Formation of triple helical nanofibers using self-assembling chiral benzene-1,3,5-tricarboxamides and reversal of the nanostructure's handedness using mirror image building blocks†

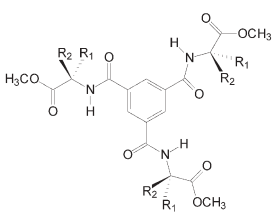
Partha Pratim Bose,[a] Michael G. B. Drew,[b] Apurba K. Das[a] and Arindam Banerjee*[a]

Received (in Cambridge, UK) 5th May 2006, Accepted 5th June 2006
First published as an Advance Article on the web 21st June 2006
DOI: 10.1039/b606371c

Intertwining triple helical nanofibers with an overall handedness have been formed from self-assembling chiral benzene-1,3,5-tricarboxamides 1, 2 and 3, whereas the achiral benzene-1,3,5-tricarboxamide 4 upon self-association gives rise to straight nanofibers without any twist and transmission electron microscopy images of chiral compounds clearly demonstrate that the handedness of the triple helical nanofibers can be reversed by using the enantiomeric benzene-1,3,5-tricarboxamide building blocks.

Nanofibers are ubiquitous and are found in both self-associated biological and non-biological systems. They are observed in self-assembling short peptides with 2–3 residues,[1] medium sized peptides,[2] naturally occurring proteins,[3] *de novo* designed proteins[4] and lipids.[5] In non-biological systems, the synthetic carbon nanofiber is very common[6] and the silicon carbide nanofiber[7] is also reported in the literature. Nanofiber structures based on β-sheet forming self-assembling peptides have been widely studied.[8] Self-assembling peptide amphiphiles form nanofibers and these nanofibers can be used for biomedical applications.[9] It has been reported that self-assembling peptide amphiphiles can be reversibly self-assembled to form a nanofiber network, which results in the formation of aqueous gel through pH changes.[10] Ryadnov and Woolfson have successfully engineered the morphology of self-assembling polypeptide-based nanofibers.[11] An earlier example of the formation of helical nanofibers includes the twisted fibers obtained from supramolecular liquid crystalline species formed by polyassociation of complementary components TP_2 and TU_2 (where T is D-, L- or *meso* tartaric acid, P is a pyridine derivative and U is a uracil derivative). The chirality of the nanofibers is controlled by using different types of tartaric acids: D-, L- or the *meso* form.[12] Recent studies of the formation of helical nanofibers include the self-assembly of the molecular system based on a dithienylethene unit functionalized with (*R*)-1-phenyl ethylamine derived amides,[13] self-association of dendron rod coil molecules[14] and β-sheet forming oligopeptides.[15] The handedness of the helical nanostructures can be reversed either by using the enantiomeric form of the dendron rod coil as a molecular scaffold[14] or by using a mirror image self-assembling oligopeptide.[15] However, none of the above mentioned examples show the further assembly of helical nanofibers, *i.e.* the formation of double helical or triple helical nanofibers. Here, we present the formation of triple helical nanofibers from self-assembling chiral benzene-1,3,5-tricarboxamides **1**, **2** and **3** and reversal of the handedness of these chiral nanofibers using the enantiomeric benzene-1,3,5-tricarboxamide (compounds **1** and **3** are enantiomers). To meet the challenge of controlling the chirality at different hierarchical levels of molecular self-assembly, particularly in the formation of chiral nanofibers, discotic molecules having opposite chirality (Fig. 1) and an achiral molecular unit have also been chosen to probe whether the chiral nanostructure is formed only by chiral building blocks.

A series of compounds **1–4** were synthesized,[16] purified, characterized and studied. A single crystal X-ray diffraction study[17] of compound **1** (crystallized from a methanol–water system) reveals that the molecule has crystallographic 3-fold symmetry within a hexagonal unit cell, space group $P6_3$. This facilitates the formation of supramolecular columnar packing along the axis parallel to the unique crystallographic *c* axis (Fig. 2). The self-assembly of disc-shaped compound **1** exhibits a supramolecular triple helical structure with an overall right-handed twist and this architecture is formed through intermolecular hydrogen bonds and other non-covalent interactions including π–π stacking interactions between the central aromatic moieties (Fig. 2). The inter-ring centroid distance is 3.546 Å in the crystal, which is compatible with distances in the π-facial arrangement of various C_3-symmetrical columnar supramolecular architectures relying on hydrogen bonding and π–π stacking.[18] The present crystal structure further revealed that the individual triple helical columns are regularly aligned *via* non hydrogen bonding non-covalent



Compound **1**: R_1 = -$CH(CH_3)_2$, R_2 = -H
Compound **2**: R_1 = -$CH_2CH(CH_3)_2$, R_2 = -H
Compound **3**: R_1 = -H, R_2 = -$CH(CH_3)_2$
Compound **4**: R_1 = -CH_3, R_2 = -CH_3

Fig. 1 Schematic representation of benzene-1,3,5-tricarboxamides **1–4**.

[a]*Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India. E-mail: bcab@mahendra.iacs.res.in; Fax: +91-33-2473-2805*
[b]*School of Chemistry, The University of Reading, Whiteknights, Reading, UK RG6 6AD*

† Electronic supplementary information (ESI) available: Synthesis, characterization, ^{1}H NMR and mass spectra of all compounds, and experimental procedures for TEM studies. See DOI: 10.1039/b606371c

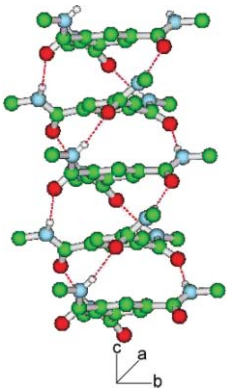


Fig. 2 Crystal packing of compound **1** showing the self-assembly of individual molecular building blocks, leads to the formation of an intermolecularly hydrogen-bonded supramolecular triple helical structure along the crystallographic *c* axis. Hydrogen bonds are shown as dotted lines. Nitrogen atoms are blue, oxygen atoms are red and carbon atoms are green. Non-hydrogen bonded hydrogen atoms and side chains of L-valine residues are omitted for clarity.

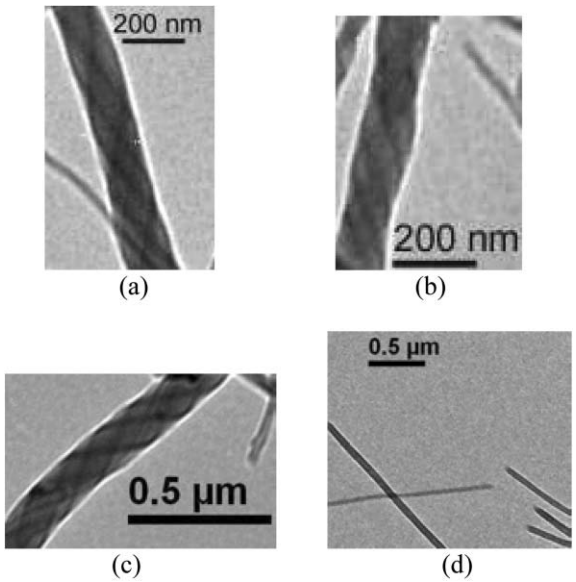


Fig. 3 Transmission electron microscope (TEM) images of (a) compound **1** and (b) compound **2** having the same chirality (*S*) giving a unique intertwining triple helical nanofiber upon self-assembly. (c) TEM image of compound **3** (having the reverse chirality (*R*) of compound **1**) illustrating the intertwining triple helical nanofiber of the reverse handedness upon self-assembly. (d) TEM picture of the achiral compound **4** showing straight nanofibers without any twist upon self-assembly. TEM micrographs were taken without any staining.

interactions to form higher ordered supramolecular arrays along the equivalent crystallographic *a* and *b* axes (ESI Fig. S10†). Transmission electron microscope studies of benzene-1,3,5-tricarboxamides were carried out using a methanol–water solution (1 : 1) of the corresponding compound (3 mg in 1 mL) on a carbon-coated copper grid (300 mesh) by slow evaporation and vacuum drying at 30 °C for two days. The transmission electron microscope (TEM) image of compound **1** shows a regular array of intertwining triple helical nanofibers 130–150 nm in diameter (width of individual nanofibers $\approx$40 nm). The TEM picture of the compound **2** also reveals that the formation of an intertwining triple helical nanofiber with 140–160 nm in diameter (width of individual nanofibers $\approx$30 nm). The TEM image of compound **3** shows that the triple helical nanofibers with a reverse chirality from that of compounds **1** and **2**, with a width of 165–185 nm (width of individual nanofibers $\approx$20 nm). However, the TEM picture of the achiral compound **4** shows that it self-assembles to form nanofibers (with a width of 45–65 nm) without any twist (Fig. 3d). Both compounds **1** and **2** have the same chirality (*S*) giving a unique intertwining triple helical nanofiber upon self-assembly (Fig. 3a and b). Compound **3** is the enantiomer of compound **1** and it self-assembles to form the intertwining triple helical nanofiber of the reverse handedness that has been observed in case of compound **1** or **2** (Fig. 3c). 3 mg of compounds **1** or **3** were dissolved in 1 mL of a methanol–water solution (1 : 1) with continuous sonication (TELSONIC TEC15, model No. 900818), 33 kHz, power 70 W) for 3 min and TEM measurements were performed by slow evaporation of benzene-1,3,5-tricarboxamide solution on a carbon-coated copper grid (300 mesh). The TEM images of the above mentioned samples upon sonication vividly demonstrate that the appearance of single stranded helical nanofibers for compounds **1** and **3** (ESI Fig. S11†) and these nanofibers appear to be of the opposite hand when the enantiomeric compounds were used. So, the construction and self-assembly of the helical nanofibers can be programmed using suitable self-assembling chiral molecular building blocks and the handedness of the single stranded helical nanofibers and triple helical nanofibers can be reversed using an enantiomeric self-assembling benzene-1,3,5-tricarboxamide building block. Helical tape structures of width 175–195 nm have been observed when compound concentrations were increased (from 3 mg mL^{-1} to 3.8 mg mL^{-1} in 1 : 1 methanol–water) (ESI Fig. S12a†). When compound concentrations were decreased (from 3 mg mL^{-1} to 2 mg mL^{-1} in 1 : 1 methanol–water), we observed narrower nanofibers (of width 60–90 nm), which are lacking the feature of a triple helical nanofiber (ESI Fig. S12b†). However, only wider nanofibers (diameter $\geqslant$180 nm) without any twist have been observed when 3 mg of compound **1** was dissolved in 1 mL of dimethyl sulfoxide–water (1 : 1) solution and was subjected to TEM experiments (ESI Fig. S12c†). So, compound concentrations and the solvent system play a definite role in the formation of single helical and triple helical nanofibers.

It is clear that compound **1** first self-assembles to form a triple helical supramolecular structure and ultimately forms triple helical nanofibers. Over and above this, the chirality is getting transmitted from the primary to secondary and from the secondary to tertiary structure of the molecular assemblage (Fig. 4). The secondary structural aspect of the self-assembled molecules has been established by single crystal X-ray diffraction studies of compound **1** and the crystal structure reveals the presence of columnar secondary structural units along the crystallographic *c* axis (Fig. 2). Each of these structural units has its own stereochemical integrity. The crystal structure analysis of compound **1** further provides insight about the tertiary assemblage of the chiral building blocks. Viewing along the crystallographic *a* and *b* axes it is evident that individual triple helical columns, *i.e.* the chiral secondary structural units, are regularly aligned *via* non-hydrogen bonding non-covalent interactions among the side-chain of the amino acid residues to form the nanofibrillar tertiary structure (single stranded

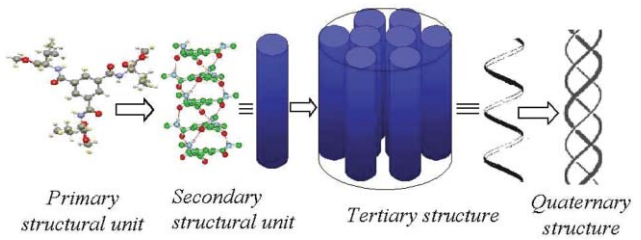


Fig. 4 Schematic representation of the molecular self-assembly of compound **1** illustrating the formation of secondary structure from the primary structure, tertiary structure from the secondary structure and quaternary structure from the corresponding tertiary structure.

helical nanofiber) (ESI Fig. S11a†). Further assembly of these single stranded helical nanofibers leads to the formation of the triple helical nanofiber (*i.e.* the quaternary structure). In this system, the supramolecular structure formed by the secondary structural unit of compound **1** produces the triple helical nanofibers observed in the TEM image.

Triple helical nanofibers can be successfully constructed using chiral molecular scaffolds (compounds **1**, **2** and **3**); the handedness of the triple helical nanofibers can also be nicely tuned by reversing the chiral nature of the molecular building blocks. The study vividly exemplifies not only the construction of self-assembled helical nanostructures but also shows the reversal of the handedness of the nanostructured assemblage using mirror image molecular building blocks. The achiral self-assembling benzene-1,3,5-tricarboxamide molecule fails to form chiral nanostructures indicating the transfer of molecular chirality into supramolecular chirality.[19,12] Functional chiral triple helical nanofiber formation using suitable molecular building blocks are yet to be explored.

This work is supported by DST, New Delhi, India Project No (SR/S5/OC-29/2003). We thank EPSRC and the University of Reading, UK for funds for Marresearch Image Plate Systems. P. P. Bose and A. K. Das wish to acknowledge the CSIR, New Delhi, India for financial assistance. Thanks are also due to the partial support from the Nanoscience and Technology Initiatives, DST, Govt. of India, New Delhi.

Notes and references

1 M. Reches and E. Gazit, *Science*, 2003, **300**, 625.
2 (*a*) M. G. Ryadnov and D. N. Woolfson, *Nat. Mater.*, 2003, **2**, 329; (*b*) N. J. Gay, L. C. Packman, M. A. Weldon and J. C. J. Barna, *FEBS Lett.*, 1991, **291**, 87; (*c*) A. Aggeli, M. Bell, N. Boden, J. N. Keen, P. F. Knowles, T. C. McLeish, M. Pitkeathly and S. E. Radford, *Nature*, 1997, **386**, 259.
3 (*a*) M. J. Van Raaij, A. Mitraki, G. Lavigne and S. Cusack, *Nature*, 1999, **401**, 935; (*b*) J. W. Kelly and W. E. Balch, *J. Cell Biol.*, 2003, 461; (*c*) J. C. Rochet and P. T. Lansbury, Jr., *Curr. Opin. Struct. Biol.*, 2000, **10**, 60; (*d*) S. Lee and D. Eisenberg, *Nat. Struct. Mol. Biol.*, 2003, **10**, 725; (*e*) T. Scheibel, R. Parthasarathy, G. Swaicki, X. M. Lin, H. Jaeger and S. L. Lindquist, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 4527.
4 (*a*) M. L. de la Paz, K. Goldie, J. Zurdo, E. Lacroix, C. M. Dobson, A. Hoenger and L. Serrano, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 16052; (*b*) W. A. Petka, J. L. Harden, K. P. McGrath, D. Wirtz and D. A. Tirrel, *Science*, 1998, **281**, 389; (*c*) A. P. Nowak, V. Breedveld, L. Pakstis, B. Ozbas, D. J. Pine, D. Pochan and T. J. Deming, *Nature*, 2002, **417**, 424; (*d*) J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis and J. Kretsinger, *J. Am. Chem. Soc.*, 2002, **124**, 15030.
5 (*a*) J. M. Schnur, *Science*, 1993, **262**, 1669; (*b*) M. S. Spector, K. R. Easwaran, G. Jyothi, J. V. Selinger, A. Singh and J. M. Schnur, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 12943.
6 (*a*) A. V. Melechko, T. E. McKnight, D. K. Hensley, M. A. Guillorn, A. Y. Borisevich, V. I. Merkulov, D. H. Lowndes and M. L. Simpson, *Nanotechnology*, 2003, **14**, 1029; (*b*) E. S. Steigerwalt, G. A. Deluga and C. M. Lukehart, *J. Phys. Chem. B*, 2002, **106**, 760.
7 Z. L. Wang, *Adv. Mater.*, 2000, **12**, 1295.
8 (*a*) C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2001, **40**, 4128; (*b*) S. G. Zhang, *Nat. Biotechnol.*, 2003, **21**, 1171; (*c*) N. Yamada, K. Ariga, M. Naito, K. Matsubara and E. Koyama, *J. Am. Chem. Soc.*, 1998, **120**, 12192; (*d*) N. Yamada, E. Koyama, T. Imai, K. Matsubara and S. Ishida, *Chem. Commun.*, 1996, 2297; (*e*) H. Yokoi, T. Kinoshita and S. Zhang, *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 8414; (*f*) S. Matsumura, S. Uemura and H. Mihara, *Chem.–Eur. J.*, 2004, **10**, 2789; (*g*) J. D. Hartgerink, E. Beniash and S. I. Stupp, *Science*, 2001, **294**, 1684; (*h*) K. L. Niece, J. D. Hartgerink, J. J. J. M. Donners and S. I. Stupp, *J. Am. Chem. Soc.*, 2003, **125**, 7146; (*i*) E. D. Sone and S. I. Stupp, *J. Am. Chem. Soc.*, 2004, **126**, 12756; (*j*) L. Li and S. I. Stupp, *Angew. Chem., Int. Ed.*, 2005, **44**, 1833.
9 (*a*) G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler and S. I. Stupp, *Science*, 2004, **303**, 1352; (*b*) M. E. Davis, M. Motion, D. A. Narmoneva, T. Takahashi, D. Hakuno, R. D. Kamm, S. Zhang and R. T. Lee, *Circulation*, 2005, **111**, 442.
10 J. D. Hartgerink, E. Beniash and S. I. Stupp, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 5133.
11 M. G. Ryadnov and D. N. Woolfson, *J. Am. Chem. Soc.*, 2004, **126**, 7454.
12 T. Gulik-Krzywicki, C. Fouquey and J. M. Lehn, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 163.
13 J. J. D. de Jong, L. N. Lukas, R. M. Kellogg, J. H. van Esch and B. L. Feringa, *Science*, 2004, **304**, 278.
14 B. W. Messmore, P. A. Sukerkar and S. I. Stupp, *J. Am. Chem. Soc.*, 2005, **127**, 7992.
15 T. Koga, M. Matsuoka and N. Higashi, *J. Am. Chem. Soc.*, 2005, **127**, 17596.
16 All reported compounds **1–4** were synthesized from methyl esters of corresponding L-valine, L-leucine, D-leucine and dimethyl glycine, respectively, and benzene-1,3,5-tricarboxylic acid using dicyclohexylcarbodiimide–1-hydroxybenzotriazole (DCC–HOBt). Detailed synthetic procedures are given in the ESI†.
17 *Crystal data for* **1**: $C_{27}H_{39}N_3O_9$, MW = 549.61, hexagonal, space group $P6_3$, a = 15.769(3), b = 15.769(3), c = 7.091(11) Å, U = 1527(5) Å^3, Z = 2, D_{calc} = 1.195 g cm^{-3}. Diffraction data were measured for compound **1** with MoKα radiation at 293 K. The crystal was positioned at 70 mm from the Image Plate. 100 frames were measured at 2° intervals with a counting time of 5 min to give 1906 independent reflections. Data analysis was carried out with the XDS program.[20] The structure was solved using direct methods with the SHELX97 program.[21] The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The structure was refined on F^2 using SHELXL. The structure contains a crystallographic 3-fold axis. The three equivalent asymmetric carbon atoms were given *S*-chirality in accordance with the synthesis. The final R values were $R1$ 0.1011 and w$R2$ 0.2557 for 1133 data with $I > 2\sigma(I)$. The largest peak and hole in the final difference Fourier were 0.75 and −0.40 e Å^{-3}. CCDC 606026. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b606371c.
18 (*a*) L. Brunsveld, H. Zhang, M. Glasbeek, J. A. J. M. Vekemans and E. W. Meijer, *J. Am. Chem. Soc.*, 2000, **122**, 6175; (*b*) A. R. A. Palmans, J. A. J. M. Vekemans, R. A. Hikmet, H. Fisher and E. W. Meijer, *Adv. Mater.*, 1998, **10**, 873.
19 M. A. Mateos-Temoneda, M. Crego-Calama and D. N. Reinhoudt, *Chem. Soc. Rev.*, 2004, **33**, 363.
20 W. Kabsch, *J. Appl. Crystallogr.*, 1988, **21**, 916.
21 G. M. Sheldrick, *SHELX97, program for crystal structure refinement*, University of Gottingen, 1997.

