Theses of the Ph.D. Dissertation

by

Jacqueline M. S. Law

Department of Chemistry and Chemical Informatics

Juhasz Gyula Teachers College

University of Szeged

Szeged, Hungary

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Abstract

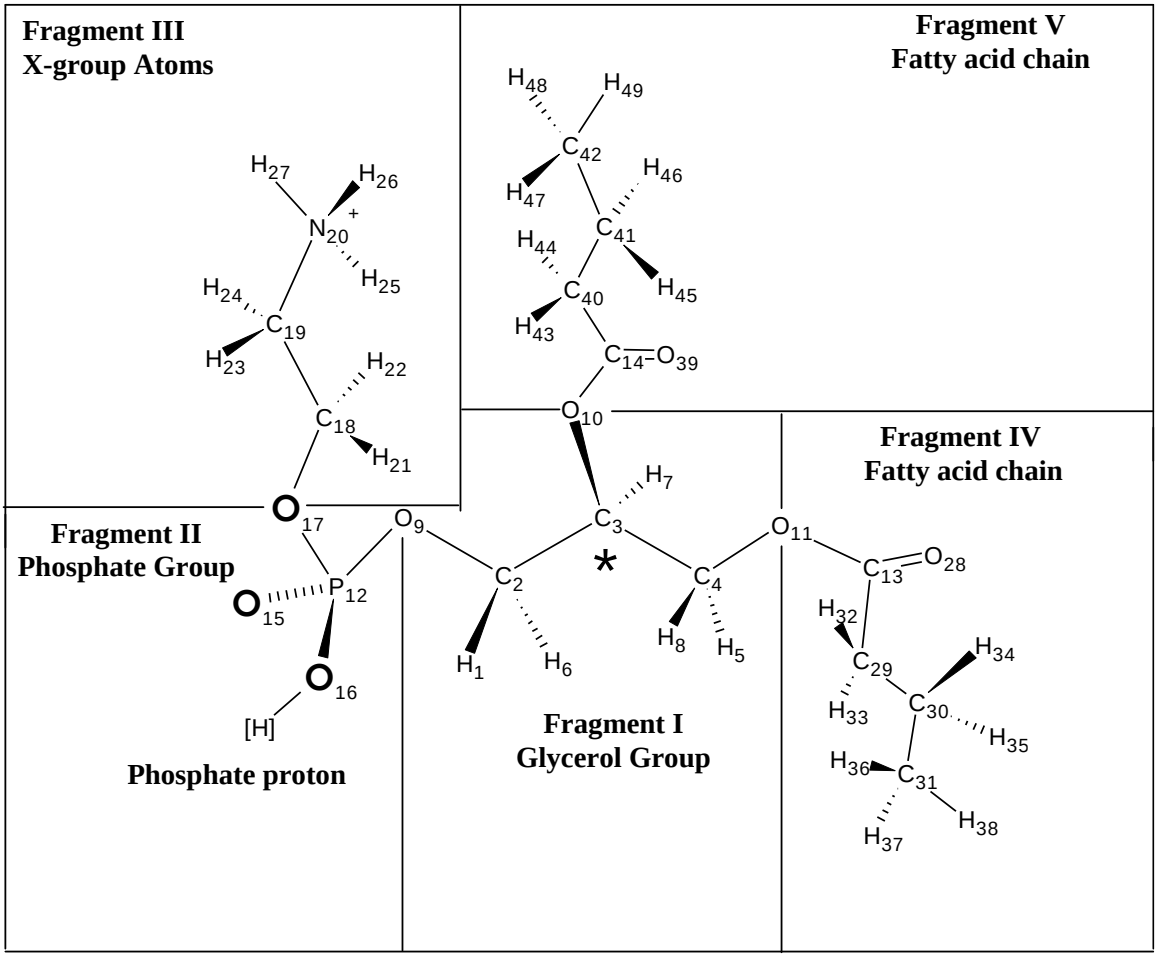
In general, the phsopholids were divided and different modular fragments and each fragment is numbered in a specific way. Such modular organization of the individual fragments would allow for the construction of a database in which different fragments can be assembled into larger models in a systematic manner. *Ab initio* geometric optimization has been carried out on the glycerol backbone at RHF/3-21G level of theory in attempt to search for a set of topologically probable conformations. Since glycerol is achiral, the energies of most conformations are paired. The study of the X-group is currently underway as a component of the full phospholipids. The fatty acid chain was analyzed in detail. Specifically, three ω-3 Polyunsaturated Fatty Acid (**PUFA**), namely **S**teari**D**onic **A**cid (**SDA**;18:4 n-3), **E**icosa**P**entaenoic **A**cid (**EPA** 20:5 n-3), and **D**ocosa**H**exenoic **A**cid (**DHA** 22:6 n-3) were chosen as the main focus of this study. Using the recurring allylic structure (hepta-2,5-diene) as the basic unit (**U**), several analogous structures with different number of units (**n**) were also constructed and computed using various methods. The *cis*- **1U PES** shows some similarity to that of the trans-MeCONH-CH_2-CONHMe. Further exhaustive search performed on the **1U** and a 2-unit structure (**2U**) yielded four straight chain structures (two *cis*- and two *trans*-) as well as 2 helical structures (one *cis*- and one *trans*-), subsequent optimizations of more complex structures and the selected **PUFA**s have also resulted in obtaining similar conformations.

Thermodynamic functions ($\Delta H, \Delta G, \Delta S$) were computed at the B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d) for the selected **PUFA**s and their analogous structures. The six conformations from the above study were analyzed here. The *trans*- to *cis*-isomerization energy is similar for all structures. This indicates that the conformers selected are consistent across all the structures. In addition, the average folding energy was found to be about 0.9 ± 0.2 kcal $mol^{-1}unit^{-1}$. However, amongst all structures, a significant difference was observed in the change of entropy of folding ($\Delta S_{folding}$), whereby the **PUFA** has the greatest rate of decrease with respect to the number of units or the degree of polymerization (**n**). Consequently, the Free Energy of Folding ($\Delta G_{\text{folding}}$) and **n** for the *cis*- and *trans*-isomers also display great variability where **PUFA** shows the highest $\frac{\Delta G_{folding}}{\Delta n}$. This suggests that the naturally occurring *cis*-isomer is less ready to fold than the *trans*-isomer since a greater degree of organization is exhibited by *cis*-isomer during the folding process.

Aim of Study

The phospholipid (**Figure 1**) is a member of the lipid family and have many roles in the biological system. Recent studies suggest that the integrity of the lipid bilayer depends on the packing between different phospholipids acyl chains, as well as the salt bridge formation between the polar head groups (X-groups) and the charged particles around them. In general, the phopsholipids can be separated into five parts: the glycerol backbone, the phosphate, the X-group and the two fatty acid chains. More importantly, it is the structural and conformational polymorphism of the fatty acid chains in phospholipids which affects the integrity of the membrane bilayer. Specific numbers are designated to specific fragments.

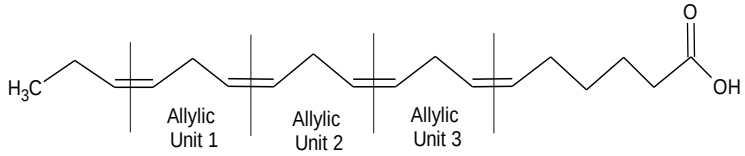
Figure 1 Phospholipid separated into 5 fragments and numbered in a specific order



Fatty acid chains come in various sizes and structures and they make up the non-polar portions of the phospholipid. A specific type of fatty acid, known as Polyunsaturated Fatty Acid (**PUFA**) contains multiple π-bonds. Stearidonic Acid (**SDA**), Eicosapentaenoic Acid (**EPA**) and Docosahexaenoic Acid (**DHA**) (**Figure**

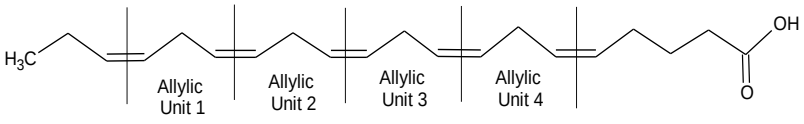
2) are ω-3 (or n-3) **PUFA**s contains multiple π-bonds forming a repeating pattern of double allylic structure (**n**).

Figure 2 Structures of SDA, EPA and DHA separated into repeating units



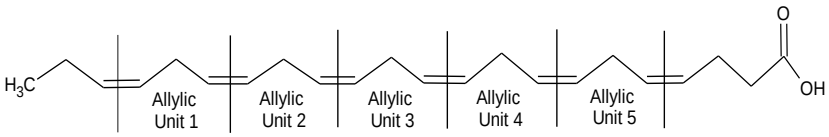
3 Double Allylic Units; n=3

SDA: Stearidonic Acid (18:4 n-3)



4 Double Allylic Units; n =4

EPA: Eicosapentaenoic Acid (20:5 n-3)

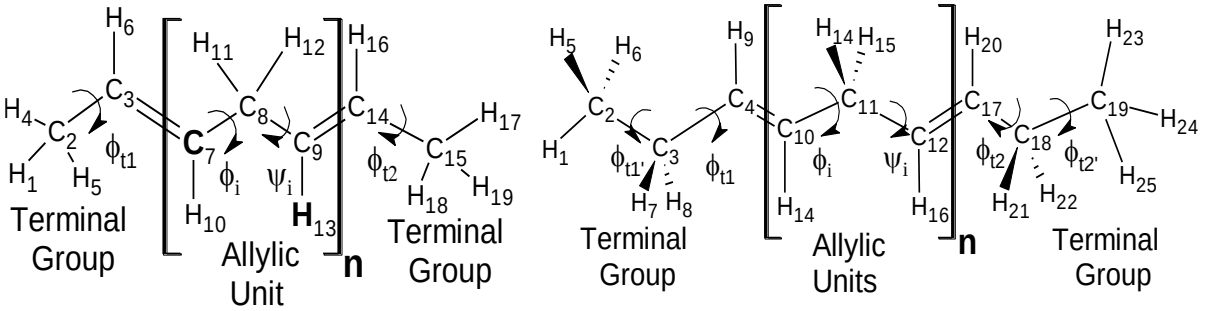


5 Double Allylic Units; n =5

DHA: Docosahexaenoic Acid (22:6 n-3)

In addition, each **PUFA** type also contains a different alkyl structure at the carboxyl end. Therefore, analogous models are designed with different structures for the ends. They are labeled **Model 0**, **Model 1** and **Model 2**. The focus of the study will be on the **PUFA**s and their analogous models. **Model 0** will be all structures with n = 1 to 6, but will contain methyl groups at the ends (**Figure 3**). In **Model 1**, n = 3, 4 and 5 while both terminal groups will be extended into ethyl fragments (**Figure 3**).

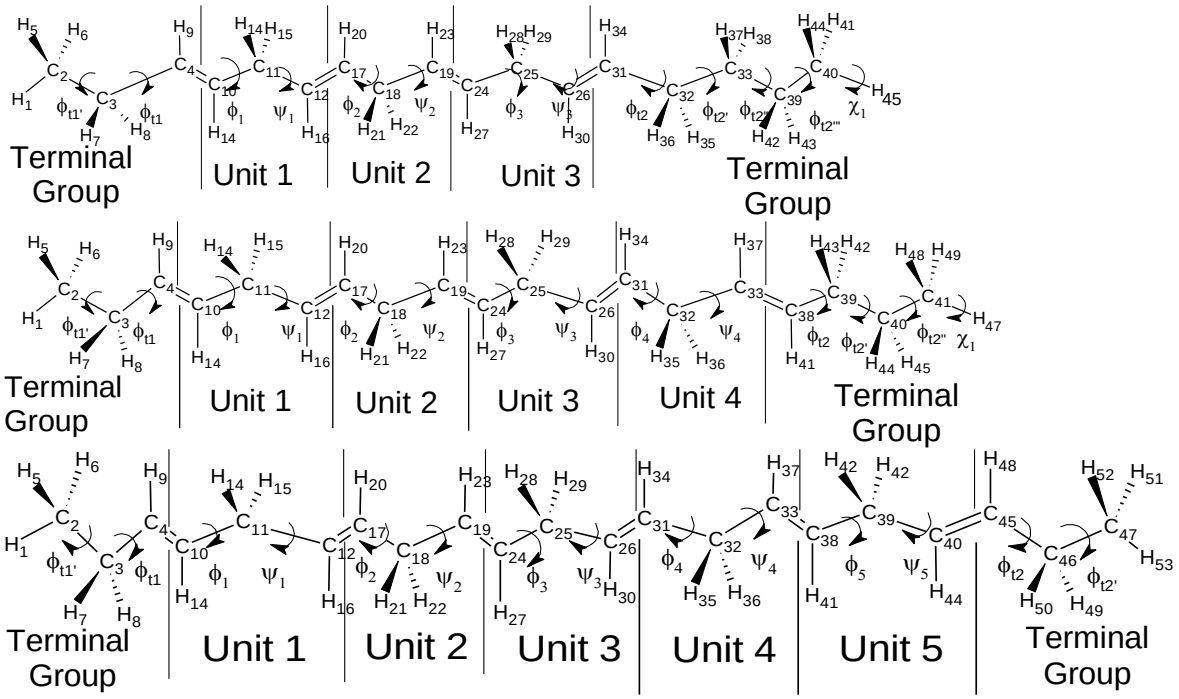
Figure 3 Numbering and definition for Model 0 (Left) and Model 1 (Right)



Model 2 is slightly more complicated because the end groups mimic that of the full **PUFA** very closely with n = 3, 4 and 5 (**Figure 4**). The only difference between **Model 2** and the **PUFAs** is that the structures in **Model 2** do not have the carboxyl

group found in that is in **PUFA**s. The general structure for **Model 2** is similar to **Model 1**. However, H_{25} will be removed and replaced by alkyl groups of different sizes (R-group).

Figure 4 Numbering of Model 2 fragments where n = 3, 4 and 5



Only the all-*trans* structures are shown above, but the numbering of the atoms in the *cis*- isomers is identical to that of the *trans*-.

Method

Preliminary Study and **Model 0**

The first part of the study of **PUFA** consists of building **Model 0**. For initial analysis of the flexibility of the allylic systems, Potentian Energy Curves and Surfaces are generated from structures of **Model 0**, where n = 0, 1, respectiviely, by scaning the dihedrals shown in (**Figure 3**). They are then compared to MeCO-NHMe and MeCO-Gly-NHMe, respectively. Then exhaustive search was carried out at lower levels of computation on the structure **Model 0**: n = 2 (2U).

Study of Molecular flexibilities by conformational analysis of higher Models

Six structures from 2U are selected to build larger analogous models. To obtain such models, the dihedral values from each 2U allylic unit are first applied repeatedly until the chain reaches the desired degree of polymerization. Then the

proper end groups are added to complete the construction of **Model 0**, **Model 1**, **Model 2** and **PUFA**s. Therefore, the new models will contain identical 2U modules repeated various times, but they would differ in their structure at the carboxyl end. Computations at a higher level of theory (B3LYP/6-31G(d)) are carried out on these larger structures in all of the models in attempt to obtain the geometric structure as well as the frequency data. In addition, single point calculations were carried out on the optimized structures at B3LYP/6-311+G(2d, p) level of theory.

Thermodynamic Analysis for **PUFA**s and Analogous Models

Frequency calculations has also been performed for the **Model 0**, **Model 1**, **Model 2** and **PUFA** at B3LYP/6-31G(d) level of theory. The purpose of such calculation is to obtain thermodynamic data for the analysis of ΔH, ΔG and ΔS as a function of structure. In general, there are three molecular motions contributing to the thermal energy, enthalpy and entropy. The motions include translation, rotational and vibration.

Results

Thesis I Flexibility Study via Conformational Analysis

The simple structure of **Model 0** had to be studied first. A potential energy curve is generated from scaning **Model 0**: n = 0 (0U) (**Figure 5, Left**). Then the Potential energy curve is compared to that of the peptide bond (**Figure 5, Right**). From this study, it was found that the minima of ϕ_{t1} and ϕ_{t2} are located in the same areas as those of ϕ_{t2} of the peptide model (**Figure 6**). However, they were 60^{o} out of phase with the peptide ϕ_{t1} minima. Therefore, this implies that although the 0U model and the peptide model are structurally similar, they have marked conformational differences.

Figure 5 Left: Structure of Model 0: n=0 (0U); Right: Structure of MeCO-NHMe

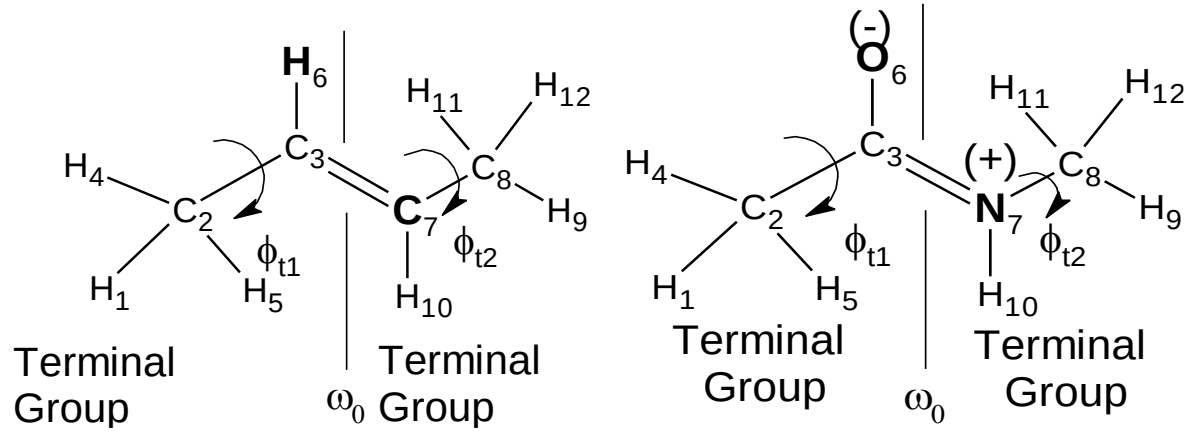
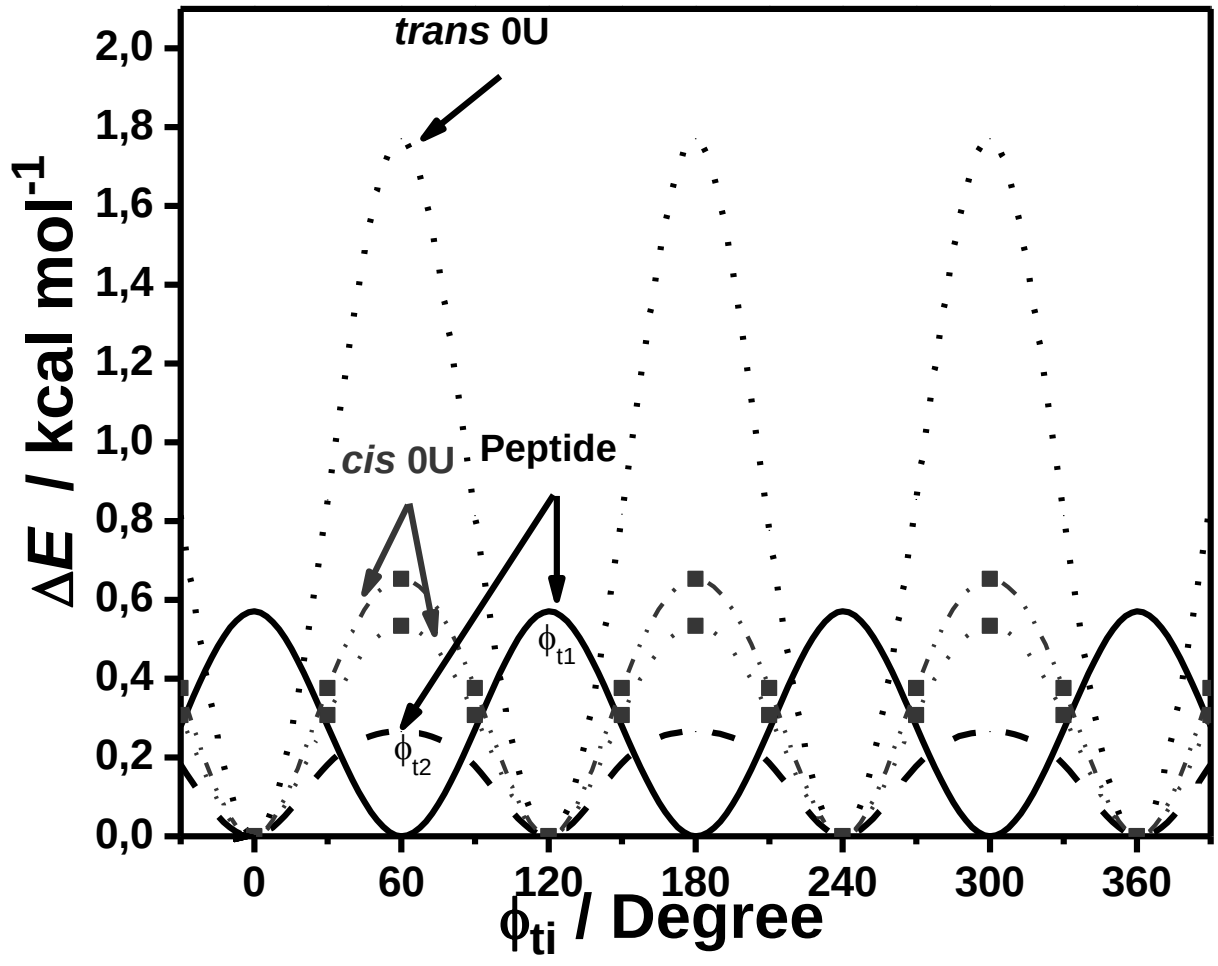


Figure 6 Potential Energy Curve of 0U and the peptide model in search of all minima

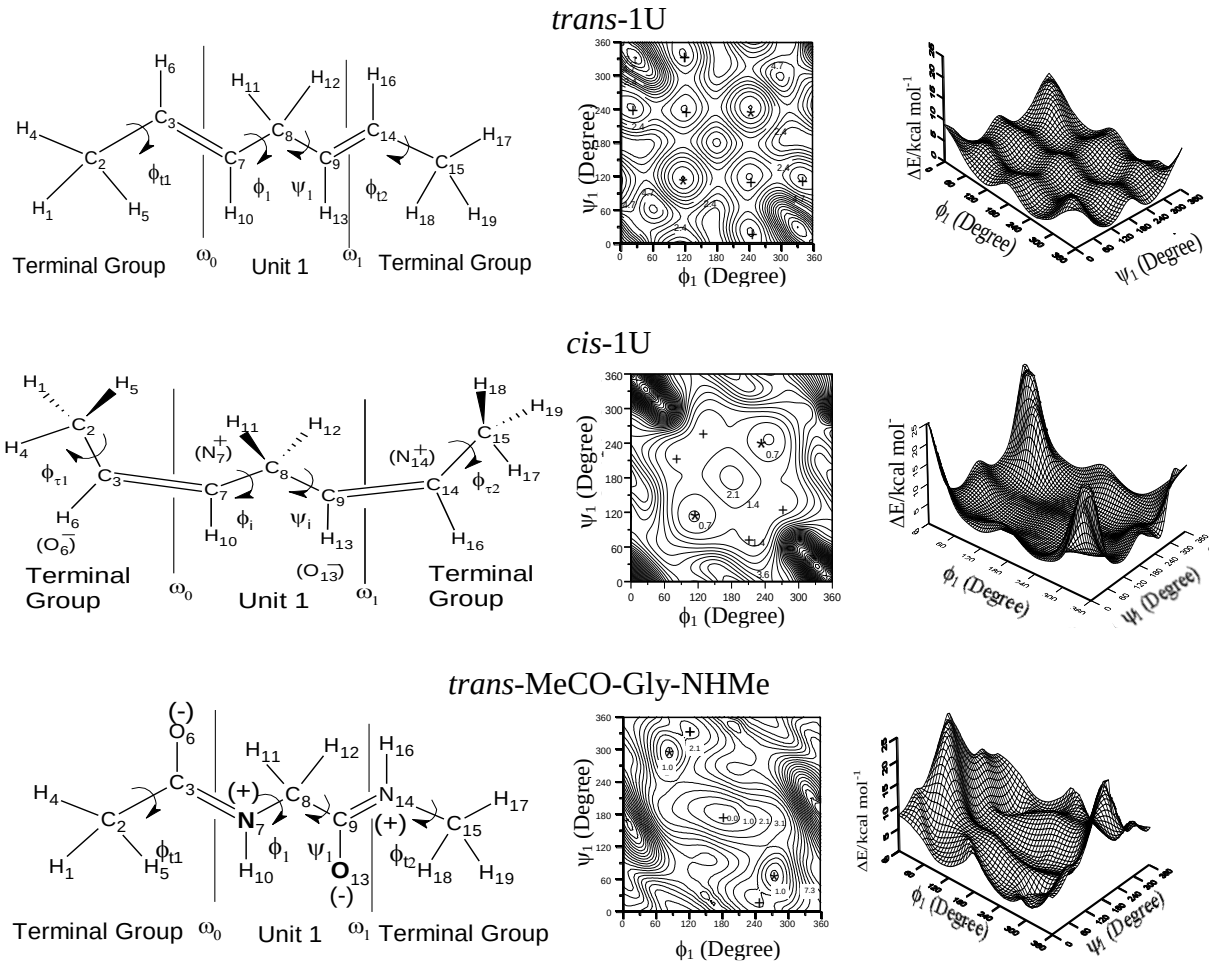


Thesis II Flexibility Analysis of 1U and MeCO-Gly-NHMe Using Potential Energy Surfaces

The Potential Energy Surfaces (**PES**s) of **Model 0**: n=1 (1U) has been studied. The **PES**s of *cis*- and *trans*- 1U are generated by scanning the ϕ and ψ dihedrals of 1U. While the **PES** for *trans*- 1U looks quite regular (**Figure 7**, **Top**), a unique **PES**s

emerged for the *cis*- 1U (**Figure 7**, **Middle**). It can be seen that in the center of the latter **PES**, there is a very flat surface. Furthermore, comparison of the *cis* -1U **PES**s to that of *trans*-MeCO-Gly-NHMe (**Figure 7**, **Bottom**) shows that they are somewhat similar. A plausible explanation for this observation may be that the barrier seen in the peptide model is the results of hydrogen bonding within the model. However, in the *cis*- 1U model, such interactions are impossible and hence this yields a **PES** with relatively low barriers. In general, it can be concluded that the *cis*- 1U is very flexible and, therefore, is able to form various structures in a short period of time.

Figure 7 PES of *cis*- and *trans*- 1U and *trans*-MeCO-Gly-NHMe

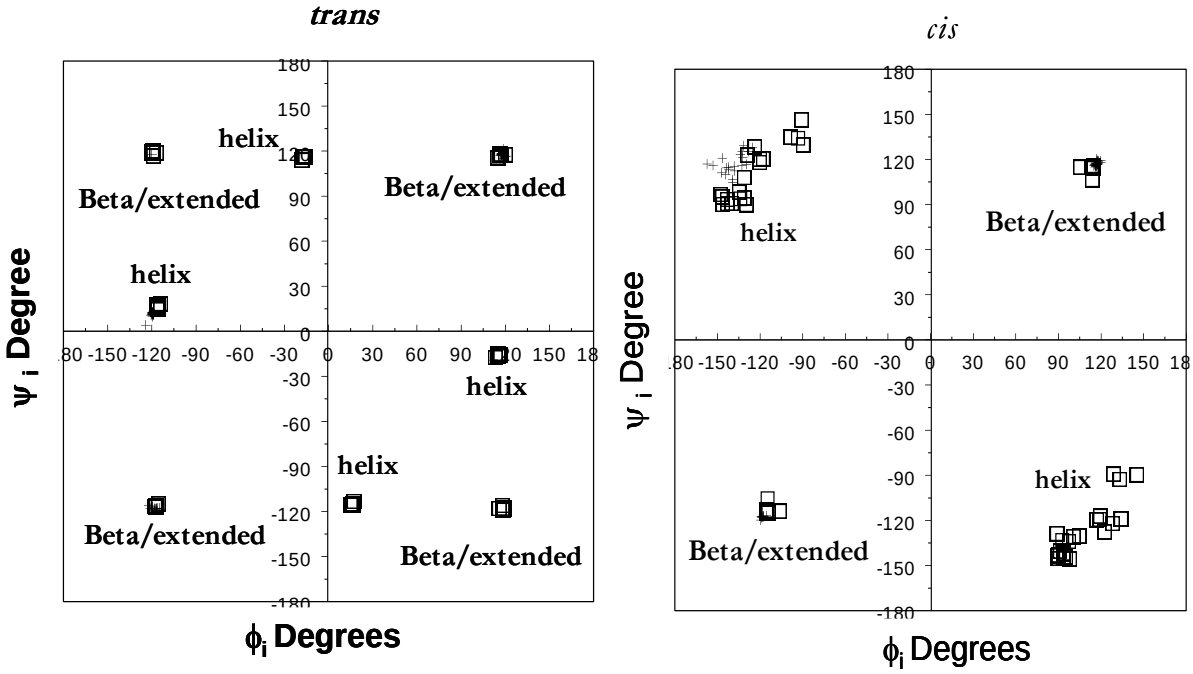


Thesis III Nanostructural Feature of **PUFA** and Hydrocarbon Models

Fully relaxed optimizations were carried out on all models. The values of the dihedrals from the selected structures were plotted on a Ramachandran type map. The location of *trans*- dihedrals can be found at one of the following values 120^0 (+), -120^0 (-) and 15^0 (S^+) and -15^0(S^-). Therefore, in each unit, the two

dihedrals may have the combination of ++; +-, -+, --, $+S^-$, $-S^+$, S^+-, and S^-+ configuration. For *cis*- structures the dihedral minima are found at 120^0 (+), -120^0 (-) and between 130^0 (a^+) and $+130^0$ (a^-). Therefore, possible combination of values within one unit may be of ++ and a^-+ and a^+-. From 2U, 6 conformations, 3 from *trans*- and 3 from *cis*- isomers, with the following conformations (quoted in order of $\phi_1\ \psi_1\ \phi_2\ \psi_2$): ++-- (*trans- beta*), ++++ (*trans- extended*), $-\ S^+-\ S^+$ (*trans- helix*), ++--(*cis- beta*), ++++ (*cis- exte*nded) and a–+a–+ (cis- helix) are especially interesting. Therefore, these pairs of units are used to extend or constructure from 3U to 6U. All conformations found from 1U to 6U are summarized below (**Figure 8**).

Figure 8 Exhaustive search for all conformations in *cis*- (left) and *trans*- (right) 2U



Thesis IV Nanostructural Analysis

Geometries of the planes of the double bonds (**Table 1**) were also analyzed for all models. Both the *extended* and *beta* structures (*cis*- and *trans*-) are rotated about 90 ± 5^0 degrees between adjacent double bond planes. *trans- helices* are rotated 104 ± 1^0 between adjacent bonds. However, a large variation is seen in the *cis-helix* bond rotations (standard deviation of ±21). These observations are consistent with the **PES**s in **Figure 9**. The data are summarized in **Table 1.**

Table 1 Values for the plane rotation of the SDA, EPA and DHA chain

Structures	Average Angle of Plane Rotation (Degrees)		Standard Deviation (σ/degree)
cis – **SDA**	Helix	25.17	20.51
	Extended	-93.05	0.07
	Beta	92.33	0.76
trans – **SDA**	Helix	-104.90	0.97
	Extended	93.40	0.34
	Beta	93.23	0.54
cis – **EPA**	Helix	15.48	10.17
	Extended	-94.20	0.26
	Beta	92.11	1.33
trans – **EPA**	Helix	-104.66	0.76
	Extended	92.58	0.34
	Beta	93.02	0.39
cis – **DHA**	Helix	30.77	14.81
	Extended	-94.12	0.44
	Beta	92.76	0.46
trans – **DHA**	Helix	-105.32	0.63
	Extended	93.36	0.25
	Beta	93.00	0.57

Thesis V Thermodynamic Function (ΔH and ΔG) for *trans*-→*cis*- isomers

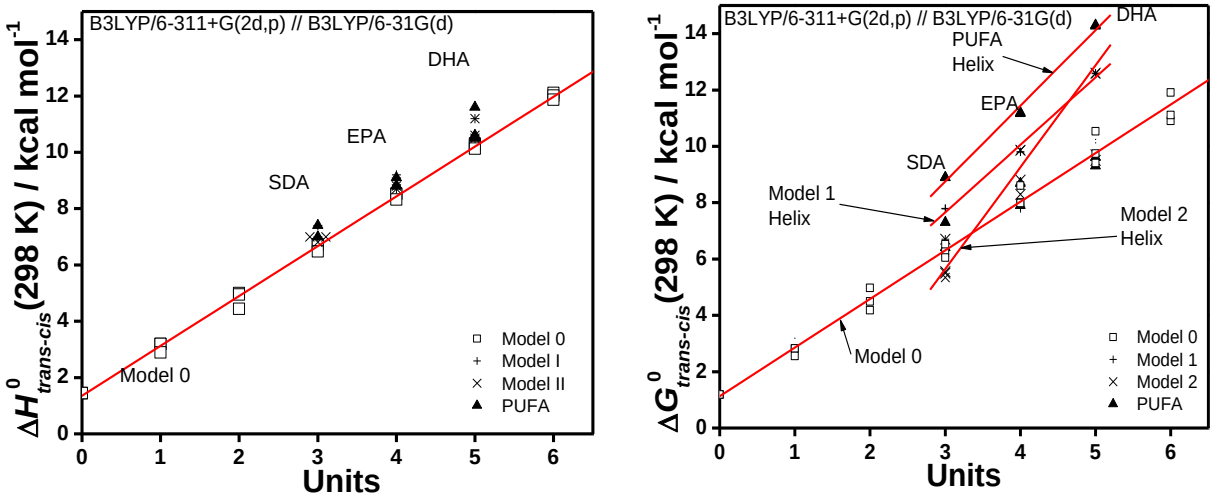
In the analysis of the *trans*- to *cis*- isomerization had thermodynamic functions (ΔH and ΔG) which varied linearly with increasing number of allylic units in the chain. It seems that both ΔH and ΔG had positive slopes (**Table 2** and **Figure 9**). The ΔH slopes of all the models are the same as other. However, for ΔG, **Model 2** and **PUFA** has a significantly larger slope than the other models. Furthermore, the line for **Model 1** and **Model 2** intersects with each other.

Table 2 Thermodynamic functions (*ΔH* and *ΔG*) for the *trans-→cis-* isomerization of all models

Process	Thermodynamic Function	Model	m	b	R^2
trans-→cis- Isomerization for Helices	*ΔH*	0	**1.8**	**1.4**	**1.00**
		1	**1.8**	**1.4**	**1.00**
		2	**1.8**	**1.4**	**1.00**
		PUFA	**1.8**	**1.4**	**1.00**
	ΔG	0	**1.7**	**1.1**	**0.99**
		1	**1.9**	**1.2**	**0.98**
		2	**3.6**	**-5.2**	**0.99**
		PUFA	**2.7**	**0.7**	**0.99**

Assessment of **Table 2** and **Figure 9** shows that the *ΔG* for **PUFA** has a higher slope than **Model 0**, but a lower slope than **Model 2**.

Figure 9 Thermodynamic functions (*ΔH* and *ΔG*) for *trans-→cis-* isomerization of all models (0, 1, 2 and PUFA) and all conformers (*Helix*, *Extended*, *Beta*)



It should also be noted that *extended* and *beta* conformers of **Model 1** and **2**, as well as **PUFA**s fall more of less on the line of **Model 0**.

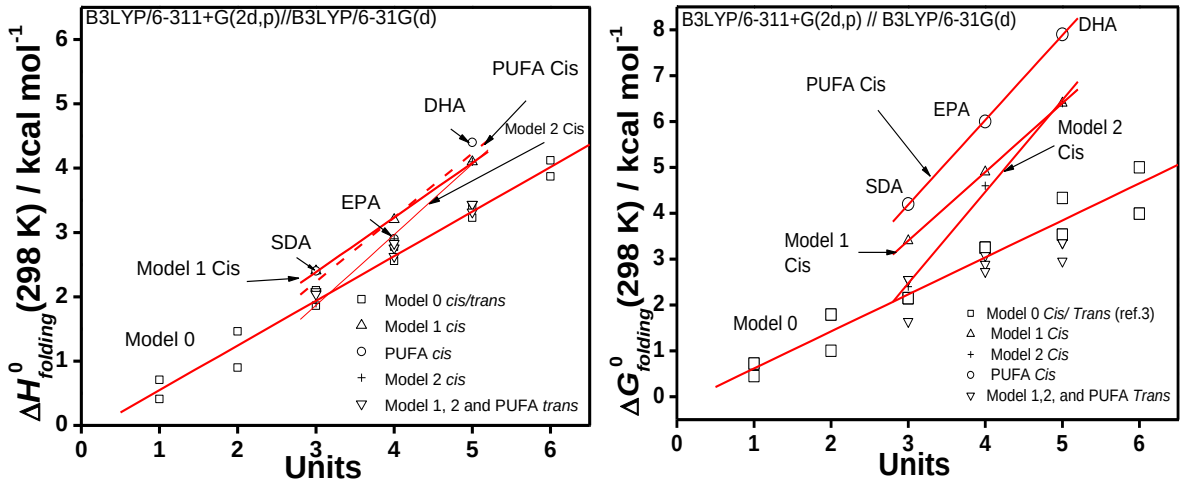
Thesis VI Thermodynamic Functions (*ΔH* and *ΔG*) for *beta→helix* folding

The *beta→helix* folding, *ΔH* and *ΔG*, also display a similar positive trend with respect to the increase of degree of polymerization (**Table 3** and **Figure 10**). Across all models, the rate of increase in *ΔH* and *ΔG* is relatively close to each other. However, the slope is smaller than the *trans-→cis-* isomerization.

Table 3 Thermodynamic functions (*ΔH* and *ΔG*) for the *trans-→cis-* isomerization of all models

Process	Thermodynamic Function	Model	m	b	R^2
beta→helix folding for *cis-*	*ΔH*	0[a]	**0.7**	**-0.1**	**0.98**
		1	**0.9**	**-0.2**	**1.00**
		2	**1.1**	**-1.4**	**0.99**
		PUFA	**1.0**	**-0.8**	**0.92**
	ΔG	0[a]	**0.8**	**-0.2**	**0.94**
		1	**1.5**	**-1.1**	**1.00**
		2	**2.0**	**-3.5**	**0.99**
		PUFA	**1.9**	**-1.4**	**1.00**

Figure 10 Thermodynamic functions (*ΔH* and *ΔG*) for *beta→helix* folding of all models



It should also be noted that *trans-* isomers of **Model 1** and **2**, as well as **PUFA**s fall more of less on the line of **Model 0**.

Thesis VII Entropy Variation for *trans-→cis-* isomerization and *beta→helix* folding

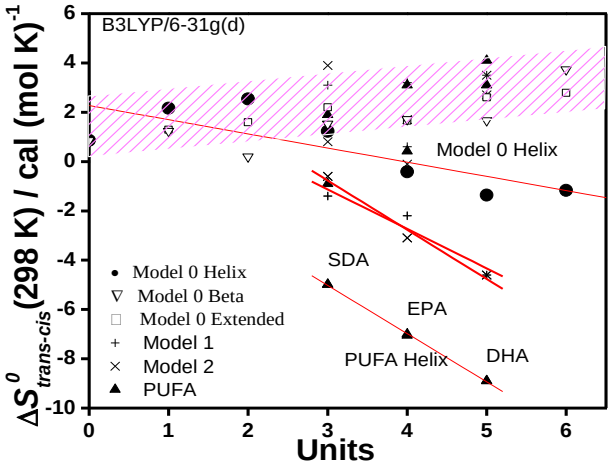
The entropy (*ΔS*) for *trans-→cis-* isomerization (**Figure 11 Left** and **Table 4**, **Top**) and *beta→helix* folding (**Figure 11**, Right and **Table 4**, Bottom) display a very different trend. All the models have different negative slopes. In the *trans-→cis-* isomerization reaction, only the *helices* display a clear linear relationship with respect to the degree of polymerization. The *extended* and *beta* do not clearly show such a relationship. **Model 0** seem to have the smallest rate of decrease while **Model 1** and **Model 2** have about the same rate. The **PUFA**s seem to display the most drastic lowering of entropy with respect to the degree of polymerization. The *ΔS* of folding with respect to the degree of polymerization displays a similar trend

to that of the *cis*- isomer. However, the *trans*-isomer displays a rather invariant relationship with the degree of polymerization. Once again, **Model 0** shows the smallest decrease while the **PUFA**s have the largest. **Model 1** and **Model 2** have the same rate of decrease.

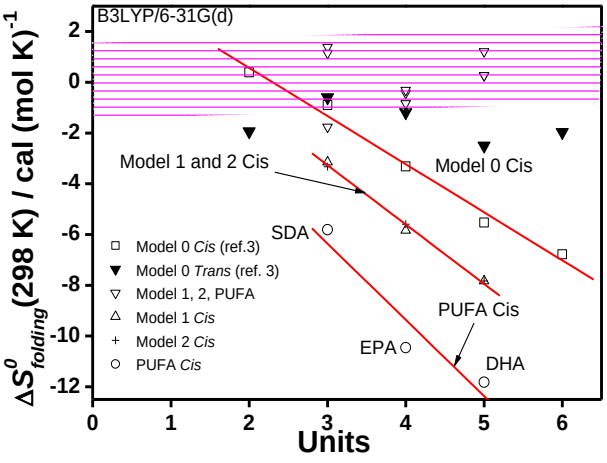
Table 4 Entropy Change (ΔS) for *trans*-→*cis*- isomerization and *beta*→*helix* folding of all models

Process	Thermodynamic Function	Model	m	b	R^2
trans-→*cis*- Isomerization for Helices	ΔS	0[a]	**-0.6**	**2.3**	**0.99**
		1	**-1.6**	**3.6**	**0.95**
		2	**-2**	**5.2**	**0.95**
		PUFA	**-2.0**	**0.8**	**1.00**
beta→*helix* Folding for *cis*- isomers	ΔS	0	**-1.9**	**4.4**	**0.99**
		1	**-2.3**	**3.8**	**0.99**
		2	**-2.3**	**3.8**	**0.99**
		PUFA	**-3.0**	**2.7**	**0.91**

Figure 11 Entropy Change (*ΔS*) for *trans*-→*cis*- isomerization (left) *beta*→*helix* folding (right) of all models



Shaded area contains the *extended* and *beta* conformers



Shaded area contains the *trans*- isomers

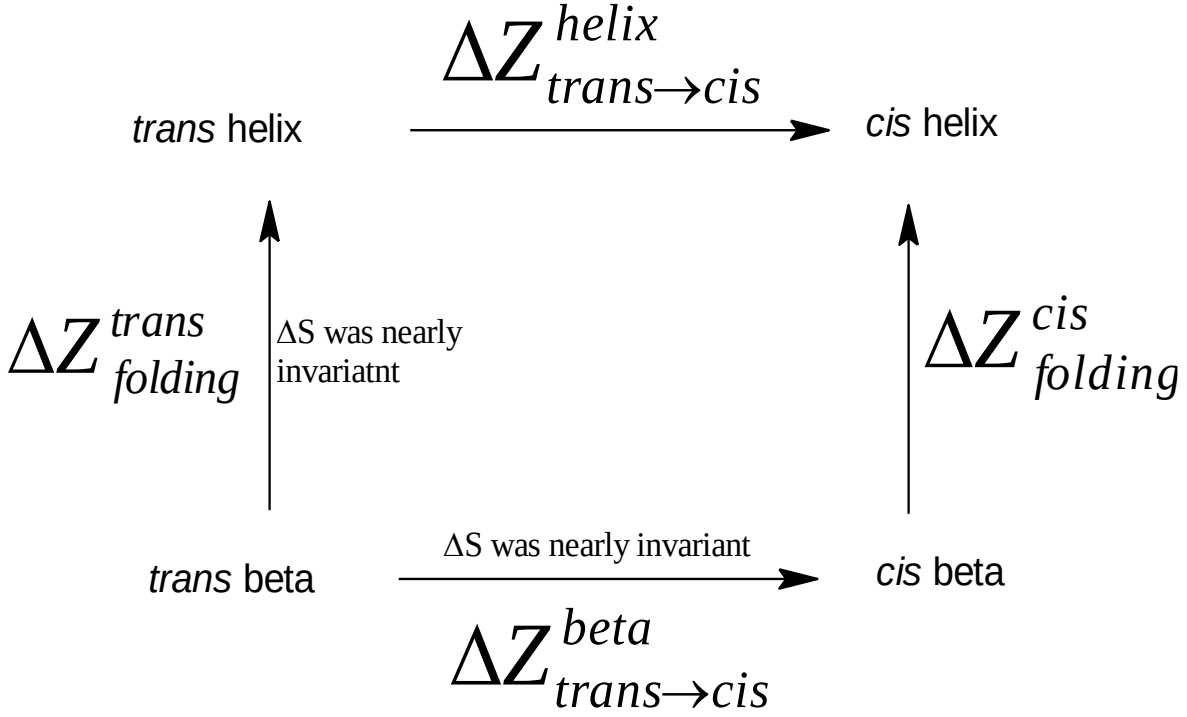
Conclusion

After some exploratory study of the various components of phospholipids, most of the work was focused on the conformational intricacies of **PUFA**s and their hydrocarbon models. The selected *cis*- and *trans*- nanostructures, all of which belong to one of the three types: *extended*, *beta* and *helix*, were studied and the flexibilities of these chains were established. It was concluded from the comparison of conformational potential energy surfaces that the *cis*- isomers of

these polyunsaturated compounds have similar flexibilities than peptide chains do.

Considering the changes in the thermodynamic functions Z (where Z may be *H*, *G* and *S*) for the *trans*- to *cis*- isomerization as well as for the *beta* to *helix* folding, thermochemical cycles may be constructed as shown in (**Figure 12**).

Figure 12 Born-Haber type cycle for configurational and conformational changes of PUFA as well as their hydrocarbon models; ΔZ may be ΔH, ΔG, ΔS



Interestingly enough, the *trans*- to *cis*- isomerization as well as *beta* to *helix* folding had thermodynamic functions, *ΔH* and *ΔG*, that varied linearly with increasing number of allylic units in the chain. This was the case not only for **PUFA**s but for their hydrocarbon models as well. In contrast to that the *ΔS* functions for *trans*- to *cis*- isomerization were almost invariant with respect to the degrees of polymerization for the *extended* and *beta* conformers. Also for the *beta* to *helix* folding the *ΔS* functions were virtually invariant for the *trans*- isomers. For all others the entropy lowering with increasing degree of polymerization was linear for **PUFA**s as well as for their hydrocarbon models.

Acknowledgment

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The Dissertation is based on the following Published Papers

1. **Jacqueline M.S. Law**, Gregory. A. Chass, Ladislau. L. Torday, Andras. Varro, Julius. Gy. Papp, Molecular Computation on Lipids: A numbering system for phospholipids and triglycerides. *J. Mol. Struct.* (THEOCHEM) **619 (2002) 1-20**
   IF : 1.014

2. **Jacqueline M. S. Law**, Joseph. C. P. Koo, Gregory. A. Chass, Imre. G. Csizmadia Molecular Orbital Computations on Lipids: Modular Numbering. *J. Mol. Struct.* (THEOCHEM) **666-667 (2003) 445-449**
   IF : 1.014

3. **Jacqueline M. S. Law**, David. H. Setiadi, Gregory. A. Chass, Imre G. Csizmadia, and B. Viskolcz, Flexibility of "Polyunsaturated Fatty Acid Chains" and Peptide Backbones: A Comparative ab Initio Study *J. Phys. Chem. A.* **109 (2005) 520-533**
   IF : 2.792

4. **Jacqueline M. S. Law** Gregory A. Chass, Andras Varro, and Julius Gy. Papp Molecular Orbital Computations on LipidsAn *ab initio* Exploratory Study on the Conformations of Glycerol and its Fluorine Congeners. THEOCHEM **722 (2005) 79-96**
   IF : 1.014

5. **Jacqueline M. S. Law**, Milan Szori, Botond Penke, Imre G. Csizmadia, Bela Viskolcz Folded and Unfolded Conformations of ω-3 Polyunsaturated FA Family: CH_3–CH_2-[CH=CH–CH_2–]$_N$–[CH_2]$_M$-COOH *J. Phys. Chem. A.* **110 (2006) 6100-6111**
   IF: 2.792

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Cumulative IF: 8.626
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Other Papers

Gregory. A. Chass, Michelle A. Sahai, **Jacqueline. M. S. Law**, Sandor Lovas, Ödon. Farkas, Andras Perczel and Imre. G. Csizmadia Toward a Computed Peptide Structure Database: The role of a Universal Atomic Numbering System of Amino Acids in Peptides and Internal Hierarchy of Database. *Int. J Quantum Chem*. **90 (2002) 933-968**

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