Designer Hybrid Cyclopeptides for Membrane Ion Transport and Tubular Structures†

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ABSTRACT
The incorporation of chosen, non-amino acids into cyclopeptides is a promising approach to designs capable of performing specific tasks. While such hybrid cyclopeptides are common in the microbial world, there is hardly any effort by synthetic organic chemists to explore such designs for creating architecturally beautiful and functionally useful macrocyclic hybrid peptides. This Account, mainly reviewing the author's own work, presents a simple design strategy enabling the crafting of a large variety of hybrid cyclopeptides and leading to the identification of excellent membrane ion carriers, units that self-assemble into tubular structures, and systems for specific guest recognition.

Creation of new molecules with exotic architecture and useful functions is an area that will continue to stimulate the imagination of synthetic chemists.[1] Among the biomolecules, peptides, constituted from twenty α-amino acids having the versatility to generate diverse (polar, hydrophobic, acidic, basic, neutral, nucleophilic, and electrophilic) environments, are particularly amenable to design and provide unlimited scope to craft new molecules with unusual properties. Additionally, the amide (NH−CO) group of the peptide chain is self-complementary in intermolecular hydrogen bonding, and this property can be exploited to create three-dimensional supramolecular structures, close to natural systems in form and function. Cyclopeptides,[2] in particular, attracted our attention as designer targets because of their demonstrated potential as antibiotics, regulators of membrane ion transport, and templates for protein design. Recent reports[3] of nanotube formation from cyclopeptides have added another dimension to their utility as new biomaterials.

Interestingly, literature reports[4] thus far have largely focused on the use of cyclopeptides as models for the study of determining preferences in protein secondary structures, and barring few exceptions,[5] there are no example of synthetic cyclopeptides containing units other than the natural α-amino acids. On the other hand, microbial organisms of land as well as sea origin display their best forms of art in the creation of cyclopeptides with unusual and exotic non-amino acid structural inserts in the ring framework.[6] These hybrid cyclopeptides, most of which contain thiazole and oxazoline amino acids, act as potent cytotoxic, antineoplastic, or antiviral agents of great therapeutic value.[6]

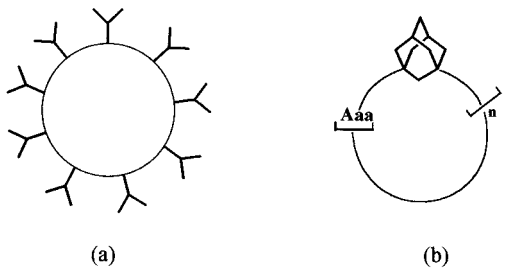

FIGURE 1. (a) Totally hydrophobic exterior of Valinomycin provided by a large number of isopropyl groups. (b) Proposed design for creating a ball-like hydrophobic exterior in cyclopeptides by arranging alternating repeats of adamantane and amino acid units.

Inspired by nature to create an entirely new class of cyclopeptides, tailored to perform specific tasks, we explored the design of hybrid cyclopeptides by incorporating non-amino acid units into the ring framework of cyclopeptides composed of natural α-amino acids. These crafted hybrid cyclopeptides[7] were expected to exhibit properties largely dictated by the nature of the hybrid unit. This Account, mainly reviewing our own work, has as its major focus the design of a special class of cyclopeptides for membrane ion transport, host−guest complexation, and nanotube formation. The design strategy employed for the synthesis of hybrid cyclopeptides was essentially property-directed in the sense that choice of the hybrid unit was mainly governed by the specific property or the particular function the hybrid cyclopeptide was expected to display.

The synthesis essentially involved a one-step condensation of the hybrid unit 1,ω-dicarbonyl dichloride with suitably crafted, peptide derived, 1,ω-diamine and turned out to be highly flexible with respect to the ring size (which could be varied from 13- to 78-membered), the choice of the amino acid (almost all the coded α-amino acids could be used), and the choice of the hybrid unit (from highly rigid nonaromatic polycyclic cagelike structures to flat aromatic units to flexible polymethylene bridges). An additional advantage is provided by the built-in handles (in the form of protected COOH and NH_2 groups) that can be ligated via peptide chemistry to a variety of subunits, such as long alkyl chains, polysaccharide units, oligonucleotide segments, protein secondary structural elements, peptide-based dendrons, and metal-complexing ligands, leading to attractive models for novel, artificial protein design, membrane ion transport, and ionophoric studies.

† Dedicated to Dr. Isabella L. Karle on the occasion of her 80th birthday.

Darshan Ranganathan was born on June 4, 1941, in Delhi, India, and sadly, when this Account was being processed for publication, passed away on June 4, 2001, from metastasis of cancer, at the crest of her career, in Hyderabad. After receiving her Ph.D. degree from Delhi University in 1967 under the guidance of Professor T. R. Seshadri, she spent two postdoctoral years with Sir D. H. R. Barton. She carried out her own research at the Indian Instititue of Technology, Kanpur (1970−1992), the Regional Research Laboratory, Trivandrum (1993−1998), and the Indian Institute of Chemical Technology, Hyderabad (1998−2001). She has won international acclaim, through her prolific contributions during the past three decades, on a variety of topics. She is largely responsible for the concept, design, and synthesis of hybrid peptides that have special properties of assembling into nanotubes and/or transporting ions, which form the theme of the present Account.

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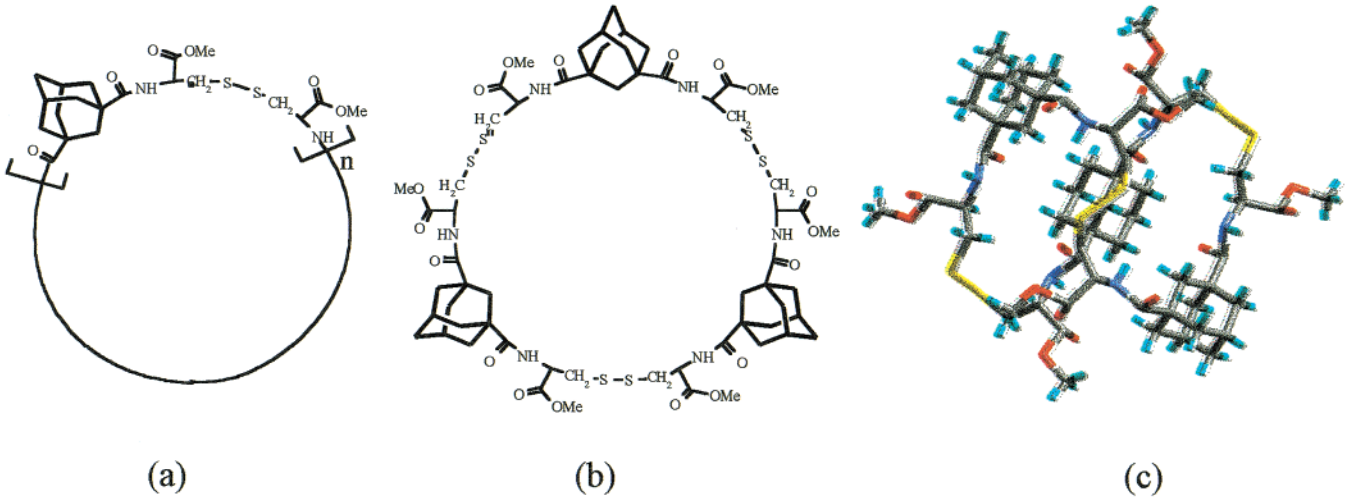


FIGURE 2. (a) Cyclo(Adm-Cyst)$_n$ ($n = 2-5$) oligomers formed in a single-step condensation of cystine-di-OMe with 1,3-adamantane dicarbonyl dichloride. (b) Cyclotrimer with threefold symmetry. (c) Figure eight motif adopted by the 39-membered macrocycle cyclo(Adm-L-Cyst)$_3$ in the solid state.

Designer Cyclopeptides for Membrane Ion Transport

(a) Cyclopeptides on Adamantane Building Block. For designing membrane ion transporting cyclopeptides, our inspiration was valinomycin, a 36-membered cyclododecadepsipeptide containing three repeats of a four residue series (D-Val-L-Lac-L-Val-D-HyV), which is a natural membrane ion carrier for selective transport of K^+ ions.[8] Valinomycin has a ball-like hydrophobic exterior provided by a large number of isopropyl groups of Val and hydroxy Val, capable of penetrating the bilayer membrane, and a polar interior provided by a large number of carbonyl groups that harbor K^+ ions. Nature seems to have taken particular care to provide repeated bends in valinomycin by arranging an alternating sequences of DD and LL amino acids creating a tennis ball seam arrangement without imposing much rigidity on the structure, a prerequisite for ionophores, because not only must a metal ion be bound and transported, but it must also be released after transport.[9]

Thus, with valinomycin as the model, our first task was to create a ball-like hydrophobic exterior (Figure 1a). It was envisaged that incorporation of small molecular weight, highly lipophilic, cagelike polycyclic units in alternating sequence with α-amino acids would create the same effect. For this purpose, the adamantane unit (a diamondoid building block which is highly lipophilic, has a small molecular weight, and contains four chair forms of cyclohexane fused together in a cagelike framework) appeared as the ideal choice (Figure 1b).

(i) Cystine-Based Cyclopeptides as Membrane Ion Carriers. As a first illustration of this concept, a large family of adamantane-containing cystine-based cyclopeptides (Figure 2a) containing two, three, four, and five cyclic repeats of the Adm-Cyst (Adm = 1,3-adamantanedicarbonyl; Cyst = L-cystine dimethyl ester) unit in 26-, 39-, 52-, and 65- membered rings was constructed in a single step by the reaction of L-cystine dimethyl ester with 1,3-adamantanedicarbonyl dichloride.[10] Membrane ion transport studies showed that while the 26-membered cyclic dimer (Figure 2a; $n = 2$) transported Na^+ in preference to K^+ and the 39-membered cyclic trimer (Figure 2a; $n = 3$) was more selective toward K^+, the 52- and 65-membered macrocycles (Figure 2a; $n = 4$ and $n = 5$, respectively) showed only negligible transport properties for both ions. Structural studies suggested that the movement of alkali metal ions across the lipid bilayer membranes operated by a carrier type of ion-transport mechanism similar to that of valinomycin. The crystal structure[11] of the 39-membered cyclo(Adm-Cyst)$_3$ showed that although the macrocycle has threefold symmetry in its molecular formula (Figure 2b), it adopts a geometrically unique "figure eight" (double-helical) motif in the solid state (Figure 2c). The exclusive right-handedness of the top strand at the point of intercrossing is predetermined by the chirality of the starting cystine. The mirror-image isomer with the left-handed helix on the top was prepared by using D-cystine.[12]

Both L- and D-mirror-image isomers of cyclo(Adm-Cyst)$_3$ transported K^+ ions with equal ease. In the absence of the crystal structure of the K^+ complex of cyclo(Adm-Cyst)$_3$, a model was constructed with threefold symmetry, placing the K^+ ion in an octahedron, hexacoordinated with the six amide carbonyl oxygens with the K^+-O distance of ~2.8 Å in a cavity of ~13 Å. The complex possesses a polar interior with a highly hydrophobic periphery (cf., valinomycin) lined with three Adm units. The six CO_2Me groups form a protective cap for the K^+ ion, shielding it from solvation, resulting in high membrane ion transport efficiency.

(ii) Serine-Based Membrane Ion-Transporting Cyclodepsipeptides. Interestingly, while hydroxy acids such as lactic and hydroxy valeric are commonly employed by nature for depsibond formation in cyclodepsipeptides, hydroxy amino acids are rarely used, and there are hardly any examples where proteinous amino acids such as

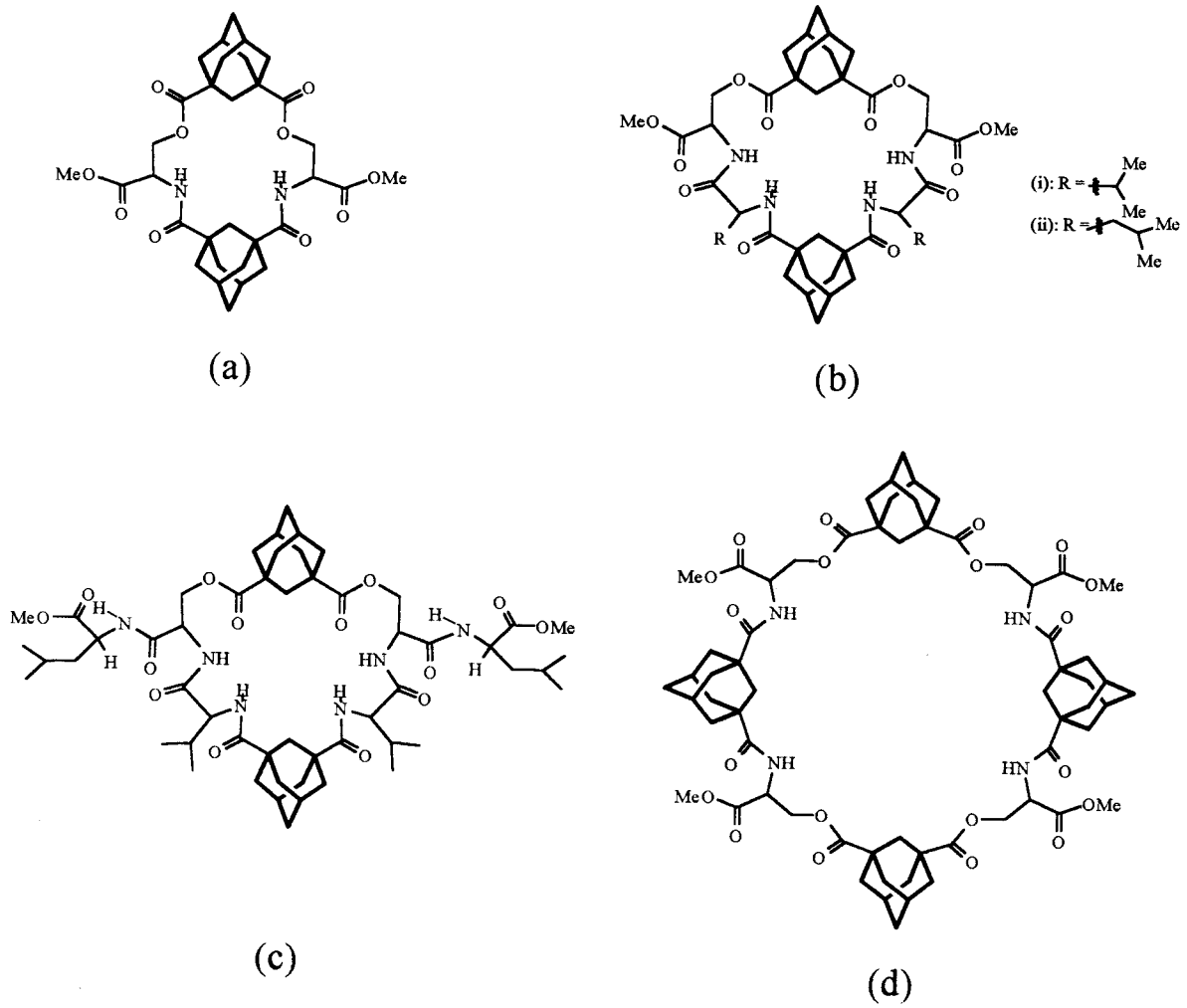


FIGURE 3. Serine-based cyclodepsipeptides prepared in a two-step synthesis are acting as efficient membrane ion carriers.

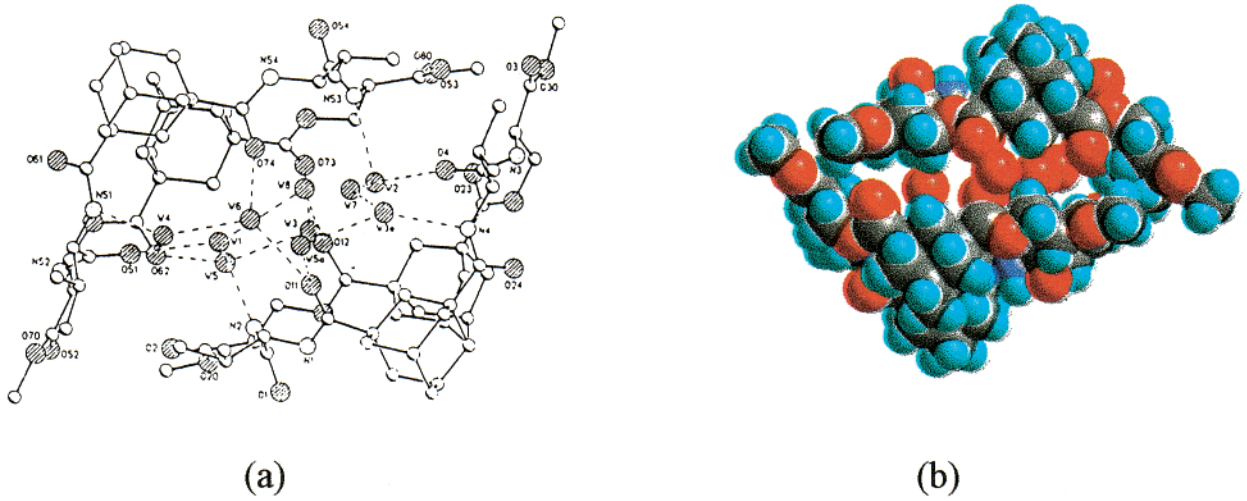


FIGURE 4. (a) Dimeric structure with water-filled channel, formed by the head-to-head linking of cyclo(Adm-Ser-Val)$_2$ (Figure 3bi). (b) Space-filling diagram of the water-filled channel.

serine and tyrosine are exploited either by nature or in the laboratory for the construction of cyclodepsipeptides.[2]

With the main aim of simulating the alternating ester/amide arrangement of the valinomycin ring backbone, for increasing the conformational flexibility and consequently the membrane ion transport efficiency, we envisaged the use of serine for constructing a new class of cyclodepsipeptides wherein the side chain CH_2OH of serine is used for the ester and the α-amino function for the amide bond formation, thus providing, in a two-step synthetic strategy, a large family of serine-based cyclodepsipeptides on an adamantane building block with ring size varying from

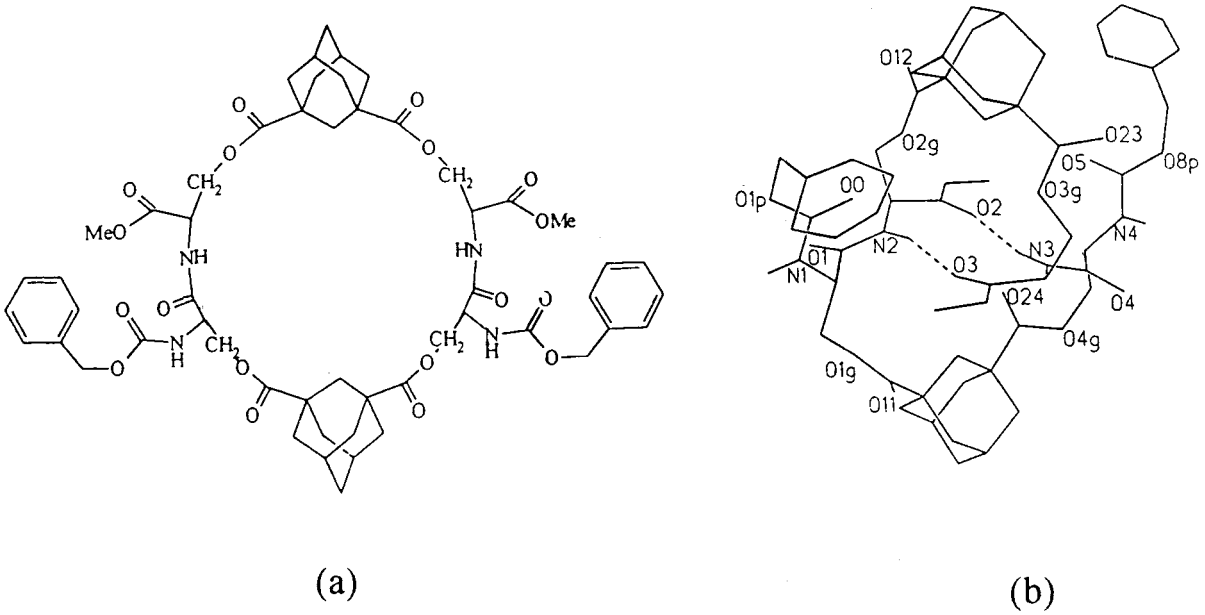
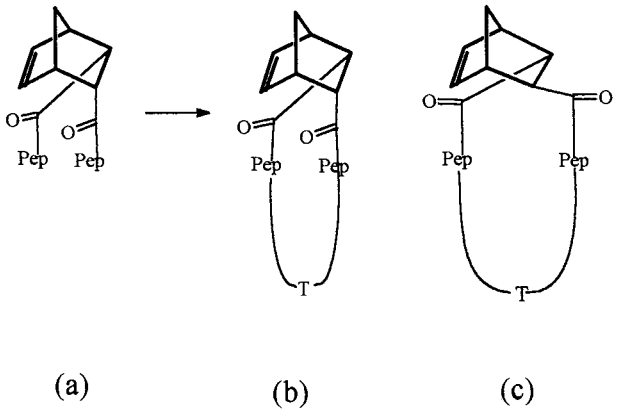


FIGURE 5. (a) Cyclo(Adm-Ser-Ser)$_2$. (b) Crystal structure showing an intramolecular hydrogen-bonded antiparallel β-sheet.

18- to 36-membered.[13] The design was highly flexible with respect to the choice of an amino acid, which could be either incorporated as part of the cyclic backbone or attached as pendant on the exterior through carboxylic handles. Figure 3 presents some selected members of serine-based cyclodepsipeptides with adamantane building blocks. Ion transport studies in model membranes showed that while the 18-membered cyclodepsipeptide in Figure 3a showed a near absence of any ion transport capability, the larger macrocycles were all found to be very effective in translocating Na^+, Mg^{2+}, and Ca^{2+} ions across bilayer membranes. Among these macrocycles, the cyclodepsipeptide in Figure 3c, with two leucine residue pendants on the exterior of the ring, showed maximum efficiency and was demonstrated to be almost as efficient as valinomycin (with respect to the lipid:ionophore ratio). However, no selectivity was observed in membrane ion transport.

The crystal structure[14] of the 24-membered cyclo(Adm-Ser-Val)$_2$ (Figure 3bi) showed a head-to-head assembly of two closely related conformers, to provide a highly hydrophobic exterior and a polar interior filled with ordered water molecules which act as bridges and stabilize the dimer through O−H···O and N−H···O hydrogen bonds (Figure 4a,b). A possible explanation for the highly efficient but nonselective nature of ion transport by these macrocycles could be that transport occurs by the harboring of metal ions in the flexible interior and by displacement of water molecules followed by transportation across the bilayer membranes.

The 26-membered cyclodepsipeptide (Figure 5a) containing four serine residues connected to adamantane units through ester linkages showed a unique fold in its crystal structure. The antiparallel sheet structure exhibited by this molecule, to our knowledge, is the first example of a cyclodepsipeptide making a reverse fold to create an intramolecularly hydrogen bonded 10-membered ring by



T = Cystine/ Adamantane / Norbornene

FIGURE 6. Constrained cyclic β-sheets (a) using norbornene carbonyl unit and (b) as the turn element by capping with template T. (c) Related strategy for wider rings with trans isomer.

complementary NH···O=C hydrogen bonding between the side chain and the backbone groups (Figure 5b).[14]

The solution state conformation of adamantane-containing cystine- and serine-based cyclodepsipeptides, examined by ^{1}H NMR (rotating-frame Overhauser enhancement spectroscopy (ROESY) and variable temperature amide proton shifts) and circular dichroism (CD) studies, was in good agreement with solid-state structures. Characteristic cross-peaks attributed to β-turn-type structures were seen in the ROESY spectra of adamantane-containing cystine- and serine-based cyclopeptides. CD spectra provided further support for the presence of the turn-type conformation. The mirror-image relationship of L- and D-isomers of cyclo(Adm-Cyst)$_3$ was beautifully reflected in their CD spectra.[12]

(b) Cyclopeptides and Cyclodepsipeptides on a Norbornene Building Block for Membrane Ion Transport.

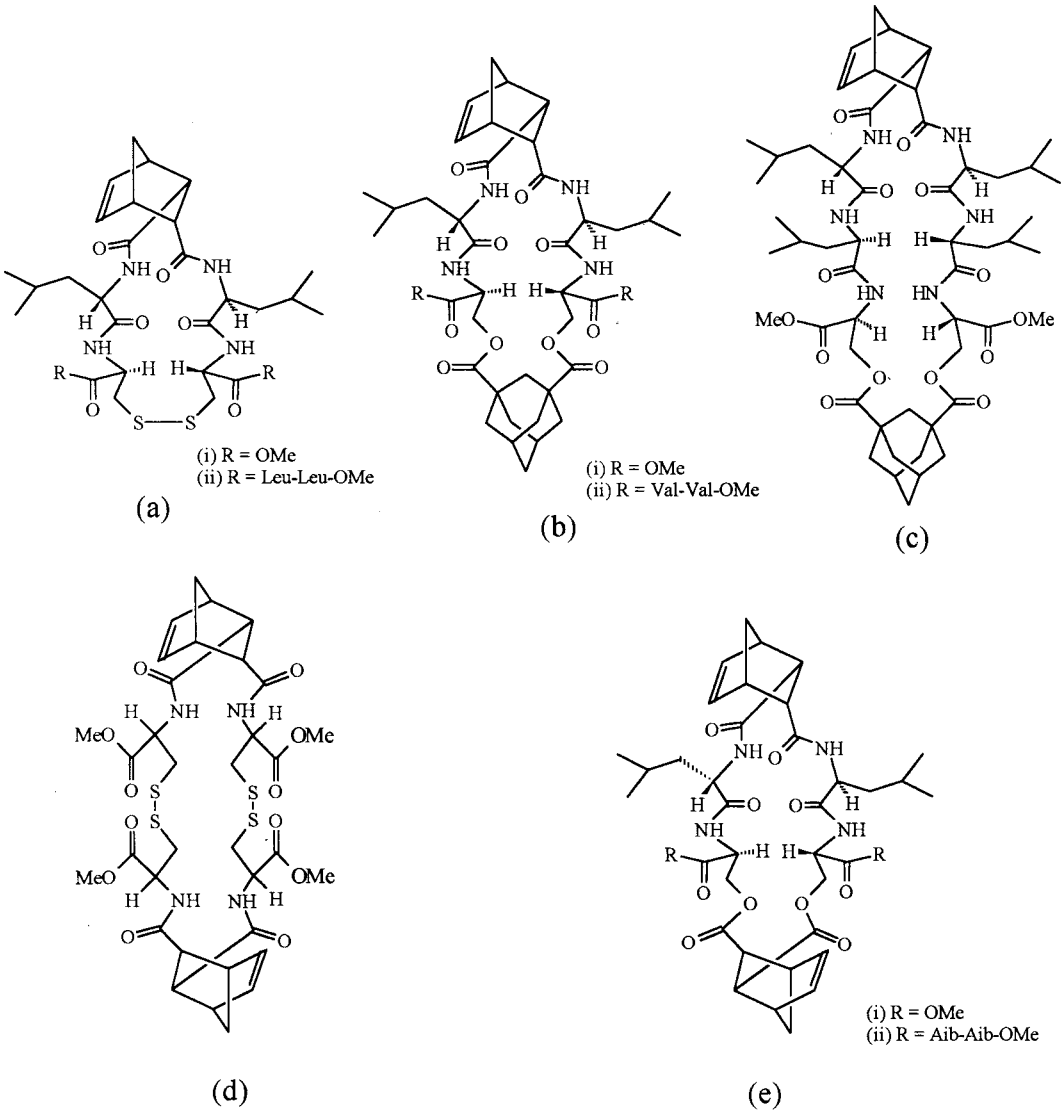


FIGURE 7. Norbornene-bridged cyclopeptides with hairpin architecture.

The flexible design permitted the synthesis of a novel class of hybrid cyclopeptides, anchored on norbornene to craft cyclopeptides with hairpin architecture that may show high selectivity in membrane ion transport. Our recent demonstration[15] of the *endo-cis*-(2*S*,3*R*)-norbornene dicarbonyl unit as an efficient turn element in the design of simple models of two-stranded parallel β-sheets (Figure 6a) suggested an exciting possibility for the creation of constrained cyclic β-sheets by simply tying the free ends of the two peptide chains together either by another norbornene unit or an equivalent turn template T (Figure 6b). It was envisaged that while the *endo-cis*-(2*S*,3*R*)-norbornene dicarbonyl unit would generate a narrow hairpin structure, the 2,3-trans isomer would lead to wider rings (Figure 6c) that may show modified behavior in membrane ion transport.

The design draws a parallel with nature's strategy for the construction of gramicidin S.[16] Thus, while nature uses two proline residues at almost opposite poles to create a hairpin architecture in gramicidin S, the present design[17] deploys norbornene units for the same purpose.

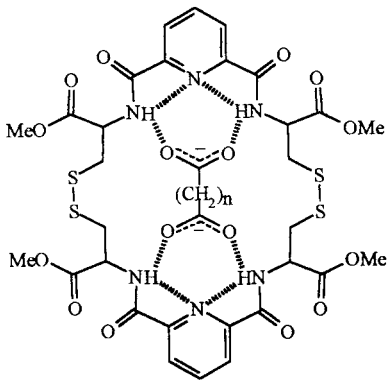


FIGURE 8. Pyridine-bridged cystinophanes act as excellent hosts for 1,ω-alkane dicarboxylates.

Figure 7 presents some selected members of norbornene: constrained hairpin cyclopeptides and depsipeptides with assorted ring sizes. The presence of built-in handles (as protected COOH groups) permitted the attachment of a variety of peptide subunits as illustrated with the ligation of Leu-Leu, Val-Val, or Aib-Aib (Aib = α-aminoisobutyric acid) pendants. ^{1}H NMR (ROESY, VT),

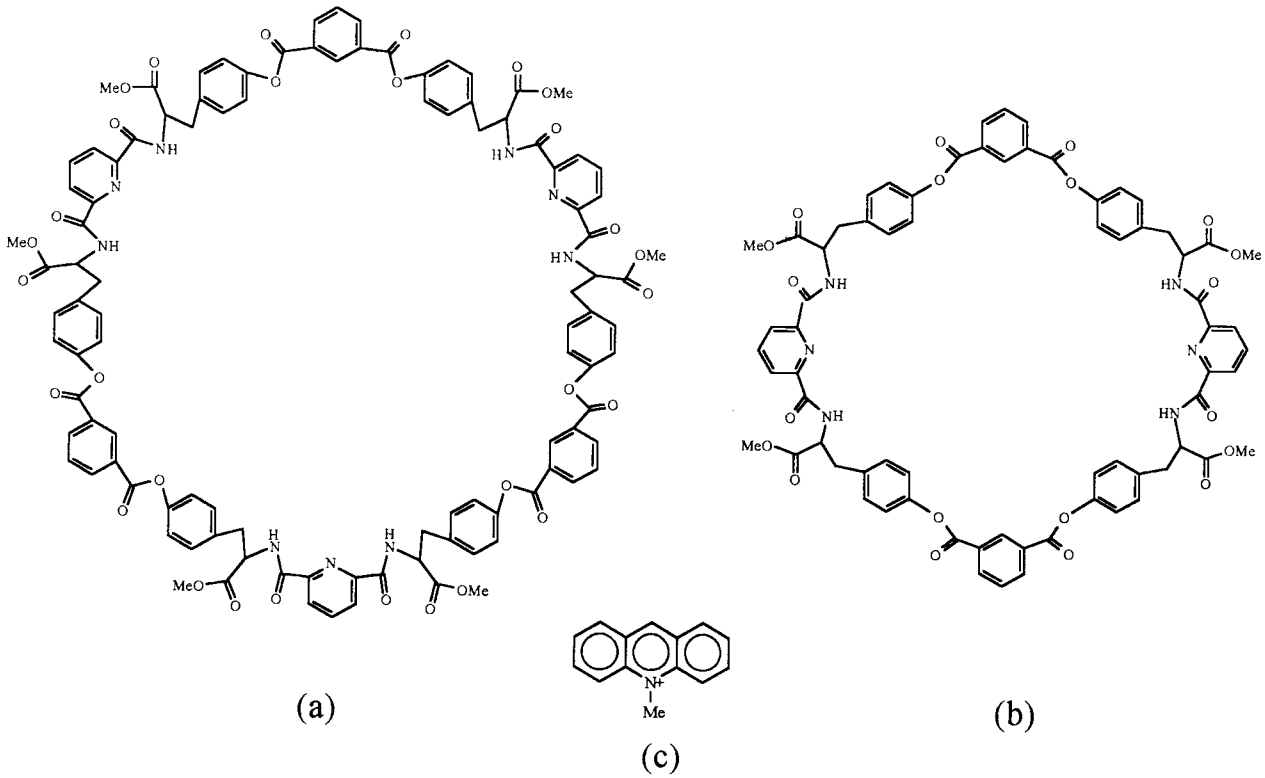


FIGURE 9. Tyrosinophanes with large numbers of aromatic units in their ring framework are useful hosts for studying cation−π interactions.

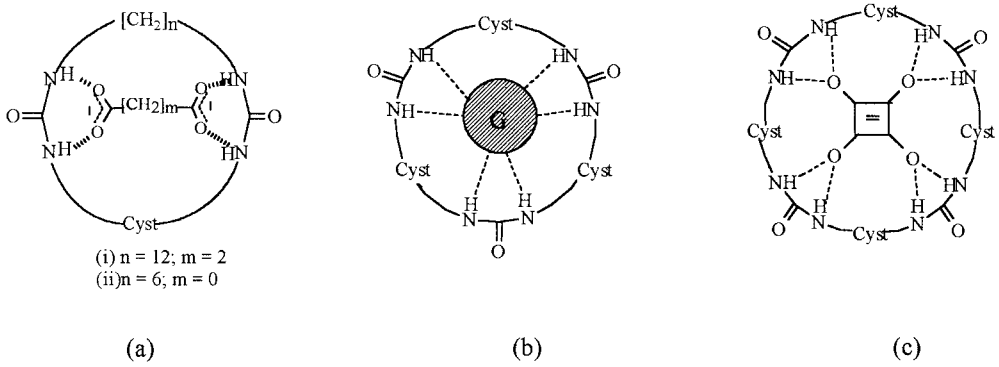


FIGURE 10. (a) Cyclobisureas act as efficient hosts for 1,ω-alkane dicarboxylate anions. (b) Cyclotriurea with threefold symmetry in its structure complexes with trigonal planar nitrate anions and spherical halide anions with equal ease (G = $Cl^-/Br^-/NO_3^-$, Cyst = L-cystine unit). (c) Cyclotetraurea can trap the tetragonal planar squarate dianion with modest efficiency. The binding takes place exclusively through hydrogen bonds.

FT-IR, and CD studies strongly indicated that, while the simple 18-membered cystine-bridged norbornene-constrained cyclopeptides adopted β-turn-type structures, the higher norborneno cyclopeptides with adamantane as the bridging template exhibited largely β-sheet conformations. Membrane ion transport studies showed that only those norborneno cyclopeptides that contained dipeptide (Leu-Leu or Val-Val) segments anchored on the exterior of the ring were able to transport metal ions across membranes. Thus, norbornene-constrained cyclopeptides containing either cystine (with Leu-Leu pendants) or adamantane (with Val-Val pendants) units as the bridging template showed high efficiency in transporting selectively only the monovalent metal ions in model membranes. Although the efficiency in transporting Li^+, Na^+, and K^+ ions was almost equal to that of valinomycin, the norbornene-constrained peptides were found totally incapable of translocating divalent metal ions Ca^{2+} or Mg^{2+} across bilayer membranes. The charge specific selectivity of metal ion transport may be attributed to the high solvation effect of divalent metal ions that makes them unsuitable for narrower hairpinlike carrier ionophores.

To assess the role of an adamantane in membrane ion transport, a large family of cystine-based macrocycles containing flexible polymethylene bridges ($(CH_2)_n$, n = 3−20) was constructed.[3e] Another set of cystine-[18] and serine-based[13] macrocycles containing a single adamantane provided additional examples as controls. Membrane ion transport studies showed that none of these were capable of appreciable ion transport across model membranes. That rigidity alone is not sufficient was shown by the total incapability of membrane ion transport of

cystine- and serine-based macrocycles containing small rigid aromatic (Ph or Pyr) units in place of the adamantane block as part of the ring backbone. On the basis of the above data, it appears that the minimum criterion for a cyclopeptide or a depsipeptide to be an efficient membrane ion transporter is the presence of at least two adamantane units or a combination of an adamantane and a norbornene unit in a minimum of a 24-membered ring framework containing an adequate number (preferably six) of carbonyl groups as amides or a combination of amide and ester functions. The presence of hydrophobic ligands attached as pendants on the ring is shown to improve the membrane penetration.

Hybrid Cyclopeptides and Cyclodepsipeptides for Host−Guest Complexation

The present strategy for hybrid cyclopeptides provided an easy entry into a host of macrocyclic peptides that were efficient receptors for specific recognition of a variety of anionic and cationic guests.

(a) Pyridine-Bridged Cystine-Based Cyclopeptides as Hosts for Molecular Recognition of 1,ω-Alkane Dicarboxylate Guests. Cystine-based 26-membered hybrid cyclopeptides containing two cyclic repeats of Pyr-Cyst (Pyr = 2,6-pyridine dicarbonyl, Cyst = cyst-diOMe) units (prepared in a single step from cystine-diOMe and 2,6-pyridine dicarbonyl dichloride) were shown[18] to bind to a number of 1,ω-alkane dicarboxylic acids [$(CH_2)_n$-$(COOH)_2$, n = 1−4] with maximum affinity (K_{assoc}= 3.69 × 10^2 M^{-1}) and selectivity for glutaric acid (n = 3) tetrabutylammonium (TBA) salt (Figure 8).

(b) Tyrosine-Based Aromatic-Bridged Cyclodepsipeptides for Molecular Recognition of Pyridinium Cationic Guests. The 78- and 52-membered tyrosine-based aromatic-bridged cyclodepsipeptides, with large open pores (Figure 9a,b) (prepared[19] by sequence, condensation of an N,C-protected tyrosine with benzene 1,3-dicarbonyl dichloride, N-deprotection, and coupling with pyridine 2,6-dicarbonyl dichloride) containing unusually large (8−12) numbers of aromatic units, appeared to be particularly attractive hosts for studying cation−π interactions, known to play an important role in protein structure, binding, and catalysis. Fluorescence studies showed[19] strong interaction of the cationic pyridinium guest, *N*-methyl acridinium hexafluorophosphate (Figure 9c), with both macrocyclic aromatic hosts with a K_{assoc} value of 8.95 × 10^3 M^{-1} for the 52-membered macrocycle.

(c) Cystine-Based Macrocyclic Oligoureas as Artificial Receptors for Anionic Guests. Cystine-based macrocycles containing multiple urea functions as part of the ring, positioned equidistant from each other, prepared in a single step by condensation of cystine-diOMe with either 1,ω-alkane diisocyanates[3f] to give polymethylene-bridged macrocyclic bisureas with adjustable ring size or with triphosgene (a commercially available precursor for phosgene) to give homo-oligocystino cyclic ureas[20] were demonstrated to be specific hosts for anionic guests. For example, 1,ω-alkane dicarboxylates, spherical halides, and planar polyoxy anions bind to these hosts with moderate to high K_{assoc} values (Figure 10).

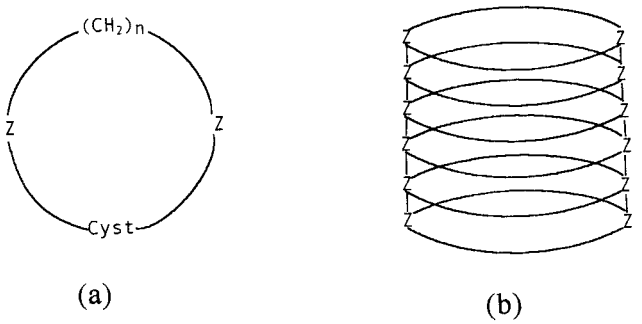


FIGURE 11. Polymethylene-bridged cystine-based macrocycles (a) with a pair of self-complementary hydrogen-bonding groups (Z = NH−CO or NH−CO−NH) placed at almost opposite poles of the ring can stack on top of one another to form hydrogen-bonded nanotubes (b).

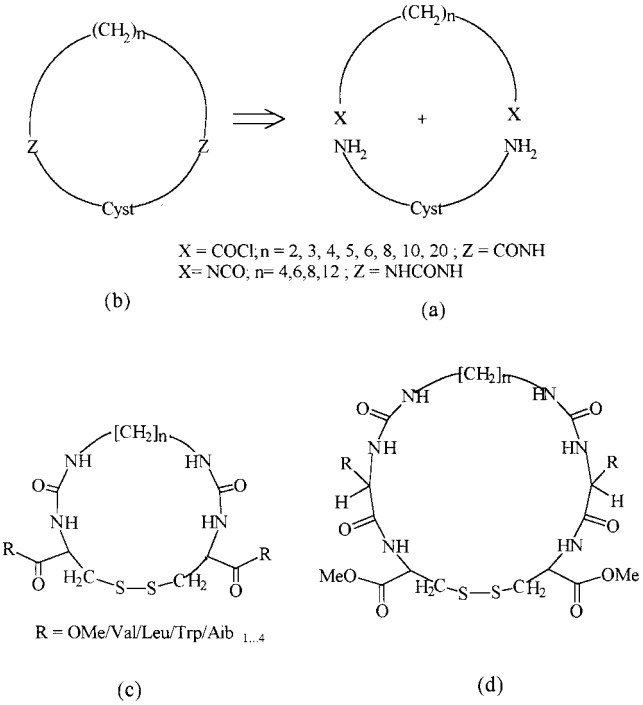


FIGURE 12. Synthesis of macrocyclic bisamides and bisureas by one-step condensation of 1,ω-alkane dicarbonyl dichloride and diisocyanate with cystine-derived C, C′- or N, N′-extended bispeptides.

Designer Cyclopeptides for Self-Assembled Tubular Structures

Creation of hollow tubular structures by noncovalent self-assembly of organic molecules has been the subject of considerable research in recent years.[3h] Tubular structures constructed from chiral amino acids are particularly important, as models for biological channels, as transport vehicles in drug delivery systems, and in the design of nanostructured biomaterials.[3a,h] The stacking of cyclopeptides for the construction of hydrogen-bonded, open-ended, hollow peptide tubes is well studied.[21] For example, cyclopeptides composed of an equal number of α- and

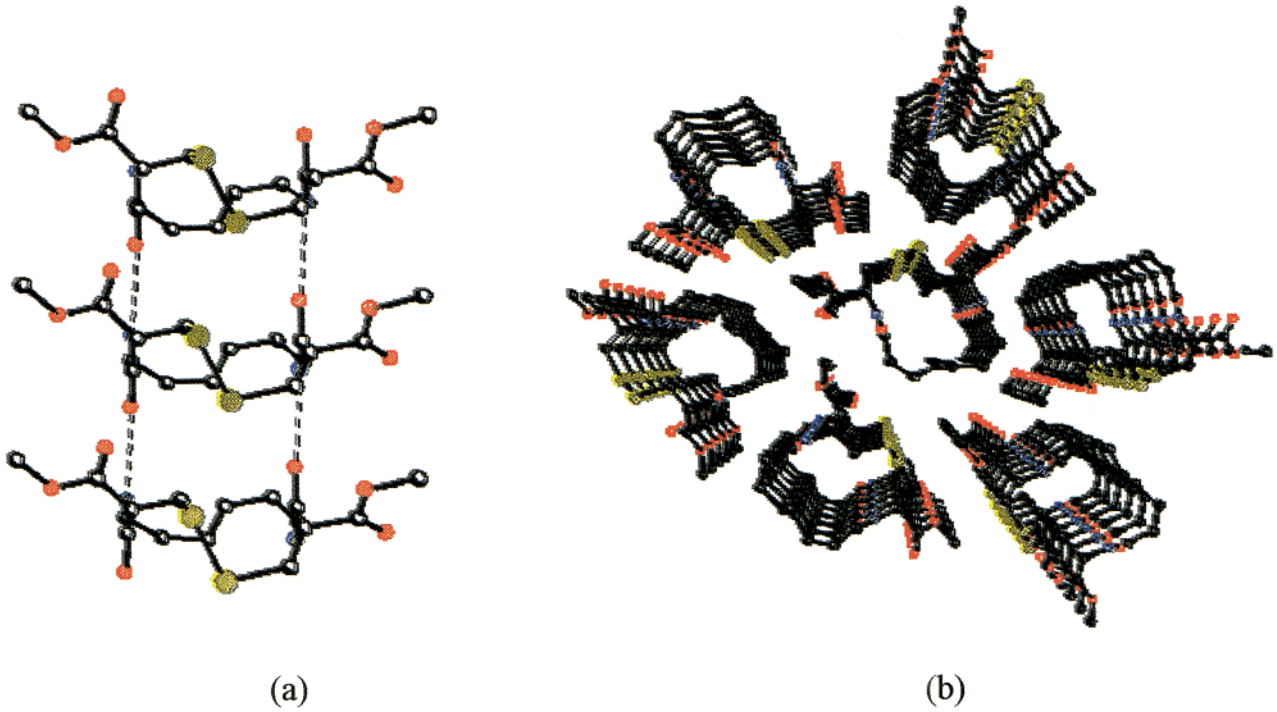


FIGURE 13. (a) Hydrogen-bonded vertical stack of hexamethylene-bridged cystine-based cyclobisamide in the solid state. The molecules of the cyclobisamide aligned in a parallel fashion stack on top of one another and form strings of hydrogen bonds (NH···O═C) on either side of the stack extending into a tubular structure. The tubes are empty and extend to infinity. (b) Side-by-side packing of nanotubes of the cyclobisamide.

β-amino acids,[3b] or all β-amino acids,[3c,22] or even numbers of alternating D- and L- amino acids[3a,23] 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 nearly flat ring conformation by the cyclopeptide with side chains extending outward and amide groups perpendicular to the ring. The peptide rings in this conformation are poised to form contiguously hydrogen-bonded β-sheetlike tubular ensembles by stacking on top of one another.

(a) Cystine-Based Polymethylene-Bridged Macrocyclic Bisamides and Bisureas for Hydrogen-Bonded Peptide Nanotubes. Based on model building, we have evolved a simple and rational design strategy[3e] for the construction of polymethylene-bridged cystine-based macrocycles that can be persuaded to form tubelike structures by stacking one on top of another through hydrogen bonds. A key structural feature of the design is the placement of a pair of self-complementary hydrogen-bonding functions, such as an amide (NH−CO)[3e]/urea (NH−CO−NH)[3f] (Z) at almost opposite poles of the ring (Figure 11).

The synthesis involves closing the polymethylene chain with cystine-diOMe or its bispeptide and was accomplished in a single step by the condensation of 1,ω-alkane dicarbonyl dichloride or diisocyanate[$(CH_2)_nX_2$; X = COCl or NCO, n = 2−20] with either the simple cystine dimethyl ester or its extended C,C′- or N,N′-bispeptide to give macrocyclic bisamides (Figure 12b, Z = CONH) and bisureas (Figure 12b, Z = NHCONH) with adjustable ring size. The design permits the incorporation of a variety of amino acid residues either as part of the ring (Figure 12d) or as pendants on the exterior of the ring (Figure 12c).

X-ray crystallographic studies of a large number of cyclobisamides have shown that these macrocycles possess an inherent property of self-assembling into tubelike structures by stacking one on top of another through contiguous amide−amide hydrogen bonds which appear as a pair of strings on either side (Figure 13a). The tubes are hollow, open-ended, and extend to infinity (Figure 13b). In the macrocyclic bisamides of which the solid-state structure was examined (with n = 4, 6, 8, 10), the diameter of the tube varied between ∼5 Å (n = 4) and ∼10 Å (n = 10). Figure 13 presents a typical X-ray picture of a cyclobisamide (n = 6) stacking into a hydrogen-bonded tubular structure.

The cystine-based polymethylene-bridged cyclobisamide tubes possess a largely hydrophobic interior with a polarity comparable to that of a nonpolar organic solvent. Consequently, these tubes can create a microenvironment suitable for encapsulating highly lipophilic substances by selective host−guest complexation. Fluorescence experiments have shown that the cyclobisamide tubes of appropriate diameter do the following: (a) enhance the solubility of extremely insoluble aromatic polycycles, namely pyrene and perylene, in water, (b) bind to fluorescent probe dyes such as Nile red, and (c) induce an ordered secondary structure in linear peptides as demonstrated with 26-residue bee venom peptide mellitin, adopting largely a α-helical structure in the 30-membered cystine tubule.[3e]

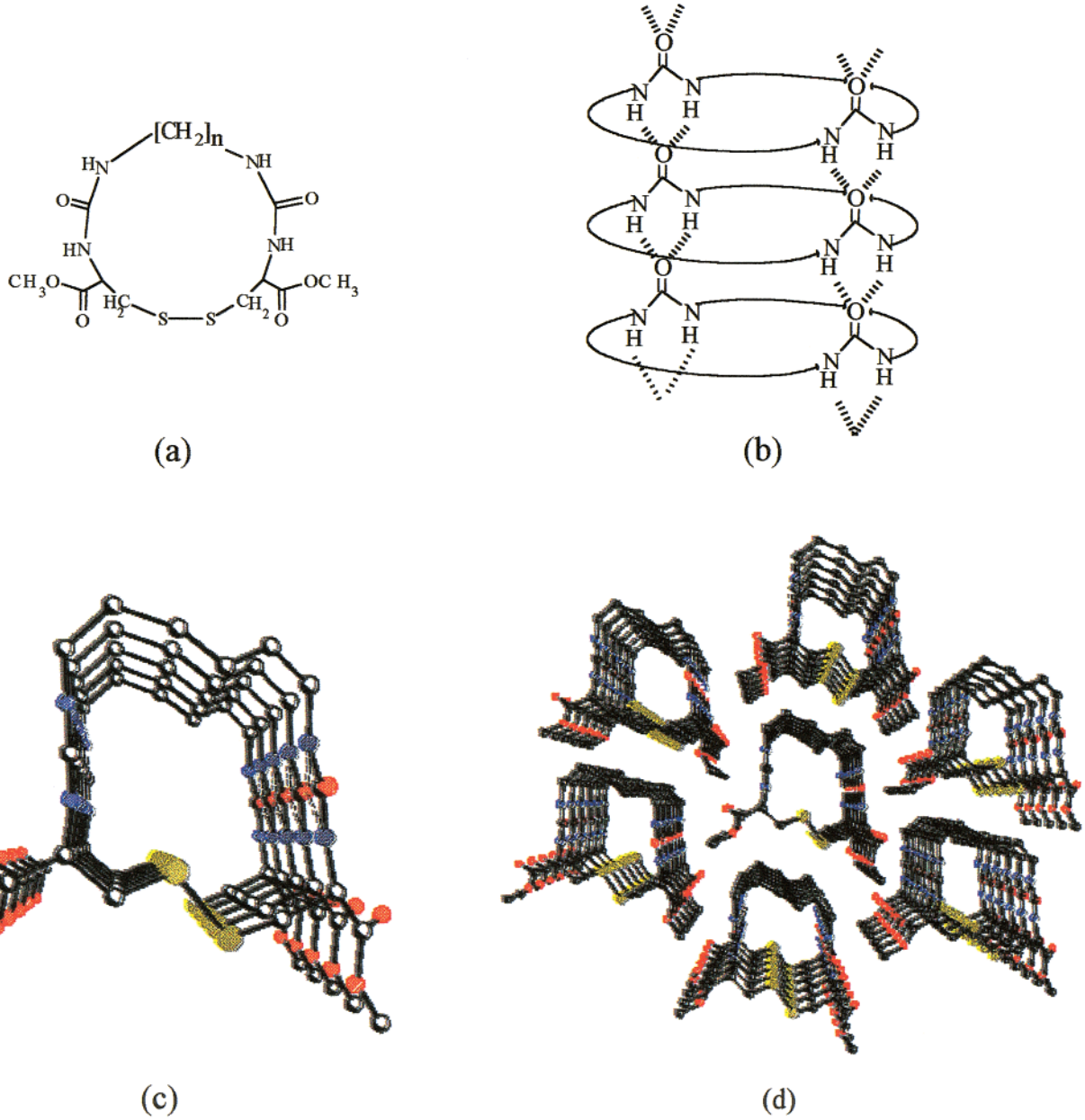
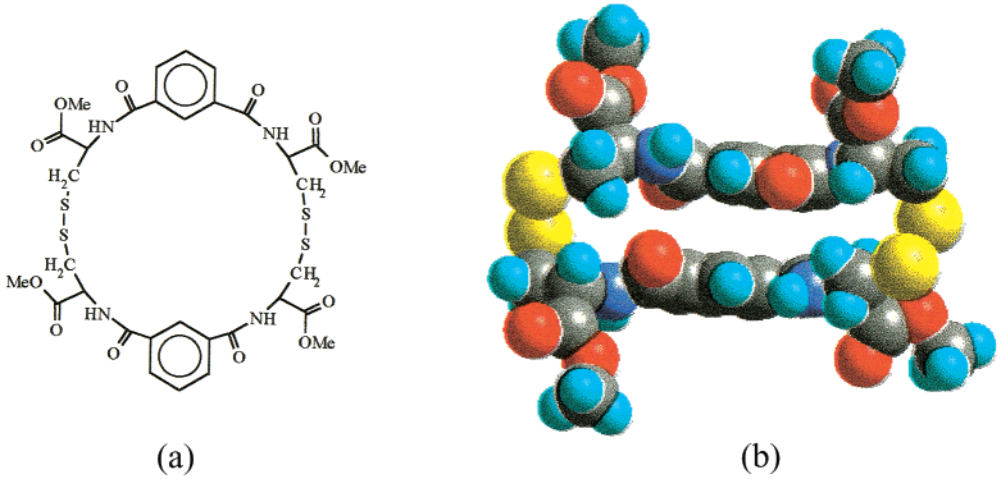


FIGURE 14. (a) Hexamethylene-bridged ($n = 6$) cystine-based cyclobisurea. (b) Chemical representation of the hydrogen-bonded stack observed in the crystal of cyclobisurea molecule. The hollow tubular ensemble is held on either side by a string of hydrogen bonds, which are of the typical type for urea. (c) A view into the cavity of the cyclobisurea tube. (d) Three-dimensional view showing side-by-side stacking of bisurea tubes; the tubes are held in the pack only by the hydrophobic interactions.

Interestingly, the cystine-based polymethylene-bridged cyclobisureas showed remarkably similar profiles in their self-assembly behavior.[3f] Thus, as demonstrated by the crystal structure of the 18-membered cyclobisurea, the crown-shaped rings of the macrocycle aligned in a parallel fashion, stacked on top of one another, maintaining perfect registry between the subunits, generating an open-ended hollow nanotube that extends to infinity. The hollow tubular ensemble is held on either side by a string of typical urea-type hydrogen bonds.(Figure 14).

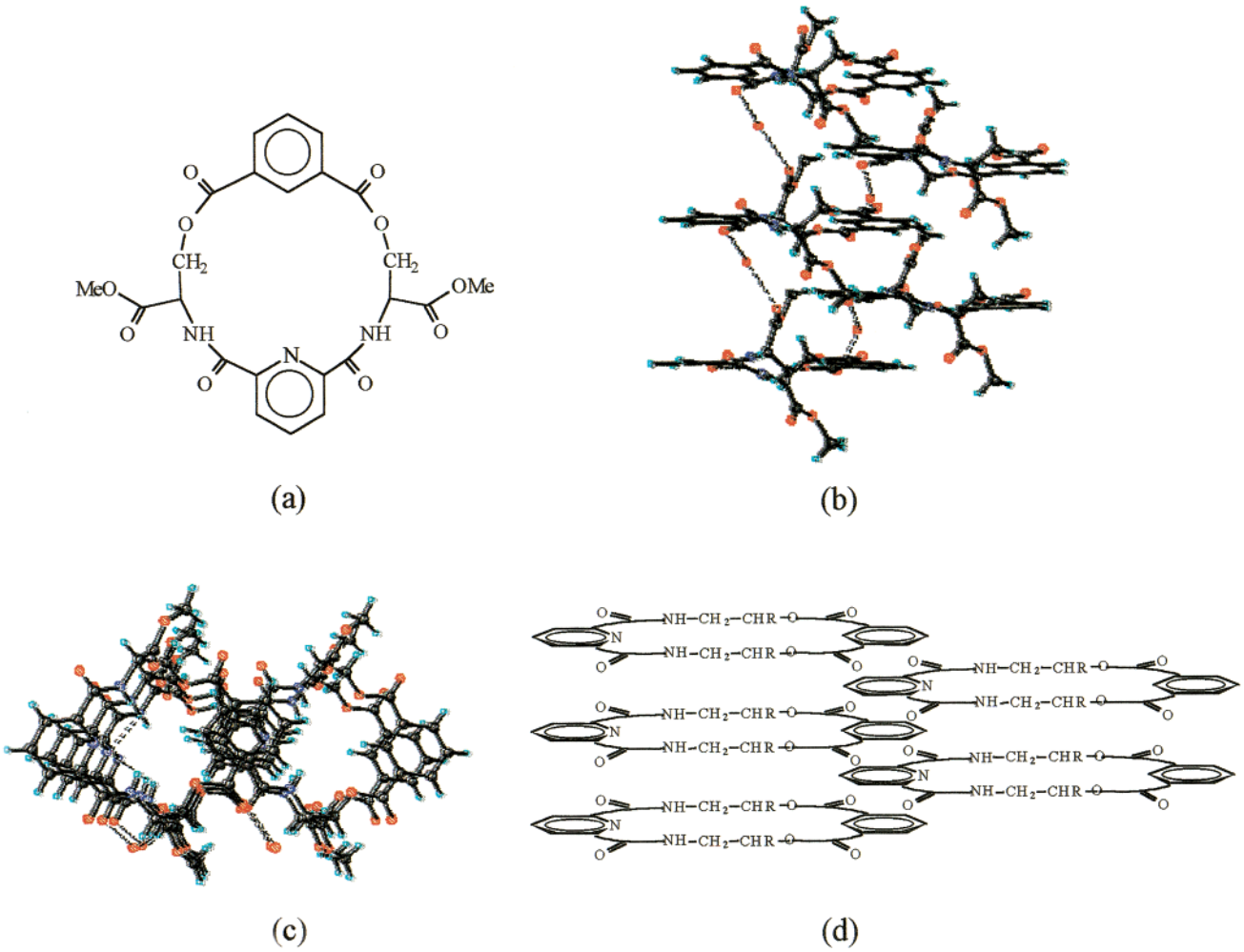
The cystine-based cyclic oligoureas serve as excellent receptors for anionic guests and show specificity according to size complementarity of the host−guest molecules. Thus, appropriately crafted cystine-based polymethylene-bridged cyclobisamides and bisureas possess an intrinsic property of self-assembly into hydrogen-bonded, open-ended hollow tubes and hold promise as artificial receptors for guest molecules, with potential application in inclusion chemistry, catalysis, and drug delivery.

To extend the scope of the design and to understand the role of cystine in facilitating vertical stacking, we prepared a large number of simple polymethylene-bridged cyclobisamides and bisureas by direct condensation of commercially available 1,ω-alkane diamines with, respectively, 1,ω-alkane dicarbonyl dichlorides and diisocyanates. Several of these macrocycles crystallized. Interestingly, while the simple polymethylene-bridged cyclobisamides with the general structure cyclo[(−CONH−$(CH_2)_n$−



(a) (b)

FIGURE 15. (a) 26-Membered cystinophane. (b) Crystal structure shows a collapsed ring conformation with a near parallel, face-to-face orientation of the two aromatic rings.



(a) (b) (c) (d)

FIGURE 16. (a) 18-Membered serinophane containing pyridyl and phenyl rings as bridges. (b) Vertical parallel stack in the solid-state self-assembly of serinophane. The pyridine and phenyl rings interdigitate from one column to the other with ~3.5 Å separation between the planes of the rings. There are no direct hydrogen bonds between the neighboring stacks. The main organizing force for the formation of the tubular structure is the aromatic $\pi-\pi$ interactions. (c) Top view of a portion of a layer containing the interdigitated stacks in serinophane with 2.35 Å open channel through the assembly. (d) Schematic representation of the multiple $\pi-\pi$ stacks in the self-assembly.

NHCO$-(CH_2)_m-$); $n = 6$, $m = 4$; $n = 6$, $m = 8$; $n = 12$, $m = 10$] showed nanotube formation in the solid state in much the same way as in cystine-based cyclobisamides, the analogous cyclobisureas were unable to form tubular stacks. The importance of cystine residues in forming tubular structures, particularly in bisureas, was clearly

brought out in the X-ray structure of cystamine-based 24- and 18-membered cyclic bisureas, which failed to form any tubular assemblies. The absence of CO_2Me groups in cystamine-based cyclobisureas offsets the parallel alignment, disturbing the registry of the subunits resulting in horizontal assemblies rather than vertical stacking.[3g]

(b) Aromatic-Bridged Serine-Based Cyclopeptides for $\pi-\pi$-Stacked Nanotubes. The placement of a pair of self-complementary hydrogen bonding functions at almost opposite poles of the cyclopeptide ring was inherent in the self-assembly of peptide nanotubes described thus far.[3e−g] A modification of the design strategy, wherein a pair of aromatic units replaced the hydrogen bonding groups at the two poles, provided a variety of hybrid cyclopeptides with aromatic $\pi-\pi$ stacking potential for nanotube formation.

Interestingly, the 26-membered cystine-based cyclopeptides with aromatic (a phenyl or a pyridyl ring) units as the bridges proved to be unsuccessful, as demonstrated by the crystal structure of phenyl-bridged cystine cyclopeptide which showed a collapsed ring conformation due to internal aromatic face-to-face $\pi-\pi$ stacking[18] (Figure 15).

Substituting cystine with tyrosine[19] was also of no help. Our expectation of $\pi-\pi$ stacked nanotube formation was eventually realized[3d] in the structure of 18-membered serine-based macrocycles containing alternating repeats of serine and pyridyl/phenyl units in the cyclic backbone (Figure 16). A crucial requirement for $\pi-\pi$ stacking is the presence of a pyridyl unit at the amide end, which locks the amide NHs in NH···N hydrogen bonding, creating an almost flat ring conformation, a prerequisite for vertical stacking.

While the search is still on for an efficient system for aromatic $\pi-\pi$ stacking, the present results have clearly shown the feasibility of our design strategy for $\pi-\pi$ stacked nanotube formation in cyclopeptides.

Concluding Remarks

A new class of cyclopeptides designed to perform specific tasks is described. These hybrid cyclopeptides are readily accessible, essentially in one step, by the condensation of the 1,ω-dicarbonyl equivalent of the chosen hybrid unit with an appropriately crafted peptide-derived 1,ω-diamine. The choice of the hybrid unit was mainly dictated by the specific function desired. Using this concept, a large variety of membrane ion-transporting cyclopeptides were constructed, a key feature of which was the incorporation of rigid, low molecular weight, highly lipophilic alicyclic units in the ring backbone. Among the hybrid units used, the adamantane was found to be the best choice for the desired membrane permeability and conformational constraint for efficient and selective ion transport across bilayer membranes. Design, synthesis, crystal structure, and membrane ion-transport properties of a large number of adamantane-containing cystine- and serine-based cyclopeptides and cyclodepsipeptides are described. The use of norbornene provided hybrid cyclopeptides with hairpin architecture possessing a narrower cavity that showed membrane ion transport selectivity for monovalent cations. A slight modification of the design incorporating simple aromatic groups (preferably pyridyl) as hybrid units provided cyclopeptides with excellent host properties for a variety of anionic guests. Tyrosine-based cyclodepsipeptides with unusually large numbers of aromatic rings were demonstrated to act as simple models for studying aromatic $\pi-$cation interactions.

Perhaps the most significant outcome of the present synthetic strategy is the delineation of a simple design for a facile and direct entry into hydrogen-bonded peptide nanotubes as demonstrated in this Account 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 N, N′- or C, C′-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 largely hydrophobic interior of the cyclobisamide tubes creates a microenvironment 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 peptide rings through aromatic $\pi-\pi$ interactions.

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