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

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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 a well-defined folded conformation featuring intramolecular mixed hydrogen bonding (7/11) and intra-residual (6/5) H-bonding interactions, respectively.

Modulation and control of the 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 homo-oligomer 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 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

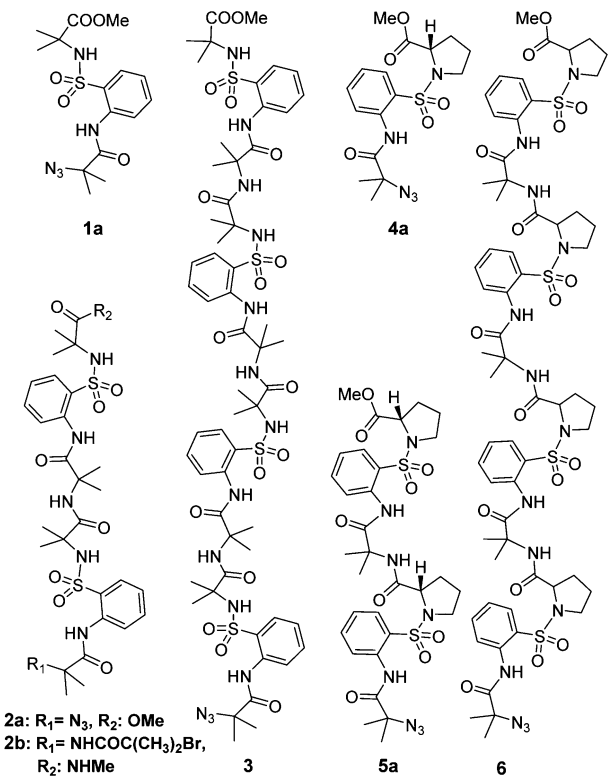

Fig. 1 Molecular structures of oligomers described in this work.

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† 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. CCDC 931177 and 931178. For the ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc48850k

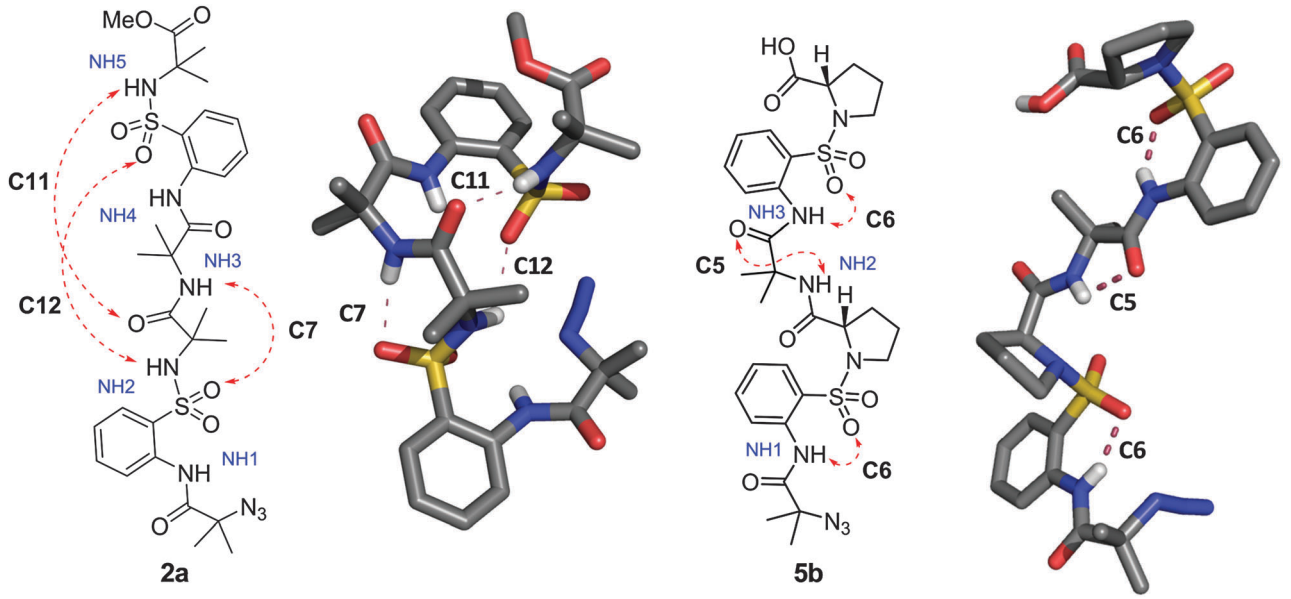

Fig. 2 PyMOL-rendered crystal structures of **2a** (a) and **5b** (b). Hydrogens, other than the polar amide ones, have been removed for clarity. The H-bonding interactions are indicated by double-headed arrows in the molecular structures.

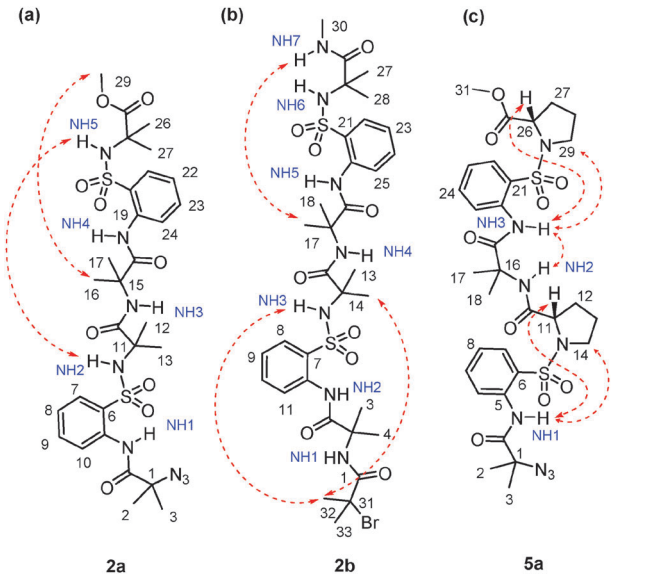

Fig. 3 Selected NOE extracts from the 2D NOESY data of **2a**, **2b** and **5a** ($CDCl_3$, 40 mM, 500 MHz). Detailed analyses, including 2D NMR plots, dilution and variable temperature NMR data, are given in the ESI‡ (S31–S39).

patterns (Fig. 3a): (i) 11-membered 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 torsional 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 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 an angle ϕ of 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 the Aib residues. 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 angles ϕ and ψ of about 180° (176.8° and −177.7°), suggesting planarity of the Aib residue, which leads to intra-residual C5 H-bonding. The ^{S}Ant rings display torsional angles ψ and ω of about 92° and 69°, respectively. The torsional angle θ of the ^{S}Ant is close to zero (7.0° and 9.9°). The Pro residues show a torsional angle ϕ of about 90°. It is noteworthy that the oligomer **5b** is devoid of any inter-residual H-bonding. Presumably, it is the torsional constraints of the inner Aib residues that force the molecules to adopt intra-residual C6 and C5 H-bonding, instead of other inter-residual H-bondings.

X-ray analysis of **2a** revealed the absence of H-bonding at the N-terminus due to the absence of a 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 a 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 were 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 nOe 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

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

	$\Delta\delta$ (ppm)						
Comp	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	—	—	—	—

NMR data of **2b** (Fig. 3b) shows an inter-residual nOe between C17H *vs.* NH6 and C17H *vs.* NH7, suggesting a similar conformation as observed in **2a**. It is noteworthy that the inter-residual nOe between C32H *vs.* C14H and C32H *vs.* NH3 observed in **2b** confirms the existence of 11-membered ring H-bonding between the N-terminus carbonyl group and NH3. The crystal structure analysis of **5b** suggested that the characteristic long-range nOe in the solution-state that would support the helical conformation would be the 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 the 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** was supported by DMSO titration experiments. The amide NHs involved in intramolecular H-bonding show negligible changes in the chemical shift (Table 1). However, the amide NH2 of **2a** involved in C12 H-bonding displays a considerable chemical shift (1.26 ppm), suggesting a weak hydrogen-bonding in the solution-state. The variable temperature studies also support intramolecular H-bonding in both peptides **2a** and **2b** [temperature coefficients for **2a** ($\Delta\delta/\Delta T$) < -3 and for **2b** ($\Delta\delta/\Delta T$) < -3.3, ESI,‡ S32 and 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 [for NH1 ($\Delta\delta/\Delta T$) < -1.45, for NH2 ($\Delta\delta/\Delta T$) < -1.64 and for NH3 ($\Delta\delta/\Delta T$) < -1.45], suggestive of intramolecular H-bonding.

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-amino-benzenesulfonic 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 a folded screw-sense inversion conformation with mixed hydrogen bonding networks (C11 and C7), the (Aib-^{S}Ant-Pro)$_n$ oligomers display a 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.

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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 and 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 and 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. Biodiversity*, 2008, **5**, 1238.

6 (*a*) R. Gessmann, H. Bruckner and K. Petratos, *J. Pept. Sci.*, 2003, **9**, 753; (*b*) M. Vlassi, H. Brueckner and M. Kokkinidis, *Acta Crystallogr., 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. U. S. A.*, 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 and 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** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. 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.

