Synthesis of (*R*)-(−)-Carnitine Chloride (3) (*R*)-(+)-isobutyl 3,4-epoxybutyrate (3.3 g, 20.9 mmol) of 95% ee was suspended in 30 mL of 0.003 N phosphate buffer at 35 °C and pH 8 and treated with alcalase 2.0 T (0.4 g, 0.86 unit). The pH was maintained at 8 with 1 N aqueous NaOH by using a pH-stat until the consumption of base stopped (15 h). The reaction mixture was extracted with methylene chloride (2 × 10 mL) and reacted for 2 h at 45 °C with 5 N trimethylamine (5 mL, 25 mmol). After evaporation to dryness to remove unreacted trimethylamine, concentrated hydrochloric acid (3 mL) was added, and the resulting solution was evaporated to dryness. The residue was crystallized from isopropyl alcohol to give 3.28 g of (*R*)-(−)-carnitine chloride (3) (79% yield); $[\alpha]^{25}_D$ −22.2° (*c* 1, H_2O). The ee, determined by comparison of the optical activity with the literature value ($[\alpha]^{25}_D$ −23.7°),[13] was 94%.

Note added in proof: After submission of this manuscript for publication, a highly enantioselective PLE-catalyzed hydrolysis of methyl 3,4-epoxybutyrate was reported (Mohr, P.; Rösslein, L.; Tamm, C. *Helv. Chim. Acta* **1987**, *70*, 142).

(13) Strack, E.; Lorenz, J. *Z. Physiol. Chem.* **1960**, *318*, 129.

Solution and Solid-State Conformations of Ascidiacyclamide, a Cytotoxic Cyclic Peptide from Ascidian

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The solution and solid-state conformations of ascidiacyclamide, a cytotoxic cyclic peptide from ascidian, were determined by ^1H NMR spectroscopy and X-ray diffraction. The measurement of solvent and temperature dependences showed that the peptide NH protons of C_2-symmetric ascidiacyclamide are all solvent-shielded, suggesting the locations of these protons in the interior of the ring structure of ascidiacyclamide. On the basis of coupling constants and the known stereochemical preferences, a saddle-shaped conformation was proposed as being favored in solution. X-ray diffraction analysis revealed a C_2-symmetric saddle-shaped conformation, almost compatible with the NMR data. Two benzene molecules per one ascidiacyclamide molecule were co-crystallized and stabilized by the van der Waals contact with D-valine and L-isoleucine side chains, respectively. No hydrogen bond was observed in the crystal packing. The analysis of the thermal behavior of the crystals, with the aid of their IR spectra, showed the thermal stability of the ring conformation observed in the crystal. The conformation for the related cyclic peptides, along with its biological implications, was discussed on the basis of the present results.

Introduction

Lipophilic cyclic peptides from marine organisms have been receiving increasing interest, due largely to the high potency of their antineoplastic and/or cytotoxic activities.[1,2] Several cyclic peptides that contain unusual thiazole and oxazoline amino acids have been isolated from ascidian (Chart I): ulithiacyclamide (1),[3] ulicyclamide (2),[3a,4] patellamides A (3),[5] B (4),[4,6] and C (5),[4,6a,b] and ascidiacyclamide (6).[7] These cyclic peptides, which exhibit potent cytotoxic activites,[5a,7a] all have a common (1, 3–6) or related (2) ring structure. Therefore this ring formation may be necessary for the cytotoxic activity, although up to now little has been known about the mechanism of its biological action.

Study of the active conformation of the biologically important molecule is very useful for understanding the mechanism of action at the atomic level. As part of a program to elucidate the active forms of these cyclic

Table I. NMR Parameters of NH and $C_\alpha H$ Resonances

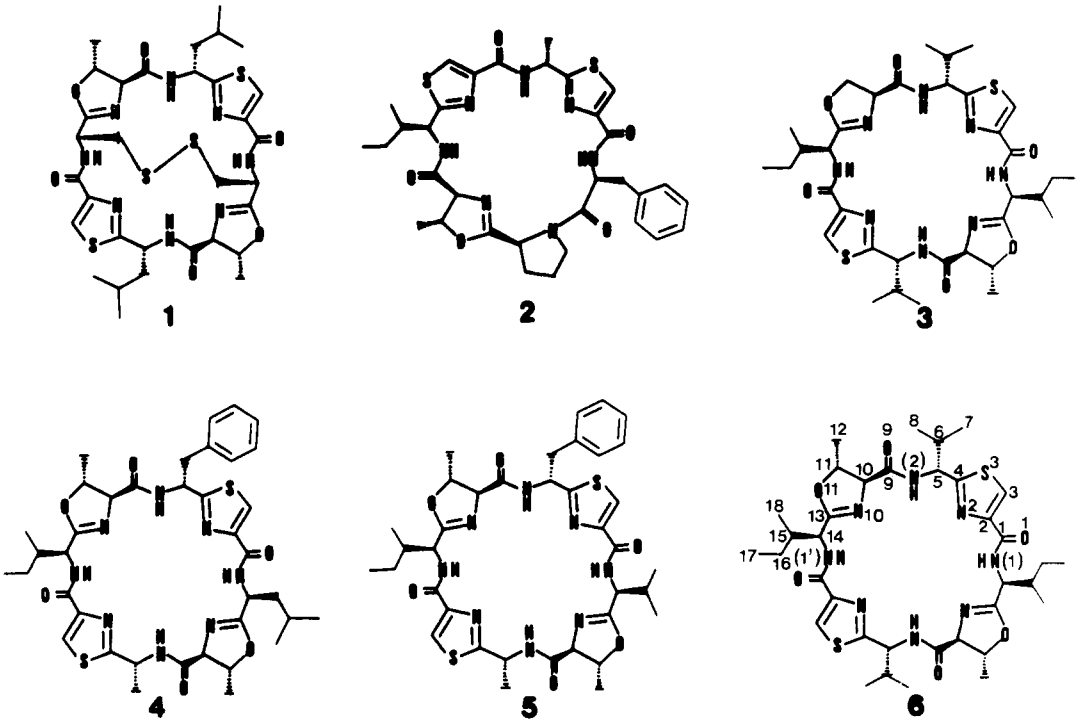
		δ (ppm) at 24 °C	$d\delta/dT \times 10^4$ (ppm/deg)	$J_{HNC_\alpha H}$ (Hz) at 24 °C	$J_{HNC_\alpha H}$ (Hz) at 61 °C
NH(1)	C_6D_6	8.330	17	7.58	7.91
	$CDCl_3$	7.951	−7	7.91	7.91
	$(CD_3)_2SO$	7.904	5	8.71	8.24
NH(2)	C_6D_6	7.587	−2	9.89	9.81
	$CDCl_3$	7.380	−15	10.55	10.22
	$(CD_3)_2SO$	7.323	10	9.85	10.29

		$J_{\alpha\beta}$ (Hz) at 24 °C	$J_{\alpha\beta}$ (Hz) at 61 °C
H5	C_6D_6	7.25	7.36
	$CDCl_3$	5.94	6.37
	$(CD_3)_2SO$	6.61	6.56
H14	C_6D_6	4.45	4.10
	$CDCl_3$	5.90	5.30
	$(CD_3)_2SO$	5.50	4.45

(1) (a) Faulkner, D. J. *Nat. Prod. Rep.* **984**, *1*, 551–598. (b) Faulkner, D. J. *Nat. Prod. Rep.* **1986**, *3*, 1–33.
(2) Kobayashi, J. *Kagaku to Seibutsu* **1985**, *23*, 119–123.
(3) (a) Ireland, C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688–5691. (b) Kato, S.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1986**, *27*, 2653–2656. (c) Schmidt, U.; Weller, D. *Tetrahedron Lett.* **1986**, *27*, 3495–3496.
(4) (a) Wasylyk, J. M.; Biskupiak, J. E.; Costello, C. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 4445–4449. (b) Schmidt, U.; Gleich, P. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 569–571.
(5) (a) Ireland, C. M.; Durso, A. R., Jr.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807–1811. (b) Biskupiak, J. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 2302–2304. (c) Hamada, Y.; shibata, M.; Shioiri, T. *Tetrahedron Lett.* **1985**, *26*, 6501–6504.
(6) (a) Hamada, Y.; Shibata, M.; Shioiri, T. *Tetrahedron Lett.* **1985**, *26*, 5155–5158. (b) Hamada, Y.; Shibata, M.; Shioiri, T. *Tetrahedron Lett.* **1985**, *26*, 5159–5162. (c) Schmidt, U.; Griesser, H. *Tetrahedron Lett.* **1986**, *27*, 163–166.
(7) (a) Hamamoto, Y.; Endo, M.; Nakagawa, M.; Nakanishi, T.; Mizukawa, K. *J. Chem. Soc., Chem. Commun.* **1983**, 323–324. (b) Hamada, Y.; Kato, S.; Shioiri, T. *Tetrahedron Lett.* **1985**, *26*, 3223–3226.

0022-3263/88/1953-0107$01.50/0

Chart I



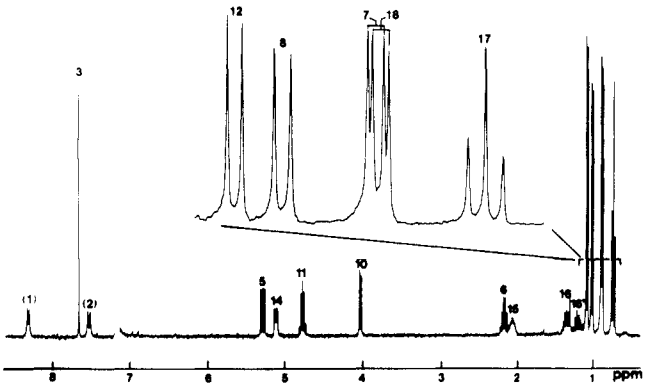


Figure 1. ^1H NMR spectrum of ascidiacyclamide in C_6D_6 solution at 61 °C.

peptides, we have studied the stereostructure of ascidiacyclamide (**6**) in solution and in the solid state.[8]

Results and Discussion

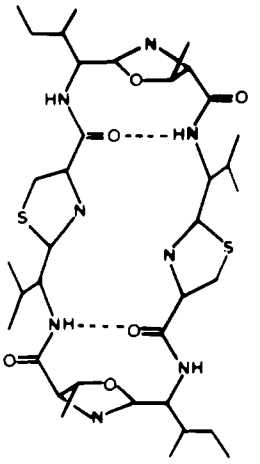
NMR Studies of Solution Conformation of Ascidiacyclamide. The atomic numbering scheme used for this paper is shown in Chart I. The ^1H NMR spectrum of **6** in C_6D_6 solution at 61 °C is shown in Figure 1.

C_2 Symmetry. All resonances in various solvents showed that the dominant form of **6** is C_2 symmetry in the NMR time average. A coupling between H10 and H14 protons was observed and became clear as the temperature was increased. This could be interpreted as the homoallylic effect:[7a] the higher the temperature, the larger the effect. Similar couplings has also been reported for the oxazoline-cysteine protons in **1**.[3a]

Solvent and Temperature Dependences of NH Protons. The participation of NH protons in intramolecular hydrogen bonds was examined by the temperature dependence of the NH chemical shifts in several solvents.

(8) Preliminary X-ray results were reported: Ishida, T.; Inoue, M.; Hamada, Y.; Kato, S.; Shioiri, T. *J. Chem. Soc., Chem. Commun.* **1987**, 370-371.

Chart II



The results are given in Table I.

Two kinds of basic conformations could be considered as possible forms of **6** from the model designs: a rectangular form stabilized by two intramolecular hydrogen bonds between NH and C=O groups (type I) shown in Chart II, and a square one without the hydrogen bond (type II) illustrated in Chart I. If **6** prefers the conformation of type I, it could be expected that the NH(1) proton of isoleucine residue exhibits different solvent and temperature dependences from the NH(2) proton of the valine residue. Similar behavior could be expected in a conformation of type II. As is obvious from Table I, the results suggested the behavior of the two NH protons to be nearly the same: (1) the protons undergo an upfield shifts of 0.3 to 0.4 ppm upon changing the solvent from C_6D_6 to $(CD_3)_2SO$; (2) the temperature coefficients ($d\delta/dT$) of the NH shifts showed no significant difference from each other (<0.002 ppm/deg); (3) upon five fold dilution of **6** in these solvents, no significant change was observed for both NH chemical shifts; (4) the rates of deuterium exchange of the NH protons (upon addition of CD_3OD to $CDCl_3$ solution of **6** were both slow ($t_{1/2}$ of 2 days). When

these data are compared with those of other related peptides,[9] it could be supposed that the NH protons are both located in the interior of the ring structure of **6**, where the NH groups are sterically shielded from solvent. Thus, the NMR data, together with consideration of the space filling (CPK) model, suggest the type II form as the major solution conformation of **6**.[10]

On the other hand, the chemical shifts and coupling constants were almost independent of the solvent and temperature variations: the maximum δ change observed for any proton was 0.468 ppm for H12 on going from C_6D_6 to $CDCl_3$, and the maximum $d\delta/dT$ observed for any proton in any solvent was 0.002 ppm/deg for H3 in C_6D_6. This would reflect the rigidity of the stereostructure of **6**.

Conformation around N–C_α and C_α–C_β Bonds. $J_{HNC_\alpha H}$ and $J_{HC_\alpha C_\beta H}$ are also listed in Table I. A possible conformation can be estimated from these coupling constants, based on eq A and B shown in the Experimental Section. The $^3J_{HNC_\alpha H}$ value for the valine residue corresponds with the torsion angle of H–N–C_α–H close to 180°, while the torsion angle of isoleucine residue is near to 150°.[11] Among the possible H–C_α–C_β–H torsion angles of valine (~±40° and ~±130°) and isoleucine (~±50° and ~±120°) residues, CPK model considerations of the type II form suggest ±40° for the former and 50° for the latter residue as being most favorable, provided that the χ^{21} conformation is trans in isoleucine, which is the most frequently observed form.[12]

Since the absolute configuration about the asymmetric carbons in ascidiacyclamide (**6**) have already been determined by the synthetic method,[7b] it is possible to build the probable conformation of **6** in solution from these available NMR data. Prior to the model building, the following theories were postulated: (1) the peptide groups are trans planar; (2) the peptide group neighboring isoleucine is coplanar with the thiazole ring because of the resonance effect, and this has been observed in the crystal structure of nosiheptide,[13] a related cyclic peptide. As a result, a saddle-shaped conformation with valine side chains protruding from the two parallel sides of a rectangular ring chain was constructed, with of isoleucine turning below from the other side. The thiazole and oxazoline rings were located at the corners, respectively.

In order to substantiate the conclusions of these NMR studies and to further define the conformational characteristics of **6**, the X-ray diffraction analysis of its single crystal was carried out.

X-ray Crystallography

The refined atomic coordinates of non-hydrogen atoms are available in the supplementary material.

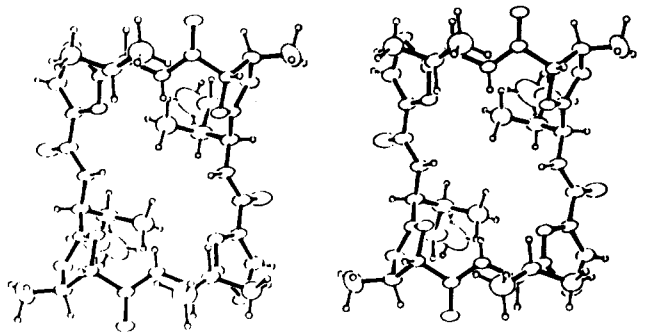


Figure 2. Stereoscopic drawing of ascidiacyclamide, viewed along the C_2 symmetry axis.

Table II. Conformational Torsion Angles (deg) of Ascidiacyclamide

	thiazole	isoleucine	oxazoline	valine
ϕ		−139.6 (4)		129.4 (4)
ψ	1.5 (6)[a]	−25.4 (5)	23.7 (5)[b]	46.6 (5)
ω	177.1 (3)		177.0 (3)	
χ^{11}		−162.4 (5)		58.5 (5)
χ^{12}		72.4 (6)		−176.2 (4)
χ^{21}		164.5 (8)		

[a] N(1)–C1–C2–N2. [b] N10–C10–C9–N(2).

Molecular Dimensions. Tables of the bond lengths and angles between non-hydrogen atoms are available in the supplementary material. The averaged standard deviations for the lengths and angles are 0.006 Å and 0.4° for **6**, respectively. These bonding parameters are in the acceptable range and compare well with the average values in the literature[14] for the experimental error, except for the values for the isoleucine end atoms and the benzene atoms (solvents of crystallization) that show high thermal motions. The bonding geometries around C10 and C14 atoms, where the homoallylic coupling between H10 and H14 protons was observed, are also normal. The molecular dimensions of the thiazole and oxazoline rings are in the usual range, and are almost planar, with a maximum shift of 0.016 (6) Å at the C3 atom and 0.088 (6) Å at the C11 atom from their respective best-fit planes: the root-mean-square deviations of the respective five non-hydrogen atoms from their best planes are 0.006 Å for the thiazole ring and 0.02 Å for the oxazoline ring.

Molecular Conformation. Stereoscopic drawing (ORTEP[15]) viewed along C_2 symmetry axis is shown in Figure 2. The selected torsion angles are listed in Table II. The molecular conformation of **6** takes a saddle-shaped form, and the valine and isoleucine side chains protrude over and under the ring chain, respectively. The ring structure is cylindricaly curved along the line joining two N(1) atoms related by C_2 symmetry. The radius of the curvature is about 4 Å. The peptide groups are all trans and are nearly planar, with 3.0 (3)° as the maximum value of $\Delta\omega$ at the oxazole–valine peptide bonds. The peptide group adjacent to the isoleucine chain is almost coplanar with the thiazole ring (their dihedral angle is 0.8(5)°) and forms a slightly warped plane between the thiazole and oxazoline rings.[16] The direction of this plane makes approximately a right angle with that of the other peptide plane.[17] Consequently

(9) (a) Kopple, K. D.; Ohnishi, M.; Go, A. J. *J. Am. Chem. Soc.* **1969**, *91*, 4264–4272. (b) Pitner, T. P.; Urry, D. W. *J. Am. Chem. Soc.* **1972**, *94*, 1399–1400. (c) Hruby, V. J. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weistein, B., Ed.; Dekker: New York, 1974; Vol. 3, pp 1–188. (d) Wuthrich, K. *NMR in Biological Research: Peptides and Proteins*; North-Holland: Amsterdam, 1976. (e) Mauger, A. B.; Stuart, O. A.; Highet, R. J.; Silverton, J. V. *J. Am. Chem. Soc.* **1982**, *104*, 174–180. (f) Ravi, A.; Prasad, B. V. V.; Balaram, P. *J. Am. Chem. Soc.* **1983**, *105*, 105–109. (g) Balaram, H.; Prasad, B. V. V.; Balaram, P. *J. Am. Chem. Soc.* **1983**, *105*, 4065–4071. (h) Degelaen, J. P.; Pham, P.; Blout, E. R. *J. Am. Chem. Soc.* **1984**, *106*, 4882–4890.

(10) The possibility of all NH protons participating in intramolecular hydrogen bonds could not be ruled out only from these NMR data. But model building was impossible.

(11) The other possible torsion angle of −150° causes the strict steric hindrance between the C_2-related side chains of isoleucine residues.

(12) Cody, V. In *Chemistry and Biochemistry of the Amino Acids*; Barrett, G. C., Ed.; Chapman and Hall: London, New York, 1985; pp 625–653.

(13) Pascard, C.; Ducruix, A.; Lunel, J.; Prange, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418–6423.

(14) (a) Benedetti, E. In *Peptides, Proceedings of the 5th American Peptide Symposium*; Goodman, M., Meinhofer, J., Eds.; Wiley: New York, 1977; pp 257–274. (b) Gould, R. O.; Gray, A. M.; Taylor, P.; Walkinshaw, M. D. *J. Am. Chem. Soc.* **1985**, *107*, 5921–5927.

(15) Johnson, C. K. ORTEP, Oak Ridge National Laboratory Report ORNL-3794, 1965.

(16) The dihedral angle between both the rings is 139.4 (2)°.

(17) The dihedral angle between these two peptide planes is 45.8 (7)°.

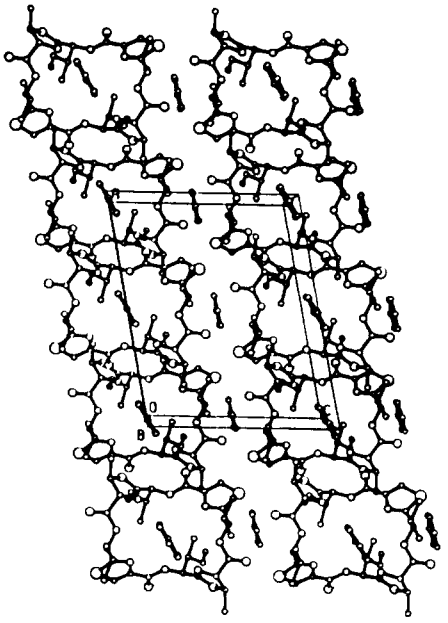


Figure 3. Crystal packing of ascidiacyclamide, viewed along the *b* axis.

the thiazole and oxazoline rings are alternately located at each corner of the rectangular ring chain: the long and small axes are about 7.5 and 6.1 Å, respectively, and the latter value corresponds to the periodicity usually observed in a pleated β-sheet structure.[18]

The conformational angles of valine and isoleucine backbone chains deviate slightly from the most stable region in a (ϕ, ψ) Ramachandron plot.[19] This would mainly be due to the respective transformations of cysteine and threonine residues to thiazole and oxazoline rings and to the formation of a cyclic structure. On the other hand, the side conformations of valine and isoleucine residues appear to be stable under conditions when the respective residues are placed. Although the (χ^{11}, χ^{12}, χ^{21}) value of isoleucine is mostly in a (gauche$^-$, trans, trans) region,[12,14a] respectively, this side chain takes a (*trans, gauche, trans*) conformation as a result of the steric restriction due to the close contacts between the C_2 symmetry-related isoleucine residues. This conformation corresponds to the second most commonly observed form. The (χ^{11}, χ^{12}) angles of D-valine are in a stable (*gauche, trans*) region.[12,14a]

The most important feature observed in this molecular conformation appears to be that the peptide NH groups are all directed toward the interior of the ring structure, while all oxygen atoms of carbonyl groups and thiazole and oxazoline rings are directed away from the ring. Furthermore, it is of interest to note that the NH groups of peptide groups and the N atoms of the thiazole and oxazoline rings are alternatively arranged around the ring structure with nearly the same intervals [N(1)–N2 = 2.841 (4), N2–N(2) = 2.853 (4), N(2)–N10 = 2.756 (4), and N10–N(1′) = 2.707 (4) Å]. The directions of N–H bonds and N lone pairs are both toward the same point of the ring structure.

Molecular Packing and Interaction with Benzene Molecules. Crystal packings viewed from the *b* axis are shown in Figure 3, where hydrogen atoms are omitted for the sake of clarity. A characteristic of the crystal structure is that there is no hydrogen bond, and the cavities formed by the molecular packing of **6** are occupied by benzene molecules. Two crystallographically independent benzene molecules, which are both located on the diad symmetries of the crystal lattice, exist in the ring structure of the molecule and between the neighboring molecules, respectively, and stabilize the crystal packing of **6**. The interaction mode between **6** and benzene molecules is shown in Figure 4. Ascidiacyclamide (**6**) is holding one of the two benzene molecules (named benzene A) with its warped ring conformation. Therefore, this molecule may be characterized as a kind of inclusion compound. The benzene molecule is mainly kept by van der Waals interactions with two hydrophobic valine side chains: the averaged interatomic distance is 4.0 Å. On the other hand, the other benzene molecule (named benzene B) is stabilized by the van der Waals contacts with two isoleucine side chains that are intermolecularly related by a diad symmetry: the averaged interatomic distance is 4.3 Å. Since these interatomic distances of valine and isoleucine side chains with benzene molecules are somewhat longer than the van der Waals separation distance (3.4 Å), their interaction forces would not be so strong. Indeed, colorless transparent crystals of **6** obtained from benzene solution became whitish opaque ones by being left in the air for about 1 day.

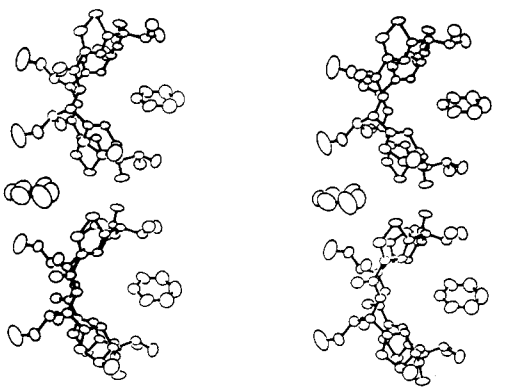


Figure 4. Stereoscopic drawing of the interaction mode between ascidiacyclamide and benzene molecules.

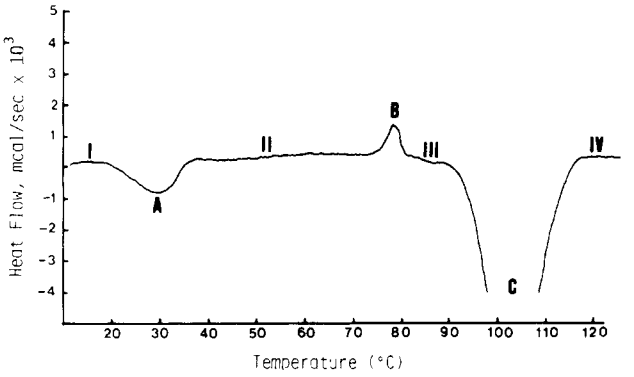


Figure 5. DSC profile of ascidiacyclamide crystals obtained from benzene solution.

Thermal Behavior of Crystal Structure. In order to investigate the thermal behavior of crystals of **6**, the DSC profile accompanying the increase of temperature was measured. Figure 5 shows the results. For the following discussion, the designations: states I, II, III, and IV were assigned to the states of <15 °C, 38–73 °C, 82–90 °C, and >116 °C, respectively. The endothermic change accompanying the release of benzene molecules from the crystals took place during 15–38 °C (peak A: T_{max} = 32 °C, $-\Delta H$ = 0.25 (1) kcal/mol). Interestingly, an exothermic peak was revealed in the temperature range from 74 °C to 81 °C (peak B: T_{max} = 78° C, ΔH = 60 (5) cal/mol). The appearance of peak B could be interpreted as the structural

(18) Ashida, T.; Yamane, T.; Tanaka, I. *J. Cryst. Soc. Jpn.* **1980**, *22*, 187–202.

conversion of the crystalline state to the amorphous one. This is based on the observations of the X-ray powder patterns measured at states II and III: the powder of state III showed no diffractional peak, while state II diffracted the X-ray powder patterns similarly to, but not identical with, state I. The third peak revealed from 90 °C to 116 °C would be an endothermic one corresponding to the melting (peak C: T_{max} = 103 °C, $-\Delta H$ = 6.42 (1) kcal/mol).[20]

It is interesting to note that these thermal changes are able to be interpreted from the crystal packing shown in Figure 3. Under the sample conditions packed in a sealed cell,[21] the DSC measurement of state II samples from 0 °C, which was prepared from state I by heating it to 50 °C and then by cooling, revealed the same endothermic peak ($-\Delta H$ = 0.23 (1) kcal/mol) as peak A shown in Figure 5, while the measurements of states III and IV gave $-\Delta Hs$ of about 0.12 (1) kcal/mol, which corresponds to half of peak A, respectively. This would be interpreted as follows: state II has still the same crystal lattice as state I, and the released benzene molecules revert to the same state as I by being cooled below 15 °C. On the other hand, the transformation of state II to III results in the loss of cavities among the molecules of **6** existing in the crystal structure of state I. Consequently, one of two crystallographically independent benzene molecules (corresponding benzene B) is no longer reversible. Samples of state III are energetically more stable than those of II by 60 cal/mol, and therefore the cooling and reheating of state III shows no exothermic peak corresponding to Peak B.

IR spectra of states I–IV measured by using the KBr disk method showed identical patterns to one another. This clearly implies that the ring conformation of **6** observed in the crystal structure is energetically stable and that no conformational change takes place through the heating up to the decomposition. As a result, the endothermic peak corresponding to the van der Waals interacton of a benzene molecule with the interior of the ring structure is always observed during 15–38 °C in the DSC measurement of each sample of states I–IV.

Comparison of Solution and Crystal Conformations of Ascidiacyclamide and Biological Implication. The C_2-symmetric structure of **6** observed in the crystal structure appears to be essentially the same as the proposed one from the solution NMR measurements. All NH bonds of peptide groups are directed to the interior of the ring structure. When $\phi_{HNC_\alpha H}$ values of valine and isoleucine backbone chains observed in the crystal are compared with those proposed from the solution conformation, agreement is good: $\phi_{isoleucine}$ = ~150° (NMR), 156° (X-ray); ϕ_{valine} = ~180° (NMR), −170° (X-ray). However, a large deviation is observed in the $\theta_{HC_\alpha C_\beta H}$ angle of the valine side chain, while the conformation of the isoleucine side chain is in good agreement: θ_{valine} = ~±40° (NMR), −172° (X-ray); $\theta_{isoleucine}$ = ~50° (NMR), 72° (X-ray). Although the reason why the solution conformation around the valine C_α–C_β bond is in an energetically unfavorable state[14a] is unclear at present, eq B may be unsuitable to estimate the possible conformation of the D-valine side chain. Judging from NMR, X-ray, and thermal data, it was concluded that the saddle-shaped ring conformation shown in Figure 2 is energetically stable and is the predominant form in the solution and solid states. As is obvious from Chart I, patellamides A, B, and C, isolated from the tunicate *Lissoclinum patella*, have the same molecular configuration as **6** and show similar cytotoxic activities.[5a,7a] Therefore, their favorable conformations are conceivably the same as that of **6**. Although the mechanisms of their biological reactions are unclear at present, the observed conformation in **6** would be related to the emergence of the biological activity. It is interesting to consider the possible conformatin of ulithiacyclamide (**1**), because this molecule shows the most potent cytotoxic activity among the compounds shown in Chart I.[5a] The most significant difference of **1**, compared with **6**, is the existence of the L-cystine residue formed from two C_2-symmetric L-cysteine residues. The formation of a disulfide bond plays a role in the stabilization of the ring conformation. CPK model building led to a U-shaped conformation with all NH bonds of peptides directing to the interior of the ring structure. This conformation was also similar to the saddle-shaped ring structure of **6**.[22]

It is interesting to speculate about which biomolecules can specifically interact with these cyclic peptides. There is no direct evidence of the interaction of these peptides with metal ions. On the other hand, preliminary thermal studies indicated that phospholipids and nucleic acids are affected by **6**; for example, the DSC profile of the phase transition of dipalmitoylphosphatidylcholine, a model compound of a phospholipid, dissolved in cacodilate buffer (39–43 °C, $-\Delta H$ = 11.4 cal/g) is meaningfully broadened (37–45 °C, $-\Delta H$ = 10.8 cal/g), and the melting curve of polyadenylic acid shifts to the higher temperature side by the existence of **6**. The chemical group commonly existing in these biomolecules is phosphate. The specific interaction between the phosphate group and the **6** molecule appears to be possible via some NH···O hydrogen-bond formations. Interaction studies with these biomolecules are now in progress.

Although the biological analyses of these cyclic peptides must be subjected to future studies, the insights obtained by the present study would provide important information for these studies.

Experimental Section

Materials. **6** was chemically synthesized[7b] and crystallized as colorless prisms from benzene solution by slow evaporation at room temperature.

NMR Measurements. ^{1}H NMR data were obtained by using a Varian XL-300 (300 MHz for protons) spectrometer equipped with fast FT and temperature-controlled units (margin of error: ±1 °C). The concentration of **6**, which was dried over a day in a dessicator under reduced pressure, was gravimetrically adjusted to ca. 7 mM. The solvents used were C_6D_6, $CDCl_3$, and $(CD_3)_2SO$ (99.8% isotropic purities, respectively). Tetramethylsilane (TMS) was employed as an internal standard, and chemical shifts were measured as downfield shifts from TMS. The estimated standard error was ±0.001 ppm and ±0.3 Hz. All the proton resonances were assigned by homonuclear decoupling, spin multiplicities, and two-dimensional spin–echo correlations and were in good agreement with the published data.[7a] The conformation of valine and isoleucine residues in **6** was estimated from the coupling constants by using eq A[23] and B,[24] where θ represents the torsion angle of

$$J_{HNC_\alpha H} = 7.9\cos^2\theta - 1.55\cos\theta + 1.35\sin^2\theta \quad \text{(A)}$$

$$J_{HC_\alpha C_\beta H} = 11.0\cos^2\theta - 1.4\cos\theta + 1.6\sin^2\theta \quad \text{(B)}$$

(19) Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. *J. Mol. Biol.* **1963**, *7*, 95–99.

(20) The complete transformation of the solid state to the solute state was not observed.

(21) The sealed cell was used to study the interaction of **6** with benzene molecules. The thermal profile of the crystals measured by using the sealed cell was nearly the same as shown in Figure 5 using the open cell.

(22) The details on the conformations of **1**–**5** are now being studied.

(23) Ramachandran, G. N.; Chandrasekaran, R.; Kopple, K. D. *Biochemistry* **1971**, *10*, 2113–2131.

Table III. Summary of Crystal Data and Data Collection

formula	$C_{36}H_{52}N_8O_6S_2 \cdot 2C_6H_6$
M_r	913.21
space group	$C2$
a, Å	15.909 (6)
b, Å	13.150 (6)
c, Å	12.754 (5)
β, deg	101.13 (2)
V, Å^3	2618 (2)
Z	2
D(measd), g·cm^{-3}	1.152 (2)
D(calcd), g·cm^{-3}	1.158
absorpt coeff, cm^{-1}	12.93
F(000)	976
crystal size, mm^3	0.3 × 0.3 × 0.4
T of data collection, °C	15
data collection method	ω–2θ scan
scan speed in 2θ, deg·min^{-1}	3
scan range in ω, deg	1.3 +0.15 tan θ
data range measd, deg	$2 \leq 2\theta \leq 130$
data collected	h, k, $\pm l$
no. of unique data measd	2346
no. of data with $F_o > 3\sigma(F_o)$	2109
no. of variables	385
R_F	0.079
R_{wF}	0.075

the H–N–C_α–H or H–C_α–C_β–H fragment for eq A or B, respectively.

X-ray Analysis. Since transparent crystals from a benzene solution become opaque in the air, they were sealed in glass capillaries containing some mother liquid. Oscillation and Weissenberg photographs indicated the crystal to be monoclinic with a space group of $C2$. Unit cell dimensions and diffracton intensities were measured with graphite-monochromated Cu K_α radiation (λ = 1.5418 Å) on a Rigaku AFC-5 computer-controlled diffractometer. Crystal data and parameters for data collection are summarized in Table III. The unit cell parameters were determined by a least-squares fit of 2θ angles for 25 reflections ($30° < 2\theta < 60°$). The crystal density was measured by the flotation method using aqueous KI solution. The value of Z, which is calculated from M_r, volume, and density values, equals 2, and this implies that the C_2 symmetric axis which **6** itself has coincides with that of the $C2$ space group, because the Z value of its space group usually equals 4. The ω–2θ scan technique was employed for the intensity recording. The peak counts were corrected with background counts for 5 s at both ends of the scan range. Four standard reflections were monitored at every 100 reflection intervals throughout the data collection and showed no significant deterioration (random fluctuation within 5%). The observed intensities were corrected for Lorentz and polarization effects. Correction of the absorption effect was also done by using an empirical method based on the ϕ scan at $\chi = 90°$.

The structure was solved by the Patterson superposition method, based on the sulfur atomic position. The positional parameters obtained were then refined by a full-matrix least-squares analysis with isotropic temperature factors and then by a block-diagonal least-squares analysis with anisotropic ones. The positions of the geometrically reasonable hydrogen atoms were determined on a difference Fourier map and included in subsequent refinements with an isotropic temperature factor (= 6.2 Å^2).

The function minimized was $\sum w(|F_o| - |F_c|)^2$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure amplitudes, respectively. The weighting scheme used for refinement is as follow: $w = a$ for $F_o = 0.0$ and $w = 1.0/[\sigma(F_o)^2 + b|F_o| + c|F_o|^2]$ for $F_o > 0.0$, where $\sigma(F_o)^2$ is the standard deviation of the intensity based on counting statistics. In the final refinements, the coefficients used were 0.60401, −0.18535, and 0.01388 for a, b, and c, respectively. The discrepancy indices R_F (= $\sum||F_o| - |F_c||/\sum|F_o|$) and R_{wF} (= $[\sum w(|F_o| - |F_c|)^2/wF_o^2]^{1/2}$) were 0.079 and 0.075 for 2346 observed reflections, respectively, and S (= $[\sum w(|F_o| - |F_c|)^2/(M - N)]^{1/2}$, where M = number of observations and N = number of variables) was 2.46. None of the positional parameters shifted more than one-fifth of their standard deviations, and maximum electron density in the final difference Fourier synthesis was 0.32 e Å^{-3}. For all crystallographic computations, the UNICS programs[25] were used, and atomic scattering factors and the terms of the anomalous dispersion correction were from International Tables for X-ray Crystallography.[26] The calculations were performed on an ACOS-1000 computer at the Computation Center of Osaka University.

Thermal Analysis. The calorimetric change accompanying the heating of the sample was monitored by a differential scanning calorimetry (DSC) instrument (DSC-8240B, Rigaku, Japan). Al_2O_3 was used as a standard compound. The heating range was −10 to 160 °C, and the heating rate was 1°/min. Samples of 1.5–4.5 mg were sealed or loaded into the aluminum cells.

IR Measurements. IR spectra were measured by the KBr disk method on a Hitachi 260-10 spectrometer (Japan).

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Supplementary Material Available: Tables of ^{1}H NMR chemical shifts and their temperature coefficients in C_6D_6, $CDCl_3$, and $(CD_3)_2SO$ solutions, final atomic coordinates of non-hydrogen atoms with isotropic equivalent of the anisotropic thermal parameters, anisotropic thermal parameters of non-hydrogen atoms, coordinates of hydrogen atoms, bond distances and angles between non-hydrogen atoms, and short contacts between ascidiacyclamide molecules and the interatomic distances with benzene molecules (7 pages). Ordering information is given on any current masthead page.

(24) Kopple, K. D.; Wiley, G. R.; Tauke, R. *Biopolymers* **1973**, *12*, 627–636.

(25) *The Universal Crystallographic Computing System-Osaka*; The Computation Center, Osaka University: Osaka, Japan, 1979.

(26) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV.