# Conformational Study of the Tetrapeptide Boc-Arg-Ala-Gly-Glu-NHEt. A β Turn Locked by a Salt Bridge

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**Abstract:** The conformation of the tetrapeptide Boc-Arg-Ala-Gly-Glu-NHEt is obtained from $^1$H NMR spectra analysis in $Me_2SO$-$d_6$. An intramolecular complex with two hydrogen bonds is found between the charged side chains of arginine and glutamic acid residues. The observation of Gly[3] methylene protons as ABX spin system indicates a constrained structure. The temperature dependence of the NH protons and the hydrogen–deuterium exchange studies reveal the presence of a β turn, a ten-atom hydrogen-bonded ring involving the Glu[4] NH and Arg[1] CO, and a supplementary hydrogen bond between the Arg[1] NH and the Glu[4] CO. Structural changes observed in presence of salt or polar solvents show that the β turn is locked by the $Arg^+\cdots Glu^-$ salt bridge.

It is now well established that in globular proteins 30% of the amino acids are involved in β turns and that this structure has therefore a significant contribution to the structure of proteins. In such regions the peptide chain folds back on itself and may or may not form an hydrogen bond between the carbonyl group of residue $i$ and the peptidic NH group of residue $i + 3$. Such β turns were first characterized in detail by Venkatachalam.[1] Although several β turn structures have been already established for oligopeptides, equilibria among several conformations can exist in solution and more searching characterization of this structure and of its stabilization factors appears desirable.

A systematic study of the primary structure of β turns in proteins[1-4] has shown that although some particular residues occur more frequently at preferential positions, almost all the amino acids can be found experimentally at each position of the turn. Thus for oligopeptides other factors of stabilization of this structure are necessary. Cyclization or ionic interactions between the N- and C-terminal charges of the peptidic backbone[5] have been used. Other factors such as supplementary hydrogen bonds provided by a contiguous β sheet or ionic interactions between amino acid side chains are more representative of the protein structure.

In protein molecules the positive charges are mainly imparted by the basic guanidino and amino groups of the arginine and lysine side chains. These charges tend to be balanced by the negative charges of the carboxylate groups of the aspartyl and glutamyl residues. Salt bridges have been observed in the crystalline structure of several proteins. For example, salt bridges with two hydrogen bonds have been described in myoglobin[6] between $Arg_{45}$ and $Asp_{60}$ and in carboxypeptidase A[7] between $Arg_{272}$ and $Glu_{292}$ while salt bridges with only one hydrogen bond between arginine and aspartic or glutamic acid side chains have been found frequently in several proteins. In solution, an intramolecular complex between the arginyl and glutamyl residues side chains has also been previously reported for the dipeptide Arg-Glu with both N- and C-terminal ends blocked.[8]

The present work is based upon the idea that such a salt bridge between the side chains of arginine and glutamic acid residues in positions 1 and 4, respectively, in a tetrapeptide should lock a β bend of the peptidic backbone. Due to the predicted high probability to obtain a β turn with the sequence alanyl[2]-glycyl[3] the tetrapeptide Boc-Arg-Ala-Gly-Glu-NHEt was synthesized with its N- and C-terminal ends blocked. In spite of the fact that this sequence has not been found in the 421 turns analyzed by Chou and Fasman[4] in their survey of 26 proteins whose structures have been determined by X-ray crystallography, it must be noticed that the sequence Asp-X-Y-Arg is found four times.

Here we report a NMR investigation of the conformation of Boc-Arg-Ala-Gly-Glu-NHEt, in solution. We have examined the effects of ionic strength and solvent on this conformation.

## Experimental Section

**Materials.** All amino acids (of the L configuration) were obtained from Merck. For thin-layer chromatography (TLC) precoated silicagel plates from Merck were used. $Me_2SO$-$d_6$ was purchased from C.E.A. (France).

**Peptide Synthesis.** The starting materials Boc-Arg($NO_2$)-OH,[9] Nps-Ala-OH, DCHA,[10] Boc-Gly-OH,[11] and Nps-Glu(OBzl)-NHEt[8] were prepared by standard literature procedures. The Nps and Boc protecting groups were removed by HCl/ether and HCl/acetic acid, respectively. The classical dicyclohexylcarbodiimide method at low temperature was used for the formation of the peptide bonds. Compounds 1,3, and 5 (Table I) were further purified by preparative chromatography on silica gel with chloroform/methanol (9:1) as eluting solvent.

The hydrogenolytic cleavage of the nitro group from $N^{\omega}$-nitroarginine and the benzyl group protecting the side chain of glutamic acid was carried out simultaneously in water/methanol (1:6) solution by using palladium/activated charcoal (10% Pd) as the hydrogenation catalyst. No acid was added in these hydrogenolyses because as soon as the guanidinium groups of the arginine side chain were liberated, they were protonated by the carboxylic groups of the glutamic acid side chains.

After purification, all the peptides prepared showed a single spot when subjected to thin-layer chromatography on silica gel by using the following solvent systems: (A) chloroform/methanol/acetic acid (85:10:5), (B) 1-butanol/acetic acid/water (4:1:1), and (C) ethyl acetate/pyridine/water (20:10:11) (all ratios by volume). A summary of the reaction yields, melting points, optical rotations, and thin-layer chromatography $R_f$ values of the new compounds synthesized is given in Table I. The greatest deviation in the elementary analysis of these compounds between the experimental and the calculated values did not exceed 0.2% for C, 0.2% for H, 0.1% for N, 0.3% for O, and 0.2% for S. Great care was taken to avoid the presence of chloride and acetate anions which strongly interact with the guanidinium group.[12]

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Table I. Summary of Physical Data of the Oligopeptides Synthesized

| no. | peptide | % yield | mp, °C | $[\alpha]^{25}_{546}$, deg ($c$ = 1, MeOH) | $R_f$ value (solvent system) |
|---|---|---|---|---|---|
| 1 | Boc-Gly-Glu(OBzl)-NHEt | 85 | 86-88 | −13.5 | 0.65 (A) |
| 2 | HCl, H-Gly-Glu(OBzl)-NHEt | 95 | 162-163 | −4.2 | 0.45 (C) |
| 3 | Nps-Ala-Gly-Glu(OBzl)-NHEt | 73 | 159-160 | −47.0 | 0.56 (A) |
| 4 | HCl, H-Ala-Gly-Glu(OBzl)-NHEt | 95 | 193-195 | +1.5 | 0.46 (B) |
| 5 | Boc-Arg($NO_2$)-Ala-Gly-Glu(OBzl)-NHEt | 55 | 95-98 | −7.1 | 0.62 (B) |
| 6 | Boc-Arg-Ala-Gly-Glu-NHEt | 89 | 155-158 | +8.5 | 0.31 (B) |

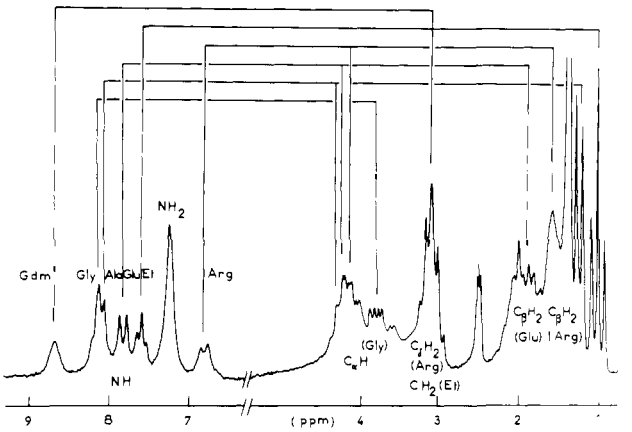


**Figure 1.** The 90-MHz spectrum of Boc-Arg-Ala-Gly-Glu-NHEt ($2 \times 10^{-2}$ M) in $Me_2SO$-$d_6$ at 295 K. The full lines show the irradiated resonance lines and the corresponding observed collapsed lines. Parts per million are expressed from tetramethylsilane ($Me_4Si$ = 0.00).

**Methods.** The $^1H$ (90-MHz) NMR spectra were recorded with a WH 90 Bruker spectrometer operating in the pulse Fourier transform mode. The temperature was controlled within ±1 °C. Samples were thoroughly dried under vacuum at 40 °C and dissolved in 99.95% $Me_2SO$-$d_6$ under dry-nitrogen atmosphere. The $^1H$ (250-MHz) NMR spectra were recorded with a Cameca 250 spectrometer operating in the pulse Fourier transform mode. All double irradiation experiments were made at 90 MHz, and the results were confirmed by 250-MHz experiments. The NOE experiments were performed in the pulsed Fourier transform mode with a 250 WH Bruker spectrometer on a degazed solution ($5.10^{-2}$ M) in $Me_2SO$-$d_6$ at 293 K. The NMR signal of interest was selectively saturated, and then the NMR spectrum was obtained by applying an observation pulse ($\pi/2$) and Fourier transforming the resulting free-induction decay (FID). Four FID of this type were accumulated following by four FID without irradiation. Each experiment was separated by 5 s and a total of 4 × 1500 FID's accumulated with and without saturation. Finally the NOE was obtained by substracting the single-resonance Fourier transform NMR spectrum from that of the double resonance. The samples of peptide in different ionization state in $Me_2SO$-$d_6$ were prepared as follows: the peptide was dissolved in water and the pH was adjusted by addition of perchloric acid, and afterward this solution was lyophilized and the residue dissolved in dimethyl sulfoxide.

## Results and Discussion

**1. Assignments and Ionization State of the Side Chains.** Figure 1 shows the 90-MHz $^1H$ resonance spectrum of Boc-Arg-Ala-Gly-Glu-NHEt in $Me_2SO$-$d_6$ at 295 K. The assignments of the different resonance lines could be partly solved by comparison with the previously reported spectra of peptides in $Me_2SO$-$d_6$[13] and with the spectra of the N- and C- protected amino acids Ac-Arg-NHEt and Ac-Glu-NHEt[8] (Table II). The $C_\alpha H$ resonance lines of Ala, Glu, and Arg were assigned by irradiation of the $C_\beta H_3$ (Ala), $C_\beta H_2$ (Glu), and $C_\beta H_2$ (Arg) resonances, respectively. The *tert*-butyloxycarbonyl group (Boc) on the N-terminal end of arginine led to an upfield shift (0.35 ppm)[8] of the $C_\alpha H$ (Arg) resonance and allowed a good resolution of the $C_\alpha H$ (Ala) and $C_\alpha H$ (Glu) resonance lines. This did not occur with the *N*-acetyl tetrapeptide where these three $C_\alpha H$ resonance lines overlapped. The typical octuplet at 3.67 ppm was assigned to the $C_\alpha H_2$ (Gly).

All the peptidic NH resonance lines were assinged by homonuclear decoupling of the corresponding $C_\alpha H$ groups. The typical triplet structure ($J$ = 5 Hz) of the NH (Et) at 7.75 ppm and the typical downfield shift[8,14,15] of NH (Arg) from 1.57 ppm when the Boc group is substituted by an acetyl group are in agreement with these assignments. The broad resonance line at 8.92 ppm could be ascribed to the $N_\epsilon H$ of the guanidinium group or to the proton of the carboxylic group (COOH) of the glutamic acid side chain, since the tetrapeptide has a total net charge equal to zero and can exist under two forms: ionized form $-NH-C^+-(NH_2)_2\cdots COO^-$ or protonated form $-N=C-(NH_2)_2\cdots COOH$. The irradiation of $C_\delta H_2$ (Arg) led to a collapse of this blurred triplet at 8.92 ppm and so this resonance line was attributed to the $N_\epsilon H$ (Arg) proton. Consequently the glutamyl residue exists in the carboxylate form in the tetrapeptide.

**2. Hydrogen Bonding between Guanidinium and Carboxylate Groups.** Evidence of association between guanidinium and carboxylate groups has been already reported in the literature. The X-ray studies of crystalline complex of L-arginine-L-glutamate show that each oxygen atom of the glutamic acid is bound by one hydrogen bond with the $NH_2$ groups of the arginine side chain.[16] More recently it was shown that substitution of the perchlorate anion by an acetate anion in AcArgNHEt perchlorate led to a downfield shift of the guanidinium amino resonance lines.[8] We have extended these results by studying the concentration dependence of the chemical shifts of amino protons of *N*-methylguanidinium acetate in $Me_2SO$-$d_6$ at 305 K. Lowering the concentration of guanidinium from $5 \times 10^{-2}$ to $10^{-3}$ M led to an upfield shift of NH (Gdm) by 1.45 ppm and of $NH_2$ (Gdm) by 0.91 ppm. For concentrations higher than $2.0 \times 10^{-2}$ M the ratio of the variation of the chemical shifts of $N_\epsilon H$ and $NH_2$ protons is not constant, that shows the existence of complexes of stochiometry different of 1:1 in solution. Analysis of these data as well as Job curves obtained by mixing sodium acetate and *N*-methylguanidinium perchlorate (the sum of the concentrations was kept constant and equal to $2 \times 10^{-2}$ M) and by using a least-square program gives the association constant $K = 220 \pm 40$ $M^{-1}$ the chemical shift at zero concentration $\delta_0(NH_2)$ 6.81 and $\delta_0(N_\epsilon H)$ 7.24 for *N*-methylguanidinium acetate and $\delta_0(NH_2)$ 7.02 and $\delta_0(N_\epsilon H)$ 7.28 for *N*-methylguanidinium perchlorate, and the variation of chemical shift between complexed and free guanidinium $\Delta\delta_c(NH_2)$ = 1.46 and $\Delta\delta_c(N_\epsilon H)$ = 2.80. As already reported,[8] two 1:1 complexes by two hydrogen bonds between guanidinium and carboxylate groups can exist and the association constants computed here represent the sum of the association constants corresponding to each 1:1 complex.

**3. Evidence of Hydrogen Bond Formation in the Tetrapeptide.** The resonance lines of the $N_\epsilon H$ and $NH_2$ protons of the guanidinium (Gdm) group were found at 7.59 and 7.07 ppm in Ac-Arg-NHEt, $HClO_4$, and at 8.92 and 7.36 pmm in Boc-Arg-Ala-Gly-Glu-NHEt, respectively (Table II). For the two amino groups of guanidinium the true variation of the chemical shift of the NH proton which is hydrogen bonded can be four times the observed variation since only the average frequency of the two amino groups is measured. The downfield shifts observed for the

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Table II. Proton Magnetic Resonance Data of Peptides ($5 \times 10^{-2}$ M) in $Me_2SO$-$d_6$ at 295 K from Tetramethylsilane ($Me_4Si$ = 0.00)

| peptide | $N_\epsilon H$ (Gdm) | NH (Gly) | NH (Ala) | NH (Glu) | NH (Et) | $NH_2$ (Gdm) | NH (Arg) | $C_\alpha H$ (Ala) | $C_\alpha H$ (Glu) | $C_\alpha H$ (Arg) | $C_\alpha H$ (Gly) | $C_\delta H_2$ (Arg) | $C_\gamma H_2$ (Glu) | $C_\beta H_2$ (Glu) | $C_\gamma H_2$ (Arg) | $C_\beta H_2$ (Arg) | $C_\beta H_3$ (Ala) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ac-Arg-NHEt, perchlorate | 7.59 | | | | 7.94 | 7.07 | 7.97 | | | 4.19 | | 3.08 | | | 1.50 | 1.48 | |
| -Ala-[a] | | | 8.04 | | | | | 4.34 | | | | | | | | | 1.22 |
| Boc-Gly-NHEt | | 6.88 | | | 7.73 | | | | | | 3.47 | | | | | | |
| Ac-Glu-NHEt, sodium | | | | 8.83 | 8.14 | | | | 4.04 | | | | 1.91 | 1.70 | | | |
| Boc-Arg-Ala-Gly-Glu-NHEt[b] | 8.92 | 8.37 | 8.21 | 7.85 | 7.74 | 7.36 | 7.11 | 4.14 | 4.06 | 3.94 | 3.67 | 3.06 | 1.96 | 1.80 | 1.55 | 1.55 | 1.22 |
| Boc-Arg($NO_2$)-Ala-Gly-Glu(OBzl)-NHEt | | 8.19 | 8.03 | 7.95 | 7.90 | | 6.95 | 4.25 | 4.25 | 3.90 | 3.70 | 3.06 | 1.96 | 1.85 | 1.51 | 1.51 | 1.20 |

[a] From $CF_3$-CO-Gly-Gly-Ala-Ala-$OCH_3$.[13] [b] For $2 \times 10^{-2}$ M concentration.

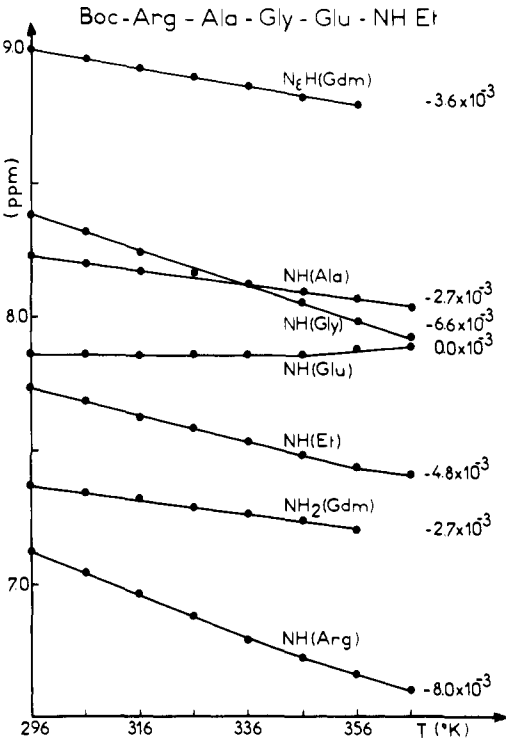


**Figure 2.** Temperature dependence of NH chemical shifts of Boc-Arg-Ala-Gly-Glu-NHEt ($2 \times 10^{-2}$ M) in $Me_2SO$-$d_6$ (slopes are in ppm by degree).

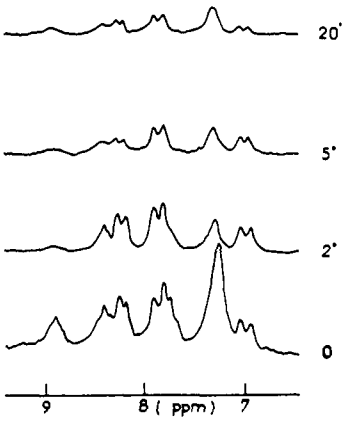


**Figure 3.** The 90-MHz spectra of exchangeable protons of Boc-Arg-Ala-Gly-Glu-NHEt ($5 \times 10^{-2}$ M) in $Me_2SO$-$d_6$ at different times after addition of $5 \times 10^{-1}$ M $D_2O$. With this ratio of concentration only 70% of protons can be exchanged. Each spectrum is obtained with 50 scans (80 s).

tetrapeptide can result from intramolecular or intermolecular hydrogen bonding.

The concentration dependence of the chemical shifts of the different NH protons of the tetrapeptides in $Me_2SO$-$d_6$ is shown in Table III. The small variations of the chemical shifts observed (0.08 ppm for $N_\epsilon H$ (Gdm) and 0.07 ppm for $NH_2$ (Gdm)) at concentrations ranging from $10^{-3}$ to $5 \times 10^{-2}$ M showed that there were only very few intermolecular associations which will be further neglected.

The temperature dependence of the chemical shifts of the NH protons of Boc-Arg-Ala-Gly-Glu-NHEt ($2 \times 10^{-2}$ M in $Me_2SO$-$d_6$) is shown in Figure 2. A linear dependence is observed with slopes given in Table IV. Since NH (Arg) is bonded to a *tert*-butyloxycarbonyl group by an urethan linkage, the temperature dependence of its chemical shift could not be compared to that of peptidic NHs. The structure of an urethan bond is indeed different from that of a peptidic bond and can be found in a cis conformation.[15] Since the temperature dependence of the chemical shift of amide protons exposed to solvent is typically $-6 \times 10^{-3}$

Table III. Concentration Dependence of the NH Chemical Shifts in Boc-Arg-Ala-Gly-Glu-NHEt at 315 K in $Me_2SO$-$d_6$

| concn, M | $N_\epsilon H$ | NH (Gly) | NH (Ala) | NH (Glu) | NH (Et) | NH (Gdm) | NH (Arg) |
|---|---|---|---|---|---|---|---|
| $5 \times 10^{-2}$ | 8.88 | 8.24 | 8.16 | 7.85 | 7.64 | 7.31 | 6.95 |
| $2 \times 10^{-2}$ | 8.85 | 8.24 | 8.16 | 7.85 | 7.64 | 7.31 | 6.95 |
| $2 \times 10^{-3}$ | 8.81 | 8.24 | 8.14 | 7.83 | 7.63 | 7.24 | 6.95 |
| $10^{-3}$ | 8.80 | 8.24 | 8.14 | 7.83 | 7.63 | 7.24 | 6.95 |
| $2 \times 10^{-2}$ + $LiClO_4$ (2 M) | 8.29 | 8.14 | 8.14 | 7.81 | 7.68 | 7.10 | 6.83 |

Table IV. Temperature Dependence of the NH Chemical Shift of Boc-Arg-Ala-Gly-Glu-NHEt ($2 \times 10^{-2}$ M) and of the Protected Tetrapeptide Boc-Arg($NO_2$)-Ala-Gly-Glu(OBzl)-NHEt ($2 \times 10^{-2}$ M) (Slopes in $10^{-3}$ ppm/deg)

| solvent | NH (Arg) | $N_\epsilon H$ (Gdm) | $NH_2$ (Gdm) | NH (Ala) | NH (Gly) | NH (Glu) | NH (Et) |
|---|---|---|---|---|---|---|---|
| $Me_2SO$-$d_6$ | −8.0 | −3.6 | −2.7 | −2.7 | −6.6 | 0.0 | −4.8 |
| $Me_2SO$-$d_6$ + $LiClO_4$ (2 M) | −6.6 | −9.5 | −4.1 | −5.7 | −5.7 | −2.0 | −5.8 |
| protected tetrapeptide, $Me_2SO$-$d_6$ | −9.5 | | | −5.7 | −6.5 | −5.6 | −6.5 |

ppm/deg,[18] the results obtained provide evidence for intramolecular hydrogen bonding of NH (Glu) in the tetrapeptide.

Conventional hydrogen–deuterium exchange experiments were also carried out to obtain information about possible intramolecular hydrogen bonds. Figure 3 shows the NH resonance spectra of the tetrapeptide ($5 \times 10^{-2}$ M) in $Me_2SO$-$d_6$ at 295 K at different times after addition of 0.5 M $D_2O$. With these concentrations only 70% of the protons could be exchanged, and since each spectrum was obtained after 80 s (50 scans), the half-life time of exchange ($t_{1/2}$) could only be evaluated. $t_{1/2}$ of $N_\epsilon H$ (Gdm) and $NH_2$ (Gdm) are less than 30 s, $t_{1/2}$ of NH (Gly), NH (Ala), and NH (Et) are in the range of 1–2 min, $t_{1/2}$ of NH (Glu) is about 4 min, and $t_{1/2}$ of NH (Arg) is about 8 min. It is well-known that the rate of exchange of NH protons is both acid and base catalyzed. So no conclusion could be reached for $N_\epsilon H$ (Gdm) and $NH_2$ (Gdm) since they belong to the positively charged guanidinium group. The rate of exchange of the NH protons of Ala, Gly, and ethylamide is fast and suggests a structure in which these protons are exposed to the solvent. The relatively long half-life time exchange of NH (Glu) and NH (Arg) is evidence of intramolecular hydrogen bonding of these protons.

The role of the salt bridge between the protonated guanidinium group and the deprotonated carboxylate on the formation of these two hydrogen bonds could be checked by chemical or physical modifications of the corresponding side chains. The chemical shifts of the exchangeable protons of the protected peptide Boc-Arg-($NO_2$)-Ala-Gly-Glu(OBzl)-NHEt in $Me_2SO$-$d_6$ solution are reported in Table II. The values of these chemical shifts as well as the temperature dependence (Table IV) and the half-life time of the H–D exchange (less than 30 s for each NH protons) show that no hydrogen bond exists in this peptide. Moreover the influence of the ionization state of the glutamic acid side chain has been studied. Figure 4 shows the NMR spectra of the NH protons of Boc-Arg-Ala-Gly-Glu-NHEt in $Me_2SO$-$d_6$ at pH 4.9 (ionized $COO^-$ group) and at pH 2.5 (protonated COOH group) (see Experimental Section). It appears that the protonation of the glutamic acid side chain led to an upfield shift of $N_\epsilon H$ (Gdm), $NH_2$ (Gdm) and NH (Arg). These results confirm that the $N_\epsilon H$ and the $NH_2$ protons of the guanidinium are hydrogen bonded to the ionized carboxylate group of the glutamyl residue.

**4. Nuclear Overhauser Experiments.** The intramolecular nuclear overhauser effect (NOE) is very sensitive to intramolecular distance between spins and has already yielded informations about the conformation of various molecules.[18] The origins of the NOE effects have been extensively investigated on heteronuclear systems and homonuclear systems.[18,19] When only dipole–dipole mechanisms contribute significantly to the two quantum transition between states at two spins, the NOE effect is related by $W/r^6$ where $W$ is the transition probability for a pair of spins separated by one unit of distance and $r$ the distance between the two nuclei considered. The NOE effect is then very sensitive to the distance ($r^{-6}$) of the two protons and was used for the quantitative evaluation of the structure of small molecules.[18]

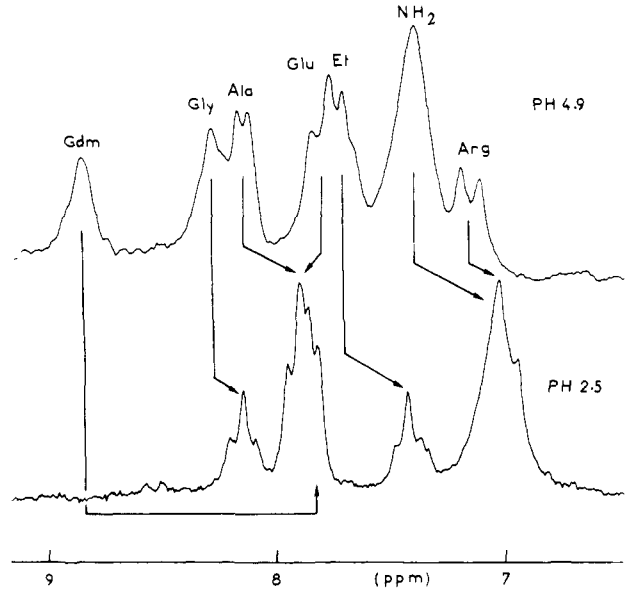


**Figure 4.** The 90-MHz spectra of NH protons of Boc-Arg-Ala-Gly-Glu-NHEt ($2 \times 10^{-2}$ M) at pH 4.9 and 2.5 (see Experimental Section) in $Me_2SO$-$d_6$ at 305 K.

When the NH (Ala) resonance of the tetrapeptide Boc-Arg-Ala-Gly-Glu-NHEt ($5 \times 10^{-2}$ M, 293 K) was saturated, NOE effects of intensity 0.036 proton and 0.049 proton were observed on $C_\alpha H$ (Ala) and $C_\alpha H$ (Arg) resonances, respectively. A weaker effect was observed on $C_\alpha H$ (Glu) resonance (0.01 proton) when the NH (Et) resonance was saturated. These data are indicative of several informations: (*i*) the proton–proton distance $C_\alpha H$ (Arg)–NH (Ala) is small and in the range of the distance $C_\alpha H$ (Ala)–NH (Ala), (ii) the proton–proton distance NH (Et)–$C_\alpha H$ (Glu) is small but greater than $C_\alpha H$ (Ala)–NH(Ala), and (iii) the structure of the tetrapeptide is rather constrained since NOE effects in the range of 0.05 proton are observed.

**5. Conformation of the Tetrapeptide.** The conformation of the tetrapeptide can be solved up by the determination of the conformational torsion angles $\phi$ obtained from the observed $^3J_{C_\alpha H\text{-}NH}$ coupling constants by using the Karplus-like relationship of Bystrov.[20] In $Me_2SO$-$d_6$ at 295 K the values of these coupling constants are 8.8 Hz for Arg, 5.0 Hz for Ala, and 8.2 Hz for Glu. Figure 5 shows the 250-MHz resonance spectra of the $C_\alpha H$ protons of Boc-Arg-Ala-Gly-Glu-NHEt in $Me_2SO$-$d_6$ at 295 and 325 K. An ABX spin pattern was observed for the methylene $C_\alpha H_2$ protons of glycine. This allowed us to estimate the $C_\alpha H$–NH torsion angles by means of the $^3J$ coupling constants. The ABX spectrum of $C_\alpha H_2$ (Gly) was simulated on a computer, and the best fits of coupling constants were obtained at 295 K for $\Delta\nu_{AB}$ = 68 Hz, $J_{AX} + J_{BX}$ = 11.0 Hz, $J_{AX}$ = 6.8 Hz, $J_{BX}$ = 4.2 Hz,

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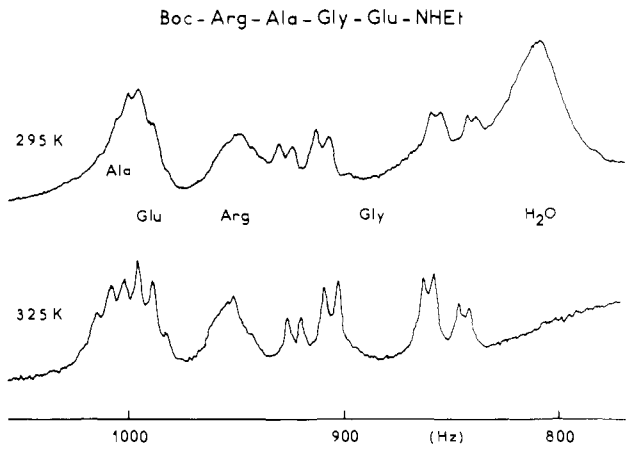


**Figure 5.** The 250-MHz spectra of $C_\alpha H$ protons of Boc-Arg-Ala-Gly-Glu-NHEt ($2 \times 10^{-2}$ M) in $Me_2SO$-$d_6$ at 295 and 325 K. The broadening of the resonance lines of the peptide at 295 K reflects the increase of the viscosity of $Me_2SO$-$d_6$ as shown by the broadening of the $Me_4Si$ resonance line.

and $J_{AB} = -16.8$ Hz and at 325 K for $\Delta\nu_{AB} = 60$ Hz, $J_{AX} + J_{BX} = 11.2$ Hz, $J_{AX} = 6.1$ Hz, $J_{BX} = 5.1$ Hz, and $J_{AB} = -16.8$ Hz. The appearance of the $C_\alpha H_2$ (Gly) protons as an ABX spin system is consistent with a constrained conformation.[21]

The Bystrov's relationship[20] provides four possible values of torsion angle for each value of $^3J$ coupling constant: $\phi$(Arg) = 60°, -90°, or -150°; $\phi$(Ala) = 20°, 100°, -70°, or -170°; $\phi$(Gly) = ±60° or ± 135°; $\phi$(Glu) = 60°, -95°, or -155°.

Moreover, the geminal H–$C_\alpha$–H coupling constant is a function of the orientation of the $\pi$ orbital of the glycyl C=O bond. The relationship of Cookson et al.[22] allowed us to compute a torsion angle $\psi = \pm(18 \pm 18°)$ or $\psi = \pm(163 \pm 17°)$ for $J = -16.8$ Hz.

Taking into account all these different values of torsion angles, the existence of the Arg-Glu salt bridge and the hydrogen bonding of NH (Glu) and NH (Arg), the examination of molecular models reveals that only one type of conformation can be obtained: a $\beta$ turn involving a Arg[1]-CO···HN-Glu[4] hydrogen bond with Ala[2] and Gly[3] at the corners. There is an additional Arg[1]-NH···OC-Glu[4] hydrogen bond, and this structure is locked by the salt bridge involving two hydrogen bonds between the carboxylate group and the $N_\epsilon H$ (Gdm) and $NH_2$ (Gdm) protons. These four hydrogen bonds are shown in Figure 6 where a type I $\beta$ turn has been represented only for illustrative purpose. Actually the measured coupling constants did not allow us to distinguish between turns of type I or type II. The NOE results support this model since they are in good agreement with the proton–proton distances measured on the Dreiding model (Figure 6): $C_\alpha H$ (Ala)–NH (Ala) = 2.4 Å, $C_\alpha H$ (Arg)–NH (Ala) = 2.2 Å, and $C_\alpha H$ (Glu)–NH (Et) = 3.1 Å.

**Effect of the Ionic Strength and of the Solvent on the Conformation of the Peptide.** The temperature dependence of the chemical shift of the peptidic NH protons of Boc-Arg-Ala-Glu-NHEt in $Me_2SO$-$d_6$ was studied in function of the concentration of lithium perchlorate (Table IV). The addition of $LiClO_4$ weakened the salt bridge of the peptide as inferred from the 0.56 ppm upfield shift of the $N_\epsilon H$ resonance line observed in 2 M of salt (Table III). In absence of salt the temperature dependence

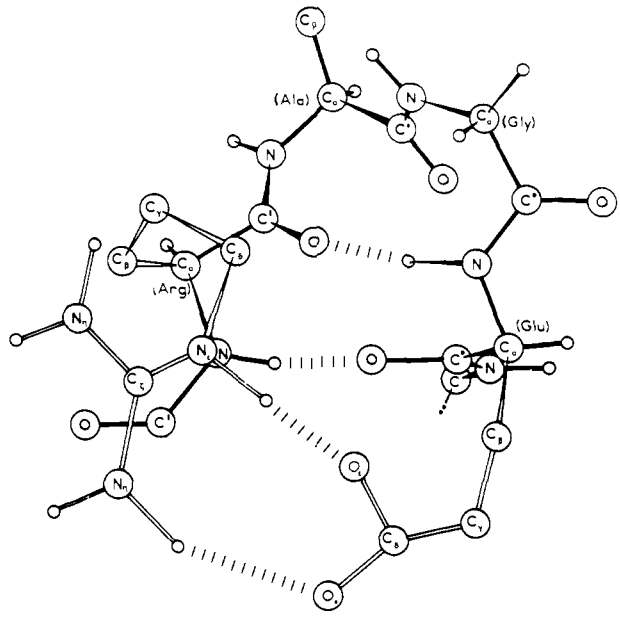


**Figure 6.** A proposed conformation of the tetrapeptide Arg-Ala-Gly-Glu obtained by Dreiding model.

of NH (Glu) was linear and equal to zero between 296 and 346 K and for higher temperatures the slope became slightly positive (Figure 2). This agrees with the existence of a bent NH (Glu)···CO (Arg) hydrogen bond at room temperature as shown in Figure 6. The increase of temperature weakens the salt bridge, and the tetrapeptide takes a conformation which allows a stabilization of this hydrogen bond in agreement with the downfield shift observed for the NH (Glu) resonance line above 346 K. On the other hand, when $LiClO_4$ is added the salt bridge is weakened and this hydrogen bond becomes more straight. In presence of salt an increase of temperature reveals only the accessibility of the solvent to the NH (Glu) proton which results in a linear temperature dependence with a negative slope of $-2 \times 10^{-3}$ ppm/deg. Moreover, in presence of $LiClO_4$, the resonance of the Gly methylene does not retained its ABX appearance. A doublet at 3.68 ppm ($J = 17$ Hz) was assigned to the two equivalent protons $C_\alpha H_2$ of glycine. This result is indicative of a great mobility of the methylene protons of glycine and agrees with the conclusion that in presence of salt, the $\beta$ turn structure was disrupted.

In hydrogen-bonding solvents such as water or alcohols the two hydrogen bonds between the Arg and Glu side chains are drastically weaker. In water the $N_\epsilon H$ resonance line is found at 7.13 ppm, and all peptidic NH protons have temperature dependence with slopes in the range of $-5 \times 10^{-3}$ to $-7 \times 10^{-3}$ ppm/deg, showing the absence of any hydrogen bonds. Therefore solvents of high polarity and hydrogen bonding ability disrupt the Arg···Glu salt bridge and break the $\beta$ turn structure.

From the results reported here it appears that the tetrapeptide Boc-Arg-Ala-Gly-Glu-NHEt is a $\beta$ turn whose structure is strongly stabilized by the salt bridge between the charged side chains of the arginyl and glutamyl residues. Moreover CPK models show that such a $\beta$ turn structure can also exist in the peptides Arg-Ala-Gly-Asp, Glu-Ala-Gly-Arg, and Asp-Ala-Gly-Arg.

**Acknowledgment.** We wish to thank Professor M. Ptak for critical discussions and NOE experiments and Professor C. Hélène and Dr. G. Spach for helpful discussions.

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