Supplementary Material Available: Tables of crystal data, atomic coordinates, bond lengths and angles, and anisotropic displacement parameters of **4a**, **22a**, and **25**; ^{1}H NMR spectra of the new compounds; final results of PM3 calculation; data of NOESY of the compounds **6**, **11a**, **11b**, **12d**, **15a**, **15c**, **15d**, **18**, **19**, **20**, **22b**, **46**, **47**, **50**, **51**, and **52** (155 pages); tables of observed and calculated structure factors (42 pages). Ordering information is given on any current masthead page.

Prediction of the Best Linear Precursor in the Synthesis of Cyclotetrapeptides by Molecular Mechanic Calculations

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Abstract: Small cyclopeptides of four to six residues are of great interest because of their biological properties (drugs, insecticides, etc.). Identification of a good precursor that is able to undergo cyclization is very important because of the lengthy synthesis of the linear precursors. Several factors are involved in the cyclization yield. In order to determine the relative influence of each factor in this reaction, molecular modeling calculations were performed using the GenMol program. The transition-state energy rather than the dimerization reaction is the determining factor in ring closure, as evidenced by calculations performed on 4-Ala-chlamydocin, HC-toxin analogues, cyclotetrapeptides of sarcosine with glycine, and 4-Ala-chlamydocin and Cyl 2 analogues.

Introduction

Small cyclopeptides of four to six residues are of great interest because of their specific properties:

(i) Cyclization of a peptide reduces the number of allowed conformations. It is of interest to determine the structure of the receptor sites of the resulting rigid molecules after identifying the active conformations.[1-3]

(ii) Cyclic peptides are more resistant "in vivo" because they are not recognized by the exoproteases. The absence of charged extremities facilitates the crossing of lipid membranes, leading to better bioavailability which can present some advantages in therapeutic applications. The cyclization of a linear biologically active peptide often leads to a derivative drug which is more specific and sometimes more efficient.[4,5]

(iii) Most natural cyclopeptides or cyclodepsipeptides present interesting biological properties. The following examples illustrate the diversity of their structures and of their biological activities: gramicidin S[6,7] is an antibiotic, dolastatin 3[8,9] is one of the most powerful antineoplastics known, peptides of the destruxin family[10,11] are very efficient insecticides, tentoxin[12-15] and HC-toxin[16-19] are phytotoxins whilst another phytotoxin, chlamydocin,[20-24] exhibits cytostatic and cancerostatic properties.

Cyclization is the limiting step in small cyclopeptide synthesis. The various techniques suggested in the literature[25,26] are often unsatisfactory. Competition between dimerization and cyclization can reduce the monocyclopeptide yields.

The rigidity of the linear precursor gives rise to the difficulty in cyclization. Peptide bonds possess strong π character and preferentially adopt a transoid conformation. The linear precursor is elongated with the terminal acid and amine functions in remote positions, this being unfavorable to intramolecular coupling.

Some structural features are observed in natural cyclopeptides. (i) The presence of N-substituted amino acids (imino acids) reestablishes the transoid–cisoid equilibrium. An experimental result concerning cyclization of tetraglycine[27] and tetrasarcosine[28] under the same conditions confirms this effect. The cyclomonomer yields are 5% and 43%, respectively. (ii) D and L amino acids in

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(1) Kessler, H.; Kutscher, B. *Tetrahedron Lett.* **1985**, *26*, 177–180.
(2) Chippens, G. I.; Mutilis, F. K.; Myshlyakova, N. V.; Musina, F. R.; Vitolina, R. O.; Klusha, V. J.; Katayev, B. S. *Int. J. Pept. Protein Res.* **1985**, *26*, 460–468.
(3) Spear, K. L.; Brown, M. S.; Reinhard, E. J.; Mc Mahon, E. G. *J. Med. Chem.* **1990**, *33*, 1935–1940.
(4) Charpentier, B.; Dor, A.; Roy, P.; England, P. *J. Med. Chem.* **1989**, *32*, 1184–1190.
(5) Spanevello, R. A.; Hirschmann, R.; Raynor, K.; Reisine, T.; Nutt, R. F. *Tetrahedron Lett.* **1991**, *32*, 4675–4678.
(6) Ovchinnikov, V. A.; Ivanov, V. T. *Tetrahedron Lett.* **1975**, *31*, 2177–2209.
(7) Izumiyia, N.; Kato, T.; Waki, M. *Biopolymers* **1991**, *20*, 1785–1791.
(8) Schmidt, U.; Utz, R.; Lieberknecht, A.; Griesser, H.; Potzoli, B.; Bhar, J.; Wagner, K.; Fischer, P. *Synthesis* **1987**, 233–236 and 236–241.
(9) Holzapfel, C. W.; Van Zyl, W. J. *Tetrahedron* **1990**, *46*, 649–660.
(10) Pais, M.; Das, B. C.; Ferron, P. *Phytochemistry* **1981**, *20*, 715–723.
(11) Morel, E.; Pais, M.; Turpin, M.; Guyot, M. *Biomed. Pharmacother.* **1983**, *37*, 184–185.
(12) Rich, D. H.; Mathiaparanam, P. *Tetrahedron Lett.* **1974**, *46*, 4037–4040.
(13) Rich, D. H.; Bathnagar, P. K.; Grant, J. A.; Tam, J. P. *J. Org. Chem.* **1978**, *43*, 296–302.
(14) Jacquier, R.; Verducci, J. *Tetrahedron Lett.* **1984**, *25*, 2775–2778.
(15) Heitz, F.; Jacquier, R.; Kaddari, F.; Verducci, J. *Biophys. Chem.* **1986**, *23*, 245–249.
(16) Kawai, M.; Rich, D. H.; Walton, J. D. *Biochem. Biophys. Res. Commun.* **1983**, *111*, 398–404.
(17) Kawai, M.; Rich, D. H. *Tetrahedron, Lett.* **1983**, *24*, 5309–5312.
(18) Jacquier, R.; Lazaro, R.; Ranirisehenо, H.; Viallefont, P. *Tetrahedron Lett.* **1986**, *27*, 4735.
(19) Jacquier, R.; Lazaro, R.; Ranirisehenо, H.; Viallefont, P. *Int. J. Pept. Protein Res.* **1987**, *30*, 22–32.
(20) Rich, D. H.; Gardner, J. *Tetrahedron Lett.* **1983**, *24*, 5305–5308.
(21) Schmidt, U.; Beutler, T.; Lieberknecht, A.; Griesser, H. *Tetrahedron Lett.* **1983**, *24*, 3573–3576.
(22) Schmidt, U.; Lieberknecht, A.; Griesser, H.; Bartowiak, F. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 318–320.
(23) Schmidt, U.; Lieberknecht, A.; Griesser, H.; Utz, R.; Beutler, T.; Bartowiak, F. *Synthesis* **1986**, 361–366.
(24) Kawai, M.; Gardner, J. H.; Rich, D. H. *Tetrahedron Lett.* **1986**, *27*, 1877–1880.
(25) Spatola, A. F.; Anwer, M. K.; Rockwell, A. L.; Gierash, L. M. *J. Am. Chem. Soc.* **1986**, *108*, 825–831.
(26) Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.* **1991**, *32*, 2639–2642.

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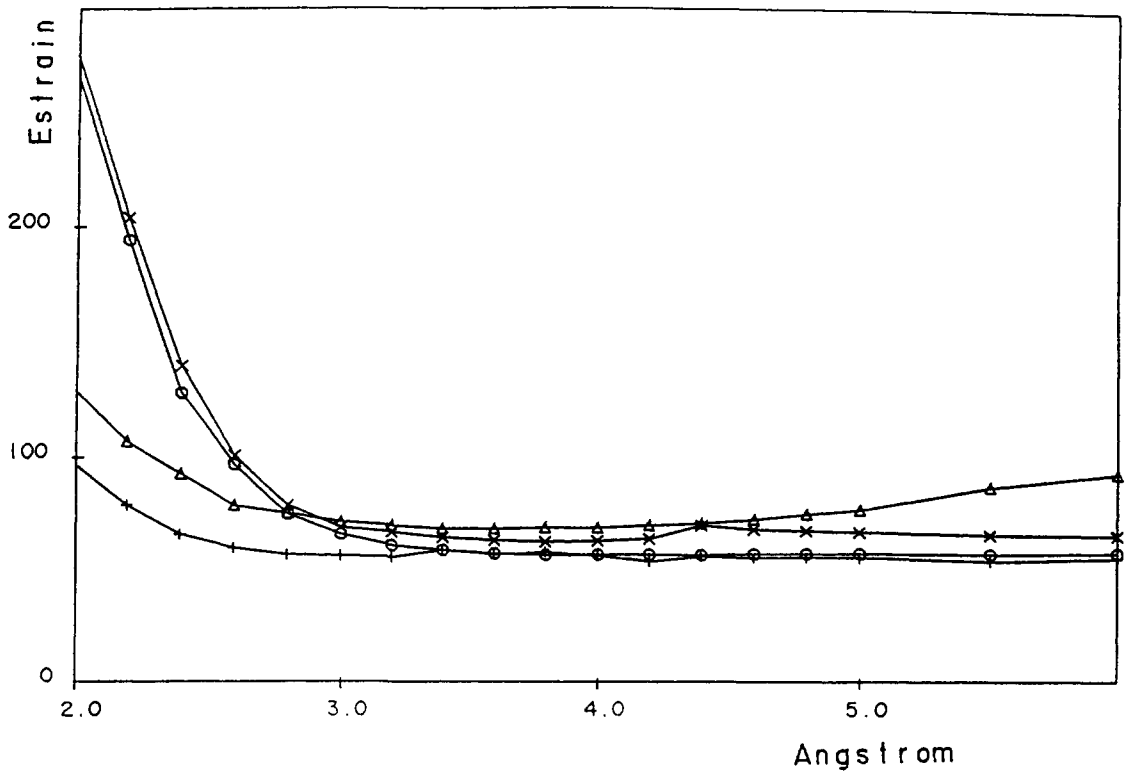

Figure 1. Reaction path for the 4-Ala-chlamydocin ring closure: (O) Phe-D-Pro-Ala-Aib-*O*-Ph; (+) D-Pro-Ala-Aib-Phe-*O*-Ph; (×) Ala-Aib-Phe-D-Pro-*O*-Ph; (Δ) Aib-Phe-D-Pro-Ala-*O*-Ph.

alternate positions[19] seem also to favor cyclization.

The ring closure site is another important factor with respect to cyclization yield. The choice of the precursor linear peptide sequence is all-important but very difficult to rationalize. In the case of cyclotetrapeptides, four linear sequences are possible: ABCD, BCDA, CDAB, and DABC. Generally only one will cyclize in acceptable yield, but it is not possible to predict "a priori" which will be the most advantageous. A few literature examples[19,20,29] show the importance of the ring closure site, but without any explanation, except in the case of Bhatnagar,[30] who tried to define the parameters responsible for the ability of a peptide to cyclize. From the tentoxin example, he suggested the need of a central cisoid bond, induced by the presence of an imino acid in the precursor at position 2. The results obtained in the 4-Ala-chlamydocin[29] synthesis contradict this hypothesis, since they clearly indicate that the unique imino acid of the sequence must be located at position 4 (C-terminal) to give the cyclotetrapeptide.

Prediction of the sequence best able to cyclize would be of great interest in order to reduce the amount of work needed in precursor synthesis. In order to shed some light on this problem, modeling calculations were performed using the GenMol program.[31]

Chlamydocin was chosen as the example because experimental data are available for this compound, these being the structural data (X-ray)[32] and experimental cyclization yields of all the precursors of the analogue 4-Ala-chlamydocin,[29] in which the Aoe group is replaced by an alanine residue (chlamydocin = c[Aib-Phe-D-Pro-Aoe]; 4-Ala-chlamydocin = c[Aib-Phe-D-Pro-Ala]; Aib = aminoisobutyric acid; Aoe = 2-amino-8-oxo-9,10-epoxydecanoic acid).

Chlamydocin Cyclization Study

In order to check the calculation coherence, we verified that substitution of the Aoe chain by a methyl group does not significantly modify the cycle geometry. The rms between the two structures (the corresponding parts) is 0.035 Å after relaxation (rms is the root mean square of the distances between equivalent atoms after performing a best molecular fit on the two molecules).

Table I. Energies[40] and Geometries of the 4-Ala-Chlamydocin Precursors

compd[a]	E_{bcy}[b]	E_{rel}[c]	ΔE[d]	rms[e]	d_{bcy}[f]	d_{rel}
1	75.4	55.2	20.2	0.3	3.81	4.27
2	73.9	54.4	19.5	1.8	4.08	7.56
3	66.8	54.5	12.3	1.3	3.89	4.31
4	67.7	52.6	15.1	1.3	4.51	5.67

[a] (1) Phe-D-Pro-Ala-Aib-*O*-Ph; (2) D-Pro-Ala-Aib-Phe-*O*-Ph; (3) Ala-Aib-Phe-D-Pro-*O*-Ph; (4) Aib-Phe-D-Pro-Ala-*O*-Ph. [b] E_{bcy} (kcal/mol) represents the energy of the conformation before the ring closure. [c] E_{rel} (kcal/mol) is the energy of the conformation when completely relaxed. [d] $\Delta E = E_{bcy} - E_{rel}$. [e] rms = root mean square between the two geometries after performing a best molecular fit. [f] d (Å) is the distance between N- and C-terminal atoms.

Several factors can influence the ability of a precursor to cyclize: steric hindrance to ring closure, kinetic competition with the dimerization reaction, and transition-state energy. We looked for specific calculations which would represent these different contributions in order to find the determining factor in ring closure, as confirmed by the experimental data.

1. Steric Hindrance to Ring Closure. (a) All the peptide bonds of chlamydocin, with their known X-ray[32] geometries, are broken successively in order to obtain the four precursors in their folded conformations before cyclization. The extremities of each linear peptide are progressively moved further away in 0.2-Å steps; at each step the molecule is relaxed. This was done from 2.8 Å (the shortest distance without atom interpenetration) to 6 Å (from 6 to 11 Å the energy stays the same); the results are displayed in Figure 1. We do not observe any significant variation of the strain energy able to explain the cyclization behavior of the various precursors.

(b) The conformation before cyclization is obtained as described previously. The terminal acid function is transformed into a phenolic ester in order to model the activating group. The phenyl group was chosen because of the steric hindrance of its electronic

(27) Titlestad, K. *Acta Chem. Scand.* **1977**, *B31*, 641–661.
(28) Dale, J.; Titlestad, K. *J. Chem. Soc. D* **1969**, 656–659.
(29) Pastuszac, J.; Gardner, J. H.; Singh, J.; Rich, D. H. *J. Org. Chem.* **1982**, *47*, 2982–2987.
(30) Bhatnagar, P. K. Ph.D. Thesis, University of Wisconsin–Madison, 1977.
(31) Pèpe, G.; Siri, D. *Stud. Phys. Theor. Chem.* **1990**, *71*, 93–101.
(32) Flippen, J. L.; Karle, I. L. *Biopolymers* **1976**, *15*, 1081–1092.

Table II. Association Energies in 4-Ala-Chlamydocin Dimers

dimer	EnT[a]	EnC[b]
1,1	−105	−94
2,2	−108	−105
3,3	−104	−96
4,4	−100	−83

[a] EnT = nonbonded interactions between the two molecules in kcal/mol. [b] EnC = Coulombic contribution to this energy (kcal/mol).

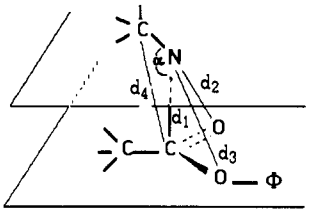

Figure 2. Geometry of the transition state under the constraints imposed in the calculations.

cloud, this being well representative of the majority of the activating groups used in peptide synthesis (trifluorophenol, pentafluorophenol, succinimide, etc.). E_{bcy} is the energy value for each molecule so modified, and E_{rel} is the new energy value after complete relaxation[33] (Table I). The difference between these two energies, which can be likened to an activation energy, was computed, and then the geometries were compared. Precursor **3** is best; the results indicate the lowest activation energy for this precursor (12.3 kcal/mol), and the molecule extremities remain close (4.3 Å instead of 3.9 Å). However, these results are not sufficiently different to provide a satisfying and general conclusion for the reasons indicated below.

2. Kinetic Competition with the Dimerization Reaction. The association of two charged molecules corresponding to the energy minimum was performed with the help of the Dock program[34] associated with GenMol. The nonbonded interactions of the complex (van der Waals, hydrogen bond, and electrostatic) were computed (Table II). As there is no energy barrier for the complex formation, this energy can be correlated to the entropy of the dimerization reaction. These energy values clearly indicate no preferential association for any precursor.

3. Transition-State Energy. The cyclization reaction involves nucleophilic attack by the terminal amine on the carboxylic group. The geometry of the best association N···C=O preceding the addition has been observed for other nonpeptide compounds in the crystal state.[35] This extremely precisely known and stable geometry led us to model the transition state as follows: the distances *d*1, *d*2, *d*3, and *d*4 were given the values 2.8, 3.05, 3.12, and 3.17 Å respectively, as displayed in Figure 2. These values were chosen in order to have an α value close to 90°, neglecting the carboxylate group deformation observed in the crystalline state.[35]

This geometry was imposed for each precursor of 4-Ala-chlamydocin built up directly from the sequence, and the corresponding strain energy was computed for the charged forms (Table III, section A). These forms were chosen in order to illustrate the nucleophilic and electrophilic partial charges of the transition state. This geometry was then relaxed by complete force field calculations[33] (section B) and optimized by rotation around all the molecule pivots,[33] followed by a new complete force field calculation in order to be sure that the preferred conformation of the molecule had been obtained (section C). The final results gathered in Table III clearly indicate that precursor **3** (the best one) is the least strained in the transition state (61.7 kcal/mol instead of 129, 106, 103, and 92.7 kcal/mol for the other precursors, respectively). The strain energy of the transition state of the precursor able to cyclize is close to the energy of the relaxed geometry, which means it will reach this state easily.

The energy difference between the transition state (section A) and the relaxed state ΔE is the lowest for this precursor (14 kcal/mol instead of 55, 54, and 54 kcal/mol for precursors **1**, **2**, and **4**, respectively. Compound **5** is equivalent to **3** after inverting the alanine absolute configuration, and so in this case too, the comparison of ΔE provides an explanation for the experimental results.

Table III. Energies in kcal/mol and Geometric Parameters in Å of the Transition States of the 4-Ala-Chlamydocin Precursors

	compd				
	1	**2**	**3**	**4**	**5**[a]
A. Charged Forms in Strained State					
En	129	106	61.7	103	92.7
*d*1	2.77	2.71	2.76	2.78	2.75
*d*2	3.05	3.07	3.08	3.03	3.08
*d*3	3.14	3.12	3.13	3.12	3.12
*d*4	3.24	3.23	3.22	3.25	3.24
α (deg)	94.5	96.3	94.7	94.5	95
B. Molecules Relaxed by Complete Force Field Calculations[33]					
En	78.4	52.7	46.9	51.4	45.8
*d*1	3.23	2.95	2.93	3.03	2.93
*d*2	3.22	3.70	3.22	3.03	3.18
*d*3	3.71	3.14	3.33	3.44	3.37
*d*4	3.60	3.70	3.57	3.95	3.80
α (deg)	91.6	108.3	102	116.2	114
C. Molecules Relaxed by Rotation around Pivots[33] Followed by a Complete Force Field Calculation					
En	73.7	52.0	47.6	48.8	46.8
*d*1	3.06	2.94	2.97	2.98	2.93
*d*2	3.13	3.74	3.15	3.22	3.12
*d*3	3.31	3.04	3.46	3.50	3.38
*d*4	3.98	3.79	3.66	4.12	3.81
α (deg)	117	113.7	105	134	115
ΔE	55	54	14	54	46
cyclizatn yield (%)	2	3	45	3	5

[a] (5) D-Ala-Aib-Phe-D-Pro-*O*-Ph.

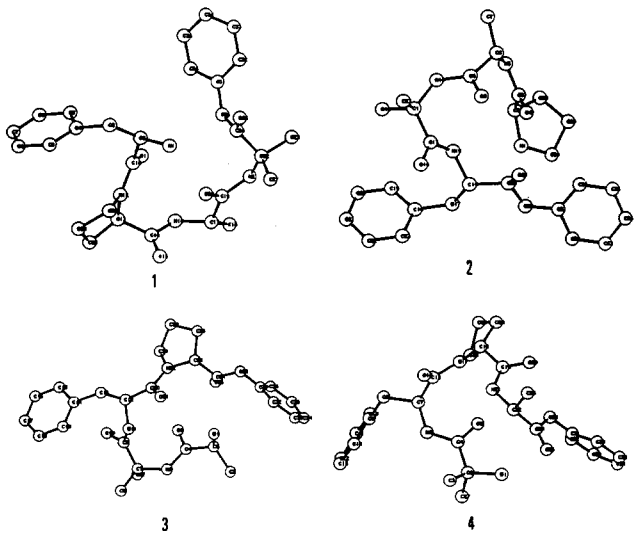

Figure 3. Ortep projections of the four precursors of 4-Ala-chlamydocin.

(33) Note: The GenMol program allows one to choose between two different algorithms to find a good molecule geometry. The complete force field calculation corresponds to the minimization of the total strain energy, involving all the molecule parameters, by the conjugate gradient method. This algorithm using derivative calculations in the same way as in Newton algorithms gives the minimum which is closest to the starting point and does not allow escape from either this local energy minimum or an energy plateau. In contrast, the "rotation around pivots" algorithm using energy differences rather than energy derivative differences allows complete modification of the folding of a molecule. It defines a hierarchy of the rotations around pivots by modifying only torsion angle values, thus giving the geometry corresponding to the overall energy minimum. The hierarchy of the rotations is determined from local energy calculations which correspond to the nonbonded interactions between the two parts of the molecule located on the extremities of each pivot.

(34) For more information, contact: Pèpe, G. CRMC2-CNRS Marseille, France.

(35) Bürgi, H. B.; Dunitz, J. D.; Shepter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065–5067.

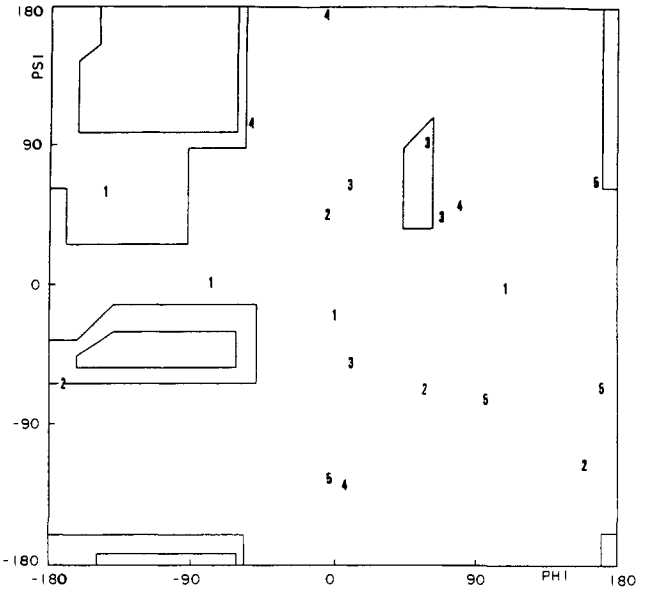

Figure 4. Ramachandran plot of the 4-Ala-chlamydocin precursors chain folding displaying homogeneous Ψ and φ values for precursor **3**, as observed in Figure 3.

Table IV. Details of the Energy Contributions (kcal/mol) to the Total Strain Energy of the Transition State and the Relaxed Geometries Displayed in Table III[a]

	compd				
	1	**2**	**3**	**4**	**5**
A. Charged Forms in Strained State					
E_{vdw}	85.20	80.48	33.60	68.10	63.70
E_H	−1.04	−1.78	−0.71	−1.09	−0.42
E_C	19.40	4.87	1.49	4.46	3.05
E_S	3.53	3.12	2.92	3.36	3.60
E_T	10.73	6.51	14.68	10.60	10.90
E_B	11.10	13.00	9.72	17.50	11.90
C. Molecules Relaxed by Rotation around Pivots[33] Followed by a Complete Force Field Calculation					
E_{vdw}	36.90	23.80	22.80	22.39	19.70
E_H	−1.09	−1.08	−1.12	−1.28	−0.47
E_C	17.40	5.87	2.02	3.61	1.82
E_S	2.47	3.38	2.86	2.72	3.48
E_T	9.20	6.53	11.75	11.40	11.30
E_B	8.82	13.50	9.33	9.94	10.60

[a] Parts A and C of this table correspond to parts A and C of the other tables in this paper. The data presented in part B of the other tables is not relevant in this case.

In Table III where the geometries of the associations between the nitrogen atoms and the carboxylic groups are given, we do not observe significant differences between the precursor geometries when they are relaxed. In Figure 3, Ortep[36] projections of the four precursors of chlamydocin indicate the greatest regularity of the principal chain geometry for precursor **3**, making it the best one. This regularity is confirmed by the Ψ and φ angles, the values of which are in the same range, characteristic of the chain folding displayed in the Ramachandran plot in Figure 4. The energy differences between the transition and the relaxed state come essentially from the van der Waals contributions as shown in Table IV, which means the molecule in the transition state is not distorted at the angle level (the valency and the torsion). As all the calculations are performed in vacuo, the solvation effect was estimated.[34] We calculated the interaction energy between the precursors of 4-Ala-chlamydocin and the pyridine molecule, which corresponds to the solvent used in the experiment. The strongest interactions involve the closure site and remain almost the same

(36) Johnson, C. K. ORTEP. Report ORNL-3794; Oak Ridge National Laboratory: Oak Ridge, TN, 1965.

Table V. Energies in kcal/mol and Geometric Parameters in Å of the Transition States of the HC-Toxin Precursors

	compd[a]				
	6	**7**	**8**	**9**	**10**
A. Charged Forms in Strained State					
En	99.2	113	206	64.4	87.9
*d*1	2.74	2.78	2.71	2.76	2.75
*d*2	3.08	3.06	3.06	3.06	3.09
*d*3	3.14	3.09	3.13	3.14	3.13
*d*4	3.24	3.27	3.20	3.23	3.21
α (deg)	94.8	95.4	94.8	93.6	94
B. Molecules Relaxed by Complete Force Field Calculations[33]					
En	62.1	62.9	58.0	54.3	51.9
*d*1	2.90	3.11	2.79	2.90	2.97
*d*2	3.10	3.37	3.64	3.00	3.05
*d*3	3.57	2.88	2.90	3.31	3.19
*d*4	3.80	3.90	3.32	3.81	3.71
α (deg)	114.8	110	97	111	107
C. Molecules Relaxed by Rotation around Pivots[33] Followed by a Complete Force Field Calculation					
En	61.9	60.2	55.7	50.8	51.8
*d*1	2.95	3.07	2.73	2.93	3.04
*d*2	3.08	3.12	3.48	3.17	2.97
*d*3	3.73	2.91	2.90	3.13	3.39
*d*4	3.94	3.86	3.38	3.86	3.89
α (deg)	120	110	102	113	113
Δ*E*	37	53	150	13	36
cyclizatn yield (%)	1	1	1	45	3

[a] (**6**) D-Ala-Ala-D-Pro-Ala-*O*-Ph; (**7**) Ala-D-Pro-Ala-D-Ala-*O*-Ph; (**8**) D-Pro-Ala-D-Ala-Ala-*O*-Ph; (**9**) Ala-D-Ala-D-Pro-*O*-Ph; (**10**) Ala-D-Ala-D-Ala-D-Pro-*O*-Ph.

in the transition state (−3.9 kcal/mol) as in the relaxed state (−5.0 kcal/mol). The same calculations performed with the DMF molecule, a common solvent for such cyclization, give −5.0 and −4.9 kcal/mol, respectively. These results allow us to neglect the solvation effect to a first approximation for this family of compounds.

Extension of the Study to Other Cyclotetrapeptides

In order to extend the study, the calculations which gave results in agreement with experimental data (those described in parts 1b and 3 of the preceding section) were applied to other cyclotetrapeptides, for which the cyclization yields of all the precursors were known. The calculations described in part 1b were abandoned because they did not allow prediction of the competent precursor for all the other examples examined from the generated geometries (X-ray structures are not available). For this reason only the transition-state energy, the significant factor in ring closure, was considered for these tetrapeptides. We also applied calculations of the type described in part 3 of the preceding section to peptides where the absolute configuration of an amino acid is inverted.

1. HC-Toxin Analogues. (a) Results concerning HC-toxin analogues (Aoe replaced by Ala) are reported in Table V; precursors **6**, **7**, **8**, and **9** correspond to the four closing sites, and compound **10** is equivalent to precursor **9** after inverting the alanine absolute configuration. The energy differences between the transition state and the relaxed geometries are 37, 53, 150, 13, and 36 kcal/mol for these peptides, respectively. As found previously, the best experimental yield[19] (45%) for precursor **9** corresponds to the lower transition state energy (64.4 kcal/mol) and the lower energy difference Δ*E* (13 kcal/mol). Also, in the case of all these compounds, the geometries of the associations between the nitrogen atoms and the carboxylic groups do not differ significantly.

(b) In the cyclization of another HC-toxin analogue (Aoe replaced by racemic Abo, Abo = 2-amino-8-(benzyloxy)octanoic acid), experimental results show that the L-enantiomer gives the best yield;[37] the energy calculations for the transition state of this

(37) Kawai, M.; Rich, D. H. *Tetrahedron Lett.* **1983**, *24*, 5309–5312.

Table VI. Energies in kcal/mol and Geometric Parameters in Å of the Transition States of the HC-Toxin Precursors

	compd[a]	
	11	**12**
A. Charged Forms in Strained State		
En	118	169
*d*1	2.75	2.75
*d*2	3.08	3.08
*d*3	3.13	3.14
*d*4	3.22	3.23
α (deg)	94	94.5
B. Molecules Relaxed by Complete Force Field Calculations[33]		
En	64.8	64.4
*d*1	2.93	2.92
*d*2	2.97	3.05
*d*3	3.42	3.39
*d*4	3.84	3.78
α (deg)	115.5	113
C. Molecules Relaxed by Rotation around Pivots[33] Followed by a Complete Force Field Calculation		
En	60.7	60.8
*d*1	2.94	2.94
*d*2	3.00	3.00
*d*3	3.45	3.48
*d*4	4.54	3.85
α (deg)	115.2	116
Δ*E*	58	108
cyclizatn yield (%)	21	4

[a] **(11)** Ala-D-Ala-L-Abo-D-Pro-*O*-Ph; **(12)** Ala-D-Ala-D-Abo-D-Pro-*O*-Ph.

Table VII. Energies in kcal/mol and Geometric Parameters in Å of the Transition States of Three Tetrapeptides of Sarcosine in Combination with Glycine

	compd[a]		
	13	**14**	**15**
A. Charged Forms in Strained State			
En	52.2	65.5	61.1
*d*1	2.78	2.77	2.74
*d*2	3.06	3.06	3.08
*d*3	3.12	3.13	3.16
*d*4	3.21	3.21	3.23
α (deg)	95.8	93	95.2
B. Molecules Relaxed by Complete Force Field Calculations[33]			
En	39.9	39.5	38.1
*d*1	3.13	2.96	2.93
*d*2	3.48	3.10	3.65
*d*3	3.38	3.52	3.88
*d*4	4.07	3.66	3.57
α (deg)	118	105	119
C. Molecules Relaxed by Rotation around Pivots[33] Followed by a Complete Force Field Calculation			
En	34.1	38.5	38.7
*d*1	2.97	2.94	2.96
*d*2	3.42	3.16	3.64
*d*3	3.13	3.53	3.22
*d*4	4.52	3.74	3.90
α (deg)	121	110	118
Δ*E*	18	27	22
cyclizatn yield (%)	10	1	0

[a] **(13)** Sar-Gly-Gly-Sar-*O*-Ph; **(14)** Sar-Sar-Gly-Gly-*O*-Ph; **(15)** Gly-Sar-Sar-Gly-*O*-Ph.

compound are in agreement with these data. In fact it is the least strained (118 instead of 169 kcal/mol), and the Δ*E* value for precursor **11** (58 kcal/mol) is lower than the Δ*E* value for precursor **12** (108 kcal/mol) (Table VI).

2. Tetrapeptides of Sarcosine in Combination with Glycine[27] (Table VII). The lowest Δ*E* value in this case also corresponds to the unique possibility of cyclization, although the cyclization yield is low (10%).

Table VIII. Effect of the Absolute Configuration of the C-Terminal Amino Acid on Ring Closure

	compd[a]	
	16	**17**
A. Charged Forms in Strained State		
En	55.0	64.0
*d*1	2.75	2.77
*d*2	3.08	3.06
*d*3	3.13	3.12
*d*4	3.23	3.23
α (deg)	94.5	93.8
B. Molecules Relaxed by Complete Force Field Calculations[33]		
En	57.9	60.7
*d*1	2.95	2.91
*d*2	3.17	3.04
*d*3	3.54	3.39
*d*4	3.87	3.84
α (deg)	116	117
C. Molecules Relaxed by Rotation around Pivots[33] Followed by a Complete Force Field Calculation		
En	57.4	61.0
*d*1	2.96	2.96
*d*2	3.20	2.99
*d*3	3.20	3.38
*d*4	4.01	4.08
α (deg)	125	128
Δ*E*	−2.4	3.0
cyclizatn yield (%)	47	0

[a] **(16)** Gly-Phe-D-Pro-L-Ala-*O*-Ph; **(17)** Gly-Phe-D-Pro-D-Ala-*O*-Ph.

Table IX. Effect of the Absolute Configuration of the N-Terminal Amino Acid on Ring Closure

	compd[a]	
	18	**19**
A. Charged Forms in Strained State		
En	109	129
*d*1	2.83	2.79
*d*2	3.02	3.07
*d*3	3.18	3.14
*d*4	3.28	3.28
α (deg)	94	95
B. Molecules Relaxed by Complete Force Field Calculations[33]		
En	109	98.0
*d*1	4.28	3.27
*d*2	4.10	3.18
*d*3	4.18	3.66
*d*4	4.25	4.06
α (deg)	79	111
C. Molecules Relaxed by Rotation around Pivots[33] Followed by a Complete Force Field Calculation		
En	101	83.7
*d*1	4.47	3.18
*d*2	4.34	3.03
*d*3	4.12	3.90
*d*4	5.22	4.11
α (deg)	112	118
Δ*E*	7	46
cyclizatn yield (%)	50	8

[a] **(18)** D-Tyr(Me)-Ile-Pro-Leu-*O*-Ph; **(19)** L-Tyr(Me)-Ile-Pro-Leu-*O*-Ph.

3. 4-Ala-Chlamydocin Analogues[38] (Aib Replaced by Gly) (Table VIII). If the absolute configuration of the C-terminal amino acid is inverted, the cyclization experimental yield increases considerably (0% to 47%). The transition-state energy of the competent precursor (L-Ala) is lower than that of the D-Ala

(38) Rich, D. H.; Jasensky, R. D.; Singh, J. *Proceedings of the International Symposium on Neurohypophyseal Peptide Hormones and Other Biologically Active Peptides*, Chicago, IL, 1980; Elsevier: Amsterdam, The Netherlands, 1981; p 49–61.

precursor (55 kcal/mol instead of 64 kcal/mol).

4. Cyl 2 Analogues[39] (Aoe Replaced by Leu) (Table IX). If the absolute configuration of the N-terminal amino acid is inverted, in the case of D-Tyr, the transition state is the least strained (109 kcal/mol instead of 129 kcal/mol for the L-Tyr compound). Also the ΔE value is the lowest, being 7 kcal/mol for this precursor while it is 46 kcal/mol for the other one.

Conclusion

The limiting factor for small peptide cyclization is the transition-state energy. Calculations performed using the GenMol program on five cyclotetrapeptides (chlamydocin, HC-toxin, cyclotetrapeptides of sarcosine in combination with glycine, 4-Ala-chlamydocin, and Cyl analogues) clearly indicate that the best precursor is the linear peptide which is the least strained in the transition state, thus corresponding to the lowest energy barrier to be crossed in order to bring the geometry of the molecule from the preferred conformation to the transition-state geometry. Our model can predict which precursor must be chosen for obtaining the best cyclization yield. To check if the model can be generalized, we are now performing calculations on larger peptides.

Registry No. 1, 143429-86-1; **1** (dimer), 143430-04-0; **2**, 143429-87-2; **2** (dimer), 143430-05-1; **3**, 143429-88-3; **3** (dimer), 143430-06-2; **4**, 143429-89-4; **4** (dimer), 143430-07-3; **5**, 143429-90-7; **6**, 143429-91-8; **7**, 143429-92-9; **8**, 143429-93-0; **9**, 143429-94-1; **10**, 143429-95-2; **11**, 143429-96-3; **12**, 143429-97-4; **13**, 143429-98-5; **14**, 143429-99-6; **15**, 143430-00-6; **16**, 143430-01-7; **17**, 143445-99-2; **18**, 143430-02-8; **19**, 143430-03-9.

(39) Yasutake, A.; Aoyagi, H.; Kato, T.; Izumiya, N. *Int. J. Pept. Protein Res.* **1980**, *15*, 113-121.

(40) Note: the energy values are approximate (+2), and in this work we only discuss the significant differences.

Molecular Design and Chemical Synthesis of Potent Enediynes. 1. Dynemicin Model Systems Equipped with N-Tethered Triggering Devices

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Abstract: In this article the molecular design and chemical synthesis of a series of enediynes (**12–19**, Chart I) related to the dynemicin A structure and carrying N-tethered triggering devices are described. The design envisioned the [(arylsulfonyl)ethoxy]carbonyl group attached at the nitrogen atom as a triggering device for the Bergman cycloaromatization reaction because of its ability to undergo β-elimination under basic conditions, liberating the labile free amine intermediate. A number of tethering groups on the aromatic ring were also installed in these systems for future incorporation of other desirable moieties such as delivery systems and solubility enhancers. The chemical synthesis of the designed systems proceeded from the corresponding quinoline intermediates **46**, **49**, and **52** (Scheme VII) through acetylide additions to quinoline (intermolecular) and carbonyl (intramolecular) functionalities as the key steps. Bergman cycloaromatization experiments under basic and acidic conditions demonstrated the abilities of these compounds to generate benzenoid diradicals. A number of potent DNA-cleaving compounds and cytotoxic agents emerged from these studies.

Introduction

The emergence of the enediyne anticancer antibiotics (Scheme I) as an exceptionally potent class of bioactive substances combining unprecedented molecular architecture and mechanism of action elicited intensive investigations in chemistry, biology, and medicine.[1] With the exception of the neocarzinostatin chromophore (**4**),[2] whose structure and mode of action represent slight variations from those of the other members of the class [calicheamicin γ_1^1 (**2**),[3] esperamicin A_1 (**3**),[4] dynemicin A (**1**)[5]], these naturally occurring substances possess a conjugated enediyne moiety embedded in a 10-membered-ring skeleton, a delivery system (carbohydrate chains or intercalating groups), and a sensitive triggering device. Upon suitable activation, these molecules enter a fascinating cascade of reactions, central to which is a Bergman cycloaromatization (**5** → **6**, Scheme II)[6] leading to a highly reactive benzenoid diradical. The potent anticancer activity of these compounds is a consequence of DNA damage by the generated reactive species which have the ability to abstract hydrogen atoms from the deoxyribose framework of one or both strands of the genetic material. The mode of action[7] of dynemicin

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(1) For a review, see: Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387.

(2) Edo, K.; Mizuyaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331. Myers, A. G.; Proteau, P. J. *J. Am. Chem. Soc.* **1988**, *110*, 1146.

(3) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466.

(4) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461. Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462.

(5) Konishi, M.; Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Oki, T.; Kawaguchi, H.; VanDuyne, G. D.; Clardy, J. *J. Antibiot.* **1989**, *42*, 1449. Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; VanDuyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715. Shiomi, K.; Iinuma, H.; Naganawa, H.; Hamda, M.; Hattori, S.; Nakamura, H.; Takeuchi, T.; Iitaka, Y. *J. Antibiot.* **1990**, *43*, 1000.

(6) Jones, R. R.; Bergman, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 660. Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25. Lockhart, T. P.; Comita, P. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4082. Lockhart, T. P.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4091.

0002-7863/92/1514-8890$03.00/0