Double-Helical Cyclic Peptides: Design, Synthesis, and Crystal Structure of Figure-Eight Mirror-Image Conformers of Adamantane-Constrained Cystine-Containing Cyclic Peptide Cyclo (Adm-Cyst)$_3$

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A large number of macrocycles containing alternating repeats of cystine diOMe(−NH-CH-(CO_2Me)-CH_2-S−)$_2$ and either a conformationally rigid aromatic/alicyclic moiety or a flexible polymethylene unit (X) in the cyclic backbone with ring size varying from 13- to 78-membered have been examined by spectral (^{1}H NMR, FT-IR, CD) and X-ray crystallography studies for unusual conformational preferences. While ^{1}H NMR measurements indicated a turnlike conformation for all macrocycles, stabilized by intramolecular NH···CO hydrogen bonding, as also supported by FT-IR spectra in chloroform, convincing proof for β-turn structures was provided by circular dichroism studies. Single-crystal X-ray studies on 39-membered cyclo (Adm-L-Cyst)$_3$ revealed a double-helical fold (figure-eight motif) for the macrocycle. Only a right-handed double helix was seen in the macrocycle constructed from L-cystine. The mirror-image macrocycle made up of D-cystine units exhibited a double helix with exactly the opposite screw sense, as expected. The enantiomeric figure-eights were stabilized by two intramolecular NH···CO hydrogen bonds and exhibited identical ^{1}H NMR and FT-IR spectra. The CD spectra of both isomers had a mirror-image relationship. The present results have clearly brought out the importance of cystine residues in inducing turn conformation that may be an important deciding factor for the adoption of topologically important structures by macrocycles containing multiple S−S linkages.

Introduction

The figure-eight motif representing the simplest form of a double-helical arrangement in a ring has recently attracted much attention not only for its unique supramolecular architecture but also for its topological implications in biological systems.[1−6] Although large cyclic peptides are known to have a natural tendency to fold their backbone, assisted by either intracyclic hydrogen bonding or aromatic $\pi-\pi$ stacking, into stabilized conformations of various secondary structural elements, for example, β-turns, β-sheets, and helical motifs, and while the structural factors responsible for inducing turns and sheets are quite well understood, the reasons for adopting a double-helical fold still remain to be identified.

Several simple models of a figure-eight motif from macrocyclic ligands[7−14] have been reported. While in metal-assisted figure eights[9,13] it is the metal ion and its preference for a particular coordination geometry that dictates the formation of a double-helical fold, in simple macrocycles, apart from the ring size, the major controlling factors appear to be the stabilizations due to hydrogen bond formation and or aromatic $\pi-\pi$ interactions.

Interestingly, while molecules with a figure-eight topology are quite well-known in the field of helicines,[15]

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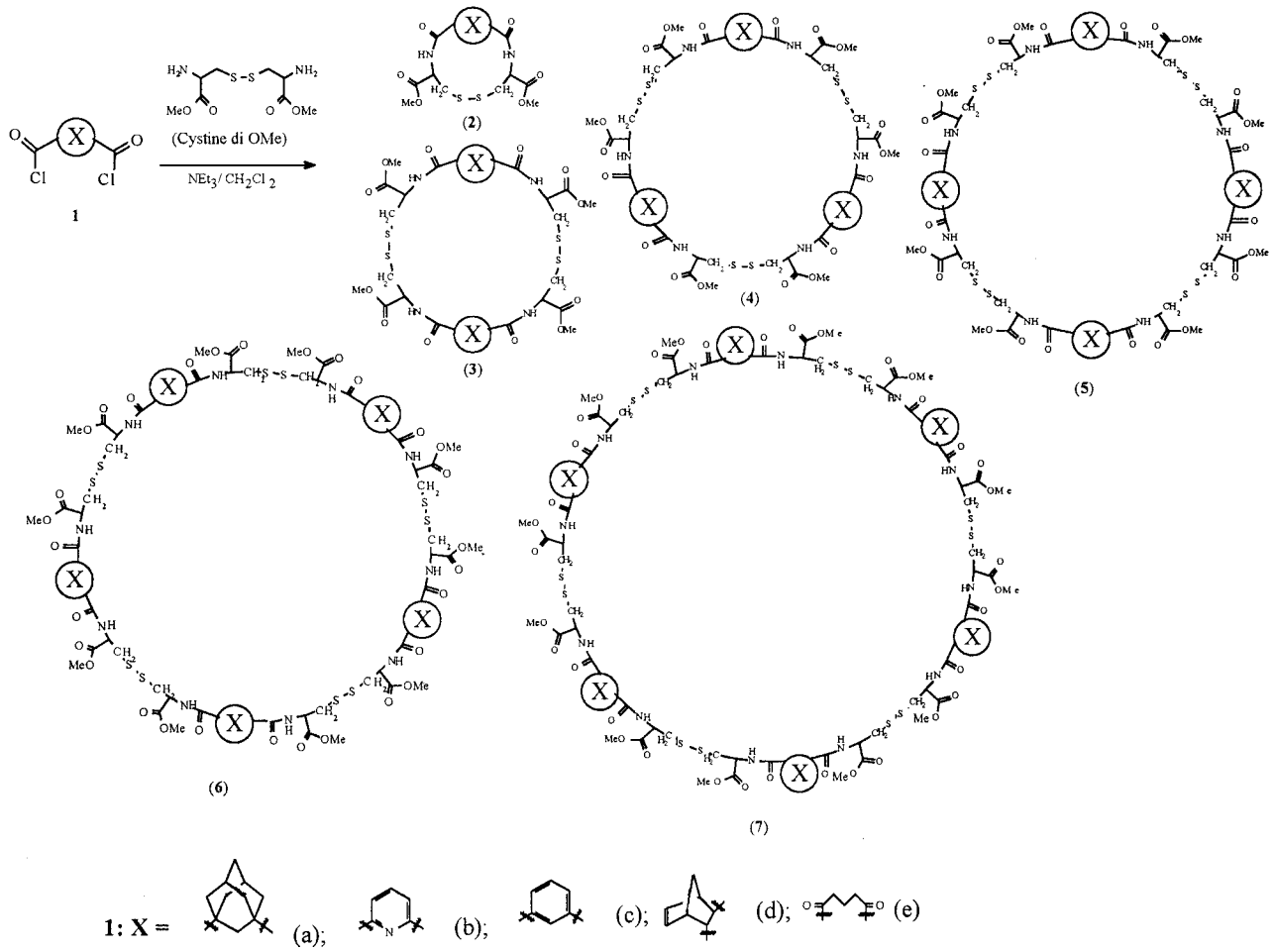

Figure 1. Synthesis of X-constrained cystine-based macrocycles.

cyclophanes,[16] and polypyrroles,[10−12] to our knowledge, this motif is rarely observed among cyclic peptides. Patellamide-D, a 26-membered cytotoxic cyclic peptide of marine origin containing an unusual combination of thiazole and oxazoline amino acids in the ring, provides a unique example of a figure-eight conformation.[17,18] Some features of figure-eight structure were also noticed in a related cyclic peptide alkaloid lissoclinamide-7, a 21-membered macrocycle containing two thiazole rings in addition to an oxazoline heterocycle.[19]

Among the non-natural cyclic peptides, a 22-membered tryptophan-derived macrocycle was demonstrated to fold into a left-handed double helix stabilized by transannular hydrogen-bonding.[7] The corresponding decarboxy analogue, however, did not show any double helical character presumably because of its much decreased tendency toward intramolecular hydrogen bonding.[8]

Our recent work[14] reporting on the design of adamantane-constrained cystine-based cyclic peptides provided important insights into the nature of factors responsible for driving the conformation of a macrocyclic peptide into a double-helical fold. While intramolecular hydrogen bonding may play only a minor role, the presence of a type-II β-turn conformation appears to be the deciding factor in the choice between an open and a double-helical structure for a cyclic peptide. Among the conformational constraints that can be used to induce type-II β turns, incorporation of cystine amino acid was shown by us[20] to be particularly reliable. In the present work, a large variety of cystine-containing cyclic peptides with the general structure cyclo (X-Cyst−)$_n$ wherein cystine (Cyst = (HN-CH(CO_2Me)-CH_2-S−)$_2$) residue alternates with a conformationally rigid alicyclic (1,3-adamantanedicarbonyl or 2,3-*trans*-norbornenedicarbonyl) or aromatic (1,3-benzene- or 2,6-pyridinedicarbonyl) or flexible (1,3-propanedicarbonyl) unit X in a ring with adjustable size (13- to 78-membered; n = 1, 2, 3...6) were prepared and examined for unusual folds and conformational preferences (Figure 1).

Results and Discussion

The common synthetic strategy used for the preparation of cystine-based macrocycles was one-step condensation of cystine-diOMe (generated in situ from dihydrochloride and triethylamine) with the corresponding

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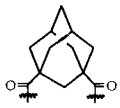
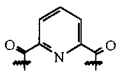
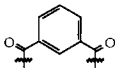
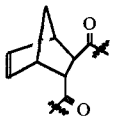
Table 1. Yield Distribution of Products in the Condensation of Cystine DiOMe with Various Templates (X)

	Mode of cyclization (ring size)					
Template	1+1 (13)	2+2 (26)	3+3 (39)	4+4 (52)	5+5 (65)	6+6 (78)
	–	55	15	12	2	–
	–	51	12	4	–	–
	–	52	–	–	–	–
	25	23	2	–	–	–
	9.3	20.3	5.4	1.5	0.3	0.2

dicarbonyl dichloride X $(COCl)_2$ in the presence of triethylamine in dry CH_2Cl_2 under high dilution conditions. The products were separated by careful chromatography on silica gel columns using gradient elution with a mixture of chloroform and methanol and characterized by FAB MS or ES MS for oligomeric nature.

Interestingly, none of the condensation reactions (with the sole exception of **1c**) resulted in a single macrocycle. Thus, 1,3-adamantane dicarbonyl dichloride (**1a**) yielded a mixture of four macrocycles identified as 2 + 2 (**3a**), 3 + 3 (**4a**), 4 + 4 (**5a**), and 5 + 5 (**6a**) condensation products, cystine diOMe and 1,3-pyridinedicarbonyl dichloride (**1b**) afforded three macrocyclic products identified as 2 + 2 (**3b**), 3 + 3 (**4b**), and 4 + 4 (**5b**), and only one macrocycle characterized as 2 + 2 product (**3c**) was obtained in the case of 1,3-benzenedicarbonyl dichloride (**1c**). With norbornene *trans*-2,3 dicarbonyl dichloride (**1d**), the condensation of cystine diOMe led to the isolation of three products identified as arising from 1 + 1 (**2d**), 2 + 2 (**3d**), and 3 + 3 (**4d**) cyclization. As anticipated, the use of flexible 1,3-propanedicarbonyl dichloride (**1e**) afforded six macrocycles (**2e**−**7e**) starting from the minimum size 13-membered to maximum 78-membered. Table 1 presents the yield distribution of products in the condensation of cystine diOMe with various templates (X).

In another series of experiments, in order to evaluate the role of S−S linkage in facilitating macrocyclization, it was envisaged to replace cystine with several 1,ω-diamines of approximately equal chain length, for example, cystamine (achiral, contains S−S), lysine (chiral, lacks S−S), and simple 1,6-diaminohexane (achiral, lacks S−S linkage). Condensation reaction of thus selected 1,ω-diamines with 1,3-adamantanedicarbonyl dichloride under identical conditions afforded cyclic products varying in total yield of 10−59% (Figure 2). For example, while cystamine produced two macrocycles arising from 2 + 2 (**12**) and 3 + 3 (**13**) cyclization reaction in yields of 46 and 13%, respectively (not very different from cystine case), the non S−S diamines gave poor yields of cyclization products. Thus, while L-lysine in a two-step reaction afforded a 24-membered macrocycle (**11**) in a total yield of ∼20%, the simple 1,ω-diamino hexane on direct condensation with 1,3-adamantane dicarbonyl dichloride led to two cyclic products **8** and **9** (arising from 2 + 2 and 4 + 4 cyclization, respectively) in abysmally poor total yield of ∼10%, with the 26-membered 2 + 2 macrocycle as the major component (8%) (Figure 2). The above results support the notion that within a narrow window of reaction conditions the disulfide linkage manifests itself in increased macrocyclization yield.

In their ^{1}H NMR spectra, regardless of the ring size and the nature of "X", all cystine-based macrocycles exhibited a single set of resonances for Cyst and X units indicating the presence of high symmetry in the rings. The presence of strong Cyst NH-CβH$_2$ cross-peaks in their ROESY NMR spectra (Supporting Information) supported β-turn features. The other prominent cross-peaks were between Cyst NH and X protons indicating trans-orientation of the X-carbonyls. FT-IR studies (Supporting Information) in chloroform solution (at 298 K) indicated considerable population of intramolecularly hydrogen-bonded conformations as shown by the presence of a concentration-independent broad band at 3310−3330 cm^{-1} in the NH stretch region of most cystine-based cyclic peptides attributed to hydrogen-bonded NH groups. ^{1}H NMR VT studies (conducted in DMSO-d_6 between 303 and 343 K) corroborated FT-IR results. Most cystine-based macrocycles exhibited relatively low values of temperature coefficients (dδ/dT varying from ∼−1 to −5 ppb/K) indicating intramolecularly hydrogen-bonded NH groups (Supporting Information).

Convincing support for β-turn-type structures was provided by circular dichroism (CD) spectra.[21] CD studies were carried out in trifluoroethanol (TFE) solution at 298

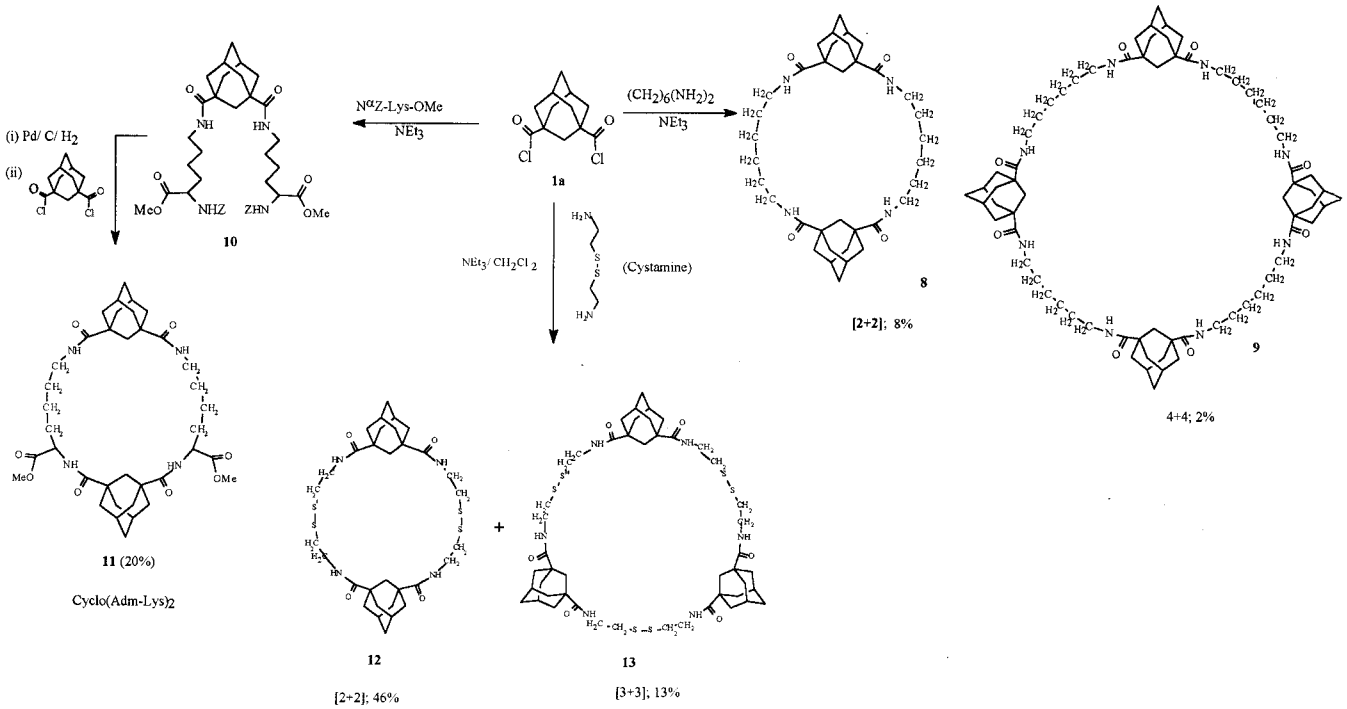


Figure 2. Synthesis of adamantane-containing noncystine-based macrocycles.

K at a concentration of ~0.5 μM. The presence of a prominent positive band at ~213 nm in cystine-containing macrocycles with X = Adm, at 218 nm with X = Pyr, at 229 nm with X = Ph, and at 217 nm with X = $(-CH_2-)_3$ strongly suggested type-II β-turn conformation. A comparison of CD spectra in pyridine-containing cystine-based 3 + 3 and 4 + 4 macrocycles of 39- and 52-membered rings, respectively, is presented in Figure 3. The adamantane-containing macrocycles of cystine in 26-, 39-, 52-, and 65-membered rings showed CD bands as presented in Figure 4. The same trend was shown with cystine macrocycles containing 1,3-propanedicarbonyl unit in 26-, 39-, and 52-membered rings (Figure 5). The turn structure became highly pronounced in norbornene containing cystine macrocycles as shown by the presence of a sharp CD maximum at 208 nm in 12-membered cyclo (NBE-Cyst) and at 222 nm in 24-membered cyclo (NBE-Cyst)$_2$ (Supporting Information).

Suitable crystals could be obtained only with adamantane-containing 39-membered cystine cyclic peptide **4a**. The crystal structure revealed a figure-eight conformation with a double cavity. Interestingly, despite the 3-fold symmetry in its formula (Figure 7a), the cyclo (Adm-L-Cyst)$_3$ molecule crystallizes with a 2-fold rotation axis that passes through one adamantyl group and through the center of the opposing S−S bond (Figure 7b). As seen in Figure 7b, while the two symmetry equivalent adamantane units have their carbonyl units anti to each other (see O6 and O7), the carbonyl groups in the central

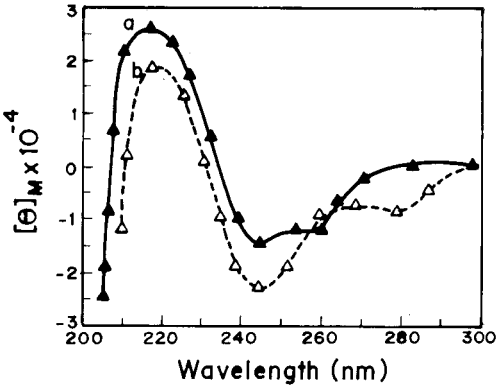


Figure 3. CD spectra of cyclo (Pyr-Cyst)$_3$ (a) and cyclo (Pyr-Cyst)$_4$ (b).

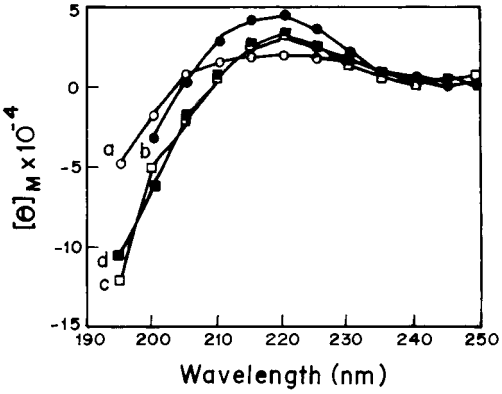


Figure 4. CD spectra of cyclo (Adm-L-Cyst)$_2$ (a), cyclo (Adm-L-Cyst)$_3$ (b), cyclo (Adm-L-Cyst)$_4$ (c), and cyclo (Adm-L-Cyst)$_5$ (d).

(21) The CD studies for all cyclopeptides reported in this paper were carried out in the wavelength region of 200−250 nm. The S−S chromophore of cystine unit is known to absorb at ~260 nm (Woody, R. W.; Dunker, A. K. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York, 1966; pp 109−157) and does not interfere in this region. The C=C chromophore of norbornene unit also does not absorb in this region as shown by us recently (*J. Am. Chem. Soc.* **1998**, *120*, 8448) in the comparison of olefinic and the dihydro analogues of norborneno peptides, wherein it was found that both exhibit a prominent positive CD band at ~211 nm. A large number of cystine-containing cyclic peptides have been studied by us (*Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1105; *J. Am. Chem. Soc.* **1996,** *118*, 10916; *J. Am. Chem. Soc.* **1998**, *120*, 2695) for CD spectra and are found to absorb in the range of 210−230 nm, the peptide chromophore region. We believe therefore that the CD bands exhibited by cystine-containing cyclic peptides described in the present work are largely due to normal peptide chromophores.

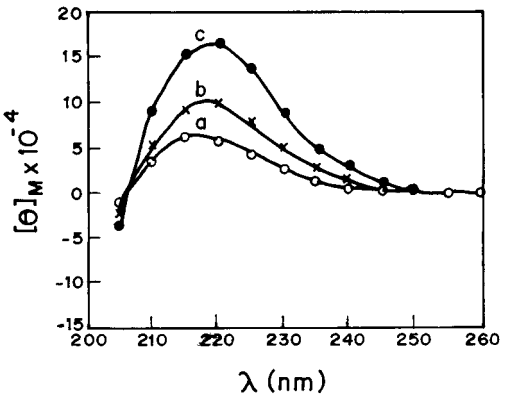


Figure 5. CD spectra of cyclo (Glut-Cyst)$_2$ (a), cyclo (Glut-Cyst)$_3$ (b), and cyclo (Glut-Cyst)$_4$ (c).

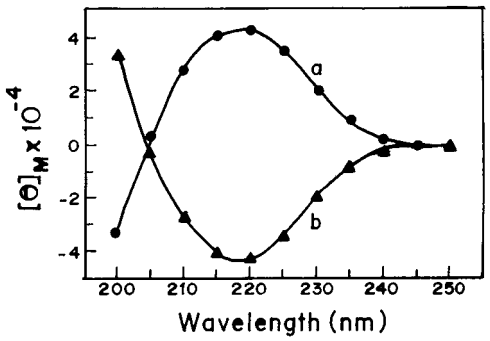


Figure 6. Comparison of CD spectra of cyclo (Adm-L-Cyst)$_3$ (a) and cyclo (Adm-D-Cyst)$_3$ (b). All spectra were taken in TFE solution at 298 K with 0.5 μM concentration.

adamantane unit (see O1 and O7) adopt a syn conformation that is in a favorable orientation to form intramolecular NH···OC bonds (N(3)···O(1a) 3.027 Å; H···O = 2.21 Å) with the amide NHs of the middle cystine unit (Figure 8a) that stabilizes the figure-8-fold in cyclo (Adm-L-cyst)$_3$.

In principle, the figure-eight (double-helical arrangement) motif could have a left or a right disposition of the intercrossing helices (Figure 9, parts a and b, respectively). The fact that only one arrangement (Figure 9a) is seen in cyclo (Adm-L-Cyst)$_3$ suggests control by the L-chirality of the cystine unit in the macrocycle formation. The choice of "a" over the alternate form "b" (Figure 9) is analogous to the choice of right- or left-handed helices in linear peptides where the backbone atoms form a right-handed helix when the side chains have the L-configuration at the C$^\alpha$ atoms and a left-handed helix when the side chains have the D-configuration at the C$^\alpha$ atoms. In cases where the C$^\alpha$ atoms are rendered achiral by the presence of two CH_3 groups (as in Aib residue), both right- and left-handed helices occur as shown, for example, in the crystal structure analyses of X-(Aib)$_n$-Y peptides.[22] In the present case, there are six chiral C$^\alpha$ atoms, two for each L-cystine residue, that predispose the figure-eight backbone to assume the conformation "a" in Figure 9, rather than conformation "b". Conformation "b" is not favored, if not impossible, because the six $COOCH_3$ moieties that extend outward (Figure 7b) would have to turn inward for "b" and cause considerable steric interference. The question would arise then that if chirality at C$^\alpha$ atom predetermines the helical sense, would the D-Cyst containing macrocycle, cyclo (Adm-D-Cyst)$_3$, adopt the mirror image form (conformation b in Figure 9) and furthermore would two forms "a" and "b" be equally present in an achiral, cystamine-based macrocycle? While cystamine-based adamantane-constrained macrocycles **12** and **13**, prepared in an analogous manner by the reaction of 1,3-adamantanedicarbonyl dichloride with cystamine under high dilution conditions did not yield suitable crystals for X-ray studies, the 39-membered D-cystine-based macrocycle cyclo (Adm-D-Cyst)$_3$ crystallized in colorless prisms. The presence of the dimer cyclo (Adm-D-Cyst)$_2$ and the tetramer cyclo (Adm-D-Cyst)$_4$ was confirmed by FAB-MS spectra. As in the L-Cyst case, the yield distribution was ~50:20:5 in the D-Cyst-based macrocycles of dimer, trimer, and tetramer structure. Interestingly, in both L- and D-Cyst series, it was only the 3 + 3 cyclic trimer that showed any tendency to crystallize.

The crystal structure of enantiomeric cyclo (Adm-D-Cyst)$_3$ was shown (Figure 7c) to be the exact mirror image of the L-Cyst-based macrocycle, including the disorder at S3−S3a. The result had been expected, although there are rare exceptions in which, under the same conditions, enantiomers of peptides have crystallized in different space groups and exhibited different orientations in the side chains (Balaram and Karle, to be published). A comparison of the two structures (Figure 7b,c) shows that the only difference between the two enantiomers is the sign of the handedness at the interstrand crossing. While in L-Cyst macrocycle it is the right-handed helix on the top, in the D-isomer the top helix assumes the left-handed conformation. The space-filling diagrams of the two enantiomers (L and D) are compared in Figure 10, looking down the 2-fold axes of the molecules. The D-Cyst enantiomer also shows two internal hydrogen bonds (Figure 8b).

The D-cyst enantiomer showed almost identical ^{1}H NMR spectra. The appearance of only a single set of resonances for cystine and adamantane protons coupled with the observation that, like in L-Cyst, these remain unaffected in the temperature range of 20−90 °C indicated that the molecule has a single conformation in solution. The anti arrangement for the 1,3-adamantane carbonyls seen for the two symmetry equivalent adamantane units in the crystal structure was also suggested by the enhanced ROE between the NH and the adamantane methylene protons in the ROESY spectrum of both L- and L-enantiomers of cyclo(Adm-Cyst)$_3$ (Supporting Information). FT-IR and ^{1}H NMR VT studies indicate only modest amount of intramolecular hydrogen bonding (Supporting Information).

The circular dichroism spectrum of the D-isomer confirmed the enantiomeric nature of the molecule. Comparison of the CD spectra of L- and D-isomers shows the exact mirror-image relationship (Figure 6). The type-II β-turn structure in both enantiomers was indicated by the presence of an intense CD band at ~213 nm. Thus, collectively ^{1} H NMR, FT-IR, and CD studies supported the enantiomeric Figure-8-fold for the L- and D-macrocycles in solution.

Although the adoption of the figure-eight motif has been demonstrated only in the 39-membered cyclo (Adm-Cyst) trimer, the possibility of presence of extended double-helical structures in higher (52- and 65-membered cyclicoligomers) cannot be ruled out. The 26-membered

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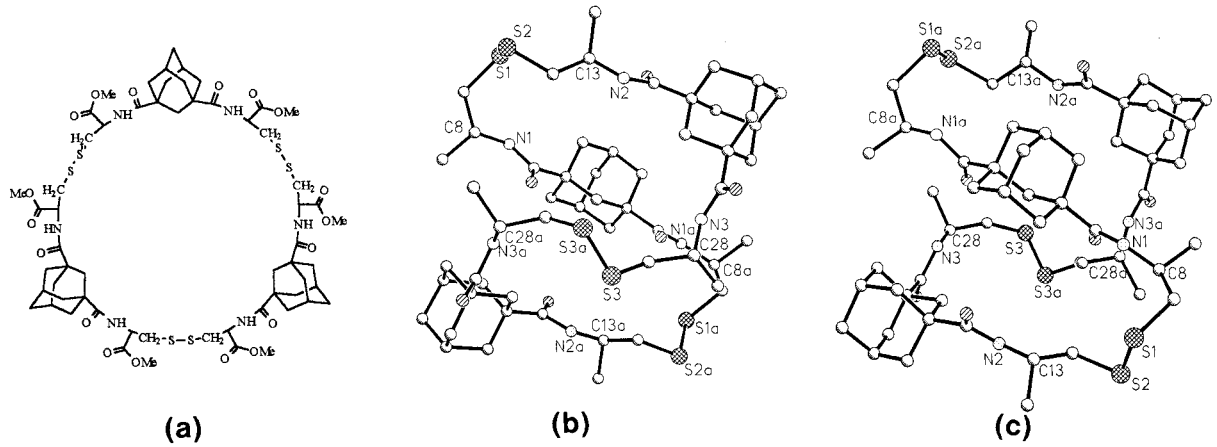

Figure 7. (a) Chemical structure of cyclo (Adm-Cyst)$_3$. (b) X-ray structure of cyclo (Adm-L-Cyst)$_3$. A 2-fold rotation axis passes through C(1) and C(6) and halfway between S(3) and S(3a). The view is offset somewhat from being down the *x* axis in order to illustrate better the Figure 8. Several interatomic distances that indicate the size of the cavity are as follows: C(1)−S(3), 5.8 Å; N(2)−C(4), 4.36 Å; N(2)−S(3a), 6.20 Å; N(1)−C(12), 3.94 Å; C(18)−C(3a), 4.77 Å; O(6)−C(5), 3.85 Å. (c) X-ray structure of cyclo (Adm-D-Cyst)$_3$.

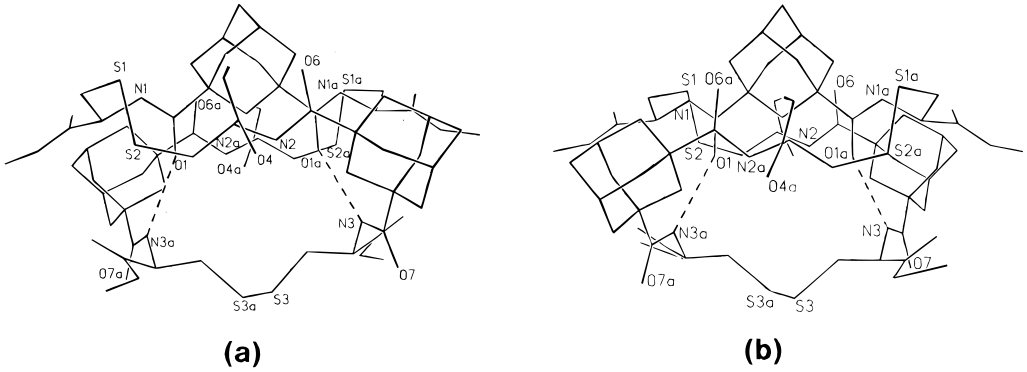

Figure 8. (a) View of cyclo (Adm-L-Cyst)$_3$ down the *z* axis. Only the most prevalent of the disordered sites for S(3)−S(3a) and the bonded CH_2's (at the bottom of the macrocycle) are shown in all the diagrams. The dotted lines indicate the only two intramolecular hydrogen bonds, N(3)···O(1a) and N(3a)···O(1) with an N···O distance of 3.03 Å. (b) View of cyclo (Adm-D-Cyst)$_3$ down the *z* axis.

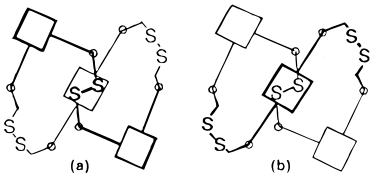

Figure 9. Presence of L-substituents on chiral C^{α} atoms (designated by O) results in the preference of (a) for the figure-eight conformation of the backbone of (Adm-L-Cyst)$_3$ rather than (b). The heavy lines are above the plane of the paper. If there were no chiral atoms in the macrocycle, then conformation (a) could be twisted into (b).

lower homologue, however ,appears to be less likely to have a figure-eight motif, since the crystal structure of related 26-membered 2 + 2 macrocycle containing alternating repeats of *m*-phenylene and cystine units was shown to adopt a collapsed open-ring structure.[20] Thus, apart from the ring size, the factors that may control the figure-eight formation in macrocyclic peptides appear to be the presence of turn-inducing units. In the present example, the presence of three cystine residues promotes the attainment of a double-helical fold. Interestingly, a survey[23] has shown the significance of the presence of multiple disulfide (S−S) bonds in protein and polypeptides with topologically important features. A rotaxane-like motif of three cystine residues present in various growth factors and related proteins has been referred to as a knot.[24−26]

Conclusion

The cystine-containing macrocycles reported here represent a novel class of cyclic peptides capable of adopting topologically important conformations. ^{1}H NMR, FT-IR, and CD studies have shown these cystine-based macrocycles to adopt β-turn-like features in their structures which are largely stabilized by intramolecular hydrogen bonds. The 39-membered cyclo (Adm-L-Cyst)$_3$ and its enantiomer cyclo(Adm-D-Cyst)$_3$ are shown by their X-ray crystal structure to adopt figure-eight conformations with exact mirror image relationship, and the spectroscopic measurements on these molecules confirm that each enantiomer has a single conformation in solution. The increasing number of cystine residues in these macrocycles are likely to be of consequence in designing macrocycles with extended double helical structures that

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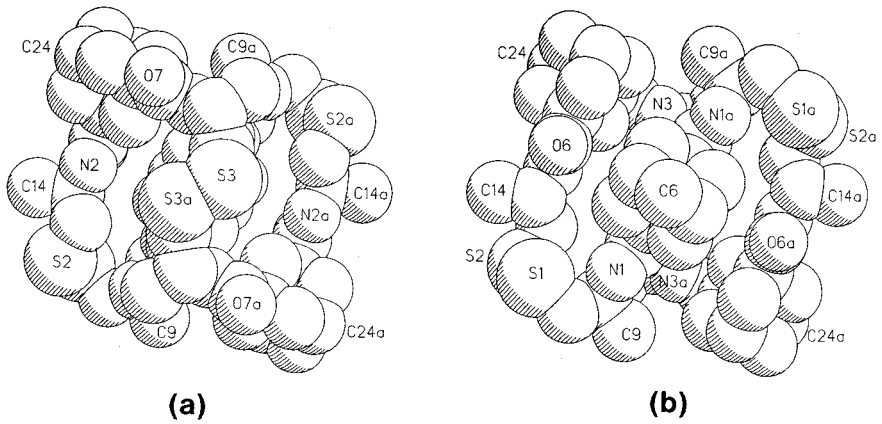

Figure 10. Space-filling diagrams of (a) cyclo (Adm-L-Cyst)$_3$ and (b) cyclo (Adm-D-Cyst)$_3$.

may have relevance in providing new insights into protein folding mechanism and in the design of topologically important macrocycles.

Experimental Section

All amino acids used were of the L-configuration [except for (Adm-D-Cyst)$_n$]. Melting points are uncorrected. ^{1}H NMR ROESY experiments were performed using 0.2 and 0.3 s mixing time with pulse spin locking with 30 pulses and 2 kHz spin locking field. FAB MS were obtained using *m*-nitrobenzyl alcohol as a matrix. The circular dichroism (CD) spectra were recorded in quartz cells of 1 mm path length at 25 °C. Reactions were monitored wherever possible by TLC. Silica gel (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 hexane or a mixture of hexane and ethyl acetate. Products were eluted with either a mixture of ethyl acetate/hexane or chloroform/methanol.

General Procedure for the Preparation of Cyclo [(X-Cyst)$_n$]. A solution of freshly prepared X $(COCl)_2$ (X = 1,3-adamantane, 1,3-benzene, 2,6-pyridine, 2,3-*trans*-norbornene, and 1,3-propane unit) (1 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise over 0.5 h to a well-stirred solution of L- or D-cystine dimethyl ester hydrochloride (1 mmol) and triethylamine (4.5 mmol) in dry CH_2Cl_2 (∼150 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4−6 h and monitored by TLC. The reaction mixture was washed sequentially with ice-cold 2 N H_2SO_4, H_2O, and 5% $NaHCO_3$ (∼20 mL each). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on a column of silica gel using either a mixture of ethyl acetate/hexane or chloroform/methanol as eluent to afford the X-constrained cystine macrocycles. A similar procedure was followed for the preparation of cystamine-based macrocycles.

Selected Data. Detailed spectral and analytical data have been reported for cyclo[(Adm-L-Cyst)$_n$; n = 2, 3, 4, 5], cyclo-[Ar-L-Cyst)$_n$; n = 2, 3, 4; Ar = Pyr or Ph], cyclo[(Glut-L-Cyst)$_n$; n = 1, 2...6; Glut = −CO−CH_2−CH_2−CH_2−CO-], and cyclo-[(NBE-L-Cyst-)$_n$; n = 1, 2, 3; NBE = 2,3-trans norbornene dicarbonyl unit] in refs 20 and 27−29, respectively. Selected data for all new compounds reported in the present paper are given below.

Cyclo(Adm-cysta)$_2$ (12): yield 46%; mp 297−298 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.60−2.32 (m, 28H), 2.95 (m, 8H), 3.50 (m, 8H), 7.85 (brd, 4H); FAB-MS *m*/*z* 681 (64) [M + H]$^+$. Anal. Calcd for $C_{32}H_{48}N_4S_4O_4$ (mol wt 680): C, 56.47; H, 7.05; N, 8.23. Found: C, 56.63; H, 7.13; N, 8.30.

Cyclo(Adm-cysta)$_3$ (13): yield 13%; mp 142−143 °C; ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) δ 1.60−2.28 (m, 42H), 2.85 (m, 12H), 3.50 (m, 12H), 7.32 (brd, 6H); FAB MS *m*/*z* 1022 (62) [M + H]$^+$. Anal. Calcd for $C_{48}H_{72}N_6O_6S_6$ (mol wt 1020): C, 56.47; H, 7.05; N, 8.23. Found: C, 56.19; H, 6.98; N, 8.19.

Cyclo(Adm-D-Cyst)$_2$: yield 50%; mp 118−120 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57−2.32 (m, 28H), 3.13 (m, 4H), 3.28 (m, 4H), 3.78 (s, 12H), 4.81 (m, 4H), 6.61 (d, J = 6.71 Hz, 4H); FAB MS (*m*/*z*) 913 (100) [M + H]$^+$. Anal. Calcd for $C_{40}H_{56}N_4O_{12}S_4$ (mol wt 912): C, 52.63; H, 6.14; N, 6.14. Found: C, 52.70; H, 6.23; N, 6.09.

Cyclo(Adm-D-Cyst)$_3$: yield 15%; mp 173−175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67−2.28 (m, 42H), 3.18 (m, 12H), 3.78 (s, 18H), 4.80 (m, 6H), 6.72 (d, J = 7.18 Hz, 6H); FAB MS *m*/*z* 1369 (63) [M + H]$^+$. Anal. Calcd for $C_{60}H_{84}N_6O_{18}S_6$ (mol wt 1368): C, 52.63; H, 6.14; N, 6.14. Found: C, 52.90; H, 6.23; N, 6.21.

Reaction of 1, 6-Diaminohexane with 1,3-Adamantanedicarbonyl Dichloride. (a) Synthesis of Macrocycles 8 and 9. To an ice-cooled and stirred solution of 1,6-diaminohexane (1 mmol) in dry CH_2Cl_2 (150 mL) containing triethylamine (2.5 mmol) was added 1,3-adamantanedicarbonyl dichloride (1 mmol in 50 mL dry CH_2Cl_2) over a period of 0.5 h and the mixture stirred for 12 h (TLC). The reaction mixture was worked up by washing, sequentially, with ice-cold 2 N H_2SO_4, H_2O, and 5% $NaHCO_3$ (∼20 mL each), drying the organic layer with anhyd $MgSO_4$, and evaporating in vacuo. The residue was chromatographed on a column of silica gel, and elution with a mixture of chloroform/methanol yielded two products which were identified as macrocycles **8** and **9**.

8: yield 8%; mp 360 °C dec; IR (KBr) 3386, 3314, 1644, 1552, 1526 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00−2.12 (m, 44H), 2.98 (m, 8H), 7.35 (brs, 4H); FAB MS (*m*/*z*) 609 (100) (MH)$^+$. Anal. Calcd for $C_{36}H_{56}N_4O_4$ (mol wt 608): C, 71.05; H, 9.21; N, 9.21. Found: C, 70.98; H, 9.09; N, 9.33.

9: yield 2%; IR (KBr) 3352, 1644, 1552, 1533 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.09−2.09 (m, 88H), 3.00 (m, 16H), 7.35 (brs, 8H); FAB MS (*m*/*z*) 1217 (72) (MH)$^+$. Anal. Calcd for $C_{72}H_{112}N_8O_8$ (mol wt 1216): C, 71.05; H, 9.21; N, 9.21. Found: C, 71.13; H, 9.09; N, 9.30.

Synthesis of Lysine-Based Macrocycle (11). (a) Synthesis of N$^\alpha$-Z-Lys-Adm (10). A solution of freshly prepared 1,3-adamantanedicarbonyl dichloride (1 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise to a well-stirred solution of N$^\alpha$Z-LysOMe·HCl (2 mmol) in dry CH_2Cl_2 (75 mL) containing triethylamine (4 mmol) at 0 °C over 0.5 h and mixture stirred at room temperature for 12 h (TLC). The reaction mixture was worked up by washing, sequentially, with ice-cold 2 N H_2SO_4, H_2O, and 5% $NaHCO_3$ (∼20 mL each), drying the organic layer with anhyd $MgSO_4$, and evaporating in vacuo. The residue was

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chromatographed on silica gel with a mixture of ethyl acetate/hexane as eluents.

Selected data: yield 57%; oily; $[\alpha]^{26}_{D} = -4.36$ (*c* 4.3, $CHCl_3$); IR (KBr) 3379 (br), 1749, 1730, 1710, 1639, 1560, 1540, 1532 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.10−2.32 (m, 26H), 3.24 (m, 4H), 3.72 (m, 6H), 4.30 (m, 2H), 5.10 (s, 4H), 5.75 (m, 4H), 7.35 (brs, 10H).

(b) Synthesis of Cyclo(Lys-Adm) (11). A solution of freshly prepared 1,3-adamantanedicarbonyl dichloride (2 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise to a well-stirred solution of 2 mmol of N-deprotected $(H_2N^{\alpha}\text{-Lys})_2$Adm (2 mmol) in dry CH_2Cl_2 (100 mL) containing triethylamine (4 mmol) at 0 °C over 0.5 h and mixture stirred at room temperature for 24 h (TLC). The reaction mixture was worked up by washing, sequentially, with ice-cold 2 N H_2SO_4, H_2O, and 5% $NaHCO_3$ (∼20 mL each), drying the organic layer with anhyd $MgSO_4$, and evaporating in vacuo. The residue was chromatographed on silica gel using a mixture of ethyl acetate/hexane as eluents.

Selected data: yield 20%; mp 272 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30−2.29 (m, 40H), 3.09 (m, 2H), 3.43 (m, 2H), 3.73 (s, 6H), 4.53 (m, 2H), 6.11 (brs, 2H), 6.46 (d, $J = 9.0$ Hz, 2H); FAB MS *m*/*z* 697 $[M + H]^+$.

Crystal Structure Analysis. (a) (Adm-L-Cyst)$_3$, $C_{60}H_{84}N_6O_{18}S_6$. $C_4H_8O_2$, space group $C222_1$, $a = 16.587(1)$ Å, $b = 16.945(1)$ Å, $c = 25.882(1)$ Å, $V = 7274.6$ Å^3, $d_c = 1.331$ g/cm^3, $Z = 4$, Cu radiation, $\lambda = 1.5418$ Å. Crystals were obtained from chloroform and ethyl acetate. The molecule lies on a 2-fold axis. The structure was solved by direct phase determination and refined by full-matrix anisotropic least squares. Distance restraints were applied to the disordered ethyl acetate molecule that lies on a 2-fold axis, to one methyl ester group with high mobility and one C−S−S−C group highly disordered around a crystallographic 2-fold axis. $R = 9.1$ for 433 parameters and 2130 independent data ($>3\sigma(F)$). Absolute configuration determined by the anomalous scattering of the S atoms. (b) (Adm-D-Cyst)$_3$, the same as above except $a = 16.536(2)$ Å, $b = 16.946(1)$ Å. $c = 26.080(2)$ Å; $R = 9.7$ for 417 parameters and 2793 independent data ($>3\sigma(F)$).

Supporting Information Available: X-ray diffraction analyses: tables of atomic coordinates, bond lengths and bond angles, anisotropic thermal parameters, hydrogen atom coordinates, and torsion angles for (Adm-L-Cyst)$_3$ and (Adm-D-Cyst)$_3$. Solution data: ^{1}H NMR, ROESY NMR, VT NMR, FAB MS, and CD spectra for (X-Cyst)$_n$ where $n = 2-5$. This material is available free of charge via the Internet at http://pubs.acs.org.

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