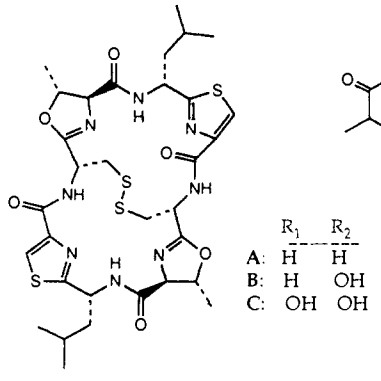
Studies on the Solution- and Solid-State Structure of Patellin 2

T. Mark Zabriskie,[†] Mark P. Foster,[†] Thomas J. Stout,[‡] Jon Clardy,*[,‡] and Chris M. Ireland*[,†,1]

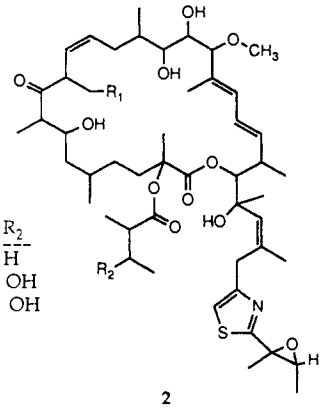
Contribution from the Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112, and Department of Chemistry, Cornell University, Ithaca, New York 14853.
Received February 16, 1990

Abstract: The major component of a new family of novel cyclic peptides has been characterized from the Fijian marine tunicate (ascidian) *Lissoclinum patella* (Gottschaldt, 1898). Patellin 2 (**4**) lacks the characteristic thiazole and oxazoline amino acids present in the previously reported *Lissoclinum* peptides and contains a thiazoline and two threonine residues modified as dimethylallyl ethers. Patellin 2 is shown to exist in different conformations in solution and in the crystal on the basis of X-ray crystallography, NMR spectroscopy, and molecular modeling. Cis–trans isomerization of the Val–Pro amide bond leads to two distinct conformers in solution, while the crystalline conformers (with the more stable *trans*-Val–Pro amide bond) differ by inversion of the thiazoline ring.

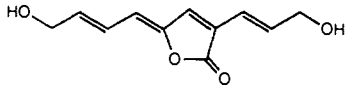
The marine tunicate (ascidian) *Lissoclinum patella* collected in Micronesia or the Great Barrier Reef has previously been shown to produce a variety of cytotoxic cyclic peptides, all of which contain thiazole(s) and usually oxazoline amino acids.[2] Ulithiacyclamide (**1**) also exhibited in vivo activity against the murine



1



2

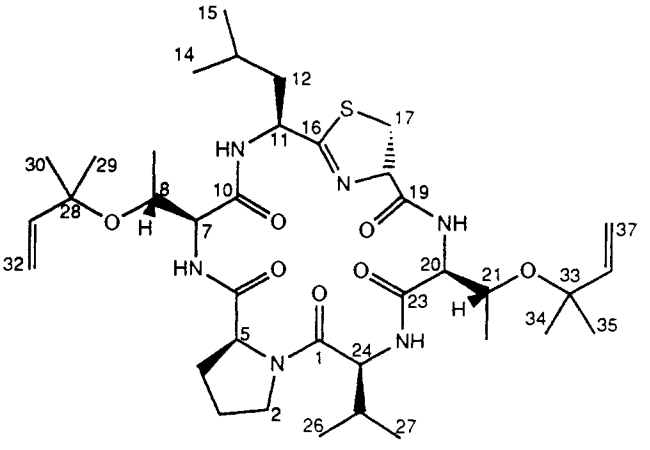


3

leukemia P1534J with a T/C of 178 at 10 mg/kg.[3] The same species from the Fijian Island of Ndravuni yielded the thiazole-containing macrolides, patellazoles A–C (**2**),[4] and lissoclinolide (**3**), the first nonnitrogenous metabolite reported from a *Lissoclinum* species. The patellazoles exhibited cytotoxicity against the human colon cancer HT 29 at 10^{-6} μg/mL. We now report the isolation and structure of patellin 2 (**4**), a modified cyclic peptide found in *L. patella* collected at the Fijian Island of Viti Levu.[5] This unique metabolite lacks the characteristic aromatic amino acids found in the previous *Lissoclinum* peptides and contains two novel threonine residues modified as dimethylallyl ethers (Dat: dimethylallylthreonine) as well as a thiazoline (Tzn) amino acid. Patellin 2 has been shown by use of X-ray, NMR, and molecular modeling data to exist in multiple conformations in both the crystal and solution state.

Discussion and Results

Preliminary NMR studies suggested that patellin 2 was a mixture of either two closely related compounds or multiple conformers of a single compound, as indicated by doubling of nearly every signal in the spectra. The observation that the ratio of doubled signals varied with temperature and solvent confirmed



Patellin 2 (**4**)

the latter. Spectra obtained in solvents with high dielectric constants (e.g., acetone or acetonitrile) showed the greatest difference in conformer distribution, while using solvents with low dielectric constants (e.g., methylene chloride or chloroform) results in spectra displaying nearly equal conformer populations (Figure 1). Additionally, temperature dependence studies in acetone-d_6 (ϵ_r 20.7) indicated that the ratio of signals decreased by approximately a factor of 3 over the temperature range −60 to +23 °C (Figure 2). The ^{1}H and ^{13}C NMR assignments of the major conformer of **4** obtained in acetone-d_6 are given in Table I.

Although the NMR spectra were simplified in acetone-d_6, the minor conformer was still clearly visible and served to complicate all 2D correlation experiments. Fortunately, patellin 2 crystallized as flat plates from aqueous methanol, and X-ray analysis defined the structure of patellin 2 with relative stereochemistry. A computer-generated perspective drawing is shown in Figure 3. The absolute configuration of the peptide was not independently defined by X-ray analysis and was determined by use of Marfey's procedure for determining the chirality of amino acids as their 1-fluoro-2,4-(dinitrophenyl)-5-L-alanine amide (FDAA) diastereomers.[6] This analysis unequivocally demonstrated that valine, proline, leucine, and the threonines possessed the L configuration

[†] University of Utah.
[‡] Cornell University.

(1) NCI Career Development Awardee 1987–1992.
(2) For a review of peptides from marine organisms, see: Ireland, C. M.; Molinski, T. F.; Roll, D. M.; Zabriskie, T. M.; McKee, T. C.; Swersey, J. C.; Foster, M. P. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer-Verlag: Berlin, 1989; Vol. 3, pp 1–46.
(3) Sesin, D. F.; Gaskell, S. J.; Ireland, C. M. *Bull. Soc. Chim. Belg.* **1986**, *95*, 853 and references cited therein.
(4) Zabriskie, T. M.; Mayne, C. L.; Ireland, C. M. *J. Am. Chem. Soc.* **1988**, *110*, 7919.
(5) The tunicate was identified as *L. patella* (Gottschaldt, 1898) by Dr. Françoise Monniot, Museum National d'Histoire Naturelle Paris, France.
(6) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.

0002-7863/90/1512-8080$02.50/0

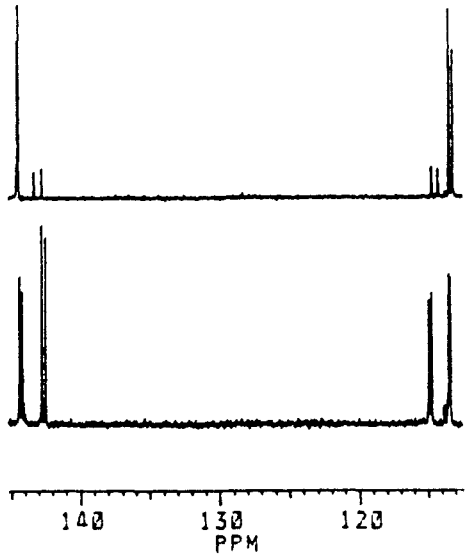


Figure 1. Effect of solvent on conformer populations. Olefinic region of the ^{13}C NMR spectrum of patellin 2 (50 MHz, 0.14 M sample, 0.5-Hz line broadening). Top spectrum acquired in acetone-d_6, bottom $CDCl_3$.

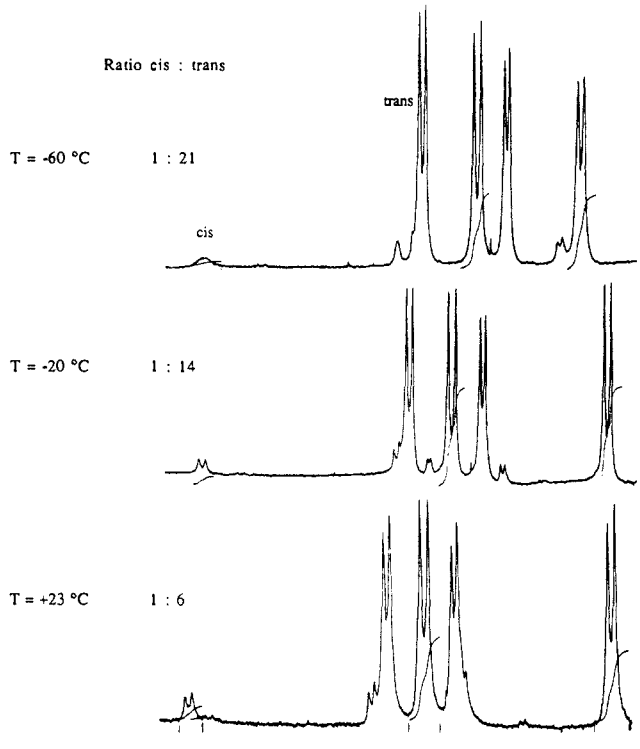


Figure 2. Temperature dependence of conformer populations. Comparison of the amide signals of cis and trans conformers (200 MHz, acetone-d_6).

and served to define the absolute configuration of patellin 2 as 5*S*,7*S*,8*R*,11*S*,18*R*,20*S*,21*R*,24*S*, allowing assignment of the absolute stereostructure as **4**.

One of the most striking overall features of the crystal structure is that the cyclic peptide has all of the nonpolar residues on one side while the thiazoline and many of the carbonyls are on the other side. Additionally, the thiazoline ring was disordered in the solid state and had to be modeled with two distinct conformations. The simplest analysis, illustrated in Figure 3, involved disordering two atoms, S1 and C17, resulting in two different C16–S1–C17–C18 torsional angles of 21.1 and −31.1°. Preliminary molecular modeling studies with MACROMODEL[7] also indicated two distinct minima with torsional angles essentially the same as those in the solid state. This analysis disclosed that these two conformations differed by only 0.15 kcal/mol, and the presumed intermediate interconverting the two conformations was only 0.20 kcal/mol above the higher energy conformer. This energy barrier can not account for the observation of two conformations on the NMR time scale; therefore, another type of conformational interconversion must be responsible for the solvent-dependent doubling of the signals in the NMR spectra.

The solution-phase conformers of patellin 2 were ultimately shown to arise from cis–trans isomerization of the Val–Pro amide bond, based on evidence from NMR and molecular modeling studies. Proline cis–trans isomers can be distinguished in solution by the chemical shift differential of the β- and γ-carbons. Pook demonstrated, with a series of *cis*- and *trans*-proline models, that the chemical shift differential for these two carbons increases linearly with increasing angle ψ(Pro), and in cis X–Pro these signals are further separated than in trans X–Pro.[8] The chemical

Table I. ^1H and ^{13}C NMR Assignments of Patellin 2 (**4**)[a]

C	^{13}C:[b] δ (mult)	^1H:[c] δ (mult, *J*(Hz))
1	174.46 (s)*	
2	48.94 (t)	A-3.97 (m)
		B-3.74 (m)
3	25.85 (t)	2.05 (m)
4	30.76 (t)	A-2.38 (m)
		B-1.93 (m)
5	64.99 (d)	4.19 (bd, 10.8)
6	173.87 (s)*	
	N2*H*† 6.28 (d, 9.3)	
7	59.45 (d)†	4.29 (dd, 9.3, 1.7)
8	68.10 (d)†	4.46 (dq, 6.3, 1.7)
9	21.49 (q)†	1.10 (d, 6.3)
10	171.42 (s)*	
	N3*H* 7.72 (d, 8.2)	
11	50.45 (d)	4.75 (m)
12	44.06 (t)	A-2.08 (m)
		B-1.44 (m)
13	25.38 (d)	1.79 (spt, 6.6)
14	23.27 (q)	0.95 (d, 6.6)
15	22.69 (q)	0.90 (d, 6.6)
16	170.96 (s)*	
17	34.20 (t)	A-3.77 (dd, 11.1, 8.1)
		B-3.54 (dd, 11.1, 10.2)
18	79.35 (d)	5.42 (ddd, 10.2, 8.1, 2.0)
19	170.54 (s)*	
	N5*H*§ 7.48 (d, 10.0)	
20	60.94 (d)§	4.18 (dd, 10.0, 1.4)
21	68.45 (d)§	4.37 (dq, 6.2, 1.4)
22	21.29 (q)§	1.21 (d, 6.2)
23	170.21 (s)*	
	N6*H* 7.29 (d, 7.2)	
24	56.80 (d)	4.54 (dd, 7.2, 1.8)
25	31.52 (d)	2.38 (m)
26	21.10 (q)	1.09 (d, 6.8)
27	18.02 (q)	0.88 (d, 6.8)
28	76.29 (s)•	
29	28.01 (q)•	1.28 (s)
30	28.01 (q)•	1.18 (s)
31	145.93 (d)•	5.93 (dd, 17.62, 10.81)
32	113.36 (t)•	A-5.17 (dd, 17.62, 1.13)
		B-5.05 (dd, 10.81, 1.13)
33	76.11 (s)#	
34	26.77 (q)#	1.28 (s)
35	26.32 (q)#	1.18 (s)
36	145.84 (d)#	5.87 (dd, 17.64, 10.83)
37	113.64 (t)#	A-5.10 (dd, 17.64, 1.22)
		B-5.02 (dd, 10.83, 1.22)

[a] Signals marked with * are interchangeable. The spin system marked with † is interchangeable with that marked §, and the system denoted by • is not distinguished from the corresponding system marked with #. [b] Measured at 100 MHz and referenced to internal acetone-d_6. Multiplicity determined by DEPT experiment. [c] Measured at 400 MHz and referenced to residual acetone-d_5.

(7) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrikson, T.; Still, W. C. *J. Comput. Chem* **1990**, *11*, 440.

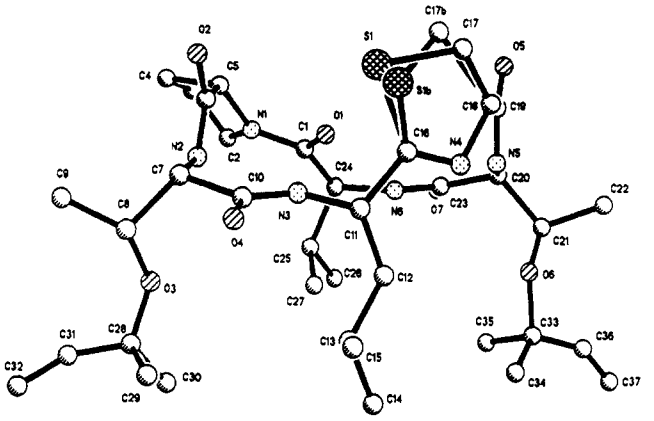


Figure 3. Computer-generated perspective drawing of patellin 2. Hydrogens have been omitted for clarity. Both conformations of the thiazoline are superimposed.

Table II. Backbone Dihedral Angles (Degrees) for Lowest Energy Conformer of *trans*-Patellin 2

residue	ϕ	ψ	ω
leu	−76	−149	174
tzn	−94	−48	174
dat_1	−61	−60	172
val	−79	167	−177
pro	−49	−53	178
dat_2	−65	−54	177

Table III. Backbone Dihedral Angles (Degrees) for Lowest Energy Conformer of *cis*-Patellin 2

residue	ϕ	ψ	ω
leu	−82	−66	−178
tzn	−144	−65	175
dat_1	−77	−37	−175
val	−44	−42	−3
pro	−101	45	171
dat_2	−60	−53	168

shifts of the proline β- and γ-carbons in the major conformer of patellin 2 (C3 and C4 in Table I) differ by 4.91 ppm. The corresponding carbon signals of the minor conformer (22.45 and 30.10 ppm, respectively) differ by 7.65 ppm, thus indicating that the predominant signals arise from the trans conformer.

Molecular modeling studies with use of QUANTA/CHARMM[9,10] resulted in two local minima with opposite configurations about the Val–Pro amide bond (Figure 4). The molecular modeling protocol is primarily a variation on the minimization–dynamics–minimization (min–md–min) procedure.[11,12] Structures resulting from minimization of dynamics data sets resulted in good convergence to the lowest energy structure for both *trans*- and *cis*-patellin 2 (Figure 5). The barrier to inversion of the amide bond was calculated to be 21 kcal/mol. Structures modeled with a constant dielectric of 20 (ϵ_r of acetone is 20.7 at 20 °C) resulted in a cis conformer that was destablized relative to the trans conformer when compared to results obtained with a constant dielectric of 1 (in vacuo). This parallels the NMR observation that in acetone the trans conformer predominates. The Pro ψ angles obtained from modeling match those expected from Pook's analysis (see Tables II and III). The NH–α-C dihedrals of the resulting trans conformer (shown by NMR to be the most stable conformer in acetone) were compared to the values obtained by a Karplus-type analysis of the $^3J_{HNCH}$ and were found to match within 25°.[13]

Small, proline-containing cyclic peptides have previously been reported to exist in multiple solution conformations due to cis–trans X–Pro isomerization and, like patellin 2, many of these peptides exhibit only trans peptide bonds in the crystal structures.[14] Also, the thiazoline inversion seen in the crystal structure of **4** presumably occurs in solution as well; dynamics simulations showed that interconversion proceeds on the picosecond time scale (see Figure 5a), in agreement with the 0.2 kcal/mol barrier determined with MACROMODEL.

Experimental Section

NMR spectra were recorded on either an IBM AF 200, a Varian XL-400, or a Varian VXR-500 spectrometer. Chemical shifts are reported on the δ scale and referenced to solvent. Infrared spectra were recorded either on a Beckman FT-2100 or on a Perkin-Elmer 1600 Fourier transform spectrophotometer. Ultraviolet spectra were obtained with a Beckman DU-8 spectrophotometer with methanol as solvent. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. Melting point was determined on a Hoover capillary melting point apparatus and was uncorrected. High- and low-resolution fast atom bombardment mass measurements were performed on a Varian MAT-731 equipped with an Ion Tech atom gun. Preliminary energy calculations with MACROMODEL were done on a Micro Vax II. Rigorous dynamics and energy calculations were done with use of QUANTA/CHARMM on Silicon Graphics Iris workstations.

Isolation Procedures. Frozen *L. patella* (1069 g) was lyophilized to give 220 g of dried animal, which was then homogenized and extracted in 2.2 L of MeOH. With use of a modified Kupchan solvent-partitioning scheme, the crude homogenate (reduced to 700 mL) was separated into increasingly polar fractions by extracting first with hexanes (3 × 500 mL), followed by addition of 60 mL of H_2O and extraction with CCl_4 (3 × 500 mL), and finally by adding 120 mL of H_2O and extracting with $CHCl_3$ (3 × 500 mL). The resulting 2.418 g of CCl_4 extract was subjected to gravity silica gel chromatography (column, 2.8 × 50 cm; silica gel 62; stepped gradient elution, EtOAc, 9:1 EtOAc/MeOH, 1:1 EtOAc/MeOH, 100 % MeOH) followed by reversed-phase HPLC (Whatman Patisil-10 ODS-3; 1 × 50 cm; 15% aqueous MeOH; 2.0 mL/min) to yield patellin 2 (**4**) as white crystals: 134 mg, 0.06% of dry weight; $[\alpha]^{25}_D$ −110° (*c* 1.48, MeOH); mp 128–130 °C; UV(MeOH) λ_{max} 210 (ϵ 8400), 248 nm (sh, ϵ 2450); IR (neat film) ν_{max} 3327, broad absorptions centered at 1670, 1627, 1520, 1491 cm^{-1}. Positive- and negative-ion FAB mass spectrometry established a molecular weight of 732. The molecular formula of $C_{37}H_{60}N_6O_7S$ was determined by high-resolution FABMS measurement of the more prominent *m/z* 597 ion (733 − 2(C_5H_8), corresponding to facile loss of the dimethylallyl groups): found 597.3069; $C_{27}H_{45}N_6O_7S$ requires 597.3071.

Stereochemistry of Patellin 2. Hydrolysis of **4** was carried out in 6 N HCl under N_2 at 130 °C for 24 h. The resulting hydrolysate was derivatized with 1-fluoro-2,4-(dinitrophenyl)-5-L-alanine amide (FDAA) (Pierce) as per Marfey's procedure.[6] Thin-layer chromatography (Whatman precoated $C_{18}F_{254}$; 1:1 H_2O/MeOH) of the FDAA-derivatized total acid hydrolysate against like-derivatized standards unequivocally showed that the valine and leucine residues possessed the L configuration. These assignments were further confirmed by reversed-phase HPLC (Waters NOVAPAK C_{18}; 4.6 × 100 mm column; gradient elution of 50 mM triethylammonium phosphate (pH 3.0)/90:10 MeCN ramped to 60:40 in 45 min; 2.0 mL/min; UV detection λ = 340 nm), which also served to assign the L stereochemistry to the threonines (hydrolysis removes the dimethylallyl groups) and proline.

Single-Crystal X-ray Diffraction Analysis of Patellin 2. Crystals were grown from aqueous methanol, and an irregularly shaped crystal (0.2 × 0.4 × 0.5 mm) was mounted in a capillary to prevent loss of solvent. Preliminary diffraction photographs displayed monoclinic symmetry, and accurate lattice constants of a = 12.353 (4) Å, b = 10.836 (3) Å, c = 16.439 (4) Å, and β = 95.63 (11)° were determined from least-squares fit of 20 2θ values. Systematic extinctions and the observed optical activity uniquely defined the space group as $P2_1$, and the density considerations indicated Z = 2 for a molecular formula of $C_{37}H_{60}N_6O_7 \cdot H_2O$. All unique diffraction maxima with $2\theta \leq 114°$ were collected from $2\theta{:}\theta$ scans with the graphite-monochromated Cu Kα radiation (1.541 78 Å). Of the 2930 reflections measured, 1824 (62%) were judged observed ($|F_o| \geq 4\sigma(F_o)$).

(8) Siemion, I. Z.; Wieland, T.; Pook, K. H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 702.
(9) Polygen Corp. 200 Fifth Ave., Waltham, MA 02254.
(10) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
(11) Brown, F. K.; Hempel, J. C.; Dixon, J. S.; Amato, S.; Mueller, L.; Jeffs, P. W. *J. Am. Chem. Soc.* **1989**, *111*, 7328.
(12) Howard, A. E.; Kollman, P. A. *J. Med. Chem.* **1989**, *31*, 1669.
(13) Bystrov, V. F. *Prog. Natl. Magn. Reson. Spectrosc.* **1976**, *10*, 41.
(14) Romanowska, K.; Kopple, K. D. *Int. J. Pept. Protein Res.* **1987**, *30*, 289 and references therein.

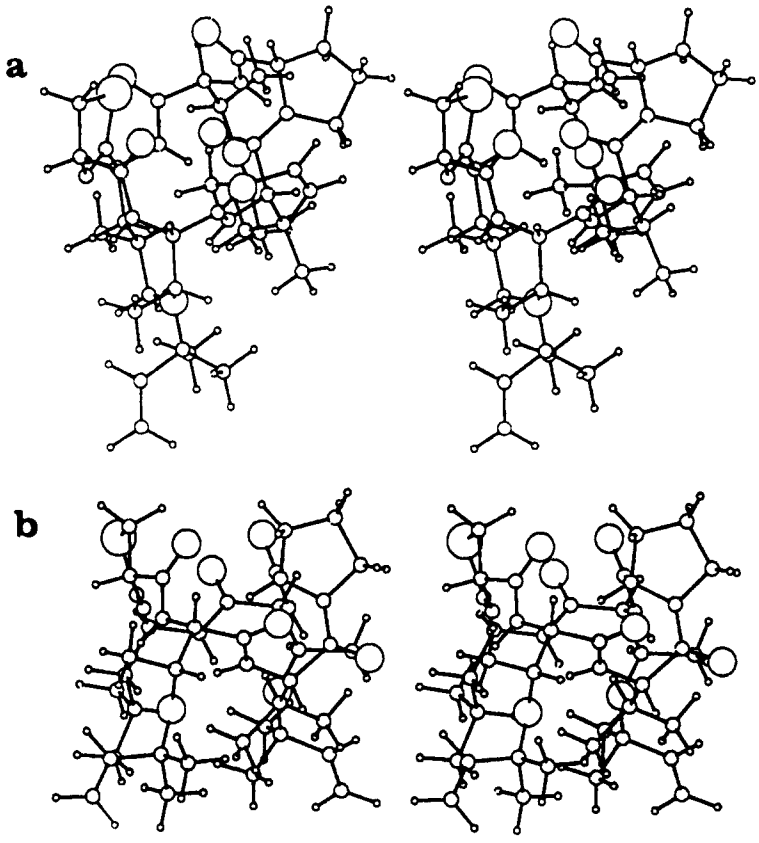


Figure 4. Stereodrawings (cross-eyed) of minimized (a) trans and (b) cis conformers of patellin 2.

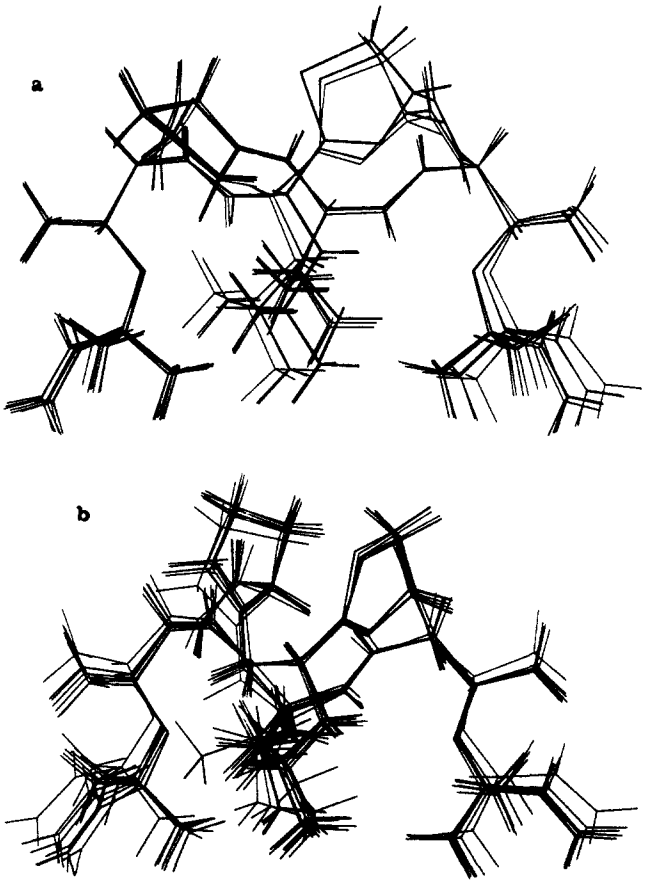


Figure 5. Superimposed minimized dynamics datasets of (a) *trans*- and (b) *cis*-patellin 2 showing good convergence. For (a), note opposite conformations of thiazoline ring.

The structure was solved by direct methods, and the initial phasing model revealed the macrocyclic ring and most of the side chains. Tangent formula recycling revealed the remaining heavy atoms with the exception of C37, which was extensively disordered. The atom C37 had to be located on a difference map and constrained to a reasonable geometry with fixed isotropic thermal parameters. The final model also included disorder of the thiazoline ring; the sulfur atom and C17 had to be placed at two different sites. Full-matrix least-squares refinements have presently converged to a conventional crystallographic discrepancy index of 0.068 for the observed reflections.

Molecular Modelling. Molecular dynamics (MD) calculations were done with QUANTA/CHARMM on Silicon Graphics Iris 3130 or Personal Iris 4D workstations. All energy terms were calculated. Minimization protocol involved the use of an adopted basis Newton–Raphson algorithm followed by conjugate gradient minimization. Minimizations were terminated when the energy value gradient between cycles was less than 0.001 kcal/mol. Molecular dynamics were run at 300 K and involved 300 steps (1 step = 1 fs) of heating, 300 steps of equilibration, and 5000 steps (5 ps) of simulation, saving coordinates every 100 steps (56 datasets). Except where mentioned in the following text, no constraints were applied.

The X-ray coordinates were entered and the structure was modeled with use of the all atom representation. Preliminary minimization with use of the steepest descents algorithm was followed by a regimen of min–md–min with a constant dielectric of 1. The structure generated by this procedure resembled the X-ray structure with all amide bonds in the trans conformation.

To generate the cis conformer, the Val–Pro amide bond was constrained to 0° and subjected to the min–md–min procedure described previously. The dihedral constraint was removed after the first series of minimizations. The resulting structure maintained its cis amide bond, and its energy was comparable to that of the trans conformer. Other amides were constrained in the same manner, but none resulted in stable cis conformers.

To simulate the effect of changing solvent, the dielectric constant was set to 20 and each structure was resubmitted to the min–md–min procedure. Convergence tests involved minimization of every second data set and comparison to the lowest energy structure achieved. Running dynamics at 600 K for 10 ps resulted in structures with the same conformation of the cyclic peptide.

Preliminary molecular modeling analysis with MACROMODEL[7] involved application of torsional constraints to the Tzn dihedral defined by C16–S–C17–C18 to achieve those conformations observed in the crystal structure: +21 and −31°. The constraints were then removed and the conformational energies minimized. Both conformations were found to be true minima, differing in energy by 0.15 kcal/mol. The 0.2 kcal/mol

barrier to inversion was determined by setting the dihedral angle to 0° (the presumed intermediate).

Acknowledgment. This work was supported by funding from the National Institutes of Health (CA 36622, CA 01179 (C.M.I.), and CA 24487 (J.C.)) and the New York State Sea Grant (J.C.). T.M.Z. thanks the American Foundation for Pharmaceutical Education and the University of Utah Research Committee for financial support. Dr. Chad Nelson is thanked for performing the mass spectrometric measurements.

Registry No. 4, 129216-76-8.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, and bond angles for crystalline patellin 2 (**4**) (6 pages). Ordering information is given on any current masthead page.

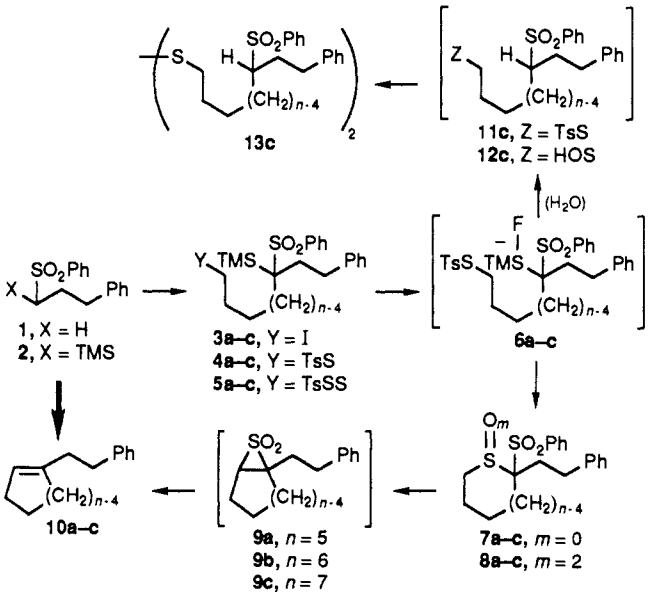
Fluoride Ion Mediated Intramolecular Sulfenylation of α-Silyl Sulfones: Ramberg–Bäcklund Annulation to Exocyclic Fused Olefins

David Scarpetti and Philip L. Fuchs*

Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907. *Received March 30, 1990*

Abstract: A series of α-trimethylsilyl-substituted phenyl sulfones bearing an ω-*p*-toluenethiosulfonyl α-substituent were synthesized and subjected to treatment with tetra-*n*-butylammonium fluoride to provide the corresponding monocyclic sulfides (6-, 7-, and 8-membered rings). This intramolecular sulfenylation reaction was also used in an annulation sequence. The cyclic sulfides obtained were oxidized to the bis(sulfone) derivatives and subjected to Ramberg–Bäcklund ring contraction to give the monocyclic olefins and an exocyclic fused olefin.

In connection with our synthetic program,[1] we needed an efficient synthesis of a fused bicyclic olefin. α-Sulfonyl sulfones have been utilized by us[2] and several other groups[3] for the Ramberg–Bäcklund olefination reaction. Our recent success in refunctionalizing the α-silyl sulfone moiety[4] coupled with the ability to use thiosulfonates as sulfenylating reagents[2,5] prompted our investigation of the intramolecular sulfenylation of **3a–c** to synthesize cyclic α-sulfide sulfones **7a–c**.[6]



Metalation of 1-phenyl-3-(phenylsulfonyl)propane (**1**)[7] with *n*-BuLi in tetrahydrofuran (THF) at −78 °C for 15 min followed by quenching of the α-sulfonyl anion with an excess of acid-free trimethylsilyl chloride[8] ((TMS)Cl, 1.4 equiv) afforded crystalline (mp 82–84 °C) α-silyl sulfone **2** in 76% yield after chromatography. Subsequent metalation of **2** with *n*-BuLi in THF at −78 °C for 15 min followed by inverse addition of this anion to a solution of excess α,ω-diiodide (4 equiv) at 0 °C produced homologous iodides **3a–c** in 67–77% yields. By use of a modification of existing procedures,[9] iodides **3a–c** were treated with potassium *p*-toluenethiosulfonate (4 equiv) in 20% aqueous acetone for 20 h at 25 °C followed by brief treatment (10 min) with sodium *p*-toluenesulfinate (4 equiv) to provide **4a–c** in greater than 85% isolated yield.

Initial attempts at mediating the intramolecular sulfenylation of **4a–c** employed tetra-*n*-butylammonium fluoride[10] ((TBA)F)

(1) Synthesis via Vinyl Sulfones. 38. Cytochalasin Support Studies. 12. For the previous paper in the cytochalasin series, see ref 4. For a review of the vinyl sulfone strategy, see: Fuchs, P. L.; Braish, T. F. *Chem. Rev.* **1986**, *86*, 903.

(2) Ranasinghe, M. G.; Fuchs, P. L. *J. Am. Chem. Soc.* **1989**, *111*, 779.

(3) (a) Hendrickson, J. B.; Palumbo, P. S. *J. Org. Chem.* **1985**, *50*, 2110. (b) Hendrickson, J. B.; Boudreux, G. J.; Palumbo, P. S. *J. Am. Chem. Soc.* **1986**, *108*, 2358. (c) Matsuyama, H.; Miyazawa, Y.; Takei, Y.; Kobayashi, M. *J. Org. Chem.* **1987**, *52*, 1703.

(4) Anderson, M. B.; Fuchs, P. L. *J. Org. Chem.* **1990**, *54*, 337.

(5) (a) Trost, B. M. *Chem. Rev.* **1978**, *78*, 363. (b) Caputo, R.; Ferreri, C.; Palumbo, G. *Synthesis* **1989**, 464.

(6) The utility of α-sulfide phenylsulfones is noteworthy; (methylthio)-methyl aryl sulfones have been demonstrated as valuable synthons. (a) Simpkins, N. *Tetrahedron Lett.* **1988**, *29*, 6787. (b) Ogura, K.; Uchida, T.; Tsurda, T.; Takahashi, K. *Tetrahedron Lett.* **1987**, *28*, 5703.

(7) Julia, M.; Uguen, D. *Bull. Soc. Chim. Fr.* **1976**, 513.

(8) Hutchinson, D. K. *Aldrichimica Acta* **1986**, *19(3)*, 58.

(9) Although the synthesis of thiosulfonate esters by alkylation of thiosulfonate salts has ample precedent in the literature ((a) Chandra, R.; Field, L. *J. Org. Chem.* **1986**, *51*, 1984. (b) Macke, J. D.; Field, L. *J. Org. Chem.* **1986**, *51*, 1844. (c) Woodward, R. B.; Pochter, I. J.; Scheinbaum, M. L. *J. Org. Chem.* **1972**, *37*, 333. (d) Kozikowski, A. P.; Ames, A.; Wetter, H. *J. Organomet. Chem.* **1979**, *164*, c33. (e) Takano, S.; Hiroya, K.; Ogasawara, K. *Chem. Lett.* **1983**, 255.), we observed the formation of **5a** when **3a** was treated with potassium *p*-toluenethiosulfonate (4 equiv) in the absence of any sulfinate salt (**4a**:**5a** = 4:1 in 91% yield). Addition of the sodium sulfinate produced a mixture of **4a**:**5a** = 16:1 in 98% yield. We have found no precedence for this type of reaction in the literature. Apparently, the added sulfinate anion serves to convert unwanted sulfonyl disulfide **5a** (produced via attack of thiosulfonate anion on **4a**) back to **4a**. Support for this claim has been derived from the control reaction where it was shown that treatment of pure **5a** with sodium *p*-toluenesulfinate (5 equiv) converted **5a** to a mixture of **4a** and **5a** in quantitative yield in a ratio of approximately 13:1, respectively. In other cases (**4b** and **4c**), evidence of sulfonyl disulfide formation was observed from the TLC of the reaction; a slightly higher R_f spot was seen. In these cases, the putative sulfonyl disulfide was not isolated, but the higher R_f spot converted to the lower spot upon addition of the sulfinate salt.

(10) (TBA)F (1.0 M in THF) and powdered 4-Å molecular sieves were purchased from Lancaster Synthesis. (TBA)F·3H$_2$O was purchased from Aldrich.

0002-7863/90/1512-8084$02.50/0