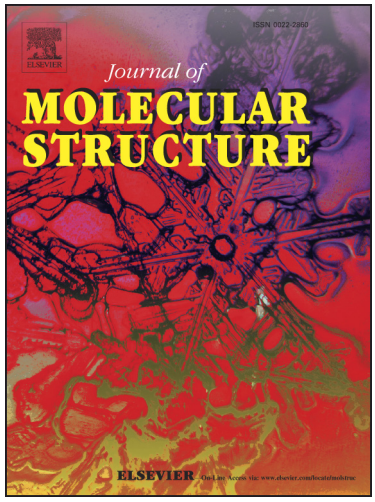



# $^{1}$H NMR and theoretical studies on the conformational equilibrium of tryptophan methyl ester

Claudimar J. Duarte, Rodrigo A. Cormanich, Lucas C. Ducati, Roberto Rittner

PII: S0022-2860(13)00629-7
DOI: http://dx.doi.org/10.1016/j.molstruc.2013.07.024
Reference: MOLSTR 19899

To appear in: *Journal of Molecular Structure*

Received Date: 13 June 2013
Revised Date: 15 July 2013
Accepted Date: 15 July 2013

Please cite this article as: C.J. Duarte, R.A. Cormanich, L.C. Ducati, R. Rittner, $^{1}$H NMR and theoretical studies on the conformational equilibrium of tryptophan methyl ester, *Journal of Molecular Structure* (2013), doi: http://dx.doi.org/10.1016/j.molstruc.2013.07.024

Manuscript submitted for publication in:

**Journal of Molecular Structure**

**Title:** $^{1}$H NMR and theoretical studies on the conformational equilibrium of tryptophan methyl ester

**Contents:** 15 pages, 3 tables and 3 figures

Supporting Information : 2 tables and 4 figures

Correspondence to:

Professor Roberto Rittner
Instituto de Química – UNICAMP
Caixa Postal 6154
13083-970 – CAMPINAS - S.P.
BRAZIL
*E-mail*: rittner@iqm.unicamp.br
fax: +55-19-3521-3023
telephone/fax: +55-19-3521-3150

# $^{1}$H NMR and theoretical studies on the conformational equilibrium of tryptophan methyl ester

Claudimar J. Duarte,[a] Rodrigo A. Cormanich,[a] Lucas C. Ducati[b] and Roberto Rittner[a,*]

[a]Chemistry Institute, State University of Campinas, P.O. Box 6154, 13083-970, Campinas, SP, Brazil
[b]Institute of Chemistry, University of São Paulo, P.O. Box 26077, 05513-970 São Paulo, SP, Brazil

* E-mail: rittner@iqm.unicamp.br

**Abstract**

Selected $^{3}J_{HH}$ coupling constants and theoretical calculations were used to explain the conformational equilibrium of *L*-tryptophan methyl ester (Trp-OMe) in several solvents. The obtained $^{3}J_{H\alpha H\beta}$ values did not exhibit any significant variability and thus indicate that there are no conformational population variations for the side chain of the Trp-O-Me depending on the solvent. Moreover, the potential energy surfaces obtained at the B3LYP/cc-pVDZ theoretical level produced eight energy minima that were analysed by QTAIM and NBO methods. It was possible to conclude that the Trp-OMe conformational preferences were due to hyperconjugative effects involving the nonbonding electron pairs of the main chain nitrogen atom and certain antibonding orbitals ($\sigma^{*}_{C4-C13}$, $\sigma^{*}_{C1-C4}$and $\sigma^{*}_{C4-H12}$) and also to the steric effects from the nonbonding electron pairs of oxygen atoms and the main and side chain of this system.

Keywords: tryptophan methyl ester, conformational analysis, hydrogen bonding, stereoeletronic interactions.

## 1. Introduction

Amino acids play an important role in nature as the building blocks of proteins [1]. Indeed, the conformational analysis of amino acids has gained special attention with an increasing number of experimental and theoretical studies reported in the literature [2-16]. Amino acids in neutral form ($NH_2$-CHR-COOH; R = amino acid side chain) predominate in the gas phase, while ionic or zwitterionic forms ($^+NH_3$-CHR-$COO^-$) are observed in aqueous solution and crystal structures. However, amino acids are thermally unstable solids with high melting points and low vapour pressures [7]. Despite these characteristics of amino acids, which generate many gas-phase experimental-based approach difficulties, these studies have gained increasing popularity in the literature, while the conformational analysis of these compounds in water and organic solvents is rare, due to either the zwitterionic form or low solubility. Moreover, amino acid zwitterionic structures are distinct from the neutral ones present in polypeptide chains. Therefore, most studies are performed in the gas phase using both experimental [4,7,17-20] and theoretical [1, 9,21-26] approaches. An alternative method to avoid these difficulties, as recently reported for the valine methyl ester [27], is to use amino acid derivatives obtained by esterification or acetylation [28,29].

Tryptophan is an important serotonin [30] precursor neurotransmitter, whose conformational analysis has been extensively studied in the literature [2, 17, 18, 23, 26, 31-36]. In general, the conformational preferences of tryptophan and other amino acids have been attributed to intramolecular hydrogen bonding (HB) between the carboxylic acid (COOH) and the amino ($NH_2$) functional groups, while classic and quantum effects have been ignored. However, in recent publications, it was shown that the ubiquitous steric and hyperconjugative interactions, and not HB, are the forces responsible for glycine [37], alanine [38], sarcosine and *N*,*N*-dimethylglycine [39] and valine methyl ester [27] conformational preferences.

In the present study, we performed the conformational analysis of *L*-tryptophan methyl ester (Trp-OMe) (Fig.1), a compound that is soluble in certain organic solvents and that does not present the zwitterionic structure in this media, using $^{1}$H NMR spin-spin coupling constants ($^{3}J_{HH}$) together with DFT theoretical calculations, the quantum theory atoms in molecules (QTAIM) [40-44] and natural bond orbital (NBO) [45] methods.

## 2 Experimental

### *2.1 Synthesis*

Trp-OMe was prepared by the deprotonation of *L*-tryptophan methyl ester hydrochloride (from Sigma-Aldrich Company) in THF (tetrahydrofuran) using zinc powder according to a described procedure [46]. To a suspension of the Trp-OMe.HCl (4 mmol; 1.015 g) in 40 mL of THF, commercial zinc powder (400 mg) was added in one portion. The mixture was stirred for *ca.* 10 minutes. Subsequently, the mixture was filtered, and the solvent was evaporated. The Trp-OMe was obtained as a free ester crystalline solid (0.687 g; 79.0 % yield). Experimental monoisotopic molecular ion $M^{+}$ = 218.1055; calcd. for $C_{12}H_{14}N_2O_2$ $M^{+}$ = 218.10553.

### *2.2 NMR Experiments*

The solvents (acetonitrile-$d_3$, methanol-$d_4$, pyridine-$d_5$ and DMSO-$d_6$) were commercially available and used without further purification. $^{1}$H NMR spectra were recorded on a Bruker Avance III spectrometer equipped with a 5-mm probe operating at 600 MHz for $^{1}$H (Fig. 2). The internal reference used was TMS. Typical conditions for $^{1}$H spectra were as follows: eight transients, spectral width 5102Hz with 48076 data

points, giving an acquisition time of 4.71 s. The resolution of spectra obtained by these parameters is 0.1 Hz.

*2.3 Theoretical calculations*

Potential energy surfaces (PESs) for the Trp-OMe compound were built at the B3LYP/cc-pVDZ theoretical level by scanning the $\chi_1$ [N-Cα-Cβ-Cγ] torsion angle from 0º to 360º in steps of 10º, while the $\phi$ [LP-N-$C_\alpha$-C(O)] (LP represents the nonbonding electron pair of the nitrogen atom) and $\psi$ [N-$C_\alpha$-C=O] dihedral angles were kept static (Fig. 1). The $\psi$ and $\phi$ dihedral angles have previously been optimised for the alanine methyl ester [27], which provided 6 stable conformers. Thus, the 6 alanine methyl ester main chain geometries were used as starting points to build the $\chi_1$ Trp-OMe dihedral angle PES by exchanging an alanine methyl ester hydrogen atom for an indolyl group. In addition, the dihedral angle [O=C-O-$CH_3$] was maintained in the *cis* form because this form is the most stable O=C-O-$CH_3$ dihedral angle geometry, as demonstrated for several methyl esters [47]. Each minimum obtained from the Trp-OMe PES was fully optimised at the B3LYP/aug-cc-pVDZ theoretical level, which already showed good agreement to CCSD/CBS and experimental data for both glycine and alanine conformers [48,49]. Some of the $\phi$ [LP-N-$C_\alpha$-C(O)] and $\psi$ [N-$C_\alpha$-C=O] dihedral angles were similar to the ones from the starting geometries of alanine methyl esters, but this procedure did not interfere with the results, since a final full optimization was performed for the geometries of the methyl ester of tryptophan. To these calculated energies, the zero point energy (ZPE) corrections were added. Subsequently, each Trp-OMe energy minimum was optimised by considering solvent effects through the IEF-PCM implicit solvent model [50] in solvents with different ($\varepsilon$) dielectric constants (methanol, pyridine and DMSO). The NBO analysis was performed over the wave functions obtained from the B3LYP/aug-cc-pVDZ optimisations in the same level of theory. The spin-spin

coupling constant calculations were performed at the B3LYP functional and with the EPR-III (for C and H) and the aug-cc-pVDZ (for O and N) basis sets. All calculations were performed using the Gaussian 09 package of programmes [51].

Moreover, topological analyses and the evaluation of integral properties over the atomic basins ($\Omega$) were conducted with the AIMALL programme [52] over the electron density obtained from the B3LYP/aug-cc-pVDZ optimisation calculations. As usual, the accuracy of these calculations was checked by the integral values of the Laplacian of the charge density in each atom, which did not show absolute values larger than $10^{-3}$ atomic units (au) [27, 37-39].

## 3 Results and Discussion

The side chain rotational isomerism of amino acids and their derivatives is usually represented in terms of a mixture of three staggered rotamers ("a", "b" and "c") as shown in Fig. 3 for Trp-OMe. In this context, the $^{3}J_{HH}$ vicinal coupling constant between the $\alpha$ and β hydrogen atoms is one important parameter, which is dependent on the dihedral angle of rotation around the $C_{\alpha}$-$C_{\beta}$ single bond and is useful to evaluate the relationship between the side and main chain of these compounds. Thus, to understand the conformational changes induced by each solvent, $^{1}$H NMR spectra were recorded in solvents of varying dielectric constant values: acetonitrile, methanol, pyridine and DMSO (Table 1). The $^{3}J_{H\alpha H\beta}$ values did not show significant changes (values between 6.39 and 7.14 Hz for $^{3}J_{H\alpha H\beta1}$ and between 5.57 and 5.86 Hz for $^{3}J_{H\alpha H\beta2}$) and thus indicate that there are no conformational population variations concerning the side chain of Trp-O-Me caused by changing the solvent. Once internal rotation is sufficiently rapid, the observed spin-spin coupling constant values are weighted averages obtained for the three rotamers, and thus experimental data from NMR alone does not explain the Trp-OMe rotational isomerism.

To interpret the experimental results, theoretical calculations were performed. PESs were built for the Trp-OMe compound (ggg. S1, Supporting Information) by using each of the 6 stable conformers of alanine. Although 18 energy minima have been identified (3 from each PES), only 8 conformers (named according to the increasing order of energy, **Trp1** to **Trp8**) converged to stable structures at the B3LYP/aug-cc-pVDZ level (Fig. S2, Supporting Information). Subsequently, each energy minimum was optimised by including solvent effects using the IEF-PCM implicit solvation model for the solvents methanol, pyridine and DMSO. The contribution of each "i" conformer in a given solvent for the observed $^3J_{H\alpha H\beta}$ value may be obtained by the equation:

$$^3J_{H\alpha H\beta 1} = \sum_{i=1}^{n} \frac{n_i}{n_T} {}^3J_i \qquad \textbf{(eq. 1)}$$

where $n_i/n_T$ and $J_i$ are the fractional population and the intrinsic coupling constant of conformer "i", respectively. The population for each conformer may be easily obtained by the Boltzmann distribution:

$$\frac{n_i}{n_T} = e^{-\frac{\Delta E_i}{RT}} \qquad \textbf{(eq. 2)}$$

where $\Delta E_i$ is the relative energy of the conformer "i", R is the Boltzmann constant, and T is the temperature. The values of $n_i/n_T$ x $^3J_{H\alpha H\beta 1}$ for the Trp-OMe conformers calculated in DMSO, pyridine and methanol are shown in Fig. S3 (Supporting Information). The values of $^3J_{H\alpha H\beta 1}$ do not vary considerably with increased dielectric constant, which is in good agreement with the experimental data (Table 1).

Hydrogen bond (HB) formation is commonly assumed to govern amino acid relative energy values. In fact, several rotamers of Trp-OMe exhibit suitable geometry for possible HB formation. However, other interactions such as steric hindrance and hyperconjugative effects may operate in these systems. Thus, to investigate the

interactions that rule the conformational equilibrium of Trp-OMe, QTAIM [41-45] and (NBO) methods [46] were employed.

Popelier criteria [45], which use QTAIM parameters, are useful for HB characterisation. According to Popelier's first criterion, if a HB is formed, atomic interaction lines [called bond paths (BP) when the conformer is in the equilibrium geometry] must be formed connecting the hydrogen atom and the hydrogen acceptor atom, along with a bond critical point (BCP). The molecular graphs for all conformers studied are shown in Fig. S4 (Supporting Information). Only conformers **Trp2**, **Trp4**, **Trp6**, **Trp7** and **Trp8** exhibit BPs and BCPs relative to an unusual HB [53] involving the Trp-OMe main and side chain. Because the presence of a BCP and a BP is the necessary condition for atoms to be bonded from the QTAIM standpoint, only these rotamers could show stable HBs.

To evaluate the remaining criteria, the electronic density ($\rho_{BCP}$) and its Laplacian ($\nabla^2\rho_{BCP}$) values in the HB and BCP were obtained as well as the hydrogen atomic energy *E*(H), hydrogen atomic charge *q*(H), hydrogen dipole moment $M_1$(H) and hydrogen atomic volume *V*(H) for the hydrogen atoms possibly involved in a HB (Table 2). In addition, the conformer **Trp1**, which does not show any HBs, was used as reference. In line with Popelier's criteria, the analysis of Table 2 shows that only the conformation **Trp8** exhibits hydrogen bonding. The **Trp2**, **Trp4**, **Trp6** and **Trp7** conformers show $\nabla^2\rho$ outside of the 0.0024-0.139 au range. According to Popelier's criteria, a hydrogen atom involved in a HB should lose *q*(H) while the $M_1$(H) and V(H) decreases and the *E*(H) increase in magnitude, when compared with an isolated, HB-uninvolved hydrogen atom. Therefore, only **Trp8** fully meets all criteria. However, this conformer was found to be more unstable than the remaining conformers, and thus hydrogen bonding is not the interaction responsible for the stability of Trp-OMe.

NBO analysis was performed to evaluate the steric and hyperconjugative contributions for Trp-OMe conformational preferences. In this way, all interactions

involving antibonding and Rydberg orbitals were deleted, *i.e.*, conformer energies were computed as a Lewis structure, which does not account for hyperconjugative interactions. In this hypothetical situation, it is possible to obtain the contributions of steric and hyperconjugation effects because the conformer energies before and after hyperconjugation deletion are known (Table 3). In the real system ($E_{Full}$), **Trp1** is the most stable conformer. However, when the hyperconjugation is removed, **Trp5** is the most stable conformation by an energy difference greater than 14 kJ mol$^{-1}$ ($E_{Lewis}$). These results indicate that **Trp5** is the conformer that experiences the least steric effects, but they also indicate that hyperconjugation has marked importance for the stabilisation of **Trp1**. The contribution of hyperconjugative effects is 66.13 kJ mol$^{-1}$ greater for conformer **Trp1** in comparison to conformer **Trp5** (Table 3), which is the least stabilised conformer by hyperconjugative effects, followed by **Trp3** and **Trp7**.

The importance of hyperconjugation is highlighted by the values shown in Table S1 (Supporting Information), which shows the main orbital interactions involving the nitrogen and oxygen lone pairs for the Trp-OMe conformers. The **Trp1**, **Trp3** and **Trp6** conformers present $LP_{(N5)}$ directed to the $\sigma^*_{C4\text{-}C13}$ antibonding orbital ($\phi \approx 300^o$), resulting in the interaction energies ($LP_{(N5)} \rightarrow \sigma^*_{C4\text{-}C13}$) contributing to the effective stabilisation of these conformers. Indeed, for the first conformer, one additional interaction involving $LP_{(N5)}$ and $\sigma^*_{C4\text{-}H12}$ is also predominant (12.46 kJ mol$^{-1}$) when compared with **Trp3** and **Trp6**, although this interaction shows larger values for **Trp4** and **Trp8** (35.20 and 26.75 kJ mol$^{-1}$, respectively), due to a favourable dihedral angle ($\phi \approx 90^o$). Therefore, the hyperconjugative interactions that reinforce the stability of **Trp1**, in relation to the remaining conformers, are the sum of $LP_{(N5)} \rightarrow \sigma^*_{C4\text{-}C13}$ and $LP_{(N5)} \rightarrow \sigma^*_{C4\text{-}H12}$. Moreover, the **Trp2**, **Trp5** and **Trp7** conformers ($\phi \approx 180^o$) are stabilised by the interaction $LP_{(N5)} \rightarrow \sigma^*_{C1\text{-}C4}$ with a larger influence for **Trp2** because it has a better spatial arrangement between these two orbitals. Additionally, for the **Trp2** conformer, the $LP_{(N5)}$ is distant from $LP_{(O2)}$, minimising the steric repulsion between these lone pairs. Therefore, the $LP_{(N5)}$ orientation plays an important role in Trp-OMe orbitals' interaction

energies and contributes effectively to the conformational equilibrium of this compound. For the hyperconjugative interactions involving non-bonding electrons of oxygen atoms, the values observed are practically identical for all conformers.

In addition, to evaluate the importance of steric effects for this system, the AIM electronic population $N(\Omega)$ and atomic electronic energy $E(\Omega)$ parameters were obtained. As noted previously [37,38], the loss of $N(\Omega)$ of an atom $\Omega$ from one conformer (which experiences less steric effects) to another, may be understood as an atomic electronic density rearrangement involved in steric effects. Consequently, this rearrangement affects the atomic energies $E(\Omega)$ of the atoms, which lose and receive $N(\Omega)$. Table S2 (Supporting Information) shows the main $N(\Omega)$ and $E(\Omega)$ variations for the conformers of Trp-OMe relative to **Trp5**, the conformer that shows the smaller Lewis energy (Table 3). The **Trp1** conformer shows a decrease in $N(\Omega)$ to C1, C4 and H6 atoms, while the inverse may be observed to N5. These $N(\Omega)$ variations can be explained by high steric hindrance resulting from the interactions between H6 and $LP_{(O2)}$, C4 and $LP_{(O3)}$ and C1 and $LP_{(N5)}$, which are closer in space relative to the **Trp5** conformer. For these interactions, N5 acts as an electronic density acceptor from H6, C1 and C4 atoms, and **Trp1** therefore shows high $N$(N5) and low $E$(N5) when compared to **Trp5**. Because **Trp1** shows higher $\Delta E_{Lewis}$ (Table 3), these interactions should be the main sources of instability for this conformer. However, these steric effects are compensated by the larger hyperconjugation energy when compared with the remaining conformers, and thus **Trp1** represents the most stable form of Trp-OMe. For **Trp2** and **Trp6**, the loss of $N$(H25), together with an increase of $N$(O2), can be explained by transferring electron density involving these atoms, due the proximity between the main and side chains. Additionally, this last conformer seems to experience steric effects for the interaction between C1 and $LP_{(N5)}$. A similar effect present in **Trp2** and **Trp6**, involving the side and main chain, is observed for the **Trp8** conformer, though it is mediated by H22 and $LP_{(N5)}$. Indeed, a steric repulsion occurs between H7 and C1 atoms, and thus the electron density from these atoms is

transferred to the N5 acceptor atom. Therefore, although **Trp8** forms an intramolecular HB that contributes to its stabilisation, the repulsions between $LP_{(N5)}$ and H22 and between H7 and C1 increase its energy, making it more unstable than the other conformers. In the **Trp4** conformer, the main *N*(Ω) and *E*(Ω) variations are observed for C1 and H7 and N5 and H25, which interact repulsively with each other.

Moreover, from the QTAIM point view, **Trp3** does not show significant changes of *N*(Ω) and *E*(Ω), except for *N*(C1) and *N*(N5), when compared to **Trp5**. The **Trp3** conformer exhibits low $\Delta E_{Lewis}$ (Table 3) due to the perpendicular relationship between $LP_{(N5)}$ and $COOCH_3$. This spatial arrangement minimises the repulsion between $LP_{(N5)}$ and the nonbonding electron pairs of the oxygen atoms, and this result corroborates the NBO analysis. **Trp7** shows decreased *N*(H7) and *N*(H25) values and a slight increase in the *N*(N5) value. Thus, the repulsion between H7 and O2 and between $LP_{(N5)}$ and H25 atoms results in a transfer of electron density to N5, which in turn decreases its *E*(N) value.

These results indicate that the Trp-OMe spatial arrangement cannot be attributed to the formation of an intramolecular hydrogen bond, but due to hyperconjugation and steric effects together, which have remarkable importance for the Trp-OMe conformational isomerism.

## 4. Conclusions

In summary, using experimental NMR parameters together with theoretical calculations and NBO and QTAIM analysis, it was demonstrated that both hyperconjugation and steric effects are responsible for the conformational isomerism of Trp-OMe. It was also demonstrated that an intramolecular HB is not an important interaction for the Trp-OMe conformational preferences because this interaction is observed only in one relatively unstable conformer.

**Acknowledgments**

The authors thank a grant # 2012/03933-5, São Paulo Research Foundation (FAPESP) for providing financial support for this research, for a scholarship [to C.J.D. # 2011/11098-6 and to R.A.C. #2011/01170-1, FAPESP) and for a fellowship (to L.C.D. #2010/15764-4, FAPESP), and the authors thank CNPq for a fellowship (to R.R. #300379/2009-9).

**References**

[1] M. A. Scotto, S. Antonczac, J. H. Bredehöft, S. V. Hoffmann and U. J. Meierhenrich, Symmetry 2 (2010) 935-949.

[2] J. S. Anderson, G. S. Bowitchand R. L. Brewster, Biopolymers 22 (1983) 2459-2476.

[3] M. L. Ceci, M. A. L. Verrilli, S. S. Vallcaneras, J. A. Bombasaro, A. M. Rodrıguez, B. Penke and R. D. Enriz, J. Mol. Struct. (THEOCHEM) 631 (2003) 277-290.

[4] E. J. Cocinero, A. Lesarri, J. Grabow, J. C. López, and J. L. Alonso, ChemPhysChem 8 (2007) 599-604.

[5] T. Emmler, S. Gieschler, H. H. Limbach and G. Buntkowsky, J. Mol. Struct. (THEOCHEM) 700 (2004) 29-38.

[6] B. Yogeswari, R. Kanakaraju, A. Abiram and P. Kolandaivel, Comput. Theor. Chem. 967 (2011) 81-92

[7] A. Lesarri, E. J. Cocinero, J. C. López, and J. L. Alonso, Angew. Chem. Int. Ed., 43 (2004) 605-610.

[8] J. R. A. Moreno, M. M. Q. Moreno, F. P. Ureña and J. J. L. González, Tetrahedron: Asymmetry 23 (2012) 1084-1092.

[9] U. Purushotham, D. Vijay and G. N. Sastry, J. Comp. Chem. 33 (2011) 44-59.

[10] B. Boeckx and G. Maes, J. Phys. Chem. A 116 (2012) 1956-1965.

[11] F. Jiang, W. Han and Y. Wu. J. Phys. Chem. B. 114 (2010) 5840-5850.

[12] N. T. Moldogazieva, K. V. Shaitan, M. Y. Antonov, I. K. Vinogradova and A. A. Terentiev, Biochemistry (Moscow) 76 (2011) 1321.

[13] B. Yogeswari, R. kanakaraju, A. Abiram, P. Kolandaivel, Comp. Theor. Chem. 967 (2011) 81-92.

[14] G. Chouhan and K. James, Org. Lett. 15 (2013) 1206-1209.

[15] S. Toal, D. Meral, D. Verbaro, B. Urbanc and R. S. Stenner, J. Phys Chem. B 117 (2013) 3689-3706.

[16] S. A. Pless and C. A. Ahern, Annu. Rev. Pharmacol. Toxicol. 53 (2013) 211-229.

[17] F. Piuzzi, I. Dimicoli, M. Mons, B. Tardivel and Q. Zhao, Chem. Phys. Lett. 320 (2000) 282-288.

[18] I. Compagnon, F. C. Hagmeister, R. Antoine, D. Rayane, M. Broyer, P. Dugourd, R. R. Hudgins and M. F. Jarrold. J. Am. Chem. Soc. 123 (2001) 8440-8441.

[19] A. Lessari, S. Mata, E. J. Cocinero, S. Blanco, J. C. Lópes and J. L. Alonso, Angew. Chem. Inst. Ed. 41 (2002) 4673-4676.

[20] S. Blanco, M. E. Sanz, J. C. Lópes and J. L. Alonso, Proc. Natl. Acad. Sci. U. S. A. 18 (2007) 20183-20188.

[21] A. G. Császár, J. Am. Chem. Soc. 114 (1992) 9568-9575.

[22] S. Gronert and R. A. J. O'Hair, J. Am. Chem. Soc. 117 (1995) 2071-2081.

[23] E. Yurtsever, D. Yuret and B.Erman, J. Phys. Chem. A 110 (2006) 13933-13938.

[24] G. Yang, L. Zhou, Y. Zu, Y. Fu, R. Zhu and C Liu, J. Mol. Struct.: THEOCHEM 901 (2009) 81-87

[25] K. Y. Baek, M. Hayashy, Y. Fujimura, S. H. Lin and S. K. Kin, J. Phys. Chem. 114 (2010) 7583-7589.

[26] K. Y. Baek, Y. Fujimura, M. Hayashi, S. H. Lin, and S. K. Kim, J. Phys. Chem. A 115 (2011) 9658-9668.

[27] R. A. Cormanich; L. C.Ducati; C. F.Tormena and R. Rittner, J. Phys. Org. Chem. 2013 (In press).

[28] G. M. S. Pinheiro, E. A. Basso, W. F. Costa, F. Cendes, R. Rittner, A. N. Oliveira and N. F. Höehr, Clin. Chim. Acta 404 (2009) 166-168.

[29] U. F. H. Engelke, J. O. Sass, R. N. V. Coster, E. Gerlo, H. Olbrich, S. Krywawych, J. Calvin, C. Hart, H. Omran and R. A. Wevers, NMR Biomed. 21 (2008) 138-147.

[30] S. Lima, S. Kumar, V. Gawandu, C. Momany, R. S. Phillips, J. Med. Chem. 52 (2009) 389-396.

[31] H. Sun and E. Oldfield, J. Am. Chem. Soc. 126 (2004) 4726-4734.

[32] O. Guvench and C. L. Brooks, J. Am. Chem. Soc. 127 (2005) 4668-4674.

[33] M. N. Blom, I. Compagnon, N. C. Polfer, G. Helden, G. Meijer, S. Suhai, B. Paizs and J. Oomens, J. Phys. Chem. A 111 (2007) 7309-7316.

[34] A. Kaczor, I. D. Reva, L. M. Proniewicz, and R. Fausto, J. Phys. Chem. A 111 (2007) 2957-2965.

[35] E. Fukumura, H. Sugimoto, Y. Misumi, T. Ogura and Y. Shiro, J. Biochem. 145 (2009) 505-515.

[36] P. Garidel, M. Hegyi, S. Bassarab and M. Weichel, Biotechnol. J. 3 (2008) 1201-1211.

[37] R. A. Cormanich, L. C.Ducatti, R.Rittner, J. Chem. Phys. 387 (2011) 85-91.

[38] R. A. Cormanich, L. C.Ducatti, R. Rittner, J. Mol. Struc. 1014 (2012) 12-16.

[39] R. A. Cormanich,; L. C. Ducati, C. F.Tormena,; R. Rittner, Chem. Phys. 2013 (In press).

[40] R. F. W. Bader, Atoms in Molecules: A Quantum Theory; Oxford University Press: Oxford, 1990.

[41] R. F. W. Bader, Chem. Rev. 91 (1991) 893-928.

[42] R. F. W. Bader, J. Phys. Chem. A 102 (1998) 7314-7323.

[43] S. J. Grabowski, Chem. Rev. 111 (2011) 2597-2625.

[44] U. Koch, P. L. A. Popelier, J. Phys. Chem. 99 (1995) 9747-9754.

[45] NBO 5.0, E.D. Glendening, J.K. Badenhoop, A.E. Reed, J.E. Carpenter, J.A.Bohmann, C.M. Morales, F. Weinhold, Theoretical Chemistry Institute,University of Wisconsin, Madison, 2001.

[46] K. Ananda, V.V. S.Babu, J. Pept. Res. 57 (2001) 223-226.

[47] C. F. Tormena,T. R. F. Yoshinaga, R. Rittner, Spectrochim. Acta A63 (2006) 511-517.

[48] A. G. Császár, J. Phys. Chem. 100 (1996) 3541-3551.

[49] R. M.Balabin, Comput. Theor. Chem. 965(2011)15-21.

[50] E. Cances; B. Mennucci; J. Tomasi, J. Chem. Phys. 107 (1997) 3032-3041.

[51]Gaussian 09, Revision B.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

[52] T.A. Keith, AIMAll (Version 11.12.19), 2011.

[53] G. Gilli, P. Gilli, The Nature of the Hydrogen Bond: Outline of a Comprehensive Hydrogen Bond Theory. First Ed., Oxford University Press, New York, 2009.

Figure Captions

Figure 1: Rotation of dihedral angle $\chi$ [N-C$\alpha$-C$\beta$-C$\gamma$] provided the conformers of Trp-OMe.

Figure 2: 600 MHz $^{1}$H NMR spectra of tryptophan methyl ester in DMSO-$d_6$ (a); expanded spectral region of 3-4 ppm in: DMSO-$d_6$ (b), MeOD (c), pyridine-$d_5$ (d) and acetonitrile-$d_3$ (e).

Figure 3: Representation of staggered rotamers relative to the side chain of Trp-O-Me.

Highlights

The conformational equilibrium of tryptophan methyl ester was analysed.

Experimental $^{3}J_{HH}$ and theoretical calculations were employed.

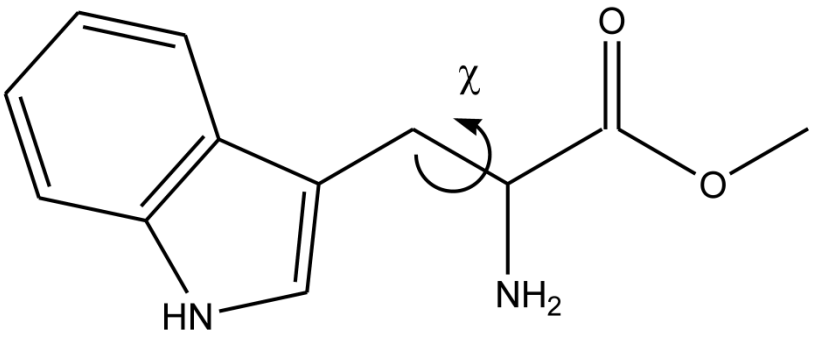
Conformer populations were not dependent on the solvent.

Hyperconjugation and steric effects govern the ester's conformational isomerism.

## Graphical Abstracts

### $^{1}$H NMR and theoretical studies on the conformational equilibrium of Tryptophan methyl ester

Claudimar J. Duarte, Rodrigo A. Cormanich, Lucas C. Ducati and Roberto Rittner



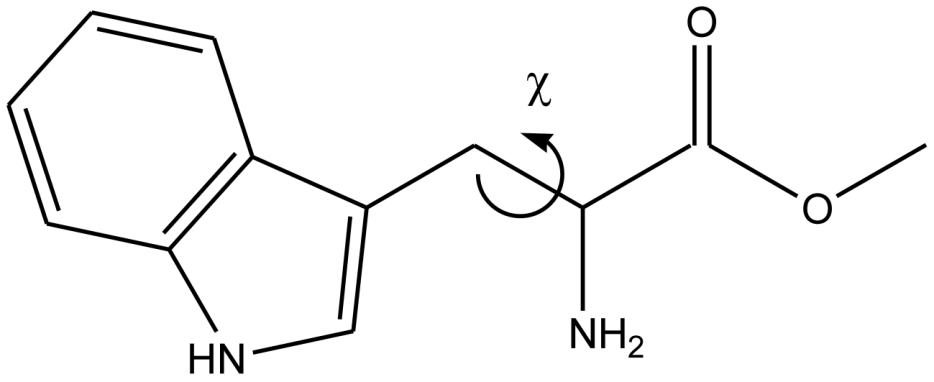



Figure 1

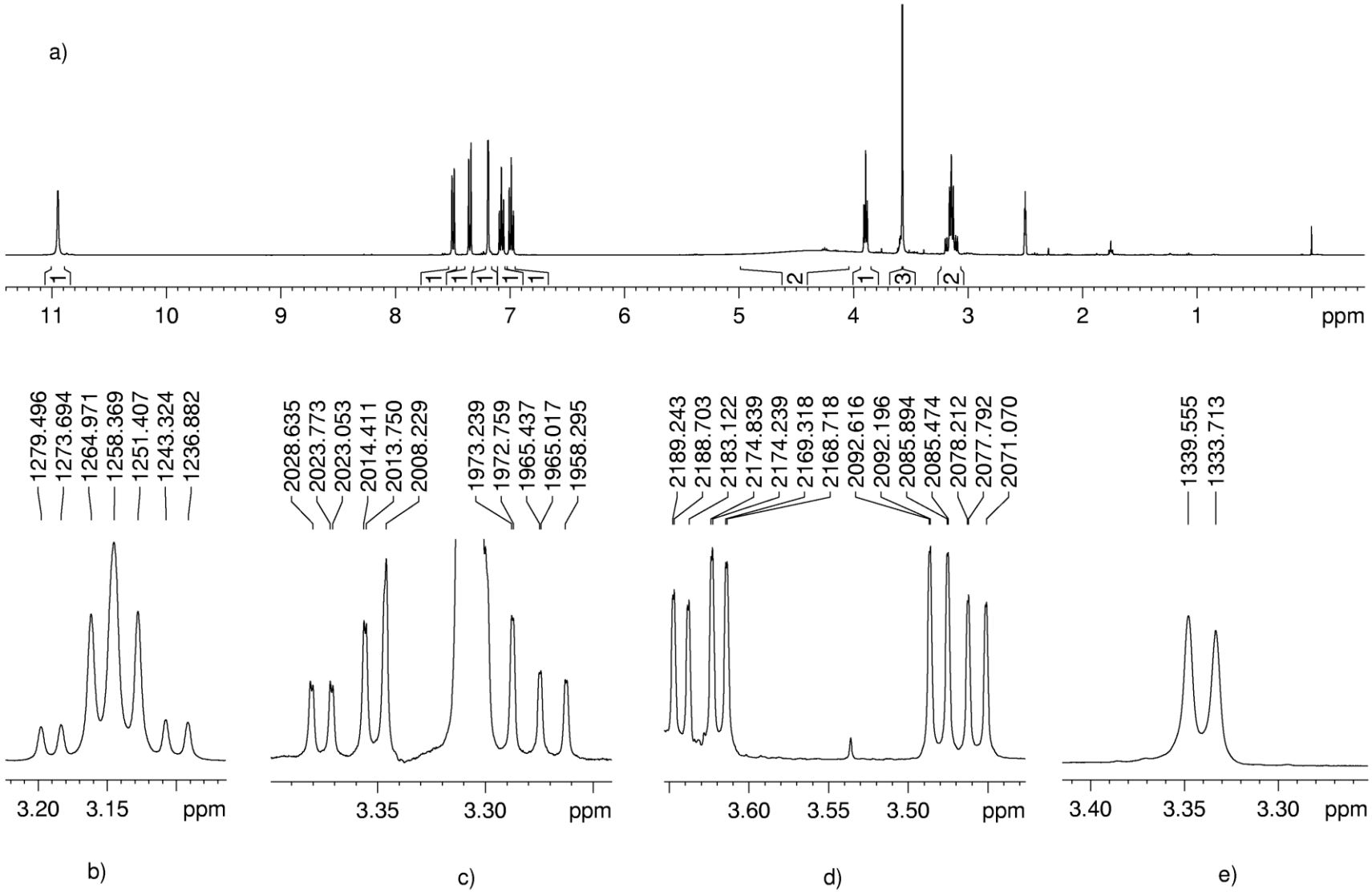



Figure 2

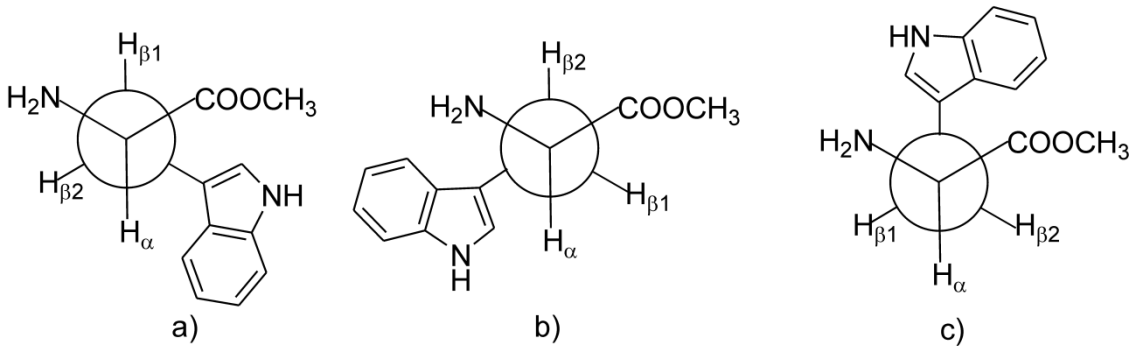
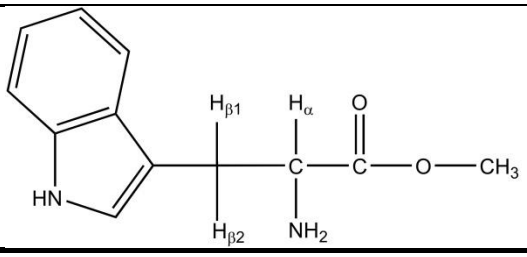



Figure 3

Table 1
Coupling constants (*J*, in Hz) and Chemical shifts (δ, in ppm) to the Trp-O-Me rotamers in solvents of different dielectric constants (ε).




| **Solvent** | ε | $\delta_\alpha$ | $\delta_{\beta1}$ | $\delta_{\beta2}$ | $^3J_{\alpha\beta1}$ | $^3J_{\alpha\beta2}$ |
|---|---|---|---|---|---|---|
| Pyridine-$d_5$ | 12.3 | 3.89 | 3.12 | 3.18 | 6.46 | 5.78 |
| MeOD | 32.7 | 4.13 | 3.28 | 3.37 | 7.14 | 5.57 |
| $CD_3CN$ | 37.5 | 4.12 | 3.34 | 3.34 | ------ | ------ |
| DMSO-$d_6$ | 46.7 | 3.89 | 3.11 | 3.17 | 6.39 | 5.86 |

Table 2
Electronic density ($\rho_{BCP}$) and electronic density Laplacian ($\nabla^2\rho_{BCP}$) at the H-bond BCP and integrated atomic properties in au units.

| | | Parameter | | | | | |
|---|---|---|---|---|---|---|---|
| **Conformer** | H | $\rho_{BCP}$ (H) | $\nabla^2\rho_{BCP}$ (H) | q(H) | E(H) | M1(H) | V(H) |
| **Trp1** | H(9) | --- | --- | +0.020 | -0.605 | 0.135 | 47.84 |
| | H(10) | --- | --- | +0.025 | -0.603 | 0.134 | 47.47 |
| | H(22) | --- | --- | +0.032 | -0.596 | 0.130 | 49.40 |
| | H(25) | --- | --- | -0.003 | -0.612 | 0.129 | 51.40 |
| **Trp2** | H(10) | 0.002 | +0.007 | +0.030 | -0.603 | 0.132 | 49.06 |
| | H(25) | 0.006 | +0.020 | +0.022 | -0.606 | 0.118 | 47.59 |
| **Trp4** | H(10) | 0.002 | +0.007 | +0.030 | -0.603 | 0.132 | 49.06 |
| | H(25) | 0.006 | +0.020 | +0.022 | -0.606 | 0.118 | 47.59 |
| **Trp6** | H(9) | 0.003 | +0.008 | +0.030 | -0.603 | 0.131 | 48.72 |
| | H(25) | 0.005 | +0.017 | +0.016 | -0.608 | 0.121 | 48.72 |
| **Trp7** | H(25) | 0.007 | +0.022 | +0.020 | -0.606 | 0.129 | 48.15 |
| **Trp8** | H(22) | 0.009 | +0.029 | +0.066 | -0.585 | 0.129 | 45.43 |

Table 3

Total energy ($\Delta E_{FULL}$), Lewis energy ($\Delta E_{Lewis}$) and hyperconjugative energy ($\Delta E_{hyper}$) for Trp-OMe conformers (in kJ $mol^{-1}$) at the B3LYP/aug-cc-pVDZ theoretical level.

| | **Conformer** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Trp 1 | Trp 2 | Trp 3 | Trp 4 | Trp 5 | Trp 6 | Trp 7 | Trp 8 |
| **$\Delta E_{total}$** | 0.00 | 2.17 | 3.85 | 4.22 | 4.81 | 5.35 | 5.43 | 5.52 |
| **$\Delta E_{Lewis}$** | 61.36 | 52.25 | 14.63 | 21.15 | 0.00 | 48.66 | 18.60 | 49.87 |
| **$\Delta E_{hyper.}$** | 66.13 | 54.88 | 15.59 | 21.74 | 0.00 | 48.11 | 17.89 | 49.12 |