INFLUENCE OF SALT BRIDGE FORMATION ON HELIX STABILITY

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Submitted in fulfillment

of the requirements for

Honors in the Department of Chemistry

Union College

June, 1995

ABSTRACT

NICOLETTA, ANGELO Influence of Salt Bridge Formation on Helix Stability. Department of Chemistry, June 1995

One of the most common structural elements in proteins is the a-helix. The main goal of this study was to see if the helical properties of short peptide chains could be increased by the formation of salt bridges between side chains. It has been shown that i,i+4 peptides posses helical character. The research that has been done was to make a hybrid peptide chain, that had partial i,i+4 and partial i,i+3 salt bridge formation. The hybrid peptide was a i+4, i+3, i+4 peptide chain. The overall purpose of making this type of hybrid peptide was to make the approximate number of residues in a turn of the helix closer to the 3.6 residues, which is the number of residues in a natural turn of the helix. The charged amino acids used were positive lysine residues and negative glutamate residues. The peptides were constructed through solid-phase synthesis and purified using HPLC. The stability of the peptides were examined theoretically using molecular modeling and experimentally the helicity was tested by Circular Dichroism. The experimental results of the mixed spaced peptides were compared to Stellwagon's and Baldwin's uniformly spaced peptides. This study found that the mixed spaced peptides were at least as stable as the uniformly spaced peptides. A version of the uniformly spaced peptide was also synthesized, but assumed a b-sheet configuration, which is currently under investigation through both experimental and theoretical studies.

Acknowledgements:

I would like to thank the following people and institutions for their guidance and support.

Union College

N.Y.U.

Dr. J.S. Anderson

Dr. L.A. Hull

Mr. and Mrs. Angelo Nicoletta

Angelo Carmine Nicoletta

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Chapter 1 - Introduction:

Background:

The secondary structures of proteins have been of interest to scientists for years. The secondary structure of a protein gives a substantial amount of information and foresight to the protein's function in a given biological system. Proteins are biomolecules composed of amino acids. Amino acids are the building blocks for proteins; there may be up to as many as several thousand amino acids in a single protein.

There are twenty different natural amino acids. Natural amino acids are those amino acids that are found in living organisms. These twenty natural amino acids are differentiated only by the side chain on the α-carbon of the carboxylic portion of the amino acids. The general structure of an amino acid is an amine group bonded to the α-carbon of an ethanoic acid. According to their side chains amino acids have been grouped into six general categories. An example of this grouping is the system used by Loudon[1]:

[1] Loudon, G.M., Organic Chemistry, The Benjamin/Cummings Publishing Company, Inc., USA, 1984. Chapter 26

1. Amino Acids with either a hydrogen or aliphatic hydrocarbon side chain

2. Amino Acids with an aromatic side chain

3. Amino Acids whose side chains contain either a SH, SCH3, or OH group

4. Amino Acids whose side chains contain either a carboxylic acid or amide group

5. Amino Acids with basic side chains

6. Proline is placed in its own class because of its unique structure

Proteins contain several levels of structure each more intricate then the next. The actual linking of the amino acids in a protein are through what are referred to as peptide bonds. A peptide bond is a bond formed between the amine nitrogen of one amino acid and the carbonyl of another. The actual sequence of amino acids in a protein is the protein's primary structure, which is also called the backbone of the protein. By convention, peptide chains are always named starting at the amino or N-terminus and ending at the carboxy or C-terminus.

The protein will fold up according to its specific primary structure, part of this folded structure is called the secondary structure. The secondary structure of a protein is determined by the hydrogen bonding that occurs within the backbone of the protein. The secondary structure hydrogen bond is formed between the amine hydrogen on one amino acid and the oxygen on the carbonyl group of another amino acid.

The secondary structure of a protein can be in one of three subclasses:

1. The α-Helix

2. The β-Pleated Sheet

3. The Random Coil

The first subclass, the α-helix is the secondary of most concern to this study. In the α-helix structure, the side chains are bonded on the outside of the structure. This structure gives the appearance of a spiral staircase. The hydrogen bonds that stabilize this secondary structure are between amino acids that are four residues apart. The second subclass, the β-pleated sheet, is held together through hydrogen bonding between adjacent parallel peptide chains. β-pleated sheets can be either parallel or anti-parallel; this

depends on the way the adjacent peptides backbones are linked together. The third and final subclass, the random coil, will be of interest in this study. The random coil is important in studying helix formation in short peptide chains, due to the fact that the peptide chain will fluctuate between a random coil structure and an α-helical structure.

A protein's tertiary structure is formed from interactions between amino acid side chains on the same monomeric protein. The tertiary structure is what will be manipulated in this study to cause a greater stabilization of the protein's secondary structure.

The quaternary structure of a protein is determined by the interactions formed between two or more monomeric proteins. Therefore, quaternary structure is only applicable to multimeric proteins and is not of concern to this study in helix formation in short peptides.

The secondary structure subclass of the a-helix was characterized in 1955 by Pauling[2]. To better understand the α-helix structure, studies were done on the physical properties of the peptide chain. Schellman was at the forefront of this research. In studying the hydrogen bonding of the α-helix Schellman[3] estimated the enthalpy per residue to be roughly -1.5kcal/mol; he also found by changing the entropy term to -R ln j (where j is the number of equivalent torsional conformations of the peptide's primary structure), he

[2] Pauling, L., Carey, R.B., Bransin, H.R. 1951. *Proc. Natl. Acad. Sci.*, USA 205-211
[3] Schellman, J.A. 1955. *C.R. Trav. Lab. Carlsberg Ser. Chim.* **29**: 223-29

estimated the entropy per residue to be roughly -1.4 kcal/mol[4]. Schellman concluded from his results that it was possible to form a somewhat stable helix in an aqueous solvent.

Schellman's research got others curious in protein secondary structure, one researcher in particular was Baldwin. Baldwin[5] wanted to learn more about the formation of α-helices, specifically what the enthalpy of peptide hydrogen bond formation was in water; what the actual j value was and how much of the side chains contribute to it; and how the hydrophobic interaction affects the entropy and enthalpy. For a long time these questions troubled scientists because they had many problems working with large polypeptides in aqueous environments. By using shorter peptides with defined sequences many of the problems first encountered were overcome.

Around 1958 statistical mechanical models of α-helix formation were proposed. In these models helix formation was a two step process: helix nucleation can take place at random locations and only after a helical nucleus has been formed, propagation can occur. Scientists had hoped to use these models to aid their study of α-helix formation. The most common statistical theory is the Zimm-Bragg Theory. In this theory, the first amino acid in the peptide is given a statistical weight (σs), and then every succeeding amino acid is given a statistical weight. The nucleation

[4] Schellman, J.A. 1955 *C.R. Trav. Lab. Carlsberg Ser. Chim.* **29**:230-59
[5] Baldwin, R.L., Scholtz, J.M., 1992. *Annu. Rev. Biophys. Biomol. Struct.* **21**: 95-118

parameter (σ) is the theoretical possibility that the first residue is in the correct conformation to initiate α-helix formation. The (s) term is a measure of each amino acids ability to stabilize or destabilize α-helix formation.

The Zimm-Bragg theory was tested in 1959, by Zimm et al[6]. He tested a series of polypeptides differing in average chain length, by fitting the helix-coil transitions from the theory. The polypeptides were polymers of γ-benzyl-L glutamate. He found the enthalpy through calorimetry calculations, which were later confirmed by Ackerman[7] who also used calorimetry. At the temperature where (s) = 1 was the temperature midpoint on the transition curve. The enthalpy per residue was compared to the Van't Hoff change in the equation:

$$\Delta H^{\circ}_{VH} = \Delta H^{\circ} / s^{1/2}$$

Measuring the ratio of $\Delta H^{\circ}_{VH} / \Delta H^{\circ}$ gives the average number of residues in a helical segment at the temperature midpoint. This first experiment to test the helix-coil theory, using a nonionizing amino acid and organic solvent system, worked well showing good agreement between the theoretical results and the actual ones. The next step was to study helix formation in an aqueous solvent system. The largest difficulty encountered was that helix-forming amino acids such as, methionine and alanine, create polypeptides that are insoluble in water. Also the amino acids that were not helix-forming, such as

[6] Zimm, B.H., Doty, P., Iso, K. 1959. *Proc. Natl. Acad. Sci.* USA **45**: 1601-7
[7] Ackerman, T., Neumann, E. 1967. *Biopolymers* **5**: 649-62

serine and histidine, were soluble in water, but only under certain conditions. The conditions that the uncharged amino acids formed the helix and the charged amino acids did not. Further investigation led to the discovery that the (s) value for both glutamic acid and lysine was 0.0025[8].

The next experiments performed by Scheraga[9,10,11,12]. For all natural amino acids Scheraga and his coworkers determined the statistical weight of the propagation parameter. The studies performed by Scheraga were guest-host studies. In these experiments Scheraga used a water soluble and nonionizing copolymer as the host and one of the twenty amino acids was the guest. The helix-coil transition curves were generated based on the assumption that the copolymer sequences are random. Any deviations from randomness would have had serious repercussions on the outcome of the experiment. All twenty amino acids have been given host-guest values of (s) at 20°C[12]. For all twenty amino acids most of their values were approximately 1(+/- 20%), and the value s=1 signifies helix indifference on the part of the amino acid. Also the host-guest values of (s) are significantly different in water opposed to organic solvents. In organic solvents (s) values indicate

[8] Ptitsyn, O.B. 1972. *Pure Appl. Chem.* **31**: 227-44
[9] Chou, P.Y., Wells, M., Fasman, G.D. 1972. *Biochemistry* **11**: 3028-43
[10] Seuki, M., Lee, S., Powers, S.P., Denton, J.B., Konishi, Y., Scheraga, H.A. 1984. *Macromolecules* **17**: 148-55
[11] Von Dreele, P.H., Lotan, N., Ananthanarayanan, V.S., Andreatta, R.H., Poland, D., Scheraga,H.A. 1971. *Macromolecules* **4**: 408-417
[12] Wojcik, J., Altman, K.H., Scheraga, H.A. 1990. *Biopolymers* **30**: 121-34

that β-branched amino acids are helix destabilizing[13]. The dependence of the (s) value on temperature for each amino acid using the host-guest theory are noticeable because of their variability.

This host-guest method was further investigated by Stellwagen[14] using peptides with a determined sequence. In this study (s) values for the amino acids were found to range between 0.51 and 1.47; the (σ) values were found to range from 0.00001 to 0.0210. The ranges found by Stellwagwen suggest that the nucleation parameter is more influential to α-helix formation then the propagation parameter.

At this point the main instrument used to determine the helical content of a protein was through circular dichroism (CD) measurements. Spectra were taken between 180nm and 260nm because the α-helicies have two characteristic minima peaks (one at 208nm and another at 222nm)[15]. There were some problems encountered with these measurements. The main one was that it was next to impossible to distinguish between, when 50% of the chains were fully helical and 50% were fully random coil and when each chain was 50% helical and 50% random coil. As a result another method of helix determination needed to be used to confirm CD data.

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[13] Blout, E.R. 1962. -Stahmann, M.A. *Polypeptides and Proteins*. Madison: Univ. Wis. Press, 1962.
[14] Park, S., Shalongo, W., Stellwagen, E. 1993 *Biochemistry*, **32**: 7048-53
[15] (a) Greenfield, N., Fasman, G.D. 1969. *Biochemistry* **8**: 4108. (b) Johnson, N.C., Jr. 1988. *Annu. Rev. Biophys. Biophys. Chem.* **17**: 145

Scientists began using NMR to confirm their results. NMR allows one to determine the amino acids in a peptide chain that displays helical properties[16]. In protein NMR four conditions are sought for these spectra: (1) strong NOE signals between adjacent NH protons[17], (2) the NOE signals between peptide NH protons and CαH three amino acids apart[18], (3) values of three-bond $^3J_{\alpha N}$ coupling constants[19], and (4) values of the CαH chemical shifts[20]. Recently, experiments to determine secondary structure content using fourier-transform infrared spectroscopy have been improved to give better resolution[21]. There are known IR spectral ranges for both α-helix and β-pleated sheet structures at 1650-1660cm-1 and 1630-1640cm-1 respectively. It was found that by employing deconvolution techniques and taking the second derivative of the spectra it will, to a certain extent, separate overlapping components to give a more defined spectra[21,22].

The first peptides found to show some helix formation at low temperatures were the C and S peptides of ribonuclease A, discovered by Brown and Klee[23]. The C-peptide is the first thirteen amino acids in the

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[16] Kallenbach, N.R., Liff, M.I., Lyu, P.C. 1991. Journal of the Am. Chem. Society : 113

[17] (a) Billeter, M., Braun, W., Wuthrich, K. 1982. *Mol. Bio.* **155**: 321 (b) Wuthrich, K., Wider, G., Braun, W. 1982. *J. Mol. Biol.* **155**: 371

[18] Wuthrich, K., Billeter, M., Braun, W. 1984. *J. Mol. Biol.* **180**: 715

[19] (a) Bystrov, V.F. 1976. *Prog. Nucl. Magn. Reson. Spectros.* **10**: 41 (b) Bystrov, V.F., Arsenier, A.S., Garrilov, Yu, D. 1978. *J. Magn. Reson.* **30**: 151 (c) Pardi, A., Billeter, M., Wuthrich, K. 1984. *J. Mol.Biol.* **180**: 741

[20] Pardi, A., Wanger, G., Wuthrich, K. 1983. *Eur. J. Biochem.* **137**: 445

[21] Susi, H., Byler, D.M. 1986. *Methods Enzymol.* **130**: 290-311

[22] Chapman, D., Haris, P.I. 1992. *Biochem. Sci.* **17**: 328-33

[23] Brown, J.E., Klee, W.A. 1971. *Biochemistry* **10**: 470-76

ribonuclease and the S-peptide is the first twenty. Brown and Klee used CD measurements to determine helical content. The C-peptide was found to be only about 25% helical at 0°C and at 25°C it undergoes thermal unfolding so its helicity is relatively small. Bierzynski et al[24] further investigated helix formation of the C-peptide in water. NMR and CD measurements were used to confirm the work done earlier by Brown and Klee.

Bierzynski[22] also found the helicity of these peptides were dependent on pH. The helical content of the peptides follows a bell-shaped curve containing a maximum at around a pH of 5, which is when the peptide is most helical. The maximum helical stability of the peptide is dependent on the presence of two ionized side chains, one with a pKa close to 3.5 and one with a pKa close to 6.5. This observation implies that side chain interactions are stabilizing the helix. In the C-peptide the two ionized amino acids are thought to be glutamic acid (pKa near 3.5) and histidine (pKa near 6.5). The fact that helicity in a short peptide can be measured contradicts the host-guest theory. This contradiction is justified by the peptides helicity being dependent upon ionized side chain interactions.

Helix content in proteins are on average eleven amino acids long. Therefore, proteins must have some form of helix termination signal. Rico[25]

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[24] Bierzynski, A., Kim, P.S., Baldwin, R.L. 1982. *Proc. Natl. Acad. Sci.* USA **79**: 2470-74

[25] Rico, M., Nieto, J.L., Santor, J., Bermejo, F.J., Herranz, J., Gallego, E. 1983. *FEBS Lett.* **162**: 314-19

and Baldwin[26] both independently discovered termination of helix formation in the S-peptide, through NMR. Both found that the methionine in the thirteenth position stops further helix formation. Further studies of the C-peptide[27] led researchers to find that it in fact was the charged glutamic acid residue in the second position and the charged histidine in the twelfth position that maximize the helix stability. This was somewhat surprising because of the distance between these two residues in the helix and substitution studies[28] have shown that each of these two amino acids act independently of one another. It was thought that the two amino acids may stabilize the helix by interaction with the helicies natural dipole because they are at the ends of the helix.

This was tested by Shoemaker et al.[29] who confirmed that there was a charged helix-dipole interaction. They also found that acidic residues were usually found near the C-terminus. Their work showed that it was possible to significantly increase helix formation by changing a few amino acids so favorable interactions are made, which more easily allows the formation of short peptide α-helicies in aqueous solutions. These short peptides could now be studied for side chain interactions.

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[26] Kim, P.S., Baldwin, R.L. 1984. *Nature* (London) **307**: 329-33

[27] Shoemaker, K.R., Kim, P.S., Brems, D.N., Marqusee, S., York, E.J., et al. 1985. *Proc. Natl. Acad. Sci.* USA **82**: 2349-53

[28] Rico, M., Gallego, E., Santor, J., Bermejo, F.J., Herranz, J. 1984. *Biochem. Biophys. Res. Commun.* **123**:757-63

[29] Shoemaker, K.R., Baldwin, R.L., Kim,P.S., York, E.J., Stewart, J.M. 1987. *Nature* **329**: 563-67

To this point the studies done on α-helix formation led to the discovery that the amino acid side chains are interacting in some way with the dipole of the helix to stabilize this conformation. Interested in these interactions Baldwin[30] decided to investigate the affects of possible salt bridge formation in short peptide to stabilize α-helix formation. For this study he created four alanine-based peptides. Each peptide contained three glutamic acid residues and three lysine residues, was sixteen or seventeen amino acids long and had blocked α-NH_2 and α-COOH groups. Each of the four peptides had slightly different arrangements, the charged groups were spaced four residues apart (i,i+4 chain) or three residues apart (i,i+3 chain). He also alternated the positively and negatively charged amino acids. He developed two subclasses of peptides:

1. **(i,i+4) 4.0 peptides:**
   - **a.** K,E : Ac-A-K-AAA-E-K-A-A-A-E-K-A-A-A-E-A-NH_2
   - **b.** E,K : Ac-A-E-A-A-A-K-E-A-A-A-K-E-A-A-A-K-A-NH_2
2. **(i,i+3) 3.0 peptides:**
   - **a.** K,E : Ac-A-K-A-A-E-A-K-A-A-E-A-K-A-A-E-A-NH_2
   - **b.** E,K : Ac-A-E-A-A-K-A-E-A-A-K-A-E-A-A-K-NH_2

These are the four peptides created by Baldwin, the ends of the chains are blocked by an acetyl group on the N-terminus and an amine group on the

[30] Marqusse, S., Baldwin, R.L. 1987. *Proc. Natl. Acad. Sci.* USA **84**: 8898-902

C-terminus end. Baldwin choose these two spacing arrangements because one turn of the helix is approximately 3.6 amino acids and the (i,i+4) spacing will form a salt bridge between amino acids four residues away (4.0 amino acids), which slightly overturns the helix and the (i,i+3) spacing will form a salt bridge between amino acids three residues away (3.o amino acids), which slightly underturns the helix. Baldwin found through CD measurements that of the four peptides the E,K peptides were more stable then the K,E peptides and that the (i,i+4) peptides were more stable then the (i,i+3) peptides.

Once he gathered this preliminary data Baldwin began to make variations on these peptides. An early variation done on the peptide sequence was using aspartic acid as the negatively charged amino acid instead of glutamic acid. The positively charged lysine residue was also replaced with an arginine residue. Approximately the same results of α-helix stability was seen for the two substitutions in regard to the original Baldwin peptides[31] . Other variations of this study were done by Stellwagen[14]. Stellwagen used the host-guest theory using two peptides:

1. Ac-Y-E-A-A-A-K-E-A-X-A-K-E-A-A-A-K-A-NH_2
2. Ac-Y-E-A-A-A-E-K-A-X-A-K-E-A-A-A-K-A-NH_2

[31] Baldwin, R.L., Huyghues-Despoints, B.M.P., Sholtz, J.M. 1992. *Protein Science* 2: 80-85

One chain contains three (i,i+4) ion pairs and the other chain contains two potential antagonistic (i,i+4) ion pairs. The guest residue became the residue in the ninth position (the X residue in the above chains). He found that central ionic interactions affect helix stability more than peripheral ionic interactions. The complementary central ionic interactions stabilized the helix by approximately 0.4 kcal/mol and the antagonistic central ionic interactions destabilized the helix by approximately 0.2 kcal/mol. Therefore, changing the central acids in an α-helix can significantly affect the helix stability.

Synthesis Background:

Synthesis of peptides can be done by a variety of different methods. Within these methods are two general types of synthesis blocking groups. The first type is FMOC synthesis[32]. In this type of synthesis the N-terminus of the amino acid is blocked by an FMOC (9-flourenylmethoxycarbonyl) group, which is a dibenzo cyclopentadiene derivative with a carbonate derivative attached to the cyclopentadiene ring. The FMOC group is linked to the amino terminus through the carbonate derivative. Synthesis using FMOC groups to protect the backbone of the amino acid will be employed. Amino acids synthesized using the FMOC method are generally stable

[32] Jones, J. Amino Acid and Peptide Synthesis, Oxford Univ. Press, USA, 1992.

crystalline solids. The second type is Boc synthesis[30]. The Boc protecting group is a t-Butoxycarbonyl that protects the side chains on the amino acids. Boc groups are attached to the side chains through the carbonyl. These two protection groups have been chosen because they are orthogonal to one another. This is because the FMOC protection group is removed under slightly basic conditions and the Boc protection group is removed under slightly acidic conditions. This makes them perfect compliments for protecting both the side chain and the backbone of the amino acids in the same synthesis.

Once synthesized the short peptide chains will need to be purified and concentrated for accurate CD and NMR spectrum. The synthesis is not 100% efficient, therefore some impurity will be in the peptide that will need to be removed before spectra are acquired. The peptides will be purified using gradient HPLC and the purity of the peptide will be confirmed through electrospray mass spectrometery. The helicity of the peptide chains will then be resolved through CD measurements, NMR experiments and possibly IR spectroscopy. Also computer modeling will be employed to find the theoretical energy of the stabilized helix.

The purpose of this study is to examine the influence of non-homogeneous spacing in the Baldwin peptides. For instance combining

both (i,i+3) and (i,i+4) spacing in the same peptide chain. Two new peptides are created from this (i,i+3), (i,i+4) hybridization:

1. **E,K 3.3 peptide:**

   Ac-Y-E-A-A-K-A-A-E-A-A-A-K-A-A-E-A-A-A-K-NH_2[33,34]

2. **E,K 3.7 peptide:**

   Ac-Y-E-A-A-A-K-A-E-A-A-K-A-E-A-A-A-K-A-NH_2

By making hybrid chains the average number of amino acids per salt bridge formation is closer to the actual number of amino acids in one turn of the helix. This closer approximation to the actual value should increase the overall stability of α-helix formation in the peptide. The helicity of the E,K 3.3 and E,K 3.7 peptides would be compared to one another and to a Baldwin type E,K 4.0 homogeneously spaced peptide.

[33]Berger, J.S. Synthesis of a Hybrid Baldwin Peptide, June 1994. Union College, Schenectady, NY

[34] Ernst, J.A NMR Studies of Short Salt Bridged Peptides, June 1994. Union College, Schenectady, NY

Chapter 2 - Experimental:

Synthesis:

The short peptide chains that were used in this study were synthesized utilizing a standard protocol for solid-phase 9-Fluorenylmethoxycarbonyl (FMOC) type of synthesis. The protocol used was a modified version of the FMOC synthesis found in *Amino Acid and Peptide Synthesis*[35]. Our modified version is unusual from normal peptide synthesis in several ways. The most unusual variation is that we are synthesizing the amide form of the peptide chain.

The synthesis was based on the Rink Amide polymer resin, [4-(2'-4'-Dimethoxyphenyl-FMOC-aninomethyl)-phenoxy resin], which was purchased through Nova BioChem[36]. The structure of the resin and linker is shown in Figure 1. As can be seen the amide linker is blocked by an FMOC group and when removed the C-terminus of the amino acid adds to the linker. All peptides were synthesized using approximately 0.5g of the resin. The substitution level of the resin is 0.39 mmol/g; therefore approximately 0.195 mmol of peptide can theoretically be produced provided each of the amino acid coupling reactions goes to completion.

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[35] Jones, J., *Amino Acid and Peptide Synthesis*, Oxford Univ. Press, USA, **1992**.
[36] King, D.S. et al 1990. *Int. J. Pept. Prot. Res.* **36**:255

The short peptide chains that were synthesized are composed of four different amino acids. All amino acids were purchased through Bachem California. The most abundant amino acid found in the peptides is alanine. The alanine purchased from Bachem California was N-FMOC-L-Alanine, in which the N-terminus of the amino acid is blocked by an FMOC group. The positively and negatively charged amino acids used were lys ıd glutamic acid respectively. The actual lysine amino acid purchased from Bachem California was N-ε-Boc-α-FMOC-L-Lysine, where the N-terminus is blocked by the FMOC group and the amine side chain of the amino acid is blocked by a t-Butyoxycarbonyl (Boc) group. The glutamic acid residue that was purchased was N-FMOC-L-Glutamic Acid-γ-t-Butyl ester, where again the N-terminus is blocked by an FMOC group and the acid side chain was blocked with a t-Butyl ester. The final amino acid used was the marker, tyrosine. The tyrosine amino acid purchased was N-FMOC-O-t-Butyl-Tyrosine, where the N-terminus is blocked by an FMOC group and the phenol is blocked with an O-t-Butyl ether group.

Amino acid coupling reactions were carried out in a reaction flask that was fitted with a glass sintered filter, which allowed easy removal of excess reagents and soluble by-products through suction filtration. The reaction flask used is illustrated in Figure 2. The reaction flask was inserted into a

Burrel wrist action shaker to agitate the mixture and increase the rate of the reaction.

Deprotection:

The first step in the synthesis was the removal of the FMOC protection group from the N-terminus. The resin was first soaked in 15ml of N,N-Dimethylformamide (DMF), for 15 minutes to wash off any impurities on the resin that might have been picked up in transferring it to the reaction flask and to expand the resin thereby increasing its surface area. FMOC blocking groups on the resin linker and the N-terminus of the resin-peptide complex were removed in the following fashion:

Step 1: 15ml of a 30% by volume Piperdine(Pip)/ DMF solution is added to the reaction flask and the mixture is shaken for one minute
Step 2: the reagents in the reaction flask are removed by suction filtration
Step 3: another 15ml of the Pip/DMF solution is added to the reaction mixture and shaken for ten minutes
Step 4: after the ten minutes the reagents are removed by suction filtration
Step 5: the now deprotected complex is washed five consecutive times with 15ml of DMF for one minute each to remove any excess reagents

The 30% by volume Pip/DMF solution needed to be replaced every four to five days to make sure it was fresh and reactive solution.

Coupling:

In this step the peptide chain is extended by adding an amino acid to the deprotected peptide or resin. The mmol amount of all amino acids were added in five fold excess of the 0.195 mmols resin used, which was also five times the mmol amount of peptide (0.975 mmols), that should theoretically be produced if all coupling reactions go to completion. The first part of the coupling step was activating an amino acid. This was accomplished by adding the amino acid to the coupling solution.

Coupling: part I

Step 1: mix 5ml of DIEA/DMF with 5ml of 5ml DMF
Step 2: add five fold excess amounts of BOP, HOBt, and the amino acid to be activated
Step 3: agitate mixture for approximately three minutes

All reagents added to the coupling solution were in five fold molar excess of the resin. The reagent BOP is Benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate, HOBt is N-Hydroxybenzotriazole and DIEA is N,N-Diisopropyylethylamine. The DIEA/DMF solution was prepared by mixing 3.4ml of DIEA with 96.6ml of DMF, which was a 1.95mM DIEA solution. Table 1 shows the amounts of each reagent and amino acid added to the coupling solutions:

Table 1:

Amino Acid	Res. M.W.	M.W.	mmol	Amount Added
Lys(Boc)	128.2	468.6	0.975	0.4569g
Tyr(tButyl)	163.2	459.5	0.975	0.4480g
Glu(tButyl)	129.1	425.5	0.975	0.4149g
Ala	71.1	311.3	0.975	0.3035g
Reagent				
BOP	----	442.5	0.975	0.431g
HOBt	----	135.1	0.975	0.132g
DIEA/DMF	----	129.25	0.975 of DIEA	5.0ml

After the amino acid is activated it can be added to the deprotected peptide or resin:

Coupling: part II

Step 1: add coupling solution with activated amino acid to reaction flask

Step 2: allow activated amino acid to react for approximately one hour by agitating in a Burrel wrist action shaker, (usually a little longer for the first amino acid addition)

Step 3: after the hour is over the coupling reagents, side products, and waste are removed by suction filtration

Step 4: the now lengthened resin-peptide complex is washed five consecutive times with 15ml portions of DMF for one minute each

Step 5: Kaiser test[37] is done for free amine detection

[37] Stewart, J.M., Young, J.P., *Solid Phase Peptide Synthesis*, Pierce Chemical Company, Illinois, 1984.

The Kaiser test done in step 5 is to detect free amines still present in the reaction flask. The reaction is run by placing a few grains of the resin-peptide complex in a test tube and two drops of each of three Kaiser reagents are added (0.215M Ninhydrin in n-BuOH, 4.25M Phenol in n-BuOH, and 0.0002M KCN in Pyridine). The test tube with the resin-peptide and Kaiser reagents and a control test tube with only Kaiser reagents are placed in a beaker of boiling water for five minutes. After five minutes if the color of the resin-peptide gains even a slight blue color there are too many free amines present and the coupling step is repeated. The deprotection step is not repeated before doing the coupling step over, however a new coupling mixture needs to be prepared to activate more of the amino acid. The second coupling reaction is allowed to go for 30-45 minutes.

Capping:

In this step the resin-peptide complex is capped at the N-terminal end after the last amino acid is added. The resin-peptide was capped with an acetyl group in a process which is similar to the coupling step mentioned earlier.

Step 1: FMOC protection group is removed from the N-terminus, just as if coupling another amino acid

Step 2: 92μl of acetic anhydride is mixed with approximately 0.431g of BOP and 0.132g of HOBt in 10ml of DMF, and allowed to mix for three minutes prior to adding it to the reaction flask.

Step 3: the capping mixture is added to the reaction flask and allowed to react for one hour

Step 4: capping reagent are removed after one hour by suction filtration

Step 5: excess capping reagents are removed by five consecutive washes of 15ml DMF for one minute each

Step 6: after the five washes the capped resin-peptide is tested for free amines using the Kaiser test

All reagents were added in five fold excess of 0.975mmol, (the theoretical amount of peptide that will be synthesized). Since the molecular weight of acetic anhydride is 102g/ mol, 92μl are needed for a five fold excess. Also the BOP and HOBt reagents were not required for this step, but their presence did not seem to cause any unforeseen problems.

Cleavage:

After the resin-peptide complex is capped with an acetyl group the peptide needs to be cleaved from the polymer resin in the following process:

Step first the resin-peptide complex is washed five consecutive times with 15ml of DMF for one minute each

Step 2: the resin-peptide complex is physically removed from the reaction flask and placed in a polymer test tube

Step 3: the test tube with the resin-peptide complex is placed in a vacuum desicator and dried overnight

Step 4: a cleavage solution is prepared from TFA, Anisole and Thioanisole

Step 5: 15ml of the cleavage solution is added to the resin-peptide complex and stirred in the hood at room temperature for two hours using a magnetic stir bar

Step 6: after two hours the resin is removed by suction filtration through a glass sintered filter, *the peptide is dissolved in solution, so the filtrate is not to be discarded*

Step 7: the resin is washed three times with 5ml portions of the cleavage solution to make sure all the peptide is transferred to the filtrate

Step 8: the filtrate is evaporated down to approximately 10ml using a rotovap with a dry-ice and acetone trap

Step 9: the peptide is precipitated out of the filtrate by the addition of 125ml of cold, anhydrous ether, in a drop wise fashion

Step 10: the peptide-ether solution is kept in the refrigerator overnight to ensure all the peptide precipitates out of solution

Step 11: the peptide is removed by suction filtration through a glass sintered filter

Step 12: the peptide precipitate is washed by three consecutive 5ml portions of cold anhydrous ether

Step 13: the peptide is placed in a plastic test tube which is put in a vacuum desicator with a liquid nitrogen trap, to dry overnight

The cleavage solution that was prepared and used in steps 4, 5, and 7, was composed of 90% Trifluoroacetic Acid (TFA) by weight, 5% Anisole by weight and 5% Thioanisole by weight. A 35ml supply of cleavage solution was prepared by adding 30.41ml of TFA, 2.51ml of Anisole, and 2.36ml of Thioanisole. It is important to note that in step 6 when the resin is removed by suction filtration that the peptide is dissolved in the filtrate and the filtrate is to be collected in a flask or test tube. The product yields of the different peptides are listed in Table 2:

Table 2: Percent Yields for the Synthesized Peptides

Peptide	mass collected(mg)	theo. weight(mg)	% yield
E,K4.0*	360	418	87
E,K3.3*	390	418	93
E,K3.7	289	346	84

* synthesis of these peptides done by Jay Berger and James Emst

Molecular Weight Determination:

The purity of the E,K 4.0, E,K 3.3, and E,K 3.7 peptides was analyzed and confirmed through electrospray mass spectrometry performed at NYU.

Purification:

The E,K 4.0 and E,K 3.7 were purified by reverse phase high performance liquid chromatography at NYU. The E,K 3.3 peptide was purified at Union College[38] by gradient reverse phase high performance liquid chromatography. The conditions used in the purification experiments are listed in Table 3:

Table 3: HPLC Gradient Program

Time	%Water	%Acetonitrile
0	95	5
15	95	5
20	90	10
67	90	10
75	20	80
95	20	80
100	95	5

The water contained 1% acetonitrile to kill off any bacteria that may flourish in the reservoir's environment. Also both the water and the acetonitrile contained 0.1% TFA to maintain slight acidic conditions. The flow rate was 3ml / min and the column used was a semi-prep reverse phase C-8 column with 10μ packing. Purification samples were approximately 200μl and was

[38] Purification of E,K 3.3 peptide done by Jay Berger at Union College

detected with a UV detector set at 275nm. After collection the samples were then frozen and placed in a vacuum desicator with a liquid nitrogen trap, to remove all the solvents. The purification gave a percent yield of 14.6[39]

Circular Dichroism:

The helicity of the three peptides was tested through Circular Dichroism (CD) measurements performed at NYU. The CD measurements were taken at four degrees Celsius and at a pH of 7. The data obtained from the CD measurements at 222nm are shown in Table 4:

Table 4: CD Theta Values of Peptides

Peptide	-Theta ($^{\circ}cm^2dmol^{-1}$)
E,K 3.3	26,000
E,K 3.7	22,000

Typical CD spectra are shown in Figure 3. The characteristic absorption minima of the α-helix is at 208nm and 222nm, and 218nm for the β-sheet.

[39] Berger, J., *Synthesis of a Hybrid Baldwin Peptide: A Study In the Factors That Influence Helix Formation*, Union College, 1994.

Molecular Modeling:

The three models devised that suggest the most α-helical character were used in theoretical computer modeling experiments. The three peptide models chosen were the two hybrid Baldwin peptides, the EK3.7 (i,i+4...i,i+3...i,i+4) and the EK3.3 (i,i+3...i,i+4...i,i+3), and the Baldwin type EK4.0 peptide (i,i+4).

EK3.3 Ac-Y-E-A-A-K-A-AE-A-A-A-K-A-A-E-A-A-K-NH2

EK3.7 Ac-Y-E-A-A-A-K-A-E-A-A-K-A-E-A-A-A-K-A-NH2

EK4.0 Ac-Y-E-A-A-A-K-A-E-A-A-A-K-A-E-A-A-A-K-NH2

All computational work was done on a VAX 6220 system. The three peptides were constructed using the computer program MacroModel v3.0, which is a VAXCluster program. MacroModel is a graphically-oriented molecular modeling program that allows drawing, manipulation, and energy calculations to be performed on complex molecules. The actual calculations for the energy minimizations were carried out on the MacroModel cooperative program BatchMin v3.1. The BatchMin program is primarily used as a tool for conformational searches, large structure energy minimizations and

molecular dynamics calculations. The peptides were constructed on the MacroModel program by the following procedure:

Peptide Construction:

Step 1: enter Macromodel v3.0

Step 2: select the <peptide> option

Step 3: once inside the peptide option select <grow>, which allows the amino acids to be added in sequence from the N-terminus to the C-terminus

Step 4: the <a-helix> peptide geometry option needs to be selected, so the amino acids are added in an a-helical formation.

Step 5: the peptide construct is then saved as a .dat file (see Appendix A), by selecting <write> option

All three peptides were assembled in this fashion. Once in the .dat file form the peptide was ready to be submitted for a BatchMin conformational energy minimization. The .dat file was entered as the input file in a .com file, which gave BatchMin all the parameters to be used in the minimization. A .com file is created by selecting all the parameters necessary to the type of minimization experiment to be performed. Appendix B shows an example of the .com file used for submitting .dat files for energy minimization calculations. In the .com file all the parameters for the minimization are set,

force field specifications, new .dat file name and number of iterations are selected and sent to BatchMin.

Submitting Batch Files:

Step 1: enter .dat file to be minimized into the .com file

Step 2: name the output .dat file for the minimized structure, in the .com file

Step 3: submit .com file to BatchMin by typing <submit (name of .com file) \keep\notify \noprintout>.

Typing <submit> tells the program to start the minimization calculations for the .dat file using the parameters specified in the .com file. The <keep> command tells the VAX to save the .log file and .dat file that is created, in the users VAX account, so it can be viewed by the user. Otherwise the .log file that is created will be deleted and if the <noprintout> command is also used no hard copy will be produced. The <notify> command tells the VAXCluster to notify the user when the minimization calculations were completed for the specified number of iterations. Typing <noprintout> notifies the VAXCluster not to make a hard cc py of the new .dat and .log file. After a minimization is completed BatchMin creates three new files. The first file is the output or new .dat file, or the output file, which has the new minimized atomic coordinates for the peptide. The next file created is a .log file (see Appendix C). The .log file contains the VAX settings that were to be programmed into the account during the calculations, such as turning the

"phone" option off, so the batch job was not interrupted and caused to terminate before the calculations were completed. It also contains the .com file parameters, names of the input and output files, the translation of the .com file parameters, number of iterations completed, minimized energy, time batch job was terminated, CPU time and real time elapsed. The last file that is created by BatchMin is the .mmo file (Appendix D). The .mmo file contains all the energy minimization calculations for the run. The types energies that were examined in the run are in the .mmo file. It contains the overall total force field energy in kJ/mol and kcal/mol. Also in the .mmo file is the starting and finishing energies for the assorted types of energies examined such as Van der Waals and torsional energy strains. Maximum Van der Waals, electrostatic, and hydrogen bond distances are listed, as well as the molecular dielectric constant, RMS gradient, and CPU time. Atomic charges, minimized atomic coordinates and residue connectivity are also included in the .mmo file.

Once the calculations are completed the new .dat file, or output file, is in assembly language and needs to be converted to English if coordinates are to be recognized. The VAX program mmformat does this conversion. To convert a assembly file:

Translating Assembly Files:

Step 1: type <run [.macromodel.mmv30.run]mmformat> at vax prompt

Step 2: at next prompt type <(name of .dat file to be converted)>

Step 3: to stop the mmformat program just hit the <return> key at the prompt

Once converted to English the coordinates are in numbers that can be easily recognized.

When the new .dat file is created the corresponding .mmo file is examined to see if the peptide has been minimized to an acceptable degree. The RMS gradient is used to measure the conformational minimzations of the peptides. An RMS gradient of 0.01 kJ/mol was determined to be the minimum acceptable value for the energy calculations. If the RMS gradient was greater than 0.01 kJ/mol the peptide was resubmitted to BatchMin for further energy minimizations. The new .dat file became the input file and another output file was defined. This process was repeated until an RMS gradient of 0.01kJ/mol or lower was achieved.

After the appropriate RMS gradient was reached the .dat file was converted to a .pdb file (protein database file, see Appendix E). Using another VAXCluster program rmmodpdb, the .dat file can be converted by:

Creating .PDB Files:

Step 1: type <run rmmodpdb> at vax prompt

Step 2: at the next prompt the .dat file to be converted is typed, <(name of .dat file)>

Step 3: at the next prompt the .pdb file is named, <(name of .pdb file)>

Step 4: the program can be stopped by pressing the <return> key.

The .dat file needs to be converted to a .pdb file so it can be examined in a graphics program. Two different graphics programs were utilized for visualization of the peptides. The first program used was Chem 3-D Plus for the Macintosh. The second program used to visually examine the peptides was CaChe. CaChe was used for most of the visualization and to find the approximate atomic distances where proposed ionic interactions existed. Transferring the .pdb file from the VAXCluster to the graphics program was accomplished through the use of the Fetch 2.1 program:

Transferring .PDB Files to CaChe:

Step 1: by opening the Fetch 2.1 program the VAX account was entered

Step 2: the .pdb file to be transferred was selected and saved to the appropriate folder on the computer's hard drive

Step 3: from the hard drive the .pdb file cold be brought into the CaChe program

Once on the hard drive the .pdb file could be accessed by any program on the computer. When the .pdb file was transferred to the CaChe program folder it needed to be converted to a CaChe file to be opened by the program. The .pdb file is in a Brookhaven format and needs to be converted to a CaChe format. Using the CaChe translator program the .pdb file is easily converted from its Brookhaven form to Cache form. Once in CaChe form the file can be examined and manipulated using the CaChe editor program. Approximate atomic distances can be calculated in CaChe by first selecting the two atoms of interest, then clicking on the <Adjust> option at the top of the screen with the mouse and finally dragging the mouse down to atom distance and selecting it. Using the CaChe editor the peptides could easily be manipulated to observe the potential ionic interactions between amino acid side chains.

Chapter 3 - Results and Discussion:

Peptides:

Three peptides have been synthesized and investigated in this study. Taking Baldwin's i,i+4 and i,i+3 short peptide models and modifying them we created two hybrid peptides:

E,K3.3

Ac-Y-E-A-A-K-A-A-E-A-A-A-K-A-A-E-A-A-K-NH2

E,K3.7

Ac-Y-E-A-A-A-K-A-E-A-A-K-A-E-A-A-A-K-A-NH2

The i,i+4 peptide was slightly modified and synthesized to simulated the i,i+4 peptides that Baldwin and Stellwagon examined in their studies:

E,K4.0

Ac-Y-E-A-A-A-K-A-E-A-A-A-K-A-E-A-A-A-K-NH2

The slight modification on the i,i+4 peptide that we synthesized is that between the glutamic acid and lysine amino acids an alanine amino acid has been inserted at positions 7 and 13. The addition of the alanine amino acids

was to retain the same number of amino acids in the short peptide chain as Baldwin and Stellwagon, their placement in the peptide chain was arbitrary. This peptide was synthesized to simulate Baldwin's original E,K i,i+4 peptide so that the results of the two hybrid peptides that were created could be compared to the previously used model of the most stable helical peptide. The E,K3.3 and E,K3.7 peptides were synthesized to compare the α-helix stability of Baldwin's uniformly spaced i,i+3 and i,i+4 peptides to mixed spaced peptide chains. In all three peptide chains synthesized the number of amino acids and their nature were strictly conserved, so that variation in chain length and type of amino acids would not be a factor when comparing helix stability. The E,K3.3 peptide is a mixed spaced peptide having an i,i+3 ion-pair, an i,i+4 ion-pair, and an i,i+3 ion-pair. The E,K3.7 peptide has a mixed spacing of ion-pairs that are i,i+4, i,i+3, and i,i+4. It has been shown from X-ray data that there are approximately 3.6 amino acids per helical turn[40]. The reason these mixed spaced peptides were synthesized was because it has been theorized that the mixed spacing of the E,K3.3 peptide will, overall, slightly under turn the helix having approximately 3.3 amino acids per turn and the E,K3.7 peptide will overall slightly over turn the helix having approximately 3.7 amino acids per turn. Therefore, both the E,K3.3 and E,K3.7 should both be more stable than the E,K4.0 and i,i+4 peptides,

[40] Maxfirld,F., Scheraga,H.A., 1975. *Macromolecules*, **8**: 491

since the i,i+4 peptide and the E,K4.0 peptide over turn the helix by more then the E,K3.3 under turns the helix, and the E,K3.7 over turns the helix. All synthesized peptides have a tyrosine amino acid in the first position. The tyrosine amino acid has a phenol group as the side chain. The phenol group is UV active at 256nm, therefore the tyrosine amino acid serves as a marker for the short peptide. Having the tyrosine as a marker allows the peptide chain to easily be detected during HPLC purification and also allows for the concentration of the peptide to be determined using spectroscopic methods. However, it has been shown by Stellwagon in his guest-host studies that tyrosine is an α-helix destabilizing amino acid[41]. In addition to having the tyrosine amino acid as a marker all the peptide chains are alanine based. There are two important reasons that the peptides are alanine based. The first is that in guest-host studies it has been found that the amino acid alanine tends to stabilize α-helix formation in short peptide chains[42]. The second reason is that the side chain of the alanine amino acid is composed of a single methyl group. Since the methyl group is so small it does not interfere with the salt bridge formation between the glutamic acid and lysine amino acid side chains.

Other modifications to the backbone of the peptide chain have been made to compensate for natural destabilizing factors. One modification to

[41] Merutka,G., Lipton,W., Shalongo,W., Park,S.H., Stellwagen.E., 1990. *Biochemistry* **28**: 7511-15
[42] Marqusse,S., Baldwin,R.L., 1987. *Proc. National Acad. Sci.* U.S.A. **84**: 8898-902

the peptide chain was the addition of an acetyl group to cap off the N-terminus of the peptide. By capping the N-terminus with an acetyl cap the addition of unwanted amino acids to the N-terminus of the peptide chain is prevented. It also stabilizes the dipole of the helix by doing two things. First, it prevents a helix destabilizing positive charge to build up at the N-terminus by preventing the amine group to be protonated. Secondly, the acetyl group can act as a hydrogen bond acceptor therefore, the α-helix can be further stabilized through hydrogen bonding of the acetyl group. Another similar modification to the peptide chain was the addition of an amine group to the C-terminus of the peptide to form the amide. The amine group stabilizes the α-helix by preventing the α-helix destabilizing action of deprotonating the carboxylic acid of the C-terminus. The amide group does this by preventing a negative charge at the negative net dipole of the helix. The amide group, as opposed to the acetyl group, is a hydrogen bond donor, which also stabilizes the α-helix through hydrogen bonding[43].

It has been determined in previous studies that the arrangement of the amino acids with charged side chains relative to the dipole moment of the helix has an impact on the stability of the helix. Baldwin found that positioning the amino acid with the negatively charged side chain at the N-terminal end of the peptide and the amino acid with the positively charged

[43] Marqusse,S., Baldwin,R.L., 1987. *Proc. National Acad. Sci.* U.S.A. **84**: 8898-902

side chain at the C-terminal end reduces the dipole moment of the helix, which further stabilizes its formation[44]

FMOC Removal:

The FMOC groups are blocking the backbone of the amino acids. Their removal from the peptide backbone is the first step in the amino acid coupling step. The FMOC groups are removed with the addition of a piperdine based solution. Piperdine is a basic secondary amine that is sufficiently basic to cleave the FMOC group[45] (Figure 4). When cleaved the FMOC group is in it's salt form which is soluble in DMF and therefore easily removed with other excess reagents and other waste products.

Coupling:

After the FMOC group on the backbone of the peptide is removed the amino acid to be coupled must first be activated before it can be coupled to the resin-peptide complex. The amino acid is activated by mixing it with HOBt and BOP in a 0.975mM DIEA/DMF solution. The amount of each reagent added is 0.975 mmol, this is in accordance to five times the substitution level of the starting resin. A five fold excess is added to insure that the reaction goes to approximately 100% completion. The activating

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[44] Marqusse,S., Baldwin,R.L., 1987. *Proc. National Acad. Sci.* U.S.A. **84**: 8898-902

[45] Jones,J., *Amino Acid and Peptide Synthesis*, Oxford Univ. Press, U.S.A., **1992**.

reagents are mixed and agitated for three minutes, (the reaction taking place is shown in Figure 5). The activation step is not essential in order for the amino acid to couple to the resin-peptide, but it increases to reactivity rate making the reaction more efficient and reducing the production of undesired products.

Once activated, the amino acid is added to the resin-peptide complex and is agitated in the Burrel wrist action shaker for approximately an hour, the coupling reaction that takes place is illustrated in Figure 6. In this reaction DIEA is the base and not piperdine. DIEA is a tertiary, sterically hindered base and is used instead of piperdine because DIEA is not strong enough to remove the FMOC group on the amino acid being coupled thereby reducing the possibility of creating unwanted side products.

The extent of the amino acid coupling reaction is checked with the use of the Kaiser test for free amine groups on the resin-peptide complex[46] . If there is a significant number of free amines present the Kaiser test solution will change to a blue color. If this is the case then the coupling step is repeated, however the resin-peptide complex is not deprotected a second time. If the Kaiser test solution does not turn blue then the coupling reaction has gone to nearly 100% completion. The Kaiser test, although a necessary

[46] Stewart,J.M., Young,J.P., *Solid Phase Peptide Syntyhesis*, Pierce Chemical Company, Illinois, 1984.

tool in determining the extent of reaction, destroys a portion of the peptide and slightly reduces the overall percent yield of the synthesis.

Cleavage:

After the resin-peptide complex is capped and completed the resin is removed from the complex. The resin is removed by adding a TFA solution and allowing the mixture to react for approximately two hours[47], (Figure 7). The tri-trifluoroacetic salts of the peptides are formed and are soluble in the cleavage solution. Since the peptide salts become soluble and the resin does not the resin is easily separated from the peptide. Adding the TFA solution also causes the cleavage of the Boc and T-Butyl blocking groups on the amino acid side chains, (see Figure 8).

Molecular Weight Determination:

Using electrospray mass spectrometry the molecular weights of the peptides could be determined to see if the desired peptide was synthesized. The cationic form of the peptide is analyzed and the mono, di, and tri-protonated forms of the peptide can then be detected. The neutral for of the peptide has a molecular weight of 1776. The mono-protonated peptide has a molecular weight of 1777,(1776+1/1), the di-protonated peptide has a

[47] King,D.S., Fields,C.G., Fields,G.B., 1990. *Int. J. of Protein Res.* **36**: 255-66

molecular weight of 889, (1776+2/2) and the tri-protonated peptide has a molecular weight of 593, (1776+3/3)[48]. The few impurities that were shown to be present in the spectra were easily removed by HPLC purification.

Computer Modeling:

All three peptides were minimized to a RMS gradient of at least 0.01kJ/mol and transferred to CaChe for visual examination. Once in CaChe the first thing that was looked for was the proximity of the amino acid side chains for confirmation of salt bridge formations. Two main views of the peptide were examined: the first view was down the barrel of the helix to look at where the side chains migrated; the second view was the side view of the peptide to observe the proximity of the side chains. The ΔH for the peptides are listed in Table 4:

[48] Berger,J.S., Synthesis of a Hybrid Baldwin Peptide: *A Study in the Factors that Influence Helix Formation*, June 1994. Union College, Schenectady, NY

Table 4: Theoretical Energies of Minimized Helical Peptides

Peptide	ΔH(kJ/mol)
EK3.3	-1626.13
EK3.3FIX	-1674.44
EK3.7	-1670.84
EK3.7FIX	-1553.50
EK4.0	-1469.84

Upon viewing the EK3.3 and EK3.7 peptides it seemed there were interactions with the tyrosine residue and the side chains or the peptide was thought to be stuck in a local minima, so they were manipulated within the MacroModel v3.0 program and resubmitted as EK3.3FIX and EK3.7FIX respectively. There are three potential salt bridges per peptide, in Table 5 they are listed as ion-pairs 1 through 3 with ion-pair 1 being the interaction at the N-terminus and ion-pair 3 the interactions between side chains at the C-terminus.

Table 5: Approximate Salt Bridge Distances

EK3.3		EK3.3FIX	
(ion-pair)	Distance(A)	(ion-pair)	Distance(A)
1w/Y*	1.843	1	1.687
2	1.624		1.710
3	1.653	2	1.668
	1.641		1.728
		3	1.632

EK3.7		EK3.7FIX	
(ion-pair)	Distance (A)	(ion-pair)	Distance (A)
1	1.688	1	1.717
	1.705		1.684
2	1.625	2	1.618
w/Y*	2.604	3	1.733
3	1.679		1.680
	1.734		

EK4.0	
(ion-pair)	Distance (A)
1	1.729
	1.701
2	1.641
3	1.728
	1.691

* distance was measured between carboxylate and tyrosine oxygens

** ion-pairs with two distances have distance approximations for two of the three hydrogens on the ammonium group on lysine's side chain

From the .mmo files created the minimized energy for the peptides Van der Waals interactions, hydrogen bonding, and electrostatic interactions were calculated as shown in Table 6 for the lowest energy configurations of the three peptides.

Table 6: Van der Waals, Hydrogen bonding, and Electrostatic Energies

Peptide	Van der Waals	H-bonding	Electrostatic
E,K 4.0	-87.92 kJ/mol	-32.40 kJ/mol	-1534.02 kJ/mol
E,K 3.3FIX	-164.58 kJ/mol	-21.36 kJ/mol	-1610.24 kJ/mol
E,K 3.7	-180.64 kJ/mol	-20.45 kJ/mol	-1622.16 kJ/mol

As can be seen from Table 6 the electrostatic interactions in the peptide have the largest contribution to the overall energy of the peptides.

EK 3.3 Peptide:

The EK3.3 peptide as illustrated in Table 3 had the lowest ΔH of the three peptide models, which suggests that it would be the most α-helical. Figure 9 shows the side and barrel views of the EK3.3 peptide. Upon examining the side view it seemed as if the tyrosine -OH group was hydrogen bonding with the lysine $-NH_3^+$ hydrogens. The atomic distance between the tyrosine oxygen atom and the nearest lysine ammonium

hydrogen was 1.843 A (see Table 5). This would suggest that there is some hydrogen bonding occurring between these two side chains. This was unexpected and undesired so the EK3.3 peptide was manipulated to move the tyrosine side chain away from the lysine side chain and the lysine side chain closer to the glutamic acid side chain. It also seemed as though the 2 and 3 lysine residues were interacting with the 3 glutamic acid residue. Therefore, the 2 lysine was moved closer to the 2 glutamic acid. The manipulated EK3.3 peptide was resubmitted as the EK3.3FIX peptide.

EK3.3FIX Peptide:

The EK3.3FIX was the version of the EK3.3 peptide with the lowest ΔH value. Figure 10 shows the side and barrel view of the EK3.3FIX minimized peptide respectively. The side view in Figure 10 shows that the tyrosine residue is no longer interfering with side chain interactions at bridge site 1. The EK3.3FIX has more possible side chain interactions between the lysine and glutamic acid residues at ion-pairs 2 and 3, then the EK3.3 peptide. The side view also shows that the peptide forms an α-helix. Looking at Figures 6 and 7 the barrel views of the EK3.3 and EK3.3FIX respectively, it can be seen that all the salt bridges are forming on one side of the α-helix. This opens a huge hydrophobic surface on the other side of the peptide. This would allow for possible hydrophobic interactions between α-helices or

possible α-helix bundle formations, which is a common substructural motif found in many proteins.

EK3.7 Peptide:

The EK3.7 peptide was marginally less stable then the EK3.3FIX peptide, (see Table 4). This suggests that the EK3.7 peptide is also energetically stable in the α-helix conformation. Figure 11 illustrate the side and barrel views of the EK3.7 peptide respectively. When observing the side view of the EK3.7 peptide in Figure 11 it looks as if the tyrosine residue is again interacting with the salt bridge at ion-pair 1. There seems to be hydrogen bonding between the tyrosine hydroxyl and the glutamic acid carboxylate. The approximate atomic distance between the tyrosine hydroxide oxygen and the nearest carboxylate oxygen was 2.604 A (see Table 5). Therefore, the distance between the hydroxyl hydrogen and the nearest carboxylate oxygen is even closer which would lead us to believe there is definitely some kind of interaction there. However, it seemed that there were salt bridges forming at all 3 sites. The EK3.7 was also manipulated in MacroModel to orient the tyrosine residue away from the glutamic acid carboxylate. The manipulated EK3.7 peptide was resubmitted to BatchMin for geometrical energy minimizations as the EK3.7FIX peptide.

EK3.7FIX Peptide:

The side and barrel views of the EK3.7FIX peptides are illustrated in Figure 12 respectively. The side view of the EK3.7 in Figure 11 and the EK3.7FIX in Figure 12 seem identical with the exception of the tyrosine interaction, which is absent in the EK3.7FIX peptide. The atomic distances in both peptides are approximately the same. However, the ΔH for the EK3.7 is significantly lower then the ΔH for the EK3.7FIX. This would suggest that the tyrosine interaction in the EK3.7 is important to the overall energy distribution of this geometrical conformation. As with the two EK3.3 barrel views both EK3.7 peptide barrel views in Figure 11 and 12 (for EK3.7 and EK3.7FIX respectively), have similar motifs in its appearance. The most striking is the approximate 180° separation between salt bridges. Having this 180° separation gives the peptide four different external faces alternating between hydrophobic/hydrophilic properties. Again the four faces on one α-helix would be available to interact either hydrophobically or ionically with another peptide. This external face conformation would also allow for α-helix bundle formation.

EK4.0 Peptide:

The EK4.0 peptide in its side view and its barrel view is illustrated in Figure 13. The side view of this peptide shows that the interactions between the glutamic acid and lysine side chains. It seems that the EK4.0 peptide has a significantly lower ΔH than both the EK3.3 peptides and the EK3.7 peptides. However, looking at the barrel view in Figure 13 all salt bridge formations are approximately 120° apart around the barrel of the helix. This conformation seems to have the least amount of torsional strain on the barrel of the helix. When the barrel views of all the peptides are examined the EK4.0 view looks to have the most even α-helical structure. With 120° separation there are three hydrophobic faces that are exposed on the external helix surface. These faces would also be available for hydrophobic interactions with other α-helices. There is most probably not enough ionic character on the exterior of the helix for intermolecular interactions.

CD Measurements:

The CD spectra taken at NYU are illustrated in Figure 3. As can be seen there are absorptions at 208 and 222nm in the EK 3.3 and EK 3.7 spectra which is characteristic of α-helical structure. The spectrum for the EK 4.0 peptide shows a single absorption at 218nm which is characteristic of β-sheet structure. Table 7 lists the Theta values for the EK3.3 and EK 3.7

peptides that were synthesized, the i,i+3 and i,i+4 peptides that Baldwin synthesized and the i,i+4 peptide that Stellwagon synthesized.

Table 7[49]: Theta values at 222nm

Peptide	-Theta value ($^{\circ}cm^{2}dmol^{-1}$)
EK3.3	26,000*
EK3.7	22,000*
Baldwin (i+4)	29,000**
Baldwin (i+3)	17,600**
Stellwagen	24,300***

* 4°C, this work

**1°C, Marquesse, S., Baldwin,R., 1987. Proc. National Acad. Sci. USA 84, 8898

***0°C, Park,S., Shalongo,W., Stellwagen,E., 1993, Biochemistry 32, 7048

As can be seen from Table 7 Baldwin's i,i+4 peptide is the most α-helical, however the EK3.3 peptide is only slightly less stable than Baldwin's and more stable than Stellwagen's peptide. Baldwin and Stellwagon both carried out their CD measurements at lower temperatures than in this study. Even though our peptides were tested at a higher temperature the EK3.3 is still more helical than Stellwagon's peptide. Also like Stellwagen's peptide the EK3.3 and EK3.7 peptides contain a tyrosine amino acid which has been determined to be a helix destabilizing amino acid as stated earlier.

[49] Berger,J.S., Ernst,J.A., Nicoletta,A., Hull,L.A., *Helix Stability of Mixed Spaced Salt Bridged Short Peptides*, 1995. Union College, Schenectady, NY

Therefore, it is believed that the peptides that we have synthesized are at least as stable if not more stable than uniformly spaced i,i+4 peptide.

E,K 4.0 β-Sheet:

We believe that the E,K 4.0 formed a β-sheet configuration. This is evident when the peptide is stretched into a β-sheet configuration as shown in Figure 15. It can be seen that when stretched out into a β-sheet type configuration the amino acids with charged side chains are all on one side of the β-sheet. Therefore, when two sheets are laid next to each other they can aggregate in such a way that β-sheet will be the configuration of lower energy.

Conclusion:

Several conclusions can be drawn from this study. The first is that peptides with mixed spacing are at least as stable as the uniformly spaced i,i+4 peptides. This is clearly illustrated in the CD data in Table 7. The theta values show that the mixed spaced E,K 3.3 peptide is more energetically stable then Stellwagen's uniformly spaced i,i+4 peptide and almost as stable as Baldwin's i,i+4 peptide. Secondly, the overall stability of the peptide is not dependent on the number of i,i+4 salt bridges. Again as can be seen in the CD data from Table 7 the E,K 3.3 peptide that contains only one i,i+4 salt

bridge and is more energetically stable than Stellwagen's peptide that contains three i,i+4 salt bridges. Finally, it can be said that the stability of i,i+3 salt bridges relative to i,i+4 salt bridges is dependent on the surrounding environment of the salt bridge. Basically the stability of a given salt bridge is strongly influenced by it's immediate environment. Also because of this final conclusion it can be said that these isolated tests on short peptide chains are not entirely conclusive as to how these substructures may act in a full sized protein. Also the shapes of the mixed spaced peptides offer hydrophobic faces that may add to site specific stability of the helix. However, it does give a good basis for estimations of protein folding in a complex system.

Future Work:

Some of the work that is planned is to further study the E,K 4.0 peptide that was synthesized. Physical measurements and studies will be done to determine if it is a feasible way to predict β-sheet formation in protein folding mechanisms. The E,K 4.0 will be submitted in molecular modeling in it's β-sheet configuration to determine the theoretical stability of this structure. Also Scanning Tunneling Microscopy is being done on the E,K 4.0 to see if it actually took a β-sheet configuration. Finally, the E,K 4.0 should be resynthesized without alanine spacers to determine if α-helical character will return if the charged amino acids are not all on the same side of the β-sheet.

Protocol for Peptide Synthesis

(starting with 0.5g FMOC-AA-Polymer 0.39mmol/g = 0.195mmol)
Fmoc-AA = _____ Sequence # = _____
Done by:_____ Date__/___/__

Wash I:
_____1. wash 100% DMF (15ml for 1 min. five times)a.___b.___c.___d.___e.___
_____1a. Kaiser test (safe to leave overnight).

Deblock:
____ 2. 30% Pip/DMF (15ml for 1 min, one time)
____ 3. 30% Pip/DMF (15ml for 10 min, one time)
Wash II:
____ 4. wash 100%DMF (15ml for 1min. 5 times) a. ___ b. ___ c.___ d.___ e.___
Coupling: Fmoc-AA = _____ Sequence # = _____
____ 5. 5eq. (0.975 mm) of BOP (0.431 g), HOBt (0.132 g), FMOC-AA (_____), weigh into flask
____ 6. Add to bottle 5 mL DIEA/DMF, 0.195 mm/mL (5 eq of DIEA) and 5 mL of DMF
____ 7. Swirl & dissolve for 3 mins
____ 8. Add solution to resin and let sit from 30-60min.
start time_____stop time_____

Repeat (go to Wash I, step 1)

Protocol for Peptide Cleavage:

Cleavage:

1. Wash and Dry resin
2. Add 15ml 95/5% TFA Phenol let sit with stirring for 2hr

Wash:

3. Filter TFA solution with vacuum filtration and stinted glass funnel into 500ml
   round bottom flask
4. Wash resin 3x 5ml 90/5/5% TFA/Anisole/Thioanisole mixture and filter TFA
   solution to rest of TFA solution

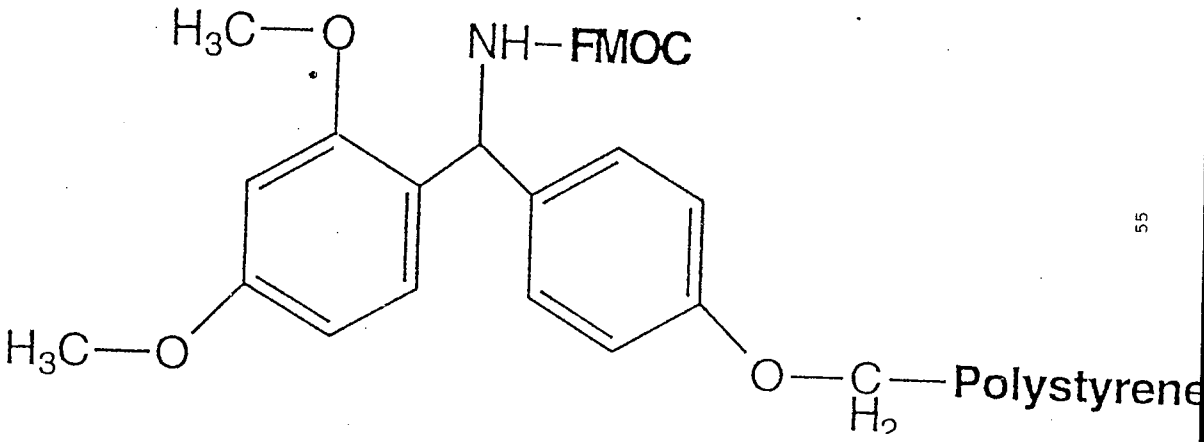
Evaporation:

5. Evaporate TFA solution down to ~10ml on rotovap with dry ice acetone trap

Precipitation:

6. Add drop wise a ~125ml of cold dry ether
7. Place in refrigerator over night
8. Filter precipitate from ether with sintered glass filter
9. Wash 500ml flask and precipitate 3x with cold ether
10. Remove precipitate and place in vacuum desiccator

FIGURE 1



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Berger, J., Synthesis of a Hybrid Baldwin Peptide, June 1994. Union College

FIGURE 2

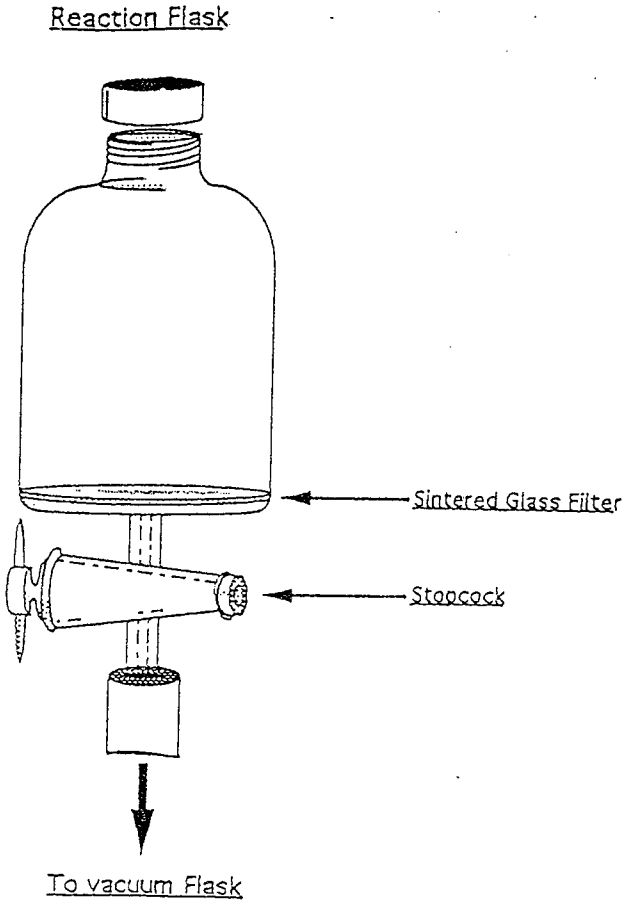

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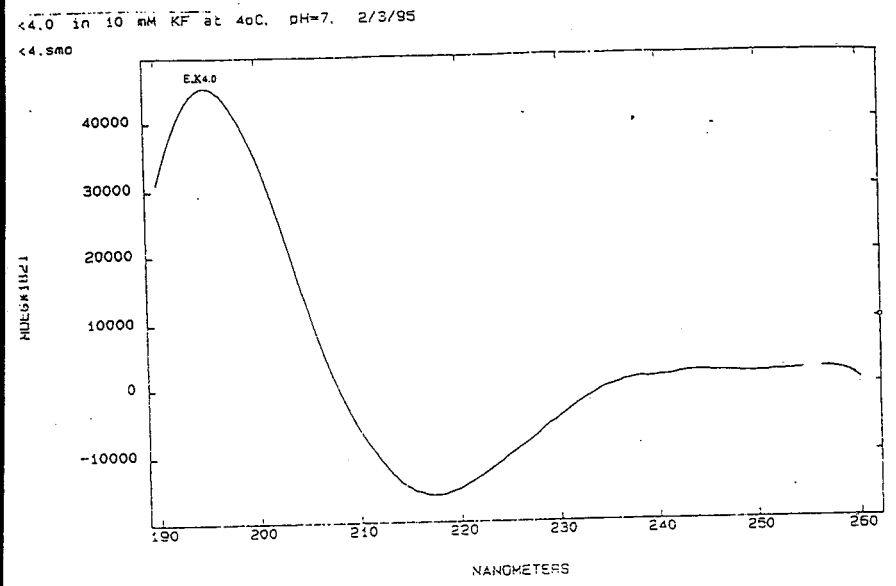
FIGURE 3


<4.0 in 10 mM KF at 4oC. pH=7. 2/3/95
<4.smo
E.X4.0
40000
30000
20000
10000
0
-10000
190
200
210
220
230
240
250
260
NANOMETERS

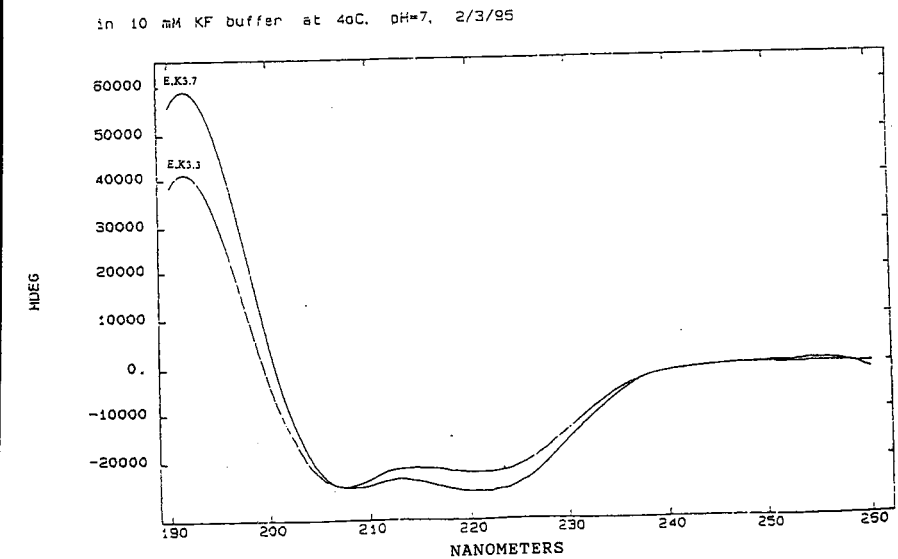

in 10 mM KF buffer at 4oC. pH=7. 2/3/95
E.K3.7
E.K3.3
60000
50000
40000
30000
20000
10000
0.
-10000
-20000
190
200
210
220
230
240
250
NANOMETERS

FIGURE 4

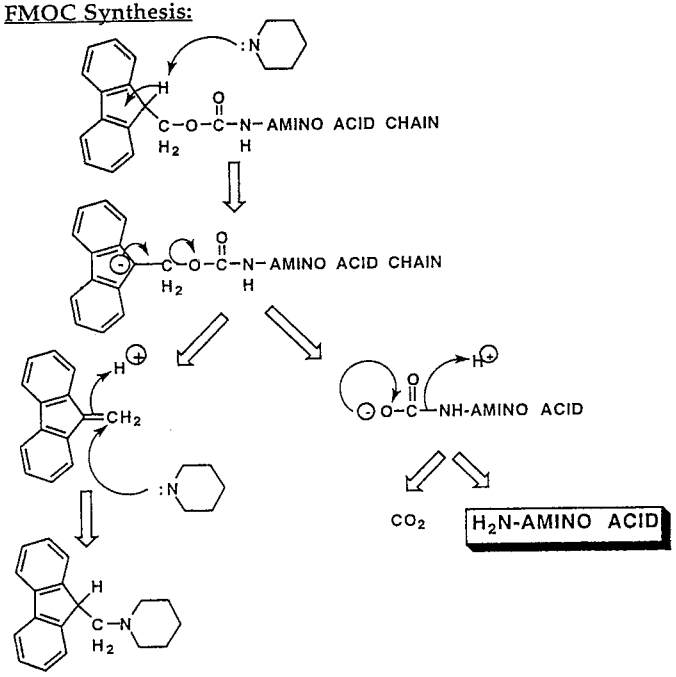


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FIGURE 5

Activation of Amino Acid:

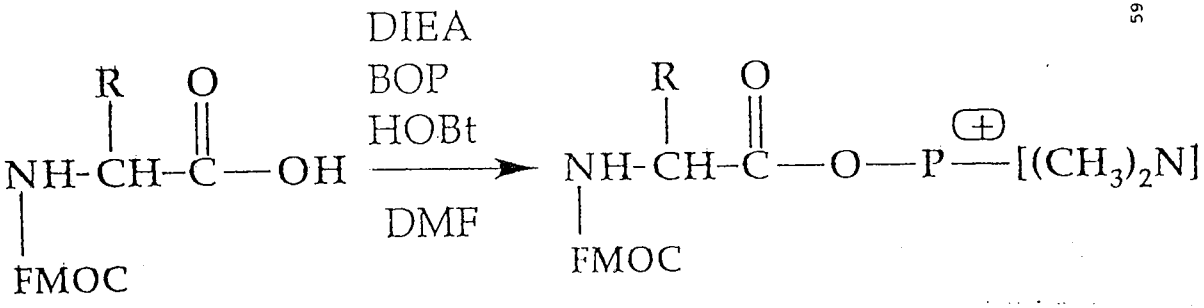

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FIGURE 6

Coupling Reaction Step:

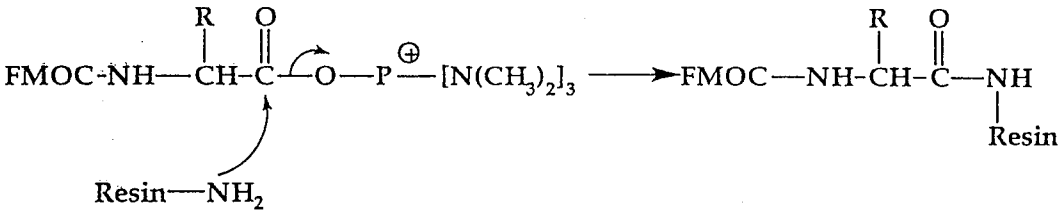

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FIGURE 7

Cleavage of Peptide from Resin Support:

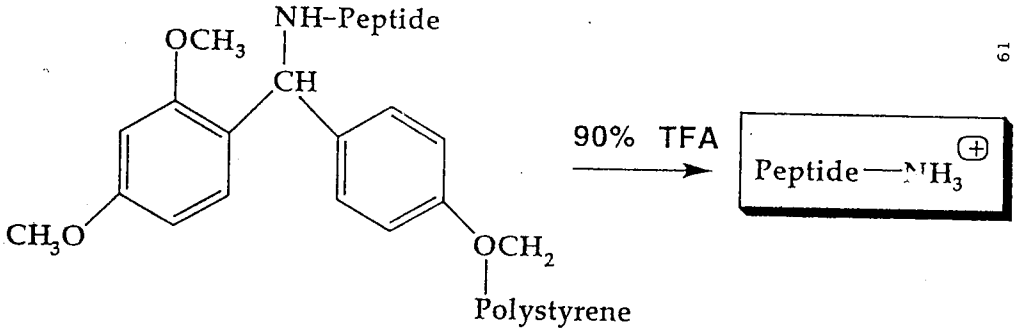

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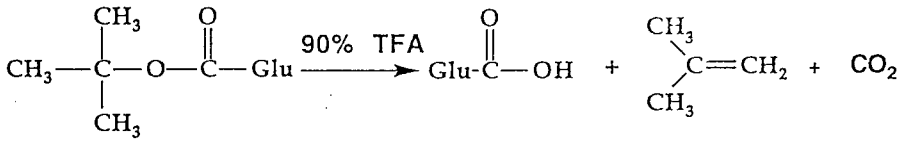
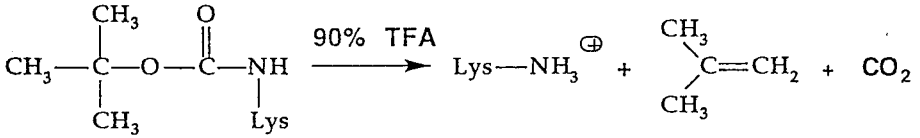
FIGURE 8

Cleavage of Side Chain Protection Groups:

Glutamic Acid Protection Group:



Lysine Protection Group:



Tyrosine Protection Group:

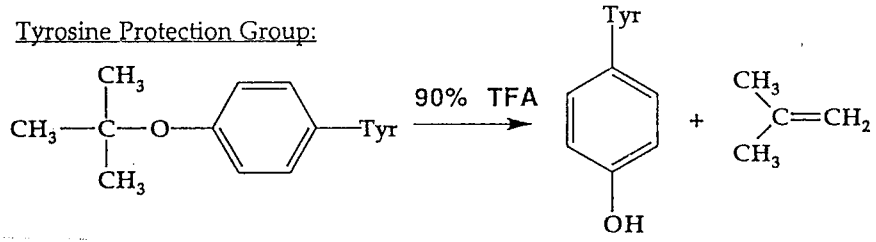


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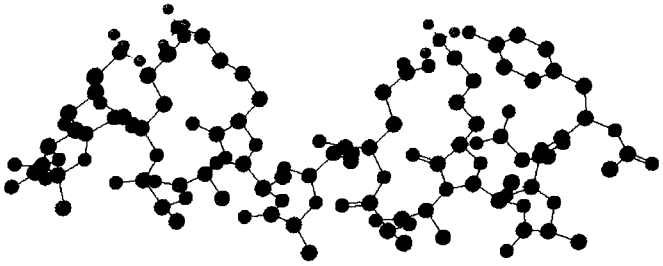
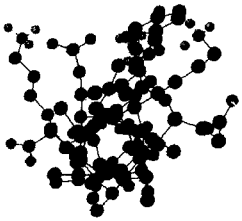
FIGURE 9

E,K3.3



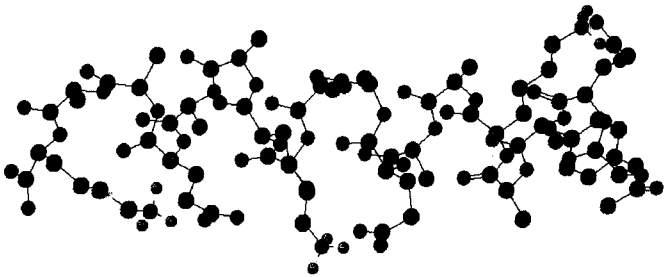
side view



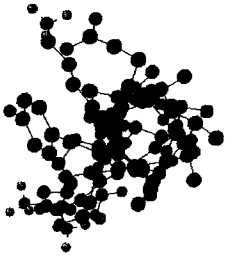
barrel view

FIGURE 10

E,K 3.3FIX



side view



barrel view

UN82 NICOLETTA, ANGELO INFLUENCE OF SALT BRIDGE FORMATION, ETC.
N643i/1995 DEPT. OF CHEMISTRY HRS 6/95 2/2

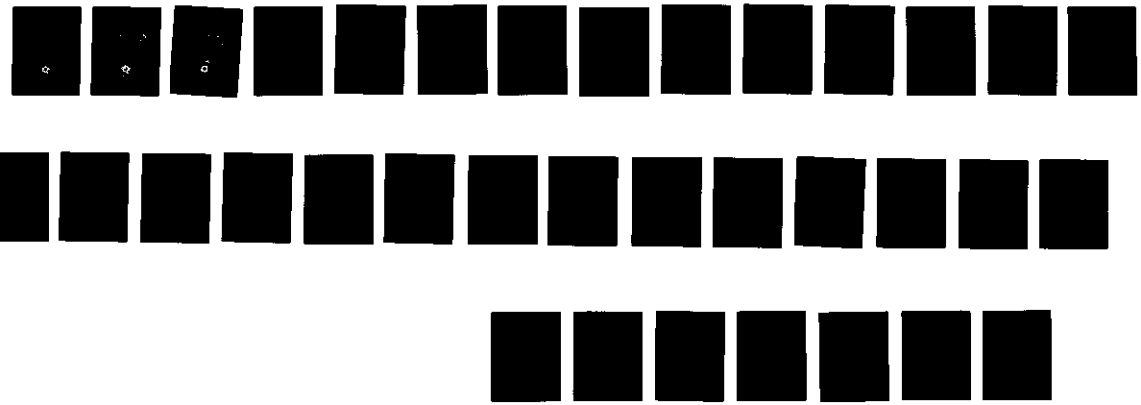
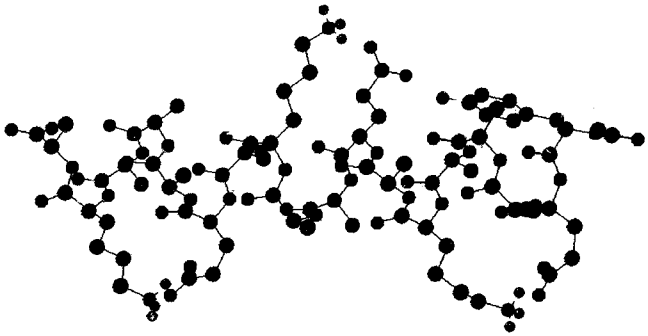
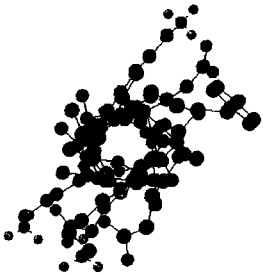


FIGURE 11

E,K3.7



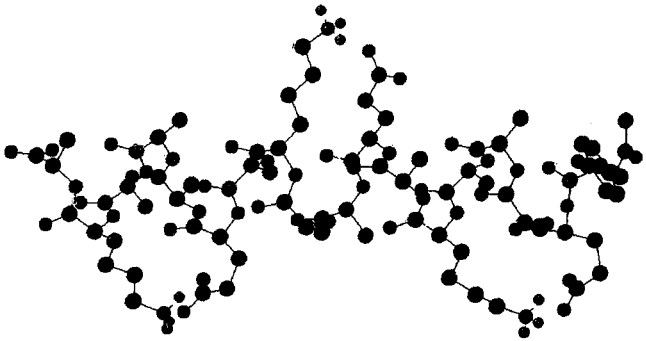
side view



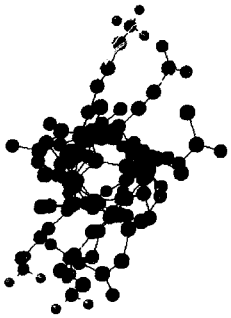
barrel view

FIGURE 12

E.K 3.7FIX



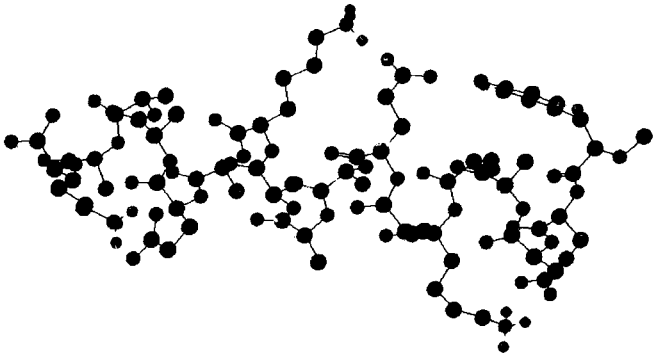
side view



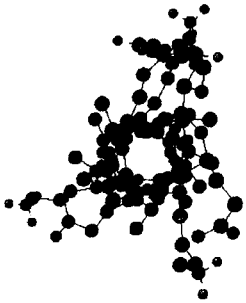
barrel view

FIGURE 13

E,K4.0



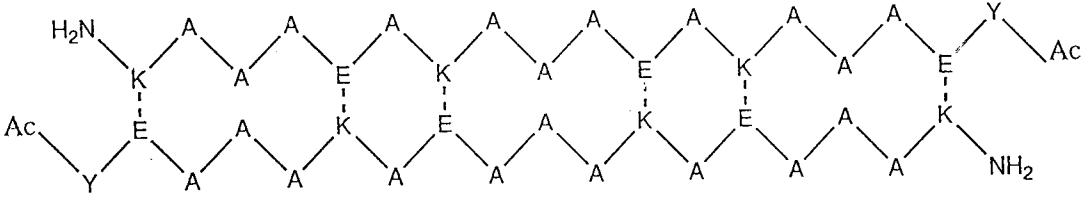
side view



barrel view

FIGURE 14

E,K4.0 BETA SHEET CONFIGURATION



APPENDIX A:

249	james' peptide		10-27-94						-1384.	kJ/mol	
3	2 1	3 1	4 1	248 1	0 0	0 0	-1.019114	-1.407921	-1		
.874496	0XX 10										
41	1 1	0 0	0 0	0 0	0 0	0 0	-0.758932	-0.353409	-1		
.968315	0XX 10										
41	1 1	0 0	0 0	0 0	0 0	0 0	-1.557932	-1.715135	-2		
.771442	0XX 10										
41	1 1	0 0	0 0	0 0	0 0	0 0	-0.100349	-1.986486	-1		
.787167	0XX 10										
3	248 1	6 1	8 1	193 1	0 0	0 0	-2.336498	-2.953221	-0		
.507992	1YX 10										
2	5 1	7 2	16 1	0 0	0 0	0 0	-3.599665	-2.916179	0		
.368782	1YX 10										
15	6 2	0 0	0 0	0 0	0 0	0 0	-3.933057	-1.871236	0		
.930942	1YX 16										
3	5 1	9 1	194 1	195 1	0 0	0 0	-1.231633	-3.850893	0		
.087518	1YX 10										
2	8 1	10 1	14 2	0 0	0 0	0 0	-1.642098	-5.289927	0		
.338354	1YX 10										
2	9 1	11 2	196 1	0 0	0 0	0 0	-1.728477	-5.790192	1		
.652050	1YX 10										
2	10 2	12 1	197 1	0 0	0 0	0 0	-2.331230	-7.038511	1		
.887550	1YX 10										
2	11 1	13 2	15 1	0 0	0 0	0 0	-2.860869	-7.771885	0		
.811102	1YX 10										
2	12 2	14 1	198 1	0 0	0 0	0 0	-2.607456	-7.359262	-0		
.507733	1YX 10										
2	13 1	9 2	199 1	0 0	0 0	0 0	-1.995621	-6.116360	-0		
.744833	1YX 10										
16	12 1	16 1	0 0	0 0	0 0	0 0	-3.652502	-8.860728	1		
.040595	1YX 16										
42	15 1	0 0	0 0	0 0	0 0	0 0	-4.299252	-8.732629	1		
.761198	1YX 10										
43	18 1	0 0	0 0	0 0	0 0	0 0	-3.947656	-4.854687	-0		
.040593	2EX 10										
25	17 1	19 1	6 1	0 0	0 0	0 0	-4.299245	-4.056219	0		
.466352	2EX 4										
3	18 1	20 1	22 1	200 1	0 0	0 0	-5.470676	-4.294096	1		
.305258	2EX 10										
2	19 1	21 2	28 1	0 0	0 0	0 0	-5.368982	-3.508663	2		
.612866	2EX 10										
15	20 2	0 0	0 0	0 0	0 0	0 0	-6.277983	-2.764791	2		
.970185	2EX 16										
3	19 1	23 1	201 1	202 1	0 0	0 0	-5.539249	-5.798656	1		
.611473	2EX 10										
3	22 1	24 1	203 1	204 1	0 0	0 0	-6.756268	-6.246609	2		
.434804	2EX 10										
2	23 1	25 2	26 1	0 0	0 0	0 0	-6.485645	-7.560142	3		
.163892	2EX 10										
15	24 2	0 0	0 0	0 0	0 0	0 0	-7.178774	-7.792958	4		
.179154	2EX 16										
18	24 1	0 0	0 0	0 0	0 0	0 0	-5.573956	-8.294514	2		
.725201	2EX 16										
43	28 1	0 0	0 0	0 0	0 0	0 0	-3.549213	-4.298408	2		
.912303	3AX 10										
25	27 1	29 1	20 1	0 0	0 0	0 0	-4.245405	-3.689295	3		
.313628	3AX 4										
3	28 1	30 1	32 1	205 1	0 0	0 0	-3.966166	-3.091433	4		
.606451	3AX 10										
2	29 1	31 2	34 1	0 0	0 0	0 0	-4.294172	-1.598048	4		
.644143	3AX 10										
15	30 2	0 0	0 0	0 0	0 0	0 0	-4.854871	-1.124326	5		
.627782	3AX 16										
3	29 1	206 1	207 1	208 1	0 0	0 0	-2.496546	-3.327484	4		
.961986	3AX 10										

43 34 1	0 0	0 0	0 0	0 0	0 0	-3.630918	-1.308539	2
.741080	4AX 10							
25 33 1	35 1	30 1	0 0	0 0	0 0	-3.948124	-0.851352	3
.591955	4AX 4							
3 34 1	36 1	38 1	209 1	0 0	0 0	-4.150862	0.588341	3
.571355	4AX 10							
2 35 1	37 2	40 1	0 0	0 0	0 0	-5.645234	0.911228	3
.571319	4AX 10							
15 36 2	0 0	0 0	0 0	0 0	0 0	-6.136511	1.627546	4
.442280	4AX 16							
3 35 1	210 1	211 1	212 1	0 0	0 0	-3.448988	1.192556	2
.351820	4AX 10							
43 40 1	0 0	0 0	0 0	0 0	0 0	-5.915839	-0.282692	1
.964719	5AX 10							
25 39 1	41 1	36 1	0 0	0 0	0 0	-6.371051	0.366122	2
.593309	5AX 4							
3 40 1	42 1	44 1	213 1	0 0	0 0	-7.797672	0.612411	2
.439952	5AX 10							
2 41 1	43 2	46 1	0 0	0 0	0 0	-8.570512	0.073089	3
.644981	5AX 10							
15 42 2	0 0	0 0	0 0	0 0	0 0	-9.551549	0.664030	4
.084675	5AX 16							
3 41 1	214 1	215 1	216 1	0 0	0 0	-8.287340	-0.042709	1
.146864	5AX 10							
43 46 1	0 0	0 0	0 0	0 0	0 0	-7.319757	-1.502521	3
.747001	6KX 10							
25 45 1	47 1	42 1	0 0	0 0	0 0	-8.131226	-1.066778	4
.172721	6KX 4							
3 46 1	48 1	50 1	217 1	0 0	0 0	-8.751862	-1.744736	5
.294298	6KX 10							
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.724573      6KX  10
41    53 1        0 0      0 0      0 0      0 0      0 0    -9.937402    -6.522053     7
.135686      6KX  10
41    60 1        0 0      0 0      0 0      0 0      0 0    -7.270336    -0.082182     8
.847174      7AX  10
41    63 1        0 0      0 0      0 0      0 0      0 0    -5.291603     1.396120     8
.795364      7AX  10
41    63 1        0 0      0 0      0 0      0 0      0 0    -4.941424    -0.196973     8
.098954      7AX  10
41    63 1        0 0      0 0      0 0      0 0      0 0    -5.187502     1.207211     7
.033043      7AX  10
41    66 1        0 0      0 0      0 0      0 0      0 0    -8.280225     4.310306     6
.984416      8EX  10
```

```
 41    69 1      0 0      0 0      0 0      0 0      0 0    -7.547703     4.006665     4
.562486      8EX  10
 41    69 1      0 0      0 0      0 0      0 0      0 0    -9.086998     3.195192     4
.264750      8EX  10
 41    70 1      0 0      0 0      0 0      0 0      0 0   -10.123145     5.394214     5
.352893      8EX  10
 41    70 1      0 0      0 0      0 0      0 0      0 0    -8.555942     6.093531     4
.933611      8EX  10
 41    79 1      0 0      0 0      0 0      0 0      0 0   -13.450761     0.235196     5
.788895      9AX  10
 41    79 1      0 0      0 0      0 0      0 0      0 0   -12.254539     0.669757     4
.551465      9AX  10
 41    79 1      0 0      0 0      0 0      0 0      0 0   -11.768561    -0.310407     5
.952607      9AX  10
 41    82 1      0 0      0 0      0 0      0 0      0 0   -12.593334     0.138885    10
.185894     10AX  10
 41    85 1      0 0      0 0      0 0      0 0      0 0   -10.640049    -0.466890    11
.567139     10AX  10
 41    85 1      0 0      0 0      0 0      0 0      0 0   -10.578122    -1.239483     9
.969302     10AX  10
 41    85 1      0 0      0 0      0 0      0 0      0 0    -9.547700     0.164612    10
.316662     10AX  10
 41    88 1      0 0      0 0      0 0      0 0      0 0   -10.458776     3.776765    12
.450870     11AX  10
 41    91 1      0 0      0 0      0 0      0 0      0 0    -8.923749     5.529321    11
.632396     11AX  10
 41    91 1      0 0      0 0      0 0      0 0      0 0    -8.267692     3.905655    11
.332412     11AX  10
 41    91 1      0 0      0 0      0 0      0 0      0 0    -8.987281     4.832911     9
.997643     11AX  10
 43   151 1      0 0      0 0      0 0      0 0      0 0   -17.807238     9.336741    15
.287271     18XX  10
 43   151 1      0 0      0 0      0 0      0 0      0 0   -18.373932    10.787964    16
.104023     18XX  10
 26     1 1      5 1    249 1      0 0      0 0      0 0    -1.851785    -1.578179    -0
.704231      0XX   4
 43   248 1      0 0      0 0      0 0      0 0      0 0    -2.686540    -1.019160    -0
.825473      0XX  10
```

APPENDIX B:

```
$ RUN [nicoleta.macromodel.mmv30.inc1]BATCHMIN
 input file
 output file
 FFLD       3                                1.0000
 READ
 CONV       2       2
 MINI       2       0     500
 ELST       0
[EOB]
```

APPENDIX C:

```
The date and time are:  20-FEB-1995 16:48:46.61

$!
$ SET NOON
$ SET TERM/INSERT
%SET-W-NOTSET, error modifying SKIPPY$DUA0:
-CLI-E-IVDEVTYPE, invalid device type - specify a mailbox device
$ SET TERM/DEV=VT100
%SET-W-NOTSET, error modifying SKIPPY$DUA0:
-CLI-E-IVDEVTYPE, invalid device type - specify a mailbox device
$ SET BROADCAST=(noPHONE,MAIL)
%SET-W-READERR, error reading broadcast classes
-SYSTEM-F-NOPRIV, no privilege for attempted operation
$ TIME :== SHOW TIME
$ CHE  :== SET DEF [NICOLETA.CHELP]
$ NORM :== SET DEF [NICOLETA]
$ DEFINE INCLOC  95$DISK:[NICOLETA.MACROMODEL.MMV30.MMSOURCE]
$ DEFINE INCLOC1 95$DISK:[NICOLETA.MACROMODEL.MMV30.INC1]
$ DEFINE INCLOC2 95$DISK:[NICOLETA.MACROMODEL.MMV30.INCLOC2]
$ DEFINE INCLOC3 95$DISK:[NICOLETA.MACROMODEL.MMV30.INCLOC3]
$ ASSIGN SYS$COMMAND PLOTOUT
$ RMMOD :== RUN INCLOC:MMOD
$ PURGE/KEEP=1 /EXCLUDE=MAIL.DIR *.*
$ set prompt = "32;1mSQUIGGY -->46m"
$ RUN [nicoleta.macromodel.mmv30.inc1]BATCHMIN
BatchMin V3.1c                    Starting Time 20-FEB-95  16:48:54
 ek37fix1e.dat
 ek37fix1f.dat
 FFLD       3                              1.0000
 READ
 CONV       2       2
 MINI       2       0     500
 ELST       0

Input filename:   ek37fix1e.dat

Output filename:  ek37fix1f.dat

Force field: amber.fld
Read structure. Name =                                                    -15
Iterations =          10
CONF     1 E = -1553.516 (  0.006) kJ/mol
Total Energy =    -1553.516 kJ/mol
BatchMin normal termination
Total number of structures minimized =           1
FORTRAN STOP
  NICOLETA      job terminated at 20-FEB-1995 16:49:59.28

  Accounting information:
  Buffered I/O count:              131         Peak working set size:    2431
  Direct I/O count:                100         Peak page file size:     49890
  Page faults:                    9748         Mounted volumes:             0
  Charged CPU time:          0 00:00:55.08     Elapsed time:     0 00:01:15.59
```

APPENDIX D:

TIME: 16:53:46 DATE: 17-NOV-94

```
** MacroModel BatchMin Version 3.1 ******************
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*                                                     *
*******************************************************
*                                                     *
* Structure name:                                     *
*                                                     *
* Datafile name:                                      *
*                                                     *
************************ Columbia University 1990 **
```

Amber Force Field
P. Kollman, JACS, 106, 765 (1984); J. Comp. Chem., 7, 230 (1986)

Energy Equations in Use:

Bond Length	- 1: Harmonic stretch
Bond Angle	- 1: Harmonic bend
Stretch-Bend	- 0: None
Torsional	- 1: 1-3 Fold cosine function
Improper Torsion (OPB)	- 2: Improper torsion
Van der Waals	- 2: Softcut 6,12-Lennard Jones (R,Eps)
1,4-Van der Waals	- 3: multiplier 0.50
1,4-Electrostatics	- 3: multiplier 0.50
Electrostatic	- 2: Point charge with distance-dependent dielectric
Hydrogen-Bonding	- 1: Angle-independent 10,12-Lennard Jones
Fixed Atoms	- 1: Harmonic anchoring
Angular H-Bonds (A-B-C)	- 0: None
Angular H-Bonds (A-B-C-D)	- 0: None

Alternative parameter sets selected:

Number	Label	Description
1	"b"	United atom field charges
2	"Z"	Zinc

Parameter qualifier sets selected:

Column	Label	Description
1	"O"	Original AMBER params
1	"M"	Modified params
1	"A"	Added params
2	"1"	Specific, high quality params
2	"2"	Tentative values for params
2	"3"	Generalized, low quality param

Total amber.fld energy is -1384.441 kJ/mol (-330.889 kcal/mol)

Van der Waals	-92.341 (-22.070)	Stretch	11.141 (2.663)
Torsion	71.981 (17.204)	Bend	80.926 (19.342)
Improper Torsion	1.989 (0.475)	Stretch-Bend	0.000 (0.000)
Hydrogen Bond	-19.510 (-4.663)	Electrostatic	-1438.627 (-343.840)

Dipole moment of total system = 2.436 debyes

Maximum van der Waals distance = 7.0 Angstroms
Maximum electrostatic distance = 12.0 Angstroms
Maximum hydrogen bond distance = 4.0 Angstroms
Molecular dielectric constant = 1.00

RMS Gradient = 0.0098 kj/A-mol

CPU Time = 104.40 seconds

Time: 16:53:46 Date: 17-NOV-94

Connection Table

Atomic Charges, Coordinates and Connectivity

Atom Type	Atoms & Bonds Number	Charge	Coordinates X	Y	Z	Residue		Attached	
C3	(1)	0.085	-1.0191	-1.4079	-1.8745	UNK	0X	2-	3-
4-	248-								
H1	(2)	0.038	-0.7589	-0.3534	-1.9683	UNK	0X	1-	
H1	(3)	0.038	-1.5579	-1.7151	-2.7714	UNK	0X	1-	
H1	(4)	0.038	-0.1003	-1.9865	-1.7872	UNK	0X	1-	
C3	(5)	0.025	-2.3365	-2.9532	-0.5080	TYR	1X	248-	6-
8-	193-								
C2	(6)	0.526	-3.5997	-2.9162	0.3688	TYR	1X	5-	7=
18-									
O2	(7)	-0.500	-3.9331	-1.8712	0.9309	TYR	1X	6=	
C3	(8)	-0.054	-1.2316	-3.8509	0.0875	TYR	1X	5-	9-
194-	195-								
C2	(9)	-0.001	-1.6421	-5.2899	0.3384	TYR	1X	8-	10-
14=									
C2	(10)	-0.099	-1.7285	-5.7902	1.6521	TYR	1X	9-	11=
196-									
C2	(11)	-0.002	-2.3312	-7.0385	1.8875	TYR	1X	10=	12-
197-									
C2	(12)	-0.121	-2.8609	-7.7719	0.8111	TYR	1X	11-	13=
15-									
C2	(13)	-0.002	-2.6075	-7.3593	-0.5077	TYR	1X	12=	14-
198-									
C2	(14)	-0.099	-1.9956	-6.1164	-0.7448	TYR	1X	13-	9=
199-									
O3	(15)	-0.368	-3.6525	-8.8607	1.0406	TYR	1X	12-	16-
H2	(16)	0.339	-4.2993	-8.7326	1.7612	TYR	1X	15-	
H3	(17)	0.248	-3.9477	-4.8547	-0.0406	GLU	2X	18-	
N2	(18)	-0.520	-4.2992	-4.0562	0.4664	GLU	2X	17-	19-
6-									
C3	(19)	0.198	-5.4707	-4.2941	1.3053	GLU	2X	18-	20-
22-	200-								
C2	(20)	0.526	-5.3690	-3.5087	2.6129	GLU	2X	19-	21=
28-									
O2	(21)	-0.500	-6.2780	-2.7648	2.9702	GLU	2X	20=	
C3	(22)	-0.184	-5.5392	-5.7987	1.6115	GLU	2X	19-	23-
201-	202-								
C3	(23)	-0.350	-6.7563	-6.2466	2.4348	GLU	2X	22-	24-
203-	204-								
C2	(24)	0.620	-6.4856	-7.5601	3.1639	GLU	2X	23-	25=
26-									
O2	(25)	-0.706	-7.1788	-7.7930	4.1792	GLU	2X	24=	
OM	(26)	-0.706	-5.5740	-8.2945	2.7252	GLU	2X	24-	
H3	(27)	0.248	-3.5492	-4.2984	2.9123	ALA	3X	28-	
N2	(28)	-0.520	-4.2454	-3.6893	3.3136	ALA	3X	27-	29-
20-									
C3	(29)	0.167	-3.9662	-3.0914	4.6065	ALA	3X	28-	30-
32-	205-								
C2	(30)	0.526	-4.2942	-1.5980	4.6441	ALA	3X	29-	31=
34-									
O2	(31)	-0.500	-4.8549	-1.1243	5.6278	ALA	3X	30=	
C3	(32)	-0.083	-2.4965	-3.3275	4.9620	ALA	3X	29-	206-
207-	208-								
H3	(33)	0.248	-3.6309	-1.3085	2.7411	ALA	4X	34-	
N2	(34)	-0.520	-3.9481	-0.8514	3.5920	ALA	4X	33-	35-
30-									
C3	(35)	0.167	-4.1509	0.5883	3.5714	ALA	4X	34-	36-
38-	209-								
C2	(36)	0.526	-5.6452	0.9112	3.5713	ALA	4X	35-	37=
40-									

```
O2  (  37)  -0.500  -6.1365   1.6275   4.4423  ALA  4X  36=
C3  (  38)  -0.083  -3.4490   1.1926   2.3518  ALA  4X  35-  210-
 211-  212-
H3  (  39)   0.248  -5.9158  -0.2827   1.9647  ALA  5X  40-
N2  (  40)  -0.520  -6.3711   0.3661   2.5933  ALA  5X  39-  41-
 36-
C3  (  41)   0.167  -7.7977   0.6124   2.4400  ALA  5X  40-  42-
 44-  213-
C2  (  42)   0.526  -8.5705   0.0731   3.6450  ALA  5X  41-  43=
 46-
O2  (  43)  -0.500  -9.5515   0.6640   4.0847  ALA  5X  42=
C3  (  44)  -0.083  -8.2873  -0.0427   1.1469  ALA  5X  41-  214-
 215-  216-
H3  (  45)   0.248  -7.3198  -1.5025   3.7470  LYS  6X  46-
N2  (  46)  -0.520  -8.1312  -1.0668   4.1727  LYS  6X  45-  47-
 42-
C3  (  47)   0.179  -8.7519  -1.7447   5.2943  LYS  6X  46-  48-
 50-  217-
C2  (  48)   0.526  -8.5349  -0.9425   6.5802  LYS  6X  47-  49=
 59-
O2  (  49)  -0.500  -9.4721  -0.7435   7.3528  LYS  6X  48=
C3  (  50)  -0.037  -8.1699  -3.1618   5.3449  LYS  6X  47-  51-
 218-  219-
C3  (  51)  -0.179  -8.8449  -4.1296   6.3238  LYS  6X  50-  52-
 220-  221-
C3  (  52)  -0.196  -8.4809  -5.5483   5.8600  LYS  6X  51-  53-
 222-  223-
C3  (  53)   0.022  -8.8955  -6.6525   6.8373  LYS  6X  52-  54-
 224-  225-
N5  (  54)  -0.272  -8.7284  -7.9800   6.2107  LYS  6X  53-  55-
 56-  57-
H4  (  55)   0.311  -8.7090  -8.7271   6.8871  LYS  6X  54-
H4  (  56)   0.311  -9.4551  -8.1465   5.5314  LYS  6X  54-
H4  (  57)   0.311  -7.8657  -7.9943   5.6577  LYS  6X  54-
H3  (  58)   0.248  -6.5674  -0.6459   6.1343  ALA  7X  59-
N2  (  59)  -0.520  -7.3094  -0.4569   6.8021  ALA  7X  58-  60-
 48-
C3  (  60)   0.167  -7.0016   0.4232   7.9181  ALA  7X  59-  61-
 63-  226-
C2  (  61)   0.526  -7.8147   1.7122   7.8126  ALA  7X  60-  62=
 65-
O2  (  62)  -0.500  -8.4188   2.1373   8.7990  ALA  7X  61=
C3  (  63)  -0.083  -5.5023   0.7274   7.9603  ALA  7X  60-  227-
 228-  229-
H3  (  64)   0.248  -7.3054   1.9282   5.8481  GLU  8X  65-
N2  (  65)  -0.520  -7.8413   2.3198   6.6178  GLU  8X  64-  66-
 61-
C3  (  66)   0.198  -8.6574   3.4956   6.3642  GLU  8X  65-  67-
 69-  230-
C2  (  67)   0.526 -10.0984   3.1887   6.7656  GLU  8X  66-  68=
 75-
O2  (  68)  -0.500 -10.6527   3.8664   7.6219  GLU  8X  67=
C3  (  69)  -0.184  -8.5849   3.9319   4.8915  GLU  8X  66-  70-
 231-  232-
C3  (  70)  -0.350  -9.2631   5.3001   4.6925  GLU  8X  69-  71-
 233-  234-
C2  (  71)   0.620  -9.7644   5.5000   3.2687  GLU  8X  70-  72=
 73-
O2  (  72)  -0.706 -10.8812   6.0527   3.1377  GLU  8X  71=
OM  (  73)  -0.706  -9.0345   5.1031   2.3391  GLU  8X  71-
H3  (  74)   0.248 -10.1703   1.6364   5.4714  ALA  9X  75-
N2  (  75)  -0.520 -10.6988   2.1638   6.1570  ALA  9X  74-  76-
 67-
C3  (  76)   0.167 -12.0808   1.7860   6.3909  ALA  9X  75-  77-
 79-  152-
C2  (  77)   0.526 -12.3548   1.6134   7.8837  ALA  9X  76-  78=
```

81-								
O2 (	78)	-0.500	-13.3257	2.1627	8.3980	ALA	9X	77=
C3 (	79)	-0.083	-12.4084	0.5061	5.6184	ALA	9X	76- 235-
236-	237-							
H3 (	80)	0.248	-10.7043	0.4478	8.1004	ALA	10X	81-
N2 (	81)	-0.520	-11.4974	0.8646	8.5816	ALA	10X	80- 82-
77-								
C3 (	82)	0.167	-11.6396	0.6392	10.0098	ALA	10X	81- 83-
85-	238-							
C2 (	83)	0.526	-11.6424	1.9628	10.7806	ALA	10X	82- 84=
87-								
O2 (	84)	-0.500	-12.5923	2.2605	11.5034	ALA	10X	83=
C3 (	85)	-0.083	-10.5229	-0.2875	10.4980	ALA	10X	82- 239-
240-	241-							
H3 (	86)	0.248	-9.8563	2.4993	9.9681	ALA	11X	87-
N2 (	87)	-0.520	-10.5802	2.7591	10.6356	ALA	11X	86- 88-
83-								
C3 (	88)	0.167	-10.4236	4.0005	11.3833	ALA	11X	87- 89-
91-	242-							
C2 (	89)	0.526	-11.5451	4.9901	11.0547	ALA	11X	88- 90=
93-								
O2 (	90)	-0.500	-12.0690	5.6770	11.9275	ALA	11X	89=
C3 (	91)	-0.083	-9.0564	4.6088	11.0631	ALA	11X	88- 243-
244-	245-							
H3 (	92)	0.248	-11.4254	4.4457	9.1284	LYS	12X	93-
N2 (	93)	-0.520	-11.8979	5.0666	9.7757	LYS	12X	92- 94-
89-								
C3 (	94)	0.179	-12.8930	5.9665	9.2261	LYS	12X	93- 95-
97-	153-							
C2 (	95)	0.526	-14.2832	5.5710	9.7253	LYS	12X	94- 96=
106-								
O2 (	96)	-0.500	-15.0645	6.4334	10.1195	LYS	12X	95=
C3 (	97)	-0.037	-12.7419	5.9088	7.7014	LYS	12X	94- 98-
154-	155-							
C3 (	98)	-0.179	-13.5321	6.9138	6.8571	LYS	12X	97- 99-
156-	157-							
C3 (	99)	-0.196	-12.8379	6.9312	5.4839	LYS	12X	98- 100-
158-	159-							
C3 (	100)	0.022	-13.6099	7.6663	4.3851	LYS	12X	99- 101-
160-	161-							
N5 (	101)	-0.272	-12.7811	7.7760	3.1663	LYS	12X	100- 102-
103-	104-							
H4 (	102)	0.311	-13.3241	8.0104	2.3501	LYS	12X	101-
H4 (	103)	0.311	-12.0432	8.4514	3.2949	LYS	12X	101-
H4 (	104)	0.311	-12.2825	6.8957	3.0009	LYS	12X	101-
H3 (	105)	0.248	-13.9064	3.5960	9.4028	ALA	13X	106-
N2 (	106)	-0.520	-14.5876	4.2702	9.7414	ALA	13X	105- 107-
95-								
C3 (	107)	0.167	-15.8236	3.7567	10.3099	ALA	13X	106- 108-
110-	162-							
C2 (	108)	0.526	-15.8850	4.0543	11.8087	ALA	13X	107- 109=
112-								
O2 (	109)	-0.500	-16.9078	4.5335	12.2988	ALA	13X	108=
C3 (	110)	-0.083	-15.9396	2.2534	10.0473	ALA	13X	107- 163-
164-	165-							
H3 (	111)	0.248	-13.9875	3.3702	12.0514	GLU	14X	112-
N2 (	112)	-0.520	-14.7902	3.7738	12.5270	GLU	14X	111- 113-
108-								
C3 (	113)	0.198	-14.6757	4.0414	13.9544	GLU	14X	112- 114-
116-	166-							
C2 (	114)	0.526	-15.0083	5.5134	14.2052	GLU	14X	113- 115=
122-								
O2 (	115)	-0.500	-15.9712	5.8242	14.9039	GLU	14X	114=
C3 (	116)	-0.184	-13.2730	3.6286	14.4455	GLU	14X	113- 117-
167-	168-							
C3 (	117)	-0.350	-13.1573	3.3959	15.9646	GLU	14X	116- 118-

169-	170-								
C2	(118)	0.620	-12.6397	4.5746	16.7822	GLU	14X	117-	119=
120-									
O2	(119)	-0.706	-12.3284	5.6497	16.2215	GLU	14X	118=	
OM	(120)	-0.706	-12.5353	4.4139	18.0186	GLU	14X	118-	
H3	(121)	0.248	-13.5004	6.0722	12.9828	ALA	15X	122-	
N2	(122)	-0.520	-14.2629	6.4129	13.5592	ALA	15X	121-	123-
114-									
C3	(123)	0.167	-14.4613	7.8480	13.6496	ALA	15X	122-	124-
126-	171-								
C2	(124)	0.526	-15.9036	8.2395	13.3169	ALA	15X	123-	125=
128-									
O2	(125)	-0.500	-16.5248	8.9967	14.0597	ALA	15X	124=	
C3	(126)	-0.083	-13.4738	8.5577	12.7206	ALA	15X	123-	172-
173-	174-								
H3	(127)	0.248	-15.8847	7.1130	11.6253	ALA	16X	128-	
N2	(128)	-0.520	-16.4396	7.7399	12.2006	ALA	16X	127-	129-
124-									
C3	(129)	0.167	-17.7817	8.0769	11.7512	ALA	16X	128-	130-
132-	175-								
C2	(130)	0.526	-18.8315	7.7039	12.7995	ALA	16X	129-	131=
134-									
O2	(131)	-0.500	-19.6796	8.5234	13.1435	ALA	16X	130=	
C3	(132)	-0.083	-18.0753	7.3895	10.4156	ALA	16X	129-	176-
177-	178-								
H3	(133)	0.248	-18.0391	5.8428	13.0049	ALA	17X	134-	
N2	(134)	-0.520	-18.7832	6.4694	13.3053	ALA	17X	133-	135-
130-									
C3	(135)	0.167	-19.7354	5.9974	14.3020	ALA	17X	134-	136-
138-	179-								
C2	(136)	0.526	-19.5763	6.7627	15.6195	ALA	17X	135-	137=
140-									
O2	(137)	-0.500	-20.5524	7.0720	16.2980	ALA	17X	136=	
C3	(138)	-0.083	-19.5270	4.4972	14.5212	ALA	17X	135-	180-
181-	182-								
H3	(139)	0.248	509	6.7062	15.3739	UNK	18X	140-	
N2	(140)	-0.520	18.3256	7.0301	15.9873	UNK	18X	139-	141-
136-									
C3	(141)	0.179	-17.9186	7.6426	17.2414	UNK	18X	140-	150-
143-	183-								
O2	(142)	-0.500	-18.5850	9.6862	18.3085	UNK	18X	150=	
C3	(143)	-0.037	-16.4205	7.3710	17.3278	UNK	18X	141-	144-
184-	185-								
C3	(144)	-0.179	-15.5814	7.8699	18.5065	UNK	18X	143-	145-
186-	187-								
C3	(145)	-0.196	-14.2674	7.0990	18.3021	UNK	18X	144-	146-
188-	189-								
C3	(146)	0.022	-13.0018	7.6458	18.9577	UNK	18X	145-	147-
190-	191-								
N5	(147)	-0.272	-11.8560	6.8325	18.5012	UNK	18X	146-	148-
149-	192-								
H4	(148)	0.311	-10.9584	7.1444	18.8315	UNK	18X	147-	
H4	(149)	0.311	-12.0117	5.8486	18.7419	UNK	18X	147-	
C2	(150)	0.526	-18.2473	9.1358	17.2652	UNK	18X	141-	151-
142=									
N2	(151)	-0.688	-18.1367	9.8114	16.1260	UNK	18X	150-	246-
247-									
H1	(152)	0.048	-12.7218	2.5837	6.0125	ALA	9X	76-	
H1	(153)	0.048	-12.6709	6.9807	9.5614	LYS	12X	94-	
H1	(154)	0.038	-11.6871	6.1038	7.5044	LYS	12X	97-	
H1	(155)	0.038	-12.9840	4.9021	7.3569	LYS	12X	97-	
H1	(156)	0.116	-14.5706	6.5875	6.7772	LYS	12X	98-	
H1	(157)	0.116	-13.4920	7.9056	7.3111	LYS	12X	98-	
H1	(158)	0.122	-11.8547	7.3935	5.6008	LYS	12X	99-	
H1	(159)	0.122	-12.6820	5.9000	5.1541	LYS	12X	99-	
H1	(160)	0.098	-14.5152	7.1000	4.1572	LYS	12X	100-	

H1	(161)	0.098	-13.8923	8.6643	4.7255	LYS	12X	100-
H1	(162)	0.048	-16.6656	4.2495	9.8216	ALA	13X	107-
H1	(163)	0.038	-16.8691	1.8803	10.4783	ALA	13X	110-
H1	(164)	0.038	-15.9476	2.0660	8.9733	ALA	13X	110-
H1	(165)	0.038	-15.1004	1.7242	10.4996	ALA	13X	110-
H1	(166)	0.048	-15.4183	3.4243	14.4620	GLU	14X	113-
H1	(167)	0.092	-13.0506	2.6657	13.9859	GLU	14X	116-
H1	(168)	0.092	-12.5158	4.3374	14.1107	GLU	14X	116-
H1	(169)	0.071	-14.1163	3.0765	16.3717	GLU	14X	117-
H1	(170)	0.071	-12.4420	2.5886	16.1239	GLU	14X	117-
H1	(171)	0.048	-14.2438	8.1584	14.6723	ALA	15X	123-
H1	(172)	0.038	-13.6051	9.6369	12.8046	ALA	15X	126-
H1	(173)	0.038	-12.4527	8.2999	13.0034	ALA	15X	126-
H1	(174)	0.038	-13.6462	8.2557	11.6874	ALA	15X	126-
H1	(175)	0.048	-17.8293	9.1552	11.5907	ALA	16X	129-
H1	(176)	0.038	-19.0814	7.6512	10.0864	ALA	16X	132-
H1	(177)	0.038	-17.3598	7.7222	9.6635	ALA	16X	132-
H1	(178)	0.038	-18.0070	6.3074	10.5254	ALA	16X	132-
H1	(179)	0.048	-20.7491	6.1531	13.9290	ALA	17X	135-
H1	(180)	0.038	-20.2467	4.1317	15.2544	ALA	17X	138-
H1	(181)	0.038	-19.6720	3.9636	13.5814	ALA	17X	138-
H1	(182)	0.038	-18.5164	4.3097	14.8868	ALA	17X	138-
H1	(183)	0.048	-18.4237	7.1497	18.0738	UNK	18X	141-
H1	(184)	0.038	-16.3243	6.2839	17.3049	UNK	18X	143-
H1	(185)	0.038	-15.9744	7.7932	16.4269	UNK	18X	143-
H1	(186)	0.116	-15.4394	8.9493	18.4304	UNK	18X	144-
H1	(187)	0.116	-16.0446	7.6138	19.4608	UNK	18X	144-
H1	(188)	0.122	-14.4209	6.0673	18.6295	UNK	18X	145-
H1	(189)	0.122	-14.0593	7.0759	17.2301	UNK	18X	145-
H1	(190)	0.098	-12.8530	8.6832	18.6535	UNK	18X	146-
H1	(191)	0.098	-13.0919	7.5949	20.0441	UNK	18X	146-
H4	(192)	0.311	-11.8646	6.7403	17.4790	UNK	18X	147-
H1	(193)	0.048	-2.6429	-3.3547	-1.4751	TYR	1X	5-
H1	(194)	0.038	-0.3786	-3.8762	-0.5897	TYR	1X	8-
H1	(195)	0.038	-0.8994	-3.4030	1.0252	TYR	1X	8-
H1	(196)	0.064	-1.3795	-5.2007	2.4870	TYR	1X	10-
H1	(197)	0.102	-2.4522	-7.3991	2.8994	TYR	1X	11-
H1	(198)	0.102	-2.9405	-7.9669	-1.3369	TYR	1X	13-
H1	(199)	0.064	-1.8537	-5.7751	-1.7600	TYR	1X	14-
H1	(200)	0.048	-6.3674	-3.9908	0.7633	GLU	2X	19-
H1	(201)	0.092	-5.5146	-6.3814	0.6897	GLU	2X	22-
H1	(202)	0.092	-4.6595	-6.0465	2.1975	GLU	2X	22-
H1	(203)	0.071	-6.9940	-5.4995	3.1893	GLU	2X	23-
H1	(204)	0.071	-7.6187	-6.3687	1.7797	GLU	2X	23-
H1	(205)	0.048	-4.5803	-3.5957	5.3538	ALA	3X	29-
H1	(206)	0.038	-2.2867	-2.9024	5.9442	ALA	3X	32-
H1	(207)	0.038	-2.2883	-4.3976	4.9870	ALA	3X	32-
H1	(208)	0.038	-1.8518	-2.8515	4.2220	ALA	3X	32-
H1	(209)	0.048	-3.7022	1.0211	4.4673	ALA	4X	35-
H1	(210)	0.038	-3.5831	2.2748	2.3552	ALA	4X	38-
H1	(211)	0.038	-2.3831	0.9662	2.3913	ALA	4X	38-
H1	(212)	0.038	-3.8658	0.7859	1.4301	ALA	4X	38-
H1	(213)	0.048	-7.9678	1.6885	2.3675	ALA	5X	41-
H1	(214)	0.038	-9.3528	0.1529	1.0212	ALA	5X	44-
H1	(215)	0.038	-7.7469	0.3722	0.2955	ALA	5X	44-
H1	(216)	0.038	-8.1233	-1.1204	1.1878	ALA	5X	44-
H1	(217)	0.048	-9.8236	-1.8195	5.1036	LYS	6X	47-
H1	(218)	0.038	-8.2935	-3.5755	4.3432	LYS	6X	50-
H1	(219)	0.038	-7.1032	-3.1118	5.5677	LYS	6X	50-
H1	(220)	0.116	-8.4834	-3.9368	7.3356	LYS	6X	51-
H1	(221)	0.116	-9.9281	-3.9989	6.2938	LYS	6X	51-
H1	(222)	0.122	-8.9588	-5.7270	4.8935	LYS	6X	52-
H1	(223)	0.122	-7.3990	-5.6070	5.7139	LYS	6X	52-
H1	(224)	0.098	-8.2625	-6.5873	7.7246	LYS	6X	53-
H1	(225)	0.098	-9.9374	-6.5221	7.1357	LYS	6X	53-
H1	(226)	0.048	-7.2703	-0.0822	8.8472	ALA	7X	60-

H1	(227)	0.038	-5.2916	1.3961	8.7954	ALA	7X	63-	
H1	(228)	0.038	-4.9414	-0.1970	8.0990	ALA	7X	63-	
H1	(229)	0.038	-5.1875	1.2072	7.0330	ALA	7X	63-	
H1	(230)	0.048	-8.2802	4.3103	6.9844	GLU	8X	66-	
H1	(231)	0.092	-7.5477	4.0067	4.5625	GLU	8X	69-	
H1	(232)	0.092	-9.0870	3.1952	4.2647	GLU	8X	69-	
H1	(233)	0.071	-10.1231	5.3942	5.3529	GLU	8X	70-	
H1	(234)	0.071	-8.5559	6.0935	4.9336	GLU	8X	70-	
H1	(235)	0.038	-13.4508	0.2352	5.7889	ALA	9X	79-	
H1	(236)	0.038	-12.2545	0.6698	4.5515	ALA	9X	79-	
H1	(237)	0.038	-11.7686	-0.3104	5.9526	ALA	9X	79-	
H1	(238)	0.048	-12.5933	0.1389	10.1859	ALA	10X	82-	
H1	(239)	0.038	-10.6400	-0.4669	11.5671	ALA	10X	85-	
H1	(240)	0.038	-10.5781	-1.2395	9.9693	ALA	10X	85-	
H1	(241)	0.038	-9.5477	0.1646	10.3167	ALA	10X	85-	
H1	(242)	0.048	-10.4588	3.7768	12.4509	ALA	11X	88-	
H1	(243)	0.038	-8.9237	5.5293	11.6324	ALA	11X	91-	
H1	(244)	0.038	-8.2677	3.9057	11.3324	ALA	11X	91-	
H1	(245)	0.038	-8.9873	4.8329	9.9976	ALA	11X	91-	
H3	(246)	0.344	-17.8072	9.3367	15.2873	UNK	18X	151-	
H3	(247)	0.344	-18.3739	10.7880	16.1040	UNK	18X	151-	
N3	(248)	-0.500	-1.8518	-1.5782	-0.7042	UNK	0X	1-	5-
249-									
H3	(249)	0.200	-2.6865	-1.0192	-0.8255	UNK	0X	248-	

Total charge on system = 0.000

APPENDIX E:

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ATOM      1  C01 UNK X   0      -1.019  -1.408  -1.874  1.00 0.0000   0
ATOM      2  H02 UNK X   0      -0.759  -0.353  -1.968  1.00 0.0000   0
ATOM      3  H03 UNK X   0      -1.558  -1.715  -2.771  1.00 0.0000   0
ATOM      4  H04 UNK X   0      -0.100  -1.986  -1.787  1.00 0.0000   0
ATOM      5  C01 TYR X   1      -2.336  -2.953  -0.508  1.00 0.0000   0
ATOM      6  C02 TYR X   1      -3.600  -2.916   0.369  1.00 0.0000   0
ATOM      7  O03 TYR X   1      -3.933  -1.871   0.931  1.00 0.0000   0
ATOM      8  C04 TYR X   1      -1.232  -3.851   0.088  1.00 0.0000   0
ATOM      9  C05 TYR X   1      -1.642  -5.290   0.338  1.00 0.0000   0
ATOM     10  C06 TYR X   1      -1.728  -5.790   1.652  1.00 0.0000   0
ATOM     11  C07 TYR X   1      -2.331  -7.039   1.888  1.00 0.0000   0
ATOM     12  C08 TYR X   1      -2.861  -7.772   0.811  1.00 0.0000   0
ATOM     13  C09 TYR X   1      -2.607  -7.359  -0.508  1.00 0.0000   0
ATOM     14  C10 TYR X   1      -1.996  -6.116  -0.745  1.00 0.0000   0
ATOM     15  O11 TYR X   1      -3.653  -8.861   1.041  1.00 0.0000   0
ATOM     16  H12 TYR X   1      -4.299  -8.733   1.761  1.00 0.0000   0
ATOM     17  H01 GLU X   2      -3.948  -4.855  -0.041  1.00 0.0000   0
ATOM     18  N02 GLU X   2      -4.299  -4.056   0.466  1.00 0.0000   0
ATOM     19  C03 GLU X   2      -5.471  -4.294   1.305  1.00 0.0000   0
ATOM     20  C04 GLU X   2      -5.369  -3.509   2.613  1.00 0.0000   0
ATOM     21  O05 GLU X   2      -6.278  -2.765   2.970  1.00 0.0000   0
ATOM     22  C06 GLU X   2      -5.539  -5.799   1.611  1.00 0.0000   0
ATOM     23  C07 GLU X   2      -6.756  -6.247   2.435  1.00 0.0000   0
ATOM     24  C08 GLU X   2      -6.486  -7.560   3.164  1.00 0.0000   0
ATOM     25  O09 GLU X   2      -7.179  -7.793   4.179  1.00 0.0000   0
ATOM     26  O10 GLU X   2      -5.574  -8.295   2.725  1.00 0.0000   0
ATOM     27  H01 ALA X   3      -3.549  -4.298   2.912  1.00 0.0000   0
ATOM     28  N02 ALA X   3      -4.245  -3.689   3.314  1.00 0.0000   0
ATOM     29  C03 ALA X   3      -3.966  -3.091   4.606  1.00 0.0000   0
ATOM     30  C04 ALA X   3      -4.294  -1.598   4.644  1.00 0.0000   0
ATOM     31  O05 ALA X   3      -4.855  -1.124   5.628  1.00 0.0000   0
ATOM     32  C06 ALA X   3      -2.497  -3.327   4.962  1.00 0.0000   0
ATOM     33  H01 ALA X   4      -3.631  -1.309   2.741  1.00 0.0000   0
ATOM     34  N02 ALA X   4      -3.948  -0.851   3.592  1.00 0.0000   0
ATOM     35  C03 ALA X   4      -4.151   0.588   3.571  1.00 0.0000   0
ATOM     36  C04 ALA X   4      -5.645   0.911   3.571  1.00 0.0000   0
ATOM     37  O05 ALA X   4      -6.137   1.628   4.442  1.00 0.0000   0
ATOM     38  C06 ALA X   4      -3.449   1.193   2.352  1.00 0.0000   0
ATOM     39  H01 ALA X   5      -5.916  -0.283   1.965  1.00 0.0000   0
ATOM     40  N02 ALA X   5      -6.371   0.366   2.593  1.00 0.0000   0
ATOM     41  C03 ALA X   5      -7.798   0.612   2.440  1.00 0.0000   0
ATOM     42  C04 ALA X   5      -8.571   0.073   3.645  1.00 0.0000   0
ATOM     43  O05 ALA X   5      -9.552   0.664   4.085  1.00 0.0000   0
ATOM     44  C06 ALA X   5      -8.287  -0.043   1.147  1.00 0.0000   0
ATOM     45  H01 LYS X   6      -7.320  -1.503   3.747  1.00 0.0000   0
ATOM     46  N02 LYS X   6      -8.131  -1.067   4.173  1.00 0.0000   0
ATOM     47  C03 LYS X   6      -8.752  -1.745   5.294  1.00 0.0000   0
ATOM     48  C04 LYS X   6      -8.535  -0.943   6.580  1.00 0.0000   0
ATOM     49  O05 LYS X   6      -9.472  -0.744   7.353  1.00 0.0000   0
ATOM     50  C06 LYS X   6      -8.170  -3.162   5.345  1.00 0.0000   0
ATOM     51  C07 LYS X   6      -8.845  -4.130   6.324  1.00 0.0000   0
ATOM     52  C08 LYS X   6      -8.481  -5.548   5.860  1.00 0.0000   0
ATOM     53  C09 LYS X   6      -8.895  -6.652   6.837  1.00 0.0000   0
ATOM     54  N10 LYS X   6      -8.728  -7.980   6.211  1.00 0.0000   0
ATOM     55  H11 LYS X   6      -8.709  -8.727   6.887  1.00 0.0000   0
ATOM     56  H12 LYS X   6      -9.455  -8.147   5.531  1.00 0.0000   0
ATOM     57  H13 LYS X   6      -7.866  -7.994   5.658  1.00 0.0000   0
ATOM     58  H01 ALA X   7      -6.567  -0.646   6.134  1.00 0.0000   0
ATOM     59  N02 ALA X   7      -7.309  -0.457   6.802  1.00 0.0000   0
ATOM     60  C03 ALA X   7      -7.002   0.423   7.918  1.00 0.0000   0
ATOM     61  C04 ALA X   7      -7.815   1.712   7.813  1.00 0.0000   0
ATOM     62  O05 ALA X   7      -8.419   2.137   8.799  1.00 0.0000   0
ATOM     63  C06 ALA X   7      -5.502   0.727   7.960  1.00 0.0000   0
ATOM     64  H01 GLU X   8      -7.305   1.928   5.848  1.00 0.0000   0
ATOM     65  N02 GLU X   8      -7.841   2.320   6.618  1.00 0.0000   0
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ATOM     66  C03 GLU X   8      -8.657   3.496   6.364  1.00 0.0000   0
ATOM     67  C04 GLU X   8     -10.098   3.189   6.766  1.00 0.0000   0
ATOM     68  O05 GLU X   8     -10.653   3.866   7.622  1.00 0.0000   0
ATOM     69  C06 GLU X   8      -8.585   3.932   4.891  1.00 0.0000   0
ATOM     70  C07 GLU X   8      -9.263   5.300   4.692  1.00 0.0000   0
ATOM     71  C08 GLU X   8      -9.764   5.500   3.269  1.00 0.0000   0
ATOM     72  O09 GLU X   8     -10.881   6.053   3.138  1.00 0.0000   0
ATOM     73  O10 GLU X   8      -9.034   5.103   2.339  1.00 0.0000   0
ATOM     74  H01 ALA X   9     -10.170   1.636   5.471  1.00 0.0000   0
ATOM     75  N02 ALA X   9     -10.699   2.164   6.157  1.00 0.0000   0
ATOM     76  C03 ALA X   9     -12.081   1.786   6.391  1.00 0.0000   0
ATOM     77  C04 ALA X   9     -12.355   1.613   7.884  1.00 0.0000   0
ATOM     78  O05 ALA X   9     -13.326   2.163   8.398  1.00 0.0000   0
ATOM     79  C06 ALA X   9     -12.408   0.506   5.618  1.00 0.0000   0
ATOM     80  H01 ALA X  10     -10.704   0.448   8.100  1.00 0.0000   0
ATOM     81  N02 ALA X  10     -11.497   0.865   8.582  1.00 0.0000   0
ATOM     82  C03 ALA X  10     -11.640   0.639  10.010  1.00 0.0000   0
ATOM     83  C04 ALA X  10     -11.642   1.963  10.781  1.00 0.0000   0
ATOM     84  O05 ALA X  10     -12.592   2.261  11.503  1.00 0.0000   0
ATOM     85  C06 ALA X  10     -10.523  -0.287  10.498  1.00 0.0000   0
ATOM     86  H01 ALA X  11      -9.856   2.499   9.968  1.00 0.0000   0
ATOM     87  N02 ALA X  11     -10.580   2.759  10.636  1.00 0.0000   0
ATOM     88  C03 ALA X  11     -10.424   4.000  11.382  1.00 0.0000   0
ATOM     89  C04 ALA X  11     -11.545   4.990  11.055  1.00 0.0000   0
ATOM     90  O05 ALA X  11     -12.069   5.677  11.927  1.00 0.0000   0
ATOM     91  C06 ALA X  11      -9.056   4.609  11.063  1.00 0.0000   0
ATOM     92  H01 LYS X  12     -11.425   4.446   9.128  1.00 0.0000   0
ATOM     93  N02 LYS X  12     -11.898   5.067   9.776  1.00 0.0000   0
ATOM     94  C03 LYS X  12     -12.893   5.967   9.226  1.00 0.0000   0
ATOM     95  C04 LYS X  12     -14.283   5.571   9.725  1.00 0.0000   0
ATOM     96  O05 LYS X  12     -15.065   6.433  10.119  1.00 0.0000   0
ATOM     97  C06 LYS X  12     -12.742   5.909   7.701  1.00 0.0000   0
ATOM     98  C07 LYS X  12     -13.532   6.914   6.857  1.00 0.0000   0
ATOM     99  C08 LYS X  12     -12.838   6.931   5.484  1.00 0.0000   0
ATOM    100  C09 LYS X  12     -13.610   7.666   4.385  1.00 0.0000   0
ATOM    101  N10 LYS X  12     -12.781   7.776   3.166  1.00 0.0000   0
ATOM    102  H11 LYS X  12     -13.324   8.010   2.350  1.00 0.0000   0
ATOM    103  H12 LYS X  12     -12.043   8.451   3.295  1.00 0.0000   0
ATOM    104  H13 LYS X  12     -12.283   6.896   3.001  1.00 0.0000   0
ATOM    105  H01 ALA X  13     -13.906   3.596   9.403  1.00 0.0000   0
ATOM    106  N02 ALA X  13     -14.588   4.270   9.741  1.00 0.0000   0
ATOM    107  C03 ALA X  13     -15.824   3.757  10.310  1.00 0.0000   0
ATOM    108  C04 ALA X  13     -15.885   4.054  11.809  1.00 0.0000   0
ATOM    109  O05 ALA X  13     -16.908   4.533  12.299  1.00 0.0000   0
ATOM    110  C06 ALA X  13     -15.940   2.253  10.047  1.00 0.0000   0
ATOM    111  H01 GLU X  14     -13.987   3.370  12.051  1.00 0.0000   0
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ATOM    130  C04 ALA X  16     -18.832   7.704  12.799  1.00 0.0000   0
ATOM    131  O05 ALA X  16     -19.680   8.523  13.143  1.00 0.0000   0
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ATOM    144  C06 UNK X  18     -15.581   7.870  18.506  1.00 0.0000   0
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ATOM    146  C08 UNK X  18     -13.002   7.646  18.958  1.00 0.0000   0
ATOM    147  N09 UNK X  18     -11.856   6.832  18.501  1.00 0.0000   0
ATOM    148  H10 UNK X  18     -10.958   7.144  18.832  1.00 0.0000   0
ATOM    149  H11 UNK X  18     -12.012   5.849  18.742  1.00 0.0000   0
ATOM    150  C12 UNK X  18     -18.247   9.136  17.265  1.00 0.0000   0
ATOM    151  N13 UNK X  18     -18.137   9.811  16.126  1.00 0.0000   0
ATOM    152  H07 ALA X   9     -12.722   2.584   6.013  1.00 0.0000   0
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ATOM    154  H15 LYS X  12     -11.687   6.104   7.504  1.00 0.0000   0
ATOM    155  H16 LYS X  12     -12.984   4.902   7.357  1.00 0.0000   0
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ATOM    157  H18 LYS X  12     -13.492   7.906   7.311  1.00 0.0000   0
ATOM    158  H19 LYS X  12     -11.855   7.394   5.601  1.00 0.0000   0
ATOM    159  H20 LYS X  12     -12.682   5.900   5.154  1.00 0.0000   0
ATOM    160  H21 LYS X  12     -14.515   7.100   4.157  1.00 0.0000   0
ATOM    161  H22 LYS X  12     -13.892   8.664   4.725  1.00 0.0000   0
ATOM    162  H07 ALA X  13     -16.666   4.249   9.822  1.00 0.0000   0
ATOM    163  H08 ALA X  13     -16.869   1.880  10.478  1.00 0.0000   0
ATOM    164  H09 ALA X  13     -15.948   2.066   8.973  1.00 0.0000   0
ATOM    165  H10 ALA X  13     -15.100   1.724  10.500  1.00 0.0000   0
ATOM    166  H11 GLU X  14     -15.418   3.424  14.462  1.00 0.0000   0
ATOM    167  H12 GLU X  14     -13.051   2.666  13.986  1.00 0.0000   0
ATOM    168  H13 GLU X  14     -12.516   4.337  14.111  1.00 0.0000   0
ATOM    169  H14 GLU X  14     -14.116   3.076  16.372  1.00 0.0000   0
ATOM    170  H15 GLU X  14     -12.442   2.589  16.124  1.00 0.0000   0
ATOM    171  H07 ALA X  15     -14.244   8.158  14.672  1.00 0.0000   0
ATOM    172  H08 ALA X  15     -13.605   9.637  12.805  1.00 0.0000   0
ATOM    173  H09 ALA X  15     -12.453   8.300  13.003  1.00 0.0000   0
ATOM    174  H10 ALA X  15     -13.646   8.256  11.687  1.00 0.0000   0
ATOM    175  H07 ALA X  16     -17.829   9.155  11.591  1.00 0.0000   0
ATOM    176  H08 ALA X  16     -19.081   7.651  10.086  1.00 0.0000   0
ATOM    177  H09 ALA X  16     -17.360   7.722   9.663  1.00 0.0000   0
ATOM    178  H10 ALA X  16     -18.007   6.307  10.525  1.00 0.0000   0
ATOM    179  H07 ALA X  17     -20.749   6.153  13.929  1.00 0.0000   0
ATOM    180  H08 ALA X  17     -20.247   4.132  15.254  1.00 0.0000   0
ATOM    181  H09 ALA X  17     -19.672   3.964  13.581  1.00 0.0000   0
ATOM    182  H10 ALA X  17     -18.516   4.310  14.887  1.00 0.0000   0
ATOM    183  H14 UNK X  18     -18.424   7.150  18.074  1.00 0.0000   0
ATOM    184  H15 UNK X  18     -16.324   6.284  17.305  1.00 0.0000   0
ATOM    185  H16 UNK X  18     -15.974   7.793  16.427  1.00 0.0000   0
ATOM    186  H17 UNK X  18     -15.439   8.949  18.430  1.00 0.0000   0
ATOM    187  H18 UNK X  18     -16.045   7.614  19.461  1.00 0.0000   0
ATOM    188  H19 UNK X  18     -14.421   6.067  18.630  1.00 0.0000   0
ATOM    189  H20 UNK X  18     -14.059   7.076  17.230  1.00 0.0000   0
ATOM    190  H21 UNK X  18     -12.853   8.683  18.653  1.00 0.0000   0
ATOM    191  H22 UNK X  18     -13.092   7.595  20.044  1.00 0.0000   0
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ATOM    194  H14 TYR X   1      -0.379  -3.876  -0.590  1.00 0.0000   0
ATOM    195  H15 TYR X   1      -0.899  -3.403   1.025  1.00 0.0000   0
ATOM    196  H16 TYR X   1      -1.379  -5.201   2.487  1.00 0.0000   0
ATOM    197  H17 TYR X   1      -2.452  -7.399   2.899  1.00 0.0000   0
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ATOM    199  H19 TYR X   1      -1.854  -5.775  -1.760  1.00 0.0000    0
ATOM    200  H11 GLU X   2      -6.367  -3.991   0.763  1.00 0.0000    0
ATOM    201  H12 GLU X   2      -5.515  -6.381   0.690  1.00 0.0000    0
ATOM    202  H13 GLU X   2      -4.659  -6.046   2.197  1.00 0.0000    0
ATOM    203  H14 GLU X   2      -6.994  -5.500   3.189  1.00 0.0000    0
ATOM    204  H15 GLU X   2      -7.619  -6.369   1.780  1.00 0.0000    0
ATOM    205  H07 ALA X   3      -4.580  -3.596   5.354  1.00 0.0000    0
ATOM    206  H08 ALA X   3      -2.287  -2.902   5.944  1.00 0.0000    0
ATOM    207  H09 ALA X   3      -2.288  -4.398   4.987  1.00 0.0000    0
ATOM    208  H10 ALA X   3      -1 852  -2.852   4.222  1.00 0.0000    0
ATOM    209  H07 ALA X   4      -3.702   1.021   4.467  1.00 0.0000    0
ATOM    210  H08 ALA X   4      -3.583   2.275   2.355  1.00 0.0000    0
ATOM    211  H09 ALA X   4      -2.383   0.966   2.391  1.00 0.0000    0
ATOM    212  H10 ALA X   4      -3.866   0.786   1.430  1.00 0.0000    0
ATOM    213  H07 ALA X   5      -7.968   1.689   2.368  1.00 0.0000    0
ATOM    214  H08 ALA X   5      -9.353   0.153   1.021  1.00 0.0000    0
ATOM    215  H09 ALA X   5      -7.747   0.372   0.296  1.00 0.0000    0
ATOM    216  H10 ALA X   5      -8.123  -1.120   1.188  1.00 0.0000    0
ATOM    217  H14 LYS X   6      -9.824  -1.820   5.104  1.00 0.0000    0
ATOM    218  H15 LYS X   6      -8.294  -3.576   4.343  1.00 0.0000    0
ATOM    219  H16 LYS X   6      -7.103  -3.112   5.568  1.00 0.0000    0
ATOM    220  H17 LYS X   6      -8.483  -3.937   7.336  1.00 0.0000    0
ATOM    221  H18 LYS X   6      -9.928  -3.999   6.294  1.00 0.0000    0
ATOM    222  H19 LYS X   6      -8.959  -5.727   4.894  1.00 0.0000    0
ATOM    223  H20 LYS X   6      -7.399  -5.607   5.714  1.00 0.0000    0
ATOM    224  H21 LYS X   6      -8.262  -6.587   7.725  1.00 0.0000    0
ATOM    225  H22 LYS X   6      -9.937  -6.522   7.136  1.00 0.0000    0
ATOM    226  H07 ALA X   7      -7.270  -0.082   8.847  1.00 0.0000    0
ATOM    227  H08 ALA X   7      -5.292   1.396   8.795  1.00 0.0000    0
ATOM    228  H09 ALA X   7      -4.941  -0.197   8.099  1.00 0.0000    0
ATOM    229  H10 ALA X   7      -5.188   1.207   7.033  1.00 0.0000    0
ATOM    230  H11 GLU X   8      -8.280   4.310   6.984  1.00 0.0000    0
ATOM    231  H12 GLU X   8      -7.548   4.007   4.562  1.00 0.0000    0
ATOM    232  H13 GLU X   8      -9.087   3.195   4.265  1.00 0.0000    0
ATOM    233  H14 GLU X   8     -10.123   5.394   5.353  1.00 0.0000    0
ATOM    234  H15 GLU X   8      -8.556   6.094   4.934  1.00 0.0000    0
ATOM    235  H08 ALA X   9     -13.451   0.235   5.789  1.00 0.0000    0
ATOM    236  H09 ALA X   9     -12.255   0.670   4.551  1.00 0.0000    0
ATOM    237  H10 ALA X   9     -11.769  -0.310   5.953  1.00 0.0000    0
ATOM    238  H07 ALA X  10     -12.593   0.139  10.186  1.00 0.0000    0
ATOM    239  H08 ALA X  10     -10.640  -0.467  11.567  1.00 0.0000    0
ATOM    240  H09 ALA X  10     -10.578  -1.239   9.969  1.00 0.0000    0
ATOM    241  H10 ALA X  10      -9.548   0.165  10.317  1.00 0.0000    0
ATOM    242  H07 ALA X  11     -10.459   3.777  12.451  1.00 0.0000    0
ATOM    243  H08 ALA X  11      -8.924   5.529  11.632  1.00 0.0000    0
ATOM    244  H09 ALA X  11      -8.268   3.906  11.332  1.00 0.0000    0
ATOM    245  H10 ALA X  11      -8.987   4.833   9.998  1.00 0.0000    0
ATOM    246  H24 UNK X  18     -17.807   9.337  15.287  1.00 0.0000    0
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CONECT  158   99
CONECT  159   99
CONECT  160  100
CONECT  161  100
CONECT  162  107
CONECT  163  110
CONECT  164  110
CONECT  165  110
CONECT  166  113
CONECT  167  116
CONECT  168  116
CONECT  169  117
CONECT  170  117
CONECT  171  123
CONECT  172  126
CONECT  173  126
CONECT  174  126
CONECT  175  129
CONECT  176  132
CONECT  177  132
CONECT  178  132
CONECT  179  135
CONECT  180  138
CONECT  181  138
CONECT  182  138
CONECT  183  141
CONECT  184  143
CONECT  185  143
CONECT  186  144
CONECT  187  144
CONECT  188  145
CONECT  189  145
CONECT  190  146
CONECT  191  146
CONECT  192  147
CONECT  193    5
CONECT  194    8
CONECT  195    8
CONECT  196   10
CONECT  197   11
CONECT  198   13
CONECT  199   14
CONECT  200   19
CONECT  201   22
CONECT  202   22
CONECT  203   23
CONECT  204   23
CONECT  205   29
CONECT  206   32
CONECT  207   32
CONECT  208   32
CONECT  209   35
CONECT  210   38
CONECT  211   38
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CONECT  212   38
CONECT  213   41
CONECT  214   44
CONECT  215   44
CONECT  216   44
CONECT  217   47
CONECT  218   50
CONECT  219   50
CONECT  220   51
CONECT  221   51
CONECT  222   52
CONECT  223   52
CONECT  224   53
CONECT  225   53
CONECT  226   60
CONECT  227   63
CONECT  228   63
CONECT  229   63
CONECT  230   66
CONECT  231   69
CONECT  232   69
CONECT  233   70
CONECT  234   70
CONECT  235   79
CONECT  236   79
CONECT  237   79
CONECT  238   82
CONECT  239   85
CONECT  240   85
CONECT  241   85
CONECT  242   88
CONECT  243   91
CONECT  244   91
CONECT  245   91
CONECT  246  151
CONECT  247  151
CONECT  248    1    5  249
CONECT  249  248
END
```