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A Helical, Aromatic, Peptide Nanotube

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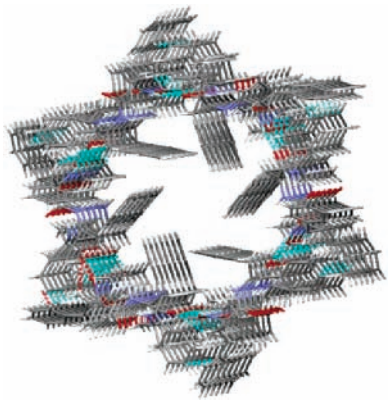
ABSTRACT



The self-assembly in the crystal state of the terminally protected, linear dipeptide Boc-(*S*,*S*)$c_3$diPhe-(*R*,*R*)$c_3$diPhe-NH^{i}Pr (1) through intermolecular hydrogen bonds leads to the formation of a supramolecular helix of large diameter (18 Å), internally decorated with phenyl rings. As a result, a hollow helical channel large enough to accommodate guest molecules is observed. This supramolecular structure differs from previous examples of peptide nanotubes. Compound 1 incorporates a highly restricted cyclopropane phenylalanine analogue ($c_3$diPhe) with remarkable conformational properties.

The synthesis and characterization of 3D structures based on the supramolecular assembly of oligopeptide-based organic molecules has become a research area of increasing interest. In particular, supramolecular structures known as peptide nanotubes have been the subject of numerous recent studies[1] because of their potential utility[2] in chemical, biological, and materials science fields, having proven useful in the design of porous materials[3] and size-selective transmembrane channels[4] and in the fabrication of inorganic nanocluster composites.[5] Another distinctive feature of these supramolecular architectures lies in the chiral character of their building blocks, which could result in their application in chiral recognition or separation.[6]

The feasible arrangement of a cyclic peptide in a hollow tubular structure was suggested in 1974 on the basis of

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(1) (a) Kol, N.; Adler-Abramovich, L.; Barlam, D.; Shneck, R. Z.; Gazit, E.; Rousso, I. *Nano Lett.* **2005**, *5*, 1343−1346. (b) Okamoto, H.; Takeda, K. *J. Drug Delivery Sci. Technol.* **2005**, *15*, 97−107. (c) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988−1011. (d) Hartgerink, J. D.; Clark, T. D.; Ghadiri, M. R. *Chem. Eur. J.* **1998**, *4*, 1367−1372. (e) Ghadiri, M. R. *Adv. Mater.* **1995**, *7*, 675−677.

(2) (a) Gao, X. Y.; Matsui, H. *Adv. Mater.* **2005**, *17*, 2037−2050. (b) Rajagopal, K.; Schneider, J. P. *Curr. Opin. Struct. Biol.* **2004**, *14*, 480−486. (c) Martin, C. R.; Kohli, P. *Nat. Rev.* **2003**, *2*, 29−37.

(3) (a) Hartgerink, J. D.; Granja, J. R.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 43−50. (b) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 6011−6012. (c) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324−327.

(4) (a) Matile, S.; Som, A.; Sorde, N. *Tetrahedron* **2004**, *60*, 6405−6435. (b) Sanchez-Quesada, J.; Isler, M. P.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2002**, *124*, 10004−10005. (c) Clark, T. D.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651−656. (d) Granja, J. R.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 10785−10786.

(5) (a) Banerjee, I. A.; Yu, L.; Matsui, H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14678−14682. (b) Djalali, R.; Chen, Y. F.; Matsui, H. *J. Am. Chem. Soc.* **2003**, *125*, 5873−5879.

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theroretical analysis,[7] although the first crystalline structure showing a nanotube formation was described by Ghadiri and co-workers in 1993, by ring stacking of cyclic peptides incorporating alternating D- and L-α-amino acids.[3c] Since then, the self-association of cyclic peptides into open-ended nanotubes has been studied in great detail. In these structures, the oligopeptide backbone adopts an essentially flat shape, allowing the stacking of successive rings through intermolecular hydrogen bonding between amide bonds. In recent years, nanotubes have been obtained from cyclic peptides incorporating also β- or γ-amino acids, or pseudopeptide units.[4c,8]

The other category of peptide nanotubes described in the literature is formed by linear peptides. These can be classified into two subtypes, based on whether their terminal groups are protected or free, i.e., the peptides are charged or not. Almost all of the examples reported refer to non-protected dipeptides incorporating hydrophobic amino acids such as Ala, Val, Ile, Leu, or Phe.[9] In these supramolecular structures the peptide chains are interconnected through hydrogen bonds involving the terminal COO^- and NH_3^+ groups. The self-assembly of the Phe−Phe dipeptide in nanotubes[9b,d,h] is of particular interest as this sequence is the core recognition element in the β-amyloid polypeptide that forms amyloid fibrils in Alzheimer's disease.

In contrast, there are very few examples of peptide nanotubes formed by terminally protected linear peptides. A recent report[10] describes the X-ray crystalline structure of Boc-Tyr-Xaa-Tyr-OMe (Xaa = Val or Ile). Both tripeptides self-assemble into tubular structures, with intermolecular hydrogen bonds involving the phenolic OH groups from the tyrosine residues. Despite its acyclic character, the supramolecular organization giving rise to the nanotube formation resembles that of cyclic peptides. Thus, each peptide molecule adopts a flat open-ring shape, the CO and NH groups being oriented nearly perpendicular to the plane defined by this ring. The stacking of these peptide subunits through intermolecular hydrogen bonds to generate the cylindrical structure is therefore equivalent to the assembly of cyclic peptides described above.

As part of our ongoing study of the conformational preferences of the restricted Phe analogue $c_3$diPhe (1-amino-*c*-2,*t*-3-diphenylcyclopropane-*r*-1-carboxylic acid),[11] the heterochiral dipeptide Boc-(*S*,*S*)$c_3$diPhe-(*R*,*R*)$c_3$diPhe-NH^{i}Pr (**1**, Figure 1) was synthesized. The stereochemical properties of this cyclopropane α-amino acid are peculiar in that it bears two phenyl substituents on adjacent side-chain β-carbons in a *trans* relative disposition and is therefore characterized by an achiral α-carbon and two chiral β-carbons, with two enantiomeric forms being possible: (*R*,*R*)- and (*S*,*S*)$c_3$diPhe.

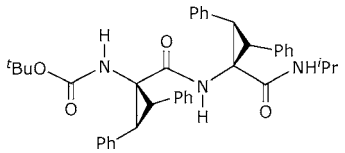

Figure 1. Dipeptide Boc-(*S*,*S*)$c_3$diPhe-(*R*,*R*)$c_3$diPhe-NH^i Pr (**1**).

Dipeptide **1** was obtained in excellent yield after 4 days of reaction between Boc-(*S*,*S*)$c_3$diPhe-OH (Boc, *tert*-butyloxycarbonyl) and H-(*R*,*R*)$c_3$diPhe-NH^{i}Pr (^{i}Pr, isopropyl) using HOAt (1-hydroxy-7-azabenzotriazole)/HATU {*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide} as coupling agents[12] (see Supporting Information).

Single crystals of **1** suitable for X-ray diffraction analysis[13] were obtained from a hexanes−dichloromethane solvent mixture and were stable in the absence of mother liquor.

(6) Zhang, J.; Albelda, M. T.; Liu, Y.; Canary, J. W. *Chirality* **2005**, *17*, 404−418.

(7) De Santis, P.; Morosetti, S.; Rizzo, R. *Macromolecules* **1974**, *7*, 52−58.

(8) (a) Brea, R. J.; Amorín, M.; Castedo, L.; Granja, J. R. *Angew. Chem., Int. Ed.* **2005**, *44*, 5710−5713. (b) Leclair, S.; Baillargeon, P.; Skouta, R.; Gauthier, D.; Zhao, Y.; Dory, Y. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 349−353. (c) Horne, W. S.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 9372−9376. (d) Semetey, V.; Didierjean, C.; Briand, J.-P.; Aubry, A.; Guichard, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1895−1898. (e) Gauthier, D.; Baillargeon, P.; Drouin, M.; Dory, Y. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 4635−4638. (f) Gademann, K. Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 957−962. (g) Fujimura, F.; Fukuda, M.; Sugiyama, J.; Morita, T.; Kimura, S. *Org. Biomol. Chem.* **2006**, *4*, 1896 −1901.

(9) (a) Görbitz, C. H. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **2006**, *62*, o328−o330. (b) Soldatov, D. V.; Moudrakovski, I. L.; Grachev, E. V.; Ripmeester, J. A. *J. Am. Chem. Soc.* **2006**, *128*, 6737−6744. (c) Görbitz, C. H.; Nilsen, M.; Szeto, K.; Tangen, L. W. *Chem. Commun.* **2005**, 4288−4290. (d) Song, Y.; Challa, S. R.; Medforth, C. J.; Qiu, Y.; Watt, R. K.; Peña, D.; Miller, J. E.; Swol, F. V.; Shelmutt, J. A. *Chem. Commun.* **2004**, 1044−1045. (e) Soldatov, D. V.; Moudrakovski, I. L.; Ripmeester, J. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 6308−6311. (f) Reches, M.; Gazit, E. *Science* **2003**, *300*, 625−627. (g) Görbitz, C. H. *New J. Chem.* **2003**, *27*, 1789−1793. (h) Birkedal, H.; Schwarzenbach, D.; Pattison, P. *Angew. Chem., Int. Ed.* **2002**, *41*, 754−755. (i) Görbitz, C. H. *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, *58*, 849−854. (j) Görbitz, C. H. *Chem. Eur. J.* **2001**, *7*, 5153−5159. (k) Görbitz, C. H.; Gundersen, E. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1996**, *52*, 1764−1767.

(10) (a) Ray, S.; Drew, M. G. B.; Das, A. K.; Banerjee, A. *Tetrahedron* **2006**, *62*, 7274−7283. (b) Ray, S.; Haldar, D.; Drew, M. G. B.; Banerjee, A. *Org. Lett.* **2004**, *6*, 4463−4465.

(11) (a) Crisma, M.; De Borggraeve, W. M.; Peggion, C.; Formaggio, F.; Royo, S.; Jiménez, A. I.; Cativiela, C.; Toniolo C. *Chem. Eur. J.* **2006**, *12*, 251−260. (b) Royo, S.; De Borggraeve, W. M.; Peggion, C.; Formaggio, F.; Crisma, M.; Jiménez, A. I.; Cativiela, C.; Toniolo, C. *J. Am. Chem. Soc.* **2005**, *127*, 2036−2037. (c) Jiménez, A. I.; Cativiela, C.; Marraud, M. *Tetrahedron Lett.* **2000**, *41*, 5353−5356.

(12) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(13) **Crystal data for peptide 1:** $C_{40}H_{43}N_3O_4$, $M = 629.77$, hexagonal, space group $P6_5$, $a = b = 23.721(3)$ Å, $c = 12.930(3)$ Å, $V = 6300.8(18)$ Å^3, $Z = 6$; $d_{calc} = 0.996$ g cm^{-3}. Intensity data were collected at room temperature with Cu Kα radiation ($\lambda = 1.54178$ Å) using a Philips PW 1100 diffractometer. The structure was solved by direct methods with the SIR 2002 program. The choice between the enantiomorphic $P6_5$ and $P6_1$ space groups was based upon the known configurations of the constituent amino acid residues. Refinement was carried out on F^2 by the full-matrix block least-squares procedure, using all data, by application of the SHELXL 97 program. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were calculated at idealized positions and refined using a riding model. All phenyl groups were constrained to the idealized geometry. The final R values were $R_1 = 0.0605$ for 2467 data with $I > 2\sigma(I)$ and $wR_2 = 0.1708$ for all 3281 data. The largest peak and hole in the final difference Fourier map were 0.33 and −0.17 e Å^{-3}. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre with reference number CCDC 623144. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif. *SIR 2002*: Burla, M. C.; Camalli, M.; Carrozzini, B.; Cascarano, G. L.; Giacovazzo, C.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **2003**, *36*, 1103. *SHELXL 97*: Sheldrick, G. M. *SHELXL 97. Program for the Refinement of Crystal Structures*; University of Göttingen: Göttingen, Germany, 1997.

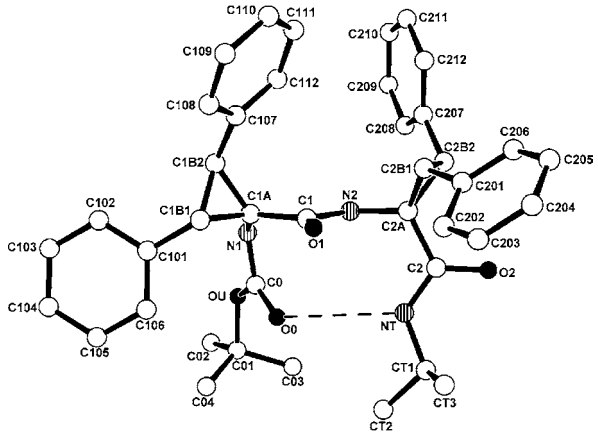

Figure 2. X-ray diffraction structure of peptide **1** with atom numbering. The intramolecular H-bond is shown as a dashed line.

The 3D structure of this compound in the crystal state is depicted in Figure 2. The molecule is folded in a type-I′ β-turn conformation (Table 1), stabilized by a weak intramolecular (Boc) C═O···H−N (NH^{i}Pr) hydrogen bond, which closes a 10-membered atom ring (Table 2).

Table 1. Selected Torsion Angles[a] for Peptide **1**

torsion angles (deg)	peptide **1**
θ^1	−173.1(6)
ω_0	162.5(4)
ϕ_1	64.7(5)
ψ_1	33.4(5)
ω_1	176.4(4)
ϕ_2	88.8(5)
ψ_2	−16.3(5)
ω_2	−170.6(4)

[a] Ideal values for a type I′ β-turn: $(\phi,\psi)_1 = (60,30)$; $(\phi,\psi)_2 = (90,0)$.[14]

Table 2. Hydrogen Bond Parameters for Peptide **1**

D−H···A	H···A (Å)	D···A (Å)	D−H···A (deg)
NT−HT···O0	2.41	3.246(5)	164
N1−H1···O2[a]	2.15	2.971(4)	158

[a] Symmetry equivalence x − y, x, z − 1/6.

In the crystal, the peptide molecules are connected by intermolecular hydrogen bonds between the [(*S*,*S*)c$_3$diPhe] N−H and the [(*R*,*R*)c$_3$diPhe] C═O groups. The self-assembly of the peptide molecules through this intermolecular hydrogen-bonding scheme results in a supramolecular, left-handed, helical structure, the screw axis of which is the crystallographic *c*-axis (a 6_5 axis). This helical assembly generates an open-ended cylinder of an average diameter of 18 Å (Figure 3), with a turn of the helix being completed every six peptide molecules, that is 12.9 Å.

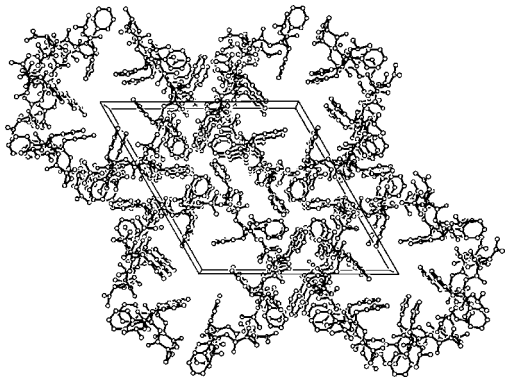

Figure 3. Top view of the nanotubular ensemble of the crystalline structure of peptide **1**.

One of the phenyl groups of the (*R*,*R*)c$_3$diPhe residue is directed toward the inner part of the helix, which as a result has a hydrophobic, electron-rich character. Due to the remarkable high diameter of the supramolecular helix and despite the bulkiness of the aromatic rings it hosts, the interior of the tube shows a hollow cylinder of an approximate diameter of 7 Å. Furthermore, as the edge-to-edge distance between phenyl rings spaced by one complete helical turn is 8.3 Å, additional room to accommodate guest molecules is located along the helical path surrounding the central hollow cylinder.[15] Nevertheless, the crystal structure determination showed no residual electron density from solvent molecules inside the channel.

Another remarkable feature of this crystalline structure is its calculated density (0.996 g/cm^3), which is significantly low in comparison to the values usually encountered for organic crystal structures of similar composition. This finding provides evidence for the high percentage of free space available in the crystal, that is, for the great porosity of the solid.

The tubular structure formed by dipeptide **1** may be considered somewhat unique, showing distinctive features that make it different from all of the categories of peptide nanotubes described so far. Compound **1** incorporates two hydrophobic residues, similarly to the previously studied linear, non-protected dipeptides containing Ala, Val, Ile, Leu, or Phe.[9] However, the intermolecular hydrogen-bonding scheme that supports the supramolecular structure of **1** is clearly different, since both termini are blocked, so that the peptide molecules are connected through hydrogen bonds

(14) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425−1436.
(15) By assuming an average van der Waals' volume of 19 Å^3 per non-hydrogen atom, the observed unit cell volume (6301 Å^3) would correspond to 332 non-hydrogen atoms. As the six peptide molecules in the unit cell sum up to 282 non-hydrogen atoms, there is room left for up to 50 additional, non-hydrogen atoms per unit cell (8 atoms per peptide molecule).

involving the backbone amide groups. Thus, the hydrogen-bonding network in **1** is somehow reminiscent of that of cyclic peptides and of the protected tripeptides presented by Banerjee and co-workers.[10] However, at variance with the aforementioned examples, the supramolecular structure generated by dipeptide **1** gives rise to a *helical*, hollow channel with a left-handed screw sense. In principle, this property may make it possible to exploit this material as a nanosolenoid, provided that charged species of suitable size can be forced to travel along the helical, aromatic channel.

The singularity of the supramolecular self-assembly of dipeptide **1** molecules, together with the unusually large diameter of the supramolecular helix and the high porosity of the crystal, make this 3D structure very significant. Quite recently, the non-protected Phe−Phe sequence has been shown to form nanostructures in the solid state,[16] highlighting the ability of aromatic homo-dipeptides to self-assemble into well-ordered structures in the nanometric scale. The results described herein on the terminally protected, heterochiral, homo-dipeptide **1**, incorporating a restricted Phe analogue with two phenyl groups,[11] further support the view that aromaticity might be instrumental in dictating peculiar self-assembling properties to short peptides.

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Supporting Information Available: Experimental procedures, spectral characterization and crystallographic data in CIF format for peptide **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) (a) Reches, M.; Gazit, E. *Phys. Biol.* **2006**, *3*, S10− S19. (b) Görbitz, C. H. *Chem. Commun.* **2006**, 2332−2334. (c) Reches, M.; Gazit, E. *Isr. J. Chem.* **2005**, 363−371.