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Analysis of N-*tert*-Butoxy-Carboynl Anyhydride Formation Using Molecular Simulation

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Abstract: Three dimensional structures of peptides: A, AG, AGA, AGAG and their cyclisation structures such as A-Cl, AG-Cl and their NCAs' were drawn into a computer. Properties such as distance, angles, state surfaces were obtained and optimized to find the potential energy surface (PES), using a Gaussian program. These values were used to simulate the reaction of NCA formation from the precursor structures using their thermodynamic parameters' (ΔG, ΔH and ΔS) as a function of temperature, structure and length of the NCA precursor. The thermodynamic feasibility of the processes was investigated to augment work in the laboratory in view of the large number of applications including bio-organic synthesis and tissue engineering. Spontaneity of the reactions to form stable NCAs' was found to increase with peptide length. The changes in thermodynamics, revealed more about the changes in energy with conformation in the reaction indicating that emphasis should be put on the general trends in the reaction, such that the increasingly negative values with length of molecules show increasing spontaneity of the reaction.

Keywords: Molecular Dynamics, QMMM, Conformational Change, N-*tert* Butoxy-Carboynl Anhydride, Peptides, Acid Chloride

1. Graphical Abstract

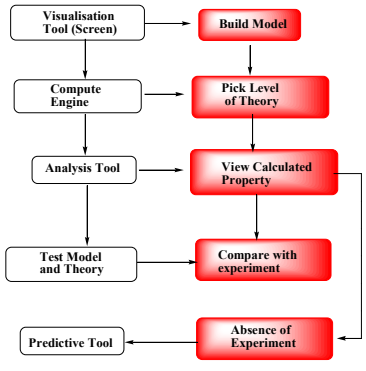


Figure 1. *Flow chart of molecular simulation of NCA formation.*

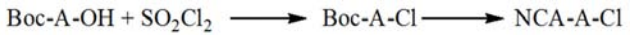
2. Introduction

Extensive research went into understanding the synthesis of NCAs' in the 1920s', first by Wesley and more recently by Deming et al "[1], [2]" who went further to polymerize the NCAs with Nickel and cobalt catalysts and yielded polypeptides of

25000Da and low polydispersities "[3], [4]". However, NCAs' were mostly synthesized from one amino acid and only polymerized to form polypeptides and block polypeptides and their methods of synthesis are well documented in literature "[5], [6]".

NCAs' are synthesized using three known methods, namely; Fuch Farthing "[7], [8], [9]" Leuch's "[10], [11], [12]" and the Silyazide method "[13], [14]". Leuch's and

Fuch Farthing are more commonly used; however, we modeled and used Leuch's method due to the nature of our amino acids and their relative ease in purification after synthesis "[15]". Synthesis using Leuch's method involves the use of Lewis acids and proceeds with chlorination of the amine, followed by an electrophilic attack of the 'boc' carbonyl, which then cleaves carbon monoxide and 2,2 dimethyl propanol (see Scheme 1).



Scheme 1. *Formation of NCA-A-Cl precursor.*

There is a growing interest in NCAs' that stems from applications of their polymers in biomaterials "[16]". Some of these applications from the homo and co-polypeptides include preparation of drug delivery, synthetic wool or wool substitutes and tissue scaffolding "[17]". The use of polypeptides in tissue scaffolding as a framework for repair and regeneration is increasingly important in organs such as the heart, whose tissue does not functionally restore itself after damage. "[18]" It would be advantageous if biodegradable materials on the market could encourage heart cells to regenerate so that their by-products would then come out through the normal excretive channels and eliminate the need for invasive surgery. This can be done by synthesizing polypeptides with similar sequences to the cells, which encourage cell regeneration in other organs. However, synthesis of stereo-specific polypeptides necessitates the use of complex monomers, which will be modeled here.

Computational methods were used to simulate the successful synthesis of small peptides to acid chlorides then NCA synthesis. Computer annotations of similar reactions, where the trans-phosphorylation reaction in fragment models was constructed, to mimic ribozyme catalytic reactions, have been reported by Gregerson et al. "[19]" They found that 'iniline attack' mechanisms are associative, in character, and proceed via two concerted transition states. Furthermore, the relative heights of barriers were found to be dependent on contexts such as native' vs. thio and the substituted fragments. Some authors reported kinetic isotropic effects "[20]" and psuedoration in the oxylphosphoranes "[21]". Where-as most studies here were concerned with the mechanism and kinetics of the reactions, we use other parameters for our study.

In this article, we employ several computational protocols with the objective of gaining some insight into the effect of NCA formation as a function of temperature, structure and length of NCA. In particular, we hope to gain some insight into probability of these reactions proceeding from peptide to NCA and the stability of the structures once formed, based on the classical molecular dynamics and mixed quantum mechanics (QMMM) "[22]" force-fields, we study possibility of NCA formation a function of the aforementioned parameters.

Although the parameters used are not unique to organic synthesis, the significance of finding the most optimum configuration for synthesizing each molecule will uncover a method for simulating the synthesis of NCA monomers that can then be polymerized into stereo-regular polymers whose physical and chemical characteristics can be controlled. Our computational methods suggest a relative ease in NCA formation of dimer and trimers and an increase in stability of their NCA.

NCAs' from amino acids will be simulated as a reference, since the success of their synthesis is well known and documented. NCAs' of dimers, trimers and tetramers will then be simulated as a function of temperature, structure and NCA length.

3. Results and Discussion

3.1. Results

Thermodynamic data of synthesis of the acid chlorides are presented in Table 1.

Table 1. *Thermodynamic data of the synthesis of peptide chlorides.*

Amino acid/peptide	Electronic energy (ZPE)	Enthalpy ΔH (J mol $^{-1}$)	Entropy ΔS (J mol $^{-1}$)	Gibbs free energy ΔG (J mol $^{-1}$)
Boc-A-Cl	-1053.72	-1053.70	0.000201	-1053.76
Boc-AG-Cl	-1261.68	-1261.65	0.000246	-1261.72
Boc-AGA-Cl	-1469.63	-1469.60	0.000286	-1469.69

We sought to find a relationship between length of acid chloride and NCA vs. changes in electronic energy, enthalpy, entropy and Gibbs free energy. In order to explore these relationships, we conducted simulations for chlorination and cyclisation reactions by varying the lengths of the molecules but keeping other parameters such as temperature and pressure constant. The relationships between the chlorination and electronic thermodynamic are listed in Table 1 whilst those between the NCA formation and the electronic thermodynamics are listed in Table 2.

The results in Table 2, 'chlorination' indicate, that the chlorination reaction should proceed spontaneously. There is an increase in spontaneity of this reaction with successive increases additions of amino acid to the peptide. There is also an increase in entropy indicating a more feasible reaction with stable conformations, as the molecule increases in length. However, the electronic energy of peptide-chloride decreases with the successive increases of amino acids in the peptide. However, emphasis should be put on the general trends in the reaction, such that the increasingly negative values with length of molecules that indicate increasing spontaneous reaction without added energy.

Table 2. *Electronic, enthalpy, entropy and Gibbs free energy for preparation of NCAs' from peptides.*

NCAs'	Electronic energy (ZPE)	Enthalpy (ΔH)	Entropy (ΔS)	Gibbs free Energy (ΔG)
A	-435.791	-435.783	0.000134	-435.823
AG	-643.719	-643.707	0.000164	-643.756
AGA	-851.661	-851.645	0.000194	-851.703

ΔG increases with the increase in the size of NCA molecule. This indicates an increase in spontaneity of the reaction with peptide length. However, the marginal increase in entropy indicated that NCAs were equally stable conformations once formed and there was a slight increase in stability with an increase in length of peptide use for NCA formation. Values for the second derivation were drawn up for the formation of the NCA. Thermodynamics with this second derivative for the NCA formation reaction were not particularly encouraging. The change's in entropy and Gibbs free energy was not favorable for the forward reaction to proceed spontaneously with increase in length of the peptide, as is shown in Table 2 and Table 3. The reaction is less spontaneous as the length of the peptide increases. An increasing amount of energy is necessary for the reaction to proceed. Entropy also decreases with the increase in the peptide length. This may be attributed to less strain imposed by larger structures and therefore wider angles and longer bonds, which lower the internal energy, and stabilize the conformation. However more research needs to be done on this particular subject. Increase in Gibbs free energy at 25°C is an indication that some energy needs to go into this reaction for it to go ahead, see Table 3 below.

Table 3. *Values of ΔH, ΔS, ΔG and their derivatives for the NCA formation reaction.*

NCAs'	Elect—ronic Energy (ZPE)	ΔH (J mol^{-1})	ΔS (J mol^{-1})	ΔG (J mol^{-1})	dH (Kcal/mol)	dS (Kcal/molK)	dG (Kcal/mol)
A	617.94	617.93	0.000124	617.97	-4.63	0.036	-15.37
AF	617.94	617.93	0.000124	617.97	11.80	0.027	3.75
AGA	617.94	617.93	0.000124	617.97	20.16	0.021	14.33
AGAG	617.94	617.93	0.000124	617.97	20.16	0.021	14.33

The cyclization reaction of NCA-Ala was simulated over a temperature range of 25°C to 300°C and 1 atm. Other parameters such as the polar solvent and appropriate proton grabbing amine were already optimized in the reaction. The reaction was a two, step process, where the OH end group of protected amino acid/ peptide, was replaced with the halogen, followed by a nucleophilic attack of the polarized carboxyl group by the carbonyl on the Boc protecting group, producing hydrochloric acid, carbon monoxide and an alcohol as by-products. The by-products were not taken into account, for the simulation either, see equation 1.

$$\text{Boc-Ala-OH(s)} + CO_2Cl_2\text{(l) Boc-Ala-Cl} \rightarrow \text{NCA-Ala} + \text{HCl} + \text{CO} + C_{11}H_9O$$

Equation 1: Cyclization of Boc-Ala/ Boc-A

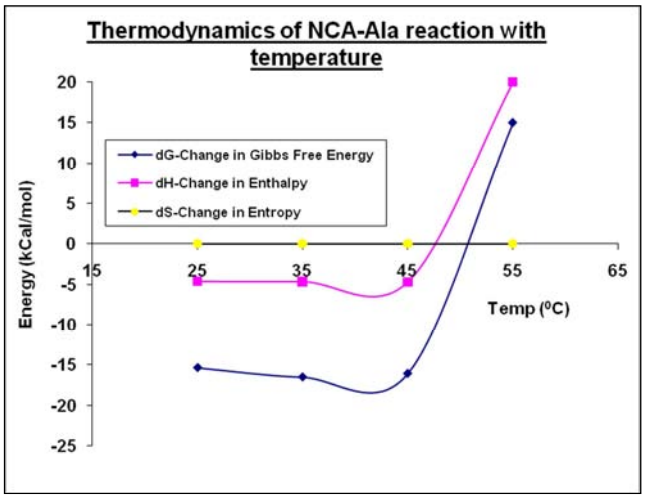


Figure 2. *Thermodynamics of NCA reaction with temperature.*

Changes in enthalpy and Gibbs free energy were negative and fairly stable up to 30°C, where a trough appears in the graph, followed by a steep increase in both energies till 300°C. In conclusion, the 'G' energy increased with the enthalpy, indicating temperature too was needed to drive the reaction forward. Results showed an increase in the temperature was one of the driving forces of the reaction (see Figure 2).

NCA formation reaction was to give NCA-Ala, NCA-AG, NCA-AGA "[23]" and NCA-AGAG.

The changes in enthalpy, entropy and Gibbs free energy were calculated over a temp range. NCA-Ala had a Gibbs free energy higher than zero from temps of 0-200°C. Changes in Gibbs free energy, 'dG' of NC-Ala & NCA-AGA increases with temp. NCA-Ala has a steeper increase in 'dG' with temp. NCA-AG & NCA-AGA show a decrease in 'dG' with temperature (See Figure 3).

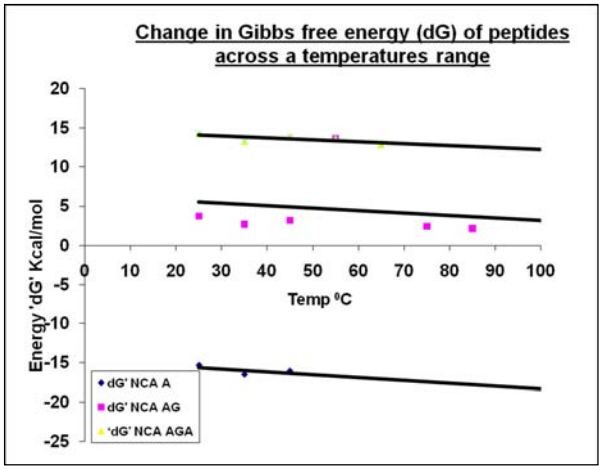


Figure 3. *Changes in Gibbs free energy (dG) with Temperature °C range.*

Changes in enthalpy, entropy and Gibbs free were calculated as a function of temperature for NCA-A, NCA-AG "[24]" and NCA-AGA. All three NCA-single, dimer and trimer showed a decrease in entropy with an increase in temp. NCA-AGA and NCA-A had a steeper decline in entropy. Boc-AGA and Boc-A proceeded with increase in energy. Boc-AG needs a lot of energy to get the reaction going (see Figure 4).

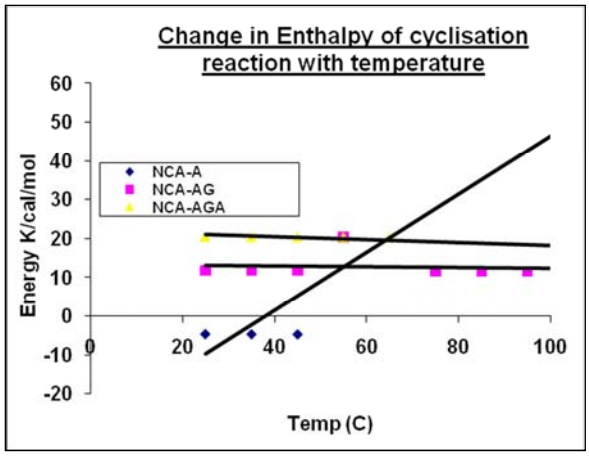


Figure 4. *Change in Entropy of cyclisation reaction with temperature.*

3.2. Discussion

The experiment carried out was the modeling of NCA formation of peptides from single amino acid namely: NCA-Ala, NCA Ala-Gly, NCA Ala-Gly-Ala and NCA Ala-Gly-Ala-Gly.

1 The computer simulation model illustrated, that all three NCA-peptides showed a decrease in entropy with increase in temperature.

NCAs' from Boc-AGA-OH and Boc-A-OH were likely to proceed with an increase in energy; however NCAs' from Boc-AG-OH needed more energy.

2 The most optimum solvent for cyclisation was found to be DMF, which was the most polar solvent and favored a forward reaction.

Optimum temperatures were -20°C initially to 300ºC for the dimer and trimer cyclisation.

The thermodynamics of the reaction from the changes in enthalpy, entropy and Gibbs free energy for all the peptides was calculated as a function of temperature. The resulting model illustrated the change in entropy of the NCA- peptide reactions as a function of temperature and all three NCA-peptides showed a decrease in entropy with increase in temperature. However, the NCA Ala-Gly-Ala followed by NCA Ala showed a steeper decline in entropy. Hence reactions for both Boc Ala-Gly-Ala and Boc Ala were most likely to proceed with an increase in energy since states of low strain in the peptides can be reached without much energy input, which may otherwise compromise the structure of the peptide. This was not the case for Boc Ala-Gly, which would need considerable energy to get the reaction going.

4. Experimental

4.1. Method of Analysing NCA Formation Using Molecular Simulation Optimization of NCAs'

3D structures of Alanaine (A), Alanine-Glycine (AG)/dimer, AGA / trimer and AGAG/tetramer and their cyclisation structures 2-aminopropanoyl chloride (A-Cl), 2-(2-aminopropanamido) acetylchloride (AG-Cl), 2-(2-(2-aminopropanamido) acetamido)propanoyl chloride (AGA-Cl) and 2-(2-(2-(2-aminopropanamido) acetamido) propanamido) propanoyl chloride (AGAG-Cl) were drawn into 3D structures and measurements of distance, angles, state surfaces and other properties were taken. These properties were incorporated into an algorithm and locally minimized, with the Gaussian program, to find a local minimum on the potential energy surface (PES). This information was then used to find to calculate their thermodynamic parameters. That information was then used in addition to other thermodynamic data to build a simulation model of NCA formation. The grey spots denote the carbon atoms, white spots are the hydrogen and blue spots are the nitrogen and red spots are the oxygen (see Figure 5-8).

Figure 5 through 8 were the optimized (optimized interaction parameters) structures of 'NCA A, NCA AG, NCA AGA and NCA AGAG.' They were locally minimized with the Gaussian program.

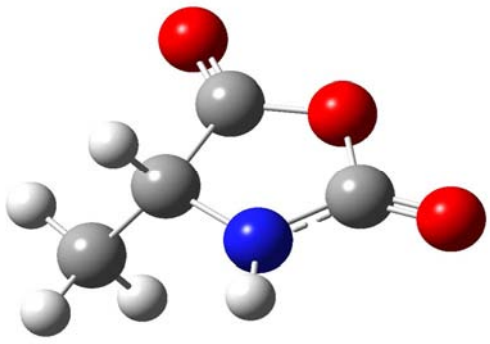


Figure 5. *Model of an optimized NCA-A.*

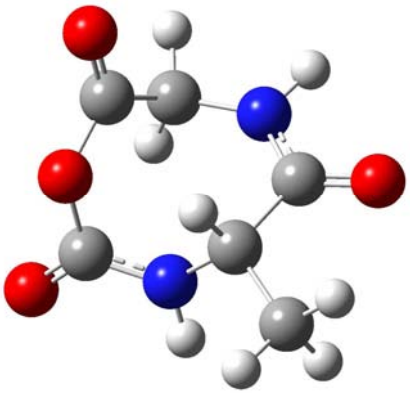


Figure 6. *Model of optimised NCA-AG.*

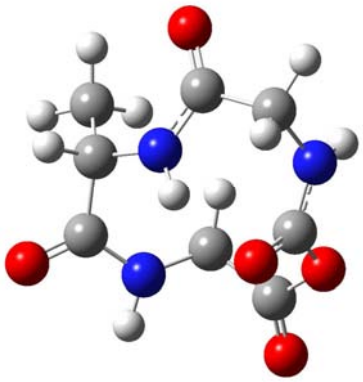


Figure 7. *Model of optimised NCA-AGA.*

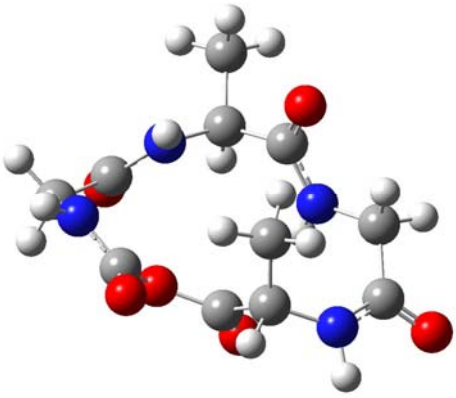


Figure 8. *Model of optimised NCA-AGAG.*

The electronic energies of the single amino acid, dimer, trimer and tetramer chlorides and their corresponding formation of NCAs,' in the reaction were calculated using the DFT (B3LYP) approximation and 6 to 31G (d) as the basis set. "[25]" Berny algorithm was used with tight optimization conditions. Vibrational analysis of the geometries was used to obtain the local minima. The value was used at the minimum to calculate the thermodynamic parameters (ΔG, ΔH and ΔS) for the single amino acid, dimer, trimer and tetramer in their chlorides formed as well as their corresponding NCAs at a selected range of temperatures. It is important to note that only internal energy terms for vibrational energy based on quantum mechanics are vibrational and the values are used to obtain electronic energies. "[26]" The rotational and translational energies were calculated from simple formulas, shown below. See equation 2.

The first step was to obtain figures for electronic energies of the peptide chlorides after the minimization was obtained. The type of peptides, reactants, intermediates are shown in Table 4. Equation 2 shows cyclisation reaction.

$$\text{Boc-A-OH} + SO_2Cl_2 \longrightarrow \text{Boc-A-Cl} \longrightarrow \text{NCA-A-Cl}$$

Equation 2: Cyclizing of single amino acid

Table 4. *Summary of structures from single amino acid, dimer, trimer and tetra chlorides to NCAs'.*

Type of Peptide	Reactant intermediate (NCA precursor)	Product
Single amino acid	Boc-A-Cl	NCA-A
Dimer	Boc-AG-Cl	NCA-AG
Trimer	Boc-AGA-Cl	NCA-AGA
Tetramer	Boc-AGAG-Cl	NCA-AGAG

The electronic energy was then used together with the Massieu bridging equation to calculate the changes in entropy, enthalpy and Gibbs free energy of the amino acid, dimer, trimer and tetramer chloride. These results are displayed in the Table 5.

Table 5. *Thermodynamic data of synthesis of peptide Chlorides.*

Amino acid or peptide	Electronic energy (ZPE)	Enthalpy ΔH (J mol $^{-1}$)	Entropy ΔS (J mol $^{-1}$)	Gibbs free energy ΔG (J mol $^{-1}$)
Boc-A-Cl	-1053.72	-1053.70	0.000201	-1053.76
Boc-AG-Cl	-1261.68	-1261.65	0.000246	-1261.72
Boc-AGA-Cl	-1469.63	-1469.60	0.000286	-1469.69

The results in Table 5 indicate that the reaction in which the halogen replaces the OH end group of the carboxylic acid end proceeds spontaneously. There is an increase in spontaneity of this reaction with successive increases in addition of an amino acid to the peptide. There is also an increase in entropy, which is a welcome sign of a feasible reaction and has stable conformations with the successive increases in addition of amino acids in the peptides. The electronic energy of peptide-chloride decreases with the successive increases of the amino acid in the peptide.

However, there are few points for consideration. One is that an increased likelihood of the reaction proceeding spontaneously without added energy is described by a negative sign, so that the more negative the result is the more

likely it is that the reaction will proceed spontaneously. Therefore a reaction that would need more energy to go forward will be expressed with positive values. Secondly, the Gibbs free energy, enthalpy and entropy are not precise and have a fair margin of error; so more emphasis should be placed on the trend in values themselves. Lastly. Factors such as unfavorable thermodynamics alone do not restrict reactions from proceeding spontaneously. Some reactions can be kinetically possible even though the thermodynamics are unfavorable.

The Gaussian program was run for the preparation of NCA amino acid from the peptides and the results are tabulated in Table 3. The reaction from the coupled amino acids to the monocyclic dimer and tricyclic monomer is an isodesmic reaction, where the type of chemical bonds in the reactant and products are the same, so there is little change in energy. These 'class' of reactions are called the 'isogyric' reactions, were transformations in which reactants and products yield the same electrons pairs, as shown in equation 3. "[27]"

For example:

$$\text{Boc-Leu} + CO_2Cl_2 \rightarrow \text{Boc-Leu-O-Cl} + \text{NCA-Leu} + CO + HCl + C_4H_9O$$

Equation 3: Cyclisation of the single amino acid leucine (Leu) to NCA-L

Boc-Leu-OH has 7(C-C), 19(C-H), 1(N-H), (C-OH), 2(C=O) and 2(C-O) bonds present in the reactant, whilst NCA Leu product has 5(C-C), 10(C-H), 1(N-H) and 2(C-O) are the bonds. This is one of the easier reactions to work within thermo-chemistry and the calculations are more accurate.

In this case the change in enthalpy, entropy, electronic energy levels and Gibbs free energy levels was calculated for the NCAs at a constant temperature and atmospheric pressure, (see Table 6).

Table 6. *Electronic, enthalpy, entropy and Gibbs free energy for preparation of NCAs' from peptides.*

NCA	Electronic energy (ZPE)	Enthalpy (ΔH)	Entropy (ΔS)	Gibb's free Energy (ΔG)
NCA-A	-435.791	-435.783	0.000134	-435.823
NCA-AG	-643.719	-643.707	0.000164	-643.756
NCA-AGA	-851.661	-851.645	0.000194	-851.703

ΔG increases with the increase in peptide length. This indicates an increase in spontaneity of the reaction with peptide length. However, the marginal increase in entropy indicated that NCAs were equally stable conformations once formed and there was a slight increase in stability with an increase in length of peptide use for NCA formation. The values for the second derivation were drawn up for the formation of the NCA. The thermodynamics with this second derivative for the NCA formation reaction were not particularly encouraging. The change in entropy and Gibbs free energy were not favorable for the forward reaction to proceed spontaneously with increase in length of the peptide, as is shown in Table 5 and Table 6. The reaction is less spontaneous as the length of the peptide increases. An increasing amount of energy is necessary for the reaction to proceed. Entropy also decreases with the increase in the peptide length. This may be attributed to less strain imposed by larger structures and therefore wider angles and longer bonds, which lower the internal energy, and stabilize the conformation. However, more research needs to be done on this particular subject. Increase in Gibbs free energy at 25 °C is an indication that some energy needs to go into this reaction for it to go ahead (see Table 7).

Table 7. *Values of ΔH, ΔS, ΔG and their derivatives for the NCA formation reaction.*

NCAs'	Electronic Energy (ZPE)	ΔH (J mol^{-1})	ΔS (J mol^{-1})	ΔG (J mol^{-1})	dH (kcal/mol)	dS (Kcal/molK)	dG (Kcal/mol)
A	617.94	617.93	0.000124	617.97	-4.63	0.036	-15.37
AG	617.94	617.93	0.000124	617.97	11.80	0.027	3.75
AGA	617.94	617.93	0.000124	617.97	20.16	0.021	14.33
AGAG	617.94	617.93	0.000124	617.97	20.16	0.021	14.33

4.2. Computer Simulation

The cyclization reaction was simulated at 25°C and 1 atm. Other parameters such as the polar solvent and appropriate proton grabbing amine were already optimized in the reaction. The reaction was a two, step process, where the OH end group of protected amino acid/ peptide, was replaced with the halogen, followed by a nucleophilic attack of the polarized carboxyl group by the carbonyl on the 'Boc' protecting group, producing hydrochloric acid, carbon monoxide and an alcohol as by-products. The by-products were not taken into account for the simulation either. See Equation 3.

$$\text{Boc-A-OH(s)} + CO_2Cl_2\text{(l)} \rightarrow \text{Boc-A-Cl} \rightarrow \text{NCA-A} + HCl + CO + C_{11}H_9O$$

Equation 3: Cyclization of Boc-Ala

5. Conclusion

There are few points for consideration. One is that an increased likelihood of the reaction proceeding spontaneously without added energy is described by a negative sign, so that the more negative the result is the more likely it is that the reaction will proceed spontaneously. Therefore a reaction that would need more energy to go forward will be expressed with positive values. Secondly, the

Gibbs free energy, enthalpy and entropy are not precise and have a fair margin of error; so more emphasis should be placed on the trend in values themselves. Lastly, factors such as unfavorable thermodynamics alone do not restrict reactions from proceeding spontaneously. Some reactions can be kinetically possible even though the thermodynamics are unfavorable.

Acknowledgments

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