Tetrahedron Vol 44, No 3, pp. 895 to 924, 1988
Printed in Great Britain

0040-4020/88 $3.00 + .00
Pergamon Journals Ltd

Conformational Analysis of Constrained Dilactam-bridged Tetrapeptides

Rebecca Rone, Nick Manesis, Moises Hassan, and Murray Goodman*

Department of Chemistry, B-014
University of California, San Diego
La Jolla, California 92093

Arnold T. Hagler, David H. Kitson, and Victoria A. Roberts

The Agouron Institute
La Jolla, California 92037

(*Received in USA 27 July* 1987)

Summary

In order to probe constrained regions in polypeptides, a series of lactam-bridged derivatives were prepared:

Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe (**1**),

Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe (**2**),

Ac-L-Lys-L-Glu-NHMe (**3**),

Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe (**4**),

Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe (**5**), and

Ac-L-Glu-L-Lys-NHMe (**6**).

Our approach has been to limit mobility of the peptide backbone by coupling side chains on adjacent residues. This allows rotation about the torsion angles ψ_2 and ϕ_3 in the tetrapeptides. These model compounds, therefore, can mimic turns in polypeptides. The conformations of these peptides has been investigated by circular dichroism and nuclear magnetic resonance spectroscopy as well as via molecular dynamics simulations and energy minimization calculations. The results from these studies are consistent with the presence of a β-turn in the "LLDD LysGlu" tetrapeptide (compound **1**) and with a variety of C_7 structures for the other tetrapeptides (compounds **2**, **4**, and **5**).

*To whom correspondence should be addressed.

Introduction

One of the goals of peptide chemistry is to understand the relationship between structure and biological activity. The method of choice in our laboratory for studying secondary structure is the synthesis of compounds with constrained geometry, in order to reduce the conformational space accessible to the molecule. We then compare conformers indicated by spectroscopic results with conformers calculated by molecular simulations. A variety of experimental conditions, including changes in solvent and temperature, is used in the accumulation of the spectroscopic data. This data is compared to computer simulations of molecular dynamics and energy minima, in order to delineate the important structural features of the peptide under study. This information can then be used to elucidate probable conformations.

Synthetic oligopeptides with constrained geometry have been used in this and other laboratories as model compounds for investigating the secondary structures of polypeptides.[1–3] The constraints imposed are often designed to limit the degree of conformational freedom about the peptide backbone,[4,5] thereby aiding the interpretation of spectroscopic data by reducing the number of conformers. These peptides have usually been constrained either by incorporating bulky side groups or by cyclization through: (1) normal backbone coupling, (2) coupling backbone to side chain, or (3) introducing some "spacer" group within the ring.[6,7]

An alternate method of constraining the peptide backbone is to covalently link side chains of specific residues. These bridged peptides exist in nature and are present as disulfide bridges in cystine-containing compounds such as oxytocin,[8] vasopressin,[9] and insulin[10] and as monosulfide bridges found in lanthionine-containing peptides, i.e. nisin,[11,12] ancovenin,[12] and epidermin.[13] An exciting example of a bridged structure has been reported where the alpha C of a residue is bridged to the NH of the next residue forming a pyrrolidine (a γ-lactam). In the specific case of leutenizing hormone-releasing hormone (LH-RH) such a constrained analog led to a more-active agonist than the native LH-RH.[14]

Our approach has been to limit mobility of the peptide backbone by coupling side chains on adjacent residues through lactam bonds. Although this constrains the peptide backbone, there is still rotation about the torsion angles ψ_2 and ϕ_3 in the

tetrapeptides. This swivel-like effect can allow the peptides to adopt low energy conformers characteristic of turns found in polypeptides (i. e. β- or γ-turns). Because of this flexibility, the peptide backbone of the bridged compounds realistically mimics folded regions in naturally occurring molecules, such as valinomycin.[15] We have prepared a set of bridged compounds, incorporating lysine and glutamic acid residues, designed to introduce constraints that induce folding in the tetrapeptide derivatives:

Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe (1)
("LLDD LysGlu")

Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe (2)
("LLLL LysGlu")

Ac-L-Lys-L-Glu-NHMe (3)
("LL LysGlu")

Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe (4)
("LLDD GluLys")

Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe (5)
("LLLL GluLys")

Ac-L-Glu-L-Lys-NHMe (6)
("LL GluLys")

On the basis of energy minimization studies,[2] we selected the "LLDD" compounds for synthesis, since they had a high probability of forming turns. Although they are not strictly biomimetic they could prove useful in host-guest studies in polypeptides whose activity depends upon a flexible turn element. The dipeptides and the "LLLL" compounds were synthesized for purposes of comparison.

Of the two "LLDD" compounds, "LLDD LysGlu" (compound **1**) is indeed found to exhibit the predicted conformation, a β-turn. This is entirely in keeping with Venkatachalam's theories of β-turns for LLDD combinations of tetrapeptides,[16] and similar in nature to experimental findings for valinomycin, a naturally occurring cyclic dodecadepsipeptide with an LLDD sequence[15,16] and its synthetic peptidic analog.[18] Other peptides with LD sequences at the swivel point exhibit similar structures.[19,20] The "LLDD GluLys" tetrapeptide (compound **4**) is found to have alternate folding

patterns (i.e. C_7 structures). Both structures are represented by a hydrogen bond (1 ← 4 for β-turns or 1 ← 3 for C_7 turns) defined to be less than 2.5 Å in length and without angular dependence. This definition is consistent with the force field used, where the non-bond interaction of donor hydrogen with an acceptor is a function of distance only and not angle. It is also sufficient for our applications, since close approach of the amide proton with the carbonyl oxygen acceptor establishes solvent-shielding (shielding from the solvent) as measured in the NMR studies.

Experimental

Syntheses

The preparation of compounds **1** - **6** were carried out by conventional solution methods. The detailed experimental procedures have been accepted for publication, elsewhere.[21]

Circular Dichroism

Circular dichroism (CD) spectra were obtained on a Cary 61 spectrophotometer equipped with a 50 KHz photoelastic modulator (FS-5/PEM-80, Hinds International, Inc.) used in conjunction with a lock-in amplifier (128A, EG&G Princeton Applied Research). System automation, signal averaging, and baseline subtraction were accomplished using a DEC 11/02 computer. The system software and custom hardware interfaces were designed by Allen MicroComputer Services, Inc.

Spectra of the peptides were measured in water and 2,2,2-trifluoroethanol (TFE; Aldrich Chemical Co.). Water was purified via simple distillation of deionized water using a quartz immersion heater while TFE was purified by distilling the stock over sodium bicarbonate and collecting the fraction boiling at 72 - 73° C (ca 750 mm). The CD spectra of all the compounds used in this study (compounds **1** - **6**) were measured in TFE. In water, only spectra of "LLDD LysGlu" (compound **1**), "LL LysGlu" (compound **3**), "LLDD GluLys" (compound **4**), and "LL GluLys" (compound **6**) were obtained. The CD spectra of "LLLL LysGlu" (compound **2**) and "LLLL GluLys" (compound **5**) were not measured in water because of poor solubility. The peptides were studied at concentrations of approximately 1 - 3 mM at 20° C. The molar

ellipticities ([θ]) are given in units of deg cm^2 $decimol^{-1}$ and are normalized to the dipeptide segment.

Nuclear Magnetic Resonance

Proton NMR spectra were obtained on a 360 MHz NMR (in the Fourier-transform mode) spectrometer built in-house from a continuous-wave Varian console equipped with an Oxford superconducting magnet and a Nicolet 1280 computer. The spectrometer is equipped with a variable temperature controller and an extra frequency synthesizer for double irradiation (decoupling) experiments.

Spectra were obtained in 99.9% dimethylsulfoxide-d_6 (DMSO-d_6, MSD Isotopes), water, and TFE. Assignments were made based on one-dimensional decoupling and on two-dimensional homonuclear shift correlation, COSY[22] and relayed coherence transfer spectroscopy.[23,24]

In order to verify that the molecules were not associated, concentration studies were performed. Proton NMR spectra of "LLDD LysGlu" (compound **1**), "LL LysGlu" (compound **3**), "LLDD GluLys" (compound **4**), and "LL GluLys" (compound **6**) were acquired at concentrations of 1.0 to 45 mM in each solvent: DMSO-d_6, water, and TFE. Because of poor solubility in DMSO-d_6 and water, concentration studies of "LLLL LysGlu" (compound **2**) and "LLLL GluLys" (compound **5**) were only carried out in TFE. In DMSO-d_6, it was estimated that compounds **2** and **5** are soluble to the extent of 0.1 mg/0.4 ml DMSO-d_6 (0.45 mM). No spectra of these compounds were measured in water. During these concentration studies, the spectra showed no appreciable change in peak widths or positions. It is, therefore, concluded that aggregation does not occur under the experimental conditions of these studies.

Temperature coefficients of the amide NH groups were determined in the three solvent systems: DMSO-d_6, water, and TFE. The studies were typically carried out at 1 mg/0.4 ml (4.5 mM). The temperature readings were accurate to $\pm$ 0.2 ° C. In DMSO-d_6, the coefficients were calculated from 5 - 7 data points over the range 20 - 75 ° C. In TFE, the temperature coefficients were calculated using 5 data points over the range 20 - 55 ° C. Because of chemical exchange with the protons in water and subsequent line broadening, temperature coefficents measured in water were calculated

from as many data points as possible. All temperature coefficients were determined using a linear regression analysis. The coefficients are reported in parts per billion per degree (ppb/° C). In the water spectra, the HOD peak was suppressed by using the 1331 pulse suppression sequence.[25] In the TFE spectra, the -OH peak was also suppressed by using the 1331 pulse sequence while the $-CH_2-$ peak was irradiated with the decoupler.

Solvent titration studies were carried out by monitoring the chemical shifts of the amide NH's as a function of solvent composition. Two binary solvent systems were studied: DMSO-d_6/water and DMSO-d_6/TFE. Proton NMR spectra were measured in 0.1 mole fraction (χ) increments over the entire range $\chi_{\text{DMSO-d}_6} = 1.0$ to $\chi_{\text{DMSO-d}_6} = 0.0$. The mole fraction of water present in stock DMSO-d_6 was estimated to be approximately $2 \bullet 10^{-4}$. The concentration of peptide (typically 4.5 mM) was kept constant throughout the entire range. All compounds (**1** - **6**) were studied in DMSO-d_6/TFE while DMSO-d_6/water studies involved only "LLDD LysGlu" (compound **1**), "LL LysGlu" (compound **3**), "LLDD GluLys" (compound **4**), and "LL GluLys" (compound **6**). The "LLLL LysGlu" tetrapeptide (compound **2**) and "LLLL GluLys" (compound **5**) were not studied in DMSO-d_6/water because of poor solubility.

Computer Simulations

Calculations were performed using a valence force field developed by Hagler *et al.*[26] The molecular dynamics of the system is simulated by numerical integration of Newton's equations of motion. A Gear predictor-corrector algorithm[27] is applied for this integration using a time step of 0.5 fsec. A short time step for the integration is necessary in order to examine the details of motional properties on a microscopic scale. An equilibration period of 2.5 psec is used to begin the simulations, which last 20 psec. During equilibration the temperature is adjusted to room temperature by increasing the atomic velocities in small steps, keeping the total momentum equal to zero to prevent translation or rotation of the molecule. This thermal energy is equipartitioned between potential and kinetic energies. The total energy, the sum of the potential and kinetic energies, increases accordingly during this period and is practically constant during the remainder of the simulation. All of the atoms in the molecule are allowed to

move without restriction. A VAX 11/780 is employed to perform the calculations. Each picosecond of the simulation requires approximately four hours of CPU time. The atomic trajectories are displayed on an IRIS graphic system (Silicon Graphics).

Initial conformations for energy minimization studies are selected from the molecular dynamics simulations by taking the coordinates of the molecule at selected intervals of 1.0 or 1.5 psec. These initial conformers are energy minimized with respect to all cartesian coordinates of the molecule using the steepest descent method followed by a modified Newton-Raphson method[27] until all the derivatives are less than 0.01 kcal/mole.

Results

Circular Dichroism

Spectra of the "LysGlu" series (compounds **1-3**) in TFE are shown in Figure 1.

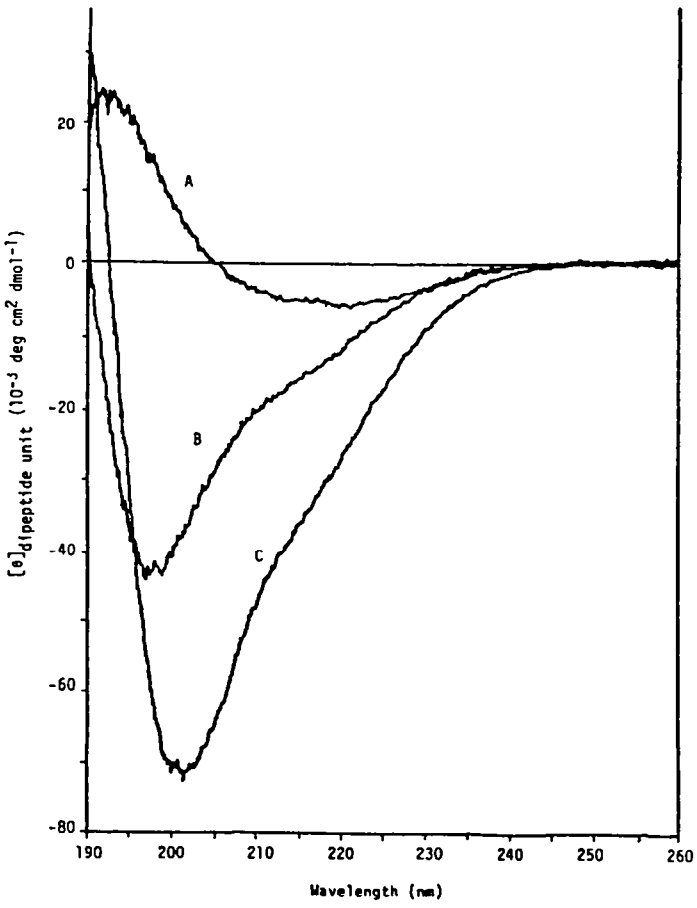

Figure 1. CD Spectra of A: Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe (1.8 mM); B: Ac-L-Lys-L-Glu-NHMe (3.0 mM); and C: Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe (1.8 mM) in TFE at 20° C.

The CD curves for the "LL" and "LLLL" peptides (compounds **2** and **3**) are quite similar, but the CD spectrum of "LLDD" (compound **1**) is entirely different. The CD curves for the "LL" and "LLDD LysGlu" peptides (compounds **1** and **3**) in water are similar to each other, and give rise to negative bands at 197 nm and 195 nm, respectively.

The CD curves for the "GluLys" series in TFE (compounds **4-6**; see Figure 2) are similar to those obtained for the "LL" and "LLLL LysGlu" peptides in the same solvent system. The CD spectra for the "LL" and "LLDD GluLys" compounds in water resemble those obtained for the "LysGlu" peptides.

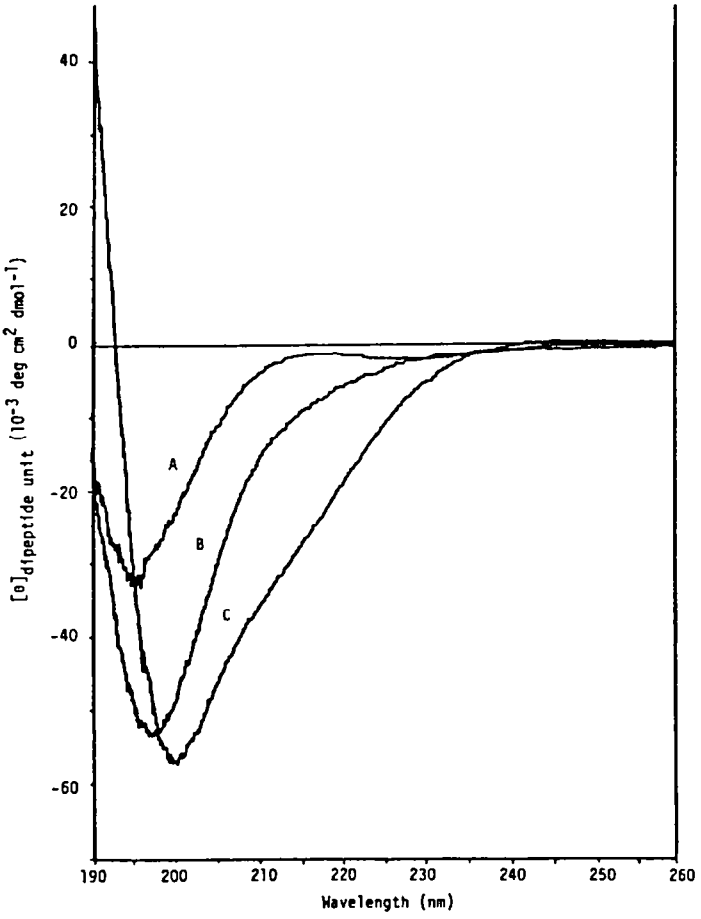

Figure 2. CD Spectra of A: Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe (1.8 mM);
B: Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe (1.8 mM); and
C: Ac-L-Glu-L-Lys-NHMe (3.0 mM) in TFE at 20 ° C.

Nuclear Magnetic Resonance

Assignment of Proton NMR Spectra. Two-dimensional NMR spectroscopy[28] was used to follow connectivity of the atoms in the molecules. Correlation spectroscopy

was used to detect 3-bond proton couplings (^{3}J) whereas RELCO spectroscopy detected 4-bond proton couplings (^{4}J). All amide NH groups are well resolved.

In order to differentiate L-Lys and L-Glu from the D-Lys and D-Glu residues in "LLDD LysGlu" (compound **1**), an isotopically-labelled analog was synthesized.[21] The analog incorporated deuterium at the C^{α} positions in the first two residues (L-Lys and L-Glu) and also contained ^{15}N in the N-terminal Lys side chain. By labelling the peptide with these isotopes and comparing splitting patterns with those in the unlabelled analog, unambiguous assignments of the amide NH's were made. Assignments of the proton resonances in the "LLDD LysGlu" tetrapeptide are shown in Figure 3.

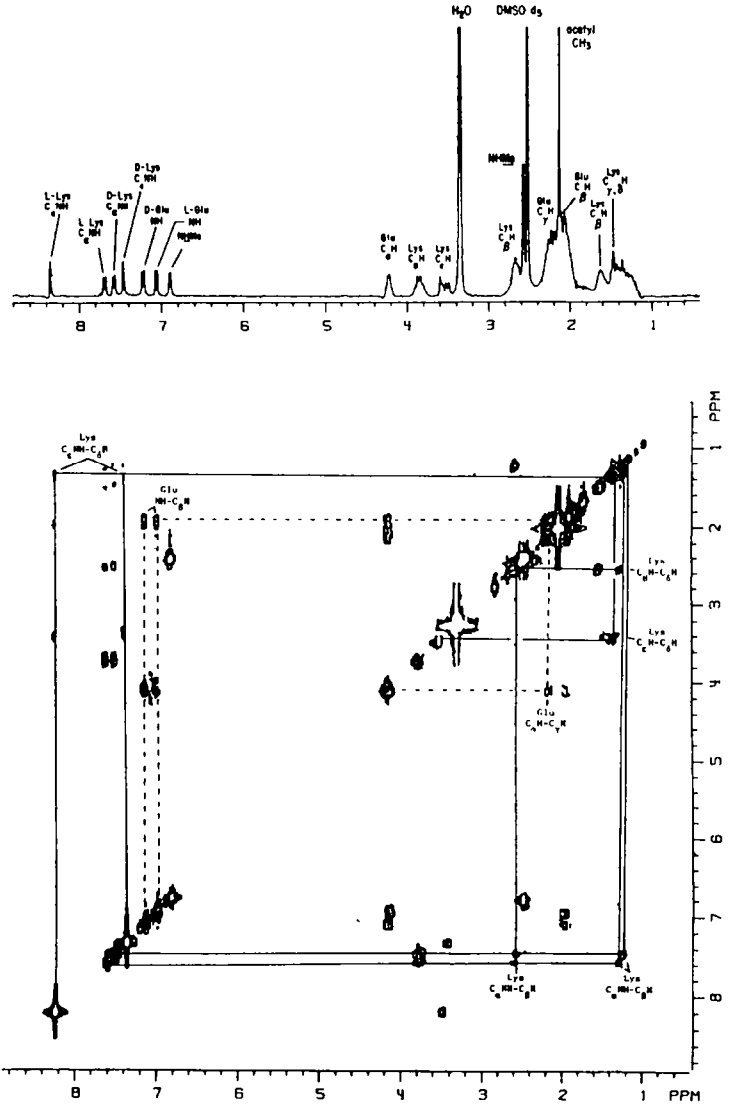

Figure 3. Top: Assigned 1-D Spectrum of Ac-L-Lys-L-Glu-D-Lys-D-Glu-MHMe. Bottom: RELCO Spectrum taken in DMSO-d_6 (7.3 mM) at 21 ° C. Solid line: Lys proton couplings; Dashed line: Glu protons couplings.

Temperature Studies. Temperature dependence of the amide NH's yield useful information regarding the existence of solvent-shielded or intramolecular H bonded groups. The proton NMR spectra of the amide NH regions in the model compounds were taken at various temperatures. The temperature coefficients calculated in DMSO-d_6, TFE, and water for the "LysGlu" and "GluLys" series are reported in Tables I and II, respectively. Most amide proton resonances shift upfield with increasing temperature. This is consistent with the behavior of other peptides in hydrogen bonding solvents.[29,30] Only two amide NH's shift downfield with increasing temperature. These correspond to the D-Lys C^{ϵ}NH and N-methylamide proton in "LLDD Lys-Glu" (compound **1**) in DMSO-d_6. Downfield shifts have also been reported for the ornithine (Orn) residue in alumichrome and alumichrome C.[31] All plots showed linear

Table I: Temperature Coefficients for the Amide NH's in the LysGlu Series in Various Solvents

Compound	Temperature Coefficients (ppb deg^{-1}) DMSO-d_6	TFE	Water
Ac-L-Lys-L-Glu-NHMe			
Lys C_{α}NH	-4.07	-7.62	-7.86
Lys C_{ϵ}NH	-4.88	-9.71	-8.90
Glu NH	-3.24	-1.71	-5.09
NHMe	-3.60	-5.21	-3.75
Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe			
L-Lys C_{α}NH	-2.77	-7.67	-6.39
D-Lys C_{α}NH	-2.32	-8.70	-6.41
L-Lys C_{ϵ}NH	-4.76	-8.56	-7.18
D-Lys C_{ϵ}NH	+0.21	-6.18	-3.17
L-Glu NH	-4.17	-2.97	-4.37
D-Glu NH	-1.29	-2.81	-3.80
NHMe	+1.31	-4.14	-3.94
Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe			
Lys C_{α}NH (1)*	-2.74	-4.71	
Lys C_{α}NH (2)	-3.22	-5.77	
Lys C_{ϵ}NH (1)	-5.57	-9.76	
Lys C_{ϵ}NH (2)	-3.13	-7.80	
Glu NH (1)	-4.35	-7.35	
Glu NH (2)	-4.16	-1.18	
NHMe	-1.47	-4.61	

*numbers in parentheses refer to relative chemical shift positions of amide NH's in DMSO-d_6

Table II: Temperature Coefficients for the Amide NH's in the GluLys Series in Various Solvents

Compound	Temperature Coefficients (ppb deg^{-1}) DMSO-d_6	TFE	Water
Ac-L-Glu-L-Lys-NHMe			
Glu NH	-3.67	-5.92	-7.26
Lys C_αNH	-4.07	-3.80	-7.04
Lys C_ϵNH	-5.18	-5.03	-9.76
NHMe	-3.65	-6.77	-3.59
Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe			
Glu NH (1)*	-5.05	-6.11	-7.11
Glu NH (2)	-0.98	-2.48	-5.79
Lys C_αNH (1)	-1.77	-6.01	-3.98
Lys C_αNH (2)	-3.55	-3.05	-9.14
Lys C_ϵNH (1)	-6.36	-8.34	-9.14
Lys C_ϵNH (2)	-4.75	-6.20	-9.14
NHMe	-4.47	-6.10	-4.29
Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe			
Glu NH (1)	-4.33	-3.50	
Glu NH (2)	-2.02	-7.51	
Lys C_αNH (1)	-3.12	-1.61	
Lys C_αNH (2)	-1.23	-2.53	
Lys C_ϵNH (1)	-5.21	-14.3	
Lys C_ϵNH (2)	-4.47	-6.57	
NHMe	-3.40	-4.52	

*numbers in parentheses refer to relative chemical shift positions of amide NH's in DMSO-d_6

relationships between amide NH chemical shifts and temperature except for one of the Lys C^αNH's in "LLDD GluLys" (compound 4). It is believed that a linear relationship suggests no conformational transition throughout the entire temperature range. However, nonlinear behavior has also been seen for the Orn[1] NH of alumichrome C in DMF.[31] and for one of the amides of deferriferrichrysin in DMSO-d_6,[32] despite the absence of conformational transitions. A conformational change, therefore, in "LLDD GluLys" (compound 4) cannot be substantiated by the nonlinearity of the temperature behavior of the Lys C^αNH.

Solvent Titration Studies. Solvent titration spectra of the model compounds were measured for three reasons: (1) for assignment of the amide proton resonances in

TFE and water, (2) to detect solvent-shielded and solvent-exposed NH groups, and (3) to detect conformational transitions. Plots of chemical shift vs mole fraction DMSO-d_6 were generated for each model compound.

During titration, *most* amide proton resonances shift downfield, then undergo a relatively large chemical shift change, and finally shift upfield with increasing TFE concentration. In general, the shifts downfield occur in the region $\chi_{\text{DMSO-d}_6} = 1.0 - 0.6$ and appear to be mostly linear. The region where the chemical shifts are extremely sensitive to solvent perturbation occur at $\chi_{\text{DMSO-d}_6} = 0.6 - 0.3$. The shifts upfield occur in the region $\chi_{\text{DMSO-d}_6} = 0.3 - 0.0$ and are mostly linear. This indicates that a conformational change occurs in going from DMSO-d_6 to TFE.

In "LLDD LysGlu" (compound **1**), the L-Lys $C^{\epsilon}NH$ and Lys $C^{\alpha}NH$'s shift upfield while the D-Lys $C^{\epsilon}NH$, Glu NH's, and the N-methylamide proton shift downfield or remain approximately unchanged on going from pure DMSO-d_6 to pure TFE. Llinas and Klein (1972) showed that internal or solvent-shielded NH groups shifted downfield as the solvent acidity increased.

In water, the amide protons are less sensitive to solvent changes than in the DMSO-d_6/TFE solutions. Most amide proton resonances shift downfield with increasing water concentration. Two regions are evident, one occurring in the range $\chi_{\text{DMSO-d}_6} = 1.0 - 0.5$ and the other in $\chi_{\text{DMSO-d}_6} = 0.5 - 0.0$. The plots approximate linear relationships in both regions, however, having different slopes. The relative shielding of the NH groups is generally maintained in each solvent and few crossovers allow relatively easy assignments in water.

Molecular Dynamics Simulations

Molecular dynamics simulations were carried out for "LLDD LysGlu" (compound **1**) starting from two different conformations. In one simulation the initial structure was a conformation resembling a β-turn (see Figure 4A).

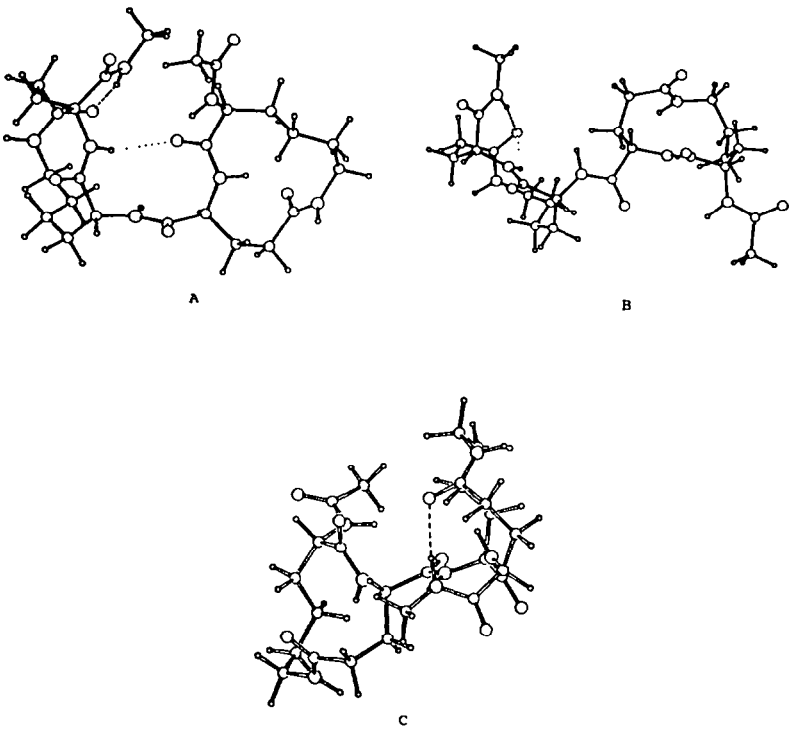

Figure 4. Starting Conformers for Molecular Dynamics of Compounds **1** and **2**.

This conformation was obtained in previously executed minimization studies[2] in which the amide bonds in both lactam rings were initially in the *trans* conformation. The starting conformation for the other simulation consisted of an extended structure in which the amino and carboxyl ends were well separated (see Figure 4B). This conformation was obtained from the initial folded conformation by forcing the torsion angles Glu ψ and D-Lys ϕ (i.e. ψ_2, ϕ_3) to 180±20° and then allowing the molecule to relax using 200 cycles of steepest descent minimization (see Table III). The purpose of the two simulations was to study the motional properties of the molecule starting from different conformations. The initial conformation for the molecular dynamics simulation on the other three tetrapeptides was generated in the same manner as the extended conformer for "LLDD LysGlu" (compound **1**). The dipeptide derivatives were not simulated.

Table III: Energy Minima from the Molecular Dynamics Simulation of Ac-Lys-Glu-D-Lys-D-Glu-NMe

Time(psec)	Energy(kcal/mole)	Hydrogen bonds		
0.0	61.8	Lys CO	←	D-Lys NH
		D-Lys C^{α} CO	←	D-Glu NH
		D-Lys C^{α} CO	←	NMe NH
1.0	64.3	D-Glu CO	←	Lys NH
		Lys CO	←	D-Lys NH
		D-Lys C^{α} CO	←	NMe NH
2.0	70.5	D-Lys CO	←	Lys NH
		Lys CO	←	D-Lys NH
4.0	64.1	Lys CO	←	D-Lys NH
		Ac CO	←	NMe NH
5.0	60.3	Lys CO	←	D-Lys NH
		D-Glu CO	←	D-Lys C^{α} NH
		Ac CO	←	NMe NH
6.0	61.7	D-Lys CO	←	Lys NH
		Lys CO	←	D-Lys NH
		D-Glu CO	←	D-Lys C^{α} NH
7.5	63.5	D-Glu CO	←	D-Lys C^{α} NH
13.0	61.6	D-Glu CO	←	D-Lys C^{α} NH
		Ac CO	←	NMe NH
14.0	59.2	Lys CO	←	D-Lys NH
		D-Glu CO	←	D-Lys C^{α} NH
		Ac CO	←	NMe NH
1-20*	61.6	Lys CO	←	D-Glu NH
		D-Lys C^{α} CO	←	NMe NH

*Simulation starting from the folded conformation.

Extended Conformer for Compound **1**. During the simulation of the extended conformer for "LLDD LysGlu" (compound **1**) the molecule folds as a consequence of changes in the torsion angles with time. This folding is indicated by a decrease in the distance between the end groups, from ~12 Å to ~3 Å, and a change in the torsion angles Glu$^2\psi$ and D-Lys$^3\phi$ from ~180 ° to ~100 ° (see Figure 5A).

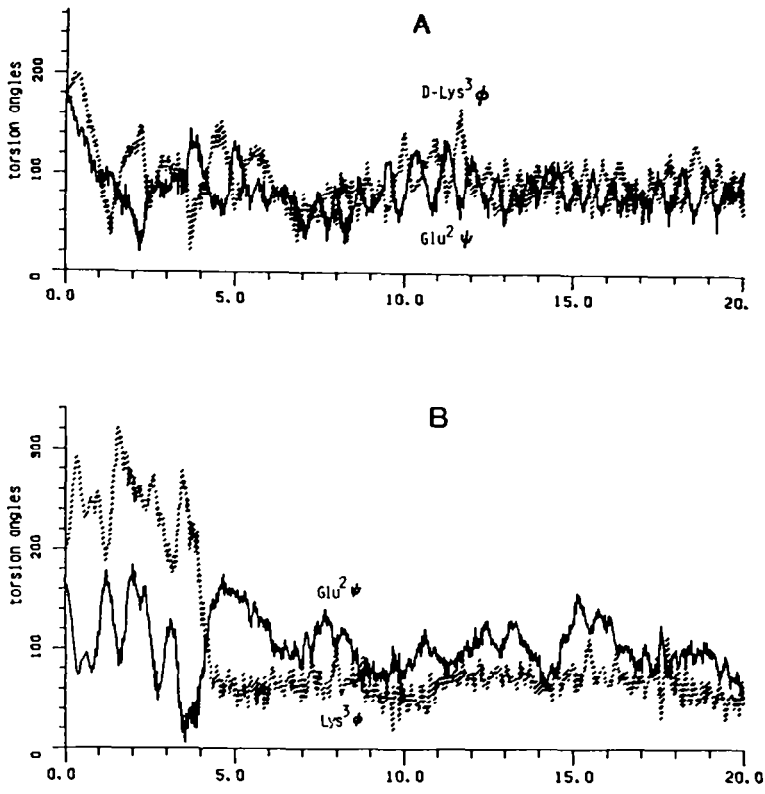

Figure 5. Time Variation During the Molecular Dynaimcs Simulation of
A: Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe and
B: Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe from the Extended Structure.

All of the pairs of ψ, ϕ angles on each side of the peptide units show correlated transitions. This correlation has been observed in previous molecular dynamics simulations of peptide systems. There is also coupling between pairs of side chain angles (χ). These concerted motions are an indication of the conformational constraints imposed on the side chains by the cyclization forming the 12-membered ring systems.

These dynamic transitions are correlated with changes in the molecular conformation as indicated from the energy minimization studies and graphical displays of the dynamic simulation. These conformations exhibit several hydrogen bonds which stabilize the folded structure and which continuously break and reform during the simulation.

Folded Conformer for Compound **1**. During the simulation of the folded conformer for "LLDD LysGlu" (compound **1**), most of the motions of the molecule arise from fluctuations of the torsion angles. These fluctuations show small values ($\sigma < 22°$), indicating that no conformational transitions among different equilibrium states have occurred. Although the motion of the molecule gives rise to different intramolecular hydrogen bond patterns, the overall shape of the molecule is the same. It remains in the folded conformation from which the simulation commenced.

Extended Conformer for Compound **2**. The simulation of the extended conformer for "LLLL LysGlu" (compound **2**) also shows folding with hydrogen bonding of the endgroups prevalent from 11 psec. to 18 psec. (a change in distance from over 10 Å to ~3 Å). As in the case of the extended conformer for "LLDD LysGlu" (compound **1**), changes in the angles ψ_2, ϕ_3 are responsible for most of the folding (see Figure 5B). Examining the trajectory of "LLLL LysGlu" (compound **2**) several C_7 structures are found. The majority of these occur around Lys^3.

Extended Conformers for Compounds **4** and **5**. The simulations for "LLDD GluLys" and "LLLL GluLys" (compounds **4** and **5**, respectively) are similar to the simulation for "LLLL LysGlu" (compound **2**) and show a variety of C_7, both axial and equatorial, and other structures. Folding occurs as a consequence of changes in the dihedral angles, principally ψ_2, ϕ_3. Hydrogen bonding of the end groups occurs as well.

Energy Minimizations

Extended Conformer for Compound **1**. The large standard deviation of the torsional angles observed in this simulation indicate that the molecule is traversing various conformational states not just undergoing fluctuations about a single minimum. The energy minimizations confirmed this result. Structures taken at regular intervals during the simulation show several different minima with a variety of hydrogen bonding patterns which help to stabilize these minima (see Figure 6). The values of the torsion angles ϕ and ψ of residues L-Glu and D-Lys are shown in Table IV. All the minima, except VII, exhibit a distance of less than 7 Å between the C^α atoms of residues 1 and 4, indicating the presence of folded conformations.

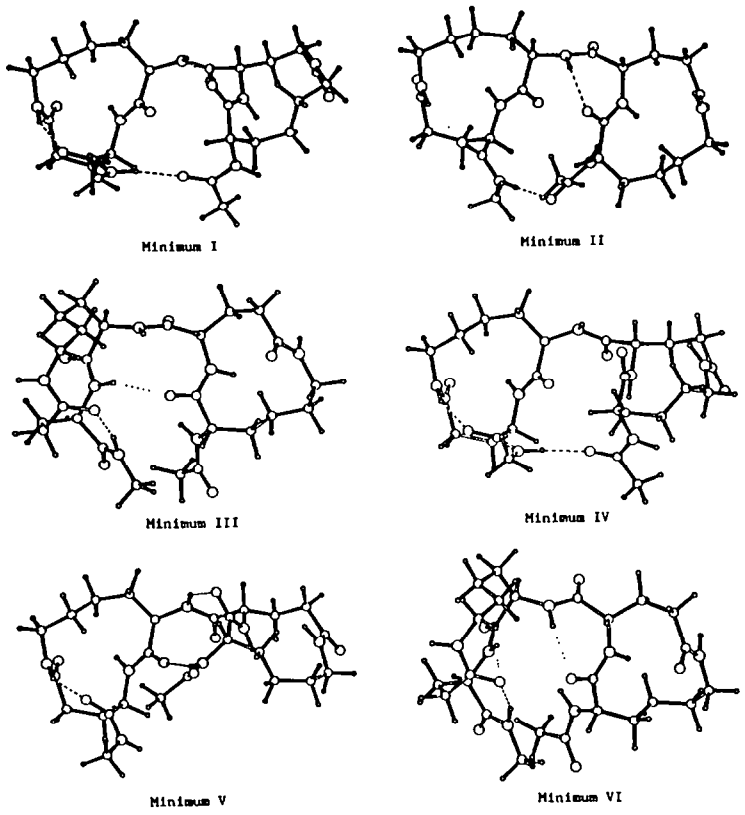

Figure 6. Representative Energy Minima of Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe Calculated from the Molecular Dynamics Simulations.

Even the minimum obtained when the energy of the initial extended structure was fully minimized, minimum VI, shows a distance of 5.4 Å. Thus, no energy minima were found in the extended region.

Dihedral angles of the peptide backbone around the swivel point are shown in Table IV. Figures of the energy minimum obtained from the simulation starting from the folded structure (minimum III), and of the minima obtained from the simulation starting from the extended structure are presented in Figure 6.

Table IV: Conformational Properties of the Energy Minima for the LysGlu Series

Minimum	Energy (kcal/mole)	ϕ^2 (°)	ψ^2 (°)	ϕ^3 (°)	ψ^3 (°)
LLDD LysGlu					
I	59.2	-84	85	90	-149
II	60.3	-79	77	86	107
III	61.6	-79	100	92	31
IV	61.6	-114	79	80	-146
V	61.7	-89	71	72	-151
VI	61.8	-85	83	111	58
VII	63.5	-121	62	68	-160
VIII	64.1	-78	87	94	71
IX	64.3	-79	90	66	64
X	70.5	-88	71	83	-170
LLLL LysGlu					
I	65.4	-86	123	66	-68
II	65.6	-79	110	67	-66
III	65.8	-81	80	67	-78
IV	66.7	-79	102	74	-100
V	67.2	-90	75	-173	-75
VI	68.0	-89	80	53	-113
VII	68.7	-83	80	-168	-72
VIII	70.3	-83	100	63	-81
IX	70.4	-103	72	48	-117

Folded Conformer for Compound **1**. Structures taken at regular time intervals and fully minimized revealed only one minimum (see minimum III, Figure 6). This minimum has the same structure as the minimized initial conformation. Thus, the minimum corresponding to the β-turn conformation seems to be located in a relatively deep well from which the molecule was unable to escape with the thermal energy available during the simulation.

Extended Conformer for Compound **2**. The extended conformer for "LLLL LysGlu" (compound **2**) is traversing a variety of conformational states as shown by the various minima (see Tables IV and V). Minima III, V, VII, and VIII show C_7^{eq} structures around Glu^2. Minima I, II, III, IX show C_7^{ax} structures around Lys^3. Calculations of Lewis *et al.*[33] indicate that the C_7^{ax} structure for a linear L-polypeptide is

higher in energy than the C_7^{eq}. Adoption of the C_7^{ax} structure during this simulation is probably due to the restrictions imposed by the side chain cyclization. These findings are similar to the findings of Hagler *et al.* in the case of vasopressin.[34] Minima I and III show both types of C_7 structure, with C_7^{ax} around Lys^3 ϕ, C_7^{eq} around Glu^2 ψ for minimum III and C_7^{eq} around Glu^4 ψ for minimum I. Minimum VII shows the 1 → 3 and 1 ← 3 hydrogen bonding pattern that has been characterized as a gamma turn.

Table V: Energy Minima from the Molecular Dynamics Simulation of Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe

Time(psec)	Energy(kcal/mole)	Hydrogen bonds		
0.0	67.4	Lys^1 CO	←	Lys^3 NH
		Glu^4 C^7 CO	←	Glu^4 NH
		Glu^4 C^7 CO	←	NMe NH
3.0	68.7	Lys^1 CO	←	Lys^3 NH
		Glu^4 C^7 CO	←	Glu^4 NH
		Glu^4 C^7 CO	←	NMe NH
4.0	67.2	Lys^1 CO	←	Lys^3 NH
		Glu^4 C^7 CO	←	NMe NH
5.0	70.3	Glu^3 CO	←	Glu^4 NH
6.0	65.4	Glu^4 CO	←	Lys^1 NH
		Glu^2 CO	←	Lys^3 C^ϵ NH
		Glu^2 CO	←	Glu^4 NH
		Lys^3 CO	←	NMe NH
7.0	65.6	Glu^4 CO	←	Lys^1 NH
		Glu^2 CO	←	Lys^1 C^ϵ NH
		Glu^2 CO	←	Glu^4 NH
8.0	65.8	Lys^1 CO	←	Lys^3 NH
		Glu^4 CO	←	Lys^3 C^ϵ NH
		Glu^2 CO	←	Glu^4 NH
		Ac CO	←	NMe NH
10.0	70.4	Glu^4 CO	←	Lys^1 C^ϵ NH
12.0	66.7	Ac CO	←	NMe NH
19.0	68.0	Lys^3 CO	←	Lys^1 NH
		Lys^1 CO	←	Lys^3 NH
		Glu^4 CO	←	Lys^3 C^ϵ NH

Extended Conformers for Compounds **4** and **5**. The simulation for "LLDD GluLys" and "LLLL GluLys" (compounds **4** and **5**) show C_7 structures that are generally the reverse of those shown by "LLLL LysGlu" (compound **2**). For "LLDD GluLys" (compound **4**) all the minima show C_7^{ax} around Glu^2 and minimum III is also C_7^{ax} around Lys^3. For "LLLL GluLys" (compound **5**) many of the minima show C_7^{ax} around Glu^2 and C_7^{eq} around Lys^3. These various conformational states are described in Tables VI - VIII.

Table VI: Energy Minima from the Molecular Dynamics Simulation of Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe

Minimum	Energy (kcal/mole)	ϕ_2	ψ_2	ϕ_3	ψ_3
Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe					
IA	53.8	70°	-67°	96°	-123°
IB	53.9	70	-68	143	-126
IC	53.9	70	-68	124	-125
II	54.7	78	-50	168	-127
III	55.2	69	-68	85	-78
IV	55.8	77	-54	166	-129
V	56.0	78	-47	174	-134
VI	58.0	75	-67	101	-122
VII	62.3	61	-79	160	60
Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe					
I	56.5	68	-87	-98	84
II	65.0	75	-69	-81	89
III	66.5	72	-62	-77	152
IV	66.5	74	-72	77	98
V	66.8	77	-67	74	99
VI	68.3	76	-70	-90	148
VII	71.1	-55	-67	-150	-94

Table VII: Conformational Properties of the Energy Minima of the GluLys Series

Time(psec)	Energy(kcal/mole)	Hydrogen bonds		
0.0	62.3	Ac CO	←	Lys NH
		D-Lys CO	←	Lys C^{α} NH
		Glu CO	←	D-Glu NH
		Glu CO	←	D-Lys NH
1.5	55.2	Ac CO	←	Lys NH
		Glu CO	←	D-Glu NH
		Lys CO	←	D-Lys NH
		D-Glu CO	←	NMe NH
3.0*	53.9	Ac CO	←	Lys NH
5.5	53.9	Glu CO	←	D-Glu NH
6.0	53.8	D-Glu CO	←	NMe NH
7.0	54.7	Glu CO	←	D-Glu NH
		D-Glu CO	←	NMe NH
8.0	58.0	Glu C^{γ} CO	←	Lys NH
		Glu CO	←	D-Glu NH
		D-Glu CO	←	NMe NH
13.0	56.0	Glu CO	←	D-Glu NH
		D-Glu C^{γ} CO	←	D-Lys NH
19.0	55.8	Glu C^{γ} CO	←	Lys NH
		Glu CO	←	D-Glu NH
		D-Glu CO	←	NMe NH

*These three minima have the same hydrogen bonding pattern but different ϕ, ψ angles.

Discussion

The CD spectrum of "LLDD LysGlu" (compound **1**) in TFE is similar to that for other model systems having a preferred secondary structure in solution. The "LLDD LysGlu" tetrapeptide (see Figure 1, curve A) has positive and negative extrema which are considerably different, both in intensity and wavelength, from the "LL" dipeptide (curve B) and the "LLLL" compound (curve C). This spectrum is similar to that found in other laboratories for other β-turn models.[1,35,36] The CD curve obtained for the "LLDD LysGlu" tetrapeptide resembles a class-A β-turn spectrum as calculated by Woody.[37]

Table VIII: Energy Minima from the Molecular Dynamics Simulation of Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe

Time(psec)	Energy(kcal/mole)	Hydrogen bonds
0.0	68.2	Ac CO ← Lys^3 NH
		Glu^5 CO ← Lys^4 NH
1.0	71.1	Glu^5 CO ← Lys^3 C^α NH
2.0	66.5	Ac CO ← Lys^3 NH
		Glu^1 CO ← Glu^5 NH
3.0	68.3	Glu^1 CO ← Glu^5 NH
13.0	66.5	Glu^1 CO ← Lys^3 C^α NH
		Glu^1 CO ← Glu^5 NH
14.0	66.8	Glu^1 CO ← Lys^3 C^α NH
		Glu^1 CO ← Glu^5 NH
		Lys^3 CO ← Lys^4 NH
15.0	65.0	Glu^1 CO ← Lys^3 C^α NH
		Glu^1 CO ← Glu^5 NH
		Lys^3 CO ← Lys^4 NH
		Glu^5 CO ← NMe NH
16.0	56.5	Lys^4 CO ← Glu^1 NH
		Ac CO ← Lys^3 NH
		Glu^1 CO ← Glu^5 NH
		Glu^5 CO ← Lys^4 C^α NH
		Glu^5 CO ← NME NH

As seen in the simulations, the torsion angles about the swivel point (see Table IV) define the types of turns responsible for the folding of the molecule. Minimum III, obtained from the simulation from the folded structure, is a type II β-turn. For all the minima, except IV and VII, the values of the torsion angles Glu^2 ϕ, Glu^2 ψ, and D-Lys^3 ϕ are in agreement with the values corresponding to a type II β-turn. The torsion angle D-Lys^3 ψ exhibits deviations from the expected value of 0° for type II β-turns. These minima are classified as type IV β-turns, according to Lewis *et al.*[33]

In DMSO-d_6, temperature coefficients greater than 4.0 ppb/deg are considered evidence for solvent-exposed NH groups while those having coefficients less than 2.0 ppb/deg, in absolute value, are considered as solvent-shielded.[38] Intermediate

temperature coefficients may indicate weakly hydrogen bonded NH groups or equilibria between hydrogen bonded and nonbonded conformers. For "LLDD LysGlu" (compound **1**) in DMSO-d_6, three amide protons have considerably lower temperature coefficients and are considered solvent-shielded, D-Lys $C^{\epsilon}NH$, the D-Glu NH, and the NHMe. Since the tetrapeptide is relatively small (MW ~ 550), it is reasonable to assume that solvent-shielded NH groups are stabilized via intramolecular hydrogen bonding and not shielded because of inaccessibility to solvent arising from steric effects. The Lys $C^{\alpha}NH$'s are less solvent-shielded while the L-Lys $C^{\epsilon}NH$ and the L-Glu NH are the most solvent-exposed groups in the molecule.

The temperature coefficients for the "LysGlu" compounds are, in general, larger in TFE than in DMSO-d_6. This trend was also observed by Llinas and Klein (1972) for molecules that show no conformational change between solvents. We therefore compare relative temperature coefficients in order to examine H-bonding and identify crossovers to examine conformational differences between solvents. For "LLDD LysGlu" (compound **1**), two of the amide protons, those of L-Glu and D-Glu, have noticeably lower temperature coefficients in TFE. The D-Glu amide proton has relatively low temperature coefficients in both DMSO-d_6 and TFE, indicating intramolecular hydrogen bonding. The N-methylamide proton has one of the three lowest coefficients in DMSO-d_6 and in TFE. The Lys $C^{\alpha}NH$'s and the L-Lys $C^{\epsilon}NH$ remain solvent exposed in both DMSO-d_6 and TFE. The D-Lys $C^{\epsilon}NH$ shows the most variation, since it is strongly solvent-shielded in DMSO-d_6, while in TFE its temperature coefficient suggests solvent exposure.

Since the temperature coefficients for the "LysGlu" series in water are generally higher than those obtained in DMSO-d_6 (Table I), no case can be made for strongly solvent-shielded amide NH groups in water. However, the four amide protons with the lowest temperature coefficients in water (L-Glu NH, D-Glu NH, D-Lys $C^{\epsilon}NH$, and the N-methylamide proton) are solvent-shielded according to the solvent titration data for "LLDD LysGlu" (compound **1**) in DMSO-d_6/TFE. This list represents a combination of the amide protons that according to temperature studies are solvent-shielded in TFE, with those that are solvent-shielded in DMSO-d_6. It appears that a conformational transition, or a change in equilibrium distribution of a set of conform-

ers, is taking place on going from DMSO-d_6 to TFE as well as from DMSO-d_6 to water. However, it seems that the environment of the D-Glu NH remains relatively constant in both DMSO-d_6 and in TFE.

In simulations for "LLDD LysGlu" (compound 1), hydrogen bonding involving the D-Glu and NHMe NH groups is found in the energy minimum from the simulation of the folded conformer (minimum III). This conformer exhibits a β-turn with the typical hydrogen bonding pattern, L-Lys CO ← D-Glu NH (1 ← 4), plus the hydrogen bond D-Lys $C^{\epsilon}CO$ ← NHMe NH. The temperature coefficients for these protons, as obtained in the NMR study of the compound, are, in all solvents, two of the three lowest values seen. This is supported by the evidence of a β-turn in TFE found by the CD study. The correlation between experimentally derived and computer simulated conformers occurs even though the calculations are performed on a model *in vacuo*. This data suggests that the relative free energy of minimum III, the β-turn conformer is lower in TFE, where a β-turn is experimentally indicated, than its relative free energy in water. The slightly higher temperature coefficient in water for these protons may indicate an equilibrium between hydrogen bonded and nonhydrogen bonded conformers. Calculation of the solvated free energy in each solvent is proposed. In addition, the low temperature coefficient in TFE for a proton not found as a hydrogen donor in the minima (L-Glu), suggests that not all of the low-energy minima have been shown by the simulation to date.

Interpretation of the data in DMSO-d_6 compared to the calculated conformers is more difficult, and leads us to propose a set of conformers composed of minima I-III as major contributors with possible lesser contributions from other conformers. In support of this, the coupling constants of ϕ_2 and ϕ_3, 9.41 and 10.1 Hz respectively, make minima IV-VII and IX unlikely as major contributors. Likewise, the calculated energy values make minima VIII and X unlikely as major contributors. This set of conformers, I-III, would also explain the low temperature coefficients for D-Lys Cα NH, D-Lys Cϵ NH, D-Glu NH, and the N-methylamide NH in DMSO-d_6. However, it would not explain why D-Lys Cϵ NH is extremely low. The explanation for that may lie in steric effects introduced by the side-chain lactam closure. This measured effect may also signify a reduction in the size of the lactam ring on the "DD" dipeptide por-

tion relative to the "LL" dipeptide side, due to more concise folding These possibilities are the subject of ongoing calculations for this tetrapeptides and its analogs.

The effect of the side chain cyclization can partially be assessed by examining the participation of the carbonyl oxygen or amide proton in hydrogen bonding in the minima, or by examining the values of the temperature coefficient for the amide proton. Employing either criteria one sees that the side chain lactam for the "D-side" of "LLDD LysGlu" (compound 1) restricts the motion of the "DD dipeptide" portion by hydrogen bonding either across the ring or with the NMe end group so that no hydrogen bonds for the side chains are formed across the swivel point. This perhaps aids the formation of the β-turn since it stabilizes the relative positions of residues 3 and 4.

In "LLLL LysGlu" (compound 2), the N-methylamide proton is the most solvent-shielded in DMSO-d_6. Five of the nine minima from the molecular dynamics simulation (see Table IV) of this molecule show hydrogen bonding for this amide proton. The moderately low temperature coefficient for both Lys NH's corresponds favorably with the occurrence of several hydrogen bonding patterns for these protons in the minima. The moderately low temperature coefficient for one of the lactam amide protons of the compounds in DMSO-d_6 compares to the preponderance of hydrogen bonding for the Lys^3 $C^{\epsilon}NH$ in the minima.

The effect of side chain cyclization can be judged as before. The temperature coefficients of Lys^3 $C^{\epsilon}NH$ in both DMSO-d_6 and TFE are higher than for "LLDD" (compound 1) and the hydrogen bonding list of the minima shows a wider variety of possible pairings. In fact, most of the other amide protons also show a higher temperature coefficient and a wider variety of hydrogen bond pairings. This implies greater flexibilty than in the "LLDD" case.

For "LLDD GluLys" (compound 4) the amide protons with the four lowest temperature coefficients compare to the hydrogen bond donor list of the five lowest minima. The D-Glu amide proton shows as hydrogen bonded in all the minima, and one of the Glu amide protons shows a low temperature coefficient in TFE, and DMSO-d_6. The large increase in the temperature coefficient for Lys C^{α} NH (1) in TFE compared to its coefficient in DMSO-d_6 may indicate a change in the distribution between minimum I, where Lys^1 C^{α} NH is hydrogen bonded and minimum II, where it

is not. As with the "LLDD LysGlu" tetrapeptide (compound **1**), the lowest minimum corresponds more closely to the DMSO-d_6 data, even though all calculations are performed on a model *in vacuo*.

Side chain cyclization, in the case of "LLDD GluLys" (compound **4**), does not result in the same restriction of motion as with "LLDD LysGlu" (compound **1**). The three lowest energy minima do not show hydrogen bonding for the side chain lactams, and the higher minima show hydrogen bonding for either lactam, not one side exclusively. Also, neither lactam closure proton shows a low temperature coefficient. The result is that the β-turn conformer found in previously executed studies[2] is higher in energy than minimum I by 10.3 kcal/mole, and is not evidenced in the CD studies.

The lowest energy minimum for "LLLL GluLys" (compound **5**) contains so many hydrogen bonds that it is difficult to discuss this compound in the same way as the others. Temperature coefficients are approximately the same range as in the "LLLL LysGlu" (compound **2**) case.

Conclusion

Preliminary calculations of potential energy minima were performed on a survey group of 62 dilactam-bridged tetrapeptides.[2] These calculations predicted β-turns for "LLDD LysGlu" (compound **1**) and "LLDD GluLys" (compound **4**), but not the "LLLL" isomers. We have now synthesized, measured the CD and NMR, and refined the calculations for all four tetrapeptides. One of the two "LLDD" compounds (Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe) appears to favor the β-turn conformer, while the other folds into C_7 structures resembling γ-turns. This correspondence occurs despite the fact that the minima are calculated *in vacuo*.

In contrast to "LLDD LysGlu" (compound **1**), the other three tetrapeptides exhibit greater flexibility, as shown by the temperature coefficients and by the computer simulations. This greater flexibility for "LLLL LysGlu", "LLDD GluLys", and "LLLL GluLys" (compounds **2**, **4**, and **5**, respectively) may explain why no secondary structures are seen in the CD studies for these compounds.

The CD curve for "LLDD LysGlu" (compound **1**) in TFE resembles spectra taken of other proposed β-turn models. From the simulations of this molecule, minimum III is the only type II β-turn conformer with the classic 1 $\leftarrow$ 4 H-bond. How-

ever, most of the other minima fit the more variable type IV classification. The type II β-turn is also supported by the temperature coefficients in TFE. Coupling constants in TFE and NOE's in all solvents are not well resolved at 360 MHz and await further study.

A set of conformers composed of minimas I and II, type IV β-turns, and minima III, type II β-turn, for compound 1 is proposed in order to explain the temperature coefficients in DMSO-d_6. The minima are supported by the coupling constants found in DMSO-d_6. The very low temperature coefficient for D-Lys C^{ϵ} NH in DMSO-d_6 indicates an extreme in solvent shielding that provides an additional H-bonding constraint. The lactam bond in this molecule produces an unusual effect not seen for the lactam amides in the other three tetrapeptides or on the "LL" dipeptide portion of compound 1.

The two lactam bonds create two constrained regions separated by a -Cα-NH-CO-Cα- region which retains flexibility. The effect of this constraint is being further explored by simulations on ester-bonded analogs and on free side chain analogs of these sequences. The most interesting of these compounds will be synthesized and analyzed spectroscopically, in order to delineate the salient features required to induce specific folding in this group of constrained molecules. Further studies on the effect of ring size are also under consideration. These studies will also be examined in relationship to ongoing studies of the lanthionine-containing compounds nisin and epidermin, which are of current interest as novel naturally occurring structures that contain regions constrained by side-chain cross-linking through sulfur.

In the near future we plan to calculate similar minima with solvent included, and to extend the time of the molecular dynamics to determine the presence or absence of more complex spatial movements. Future plans also call for the measurement of NOE spectra at 500 MHz which will serve both as experimental confirmation of minimum energy conformers and as restraints for molecular dynamics simulations. Minimum energy conformers will also be examined by incorporation of electron and energy transfer groups at the terminii of the bridged tetrapeptides. Experimentally, these derivatives could provide independent evidence for folding because of internal donor-acceptor interactions.

Acknowledgements

We gratefully acknowledge support from the National Institutes of Health through Grant GM-18694. We also acknowledge D.F. Mierke of the UCSD Dept. of Chemistry, and F. Avbelj, C.L. Fisher, and J.S. Garavelli of the Agouron Institute for valuable advice and discussion.

References

1. Deslauriers, R.; Evans, D.J.; Leach, S.J.; Meinwald, Y.C.; Minasian, E.; Nemethy, G.; Rae, I.D.; Scheraga, H.A.; Samarjai, R.L.; Stimson, E.R.; van Nispen, J.W.; Woody, R.W. *Macromol.* **1981**, *14*, 985.

2. Manesis, N.; Hassan, M.; Glaser, R.; Goodman, M. *Biopolymers* **1986**, *25*, S97.

3. Goodman, M.; Saltman, R.P. *Biopolymers* **1981**, *20*, 1929.

4. Veber, D.F.; Strachan, R.G.; Bergstrand, S.J.; Holly, F.W.; Homnick, C.F.; Hirschman, R.; Torchiana, M.L.; Saperstein, R. *J. Am. Chem. Soc.* **1976**, *98*, 2367.

5. Veber, D.F.; Holly, F.W.; Nutt, R.F.; Bergstrand, S.J.; Brady, S.F.; Hirschman, R.; Glitzer, M.S.; Saperstein, R. *Nature* **1979**, *280*, 512.

6. Berman, J.F.; Goodman, M. *Int. J. of Pept. and Prot. Res.* **1984**, *23*, 610.

7. Nemethy, G.; McQuie, J.R.; Pottle, M.S.; Scheraga, H.A. *Macromol.* **1981**, *14*, 975.

8. du Vigneaud, V.; Ressler, C.; Swan, J.M.; Roberts, C.W.; Katsoyannis, P.O.; Gordon, S. *J. Am. Chem. Soc.* **1953**, *75*, 4879.

9. Smith, C.W. *Dev. Endocrin.* **1981**, *13*, 23.

10. Hruby, V.J. in *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Marcel-Dekker, Inc.: New York, 1974; Vol. 3, p 1.

11. Gross, E.; Morell, J.L. *J. Am. Chem. Soc.* **1971**, *93*, 4634.

12. Shiba, T.; Wakamiya, T.; Fukase, K.; Sano,A.: Shimbo, K.; Ueki, Y. *Biopolymers*

13. Allgaier, H.; Gunther, J.; Werner, R. G.; Schneider, U.; Zahner, H. *Angew. Chem. Int. Ed. Eng.* **1985**, *24*, 1051.

14. Freidinger, R. M., Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656.

15. Karle, I.L. *J. Am. Chem. Soc.* **1975**, *97*, 4379.

16. Venkatachalam, C.M. *Bioploymers* **1968**, *6*, 1425.

17. Ivanov, V.T. in *Peptides: Proceedings of the Fifth American Peptide Symposium*; Goodman, M., Meienhofer, J., Eds.; 1977, p 307.

18. Davis, D.G.; Gisin, B.F.; Tosteson, D.C. *Biochemistry* **1976**, *15*, 768.

19. Brown, J.N.; Teller, R.G. *J. Am. Chem. Soc.* **1976**, *98*, 7565.

20. Kostansek, E.C.; Lipscomb, W.N.; Thiessen, W.E. *J. Am. Chem. Soc.* **1979**, *101*, 834.

21. Manesis, N.J.; Goodman, M. *J. Org. Chem.*, **1987**, accepted for publication.

22. Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; Delft University Press: Dordrecht, 1981.

23. Eich, G.; Bodenhausen, G.; Ernst, R.R. *J. Am. Chem. Soc.* **1982**, *104*, 3732.

24. King, G.; Wright, P. *J. Magn. Res.* **1983**, *54*, 328.

25. Hore, P. *J. Magn. Res.* **1983**, *55*, 283.

26. Hagler, A.T.; Dauber, P.; Lifson, S. *J. Am. Chem. Soc.* **1983**, *101*, 5131.

27. Fletcher, R. *Practical Methods of Optimization*; Wiley & Sons, Inc.: New York, 1980.

28. Jelinsky, L.W. *Chem. and Engin. News*, Nov. 5, 1984, 26.

29. Bovey, F.A.; Brewster, A.I.; Patel, D.J.; Tonelli, A.E.; Torchia, D.A. *Accts. Chem. Res.* **1972**, *5*, 193.

30. Kopple, K.D.; Ohnishi, M.; Go, A. *J. Am. Chem. Soc.* **1969**, *91*, 4264.

31. Llinas, M.; Klein, M.P. *J. Am. Chem. Soc.* **1975**, *97*, 4731.

32. Llinas, M.; Klein, M.P.; Neilands, J.B. *Int. J. Pep. Prot. Res.* **1972**, *4*, 157.

33. Lewis, P.N.; Momany, F.A.; Scheraga, H.A. *Biochim. Biophys. Acta* **1973**, *303*, 211.

34. Hagler, A.T.; Osguthorpe, D.J.; Dauber-Osguthorpe, P.; Hempel, J.C. *Science* **1985**, *227*, 1309.

35. Bush, C.A.; Sarkar, S.K.; Kopple, K.D. *Biochem.* **1978**, *17*, 4951.

36. Crisma, M.; Fasman, G.D.; Balaram, H.; Balaram, P. *Int. J. Pept. Prot. Res.* **1984**, *23*, 411.

37. Woody, R.W. in *Proceedings of the Rehovot Symposium on Poly(Amino Acids), Polypeptides, and Proteins, and Their Biological Implications*; Blout, E. T., Bovey, F. A., Eds.; John Wiley & Sons, Inc.; New York, 1974; p 338.

38. Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 512.