Conformational Studies and Structure−Activity Analysis of Lissoclinamide 7 and Related Cyclopeptide Alkaloids

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Abstract: The macrocyclic arrays of heterocycles and amides present in *Lissoclinum* peptides can serve as templates for selective metal ion binding and as lead structures for the design of conformationally preorganized peptides and peptidomimetics. The currently available secondary structure information for this class of marine alkaloids, however, is limited to 18- and 24-membered ring isomers. This work provides the first information on the solid state and solution conformation of the 21-membered class of *Lissoclinum* cyclopeptide alkaloids. The folding of lissoclinamide 7, both in the solid and in the solution state, is dominated by a combination of a type II β-turn formed at the prolyloxazoline moiety and a β-loop segment stabilized by intramolecular five-membered hydrogen bonds at the thiazoline−D-phenylalanine−thiazoline moiety. The resulting rigid backbone geometry controls the preferred stereochemistry at the stereogenic α-carbons, and the natural compound represents the thermodynamically most favorable stereoisomer. In addition to these structural studies, we have compared the relative cytotoxicity of lissoclinamide 7 with analogues with selective oxazoline and thiazoline heterocycle replacements. On the basis of in vitro cell toxicity assays, we can conclude that the substitution of thiazolines in the natural product with oxazoline rings leads to a general decrease in cellular toxicity. However, changes in the stereochemistry of the parent macrocycle also influence cytotoxicity.

Introduction

Marine flora and fauna continue to provide rich sources of pharmacologically attractive and structurally unique secondary metabolites.[1] Due to the difficulties in the isolation of significant quantities of marine natural products, synthetic chemistry serves an important role in their structural assignment and biological evaluation.[1,2] We have recently reported the total synthesis and stereochemical assignment of lissoclinamide 7 (**1**),[3] a cyclopeptide metabolite from the aplousobranch ascidian *Lissoclinum patella*.[4] This 21-membered macrocycle contains two thiazoline rings in addition to the oxazoline heterocycle and is structurally closely related to a group of ca. 30 18−24-membered cyclopeptides characterized by an alternating sequence of five-membered heterocycles and hydrophobic amino acid residues (Figure 1).[5]

As a result of our total synthesis, we were able to study the secondary structure as well as the biological activity of lissoclinamide 7 and structural analogues. Upon introduction of five-membered heterocycles into the cyclopeptide sequence, the conformationally flexibility of the 18−24-membered macrocycles is considerably reduced, and mostly a single secondary structure is preferred in the solid state and in solution. Accordingly, a thorough understanding of the effects of amino acid-derived thiazoline and oxazoline heterocycles on the conformation of cyclopeptides can facilitate the *de novo* design of 3-dimensionally preorganized macrocycles.[5,6] In particular, selective metal-ion chelation[7] and protein-binding properties[8] can be optimized in addition to other biological activities.

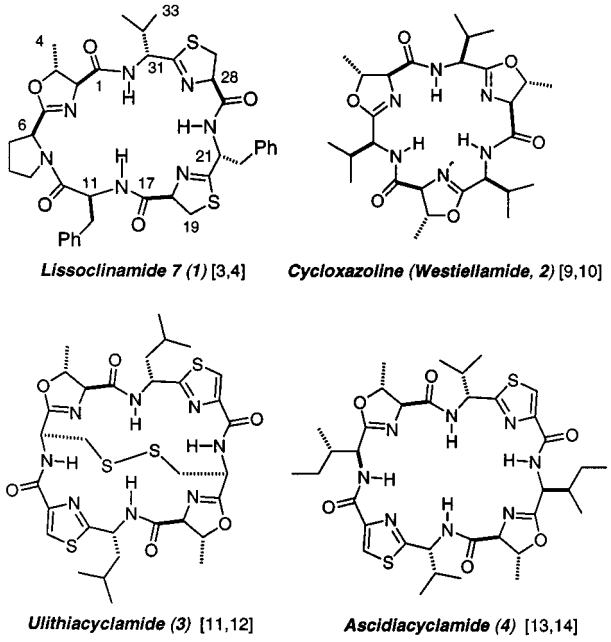

Figure 1. Representative *Lissoclinum* peptides.

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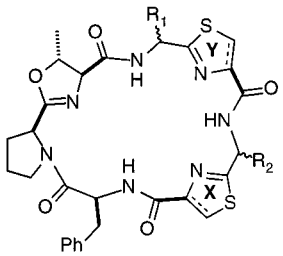
Table 1. Cytotoxic Activity of 21-Membered *Lissoclinum* Peptides and Ulithiacyclamide (IC_{50} [μg/mL])



	R_1	R_2	X	Y	T24 cells	MRC5CV1 cells	other cell lines	refs.
Lissoclinamide 1	L-Val	D-Ile	thiazole	thiazole			>10	[17]
Lissoclinamide 2	D-Ile	D-Ala	thiazole	thiazoline			>10	[17]
Lissoclinamide 3	D-Ile	L-Ala	thiazole	thiazoline			>10	[17]
Lissoclinamide 4	L-Val	D-Phe	thiazole	thiazoline	1	1	12	[18,19]
Lissoclinamide 5	L-Val	D-Phe	thiazole	thiazole	10	15	10, 20	[19,4]
Lissoclinamide 6	D-Val	D-Phe	thiazole	thiazoline			7	[19]
Lissoclinamide 7 (1)	D-Val	D-Phe	thiazoline	thiazoline	0.06	0.04	0.08	[4]
Lissoclinamide 8	Val	Phe	thiazole	thiazoline	6	1	8	[4]
Ulicyclamide	L-Ile	D-Ala	thiazole	thiazole			7	[20]
Cyclodidemnamide	-	-	-	-			16	[21]
Ulithiacyclamide (3)	-	-	-	-	0.15	0.2	0.01	[18,20]

Most *Lissoclinum* peptides display a moderate to high level of cytotoxicity that has been linked to the presence of oxazoline functions.[15,16] Table 1 illustrates the structure−activity relationships that are currently available for class B[5a] (21-membered) *Lissoclinum* peptides in comparison to ulithiacyclamide, a class C[5a] (24-membered) macrocycle with particularly high cytotoxic activity.

Solid State and Solution Structure of Lissoclinamide 7. Extensive prior work on the structural analysis of *Lissoclinum* peptides has focused almost exclusively on 18- and 24-membered macrocycles.[5] These derivatives assume unique molecular "triangle", "square", and "twisted eight" conformations in solution and in the solid state (Figure 2). Currently, the structural features that influence the transition between these conformational states are still poorly understood. A rectangular, saddle-shaped conformation was first observed for ascidiacyclamide (**4**) and other 24-membered macrocycles.[22] In contrast, a severely twisted secondary structure was observed in the solid state of the structurally closely related patellamide D.[19] Later,

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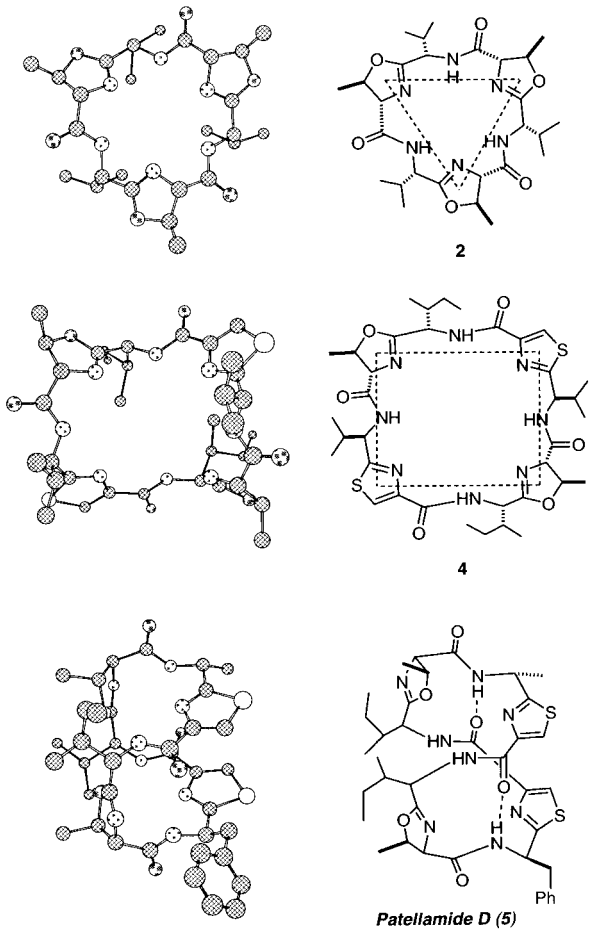

Figure 2. Cycloxazoline (westiellamide, **2**),[9] ascidiacyclamide (**4**),[27] and patellamide D[19] (**5**) X-ray structures as examples for pertinent molecular "triangle", "square", and "twisted eight" secondary structures of 18- and 24-membered *Lissoclinum* peptides.

"figure eight" conformations were also detected for patellamide B and C in solution[23] and tawicyclamide B in the solid state.[24] According to one hypothesis, the decrease in the C_2-symmetry of the macrocycle is responsible for this change in secondary structures between closely related sequences.[22,23] However, the presence of β-branched amino acid side chains and the formation of an intramolecular bifurcated hydrogen-bond network can also lead to a steric restriction of the backbone geometry into a flattened, saddle-shaped conformation.[25] Since 21-membered *Lissoclinum* peptides share structural features of both the 18- and the 24-membered analogues in an intrinsically nonsymmetrical arrangement, an elucidation of the conformational preferences of the 21-membered macrocycles could contribute greatly to the understanding of the general folding principles in this family of cyclopeptide alkaloids.[5a] To this extent, the only structural information that has become available is an ^{1}H NMR NOE/force field study of cyclodidemnamide,[21] which assigned a distorted rectangular geometry to this cycloheptapeptide. However, due to the ambiguity regarding the primary structure of cyclodidemnamide,[26] no conclusions relevant to other *Lissoclinum* peptides can be drawn.

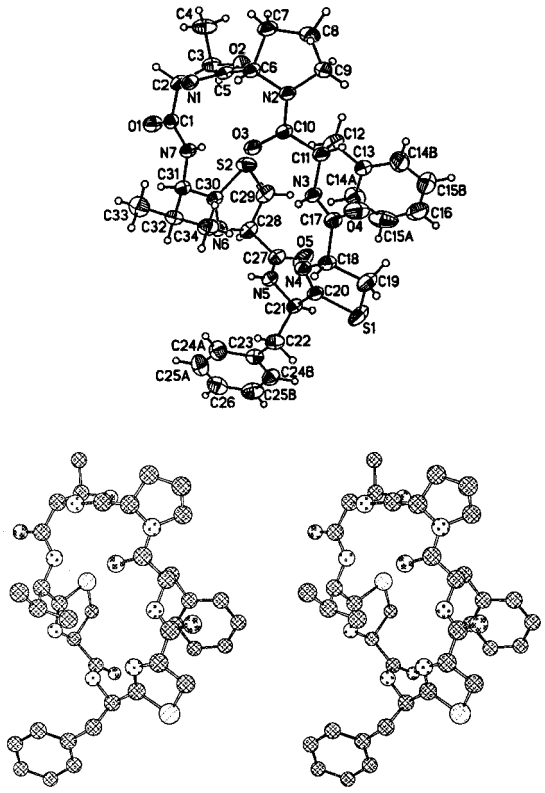

Figure 3. Thermal ellipsoid plot (35% probability ellipsoids) and stereoview of the solid-state structure of lissoclinamide 7.

Crystallization of synthetic lissoclinamide 7 from methanol and X-ray analysis allowed us to obtain the first detailed structural information on 21-membered *Lissoclinum* peptides (Figure 3).[28] The macrocycle backbone is surprisingly planar. With the exception of C(29), the maximum deviation of all the backbone chain atoms from the mean plane is less than 1.5 Å. The angles between the planes of the heterocyclic rings and the macrocycle perimeter are, however, close to orthogonal for the oxazoline and the valine-derived thiazoline and only slighted tilted from coplanarity for the phenylalanine-derived thiazoline and the pyrrolidine. None of the amide linkages is distorted, the largest deviation from planarity is observed at the N(3)−C(17) thiazolyl-phenylalanine linkage with $\omega = -167.2°$. The proline amide shows a trans orientation with a dihedral angle of $\omega = 179.0°$. Additional selected dihedral angles are given in Table 2.

An intriguing feature of the secondary structure of lissoclinamide 7 is the combination of a β-turn at the prolyl-oxazoline segment and a wider loop around the thiazoline-phenylalanyl-thiazoline moiety. Dihedral angles of $-59.3°$ (ϕ_2) and 127.0° (ψ_2), and 110.9° (ϕ_3) and $-24.7°$ (ψ_3) for proline and the threonine-derived oxazoline, respectively, are in close agreement with an ideal type II β-turn.[29] Additional stabilization of the β-turn by a tight intramolecular hydrogen bond is indicated by an N(7)−O(3)-distance of 3.02 Å. The type II β-turn is also found at the isoleucyl-oxazoline segments of the C_2-symmetric

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(29) Bend types are classified according to the dihedral angles of the two central residues. An ideal type II β-turn has backbone dihedral angles of $-60°$ (ϕ_2) and 120° (ψ_2), and 80° (ϕ_3) and 0° (ψ_3): Chou, P. Y.; Fasman, G. D. *J. Mol. Biol.* **1977**, *115*, 135−75.

Table 2. Selected Backbone Dihedral Angles for Lissoclinamide 7 [deg]

C(2)−N(1)−C(5)−O(2)	2.7(3)	C(21)−N(5)−C(27)−C(28)	−178.4(3)
C(2)−N(1)−C(5)−C(6)	−176.5(2)	C(27)−C(28)−C(29)−S(2)	−115.7(2)
C(5)−C(6)−N(2)−C(10)	−59.3(3)	C(27)−C(28)−N(6)−C(30)	118.3(3)
C(6)−N(2)−C(10)−C(11)	179.0(2)	C(30)−C(31)−N(7)−C(1)	99.3(3)
C(10)−C(11)−N(3)−C(17)	−123.9(2)	C(31)−N(7)−C(1)−C(2)	−179.6(2)
C(11)−N(3)−C(17)−C(18)	−167.1(3)	N(1)−C(5)−C(6)−N(2)	127.0(3)
C(17)−C(18)−C(19)−S(1)	−145.8(2)	N(3)−C(17)−C(18)−N(4)	4.9(4)
C(17)−C(18)−N(4)−C(20)	139.1(3)	N(5)−C(27)−C(28)−N(6)	6.2(4)
C(20)−C(21)−N(5)−C(27)	101.0(3)	N(6)−C(30)−C(31)−N(7)	173.2(2)

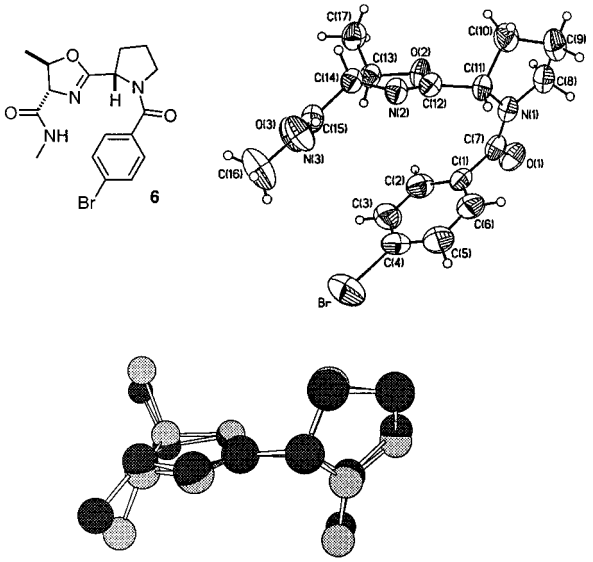

Figure 4. X-ray structure of *p*-bromobenzoyl-prolyl-oxazoline-*N*-methyl amide (**6**, black) and overlay with the prolyl-oxazoline fragment of lissoclinamide 7 (gray).

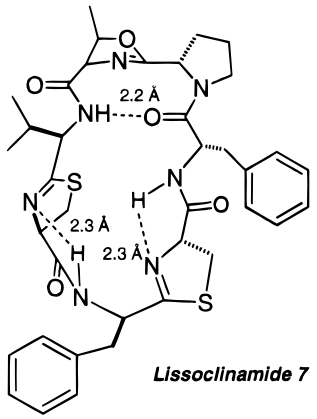

Figure 5. Graphical illustration of the hydrogen-bonding network in the β-loop and β-turn regions of the solid-state structure of lissoclinamide 7.

patellamide D[19] (−76.4° (ϕ_2) and 121.0° (ψ_2), and 101.5° (ϕ_3) and −14.3° (ψ_3)).

An interesting question is if the formation of this β-turn is simply a consequence of the cyclic structure of these peptide alkaloids or actually due to intrinsic conformational preferences of the amino acid-oxazoline sequence. An X-ray structure of the dipeptide *p*-bromobenzoyl-prolyl-oxazoline-*N*-methyl amide (**6**) obtained in our laboratory[30] showed a backbone conformation closely related to the corresponding cyclic peptide fragments (Figure 4).[28] Dihedral angles (−46.9° (ϕ_2) and 141.9° (ψ_2), and 134.5° (ϕ_3) and 45.4° (ψ_3)) observed for **6** still allow a classification of this turn as a type II β-turn,[29] and the only major derivation at ψ_3 can be explained by the formation of an inter- rather than an intramolecular hydrogen bond in the solid state of **6** (dO(3)···H−N(3′) = 2.56 Å). In $CDCl_3$ solution, the prolyloxazoline methyl amide N−H shows a temperature coefficient ($\Delta\delta/\Delta T$) of −3.1 ppb/K,[31] a reasonable indication of intramolecular hydrogen bonding.[32,35] In contrast, the parent acylated prolyl-threonine methyl amide has a much larger temperature coefficient of −9.6 ppb/K.[31] Therefore, we believe that a prolyl-oxazoline segment, or, in general, an oxazoline-containing dipeptide unit as found in lissoclinamide 7 or patellamide D is intrinsically conformationally preorganized toward a β-turn. We expect that this important structural characteristic can be used in the future *de novo* design of peptidomimetics and peptide ligands for receptor and enzyme interactions.

Another interesting feature of the solid-state structure of lissoclinamide 7 is the formation of a tight β-loop region at the thiazolyl-D-phenylalanyl-thiazoline segment of the cyclopeptide alkaloid. The X-ray structure shown in Figure 3 suggests a network of two stabilizing intramolecular hydrogen bonds between thiazoline (imine) nitrogens and phenylalanine amide hydrogens that is graphically depicted in Figure 5. The resulting five-membered hydrogen bond structure is related to the 2→2 intramolecular H-bonded peptide conformation.[33] The latter five-membered hydrogen bonds are usually quite weak and difficult to observe.[33] We were therefore surprised to learn that the solution conformation of lissoclinamide 7 also provides strong evidence for a five-membered hydrogen-bond network at the loop segment in $CDCl_3$, CD_3CN, and in DMSO-d_6 (Figure 6).[34] In addition, extraordinarily small temperature coefficients[35] of the phenylalanine amide hydrogens confirm that these intramolecular hydrogen bonds are relevant for the solution conformation of lissoclinamide 7 and might also contribute to the relative rigidity of the cyclopeptide backbone (Table 3). We are currently investigating the general relevance of this novel β-loop stabilization effect for other azole cyclopeptides.

The lack of any major backbone conformational changes observed in the solution structure of lissoclinamide 7, which is practically identical with the X-ray structure, suggests that both β-turn and β-loop segments of the cyclopeptide alkaloid act in synergism to stabilize the 3-dimensional architecture. Accord-

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(36) In CD_3OD solution, deuterium-exchange kinetics of this amide hydrogen provide a $\tau_{1/2}(^1H-^2H)$ of 35 h. The $\tau_{1/2}(^1H-^2H)$ for the D-Phe-NH and Phe-NH amide hydrogens was even lower (>5 d). The slow time scale of this exchange provides further support for an intramolecularly hydrogen-bonded conformation in solution.[37]

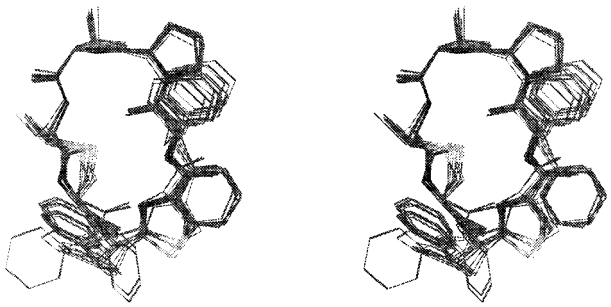


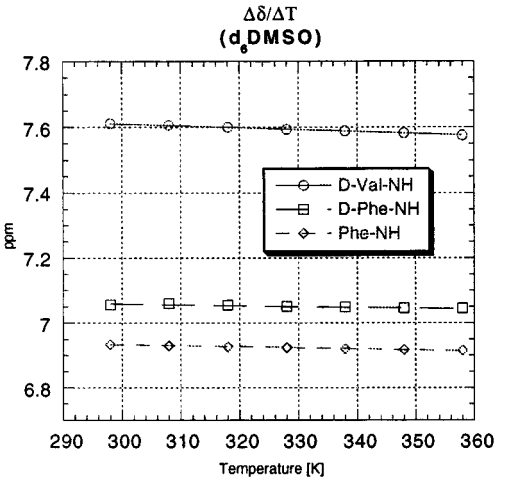
Figure 6. Stereoview of the lowest energy solution conformations of lissoclinamide 7 in $CDCl_3$ and DMSO-d_6 as determined by NMR restraints based on gradient COSY, TOCSY, NOESY, and ROESY experiments (all at 500 MHz) used in conjunction with Monte Carlo conformational search methods.

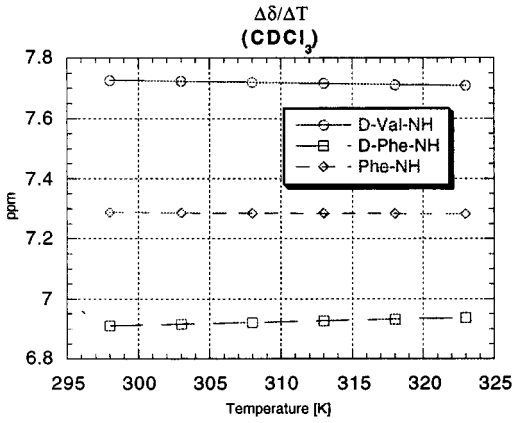
ingly, both features could be used for the design of conformationally preorganized compounds. In addition, the conformational effects of β-turn and β-loop segments should be correlated to the stereochemistry at the readily epimerizable C(31) and C(21) asymmetric carbons; e.g., the configuration present in the natural product should be the most favorable for the stabilization of the overall conformation of the molecule. To test this hypothesis, we subjected (31*S*)-lissoclinamide 7 (=[L-Val]-lissoclinamide 7)[3] to 5−10 equiv of pyridine in $CDCl_3$. Indeed, a first-order conversion was observed at 60 °C that led to the exclusive formation of the natural (31*R*)-isomer (Figure 7). Only traces of the starting material or any other isomers could be observed by NMR- and HPLC-analysis of the crude reaction mixture.[37] In contrast, lissoclinamide 7 remained unchanged under the same conditions for at least 7 d. This experiment confirms the suspected strong influence of the conformation of the cyclopeptide backbone on the configuration at the epimerizable α-carbons and sheds light on the observation that despite the multiple stereoisomers that have been isolated for *Lissoclinum* peptides with a single thiazoline ring (see Table 1), only a single stereoisomer is known for the two thiazoline-containing natural lissoclinamide 7 sequence.[38]

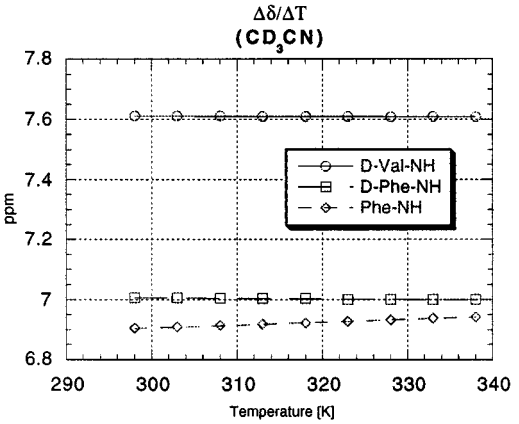
Cytotoxic Activity of Lissoclinamide 7 and Its Analogues. With reported IC_{50} values of 40, 60, and 80 ng/mL in MRC5CV1, T24, and lymphocytes cell assays,[4] respectively, lissoclinamide 7 (**1**) is, next to the 24-membered ulithiacyclamide, the most cytotoxic *Lissoclinum* derivative (Table 1). It is also the only *L. patella* metabolite that contains two thiazoline rings in addition to the oxazoline heterocycle. A comparison of the cytotoxic effects of naturally occurring *Lissoclinum* peptides, synthetic cyclic peptides and relatively short linear segments, served as the basis for the hypothesis that the oxazoline function is essential for cytotoxicity and that a cyclic skeleton might not be needed.[39] While this hypothesis has found considerable support,[40] no conclusive evidence for or against it, and no molecular rationalization for an oxazoline-induced cytotoxicity is available to date.[5,41] Since any mechanistic link between the presence of oxazolines or thiazolines and the cytocidal activity is unclear, we prepared analogues of lissoclinamide 7 with selective replacements of the heterocyclic moieties.[3] The results of a broad screening assay of lissoclinamide 7 (**1**), (31*S*)-lissoclinamide 7, trisoxazoline **7**, and

Table 3. Tabular and Graphical Display of Amide Proton Temperature Coefficients ($\Delta\delta/\Delta T$) for Lissoclinamide 7 in DMSO-d_6, $CDCl_3$, and CD_3CN [ppb/K]

	D-Val-NH[36]	D-Phe-NH	Phe-NH
DMSO-d_6	−0.6	−0.3	−0.3
$CDCl_3$	−0.7	1.1	−0.2
MeCN-d_3	−0.09	−0.2	0.9







(37) We thank Dr. Hidenori Takahashi (University of Pittsburgh) for performing these experiments.

(38) In this context, it is also interesting to note that one of our earlier synthetic approaches toward lissoclinamide 7 that used an L-valine-derived (31*S*)-stereocenter led unintentionally to the (31*R*)-natural product after heterocycle formation and macrocyclization. It is tantalizing, but currently experimentally not supported, to speculate that the (31*S*)-isomer can also be selectively epimerized during the isolation procedure from the ascidian.

(39) Shioiri, T.; Hamada, Y.; Kato, S.; Shibata, M.; Kondo, Y.; Nakagawa, H.; Kohda, K. *Biochem. Pharm.* **1987**, *36*, 4181.

(40) McDonald, L. A.; Foster, M. P.; Phillips, D. R.; Ireland, C. M.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1992**, *57*, 4616.

(41) For a structure−activity analysis of a tris-oxazoline analogue vs the tris-thiazoline-containing natural product thiangazole, see: Wipf, P.; Venkatraman, S. *Synlett* **1997**, 1.

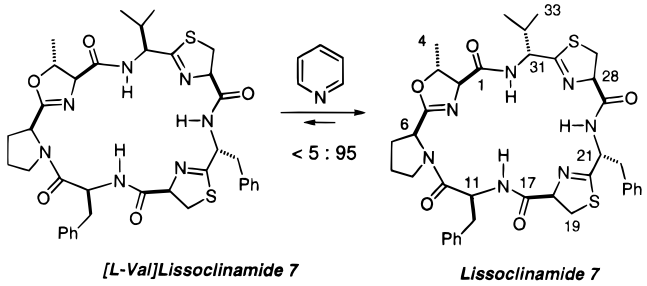
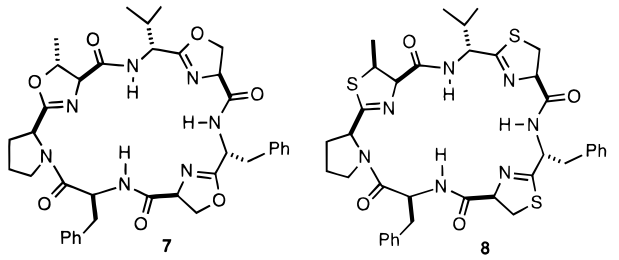


Figure 7. Thermodynamic equilibration of [L-Val]-lissoclinamide 7.

Table 4. GI_{50} Data[42] [μg/mL] for *Lissoclinum* Peptides against Selected Cell Types



7 **8**

cell type	**1**	(31*S*)-**1**	**7**	**8**
HL-60	>75	6	>75	0.07
K-562	70	8	>75	3
RPMI-8226	15	8	>75	6
HCC-2998	16	16	>75	8
OVCAR-4	30	15	>75	8
PC-3	30	16	>75	11
MDA-MB-435	40	30	>75	10

tristhiazoline **8** by the NCI Developmental Therapeutics Program[42] are summarized in Table 4.

Surprisingly, in these assays synthetic lissoclinamide 7 (**1**) shows a lower than expected level of cytotoxicity.[43] Differences in the type of assay, as well as the cell types, could account for this discrepancy with the earlier[4] data. In addition, the presence of contaminating cytotoxic impurities is a major concern with natural product samples.[44] Nonetheless, it is clear from comparison of lissoclinamide 7 (**1**) and trisoxazoline **7** that replacement of the thiazoline rings with oxazolines leads to a significant decrease in cytotoxicity. Further support for the relative importance of the thiazoline structure in causing cytotoxicity in these cell assays is provided by the general increase in toxicity observed in the tris-thiazoline analogue **8**. The high activity of **8** in the human leukemia cell line HL-60, however, is unexpected, and we currently have no explanation for this specific effect.

The biological data obtained in this cross-comparison of heterocycle isomers of *Lissoclinum* peptides represents the first systematic structure−activity investigation in this class of natural products but does not necessarily invalidate the earlier pharmacological hypothesis[39] that certain levels of cytotoxicity can be correlated with the presence of oxazoline functions. However, it provides strong evidence that either heterocyclic building block in general is not solely responsible for cytotoxicity and more subtle structural features of the macrocycle also have to be considered in SAR analyses. In this context, it is important to note that a simple stereoisomer of lissoclinamide 7 (**1**), the (31*S*)-compound with the L-Val substitution for D-Val, displayed increased cytotoxicity that almost rivals the level of activity found for the most potent analogue, tristhiazoline **7** (Table 4).

(42) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91 and references therein.

(43) Due the scarcity of natural material, no direct comparison with synthetic compound could be made in our bioassays.

(44) See, for example: Pettit, G. R.; Taylor, S. R. *J. Org. Chem.* **1996**, *61*, 2322.

Conclusions

One of the major attractive features of *Lissoclinum* peptides is their potential to serve as lead structures for the design of conformationally preorganized peptide and peptidomimetic sequences. A better understanding of the effects of amino acid-derived thiazoline and oxazoline heterocycles on the secondary structure of peptide chains might facilitate the rational engineering of short, intrinsically spatially organized peptide−heterocycle hybrid molecules and lead to practical applications of metabolically more stable peptide analogues.

We have determined the X-ray structure and the solution conformation of synthetic lissoclinamide 7, and thus established the first structural information for a 21-membered macrocycle in this family of marine peptide alkaloids. The conformational preferences both in the solid and in the solution state are dominated by a type II β-turn formed by the prolyloxazoline moiety and a β-loop segment stabilized by intramolecular five-membered hydrogen bonds at the thiazoline-D-phenylalanine-thiazoline sequence. These are novel design features that are of general significance, in particular since the type II β-turn is closely preserved in the small acyclic dipeptide derivative **6**. Compared to other structural types identified for 18- and 24-membered *Lissoclinum* peptides, the lissoclinamide 7 structure most closely resembles the "twisted figure eight" conformation of patellamide D. However, the later compound does not have a β-loop region, and lissoclinamide 7 is much flatter and completely devoid of any apparent symmetry elements. The extensive conformational rigidity of lissoclinamide 7 as a consequence of the presence of both β-turn and β-loop regions facilitates epimerization at labile stereocenters within the macrocycle. The natural product appears to be the thermodynamically clearly favored stereoisomer.

We have also been able to probe the structural basis for biological activity observed with many *Lissoclinum* peptides in a systematic fashion. Contrary to a current hypothesis that correlates cytotoxicity with the presence of oxazoline heterocycles in the backbone of *Lissoclinum* peptides, we have found that the replacement of thiazolines in lissoclinamide 7 with oxazolines leads to a general decrease in cellular toxicity. In contrast, replacement of the single oxazoline ring of the natural product with a thiazoline heterocycle increases cytotoxicity. Therefore, we have been able to engineer for the first time a more active *Lissoclinum* peptide derivative. However, we have also found that changes in the stereochemistry of the parent macrocycle can influence cytotoxicity. Accordingly, the biological activity of *Lissoclinum* peptides has to be considered to encompass all structural features of these natural products.

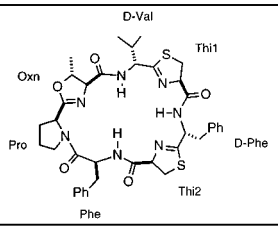
Experimental Section

Crystal data for 1: $C_{38}H_{45}N_7O_5S_2$, $M_r = 743.93$, triclinic, space group $P\bar{1}$, $a = 7.921(2)$ Å, $b = 10.277(2)$ Å, $c = 12.448(3)$ Å, $\alpha = 71.79(3)°$, $\beta = 82.98(3)°$, $\gamma = 79.65(3)°$, $V = 944.5(3)$ Å^3, $Z = 1$, $d_{calcd} = 1.308$, $F(000) = 3942$; 5616 independent reflections were collected and used in structure solution and subsequent least-squares refinement on F^2; $R_F = 0.0416$ (all data), $wR_F = 0.1112$ (all data), GOF = 1.051.[28]

Crystal data for 6: $C_{17}H_{20}BrN_3O_3$, $M_r = 394.27$, monoclinic, space group $P2_1$, $a = 5.3940(10)$ Å, $b = 20.120(4)$ Å, $c = 8.847(2)$ Å, $\beta =$

Table 5. NMR Constraints Used for Monte Carlo Conformational Searches of Lissoclinamide 7[a]

	$CDCl_3$			d_6-DMSO		
	D-Val	Phe	D-Phe	D-Val	Phe	D-Phe
$^3J_{NH,\alpha H}$	10 Hz	6.9 Hz	9.2 Hz	10 Hz	9.8 Hz	8.7 Hz
calc. Φ angle(s)[56]	120°	-165°, 80°	- 140°, 100°	120°	-120°	150°, 90°
constraint used	120±20°	none	120±40°	120±20°	-120±20°	120±50°
secondary filter	none	-165±20°, -80±20°	none	none	none	-165±20°, -80±20°



NOE and ROE constraints used:

$CDCl_3$	d_6-DMSO
D-Valα-D-Valγ	D-PheNH-D-Valγ
D-PheNH-D-Valβ	D-Phe*ortho*H-D-Valγ
D-PheNH-D-Valγ	
D-Phe*ortho*H-Thi1α	
D-Val-NH-Proα	
Phe*ortho*H-Thi1β	

[a] Protons were not stereospecifically assigned. When ambiguous, the constraint was made to the carbon atom and 1 Å was added to the constraint.

106.42(2)°, $V = 921.0(3)$ Å^3, $Z = 2$, $d_{calcd} = 1.422$, $F(000) = 404$; of 2953 reflections collected, 1503 independent reflections were used in structure solution and subsequent least-squares refinement on F^2; $R_F = 0.0515$ (all data), $wR_F = 0.0545$ (all data), GOF = 0.849.[28]

Data for **6** were collected on a Siemens P3 diffractometer with Mo Kα radiation at 293 K. Compound **6** crystallizes in noncentrosymmetric $P2_1$. Data for **1** were collected on a Rigaku AFC5R diffractometer using Ni-filtered Cu radiation at 293 K. Compound **1** crystallizes in the noncentrosymmetric space group $P\bar{1}$. Both data sets were corrected for absorption and extinction effects. Direct methods solutions yielded positions of all non-hydrogen atoms which were refined anisotropically. Hydrogen atom positions were calculated (d(C−H) = 0.96 Å), except for nitrogen hydrogen positions, which were located and refined isotropically. Refinement of the Flack parameter (0.01(2) for **1**, −0.05-(2) for **6**) confirmed the absolute configuration for both structures.

NMR Spectroscopy: General. Approximately 1 mg of synthetic lissoclinamide 7 was dissolved in 700 μL of each of the following solvents, $CDCl_3$, DMSO-d_6, and CD_3CN.[45] 1D and 2D ^{1}H NMR spectra were recorded and processed at 25 °C on a Varian Unity Plus 500 spectrometer. States methodology[46] was used to obtain phase-sensitive spectra. Temperature studies were performed in 5° increments for $CDCl_3$ from 25 to 50 °C and in 10° increments from 25 to 85 °C for DMSO-d_6. Spectra were referenced to $CDCl_3$, DMSO-d_6, and CD_3CN at 7.26, 2.49, and 1.93 ppm, respectively.

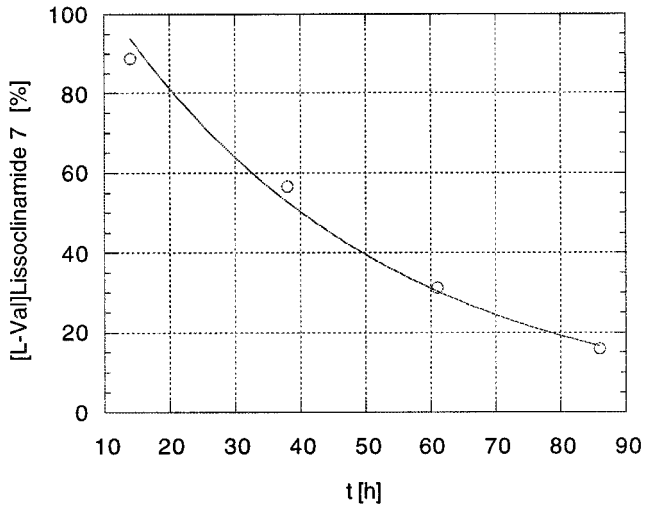


Figure 8. Kinetics of conversion of [L-Val]-lissoclinamide 7 to lissoclinamide 7.

Gradient COSY[47] **(500 MHz):** 512 experiments of 32 scans each with a relaxation delay of 1.3 s, an acquisition time for one scan of 0.253 s, a size of 2 K, a spectral width in F2 and F1 of ∼4000 Hz ppm, zero filling in F2 and F1 to 2 K, and apodization in both dimensions with a sinebell.

TOCSY (500 MHz): pulse sequence according to Bax and Davis with MLEV-17 spin-lock,[48,49] 512 experiments of 32 scans each, relaxation delay of 1.7 s, mixing time of 65 ms, acquisition time for one scan 0.206 s, size 2 K, spectral width in F2 and F1 ∼4000 Hz, zero filling in F2 and F1 to 2 K, apodization in both dimensions with squared sinebell shifted by $\pi/2$.

NOESY (500 MHz): Pulse sequence according to Bodenhausen et al.,[50] 2 s relaxation delay, 500 ms mixing time, acquisition time of 0.142 s, size 2 K, spectral width in F2 and F1 ∼4000 Hz, apodization in both dimensions with squared sinebell shifted by $\pi/2$.

ROESY (500 MHz): pulse sequence according to Kessler et al.[51] with a pulsed 2 kHz spin lock, 2 s relaxation delay, 15% duty cycle, 250 ms mixing time, acquisition time of 0.348 s, size 2 K, spectral width in F2 and F1 ∼4000 Hz, apodization in both dimensions with squared sinebell shifted by $\pi/2$.

Molecular Modeling. All modeling was performed using MacroModel/Batchmin software version 6.0.[52] Monte Carlo searches (2000 iterations) and minimizations were run with the constraints shown in Table 5. The NOEs and ROEs were used as qualitative restraints only, i.e., protons showing an ROE and/or NOE were constrained to be <5 Å apart. Two searches were performed for the DMSO-d_6 and the $CDCl_3$ constraints; calculations were not performed for CD_3CN due to the similarity of the constraints with DMSO-d_6. Energy minimizations were carried out with MacroModel's TNCG method, using the Amber*[53] force field until gradient convergence was achieved. GB/SA solvation[54,55] was used with the water parameters for DMSO-d_6 and the

(45) At this concentration (approximately 1.9 mM), no indication of aggregation was noted. Line widths were very sharp in all solvents. D_2O and D_2O−organic solvent mixtures were not used due to (a) the very low solubility of lissoclinamide 7 in aqueous environments and (b) the known problems with viscosity effects in NOE studies in DMSO− or acetonitrile−water mixtures: Amodeo, P.; Motta, A.; Picone, D.; Saviano, G.; Tancredi, T.; Temussi, P. A. *J. Magn. Reson.* **1991**, *95*, 201.

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(56) The corresponding Φ angles were determined according to the following: Pardi, A.; Billeter, M.; Wüthrich, K. *J. Mol. Biol.* **1984**, *80*, 741.

chloroform parameters for $CDCl_3$. The structures were superimposed using the backbone atoms and were then filtered further for agreement with the angle constraints (see Table 5).

Conversion of [L-Val]-lissoclinamide 7 into lissoclinamide 7. A solution of 2.0 mg (2.7 μmol) of [L-Val]-lissoclinamide 7 in 0.5 mL of $CDCl_3$ was treated with 3 μL (37 μmol) of pyridine and warmed to 60 °C. A first-order half-life of 40 h for the conversion to lissoclinamide 7 was detected by ^{1}H NMR integration of the two doublets at 0.94 and 0.92 ppm for [L-Val]-lissoclinamide 7 vs the doublet at 0.64 for lissoclinamide 7 (Figure 8).[3,37]

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