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Self-Assembling, Cystine-Derived, Fused Nanotubes Based on Spirane Architecture: Design, Synthesis, and Crystal Structure of Cystinospiranes

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Abstract: A novel family of cystine-based spirobicyclic peptides (cystinospiranes) has been synthesized by a single-step procedure involving condensation of pentaerythritol-derived tetrachloride with either the simple L-cystine dimethyl ester or its C,C′-extended bispeptides leading to a variety of 19-membered spirobicyclic peptides or its N,N′-extended bispeptides affording the ring-expanded 25-membered cystinospiranes. The design is flexible with respect to the ring size that can be adjusted depending upon the length of the N,N′-extended cystine bispeptide, and the choice of an amino acid, as illustrated here with the preparation of a large number of cystinospiranes containing a wide variety of amino acids. X-ray crystal structure of the parent spirane (**5a**) revealed nanotube formation by vertical stacking of relatively flat spirobicyclic molecules through contiguous NH- - -O═C hydrogen bonding. The fused pair of parallel nanotubes is open-ended, hollow, and extends to infinity. Crystallographic parameters are the following: $C_{33}H_{52}N_4O_{16}S_4$, space group *C*2, $a = 42.181(3)$ Å, $b = 5.1165(7)$ Å, $c = 11.8687(9)$ Å, $\beta = 106.23(1)°$.

Introduction

Creation of new molecules with exotic architecture and useful functions is an area that will continue to stimulate the imagination of synthetic chemists. Cyclic peptides are particularly important because of their demonstrated potential as antibiotics, regulators of membrane ion transport, and templates for protein design. Nanotube formation[1] from cyclic peptides has added another dimension to the utility of peptides as new materials. Our continuing interest in developing designer hybrid cyclic peptides[2] and peptide dendrimers[3] as new artificial protein materials introduced us to the use of the tetra-functional Pentaerythritol molecule[4] as the basic core for designing tetrahedrally fused spirobicyclic peptides, peptidospiranes (a new class of cystine-based hybrid cyclic peptides).

Results and Discussions

The design of peptidospiranes had its genesis in the crystal structure of a simple tetra Aib derivative (**3**) of the pentaeryth-

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(1) Hartgerink, J. D.; Clark, T. D.; Ghadiri, M. R. *Chem. Eur. J.* **1998**, *4*, 1367 and references therein. Karle, I. L.; Handa, B. K.; Hassal, C. H. *Acta Crystallogr.* **1975**, *B31*, 555. Seebach, D.; Matthews, J. L.; Meden, A.; Wessels, T.; Baerlocher, C.; McCusker, J. B. *Helv. Chim. Acta* **1997**, *80*, 173. Ranganathan, D.; Haridas, V.; Gilardi, R.; Karle, I. L. *J. Am. Chem. Soc.* **1998**, *120*, 10795. Ranganathan, D.; Haridas, V.; Sundari, C. S.; Balasubramanian, D.; Madhusudanan, K. P.; Roy, R.; Karle, I. L. *J. Org. Chem.* **1999**, *64*, 9230. Ranganathan, D.; Lakshmi, C.; Karle, I. L. *J. Am. Chem. Soc.* **1999**, *121*, 6103. Ranganathan, D.; Lakshmi, C.; Haridas, V.; Gopikumar, M. *Pure Appl. Chem.* **2000**, *72*, 365.

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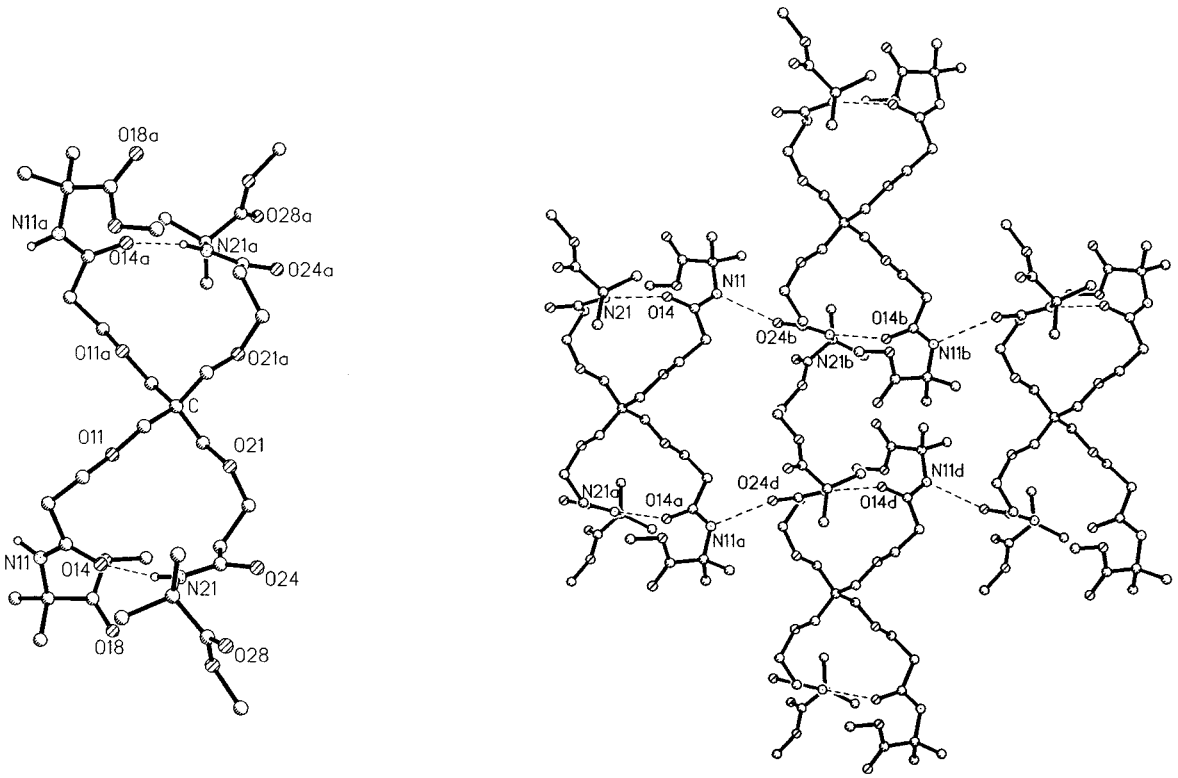
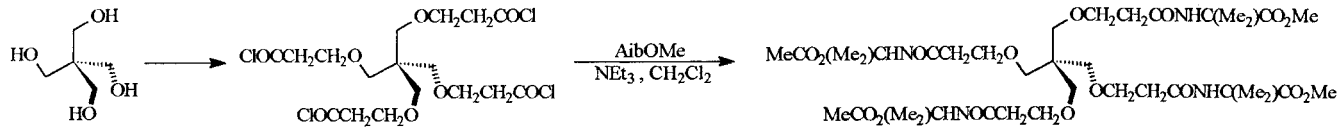


Figure 1. (a) Crystal structure of pentaerythritol-derived tetra Aib peptide **3**. The hydrogen-bonded spirobicyclic framework of 14-membered rings fused at the tetrahedral carbon is generated by the participation of Aib NH of one chain in intramolecular NH- - -O═C (N- - -O, 2.87 Å, H- - -O, 1.98 Å) hydrogen bonding with the CO group of the other. (b) The layered assembly generated by intermolecular NH- - -O═C hydrogen bonding between the neighbors. Four molecules of compound **3** are shown.

Scheme 1. Synthesis of Pentaerythritol-Derived Tetra Aib Peptide (**3**)



1 **2** **3**

ritol-based core. The single-step preparation of the branched tetra Aib peptide (**3**) involved the condensation of tetrachloride **2**, derived[5] from commercially available Pentaerythritol core (**1**), with AibOMe in dry CH_2Cl_2 in the presence of triethylamine (Scheme 1).

Suitable crystals for X-ray diffraction were obtained for **3** by slow evaporation of a chloroform solution. The crystal structure (Figure 1a) revealed a novel hydrogen-bonded spirobicyclic framework for the tetra Aib molecule **3** showing an exact 2-fold rotation axis about the tetrahedral carbon. The spirobicyclic framework is generated by two intramolecular NH- - -O═C bonds involving AibNH of one chain in hydrogen bonding with the core CO group of the other. The newly generated, two 14-membered hydrogen-bonded rings fused at the tetrahedral carbon give the appearance of a spirane framework (Figure 1a). Further participation of unutilized NH and CO groups in molecule **3** generated an extended layered structure through a network of intermolecular NH- - -O═C hydrogen bonds (Figure 1b). Hydrogen bonds and torsional angle values are listed in Tables 1 and 2, respectively.

The crystal structure of **3** opened-up exciting possibilities for creating peptidospiranes from a Pentaerythritol core and appropriately crafted peptide-based 1,ω-diamines. We describe

(2) Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B.; Sukhaswami, M. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1105. Karle, I. L.; Ranganathan, D.; Haridas, V. *J. Am. Chem. Soc.* **1996**, *118*, 10916. Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B.; *J. Am. Chem. Soc.* **1997**, *119*, 11578. Ranganathan, D.; Haridas, V.; Karle, I. L. *J. Am. Chem. Soc.* **1998**, *120*, 2695. Karle, I. L.; Ranganathan, D.; Haridas, V. *J. Am. Chem. Soc.* **1998**, *120*, 6903. Ranganathan, D.; Haridas, V.; Gilardi, R.; Karle, I. L. *J. Am. Chem. Soc.* **1998**, *120*, 10795. Ranganathan, D.; Thomas, A.; Haridas, V.; Kurur, S.; Madhusudanan, K. P.; Roy, R.; Kunwar, A. C.; Sarma, A. V. S.; Vairamani, M.; Sarma, K. D. *J. Org. Chem.* **1999**, *64*, 3620. Ranganathan, D.; Haridas, V.; Kurur, S.; Nagaraj, R; Bikshapaty, E.; Kunwar, A. C.; Sarma, A.V. S.; Vairamani, M. *J. Org. Chem.* **2000**, *65*, 365. Ranganathan, D.; Haridas, V.; Nagaraj, R.; Karle, I. L. *J. Org. Chem.* **2000**, *65*, 4415.

(3) Ranganathan, D.; Kurur, S.; Gilardi, R.; Karle, I. L. *Biopolymers* **2000**, *54*, 289. Ranganathan, D.; Kurur, S.; Madhusudanan, K. P.; Roy, R.; Karle, I. L. *J. Peptide Res.* **1998**, *51*, 297. Ranganathan, D.; Kurur, S. *Tetrahedron Lett.* **1997**, *38*, 1268.

(4) Cheng, X. H.; Diele, S.; Tschierske, C. *Angew. Chem., Int. Ed.* **2000**, *39*, 592. Wörl, R.; Köster, H. *Tetrahedron* **1999**, *55*, 2941. Armspach, D.; Cattalini, M.; Constable, E. C.; Housecroft, C. E.; Phillips, D. *Chem. Commun.* **1996**, 1823. Kuzdzal, S A.; Monning, C. A.; Newkome, G. R.; Moorefield, C. N. *J. Chem. Soc., Chem. Commun.* **1994**, 2139. Young, J. K.; Baker, G. R.; Newkome, G. R.; Morris, K.; Johnson, C. S., Jr. *Macromolecules* **1994**, *27*, 3464. Newkome, G. R.; Young, J. K.; Baker, G. R.; Potter, R. L.; Audoly, L; Cooper, D.; Weis, C. D.; Morris, K. F.; Johnson, C. S.,Jr. *Macromolecules* **1993**, *26*, 2394. Newkome, G. R.; Lin, X. F. *Macromolecules* **1991**, *24*, 1443.

(5) The reported procedure for the preparation of **2** (Newkome, G. R.; Lin, X. F. *Macromolecules* **1991**, *24*, 1443) was modified in the last step by using oxalyl chloride with traces of dry pyridine in dry benzene in place of $SOCl_2$.

Table 1. Hydrogen Bonds

compd	type	donor	acceptor	N···O, Å	H···O, Å	C═O···N angle, deg
3	intermol.	N11	O24[a]	2.843	1.97	138
	intramol.	N21	O14	2.866	1.98	149
5a	intermol.	N1	O11[b]	3.207	2.33	1.60
	intermol.	N2	O21[c]	3.022	2.14	1.74

[a] Symmetry equivalent at $-^1/_2 - x$, $^1/_2 + y$, $^1/_2 - z$. [b] Symmetry equivalent at x, $-1 + y$, z. [c] Symmetry equivalent at x, $1 + y$, z.

Table 2. Torsional Angles[a] for **3** and **5a**

3			**5a**		
angle	label	value	angle	label	value
			C2bS2S1C1b	χ_{ss}	99
			S2S1C1bC1a	χ_{12}	−52
			S1C1bC1aC1′	χ_{11}	−179
C12C13C14N11	ψ_o	−160	C13C12C11N1	ψ_o	−167
C1CN11C15	ω_o	−171	C12C11N1C1a	ω_o	−176
C14N11C15C18	ϕ_1	−57	C11N1C1aC1′	ϕ_1	−80
N11C15C18O19	ψ_1	−37	N1C1aC1′O1m	ψ_1	153
C15C18O19C19	ω_1	−179	C1aC1′O1mC1m	ω_1	−178
C14C13C12O11	a_o	70	C11C12C13O13	a_o	170
C13C12O11C11	b_o	176	C12C13O13C14	b_o	160
C12O11C11C	c_o	176	C13O13C14C25	c_o	−159
O11C11CC21	d_o	−64	O13C14C25C24	d_o	47
			S1S2C2bC2a	χ_{ss}	55
			S2C2bC2aC2′	χ_{21}	52
C22C23C24N21	ψ_{02}	137	C23C22C21N2	ψ_{02}	131
C23C24N21C25	ω_{02}	180	C22C21N2C2a	ω_{o2}	174
C24N21C25C28	ϕ_2	−51	C21N2C2aC2′	ϕ_2	−74
N21C25C28O29	ψ_2	−40	N2C2aC2′O2m	ψ_2	138
C25C28O29C29	ω_2	−174	C2aC2′O2mC2m	ω_2	176
C24C23C22O21	a_{02}	−69	C21C22C23O23	a_{02}	−78
C23C22O21C21	b_{02}	−93	C22C23O23C24	b_{02}	166
C22O21C21C	c_{02}	169	C23O23C24C25	c_{02}	156
O21C21CC11	d_{02}	−61	O23C24C25C14	d_{o2}	50

[a] Torsion angles ϕ, ψ, and ω for the backbone and χ for the side chains in peptide portions follow the convention presented in ref 6. The estimated standard deviations are near 2°.

herein the first illustration of this concept and report on the design and synthesis of a variety of cystine-based spirobicyclic peptides (cystinospiranes), a new class of hybrid bicyclic peptides, and demonstrate by single X-ray structure of the parent spirane the inherent tendency of these fused bicyclic peptides to self-assemble in hydrogen-bonded tubular structures through contiguous NH- - -O═C hydrogen bonding. The fused pair of parallel twin nanotubes is open-ended and hollow and extends to infinity.

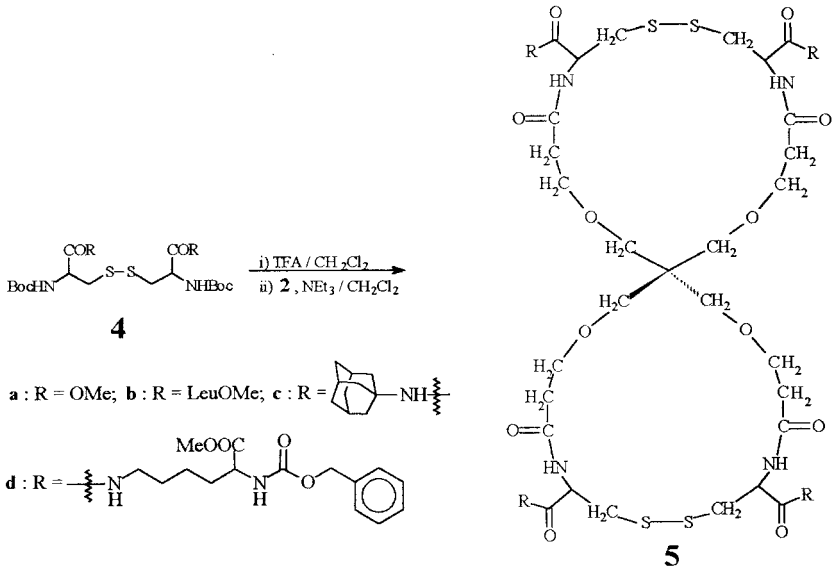
A large family of cystine-based 19-membered spirobicyclic peptides was prepared by direct condensation of pentaerythritol-derived tetrachloride **2**[5] with either the simple cystine diOMe to give the parent spirane **5a** or its C,C′-extended bispeptides (Boc-deprotected **4b**−**d**) to give amino acid functionalized spiranes (**5b**−**d**). The ring-expanded 25-membered spirane (**7**) was obtained by using N,N′-extended Boc-deprotected cystine bispeptide **6**. The present design strategy is thus highly flexible with respect to the ring size and the choice of an amino acid as illustrated here with the preparation of 19-membered (**5 a**−**d**) and 25-membered (**7**) cystinospiranes containing a variety of amino acid residues anchored on the interior and exterior of the ring framework. An additional advantage is provided by the built-in handles in **5 a**−**d** (in the form of protected COOH groups) that can be ligated via peptide chemistry to a variety of subunits, for example, a polycyclic cage-like lipophilic adamantane unit as in **5c** or a long alkylamine, a polypeptide chain, a polysaccharide unit, or even a peptide dendron, providing attractive models for novel artificial protein design and membrane ion-transport.

While the 19-membered parent cystinospirane (**5a**) was prepared by direct condensation of cystine diOMe with tetrachloride **2** in the presence of NEt_3 under high dilution conditions, the construction of amino acid-anchored spiranes (**5b**−**d**) required first the preparation of N,N′-bisBoc-C,C′-extended bispeptides (**4b**−**d**) (from bisBoc cystine and the corresponding amino acid ester using DCC/N−OH succinimide coupling) which on Boc-deprotection (TFA/CH_2Cl_2) and subsequent treatment with **2** afforded the desired peptidospiranes **5b**−**d** in good yields (Scheme 2).

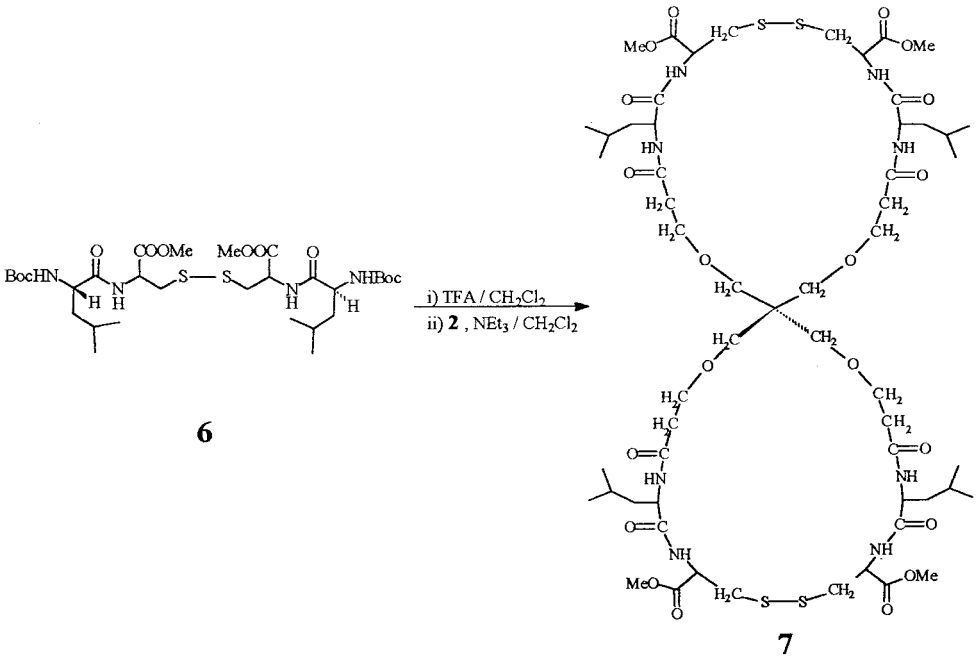
For preparation of the ring-expanded 25-membered spirane **7**, the intermediate N,N′-bis Leu (N^{α} -Boc) cystine diOMe (**6**), prepared from cystine diOMe and Boc-Leu using DCC/N−OH succinimide coupling, was Boc-deprotected (TFA/CH_2Cl_2) and coupled with **2** to give the ring-expanded sprirane **7** in moderate yields, (Scheme 3). All cystinospiranes (**5a**−**d** and **7**) required purification through a short column of silica gel with chloroform/MeOH as eluents and were fully characterized.

In ^{1}H NMR spectra, the presence of only a single set of

Scheme 2. Synthesis of 19-Membered Cystinospiranes (**5a**−**d**)



Scheme 3. Synthesis of 25-Membered Cystinospirane (**7**)



6 **7**

resonances for the cystine unit, the pendants groups, and the pentaerytritol-derived core in **5a**−**d** and **7** indicated the highly symmetrical nature of the spirobicycles (Supporting Information). The presence of strong Cyst NH−C$^{\beta}$H$_2$ cross-peaks in their ROESY NMR spectra suggested formation of a β-turn-type structure. In ^{1}H NMR variable-temperature (VT) studies, conducted in DMSO-d_6 between 303 and 343 K for **5a**, there was no indication of any intramolecular hydrogen bonding involving Cyst NHs as shown by high (−5.9 ppb/K)-temperature coefficient values for these protons (Supporting Information). The observed NMR data are thus consistent with the crystal structure.

A suitable crystal for X-ray diffraction was obtained for the parent spirobicycle **5a** from chloroform solution by slow diffusion of hexane vapor. The crystals had a strong tendency to crumble when exposed to air. All X-ray studies were therefore carried out at −60 °C with the crystal covered with microscope immersion oil.

The crystal structure of **5a** (Figure 2a,b) revealed a spirobicyclic framework with 2-fold rotation symmetry about the tetrahedral (C_{25}) carbon. All four carbomethoxy groups extend outward and there are no internal hydrogen bonds. The amide groups are all trans and lie nearly perpendicular to the plane of the spirobicyclic framework. In this conformation the spirobicyclic rings have a parallel alignment and stack on top of one another through contiguous intermolecular NH- - -C═O hydrogen bonding, maintaining perfect registry between the molecules, generating a fused pair of tubes that are hollow, open-ended, and extend to infinity. The planes of the fused rings in the tube are inclined at an angle of 22.6°. Each bicyclic molecule in the tube makes eight NH- - -O═C hydrogen bonds (average N- - -O, 3.12 Å, H- - -O, 2.24 Å) with the two neighbors above and below. The average inter-ring distance between the subunits is ∼5.12 Å, Figure 3. In the crystal packing (Figure 4), the spirobicyclic tubes are held together only by van der Waals forces.

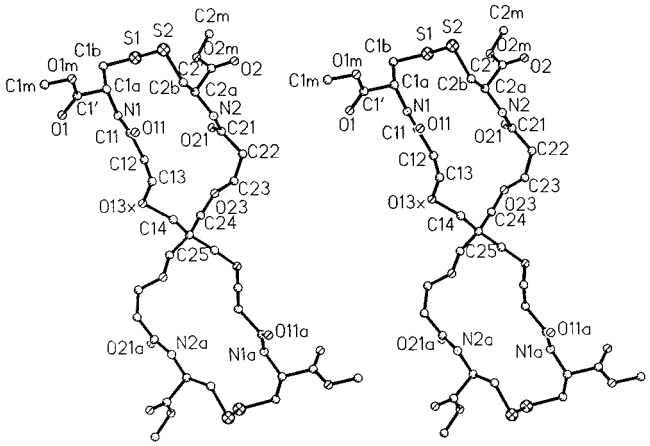


Figure 2. (a) Stereodiagram of the crystal structure of cystinospirane **5a** Torsional angles are listed in Table 2. The molecule exhibits a 2-fold rotation symmetry about the tetrahedral (C_{25}) carbon. Cavity size: S1−O23, 7.56 Å; C12−C21, 4.30 Å. The molecule is relatively rigid at either end that encompasses the cystine moieties and the intermolecular hydrogen bonds, but exhibits considerable motion for the Pentaerythritol core, and has distinct disorder at C13 and O13 (not shown). The reduction of the space group symmetry to triclinic *P*1 did not improve the fit to the experimentally measured reflections or the disorders in the structure.

The crystal structure further revealed parallel columns of empty space between the stacks. This space is surrounded by hydrophobic groups from the main molecules and is occupied by disordered solvent molecules that have not been resolved or identified.

Conclusions

In summary, the present work introduces a new class of hybrid cyclic peptides: the cystine-based spirobicyclic peptides (cystinospiranes). A single-step synthetic strategy involving direct condensation of a Pentaerythritol-derived core (as tetra-

(6) IUPAC-IUB Commission on Biomedical Nomenclature. *Biochemistry* **1970**, *9*, 3471.

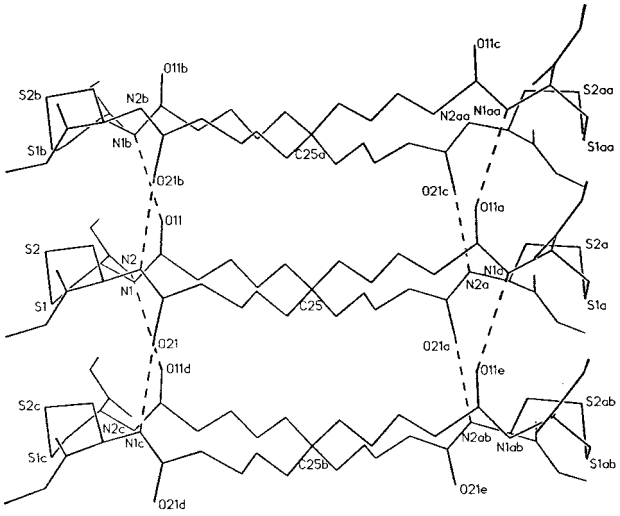


Figure 3. The molecules of **5a** aligned in a parallel fashion stack on top of one another. Each molecule participates in eight NH- - -O═C hydrogen bonds (average N- - -O, 3.12 Å, H- - -O, 2.24 Å).

chloride) with cystine diOMe or its N,N′- or C,C′-extended bispeptides leads to a variety of spirobicyclic peptides fused at the tetrahedral carbon. The design is flexible with respect to ring size and nature of the amino acid as illustrated here with the preparation of 19- and 25-membered cystinospiranes containing a wide choice of amino acids present as part of the spirane ring or attached as pendants on the exterior of the bicyclic framework. In the crystal structure, the parent cystinospirane shows an inherent tendency to self-assemble into tube-like structure by packing atop one another through pairs of contiguous NH- - -O═C hydrogen bonds on either side. The fused pair of parallel nanotubes is open-ended and hollow and extends to infinity. Although the biological and material properties of these fused cystine-based, twin nanotubes is yet to be examined, the design reported here, to our knowledge, represents the first example of a fused or a junctioned pair of peptide nanotubes that is likely to open up an exciting area of nanotube engineering.

Experimental Section

All amino acids used were of L-configuration. Melting points were recorded on a Fisher−Johns melting point apparatus and are uncorrected. Optical rotations were measured with an automatic JASCO DIP-370 digital polarimeter; concentrations are given in g/100 mL. Infrared spectra were recorded on a Nicolet 740-FT spectrometer as KBr pellets. ^{1}H NMR spectra were recorded on GEMINI-200 MHz, Varian UNITY-400 MHz, and UNITY INOVA-500 MHz instruments. The chemical shifts are reported in δ (ppm) with TMS at 0.00 as an internal reference. ROESY experiments were performed using 0.2 s mixing time with pulsed spin locking with 30° pulses and 2 kHz spinlocking field. FAB and ES mass spectra were recorded on a VG Autospec (with *m*-nitrobenzyl alcohol as the matrix) and micromass Quattro LC instruments, respectively. Reactions were monitored wherever possible by TLC. Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100−200 mesh) columns which were generally made from a slurry in chloroform or a mixture of ethyl acetate and hexane and products were eluted with a mixture of chloroform/methanol or ethyl acetate/hexane.

Preparation of Pentaerythritol-Derived Tetra Aib Peptide 3. Pentaerythritol (Merck) was converted into the tetraacid of **2** by the reported procedure.[5] The tetrachloride **2** (0.25 mmol, obtained in quantitative yields from the tetraacid[5]) was directly condensed with freshly generated Aib-OMe (1 mmol) in dry CH_2Cl_2 in the presence of triethylamine. After 24 h, reaction mixture was worked up by sequential washing with ice cold 2 N H_2SO_4, water, and saturated $NaHCO_3$ solution (20 mL each), drying the organic extract ($MgSO_4$), and evaporating under vacuum. The residue was purified on a short column of silica gel using a mixture of ethyl acetate/hexane as eluent to give the title compound **3**, yield 72%: mp 155−156 °C; IR (KBr) 3255, 3216, 3075, 3004, 2949, 2902, 2871, 1733, 1678 (sh), 1639, 1561, 1475, 1388 (sh), 1373, 1294 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 24H), 2.26 (t, 8H), 3.24 (s, 8H), 3.48 (t, 8H), 3.54 (s, 12H), 8.14 (s, 4H); ES MS *m*/*z* (%) 843 (100) (M + Na$^+$), 821 (34) (M + H)$^+$.

Preparation of C,C′-Extended Cystine Bispeptides 4b−d. 4b−d were prepared by coupling bisBoc cystine (1 mmol) with the corresponding amino acid methyl ester (2 mmol) or 1 amino adamantane (for **4c**) using DCC/N−OH succinimide procedure.

Selected data: 4b: yield 90%; mp 154−156 °C; $[\alpha]^{30}_D$ −58.60 (*c* 1.0, $CHCl_3$:MeOH, 1:1); IR (KBr) 3343, 2958, 2872 (sh), 1743, 1686, 1547, 1519, 1458, 1433, 1367, 1313, 1277, 1251, 1219, 1169 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92 (brd, 12H), 1.46 (s, 18H), 1.66 (m, 6H), 2.83 (m, 2H), 3.05 (m, 2H), 3.67 (s, 6H), 4.58(m, 2H), 4.85 (m, 2H), 5.51 (d, J = 5.06 Hz, 2H), 7.68 (d, J = 7.09 Hz, 2H); FAB MS *m*/*z* (%) 717 (32) (M + Na$^+$), 695 (54) (M + H)$^+$, 495 (100) (M − 2 × Boc + H)$^+$.

4c: yield 85%; mp 193−194 °C; $[\alpha]^{30}_D$ −25.44 (*c* 1.0, $CHCl_3$:MeOH, 1:1); IR (KBr) 3425 (sh), 3329, 3052 (sh), 2908, 2852, 1684, 1535, 1484, 1453, 1395, 1385, 1312 (sh), 1266 (sh), 1248, 1211 (sh), 1190 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.49 (brs, 18H), 1.70 (brd, 12H), 2.06 (brd, 18H), 2.90 (brd, 4H), 4.50 (m, 2H), 5.50 (d, J = 5.18 Hz, 2H), 6.80 (brs, 2H); ES MS *m*/*z* (%) 729 (100) (M + Na$^+$), 707(71) (M + H)$^+$.

4d: yield 61%; mp 108−109 °C; $[\alpha]^{30}_D$ −22.43 (*c* 1.0, $CHCl_3$:MeOH, 1:1); IR (KBr) 3338, 2950, 1745 (sh), 1692, 1688, 1521, 1458, 1381, 1312, 1276, 1248, 1100 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 18H), 1.51 (m, 12H), 2.92 (m, 4H), 3.24 (m, 4H), 3.73 (s, 6H), 4.28 (m, 2H), 4.69 (m, 2H), 5.08 (m, 4H), 5.60 (m, 4H), 7.32 (m, 10H), 7.68 (brd, 2H); ES MS *m*/*z* (%) 1016 (100) (M + Na$^+$), 994 (13) (M + H)$^+$.

Preparation of N,N′-Extended Cystine Bispeptide 6. 6 was prepared by coupling cystine diOMe (1 mmol) with Boc-Leu (2 mmol) using the DCC/N−OH succinimide procedure: yield 45%; mp 142−143 °C; $[\alpha]^{30}_D$ −52.09 (*c* 1.0, $CHCl_3$:MeOH, 1:1); IR (KBr) 3372, 3192 (sh), 2986, 2993 (sh), 1730, 1687, 1518, 1442 (sh), 1417, 1369 (sh), 1285, 1250, 1168 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (m, 12H), 1.40 (s, 18H), 1.58 (m, 6H), 3.00 (m, 4H), 3.72 (s, 6H), 4.26 (m, 2H), 4.80 (m, 2H), 5.26 (d, J = 5.91 Hz, 2H), 7.56 (d, J = 11.81 Hz, 2H); ES MS *m*/*z* (%) 717 (92) (M + Na$^+$), 695 (100) (M + H)$^+$.

General Procedure for the Preparation of Cystinospiranes 5 a−d and 7. A solution of Pentaerythritol-derived tetrachloride (**2**, 0.5 mmol, freshly prepared from the precursor tetracarboxylic acid by treating with oxalyl chloride (3 mmol) in dry benzene containing catalytic amounts of dry pyridine for 24 h, followed by drying in vacuo) in dry CH_2Cl_2 (10 mL) was added dropwise over a period of 0.5 h to a well-stirred and ice-cooled solution of cystine diOMe or freshly Boc-deprotected (TFA/CH_2Cl_2) free base of **4b−d** or **6** (1 mmol) in dry CH_2Cl_2 (300 mL) containing triethylamine (2 mmol) and the mixture stirred at room temperature for 12 h. The reaction mixture was washed sequentially with 20 mL each of ice cold 2 N H_2SO_4, H_2O, and 5% aqueous $NaHCO_3$, and the organic layer dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was purified on a short column of silica gel using a mixture of chloroform and methanol as eluent to afford the titled cystinospiranes in moderate to good yields.

Selected data for 5a−d and 7: 5a: yield 51%; mp, sinters at 200 °C, becomes brown at 240 °C, and decomposes at 260 °C; $[\alpha]^{30}_D$ −3.95 (*c* 0.65, $CHCl_3$:MeOH, 1:1); IR (KBr) 3327, 2960, 2923, 2876, 1748, 1651, 1526 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.25 (m, 4H), 2.41 (m, 4H), 2.85 (m, 4H), 3.07 (m, 4H), 3.24 (m, 8H), 3.50 (m, 4H), 3.60(m, 4H), 3.63 (s, 12H), 4.62 (m, 4H), 8.35 (d, J = 8.0 Hz, 4H); FAB MS *m*/*z* (%) 889 (100) (M + H)$^+$, 911 (60) (M + Na$^+$).

5b: yield 41%; mp, turns brown at 232 °C and decomposes at 245 °C; $[\alpha]^{30}_D$ −57.91 (*c* 1.0, $CHCl_3$:MeOH, 1:1); IR (KBr) 3308, 2957,

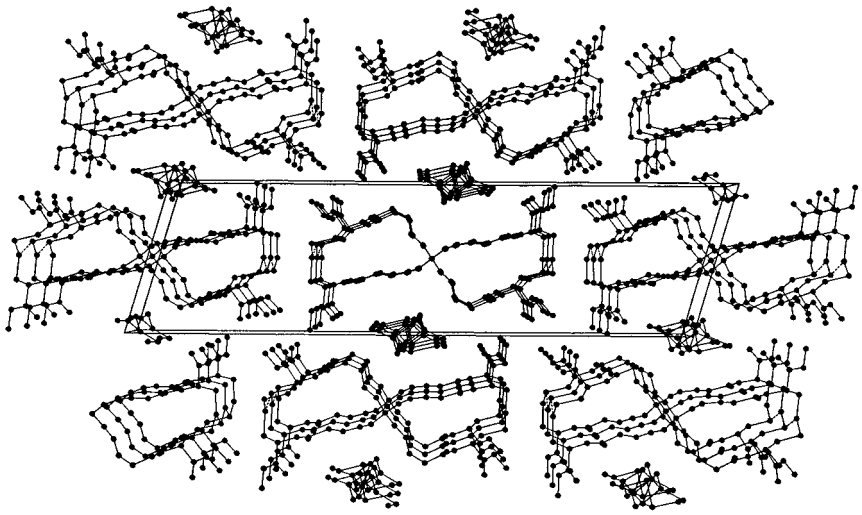


Figure 4. Side-by-side packing of fused twin tubes. Space between the twin tubes is occupied by disordered solvent.

2872, 1749, 1646, 1535 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.82 (d, 12H), 0.88 (d, 12H), 1.48 (m, 4H), 1.59 (m, 8H), 2.22 (m, 4H), 2.47 (m, 4H), 2.82 (m, 4H), 3.11(m, 4H), 3.29 (m, 8H), 3.52 (m, 4H), 3.60 (brs, 12H), 3.62 (m, 4H), 4.26 (m, 4H), 4.61(m, 4H), 8.04 (d, J = 8.4 Hz, 4H), 8.33 (d, J = 7.6 Hz, 4H); FAB MS *m*/*z* (%) 1342 (39) ($M + H)^+$, 1364 (100) ($M + Na^+$).

5c: yield 44%; mp, turns brown at 198 °C and decomposes at 210 °C; $[\alpha]^{30}_D$ +0.63 (*c* 1.0, $CHCl_3$:MeOH, 1:1); IR (KBr) 3329, 2908, 2851, 1650, 1539 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.65 (m, 30H), 2.04 (m, 30H), 2.48 (t, 8H), 3.03 (m, 4H), 3.25 (m, 4H), 3.37 (brs, 8H), 3.70 (m, 8H), 4.73 (m, 4H), 6.43 (s, 4H), 7.19 (d, J = 6.8 Hz, 4H); FAB MS *m*/*z* (%) 1366 (87) ($M + H)^+$, 1388 (52) ($M + Na^+$).

5d: yield 22%; mp 193−197 °C; $[\alpha]^{30}_D$ −13.48 (*c* 0.47, $CHCl_3$: MeOH, 1:1); IR (KBr) 3304, 2926, 2883 (sh), 1704, 1649, 1592, 1215, 1088 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.29 (m, 16H), 1.60 (m, 8H), 2.25 (m, 4H), 2.44 (m, 4H), 2.80 (m, 4H), 3.02 (m, 12H), 3.26 (m, 8H), 3.53 (m, 8H), 3.62 (s, 12H), 3.98 (m, 4H), 4.49 (m, 4H), 5.03 (m, 8H), 7.34 (m, 20H), 7.70 (d, J = 7.33 Hz, 4H), 8.02 (brs, 4H), 8.10 (d, J = 7.94 Hz, 4H); FAB MS *m*/*z* (%) 1960 (100) ($M + Na^+$).

7: yield 36%; mp, turns brown at 260 °C and decomposes at 276 °C; $[\alpha]^{30}_D$ −20.20 (*c* 1.0, $CHCl_3$:MeOH, 1:1); IR (KBr) 3312, 2954, 2930, 2870, 1739, 1640, 1539 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.84 (d, 12H), 0.88 (d, 12H), 1.43 (m, 8H), 1.58 (m, 4H), 2.25 (m, 4H), 2.41 (m, 4H), 2.91 (m, 4H), 3.09 (m, 4H), 3.20 (m, 8H), 3.48 (m, 4H), 3.52 (s, 4H), 3.62 (s, 12H), 4.40 (m, 4H), 4.61(m, 4H), 7.89 (d, J = 8.3 Hz, 4H), 8.44 (d, J = 7.6, 4H); ES MS *m*/*z* (%) 1364 (47) ($M + Na^+$).

X-ray Diffraction Analysis. X-ray data were collected at room temperature on a Bruker automated four-circle diffractometer in the $\theta/2\theta$ mode, with a constant speed of 10 deg/min, 2° scan width, and $2\theta_{max} \approx 115°$ (resolution 0.9 Å), using Cu Kα radiation (λ = 1.54178 Å) and a graphite monochromator. Structures were determined routinely with direct phase determining procedures. Full matrix, anisotropic least-squares refinement on $|F_o|^2$ was performed on the parameters for all the atoms except the H atoms. The H atoms were placed in idealized positions and allowed to ride with the C or N atom to which each was bonded. Both crystals were in the shape of elongated prisms.

3: $C_{37}H_{64}N_4O_{16}$, *C*2/*c*, a = 16.304(2) Å, b = 18.206(2) Å, c = 16.218(1) Å, β = 107.39(1)°, V = 4594.0 Å^3, d_{calcd} = 1.187 g/cm^3, R_1 = 0.0512 for 2486 $|F_o| > 4\sigma(F)$ data, wR_2 = 0.1457 for all 3159 data. The molecule contains a crystallographic 2-fold rotation axis through the spiro atom C.

5a: $C_{33}H_{52}N_4O_{16}S_4 \cdot X6$ (disordered solvent), *C*2, a = 42.181(3) Å, b = 5.1165(7) Å, c = 11.8687(9) Å, β = 106.229(9)°, V = 2459.4 Å^3, d_{calcd} = 1.295 g/cm^3, R_1 = 0.0860 for 1509 data with $|F_o| > 4\sigma(F)$, wR_2 = 0.2564 for all 1845 data. The molecule contains a crystallographic 2-fold rotation axis through the spiro atom C25. Two-positional disorder for atoms C13X and O13X was included in the refinement.

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Supporting Information Available: ^1H NMR of **3**, **5a**, **5b**, **5c**, **5d**, and **7**, 2-D ROSEY of **5a**, **5b**, **5c**, and **7**, VT NMR of **5a** and **5c**, FAB-MS of **5a**, **5b**, **5c**, and **5d**, ES-MS of **3** and **7**, and crystallographic data, coordinates, bond lengths, thermal parameters, and hydrogen bonds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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