** (Fig. 2). Analysis of the crystal data of hexapeptide **2a** revealed that (Aib-^{S}Ant-Aib)$_n$ oligomers display three different types of intramolecular hydrogen-bonding patterns (Fig. 3a): (i) 11-memberd ring H-bonding between C=O of Aib$_2$ with NH5 [d(C=O…H-N) 2.09 Å, Δ(D-H…A) 162°], (ii) 12-membered ring H-bonding between NH2 and S=O of ^{S}Ant$_2$ [d(C=O…H-N) 2.42 Å, Δ(D-H…A) 123°], and (iii) 7-membered ring H-bonding between NH2 and S=O of ^{S}Ant$_1$ [d(C=O…H-N) 2.10 Å, Δ(D-H…A) 162°]. Compound **2a** also shows two intra-residual 6-membered H-bonding, formed within ^{S}Ant itself. The

[a] *Division of Organic Chemistry, National Chemical Laboratory, Homi Bhabha Road, Pune 411 008, India; E-mail: gj.sanjayan@ncl.res.in; Web: http://nclwebapps.ncl.res.in/gjsanjayan/index.html*
[b] *Central NMR Facility, National Chemical Laboratory, India.*
[c] *Center for Materials Characterization, National Chemical Laboratory,*
‡*Dedicated to Prof. C.N. R. Rao on the occasion of his 80th birthday.*
†Electronic Supplementary Information (ESI) available: General experimental procedures, ^{1}H, ^{13}C, DEPT-135 NMR spectra and ESI mass spectra of all new compounds are included. See DOI: 10.1039/b000000x

torsion angles (N-H…O=C/S) of C11, C12 and C7 H-bonding are 83.62°, 114.63° and -74.04°, respectively. The H-bonding angles [Δ(S/C=O...N)] of C11, C12 and C7 H-bonding are 113.4°, 153.6° and 97.0°, respectively. The torsional angles ψ and ω of the ^{S}Ant rings are about 66° and 82°, respectively. The ^{S}Ant displays ϕ about 142° and the θ value is close to zero.

The single crystal x-ray diffraction data of hexapeptide **5b** revealed that (Aib-^{S}Ant-Pro)$_n$ oligomers display intra-residual hydrogen-bonded fully extended conformation which is extremely rare for Aib residues[5c] (Fig. 3b). Molecule **5b** shows six-membered hydrogen bonding (C6 H-bonding) formed within ^{S}Ant itself and five-membered hydrogen bonding (C5 H-bonding)[8] formed within Aib residue. The H-bonding distances of C6 H-bonding and C5 H-bonding are about 1.98 Å and 2.10 Å, respectively. The inner Aib residue flanked between Pro and ^{S}Ant residues shows torsional angle ϕ and ψ about 180° (176.8° and -177.7°), suggesting planarity of Aib residue which leads to intra-residual C5 H-bonding. The ^{S}Ant rings display torsional angle ψ and ω about 92° and 69°, respectively. The torsion angle θ of the ^{S}Ant is close to zero (7.0° and 9.9°). The Pro residues show torsional angle ϕ about 90°. It is noteworthy that the oligomer **5b** is devoid of any inter-residual H-bonding. Presumably, it is the torsional constraints of inner Aib residue that force the molecule to adopt intra-residual C6 and C5 H-bonding, instead of other inter-residual H-bondings.

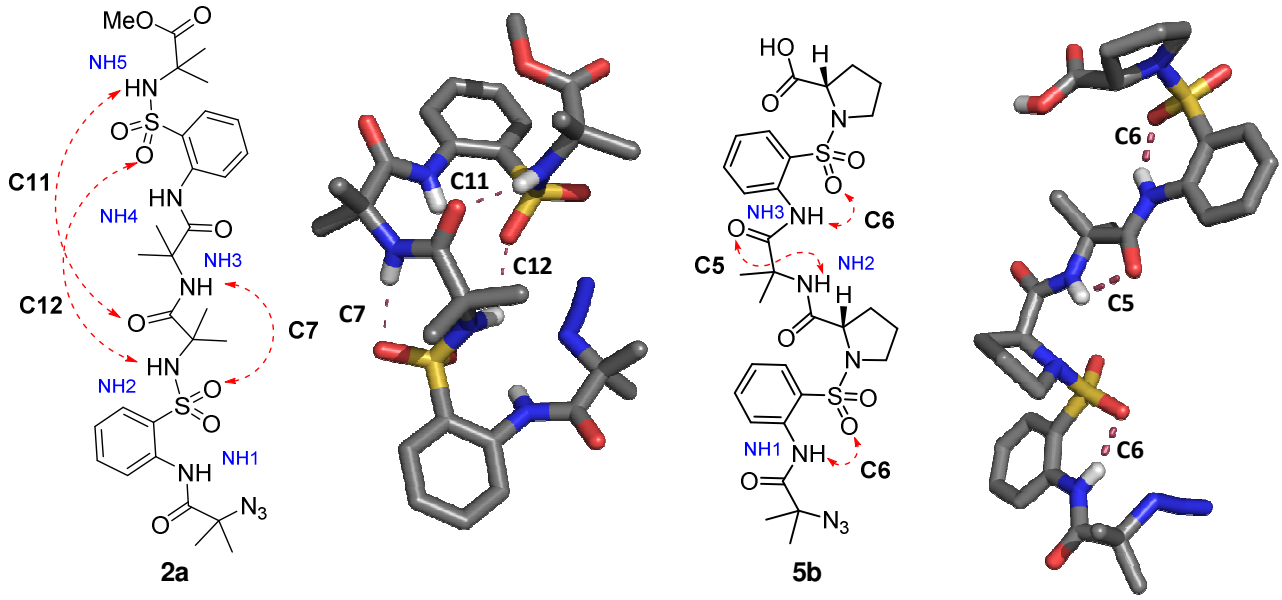


Fig. 2: PyMOL-rendered crystal structures of the **2a** (a) and **5b** (b). Hydrogens, other than the polar amide hydrogens have been removed for clarity. The H-bonding interactions are shown with double-headed arrows in the molecular structures.

X-ray analysis of **2a** revealed the absence of H-bonding at the N-terminus due to the absence of H-bond acceptor carbonyl group. In order to replicate the C11 H-bonding at the N-terminus, we synthesized **2b** (Fig. 3b, *vide infra*) featuring H-bond acceptor carbonyl at the N-terminus. The solution-state conformations of both (Aib-^{S}Ant-Aib)$_n$ oligomers and (Aib-^{S}Ant-Pro)$_n$ oligomers were investigated by NMR studies. The signal assignments of the oligomers have been done using a combination of 2D COSY, NOESY, HMBC and HSQC. The 2D NOESY analysis of **2a** revealed the existence of long-range inter-residual nOes between NH2 *vs* NH5 along with C16H *vs* C29H, NH2 *vs* NH5, NH3 *vs* NH5 and NH2 *vs* NH4 (Fig. 3a) suggesting the prevalence of folded conformation. Similarly, the analysis of 2D NMR data of **2b** (Fig. 3b) shows inter-residual nOe between C17H *vs* NH6 and C17H *vs* NH7, suggesting similar conformation as observed in **2a**. It is notworthy that inter-residual nOe between C32H *vs* C14H and C32H vs NH3 observed in **2b** confirms the existance of 11-membered ring H-bonding between N-terminus carbonyl and NH3. The crystal structure analysis of **5b** suggested that the characteristic long rang nOe in the solution-state that would support the helical conformation would be nOes between aromatic NH and consecutive α and δ Pro protons. The helical conformation of **5a**, a close analog of **5b**, is clearly evident from 2D NMR studies where we could observe characteristic inter-residual long range nOe between NH1 *vs* C11H, NH1 *vs* C14H, NH3 *vs* C26H and NH3 *vs* C29H (Fig. 3c). The conformation of the large oligomers **3** and **6** could not be ascertained owing to the difficulty in their signal assignments (due to the presence of several repetitive similar residues).

The presence of intramolecular H-bondings in **2a**, **2b** and **5a** were supported by DMSO titration experiments. The amide NHs involved in intramolecular H-bonding show negligible changes in chemical shift (Table 1). However, the amide NH2 of **2a** involved in C12 H-bonding displays considerable chemical shift (1.26 ppm) suggesting a weak hydrogen-bonding in solution-state. The variable temperature studies also support intramolecular H-bonding in both peptides **2a** and **2b** [temperature coefficients for **2a** (Δδ/ΔT) < -3 and for **2b** (Δδ/ΔT) < -3.3, ESI S32-S33]. Both DMSO titration and variable temperature experiments of **2b** support the presence of intramolecular H-bonding. In variable temperature experiments, NHs of **5a** involved in intramolecular H-bonding display temperature coefficients < -1.7 [(Δδ/ΔT) for NH1 < -1.45, (Δδ/ΔT) for NH2 < -1.64 and for NH3 (Δδ/ΔT) < -1.45], suggestive of intramolecular H-bonding.

Table 1: Chemical shift variation of amide NHs in NMR titration studies (10 mM, 400 MHz).

Comp	Δδ (ppm)						
	NH1	NH2	NH3	NH4	NH5	NH6	NH7
2a	0.16	1.26	0.04	0.32	0.09	-	-
2b	0.85	0.15	0.10	0.14	0.18	0.08	0.07
5a	0.25	0.05	0.25	-	-	-	-

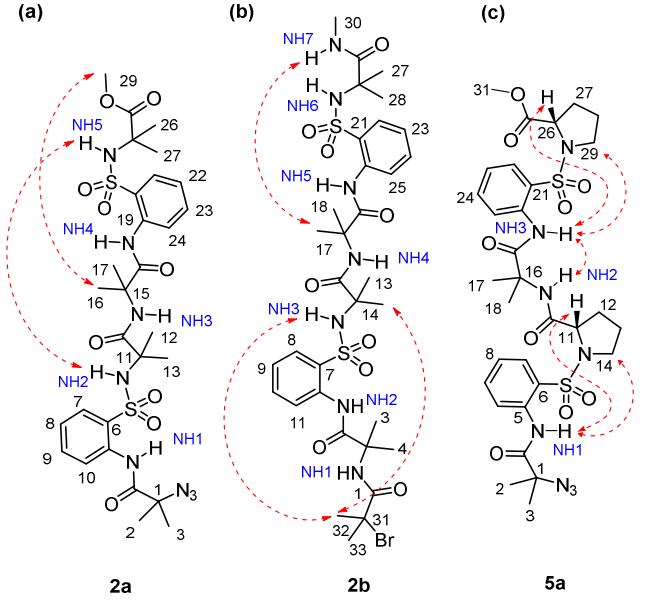


Fig. 3: Selected NOE extracts from the 2D NOESY data of **2a**, **2b** and **5a** ($CDCl_3$, 40 mM, 500 MHz). Detailed analyses, including 2D NMR plots, Dilution and variable temperature NMR data are given in the ESI (S31-S39).

The CD spectra of (Aib-^{S}Ant-Pro)$_n$ oligomers **4a**, **5a** and **6** show maxima at about 201 nm, zero-crossing at about 208 nm and strong minima at about 225 nm (ESI S40).

In conclusion, we have demonstrated the effect of 2-aminobenzenesulfonic acid (^{S}Ant) on the conformational preferences of hetero foldamers featuring Aib-^{S}Ant-Aib and Aib-^{S}Ant-Pro tripeptide building blocks. Whereas (Aib-^{S}Ant-Aib)$_n$ oligomers display folded screw-sense inversion conformation with mixed hydrogen bonding networks (C11 and C7), the (Aib-^{S}Ant-Pro)$_n$ oligomers display periodically repeating intra-residual 6-and 5-membered hydrogen-bonded fully extended conformation.[9] These findings suggest that the conformational propensities of these oligomers are primarily dictated by ^{S}Ant, whilst the other amino acids played a less prominent role. The results obtained underscore the importance of learning about the conformational propensities of newer types of unnatural amino acids, which may find application in the *de novo* design of peptides with intriguing structures and function.

SSK and RLG are thankful to CSIR, New Delhi, for a research fellowship. GJS thanks NCL-IGIB (New Delhi) for financial support.

Notes and references

1 For selected reviews, see: (a) T. A. Martinek and F. Fulop, *Chem. Soc. Rev.*, 2012, **41**, 687; (b) G. Guichard and I. Huc, *Chem. Commun.*, 2011, **47**, 5933; (c) C. M. Goodman, S. Choi1, S. Shandler and W. F. DeGrado, *Nat. Chem. Biol.* 2007, **3**, 252; (d) W. S. Horne and S. H. Gellman, *Acc. Chem. Res.*, 2008, **41**, 1399; (e) A. Roy, P. Prabhakaran, P. K. Baruah, and G. J. Sanjayan, *Chem. Commun.*, 2011, **47**, 11593; (f) P. Prabhakaran, G. Priya, and G. J. Sanjayan, *Angew. Chem. Int. Ed.*, 2012, **51**, 4006; (g) C. Tomasini, G. Angelici, N. Castellucci, *Eur. J. Org. Chem.* 2011, 3648, and references cited therein.

2 (a) Z. Hegedüs, E. Wéber, E. Kriston-Pál, I. Makra, A. Czibula, E. Monostori, and T. A. Martinek, *J. Am. Chem. Soc.,* 2013, **135**, 16578; (b) Z. E. Reinert, G. A. Lengyel, and W. S. Horne, *J. Am. Chem. Soc.,* 2013, **135**, 12528; (c) L. Guo, W. Zhang, I. A. Guzei, L. C. Spencer and S. H. Gellman, *Org. Lett.*, 2012, **14**, 2582; (d) I. V. Korendovych, S. J. Shandler, G. L. Montalvo and W. F. DeGrado, *Org. Lett.*, 2011, **13**, 3474; (e) L. Berlicki, L. Pilsl, E. Weber, I. M. Mandity, C. Cabrele, T. A. Martinek, F. Fulop and O. Reiser, *Angew. Chem. Int. Ed.* 2012, **51**, 2208; (f) Z. E. Reinert, G. A. Lengyel and W. Seth Horne, *J. Am. Chem. Soc.*, 2013, **135**, 12528; (g) G. V. M. Sharma, K. S. Reddy, S. J. Basha, K. R. Reddy and A. V. S. Sarma, *Org. Biomol. Chem.*, 2011, **9**, 8102; (h) P. Prabhakaran, V. Azzarito, T. Jacobs, M. J. Hardie, C. A. Kilner, T. A. Edwards, S. L. Warriner and A. J. Wilson. *Tetrahedron*, 2012, **68**, 4485; (i) M. D. P. Risseeuw, M. Overhand, G. W. J. Fleet, M. I. Simone, *Amino Acids*, 2013, **45**, 613; (j) C. Caumes, O. Roy, S. Faure, and C. Taillefumier, *J. Am. Chem. Soc.*, 2012, **134**, 9553.

3 (a) S. S. Kale, G. Priya, A. S. Kotmale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Chem. Commun.*, 2013, **49**, 2222; (b) V. V. E. Ramesh, S. S. Kale, A. S. Kotmale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Org. Lett.*, 2013, **15**, 1504; (c) P. Prabhakaran, S. S. Kale, V. G. Puranik, P. R. Rajamohanan, O. Chetina, J. A. K. Howard, H.-J. Hofmann and G. J. Sanjayan, *J. Am. Chem. Soc.*, 2008, **130**, 17743; (d) K. N. Vijayadas, H. C. Davis, A. S. Kotmale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Chem. Commun.*, 2012,**48**, 9747-9749; (e) R. V. Nair, S. Kheria, S. Rayavarapu, A. S. Kotmale, B. Jagadeesh, R. G. Gonnade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *J. Am. Chem. Soc.*, 2013, **135**, 11477.

4 (a) A. Choudhary and R. T. Raines, *ChemBioChem*, 2011, **12**, 1801; (b) G. Lesmaa, N. Landonia, A. Sacchettib and A. Silvani, *Tetrahedron*, 2010, **66**, 4474; (c) T. Lama, S. E. Del Valle, N. Genest and W. D. Lubell, *Int. J. Pept. Res. Ther.,* 2007, **13**, 355.

5 (a) D. F. Kennedy, M. Crisma, C. Toniolo and D. Chapman, *Biochemistry*, 1991, **30**, 6541; (b) S. Aravinda, S. Datta, N. Shamala and P. Balaram, *Angew. Chem. Int. Ed.*, 2004, **43**, 6728; (c) S. Aravinda, N. Shamala, and P. Balaram, *Chem. Biodivers*, 2008, **5**, 1238.

6 (a) R. Gessmann, H. Bruckner and K. Petratos, J. Peptide Sci., 2003, 9, 753; (b) M. Vlassi, H. Brueckner and M. Kokkinidis, *Acta Cryst. Sect. B,* 1993, **49**, 560.

7 (a) B. Di Blasio, V. Pavone, M. Saviano, A. Lombardi, F. Nastri, C. Pedone, E. Benedetti, M. Crisma, M. Anzolin and C. Toniolo, *J. Am. Chem. Soc.*, 1992, **114**, 6273; (b) I. L. Karle, J. F.-Anderson, M. Sukumart and P. Balaram, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 5087; (c) G. Valle, R. Bardi, A. M. Piazzesi, M. Crisma, C. Toniolo, G. Cavicchioni, K. Uma and P. Balaram, *Biopolymers*, 1991, **31**, 1669; (d) C. Toniolo, M. Crisma, F. Formaggio, C. Valle, G. Cavicchioni, G. Precigoux, A. Aubry and J. Kamphuis, *Biopolymers*, 1993, **33**, 1061; (e) M. Crisma, G. Valle, G.M. Bonora, C. Toniolo and G. Cavicchioni, *Int. J. Pept. Protein Res.*, 1993, **41**, 553; (f) S. A. Stoykova, A. Linden and H. Heimgartner, *Helv. Chim. Acta.*, 2012, **95**, 1325.

8 (a) E. Benedetti, C. Toniolo, P. Hardy, V. Barone, A. Bavoso, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone, *J. Am. Chem. Soc.*, 1984, **106**, 8146; (b) G. Valle, G. M. Bonora, C. Toniolo, P. M. Hardy, M. T. Leplawy and A. Redlinski, *J. Chem. Soc., Perkin Trans. 2*, 1986, 885.

9 The X-ray diffraction data of **2a** and **5b** were collected on a SMART APEX-II CCD single crystal X-ray diffractometer. Crystallographic data of **2a** and **5b** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no.CCDC- 931177 and 931178, respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.