The Dissertation is based on the following Accepted Papers

1. **Joseph C. P. Koo**, Gregory A. Chass, Andras Perczel, Ödon Farkas, Ladislaus L. Torday, Andras Varro, Julius Gy. Papp, and Imre G. Csizmadia. Exploration of the 4D-conformational potential energy hypersurface of N-acetyl-L-aspartic acid-N'-methylamide with its internally hydrogen bonded sidechain orientation, J. Phys. Chem. A, 106 (**2002**), 6999-7009.

   IF: **2.630**

2. **Joseph C. P. Koo**, Gregory A. Chass, Andras Perczel, Ödon Farkas, Ladislaus L. Torday, Andras Varro, Julius Gy. Papp, and Imre G. Csizmadia. N-acetyl-L-aspartic acid-N'-methylamide with sidechain orientation capable of external hydrogen bonding. Backbone and sidechain folding, studied at the DFT level of quantum theory, Eur. Phys. J. D, 20 (**2002**), 499-511.

   IF: **1.583**

3. **Joseph C. P. Koo**, Janice S.W. Lam, Gregory A. Chass, Salvatore J. Salpietro, R. Daniel Enriz, Ladislaus L. Torday, Andras Varro, and Julius Gy. Papp. How Reliable Could Economic Hartree-Fock Computations Be In Studying Large, Folded Peptides? A comparative HF and DFT study on N- and C-protected aspartic acid, THEOCHEM **2002** (in press)

   IF: **0.919**

4. **Joseph C. P. Koo**, Janice S.W. Lam, Gregory A. Chass, Andras Perczel, Ödon Farkas, Ladislaus L. Torday, Andras Varro, and Julius Gy. Papp. Conformational dependence of the intrinsic acidity of the aspartic acid residue sidechain in N-acetyl-*L*-aspartic acid-N'-methylamide, THEOCHEM **2002** (in press)

   IF: **0.919**

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Conformational dependence of the intrinsic acidity of the aspartic acid residue sidechain in N-acetyl-*L*-aspartic acid-N'-methylamide

Theses of the Ph.D. Dissertation by

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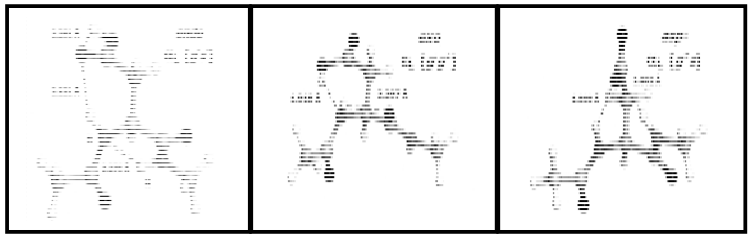
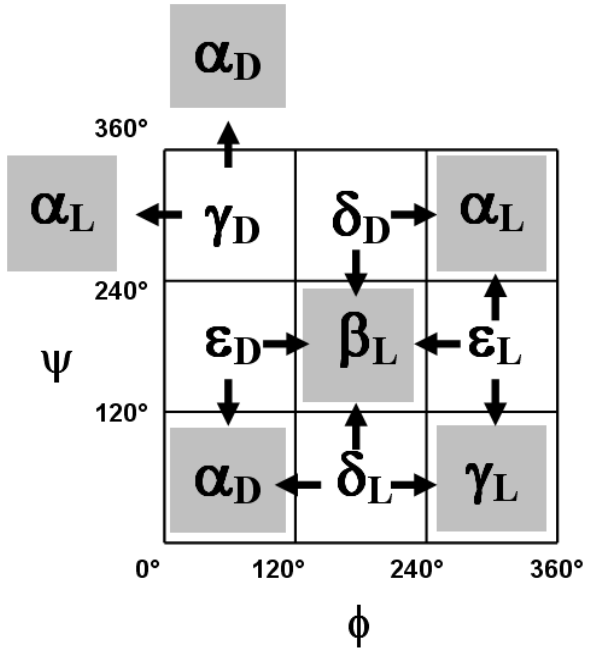
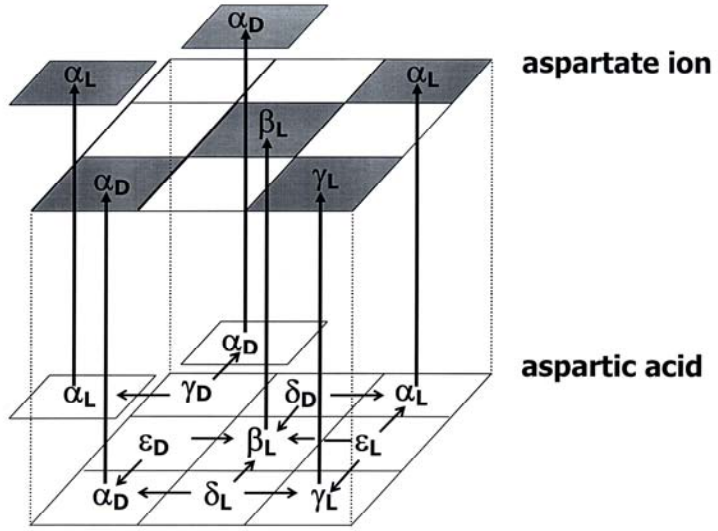
ABSTRACT

Conformational analysis has been carried out on the *endo* and *exo* forms of the carboxylic acid-containing sidechain of the aspartic acid residues as well as on their deprotonated form, the aspartate residue. Unexpected α_L and ε_L conformations were found in the cases of the aspartic acid residue. For the aspartate residue three conformers were located in the α_L conformation, one of which was the global minimum.



It seems that the aspartic acid residue would rather change conformation to the favourable backbone structures (γ_L, β_L, α_L, α_D) before it would undergo deprotonation. A conformer of the aspartic acid residue in anyone of the δ_L, ε_L, γ_D, δ_D, and ε_D backbone conformations would migrate to one of its nearest neighbours by changing either the ϕ or the ψ torsional angle prior to deprotonation.





Deprotonation Scheme of Aspartic Acid Residue to Aspartate Ion

ACKNOWLEDGEMENTS

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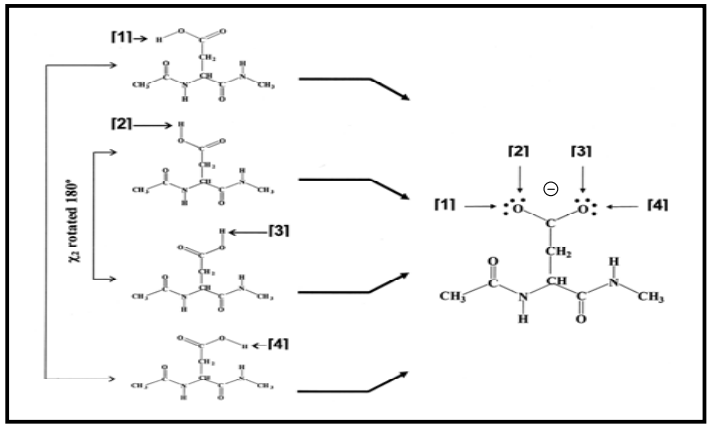
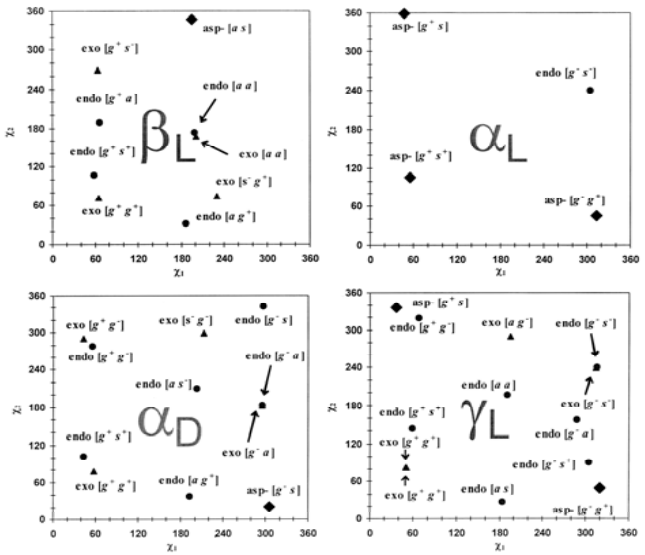


Thesis #10

I have determined that the seven stable conformers which exist for **N**-acetyl-*L*-aspartate-**N'**-methylamide have their minima located in the γ_L, β_L, α_L, and α_D backbone conformations. The aspartic acid residue would change its conformation to the favourable backbone structures (γ_L, β_L, α_L, α_D) before it would undergo deprotonation. In this case, a conformer of the aspartic acid residue in the δ_L, ε_L, γ_D, δ_D, and ε_D backbone conformations (where no stable conformers could be found for the aspartate residue) would migrate to one of its nearest neighbours (γ_L, β_L, α_L, or α_D) by changing either the ϕ or the ψ torsional angle.

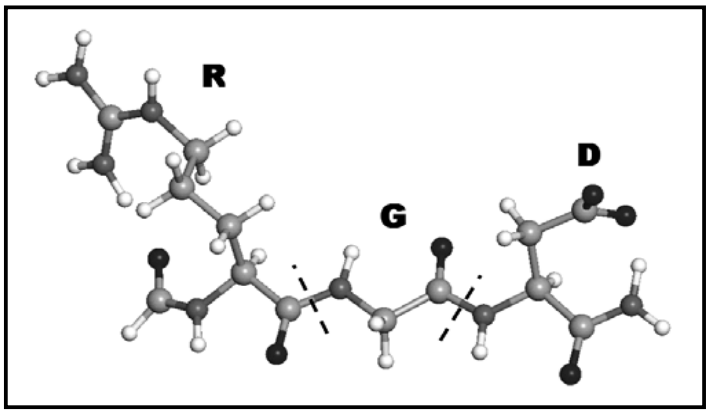


1. Introduction

1.1 Biological Background

Recently, many mutational studies were performed on receptors and channels in the biological systems. Among these investigations were reports that aspartic acid, or aspartate, is crucial in the selectivity and regulation of ion channel selectivity, ligand binding, as well as the functionality of a receptor protein.

For instance, it was reported that an aspartic acid site in the inositol 1,4,5-trisphosphate receptor is important in controlling Ca^{2+} selectivity. Also, an aspartate-rich region in the calsequestrin Ca^{2+} binding protein was found to be important in a receptor-mediated Ca^{2+} release process by acting as a direct binding site for Ca^{2+}. In another study, point mutations of the aspartate residue was also found to abolish Ca^{2+} permeation in the epithelial Ca^{2+} channel, causing the channel to be non-functional. Mutations in two aspartate regions of the human immunodeficiency virus-1 (HIV-1) *chemokine coreceptor CXCR4* were even found to be able to reduce HIV-1 entry into host cells. In these cases, the sidechain and backbone geometry of the aspartate residue will result in the formation of various stabilizing forces that may affect the binding affinity or function of proteins and receptors. The aspartate residue is also a key amino acid in many clinical studies involving probing assays, aging, cerebral injury, and enzyme activities, just to name a few.

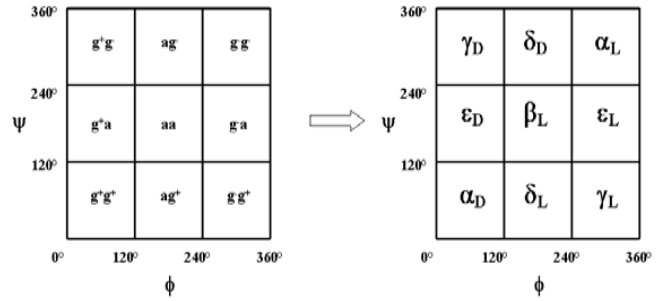


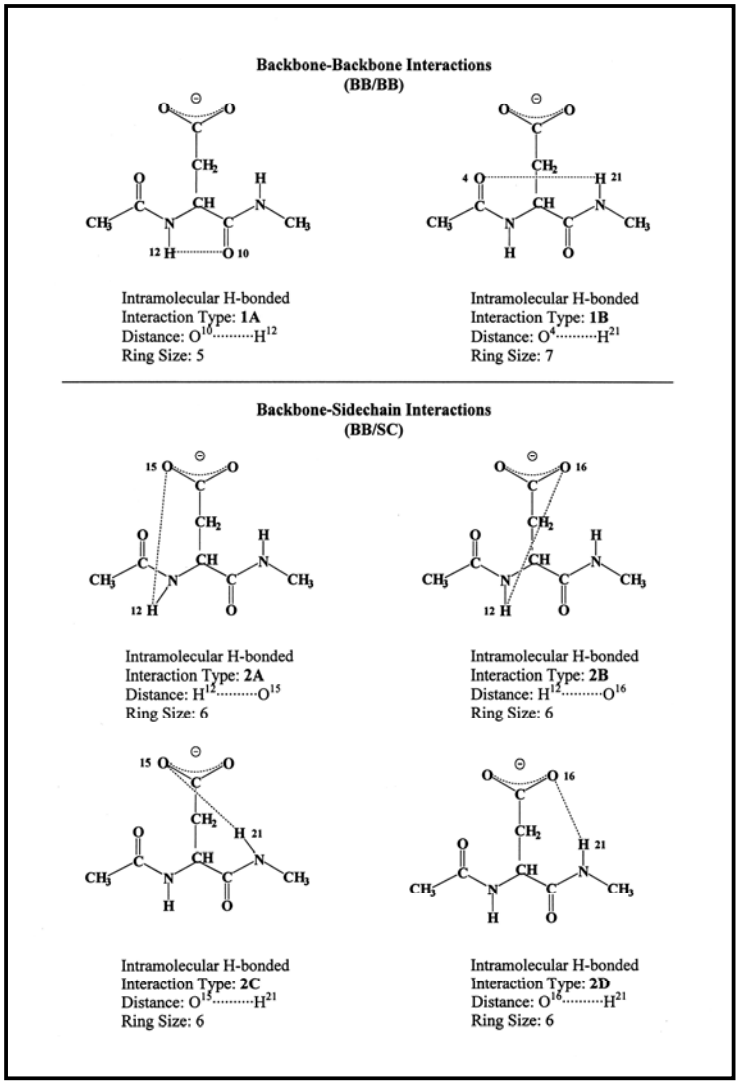
Perhaps one of its most important associations with medicine, however, lies in its connection with the RGD tripeptide. The arginine (R)-glycine (G)-aspartic acid (D) tripeptide has been intensively studied in molecular biology and medical genetics. It was shown to improve gene delivery efficiency and expand the tropism of gene-delivering vectors, and increase a drug's oral bioavailability in a body system. By studying the three dimensional molecular preferences for the aspartate residue, insights may be gained in the RGD's binding affinity and geometric characteristics. Since computational molecular modeling of molecules is an area of high interest in recent years in drug designs, *ab initio* studies on amino acids such as the aspartate residue may deem beneficial in the pharmaceutical industry; especially when exploring binding affinity or selectivity of a target receptor. In this work, all possible sidechain (SC) and backbone (BB) conformers that may exist for the aspartic acid residue were reported, using **N**-acetyl-*L*-aspartic acid-**N'**-methylamide, as well as that of its deprotonated form, as peptide models. In addition, the deprotonation and protonation preferences for the aspartate residue will be explored. The deprotonation and protonation characteristics of this aspartate will decide the various inter- and intra-residual and intermolecular hydrogen interactions that may result. In turn, these forces may directly govern the binding patterns of ligands to aspartate sites in receptors and proteins.

1.2 Conformational Background

N-acetyl-*L*-aspartate-**N'**-methylamide is the deprotonated form of either the *endo* or the *exo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide. In general, all conformations of an amino acid residue of this complexity may be characterized by at least four torsional angles: ϕ, ψ, χ_1, and χ_2, leading to a potential energy hypersurface (PEHS) consisting of four independent variables (4D) [1]:

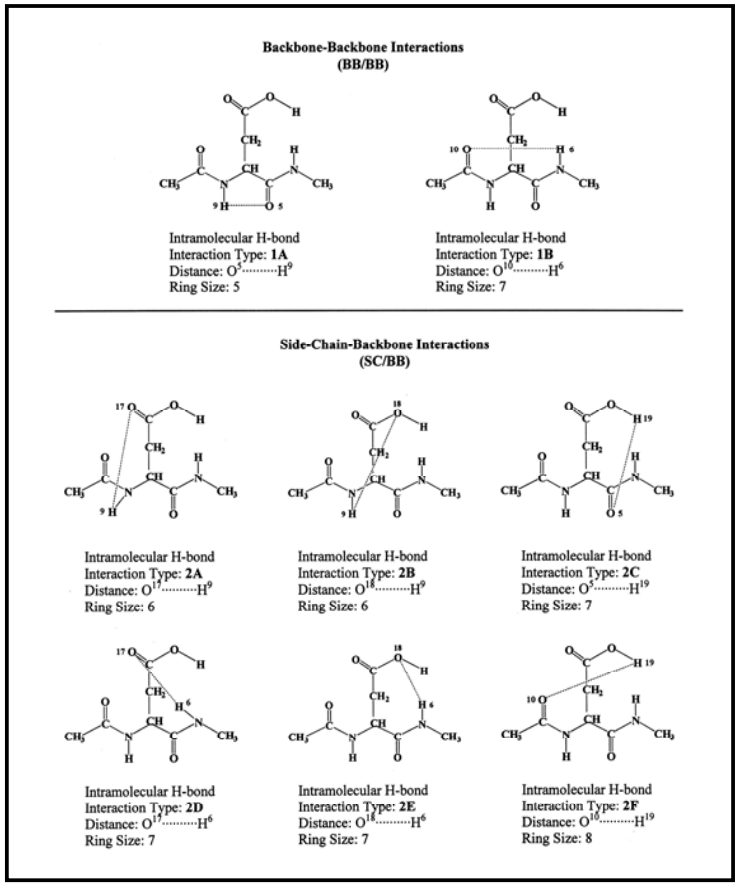
$$E = E(\phi, \psi, \chi_1, \chi_2) \qquad [1]$$





Thesis #9

I have determined the deprotonation patterns of both *endo* and *exo* forms of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide as well as the protonation patterns of **N**-acetyl-*L*-aspartate-**N'**-methylamide.



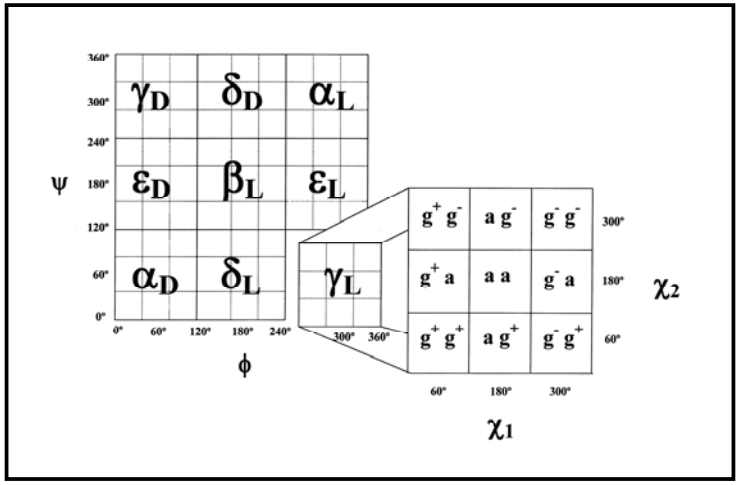
Thesis #8

I have determined that there is a total of two backbone-backbone (N-H·····O=C) and four backbone-sidechain (N-H·····$^{-}$O-C) hydrogen bond interactions for **N**-acetyl-*L*-aspartate-**N'**-methylamide. Each of the seven conformers found for the aspartate residue has at least one type of hydrogen bond interaction, suggesting that hydrogen bonding may be an important stabilizing force for the overall molecular geometry of the molecule. In addition, the aspartate residue's ability to form hydrogen bonds may be important during ligand binding or ligand recognition by a cell receptor.

In turn, the 4D PEHS can be separated into two distinctive 2D potential energy surfaces (PESs):

$$E = E(\phi, \psi) \quad [2]$$
$$E = E(\chi_1, \chi_2) \quad [3]$$

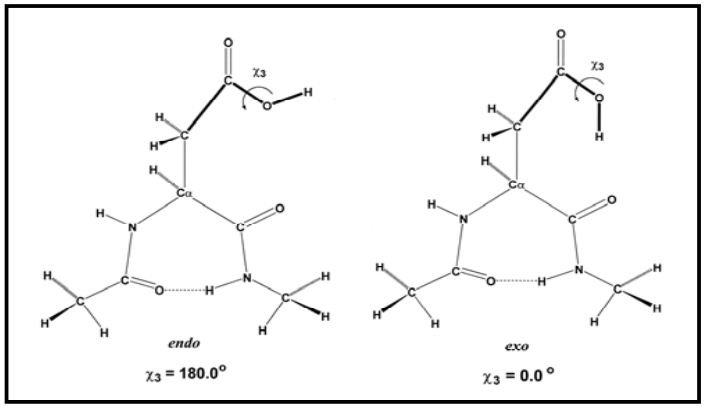
Here, equation [2] denotes the 2D PES for backbone torsional angles (Ramachandran surface) while equation [3] denotes the 2D PES for sidechain dihedral angles. Hence, for **N**-acetyl-*L*-aspartate-**N'**-methylamide, both backbone and sidechain torsional angles need to be discussed in relation to the overall PEHS so as to describe the molecular geometry of the aspartate residue.

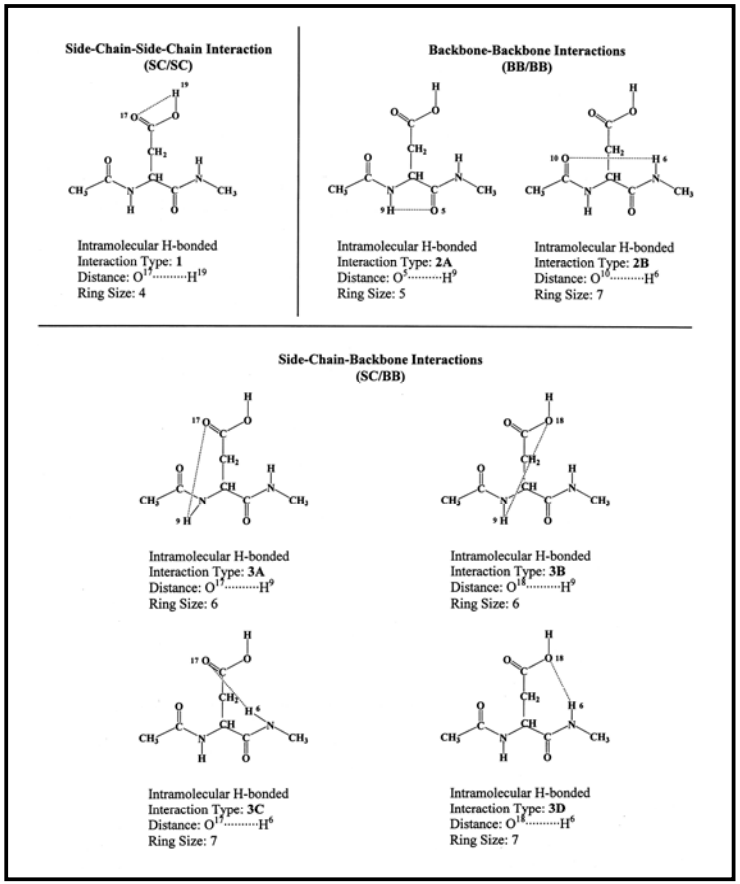


The sidechain of the aspartic acid residue can be modeled by propionic acid (CH_3-CH_2-COOH). When $\chi_3 = 180°$, the aspartic acid residue is capable of sidechain-sidechain (SC/SC) internal hydrogen bonding and it is considered to be in an *endo* orientation. When $\chi_3 = 0°$, the SC/SC interaction no longer exists and the aspartic acid sidechain is free to participate in external interactions, such as sidechain-backbone (SC/BB) hydrogen bonding. In this case, the aspartic acid residue is considered to be in *exo* orientation. Unlike aspartic acid, there are no *endo* or *exo* orientations in describing the sidechain characteristic of **N**-acetyl-*L*-aspartate-**N'**-methylamide. The aspartate anion sidechain can be modeled by propionate (CH_3-CH_2-COO^-) where the α-carbon on the aspartate is represented by CH_3.

Here, the carboxylate sidechain of aspartate may exhibit an asymmetric vibrational oscillation about its two C—O bonds, where one bond may be longer, shorter, or of equal length with respect to the other.

It is interesting to explore the protonation preference for the aspartate anion. Depending on where on the carboxylate sidechain it is protonated, an aspartate anion will transform into the *endo* or *exo* form of its aspartic acid sidechain counterpart. At the end, there could be two *endo* and two *exo* protonation location. Hence, we propose a protonation model for **N**-acetyl-*L*-aspartate-**N'**-methylamide where the H atom will protonate each conformer of the aspartate residue at a maximum of four locations. The two possible *endo* protonated forms differ from one another by a χ_2 rotation of 180°. Likewise, the two possible *exo* protonated forms differ from one another also by a χ_2 rotation of 180°. The geometric preference of the aspartate residue will be compared against those of its protonated forms, the *endo* and *exo* conformers of aspartic acid.



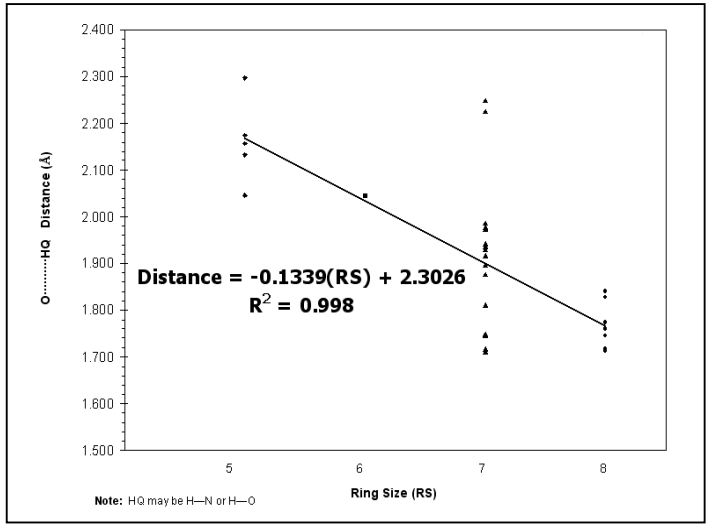


Thesis #7

I have determined that there is a total of two backbone-backbone (N-H·····O=C) and five sidechain-backbone (N-H·····O=C; N-H·····OH) hydrogen interactions found for the *exo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide at the B3LYP/6-31G(d) level of theory. Of the possible six sidechain-backbone hydrogen interactions, only five existed for *exo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide.

Thesis #5

I have determined that a correlating trend exists between hydrogen bond distances and ring sizes of the *exo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide at the B3LYP/6-31G(d) level of theory; where the shorter the hydrogen bond distance, the larger the ring size. This correlation has an overall least square value of R^2 = 0.998 and the overall equation representing this trend is Distance = -0.1339 (RS) + 2.3026.

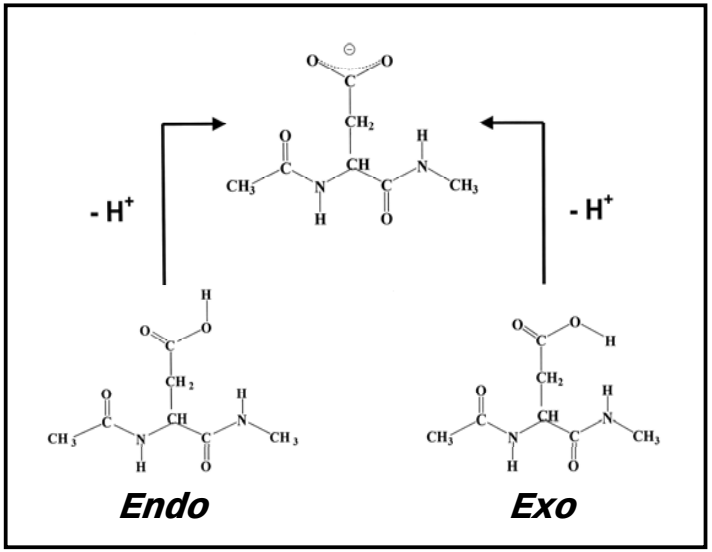


Thesis#6

I have determined that there exist a sidechain-sidechain hydrogen interaction (O-H·····O=C) in all stable conformers found for the *endo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide at the B3LYP/6-31G(d) level of theory. In addition, there is a total of two backbone-backbone (N-H·····O=C) and four sidechain-backbone (N-H·····O=C; N-H·····OH) hydrogen interactions found for the *endo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide.

3. AIM OF STUDY

In order to study the respective protonation or deprotonation patterns of the aspartate residue and the aspartic acid, **N**-acetyl-*L*-aspartate-**N'**-methylamide, along with its protonated forms, the *endo* and the *exo* forms of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide, need to be thoroughly examined.



Shown at the bottom of the diagram are the protonated *endo* and *exo* forms of the aspartic acid residue; shown at the top of the diagram is the deprotonated aspartate ion.

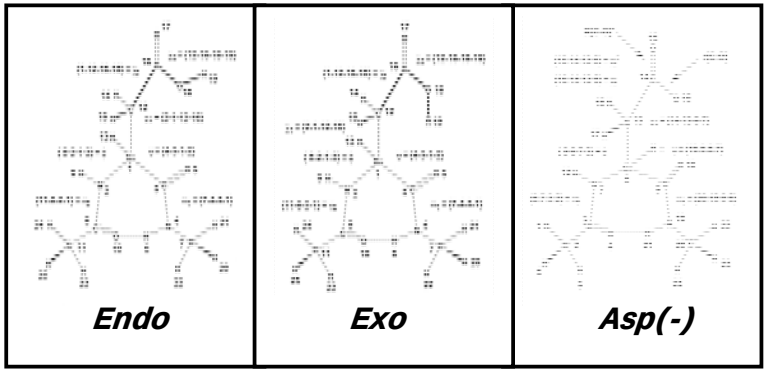
4. METHOD

Geometric optimizations were performed on all possible sidechain conformers of the *endo* and *exo* forms of aspartic acid residue as well as on the aspartate molecule. All computations were carried out using Gaussian 94 and Gaussian 98 program system. Every molecular structure was first computed at the RHF/3-21G level of theory. Subsequently, geometric optimizations were performed on converged structures at the RHF/6-31G(d) and B3LYP/6-31G(d) levels of theory.

THESES

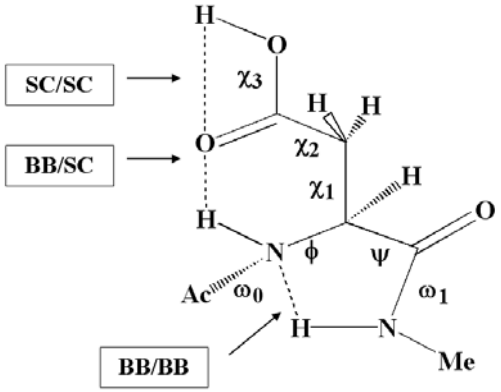
Thesis #1

I have determined that, instead of the 81 expected structures, only 37 stable conformers existed in the *endo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide and only 27 stable conformers existed in the *exo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide at the B3LYP/6-31G(d) level of theory. In contrast to that, only seven conformers were found instead of the expected 81 structures for **N**-acetyl-*L*-aspartate-**N'**-methylamide.



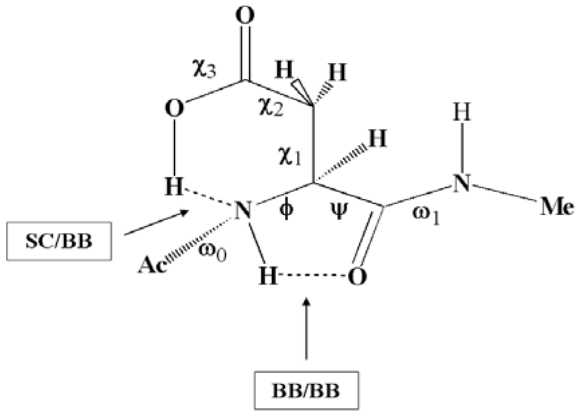
Thesis #2

I have determined that a [g^- s^-] conformer exists in the α_L backbone conformation for the *endo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide at the B3LYP/6-31G(d) level of theory, even though usually the α_L backbone conformation has no stability in the gas phase.



Thesis #3

I have determined that a [g^- g^+] conformer exists in the ε_L backbone conformation for the *exo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide at the B3LYP/6-31G(d) level of theory, even though usually the ε_L backbone conformation has no stability in the gas phase.



Thesis #4

I have determined that a correlating trend exists between hydrogen bond distances and ring sizes of the *endo* form of **N**-acetyl-*L*-aspartic acid-**N'**-methylamide at the B3LYP/6-31G(d) level of theory; where the shorter the hydrogen bond distance, the larger the ring size. This correlation has an overall least square value of R^2 = 0.8443 and the overall equation representing this trend is Distance = -0.00885 (RS) + 2.3634. The correlation for either the maximum distance or the minimum distance points has higher least square values.