Sheet-forming abiotic hetero foldamers†

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Abiotic hetero oligomers, adopting a well-defined extended self-assembled sheet-like structure, derived from conformationally constrained aliphatic and aromatic amino acid residues repeating at regular intervals are reported.

Foldamers[1] as a class of conformationally ordered synthetic oligomers have moved into prominence primarily due to their enormous potential for the creation of unnatural oligomers adopting conformational features, akin to biopolymers. Investigations by several groups have resulted in the generation of several such synthetic oligomers with diverse backbone structures and conformations.[2] Of late, increasing attention has been given to the exploration of abiotic aromatic foldamers that display novel functions, properties and conformational features, as has been independently demonstrated by various research groups.[3] An outstanding example is the development of a foldamer, by Hamilton's group, that exerts a strong influence on the shape of growing calcite crystals *via* specific interactions between the functional groups attached to the foldamer and the newly expressed faces of the growing calcite crystals.[3a] The self-assembling hetero foldamers developed by Gong's group[4] could find profound application in the development of supramolecular polymers; polymers formed exclusively from non-covalent interactions.[5]

Herein we describe a novel class of abiotic hetero foldamers derived from conformationally constrained amino acids, both aliphatic and aromatic, repeating at regular intervals (Fig. 1). The striking feature of these α-aminoisobutyric acid (Aib)-rich synthetic hetero oligomers is their ability to form self-assembled sheet-like structures through extensive intermolecular hydrogen bonding interactions from the backbone amide groups; an observation that is in stark contrast to the general finding that Aib is proven as a sheet breaking amino acid, at least in oligomers composed of α-amino acids.[6,7] It is noteworthy that sheet forming structural architectures have implications in the understanding and development of potential therapies for Alzheimer's disease, a leading cause of dementia in the elderly, which is pathologically defined by the occurrence of amyloid plaques, composed of the amyloid β-protein, and neurofibrillary tangles.[8] Indeed, scanning of the morphological architecture of the large hexadecapeptide foldamer **4** shows fibril piles composed of entangled nanofibrils, a morphological signature of extensive self-assembly,[9] which is also substantiated by the results of crystal structure studies (*vide infra*).

The foldamers, derived from conformationally constrained Aib and 3-*a*mino-5-bromo-2-*m*ethoxy *b*enzoic acid (Amb)[3i] residues (Aib–Amb motif **1**), were designed anticipating that the corresponding oligomers **2**, **3**, and **4** would adopt a well-defined, compact, three dimensional structure, governed by a combined conformational restriction imposed by the constrained Aib and Amb residues.

Whereas the achiral Aib residue is known to play a key role[10] in the conformational restriction of polypeptides due to its overwhelmingly constrained phi ($\phi \pm 60^\circ$) and psi ($\psi \pm 30^\circ$) torsion

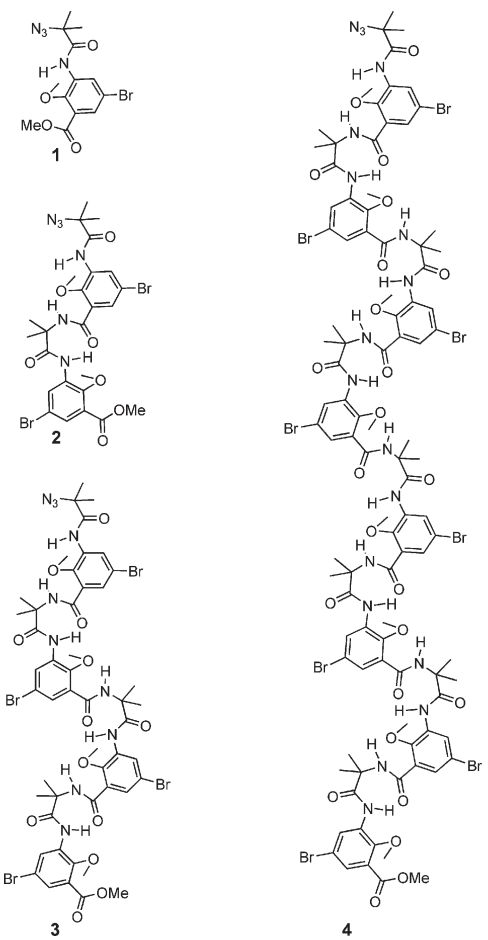

Fig. 1 Molecular structure of dipeptide **1**, tetrapeptide **2**, octapeptide **3**, and hexadecapeptide **4** foldamers synthesized in this study.

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angles, the backbone-rigidified aromatic amino acid residue Amb and its analogs[2c] are known to induce a crescent conformation in its oligomers *via* localized S(5) and S(6) type[11] hydrogen bonding interactions. Thus, we reasoned that hetero-oligomers made of Aib–Amb repeat motifs might also display conformational rigidity; which was indeed realized, as evident from structural investigations (*vide infra*). The bromine atoms on the periphery of the aromatic nuclei in the foldamers were meant to aid crystallization and to improve the quality of crystal data, due to the presence of heavy bromine atoms.[12] It is noteworthy that one of the biggest challenges in the structural investigation of large synthetic oligomers is their resistance to yield to crystal formation.

The Aib–Amb motif-based foldamers **2**–**4** were assembled from N_3–Aib–Amb–OMe building block **1**, using a segment doubling strategy (experimental details in the ESI†). Curiously enough, all efforts to couple BOC–Aib–OH with H–Amb–OMe were unsuccessful, under a variety of coupling conditions, which prompted us to use the azide moiety as the masked amine surrogate. The solubility profiles of the oligomers in non-polar solvents were noted to progressively deteriorate with an increase in size of the oligomer, a fact that is presumably due to extensive aggregation in higher order oligomers. This fact is also vindicated by the results of investigation of the oligomers **2**–**4** by transmission electron microscopy (TEM), (*vide infra*). It is noteworthy that an increasing tendency of oligomers to self-aggregate is known to be paralleled by a decrease in their solubility profile.[13] The hexadecapeptide foldamer **4** was only sparingly soluble in DMSO. Due to this reason, the synthesis of oligomers larger than the hexadecapeptide foldamer **4** could not be undertaken.

Among the oligomers, the tetrapeptide foldamer **2** crystallized from methanol in monoclinic space group $P2_1/c$,‡ although the higher order oligomers did not yield crystals suitable for single crystal X-ray studies, despite best efforts. Investigation of the crystal structure of **2** (Fig. 2) revealed that the intrinsically constrained Aib residues imposed significant twist on the foldamer backbone, as expected, with ϕ and ψ torsion angles of the central Aib residue close to 52°.

This effect was also noted in our previously reported hetero foldamers containing Aib residues.[14] Detailed analysis of the crystal structure further revealed self-assembly of the individual strands of the oligomer **2**. The self-complementary individual strands of **2** undergo self-assembly through intermolecular N–H···O=C hydrogen bonding interactions, with an average D–H···A length: 2.251 Å, to afford an extended sheet-like structure, an observation that could provide insights for developing such templates into potential protein-β-sheet mimetics.[2b] It is noteworthy that an extended sheet structure has been disclosed recently in certain cyclopropane γ-peptides stabilized by C–H···O hydrogen bonds,[15] and also in isotactic acrylamide oligomers.[13]

The extended conformation of the individual strands, as noted in the crystal structure of **2**, is prevalent in solution-state as well, as evidenced from 2D NOESY NMR studies of the oligomers **2** and **3** (Fig. 3).

However, the poor solubility of the hexadecapeptide foldamer **4**, presumably due to extensive aggregation (self-assembly), rendered its conformational studies by solution-state NMR spectroscopy difficult. One of the most characteristic NOE interactions that can be anticipated for an extended conformation, as seen in the crystal structure of **2**, would be the requirement of sequential dipolar couplings between the protons of adjacent residues of Aib–Me, Ar–NH, Ar–OMe, and Aib–NHs. Such a sequential interaction is clearly observed for both tetrapeptide **2** and octapeptide **3**, suggesting an extended conformation for the individual strands, similar to the one observed for **2** in its crystal structure (observed

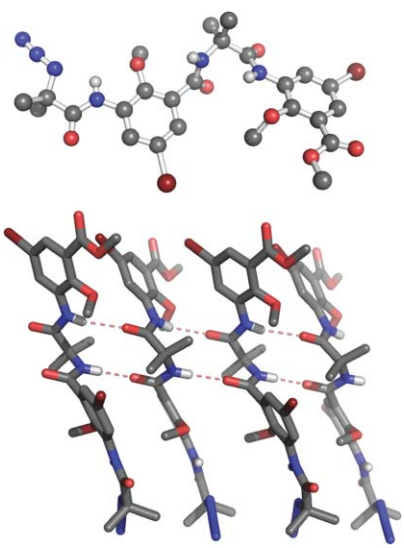

Fig. 2 Crystal structure of foldamer **2** (top, ball and stick representation) and its H-bond-mediated self-assembled structure (bottom, capped stick representation). Hydrogens, other than at the hydrogen bonding sites, have been omitted for clarity.

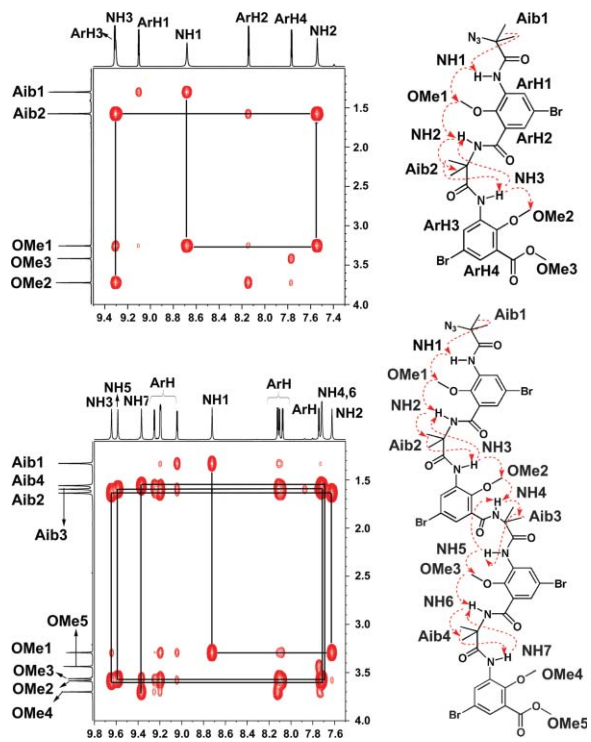

Fig. 3 Partial 2D NOESY spectra of **2** (top) and **3** (bottom) showing characteristic NOEs. For enabling assignments, the molecular structure with selected numbered atoms are also shown. The dipolar couplings are indicated with red dotted arrows.

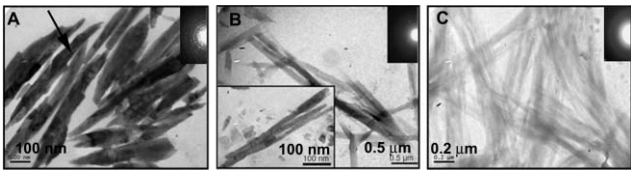

Fig. 4 (A), (B), and (C): TEM images of the oligomers **2**, **3**, and **4**, respectively, deposited on carbon coated polymer film. Corresponding selected area electron diffraction (SAED) patterns are shown in the inset. *Note*: Full-size figures are available in the ESI (S25, S26).†

NOEs for **2**: Aib1 *vs*. NH1 *vs*. OMe1 *vs*. NH2 *vs*. Aib2 *vs*. NH3 *vs*. OMe2). Further, we also note a characteristic dipolar coupling between the NHs of adjacent residues (S24, ESI†) in both **2** and **3**. Indeed, closer inspection of the crystal structure of **2** reveals the close proximity of NHs of adjacent residues in the extended conformation (*d*: 2.8 Å). In order to provide insights into the hydrogen-bonding interactions in solution, we also performed [D_6]DMSO titration studies on the oligomer **3** (details in ESI, S27†). The titration results reveal that all the NHs show downfield shifts upon increasing the concentration of [D_6]DMSO (from 0 to 50 μL), suggesting their role in intermolecular interactions. The effect is particularly pronounced for all the Aib NHs of the octapeptide **3** ($\Delta\delta \sim 1$ ppm).

To investigate the morphological architecture and its effect on the length of the oligomer, we analyzed the self-assembled structures by TEM. Fig. 4A, B, and C show the TEM images of the oligomers **2**, **3**, and **4**, respectively, deposited on carbon coated polymer film.

Comparison of the photomicrographs of the oligomers: tetrameric foldamer **2** *vs*. octamer **3** *vs*. hexadecamer **4**, reveals an interesting structural aspect. As the oligomer size increases, the morphological architecture transforms from crystalline needle shape (Fig. 4A) to entangled nanofibrils (Fig. 4C). In the case of the tetrameric foldamer **2**, the particles are needle shaped and are flat, as evident from the Mòire pattern seen from the overlapping of two needle shaped nanoparticles (indicated by an arrow in Fig. 4A). Further, the particles of **2** with size ≈400 nm are crystalline in nature as shown by the SAED pattern in the inset (Fig. 4A). In the case of octamer **3**, the size of the particle is ≈3 μm and the SAED pattern shown in the inset (Fig. 4B) is diffused, indicating a loss in the crystalline structure. However, the microscopic image of the hexadecamer foldamer **4** (Fig. 4C) reveals fibril piles composed of entangled nanofibrils, a morphological signature of extensive self-assembly.[9]

In summary, we have developed novel synthetic oligomers that adopt well-defined, compact, three-dimensional architectures, governed by a combined conformational restriction imposed by the individual amino acid constituents. The notable feature of these Aib-rich synthetic oligomers is their ability to form self-assembled sheet-like structures through extensive intermolecular hydrogen bonding interactions from the backbone amide groups, an observation that is in stark contrast to the general observation that Aib is proven as a sheet breaking amino acid, at least in oligomers composed of α-amino acids.[6] Therefore, these results suggest the utility of the hybrid foldamer strategy in modulating conformational preferences of individual amino acids in oligomer sequences. The results of investigation of the morphological architectures of these oligomers further suggest their potential in fabricating novel supramolecular nanoarchitectures.

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