

## Designer cyclopeptides for self-assembled tubular structures\*

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*Abstract:* A simple design strategy for a facile and direct entry into hydrogen-bonded peptide nanotubes is delineated with polymethylene-bridged cystine-based macrocycles. The key feature of the design is the placement of a pair of self-complementary hydrogen-bonding (NH–CO or NH–CO–NH) groups at almost opposite poles of the ring. A large variety of cyclobisamides and bisureas prepared in a single step by direct condensation of commercially available 1,ω-alkane dicarbonyl dichloride or diisocyanate with either cystine diOMe or its extended bispeptide were examined by X-ray crystallography and shown to possess an inherent property of self-assembling into hydrogen-bonded, open-ended, hollow tubular structures. The totally hydrophobic interior of the cyclobisamide tubes creates a micro environment capable of solubilizing highly lipophilic substances in water. The cyclic bisurea tubes are demonstrated to act as excellent receptors for selective binding to 1,ω-alkane dicarboxylates. The scope of the design is extended to the creation of tubular structures by stacking of rings through aromatic  $\pi$ - $\pi$  interactions.

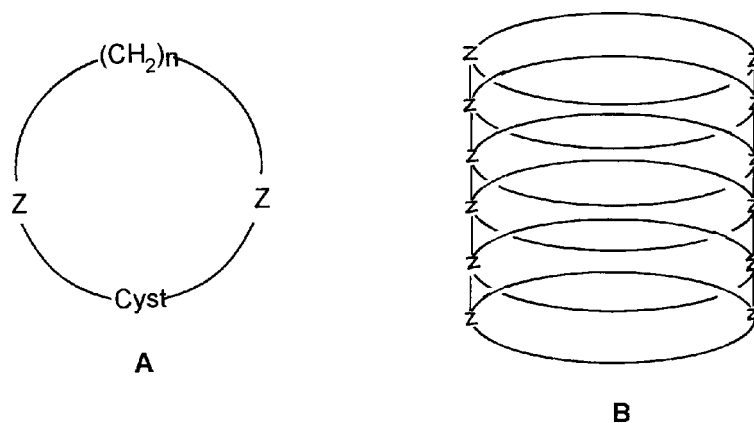
Creation of hollow tubular structures by noncovalent self-assembly of appropriately crafted organic molecules has been the subject of considerable research in recent years [1]. Tubular structures constructed [2] from chiral amino acids are particularly important because of their potential utility as models for mimicking biological channels or as transport vehicles in drug delivery systems, or they can be exploited as constrained environments to design nanostructured biomaterials. A strategy that has emerged as the most popular approach for creating hydrogen-bonded, open-ended, hollow peptide tubes is through the stacking of cyclopeptide rings [2–6]. Several designs of cyclopeptides, for example, cyclopeptides composed of an equal number of  $\alpha$ - and  $\beta$ -amino acids [4], or all  $\beta$ -amino acids [5,6], or even numbers of alternating D- and L-amino acids [7] have been reported and demonstrated to self-assemble into hollow, open-ended, tubular structures through backbone–backbone NH...O=C hydrogen bonding. A common structural feature in all these designs is the adoption of a flat or near-flat ring conformation by the cyclopeptide with side chains extending outward and amide groups lying in a plane perpendicular to the plane of the ring. The peptide rings in this conformation are poised to form contiguously hydrogen-bonded b-sheet-like tubular ensembles by stacking on top of one another.

Recently [8,9], we have evolved a rational design strategy for the construction of simple polymethylene-bridged cystine-based macrocycles that can be persuaded to form tube-like structures by stacking atop one another through hydrogen bonds. A key structural feature of the design is the placement of a pair of self-complementary hydrogen-bonding functions, for example, an amide (NH–CO) or a urea (NH–CO–NH) unit at almost opposite poles of the ring (Fig. 1).

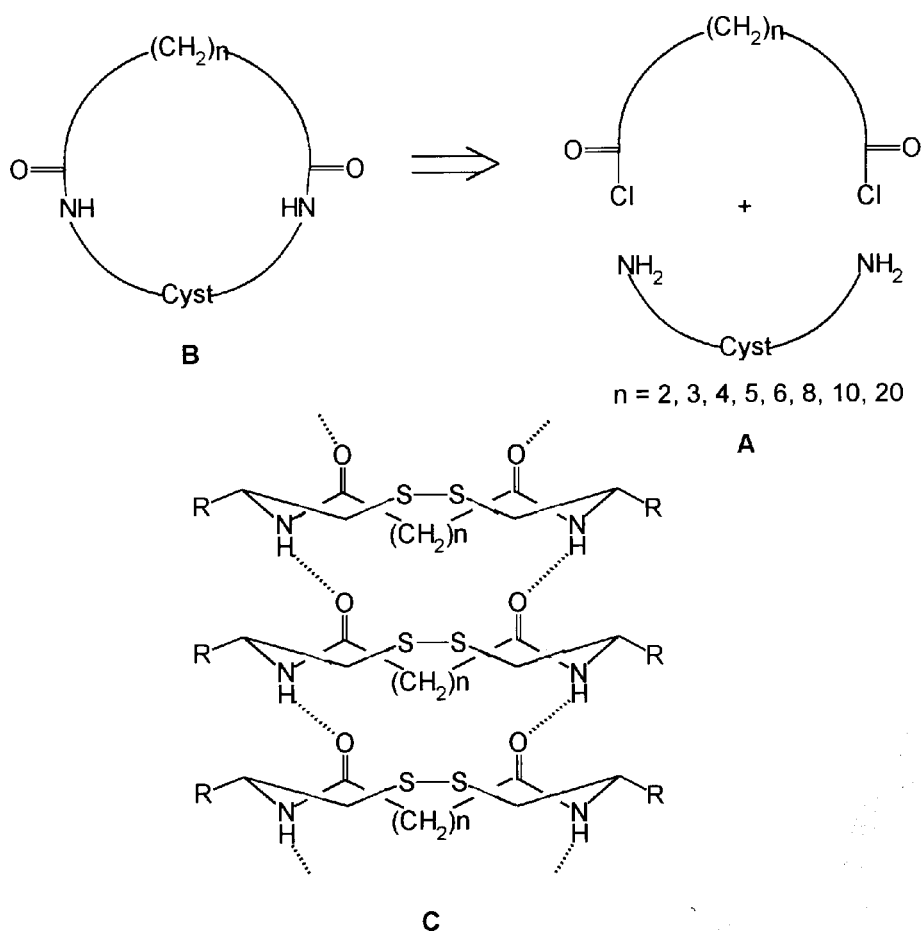
The synthesis of polymethylene-bridged cystine-based cyclobisamides (Fig. 2) and bisureas (Fig. 3)—essentially involving closing of the polymethylene chain with cystine diOMe or its bispeptide—was accomplished in a single step by the condensation of 1,ω-alkane dicarbonyldichloride or diisocyanate

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**Fig. 1** Polymethylene-bridged cystine-based macrocycles (A) with a pair of self-complementary hydrogen-bonding groups ( $Z = NH-CO$  or  $NH-CO-NH$ ) placed at almost opposite poles of the ring, can stack on top of one another to form hydrogen-bonded nanotubes (B).



**Fig. 2** Design strategy for crafting cystine-based cyclobisamide tubes. The cyclobisamides (B), constructed in a single step from 1,ω-alkane dicarbonyl dichloride and cystine diOMe (A) can stack atop one another through backbone-backbone ( $NH...O=C$ ) hydrogen bonding (C), extending into hydrogen-bonded nanotubes.

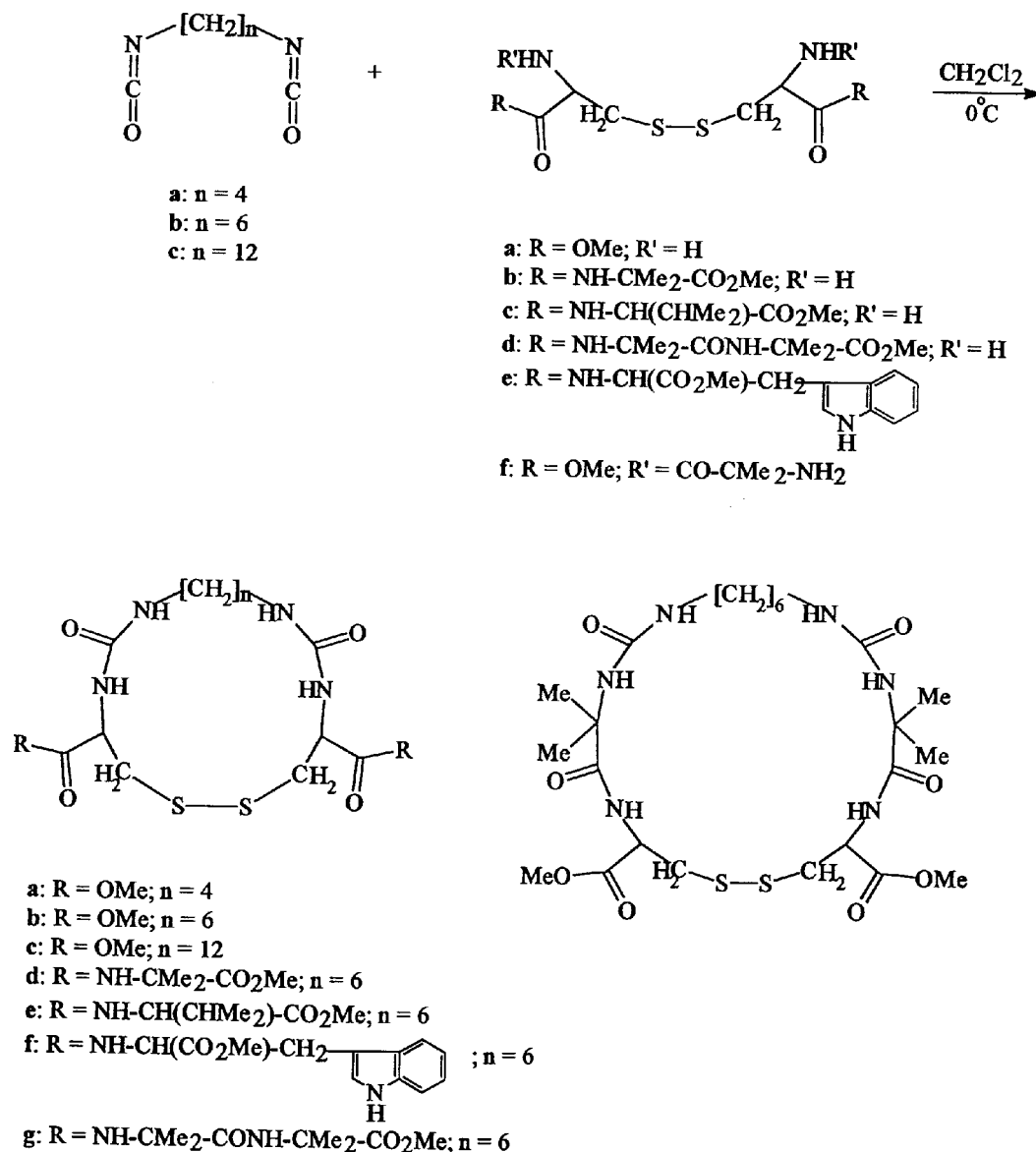
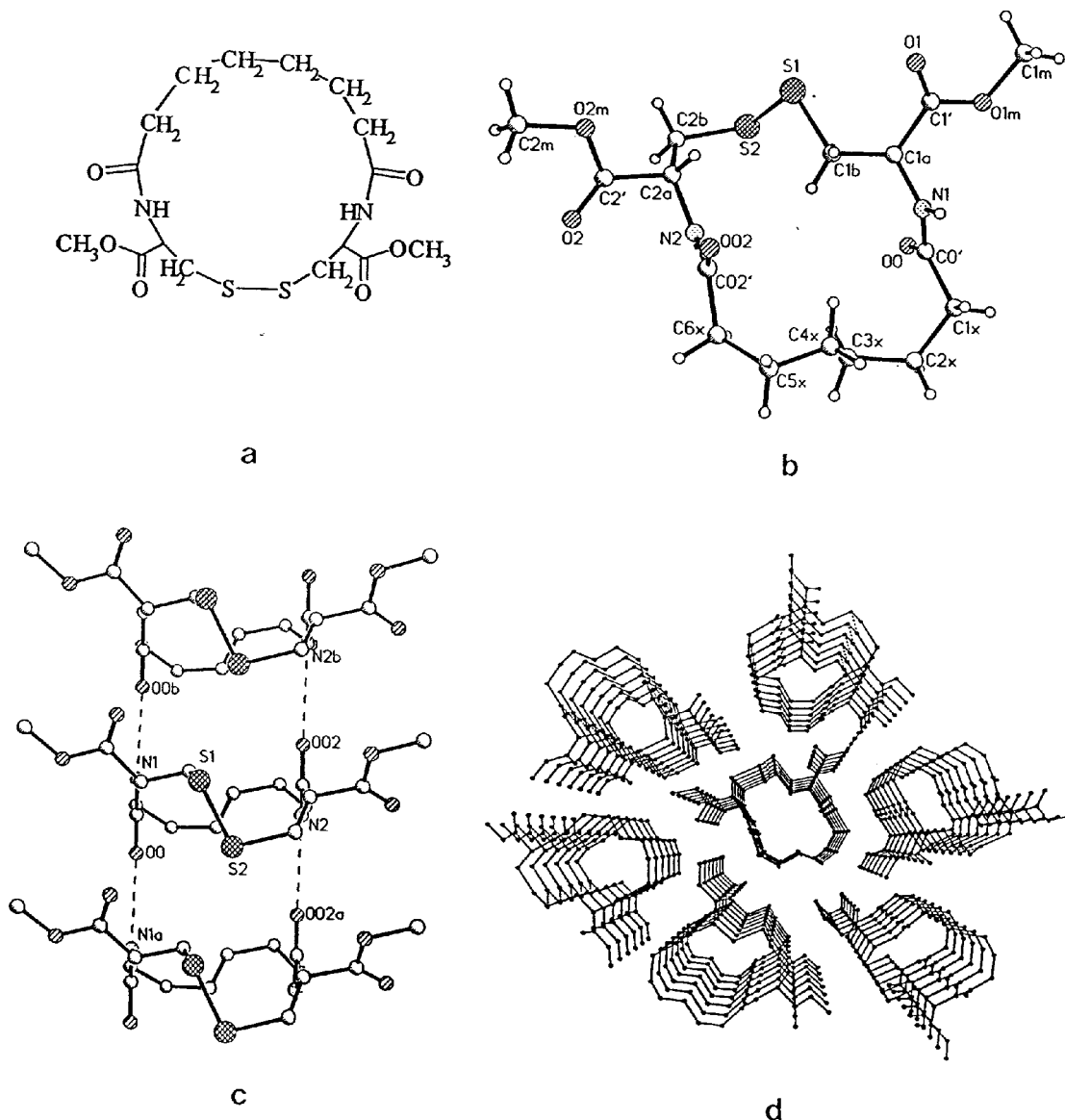


Fig. 3 Design strategy for the synthesis of cystine-based cyclic bisureas.

$[(\text{CH}_2)_n \text{X}_2; \text{X} = \text{COCl or NCO}; n = 2, 3 \dots 20]$  with either the simple L-cystine dimethyl ester or its extended C,C' or N,N'-bispeptide. The ring size of the cyclobisamide and bisurea can be adjusted by choosing dicarbonyl dichloride and diisocyanate of appropriate length as illustrated with the preparation of macrocycles ranging in ring size from 12- to 30-membered. The design is flexible with respect to the nature of the amino acid and permits the incorporation of a variety of amino acid residues either as part of the ring or as pendants on the exterior of the ring. The pendant can even be a helix, as recently shown by us, particularly useful for modifying surface properties. Interestingly, while 1 + 1 cyclization product was the only type of cyclic bisureas obtained in the condensation of diisocyanates  $[(\text{CH}_2)_n(\text{NCO})_2]$  with cystine diOMe or its bispeptide regardless of whether  $n = 4, 6,$  or  $12,$  the situation was quite different with cyclobisamides wherein the desired 1 + 1 cyclization product was invariably mixed with



**Fig. 4** Molecular formula (a) and crystal structure (b) of cyclobisamide with  $n = 6$ . cavity size : 5.29 (CO2'–CO') X 4.31 (C1b – C4x)Å. The rings stack in a vertical fashion with total registry between the subunits held together by NH–CO hydrogen bonds which appear as strings on either side (c). The tubes are empty, open-ended, and extend to infinity (d).

higher cyclic oligomers arising from 2 + 2, 3 + 3, 4 + 4, 5 + 5 and even 6 + 6 cyclization products. It was, however, gratifying to note that the yield of desired cyclobisamide (the 1 + 1 cyclization product) increases with an increase in the value of  $n$ , the optimum being 30% with  $n = 6, 8$ , or 10 indicating that ring size may be the main controlling factor in the cyclization reaction, with a 16–20 membered ring being the optimum size for maximum yield. With dodecyl ( $n = 10$ ) and docosyl ( $n = 20$ ) dicarbonyl dichlorides, the 1+1 cyclization product was the sole product of the reaction. The 1 + 1 cyclobisamides

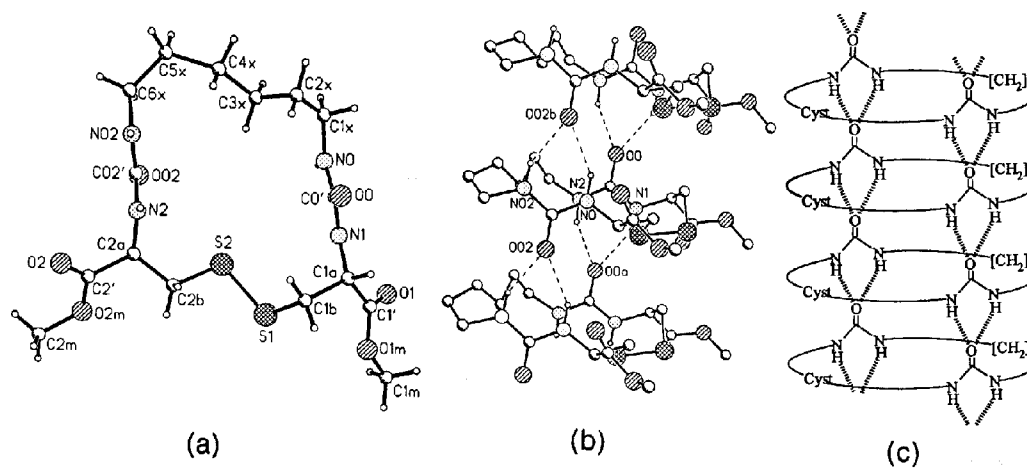
could easily be separated from cyclic oligomers by careful chromatography on silica gel column using a gradient elution with the chloroform/methanol solvent system.

X-ray crystallographic studies of a large number of cyclobisamides have shown that these macrocycles possess an inherent property of self-assembling into tube-like structures by stacking atop one another through contiguous amide–amide hydrogen bonds which appear as a pair of strings on either side. The tubes are hollow, open-ended, and extend to infinity. In the macrocyclic bisamides examined (with  $n = 4, 6, 8, 10$ ) for solid-state structure, the diameter of the tube varied between 5 Å ( $n = 4$ ) to 10 Å ( $n = 10$ ). Figure 4 presents a typical X-ray picture of a cyclobisamide ( $n = 6$ ) stacking into hydrogen-bonded tubular structures.

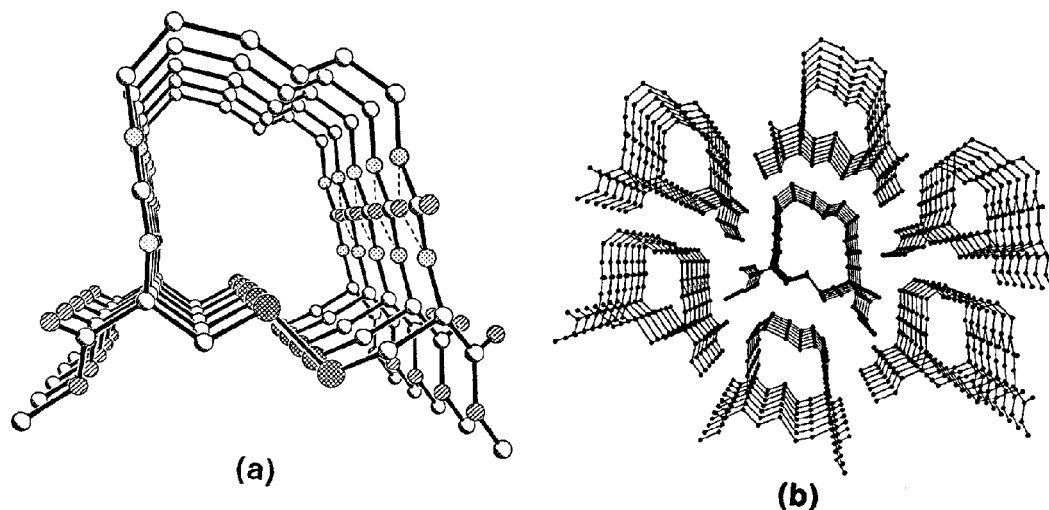
The cyclobisamide tubes possess totally hydrophobic interior comparable to a nonpolar organic solvent. Consequently, the tubes can create a microenvironment suitable for encapsulating lipophilic substances. Fluorescence experiments have shown that the cystine-based cyclobisamide tubules of appropriate diameter do the following: a) enhance the solubility of highly lipophilic arenes in water, b) bind to fluorescent probe dyes like Nile red, and c) induce an ordered structure in linear peptides as demonstrated with 26-residue bee-venom peptide melittin.

Cystine-based polymethylene-bridged cyclobisureas showed remarkably similar profile in their self-assembling behavior. Thus, as demonstrated by the single crystal X-ray structure of 18-membered cyclobisurea, the crown-shaped rings of the macrocycle aligned in a parallel fashion stack atop one another, maintaining proper registry between the subunits, generating an open-ended tube which extends to infinity. The hollow tubular-ensemble is held on either side by a string of hydrogen bonds which are of typical urea-type (Figs. 5a–5c). Figure 6a shows a view into the hydrogen-bonded cavity of the cyclobisurea. The neighboring tubes are held together only by hydrophobic interactions (Fig. 6b).

The potential of cyclobisureas to serve as tailor-made artificial receptors for 1,ω-alkane dicarboxylic acids has been demonstrated with 18- and 24-membered macrocycles which show specific binding with dianions of oxalic ( $K = 2.7 \times 10^3 \text{M}^{-1}$ ) and succinic acid ( $K = 6.21 \times 10^3 \text{M}^{-1}$ ), respectively, indicating size complementarity as the major factor for observed specificity. The 24-membered cyclobisurea was also found to exhibit high selectivity in binding to azide ion. Further host–guest com-



**Fig. 5** (a) X-ray structure of 18-membered cyclic bisurea. The urea groups are in a plane perpendicular to the plane of the ring. Consequently, the molecules aligned in a parallel fashion, stack over one another through contiguous backbone–backbone urea-type hydrogen bonding (b), making strings of hydrogen bonds on either side of the stack, leading to the generation of an extended, open-ended tubular structure with hollow interior (c).

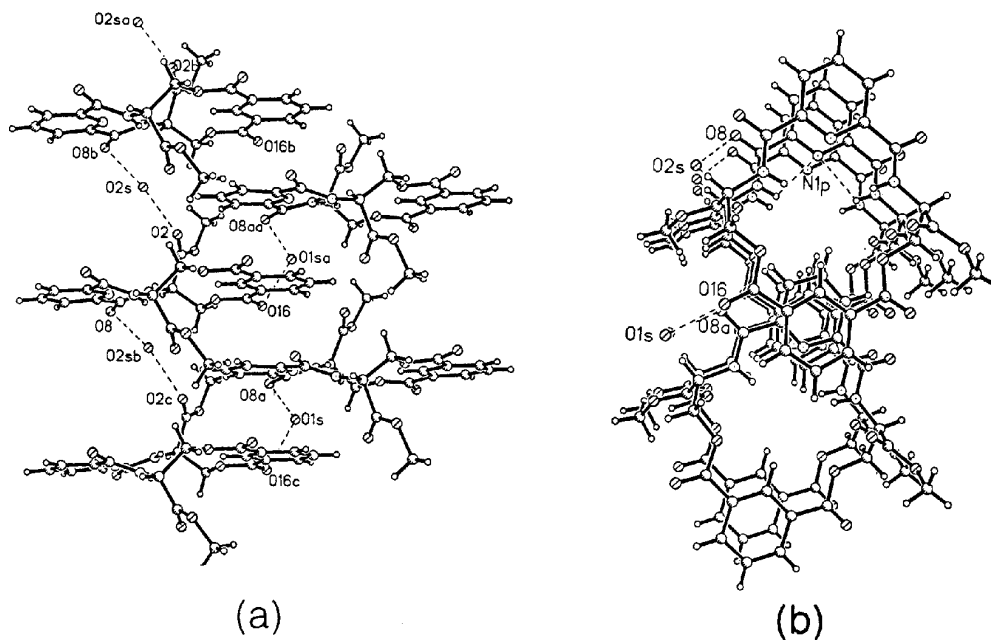


**Fig. 6** (a) A view into the cyclobisurea tube. The stack of 18-membered rings is held together by four urea-type hydrogen bonds between pairs of molecules. (b) 3D view showing side-by-side stacking of bisurea tubes. The tubes are held in the pack only by hydrophobic interactions.

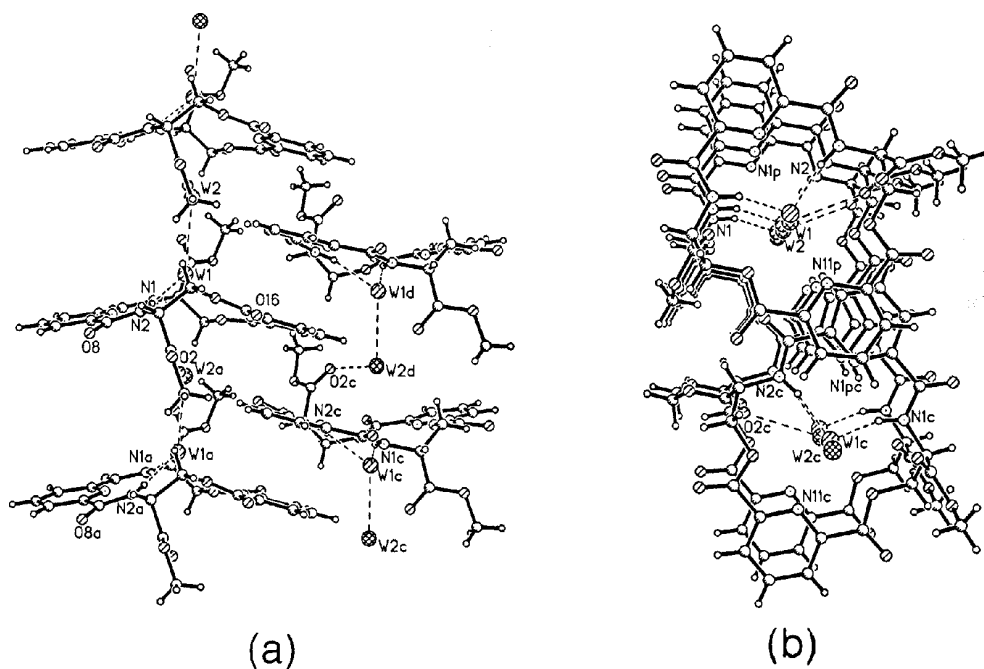
plexation studies supported the notion that cyclobisureas may serve as excellent receptors for anionic guests and would show specificity according to size complementarity of the host–guest molecules.

From the foregoing results it appeared that appropriately crafted cystine-based polymethylene-bridged cyclobisamides and bisureas would possess an intrinsic property of self-assembling into hydrogen-bonded, open-ended hollow tubes that may hold enormous promise as artificial receptors for appropriate guest molecules with potential applications in inclusion chemistry, catalysis, and drug delivery. To extend the scope of the design and to understand the role of cystine residue in facilitating vertical stacking, we prepared a large number of simple polymethylene-bridged cyclobisamides and bisureas by direct condensation of commercially available 1, $\omega$ -alkane diamines with, respectively, 1, $\omega$ -alkane dicarbonyl dichlorides and diisocyanates. Several of these macrocycles crystallized. Interestingly, while simple polymethylene-bridged cyclobisamides with the general structure  $\text{cyclo} [(-\text{CO}-\text{NH}-(\text{CH}_2)_n-\text{NH}-\text{CO}-(\text{CH}_2)_m-)]$ ;  $n = 6, m = 4$ ;  $n = 6, m = 8$  showed [10] nanotube formation in solid state by stacking of rings on top of one another much the same way as in cystine-based cyclobisamides, the analogous cyclobisureas were unable to form tubular stacks. Instead, the molecules (e.g., cyclic bisurea with  $n = 12, m = 8$ ) organize into a layered assembly [11] with checker-board motif maintaining the urea-type intermolecular hydrogen bonding. Importance of cystine residue in forming tubular structure, particularly in bisureas, was clearly brought out in the X-ray structure of cystamine-based 24-membered cyclic bisurea, which failed to form any tubular assemblies. The absence of  $\text{CO}_2\text{Me}$  groups in cystamine-based cyclobisureas off-set the parallel alignment resulting in interdigitation of macrocycles [11].

The versatility of the present design was demonstrated in the creation of peptide nanotubes through the  $\pi$ - $\pi$  stacking of aromatic-bridged macrocyclic peptides. Simple model building suggested that cyclic peptides containing small semirigid aromatic units (e.g., a phenyl or a pyridyl unit positioned at almost opposite poles of the ring) may form cylindrical stacks mainly through  $\pi$ - $\pi$  interactions of the intersubunits. This expectation was fully realized [12] in the structure of 18-membered serine-based macrocycles containing alternating repeats of serine and pyridine or phenyl units in the cyclic backbone (Figs. 7 and 8). A crucial requirement for  $\pi$ - $\pi$  stacking was the presence of a pyridine unit at the amide end, which locks the amide NHs in  $\text{NH}\dots\text{N}$  hydrogen bonding creating an almost flat ring conforma-



**Fig. 7** (a) The vertical parallel stacks in the self-assembly of cyclo(Ph-Ser-Py-Ser). Molecules 1 and 2 stack in separate columns, but the pyridine and phenyl rings interdigitate from one column to the other with a  $\sim 3.5\text{\AA}$  separation between the planes of the rings. (b) Top view of a portion of a layer containing the interdigitated stacks.



**Fig. 8** (a) The tubular self-assembly in cyclo(Py-Ser)<sub>2</sub>. The molecules stack one over the other and are connected by hydrogen bonds to two water molecules in a row. The main organizing force for the self-assembly is provided by the  $\pi$ - $\pi$  interaction between the pyridine rings. (b) Top view of a portion of a layer containing the interdigitated stacks.

tion—a prerequisite for vertical stacking. Interestingly, 26-membered cystine-based aromatic-bridged cyclic peptide showed [13] a collapsed ring conformation due to internal–aromatic face-to-face stacking. Substituting cystine with tyrosine did not improve the situation [14]. While the search is still on for an efficient system for aromatic  $\pi$ - $\pi$  stacking, the present design strategy, a key feature of which is the placement of either a pair of hydrogen-bonding, self-complementary functions or a pair of flat aromatic units at almost opposite poles of the cyclopeptide ring provides a straightforward entry into noncovalently assembled peptide nanotubes which hold enormous promise in chemical, biological, and materials science applications.

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