Conformation of Cyclic Heptapeptides: Conformational Analysis of Segetalins D and E by Distance Geometry Calculations[1)]

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Three-dimensional structures in dimethyl sulfoxide-d_6 of two cyclic heptapeptides, segetalin D(1): *cyclo* (-Gly-Leu-Ser-Phe-Ala-Phe-Pro-) and segetalin E (2): *cyclo* (-Gly-Tyr-Val-Pro-Leu-Trp-Pro-), which have been isolated from the seeds of *Vaccaria segetalis*, were elucidated by computational and NMR methods. Distance geometry calculations using nuclear Overhauser effect (NOE) constraints resulted in uniquely determined backbone conformations of segetalins D and E: each had two β-turns, a β II turn at Pro^7-Gly^1 and a β I turn at Phe^4-Ala^5 for segetalin D, and a β II turn at Pro^7-Gly^1 and a β VI turn at Val^3-Pro^4 for segetalin E, respectively. In addition, each had three intramolecular hydrogen bonds, which constructed a classical β-bulge conformation, as suggested by calculations and NMR studies.

Key words segetalin; conformation; distance geometry calculation; NMR; β-bulge; *Vaccaria segetalis*

Cyclic peptides are molecules which exhibit a wide range of biological activity. Conformational determination of such cyclic peptides is an important first step, because their biological activities are known to be closely related to their conformational states. We have reported the conformations of a series of cyclic peptides in order to clarify the relationship between their conformations and their biological activities.[2)]

Recently, we studied the conformation of cyclic heptapeptides, pseudostellarin D[3)] and yunnanin A,[4)] by a combination of X-ray diffraction, high field NMR and computational methods, and found that their conformational features were characterized by two β-turns with one β-bulge structure. Several examples of the structures and conformations of naturally occurring cyclic heptapeptides are known, such as ilamycin B_1,[5)] a dolastatin 3 analogue,[6)] cycloheptasarcosine,[7)] rhizonin A,[8)] evolidine,[9)] hymenamide[10)] and phakellistatin.[11)] However, we need to further examine the conformational preference of cyclic heptapeptides, compared with those of many cyclic penta, hexa, and octa peptides.

Recently, we isolated two cyclic heptapeptides, segetalin D(**1**): *cyclo* (-Gly-Leu-Ser-Phe-Ala-Phe-Pro-) and segetalin E (**2**): *cyclo* (-Gly-Tyr-Val-Pro-Leu-Trp-Pro-), from the seeds of *Vaccaria segetalis* (Caryophyllaceae).[12)] Here, we describe the elucidation of the solution state conformation of segetalins D and E by distance geometry (DG) calculations using nuclear Overhauser effect (NOE) constraints and NMR study, including temperature effects on NH protons.

Results and Discussion

Distance Geometry Calculation It is essential for conformational analysis to assign complete ^{1}H and ^{13}C signals. In dimethyl sulfoxide-d_6 (DMSO-d_6) solution, each single state conformation for **1** and **2** was observed. These signal assignments were performed by a combination of 2D NMR techniques such as correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) spectra, and are shown in Tables 1 and 2. In addition, to establish three-dimensional structures, it is also necessary to use distance information among each proton. We studied solution conformations of segetalins D (**1**) and E (**2**) by DG calculations using distance constraints derived from the integrated volumes of NOE correlations in phase sensitive rotating frame Overhauser enhancement spectroscopy (ROESY) spectra[13)] (Figs. 1 and 2). The intensities were classified into three ranges, 1.9—2.5, 1.9—3.5 and 1.9—5.0 Å, corresponding to strong, medium and weak ROEs, respectively. At the point of the lack of stereospecific assignments, the distance constraints were relaxed by means of pseudoatom corrections (+1.0 Å for methylene protons; +1.5 Å for methyl protons). In addition, as the presence of a *trans* amide bond, except for a *cis* amide bond, in Pro^4 of segetalin E was suggested by the NMR spectra (the *cis* amide bond was confirmed by a strong NOE correlation between Val^3-H$_\alpha$ and Pro^4-H$_\alpha$ and by a ^{13}C chemical shift difference (Δ9.1 ppm) between C$_\beta$ and C$_\gamma$ of Pro^4.), torsional constraints for amide bonds were taken into consideration. No hydrogen bonding constraints were used.

The initial structures satisfying the experimental constraints were generated by DG calculations, followed by simulated annealing (SA) calculations with the program SYBYL.[14)] Finally, the produced conformers were then subjected to constraint energy minimization with the AMBER all-atom force field.[15)]

The results of calculations are shown in Table 3. 22 structures among 287 structures embedded by the DG method for segetalin D, and 62 structures among 279 structures for segetalin E were converged [pairwise root mean square deviations (RMSD) for the backbone heavy atom is less than 0.50]. Figure 3 shows stereoscopic views of their mean structures. Backbone dihedral angles in the mean structures of segetalins D and E are summarized in Table 4.

Segetalin D RMSD between the individual structures and the mean coordinate position are 0.41 Å for the backbone heavy atoms. The mean structure adopts a type

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Table 1. ^{1}H and ^{13}C-NMR Assignments for Segetalin D (**1**) in DMSO-d_6

Position	δ_H (int.; mult.; J (Hz))	δ_C
Gly^1		
α	3.26 (1H, dd, 4.1, 16.9)	42.84
	4.16 (1H, dd, 4.3, 16.9)	
NH	8.80 (1H, dd, 4.1, 4.3)	
C=O		168.54
Leu^2		
α	4.53 (1H, m)	52.32
β	1.59 (2H, m)	42.52
γ	1.59 (1H, m)	23.99
δ	0.74 (3H, d, 5.7)	22.59
	0.78 (3H, d, 6.0)	21.59
NH	8.05 (1H, d, 10.3)	
C=O		170.22
Ser^3		
α	4.41 (1H, m)	54.43
β	3.75 (1H, m)	62.20
	4.12 (1H, m)	
NH	7.74 (1H, d, 8.6)	
C=O		170.29
Phe^4		
α	4.19 (1H, m)	56.59
β	3.04 (2H, m)	35.64
γ		137.33
δ	7.25 (2H, m)	128.18
ε	7.29 (2H, m)	128.89
ζ	7.23 (1H, m)	126.46
NH	8.47 (1H, d, 4.7)	
C=O		171.00
Ala^5		
α	4.10 (1H, m)	48.97
β	1.07 (3H, d, 7.4)	17.88
NH	8.41 (1H, d, 6.4)	
C=O		171.23
Phe^6		
α	4.73 (1H, ddd, 5.7, 8.8, 7.9)	51.81
β	2.67 (1H, dd, 5.7, 13.2)	38.51
	3.00 (1H, dd, 7.9, 13.2)	
γ		136.68
δ	7.25 (2H, m)	128.13
ε	7.29 (2H, m)	129.32
ζ	7.23 (1H, m)	126.46
NH	7.14 (1H, d, 8.8)	
C=O		168.93
Pro^7		
α	4.14 (1H, m)	61.02
β	1.73 (1H, m)	28.87
	1.83 (1H, m)	
γ	1.73 (1H, m)	24.38
	2.05 (1H, m)	
δ	2.93 (1H, m)	47.37
	3.47 (1H, m)	
C=O		170.96

II β turn at Pro^7–Gly^1 [Pro^7 ϕ, ψ (-60.2, 152.8); Gly^1 ϕ, ψ (73.9, -78.3)] and a type I β turn at Phe^4–Ala^5 [Phe^4 ϕ, ψ (-46.9, -38.4); Ala^5 ϕ, ψ (-73.3, -22.5)]. All of the amide bonds have *trans* geometry. The distances involved in the three intramolecular hydrogen bonds between Phe^6–NH and Ser^3–CO, between Ser^3–NH and Phe^6–CO, and between Leu^2–NH and Phe^6–CO are given in Table 5. Temperature dependence studies,[16] which were recorded in ten-deg. intervals over the range 300—330 K in DMSO-d_6 by NMR, suggested these intramolecular hydrogen bonds (Table 6). Low temperature coefficient values of Leu^2-, Ser^3- and Phe^6 indicated that these amide

Table 2. ^{1}H- and ^{13}C-NMR Assignments for Segetalin E (**2**) in DMSO-d_6

Position	δ_H (int.; mult.; J (Hz))	δ_C
Gly^1		
α	3.54 (1H, dd, 5.6, 17.1)	42.44
	3.80 (1H, dd, 7.0, 17.1)	
NH	8.26 (1H, dd, 5.6, 7.0)	
C=O		167.88
Tyr^2		
α	4.47 (1H, ddd, 2.9, 7.4, 10.2)	53.96
β	2.47 (1H, dd, 2.9, 13.5)	35.62
	2.66 (1H, dd, 10.2, 13.5)	
γ		128.73
δ	6.85 (2H, d, 8.4)	129.95
ε	6.55 (2H, d, 8.4)	114.59
ζ		155.32
NH	8.16 (1H, d, 7.4)	
C=O		171.50
Val^3		
α	3.83 (1H, dd, 4.8, 7.8)	58.23
β	1.91 (1H, m)	29.17
γ	0.87 (3H, d, 6.9)	18.56
	0.97 (3H, d, 6.9)	18.83
NH	8.56 (1H, d, 4.8)	
C=O		170.17
Pro^4		
α	4.58 (1H, d, 6.0)	60.49
β	1.77 (1H, m)	30.54
	2.33 (1H, m)	
γ	1.49 (1H, m)	21.44
	1.77 (1H, m)	
δ	3.14 (1H, m)	45.66
	3.43 (1H, m)	
C=O		170.12
Leu^5		
α	4.14 (1H, m)	40.05
β	1.22 (1H, m)	24.55
	1.53 (1H, m)	
γ	1.37 (1H, m)	22.68
δ	0.70 (3H, d, 6.5)	20.71
	0.74 (3H, d, 6.5)	20.71
NH	8.24 (1H, d, 8.4)	
C=O		171.26
Trp^6		
α	4.87 (1H, ddd, 4.9, 5.5, 7.0)	51.88
β	3.02 (1H, dd, 5.5, 14.7)	26.83
	3.37 (1H, dd, 4.9, 14.7)	
NH	7.57 (1H, d, 7.0)	
1 (NH)	10.84 (1H, d, 2.0)	
2	7.28 (1H, d, 2.0)	120.76
3		108.16
4	7.61 (1H, d, 7.9)	118.10
5	6.98 (1H, t, 7.9)	124.56
6	6.95 (1H, t, 7.9)	118.27
7	7.21 (1H, t, 7.9)	111.20
8		135.73
9		127.94
C=O		170.36
Pro^7		
α	4.06 (1H, m)	54.26
β	1.77 (1H, m)	28.43
	2.13 (1H, m)	
γ	1.77 (1H, m)	25.37
	1.93 (1H, m)	
δ	3.29 (1H, m)	47.11
	3.69 (1H, t, 7.6)	
C=O		171.75

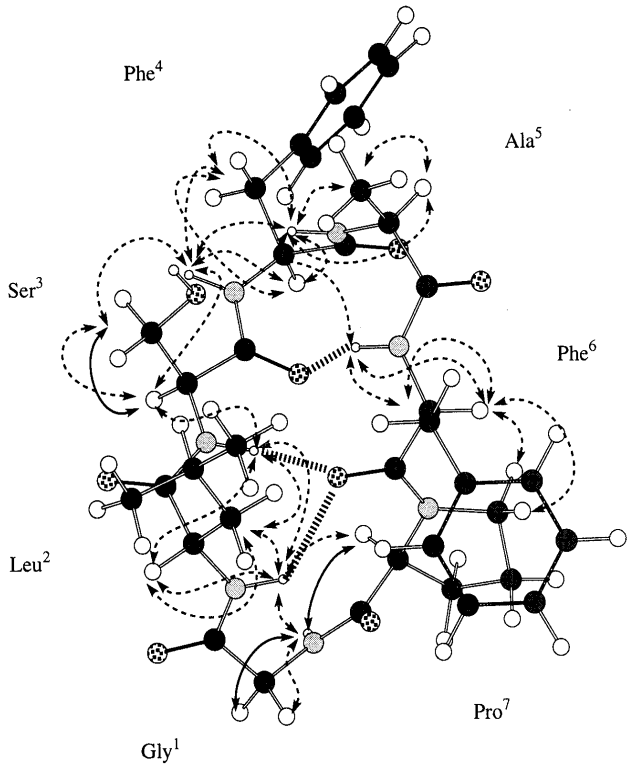

Fig. 1. Proposed Conformation of Segetalin D (**1**) in Solution

Arrows show a strong ROE relationship and broken arrows show a medium or weak ROE relationship. Three thick broken lines represent intramolecular hydrogen bonds.

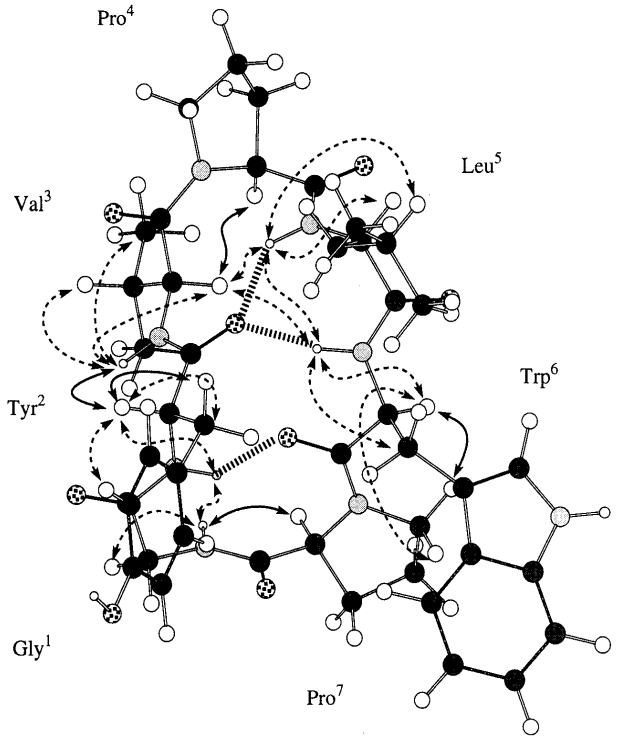

Fig. 2. Proposed Conformation of Segetalin E (**2**) in Solution

Arrows show a strong ROE relationship and broken arrows show a medium or weak ROE relationship. Three thick broken lines represent intramolecular hydrogen bonds.

protons are involved in intramolecular hydrogen bonds. In addition, Ala^5–NH is weakly hydrogen bonded with the side chain oxygen in Ser^3, and moreover, it is shielded

Table 3. Results of Distance Geometry Calculations for Segetalins D (**1**) and E (**2**)

Structural parameters	Segetalin D (**1**)	Segetalin E (**2**)
No. of constraints		
distance	35	33
torsion	7	7
No. of calculated conformers	287	279
No. of converged conformers[a)]	22	62
Mean energy (kcal/mol)	64.0752 (4.3372)	69.9309 (3.8436)
Mean RMS ROE	0.01 (0.0006)	0.02 (0.0005)
RMSD for backbone heavy atoms of means structures (Å)	0.41 (0.25)	0.26 (0.14)

a) The number of the produced conformers whose pairwise RMSD for backbone heavy atoms is less than 0.50.

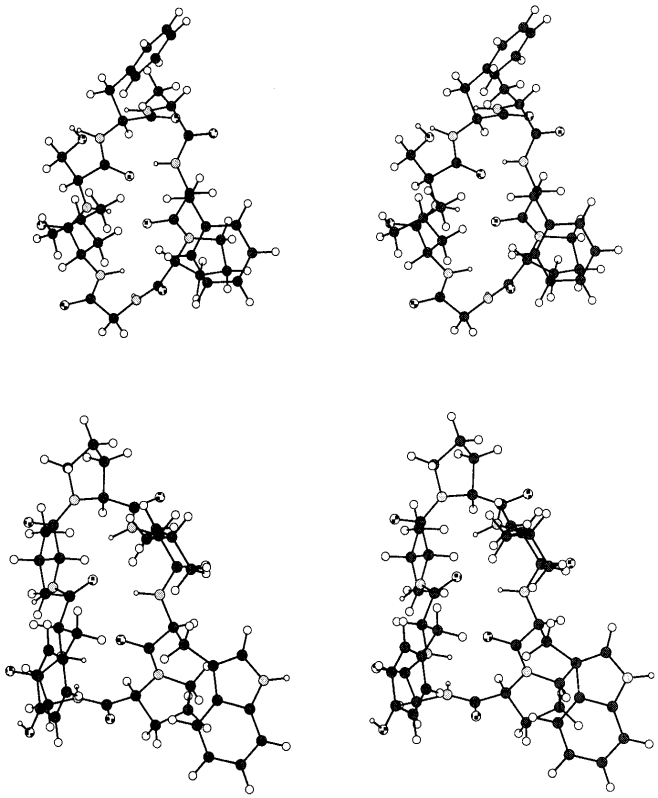

Fig. 3. Stereoscopic Views of the Mean Structures of Segetalins D and E Obtained by DG Calculation

Above: segetalin D; Below: segetalin E.

by the aromatic ring of Phe^4, corresponding to the temperature coefficient (2.0 ppb/K). The presence of a β-bulge unit, originally defined by Richardson,[14] at the Leu^2–Ser^3 residues, is caused by the two hydrogen bonds between Ser^3–NH and Phe^6–CO, and between Leu^2–NH and Phe^6–CO. This type of β-bulge is a classic type, according to Richardson, from ϕ, ψ angles of Leu^2 and Ser^3 (Leu^2: ϕ −84.7, ψ −63.2; Ser^3: ϕ −169.8, ψ −175.0). Both of the side chain conformations of Phe^4 and Phe^6 were indicated to be *trans* in the mean structure.

Segetalin E The backbone heavy atomic RMSDs between the individual structures and the mean coordinate position are 0.26 Å. The mean structure adopts a type VI β turn between Val^3 and Pro^4 residues in the *cis* con-

Table 4. Backbone Dihedral Angles in the Mean Structures of Segetalins D and E Calculated from Distance Geometry Calculations

Residues	Segetalin D[a]			Residues	Segetalin E[b]		
	ϕ	ψ	ω		ϕ	ψ	ω
Gly^1	73.9	−78.3	−164.9	Gly^1	88.4	−26.4	−179.8
Leu^2	−84.7	−63.2	−180.0	Tyr^2	−116.5	63.1	180.0
Ser^3	−169.8	−175.0	−177.3	Val^3	−67.2	134.7	180.0
Phe^4	−46.9	−38.4	179.9	Pro^4	−96.4	23.3	4.6
Ala^5	−73.3	−22.5	180.0	Leu^5	−88.9	−54.2	−173.6
Phe^6	−160.8	123.0	180.0	Trp^6	−152.8	163.2	−174.6
Pro^7	−60.2	152.8	−173.0	Pro^7	−46.3	124.6	177.4

a) The mean values of 22 converged conformers of segetalin D. *b*) The mean values of 62 converged conformers of segetalin E.

Table 5. Intramolecular Hydrogen Bonds in Mean Structures of Segetalins D and E

Compound	From	To	Distance (Å)	Angle (°)[a]
Segetalin D	Phe^6–NH	Ser^3–CO	1.918	153.3
	Ser^3–NH	Phe^6–CO	1.832	171.2
	Leu^2–NH	Phe^6–CO	2.379	128.9
Segetalin E	Tyr^2–NH	Trp^6–CO	1.861	145.4
	Trp^6–NH	Tyr^2–CO	1.907	167.8
	Leu^5–NH	Tyr^2–CO	1.888	139.7

a) Angles for N–H···O.

Table 6. Temperature Coefficients, $-d\delta/dT$ (10^{-3} ppm/K), of NH Protons of Segetalins D (**1**) and E (**2**) in Ten Intervals over the Range of 300—330 K in DMSO-d_6

Compounds	Gly^1	Leu^2	Ser^3	Phe^4	Ala^5	Phe^6
Segetalin D (**1**)	5.7	2.0	3.6	5.0	2.0	0.3

	Gly^1	Tyr^2	Val^3	Leu^5	Trp^6
Segetalin E (**2**)	7.7	3.3	5.8	2.9	−1.1

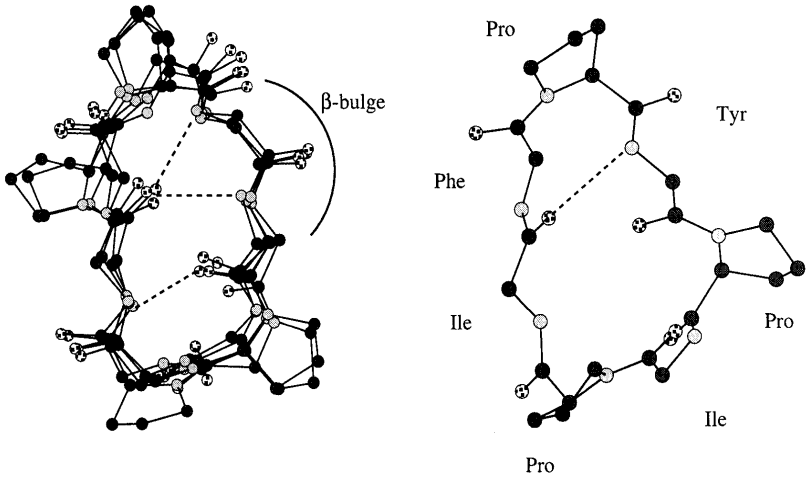

Fig. 4. Backbone Structures of Cyclic Heptapeptides

Left: superimposed structures of segetalins D, E, pseudostellarin D, yunnanin A, evolidine and stylostatin 1. Pairwise backbone RMSD is 0.620 (0.167); Right: structure of phakellistatin 1. Only backbone heavy atoms including a proline ring are shown. Broken lines represent intramolecular hydrogen bonds.

figuration, and a type IIβ turn between Pro^7 and Gly^1 residues. The mean structure shows three intramolecular hydrogen bonds. Two of them are involved in 4→1 hydrogen bonds between Tyr^2–NH and Trp^6–CO, and between Leu^5–NH and Tyr^2–CO at two β-turn structures, as shown in Table 5 (type II and type VI, respectively). The temperature coefficients also correspond to the above hydrogen bonds (Table 6). An additional hydrogen bond is suggested to exist between Trp^6–NH and Tyr^2–CO, which is consistent with the low temperature gradient of the Trp^6–NH in Table 6. The backbone conformation of segetalin E contains a β bulge unit like that of segetalin D, formed by two consecutive β-type hydrogen bonds, including two residues. The type of this β-bulge is also a classic type from ϕ, ψ angles of Leu^5 and Trp^6 (Leu^5: ϕ −88.9, ψ −54.2; Trp^6: ϕ −152.8, ψ 163.2). Both of the side chain conformations of Tyr^2 and Trp^6 were indicated to be *gauche-* and *trans*, respectively, in the mean structure.

DG and SA calculations, which considered ^{1}H-NMR information (ROE effects), led to a proposal of the solution conformation for segetalins D and E. The conformational preference of the two cyclic heptapeptides, segetalins D and E, indicates that each has two β-turns, a β II turn at Pro^7–Gly^1 and a β I turn at Phe^4–Ala^5 for segetalin D, and a β II turn at Pro^7–Gly^1 and a β VI turn at Val^3–Pro^4 for segetalin E, respectively, both of which possess a β-bulge motif.

These conformational characteristics of segetalins D and E were also observed in pseudostellarin D[3] and yunnanin A[4]: these may be favorable and common features for cyclic heptapeptides consisting of all L amino acids,

whether or not the peptide contains a *cis* amide bond. Cyclic heptapeptides, such as yunnanin A, pseudostellarin D, evolidine and stylostatin, have different types of β-turns, but they have almost the same pattern of three intramolecular hydrogen bonds constituting a β-bulge motif. Superposition of the X-ray structures of pseudostellarin D, yunnanin A, evolidine,[9] stylostatin 1,[17] and the structures of segetalins D and E obtained by DG calculations are shown in Fig. 4. Their pairwise backbone RMSD is 0.620 (0.167). However, in the case of a proline residue being present at the position of the β-bulge unit, as in phakellistatin 1: *cyclo* (–Ile–Pro–Ile–Phe–Pro–Tyr–Pro–Ile–),[18] the lack of intramolecular hydrogen bond resulted in the lack of a β-bulge motif (Fig. 4).

Experimental

Material Segetalins D and E were isolated from the seeds of *Vaccaria segetalis* according to the method described previously.[12]

NMR ^{1}H and ^{13}C-NMR spectra were recorded on Varian Unity 400 spectrometers. Each 10 mg of segetalins D and E in a 5 mm tube (0.5 ml DMSO-d_6, degassed) was used for the homonuclear and heteronuclear measurements. The spectra were recorded at 300 K. A phase sensitive ROESY experiment was made with a mixing time of 200 msec. The temperature effect on NH chemical shifts was measured to assess the solvent accessibility to the amide protons at 10 intervals, over the range of 300—330 K, using linear regression analysis.

Computational Methods Computer modeling and all calculations were carried out using the molecular-modeling software package SYBYL ver. 6.22 (Tripos, Inc., St. Louis, MO) on an IRIS 4D computer. Molecular mechanics and SA calculations were performed with the AMBER all-atom force field.[15] The dielectric constant (ε) was assumed to be proportional to the interatomic distances (r) as $\varepsilon = r$. Solvent molecules were not included in the calculations. The ROE relationships shown in Figs. 1 and 2 were taken into account in calculating the constrained minimizations and dynamics, with an extra harmonic term of the form $E = 1/2k(d - d^{low})^2$ for $d < d^{low}$, $1/2k(d^{high} - d)^2$ for $d^{high} < d$ and $E = 0.0$ for $d^{low} \leq d \leq d^{high}$ added to the force field [$k = 200$ kcal/(mole)(°)^2]. Torsion constraints with an extra harmonic form of the form $E = 1/2k(\omega - \omega^0)^2$ [$k = 0.01$ kcal/(mole)(Å^2)] were also added to the force field. In SA simulation, each system was equilibrated for 5000 fs in a thermal bath at 800 K, and thereafter, successively, for 2700 fs, the temperature was decreased 54 times until a final temperature of 100 K was reached. 287 and 279 conformers for **1** and **2**, respectively, which were frozen at 100 K, were finally minimized. The converged groups for **1** and **2** were selected as those whose pairwise backbone RMSD is less than 0.50. Each energy minimization was carried out until the derivatives became less than 0.01 kcal·mol^{-2}.Å^{-1} using the MAXMIN program.

Acknowledgments The authors thank the Uehara Memorial Foundation for financial support for the Encouragement of Young Scientists. We wish to thank Mr. Hiroshi Yamashita, Sumisho Electronics Co., Ltd., for support of the molecular simulation study, and Prof. George R. Pettit, Department of Chemistry, Arizona State University, for supplying of X-ray data of phakellistatin 1 and stylostatin 1.

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