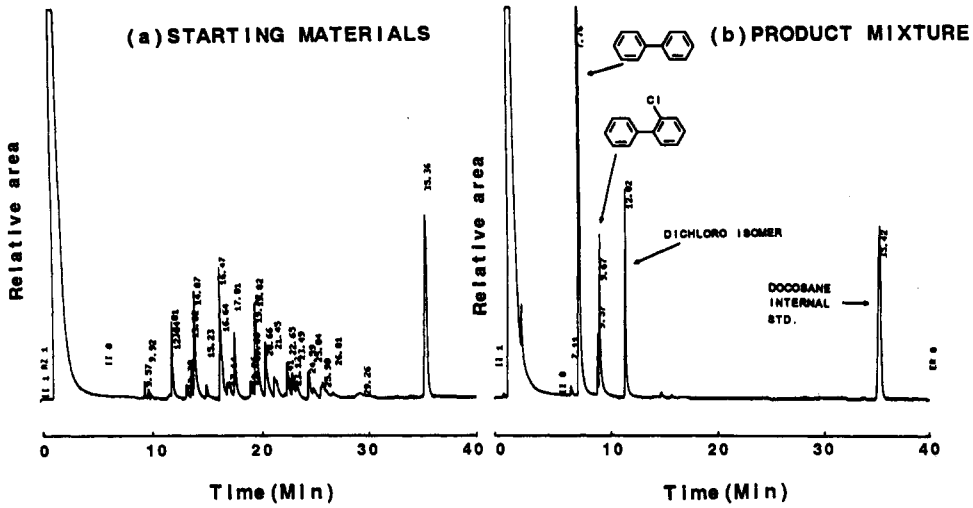

Figure 1. Gas chromatograms of Aroclor 1016 (a) before and (b) after treatment with $NiCl_2/NaBH_2(OCH_2CH_2OCH_3)_2$ in THF at 68 °C for 36 h.

Aroclor 1016 mixture after addition of 0.75 equiv of $NiCl_2$. Figure 1 shows the gas chromatograms of Aroclor 1016 (a) before and (b) after this treatment. When 1 equiv of $NiCl_2$ was slowly pumped into this Aroclor 1016 dehalogenation (other stoichiometry identical to entry 7) a 98.5 mol % yield of biphenyl was achieved. Complete dechlorination to biphenyl occurred when larger excesses of $NaBH_2(OCH_2CH_2OCH_3)_2$ or more than 1 equiv of $NiCl_2$ were used. $NaBH_4/NiCl_2$ in THF/CH_3OH (entry 5) provided significantly more effective dechlorination than $NaBH_4$ in the absence of $NiCl_2$ (entry 2).

Stoichiometry in $NiCl_2$ approached 1:1 in dechlorinations when methanol solutions of $NiCl_2$ were added dropwise or continuously pumped into the borohydride agent and substrate in THF. Phenylcyclohexane was observed in addition to biphenyl in some cases where $NiCl_2$ had been added. The rate of dechlorination caused by the borohydride reagent in the absence of added $NiCl_2$ was quite slow. Presumably active Ni(0) species must react with RCl prior to Ni(0) deactivation caused by particulate residue formation. Whether deactivation is due to diffusion limitations or chemical modifications which poison reduced Ni species is unknown.

Experimental Section

General Procedure without $NiCl_2$. Into a dry, 50-mL three-neck flask equipped with a thermometer, magnetic stirring bar, and reflux condenser were added 235 mmol (19.1 mL) of THF and 200 mg of tetradecane which was used as the internal standard for gas chromatography. Docosane was used as the internal standard in the dehalogenations of PCB mixtures since it did not overlap with any peaks in the system. Chloro-*p*-xylene (5 mmol, 0.67 mL) then was added. As the temperature was increased to 68 °C, $NaBH_4$ (20 mmol, 0.96 g) and $CH_3OCH_2CH_2OH$ (40 mmol, 3.16 mL) were added forming $NaBH_2(OCH_2CH_2OCH_3)_2$ in situ. Samples, taken at intervals using a syringe technique, were treated with dilute aqueous sulfuric acid solution and extracted with CH_2Cl_2. After being dried over anhydrous Na_2SO_4, organic samples in CH_2Cl_2 were analyzed by both GC (30 m, DB-5 capillary column, FID detector; 80 °C, 2 min, with subsequent heating at 10 °C/min to 220 °C where it was held for 9 min) and GC/MS.

Incremental $NiCl_2$ Addition. All equipment and conditions were as described in the general procedure with the exception that a suspension of $NiCl_2$ in THF was incrementally added to the reaction mixture by syringe after addition of sodium borohydride and 2-methoxyethanol was completed and the temperature had reached 68 °C. The amount of $NiCl_2$ and time between increments were varied in different reactions. Alternately, a $NiCl_2/CH_3OH$ solution was either continuously added dropwise or slowly pumped, at a constant rate, into the reaction mixture. Aliquots were removed for analysis at regular intervals before and after $NiCl_2$ incremental portions were added. The analyses were performed as described above. For reactions of Aroclor 1016 the GC temperature program employed was (130 °C held for 2 min, heated 10 °C/min to 190 °C where it was held for 1 min and then heated to 220 °C at 1 °C/min and held at 220 °C for 1 min).

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Bistratamides C and D. Two New Oxazole-Containing Cyclic Hexapeptides Isolated from a Philippine *Lissoclinum bistratum* Ascidian[1]

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Introduction

Lissoclinum spp. ascidians have become well-known as prolific producers of cytotoxic cyclic peptides with highly modified amino acid residues (e.g., ascidiacyclamide, ulithiacyclamide)[3] and macrolides with mixed polyketide/peptide biosynthesis (e.g., patellazoles,[4] bistramide A[5]). As part of our continuing effort to isolate biologically active compounds from this genus of ascidians, we collected a sample of *Lissoclinum bistratum* in the Philippines. The organism lacked any of the metabolites previously described from this organism, but contained a novel family of cyclic hexapeptides (1, 2) containing oxazole (Ozl) residues—a modified amino acid not previously seen in the vast array of modified cyclic peptides previously reported from this genus. Oxazoles from marine invertebrates were

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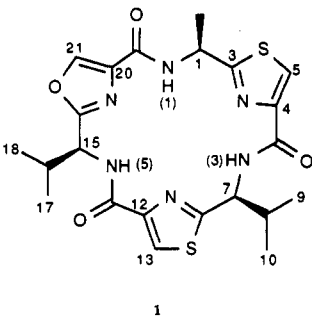
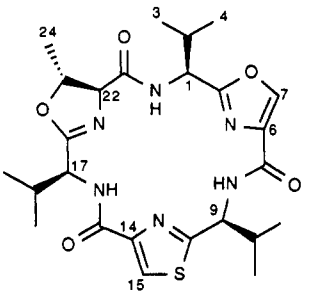


1



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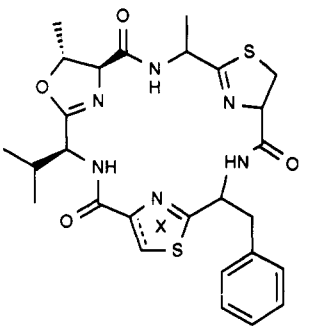
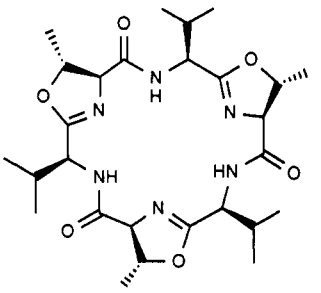


3: X = thiazoline.
4: X = thiazole.



5

first reported from nudibranch egg masses (ulapualides, halichondramides, kabiramides)[6] and subsequently from several sponges (hennoxazoles, bengazoles, calyculin A, orbiculamide A, keramamides).[7] This finding expands our understanding of the biosynthetic capabilities of *L. bistratum* ascidians (and their symbiotic unicellular alga, *Prochloron*).

Cyclic hexapeptides, bistratamides A and B (3, 4), have been reported previously from *L. bistratum* collected off the Great Barrier Reef, Australia.[8] These peptides possess the modified amino acid residues methyloxazoline (mOzn), thiazole (Tzl), and thiazoline (Tzn). In addition, a quite similar hexapeptide 5, was recently reported under two different names from both *L. bistratum* (cycloxazoline)[9] and the terrestrial cyanobacteria, *Westiallopsis prolifica* (westielamide),[10] providing circumstantial evidence that the peptides found in the ascidians may be, at least in part, synthesized by the harbored *Prochloron*. We report here the structures of the oxazole-containing hexapeptides, bistratamides C and D (1, 2). Bistratamide D showed depressant effects when introduced directly into the central nervous system of mice by intracerebral injection;[11] at a 65 μg dose, mice exhibited decreased motor activity, sluggishness, and sedation relative to control.

Results and Discussion

High-resolution positive-ion FABMS established a molecular formula for 1 of $C_{22}H_{26}N_6O_4S_2$, indicating 13 double-bond equivalents. One dimensional ^1H and 2D DQF-COSY[12] NMR experiments established three standard residues: one alanine and two valines (Table I). The hexapeptide nature was deduced from six nitrogen atoms in the formula and six sp^2 carbon signals in the amide region of the ^{13}C spectrum; a DEPT[13] experiment established the multiplicities of the carbons (Table I). The presence of the three cyclically modified amino acid residues was inferred from the absence of three amide protons, the presence of three aromatic ^1H singlets typical of thiazole (Tzl) and oxazole (Ozl) residues, and the six aro-

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Table I. NMR Assignments for Bistratamide C (1) (Cyclo-L-Ala-Tzl-L-Val-Tzl-L-Val-Ozl). Experiments Carried Out at 500 MHz in $CDCl_3$

no.	^{13}C (mult)[a]	^1H (mult, J_{HH} Hz)[b]	COSY	LR H-C ($^nJ_{CH}$, Hz)[c]
N1H		8.60 (d, 6.8)	1	22 (6.9), 1, 3 (6.5)
1	47.5 (d)	5.46 (dq, 6.8, 6.8)	N1H, 2	22, 2, 3
2	24.8 (q)	1.73 (d, 6.8)	1	1, 3
3	171.2 (s)			
4	148.5 (s)			
5	124.3 (d)	8.13 (s)		3 (5.9), 4 (4.2), 6 (2.2)[d]
6	159.6 (s)			
N3H		8.35 (d, 9.2)	7	6 (7.6), 11 (7.9)
7	55.8 (d)	5.38 (dd, 6.8, 9.2)	N3H, 8	6, 8, 9, 10, 11
8	35.3 (d)	2.30 (m)	7, 9, 10	7, 9, 10, 11
9	18.8 (q)	1.11 (d, 6.8)	8	7, 8, 10
10	18.9 (q)	1.01 (d, 6.8)	8	7, 8, 9
11	168.3 (s)			
12	149.5 (s)			
13	123.2 (d)	8.07 (s)		11 (8.2), 12 (3.9), 14 (3.9)[d]
14	160.1 (s)			
N5H		8.44 (d, 8.8)	15	14 (7.6), 19 (6.5)
15	52.8 (d)	5.28 (dd, 4.3, 8.8)	N5H, 16	14, 16, 17, 18, 19
16	33.6 (d)	2.36 (m)	15, 17, 18	15, 17, 18, 19
17	18.6 (q)	1.04 (d, 6.6)	16	15, 16, 18
18	17.9 (d)	1.01 (d, 6.9)	16	15, 16, 17
19	163.7 (s)			
20	135.4 (s)			
21	141.4 (d)	8.20 (s)		19 (7.8), 20 (14.0)
22	159.1 (s)			

[a] Determined from DEPT spectrum. [b] Assigned from HMQC, $^1J_{CH}$ = 140 Hz; ^1H–^1H couplings measured from 1D ^1H spectrum. [c] Determined from HMBC experiment, $^nJ_{CH}$ = 8 Hz, recycle time 1 s. [d] HMBC experiment optimized for $^nJ_{CH}$ = 8 Hz, recycle time 4.5 s.

matic signals in the ^{13}C spectrum.[14] These assignments account for 12 degrees of unsaturation, the thirteenth resulting from the cyclic nature of the peptide.

A $^1H\{^{13}C\}$-HMQC[15] experiment was used to assign the proton-bearing carbons, while several $^1H\{^{13}C\}$-HMBC[16] spectra provided the quaternary carbons assignments and were critical to sequencing the peptide. For example, HMBC correlations from C22 and C3 to H1 established their vicinity to the alanine α-proton, while correlations from the latter (C3) and C4 to H5 established C3 as the "carbonyl" carbon of Ala and C4 the α carbon of the Tzl.[17] Three fragments were thus assigned in a similar manner: C22 to C4, C6 to C12, and C14 to C20. The oxazole (Ozl) residue could be distinguished from the two thiazoles by the carbon chemical shifts of the α and β carbons (148 and 124 ppm for Tzl and 135 and 141 ppm for Ozl, Table I).[14] In the initial HMBC dataset, with $^nJ_{CH}$ = 8 Hz and a recycle time of 1 s, no three-bond correlations could be seen between these three fragments (e.g., across the C20–C22, C4–C6, or C12–C14 bonds). Proton T_1 measurements of 1 indicated the aromatic protons (H5, H13, H21) had very long longitudinal relaxation times (>5 s). A subsequent HMBC experiment with a 4.5-s recycle time did provide weak three-bond correlations across two of the three bonds, providing the sequencing information (C6 to H5 and C14 to H13, Table I; supplementary material).

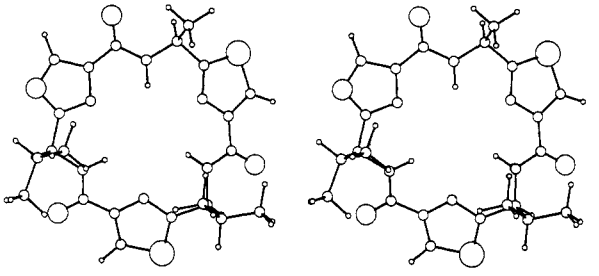

Figure 1. Stereoview of the three-dimensional structure of bistratamide C (1) resulting from molecular dynamics simulations and minimization. The three aromatic amino acids (Tzl-2, Tzl-4, Ozl-6) impose a nearly planar conformation.

High-resolution FABMS established a molecular formula of $C_{25}H_{34}N_8O_5S$ for 2, thus 12 double-bond equivalents. One-dimensional ^1H and 2D DQF-COSY spectra allowed the identification of three valine residues and a methyloxazoline (Table II). Two aromatic ^1H singlets and four olefinic ^{13}C signals were indicative of one Tzl and one Ozl residue. As above for 1, an HMQC spectrum was used to assign the proton-bearing carbons, while two HMBC experiments, acquired with $^nJ_{CH}$ = 10 and 5.26 Hz, allowed the assignment of the quaternary carbons and established the sequence. The sequence was deduced from strong two-bond ^1H–^{13}C correlations (C25 to H22) in both HMBC spectra, although a weak three-bond correlation was also observed across the C14–C16 bond in the 5.26-Hz HMBC experiment (Table II).

The absolute stereochemistries of the amino acids were established as described previously, via ozonolysis, acid hydrolysis, and derivatization.[3c,18] HPLC analysis showed that 1 contained L-Ala and L-Val, while 2 contained L-Val and L-Thr (from L-mOzn).

Due to the conformational restrictions imposed by the high degree of planarity of the Tzl and Ozl residues (conjugated π-electron systems extend from the carbonyl carbons of the preceeding residues to the amide nitrogens

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(17) We envision the Tzl residue as the product of condensation of the thiol group of a cysteine with the carbonyl carbon of the preceeding residue, followed by oxidation to the aromatic heterocycle (below). As such, the C-2 of a thiazole is the "carbonyl" carbon of the preceeding residue, while C-4 and C-5 are the "α" and "β" carbons, respectively. Tzn, Ozl, and mOzn residues can be similarly described.

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Table II. NMR Assignments of Bistratamide D (2) (Cyclo-L-Val-Ozl-L-Val-Tzl-L-Val-L-mOzn). NMR Experiments Carried Out at 500 MHz in $CDCl_3$

no.	^{13}C (mult)[a]	^1H (mult, J_{HH} Hz)[b]	COSY	LR H-C[c]
N1H		7.89 (d, 7.6)	1	25, 5, 1
1	53.1 (d)	4.96 (dd, 7.6, 5.2)	N1H, 2	25, 5, 2
2	33.2 (d)	2.22 (m)	1, 3, 4	5, 1, 3, 4
3	18.17 (q)	0.96 (d, 6.89)	2	1, 2, 4
4	17.6 (q)	0.89 (d, 6.88)	2	1, 2, 3
5	163.4 (s)			
6	135.8 (s)			
7	140.9 (d)	8.16 (s)		5, 6
8	159.2 (s)			
N3H		8.62 (d, 7.4)	9	13, 8
9	56.4 (d)	5.27 (dd, 7.4, 4.4)	N3H, 10	8, 13, 10
10	34.5 (d)	2.38 (m)	9, 11, 12	9, 13, 11, 12
11	18.15 (q)	1.03 (d, 6.9)	10	9, 10, 12
12	17.8 (q)	0.93 (d, 6.9)	10	9, 10, 11
13	167.6 (s)			
14	148.6 (s)			
15	123.6 (d)	8.10 (s)		13, 14, 16[d]
16	160.0 (s)			
N5H		8.03 (d, 9.9)	17	21, 16, 17, 14[d]
17	51.9 (d)	4.99 (dd, 9.9, 2.1, 2.4)	N5H, 18, 22	21, 16, 18
18	31.2 (d)	2.42 (m)	17, 19, 20	19, 20
19	19.0 (q)	1.06 (d, 6.9)	18	17, 18, 20
20	16.3 (q)	0.95 (d, 6.9)	18	17, 18, 19
21	169.4 (s)			
22	74.1 (d)	4.10 (dd, 9.0, 2.1)	23, 17	25, 21, 23, 24
23	82.5 (d)	4.77 (dq, 9.0, 6.3)	22, 24	25, 21
24	21.8 (q)	1.58 (d, 6.3)	23	22, 23
25	170.5 (s)			

[a] Determined from DEPT spectrum. [b] Assigned from HMQC, $^1J_{CH}$ = 140 Hz; ^1H–^1H couplings measured from 1D ^1H spectrum. [c] Determined from HMBC experiment, $^nJ_{CH}$ = 10 Hz. [d] From HMBC acquired with $^nJ_{CH}$ = 5.26 Hz.

Table III. Measured and Predicted ϕ Values for Bistratamide C

residue	$^3J_{N\alpha}$[a]	θ[b]	$\phi_{predicted}$[c]	$\phi_{measured}$[d]
Ala(1)	6.8	9, 140	69, 51, −80, −160	−155
Val(3)	9.0	162	−102, −138	−140
Val(5)	8.8	159	−99, −141	−141

[a] Measured from 1D ^1H spectrum. [b] Calculated using the modified Karplus equation: $^3J_{N\alpha} = 6.4\cos^2\theta - 1.4\cos\theta + 1.9$.[19] [c] Obtained from $\theta = |\phi - 60°|$. [d] Measured in lowest energy conformation.

of the following residues, e.g., from C3 to N3),[3c] we investigated the three-dimensional conformation of 1 by computational techniques. After building the model from a linear hexapeptide, the peptide was cyclized and the structure minimized. The resulting minimized structure was then submitted to a 10-ps dynamics simulation at 3000 K, generating several populations of structures which were subsequently minimized. A representative member of lowest energy population arising from these studies is shown in Figure 1. The ϕ dihedral angles measured from this structure are in excellent agreement with those predicted from a Karplus-type analysis of the $^3J_{N\alpha}$ ^1H–^1H couplings (Table III).[19] These values are also in excellent agreement with the population of angles observed during a room-temperature dynamics simulation of this low-energy structure. Despite the short distances between the amide nitrogens (3.3–3.7 Å), no interresidue NOEs were observed in the 300-ms ROESY spectrum of 1 (intraresidue NH-αH NOEs were observed, distances: 2.8–3.1 Å).

In summary, we have isolated two novel cyclic hexapeptides from *L. bistratum*, bistratamides C and D, each with an unprecedented oxazole amino acid, and their structures were assigned as 1 and 2; molecular modeling studies have established a planar three-dimensional structure for 1.

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Experimental Section

General. NMR experiments were carried out on a Varian Unity 500-MHz spectrometer; spectra were referenced to residual undeuteriated solvent (^1H) or to solvent signals (^{13}C). High- and low-resolution FAB$^+$ mass measurements were performed on a Finnigan MAT 95 high-resolution gas chromatograph/mass spectrometer with a MAT ICIS II operating system. UV and IR spectra were obtained on HP8452A diode array and Perkin-Elmer 16000 FTIR spectrophotometers, respectively. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Extraction and Characterization of Peptides 1 and 2. The ascidian was collected by SCUBA in Cape Bolinao, Philippines. The samples were repeatedly extracted with methanol (yield: 260 mg crude extract) and partitioned between hexane and chloroform. The chloroform layer (43 mg) was then subjected to silica gel flash chromatography (1.2 × 16-cm column, EM Science Kieselgel 60, stepped gradient elution, 100% $CHCl_3$ to 60% methanol). Normal-phase HPLC (Rainin Microsorb 0.5 × 25-cm 5-μm silica gel, 25% acetone/hexane, refractive index detection) afforded 1 (3.6 mg, 1.38% crude extract) and 2 (6.4 mg, 2.46% crude extract) as clear glasses; both were essentially inactive (IC_{50}'s 125 μg/mL) in the human colon tumor cell line HCT116 cytotoxicity assay. Bistratamides A and B (3, 4) were not detected in the extract, although several other minor component peptides were detected.

Bistratamide C (1): $C_{22}H_{26}N_6O_4S_2$, HR FAB$^+$ (M + H)$^+$ 503.153, Δ = +0.8 mmu; IR (thin film) 3392, 2968, 1676, 1538, 738 cm^{-1}; UV (CH_2Cl_2) λ_{max} 234 nm (ϵ 17 000); $[\alpha]^{25}_D$ = −65° (c = 0.42, $CHCl_3$).

Bistratamide D (2): $C_{25}H_{34}N_6O_5S$, HR FAB$^+$ (M + H)$^+$ 531.239, Δ = −0.3 mmu; IR (thin film) 3392, 2964, 1683, 1520, 753 cm^{-1}; UV (CH_2Cl_2) λ_{max} 232 nm (ϵ 13 000); $[\alpha]^{25}_D$ = −31° (c = 0.33, $CHCl_3$).

NMR Experiments. $^1H\{^{13}C\}$-HMQC experiments were optimized for a $^1J_{CH}$ = 140 Hz, with a recycle time (at + d1) of 1 s for 1 and 1.2 s for 2. Several $^1H\{^{13}C\}$-HMBC experiments were required to detect the long-range correlations required to unambiguously determine the structures: for 1, two experiments were run optimized for $^nJ_{CH}$ = 8 Hz, one with a 1-s recycle time, and in the second experiment, the spins were allowed to relax for 4.5 s before applying the next pulse train; for 2, two $^1H\{^{13}C\}$-HMBC experiments were acquired optimized for different values of $^nJ_{CH}$: 10, 5.26 Hz. Proton T_1 relaxation times for 1 were determined

with a standard inversion-recovery pulse sequence; T_1's for H5 (8.13 ppm), H13 (8.07 ppm), and H21 (8.20 ppm) were 5.05, 5.19, and 5.08 s, respectively, while T_1's for the α-protons H1, H7, and H15 (5.46, 5.38, and 5.28 ppm) were 1.97, 1.66, and 1.65 s, respectively.

Stereochemical Analaysis. A 0.5-mg portion of 1 and 2 (9.9×10^{-4} and 9.4×10^{-4} mmol) was ozonized in CH_2Cl_2 for 5 min. Solvent was removed, and the residue was dissolved in 6 N HCl and placed in a sealed bomb at 104 °C for 21 h. After removal of HCl by repeated evaporation in vacuo, the hydrolysate was resuspended in 300 μL of water, 200 μL of which was then derivatized with (1-fluoro-2,4-dinitrophenyl)-5-L-alanineamide (FDAA).[20] HPLC analysis [Waters NOVAPAK C_{18}; 4.6 × 100-mm column; linear gradient elution, triethylammonium phosphate (50 mM, pH 3.0)/MeCN, 90:10 ramped to 60:40 in 45 min; 1.5 mL/min; UV detection at λ 340 nm] of the FDAA-derivatized ozonized hydrolizates versus similarly derivatized amino acid standards established L-Val and L-Ala for 1 and L-Val and L-Thr (from L-mOzn) for 2.

Molecular Modeling. Modeling studies were carried out using Quanta/CHARMm 3.2.3 (Molecular Simulations, Inc.) on a Silicon Graphics Iris 4D/25 workstation. A CHARMm patch file containing descriptions of the cyclically modified residues was required to incorporate energy terms for these residues. Minimization and dynamics protocols approached those recently described.[3c] The first 10-ps simulation was conducted at 3000 K to probe conformational space and arrive at a population of stable structures. A second 10-ps simulation was conducted at 300 K to assess the average structure of the peptide at room temperature for evaluation of NMR data.

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Supplementary Material Available: 1D ^1H and selected traces from HMBC of 1 and 1D ^1H spectrum of 2 (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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Kinetics and Thermodynamics of Cis–Trans Isomerization of Captopril and Related Compounds

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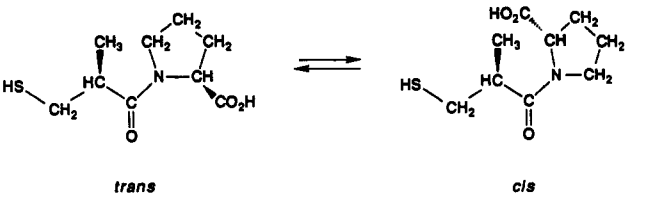
Introduction

Captopril (I), 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline, is an orally active angiotensin converting enzyme (ACE, EC 3.1.15.1) inhibitor used in the treatment of hypertension and congestive heart failure.[1,2] As shown below, captopril can exist in two conformations across the proline amide bond:



The trans conformation is the more populated. However the distribution between the cis and trans conformations is highly dependent on the protonation states of the carboxylic acid and thiol groups. The equilibrium constant for cis ⇌ trans isomerization, $K_{t/c}$ = [trans]/[cis], is 5.9, 1.45, and 3.3 for the (CO_2H, SH), (CO_2^-, SH), and (CO_2^-, S^-) forms, respectively, at 25 °C.[3] At physiological pH, captopril is present as the (CO_2^-, SH) form.[3]

Structure–activity studies have shown that captopril has the trans conformation when bound to the enzyme.[4–8] Thus, factors which govern the distribution of captopril between the active trans conformation and the cis conformation, and the kinetics and thermodynamics of interconversion between the trans and cis conformations by rotation around the amide bond, are of interest. An activation energy of 21.3 ± 0.5 kcal/mol has been reported for the cis to trans interconversion of captopril in D_2O solution.[9] Although the solution conditions were not specified, the populations reported for the cis and trans conformations suggest that captopril was present in the low pH (CO_2H, SH) form.[10]

In this paper, we report the results of detailed studies of the kinetics and thermodynamics of interconversion between the cis and trans forms of captopril and the related compounds, glycyl-L-proline (II), glycyl-4-hydroxy-L-proline (III), glycylsarcosine (IV), and glycylglycylsarcosine (V). Rate constants for the interconversion reactions were measured over a range of temperatures using the inversion–transfer NMR method,[11] from which the Gibbs free energies of activation were obtained. Differences in the equilibrium constants $K_{t/c}$ are discussed in terms of the kinetics results.

Experimental Section

The captopril was a gift from the Squibb Institute for Medical Research, Princeton, NJ. The peptides were obtained from Sigma Chemical Co. Solutions were prepared in 99.8% D_2O, and 1,4-dioxane was added as an internal chemical shift reference. Magnetization transfer measurements were made on 0.3 M solutions of captopril at pD 7.36 and 12.06; for the other systems,

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