

Tesis doctoral

AÑO 2.015

Estudio teórico de la hipoxantina y derivados

Margarita-Clara Álvarez Ros

Licenciada en Ciencias Químicas



**Universidad nacional de Educación a distancia
Facultad de Ciencias
Departamento de Ciencias y Técnicas Físicoquímicas**

**Bajo la dirección del profesor de la Universidad
Complutense de Madrid**

Dr. D. Mauricio Alcolea Palafox

Tutor: Dr. D. Arturo Horta Zubiaga

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El trabajo computacional y la redacción de la presente memoria han sido realizados por la licenciada en Ciencias Químicas Margarita - Clara Álvarez Ros, en el Departamento de Ciencias y Técnicas Físico Químicas de la Universidad Nacional a Distancia (UNED), bajo la dirección del Dr. D. Mauricio Alcolea Palafox, profesor titular del Departamento de Química Física de la Universidad Complutense de Madrid, para optar al título de Dra. en Química.

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Memoria efectuada en conexión con los laboratorios computacionales de la
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1. Lista de publicaciones de la Tesis

1. Margarita Clara Álvarez-Ros, M. Alcolea Palafox ``Molecular structure of the nucleoside analogue Inosine using DFT methods: Conformational analysis, crystal simulations and possible behaviour'' *Journal of Molecular Structure* (2013) **1047**, 358-371.
2. Margarita Clara Álvarez-Ros, M. Alcolea Palafox ``Conformational Analysis, Molecular Structure and Solid State Simulation of the Antiviral Drug Acyclovir (Zovirax) Using Density Functional Theory Methods'' *Pharmaceuticals* (2014), **7**, 295-722.
3. Margarita Clara Álvarez Ros, M. Alcolea Palafox ``Simulation of the first hydration shell of the base hypoxanthine: structures obtained using MP2 and DFT methods'' *Phys. Chem. Chem. Phys.* **Preparado.**
4. Margarita Clara Álvarez-Ros, M. Alcolea Palafox ``Simulation of the First Hydration Shell of the allopurinol: Structures Obtained Using DFT and MP2 Methods''. *Journal of Molecular Structure* (2015). **Enviado.**

1.2. Otras publicaciones

5. Ahmed A. El-Sayed, A. Tamara Molina, Margarita Clara Álvarez-Ros, M. Alcolea Palafox. ``Conformational analysis of the anti-HIV Nikavir prodrug: comparisons with AZT and Thymidine, and establishment of structure-activity relationships/tendencies in other 6'-derivatives''. *Journal of Biomolecular Structure and Dynamics*. (2015) **33** (4), 723-748.
6. S. Ortiz, Margarita Clara Álvarez-Ros, M. Alcolea Palafox, V.K. Rastogi, V. Balchandran, S. Rathor. ``FT-IR and FT-Raman spectra of 6-chlorouracil: Molecular Structure, tautomerism and solid state simulation. A comparison between 5-chlorouracil and 6-chlorouracil''. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2014) **130**, 653-668.

7. A. Hernanz, J. M. Gavira-Vallejo, M. C. Álvarez, S. Martín, S. Montero, E. A. Carter, E. Gavrilenko and H. G. Edwards, chapter 17: *Spectroscopy of Historic Textiles: a Unique 17th Century Bodice*, **Analytical Archaeometry rsabook18 chapter 17.3d** 18/7/12. The Charlesworth Group, Wakefield +44(0) 1924 204830.

1.3. Congresos

1. Margarita Clara Álvarez-Ros, M. Alcolea Palafox, M de la Fuente and N. Iza ``Conformational stability of the six main tautomers of hypoxanthine under hydration`` *Twenty-Second Austin Symposium on Molecular Structure*, **S6**, pp. 69 Austin, Texas (USA) 6-9 Marzo (2010).

NOTA NIMPORTANTE

Parte de la presente Tesis viene expuesta en las publicaciones antes citadas, principalmente en:

1. Margarita Clara Álvarez Ros, Mauricio Alcolea Palafox ``Molecular structure of the nucleoside analogue Inosine using DFT methods: Conformational analysis, crystal simulations and possible behaviour``.
2. Margarita Clara Álvarez-Ros, Mauricio Alcolea Palafox ``Conformational Analysis, Molecular Structure and Solid State Simulation of the Antiviral Drug Acyclovir (Zovirax) Using Density Functional Theory Methods``.
3. Margarita Clara Álvarez Ros, M. Alcolea Palafox ``Simulation of the first hydration shell of the base hypoxanthine: structures obtained using MP2 and DFT methods``.
4. Margarita Clara Álvarez Ros, M. Alcolea Palafox ``Simulation of the First Hydration Shell of the allopurinol: structures obtained using DFT and MP2 Methods``.

1.4. Citaciones de las publicaciones

1. M.C. Alvarez-Ros, M. Alcolea Palafox. Molecular structure of the Nucleoside Analogue Inosine using DFT methods: Conformational analysis, crystal simulations and possible behaviour. *J. of Molecular Structure*, (2013) **1047**, 358-371.
Citas: 1. Tuna, D.; Sobolewski, A.L.; Domcke, W., **Mechanisms of ultrafast excited-state deactivation in adenosine**. *J. Physical Chemistry A*, (2014) 118, 1, 122-127.
2. Prabu, S., Sivakumar, K., Swaminathan, M., Rajamohan, R. **Preparation and characterization of host-guest system between inosine and β -**

cyclodextrin through inclusion mode. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, (2015) 147, 151-157.

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2. S. Ortiz, M.C. Alvarez-Ros, M. Alcolea Palafox, V.K. Rastogi, V. Balachandran, and S.K. Rathor. FT-IR and FT-Raman spectra of 6-chlorouracil: Molecular structure, tautomerism and solid state simulation. A comparison between 5-chlorouracil and 6-chlorouracil. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **130**, 653-668(2014)

Citas: Ajaj, I., Markovski, J., Rančić, M., Mijin, D., Milčić, M., Jovanović, M., Marinković, A. **Solvent and structural effects in tautomeric 2(6)-hydroxy-4-methyl-6(2)-oxo-1-(substituted phenyl)-1,2(1,6)-dihydropyridine-3-carbonitriles: UV, NMR and quantum chemical study.** *Spectrochimica Acta A: Molecular and Biomolecular Spectroscopy*, (2015) 150, 575–585.

Te mando las citas que he encontrado

A.A. El-Sayed, A. Tamara Molina, M.C. Álvarez-Ros, and M. Alcolea Palafox. Conformational analysis of the anti-HIV Nikavir prodrug: comparisons with AZT and Thymidine, and establishment of structure-activity relationships/tendencies in other 6'-derivatives. *J. of Biomolecular Structure & Dynamics*, **33** (4) 723-748 (2015)

Citas: Brovarets, Ol'ha O.; Hovorun, Dmytro M., **How many tautomerization pathways connect Watson-Crick-like G*center**

dot T DNA base mispair and wobble mismatches?. *J. Biomolecular Structure & Dynamics*, 33 (11) 2297-2315 (2015).
 2. Brovarets, Ol'ha O.; Yurenko, Yevgen P.; Hovorun, Dmytro M. **The significant role of the intermolecular CHMIDLINE HORIZONTAL ELLIPSISO/N hydrogen bonds in governing the biologically important pairs of the DNA and RNA modified bases: a comprehensive theoretical investigation, *J. Biomolecular Structure & Dynamics*, 33 (8) 1624-1652 (2015).**

S. Ortiz, M.C. Alvarez-Ros, M. Alcolea Palafox, V.K. Rastogi, V. Balachandran, and S.K. Rathor. FT-IR and FT-Raman spectra of 6-chlorouracil: Molecular structure, tautomerism and solid state simulation. A comparison between 5-chlorouracil and 6-chlorouracil. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **130**, 653-668(2014)

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 2. Prabu, S., Sivakumar, K., Swaminathan, M., Rajamohan, R. **Preparation and characterization of host-guest system between inosine and β -cyclodextrin through inclusion mode.** *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 2015, 147, 151-157.

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2. Introducción

Desde que en 1953 J. D. Watson y F. H. C. Crick⁽⁴⁾⁷ descifraron la estructura del ácido desoxirribonucleico (DNA) y del ácido ribonucleico (RNA), el interés y los trabajos de investigación sobre estas moléculas y sus componentes han presentado un desarrollo espectacular.

Por nuestra parte, el estudio de la estructura, propiedades y aplicaciones, de los diferentes fármacos derivados de las moléculas que conforman los ácidos nucleicos, ha despertado nuestro interés, dada la demanda de nuevos medicamentos para las enfermedades que se padecen actualmente. Establecer las posibles conformaciones, su estabilidad, así como las posibles interacciones intermoleculares con otras moléculas en el cristal y con el disolvente, principalmente agua, ha centrado nuestro estudio.

Dentro de las moléculas que tienen interés por su aplicación dentro de este campo de la farmacología, la base púrica hipoxantina (HX) y sus derivados: aciclovir (ACV), y el nucleósido inosina (INO) tienen especial relevancia. Se ha incluido la molécula de alopurinol (ALO) por su analogía respecto de HX. Se ha optado por el estudio de unas moléculas relacionadas entre sí con aplicaciones análogas en Medicina y que tuviesen hoy en día una amplia utilización en la práctica médica, frente al estudio de otras moléculas con aplicaciones más reducidas.

Enfermedades como el herpes, la gota, la infección por el virus de inmunodeficiencia humano (HIV), el síndrome bipolar y otras enfermedades psiquiátricas son tratadas con este tipo de compuestos. También tiene importancia su aplicación en el tratamiento de ciertos tumores. Por ello, el principal objetivo de esta investigación es colaborar en la comprensión, conocimientos y futuras investigaciones en el vasto campo de la medicina en su vertiente farmacológica que tantos beneficios ha procurado a la humanidad.

Para llevar a cabo esta investigación se han utilizado los métodos desarrollados en la Química Cuántica (QM). Así se ha centrado nuestra atención en obtener las estructuras más estables (tautómeros y, en su caso, confórmeros) de las moléculas. Se determinaron todas las posibles estructuras estudiando con más detalle las que presentaban mayor estabilidad, y de éstas se logró la completa optimización de todos sus parámetros geométricos y se analizaron en detalle sus parámetros conformacionales (χ , β , γ , δ , ϵ , P , V máxima).

Para el estudio en el estado sólido se ha simulado su estructura a través de un dímero, trímero y tetrámero y, en ocasiones, pentámero y heptámero, comparando los datos obtenidos con aquellos publicados experimentalmente mediante rayos X.

Los espectros FT-IR y Raman se han obtenido dentro del rango $\sim 160 / 3700 \text{ cm}^{-1}$ para la HX se ha analizado e interpretado los diferentes valores, y asignándoles a los correspondientes modos normales de vibración, previa comparación con los valores experimentales en fase gaseosa.

3. Objetivos

1. Nuestro principal objetivo es analizar la estructura de la HX y sus derivados ACV, INO y del isómero ALO, principalmente mediante diversos métodos funcionales de la densidad (DFT).
2. Determinar los tautómeros: su estabilidad, sus características y su población (%) (mediante la ecuación de Boltzmann) en los niveles de cálculo B3LYP y MP2.
3. Estudiar la hidratación de los tautómeros más estables de HX y ALO, optimizando los clústeres con hasta 30 moléculas de agua colocadas de forma explícita alrededor de las moléculas de HX y ALO, y obteniendo las energías de hidratación.
4. Calcular todos los conformeros de la INO y ACV a los niveles de cálculo DFT y MP2. Analizar sus energías y la variabilidad de los parámetros estructurales característicos de la molécula/ predecir/explicar su posible comportamiento.
5. Simular el estado sólido mediante el dímero, trímero, pentámero o heptámero y comparar con los resultados obtenidos experimentalmente en el cristal, principalmente mediante difracción de rayos X.

4. Estudio de los componentes de los ácidos nucleicos

La estructura del DNA y ARN es importante y está formada por unidades próximas a la HX (base), por ello en el siguiente punto desarrollaremos la estructura de sus componentes¹⁻⁶.

4.1. Bases, nucleósidos y nucleótidos.

Los nucleótidos están formados por tres componentes: una base nitrogenada, una pentosa y un fosfato. Las bases nitrogenadas derivan de dos compuestos de partida: **pirimidina** y **purina** (Figura 4.1) dando lugar a dos tipos de bases: **pirimidínicas** y **purínicas**. Las primeras son: **citocina (C)**, **timina (T)** y **uracilo (U)** y las segundas: **adenina (A)** y **guanina (G)** (Figura 4.1).

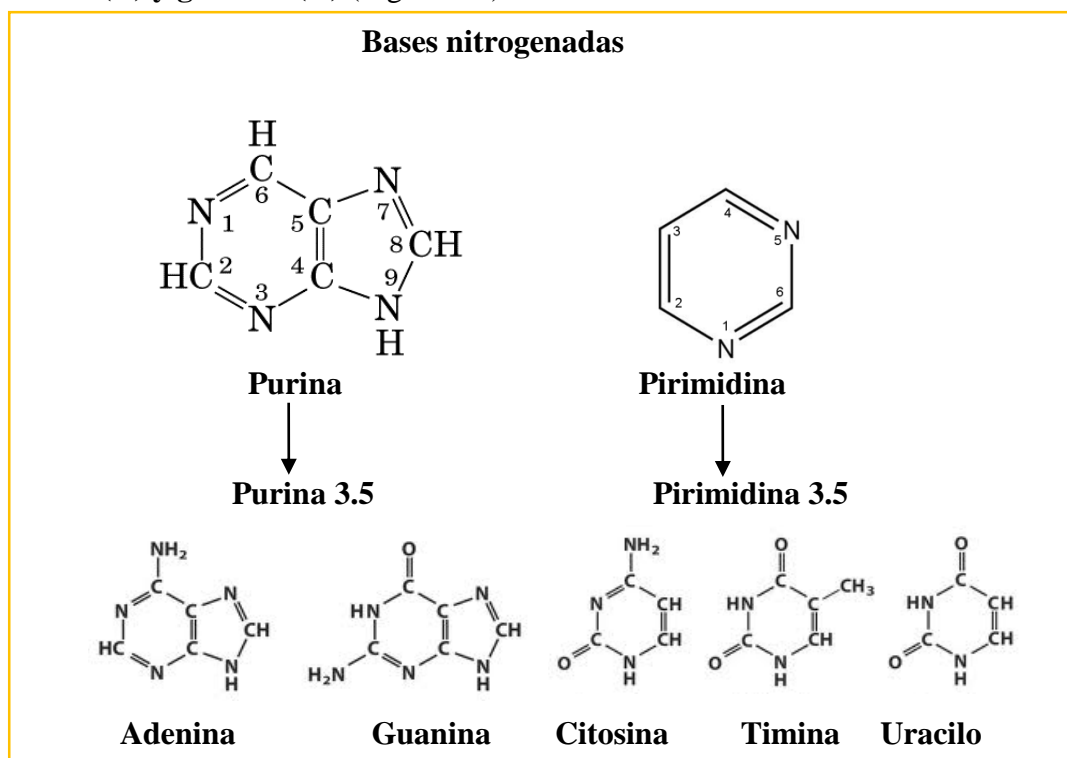


Figura 4.1. Pirimidina, purina y las bases nitrogenadas derivadas.

La pentosa, β-D-ribosa (1) o β-D-2'-desoxirribosa(2) está conectada mediante un enlace glicosídico con la base correspondiente de las antes citadas, en el átomo N1 de las pirimidinas y en el N9 en las purinas, dando lugar a un nucleósido (Figura 4.2). Cuando el nucleósido está fosforilado en el grupo 5' se tiene el nucleótido. Esta unidad es la que forma el esqueleto del DNA y ARN, obteniéndose largas cadenas unidas mediante los grupos fosfatos situados en los C 3' y 5' de la pentosa.

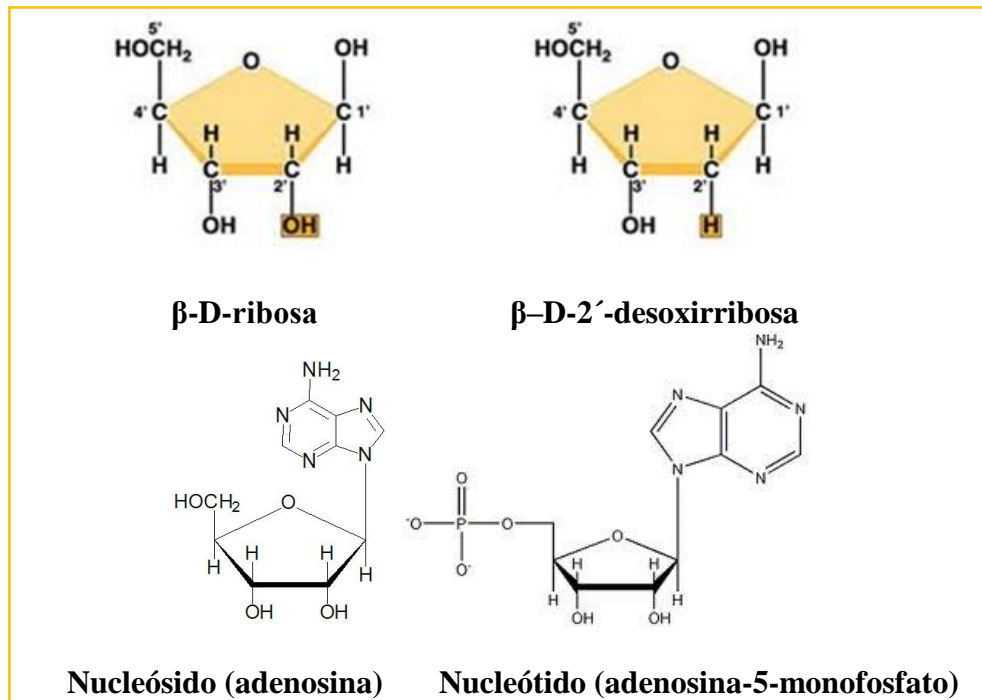


Figura 4.2. Estructura de los nucleósidos y nucleótidos.

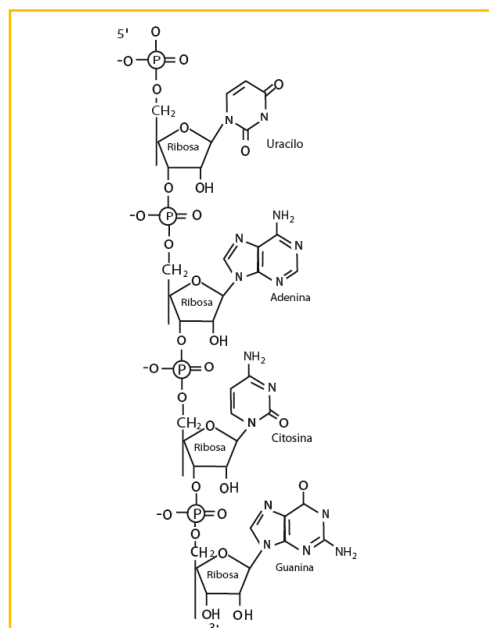


Figura 4.3. Forma esquemática de una cadena de RNA con los cuatro nucleósidos posibles⁸.

Al formarse la cadena de DNA o RNA las unidades de nucleótidos se unen mediante enlaces fosfodiéster entre el grupo 5' de un nucleótido y el grupo 3' del siguiente. Todos los enlaces fosfodiéster de las cadenas de DNA y RNA tienen la misma orientación a lo largo de la secuencia. Las pirimidinas son moléculas planas y las purinas son casi planas. Ambas son también hidrofóbicas lo cual da lugar a una interacción de apilamiento entre bases originando que las bases se sitúen con los planos de sus anillos paralelos. Entre las

bases se forman enlaces de hidrógeno entre los grupos carbonilo y amino dando lugar a la asociación de dos o más cadenas (Figura 4.3 y 4.4).

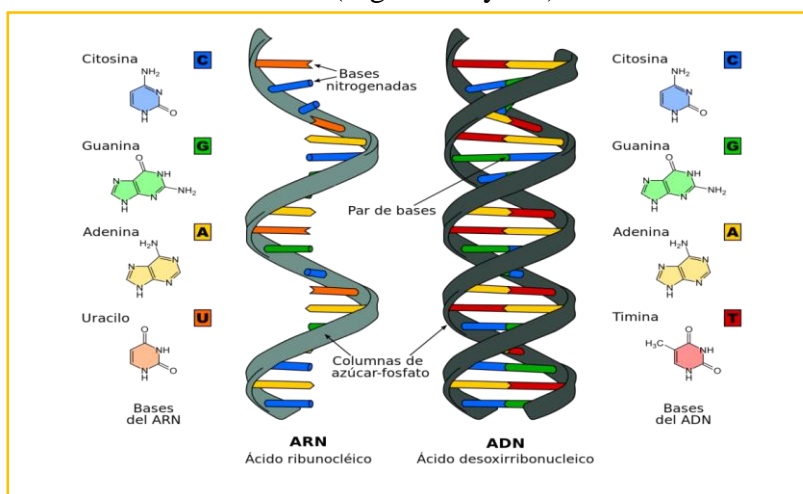


Figura 4.4. Esquema de DNA y RNA y las cuatro bases nitrogenadas⁸.

4.2. Caracterización de las moléculas: longitudes de enlace, ángulos de enlace, ángulos de torsión y ángulos diedros.

La estructura tridimensional de las moléculas se caracteriza por las longitudes de enlace, los ángulos de enlace, la rotación alrededor de un enlace central entre los átomos que se determina por el **ángulo de torsión θ** y el ángulo diedro ϕ complementario del ángulo de torsión.

Los valores de θ usados comúnmente en química orgánica propuestos por Klyne y Prelongson¹ son: ($\approx 0^\circ$) nombrado *syn*, ($\approx 80^\circ$) *anti*, ($\approx \pm 60^\circ$) \pm *synclinal*, ($\approx \pm 120^\circ$), *anticlinal*. En espectroscopía y cristalografía se emplea la notación: *cis* ($\approx 0^\circ$), *trans* ($\approx 180^\circ$), \pm *gauche* ($\approx \pm 60^\circ$) (Figura 4.5).

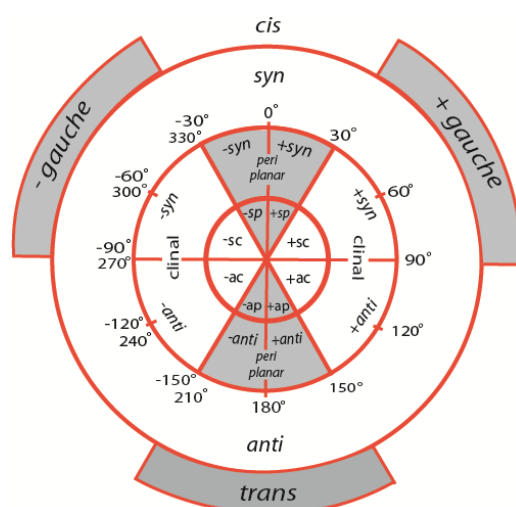


Figura 4.5. Correlación gráfica de los rangos de los ángulos de torsión empleados por los espectroscopistas orgánicos y los empleados en espectroscopía y cristalografía.

4.3. Definición de los ángulos de torsión en los nucleótidos: rangos utilizados en Química Orgánica y en cristalografía.

Los ángulos de torsión se definen según la Tabla 1:

Tabla 1.: Ángulos de torsión de los nucleótidos

Ángulo de torsión	Átomos implicados
α	$(n-1)O3' - P - O5' - C5'$
β	$P - O5' - C5' - C4'$
γ	$O5' - C5' - C4' - C3'$
δ	$C5' - C4' - C3' - O3'$
ϵ	$C4' - C3' - O3' - P$
ζ	$C3' - O3' - P - O5'_{(n+1)}$
χ	(en pirimidinas) $O4' - C1' - N1 - C2$ (en purinas) $O4' - C1' - N9 - C4$
ν_0	$C4' - O4' - C1' - C2'$
ν_1	$O4' - C1' - C2' - C3'$
ν_2	$C1' - C2' - C3' - C4'$
ν_3	$C2' - C3' - C4' - O4'$
ν_4	$C3' - C4' - O4' - C1'$

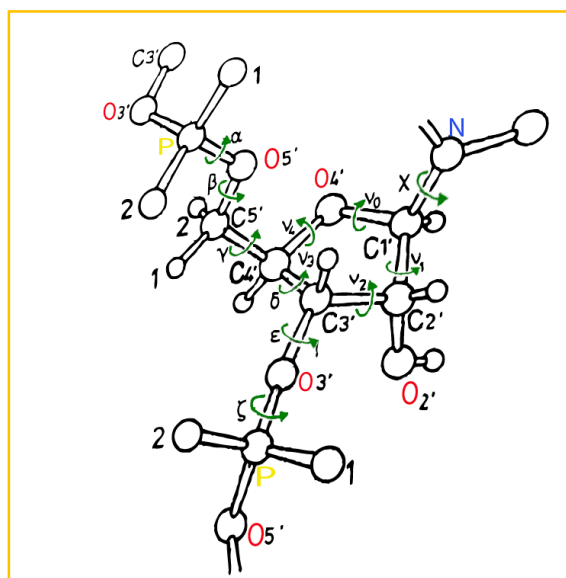


Figura 4.6. Esquemade los ángulos de torsión en los nucleósidos.

En los nucleótidos se define el ángulo pseudorrotacional de fase **P** a partir de los ángulos de torsión. (Ver en puntos siguientes).

4.4. Definición de los modos de plegado del azúcar en los nucleósidos.

El anillo de cinco átomos de la furanosa generalmente no presenta una estructura plana. Hay dos posibles formas: *envelop*, **E**, en la que se tienen cuatro átomos en un

plano y el quinto fuera del plano: *envelop* C₃-endo ³E y *envelop* C₂-endo ²E, según sea el C 3' ó 2' el que esté fuera del plano. Y *twist* (girado), **T**, con dos átomos adyacentes desplazados del plano: simétrico C₂-exo-C₃'-endo ³₂**T** y *twist* no simétrico con mayor plegamiento C₃'-endo y menor C₂-exo, ³**T**₂ (Figura 4.7).

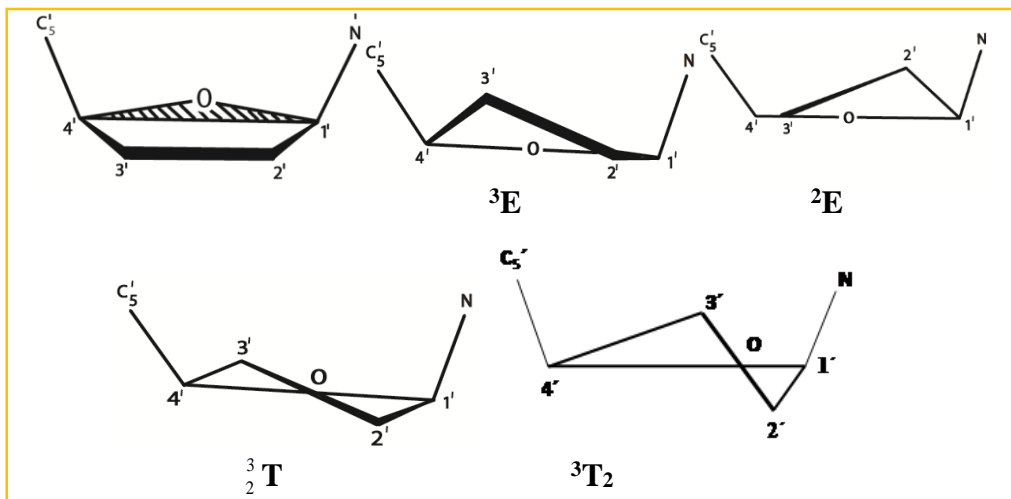


Figura 4.7. Modos de plegamiento del azúcar en los nucleósidos.

4.5. Orientación *syn/anti* del enlace glicosídico.

El enlace glicosídico tiene dos orientaciones respecto del ángulo χ (O4'-C1'-N1-C2) en las pirimidinas y (O4'-C1'-N9-C4) en las purinas. Estas dos orientaciones respecto de la furanosa se definen principalmente como: *anti* en la que la base se proyecta fuera del azúcar y *syn* en la que se proyecta sobre ésta (Figura 4.8).

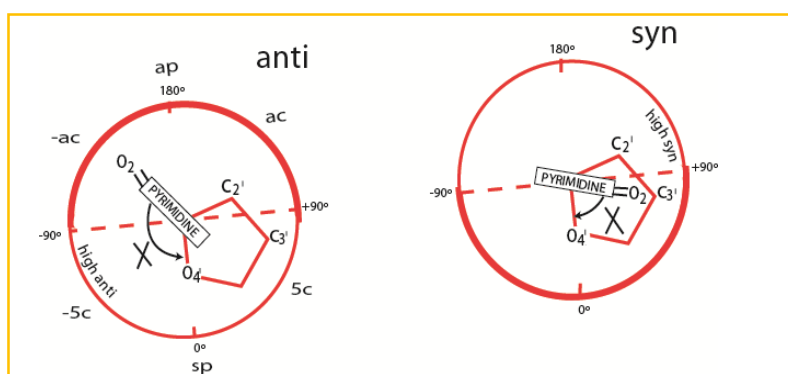


Figura 4.8. Definición de los rangos conformacionales en los nucleósidos.

4.6. Ángulo pseudorrotacional de fase P: círculo de pseudorrotación de la furanosa.

En los nucleótidos, el ángulo pseudorrotacional de fase P se calcula a partir de los ángulos endocíclicos de torsión del azúcar de la siguiente manera:

$$tgP = \frac{(v_4 + v_1) - (v_3 + v_0)}{2 \cdot (\text{sen } 32^\circ + \text{sen } 72^\circ)} \quad 4.1. F$$

El ángulo $P = 0^\circ$ se define de forma que el ángulo de torsión ν_2 tenga un valor positivo máximo, que corresponde a la forma 3_2T y la imagen en un espejo 2_3T se representa por $P = 180^\circ$ (ver Figura.4.9). El ángulo de torsión máximo ν_{max} describe el máximo movimiento fuera del plano y viene dado por:

$$\nu_{max} = \nu_2 / \cos P \quad 4.2.F$$

Para cada valor del ángulo de fase P , la suma de los ángulos de torsión positivos y negativos es cero:

$$\nu_0 + \nu_1 + \nu_2 + \nu_3 + \nu_4 = 0 \quad 4.3.F$$

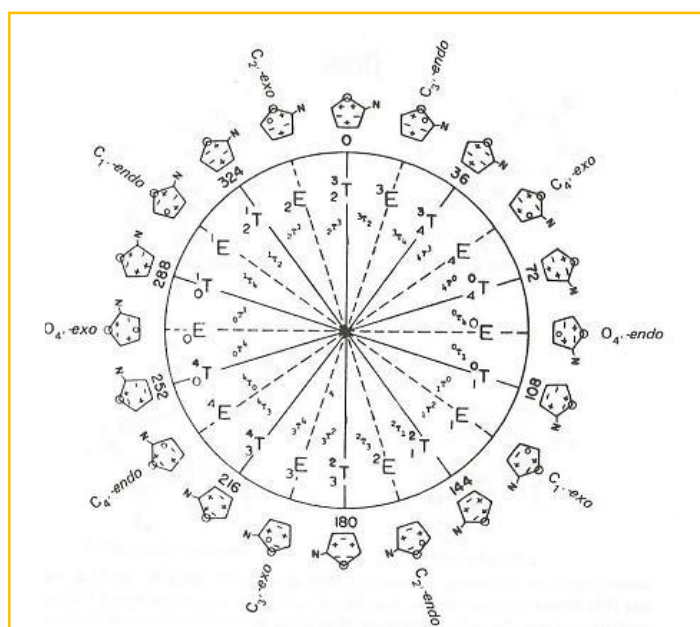


Figura 4.9. Ciclo de pseudorrotación del anillo de furanosa en los nucleósidos¹.

4.7. Referencias

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5. Cálculos mecano – cuánticos

En el estudio propuesto utilizaremos como herramienta fundamental los métodos de la Química computacional. Con el fin de una mejor comprensión de este trabajo, expondremos los métodos fundamentales desarrollados en esta ciencia, principalmente los métodos *ab initio* y los métodos basados en el funcional de la densidad, que utilizaremos en los cálculos siguientes.

5.1. Introducción a la Química computacional

La química computacional es una herramienta útil para calcular estructuras y propiedades de moléculas y de cuerpos sólidos. Se aplica para completar la información obtenida experimentalmente y en la predicción de determinados parámetros químicos y estructuras de nuevos compuestos y materiales¹. Principalmente, los cálculos realizados en el presente trabajo constan de los siguientes pasos:

- (i) Optimización de la energía de una estructura molecular determinada, es decir, la disposición en el espacio de electrones y núcleos atómicos.
- (ii) Obtención de las estructuras con los distintos ángulos de torsión y realización de optimizaciones geométricas correspondientes para determinar aquellas de menor energía. La optimización geométrica depende del gradiente de energía (la primera derivada de la energía respecto a la posición de los átomos).
- (iii) Determinación de las frecuencias de las vibraciones moleculares derivadas de las interacciones entre los átomos de la molécula. Propiedad que depende de la segunda derivada².

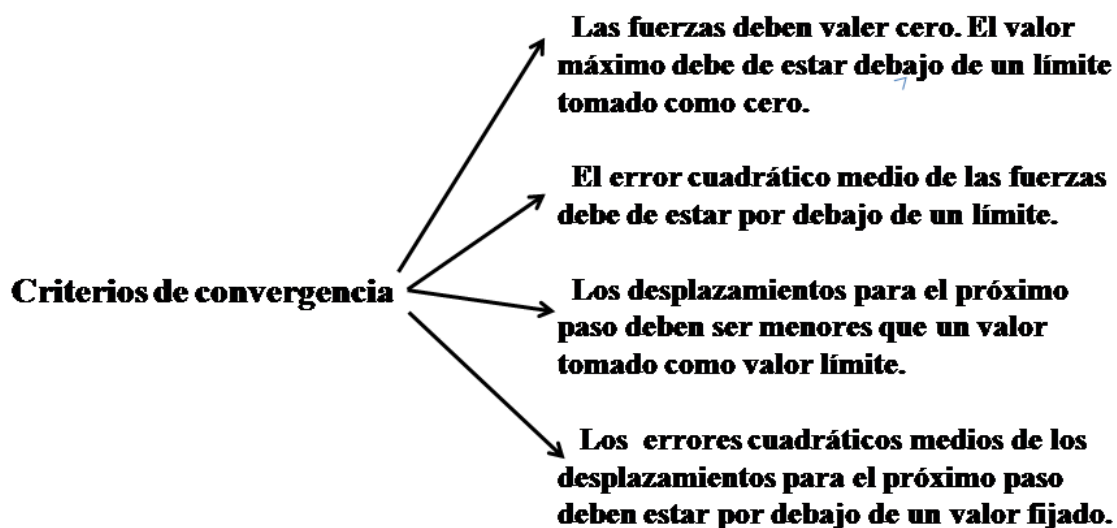
5.1.1. Optimizaciones geométricas

Las optimizaciones geométricas tienden a lograr los mínimos sobre la superficie de energía potencial (PES) que corresponden a las estructuras en equilibrio de los sistemas moleculares. También determinan las estructuras de transición. En los mínimos y en los puntos *de silla* “saddle”, la primera derivada o **gradiente** de la energía es cero y, por tanto, las fuerzas también serán cero en este punto. Un punto sobre la superficie de energía potencial en que las fuerzas son nulas recibe el nombre de **punto estacionario**.²

5.1.2. Criterios de convergencia

La optimización se ha completado cuando las fuerzas electrostáticas valen cero.

Se consideran los siguientes criterios de convergencia²

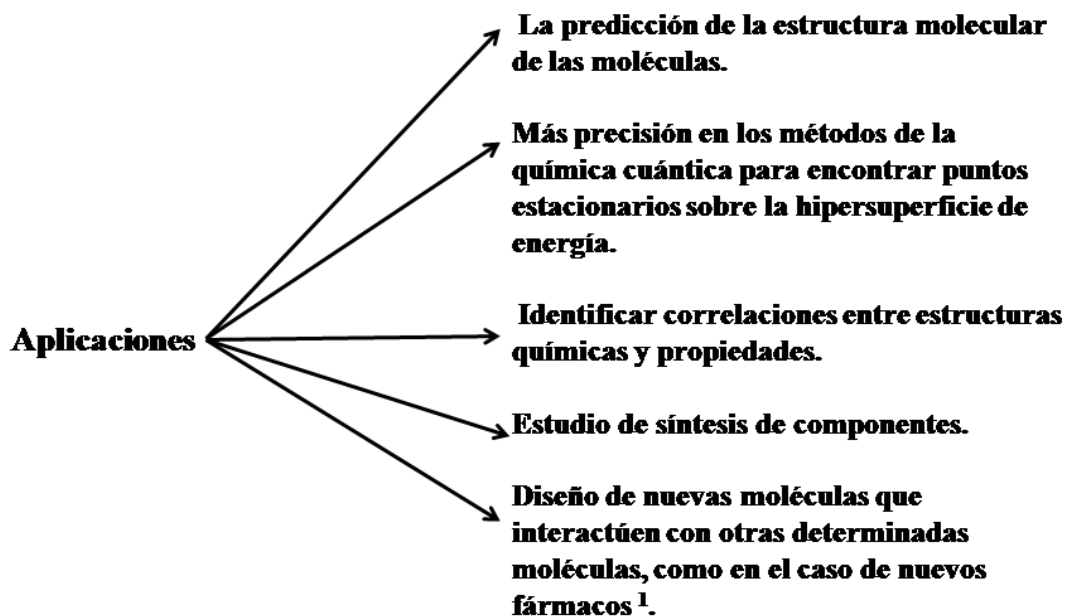


5.1.3. Cálculo de las frecuencias de vibración de las moléculas

Las frecuencias dependen de la segunda derivada de la energía respecto de la posición de los núcleos. Los cálculos deben realizarse sobre las estructuras optimizadas. Las segundas derivadas pueden calcularse por diferentes métodos teóricos²: HF, DFT (B3LYP), MP2...etc. que se expondrán más adelante.

5.1.4. Principales aplicaciones de la Química computacional

Con el avance en el desarrollo de ordenadores más rápidos y con mayor capacidad, la química computacional ha experimentado un desarrollo espectacular. Hoy en día hay numerosas aplicaciones, si bien su cálculo aún esta limitado a pequeñas estructuras. En el siguiente esquema se muestran algunas de las principales aplicaciones¹:



5.2. Métodos teóricos

El conjunto de métodos teóricos abarca un amplio abanico de metodologías computacionales las cuales difieren entre si principalmente en el grado de rigurosidad en que se aplica la teoría de la que se parte. El nivel teórico se basa en el funcional utilizado en el estudio de las interacciones de las partículas³. Los métodos más generalizados son^{1, 3, 4}:

5.2.1. Descripción de los diferentes métodos.

Los métodos cuánticos^{2, 3, 5-10} se basan en la formulación cuántica de la física molecular. En mecánica cuántica, el estado de un sistema se describe por su función de onda. La energía, magnitud física fundamental, y otras propiedades de la molécula pueden obtenerse resolviendo la ecuación de Schrödinger:

$$\mathbf{H}\Psi = \mathbf{E}\Psi \quad [5.1]$$

en donde **H** es el **operador Hamiltoniano**, definido según ciertas condiciones mecano-cuánticas, y que incluye la energía cinética y potencial de núcleos y electrones. El operador hamiltoniano, para un sistema de una partícula, es:

$$H = -\frac{\hbar^2}{2m} (\delta^2/\delta x^2 + \delta^2/\delta y^2 + \delta^2/\delta z^2) + V(x,y,z,t) \quad \hbar = \hbar/2\pi$$

siendo \hbar la constante de Planck y empleando el *operador laplaciana*:

$$\nabla^2 \equiv \delta^2/\delta x^2 + \delta^2/\delta y^2 + \delta^2/\delta z^2$$

el operador hamiltoniano para una partícula se escribe:

$$H = - (\hbar^2/2m) \nabla^2 + V$$

y para un sistema de muchas partículas:

$$H = - (\hbar^2/2m_1) \nabla_1^2 - (\hbar^2/2m_2) \nabla_2^2 - \dots - (\hbar^2/2m_n) \nabla_n^2 + V(x_1, \dots, x_n, t).$$

Esta ecuación puede escribirse según (5.1).

Ψ es la función de onda y E es la energía del sistema cuando se encuentra en el estado descrito por la función de ondas Ψ . La energía y otras propiedades de la molécula pueden obtenerse resolviendo esta ecuación. La solución exacta de la ecuación de ondas presenta dificultades para moléculas con más de tres partículas, que dan lugar a que se realicen una serie de aproximaciones.

La aproximación de Born – Oppenheimer se basa en que los electrones se mueven mucho más rápidamente que los núcleos y por ello se consideran por separado el movimiento de los electrones y el de los núcleos. Si se considera que los núcleos están fijos en el espacio, su energía cinética es cero y la energía potencial debida a la repulsión núcleo – núcleo es una constante, así el Hamiltoniano de la ecuación 5.1 se reduce al Hamiltoniano electrónico (H_{elec}). Al considerar los movimientos electrónicos se supone que los electrones se mueven respecto de una estructura fija formada por los núcleos de la molécula situados en sus posiciones de equilibrio. La energía potencial de los electrones consta de la suma total de las interacciones electrostáticas de atracción hacia los núcleos y de repulsión mutua.

Entonces la solución a la ecuación de Schrödinger es la función de ondas electrónica Ψ_{elec} (que depende solamente de las coordenadas electrónicas) y la energía electrónica E_{elec} .

$$H_{elec} \Psi_{elec} = E_{elec} \Psi_{elec} \quad [5.2]$$

Las coordenadas nucleares no aparecen explícitamente en Ψ_{elec} . La energía total E_{tot} es la suma de E_{elec} y del término de la constante de repulsión nuclear E_{nuc} .

$$E_{tot} = E_{elec} + E_{nuc} \quad [5.3]$$

La función de onda no es observable. La interpretación correcta fue dada por Max Born y sería que su cuadrado:

$$|\Psi(x_1, x_2, \dots, x_N)|^2 dx_1 dx_2 \dots dx_N \quad [5.4]$$

da la **densidad de probabilidad** de encontrar las partículas en un lugar del espacio.

Debido a que la probabilidad total de encontrar la partícula en algún sitio es 1, la función de estado está normalizada, lo que significa que la integral $|\psi|^2$ sobre todo el espacio de coordenadas es 1. La probabilidad de que los electrones 1, 2...n, se encuentren simultáneamente en los elementos $dx_1, dx_2 \dots dx_N$ y, puesto que los electrones no se distinguen unos de otros, esta probabilidad no debe cambiar si las coordenadas de dos electrones se intercambian. Se puede demostrar que, para los fermiones, la única posibilidad de que esto ocurra es que el intercambio tenga lugar con un cambio de signo. Los electrones son fermiones con spin $\frac{1}{2}$ y Ψ debe ser antisimétrica con respecto al intercambio de las coordenadas espaciales y de spin. Este principio de antisimetría representa la generalización del Principio de exclusión de Pauli. Y una consecuencia es que la integral de la ecuación 5.4, en todo el espacio de variables, sea igual a 1.

El resto de aproximaciones da lugar a las diferentes metodologías citadas en el punto anterior.

5.2.1.1. Métodos ab initio

Son los cálculos que se realizan aplicando las fórmulas de la Termodinámica Estadística y de la Mecánica Cuántica. Constan de tres etapas: 1) Aproximación a la estructura molecular. 2) Optimización para encontrar la geometría mínima. 3) Cálculo de frecuencias de vibración¹¹.

En la resolución de la Ecuación de Schrödinger no incluyen ningún parámetro empírico o semiempírico en sus ecuaciones. Todas derivan de principios teóricos, sin la inclusión de datos experimentales. En estos métodos la resolución del sistema se realiza de la forma más rigurosa posible y se aplica el principio variacional que establece que la energía de una función de onda aproximada siempre será superior a la energía exacta y, por tanto, la función de onda apropiada se logra cuando la energía es mínima^{3,10}.

Una aproximación está rigurosamente definida en base a los primeros principios (teoría cuántica) y su resolución es con un error conocido de antemano. Al aplicar métodos iterativos la solución se obtiene al iterar hasta obtener la máxima precisión que la computadora puede proporcionar.¹ Los métodos ab initio más comunes son:

5.2.1.1.1. Método de Hartree-Fock (HF)

El método de Hartree-Fock^{3,6,10,12-15} es el más sencillo y es un método iterativo para calcular la mejor solución a la ecuación de Schrödinger independiente del tiempo, para moléculas aisladas, tanto en su estado fundamental como en estados excitados. El método

se basa en suponer que los electrones se mueven independientemente unos de otros. La función de onda $\Psi_{el}(1, 2, \dots, n)$, en la que $1, 2, \dots, n$, representan las coordenadas de los n electrones de la molécula, se expresa de forma aproximada a partir de funciones que dependen de las coordenadas de un solo electrón; estas funciones son los **orbitales moleculares**. En la práctica, los electrones individuales están confinados en los orbitales moleculares, cada uno de los cuales se determina asumiendo que el electrón se mueve dentro de un campo promedio de los demás electrones. La función de onda se escribe en forma de un determinante (determinante de Slater) de orbitales de una partícula, término matemático de la Mecánica cuántica que se usa para generar funciones de ondas antisimétricas que describan los estados colectivos de varios fermiones y que cumplan el principio de exclusión de Pauli.

$$\Psi = 1/\sqrt{N} \cdot \begin{vmatrix} \chi_1(\mathbf{1}) & \dots & \chi_2(\mathbf{1}) & \dots & \dots & \dots & \chi_n(\mathbf{1}) \\ \chi_1(\mathbf{2}) & \dots & \chi_2(\mathbf{2}) & \dots & \dots & \dots & \chi_n(\mathbf{2}) \\ \vdots & & & & & & \\ \chi_1(\mathbf{n}) & \dots & \chi_2(\mathbf{n}) & \dots & \dots & \dots & \chi_n(\mathbf{n}) \end{vmatrix}$$

En el que χ_i recibe el nombre de spin orbital y es el producto de una función orbital u orbital molecular, ψ , y una función de spin α o β ¹⁵.

El punto de partida para el cálculo es un conjunto de orbitales aproximados que, para un cálculo atómico, son los orbitales de un átomo hidrogenoide y para las moléculas las funciones de onda iniciales son típicamente una combinación lineal de un número finito de conjuntos de funciones conocidas como funciones base ϕ :

Función base

$$\Psi_{\Gamma} = \sum_{\mu} (c_{\mu\Gamma} \phi_{\mu}) \quad [5.5]$$

Dónde $c_{\mu\Gamma}$ son los coeficientes de los orbitales moleculares (desconocidos). Como ϕ están centrados en las posiciones de los núcleos, se relacionan con los orbitales atómicos y la ecuación [5.5] se denomina Combinación Lineal de Orbitales Atómicos o aproximación CLOA.

Una vez se ha construido una función de ondas inicial se elige un electrón y se resume el efecto de todos los demás electrones que se usan para generar el potencial. Esto permite resolver la Ecuación de ondas para este electrón, obteniéndose una función de ondas para éste. El procedimiento se repite para cada uno de los otros electrones hasta completar un paso. Se obtiene así un nuevo potencial eléctrico y se repite el procedimiento hasta

alcanzar la convergencia (es decir, cuando el cambio entre un paso y el siguiente sea menor que un valor pequeño determinado). Este método iterativo ha recibido el nombre de **Self Consistent Field Method (SCF)**.

A continuación se describen varios conceptos:

Conjuntos base: Un conjunto base es una descripción matemática de los orbitales de un sistema molecular. Los conjuntos bases más grandes son una aproximación más exacta de los orbitales pues imponen pocas restricciones sobre la localización de los electrones en el espacio. Los conjuntos base estándar para cálculos de estructura electrónica son combinaciones lineales de funciones Gaussianas para formar cada orbital.

Funciones de base: Los conjuntos base asignan un grupo de funciones base a cada átomo en una molécula para definir los orbitales. Estas funciones base son una combinación lineal de funciones Gaussianas. A sus funciones bases se las nombra como funciones contraídas y las funciones Gaussianas componentes primitivas. Las funciones de base son combinaciones lineales de un conjunto de funciones de orbitales atómicos. Dichos orbitales pueden ser de tipo STO (Slater Type Orbital) o GTO (Gaussian Type Orbital). La diferencia entre ellos está en la función exponencial que describe el orbital. Los orbitales atómicos (OA) quedan expresados como combinación lineal de funciones de base centradas en los núcleos atómicos¹⁴.

La función de onda puede describirse mediante diferentes funciones de base. Si se asigna una sola función de base (como combinación lineal de funciones gaussianas) se tiene una *base mínima*. Las funciones denominadas *doble Z (DZ)* y *triple Z (TZ)* incluyen respectivamente dos o tres funciones de base por cada OA. En las llamadas **split – valence** los orbitales correspondientes a los electrones internos quedan descritos con una sola función de base y los de valencia con dos o más. Suelen incluirse funciones adicionales **funciones de polarización y funciones difusas**. Las primeras se indican (nominación de Pople) mediante un asterisco*. Dos asteriscos** indican que las funciones de polarización también se aplican a los átomos ligeros (hidrógeno y helio). El posible efecto de estas funciones sería, p.e. en el átomo de hidrógeno, la mínima función de base correspondiente a un orbital **s**, cuando se añade la polarización también se adiciona una función **p** a las funciones de base. Ello implica que los átomos de hidrógeno presenten asimetría respecto al núcleo, dato importante cuando se considera el enlace entre átomos. Similarmente bases **d** y **f** pueden adicionarse a funciones de base con orbitales **p** y **d** de valencia respectivamente. En este caso la notación indicando las funciones adicionales es (p,d)¹⁶.

Las **funciones difusas** hacen que la función orbital decaiga mucho más suave. Para esto se añaden contribuciones espaciales al orbital. Son muy importantes para sistemas donde los electrones están relativamente lejos del núcleo, como moléculas cargadas, y sistemas en estado excitado¹². Son versiones de gran tamaño de los orbitales atómicos tipos s y p en contraste con las funciones normales. Permiten que los orbitales ocupen una amplia región⁹. Se indican por un signo más (+). Si se tienen dos signos más (++) indica que la función difusa se adiciona también a los átomos ligeros (hidrógeno y helio).

Notación: a) Funciones de base mínimas: La forma más común es STO-nG, donde **n** es un entero que representa el número de funciones Gaussianas primitivas que comprende una simple base. Las bases mínimas dan valores poco precisos para trabajos de investigación. Parten de la teoría de Hartree-Fock pero introducen simplificaciones en la expresión del Hamiltoniano. Entre éstas aparece el uso de una base mínima para los electrones internos en el *core* y la aproximación ZDO (*Zero differential overlap*) que se basa en suponer que el producto de dos orbitales atómicos es cero en todo el espacio si los orbitales pertenecen a diferentes átomos. En esta aproximación se eliminan una gran cantidad de integrales y las restantes se estiman de diferentes formas a partir de datos experimentales. Una limitación de estos métodos es que b) Las bases split-valence se representan: X-YZg en donde X representa el número de Gaussianas primitivas que comprende cada función de las bases del orbital atómico **core** (interno); Y y Z indican que los orbitales de valencia están compuestos de dos funciones de base cada uno, el primero está compuesto de una combinación lineal de funciones Gaussianas primitivas Y y la otra de una combinación lineal de funciones Gaussianas primitivas Z. En este caso la presencia de dos números después del guión indica que esta base es un conjunto de bases split-valence double-zeta. Los conjuntos de bases triple- y cuádruple-zeta también se usan con la notación X-YZWg, X-YZWWg....etc.

Conjuntos Base Gaussiano

Los conjuntos base que se emplean en los cálculos realizados por el método de Hartree-Fock en los métodos que expondremos seguidamente y en los cálculos de configuración e interacción electrónica son del tipo de las funciones Gaussianas^{10,18, 20}. Estas bases constan de un polinomio cartesiano (coordenadas x, y, z) y una parte exponencial (r^2). Las funciones Gaussianas proporcionan la resolución exacta para el átomo de hidrógeno con un solo electrón.

La representación más simple de un orbital es la base mínima y comprende solamente aquellas funciones necesarias para situar todos los electrones del átomo teniendo simetría

esférica. Se tiene: Para el átomo de hidrógeno y el de helio una sola función (1s); para los átomos correspondientes a la segunda fila de la Tabla Periódica un conjunto de cinco funciones (1s, 2s, 2p_x, 2p_y y 2p_z); para los átomos de la tercera fila se precisa un conjunto de nueve funciones (1s, 2s, 2p_x, 2p_y, 2p_z, 3s, 3p_x, 3p_y, 3p_z). En los elementos de transición además de las funciones que describen ``core`` se precisan nueve funciones que describan la valencia (3d_{x²-y²}, 3d_{z²}, 3d_{xy}, 3d_{xz}, 3d_{yz}, 4s, 4p_x, 4p_y, 4p_z).

Los conjuntos de bases mínimas más comunes son:

STO-3G; STO-4G; STO-6G; STO-3G**

Los conjuntos de base **split – valence** más comunes son^{10,13,17}:

3-21G; 3-21G*; 6-31G*; 6-31G**; 6-31+G*; 6-31+G**; 6-31++G*;
6-31++G** 6-311G*; 6-311G**; 6-311+G*; 6-311+G**; 6-311++G*;
6-311++G**; cc-pVDZ; cc-pVTZ; cc-pVQZ.

Orbitales moleculares HOMO y LUMO

El orbital molecular HOMO (Highest Occupied Molecular orbital) corresponde al último orbital que se encuentra doblemente ocupado. Indica dónde se encuentra el par de electrones que puede arrancarse más fácilmente de la molécula.

LUMO (Lowest Unoccupied Molecular Orbital) es el primer orbital que se encuentra vacío. Indica el lugar dónde la molécula podrá aceptar más fácilmente un par de electrones².

Aproximaciones: En la aplicación del método se realizan cinco aproximaciones:

1. Se asume la aproximación de Born – Oppenheimer.
2. No se tienen en cuenta los efectos relativistas.
3. La solución variacional se asume que es una combinación lineal de un número finito de funciones de bases, las cuales suelen elegirse ortogonales.
4. Cada uno de los valores propios de la energía es describible mediante un determinante de Slater, un producto antisimétrico de una función de onda de un electrón (orbitales).
5. No se tienen en cuenta las desviaciones de la correlación de electrones¹⁶.

Los modelos Hartree-Fock: Restringido y no restringido (restricted y unrestricted):

Generalmente los sistemas moleculares presentan un número par de electrones, (singlete) los cuales se aparean en cada orbital con la misma energía y spin opuestos (sistema de capa cerrada). En este caso se llega al modelo restringido de Hartree-Folk (RHF). Cuando se tienen sistemas que presentan un número impar de electrones (sistemas

de capa abierta), como en algunos radicales o moléculas, el modelo HF no restringido (UHF) sitúa los electrones en orbitales separados según que el sentido del spin sea hacia arriba o hacia abajo (electrones α y β respectivamente)².

5.2.1.1.2. Métodos post-Hartree-Fock

Los métodos post-HF más empleados son los derivados del tratamiento de Møller y Plesset también llamada **Teoría de perturbaciones de Møller-Plesset** y **Teoría de perturbaciones de Rayleigh y Schrödinger**. Estos métodos dividen el hamiltoniano en dos partes H_0 , que recoge los efectos principales y corresponde al utilizado en el método HF, y para el que se conocen los vectores y los valores propios, y una perturbación menor V :

$$H = H_0 + V \quad [5.6]$$

dentro de la perturbación se incluyen los efectos de correlación electrónica.

Dentro de la teoría de la perturbación se introduce un hamiltoniano generalizado (H_λ), donde λ es un parámetro adimensional que varía entre 0 y 1:

$$H_\lambda = H_0 + \lambda V \quad [5.7]$$

La función de onda Ψ_λ y la energía E_λ correspondientes al Hamiltoniano H_λ pueden expresarse según una suma de los términos:

$$\Psi_\lambda = \Psi^{(0)} + \lambda \Psi^{(1)} + \lambda^2 \Psi^{(2)} + \dots \quad [5.8]$$

$$E_\lambda = E^{(0)} + \lambda E^{(1)} + \lambda^2 E^{(2)} + \dots \quad [5.9]$$

Las correcciones para la correlación electrónica empiezan en el segundo orden (MP2) que sirve para la optimización geométrica. Un cálculo más exacto de la energía empieza en 3º y 4º orden en el desarrollo perturbativo, obteniendo diferentes órdenes en que se valora la energía de correlación MP3, MP4etc. Estos métodos son más costosos que MP2^{3,4,17}.

5.2.1.2. Métodos semiempíricos

Los métodos de campo autoconsistente HF precisan el uso intensivo de computadoras pero en sistemas más grandes es simplificar el gasto computacional. Así aparecen los métodos semiempíricos y empíricos. Estos sólo pueden utilizarse para sistemas cuyos parámetros han sido desarrollados para todos los átomos que lo componen. Tampoco se determinan interacciones como las asociadas con los hidrógenos. El método

semiempírico más conocido es el método AM1 (Austin model 1) que introduce un conjunto de funciones gaussianas adicionales a cada átomo que permiten modular las interacciones de largo alcance y mejorar las interacciones covalentes. Es útil en el estudio de geometrías moleculares y energías de sistemas estabilizados por enlaces de hidrógeno.

En los métodos semiempíricos se utiliza una base mínima que reduce el tiempo de cálculo^{3,9}.

5.2.1.3. Métodos basados en el funcional de la densidad (DFT)

Teoría del funcional de la densidad (DFT): Esta teoría^{3,9,17,19} es una alternativa a la metodología *ab initio* en la que el punto de partida es la densidad electrónica y no la función de ondas de muchos cuerpos. La energía del estado fundamental de un sistema polielectrónico puede expresarse a partir de su densidad electrónica (Teorema de Hohenberg y Konh). La ventaja es que la densidad es un objeto mucho más simple que dicha función de ondas y, por lo tanto, más fácil de calcular. Para un sistema que comprende n electrones, $\rho(r)$ representa la densidad electrónica total en un punto particular del espacio r . De acuerdo con el formalismo de la DFT, la energía electrónica E se considera como un funcional de la densidad electrónica $E[\rho]$, en el sentido que a una función dada $\rho(r)$ corresponde una única energía. Sin embargo no se conoce de manera exacta la fórmula matemática que relaciona la energía con la densidad electrónica y se debe recurrir a aproximaciones.

En la mecánica cuántica el objeto básico es la función de onda y la energía es un **funcional** de esta función de onda. En DFT, el objeto básico es la densidad y la energía será un funcional de ésta, cumpliéndose que la energía será mínima para la densidad real del sistema. El problema es que no se conoce la forma completa de este funcional. Konh y Sham (1965) presentaron un desarrollo matemático que es la base de las actuales aplicaciones de DFT en la química computacional. Este formalismo daba una forma práctica para resolver el teorema de Hohenberg – Khon para un conjunto de electrones que interactúan entre sí a partir de un sistema virtual de electrones que no presentaban interacciones y que tenían una densidad total en el estado base igual a la densidad de algún sistema real en el que los electrones interactúan. El problema principal era la representación de la energía cinética de un sistema. Según la aproximación de Kohn y Sham, el funcional de la energía cinética de un sistema se puede dividir en dos partes: una parte que puede calcularse exactamente y que considera a los electrones como partículas

que no interactúan y un pequeño término de corrección para la interacción electrón – electrón.

Siguiendo este formalismo, en un orbital, la energía electrónica del estado base de un sistema con n electrones y N núcleos se puede escribir como suma de cuatro términos: el primer término representa la energía cinética de los electrones que no interactúan, el segundo término las interacciones nuclear – electrones, el tercer término corresponde a las repulsiones de Coulomb entre la distribución total de carga a r_1 y r_2 y el cuarto término, llamado término de intercambio – correlación, representa la corrección a la energía cinética debida a la interacción electrón – electrón.

La energía del sistema en función de la densidad electrónica se puede expresar como suma de diferentes términos $E(\rho) = E_{KE}(\rho) + V(\rho) + E_{XC}(\rho)$ [5.10]

En la que $E(\rho)$ es la energía total, $E_{KE}(\rho)$ es la energía cinética, $V(\rho)$ es la interacción con el potencial interno y $E_{xc}(\rho)$ es la energía de correlación intercambio. A partir de esta ecuación se llega fácilmente a una expresión matemática que se puede transformaren las ecuaciones de Hartree – Fock pero con un término perturbativo debido a la energía de correlación.

La corrección ZPE (zero – point energy) corresponde a la energía en el punto cero. Se considera que los átomos tienen una energía vibracional residual a muy bajas temperaturas no estando fácilmente unidos para formar un sólido estable^{17,20}.

5.2.1.4. Ventajas e inconvenientes de la teoría del funcional de la densidad

Las principales ventajas que presenta esta teoría residen en la obtención de buenos resultados tanto geométricos como en frecuencias de vibración, momentos dipolares, y buena estimación de la Termoquímica. Se obtienen buenos valores en moléculas que no se describen bien en el método de Hartree Fock. Los métodos DFT dan resultados análogos y en algunos casos mejor que los obtenidos con métodos HF, con unos requerimientos computacionales mucho menores.

Los principales inconvenientes residen en que se tratan de forma incorrecta los sistemas con un solo electrón y los que presentan enlaces débiles (van der Waals). La disociación de enlaces en especies iónicas y el tratamiento de estados excitados, y ante todo los problemas que derivan de la aplicación del potencial de correlación – intercambio cuando no da los resultados esperados, son aspectos que hay que mejorar.

5.2.1. Introducción a los métodos clásicos (MM)

En anteriores apartados he centrado mi atención en aquellos métodos que se van a aplicar en el estudio de las moléculas, que en este caso son los métodos QM basados en el funcional de la densidad y métodos semiempíricos. Sin embargo, una sencilla introducción a los métodos clásicos me pareció apropiada. En estos métodos la energía de un sistema químico se expresa únicamente en función de las posiciones de los núcleos y se incorpora la información de los electrones. El conjunto de parámetros utilizados se denomina campo de fuerzas (FF, *force-field*). La energía total de un sistema se calcula sumando la energía de los términos enlazantes (stretching, bending) y la energía de los términos no enlazantes (cálculo de las cargas asignadas a los átomos mediante la ecuación de Coulomb, término de van der Waals).

5.2.2. Sistemas en disolución

Los cálculos que hemos tratado corresponden a los sistemas en fase gaseosa (en estado aislado). Estos cálculos no son apropiados para describir el comportamiento de las moléculas en disolución en los que la presencia del disolvente afecta a las propiedades del sistema. Por otra parte hay que tener en cuenta que la mayoría de las reacciones químicas tienen lugar en disolución por lo que es importante estudiar estos sistemas.

Cuando se sumerge un soluto polar en un líquido formado por moléculas polares se produce una distorsión de la estructura del disolvente, originándose un campo eléctrico en éste que reacciona con el soluto dando lugar a fenómenos de solvatación de las moléculas del soluto y a la variación de diferentes propiedades².

Métodos teóricos: Los métodos para el estudio de los sistemas en disolución se clasifican, en una primera aproximación, en: **discretos**, que consideran al disolvente formado por moléculas y métodos **continuos**, que consideran al disolvente como un medio continuo caracterizado por la constante dieléctrica ϵ . Si se tienen en cuenta el nivel de la teoría aplicada se tienen métodos **cuánticos o clásicos**. Según se realicen los cálculos se tienen cuatro tipos de métodos que provienen de la aplicación de los anteriores: continuo – clásico, discreto – clásico, continuo - cuántico y discreto - cuántico.

Los métodos SCRF (Self-consistent Reaction Field) corresponden a los cálculos en presencia de un disolvente usando los modelos que citamos a continuación. En estos métodos el disolvente se considera como un medio continuo de constante dieléctrica ϵ uniforme (reaction field) y el soluto está situado en una cavidad dentro del solvente. Se

tienen cuatro modelos que difieren en la forma en que definen la cavidad y el campo de reacción:

1 – *Onsager*: Es el modelo más simple. Sitúa al soluto en una cavidad esférica dentro del campo de reacción del disolvente. Un dipolo en la molécula inducirá un dipolo en el medio y el campo eléctrico aplicado por el solvente volverá a actuar con el dipolo molecular estabilizando el conjunto.

2 – Polarizable continuum models (PCM): La cavidad en el solvente se crea por una serie de esferas solapadas.

3 – IPCM (Isodensity Surface Polarized Continuum Model): Define la cavidad como una superficie de densidad constante que está determinada por un proceso iterativo en el cual se realiza el ciclo SCF y converge usando *isodensity* actual de la cavidad.

4 - SCI-PCM (Self Consistent Isodensity Model): el efecto de la solvatación se enlaza en la computación iterativa SCF lo cual comprende un paso extra más. Así considera para todo el acoplamiento entre la cavidad y la densidad electrónica e incluye términos que acoplan que IPCM descuida².

5.2.3. Hidratación

Para simular teóricamente los efectos de la hidratación en una determinada molécula hay tres maneras^{22, 23, 24} usando: a) el modelo continuo, b) el "modelo discreto", en el que las moléculas de disolvente se representan solamente por cargas puntuales situadas en sus posiciones atómicas y c) el "modelo explícito" (EM) en el que se incluyen un suficiente número de moléculas de agua^{23, 24, 25, 26}.

El EM describe adecuadamente la presencia de las moléculas de agua en el conjunto agua-base y las interacciones soluto-disolvente. En este modelo se considera un determinado número de configuraciones a pesar del gran número de moléculas de agua precisas para simular una disolución diluida y el coste computacional del proceso que limita su aplicación. En la presente Tesis se ha aplicado este método, previa información²⁷ de como ocurre la hidratación con varias bases^{26, 28} obteniéndose un buen resultado en el primer nivel de hidratación, mejor que con otros métodos.

5.2.3.1. Energías de interacción

Las energías obtenidas en la hidratación de la base con un determinado número de moléculas de agua se corrigieron mediante (BSSE) usando el procedimiento (CP)²⁶. La

corrección CP en la energía de formación del complejo BW_n se calcula como suma de dos valores: la interacción *base-agua* $\Delta E_{B-(Wn)}^{CP}$ y *agua-agua* $\Delta E_{(Wn)}^{CP}$:

$$\Delta E_{B(Wn)}^{CP} = \Delta E_{B-(Wn)}^{CP} + \Delta E_{(Wn)}^{CP} \quad [5.11]$$

en la que $\Delta E_{B-(Wn)}^{CP}$ se calcula según:

$$\Delta E_{B-(Wn)}^{CP} = E_{BWn}^{(BWn)}(BW_n) - E_B^{(BWn)}(BW_n) - E_{(Wn)}^{(BWn)}(BW_n) + E_B^{(def)}(BW_n) \quad [5.12]$$

y la corrección CP de la energía de interacción *agua-agua*, correspondiente a la energía de formación de la molécula de agua n , en presencia de la base B, se calcula según:

$$E_{(Wn)}^{CP} = E_{(Wn)}^{(BWn)}(BW_n) - \sum_{n=1}^n E_{Wi}^{(BWn)}(BW_n) + \sum_{i=1}^n E_{Wi}^{def}(BW_n)$$

La energía de deformación del monómero X (X=B ó W) viene dada por:

$$E_X^{(def)}(BW_n) = E_X^{(X)}(BW_n) - E_X^{(X)}(X)$$

Los subíndices indican el sistema molecular y los superíndices indican si los cálculos se realizan con la base B, con la base de una molécula de agua (W), o con la base del sistema (B- W_n). Lo escrito dentro de los paréntesis indican si el cálculo se hace en la geometría optimizada del sistema (BW_n) o en el monómero (X).

Se han realizado estos cálculos en el monohidrato de los tautómeros HX-19 y HX-17 situando la molécula de agua en las tres posiciones posibles, y en los tautómeros 15 y 25 del ALO, colocando la molécula de agua en las cuatro posiciones posibles. Se han realizado también en el hidrato con 20 moléculas de agua de la HX-19.

Cálculos: La hidratación de los tautómeros se llevó a cabo mediante el esquema (MSM) basado en trabajo de Pullman²⁹. El cálculo vibracional se realizó en todos los hidratos obtenidos para garantizar que corresponden a estructuras estables mediante la evaluación de la 2ª derivada de la energía respecto del desplazamiento nuclear. Las energías relativas incluyeron la corrección en el punto cero (ZPE), y las cargas atómicas se calcularon mediante el procedimiento NBO^{30,31}.

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6. Metodología

El estudio teórico de las bases nitrogenadas HX y ALO y de los nucleósidos INO y ACV se ha realizado mediante cálculos mecanocuánticos usando el paquete de programas **Gaussian03**¹ y **Gaussian09**. Se han utilizado métodos **DFT** y **MP2**. En el primero usando **B3LYP** (Becke-style 3 Parameter Density Functional Theory, utilizando el funcional de correlación Lee-Yang-Parr) que se ajusta bien a los cálculos realizados y además presenta una economía computacional con respecto al método MP2. Éste, sin embargo, más costoso, obtiene unos valores de la energía más exactos.

Las funciones base utilizadas son:

6-31G(d,p): 6 indica que los electrones del core vienen definidos por 6 Gaussianas; 31 indica que los orbitales de los electrones de valencia se duplican, uno de ellos viene definido por 3 gaussianas y el otro por una; d indica polarización, (*d* y *p*) indica la inclusión de orbitales *p* al H.

6-311++G(3df,pd): pone tres funciones **d** y una **f** sobre átomos pesados, funciones **p** y **d** sobre el hidrógeno, y funciones difusas++¹¹.

Se ha seguido el siguiente procedimiento: Una vez dentro **GaussView**, (programa de gráficos de moléculas), se construye la molécula en estudio partiendo de la ventana *Nucleosides* y posteriormente *Guanosine*, en nuestros cálculos. La fórmula obtenida se modifica mediante los comandos oportunos y, en su caso, se suman las moléculas (generalmente H₂O) necesarias, obteniéndose en un primer paso la molécula o estructura deseados (Figura 6.1).

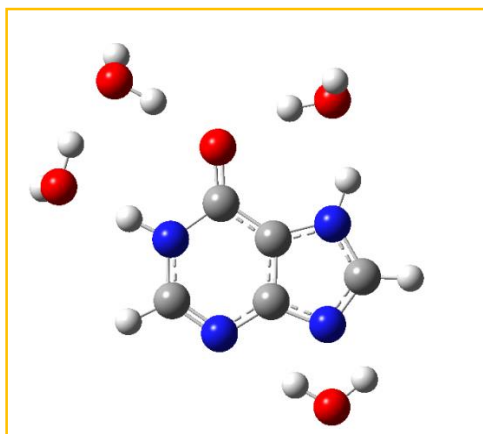


Figura 6.1. Modelo de estructura molecular obtenido utilizando el programa Gauss View

Se escriben los comandos necesarios (ver tabla adjunta) que determinan el archivo de partida (***.gjf**).

Tabla 2.: Archivo de partida.

```
%mem=85MW
```

```
%NprocShared=1
```

```
$ rungauss
```

```
# B3LYP/6-31G(d,p) opt freq
```

```
de: HX17+3aguaN7N1N1(opt)+1agua en N9: HX17+4ag3N9.
```

```
0 1
```

```
N
```

```

C      1      B1
H      2      B2      1      A1
C      2      B3      1      A2      3      D1
C      4      B4      2      A3      1      D2
O      5      B5      4      A4      2      D3
N      5      B6      4      A5      2      D4
H      7      B7      5      A6      4      D5
C      7      B8      5      A7      4      D6
N      9      B9      7      A8      5      D7
C     10     B10     9      A9      7      D8
H      9     B11     7      A10     5      D9
N      2     B12     1      A11     11     D10
H     13     B13     2      A12     1      D11
O     13     B14     2      A13     1      D12
H     15     B15    13      A14     2      D13
H     15     B16    13      A15     2      D14
H      7     B17     5      A16     4      D15
O      7     B18     5      A17     4      D16
H     19     B19     7      A18     5      D17
O     19     B20     7      A19     5      D18
H     21     B21    19      A20     7      D19
H     21     B22    19      A21     7      D20
H      1     B23     2      A22    13      D21
O      1     B24     2      A23    13      D22
H     25     B25     1      A24     2      D23

```

```

B1      1.32326742      B18      2.78150870
B2      1.08182250      B19      0.96536779
B3      2.17947459      B20      2.69049539
B4      1.42020971      B21      0.98488469
B5      1.25885432      B22      0.96522376
B6      1.38710278      B23      1.59031228
B7      1.04254835      B24      1.94829742
B8      1.37412518      B25      0.96000000
B9      1.30044837      A1      124.43007638
B10     1.36615305      A2      76.73136896
B11     1.08647408      A3      168.94349573
B12     1.36181027      A4      127.34847238
B13     1.03082573      A5      110.64500078
B14     2.76602512      A6      118.44199052
B15     0.98373123      A7      123.97059812
B16     0.96566627      A8      126.01906732
B17     3.08645642      A9      113.64077835

```

A10	114.29669443	D5	-179.24009352
A11	114.10905161	D6	0.36624438
A12	130.87633720	D7	-0.51205319
A13	150.43404329	D8	0.14970323
A14	79.51496745	D9	179.40815389
A15	114.34836344	D10	0.04662104
A16	101.28306475	D11	179.52134864
A17	119.60772512	D12	178.45774575
A18	113.02400560	D13	177.58605012
A19	86.73110258	D14	-81.13779670
A20	89.99498462	D15	177.32100244
A21	115.91655585	D16	-179.81996203
A22	108.30477159	D17	101.51489148
A23	137.27481610	D18	-6.40708191
A24	59.89721059	D19	2.84907562
D1	179.95631852	D20	109.26235334
D2	-178.89195523	D21	153.53915596
D3	-1.10803198	D22	148.08686926
D4	178.82407864	D23	-142.75419025

Dicho archivo se lanza en **ABACO**, ordenador central de cálculo de La Universidad Complutense de Madrid, (posteriormente, en QUIPU por la clausura del primero), hasta lograr la optimización y demás parámetros en estudio (energía, frecuencias...). De esta manera se obtiene el archivo (*.log) que proporciona la información solicitada, parámetros estructurales de la molécula, energía, cargas, frecuencias y demás propiedades. El estudio conformacional de las moléculas se realizó fundamentalmente en el nivel de cálculo MP2/6-31G(d,p) porque se obtienen los valores de la energía muy exactos, sin embargo, el nivel B3LYP/6-31G(d,p) se empleó en todos los casos y, muy principalmente, en aquellos clusters de gran tamaño molecular en los que no fue posible utilizar MP2 por el gran gasto computacional que ello representaba. Este método B3LYP presenta unos resultados que están en concordancia con los obtenidos con otros métodos que suponen un mayor gasto computacional (como MP2) y predice los números de ondas vibracionales de las bases del DNA, en muchos casos es mejor que métodos como HF y MP2^{2,3,4}. También se realizaron cálculos en otros niveles de mayor complejidad B3LYP/6-311++G(3df,pd) y MP2/6-311++G(2d,p).

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7. Estudio de las moléculas de hipoxantina (HX), alopurinol (ALO), inosina (INO) y aciclovir (ACV)

La base hipoxantina cuya estructura molecular aparece en la figura 7.1. tiene tres moléculas que presentan características afines: alopurinol, inosina y la molécula de aciclovir, esta última con características que recuerdan a los nucleósidos. En la figura se indica la estructura de todas ellas. Así mismo, además de su uso específico en el tratamiento de ciertas enfermedades que se describirán en los siguientes puntos, presentan propiedades terapéuticas análogas en enfermedades graves.

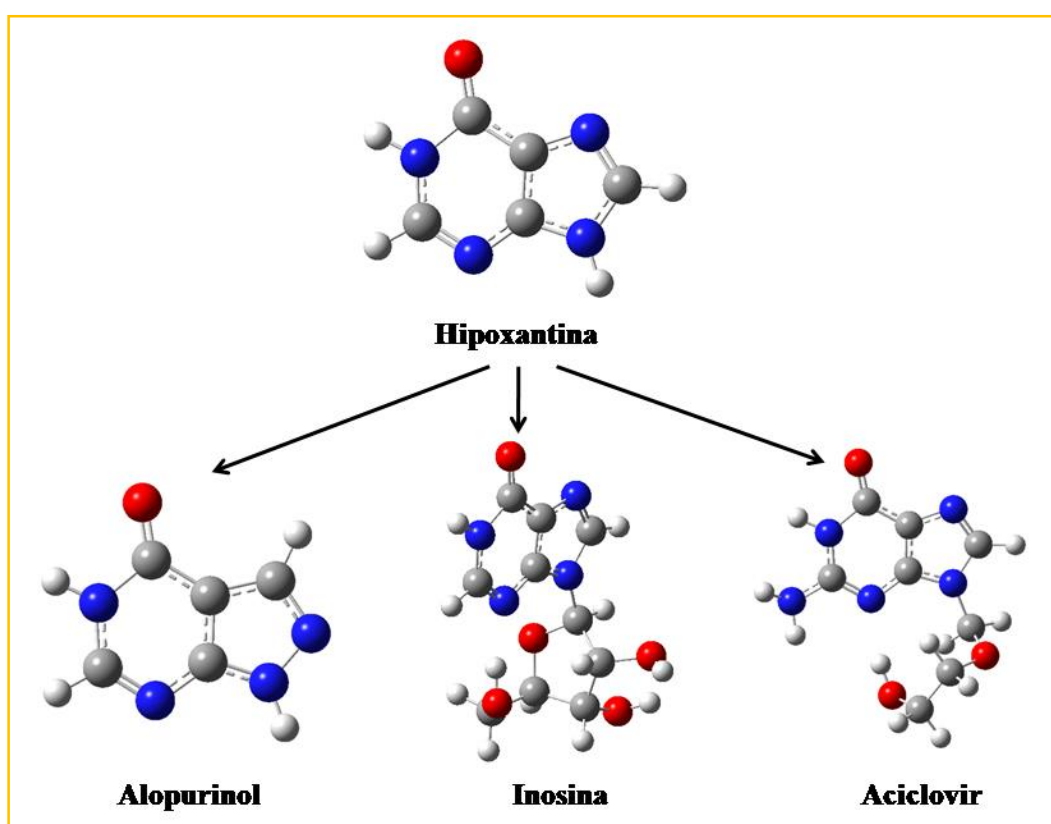


Figura 7.1. Moléculas de hipoxantina, alopurinol, inosina y aciclovir.

ALO es una base análoga a HX (isómero de HX) en la que se ha alterado la posición de los dos átomos de nitrógeno del anillo de purina, INO es el nucleósido derivado de HX y ACV incorpora una cadena lateral que confiere propiedades análogas a los nucleósidos. En la Tabla 7.1. se tienen las principales analogías entre estas moléculas.

Tabla 7.1. Analogías entre las moléculas

HX	ALO	INO	ACV
Base	Base	Nucleósido	Derivado de HX (análogo a nucleósido)
Derivada de G	Isómero de HX	Derivado de HX	Aplic. en Medicina
Aplic. en Medicina	Aplic. en Medicina	Aplic. en Medicina	Tautómeros ceto y enol
Tautómeros ceto y enol	Tautómeros ceto y enol	Tautómeros ceto y enol	Más estable forma ceto
Más estable forma ceto	Más estable forma ceto	Más estable forma ceto	Possible formación de complejos metálicos
Forma complejos con metales divalentes y Pt y Au	Forma complejos con metales (Cu, Co)		Enlaces y ángulos (en HX de ACV) no difieren significativamente de HX-19
	Enlaces y ángulos no difieren de HX	Enlaces y ángulos (en HX de INO) no difieren significativamente de HX-19	

En los siguientes apartados pasaremos a describir el estudio realizado en cada una de estas moléculas, procurando no ser repetitivo con la publicación correspondiente, poniendo así solo parte de ésta en la presente memoria, y dejando el resto en la publicación. La restante parte de la memoria está sin publicar.

7.1. Hipoxantina

La hipoxantina (HX) 1,7-dihydro-6H-purin-6-one, es una base derivada de la purina, cuya fórmula viene representada en la figura 7.1.1, junto con la notación. HX es un derivado mutagénico de la purina y sirve de llave en la síntesis *novo* de los nucleótidos de purina de los sistemas vivos. Se encuentra como constituyente de los ácidos nucleicos y del nucleósido inosina y, en menor cantidad, en RNA de transferencia. Es un producto intermedio en el metabolismo de la purina formada por degradación de los ácidos nucleicos. En el hombre, HX es oxidada a xantina y ácido úrico. HX puede depositarse en los tejidos y articulaciones y la presencia de ácido úrico origina la enfermedad de la gota¹⁻⁷.

HX tiene especial importancia en la investigación bioquímica y farmacológica, principalmente en el tautomerismo de los ácidos nucleicos y en el polimerismo del DNA^{8,9}.

Para realizar este trabajo de investigación se ha realizado un estudio previo sobre los tautómeros, la geometría, estabilidad, momento dipolar, polarizabilidad y el número de ondas de HX y purinas análogas^{6, 10-13}. También las formas catiónicas y aniónicas de los

tautómeros¹⁴⁻¹⁸, y por último sobre los cristales de cloruro de HX^{19a}, siendo abundante la información existente (lo cual indica la importancia de los mismos) y de su utilización en Biología^{19b}, en Medicina²⁰⁻²⁶, en la investigación de los ácidos nucleicos²⁷.

Es relevante la importancia de esta base en la obtención de complejos con metales divalentes^{28c}, los complejos de Pt^{28,29} con actividad anticancerígena, los complejos de Au y trifenilfosfina mono y disustituída (HLn) de composición general [Au(Ln)(PPh3)]³⁰.

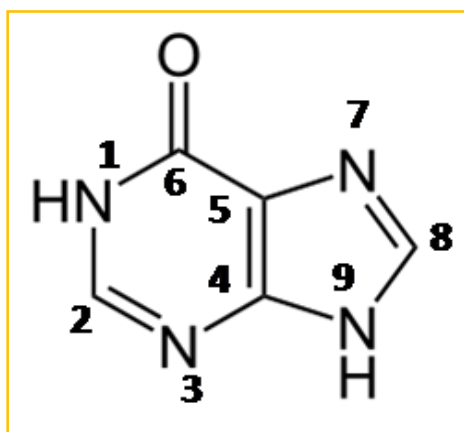


Figura 7.1.1. Molécula de HX (tautómero HX-19) y su numeración.

HX presenta una serie de tautómeros con diferente estructura, con formas ceto y formas enol. 14 formas han sido estudiadas y determinadas sus energías⁶ y en 21 formas se ha calculado su ΔG^5 . Para nombrar a los diferentes tautómeros se tienen diferentes nomenclaturas pero se ha preferido utilizar la propia por considerar que es la más sencilla y fácil de escribir. Así se tienen las formas cetónicas con el oxígeno de la molécula en forma C=O y las formas enólicas en las que el oxígeno aparece en forma OH y que se presentan en forma *cis* y en forma *trans*. Las formas cetónicas las nombraremos HX seguido de dos cifras que indican la posición de los átomos de H en la molécula. P.e. la figura 7.1.1 se leería HX-19. Las formas enólicas, también se nombrarán como HX seguido de la cifra que indica la posición del H en la molécula, el cero seguido de la terminación *cis* o *trans* (Figura 7.1.2).

Kondratyuk et al⁷ han determinado 13 tautómeros, aunque solo dos formas se han detectado experimentalmente, las formas cetónicas: (HX-17) (Figura 7.1.2a) y (HX-19) (Figura 7.1.2b)⁶. De los restantes tautómeros, se han estudiado cuatro formas consideradas como las de menor energía respecto a HX-17: tres formas enol (dos *cis* y una *trans*) (Figura 7.1.2c,d,e) y una forma ceto HX- 37 (Figura 7.1.2f).

El tautómero HX-17 es el de menor energía⁶. En la figura 7.1.3 se tienen las estructuras de los tautómeros más estables y sus correspondientes energías calculadas en los niveles B3LYP/6-311++G(3df,pd) y MP2/6-311++G(2d,p), M052X/6-31G(d,p) y M06L/6-

31G(d,p). En la Tabla 7.1.1. se muestran las energías relativas de los seis tautómeros más estables respecto de HX-17, para todos los niveles de cálculo B3LYP y MP2, y sus correspondientes poblaciones calculadas según la ecuación de Boltzman. El tautómero HX-19 es el que tiene la energía menor respecto de HX-17 y el tautómero HX-37 es el de mayor energía.

La población se ha determinado al nivel B3LYP y a las temperaturas de de 273 K y 298 K habiendo el mayor porcentaje en HX-17 seguido de HX-19. Los demás tautómeros presentan un valor muy pequeño, principalmente en los tautómero HX-37 y HX-90 <0.005 (Tabla7.1.1.).

He centrado mi estudio en los dos tautómeros más estables, he analizado su estructura mediante los métodos computacionales B3LYP/6/31G(d,p) y MP2/6/31G(d,p), he calculado su espectro (en este caso de los seis tautómeros citados) y, finalmente me he centrado en su hidratación, trabajo que supone el mayor esfuerzo.

Se eligió el método B3LYP porque numerosos estudios indican que los datos obtenidos con este método están en concordancia con los obtenidos por métodos computacionalmente más costosos, como MP2 y además predicen los números de onda de las bases del DNA mejor que otros métodos³¹⁻³⁵.

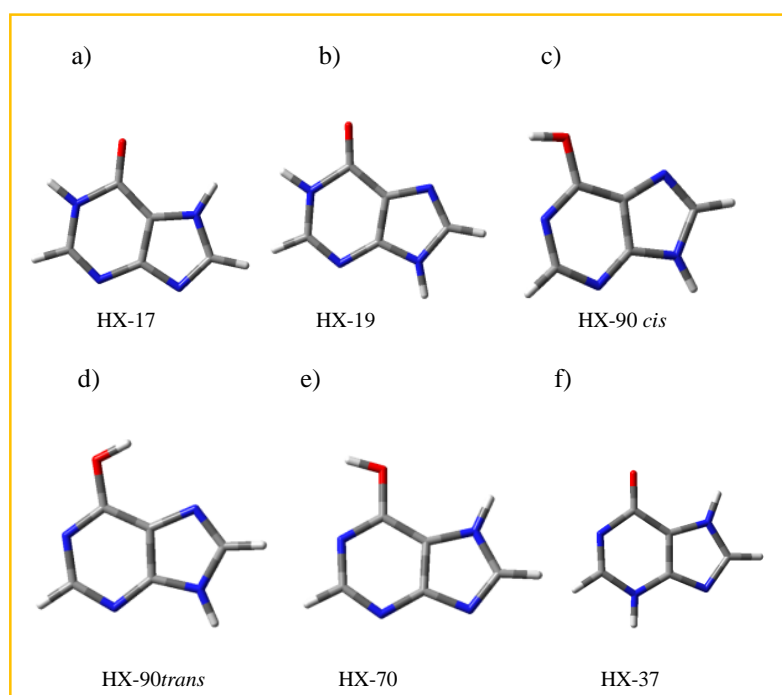


Figura 7.1.2. Los seis más estables tautómeros de la hipoxantina.

MP2 se empleó por sus buenos resultados de la energía y para confirmar los resultados obtenidos con B3LYP.

Tabla 7.1.1. Energía relativa de los seis principales tautómeros de HX (en kcal/mol) en los diferentes niveles de cálculo.

Tautómero	E(RB+HF-LYP)						Suma de la energía electrónica y la energía en el punto cero				% (273.15K)	% (298,15K)
	B3LYP	B3LYP	MP2	MP2	B3LYP	MP2	M052X	M06L	M052X	M06L		
HX-17	0 ^a	0 ^b	0 ^c	0 ^d	0 ^e	0 ^f	0 ^g	0 ^h	0 ⁱ	82.8	80.8	
HX-19	0.395	0.854	0.362	0.877	0.385	0.840	0.321	0.321	0.264	17.2	19.1	
HX-90 _{cis}	3.696	4.501	3.341	3.401	3.687	4.413	3.178	3.178	7.198	0.02	0.04	
HX-90 _{trans}	5.109	5.549	4.619	4.300	5.103	5.514	4.455	4.455	5.869	<0.005	0.01	
HX-70	6.568	6.986	6.104	5.775	6.342	6.763	5.744	5.744	7.416	<0.005	<0.005	
HX-37	7.871	7.144	8.596	7.685	7.690	7.044	8.339	8.339	7.975	<0.005	<0.005	

^a -305720.4477 kcal/mol^b -305824.5327 kcal/mol^c -304861.2348 kcal/mol^d -305048.2632 kcal/mol^e -305657.4196 kcal/mol^f -305761.1707 kcal/mol^g -304797.7089 kcal/mol^h -305625.8202 kcal/molⁱ -305631.2319 kcal/mol

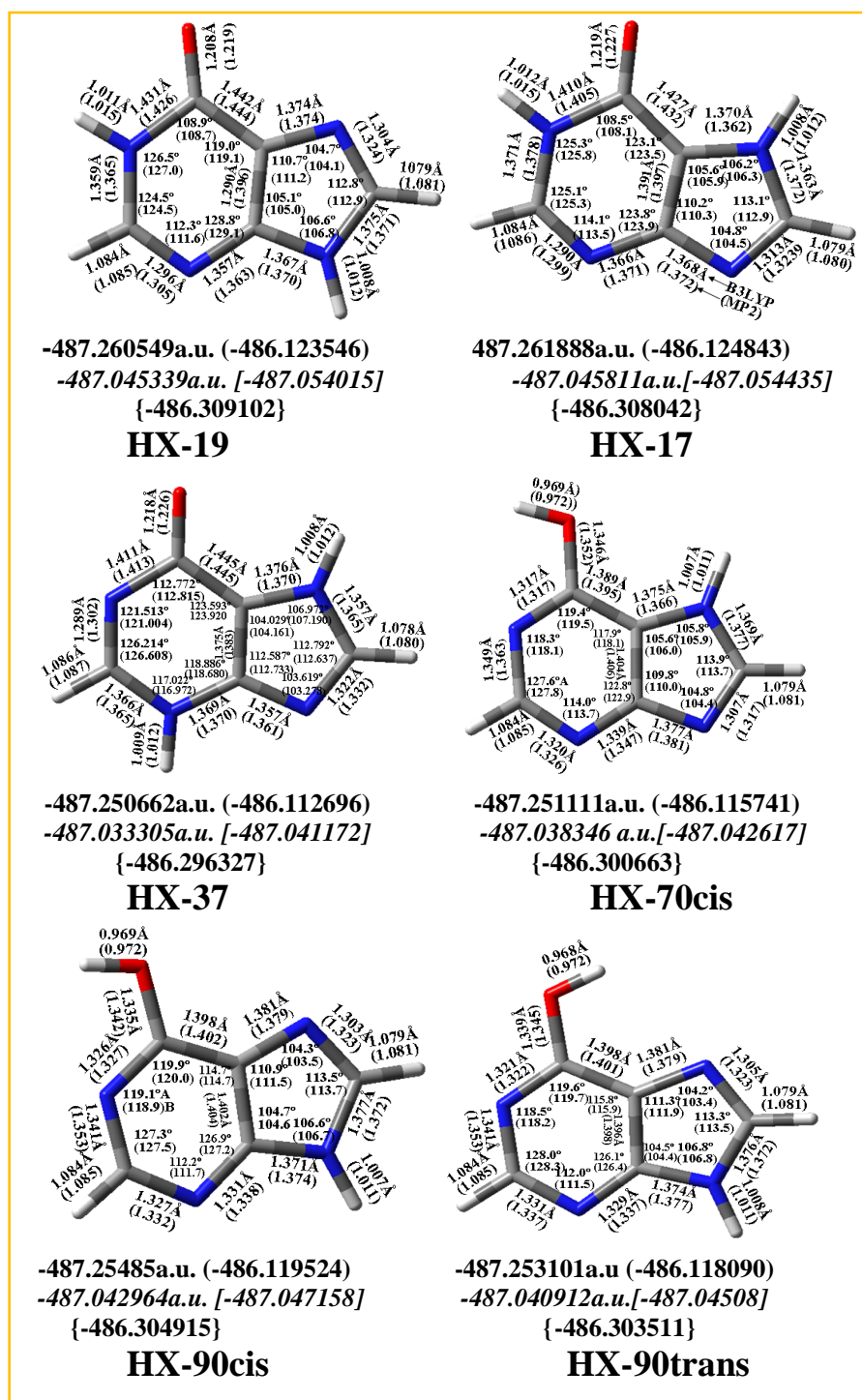


Figura 7.1.3. Longitudes de enlace, ángulos y energía de los seis principales tautómeros de HX en los niveles de cálculo B3LYP/6-311++G(3df,pd) y MP2/6-311++G(2d,p) (valores dentro del paréntesis). Y energía en los niveles M052X/6-31G(d,p), en cursiva, y M06L/6-31G(d,p), entre corchetes, en el nivel MP2/cc-pvtz entre llaves.

7.1.1. Geometría de los principales tautómeros

Se ha considerado importante estudiar la estructura molecular de los seis tautómeros más estables. En las tablas 7.1.2 - 7.1.7 y 13.7.1.8 – 13.7.1.13 (ambos en anexos) se tienen los parámetros estructurales de los seis tautómeros en todos los niveles de cálculo.

La Tabla 7.1.2. muestra los valores optimizados a los niveles B3LYP/6-31G(d,p), B3LYP/6-311++G(3df,pd), MP2/6-31G(d,p), MP2/6-311++G(2d,p) del tautómero HX-19, así como los valores experimentales en los que aparecen dos moléculas cristalográficamente independientes A y B del tautómero estudiado HX-19³. La molécula B tiene las longitudes de enlace mayores que los de la molécula A en 7 casos de los cuales 3 corresponden a enlaces entre átomos del anillo y H.

Los valores teóricos se dan en el estado aislado aunque son muy similares a los valores experimentales. Los valores que difieren más corresponden a los enlaces N-H que son más altos (entre 0.17-0.09 Å) que los experimentales. Por el contrario, los valores que más se aproximan a los experimentales son: N1-C2, en los niveles de cálculo B3LYP/6-31G(d,p) y MP2/6-311++G(2d,p) próximo a los valores de la molécula A y C2-N3, en MP2/6-311++G(2d,p), en este caso igual molécula A (1.305 Å). La distancia entre N7-C8 en el nivel B3LYP/6-31G(d,p) y el enlace C8-N9, en B3LYP/6-311++G(3df,pd) igual a la molécula B (1.311 Å y 1.374 Å respectivamente). En C5-N7 todos los valores calculados, y a todos los niveles, solo difieren en 0.002 Å, en valor absoluto, respecto de las dos moléculas A y B.

En el anillo, y en todos los niveles de cálculo, la mayor diferencia la presentan los valores teóricos de los enlaces C5-C6 y C6-N1 que son mayores que los de las moléculas A y B y C6-O que es menor que ambos enlaces experimentales. Se puede suponer que el estado físico diferente entre la molécula teórica (gas) y las experimentales afecte a los dos enlaces citados y esto influya al enlace C6-O acortándolo.

El nivel de cálculo B3LYP/6-311++G(3df,pd) presenta siete distancias que son las más próximas a los valores experimentales N3-C4, C4-C5, C5-C6, N1-H, C5-N7, N9-C4 y N9-H con respecto a las que se obtienen en los restantes niveles de cálculo, y un valor en C8-N9 que concuerda con las distancias en la molécula B. Mientras, el nivel MP2/6-31G(d,p) presenta seis valores próximos a los experimentales C6-O, C2-H, C5-N7, C8-N9, C8-H y N9-H. El enlace C2-N3 en el nivel MP2/6-311++G(2d,p) coincide con la molécula A.

El enlace C5-N7 tiene el mismo valor en los tres últimos niveles (1.374 Å) y la diferencia con los valores experimentales es 0.002 Å en ambas moléculas. El nivel B3LYP/6-31G(d,p) tiene las longitudes de enlace mayores que los otros niveles.

Tabla 7.1.2. Parámetros geométricos optimizados del tautómero HX-19: Longitudes de enlace (Å) y valores experimentales³.

Distancias	B3LYP/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)	Experimental Molecula A	Experimental Molecula B
N1 – C2	1.365	1.359	1.368	1.365	1.364	1.369
C2 – N3	1.303	1.296	1.307	1.305	1.305	1.291
N3 – C4	1.362	1.357	1.367	1.363	1.350	1.359
C4 – C5	1.396	1.390	1.396	1.396	1.381	1.388
C5 – C6	1.447	1.442	1.446	1.444	1.419	1.400
C6 – N1	1.436	1.431	1.427	1.426	1.378	1.395
C6 – O	1.217	1.208	1.225	1.219	1.246	1.247
N1 – H	1.013	1.011	1.012	1.015	0.84	0.97
C2 – H	1.087	1.084	1.083	1.085	0.95	0.98
C5 – N7	1.378	1.374	1.374	1.374	1.376	1.376
N7 – C8	1.311	1.304	1.326	1.324	1.321	1.311
C8 – N9	1.379	1.375	1.371	1.371	1.348	1.375
N9 – C4	1.371	1.367	1.371	1.370	1.365	1.365
C8 – H	1.081	1.079	1.078	1.081	0.99	1.08
N9 – H	1.009	1.008	1.008	1.012	0.86	0.98

En la Tabla 7.1.3. se muestran los ángulos del tautómero HX-19, observándose que el nivel B3LYP/6-311++G(3df,pd) tiene los valores más similares a los experimentales: N3-C4-C5, C4-C5-C6, C5-C6-N1, N9-C4-C y C4-C5-N7. Los que más se diferencian al nivel MP2/6-31G(d,p) son C5-C6-N1, con la mayor diferencia 3.0° respecto a la molécula A y 3.3° respecto de B y en C6-N1-C que difiere en 2.1° respecto de la molécula A y 3.1° respecto de B. Solamente un valor en B3LYP/6-31G(d,p) coincide con el experimental A: (C-N3-C) así como en este mismo nivel y en B3LYP/6-311++G(3df,pd) el ángulo C8-N9-C respecto de la molécula B.

Los valores más altos corresponden al nivel MP2/6-31G(d,p), con cuatro valores : N-C2-N, C4-C5-C6, C6-N1-C y C4-C5-N7. El ángulo C-N3-C en B3LYP/6-31G(d,p) tiene el mismo valor que en la molécula A y C8-N9-C en este mismo nivel y en B3LYP/6-311++G(3df,pd) concuerda con el valor del ángulo en la molécula B. Los valores más altos de los ángulos se encuentran en MP2/6-31G(d,p) y en cuatro ángulos: N-C2-N, C4-C5-C6, C6-N1-C y C4-C5-N7.

El ángulo C-N3-C calculado al nivel B3LYP/6-31G(d,p) coincide con la molécula A y el ángulo C8-N9-C a este mismo nivel y al nivel B3LYP/6-311++G(3df,pd) también concuerda con el ángulo de la molécula B.

El cálculo de la planaridad indica que la molécula es prácticamente plana presentando el mayor valor del ángulo N1-C2=N3-C4 (0.0106°), los demás son todos menores, del orden de 0.008° - 0.001°.

Tabla 7.1.3. Comparación de ángulos (en grados) del tautómero HX-19 con los valores experimentales³.

Ángulos	B3LYP/6-31G (d,p)	B3LYP/6-311++G (3df,pd)	MP2/6-31G(d, p)	MP2/6-311++G(2d,p)	Experimental Molecule A	Experimental Molecule B
N-C2-N	124.6	124.5	124.7	124.5	124.8	125.5
C-N3-C	112.0	112.3	111.2	111.6	112.0	112.1
N3-C4-C5	128.9	128.8	129.2	129.1	127.8	127.2
C4-C5-C6	119.1	119.0	119.3	119.1	118.9	119.4
C5-C6-N1	108.9	108.9	108.5	108.7	111.5	111.8
C6-N1-C	126.6	126.5	127.1	127.0	125.0	124.0
C5-N7-C	104.5	104.7	103.8	104.1	103.7	104.8
N7-C8-N	113.0	112.8	113.1	112.9	113.3	112.5
C8-N9-C	106.6	106.6	106.9	106.8	107.0	106.6
N9-C4-C	105.0	105.1	104.8	105.0	105.2	105.9
C4-C5-N7	111.0	110.7	111.5	111.2	110.8	110.2

La Tabla 7.1.4. muestra las longitudes de enlace en el tautómero HX-17. En N1-C2, N3-C4, C6-O, N7-C8 y N9-C4 se tienen valores mayores en HX-17 que los correspondientes en el tautómero HX-19 mientras que en C2-N3, C5-C6, C6-N1, C5-N7 y C8-N9 estos son menores que los de HX-19 en todos los niveles de cálculo. Ello sugiere que el cambio en la posición del átomo de H de N9 a N7 modifica el anillo imidazólico: el enlace C8-N9 se acorta y el enlace N7-C8 se alarga al formar el átomo N7 un nuevo enlace con el átomo de H y el enlace C5-N7 decrece.

Tabla 7.1.4. Parámetros geométricos optimizados del tautómero HX-17. Longitudes de enlace (Å).

Distancias	B3LYP/6-31G(d,p)	B3LYP/6-311++G (3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
N1 – C2	1.376	1.371	1.381	1.378
C2 – N3	1.297	1.290	1.302	1.299
N3 – C4	1.372	1.366	1.376	1.371
C4 – C5	1.396	1.391	1.397	1.397
C5 – C6	1.433	1.427	1.435	1.432
C6 – N1	1.416	1.410	1.406	1.405
C6 – O	1.225	1.219	1.232	1.227
N1 – H	1.013	1.012	1.012	1.015
C2 – H	1.087	1.084	1.083	1.086
C5 – N7	1.374	1.370	1.363	1.362
N7 – C8	1.368	1.363	1.372	1.372
C8 – N9	1.318	1.313	1.325	1.323
N9 – C4	1.373	1.368	1.373	1.372
N7 – H	1.009	1.008	1.009	1.012
C8 – H	1.082	1.079	1.078	1.080

Algunos enlaces de HX-17 coinciden con los de HX-19, como son en C4-C5, N1-H y C2-H al nivel de cálculo B3LYP/6-31G(d,p). También en N1-H a los niveles MP2/6-31G(d,p) y MP2/6-311++G(2d,p). En C2-H coincide además a los niveles B3LYP/6-311++G(3df,pd) y MP2/6-31G(d,p). El enlace C4-C5 varía poco en uno u otro tautómero. Todo ello indica que los cambios en el anillo imidazólico no afectan al anillo pirimidínico, siendo el cambio más importante el del incremento del enlace C=O.

La Tabla 7.1.5. corresponde a los valores teóricos de los ángulos del tautómero HX-17. Comparando éstos con los experimentales (a pesar de que son los correspondientes al tautómero HX-19) se observa que solamente un ángulo N-C2-N en el nivel de cálculo MP2/6-31G(d,p) concuerda con los valores experimentales de la molécula B. Los ángulos C-N3-C, C4-C5-C6, C6-N1-C, C5-N7-C y N9-C4-C son mayores que los de las moléculas A y B. Los ángulos N3-C4-C5, C5-C6-N1, C8-N9-C y C4-C5-N7 son menores que los de las moléculas A y B. Las principales diferencias de los valores calculados comparados con los valores experimentales aparecen en el ángulo C5-C6-N1 en el nivel de cálculo MP2/6-31G(d,p) (4.1° en relación a la molécula B) y C4-C5-N7 al nivel B3LYP/6-31G(d,p) (5.3°) respecto de la molécula A. En C5-C6-N1 y en N9-C4-C al nivel MP2/6-31G(d,p) la diferencia es 3.8° y 5.5° respectivamente. Los dos últimos valores en el tautómero HX-19 son similares a los ángulos experimentales.

Los valores mayores de cada ángulo se dan al nivel MP2/6-31G(d,p), con cinco valores en total: N-C2-N, C4-C5-C6, C6-N1-C, C5-N7-C y N9-C4-C. El nivel B3LYP/6-311++G(3df,pd) tiene tres valores mayores C-N3-C, C5-C6-N1 y C8-N9-C.

Tabla 7.1.5. Angulos (en grados) del tautómero HX-17 en todos los niveles de cálculo.

Angulos	B3LYP/6-31G(d, p)	B3LYP/6- 311++G(3df,pd)	MP2/6-31G(d, p)	MP2/6- 311++G(2d,p)
N-C2-N	125.3	125.1	125.5	125.3
C-N3-C	113.8	114.1	113.2	113.5
N3-C4-C5	123.8	123.8	123.8	123.9
C4-C5-C6	123.4	123.1	123.9	123.5
C5-C6-N1	108.2	108.5	107.7	108.1
C6-N1-C	125.5	125.3	126.0	125.8
C5-N7-C	106.1	106.2	106.4	106.3
N7-C8-N	113.4	113.1	113.1	112.9
C8-N9-C	104.5	104.8	104.2	104.5
N9-C4-C	110.5	110.2	110.7	110.3
C4-C5-N7	105.5	105.6	105.7	105.9

El cálculo de la planaridad indica que la molécula es plana presentando todos los valores de los ángulo por debajo de 0.0006°.

La Tabla 7.1.6. muestra las longitudes de enlace del tautómero HX-37. Los enlaces C2-N3, N3-C4, C5-C6, C6-O Y N7-C8 son más largos, en todos los niveles de cálculo, que los correspondientes al tautómero HX-19 y en N1-C2, C4-C5, C8-N9, N9-C4 y C8-H son

menores. Al compararlo con el tautómero HX-17 se observa que las longitudes de enlace C2-N3, N3-C4, C8-N9, C5-C6, C2-H y C5-N7 son mayores que los correspondientes a éste (N3-C4 solamente es mayor en B3LYP/6-31G(d,p) y en B3LYP/6-311++G(3df,pd)). Los enlaces N1-C2, C4-C5, N7-C8, N9-C4 Y C8-H son menores. El desplazamiento del H en N1 a N3 alarga los dos enlaces C2-N3 y N3-C4 mientras los enlaces N1-C2 Y C4-C5 se acortan. Se observa que el anillo imidazólico se modifica respecto del tautómero HX-19 pero respecto a HX-17 la modificación es menor porque la estructura de ambos tautómeros es análoga.

Tabla 7.1.6. Parámetros geométricos optimizados del tautómero HX-37: Longitudes de enlace (Å).

Distancias	B3LYP/6-31G(d, p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
N1 – C2	1.294	1.289	1.304	1.302
C2 – N3	1.373	1.366	1.368	1.365
N3 – C4	1.375	1.369	1.374	1.370
C4 – C5	1.380	1.375	1.384	1.383
C5 – C6	1.450	1.445	1.448	1.448
C6 – N1	1.418	1.411	1.416	1.413
C6 – O	1.224	1.218	1.231	1.226
C2 – H	1.089	1.086	1.085	1.087
N3 – H	1.010	1.009	1.009	1.012
C5 – N7	1.380	1.376	1.370	1.370
N7 – C8	1.361	1.357	1.366	1.365
C8 – N9	1.328	1.322	1.334	1.332
N9 – C4	1.361	1.357	1.362	1.361
N7 – H	1.010	1.008	1.009	1.012
C8 – H	1.081	1.078	1.077	1.080

La Tabla 7.1.7. muestra los ángulos del tautómero HX-37. Los valores más altos de cada ángulo se tienen en los niveles MP2/6-31G(d,p) y B3LYP/6-311++G(3df,pd). En general los valores calculados en este tautómero, en los diferentes niveles, son análogos. Seis son más altos que los experimentales A y B: N-C2-N, C-N3-C, C4-C5-C6, C5-C6-N1, C5-N7-C8 y N9-C4-C y cuatro son menores, también en todos los niveles de cálculo: N3-C4-C5, C6-N1-C, C8-N9-C y C4-C5-N7. Solamente un ángulo tiene un valor menor que la molécula A y mayores los restantes respecto de B. Las principales diferencias respecto de la molécula A son N3-C4-C5 y N9-C4-C (9.5° y 7.9°) respectivamente, ambos en MP2/6-31G(d,p) y, en relación a la molécula B, los ángulos N3-C4-C5, (8.9°) en MP2/6-31G(d,p), y C4-C5-N7 (6.3°) en MP2/6-31G(d,p) y B3LYP/6-31G(d,p).

Al comparar estos ángulos con los calculados del tautómero HX-19 se observa que hay dos valores que son iguales en ambos tautómeros: el del N7-C8-N en los niveles B3LYP/6-31G(d,p) y B3LYP/6-311++G(3df,pd). Los valores más altos o más bajos respecto de las moléculas A y B también los son respecto de los valores calculados de HX-19.

Tabla 7.1.7. Ángulos (en grados) del tautómero HX-37 en todos los niveles de cálculo.

Ángulos	B3LYP/6-31G (d, p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d, p)	MP2/6-311++G(2d, p)
N-C2-N	126.5	126.2	126.8	126.6
C-N3-C	117.0	117.0	117.0	117.0
N3-C4-C5	118.6	118.9	118.3	118.7
C4-C5-C6	123.9	123.6	124.4	123.9
C5-C6-N1	112.7	112.8	112.6	112.8
C6-N1-C	121.3	121.5	120.9	121.0
C5-N7-C8	107.0	107.0	107.3	107.2
N7-C8-N	113.0	112.8	112.8	112.6
C8-N9-C	103.3	103.6	102.9	103.3
N9-C4-C	112.9	112.6	113.1	112.7
C4-C5-N7	103.9	104.0	103.9	104.2

La Tabla 13.7.1.8. (anexo) corresponde a las longitudes de enlace del tautómero HX-70 enol. Al comparar estos valores con los del tautómero HX-19 se ve que los enlaces C2-N3, C4-C5, C6-O, N7-C8 y N9-C4 son mayores que en HX-19 y los enlaces N1-C2, N3-C4, C5-C6, C6-N1 y C8-N9 son menores. El enlace C2-H tiene el mismo valor (varía solo 0.001 Å) en los niveles B3LYP/6-31G(d,p), B3LYP/6-311++G(3df,pd) y MP2/6-311++G(2d,p). El enlace C5-N7 en los niveles de cálculo B3LYP/6-31G(d,p), y B3LYP/6-311++G(3df,pd) presenta solamente una diferencia de 0.001 Å mientras en MP2/6-311++G(2d,p) y MP2/6-31G(d,p) decrece en 0.008 y 0.007 Å, respectivamente. El nuevo enlace O-H da lugar al incremento en la longitud C6-O y a la disminución de los dos próximos C6-N1 y C5-N6. Al comparar esta estructura con la del tautómero HX-17, se tiene que el enlace N7-H es menor en 0.001 Å en todos los niveles de cálculo.

La Tabla 13.7.1.9. (anexo) muestra los ángulos de enlace del tautómero HX-70 enol. Puede observarse que los valores más altos corresponden al nivel MP2/6-31G(d,p). Hay cinco ángulos N-C2-N, N3-C4-C5, C4-C5-C6, C5-N7-C8 y N9-C4-C en estas condiciones. El nivel B3LYP/6-311++G(3df,pd) también tiene tres ángulos de mayor valor que en los otros niveles: C-N3-C, C6-N1-C y C8-N9-C. Si se comparan estos valores con los experimentales (moléculas A y B) se observa que los ángulos: N-C2-N, C-N3-C, C5-C6-N1, C5-N7-C8, N7-C8-N y N9-C4-C5 los valores mayores en todos los niveles de cálculo, mientras que los ángulos: N3-C4-C5, C4-C5-C6, C6-N1-C, C8-N9-C y C4-C5-N7 son menores.

Las principales diferencias al comparar la molécula A están en los ángulos C5-C6-N1 (8.0°) en los niveles B3LYP/6-31G(d,p) y MP2/6-311++G(2d,p) y en C6-N1-C (7.2°) en MP2/6-31G(d,p). Y respecto de la molécula B el ángulo C5-C6-N1 (7.7°) en B3LYP/6-31G(d,p) y MP2/6-311++G(2d,p), y C6-N1-C (6.2°) en MP2/6-31G.

La Tabla 13.7.1.10. (anexo) presenta las longitudes de enlace del tautómero HX-90 *cis*. Respecto del tautómero HX-19 se observa que los enlaces C2-N3, C4-C5, C6-O, C5-N7, C8-N9 y N9-C4 son mayores, los enlaces N1-C2, N3-C4, C5-C6, C6-N1 y N7-C8 y N9-H son iguales en los niveles de cálculo B3LYP/6-31G(d,p) y MP2/6-31G(d,p), mientras que son menores en los restantes niveles. Al compararlo con el ceto tautómero HX-17 se observa que el cambio en el anillo pirimidínico es igual en ambos tautómeros (HX-70 y HX-90) pero el desplazamiento del H desde N7 a N9 aumenta la longitud de los enlaces C8-N9 y N9-C4 en comparación con los del tautómero HX-19.

En la siguiente Tabla 13.7.1.11. (anexo), se tienen los ángulos del tautómero HX-90 *cis*. Cuatro ángulos son mayores que los experimentales de las moléculas A y B y que los teóricos HX-19: N-C2-N, C5-C6-N1, N7-C8-N y C4-C5-N7 y cuatro ángulos son menores que los experimentales (A y B): C-N3-C, C4-C5-C6, C6-N1-C y N9-C4-C. en cada nivel de cálculo. Los valores de los ángulos: N3-C4-C5 en los niveles B3LYP/6-31G(d,p) y MP2/6-311++G(2d,p) y C8-N9-C en los niveles B3LYP/6-31G(d,p), B3LYP/6-311++G(3df,pd) coinciden con los de la molécula B. El ángulo C8-N9-C, en todos los niveles de cálculo es menor que en el experimental A y respecto a B es igual en los niveles B3LYP/6-31G(d,p,) y B3LYP/6-311++G(3df,pd) y mayor en los dos últimos valores de cálculo. Las principales diferencias respecto de la molécula A son: C5-C6-N1 (8.7°) y C6-N1-C (6.4°) ambos en MP2/6-31G(d,p) y, respecto a la molécula B, estos mismos ángulos presentan una diferencia de (8.4°) y (5.4°) en el mismo nivel de cálculo.

Al comparar estos ángulos con los obtenidos en el ceto-tautómero HX-19 se observa que hay cuatro valores más altos: N-C2-N, C5-C6-N1, N7-C8-N y C4-C5-N7 mientras que los restantes son menores.

La Tabla 13.7.1.12. (anexos) muestra las longitudes de enlace del tautómer HX-90 *trans*. Los enlaces C2-N3, C4-C5, C6-O C5-N7, C8-N9 y N9-C4 son más largos que los del tautómero HX-19 y, en algunos también lo son comparándolo con el tautómero HX-90 *cis*. El enlace N7-C8 tiene dos valores iguales que en el tautómero HX-19 y los otros dos solamente difieren en 0.001 Å.

El enlace O-H es un poco más corto que en HX-70 en tres niveles de cálculo pero no en MP2/6-311++G(2d,p) que tiene los mismos valores en ambos tautómeros. Puede verse que, al compararlo a HX-90 *cis*, presenta algunos enlaces más largos como: C2-N3, C6-O, N7-C8 y N9-C4 y algunos más cortos como: N3-C4, C4-C5 y C6-N1. Estos valores de las longitudes de enlace indican que la influencia del H colocado sobre el átomo de O es similar en ambos HX-90 tautómeros (*cis* y *trans*).

Por último en la Tabla 13.7.1.13. (anexo) se tienen los valores de los ángulos del tautómero HX-90 *trans* en la que se observa que el nivel de cálculo MP2/6-31G(d,p) presenta cinco valores que son los mayores de cada ángulo: N-C2-N, N3-C4-C5, C6-C6-N1, N7-C8-N y C8-N9-C mientras el nivel B3LYP/76-311++G(3df,pd) presenta cuatro: C-N3-C, C6-N1-C, C5-N7-C8 y N9-C4-C. Esta tabla muestra cuatro valores mayores que los experimentales A y B en todos los niveles de cálculo: N-C2-N, C5-C6-N1, N7-C8-N y C4-C5-N7 y cinco valores menores: C-N3-C, N3-C4-C5, C4-C5-C6, C6-N1-C y N9-C4-C. Los valores mayores que los experimentales aparecen en los ángulos C5-C6-N1 (8.4°) y C6-N1-C (7.1°) ambos en MP76-31G(d,p).

En general, si comparamos los valores obtenidos en los ángulos de los seis tautómeros estudiados, se observa que los valores más altos de cada ángulo corresponden al nivel MP2/6-31G(d,p).

7.1.2. Energía de los tautómeros

La figura 7.1.3. presenta la estructura (longitudes de enlace y ángulos de enlace) de los seis tautómeros más estables de HX y sus respectivas energías calculadas en los niveles B3LYP/6-311++G(3df,pd) y MP2/6-311++G(2d,p). Como ya se ha indicado, la Tabla 9.1.1. muestra las diferentes energía de los seis principales tautómeros de HX en todos los niveles de cálculo, incluyendo la corrección de la energía en el punto cero, y en las dos últimas columnas se tienen las poblaciones de cada tautómero a las temperaturas de 273.15 K y 298.15 K.

De este gráfico y de esta tabla se deduce que el tautómero más estable es HX-17 ya que tiene los valores más bajos de la energía en todos los niveles de cálculo, seguido del tautómero HX-19 y, el menos estable el HX-90 *cis*. La mayor población la presenta HX-17, 82.8% a la temperatura de 273.15 K y 80.8% a la temperatura de 298.15 K. La población en el tautómero HX-19 es mucho menor, 17.2% y 19.1%, a las dos temperaturas antes citadas. Con mucha menor población aparece HX-90 *cis*, con 0.02% y 0.04% en ambas temperaturas.

Dado que los valores experimentales corresponden al tautómero HX-19 mientras que el valor menor de la energía, como se ha indicado anteriormente, aparece en el tautómero HX-17, he centrado mi atención preferentemente en el estudio de ambos tautómeros.

La figura 7.1.4 muestra los enlaces y los ángulos de enlace de estos dos tautómeros en los niveles de cálculo B3LYP/6-31G (d,p) y MP2/6-31G(d,p) comparándolos con los que se tienen en las formas hidratadas con 20 moléculas de agua, y PCM en ambos. En HX-

19 hay pocas diferencias en cuanto a los valores de las longitudes de enlace y ángulos de enlace en los dos niveles de cálculo antes citados. Las principales diferencias las presenta el enlace N7-C8 (0.015 Å) en HX-19, en C5-N7 (0.011 Å) en HX-17 y en C6-N (0.010 Å) también en HX-17. Las diferencias respecto a los valores de PCM son menores

Las longitudes de enlace de la molécula hidratada HX-17 con 20 moléculas de agua presentan algunas diferencias respecto a la molécula aislada, en especial en los cálculos en B3LYP/6-31G(d,p), se tiene: 0.035 Å (C=O), 0.031 Å (C6-N1), 0.021 Å (C5-C6), 0.015 Å (C8-N9) y 0.012 Å, (C2-N3) y 0.016 Å (N1-C2). Si se comparan las formas hidratadas, las mayores diferencias entre el clúster con 20 moléculas de agua y PCM aparecen en: 0.027 Å (C=O), 0.022 Å (C6-N1) y 0.019 Å (C5-C6).

El tautómero HX-19 presenta grandes diferencias entre la molécula aislada (optimizada en B3LYP) y las formas hidratadas. Así respecto del cluster con 20 moléculas de agua aparece la diferencia de 0,031 Å (C=O), 0.038 Å (C6-N1) y 0.021 Å (C5-C6), las demás diferencias son mucho menores. Con respecto a PCM, los anteriores enlaces presentan unas diferencias algo menores; se tienen los valores 0.016 Å, 0.017 Å y 0.013 Å. Estas diferencias indican que ambos tautómeros sufren modificaciones en su estructura en presencia de agua modificando las longitudes de enlace.

7.1.3. Cargas

Las cargas atómicas se determinaron mediante el procedimiento natural NBO³⁶ ya que se consideran más fiables éstas que las calculadas por otros métodos (Mulliken...). En este caso varían considerablemente con el método empleado en los cálculos.

La figura 7.1.5 muestra las cargas de los átomos en los tautómeros HX-17 y HX-19 en los niveles de cálculo B3LYP/6-31G(d,p), MP2/6-31G(d,p), B3LYP/6-311++G(3df,pd) y MP2/6-311++G(2d,p) y las cargas de las formas hidratadas, con 2, 12, 20 y 30 moléculas de agua y PCM en HX-19 y con 2, 13, 20 y 30 moléculas de agua y PCM en HX-17. Se observa que la carga negativa mayor corresponde, en todos los casos, a los átomos O6 y N1 y, en consecuencia, la mayor carga positiva a C6. Los átomos de H9 y H7 son los más positivos en el tautómero correspondiente y, por tanto, los más reactivos.

En general, las cargas de las formas hidratadas varían respecto de las formas aisladas y, salvo excepciones, los clusters con 12/13 y 20/30 moléculas de agua presentan la mayor carga, tanto positiva como negativa.

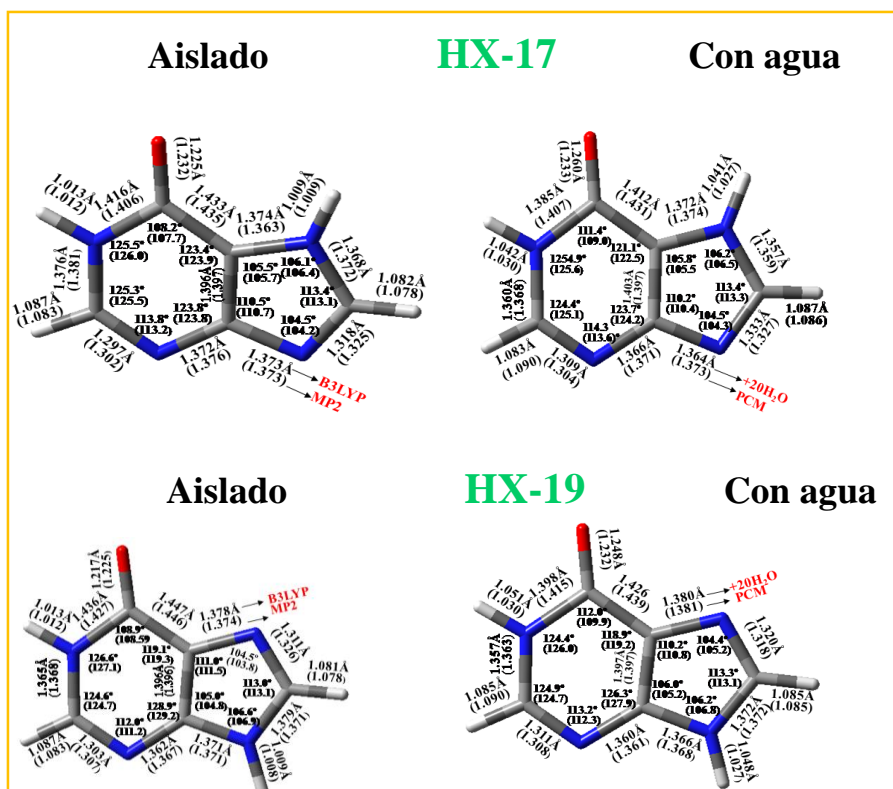


Figura 7.1.4. Longitudes de enlace en los tautómeros HX-17 y HX-19 de la hipoxantina en el estado aislado y con agua simulada de forma explícita y con el modelo PCM.

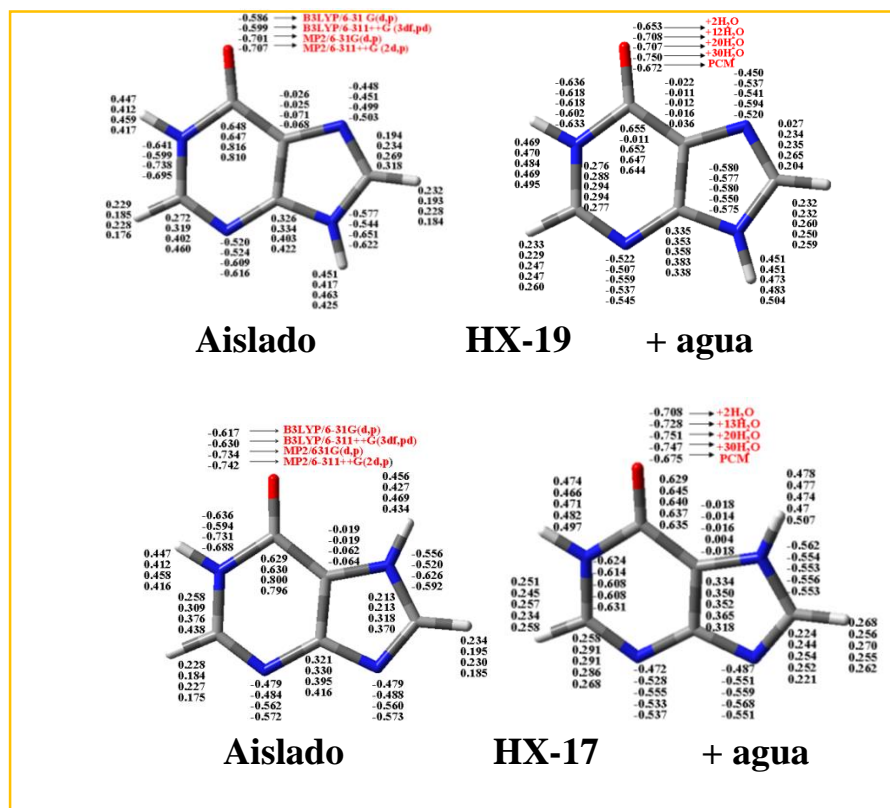


Figura 7.1.5. Cargas atómicas en los tautómeros HX-19 y HX-17 de la hipoxantina en estado aislado y con agua en los diferentes niveles de cálculo.

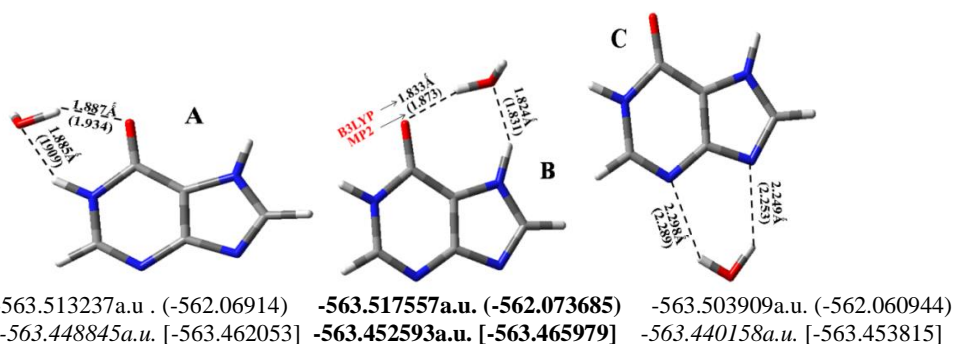
7.1.4. Hidratación

Está plenamente aceptada la presencia de moléculas de agua fuertemente unidas en la superficie del DNA^{37,38}. Mediante estudios de rayos-X se ha determinado que la hidratación del DNA tiene lugar entre los puntos hidrofílicos y electrofílicos de la molécula y se ha establecido que se forma una capa de moléculas de agua fuertemente unidas a la superficie del DNA^{39,40}. Teóricamente se han realizado diferentes estudios sobre la hidratación de las bases: adenina^{41,42}, citosina^{42,43}, uracilo^{44,45} y timina⁴⁶. También sobre nucleósidos (timidina)⁴⁷ y derivados halogenados de las bases (uracilo)⁴⁸ y sus espectros⁴⁹. Respecto de HX se ha estudiado el diferente comportamiento de los dos tautómeros HX-19 y HX-17 hidratados⁵⁰. Se ha demostrado experimentalmente la importancia del agua en uracilos y timinas en la protección del código genético frente los daños ocasionados por el medio³⁹. Por ello, adquiere capital importancia el estudio de los diferentes hidratos, su estructura y energía, y establecer las diferencias respecto de la molécula aislada.

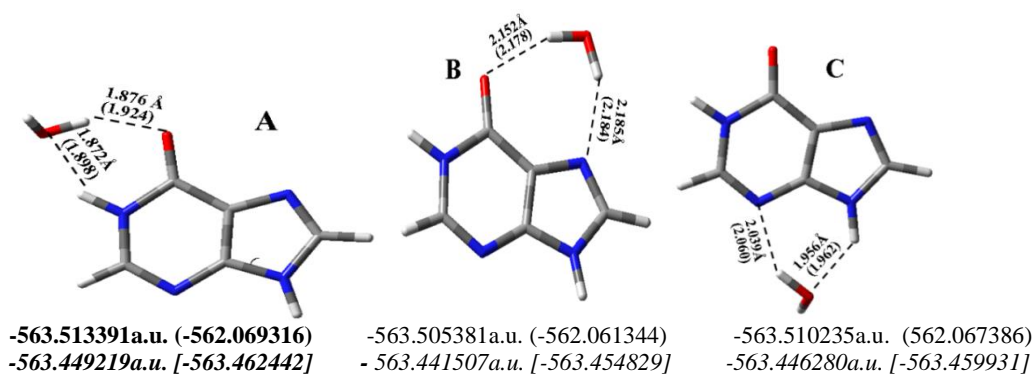
Así este trabajo se ha centrado principalmente en el estudio de la hidratación de los dos tautómeros más estables: HX-17 y HX-19, previa consulta de trabajos ya realizados⁵¹. La hidratación se ha logrado situando, en cada hidrato, una primera molécula de agua en las posiciones posibles y tomando la de menor energía (figura 7.1.6.), y situando en ésta la segunda molécula de agua, también en todas las posiciones posibles. El proceso se continúa situando la tercera molécula de agua en el dihidrato más estable, también en todas las posiciones, y de nuevo, en la de menor energía se añade una cuarta molécula de agua, de manera análoga y así sucesivamente hasta completar la capa alrededor de la base. Con la adición de nuevas moléculas de agua se obtendrán los agregados con 15, 20, 25 y 30 moléculas de agua.

Experimentalmente se ha determinado que las moléculas de agua forman enlaces de H con los oxígenos del grupo fosfato en los nucleótidos, con los oxígenos del fosfodiéster en los polinucleótidos, con O4' de la furanosa y con todos los átomos electronegativos de los pares de bases⁴⁰. Ésta tendrá lugar principalmente situando las moléculas de agua en los átomos con electrones desapareados y en los hidrógenos amino de la molécula, dado que la molécula de agua puede actuar como dador de electrones y como aceptor.

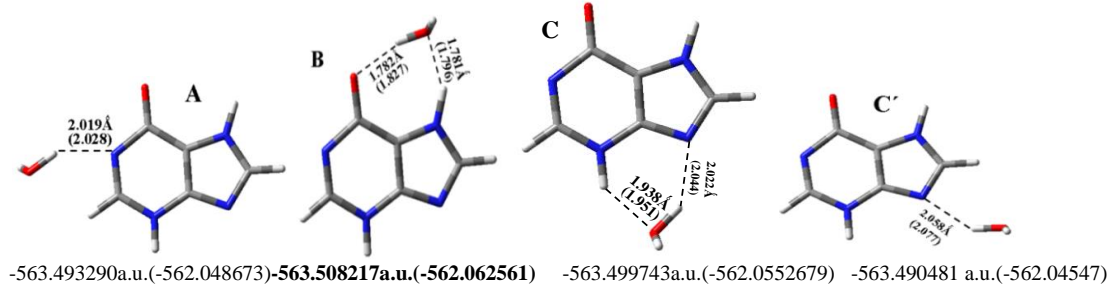
HX-17+1H₂O



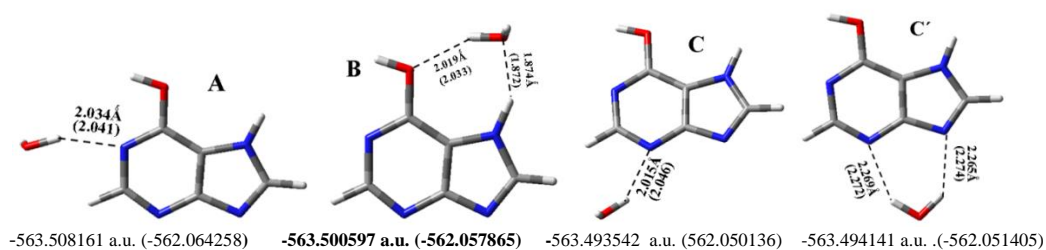
HX-19+1H₂O



HX-37+1H₂O



HX-70cis+1H₂O



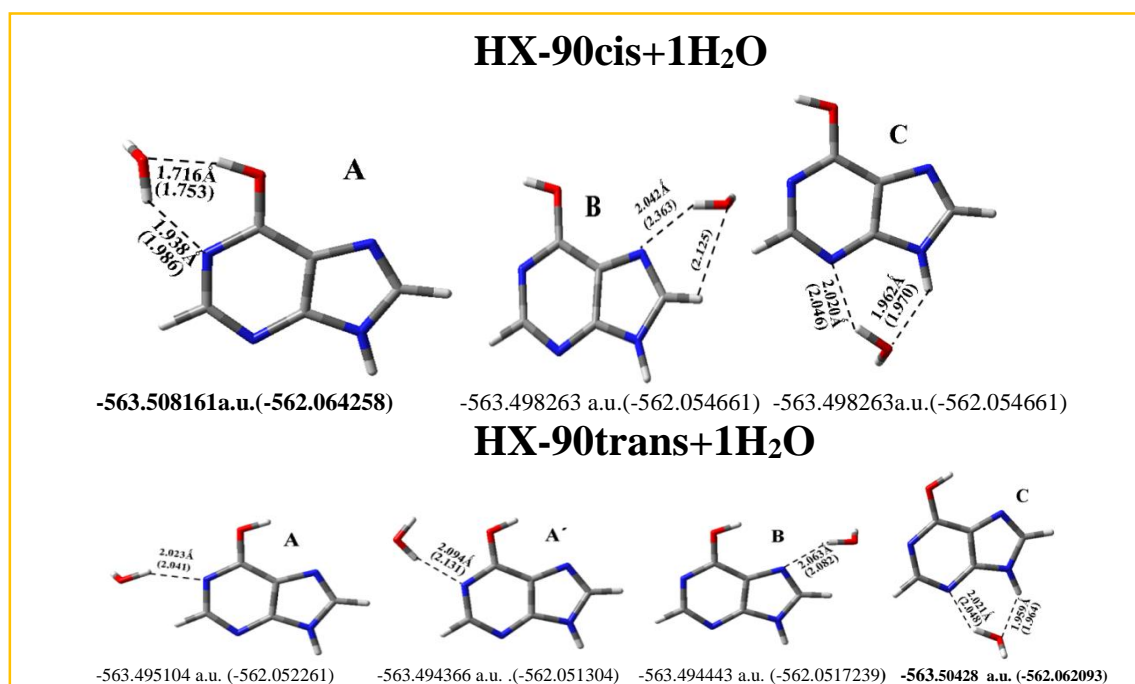


Figura 7.1.6. Posiciones óptimas de la molécula de agua en los monohidratos de los seis tautómeros más estables de HX, con los enlaces de Hidrógeno calculados en B3LYP/6-31G(d,p) y MP2/6-31G(d,p) y las energías ($E+ZPE$) en unidades atómicas. Los valores en MP2 aparecen entre paréntesis. El monohidrato con menor energía se muestra en negrita. En los tautómeros HX-17 y HX-19 se ha calculado la energía $E+ZPE$ en M052X/6-31G(d,p) (en cursiva) y M06L/6-31G(d,p) (en cursiva y entre corchetes).

La figura 7.1.6. muestra las posiciones A, B C más estables de los monohidratos de los seis principales tautómeros de HX. Los enlaces de H entre la molécula de agua y HX, y las energías $E_{total} + ZPE$ de los hidratos se han calculado en los niveles B3LYP/6-31G(d,p) y MP2/6-31G(d,p). En la figura se destacan, para cada posición del agua, el monohidrato más estable (con menor energía), con letra negrita. Puede observarse que los enlaces de hidrógeno más fuertes, para un número dado de moléculas de agua, corresponden a dicho hidrato más estable. En todos los niveles de cálculo los monohidratos con menor energía son los de HX-17.

7.1.4.1. Hidratación del tautómero HX-19

He realizado el estudio teórico de la hidratación del tautómero HX-19 mediante los métodos MP2/6-31G(d,p) y B3LYP/6-31G(d,p) partiendo de una primera molécula de agua adicionada y así hasta 30.

HX-19 + 1 H₂O

La presencia de una molécula de agua cambia la geometría y la distribución de carga en la molécula HX-19. Al comparar los valores de las longitudes de enlaces de la Tabla 7.1.2. y los de la figura 7.1.6. (HX-19+1H₂O) se observa, en el nivel B3LYP/6-31G(d,p) un aumento en la longitud del enlace C=O (0.015 Å) y un decrecimiento en N1-C6 (0.016 Å), ambos en la forma hidratada. En el nivel MP2 se tiene un aumento en C=O (0.012 Å) y disminución de N1-C6 (0.014 Å) en el monohidrato. Esta variación se debe a que la molécula de agua forma enlaces de H con O6 lo cual da lugar al alargamiento del enlace C=O y el enlace N1-C6 se reduce. Los átomos que se ven afectados por la formación de enlaces de H son los de O y N, mientras que los átomos de C no están implicados. En las cargas se tiene un aumento de la carga negativa sobre O6 de 0.050 *e* por MP2 y el incremento de la carga positiva en C4 de 0.011 *e*. En los demás átomos la diferencia es muy pequeña.

La figura 7.1.6 muestra las posiciones óptimas de la molécula de agua en los monohidratos de los seis principales tautómeros de HX y la energía+ZPE correspondiente a los niveles de cálculo B3LYP/6-31G(d,p) y MP2/6-31G(d,p) (ésta viene entre paréntesis). Esta figura muestra también los enlaces de H entre la molécula de agua y HX-19, en las tres posiciones A, B y C. Los enlaces más fuertes corresponden a la posición A con el enlace O···H-N1 que vale 1.872 Å en B3LYP y 1.898 Å en MP2, y el enlace H···O6 con 1.876 y 1.924 Å, respectivamente, en ambos niveles de cálculo. Los hidratos en posición B y C tienen los enlaces de H más débiles. Así en la posición B estos valores son: H···O6 2.152 y 2.178 Å y H···N7 2.185 y 2.184 Å, respectivamente en ambos niveles de cálculo, y en la posición C se tienen H···N3: 2.019 y 2.060 Å y O···H-N9 1.956 y 1.962 Å, todos en los niveles de cálculo ya citados. Ello está en consonancia con lo dicho anteriormente que el monohidrato más estable es el A.

En los tautómeros HX-19 y HX-17 de esta figura se indica también la energía en los niveles M052X/6-31G(d,p) (en letra cursiva) y M06L/6-31G(d,p) (entre corchetes). La menor energía se indica con letra negrita. Es sabido que los valores de la energía en el nivel MP2 son mejores que los obtenidos en B3LYP, por lo cual se ha tomado la energía en MP2 como base para el orden de estabilidad. En el tautómero HX-19, de las tres posiciones A-B-C en las que se sitúa la molécula de agua, la A es la más estable. Esta posición también presenta el mayor valor de la CP-corrección en la energía de formación del complejo (ΔE_{HX-19}^{CP}) (Tabla 7.1.14. y figura 7.1.7a.). En la posición B (figura 7.1.7b) se tiene la menor energía de deformación del HX-19 E_{HX-19}^{def} y el menor valor de la

deformación del agua E_{W1}^{def} , así como el mínimo valor de la energía de interacción, siendo esta la posición menos estable. Los enlaces de H más fuertes son los de la posición A, lo que determina la mayor estabilidad de este hidrato. Los resultados son análogos en todos los niveles de cálculo.

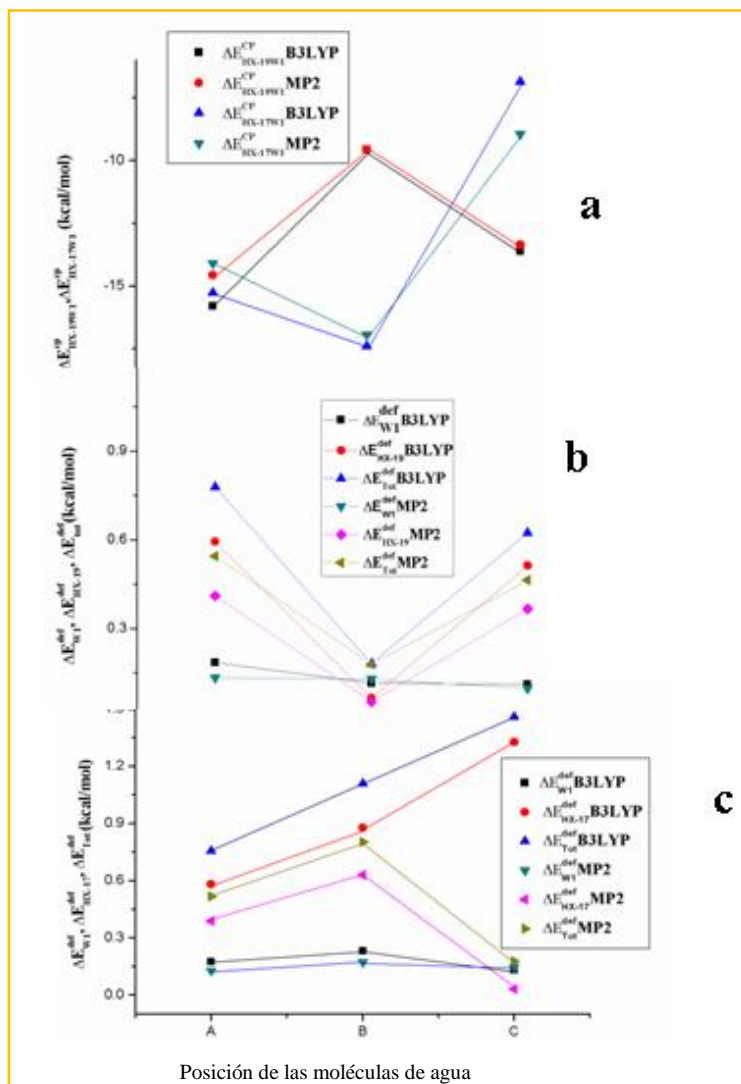


Figura 7.1.7. (a) CP corrección de energía de formación entre la molécula de los tautómeros HX-19 y HX-17 y la primera molécula de agua. (b y c) Energía de deformación de cada complejo y de la molécula de agua.

En la figura 7.1.8 se detallan las longitudes de enlace y los ángulos de la molécula hidratada, con una molécula de agua en la posición A, la más estable, del tautómero HX-19, en los dos niveles de cálculo B3LYP y MP2. Se observa que los ángulos cambian poco en ambos niveles al compararlos con los de la molécula aislada (figura 7.1.4). La diferencia mayor por MP2 es de 1.2° en el ángulo N1-C6-C5°, 0.9° en C6-N1-C2 y 0.5° en el ángulo N3-C2-N1.

Tabla 7.1.14. Corrección CP de la energía de formación del complejo: $\Delta E_{HX}^{HXW1} + \Delta E_{Total}^{def}$ en los niveles de cálculo B3LYP/6-31G(d,p) y MP2/6-31G(d,p) en todos los monohidratos de HX-19 y HX-17.

Hidrato	ΔE_{HX19W1}^{CP} (B3LYP)	ΔE_{HX19W1}^{CP} (MP2)	ΔE_{HX17W1}^{CP} (B3LYP)	ΔE_{HX17W1}^{CP} (MP2)
A	-16.58	-15.11	-16.02	-14.61
B	-9.75	-9.75	-18.51	-17.75
C	-14.25	-13.82	-8.32	-9.13

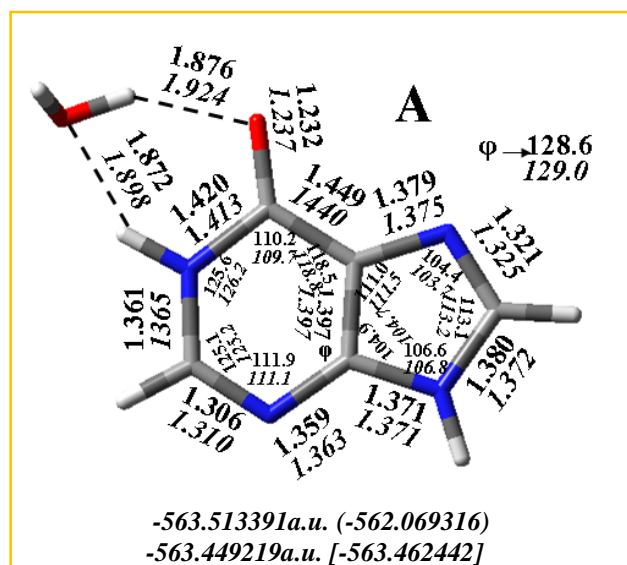


Figura 7.1.8. Longitudes de enlace Å y ángulos ($^{\circ}$) en el monohidrato A de HX-19.

HX-19 + 2 H₂O

En la figura 7.1.9. se representan los hidratos de HX-19 con dos, tres y cuatro moléculas de agua. El siguiente hidrato se obtiene añadiendo al monohidrato más estable (A) una segunda molécula de agua, en las tres posiciones A, B y C y se considera como la más estable la que presenta una menor energía en MP2. En este caso el dihidrato más estable es el que sitúa la segunda molécula en la posición A, el complejo más estable es A-A.

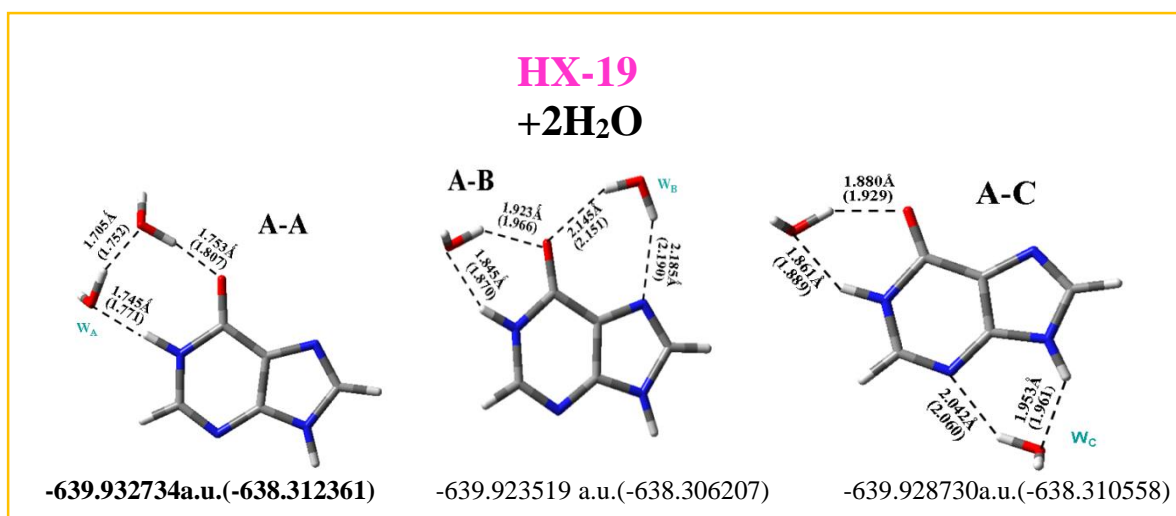
En general las longitudes de enlace calculadas en B3LYP son análogas a las obtenidas en MP2, sin embargo se tiene en la literatura⁴¹ que por B3LYP suelen ser algo más cortos, por lo cual se consideran los valores en MP2 como más exactos. Las diferencias del hidrato A-A respecto de la molécula aislada no son grandes, las mayores aparecen en MP2 en C=O 0.014 Å y N1-C6 -0.015 Å, mientras que en los ángulos son prácticamente iguales Tabla 7.1.15. y Tabla 7.1.16. El cluster A-A, es el más estable, además de tener la menor energía tiene los enlaces de H entre la molécula de agua y HX-19 más fuertes

que los otros dos dihidratos (figura 7.1.9). En ésta puede apreciarse que el orden de estabilidad de los dihidratos es:



HX19+(H₂O)₃. Siguiendo el mismo procedimiento se añade una tercera molécula de agua al dihidrato **A-A**, obteniéndose los resultados de la figura 7.1.9. en las tres posiciones. El orden de estabilidad por MP2 es: **A-A-C** > **A-A-A** > **A-A-B**. El trihidrato más estable es **A-A-C**. Comparando las longitudes de enlace de éste respecto de la molécula aislada no se observan grandes diferencias (Tabla 7.1.15. y 7.1.16.), las más significativas son: C=O, 0.017 Å en B3LYP and 0.013 Å en MP2 y N1-C6 -0.019 Å y -0.017 Å en los dos niveles de cálculo. Los ángulos varían muy poco. En la figura 7.1.9. también se observan los valores de los enlaces de H que, como en los dihidratos, los más fuertes son los correspondientes al hidrato más estable.

HX-19+(H₂O)₄. Siguiendo la misma metodología se añade la cuarta molécula de agua al hidrato **A-A-C** (MP2), en las tres posiciones ya estudiadas y se obtienen las tres posiciones óptimas del tetrahidrato. De las tres posiciones óptimas la más estable es **A-A-C-B** que, como en los casos anteriores, suele tener los enlaces de H más cortos. Al añadir la molécula de agua en la posición B se observa un marcado cambio en la molécula últimamente añadida W_c en el trihidrato **A-A-C** que conduce a la dirección de la hidratación, siendo preferente la formación de enlaces de H entre las moléculas de agua que entre agua y la molécula del tautómero.



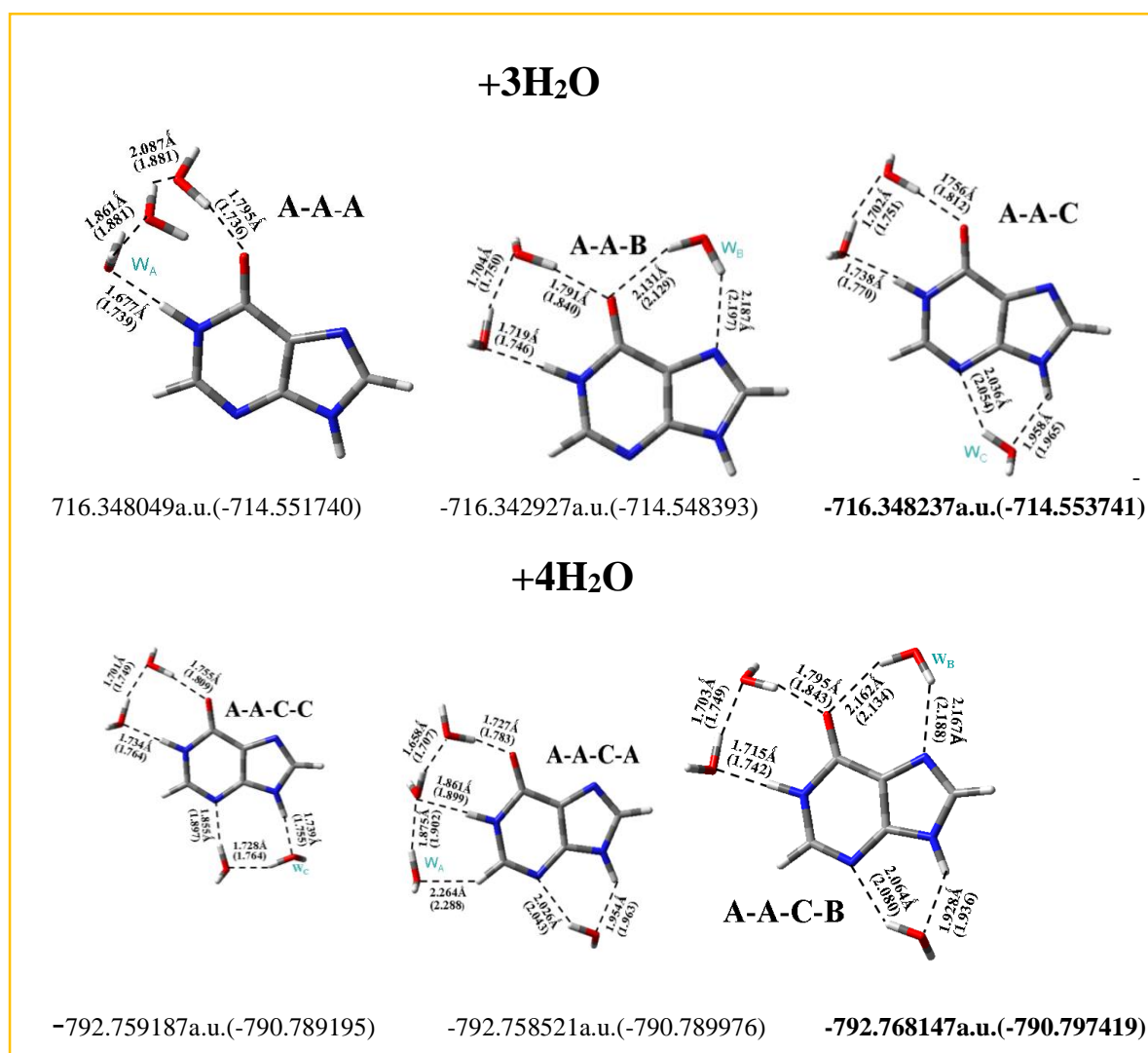


Figura 7.1.9. Las tres posiciones óptimas de la molécula de agua en los hidratos de HX-19 con 2, 3 y 4 moléculas de agua, la energía + la corrección en el cero vienen indicadas en los niveles de cálculo B3LYP y MP2. La de menor energía se escribe en letra negra.

Los cambios observados en las longitudes de enlace y en los ángulos difieren poco de la molécula aislada. Se tiene un incremento de 0.017 Å (B3LYP) y 0.014 Å (MP2) en el enlace C=O y una disminución de 0.019 y 0.017 Å en N1-C6 respectivamente en ambos niveles de cálculo, Tablas 7.1.15. y 7.1.16. Los ángulos solamente experimentan un cambio de -1.9° en N3-C4-C5.

HX-19+(H₂O)_n, n=5-13. En todos estos hidratos se emplearán las Tablas 7.1.15. y 7.1.16. como referencia. La figura 7.1.10. muestra la estructura de los hidratos **con 5 moléculas** de agua hasta 13. En este hidrato se completa la primera esfera de hidratación de HX-19. El método ha sido siempre el mismo, sumar una molécula de agua en las tres posiciones óptimas A, B y C al hidrato anterior más estable en MP2. Sin embargo en las estructuras por MP2 solamente se han logrado obtener hasta sumar la octava molécula,

ésta inclusive; a partir de la molécula de agua número nueve solamente se han obtenido resultados en B3LYP debido a limitaciones computacionales. En la figura solamente se representan los hidratos de menor energía para cada molécula de agua añadida. El hidrato con 5 moléculas de agua más estable es el correspondiente a la posición B. Para mayor simplicidad este hidrato, partiendo de la posición A-A-C-C, se ha nombrado C4, de manera que el hidrato 5 se nombra C4-B y así el de 6 moléculas de agua será C4-B-A, el de siete C4-B-A-B y, a partir de éste, se nombra B7 añadiendo el nombre de la posición de la última molécula de agua sumada: B7-B, B7-B-B, B7-B-B-B....etc.

El pentahidrato presenta una notable variación respecto de la molécula sola. Se tiene un incremento de 0.021 Å en el enlace C=O en B3LYP y 0.018 Å en MP2 y el enlace N1-C6 experimenta una variación de -0.025 y -0.023 Å en B3LYP y MP2 niveles de cálculo, respectivamente. Entre los demás enlaces las diferencias más significativa son de -0.011 Å (C5-C6), -0.009 Å (N1-C2) y 0.007 Å C2-N3. Los ángulos tienen una mayor variación, por MP2 1.8° en los ángulos que incluyen C6, 1.3° en aquellos con N1, -1.9° en N3-C4-C5 y 0.8° en N9-C4-C5. Las variaciones de los demás ángulos son menores de 0.7°.

Los enlaces de H, como puede apreciarse en la figura 7.1.10., en general son fuertes tanto entre las moléculas de agua como entre éstas y HX-19. Los enlaces más débiles corresponden a los que hay entre la molécula y el agua W_B, posiblemente la distorsión que se produce al entrar esta última molécula origina estos valores débiles de los enlaces H...O6 y H...N7.

Las diferencias en el hidrato con **6 moléculas** de agua respecto a la forma aislada son análogas a las anteriores. La variación experimentada por el hidrato con **7 moléculas de agua** no difiere de las anteriores, C=O aumenta 0.024 y 0.021 Å en los dos niveles B3LYP y MP2, respectivamente, N1-C6 varía en -0.31 y -0.027 Å, C5-C6 presenta una variación de -0.014 and 0.011 Å y únicamente N1-C2 sufre un cambio de -0.011 y 0.009 Å en los dos niveles de cálculo. Los enlaces de H son fuertes como puede apreciarse en la figura 7.1.10.

Siguiendo la misma metodología se añade una **octava molécula de agua** en las tres posiciones A, B y C, se obtiene la posición B la posición más estable, la energía en MP2 es la menor, puede apreciarse que a partir de la quinta molécula de agua no coinciden, en cuanto a menor energía B3LYP y MP2, en todos los casos se ha partido del menor valor en este último nivel de cálculo. Los hidratos con 8/10 moléculas de agua se incluyen en la figura 13.2. del Anexo. En el hidrato (H₂O)₈ se tiene un aumento del enlace C=O en 0.026 y 0.022 Å respectivamente en B3LYP y MP2 y un decrecimiento de N1-C6 en

0.032 y 0,029 Å respectivamente. Los restantes enlaces varían poco, solo C5-N6 sufre una disminución aproximadamente de 0.016 Å.

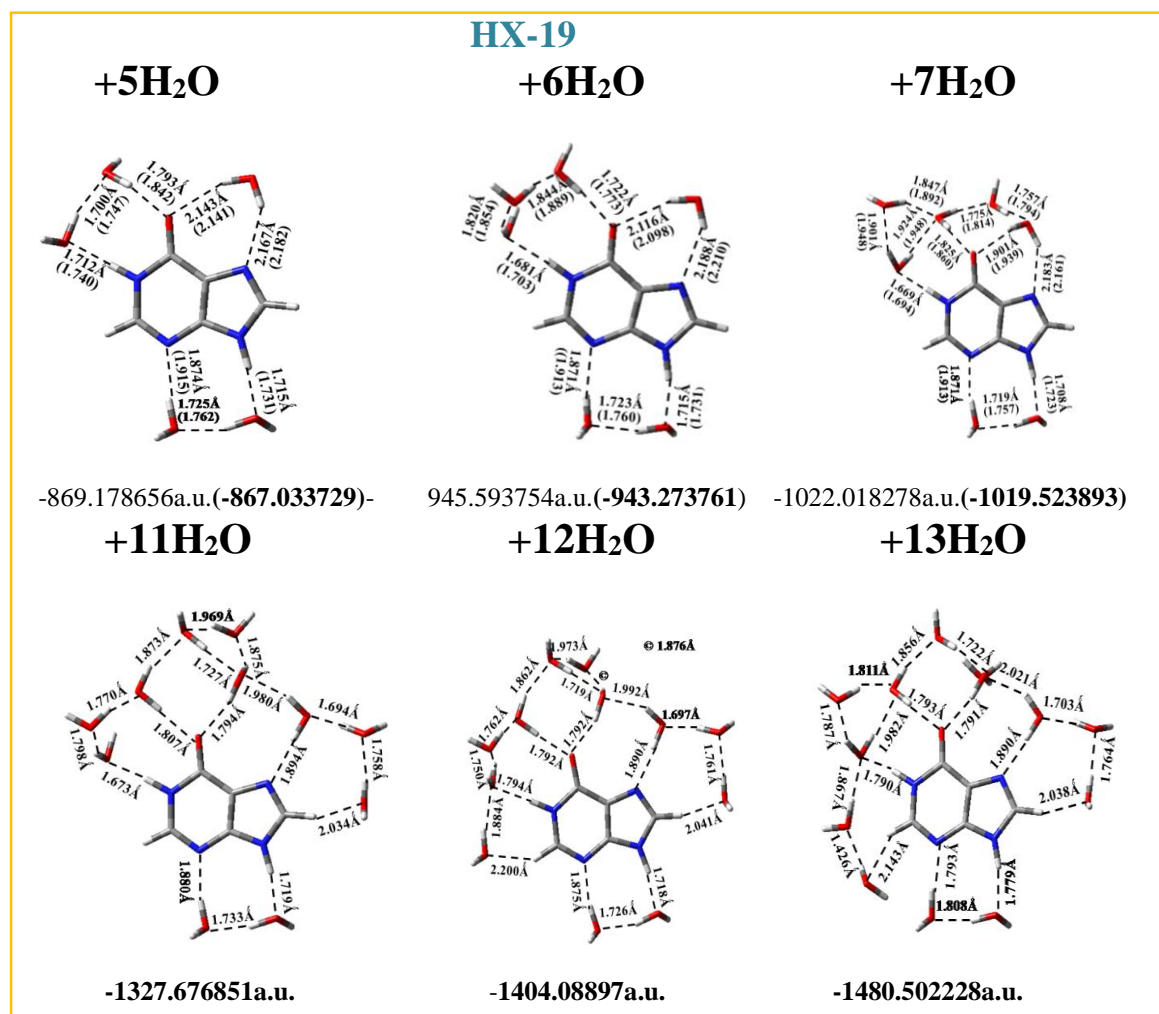


Figura 7.1.10. Estructura de los hidratos más estables de HX-19 ($n=5-13$).

Al comparar los ángulos con los de la molécula aislada se observa un aumento en C6 de 2.6° y 2.1° en cada nivel de cálculo, los demás ángulos decrecen: N1 1.8° y 1.3° respectivamente, N3-C4-C5 2.1° y 1.9° y, por último, C5-C7-N8 decrece en MP2 1.4°. Los restantes ángulos cambian por debajo de 1°. Los enlaces de H, salvo alguna excepción, son fuertes.

El hidrato (**H₂O**)₉, tiene las longitudes de enlace muy próximas a los del hidrato anterior. Los ángulos no presentan diferencias apreciables. A partir del hidrato (**H₂O**)₁₀ solamente se tienen los valores en B3LYP, dado la complejidad del cálculo en MP2 que no se logra con el ordenador del Centro de Cálculo. (**H₂O**)₁₀ (B7-B-B-B) presenta notable variación respecto a HX-19: C6-N1 (-0.036 Å), C=O (0.029 Å), C5-C6 (-0.20 Å), N1-C2 (-0.009 Å) y C2-N3 (0.009 Å). Puede apreciarse un ligero aumento de estos valores respecto de (H₂O)₉. El ángulo que sufre un mayor incremento respecto de HX-19 es C6

(2.9°). Los enlaces de H en general son fuertes a excepción de O(WB)⋯H(C8) y O⋯H (en la zona B) que muestran valores de la longitud de enlace superiores a 2 Å.

Con **(H₂O)₁₁** hay una variación análoga a la anterior en las longitudes de enlace y en los ángulos. Los enlaces de H son también fuertes a excepción de O(WB)⋯H(C8). Se aprecian unos cambios análogos a los de (H₂O)₁₀. En **(H₂O)₁₂** los valores análogos a los anteriores tanto en longitudes de enlace como en ángulos. Los enlaces de H son también fuertes salvo O⋯H-C8 y WA⋯H-C2. Los enlaces de H fuertes determinan un aumento de las cargas, principalmente sobre el átomo de O, lo cual da lugar a un aumento de la reactividad y a un aumento en las energías HOMO y LUMO.

Con 12 moléculas de agua se completa la primera esfera de hidratación de HX-19, las moléculas de agua rodean a la molécula uniéndose a ésta mediante enlaces de H con los átomos con mayor carga negativa (O y N) o mediante el O del agua y los átomos de H más positivos del tautómero. La dirección de entrada de cada nueva molécula de agua viene determinada por la posición del primer hidrato (A) y por la formación de los enlaces H₂O⋯H₂O más fuertes que entre el agua y la base. En general la óptima distribución de las moléculas de agua alrededor de dicha base concuerda con la estructura del hidrato con la molécula de uracilo⁵². Al completarse esta primera esfera se tiene un alargamiento de los enlaces C=O (0.032 Å) y N7-C8 (0.010 Å), un decrecimiento de los enlaces N1-C6 y C5-C6 (0.037 Å) y (0.021 Å) respectivamente, y una variación menor en C2-N3 (0.009 Å), C8-N9 (0.007 Å) y N9-C4 (0.006 Å).

Los cambios en los ángulos tienen lugar en C6 (+2.6°) y N3 (+1.3°) y N3-C4-C5 (-2.2°) y N1 (-1.2°). Los restantes ángulos presentan variaciones menores de 1°.

En la figura 7.1.5. se tienen las cargas en HX-19 aislado en diferentes niveles de cálculo y con 12 moléculas de agua en B3LYP, se observan incrementos en la carga negativa en O6 (0.122 *e*) y en N7 (0.089 *e*) y en las cargas positivas en C4 (0.027 *e*), C8 (0.040 *e*) y C2 (0.016 *e*) mientras decrece la carga negativa en N1 (0.023 *e*) y la carga positiva en C5 (0.015 *e*).

Al añadir la molécula de agua número 13 **(H₂O)₁₃**, estando la primera capa ya completa, ésta se sitúa en la zona A, en la posición con menor energía, y se observan las diferencias siguientes: un alargamiento, respecto de la forma anhidra, de los enlaces C=O (0.032 Å), N7-C8 (0.011 Å) y un decrecimiento de N1-C6 (0.036 Å) y C5-C6 (0.019 Å), y, con menores valores C2-N3 (0.007 Å) y N9-C4 (0.008 Å). Los ángulos C5-C4-N3 y C4-N3-C2 incrementan 2.1° y 1.5° respectivamente y C2-N1-C6 y C4-N5-C7 disminuyen en 1.2° cada uno.

HX-19+nH₂O (n=15, 20, 25, 30)

Al sumar más moléculas de agua a los hidratos hasta lograr 15-30, no se ha seguido el mismo procedimiento. Cada hidrato se ha obtenido situando las moléculas necesarias en posiciones diferentes, repartidas en la segunda capa de hidratación. La figura 7.1.11. muestra la estructura de los complejos con 15, 20, 25 y 30 moléculas de agua.

En el hidrato **(H₂O)₃₀** se observa que la variación en las longitudes de enlace respecto a los de la primera esfera de hidratación la **(H₂O)₁₂** no es significativa, las mayores diferencias son: C=O (0.013 Å), N1-C6 (-0.022 Å), 0.012 Å. C5-C6 (-0.013 Å) y C8-N9 (-0.014 Å). Respecto al hidrato **(H₂O)₁₃** los cambios más significativos son: C=O (0.011 Å), N1-C6 (-0.022 Å), C5-C6 (0.015 Å) y C8-N9 (-0.016 Å). Se aprecia que la estructura de la molécula se estabiliza a medida que aumenta el número de moléculas de agua, ello se debe a los valores de las cargas en **(H₂O)₃₀** que experimentan una variación de: N7 (0.064 *e*), O7 (+0.036 *e*), N3 (-0.032 *e*) respecto de **(H₂O)₁₂**.

Los enlaces de H en los hidratos con 15 a 30 moléculas de agua son, salvo excepciones, fuertes tanto entre el agua y la base como entre las moléculas de agua (figura 7.1.11.). Como puede apreciarse los enlaces ≥ 2.000 Å se dan preferentemente entre el agua y la molécula de HX-19.

Tabla 7.1.15. Efecto de la hidratación sobre las longitudes de enlace (Å) y ángulos (°) del tautómero HX-19 calculado en el nivel B3LYP.

Longitudes de enlace	aislada	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O	+9H ₂ O	+10H ₂ O	+12H ₂ O	+13H ₂ O
											O	O	
C=O	1.217	1.232	1.235	1.234	1.234	1.238	1.241	1.241	1.243	1.243	1.246	1.249	1.249
C6-N1	1.436	1.420	1.412	1.417	1.417	1.411	1.406	1.405	1.404	1.402	1.400	1.399	1.400
N1-C2	1.365	1.361	1.362	1.358	1.355	1.356	1.354	1.354	1.355	1.355	1.356	1.361	1.358
C2-N3	1.303	1.306	1.307	1.309	1.310	1.310	1.311	1.312	1.311	1.312	1.312	1.312	1.310
N3-C4	1.362	1.359	1.357	1.364	1.362	1.365	1.364	1.364	1.364	1.364	1.364	1.363	1.358
C4-C5	1.396	1.397	1.397	1.396	1.399	1.399	1.399	1.397	1.400	1.398	1.397	1.400	1.399
C5-C6	1.447	1.449	1.439	1.438	1.437	1.436	1.434	1.433	1.430	1.429	1.427	1.426	1.428
C5-N7	1.378	1.379	1.380	1.378	1.376	1.377	1.377	1.379	1.376	1.375	1.373	1.377	1.378
N7-C8	1.311	1.321	1.310	1.314	1.315	1.316	1.316	1.317	1.315	1.318	1.319	1.321	1.322
C8-N9	1.379	1.380	1.381	1.376	1.375	1.372	1.373	1.372	1.376	1.372	1.372	1.372	1.374
N9-C4	1.371	1.371	1.371	1.365	1.365	1.365	1.365	1.366	1.364	1.366	1.367	1.365	1.363
Ángulos de enlace													
N1-C6-C5	108.9	110.2	110.5	110.6	110.6	110.8	111.2	111.3	111.5	111.6	111.7	111.5	111.6
C6-C5-C4	119.4	118.5	118.7	118.7	119.2	119.2	119.0	119.1	118.9	119.2	119.4	118.9	118.7
C5-C4-N3	128.9	128.6	128.4	128.1	127.1	126.9	126.9	126.7	126.8	126.5	126.3	126.7	126.8
C4-N3-C2	112.0	111.7	111.7	112.1	112.7	112.7	112.7	112.7	112.7	112.8	112.9	113.3	113.5
N3-C2-N1	124.6	125.1	125.6	125.2	125.2	125.2	125.2	125.3	125.3	125.3	125.3	124.1	124.0
C2-N1-C6	126.6	125.6	125.1	125.4	125.2	125.1	125.0	124.7	124.8	124.6	124.5	125.4	125.4
C5-N7-C8	104.5	104.4	104.4	104.2	104.0	104.2	104.2	104.1	103.8	104.3	104.8	104.8	104.9
N7-C8-N9	113.0	113.1	113.1	113.4	113.9	113.7	113.7	113.7	113.9	113.4	112.9	112.7	112.6
C8-N9-C4	106.6	106.6	106.6	106.1	105.7	105.9	105.9	106.0	105.8	106.1	106.4	106.7	106.6
N9-C4-C5	105.0	104.9	105.0	105.7	105.9	105.9	105.9	105.8	105.7	105.8	105.8	105.7	106.0
C4-C5-N7	111.0	111.0	111.0	110.5	110.5	110.2	110.3	110.4	110.7	110.4	110.2	110.1	109.8

Tabla 7.1.15b (continuación).

	+14 H ₂ O	+15H ₂ O	+20H ₂ O	+25H ₂ O	+30H ₂ O
C=O	1.259	1.250	1.244	1.250	1.262
C6-N1	1.399	1.400	1.402	1.395	1.378
N1-C2	1.360	1.359	1.351	1.353	1.356
C2-N3	1.309	1.309	1.317	1.317	1.307
N3-C4	1.357	1.357	1.363	1.360	1.351
C4-C5	1.299	1.399	1.394	1.397	1.399
C5-C6	1.428	1.429	1.434	1.425	1.413
C5-N7	1.381	1.381	1.381	1.380	1.379
N7-C8	1.323	1.323	1.320	1.318	1.326
C8-N9	1.365	1.365	1.374	1.371	1.358
N9-C4	1.362	1.361	1.368	1.366	1.360

Tabla 7.1.16. Efecto de la hidratación sobre las longitudes de enlace (Å) y ángulos (°) de la molécula del tautómero HX-19 en el nivel MP2/6-31G(d,p).

Longitudes de enlace	aislada	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O
C=O	1.225	1.237	1.239	1.238	1.239	1.243	1.246	1.246	1.247
C6-N1	1.427	1.413	1.406	1.410	1.410	1.404	1.400	1.400	1.398
N1-C2	1.368	1.365	1.365	1.362	1.360	1.360	1.359	1.359	1.360
C2-N3	1.307	1.310	1.311	1.312	1.313	1.313	1.314	1.314	1.314
N3-C4	1.367	1.363	1.362	1.367	1.369	1.367	1.367	1.367	1.367
C4-C5	1.396	1.397	1.397	1.392	1.400	1.400	1.400	1.400	1.399
C5-C6	1.336	1.440	1.439	1.438	1.437	1.436	1.435	1.435	1.430
C5-N7	1.374	1.375	1.376	1.374	1.372	1.372	1.373	1.373	1.371
N7-C8	1.326	1.325	1.325	1.329	1.329	1.330	1.330	1.330	1.330
C8-N9	1.371	1.372	1.372	1.368	1.369	1.365	1.366	1.366	1.368
N9-C4	1.371	1.371	1.371	1.367	1.366	1.366	1.366	1.366	1.366

Ángulos de
enlace

N1-C6-C5	108.5	109.7	110.0	110.1	110.1	110.3	110.7	110.7	111.0
C6-C5-C4	119.3	118.8	119.0	119.4	119.4	119.4	119.2	119.3	119.3
C5-C4-N3	129.2	129.0	128.3	127.4	127.4	127.3	127.3	127.1	127.0
C4-N3-C2	111.2	111.1	111.4	112.0	112.0	112.0	112.0	112.1	112.1
N3-C2-N1	124.7	125.2	125.2	125.1	125.1	125.1	125.1	125.2	125.2
C2-N1-C6	127.1	126.2	126.1	125.9	125.9	125.8	125.7	125.5	125.3
C5-N7-C8	103.8	103.7	103.6	103.3	103.3	103.7	103.6	103.6	103.2
N7-C8-N9	113.1	113.2	113.5	114.0	114.0	113.7	113.7	113.7	114.0
C8-N9-C4	106.9	106.8	106.4	106.0	106.0	106.2	106.2	106.3	106.1
N9-C4-C5	104.8	104.7	105.4	105.6	105.6	105.7	105.6	105.6	105.4
C4-C5-N7	111.5	111.5	111.1	111.1	111.1	110.8	110.8	110.9	111.4

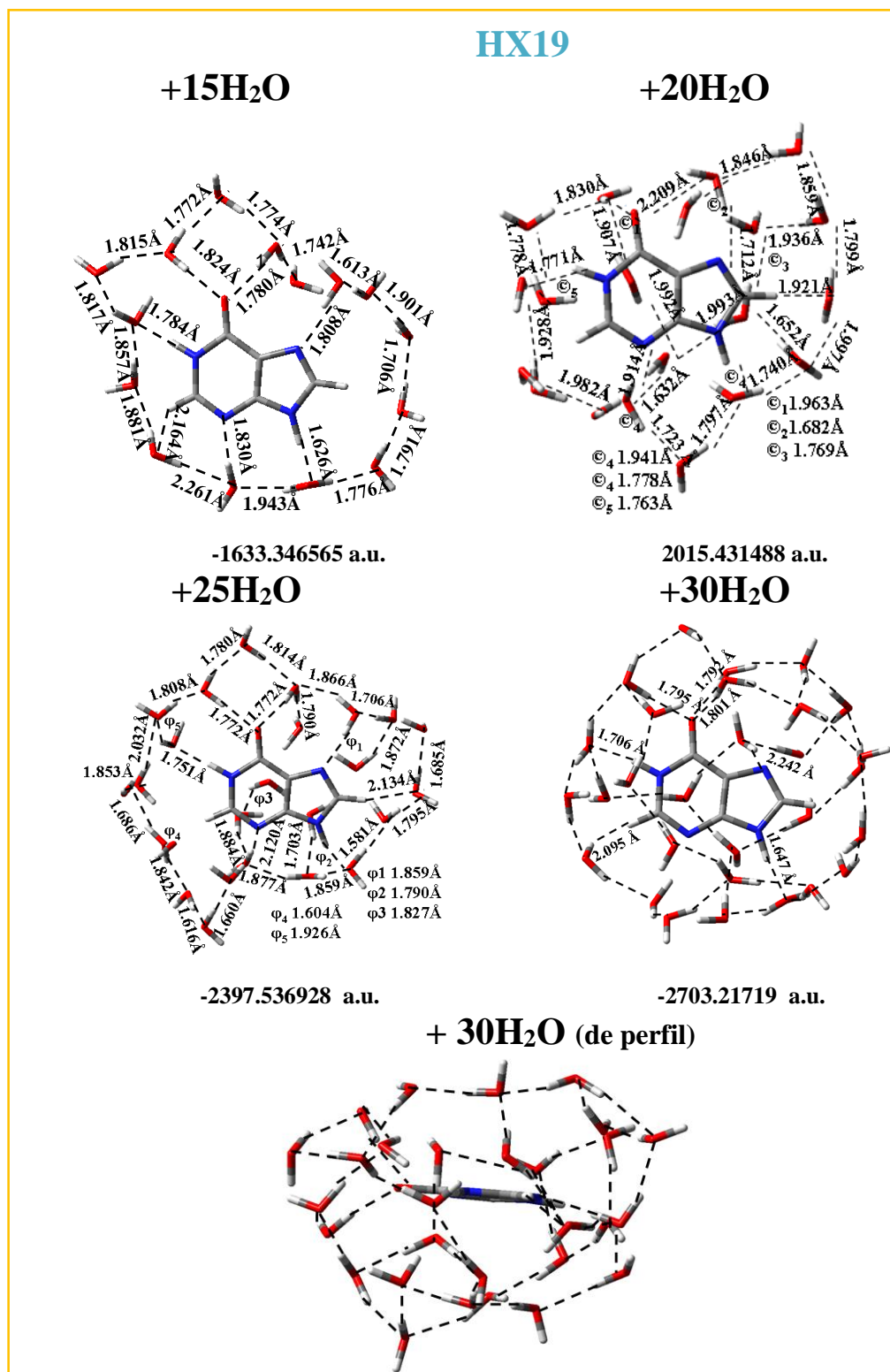


Figura 7.1.11. Estructura de los hidratos de HX-19 +15, 20, 25 y 30 moléculas de agua.

7.1.4.2. Hidratación del tautómero HX-17

Como se ha indicado en puntos anteriores el estudio de la hidratación de HX se completa con los dos tautómeros más numerosos, en este caso se estudiará el HX-17.

HX-17 + 1 H₂O

La figura 7.1.6. muestra las tres posiciones óptimas (A-B-C) del monohidrato de HX-17 en los niveles de cálculo B3LYP/6-31G(d,p), MP2/6-31G(d,p), M052X/6-31G(d,p) y M06L/6-31G(d,p), con los enlaces de H entre el agua y la base, y la energía en cada posición (E+ZPE). La más estable es la posición B, la de menor energía. La longitud de los enlaces varía poco respecto de la molécula aislada. En las posiciones A y B el enlace N1-C6 se acorta en 0.012 Å por MP2 y C6-O6 incrementa en 0.015 Å y este mismo enlace en la posición B se acorta en 0.002 Å. No se observan otras diferencias. La presencia de cada molécula de agua en la posición A y B activan los átomos próximos N1 y O6. Ello puede deberse a la distribución de las cargas que, aunque se alteran poco, O6 sufre un incremento en la carga de 0.049 *e* por MP2 en el hidrato A, de 0.051 *e*, en la posición B y de 0.026 *e* en N3. En la posición C se tienen los incrementos en la carga negativa de N3 y N9 0.029 de 0.032 *e* e igual en ambos átomos y C8 de 0.015 *e* por MP2.

En la figura 7.1.7.C se tienen los valores de la máxima deformación de energía de la molécula HX-17, ΔE_{HX-17}^{def} , en la posición C del monohidrato en B3LYP y en la posición B en MP2; la deformación del agua ΔE_{W1}^{def} tiene un cambio muy pequeño en las tres posiciones óptimas en ambos niveles de cálculo y la posición C tiene el menor valor de la energía de interacción y, por tanto, es la menos estable.

La molécula de agua en posición B tiene el valor máximo de la corrección CP de la energía de formación (ΔE_{HX17W1}^{CP}) (Tabla 7.1.14. y figura 7.1.7.A) de manera que ésta es la más estable. La posición A tiene los enlaces de H más fuertes y, aunque el valor de la corrección CP no es el más alto, ello determina su estabilidad en los dos niveles de

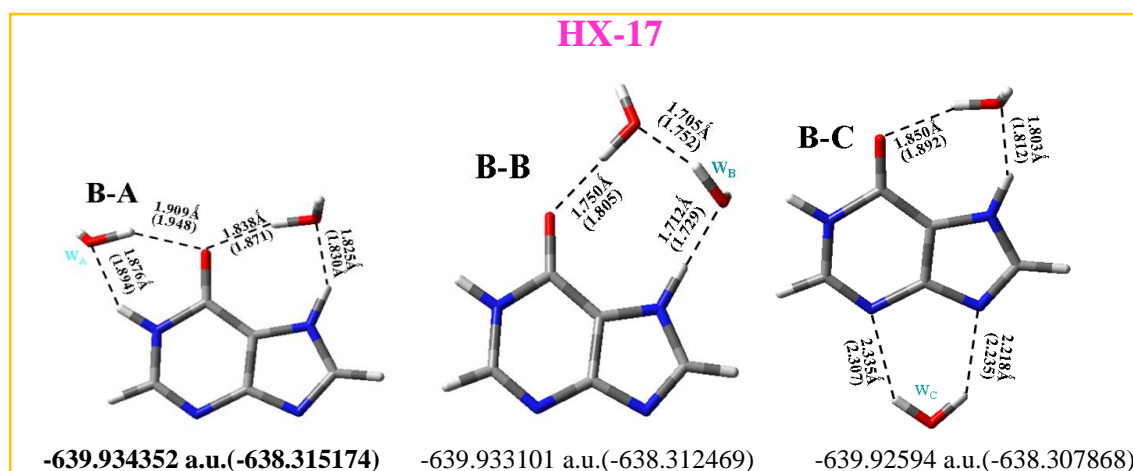


Figura 7.1.12. Las tres posiciones óptimas en los dihidratos del tautómero HX-17.

cálculo (figura 7.1.6).

HX-17 + 2H₂O

La figura 7.1.12. muestra las tres posiciones óptimas al situar una segunda molécula de agua en el tautómero HX-17 y las energías: E+ZP, en B3LYP y la energía en MP2 (entre paréntesis). Los menores valores de las mismas vienen en **negrita** y corresponden a la posición A, que será la más estable. Al comparar esta estructura con la de HX-17 aislado no se observan grandes cambios en las longitudes de enlace. Las mayores diferencias las presentan N1-C6 y C=O. El primero se acorta en 0.020 Å en MP2 y el segundo incrementa en 0.026 Å. C2-N3 también sufre un incremento 0.020 Å. Vemos que las variaciones se tienen en los enlaces entre los átomos cercanos a las moléculas de agua adicionadas. Los ángulos no presentan variaciones apreciable, la mayor es de 2.1° en C1-C6-N5, en B3LYP. En la figura se ve también que los enlaces de H son fuertes en las estructuras B-B y B-A, en este orden, lo cual da estabilidad al complejo. En la posición B-C se aprecian los enlaces de H entre la nueva molécula de agua añadida y la base que son mucho más débiles.

Las cargas presentan un gran incremento en la carga negativa de O6 de 0.091 *e*, un decrecimiento en la carga negativa de N1, 0.012 *e* y aumenta la carga positiva en C4 en 0.013 *e*, todas ellas en el nivel B3LYP; destacan también las grandes diferencias en las cargas positivas de los átomos de H que van desde un incremento en la carga positiva en H_(C8) de 0.034 *e*, al menor en H_(N7) de 0.022 *e*. En MP2 los cambios son grandes: incremento de la carga negativa en O6 de 0.095 *e*, la carga negativa en N9 aumenta 0.012 *e* y las cargas positivas de C4 y C8 aumentan en 0.019 y 0.013 *e* respectivamente, que explicarían las diferencias respecto de la molécula aislada. Por MP2 cabe destacar también los incrementos en la carga positiva de H_(N1) (0.035 *e*) y H_(N7) (0.034 *e*).

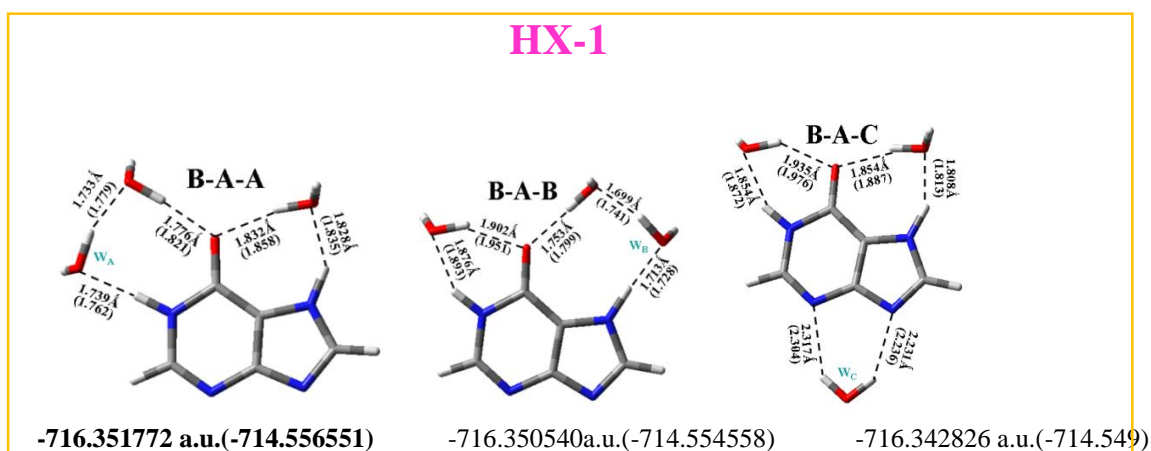


Figura 7.1.13. Las tres posiciones óptimas en los trihidratos del tautómero HX-17.

HX-17+3(H₂O).

El trihidrato, se logra al añadir una tercera molécula de agua al complejo B-A, el de menor energía en las tres posiciones A, B y C. Se obtienen las estructuras de la figura 7.1.13. en las que, como en los casos anteriores, se indican las energías B3LYP y MP2. Aquí se tienen la energía menor en B-A-B. En esta posición la longitud de enlace C=O presenta la mayor diferencia con respecto a la base aislada presentando un incremento de 0.029 Å en MP2, mientras que N1-C6 decrece en 0.026 Å, y en C5-C6 decrece en 0.013 Å y en N1-C6 se incrementa en 0.008 Å. Los ángulos prácticamente no varían.

HX-17+4(H₂O).

Cuando se adiciona una cuarta molécula de agua al hidrato B-A-B en las tres posiciones óptimas se obtienen las tres estructuras que figuran en la figura 7.1.14. La más estable, según los valores de la energía, es B-A-A-B. Se tiene: C=O aumenta 0.034 Å (B3LYP) y 0.029 Å (MP2), N1-C6 decrece 0.029 Å y 0.026 Å en los dos niveles de cálculo y C4-C5 y C5-C6 sufren una variación de 0.013 Å en B3LYP, el primero aumenta y el segundo decrece y, en MP2 la disminución del primero es de 0.026 Å y el segundo de -0.023 Å, los enlaces C4-C5 sufren un incremento, en B3LYP de 0.013 Å, en MP2 el primero se incrementa en 0.013 Å y el segundo experimenta una disminución de 0.012 Å. Los cambios tienen lugar principalmente en los enlaces próximos a las moléculas de agua adicionadas. Los ángulos prácticamente no cambian.

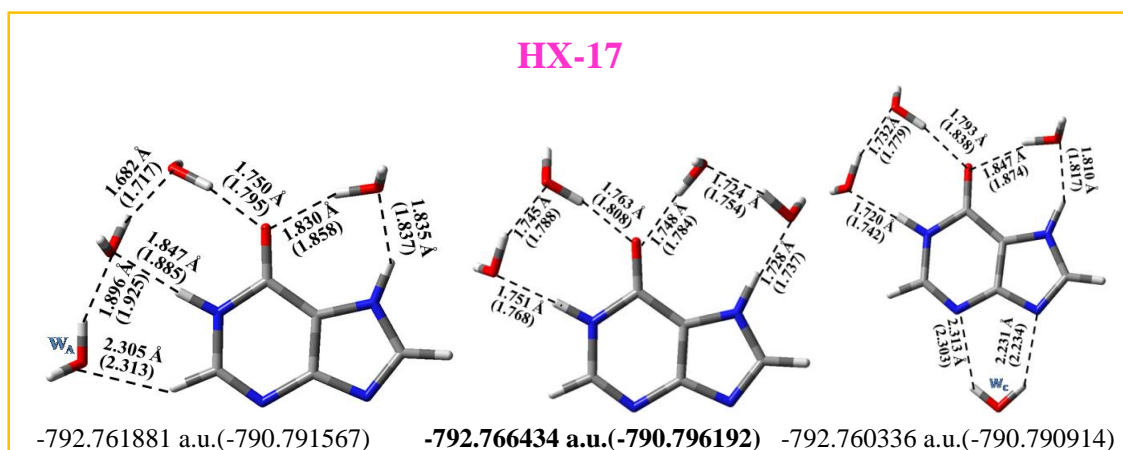


Figura 7.1.14. Las tres posiciones óptimas en los tetrahidratos del tautómero HX-17.

En la figura anterior se observan los enlaces de H fuertes en las tres posiciones pero, en B-A-A-A y B-A-A-C, se dan enlaces más débiles entre la última molécula de agua añadida y la base.

HX-17+n(H₂O). (n = 5/13)

En la figura 7.1.15. se tienen las estructuras de los hidratos con 5-13 moléculas de agua; las longitudes de enlace presentan pocas diferencias respecto de los hidratos

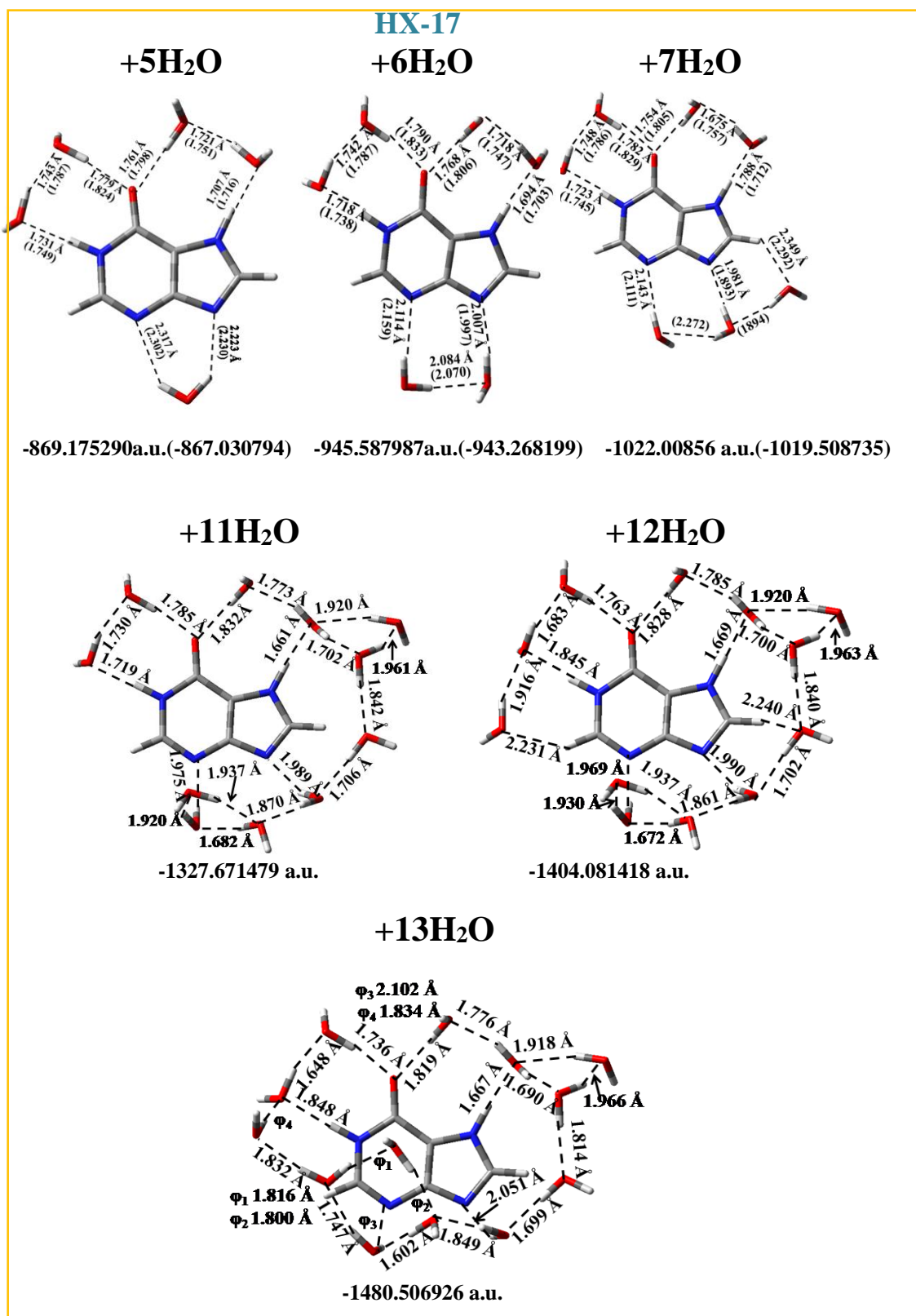


Figura 7.1.15. Las tres posiciones óptimas en los del tautómero HX-17 ($n = 5/13$).

estudiados anteriormente (Tabla 7.1.17. y 7.1.18.), C=O, N1-C6, C4-C5 y C5-C6 tienen valores análogos a los anteriores. Los ángulos prácticamente no varían.

Los enlaces de H no presentan diferencias en relación a los anteriores clusters. Se tiene la mayor diferencia en $O \cdots H_{(N1)}$ en MP2 en el hidrato con 6 moléculas de agua y, mayor con 11. Los enlaces entre el H del agua y O de la base, en algunos casos son más fuertes que los que se tienen entre las moléculas de agua. A partir de la suma de 9 moléculas de agua, no se obtuvo la optimización en MP2. Con el hidrato $(H_2O)_{13}$ se completa la primera esfera de hidratación en HX-17. A partir de éste se comienza a formar la segunda envoltura alrededor de la base.

HX-17+n(H₂O) (n =15, 20, 25, 30).

La figura 7.1.16. muestra las estructuras de los hidratos de HX-17 con 15, 20, 25 y 30 moléculas de agua, las optimizaciones se obtuvieron en B3LYP. No se observan variaciones en las longitudes de enlace al comparar con el hidrato, con la primera esfera de hidratación completa, $(H_2O)_{13}$. La mayor diferencia corresponde a $(H_2O)_{15}$ con N1-C6 (0.010 Å). Las cargas presentan grandes diferencia. Así, respecto del citado hidrato se tienen en el hidrato con 15H₂O (figura 7.1.5.): N9 (0.057 *e*), O6 (0.031 *e*), N3 (0.027 *e*) y C2 (0.029 *e*). En HX-17+20H₂O es similar. $(H_2O)_{25}$ presenta grandes diferencias en N9 (-0.056 *e*), O6 (0.019 *e* en B3LYP and 0.025 *e* in MP2) y C4, N3 y N7 presentan diferencias menores a 0.020 *e*). En la estructura con 30H₂O hay pocas diferencias: N3 (0.035 *e*) y O N3 (0.035 *e*).

7.1.4.3. PCM

En la figura 7.1.4. se tiene la estructura con el polarizable continuum modelo PCM, la estructura de la forma hidratada con 20 moléculas de agua, y la estructura de la forma aislada en los tautómeros HX-19 y HX-17, éstas en los dos niveles de cálculo B3LYP y MP2. Las longitudes de enlace con PCM en HX-19 no presentan valores muy diferentes a los de este tautómero aislado, los más significativos son: C=O, que se alarga en 0.015 Å, y N1-C6 que se acorta en 0.021 Å. Tienen diferencias acusadas los enlaces H-N1 y H-N9 con variaciones de 0.017 y 0,018 Å respectivamente. Estos enlaces sufren mayor variación en el hidrato con 20 moléculas de agua presentando las diferencias de 0.027 Å en C=O y -0.034 Å en N1-C6. Con respecto al monohidrato más estable A, C=O se alarga en éste 0.015 Å y N1-C6 se acorta en 0.016 Å. Los ángulos prácticamente no cambian en PCM y $(H_2O)_{20}$.

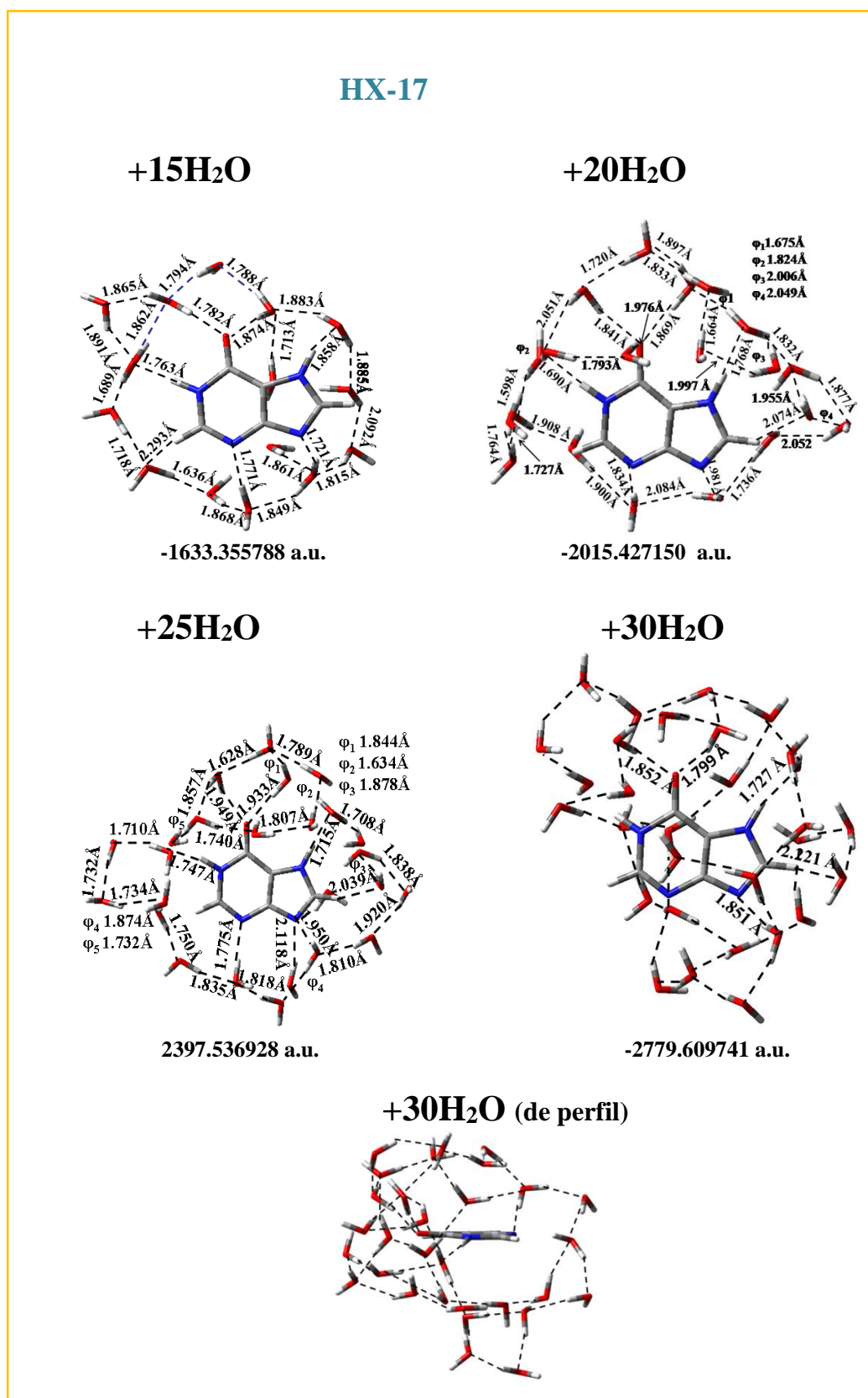


Figura 7.1.16. Estructura de los hidratos de HX-17 con 15, 20, 25 y 30 moléculas de agua.

El PCM tiene menor efecto sobre la geometría que la forma hidratada con 20 moléculas de agua. Ello está de acuerdo con el incremento en el valor de las cargas. La mayor carga negativa en $(\text{H}_2\text{O})_{20}$ corresponde a O6 (-0.694 e) que cambia en 0.0108 e , junto con N7 que varía análogamente respecto de la molécula aislada (figura 7.1.5) mientras que los restantes átomos aumentan: N3 0.093 e , C4 0.034 e , C8 0.051 e y C2 0.019 e .

Al analizar la geometría del tautómero HX-17 se observan pequeñas diferencias entre PCM y la base anhidra, tanto en las longitudes de enlace como en los ángulos a pesar de presentar diferencias notables en las cargas, incrementando la carga negativa de O6 en 0,058 e , de N3 en 0.058 e y N9 en 0.072 e y decrece la carga negativa de C5 en 0.046 e .

7.1.4.4. Momento dipolar (μ)

Los valores del momento dipolar de los tautómeros HX-19 y HX-17 y de los hidratos correspondientes se tienen en la Tabla 7.1.19., en los niveles de cálculo B3LYP y MP2. Con MP2 se obtuvieron solamente los hidratos hasta con 8 moléculas de agua, en ambos tautómeros. Aquí se han tomado los valores del momento dipolar correspondientes al hidrato de menor energía para cada número de moléculas de agua adicionadas. Las unidades de μ se dan en Debyes (D). En el tautómero HX-19 en MP2 μ varía desde 5.891 D, en el estado aislado, hasta un valor máximo de 8.214 D en el hidrato con 5 moléculas de agua, y un mínimo valor 1.950 D en el de 8 moléculas de agua.

A partir del de 9 H_2O μ se ha calculado solamente en B3LYP y sus valores van desde 5.051 D en la molécula aislada hasta 1.858 D en $(\text{H}_2\text{O})_{30}$, con un valor máximo de 7.423 D en $(\text{H}_2\text{O})_5$ y un mínimo valor de 1.245 D en $(\text{H}_2\text{O})_8$.

Los momentos dipolares de HX-17 van aumentando desde 1.426 D en el estado aislado hasta 9.227 D en $(\text{H}_2\text{O})_6$. En el nivel de cálculo B3LYP se tiene 1.758 D en el estado aislado y presenta un valor máximo de 10.048 A en el hidrato con 7 moléculas de agua y, a partir de éste, los valores fluctúan hasta 8.728 D en el hidrato con 30 moléculas de agua.

Tabla 7.1.17. Efecto de la hidratación en las longitudes de enlace (Å) y los ángulos (°) de la molécula HX-17 calculada en el nivel B3LYP/6-31G(d,p).

Longitudes de enlace	aislada	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O	+9H ₂ O	+10H ₂ O	+11H ₂ O	+12 H ₂ O	+13H ₂ O
C=O	1.225	1.240	1.255	1.259	1.259	1.256	1.255	1.254	1.256	1.253	1.254	1.254	1.256	1.256
C6-N1	1.416	1.404	1.393	1.387	1.387	1.390	1.390	1.391	1.390	1.395	1.393	1.394	1.392	1.392
N1-C2	1.376	1.372	1.374	1.374	1.372	1.369	1.367	1.367	1.368	1.364	1.369	1.365	1.370	1.370
C2-N3	1.297	1.300	1.299	1.300	1.300	1.301	1.302	1.302	1.303	1.305	1.306	1.305	1.306	1.306
N3-C4	1.372	1.369	1.368	1.366	1.366	1.366	1.365	1.365	1.365	1.368	1.367	1.367	1.366	1.366
C4-C5	1.396	1.398	1.403	1.404	1.409	1.405	1.402	1.404	1.403	1.403	1.404	1.405	1.405	1.405
C5-C6	1.433	1.427	1.421	1.420	1.420	1.421	1.423	1.423	1.421	1.422	1.420	1.420	1.418	1.418
C5-N7	1.374	1.374	1.373	1.374	1.376	1.376	1.377	1.376	1.374	1.375	1.376	1.374	1.375	1.375
N7-C8	1.368	1.368	1.362	1.362	1.361	1.358	1.356	1.354	1.356	1.353	1.353	1.352	1.352	1.352
C8-N9	1.318	1.319	1.324	1.323	1.323	1.325	1.326	1.328	1.228	1.329	1.329	1.331	1.301	1.331
N9-C4	1.373	1.374	1.369	1.369	1.367	1.367	1.366	1.366	1.367	1.365	1.366	1.365	1.365	1.365
Ángulos de enlace														
N1-C6-C5	108.2	109.2	110.3	110.0	111.0	111.0	110.9	110.9	110.9	110.8	110.7	110.8	110.7	110.7
C6-C5-C4	123.4	122.8	122.2	122.6	121.5	121.3	121.3	121.3	121.7	121.5	121.4	121.6	121.5	121.5
C5-C4-N3	123.8	123.9	123.6	123.6	123.9	124.1	124.1	124.1	123.6	123.9	124.1	123.7	123.8	123.8
C4-N3-C2	113.8	114.0	113.8	113.1	113.7	113.8	114.1	113.9	113.9	113.9	114.2	114.0	114.4	114.4
N3-C2-N1	125.3	125.2	125.6	125.9	125.7	125.5	125.4	125.4	125.5	125.4	124.5	125.4	124.5	124.5
C2-N1-C6	125.5	125.2	124.4	124.3	124.2	124.4	124.4	124.4	124.3	124.4	125.1	124.3	125.0	125.0
C5-N7-C8	106.1	105.7	105.6	105.9	105.5	105.7	105.8	106.6	105.9	105.7	105.7	105.9	105.9	105.9
N7-C8-N9	113.4	114.5	115.1	113.8	114.5	114.2	114.0	114.2	113.9	114.1	114.1	113.9	113.9	113.9
C8-N9-C4	104.5	104.2	104.2	103.9	103.9	104.1	104.3	104.2	104.0	104.1	104.2	104.2	104.3	104.3
N9-C4-C5	110.5	110.3	110.2	110.5	110.5	110.4	110.2	110.2	110.5	110.3	110.2	110.2	110.1	110.1
C4-C5-N7	105.5	105.9	105.9	105.9	105.6	105.6	105.7	105.9	105.7	105.8	105.8	105.7	105.8	105.8

Tabla 7.1.17. (continuación).

	+15H ₂ O	+20H ₂ O	+21H ₂ O	+25H ₂ O	+30H ₂ O
C=O	1.259	1.260	2.258	1.250	1.265
C6-N1	1.384	1.385	1.390	1.393	1.384
N1-C2	1.361	1.360	1.363	1.360	1.363
C2-N3	1.304	1.309	1.310	1.306	1.307
N3-C4	1.361	1.366	1.368	1.363	1.363
C4-C5	1.395	1.403	1.402	1.396	1.398
C5-C6	1.415	1.412	1.415	1.422	1.407
C5-N7	1.374	1.372	1.374	1.375	1.372
N7-C8	1.348	1.357	1.357	1.351	1.351
C8-N9	1.337	1.333	1.332	1.337	1.330
N9-C4	1.373	1.364	1.365	1.370	1.365

Tabla 7.1.18. Efecto de la hidratación en las longitudes de enlace (Å) y los ángulos (°) de la molécula HX-17 calculada en el nivel MP2/6-31G(d,p).

Longitudes de enlace	aislada	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O
C=O	1.232	1.429	1.258	1.261	1.261	1.259	1.257	1.257	1.259
C6-N1	1.406	1.395	1.386	1.380	1.380	1.382	1.382	1.385	1.385
N1-C2	1.381	1.377	1.378	1.378	1.377	1.374	1.373	1.372	1.369
C2-N3	1.302	1.305	1.305	1.306	1.305	1.306	1.307	1.307	1.309
N3-C4	1.376	1.373	1.371	1.369	1.369	1.369	1.368	1.369	1.370
C4-C5	1.397	1.298	1.404	1.404	1.423	1.405	1.404	1.404	1.404
C5-C6	1.435	1.429	1.425	1.425	1.423	1.425	1.426	1.425	1.423
C5-N7	1.363	1.364	1.363	1.363	1.364	1.364	1.365	1.365	1.362
N7-C8	1.372	1.373	1.368	1.368	1.367	1.365	1.363	1.363	1.363
C8-N9	1.325	1.324	1.328	1.328	1.328	1.329	1.330	1.332	1.332
N9-C4	1.37	1.374	1.370	1.371	1.369	1.369	1.369	1.364	1.367

Ángulos de
enlace

N1-C6-N5	107.7	108.6	109.7	110.0	110.4	110.3	110.3	110.3	110.4
C6-C5-C4	123.9	123.1	122.6	122.6	122.0	121.9	121.8	121.6	121.9
C5-C4-N3	123.8	123.9	123.8	123.6	123.9	124.1	124.1	124.4	123.7
C4-N3-C2	113.2	113.4	113.3	113.1	113.2	113.3	113.4	113.3	113.7
N3-C2-N1	125.5	125.2	125.5	125.9	126.7	125.4	125.3	125.2	125.1
C2-N1-C6	126.0	125.9	125.1	124.7	124.8	125.0	125.1	125.2	125.0
C5-N7-C8	106.4	106.0	105.9	105.9	105.7	105.9	106.0	106.4	106.4
N7-C8-N9	113.1	113.7	113.8	113.8	114.2	112.9	113.6	112.9	113.4
C8-N9-C4	104.8	103.9	103.9	103.9	103.7	103.9	104.1	104.7	104.0
N9-C4-C5	110.7	110.5	110.5	110.5	110.6	110.5	110.3	110.2	110.5
C4-C5-N7	105.7	106.0	106.0	105.9	105.9	105.9	106.0	105.8	105.7

Tabla 7.1.19. Momento dipolar (Debyes) de los tautómeros HX-17 y HX-19 aislados e hidratados.

X =	HX-17		HX-19	
	B3LYP/6-31G(d,p)	MP2/6-31G(d,p)	B3LYP/6-31G(d,p)	MP2/6-31G(d,p)
0	1.758	1.426	5.051	5.891
1	3.003	2.953	4.414	5.026
2	3.114	4.174	3.582	4.253
3	4.087	3.877	3.726	4.667
4	4.194	4.855	3.831	4.701
5	7.900	8.185	7.423	8.214
6	9.670	9.227	5.334	6.073
7	10.048	8.317	4.601	5.071
8	9.114	7.644	1.245	1.950
9	5.976	-	2.456	-
10	6.759	-	3.497	-
15	5.884	-	4.481	-
20	6.773	-	6.125	-
25	9.469	-	3.027	-
30	8.728	-	1.858	-

Longitudes de enlace N1-H y C=O y número de moléculas de agua

En la figura 7.1.17 vienen representadas las longitudes de enlace N1-H y C=O según el número de moléculas de agua de cada hidrato en los niveles de cálculo B3LYP y MP2. El primer valor corresponde a cada tautómero aislado y el último a (H₂O)₃₀. Las longitudes de enlace crecen desde el valor que tienen en la base aislada, y a partir aproximadamente, de 15 moléculas de agua tiende a estabilizarse. Posiblemente se debe al incremento en la contribución de las formas resonantes en la estructura total que da lugar a un decrecimiento de la flexibilidad del anillo⁵³.

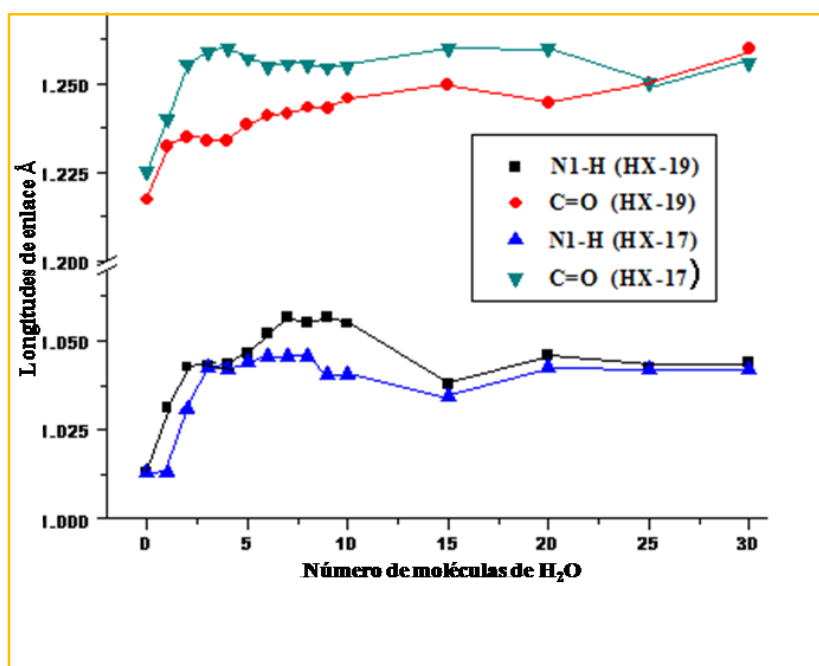


Figura 7.1.17. Longitudes de enlace N1-H y C=O en los tautómeros HX-17 y HX-19.

7.1.4.5. Energías calculadas en tautómeros HX-19 y HX-17 hidratados.

La Tabla 7.1.20. muestra los valores /calculados de las diversas energías: energía de deformación, energía de interacción y la energía de formación del complejo $\Delta E_{B(W_n)}^{CP}$ HX-19 y HX-17 con 20 y 10 moléculas de agua. Se observa que los valores de la energía de formación de los complejos de HX-19 y HX-17, con 20 y con 10 moléculas de agua respectivamente, son prácticamente iguales en ambos casos. Los valores $E_{(W_n)}^{(BW_n)}$ (BWn), presentan una acusada diferencia y, si se observa la estructura de cada hidrato, figura 7.1.11., 7.1.16. y 13.3. anexo, se tienen los enlaces de H generalmente fuertes entre las moléculas de agua y entre el agua y HX, lo cual da estabilidad al complejo.

El mayor valor de la corrección CP de energía de interacción agua-agua, $\Delta E_{W_n}^{CP}$, corresponde a los complejos con 20 moléculas de agua, siendo el mayor HX-17+20 agua dado el alto número de enlaces $H_2O \cdots H_2O$. El valor más alto de la corrección de energía $\Delta E_{B(W_n)}^{CP}$ se tiene en el complejo HX-19+20H₂O, con un alto número de enlaces de H, que dan estabilidad al conjunto.

Tabla 7.1.20. Energía de formación, interacción base-agua y de deformación, de los complejos HX-19 y HX-17 con 10 y 20 moléculas de agua..

	HX-19+20H ₂ O	HX19+10H ₂ O	HX17+10H ₂ O	HX17+20H ₂ O
$E_{(BWn)}^{(BWn)}$ (BWn) en A.U.	-2016.048	-1251.615	-1251.612	-2016.042
$E_{(B)}^{(BWn)}$ (BWn) en A.U.	-487.186	-487.195	-487.190	-487.196
$E_{(Wn)}^{(BWn)}$ (BWn) en A.U.	-1528.712	-764.403	-764.307	-1528.850
$E_B^{(def)}$ (BWn) en kcal/mol	5.987	0.034	3.932	0.0339
$\Sigma E_{Wi}^{(def)}$ (BWn) en kcal/mol	8.188	3.151	3.854	8.963
ΔE_{B-Wn}^{CP} (en kcal/mol)	-88.228	-10.428	-68.493	2.719
ΔE_{Wn}^{CP} (en kcal/mol)	-198.835	-129.049	-68.568	-285.974
$\Delta E_{B(Wn)}^{CP}$ (en kcal/mol)	-287.063	-139.477	-133.207	-283.255

7.1.5. Estudio en el estado sólido.

Para simular el estado sólido se ha tenido en cuenta la información obtenida en la bibliografía. El espectro ultravioleta indica que HX, en disolución acuosa está presente preferentemente en el tautómero HX-19, que se presenta en las dos formas A y B, y la estructura sólida revela el tautómero HX-19 como forma predominante aunque las otras formas no puedan excluirse totalmente². Los estudios realizados por Rayos X concuerdan con el espectro ultravioleta Raman de resonancia (UVRR) y el teórico NQR, y confirma que, en estado sólido, la hipoxantina se encuentra como tautómero HX-19⁵⁴. Por ello, he centrado el estudio del estado sólido, en el tautómero HX-19, calculando el dímero, trímero, tetrámero y pentámero (figura 7.1.18). Los cálculos se han realizado en el nivel B3LYP, a pie de las figuras aparece también la energía con la corrección ZPE. Se han comparado los valores de las correspondientes estructuras geométricas (las longitudes de enlace), con las de la Tabla 7.1.3., y se han estudiado las principales diferencias respecto de los valores experimentales, para HX-19.

En la Tabla 7.1.21. se tienen las longitudes de enlace de cada molécula del dímero de HX-19 en las columnas 2 y 3 y los valores experimentales A y B en las columnas 4 y 5.

En las columnas 6 y 7 se han calculado los diferenciales mayores. Las mayores diferencias respecto de los valores experimentales los presenta el enlace C6-N1, con diferencia de 0.056 Å respecto a A, en C5-C6 (0.025 Å) y en C6-O presenta un decrecimiento de 0.026 Å, también respecto de A. Respecto a B la mayor diferencia es 0.044 del enlace C5-C6. La molécula B tiene estos cambios algo menores, también respecto A: 0.043, 0.021 y -0.019 Å y con respecto a B C5-C6 0.040 Å.

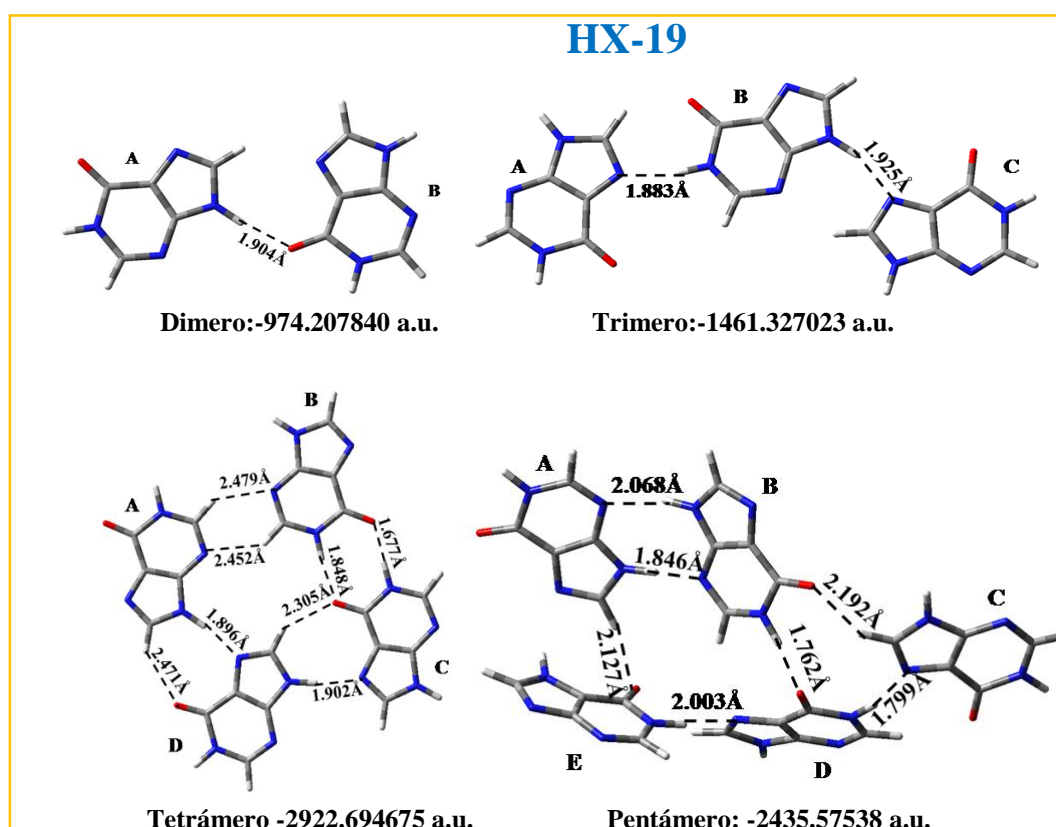


Figura 7.1.18, Dímero, trímero y pentámero del tautómero HX-19 y Energía + ZP (B3LYP).

Tabla 7.1.21. Longitudes de enlace del dímero HX-19, valores experimentales A y B y diferencias (Å). Se han omitido las diferencias menores.

Longitud de enlace (Å)	Dímero moléc. A	Dímero moléc. B	Experim. A	Experim. B	Diferencia (Å) de molec. A respecto A / B	Diferencia (Å) de molec. B respecto A / B
N1-C2	1.367	1.367	1.364	1.369		
C2-N3	1.301	1.303	1.305	1.291	/+0.010	/+0.012
N3-C4	1.365	1.360	1.350	1.359	0.015/	0.010/0.012
C4-C5	1.400	1.396	1.381	1.388	0.019/0.012	0.015/
C5-C6	1.444	1.440	1.419	1.400	0.025/0.044	0.021/0.040

C6-N1	1.434	1.421	1.378	1.395	0.056/0.039	0.043/0.026
C6-O	1.220	1.227	1.246	1.247	-0.026/-0.027	-0.019/0.020
C5-N7	1.377	1.377	1.376	1.376		
N7-C8	1.316	1.312	1.321	1.311		
C8-N9	1.374	1.378	1.348	1.375	0.026	0.030
N9-C4	1.367	1.370	1.365	1.365		

En la Tabla 7.1.22. se tienen las longitudes de enlace del trímero, moléculas A, B y C en las respectivas columnas 2, 3 y 4 y las diferencias respecto a A y B en las columnas 7, 8 y 9. Comparando la molécula A con A (exp.) las mayores diferencias corresponden a C6-N1, 0.053/ 0.036 Å, respecto a ambos valores experimentales y C5-C6, 0.024/0.043 Å, y C6-O, -0.026 y -0.027 Å.

En la molécula B los valores más altos de estas diferencias son análogamente C6-N1, C5-C6 y C6-O, 0.044/0.027, 0.023/0.042 y -0.017/-0.018 Å. Por último, en la molécula C, las mismas longitudes de enlace presentan diferencias análogas. 0.055/0.038, 0.025/0.044 y -0.028/-0.020 Å.

El tetrámero, en la Tabla 7.1.23., tiene la mayor diferencia en las longitudes de enlace C6-N1 0.057 Å de la molécula A respecto de A (exp.) y 0.050 Å de la molécula D también respecto de la molécula A (exp.) el enlace C5-C6 también presenta dos diferencias significativas 0.43 y 0.041 Å de las moléculas A y D respectivamente respecto de B. El enlace C6-O sufre un decrecimiento ~ 0.26 Å en las moléculas A y D respecto de A (exp.) y B (exp.).

La Tabla 7.1.24. presenta las longitudes de enlace de las moléculas A, B, C, D y E del pentámero, sus respectivos incrementos respecto de A (exp.) y B (exp.), en las columnas 9, 10, 11, 12 y 13. Las tres primeras moléculas, A, B y C, presentan una variación análoga a las anteriores con un máximo en C6-N1, 0.061/0.044 Å, 0.045/0.028 Å y 0.047/0.030 Å encada molécula. C5/C6 tiene 0.024/0.043 Å, 0.021/0.040 Å y 0.029/0.039 Å y C=O presenta los valores -0.029/-0.030 Å, -0.019/-0.20 Å y -0.022/-0.023 Å. Como puede observarse el enlace C=O decrece en todos los casos. Sin embargo en las moléculas D y E no presenta variación apreciable y C6-N1 solamente tiene la variación 0.023 y 0.026, muy inferior a los anteriores valores, respecto de A (exp.).

En todos los casos la obtención del dímero, trímero tetrámero o pentámero da lugar a un alargamiento de los enlaces C6-N1 y C5-C6 próximos a C=O. Ello parece lógico cuando se tiene un enlace de $>C=O \cdots H$, pues este enlace alarga los dos enlaces

próximos, (en el dímero), sin embargo, se produce el mismo efecto cuando no se forma este enlace de H (en el trímero).

Tabla 7.1.22. Longitudes de enlace del trímero HX-19, valores experimentales A y B y diferencias (Å). Se han omitido las diferencias menores.

Longitud de enlace (Å)	Trímero moléc. A	Trímero moléc. B	Trímero moléc. C	Experim. A	Experim. B	Diferencia (Å) de molec. A respecto A / B	Diferencia (Å) de molec. B respecto A / B	Diferencia (Å) de molec. C respecto A / B
N1-C2	1.366	1.358	1.366	1.364	1.369			
C2-N3	1.303	1.308	1.303	1.305	1.291		/0.017	
N3-C4	1.361	1.366	1.361	1.350	1.359		0.016/	
C4-C5	1.393	1.389	1.393	1.381	1.388			
C5-C6	1.443	1.442	1.444	1.419	1.400	0.024/0.043	0.023/0.042	0.025/0.044
C6-N1	1.431	1.422	1.433	1.378	1.395	0.053/0.036	0.044/0.027	0.055/0.038
C6-O	1.220	1.229	1.218	1.246	1.247	-0.026/-0.027	-0.017/-0.018	-0.028/-0.029
C5-N7	1.376	1.378	1.377	1.376	1.376			
N7-C8	1.316	1.315	1.316	1.321	1.311			
C8-N9	1.373	1.374	1.373	1.348	1.375	0.026		
N9-C4	1.373	1.367	1.373	1.365	1.365			

Tabla 7.1.23. Longitudes de enlace del tetrámero HX-19, valores experimentales A y B y diferencias (Å). Se han omitido las diferencias menores.

Longitud de enlace (Å)	Tetrámero Moléc. A	Tetrámero Moléc. B	Tetrámero Moléc. C	Tetrámero Moléc. D	Experim. A	Experim. B	Diferencia (Å) de molec. A respecto A / B	Diferencia (Å) de molec. B respecto A ² / B ²	Diferencia (Å) de molec. C respecto A ² / B ²	Diferencia (Å) de molec. D respecto A ² / B ²
N1-C2	1.364	1.364	1.360	1.368	1.364	1.369				
C2-N3	1.305	1.310	1.310	1.302	1.305	1.291	/0.014	/0.019	/0.019	
N3-C4	1.369	1.356	1.354	1.364	1.350	1.359				0.014
C4-C5	1.401	1.397	1.394	1.397	1.381	1.388	0.020/0.013	0.016	0.013	0.016
C5-C6	1.443	1.434	1.437	1.441	1.419	1.400	0.024/0.043	0.015/0.034	0.016/0.037	0.021/0.041
C6-N1	1.435	1.408	1.402	1.428	1.378	1.395	0.057/0.040	0.030/0.013	0.024	0.050/-0.017
C6-O	1.220	1.240	1.239	1.222	1.246	1.247	-0.026/-0.027			-0.024/-0.025
C5-N7	1.376	1.380	1.381	1.377	1.376	1.376				
N7-C8	1.317	1.310	1.313	1.319	1.321	1.311				
C8-N9	1.374	1.381	1.373	1.367	1.348	1.375	0.026/0.026	0.035/	0.025	0.019
N9-C4	1.365	1.370	1.376	1.368	1.365	1.365				

Tabla 7.1.24. Longitudes de enlace del pentámero HX-19, valores experimentales A y B y diferencias (Å). Se han omitido las diferencias menores.

Longitud de enlace (Å)	Pentámero moléc. A	Pentámero moléc. B	Pentámero moléc. C	Pentámero moléc. D	Pentámero moléc. E	Experim. A	Experim. B	Diferencia (Å) de molec. A respecto A / B	Diferencia (Å) de molec. B respecto A / B	Diferencia (Å) de molec. C respecto A ² / B ²	Diferencia (Å) de molec. D respecto A ² / B ²	Diferencia (Å) de molec. E respecto A ² / B ²
N1-C2	1.361	1.353	1.369	1.360	1.372	1.364	1.369					
C2-N3	1.305	1.311	1.303	1.312	1.299	1.305	1.291		/-0.20		/0.021	
N3-C4	1.370	1.364	1.361	1.355	1.369	1.350	1.359	0.020/	0.014/			0.019/
C4-C5	1.397	1.396	1.292	1.394	1.402	1.381	1.388	0.016/	0.015/			0.021/
C5-C6	1.443	1.440	1.439	1.434	1.430	1.419	1.400	0.024/0.043	0.021/0.040	0.029/0.039	0.015/0.034	/0.030
C6-N1	1.439	1.423	1.425	1.401	1.404	1.378	1.395	0.061/0.044	0.045/0.028	0.047/0.030	0.023/	0.026/
C6-O	1.217	1.227	1.224	1.239	1.241	1.246	1.247	-0.029/-0.030	-0.019/-0.020	-0.022/-0.023		
C5-N7	1.379	1.378	1.374	1.381	1.379	1.376	1.376					
N7-C8	1.321	1.313	1.318	1.316	1.365	1.321	1.311					
C8-N9	1.369	1.378	1.373	1.374	1.320	1.348	1.375	0.021/	0.030/	0.025/	0.026/	-0.028/-0.055
N9-C4	1.365	1.368	1.373	1.376	1.371	1.365	1.365					

Enlaces de Hidrógeno (figura 7.1.18):

En el dímero se forma un $>C=O\cdots H$ entre ambas moléculas, el O pertenece a la molécula B y el H a N9 de la molécula A pero en el trímero solamente se unen mediante dos enlaces $N7\cdots H-N1$ (entre las moléculas A y B) y $N9-H\cdots N7$ (entre las moléculas B y C).

En el tetrámero cada molécula está enlazada mediante dos enlaces de H con las dos moléculas vecinas, en algunos casos enlaces fuertes (1.674, 1.848 y 1.896 Å), lo que da estabilidad al complejo formado. Además, al comparar esta estructura con la obtenida experimentalmente³, presenta una disposición análoga a ésta.

En el pentámero, la estructura obtenida da unos enlaces de H análogos a los aportados en la bibliografía: los enlaces $C=O\cdots H$ se han formado entre las moléculas B-C, B-D y E-A, todos ellos débiles, a excepción del segundo que es fuerte. Los restantes, entre $N-H\cdots N$, cuatro en total, enlazan las moléculas A y B (un enlace débil y uno fuerte), las moléculas C y D (un enlace fuerte) y por último D y E (un enlace débil).

De lo comentado en los apartados anteriores se deduce que la estructura más próxima a la experimental es la correspondiente al tetrámero.

7.1.6. Cálculo de los espectros vibracionales.

Diversos trabajos de investigación se han elaborado respecto del espectro de HX^{6,31,55,56,57}, a pesar de ello, para completar este estudio respecto de esta base, se han realizado una serie de investigaciones teóricas sobre el espectro de los tautómeros más abundantes principalmente en los niveles de cálculo que no figuran entre los espectros ya investigados.

En la Tabla 7.1.25. se muestran los números de onda en el espectro de IR del tautómero HX-19, calculadas en el nivel de cálculo B3LYP/6-311++G(3df,pd). Las intensidades absolutas y las intensidades relativas respecto de la de mayor intensidad que, en este caso, corresponde a la frecuencia n° 32, 1803.9 cm^{-1} , cuya intensidad es 712, que se toma como valor de referencia 100. En la columna 5 se tienen las asignaciones correspondientes obtenidas al resolver el espectro con la opción y los porcentajes de cada vibración. Solamente se han tenido en cuenta los valores superiores a 10% y **consultando** la opción

Freq=Intmodes elegida por la buena aplicación en otros cálculos ya realizados³¹. En la columna nº 6 se muestran los números de ondas experimentales^{6,55} del tautómero HX-19 (en fase gaseosa) a la temperatura $T = 11$ K y, en las columnas 7 y 8 aparecen las intensidades absolutas y relativas del espectro IR. Por último en la columna 9 se dan las asignaciones correspondientes⁶. Las medidas experimentales se realizaron por el método de aislamiento de la molécula a baja temperatura en medio de Ar⁵⁸. Como puede observarse en los números de ondas nº 35 y 36 hay una perfecta concordancia entre los valores calculados y experimentales correspondientes a vibraciones N1-H y N9-H. Las asignaciones experimentales⁶ en general corresponden a vibraciones del anillo, las calculadas en la opción FREQ=INTMODES corresponden a vibraciones del anillo bien determinadas. Considerando la intensidad relativa 100 antes citada, se observa que las intensidades relativas de las diferentes frecuencias son notablemente menores, así la frecuencia nº 8, 565.9 cm^{-1} , tiene la mayor intensidad relativa (14). Las siguientes la nº 36, 3651.5 cm^{-1} (12), la nº 22, 1189.4 cm^{-1} (11). Las demás tienen valores 10 ó menores. Se observan dos hombros de baja energía a 3478 cm^{-1} y a 3428 cm^{-1} .

Posteriores determinaciones experimentales del espectro IR y Raman, realizadas por sublimación de HX sólido a 168° C y depósito sobre una ventana fría de CsI, presentan un mayor número de frecuencias⁵⁷ además de las estudiadas en esta tabla.

La Tabla 7.1.26. presenta los valores obtenidos en el cálculo de las frecuencias de HX-19 en los niveles B3LYP/6-31G(d,p) y B3LYP/6-311++G(3df,pd), con los correspondientes valores escalados [$v^{(1)} = 34.6 + 0.9447 \cdot \omega$] para el primer nivel y [$v^{(2)} = 31.9 + 0.9512 \cdot \omega$] para el segundo. En este último nivel de cálculo, en las columnas 5-12 se tienen los valores calculados: Intensidades absolutas en IR (Km/mol), intensidades relativas en IR respecto del valor máximo 712 del nº de ondas 1803.9 cm^{-1} al que se le asigna valor 100 y respecto de éste se calculan las frecuencias relativas (columna 6). En la columna 7 se tienen las intensidades relativas Raman ($\text{Å}^4/\text{AMU}$) y en la 8 las intensidades Raman relativas, calculadas respecto de la frecuencia 3661.9 cm^{-1} a cuya intensidad absoluta 140 se le asigna el valor de 100. La columna 9 presenta las masas reducidas (AMU) y la columna 10 lleva las constantes de fuerza en $\text{Dina}/\text{Å}$. En las dos últimas columnas se tienen las asignaciones experimentales en disolución acuosa a $\text{pH} = 6$ y con deuterio⁵⁶. Las asignaciones están identificadas para el tautómero HX-19⁵⁶.

En la Tabla 7.1.27. se tienen las frecuencias del tautómero HX-19 en todos los niveles de cálculo y escaladas según:

$$[\nu^{(1)} = 34,6 + 0,9447 \cdot \omega] \text{ en (B3LYP/6-31G(d,p));}$$

$$[\nu^{(2)} = 31,9 + 0,9512 \cdot \nu] \text{ en (B3LYP/6-311++G(3df,pd) y}$$

$$[\nu^{(3)} = 34,5 + 0,9372 \cdot \nu] \text{ en (MP2/6-31G(d,p)).}$$

$$[\nu^{(1)} = 0,9613 \cdot \nu] \text{ en (B3LYP/6-31G(d,p)) y}$$

$$[\nu^{(3)} = 0,9427 \cdot \nu] \text{ en (MP2/6-31G(d,p))}$$

$$\nu I [\text{factor} = 0,9679]^{59} \text{ en (B3LYP/6-311++G(3df,pd)}$$

a = frecuencias experimentales de HX (gas) y factor de escalada 0.9938⁶.

comparadas con las frecuencias experimentales⁶. Los valores obtenidos varían respecto de los experimentales y generalmente se aproximan a la frecuencia experimental en uno o dos valores escalados en cada frecuencia, en algún caso coinciden pero suelen tener una diferencia de 2 ó 3 unidades. (n^{os}: 6, 7, 9, 10, 13, 17, 21, 25 y 30).

En la Tabla 7.1.28. (anexo) se muestran los números de ondas en el espectro de IR del tautómero HX-17 calculadas en el nivel de cálculo B3LYP/6-311++G(3df,pd), las intensidades absolutas y las intensidades relativas, éstas respecto de la de mayor intensidad que, en este caso es n° 32, 1756.6 cm⁻¹, de intensidad 781 a la que se asigna 100 y, como en el tautómero HX-19, se calculan las demás intensidades respecto de ésta. También la correspondiente experimental n° 32 presenta la mayor intensidad 110 y sirve de referencia para las demás experimentales que tienen una intensidad relativa mayor que en las intensidades calculadas, así la intensidad relativa es mayor que 10 en los números de onda 36, 35, 31, 26, 25, 7 y 6. Las asignaciones calculadas se han obtenido mediante la opción **Freq=Intmodes** y se aproximan a los valores experimentales. Es de destacar la concordancia entre ambos números de ondas en los números 35 y 36. En la determinación experimental del espectro según cita 56, también se tienen un mayor número de frecuencias que las citadas en esta tabla.

En la Tabla 7.1.28. se tienen los valores de los números de onda del tautómero HX-17 escalados en los niveles B3LYP y todos los valores de las intensidades absolutas, (Km/mol), relativas, intensidades relativas Raman (Å⁴/AMU), las intensidades Raman respecto de la de referencia, las masas reducidas (AMU) y las constantes de fuerza en Dina/Å. En las dos últimas columnas se tiene la caracterización de los números de ondas experimentales en disolución acuosa a pH = 6 y en D₂O también plenamente identificada para el tautómero HX-17⁵.

Tabla 7.1.25. Número de ondas (cm^{-1}) obtenido con el método B3LYP (HX aislada) en el tautómero HX-19 y experimentales en IR.

B3LYP/6-311++G(3df,pd)				IR Experimental (cm^{-1}), ref. 6, 55				
ω (cm^{-1})	IR Intensidad absoluta	IR Intensidad relativa	Asignaciones teóricas	$\nu^{6,55}$ (cm^{-1})	Intensidad absoluta ⁵⁵ .	Intensidad relativa	Asignaciones	
1	148.8	1	0	22% $\delta(\text{C}=\text{O})$ +19% $\delta(\text{N1-H})$ +16% $\delta(\text{N9-H})$ +10% $\delta(\text{C2-H})$				
2	205.7	16	2	19% $\delta(\text{C8-H})$ + 18% $\delta(\text{N1-H})$ + 13% $\delta(\text{N9-H})$				
3	267.1	0	0	20% $\delta(\text{C2-H})$ + 12% $\delta(\text{N1-H})$ +12% $\delta(\text{N9-H})$ + $\delta(\text{C2-H})$				
4	321.9	1	0	27% $\beta(\text{C6-C5-N7})$ + 18% $\beta(\text{O-C6-C5})$ + 18% $\beta(\text{C8-H})$				
5	510.3	2	0	26% $\beta(\text{C2-N3-C4})$ +20% $\delta(\text{C5C6N1})$ +14% $\delta(\text{N9-H})$ + 12%+ $\delta(\text{N1-H})$ +12% $\delta(\text{C2-H})$.				
6	520.9	27	4	39% $\gamma(\text{C9-H})$ +21% $\gamma(\text{C8-H})$ +17% $\gamma(\text{C2-H9})$	508	3	6 (N9-H)vib.+rings vib.(omp)	
7	538.9	11	2	38% $\delta(\text{C8-H})$ +14% $\delta(\text{C}=\text{O})$.	542/534	5/5	10/10 Rings vib.	
8	565.9	99	14	23% $\delta(\text{N9-H})$ +22% $\delta(\text{C2-H})$.	558/553	17/10	34/20 (N9-H)vib.+rings vib. (omp)	
9	607.5	8	1	Rings vib.	602/598	5/3	10/6 rings vib.	
10	651.6	14	2	29% $\gamma(\text{N1-H})$	626/622	3/8	6/16 Rings+ vib/(N-H)vib.(omp)	
11	671.4	16	2	28% $\gamma(\text{C8-H})$ +22% $\gamma(\text{N9-H})$ +16% $\gamma(\text{N1-H})$	640	3	6 (N1-H) vib.+rings.vib. (omp)	
12	700.1	11	2	20% $\nu(\text{N3C4})$ +14% $\nu(\text{C6N1})$.	691	3	6 rings vib.	
13	733.4	35	5	23% $\gamma(\text{N1-H})$.	725	11	22 (N1-H)vib.+rings vib.(omp)	
14	801.2	13	2	27% $\gamma(\text{C5-C6})$ +25% $\gamma(\text{C5-C4})$ +18% $\gamma(\text{C}=\text{O})$ +10% $\gamma(\text{C8-H})$.	775		22 (C8-H) vib.+rings vib.(omp)	
15	851.5	10	1	88% $\gamma(\text{C8-H})$				
16	902.4	9	1	15% $\delta(\text{N1C2N3})$ +13% $\delta(\text{C2N3C4})$ +18% $\delta(\text{N1-C2-N5})$ +15% $\delta(\text{N1-H})$.				
17	943.9	3	0	87% $\gamma(\text{C2-H})$ +13% $\nu(\text{C2-N3})$.	860	4	8 (C2-H)vib.+pyrimidinic ring vib. (omp)	
18	949.1	6	1	36% $\beta(\text{N7-C8-N9})$ +28% $\beta(\text{C9-H})$ +19% $\beta(\text{C8-H})$.	891	5	10 rings vib.+ (N9-H) vib./(C8-H) vib.	
19	1059.1	46	6	29% $\nu(\text{C6N1})$ +23% $\beta(\text{C}=\text{O})$ +11% $\beta(\text{N1-H})$.	1054	6	12 rings vib+imidazolic-H vib (C2-H)vib.+ $\nu(\text{C}=\text{O})$	
20	1077.1	14	2	39% % $\delta(\text{N9C8})$ +31% $\nu(\text{C8-N9})$ +30% $\delta(\text{C8-H})$.				
21	1137.2	11	2	29% $\delta(\text{N1-H})$ +22% $\nu(\text{N1C2})$ +13% $\delta(\text{C2-H})$ + 12 % $\delta(\text{C5-N7})$.	1100	3	6 rings vib. +(N1-H)vib.	
22	1189.4	78	11	21% $\nu(\text{N1-C2})$ +16% $\delta(\text{C8-H})$ + 11% $\delta(\text{C2-H})$.				
23	1295.1	4	1	24% $\nu(\text{C5-N7})$ +14% $\delta(\text{C8-H})$ +13% $\delta(\text{C2-H})$.				
24	1352.5	11	2	14% (C8-H)+23% $\nu(\text{N7C5})$.				
25	1370.2	25	4	20% $\nu(\text{N7C5})$ +19% $\nu(\text{C2-H})$ +19% $\nu(\text{N7-C8})$.	1324	11	22 rings vib+(C2-H)vib	
26	1400.1	19	3	16% $\delta(\text{N9-H})$ +15% $\nu(\text{C8-N9})$.				
27	1432.5	4	1	19% $\delta(\text{N2-H})$ +16% $\nu(\text{N3-C4})$ +16% $\nu(\text{C4-N7})$.				

28	1477.5	6	1	25% $\nu(\text{N1-C2})+18\%$ $\delta(\text{N1-H})+14\%$ $\delta(\text{C8-H})+18\%$ $\nu(\text{C8-N7})+11\%$ $\nu(\text{C4-N9})$.				
29	1524.7	37	5	21% $\nu(\text{C5C4})+22\%$ $\delta(\text{C8-H})+21\%$ $\nu(\text{N3-C4})+19\%$ $\nu(\text{N9-C8})+15\%$ $\delta(\text{N1-H})+14\%$ $\nu(\text{C2-N3})$.				
30	1586.2	74	10	22% $\nu(\text{C4-C5})+20\%$ $\nu(\text{N3-C4})+20\%$ $\nu(\text{C4-N9})+13\%$ $\delta(\text{N9-H})+12\%$ $\nu(\text{N7-C8})+11\%$ $\delta(\text{C8-H})$.	1534	9	18	rings vib.+(N9-H)vib.+(C8-H)vib.
31	1627.2	90	13	24% $\nu(\text{C4-C5})+23\%$ $\delta(\text{C2-H})+22\%$ $\nu(\text{C2N3})+22\%$ $\nu(\text{N1-C2})$.	1555	11	22	rings vib.+(C2-H)vib.+ $\nu(\text{C=O})$
32	1803.9	712	100	36% $\nu(\text{C6O})+28\%$ $\nu(\text{C5C6})+26\%$ $\nu(\text{N1-C6})$.	1753	52	100	$\nu(\text{C=O})+(\text{N1-H})+\text{rings vib.}$
33	3178.8	3	0	76% $\nu(\text{C2-H})$.				
34	3247.6	0	0	76% $\nu(\text{C8-H})$.				
35	3596.8	66	9	78% $\nu(\text{N1-H})$.	Low (sh)			$\nu(\text{N1-H})$
36	3651.5	85	12	77% $\nu(\text{N9-H})$.	3478 (sh)*	10	20	$\nu(\text{N9-H})$

Las frecuencias experimentales de HX (aislada)^{6,55} a temperatura T = 11K.

ν = tensión; δ = flexión simétrica fuera del plano; τ = torsión; γ = movimiento fuera del plano; (omp) = fuera del plano molecularros. * otros autores.

Tabla 7.1.26. Número de ondas IR (cm⁻¹) de HX-19 en B3LYP y experimentales.

B3LYP/6-31G(d,p)		B3LYP/6-311++G(3df,pd)										Experimentales ^h	
Calc.		Calc.		IR int.		Raman Int.		Dep.	Dep.	Masa	Constante	en H ₂ O	en D ₂ O
ω	ω ^v	ω	ω	Abs. ^a	Rel. ^b	Abs. ^c	Rel. ^d	ratios (P) ^e	ratios (U)	reducida ^f	de fuerza ^g		
154.0	180.1	148.8	173.4	1	0	0	0	0.75	0.86	9.3	0.1		
207.0	230.2	205.7	227.6	16	2	0	0	0.75	0.86	4.6	0.1		
272.0	291.6	267.1	286.0	0	0	0	0	0.75	0.86	6.0	0.3		
319.3	336.2	321.9	338.1	1	0	2	1	0.63	0.77	9.1	0.6		
507.5	514.0	510.3	517.3	2	0	4	3	0.71	0.83	8.2	1.3		
529.3	534.6	520.9	527.4	27	4	1	1	0.75	0.86	1.8	0.3		
536.4	541.3	538.9	544.5	11	2	3	2	0.50	0.66	7.3	1.2		
571.8	574.8	565.9	570.2	99	14	1	1	0.75	0.86	1.9	0.4		
605.7	606.8	607.5	609.8	8	1	5	4	0.12	0.21	6.4	1.4		
652.7	651.2	651.6	651.7	14	2	0	0	0.75	0.86	1.9	0.5		
673.9	671.2	671.4	670.5	16	2	0	0	0.75	0.86	2.7	0.7		
702.8	698.5	700.1	697.8	11	2	19	14	0.07	0.12	8.4	2.4		
735.7	729.6	733.4	729.5	35	5	0	0	0.75	0.86	2.7	0.9		
782.3	773.6	801.2	794.0	13	2	1	1	0.75	0.86	9.1	3.4		
834.4	822.9	851.5	841.8	10	1	1	1	0.75	0.86	1.5	0.6		
901.3	886.1	902.4	890.3	9	1	7	5	0.28	0.44	7.3	3.5		
930.6	913.7	943.9	929.7	3	0	0	0	0.75	0.86	1.4	0.7		
946.1	928.4	949.1	934.7	6	1	5	4	0.23	0.37	7.6	4.0		
1063.3	1039.1	1059.1	1039.3	46	6	2	1	0.40	0.57	5.9	3.9		
1083.4	1058.1	1077.1	1056.4	14	2	4	3	0.03	0.06	1.7	1.2		
1136.9	1108.6	1137.2	1113.6	11	2	9	6	0.21	0.34	2.6	2.0		
1194.3	1162.9	1189.4	1163.3	78	11	3	2	0.12	0.21	2.6	2.2	1219	1167
1302.8	1265.4	1295.1	1263.8	4	1	15	11	0.24	0.39	1.9	1.8	1270	1256
1362.0	1321.3	1352.5	1318.4	11	2	9	6	0.66	0.80	4.1	4.4	1338	1319
1380.8	1339.0	1370.2	1335.2	25	4	23	16	0.37	0.54	2.3	2.5	1367	1352
1411.3	1367.9	1400.1	1363.7	19	3	65	46	0.35	0.52	2.3	2.6	1396	1332/1372
1439.4	1394.4	1432.5	1394.5	4	1	18	13	0.67	0.80	2.7	3.3	1424/1464	1427
1486.1	1438.5	1477.5	1437.3	6	1	43	31	0.17	0.29	3.4	4.4	1512	1495
1535.7	1485.4	1524.7	1482.2	37	5	125	89	0.25	0.40	4.7	6.4		
1601.2	1547.3	1586.2	1540.7	74	10	42	30	0.32	0.48	5.2	7.8	1575	1552
1639.8	1583.7	1627.2	1579.7	90	13	27	19	0.09	0.17	5.2	8.2	1596	1578
1833.0	1766.2	1803.9	1747.8	712	100	48	34	0.34	0.50	10.0	19.1	1694	1677
3195.2	3053.1	3178.8	3055.6	3	0	118	84	0.27	0.42	1.1	6.5		
3263.1	3117.3	3247.6	3121.0	0	0	118	84	0.27	0.42	1.1	6.8		
3604.9	3440.1	3596.8	3453.2	66	9	120	86	0.25	0.39	1.1	8.2		
3661.9	3494.0	3651.5	3505.2	85	12	140	100	0.24	0.39	1.1	8.5		

^v/Forma escalada [v⁽¹⁾ = 34.6 + 0.9447*v⁵² v²; Forma escalada [v⁽²⁾ = 31.9 + 0.9512*v] v⁵²; ^aIntensidades absolutas IR, Km/Mole; ^bIntensidades relativas IR, %;

^c Intensidades absrlutas Raman, Å⁴/AMU; ^d Intensidades relativas Raman.%; ^e Ratio despolarizacion Raman.; ^f Masas reducidas AMU; ^g Constantes de fuerza, mDyne/Å; ^h Moléculas en H₂O y D₂O. Valores experimentales⁵⁶.

Tabla 7.1.27. Número de ondas IR escaladas de HX-19 (cm^{-1}) y experimentales HX (aislada).

	B3LYP /6-31G(d,p)				B3LYP76-311++G(3df,pd)				MP2/6-31G(d,p)				Experimental ^a	
	ω	ω^a	$\omega^{v(1)}$	$\omega^{v(1)}$	ω	ω^a	$\omega^{v(2)}$	ω^{vI}	ω	ω^a	$\omega^{v(3)}$	$\omega^{v(3)}$	ν	Asignac. ^a
1	154.0	153.0	180.1	148.0	148.8	147.9	173.4	144.0	137.5	136.6	163.4	129.6		
2	207.0	205.7	230.2	199.0	205.7	204.4	227.6	199.1	197.3	196.1	219.4	186.0		
3	272.0	270.3	291.6	261.5	267.1	265.4	286.0	258.5	259.7	258.1	277.9	244.8		
4	319.3	317.3	336.2	306.9	321.9	319.9	338.1	311.6	315.9	313.9	330.6	297.8		
5	507.5	504.3	514.0	487.9	510.3	507.1	517.3	493.9	507.7	504.6	510.3	478.6		
6	529.3	526.0	534.6	508.8	520.9	517.7	527.4	504.2	515.1	511.9	517.3	485.6	508	(N9-H)vib.+rings vib.(omp)
7	536.4	533.1	541.3	515.6	538.9	535.6	544.5	521.6	538.1	534.8	538.8	507.3	542/534	Rings vib.
8	571.8	568.3	574.8	549.7	565.9	562.4	570.2	547.7	570.3	566.8	569.0	537.6	558/553	(N9-H)vib.+rings vib. (omp)
9	605.7	601.9	606.8	582.3	607.5	603.7	609.8	588.0	605.9	602.1	602.3	571.2	602/598	rings vib.
10	652.7	648.7	651.2	627.4	651.6	647.6	651.7	630.7	615.5	611.7	611.3	580.2	626/622	rings vib+(N-H)vib. (omp)
11	673.9	669.7	671.2	647.8	671.4	667.2	670.5	649.8	663.1	659.0	656.0	625.1	640	(N1-H) vib.+rings. vib. (omp)
12	702.8	698.4	698.5	675.6	700.1	695.8	697.8	677.6	680.6	676.4	672.4	641.6	691	rings vib.
13	735.7	731.1	729.61	707.2	733.4	728.9	729.5	709.9	707.9	703.5	697.9	667.3	725	(N1-H) vib.+rings. vib. (omp)
14	782.3	777.4	773.6	752.0	801.2	796.2	794.0	775.5	730.5	726.0	719.1	688.6		
15	834.4	829.2	822.9	802.1	851.5	846.2	841.8	824.2	810.4	805.4	794.0	764.0	775	(C8-H) vib.+rings vib.(omp)
16	901.3	895.7	886.1	866.4	902.4	896.8	890.3	873.4	904.6	899.0	882.3	852.8		
17	930.6	924.8	913.7	894.6	943.9	938.0	929.7	913.6	909.9	904.3	887.3	857.8	860	(C2-H)vib.+pyrimidinic ring vib. (omp)
18	946.1	940.2	928.4	909.5	949.1	943.2	934.7	918.6	942.1	936.3	917.4	888.1	891	rings vib. (N9-H) vib.+(C8-H) vib.
19	1063.3	1056.7	1039.1	1022.6	1059.1	1052.5	1039.3	1025.1	1087.2	1080.5	1053.4	1024.9	1054	rings vib+imidazoliC-H vib
20	1083.4	1076.7	1058.1	1041.5	1077.1	1070.4	1056.4	1042.5	1118.3	1111.4	1082.6	1054.2		
21	1136.9	1129.9	1108.6	1092.9	1137.2	1130.1	1113.6	1100.7	1152.6	1145.5	1114.7	1086.6	1100	rings vib. +(N1-H)vib.
22	1194.3	1186.9	1162.9	1148.1	1189.4	1182.0	1163.3	1151.2	1215.4	1207.9	1173.6	1145.8		
23	1302.8	1294.7	1265.4	1252.4	1295.1	1287.1	1263.8	1253.5	1321.0	1312.8	1272.5	1245.3		
24	1362.0	1353.6	1321.3	1309.3	1352.5	1344.1	1318.4	1309.1	1378.2	1369.7	1326.1	1299.2		
25	1380.8	1372.2	1339.0	1327.4	1370.2	1361.7	1335.2	1326.2	1423.7	1414.9	1368.8	1342.1	1324	Rings vib+(C2-H)vib.+(N1-H)vib.
26	1411.3	1402.5	1367.9	1356.7	1400.1	1391.4	1363.7	1355.2	1443.7	1434.7	1387.5	1361.0		
27	1439.4	1430.5	1394.4	1383.7	1432.5	1423.6	1394.5	1386.5	1476.6	1467.4	1418.4	1392.0		
28	1486.1	1476.9	1438.5	1428.6	1477.5	1468.3	1437.3	1430.1	1504.6	1495.3	1444.6	1418.4		
29	1535.6	1526.1	1485.3	1476.2	1524.7	1515.2	1482.2	1475.8	1553.1	1543.5	1490.1	1464.1		
30	1601.2	1591.3	1547.3	1539.2	1586.2	1576.4	1540.7	1535.3	1616.0	1606.0	1549.0	1523.4	1534	(Rings vib.)+N9-Hvib./

31	1639.8	1629.6	1583.7	1576.3	1627.2	1617.1	1579.7	1575.0	1658.4	1648.1	1588.8	1563.4	1555	(C8-H)vib. (Rings vib.)+C2- Hvib.+vC=O
32	1833.0	1821.6	1766.2	1762.1	1803.9	1792.7	1747.8	1746.0	1851.2	1839.7	1769.4	1745.1	1753	vC=O+(N1-H)+rings vib.
33	3195.2	31753.9	3053.1	3071.5	3178.8	3159.1	3055.6	3076.8	3272.7	3252.4	3101.7	3085.2		
34	3263.1	3242.9	3117.3	3136.8	3247.6	3227.5	3121.0	3143.4	3337.2	3316.5	3162.1	3146.0		
35	3604.9	3582.5	3440.1	3465.4	3596.8	3574.5	3453.2	3481.3	3663.1	3640.4	3467.6	3453.2	Low (sh)	v (N1-H)
36	3661.9	3639.2	3494.0	3520.2	3651.5	3628.9	3505.2	3534.3	3723.4	3700.3	3524.1	3510.0	3478 (sh)]	v (N9-H)

V= escalado (Palafox, M. A.; Iza, N. y Gil, M.) $[v^{(1)} = 34,6 + 0,9447*v]$ y $[v^{(2)} = 31,9 + 0,9512*v]$ y $[v^{(3)} = 34,5 + 0,9372*v]$

v = escalado (Gaussian 2003), $[v^{(1)} = 0,9613*v]$ y $[v^{(3)} = 0,9427*v]$

vI = escalado (Gaussian 2003) *Int. J Mol. Sci.* 2007, **8**, 760 – 776 [factor = 0,9679]

a= *J. Phys. Chem. A* 1997 (101) p.8309 – 8318 frecuencias experimentales de HX (gas) y factor de escalada 0.9938.

v = tensión; δ = flexion simétrica fuera del plano; τ = torsion; γ = movimiento fuera del plano; (omp) = fuera del plano molecular

Tabla 7.1.28. Número de ondas de HX-17 en B3LYP (cm^{-1}) y experimentales
 B 3LYP/6-31G(d,p) 6-311++G(3df,pd)

Calc.		Calc		IR int.		Raman Int.		Dep. ratios ^c	Dep ratios ^c	Red. (U)mass ^f	Forc. const. ^g in H ₂ O ^h in D ₂ O ^h	Experim.
ω	ω^v	ω	ω^v	Abs. ^a	Rel. ^b	Abs	Rel. ^d					
162.0	187.6	159.3	183.4	14	2	0	0	0.75	0.86	10.3	0.2	
195.5	219.3	195.4	217.8	11	1	0	0	0.75	0.86	5.1	0.1	
279.2	298.4	276.2	294.6	5	1	0	0	0.75	0.86	5.1	0.2	
301.2	319.1	307.2	324.1	16	2	2	2	0.51	0.67	7.8	0.4	
513.1	519.3	516.0	522.7	3	0	3	2	0.75	0.86	10.1	1.6	
528.7	534.1	523.7	530.0	79	10	0	0	0.75	0.86	0.2	0.2	
547.6	551.9	550.7	555.7	2	0	4	3	0.58	0.74	7.4	1.3	
561.7	565.2	564.1	568.5	26	3	1	1	0.75	0.86	3.8	0.7	
610.7	611.5	613.7	615.7	7	1	4	3	0.13	0.23	6.4	1.4	
636.0	635.4	635.6	636.5	29	4	0	0	0.75	0.86	1.7	0.4	
677.0	674.2	672.1	671.2	24	3	0	0	0.75	0.86	1.6	0.4	
711.9	707.1	710.1	707.3	3	0	20	16	0.06	0.11	9.2	2.7	
733.5	727.5	741.8	737.5	23	3	0	0	0.75	0.86	4.5	1.5	
781.0	772.4	807.0	799.5	10	1	0	0	0.75	0.86	10.9	4.2	
857.9	845.1	874.2	863.4	12	2	1	1	0.75	0.86	1.4	0.7	
899.5	884.4	902.0	889.9	3	0	6	5	0.32	0.48	7.6	3.6	
927.7	911.0	939.7	925.7	4	1	0	0	0.75	0.86	1.4	0.7	
958.1	939.7	960.8	945.8	1	0	5	4	0.18	0.31	7.1	3.9	
1079.2	1054.1	1077.8	1057.1	14	2	8	6	0.16	0.27	3.3	2.2	
1101.1	1074.8	1096.7	1075.1	40	5	4	3	0.06	0.11	1.9	1.4	
1122.8	1095.3	1121.9	1099.1	7	1	3	2	0.23	0.38	2.6	1.9	
1208.2	1176.0	1203.3	1176.5	77	10	3	2.	0.14	0.24	2.8	2.4	121.9
1300.6	1263.3	1290.1	1259.0	1	0	7	6	0.65	0.79	2.5	2.4	
1355.0	1314.7	1345.6	1311.8	35	4	15	12	0.73	0.84	2.6	2.8	128.7/128.2
1407.0	1363.8	1397.1	1360.8	84	11	86	69	0.19	0.32	4.0	4.6	
1419.3	1375.4	1410.1	1373.2	24	3	4	3	0.09	0.16	1.9	2.2	154.1
1439.7	1394.7	1433.6	1395.5	23	3	20	16	0.75	0.86	2.1	2.6	
1476.0	1429.0	1461.1	1421.7	30	4	8	6	0.57	0.73	4.4	5.5	
1551.9	1500.7	1539.2	1496.0	18	2	72	58	0.28	0.44	7.2	0.1	
1563.1	1511.3	1551.1	1507.3	25	3	9	7	0.38	0.55	3.8	5.4	
1649.2	1592.6	1633.9	1586.1	69	9	40	32	0.08	0.14	5.4	8.5	159.6
1810.7	1745.2	1765.6	1711.3	781	100	40	32	0.26	0.41	9.4	17.3	169.4
3190.4	3048.6	3176.8	3053.7	5	1	124	100	0.26	0.42	1.1	6.5	
3263.4	3117.5	3248.1	3121.5	1	0	116	93	0.28	0.44	1.1	6.8	
3607.5	3442.6	3595.0	3451.5	67	9	121	98	0.24	0.39	1.1	8.2	
3661.7	3493.8	3650.2	3504.0	97	12	109	88	0.20	0.33	1.1	8.5	

^{v1} Forma escalada $[\nu^{(1)} = 34.6 + 0.9447 \cdot \nu]^{52}$

^{v2} Forma escalada $[\nu^{(2)} = 31.9 + 0.9512 \cdot \nu]^{52}$

^a Intensidad absoluta IR. Km/Mole-

^b Intensidad relativa IR, %

^c Intensidad absoluta Raman, $\text{\AA}^4/\text{AMU}$

^d Intensidad relative Raman, %

^e Ratio despolarización Raman

^f Masas reducidas, AMU

^g Constantes de fuerza. mDyne/ \AA

^h *J. Phys. Chem. B* 2009 (113) p. 15101 – 15117.

Tabla 7.1.29. Número de ondas IR escaladas de HX-17 (cm^{-1}), experimentales y calculadas^a.

	B3LYP/ 6-31G (d,p)			B3LYP/6-311++G(3df,pd)				MP2 6-31G(d,p)			Experimental ^a			
	ω	Calc. ω^a	$\omega^{(1)}$	$\omega^{(1)}$	ω	ω^a	$\omega^{(2)}$	ω^{I}	ω	ω^a	$\omega^{(3)}$	$\omega^{(3)}$	cm^{-1}	Asignaciones
1	162.0	161.0	187.6	155.7	159.3	158.3	183.4	154.2	139.3	138.4	165.1	131.3		
2	195.5	194.3	219.3	187.9	195.4	194.2	217.8	189.1	185.8	184.6	208.6	175.2		
3	279.2	277.5	298.4	268.4	276.2	274.5	294.6	267.3	268.4	266.7	286.0	253.0		
4	301.2	299.3	319.1	289.5	307.2	305.3	324.1	297.3	298.3	296.5	314.1	281.2		
5	513.1	509.9	519.3	493.2	516.0	512.8	522.7	499.4	514.1	510.9	516.3	484.6	524	Imidazolic ring Vib+(N7-H)vib (omp)
6	528.7	525.4	534.1	508.2	523.7	520.5	530.0	506.9	520.6	517.4	522.4	490.8	542/534	rings vib.
7	547.6	544.2	551.9	526.4	550.7	547.3	555.7	533.0	547.2	543.8	547.3	515.8	558/553	rings vib.+(N1-H) vib. (omp)
8	561.7	558.2	565.2	540.0	564.1	560.6	568.5	546.0	555.2	551.8	554.8	523.4	598	rings vib.
9	610.7	606.9	611.5	587.1	613.7	609.9	615.7	594.0	611.0	607.2	607.1	576.0	602	
10	636.0	632.1	635.4	611.4	635.6	631.7	636.5	615.2	629.1	625.2	624.1	593.1	626/622	rings vib.
11	677.0	672.8	674.2	650.8	672.1	667.9	671.2	650.5	655.4	651.3	648.7	617.8	657	rings vib.+(N-H) vib.(omp)
12	711.9	707.5	707.1	684.3	710.1	705.7	707.3	687.3	681.9	677.7	673.6	642.8	691	rings vib.+N-H vib. (omp)
13	733.5	729.0	727.5	705.1	741.8	737.2	737.5	718.0	716.6	712.2	706.1	675.5	725	rings vib.
14	781.0	776.2	772.4	750.8	807.0	802.0	799.5	781.1	721.6	717.1	710.8	680.3	775	(N1-H)vib.+rings vib.(omp)
15	857.9	852.6	845.1	824.7	874.2	868.8	863.4	846.1	815.2	810.1	798.5	768.5	860	(C2-H)vib.+pyrimidinic ring vib.(omp)
16	899.5	893.9	884.4	864.7	902.0	896.4	889.9	873.0	901.4	895.8	879.3	849.7		
17	927.7	921.9	911.0	891.8	939.7	933.9	925.7	909.5	901.8	896.2	879.7	850.1		
18	958.1	952.2	939.7	921.0	960.8	954.8	945.8	930.0	958.6	952.7	932.9	903.8		
19	1079.2	1072.5	1054.1	1037.4	1077.8	1071.1	1057.1	1043.2	1090.9	1084.1	1056.9	1028.4	1054	rings vib.+ Imidazolic-H vib.
20	1101.0	1094.2	1074.7	1058.4	1096.7	1089.9	1075.1	1061.5	1117.3	1110.4	1081.6	1053.3	1062	rings vib.+ (N7-H) vib.
21	1122.8	1115.8	1095.3	1079.3	1121.9	1114.9	1099.	1085.9	1144.0	1136.9	1106.7	1078.4	1084	rings vib.+(N1-H)
22	1208.2	1200.7	1176.0	1161.4	1203.3	1195.8	1176.5	1164.7	1236.3	1228.6	1193.2	1165.5		
23	1300.6	1292.5	1263.3	1250.3	1290.1	1282.1	1259.0	1248.7	1332.4	1324.1	1283.2	1256.1		
24	1355.0	1346.6	1314.7	1302.6	1345.6	1337.3	1311.8	1302.4	1380.9	1372.3	1328.7	1301.8	1324	rings vib.+ (C2-H)vib./ (N7-H) vib./+C=O)
25	1407.0	1398.3	1363.8	1352.5	1397.1	1388.4	1360.8	1352.3	1424.8	1416.0	1369.8	1343.2	1371	rings vib.+ (C2-H)vib.+ (N7-H) vib.+v(C=O)
26	1419.3	1410.5	1375.4	1364.4	1410.1	1401.4	1373.2	1364.8	1446.0	1437.0	1389.7	1363.1	1384, 1381	rings vib.+ (N-H)vib.+v(C=O)

27	1439.7	1430.8	1394.7	1384.0	1433.6	1424.7	1395.5	1387.6	1470.6	1461.5	1412.7	1386.3	1433	rings vib.+(C8-H) vib.+ v(C=O)
28	1476.0	1466.8	1429.0	1418.9	1461.1	1452.0	1421.7	1414.2	1511.5	1502.1	1451.1	1424.9		
29	1551.9	1542.3	1500.7	1491.8	1539.2	1529.7	1496.0	1489.8	1572.6	1562.8	1508.3	1482.5		
30	1563.1	1553.4	1511.3	1502.6	1551.1	1541.5	1507.3	1501.3	1593.2	1583.3	1527.6	1501.9		
31	1649.2	1639.0	1592.6	1585.4	1633.9	1623.8	1586.1	1581.5	1657.7	1647.4	1588.1	1562.7	Band 1595	rings vib.+(C2-H)vib.+δ(N1-H)vib.+v(C=O)
32	1810.7	1799.5	1745.2	1740.6	1765.6	1754.7	1711.3	1708.9	1843.3	1831.9	1762.0	1737.7	1735	v(C=O)+rings vib.+(N1-H)vib.
33	3190.4	3170.6	3048.6	3066.9	3176.8	3157.1	3053.7	3074.8	3270.5	3250.2	3099.6	3083.1		
34	3263.4	3243.2	3117.5	3137.1	3248.1	3228.0	3121.5	3143.8	3339.0	3318.3	3163.8	3147.7		
35	3607.5	3585.1	3442.6	3467.9	3595.0	3572.7	3451.5	3479.6	3665.4	3642.7	3469.7	3455.4	Band 3428	v(N1-H)
36	3661.7	3639.0	3493.8	3520.0	3650.2	3627.6	3504.0	3533.0	3719.3	3696.2	3520.2	3506.2	Band 3464	v(N7-H)

v=escalado (Palafox, M. A.; Iza, N. y Gil, M.) $[v^{(1)} = 34,6 + 0,9447*v]$ y $[v^{(2)} = 31,9 + 0,9512*v]$ y $[v^{(3)} = 34,5 + 0,9372*v]$

v = escalado (Gaussian 2003) $[v^{(1)} = 0,9613*v]$ y $[v^{(3)} = 0,9427*v]$

vI = escalado (Gaussian 2003) *Int. J. Mol. Sci.* 2007,8, 760 – 776 [factor = 0,9679]

vII = escalado (Gaussian 2003) Bauschlicher and Partridge Energy: [factor = 0,989]

a= *J. Phys. Chem. A* 1997 (101) p.8309 – 8318// Frecuencias experimentales y teóricas de HX (gas) y factor de escalada 0.9938

v = tensión; δ = flexión simétrica fuera del plano τ = torsión; γ = movimiento fuera del plano; (omp) = fuera del plano molecular.

En la Tabla 7.1.29. se muestran todos los números de onda para todos los niveles de cálculo y escaladas en todos los valores del tautómero HX-17. Se observa que los valores más próximos a los correspondientes en fase gaseosa son los del nivel B3LYP/6-31G (d,p) escalado $v^{(1)}$ que presenta ocho valores más próximos (en cm^{-1}): 534.1 (534); 727.5 (725); 772.4 (775); 1054.1 (1054); 1429.0 (1433); 1592.6 (1595); 3442.6 (3428); 3493.8 (3464). El segundo nivel es MP2 escalado $v^{(3)}$ que presenta cuatro valores próximos o iguales a los experimentales: 607.1 (602); 624.1 (626/622); 1328.7 (1324); 1369.1 (1371).

De los espectros de los tautómeros en forma enol se encuentran pocos valores experimentales. La Tabla 13.1.30. (anexo) muestra los números de onda del tautómero HX-70 con los correspondientes valores experimentales⁵⁵. Recientes obtenciones del espectro IR y Raman de HX⁶⁰ presentan números de onda asignadas al enlace -OH: a 3620 cm^{-1} (ms), correspondiente a νOH ; 1293 cm^{-1} (s), δOH , (éste solamente en el espectro Raman) y 236 cm^{-1} (vw) asignada ωOH . Aunque no se indica el tautómero correspondiente, lógicamente pueden asignarse a los tautómeros en forma enol.

7.1.7. Conclusiones

1. Las principales diferencias entre los valores teóricos y experimentales entre las longitudes de enlace del tautómero HX-19 son el alargamiento de los enlaces C5-C6 y C6-N1 y acortamiento de C6-O en los valores teóricos respecto de los experimentales.
2. El tautómero HX-17 presenta bastantes diferencias respecto del HX-19 y ello puede considerarse debido al cambio del H desde el N9 al N7 ya que supone un cambio en el anillo imidazólico.
3. En el tautómero HX-37 los cambios son mayores respecto de HX-19 que respecto a HX-17, en este último caso debido a que ambos tautómeros tienen una estructura análoga, tienen el H en N7.
4. Las cargas negativas más altas en el tautómero HX-19 para todos los niveles de cálculo se sitúa en los átomos O6 y N1 y las positivas en los átomos C6 y C2. La presencia de una molécula de agua aumenta la carga en C6, C2 y C4. El H situado en el átomo N9 en HX-19 y en el átomo de N7 en HX-17 tiene la mayor carga positiva y son los más reactivos.
5. En la hidratación de los dos tautómeros HX-19 y HX-17 se tienen tres posiciones posibles de las moléculas de agua A, B y C en el tautómero HX-19. El más estable se

sitúa en la posición A, y en todos los niveles de cálculo es el de menor energía que generalmente da lugar a un aumento de las cargas de los átomos.

6. En el monohidrato del tautómero HX-17 la posición más estable es la B y la posición más inestable la C. Los cambios en las cargas son pequeños en las posiciones A y B pero en C se observan bastantes cambios.
7. En el tautómero HX-19 la primera esfera de hidratación se completa con 12 moléculas de agua. En todos estos hidratos la presencia del agua aumenta las cargas de los átomos O6, N1 y C5. Sin embargo, en la segunda esfera de hidratación, estas cargas prácticamente no cambian.
8. El tautómero HX-17 presenta los cambios análogos a los de la hidratación de HX-19 pero en la segunda esfera las cargas tienen un aumento espectacular.
9. Los cambios que sufren los enlaces C=O y N1-H en los dos tautómeros aumentan en la primera esfera de hidratación pero en la segunda esfera permanecen estables.
10. Comparando las longitudes de enlace del tautómero HX-19, en el estado sólido calculado, con las experimentales, los valores de C=O y C6-N1 son grandes. Las estructuras sólidas simuladas no tienen los enlaces de H fuertes.

7.1.8. Referencias

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7.2. Alopurinol

El alopurinol, 1,2-dihidropyrozolo[3,4-d]pyrimidinium-4-one (IUPAC), es un fármaco utilizado para inhibir la xantina oxidasa. Ésta es una enzima que cataliza la transformación de la hipoxantina, base estudiada en los anteriores puntos, en xantina, (3,7-Dihidropurina-2,6-dione), y ácido úrico, (7,9-Dihydro-1H-purina-2,6,8(3H)-triona) según IUPAC. Este ácido forma cristales en la sangre y ciertos tejidos depositándose en las articulaciones y en el hígado dando lugar a ataques de gota. Al inhibir esta enzima, los productos de descomposición de las purinas son xantina y hipoxantina que no dan lugar a cristales, y que son más solubles que el ácido úrico¹. La figura 7.2.1. muestra la estructura del alopurinol (ALO) y la numeración^{1,2}, en el tautómero más estable. Obsérvese la similitud con la hipoxantina (figura 7.1.1.), en donde el N7 en HX-19 (N3 en ALO) se sitúa en la posición 2.

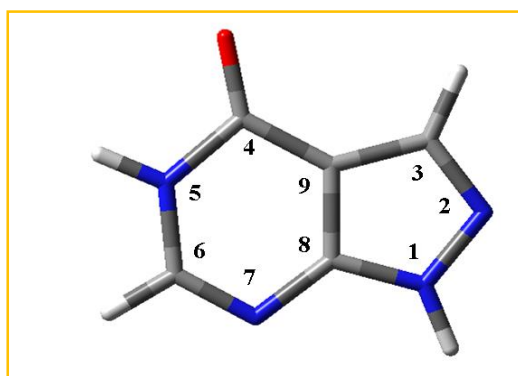


Figura 7.2.1. Molécula de alopurinol y su numeración² (tautómero ALO-15).

El mecanismo de acción del ALO como droga y el mecanismo de enlace con otras moléculas puede estudiarse a partir de su estructura electrónica y otras propiedades³. ALO se emplea, en medicina, además del tratamiento de la gota, en la hiperuricemia, paso inicial que puede desencadenar la gota, en daños en los tejidos, daños vasculares, insuficiencia cardíaca y otras enfermedades inflamatorias⁴⁻⁹. Es un protector en daños en el corazón y en las terapias anticáncer¹⁰⁻¹⁶. También se aplica en el tratamiento de la esquizofrenia¹⁷⁻¹⁹ y en la manía bipolar²⁰. También en el deterioro de los músculos y en la sarcopenia^{21,22}.

Se han estudiado las reacciones de ALO, su espectro y estructura²³⁻²⁵. La estructura se determinó previamente por difracción de rayos X^{25c}. El espectro electrónico y otros datos se midieron en disolución a diferentes pH, confirmando que en disolución ALO se presenta principalmente en forma tautomérica ceto y enol²⁴. La investigación del tautomerismo de las moléculas es importante en bioquímica y farmacología para reconocimiento de las

moléculas, y por ello hay abundante trabajo sobre el tautomerismo de las bases nucleicas^{25(a,b)}.

El estudio de ALO confirma que se presenta en 14 tautómeros posibles²⁶ neutros y 9 estructuras canónicas². Además, los cálculos de las energías relativas de los diferentes tautómeros, realizados con diferentes funcionales y conjuntos de bases²⁶, obtenidas en DFT (B3LYP) y MP2, predicen que el tautómero más estable es el cetotautómero-1,5¹ (figura 7.2.1.), seguido del cetotautómero 2,5 y, muy alejado 1-enol- cis tautómero.

Para más simplicidad se ha establecido la nomenclatura de los tautómeros de ALO partiendo de este acrónimo (ALO) seguido de los números correspondientes en la base en que se encuentran los dos átomos de H. Se tiene así en la forma ceto ALO-15 para el 1,5 ceto-tautómero (Tabla 7.2.1.). En las formas enol, de análoga forma, con el número del átomo que tiene el H seguido del número 6 y la partícula *cis* o *trans*.

El tautomerismo de ALO en fase gas y en disolución se estudió desde métodos semiempíricos, *ab initio* y los DFT, estableciéndose que la molécula de ALO existe en fase aislada principalmente en la forma ALO-15 diferenciándose de HX que se tiene principalmente como mezcla de dos tautómeros HX-19 y HX-17²⁷.

También se han realizado estudios sobre el momento dipolar y la polarizabilidad²⁸ y su comportamiento en presencia y ausencia de Cu²⁺ mediante espectroscopía vibracional y radiólisis. En estos trabajos experimentales se observa el comportamiento de ALO como ``scavenger`` del radical OH produciendo beneficios a los organismos vivos. El estudio del espectro vibracional indica que presenta facilidad para formar complejos con Cu²⁺. En éstos el metal interactúa con un átomo de N del anillo del pirazol y con C=O^{29,30}. También se han realizado trabajos sobre la síntesis, en disolución acuosa, de compuestos nuevos con aniones como Cl⁻, Br⁻, NO₃⁻ y SO₄²⁻ y estudios DFT de los complejos de ALO con Co^{31,32}.

Se han realizado estudios en (DFT) sobre ALO como anión³³, como tautómeros con dos protones en fase gaseosa y en disolución³⁴, estudios experimentales y mediante DFT de los tautómeros monoprotonados en fase gaseosa^{35a} y estudios teóricos sobre tautómeros diprotonados^{35b}. También se han realizado estudios tanto experimentales (mediante rayos X) como cuánticos sobre la geometría de ALO neutro, protonado y formando compuestos de coordinación³⁶.

Nuestro trabajo ha consistido en el estudio de la hidratación y su efecto sobre la geometría y la energía de los tautómeros, principalmente ALO-15 en los niveles de cálculo más altos,

teniendo en consideración trabajos realizados previos y sobre compuestos análogos³⁷⁻⁴². Para estudiar la estructura de los hidratos hemos partido, principalmente, de dos métodos, Becke–style 3 Parameter DensityFunctional Theory (usando el funcional de correlación Lee – Yang – Parr) (B3LYP) y Møller – Plesset de segundo orden (MP2). El conjunto de bases empleadas es 6–31G(d,p). El método MP2 se aplicó principalmente para confirmar los resultados obtenidos en B3LYP^{43,44,45}.

7.2.1. Estudio energético de los tautómeros del alopurinol

En la figura 7.2.2. se tienen los once tautómeros más estables elegidos entre los catorce posibles²¹ indicando las longitudes de enlace en Å, en los niveles de cálculo B3LYP/6-31G(d,p) y MP2/6-311++G(2d,p) (entre paréntesis) y la energía de cada tautómero en unidades atómicas, en los niveles de cálculo B3LYP/6-31G(d,p), MP2/6-311++G(2d,p) (entre paréntesis), M052X/6-31G(d,p) (en cursiva), M06L/6-31G(d,p) (entre corchetes) y MP2/cc-pvtz (entre llaves). Del estudio de estas energías se deduce que el tautómero más estable es ALO-15 y, en segundo lugar ALO-25 y, en tercer lugar ALO-16-C.

En la Tabla 7.2.1. se muestran las energías relativas de los once tautómeros antes citados respecto de ALO-15, en kcal/mol, en todos los niveles de cálculo. ALO-15, además de ser el más estable, es también el más abundante, con el 99.9 % de población, a 273.15 K, y 99.8 % a 298.15 K. El tautómero ALO-25 es el siguiente en orden de estabilidad y tiene una población de 0.1 y 0.2 % en ambas temperaturas. La población de los demás tautómeros, como puede verse en la tabla, es muy inferior. Estos resultados concuerdan con los datos experimentales que indican que las formas ceto son las predominantes^{25c}.

7.2.2. Estudio de la geometría de los tautómeros

En la Tabla 7.2.2. se muestran los valores en Å de las longitudes de enlace de los once tautómeros de ALO en los niveles de cálculo B3LYP/6-31G(d,p), MP2/6-311++G(2d,p) (en paréntesis) y MP2/6-31G(d,p) (en cursiva) y los valores experimentales^{25c}. En la Tabla 7.2.3. aparecen los ángulos de enlace en los mismos niveles de cálculo indicados anteriormente. El tautómero más estable viene en negrita.

En estas tablas se observa la gran variación que sufre el enlace C4-O en relación al valor que tiene en los datos experimentales. El mayor cambio lo presentan las formas enol, principalmente los tautómeros *cis*, aumentando la longitud de enlace de manera espectacular. Ello es lógico debido a que las formas experimentales se consideran en la forma ceto que tienen un doble enlace C=O. En cambio los tautómeros enol tienen un enlace simple de

mayor longitud. Debido a esta estructura C-O, las formas enol tienen los enlaces más cortos entre los átomos vecinos: C4-N5 y C9-N4. Se observa alguna excepción en los tautómeros enol-*trans* que, de manera análoga a las formas ceto, principalmente la longitud de estos enlaces aumenta.

La Tabla 7.2.2. muestra que las longitudes de enlace más próximas a los valores experimentales se dan en el nivel B3LYP. El enlace N1-N2, de longitud 1.374 Å (experimental), tiene los valores más próximos a éste en los tautómeros ALO-17 y ALO-12. El enlace N2-C1, de longitud 1.325 Å, tiene los valores más próximos a éste en los tautómeros ALO-17, ALO-15, ALO-56 y ALO-12. El enlace C3-C9, en los tautómeros ALO-15 y ALO-56, tienen un valor próximo al experimental (1.414 Å), todos estos valores en B3LYP. Valores análogos en el enlace C6-N7, de 1.292 Å, lo presentan ALO-25 en B3LYP, en este caso igual (1.292 Å), y MP2, y en ALO-56 en MP2. El enlace N7-C8, que experimentalmente tiene un valor de 1.379 Å tiene este mismo valor en ALO-76C, y es próximo a ALO-27, ambos en B3LYP y MP2. Las diferencias mayores las presenta el enlace C4-O: 0.123 Å en B3LYP y 0.127 Å en MP2 y C4-N5 en los niveles de cálculo B3LYP y MP2 en el tautómero ALO-26t, diferencias 0.090 y 0.093 Å, respectivamente. Por último, C6-N7 en ALO-17 (0.095 Å) en B3LYP y 0.086 Å en MP2.

En la Tabla 7.2.1. se muestra el tautómero más estable, el ALO-15 y con gran diferencia respecto de los demás tautómeros. Por ello centraremos este estudio preferentemente en este tautómero. El cálculo indica que la molécula es prácticamente plana, el mayor valor de los ángulos corresponde a N1-C8=C9-C3 0.0209°. Los demás oscilan entre 0° (C6=N7-C8=C9) y 0.019°.

Las diferencias más importantes se encuentran en el tautómero ALO-25 que presenta diferencias grandes respecto de los valores experimentales. Así en el nivel de cálculo B3LYP, el enlace C8-N1 sufre una disminución de 0.052 Å y el enlace C9-C4 un aumento de 0.053 Å. También ALO-15 presenta diferencias notables en los dos enlaces en este mismo nivel: una disminución de 0.035 Å en el primero y un aumento de 0.047 Å en el segundo. En el tautómero ALO-25 hay también diferencias acusadas en los enlaces C8-C9 (un aumento de 0.034 Å) y en C3-C9 (una disminución de 0.029 Å). Cabe también destacar el aumento en 0.032 Å del enlace C4-N5 de ALO-15.

También se observa que el enlace N1-N2 tiene los mayores valores en los tautómeros ALO-76C y ALO-56C. Posiblemente al decrecer la fuerza del enlace C4-O afecta a todo el anillo y estos dos se alargan. Este comportamiento es regular en ambos niveles de cálculo.

El enlace N5-C6 tiene su mayor valor en ALO-56. En los dos niveles de cálculo B3LYP y MP2 presenta aumentos de 0.036 y 0.031 Å respectivamente respecto del valor experimental. Los valores calculados por B3LYP y MP2 son muy parecidos, la mayor diferencia se da en los tautómeros ALO-56 y ALO-76 y en el enlace N2-C3.

Los ángulos de los tautómeros son similares a los experimentales, las diferencias mayores las presenta el ángulo C9-C4-N5: 11.0° en ALO-76C, 10.0° en ALO-16C y 9.8° en ALO-26C y 9.7° en ALO-16t, todos calculados en B3LYP y todos mayores que los experimentales. En MP2 los valores son análogos. Se observa que todos se dan en formas enol. Los ángulos C4-C5-C6 y C8-C9-C4 tienen sus mayores valores preferentemente en tautómeros ceto. Los ángulos determinados experimentalmente son similares a los obtenidos mediante cálculos teóricos principalmente los de ALO-15.

7.2.3. Cargas atómicas

Las cargas atómicas se determinaron por el procedimiento NBO⁴⁶ (natural bonding orbital). En la figura 7.2.2. vienen representadas los valores de las cargas atómicas (NBO) de los átomos en las moléculas aisladas de los tautómeros ALO-15 y ALO-25 en todos los niveles de cálculo y en la forma hidratada, con 30 moléculas de agua y PCM.

Se observa que en los dos tautómeros citados la carga negativa más alta corresponde a los átomos N5 y O, principalmente en MP2. La carga positiva más alta sobre los átomos de H la presenta H_(N5) y H_(N1) en el tautómero ALO-15 y H_(N2) y H_(N5) en ALO-25, lo cual está en concordancia con las cargas negativas en N5, N1 y N2 que son mayores que en los demás átomos. También se observa que, generalmente, las cargas en la molécula aislada son mayores que en la forma hidratada y en PCM.

7.2.4. Hidratación

En el punto 7.2.3. se ha indicado la importancia de la hidratación en los componentes de los ácidos nucleicos ya que las moléculas de agua son un importante protector de nuestro código genético, citando diferentes estudios realizados sobre la hidratación de las nucleobases. Además, en la hidratación del tautómero ALO-15, el tautómero más estable, las formas ópticamente excitadas presentan un decaimiento volviendo a las formas estables

Tabla 7.2.1. Energía relativa de los 11 tautómeros del alopurinol en diferentes niveles de cálculo respecto del menor valor en cada nivel en kcal·mol⁻¹.

Tautómeros	B3LYP/6-31G(d,p)	(ΔG) B3LYP/6-31G(d,p)	MP2/6-311++G(2d,p)	M052X/6-31G(d,p)	M06L/6-31G(d,p)	MP2/cc-pvtz	% 273.15K
1,5-ceto tautómero (ALO-15)	0 ^a	0 ^b	0 ^c	0 ^d	0 ^e	0 ^f	99.9
2,5-ceto tautómero (ALO-25)	3.694	3.717	2.709	3.845	3.620	2.716	0.1
1-enol <i>cis</i> tautómero (ALO-16C)	5.370	5.436	4.759	3.834	6.291	4.255	<0.005
1-enol <i>trans</i> tautómero (ALO-16t)	10.983	10.798	10.065	9.547	6.291	9.393	<0.005
2-enol <i>cis</i> tautómero (ALO-26C)	12.600	12.671	10.048	11.600	13.126	9.630	<0.0005
2,7-ceto tautómero (ALO-27)	12.901	12.764	12.789	13.308	13.225	13.094	<0.005
1,7-ceto tautómero (ALO-17)	17.698	17.423	18.220	18.401	18.121	18.540	<0.005
2-enol tautómero (ALO-26t)	19.344	19.485	16.313	18.671	19.790	15.698	<0.005
7-enol <i>cis</i> tautómero (ALO-76C)	25.162	25.188	23.802	25.229	25.638	24.452	<0.005
1,2-ceto tautómero (ALO-12)	34.112	33.815	50.528	35.795	34.403	39.829	<0.005
5-enol <i>cis</i> tautómero (ALO-56C)	40.004	39.619	39.047	40.867	40.934	38.871	<0.005

^a-305646.695 kcal·mol⁻¹, ^b-305666.438 kcal·mol⁻¹, ^c-305035.56 kcal·mol⁻¹

^d-305614.829 kcal·mol⁻¹, ^e-305620.917 kcal·mol⁻¹, ^f-305151.458 kcal·mol⁻¹.

Tabla 7.2.2. Longitudes de enlace en Å de los 11 tautómeros del alopurinol en B3LYP/6-31G(d,p), MP2/6-311++G(2d,p) (en paréntesis), MP2/6-31G(d,p) (en cursiva) niveles de cálculo y los valores experimentales.

Enlaces	ALO-15	ALO-25	ALO-16C	ALO-16t	ALO-26C	ALO-27	ALO-17	ALO-26t	ALO-76C	ALO-12	ALO-56C	Experimental
N1-N2	1.363 (1.351) <i>1.380</i>	1.356 (1.343) <i>1.346</i>	1.365 (1.352)	1.362 (1.350)	1.352 (1.337)	1.365 (1.352)	1.375 (1.363)	1.349 (1.334)	1.392 (1.384)	1.377 (1.360)	1.383 (1.378)	1.374
N2-C3	1.327 (1.343) <i>1.310</i>	1.352 (1.358) <i>1.359</i>	1.321 (1.339)	1.321 (1.339)	1.350 (1.361)	1.351 (1.355)	1.324 (1.337)	1.351 (1.362)	1.327 (1.347)	1.354 (1.350)	1.327 (1.347)	1.325.
C3-C9	1.417 (1.410) <i>1.390</i>	1.385 (1.387) <i>1.387</i>	1.425 (1.417)	1.428 (1.419)	1.392 (1.393)	1.388 (1.391)	1.421 (1.416)	1.395 (1.397)	1.422 (1.411)	1.377 ^o (1.383)	1.418 (1.405)	1.414
C4-N5	1.431 (1.419) <i>1.400</i>	1.414 (1.405) <i>1.407</i>	1.328 (1.320)	1.323 (1.316)	1.313 (1.309)	1.412 (1.419)	1.433 (1.429)	1.309 (1.306)	1.360 (1.350)	1.398 (1.395)	1.371 (1.358)	1.399
N5-C6	1.367 (1.369) <i>1.380</i>	1.380 (1.380) <i>1.381</i>	1.352 (1.359)	1.349 (1.357)	1.372 (1.374)	1.291 (1.297)	1.284 (1.291)	1.368 (1.370)	1.315 (1.322).	1.313 (1.320)	1.400 (1.395)	1.364

C6-N7	1.301 (1.301) <i>1.330</i>	1.292 (1.295) <i>1.298</i>	1.328 (1.326)	1.331 (1.328)	1.312 (1.316)	1.374 (1.367)	1.387 (1.378)	1.314 (1.314)	1.348 (1.341)	1.374 (1.369)	1.287 (1.295)	1.292
N7-C8	1.366 (1.368) <i>1.360</i>	1.380 (1.378) <i>1.382</i>	1.344 (1.345)	1.341 (1.342)	1.362 (1.357)	1.378 (1.374).	1.371 (1.370)	1.359 (1.354)	1.379 (1.369)	1.316 (1.321)	1.373 (1.366)	1.379
C8-C9	1.402 (1.398) <i>1.370</i>	1.429 (1.417) <i>1.418</i>	1.412 (1.345)	1.413 (1.407)	1.435 (1.422)	1.414 (1.403)	1.388 (1.385)	1.436 (1.424)	1.435 (1.428)	1.427 (1.416)	1.457 (1.450)	1.395
N1-C8	1.353 (1.355) <i>1.373</i>	1.336 (1.350) <i>1.351</i>	1.357 (1.359)	1.358 (1.360)	1.343 (1.359)	1.327 (1.340).	1.351 (1.353)	1.344 (1.360)	1.313 (1.325)	1.370 (1.368)	1.320 (1.333)	1.388
C4-O	1.219 (1.221) <i>1.230</i>	1.221 (1.223) <i>1.228</i>	1.340 (1.345)	1.347 (1.351)	1.343 (1.348)	1.220 (1.221)	1.218 (1.219)	1.351 (1.355)	1.336 (1.337)	1.226 (1.228)	1.342 (1.343)	1.228
C4-C9	1.442 (1.442) <i>1.420</i>	1.448 (1.446) <i>1.448</i>	1.405 (1.404)	1.411 (1.410)	1.415 (1.410)	1.467 (1.463)	1.461 (1.458)	1.423 (1.416)	1.385 (1.387)	1.476 (1.468)	1.368 (1.372)	1.395

Tabla 7.2.3. Ángulos de enlace (Å) de los 11 tautómeros del alopurinol en los niveles B3LYP/6-31g(d,p), MP2/6-311++G(2d,p) (en parentesis), y MP2/6-31G(d,p) (en cursiva) y los valores experimentales^{25c} (en grados).

Angulos	ALO-15	ALO-25	ALO-16C	ALO-16t	ALO-26C	ALO-27	ALO-17	ALO-26t	ALO-76C	ALO-12	ALO-56C	Experiimental
N1-N2-C3	105.3 (105.4) <i>114.0</i>	115.1 (115.5) <i>115.7</i>	102.0 (106.2)	106.6 (106.1)	115.9 (116.4)	114.5 (114.9)	105.0 (105.2)	115.9 (116.3)	110.4 (110.4)	109.1 (109.6)	110.7 (110.6)	106.4
N2-C3-C9	111.3 (110.6) <i>103.9</i>	105.1 (104.5) <i>104.5</i>	110.9 (110.2)	111.1 (110.4)	104.6 (103.9)	106.0 (105.4)	112.1 (111.5)	104.8 (104.1)	109.9 (109.4)	108.6 (107.9)	109.3 (108.8)	110.4
C3-C9-C8	104.8 (105.2) <i>110.3</i>	105.0 (105.5) <i>105.5</i>	104.9 (105.3)	104.6 (105.0)	105.1 (105.7)	103.5 (104.0)	103.7 (104.1)	104.8 (104.8)	100.9 (101.7)	107.2 (107.1)	102.3 (101.3)	104.6
C9-C8-N1	106.0 (106.0) <i>107.2</i>	111.7 (111.5) <i>111.9</i>	105.6 (105.8)	105.8 (105.9)	111.4 (111.3)	114.1 (113.8)	107.8 (107.7)	111.5 (111.4)	113.4 (113.2)	106.6 (106.6)	106.1 (110.3)	107.8
C9-C4-N5	109.6 (109.7) <i>111.4</i>	109.6 (109.6) <i>109.3</i>	121.1 (121.1)	120.8 (120.8)	120.9 (121.0)	114.4 (114.5)	114.4 (114.6)	120.6 (120.7)	122.1 (122.3)	113.6 (113.4)	117.0 (117.0)	111.1
C4-N5-C6	125.8 (126.2) <i>125.2</i>	125.3 (125.6) <i>125.8</i>	117.9 (118.1)	117.9 (118.2)	117.6 (117.8)	120.7 (120.4)	121.1 (120.8)	117.8 (118.0)	118.6 (118.6)	120.1 (120.1)	121.8 (122.3)	125.6
N5-C6-N7	125.0 (124.9) <i>123.3</i>	125.9 (125.7) <i>125.8</i>	128.1 (127.6)	128.5 (128.0)	128.7 (128.0)	127.1 (127.1)	126.4 (126.5)	129.0 (128.4)	124.2 (123.8)	131.0 (131.0)	124.3 (124.0)	125.0
C6-N7-C8	112.3 (111.9) <i>112.2</i>	113.7 (113.1) <i>112.8</i>	112.2 (112.2)	112.0 (112.0)	113.6 (113.4)	117.3 (117.0)	116.1 (115.9)	113.4 (113.1)	119.4 (119.6)	110.4 (109.9)	115.8 (115.4)	112.4
N7-C8-C9	128.0 (128.4) <i>128.6--</i>	124.5 (125.3) <i>125.3</i>	126.5 (126.6)	126.4 (126.5)	123.4 (124.1)	119.1 (119.8)	121.6 (121.9)	123.4 (124.1)	118.5 (118.9)	126.9 (127.3)	123.1 (123.8)	127.3
C8-N1-N2	112.7 (112.7) <i>104.7</i>	103.1 (101.0) <i>102.6</i>	112.5 (112.1)	112.6 (112.1)	102.9 (102.7)	101.9 (101.9)	111.4 (111.5)	103.0 (102.8)	105.4 (105.2)	108.5 (108.7)	107.0 (106.5)	110.7
C8-C9-C4	119.2 (119.0) <i>119.3</i>	120.9 (120.7) <i>121.0</i>	114.3 (114.3)	114.4 (114.5)	115.8 (115.7)	121.4 (121.2)	119.2 (121.9)	115.8 (115.8)	117.2 (116.8)	118.0 (118.3)	117.9 (117.5)	118.6

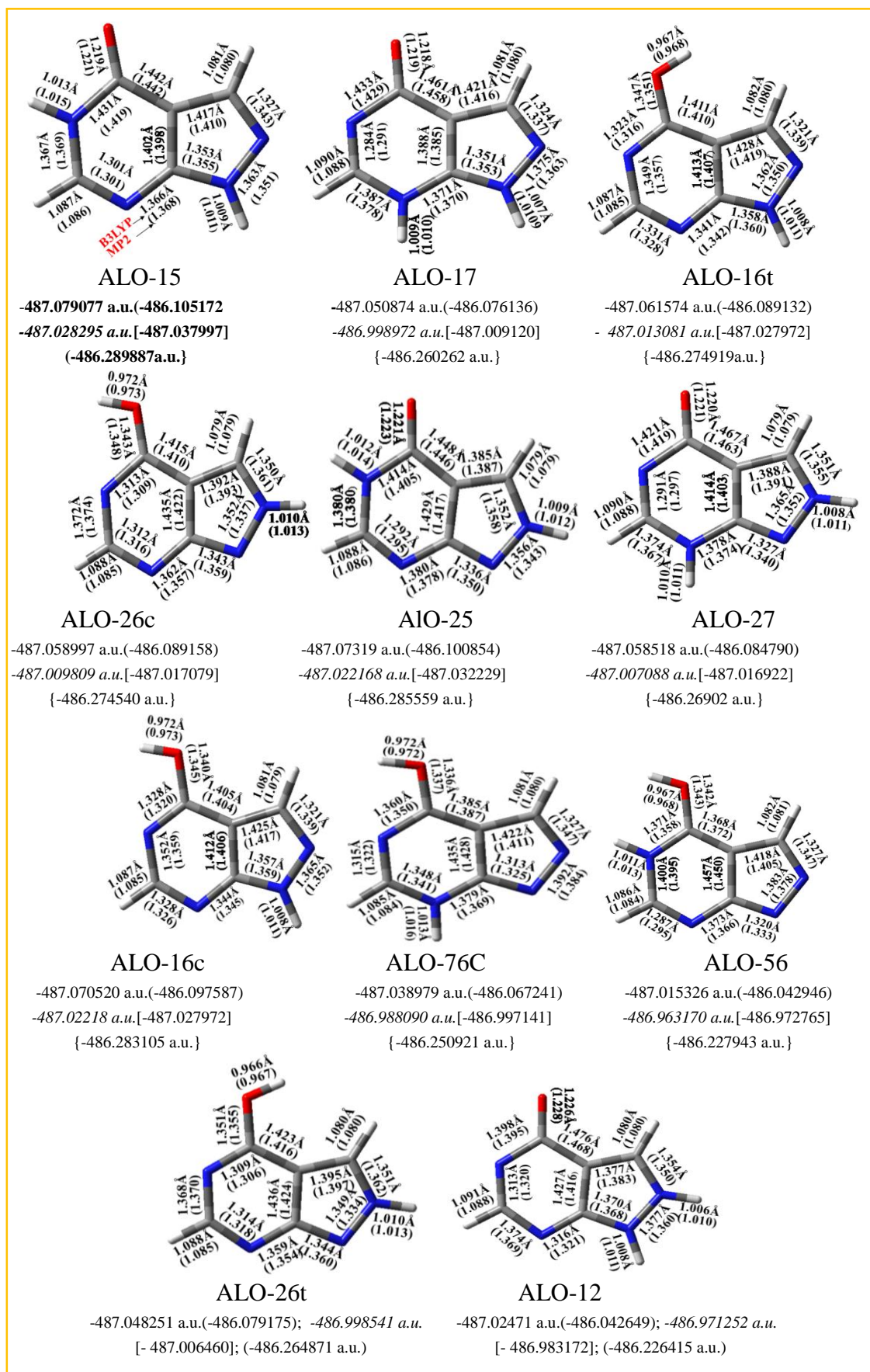


Figura 7.2.2. Tautómeros del alopurinol²⁹. Las longitudes de enlace en B3LYP/6-31G(d,p) y MP2/6-311++G(2d,p) (entre paréntesis). Las energías vienen en los niveles de cálculo B2LYP/6-31G(d,p), MP2/6-

311++G(2d,p) (entre paréntesis), M052X/6-31G(d,p) (en cursiva), M06L/6-31G(d,p) (entre corchetes) y MP2/cc-pvtz (entre llaves). La energía del tautómero más estable viene en **negrita**.

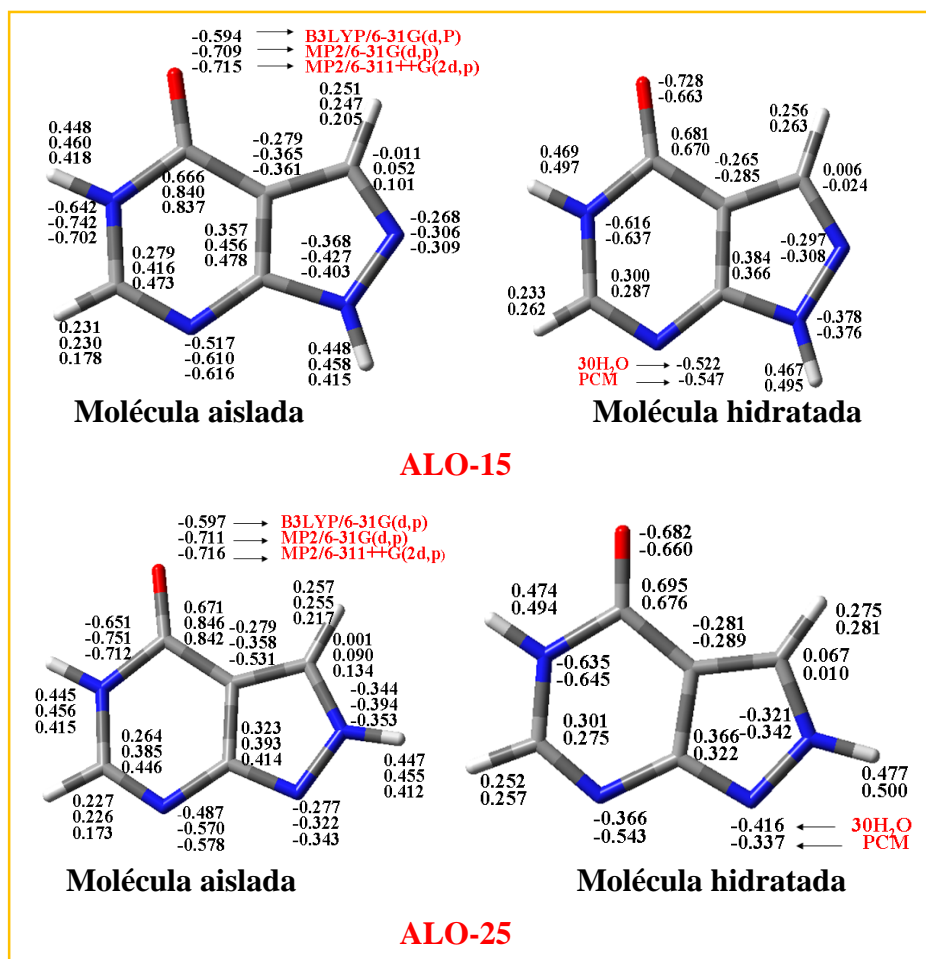


Figura 7.2.3. Cargas de los tautómeros ALO-15 y ALO-25.

diferente según la base se encuentre en estado aislado o en disolución, en este caso el decaimiento es mucho más lento⁴⁷. En la presente investigación hemos centrado nuestro estudio en las formas hidratadas de ALO-15, en B3LYP y MP2 y en ALO-25, en este caso solamente en el nivel de cálculo B3LYP, salvo algunos cálculos en MP2.

Como en el estudio de HX, el mejor método para el estudio de la hidratación de ALO se ha considerado el modelo explícito incluyendo bastantes moléculas de agua. ALO-15, tiene la menor energía entre los tautómeros de ALO, en la figura 7.2.2. vienen indicadas las energías de éstos, seguido de ALO-25. Dado que el porcentaje calculado a 273.15 K y 298.15 K para ALO-15, (Tabla 7.2.1.) es mucho mayor que el de este segundo tautómero se ha centrado en éste el estudio de la hidratación.

La primera esfera completa de la hidratación de ALO-15 se ha logrado con 12 moléculas de agua y, las medidas experimentales indican, que el número de moléculas de agua en la hidratación de los nucleótidos se completa con 12 a 20 moléculas de agua⁴⁸.

Las investigaciones sobre compuestos como D4T y timidina sitúan la primera esfera completa de hidratación en 13 moléculas de agua⁴⁹. Se han determinado cuatro regiones en la molécula de ALO favorables a la situación de las moléculas de agua: A, B, C y D (figura 7.2.4.). El método de añadir una molécula de agua y seguir añadiendo molécula a molécula a la estructura más estable se ha aplicado con buenos resultados⁵⁰. Las moléculas de agua se indican, en los gráficos adjuntos, como W_A, W_B, según la posición en que se sitúen y poder identificarlas fácilmente. En algunos casos, dado el mayor número de moléculas de agua sumadas, se indica como W₁, W₂. En las figuras se indicarán los enlaces fuertes, omitiendo los débiles.

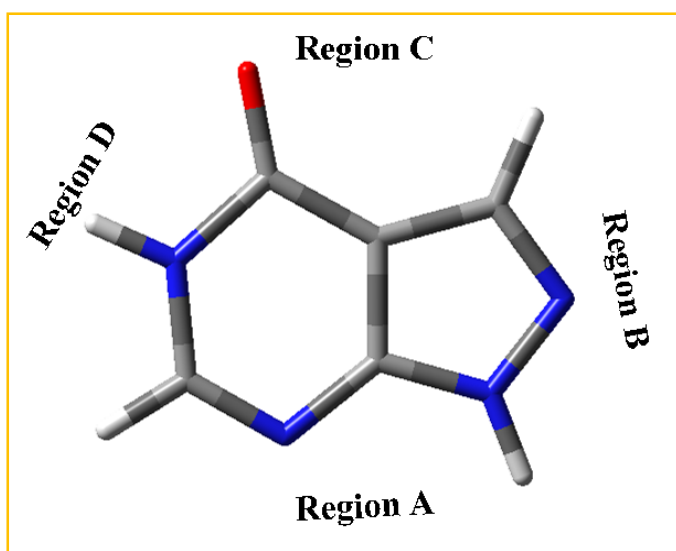
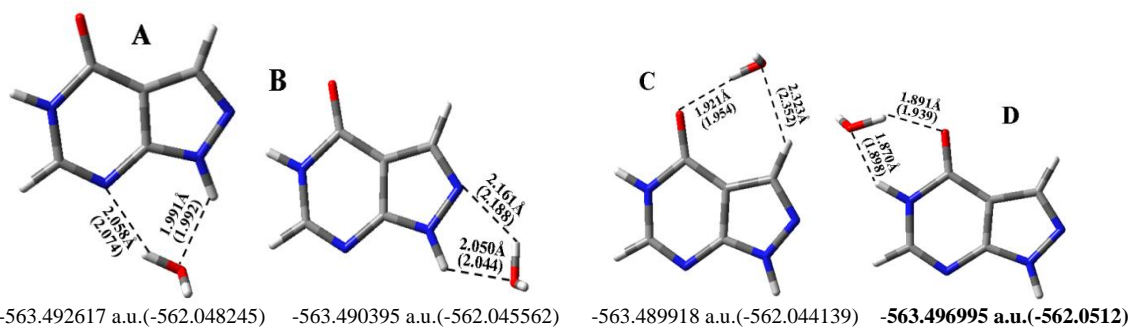
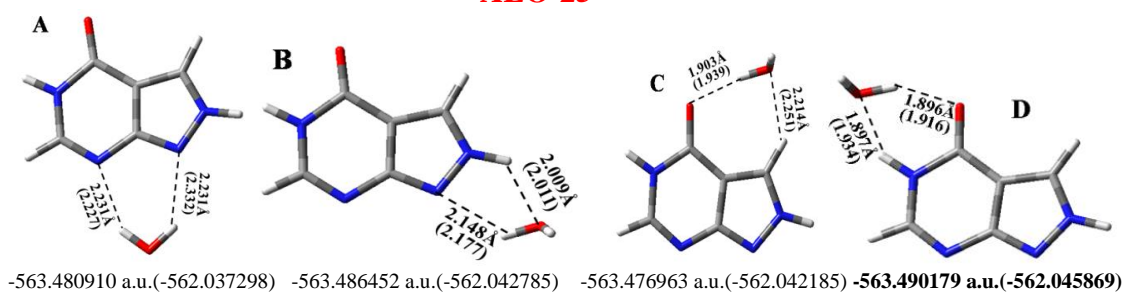
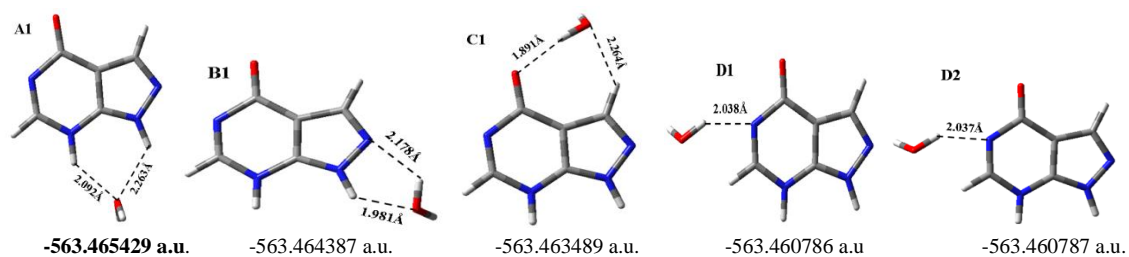
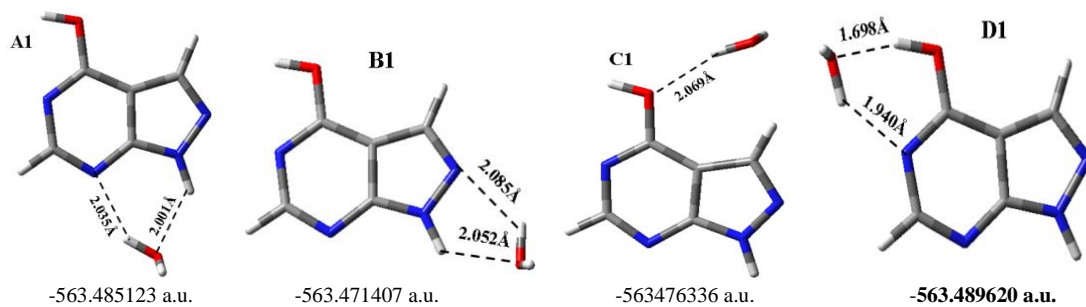


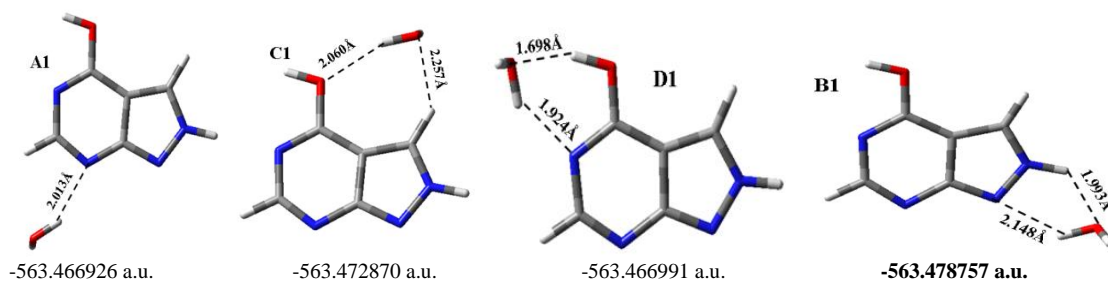
Figura 7.2.4. Regiones favorables a la situación de las moléculas de agua.

7.2.4.1. Monohidratos de ALO

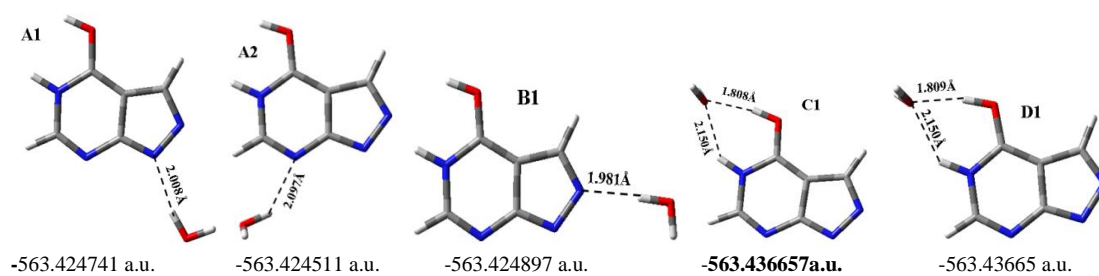
Se han calculado las cuatro posiciones óptimas de los monohidratos de los diferentes tautómeros de ALO. En la figura 7.2.5. se tienen los gráficos de los cuatro monohidratos de cada uno de los tautómeros con la energía electrónica + ZPE, en B3LYP/6-31G(d,p) y en ALO-15 y ALO-25, además de la energía en MP2. En cada tautómero el hidrato con menor energía se indica en negrita. La menor energía corresponde a ALO-15 en posición D. En la figura se tienen también los enlaces de H que en general son débiles cuando $\geq 2.000 \text{ \AA}$. El más fuerte es el correspondiente a ALO-76C, de 1.693 \AA , entre el O del agua y el H del enol, en la posición D1, y en ALO-16C. En ALO-26C es 1.689 \AA , similar al anterior, también en D1 y ALO-16t en la posición C1, de 1.789 \AA , también en la posición enol. Como puede observarse los enlaces más fuertes se dan en las formas enol, principalmente *cis* y entre el O del agua y el H del enol.

ALO-15**ALO-25****ALO-17****ALO-16C**

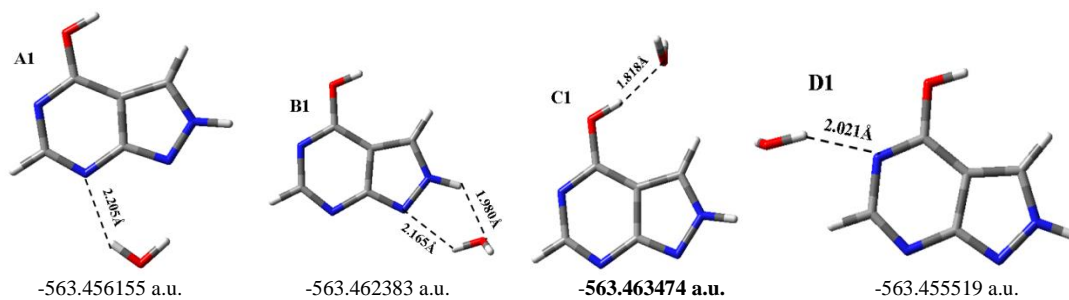
ALO-26C



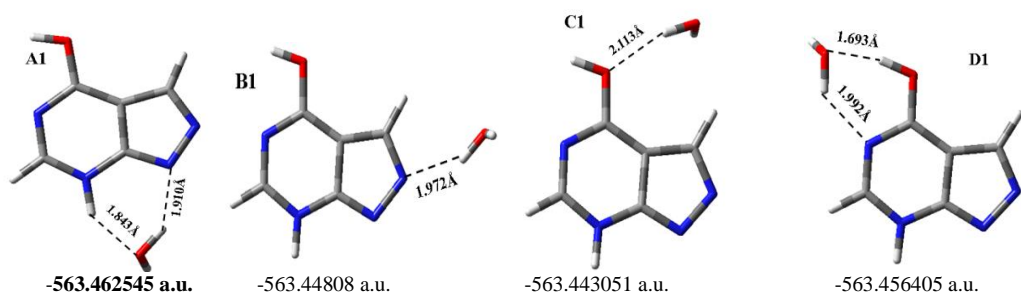
ALO-56C



ALO-26t



ALO-76C



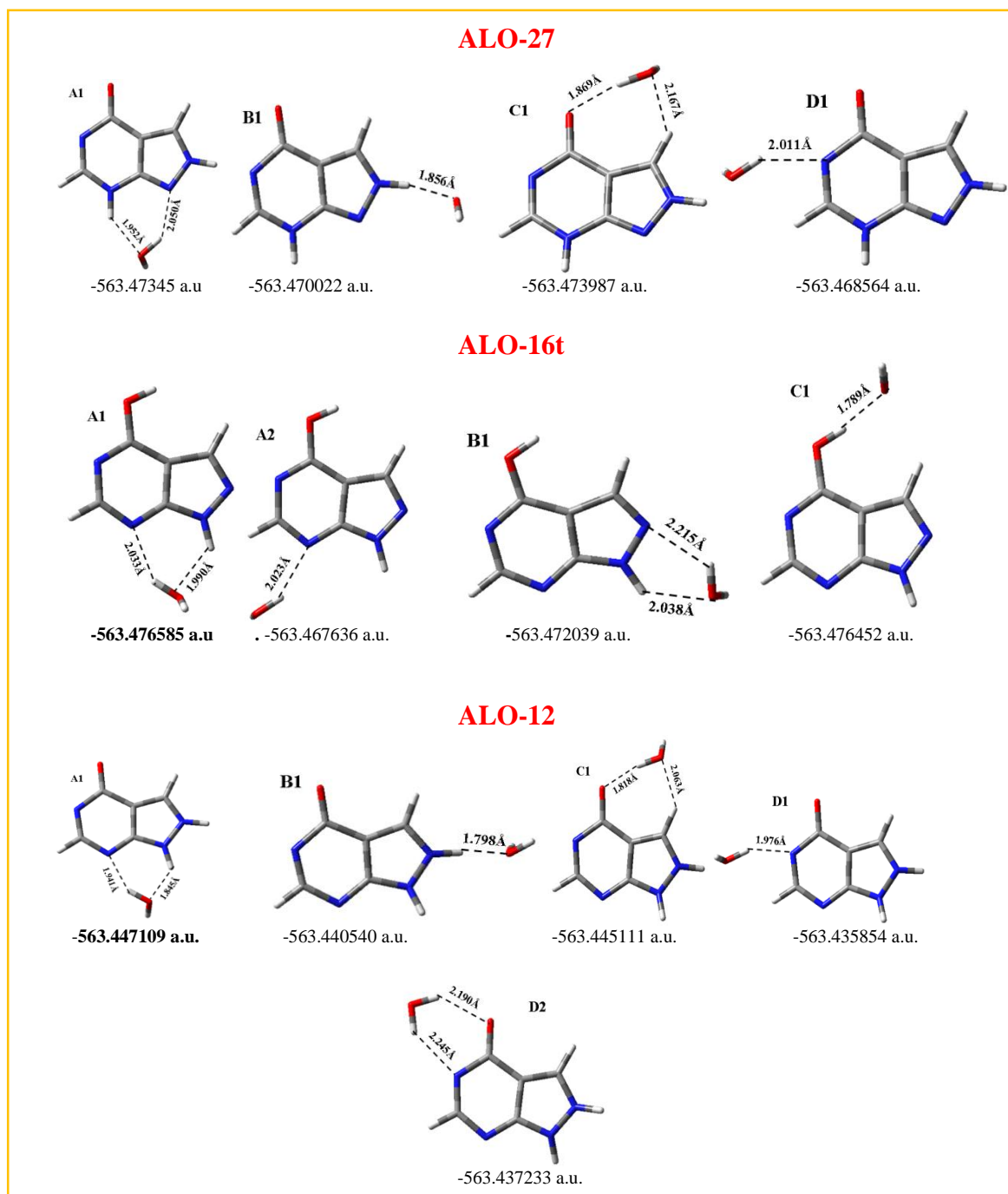


Figura 7.2.5. Posiciones óptimas de la primera molécula de agua adicionada a los tautómeros de ALO. Los enlaces de H indicados corresponden al nivel de cálculo B3LYP/6-31G(d,p). En ALO-15 y ALO-25 también se indican los valores en MP2/6-31G(d,p). La energía total (Eelectrónica + ZPE) se indica en B3LYP en unidades atómicas y en los dos tautómeros antes citados también se indica E-MP2. Para cada tautómero el monohidrato de menor energía se escribe en negrita.

7.2.4.2. Estudio de los hidratos ALO-15- (H₂O)_n y ALO-25- (H₂O)_{n=1-4}

En la figura 7.2.5. se representan las cuatro posiciones de la primera molécula de agua en el tautómero ALO-15 y la energía en los niveles de cálculo B3LYP/6-31G(d,p) y MP2/6-31G(d,p). Según el valor de la energía se tiene el orden siguiente D < A < B < C, en ambos niveles de cálculo. La posición D resulta la más estable en los dos niveles de

cálculo y, por tanto, se partirá de él en el siguiente paso de hidratación. También se representan las cuatro posiciones de la primera molécula adicionada al tautómero ALO-25. El orden de energía en B3LYP es igual que el anterior pero en MP2 hay un ligero cambio y es: (D < B < C < A). Se ha tomado la energía en el nivel de cálculo MP2/6-31G(d,p) como base, análogamente a los cálculos de la hipoxantina, para determinar el de mayor energía, dado que presenta unos datos de la energía más exactos que en B3LYP y, respecto a MP26/311++G(2d,p), el gasto computacional es mucho menor.

La molécula de ALO-15 se altera por la presencia de una molécula de agua. Si se comparan las longitudes de enlace de la posición D de la forma monohidratada con la molécula aislada se observan diferencias principalmente en MP2. Las mayores diferencias están en los enlaces N2-C3 (+0.033 Å), C8-C9 (+0.028 Å), N1-N2 (-0.026 Å), C6-N7 (-0.023 Å) y C3-C9 (+0.021 Å). También algunos enlaces de los átomos N y C con el H: el enlace H-C6 sufre una variación de 0.109 Å (en la molécula aislada es de 1.083 Å) y H-N5 (+0.025 Å). Estos incrementos se dan por los enlaces entre el H y los átomos N5 y O6 que son enlaces fuertes.

En B3LYP/6-31G(d,p) se observan pocas variaciones, el enlace C=O presenta un aumento de 0.015 Å y C9-C4 0.034 Å. Estas diferencias probablemente se deban a la formación de enlaces de H entre el agua y los átomos de O y N5 de la molécula. Los dos enlaces de H son más fuertes que los de los restantes monohidratos y contribuyen a estabilizar el grupo. Los ángulos presentan pocas diferencias respecto de la molécula aislada, se observan variaciones entre 9° y 7° en N2-C3-C9, N1-N2-C3 y C8-N1-N2, todos en el nivel de cálculo MP2.

En el tautómero ALO-25 se observan pocas diferencia respecto de la molécula aislada, en el nivel MP2 el enlace C=O presenta un aumento de 0.013 Å, una disminución de 0.011 Å en C4-C5 y incremento en 0.013 Å en el enlace H-N5.

En la Tabla 7.2.4. se tienen los valores de la energía de los complejos de ALO-15 y ALO-25 con una molécula de agua, $\Delta E_{\text{ALO15W1}}^{\text{ALO15W1}}$, $\Delta E_{\text{ALO25W1}}^{\text{ALO25W1}}$. La energía de las moléculas ALO-15 y ALO-25 en sus respectivos complejos $\Delta E_{\text{ALO15}/\text{ALO15W1-W1}}^{\text{ALO15W1}}$ y $\Delta E_{\text{ALO25}/\text{ALO25W1-W1}}^{\text{ALO25W1}}$ y la energía de la molécula de agua en cada complejo $\Delta E_{\text{W1}/\text{ALO15W1-ALO15}}^{\text{ALO15W1}}$ y $\Delta E_{\text{W1}/\text{ALO25W1-ALO25}}^{\text{ALO25W1}}$ calculado en los niveles de cálculo B3LYP y MP2. La posición D es la de menor energía en ambos tautómeros

En la figura 7.2.6., se tiene representado el valor máximo de de la corrección CP en la energía de formación del complejo ($\Delta E_{\text{ALO-15}}^{\text{CP}}$), que corresponde a la posición D en todos

los niveles de cálculo, y es el monohidrato más estable. El tautómero ALO-25 también tiene este valor máximo (ΔE_{ALO-25}^{CP}) en posición D. En B3LYP las dos posiciones B y C son similares.

En la figura 7.2.6. B viene representada la máxima deformación de energía de la molécula en los niveles de cálculo, y también la máxima deformación de energía del agua ΔE_{ALO-15}^{def} y ΔE_{W1}^{def} , que se da, en todos los niveles de cálculo en la posición D, y ΔE_{Tot}^{def} , la deformación de energía total.

La figura 7.2.6. (C) presenta estos valores respecto del monohidrato de ALO-25, E_{ALO-15}^{def} y E_{W1}^{def} , que también corresponden al hidrato en posición D. Estos valores concuerdan plenamente con la mayor estabilidad de los monohidratos de ALO-15 y ALO-25 en posición D que, además, tienen los enlaces de H más fuertes, lo cual contribuye a su estabilidad.

ALO-15 + 2H₂O

Partiendo del monohidrato de menor energía, que además es el más poblado, y tiene la primera molécula de agua adicionada en posición D, se añade una segunda molécula de agua en cada una de las posiciones óptimas, A, B, C y D, así se obtienen cuatro complejos diferentes (figura 7.2.7.). Como se observa el orden de energía es D-D < D-A < D-B < D-C, en el nivel de cálculo MP2 y también en B3LYP.

Al estudiar la geometría de los dihidratos se observan las diferencias siguientes en las longitudes de enlace respecto de la molécula aislada, calculadas en MP2: N2-C3 (0.032 Å), C8-C9 (0.028 Å), N1-N2 (-0.026 Å), C6-N7 (-0.022 Å), C3-C9 (0.021 Å), (se han tomado solamente los incrementos superiores a 0.015 Å). El enlace C6-H muestra una gran diferencia, 0.109 Å.

Los ángulos varía poco, la mayor diferencia la presentan C3-N2-N1 que decrece 9.6°, N2-N1-C8 que aumenta 8.3°, C9-C3-N2 que aumenta en 7.0° y C8-C9-C3 que disminuye en 5.0°, los cuales corresponden a los enlaces que han variado. En la figura 7.2.7. se muestran los enlaces de H del hidrato D-D que son los más fuertes.

Tabla 7.2.4. Energía de los complejos de ALO-15 y ALO-25 conteniendo una molécula de agua $\Delta E_{\text{ALO15W1}/\text{ALO15W1}}$, $\Delta E_{\text{ALO25W1}/\text{ALO25W1}}$; energía de las moléculas de ALO-15 y ALO-25 en el complejo $\Delta E_{\text{ALO15W1}/\text{ALO15}/\text{ALO15W1}-\text{W1}}$ y $\Delta E_{\text{ALO25W1}/\text{ALO25}/\text{ALO25W1}-\text{W1}}$ y de la molécula de agua en los dos complejos $\Delta E_{\text{ALO15W1}/\text{W1}/\text{ALO15W1}-\text{ALO15}}$ y $\Delta E_{\text{ALO25W1}/\text{W1}/\text{ALO25W1}-\text{ALO25}}$.
 Calculada en B3LYP/6-31G(d,p) y MP2/6-311++G(2d,p) en todos los monohidratos de ALO-15 y ALO-25.

	B3LYP			MP2		
	$\Delta E_{\text{ALO15W1}/\text{ALO15W1}}$	$\Delta E_{\text{ALO15W1}/\text{ALO15}/\text{ALO15W1}-\text{W1}}$	$\Delta E_{\text{ALO15W1}/\text{W1}/\text{ALO15W1}-\text{ALO15}}$	$\Delta E_{\text{ALO15W1}/\text{ALO15W1}}$	$\Delta E_{\text{ALO15W1}/\text{ALO15}/\text{ALO15W1}-\text{W1}}$	$\Delta E_{\text{ALO15W1}/\text{W1}/\text{ALO15W1}-\text{ALO15}}$
ALO-15W1						
A	-563.6113	-487.1794	-76.4197	-562.4119	-486.1049	-76.2899
B	-563.6159	-487.1790	-76.4200	-562.4095	-486.1049	-76.2900
C	-563.6153	-487.1792	-76.4196	-562.4081	-486.1049	-76.2900
D	-563.6231	-487.1788	-76.4195	-562.4139	-486.1047	-76.2900
ALO-25W1						
A	563.6057	-487.1737	-76.4200	-562.4024	-486.1008	-76.2900
B	563.6120	-487.1737	-76.4200	-562.4070	-486.1008	-76.2900
C	-563.6123	-487.1732	-76.4200	-562.4062	-486.1006	-76.2899
D	-563.6163	-487.1730	-76.4197	-562.4089	-486.1004	-76.2898

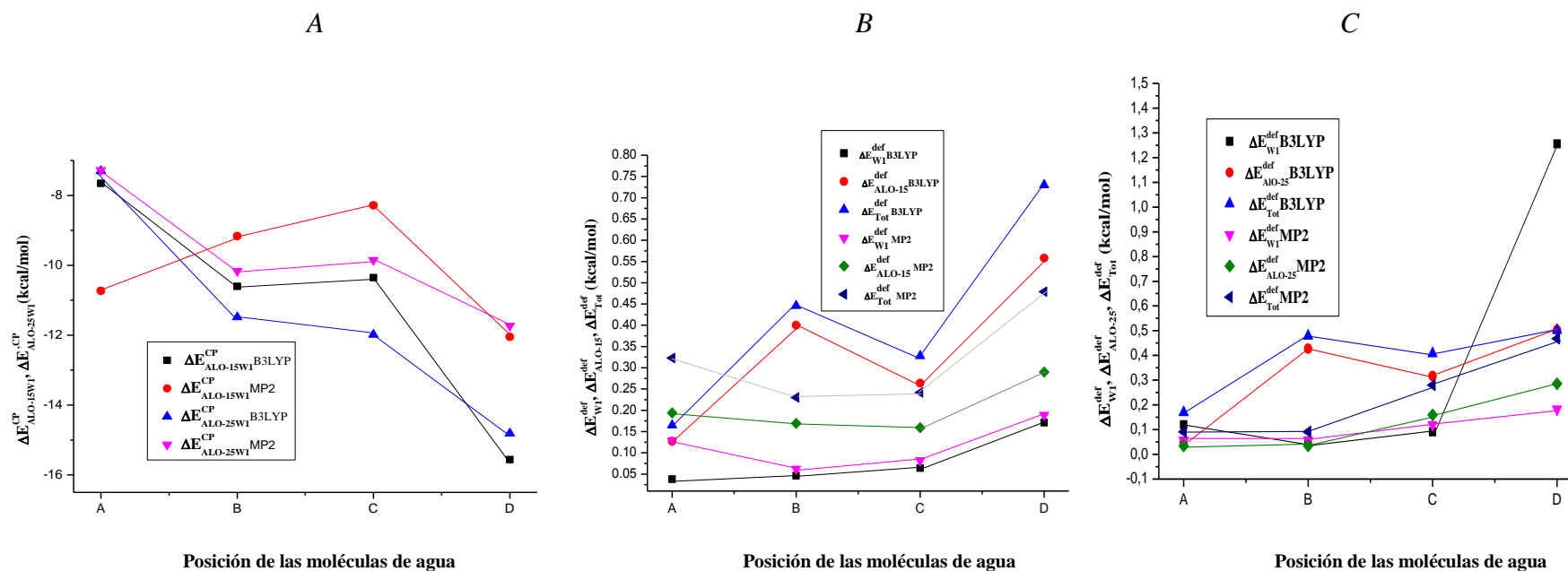


Figura 7.2.6. Posiciones de la primera molécula de agua adicionada A) $\Delta E_{\text{ALO-15W1}}^{\text{CP}}, \Delta E_{\text{ALO-25W1}}^{\text{CP}}$, B) $\Delta E_{\text{W1}}^{\text{def}}, \Delta E_{\text{ALO-15}}^{\text{def}}$ y $\Delta E_{\text{Tot}}^{\text{def}}$ y C) $\Delta E_{\text{W1}}^{\text{def}}, \Delta E_{\text{ALO-25}}^{\text{def}}, \Delta E_{\text{Tot}}^{\text{def}}$ en los niveles de cálculo B3LYP y MP2.

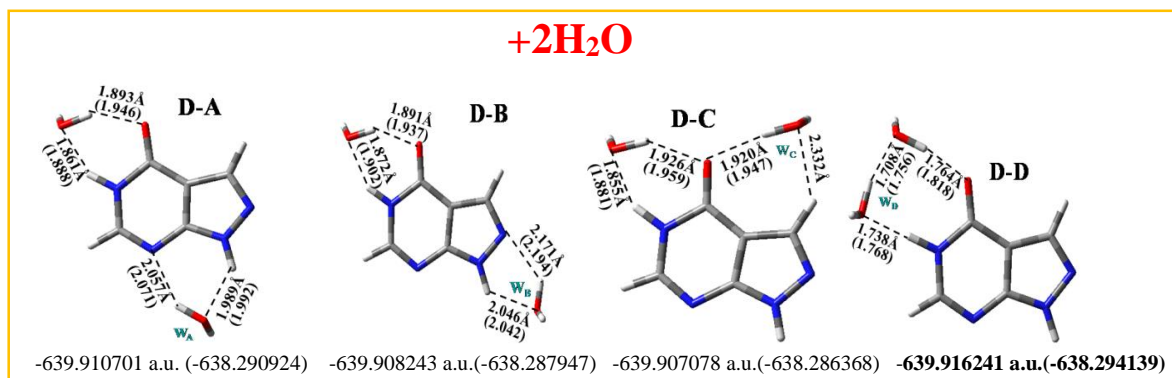


Figura 7.2.7. Las cuatro posiciones de la segunda molécula de agua asociada a ALO-15. La energía viene indicada en los dos niveles de cálculo B3LYP y MP2 (entre paréntesis).

ALO-15 + 3H₂O.

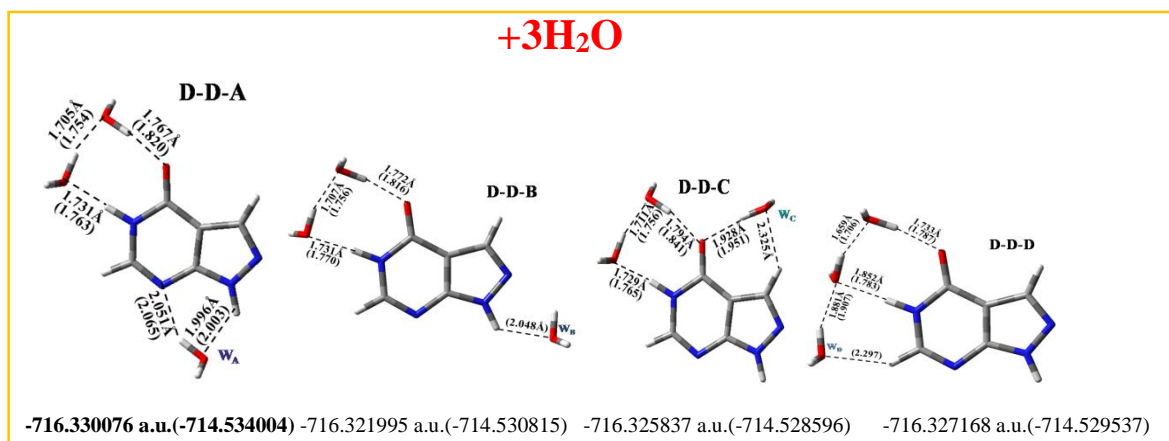


Figura 7.2.8. Las cuatro posiciones de la tercera molécula de agua asociada a ALO-15. La energía, como en el caso anterior, en B3LYP y MP2 (entre paréntesis). Se indicará en las restantes figuras de manera análoga.

La tercera molécula se asocia al dihidrato D-D, que tiene la menor energía, en las cuatro posiciones óptimas. En la figura 7.2.8. los valores de la energía tienen este orden D-D-A < D-D-B < D-D-D < D-D-C, tanto en B3LYP como en MP2; la más estable es la situada en posición D-D-A.

Los valores obtenidos en las longitudes de enlace presentan cambios no muy diferentes a los del dihidrato, en relación a la molécula aislada: N2-C3 0.034 Å, C9-C4 0.030 Å, C8-C9 0.029 Å, N1-N2 -0.028 Å, C6-N7 -0.021 Å y C3-C9 0.020 Å por MP2. El enlace C6-H aumenta en 0.109 Å, C3-H en 0.077 Å y N5-H = 0.035 Å. Los ángulos muestran las siguientes diferencias C3-N2-N1 (-8.6°), N2-N1-C8 (7.8°), C9-C3-N2 (6.9°) y C8-C9-C3 (-5.4°). Los cambios son análogos a los del dihidrato y también a los del monohidrato.

ALO-15 + 4H₂O

En la figura 7.2.8. se tienen los cuatro hidratos posibles y las correspondientes energías y enlaces de H. La posición D-D-A-A es la más estable. Se tiene el orden de energía: D-D-A-A < D-D-A-D < D-D-A-C < D-D-A-B.

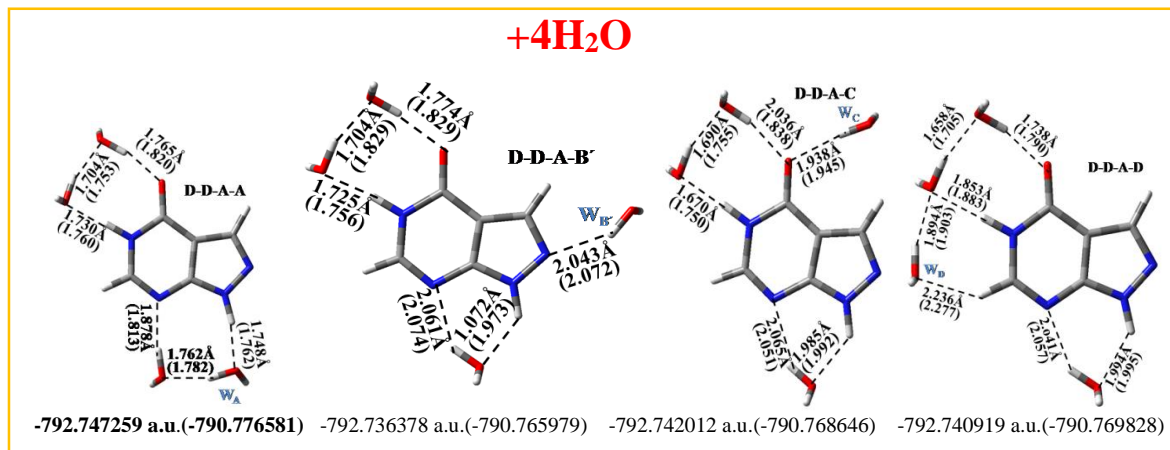


Figura 7.2.9. Las cuatro posiciones de la cuarta molécula de agua asociada a ALO-15.

Los enlaces de H presentan los siguientes cambios respecto de la molécula aislada: N2-C3 aumenta 0.034 Å, C8-C9 (0.031 Å), N1-N2 y C6-N7 disminuyen 0.027 y 0.021 Å respectivamente. C3-C9 y N5-C6 0,020 -0.017 Å, cada uno y C9-C4 se incrementa en 0.015 Å.

El enlace C6-H también aumenta en 0.109 Å y los restantes enlaces de H sufren un cambio aproximadamente de 0.036/ 0.020 Å. En la figura 7.2.9. puede observarse que el hidrato más estable tiene los enlaces de H más cortos que en las otras posiciones, principalmente con los de la molécula de agua recién añadida, lo cual le da estabilidad.

Los ángulos presentan las diferencias más acusadas en: C3-N2-N1 (-8.3°), C9-C3-N2 (6.8°), C8-C9-C3 (-5.5°), N7-C6-N5 (2.2°). Las variaciones experimentadas son análogas a las de los anteriores hidratos.

ALO-15+nH₂O n = 5-12.

El siguiente paso consiste en añadir la quinta molécula de agua en las cuatro posiciones posibles, la posición más estable en este caso es la correspondiente al pentahidrato en que la molécula de agua se sitúa en posición B. La figura 7.2.10. muestra esta estructura y la de los siguientes complejos con 6 - 12 moléculas de agua.

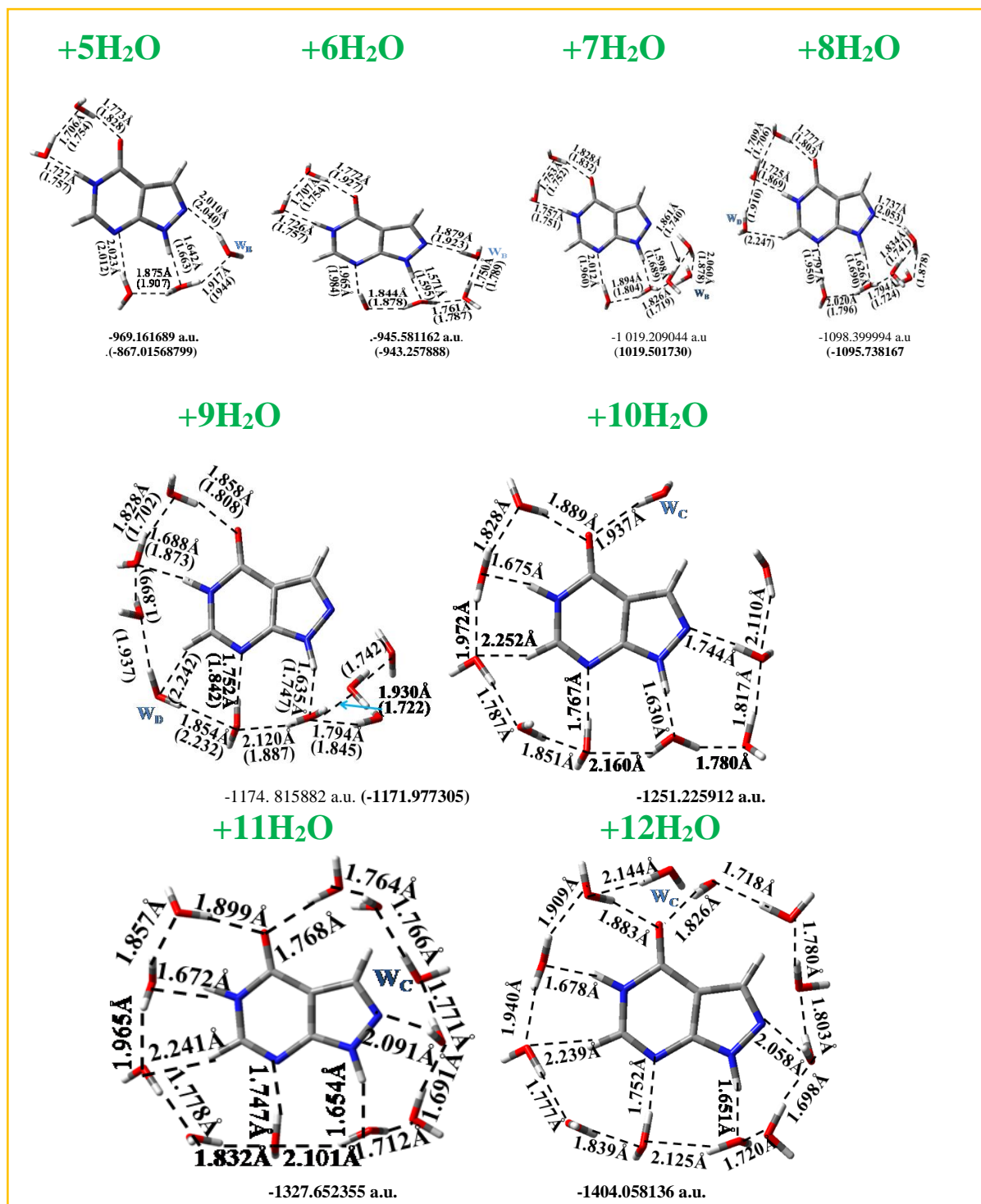


Figura 7.2.10. Los hidratos de ALO-15 más estables con 5 - 12 moléculas de agua.

En el pentahidrato la posición más estable tiene valores análogos a los de los anteriores hidratos. También los ángulos son similares a los del tetrahidrato, solamente se observa un incremento de 6.9° en N2-N1-C8. En los enlaces de H se observa que los más débiles corresponden a la última molécula de agua adicionada.

Siguiendo con el mismo método, añadiendo una nueva molécula de agua al hidrato más estable entre las cuatro posiciones posibles y tomando la posición más estable, se obtienen

los siguientes hidratos, con 6, 7 y 8 moléculas adicionales llegando a añadir 9 moléculas de agua, que es el último complejo en el que se logran los cálculos en MP2, a partir de éste solamente se pueden calcular en B3LYP.

Los hidratos con 6, 7 y 8 moléculas de agua presentan las mismas diferencias en los valores de las longitudes de enlace y ángulos que los anteriores. Los enlaces de H de estos hidratos generalmente son más cortos en la última molécula de agua sumada, análogamente al hidrato con 9 moléculas de agua.

En la misma figura pueden observarse los hidratos con 10, 11 y 12 moléculas de agua, obtenidos todos según el mismo método, con este último se tiene completa la primera esfera de hidratación. Cuando se comparan las longitudes de enlace de estos hidratos con los de la molécula aislada, solamente en el nivel de cálculo B3LYP, se observan pocas diferencias, las más significativas son: C=O 0.025, 0.033 y 0.037 Å en cada uno de los complejos y C4-N5 decrece 0.035 Å en el hidrato con 11 moléculas de H₂O y 0.037 Å en el hidrato con 12 moléculas de agua. El hidrato con 10 moléculas de agua decrece muy poco. Las anteriores variaciones son muy parecidas a las que sufre el hidrato con 9 moléculas de agua. Los ángulos prácticamente no varían. En esta misma figura la comparación de los enlaces de H, con los complejos con 10, 11 y 12 moléculas de agua muestra los más débiles que se encuentran en la última molécula de agua adicionada.

7.2.4.3. La segunda esfera de hidratación

En el punto anterior se tiene cerrada la primera esfera de hidratación con 12 moléculas de agua. A partir de esta primera envolvente los cálculos realizados en B3LYP en los tres hidratos indican que la molécula cambia muy poco por la adición de agua, lo cual puede comprobarse en la figura 7.2.12. en la que se representa la variación de los enlaces C=O y N1-N2 en los hidratos de ALO-15, desde una molécula de agua adicionada hasta 30, en los niveles de cálculo B3LYP y MP2, en este último nivel solamente hasta 9 moléculas de agua añadidas. Se observa que, al aumentar el número de moléculas de agua, las longitudes de enlace se van estabilizando.

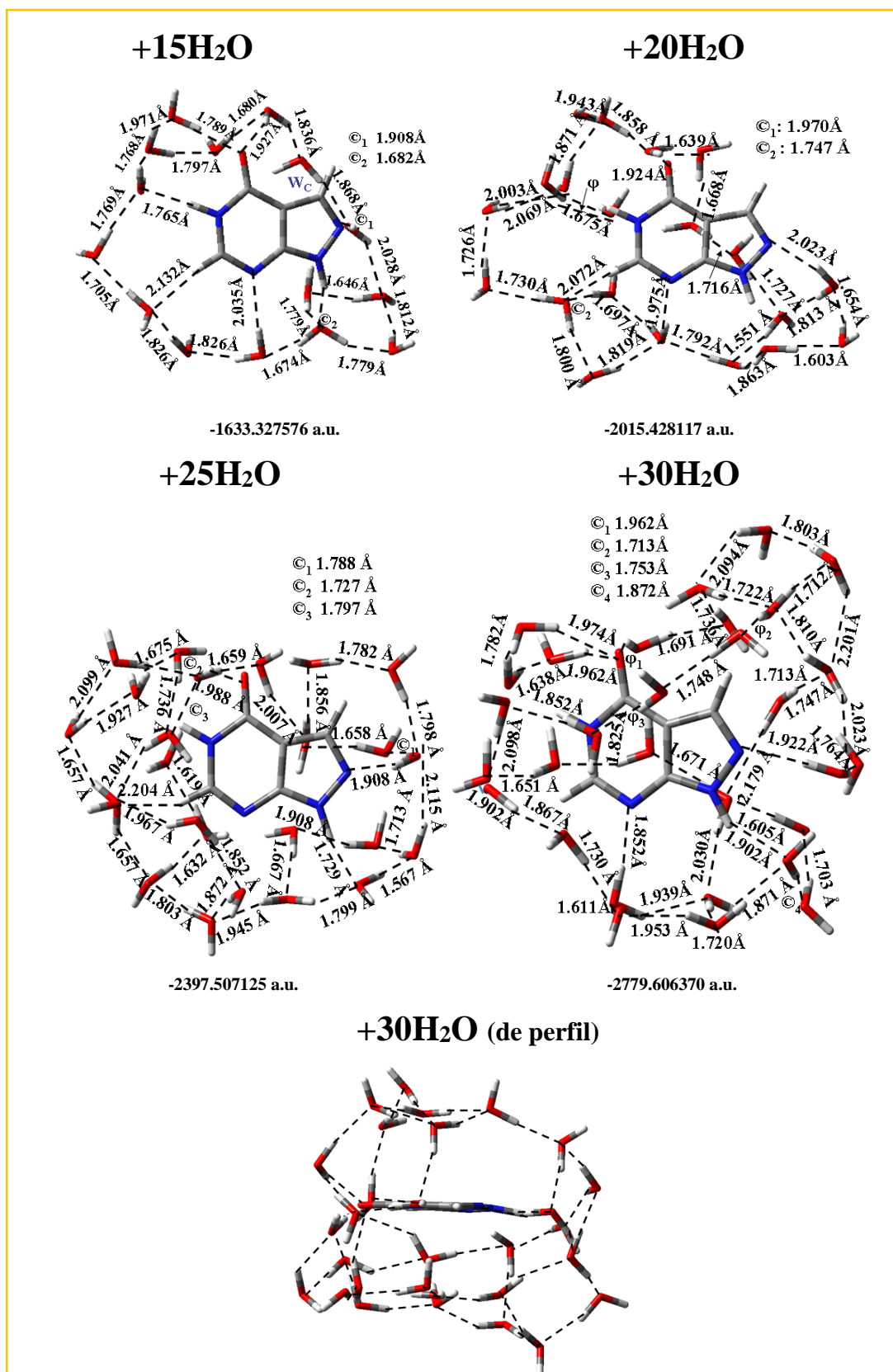


Figura 7.2.11. 2ª esfera de hidratación de ALO-15 con 15, 20, 25 y 30 moléculas de agua. Ésta también de perfil.

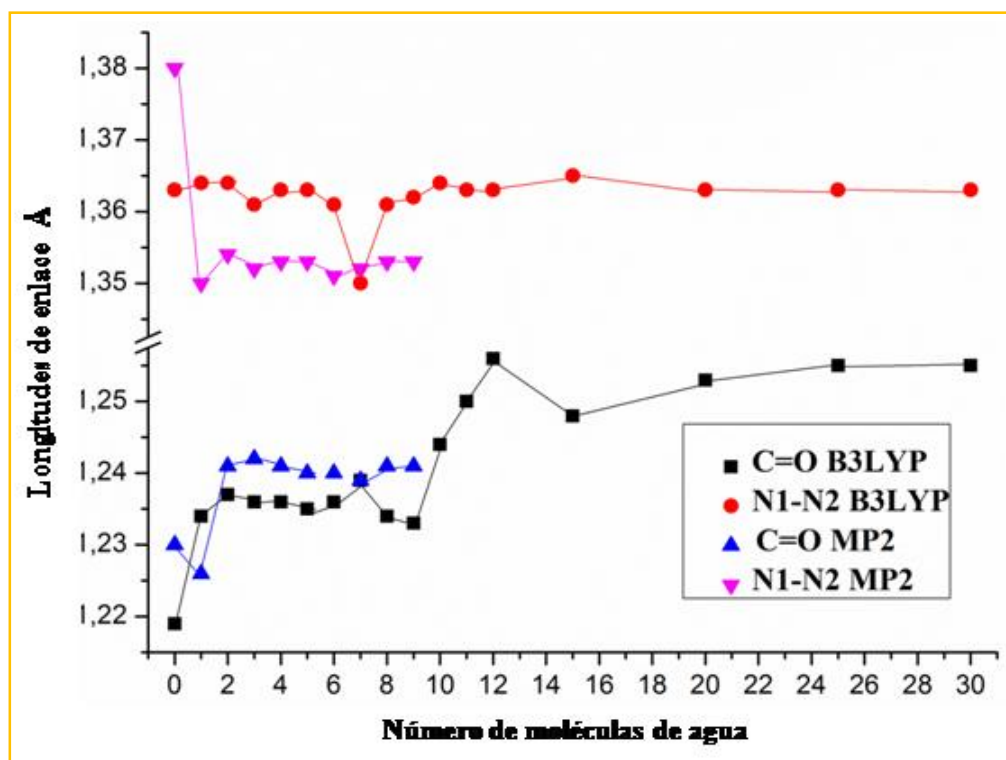


Figura 7.2.12. Longitudes de enlace C=O y N1-N2 en el tautómero ALO-15 en MP2 y B3LYP.

Tabla 7.2.5. Energías de formación, interacción base-agua y deformación del tautómero ALO-15 con 10 y con 20 moléculas de agua en el nivel B3LYP/6-31G(d,p).

	+10 H ₂ O	+20 H ₂ O
$E_{(BWn)}^{(BWn)}$ (BWn) en A.U.	-1251.578	-2016.044
$E_{(B)}^{(BWn)}$ (BWn) en A.U.	-487.171	-487.174
$E_{(Wn)}^{(BWn)}$ (BWn) en A.U.	-764.279	-1528.757
$E_B^{(def)}$ (BWn) en kcal	0.00819	0.00594
$\Sigma E_{W_i}^{(def)}$ (BWn) en A.U.	0.00568	0.0208
ΔE_{B-Wn}^{CP} (en kcal/mol)	-74.878	-67.381
ΔE_{Wn}^{CP} (en kcal/mol)	-51.180	-227.418
$\Delta E_{B(Wn)}^{CP}$ (en kcal/mol)	-126.058	-254.799

La Tabla 7.2.5. muestra las energías de formación, interacción base-agua y deformación del tautómero ALO-15 con 10 y con 20 moléculas de agua en el nivel B3LYP/6-31G(d,p).

Los valores de las energías de formación mayores corresponden al hidrato con 20 moléculas de agua y también las correcciones ΔE_{Wn}^{CP} y $\Delta E_{B(W_n)}^{CP}$. Los valores correspondientes a $E_B^{(def)}$ (BWN) (en kcal) y $\Sigma E_{Wi}^{(def)}$ (BWN) (en A.U.) son notablemente mayores los correspondientes al hidrato con 10 moléculas de agua, lo cual indica la mayor estabilidad del complejo al aumentar el número de moléculas de agua.

7.2.4.4. PCM

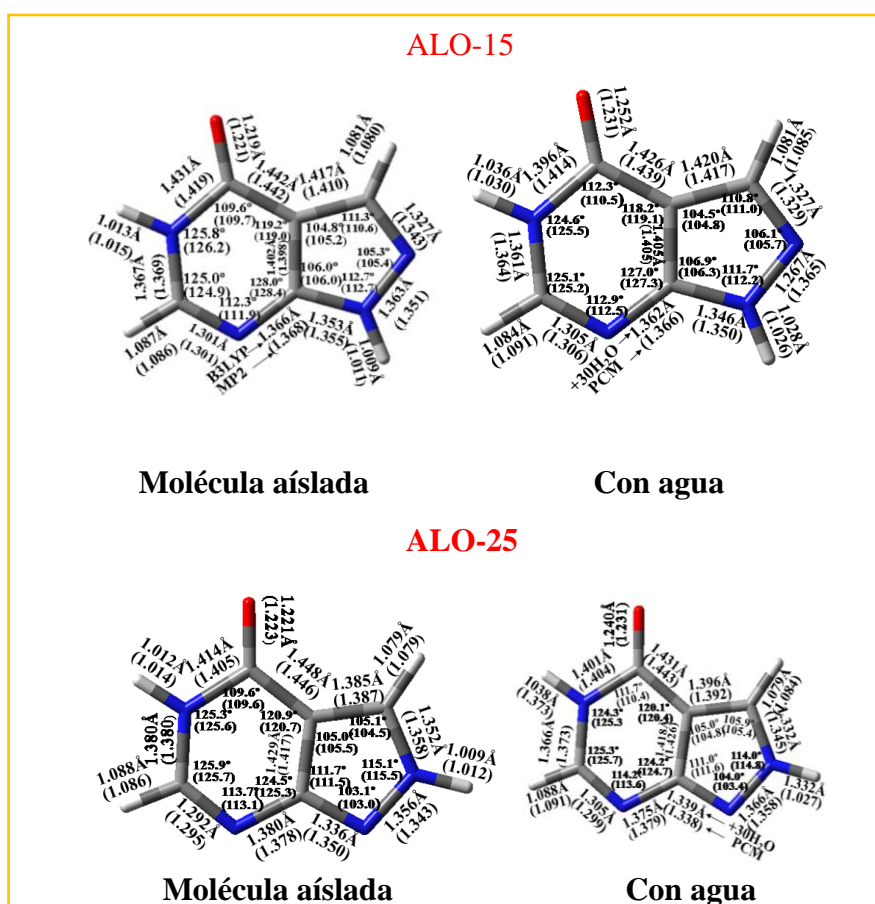


Figura 7.2.13. Comparación entre geometría de las moléculas de ALO-15 y ALO-25 aisladas e hidratadas.

La figura 7.2.13. muestra la geometría de las moléculas de ALO-15 y ALO-25 aisladas e hidratadas (+30 H₂O) en B3LYP y MP2 niveles de cálculo. El estudio de ambas opciones nos conduce a las siguientes conclusiones: las longitudes de enlace en PCM y en la forma hidratada considerada son similares. Se observan algunas variaciones en comparación con la geometría de la molécula aislada (calculada en B3LYP), principalmente en ALO-15. En este tautómero se tienen las diferencias en C4-N5 y N5-H ambas de 0.017 Å y C=O, C3-N2, N2-N1 y N1-H que presentan unas variaciones que

oscilan entre 0.012 y 0.015 Å. Si se considera el nivel de cálculo MP2 solamente se tienen las variaciones de 0.014 Å en los enlaces N2-C3 y N2-N1. En los ángulos no se aprecian cambios significativos.

En el tautómero ALO-25, comparado con la molécula aislada, solamente se tiene un cambio apreciable en el enlace N5-H y N2-H en B3LYP, 0.028 y 0.018 Å respectivamente y en MP2 N2-N1 y N2-H, en ambos casos de 0.015 Å. Lo cual nos indica que el PCM no presenta grandes cambios respecto de las moléculas aisladas y de (H₂O)₃₀. Los mayores cambios en este tautómero, en los dos niveles de cálculo, se dan en el enlace N2-H.

7.2.5. Momento dipolar (μ)

Tabla 7.2.6. Momento dipolar del tautómero de ALO-15 y ALO-25 (Debyes).

Moléculas de agua	ALO-15 B3LYP/6- 31G(d,p)	ALO-15 MP2/6- 31G(d,p)		ALO-25 B3LYP/6- 31G(d,p)	
0	3.633	4.462		0.966	
1	2.705	3.312	D	2.656	D
2	1.906	2.554	D-D	3.939	D-D
3	3.405	4.253	D-D-A	1.977	D-D-B
4	2.698	4.268	D-D-A-A	2.791	D-D-B-B
5	3.063	3.336	A4-B	3.969	B4-D
6	3.695	3.199	A4-B-B	2.387	B4-D-B
7	5.119	6.368	A4-B-B-B	5.449	B4-D-B-A
8	1.421	6.380	B7-D	5.562	A7-D
9	4.674	6.479	B7-D-D	6.076	A7-D-A
10	5.484		B7-D-D-C	7.342	A7-D-A-D
11	4.379		C10-C	5.158	D10-B
12	4.620		C10-C-C	4.825	D10-B-C
15	3.414			2.247	
20	7.756			7.809	
25	4.296			12.210	
30	13.225			10.140	

La Tabla 7.2.6. tiene los valores de μ del tautómero ALO-15 en los niveles de cálculo B3LYP y MP2 desde la molécula aislada hasta el complejo con 30 moléculas de agua (en MP2 solamente se tiene hasta el hidrato con 9 moléculas de agua); solamente se han tenido en cuenta los más estables de cada posición. Si estudiamos los valores en MP2 se tiene el primer valor de la molécula aislada de 4.262 D y los demás valores fluctúan desde 3.312 D en el monohidrato, 4.262 D, en la molécula con 4 moléculas de agua, 3.199 D cuando se han adicionado 6 moléculas de agua y aumenta hasta 6.368 D (H₂O)₆ hasta el mayor valor, 6.479 D en (H₂O)₉.

En el nivel B3LYP los valores no son muy diferentes, en la molécula aislada se tiene 3.633 D, en el monohidrato 2.705 D y el menor valor en $(\text{H}_2\text{O})_2$ 1.906 D. Luego va aumentando hasta $(\text{H}_2\text{O})_7$ cuyo valor es 5.119 D. A partir de $(\text{H}_2\text{O})_8$, que μ vale 1.421 D, fluctúa alrededor de 4.674 D presentando un valor alto en $(\text{H}_2\text{O})_{20}$ y el más elevado en $(\text{H}_2\text{O})_{30}$ 13.225 D. Este último valor es posible por el aumento de la carga en casi todos los átomos respecto de la molécula aislada (figura 7.2.2.).

7.2.6. Estado sólido

El estado sólido se ha representado mediante la figura del heptámero, que tiene la estructura análoga a la obtenida experimentalmente mediante rayos X. La estructura óptima obtenida mediante B3LYP confirma que la forma preferida en el sólido es la forma ceto (figura 7.2.14.), similar al experimental por rayos X^{25c}. Los enlaces de H se dan preferentemente entre los átomos de O y los átomos H y los átomos de N y los átomos de H ($\text{O}\cdots\text{H}_\text{C}$ y $\text{O}\cdots\text{H}_\text{N}$) y $\text{N7}\cdots\text{H}_\text{N}$ y $\text{N2}\cdots\text{H}_\text{N}$.

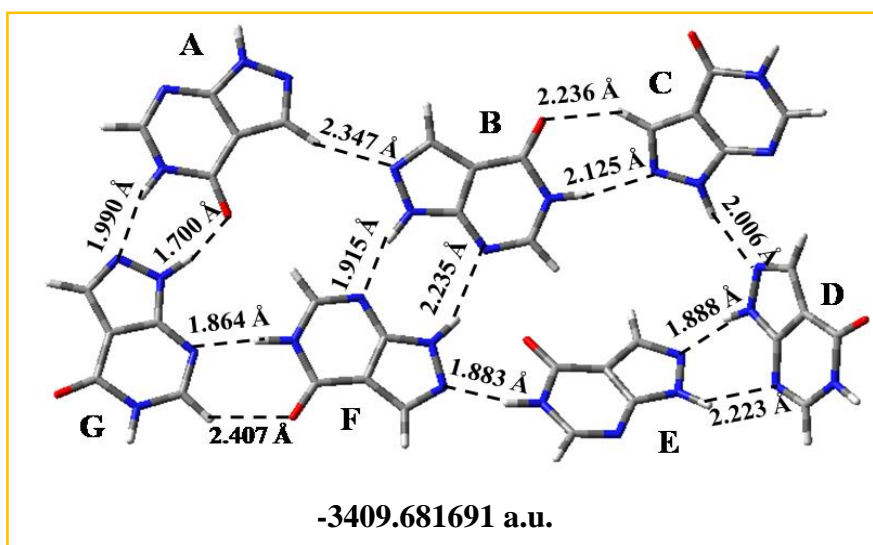


Figura 7.2.14. Estructura del heptámero del tautómero ALO-15.

Los enlaces más fuertes son $\text{O}\cdots\text{H}_{\text{N1}}$ (entre las moléculas A y G), $\text{H}_{\text{N5}}\cdots\text{N7}$ entre las moléculas (F y G), $\text{H}_{\text{N5}}\cdots\text{N2}$ (entre las moléculas E y F y D y E). La estructura experimental muestra que el sólido se encuentra en forma de apilamientos de láminas paralelas de las bases unidas mediante enlaces de H recubriendo parcialmente los anillos.

Tabla 7.2.7. Longitudes de enlace de ALO-15 sólido y valores experimentales.

Longitudes de enlace	Heptámero							Experimental ^{25c}
	Molécula	A	B	C	D	E	F	
N1-N2	1.366	1.366	1.366	1.359	1.366	1.363	1.361	1.374
N2-C3	1.325	1.331	1.331	1.330	1.328	1.327	1.330	1.325
C3-C9	1.423	1.416	1.415	1.416	1.411	1.411	1.416	1.414
C4-N5	1.409	1.426	1.433	1.435	1.413	1.416	1.436	1.399
N5-C6	1.368	1.363	1.365	1.363	1.361	1.357	1.361	1.364
C6-N7	1.302	1.307	1.301	1.303	1.307	1.309	1.305	1.292
N7-C8	1.362	1.368	1.370	1.373	1.364	1.368	1.366	1.379
C8-C9	1.406	1.402	1.403	1.404	1.404	1.405	1.402	1.395
C8-N1	1.350	1.348	1.349	1.346	1.353	1.351	1.366	1.388
C4-O	1.244	1.224	1.219	1.217	1.228	1.228	1.217	1.228
C9-C4	1.430	1.440	1.443	1.442	1.440	1.441	1.440	1.395

Tabla 7.2.8. Ángulos (°) de ALO-15 sólido y valores experimentales.

Ángulos	Heptámero							Experimental ^{25c}
	Molécula	A	B	C	D	E	F	
N1-N2-C3	106.8	105.9	106.1	106.3	106.2	106.6	106.3	106.4
N2-C3-C9	110.6	106.9	110.6	110.5	110.7	110.6	110.6	110.4
C3-C9-C8	105.1	105.1	104.8	104.7	105.2	104.7	104.4	104.6
C9-C8-N1	106.9	106.8	106.8	106.8	106.3	107.0	107.2	107.8
C9-C4-N5	111.6	110.4	109.6	109.6	110.8	110.5	109.8	111.1
C4-N5-C6	124.5	125.0	125.8	126.0	124.6	125.4	126.3	125.6
N5-C6-N7	125.5	125.5	125.1	124.8	126.0	125.0	123.8	125.0
C6-N7-C8	112.4	112.5	112.5	112.5	112.2	113.3	113.7	112.4
N7-C8-C9	127.7	127.3	127.6	127.7	127.1	126.3	127.3	127.3
C8-N1-N2	112.6	110.9	111.6	111.7	112.2	111.1	111.5	110.7
C8-C9-C4	118.4	119.3	119.4	119.2	119.2	119.5	119.1	118.6

En la Tabla 7.2.7. se tienen las longitudes de enlace de cada molécula del heptámero y los valores experimentales. Los valores no difieren respecto de los experimentales, las diferencias más significativas aparecen en: C9-C4, C8-N1, C4-N5 y N7-C8 que presentan una variación de 0.048 Å a 0.017 Å. La Tabla 7.2.8. presenta los valores de los ángulos, observándose pocas diferencias con los valores experimentales.

7.2.7. Conclusiones

1. El tautómero más estable del alopurinol, entre los catorce posibles, es ALO-15 y es también el de mayor población 99%. El siguiente es el tautómero ALO-25, con una población mucho menor, cerca del 1%.
2. Al comparar la longitud de enlace C=O del tautómero calculado con el experimental se observa que es mucho mayor la primera, principalmente en las formas enol, lo cual origina un acortamiento de los enlaces C4-N5 y C9-N4.
3. Algunas longitudes de enlace de los tautómeros ceto se parecen más a las experimentales que las enol y los ángulos calculados no difieren de los experimentales.

4. En la molécula aislada las cargas negativas más altas están en los átomos de O y N5 para todos los niveles de cálculo. En la forma hidratada con 30 moléculas de agua y en el PCM, el átomo de O tiene la carga mayor que en la forma aislada.
5. Los tautómeros ALO-15 y ALO-25 tienen cuatro posiciones posibles (A, B, C y D) dónde se colocan las moléculas de agua para formar los monohidratos.
6. En los dos tautómeros citados la posición D es la de menor energía. El monohidrato de ALO-15, en el nivel de cálculo MP2, presenta las diferencias mayores de las longitudes de enlace respecto de la molécula aislada. Es de destacar el gran incremento del enlace H-C6.
7. En el tautómero ALO-25, en el nivel de cálculo B3LYP, las diferencias respecto de la molécula aislada son menores. En ambos tautómeros el valor máximo de la corrección-CP de energía está en la posición D para todos los niveles de cálculo, siendo éste el hidrato más estable.
8. Los enlaces de H entre la molécula de agua y la base son mayores en la posición D, lo cual contribuye a su estabilidad. Los restantes hidratos con 2, 3,hasta 9 moléculas de agua se han calculado en MP2 y presentan una variación análoga a la que presenta el monohidrato.
9. En B3LYP cuando se llega a las 12 moléculas de agua adicionadas se tiene la primera esfera de hidratación completa.
10. Todos estos hidratos de ALO-15 presentan una estructura similar. En general las longitudes de enlace aumentan al aumentar la hidratación pero a partir de la molécula 15 la estructura se estabiliza y las longitudes de enlace no varían. Los hidratos del tautómero ALO-25, (solamente en el nivel de cálculo B3LYP) tiene una variación análoga a la de ALO-15.
11. El estado sólido se simuló mediante la estructura del heptámero de ALO-15 que presenta unos valores geométricos similares al sólido experimental. Los enlaces de H: $O \cdots H_{N1}$, $N7 \cdots H_{N5}$ y $N2 \cdots H_{N7}$ son los más fuertes.

7.2.8. Referencias

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7.3. Inosina

La inosina (INO) y sus derivados son nucleósidos derivados de la hipoxantina. La inosina se tiene cuando la hipoxantina se une a un anillo de ribofuranosa mediante un enlace β -N₉-glicosídico. Esta estructura indica que son posibles varias formas tautoméricas. Según IUPAC se nombra 9-[(2R, 3S, 4R, 5R)-3,4-dihidroxy-5-((hidroxymethyl)oxolan-2-yl)-6,9-dihydro-3H-purin-6-one. La estructura molecular viene indicada en la figura 7.3.1. Al compararla con la guanosina se observa que carece del grupo NH₂ en la posición C2. Es un nucleósido de considerable interés pues es uno de los componentes de los ácidos nucleicos¹. INO puede obtenerse por desaminación de la A a partir de RNA². Biológicamente se encuentra en el tDNA y es un producto que proviene de éste, el cual debe repararse para su mantenimiento³. Actúa como BDZ-receptor.

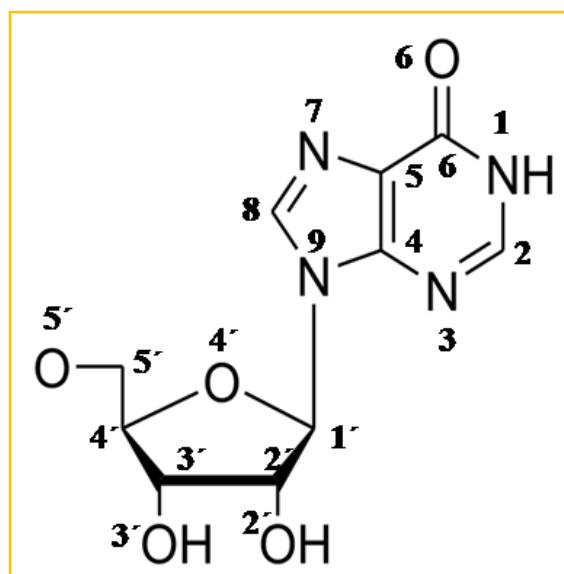


Figura 7.3.1. Estructura molecular de la inosina^{4b}.

Mediante enlaces de H débiles con las cuatro bases naturales del DNA, INO es útil para detectar y analizar las hebras del DNA que presentan ambigüedades⁵. Uno de sus derivados el 2'-deoxiinosina (2-dI), incrementa la actividad antitumoral del 5-fluorouracilo (5-FU) cuando se combina con éste compuesto en determinados tratamientos médicos. Con el desarrollo de la química de los nucleósidos se sintetizaron un número de derivados de INO con propiedades neuroprotectoras⁴ para el tratamiento de los daños de la columna vertebral⁶ en los accidentes, o mejorando algunas actividades motoras⁷. INO tiene importantes aplicaciones en la glicolisis y contracción durante la isquemia en los ataques

del corazón y ha sido muy útil al mejorar el funcionamiento del corazón y preservando la isquemia de ATP⁸⁻¹⁰.

Actualmente el isomerismo estructural de los componentes del DNA tiene especial interés habiéndose realizado muchos estudios teóricos^{5,11-14}. El espectro vibracional Raman indica que INO y los metilados análogos se dan preferentemente en la forma ceto, en disolución acuosa neutra¹⁵. La forma enol de INO es bastante estable y puede formar pares de base de Watson - Crick con la uridina¹⁶. El espectro IR también indica que la forma ceto es la predominante¹⁶.

Pueden aumentar las mutaciones espontáneas a partir del "misparing" debido a la formación de tautómeros raros, siendo diferente que los residuos ácidos de INO puedan formar pares de bases con residuos ácidos uridílicos sin romper la normal relación entre los enlaces glicosídicos¹⁶.

El espectro Raman de INO en ²H₂O indica que la desprotonación de INO tiene lugar en N1 en las disoluciones básicas, y la protonación en las disoluciones ácidas tiene lugar en N3 y N7, de manera análoga que en el nucleótido INO-5'-P; estos resultados son útiles para la identificación y caracterización de las estructuras de los biopolímeros naturales¹⁷.

En este trabajo se ha realizado el estudio de los tautómeros de INO centrándonos en los conformeros posibles y principalmente en el más estable, dejando para futuros trabajos los restantes. Los resultados se han comparado con G. Así se analizaron las estructuras, estabilidad e interacciones intramoleculares de sus conformeros. Es importante el conocimiento de conformeros de los nucleósidos naturales, diferentes autores los han analizado¹⁸⁻²³. Además, INO actúa con posibilidades de apareamiento universales en las secuencias de t-RNA²⁴.

Se han sintetizado nucleósidos análogos y estudiadas todas sus características y aplicaciones en medicina²⁵ y diferentes autores han realizado un análisis de nucleósidos naturales análogos²⁶⁻³⁰. Ello nos ha motivado para estudiar las características de este compuesto.

En primer lugar se obtuvieron cinco tautómeros de INO: N1, N3, N7, OHC y OHT según la posición del átomo de H en las posiciones 1, 3, 7, enol (*cis*) y enol (*trans*). Los respectivos valores de la energía + ZPE, en los niveles de cálculo MP2 y B3LYP, indican que el tautómero N1 es el de menor energía, y el más abundante (Tabla 7.3.1.). Por ello hemos centrado este estudio en este tautómero, dejando para siguientes estudios las demás moléculas.

Tabla 7.3.2. Longitudes de enlace (Å), correspondientes a la parte de la molécula HX, de los tautómeros de INO en B3LYP/6-31G(d,p).

Tautómero	N1	N3	N7	OHtrans	OHcis
N(1) – C(2)	1.358	1.286	1.317	1.341	1.346
C(2) – N(3)	1.305	1.382	1.366	1.340	1.333
N(3) – C(4)	1.369	1.372	1.337	1.342	1.337
C(4) – C(5)	1.395	1.382	1.383	1.400	1.407
C(5) – C(6)	1.446	1.464	1.454	1.403	1.403
C(6) – N(1)	1.441	1.436	1.396	1.331	1.334
C(6) – O(6)	1.215	1.217	1.230	1.339	1.340
C(5) – N(7)	1.373	1.384	1.382	1.381	1.385
N(7) – C(8)	1.313	1.307	1.336	1.313	1.310
C(8) – N(9)	1.384	1.394	1.349	1.386	1.387
N(9) – C(4)	1.374	1.369	1.400	1.378	1.379
N(9) – C(1')	1.468	1.459	1.471	1.465	1.450

Tabla 7.3.3. Ángulos de enlace (°), correspondientes a la parte de la molécula HX, de los tautómeros de INO en el nivel de cálculo B3LYP/6-31G(d,p).

Tautómero	N1	N3	N7	OHtrans	OHcis
N(1) – C(2) – N(3)	124.1	126.2	130.1	128.1	127.8
C(2) – N(3) – C(4)	112.9	115.0	110.3	112.3	111.9
N(3) – C(4) – C(5)	127.9	123.3	125.6	125.5	127.0
C(4) – C(5) – C(6)	119.4	119.8	121.2	115.9	114.5
C(5) – C(6) – N(1)	109.0	112.7	111.7	120.0	120.3
C(6) – N(1) – C(2)	126.7	122.0	121.0	118.2	118.5
C(5) – C(6) – O(6)	131.2	125.8	122.8	122.5	121.4
N(1) – C(6) – O(6)	119.8	120.6	125.4	117.5	118.4
C(6) – C(5) – N(7)	129.8	130.8	132.0	132.6	134.4
C(4) – C(5) – N(7)	110.8	109.3	106.8	111.4	111.1
C(5) – N(7) – C(8)	104.5	105.4	109.6	103.8	104.0
N(7) – C(8) – N(9)	113.4	112.9	108.6	114.0	114.1
C(8) – N(9) – C(4)	105.6	104.8	108.4	105.8	105.9
C(8) – N(9) – C(1')	127.0	128.8	125.6	127.0	127.3
N(3) – C(4) – N(9)	126.4	129.2	127.9	129.4	128.0
N(9) – C(4) – C(5)	105.7	107.5	106.5	105.1	105.0
C(4) – N(9) – C(1')	127.2	126.3	125.8	126.9	126.8

Tabla 7.3.1. Población de los tautómeros de INO a 273.15 K.

Tautómero	N1	N3	N7	OHtrans	OHcis
%	99.8	$2.5 \cdot 10^{-15}$	$1.4 \cdot 10^{-12}$	0.4	$1.3 \cdot 10^{-4}$

En las Tablas 7.3.2. y 7.3.3. se comparan las estructuras geométricas, correspondientes a la parte de la molécula HX, de los cinco tautómeros en el nivel de cálculo B3LYP/6-31G(d,p).

Se observa que las diferencias de las longitudes de enlace en los distintos tautómeros no siguen ninguna regla especial. En general, las diferencias entre los enol-tautómeros

son menores que las que se dan entre las formas ceto, que presentan diferencias del orden de 0.077 (Å) entre los enlaces C2-N3 de los tautómeros N1 y N3, 0.061 (Å) en este mismo enlace en los tautómeros N1 y N7, 0.032 0.072 (Å) en los enlaces N1-C2 entre N1/N3 y N3/N7 respectivamente y 0.045 (Å) en el enlace C8-N9 entre los tautómeros N3/N7 y también 0.045 (Å) en el enlace C6-N1 entre los tautómeros N1/N7. Posiblemente la presencia del H sobre N1 y, en su caso, N3 da lugar a las variaciones en las longitudes de enlace próximas.

Tabla 7.3.4. Longitudes de enlace (Å), correspondientes a la parte de la molécula de azúcar de los tautómeros de INO en B3LYP/6-31G(d,p).

Tautómero	N1	N3	N7	OHtrans	OHcis
C(1')-C(2')	1.545	1.539	1.545	1.544	1.541
C(2')-C(3')	1.536	1.538	1.538	1.537	1.540
C(3')-C(4')	1.536	1.536	1.538	1.538	1.536
C(4')-O(4')	1.459	1.449	1.466	1.461	1.442
C(4')-C(5')	1.524	1.521	1.523	1.524	1.521
C(5')-O(5')	1.419	1.422	1.419	1.419	1.428
C(2')-O(2')	1.404	1.433	1.396	1.402	1.422
C(3')-O(3')	1.417	1.415	1.416	1.417	1.415
O(4')-C(1')	1.409	1.403	1.406	1.410	1.417

Tabla 7.3.5. Ángulos de enlace (°), correspondientes a la parte de la molécula de azúcar de los tautómeros de INO en B3LYP/6-31G(d,p).

Tautómero	N1	N3	N7	OHtrans	OHcis
C(1')-C(2')-C(3')	101.4	101.8	99.6	100.1	102.2
C(2')-C(3')-C(4')	103.5	102.0	103.8	103.6	102.4
C(3')-C(4')-O(4')	106.1	105.9	106.0	106.1	106.3
C(4')-O(4')-C(1')	109.8	111.0	108.9	109.2	111.2
O(4')-C(4')-C(5')	105.8	109.6	107.4	107.1	109.6
C(1')-C(2')-O(2')	114.8	109.2	114.8	115.0	109.4
O(2')-C(2')-C(3')	109.4	112.9	109.9	109.7	112.4
O(3')-C(3')-C(4')	108.5	108.5	108.3	108.4	108.9
C(3')-C(4')-C(5')	115.6	114.9	115.7	115.6	114.9
C(4')-C(5')-O(5')	112.3	108.6	112.5	112.2	109.1
C(8)-N(9)-C(1')	128.1	128.8	125.6	127.0	127.3
N(9)-C(1')-O(4')	108.4	109.2	107.4	107.5	109.1
C(2')-C(3')-O(3')	109.8	110.1	109.5	109.7	109.7
O(4')-C(4')-C(5')	107.3	109.6	107.4	107.1	109.6

Las Tablas 7.3.4. y 7.3.5. muestran las longitudes de enlace y los ángulos de la parte de azúcar de la molécula de INO, y en los cinco tautómeros. No se observan diferencias grandes en los enlaces correspondientes a cada tautómero. La mayor variación se tiene en el enlace C2'-O2' que presenta una diferencia de 0.037 Å, entre N3 y N7, y de 0.029 Å entre N1 y N3. También en las formas enol se observa una variación de 0.020 Å en este

enlace, y en C4'-O4' entre N3 y N7 se tienen una diferencia de 0.017 Å y de 0.019 Å entre las dos formas enol. Se observa que los que varían más son los enlaces entre C y O. Los ángulos varían poco, el que presenta mayores diferencias en todos los tautómeros es C1'-C2'-O2' y O2'-C2'-C3' que en N3 y N7 y entre las dos formas enol presentan diferencias del orden de 0.017 y 0.019 Å. Como se observa estos ángulos son adyacentes a los enlaces que presentan mayores diferencias.

7.3.1. Confórmeros de la inosina

Es importante un conocimiento de las propiedades conformacionales de los nucleósidos, y por ello diferentes autores han estudiado determinados nucleósidos naturales¹⁹⁻²². Nuestro trabajo se centró en el estudio conformacional del tautómero N1, que se realizó en varios niveles de cálculo, y 3D PES por rotación de los ángulos exocíclicos y endocíclicos χ , γ , β , ε y ε' (ver 4.3) en pasos de 60°. Se obtuvieron 69 óptimos confórmeros estables que se clasificaron según los valores del ángulo χ : A (*high-anti*) (los más estables) $\chi \sim 170^\circ$, B (*syn*) $\chi \sim 70^\circ$ y C (*anti*) $\chi \sim 120^\circ$.

Los cálculos se han realizado de la misma forma a lo realizado en nucleósidos análogos³²⁻³⁵. Se obtuvieron los valores: χ , β , γ , δ , ε , ε' , ν_0 , ν_1 , ν_2 , ν_3 , ν_4 , P , S , ν_{\max}^c , μ , ΔE y ΔG . La notación empleada es la indicada en la referencia 36. Los resultados obtenidos presentan los valores de $\Delta E + ZPE$ y ΔG más favorables en los confórmeros A: A1 es el de menor energía en todos los niveles de cálculo, solamente en *el single point en MP2 aparece como menor energía el confórmero B1*.

La energía considerada en cada confórmero fué $\Delta E + ZPE$ y la energía de Gibbs ΔG . La Tabla 7.3.6. muestra todos los valores que determinan cada confórmero. Se tiene así la columna 1 que muestra cada confórmero A, B y C, según orden ya indicado. Las columnas 2 - 7 dan los valores correspondientes a los ángulos exocíclicos, y las columnas 8 - 12 a los ángulos endocíclicos. Las columnas 13 - 16 calculan el ángulo P , S , ν_{\max} y el momento dipolar μ y las dos últimas ΔE y ΔG .

Los confórmeros generalmente difieren poco en los valores de la energía. Así los 69 confórmeros optimizados se encuentran en un rango de energía $\Delta E = 0-10.7$ kcal/mol y la energía de Gibbs $\Delta G = 0-9.6$ kcal/mol en B3LYP/6-31G(d,p) algo mayor que en T²⁰. (Ver publicación n° 1).

En la Tabla 7.3.6. se tienen los 7 confórmeros más estables de N1, ordenados según los valores de ΔE , en un rango entre 0 y 2.155 kcal/mol, cuyos valores más bajos corresponden a A1, B1 y B2. Ningún confórmero en C se presenta entre estos rangos.

Tabla 7.3.6. Los 7 conformeros de menor energía, ΔE y ΔG , en los niveles de cálculo cálculo B3LYP/6-31G(d,p); B3LYP/6-311++G(3df,pd) (en negrita); O3LYP/6-31G(d,p) (entre corchetes); B3LYP/cc-pVDZ (entre paréntesis); B3PW91/6-31G(d,p) (entre comillas) y B972/6-31G(d,p) (en cursiva). Los ángulos torsionales endocíclicos y exocíclicos en grados, el ángulo pseudorrotacional P en grados y los incrementos de energía en kcal/mol.

Conf.	χ	β	γ	δ	ϵ	ϵ'	ν_0	ν_1	ν_2	ν_3	ν_4	P^a	S^b	ν_{\max}^c	μ	ΔE	ΔG
A1	165.0	70.8	55.1	136.9	152.4	60.1	-29.6	37.5	-30.9	15.0	9.1	147.2	2E	36.7	3.852	0.001	
	167.4	65.0	57.1	145.3	150.7	59.4	-25.9	37.4	-34.1	20.5	3.3	156.3		37.2	3.859	0 ^d	0 ^e
	[167.3]	[66.2]	[56.4]	[142.1]	[151.8]	[58.6]	[-27.4]	[37.9]	[-33.5]	[19.2]	[5.1]	[153.6]		[37.4]	[3.804]	[0] ^f	[0] ^h
	(167.9)	(66.0)	(57.3)	(146.1)	(149.3)	(58.7)	(-25.0)	(37.2)	(-34.6)	(21.6)	(2.0)	(158.2)		(37.3)	(3.725)	(0) ⁱ	(0) ^j
<i>166.0</i>	<i>64.2</i>	<i>57.2</i>	<i>144.7</i>	<i>150.0</i>	<i>59.3</i>	<i>-27.7</i>	<i>38.5</i>	<i>-34.2</i>	<i>19.7</i>	<i>4.9</i>	<i>154.0</i>	<i>38.1</i>	<i>3.856</i>	<i>0</i> ^k	<i>0</i> ^l		
166.9	65.7	56.9	144.4	151.1	59.7	-26.3	37.5	-34.0	20.3	3.6	155.8	37.3	3.917	0 ^m	0 ⁿ		
A2	175.6	171.6	50.3	144.1	149.8	55.3	-18.0	32.1	-33.0	23.9	-3.8	167.6	3T	33.8	4.307	0.002	
	178.1	172.9	51.8	149.5	146.8	55.0	-13.5	29.9	-33.8	27.4	-8.9	176.1		33.9	3.908	1.135	0.784
	[179.9]	[173.4]	[52.1]	[146.8]	[147.6]	[53.0]	[-14.8]	[30.6]	[-33.5]	[26.5]	[-7.5]	[173.7]		[33.7]	[3.932]	[1.203]	[1.190]
	(178.0)	(172.7)	(52.3)	(150.6)	(145.4)	(55.1)	(-12.2)	(29.5)	(-34.2)	(28.6)	(-10.4)	(178.5)		(34.2)	(3.812)	(0.895)	(0.662)
<i>177.3</i>	<i>173.1</i>	<i>51.1</i>	<i>148.9</i>	<i>146.3</i>	<i>54.3</i>	<i>-16.1</i>	<i>31.9</i>	<i>-34.4</i>	<i>26.7</i>	<i>-6.9</i>	<i>172.3</i>	<i>34.7</i>	<i>3.947</i>	<i>1.237</i>	<i>0.925</i>		
178.7	172.8	52.0	148.9	147.2	54.5	-13.7	30.1	-33.8	27.4	-8.8	175.8	33.9	3.996	1.118	0.873		
A3	168.0	-57.6	177.1	140.4	152.9	57.0	-27.8	37.6	-32.8	17.9	6.1	152.0	2E	37.1	5.365	0.003	
	169.1	-54.8	174.2	146.7	151.2	57.3	-24.7	37.2	-35.0	22.1	1.5	159.3		37.4	5.422	1.466	1.120
	[171.4]	[-57.7]	[177.2]	[144.1]	[151.8]	[54.9]	[-25.4]	[37.3]	[-34.3]	[21.2]	[2.5]	[157.4]		[37.2]	[5.195]	[1.200]	[1.086]
	(170.5)	(-53.5)	(174.7)	(146.3)	(149.4)	(55.8)	(-24.7)	(37.1)	(-34.8)	(21.9)	(1.6)	(158.8)		(37.3)	(5.143)	(1.568)	
<i>167.9</i>	<i>-54.9</i>	<i>173.9</i>	<i>147.1</i>	<i>150.4</i>	<i>57.6</i>	<i>-25.3</i>	<i>37.9</i>	<i>-35.4</i>	<i>22.3</i>	<i>1.8</i>	<i>158.6</i>	<i>38.0</i>	<i>5.442</i>	<i>1.365</i>	<i>0.274</i>		
168.8	-56.1	175.1	147.0	151.6	57.8	-24.0	37.0	-35.2	22.8	0.6	160.3	-37.4	5.471	1.354	0.984 ^o	1.003	
A4	170.9	-70.1	-67.4	145.0	152.8	56.3	-22.8	35.5	-34.0	22.1	0.3	160.8	2E	36.0	3.880	0.004	
	172.6	-67.9	-65.5	149.3	150.6	56.0	-20.5	35.0	-35.3	24.9	-2.9	165.7		36.4	3.821	2.027	1.689
	[174.6]	[-67.5]	[-66.0]	[147.5]	[151.4]	[54.3]	[-20.6]	[34.9]	[-34.9]	[24.6]	[-2.7]	[165.3]		[36.1]	[3.852]	[1.610]	[1.404]
	(172.3)	(-65.2)	(-64.0)	(148.7)	(148.9)	(55.9)	(-20.6)	(35.1)	(-35.2)	(24.9)	(-2.8)	(165.5)		(36.4)	(3.777)	(2.046)	(1.805)
<i>171.8</i>	<i>-67.9</i>	<i>-65.5</i>	<i>149.8</i>	<i>149.8</i>	<i>56.2</i>	<i>-20.7</i>	<i>35.4</i>	<i>-35.7</i>	<i>25.4</i>	<i>-3.1</i>	<i>165.9</i>	<i>36.8</i>	<i>3.818</i>	<i>1.923</i>	<i>1.609</i>		
172.8	-68.2	-65.7	149.8	151.0	56.3	-19.4	34.5	-35.5	25.8	-4.2	167.7	36.3	3.910	1.802	1.514		
A5	175.8	171.1	50.3	144.1	149.7	55.0	-17.9	32.0	-33.0	23.9	-3.9	167.7	2E	33.8	4.282	0	
	177.1	-88.4	49.8	148.0	147.7	52.8	-18.9	33.8	-34.9	25.4	-4.3	167.9		35.7	5.516	2.105	1.941
	[179.7]	[-86.9]	[51.8]	[145.9]	[148.2]	[50.2]	[-19.0]	[33.6]	[-34.4]	[25.0]	[-3.9]	[161.2]		[35.3]	[5.505]	[2.016]	[2.014]
	(176.1)	(-87.1)	(49.3)	(148.1)	(146.8)	(53.2)	(-19.1)	(34.1)	(-35.3)	(25.8)	(-4.4)	(168.0)		(36.1)	(5.337)	(1.860)	(1.790)
<i>176.4</i>	<i>-87.5</i>	<i>49.8</i>	<i>148.0</i>	<i>147.1</i>	<i>52.4</i>	<i>-20.3</i>	<i>34.9</i>	<i>-35.3</i>	<i>25.2</i>	<i>-3.3</i>	<i>166.2</i>	<i>36.4</i>	<i>5.537</i>	<i>2.152</i>	<i>2.050</i>		
178.1	-88.4	51.1	147.8	147.9	52.1	-18.2	33.4	-34.8	25.8	-4.9	169.0	35.5	5.621	2.111	1.979		
B1	56.1	63.4	48.7	145.0	153.4	141.3	-21.3	34.4	-33.8	22.5	-1.0	162.8	2E	35.4	4.816	0	
	54.1	66.9	48.2	160.3	149.2	151.5	-21.9	35.8	-35.4	23.9	-1.5	163.5		36.9	4.578	0.212	0.705
	[53.8]	[65.5]	[49.2]	[146.4]	[149.8]	[151.4]	[-22.0]	[35.5]	[-34.7]	[23.4]	[-1.1]	[162.8]		[36.3]	[4.572]	[0.452]	[1.096]
	(53.8)	(67.4)	(47.4)	(149.3)	(147.6)	(152.9)	(-20.2)	(34.9)	(-35.6)	(25.1)	(-3.4)	(166.4)		(36.6)	(4.408)	(0.064)	(0.577)

	54.2	66.1	48.6	148.7	148.4	151.6	-22.4	36.3	-35.5	23.9	-1.2	162.9	37.1	4.611	0.338	0.857
	54.1	65.9	48.8	147.7	149.4	150.6	-22.5	36.2	-35.2	23.5	-0.9	162.4	36.9	4.670	0.242	0.747
B2	56.6	62.4	47.6	146.5	-89.1	-86.4	-20.8	32.9	-31.9	21.1	-0.2	161.7	33.6	7.059	0	
	54.9	65.8	46.6	148.5	-85.5	-86.0	-21.3	33.8	-33.0	22.0	-0.6	162.3	34.6	6.709	0.244	0.585
	[54.4]	[64.2]	[47.7]	[147.0]	[-90.6]	[-86.2]	[-21.5]	[33.5]	[-32.1]	[21.2]	[0.0]	[161.2]	[34.8]	[6.697]	[0.403]	[0.870]
	(54.6)	(66.1)	(45.9)	(150.7)	(-82.3)	(-85.4)	(-19.8)	(33.3)	(-33.5)	(23.4)	(-2.4)	(165.2)	(34.7)	(6.425)	(0.031)	(0.459)
	54.8	64.9	46.9	149.3	-86.9	-86.1	-21.9	34.3	-33.0	21.8	0.0	161.3	34.8	6.795	0.373	0.729
	54.8	64.8	47.1	148.6	-86.3	-85.8	-21.9	34.3	-32.9	21.7	0.0	161.1+	33.9	6.832	0.276	0.628

^aDefinición: $tgP = \frac{(v_4 + v_1) - (v_3 + v_0)}{2 v_2 (\sin(36) + \sin(72))}$

Cuando v_2 is negativo se adicionan 180° al valor calculado de P. ref. [42d].

^bNotacion empleada ref. [44]., ^c $v_{max} = \frac{v_2}{\cos P}$, ^d $\Delta E = 0 = -983.184779$ a.u. en el nivel B3LYP/6-31G(d, p), ^e $\Delta G = 0 = -983.228991$ a.u., ^f $\Delta E = 0 = -983.766372$ a.u. en el nivel

B3LYP/6-311++G(3df, pd); ^g $\Delta E = 0 = -983.852341$ a.u. en el nivel O3LYP-31G(d,p); ^h $\Delta G = 0 = -982.896698$ a.u.; ⁱ $\Delta E = 0 = -983.234338$ a.u. en el nivel B3LYP/6/cc-pVDZ ; ^j $\Delta G = 0 = -983.278235$ a.u.; ^k $\Delta E = 0 = -982.819307$ a.u. en el nivel B3PW91/6-31G(d,p); ^l $\Delta G = 0 = -982.863160$ a.u.; ^m $\Delta E = 0 = -982.850820$ a.u. en el nivel B972/6-31G(d,p); ⁿ $\Delta G = 0 = -982.894695$ a.u.

7.3.2. Anillo de la hipoxantina en la inosina.

La molécula de INO está formada por HX unida mediante el enlace glicosídico N9-C1' con la furanosa. La parte de HX presenta una muy pequeña no planaridad. Al analizar los seis conformeros más estables, se observa una no-planaridad muy baja de la base heterocíclica, con un ángulo torsional menor que 1°. Entre los 11 ángulos diedros que describen la no-planaridad de la base se tienen: C5-C6-N1-C2, C6-N1-C2=N3, N1-C2=N3-C4, C2=N3-C4=C5, N3-C4=C5-C6, C4=C5-C6-N1, N9-C8=N7-C5, C8=N7-C5-C4, N7-C5=C4-N9 y C5=C4-N9-C8. Las tres primeras son las que más dependen de la conformación del nucleósido. Los valores más altos de las desviaciones son C4-N9-C8=N7 (-0.94°), C2=N3-C4=C5 y N3-C4=C5-C6 (-0.95° y -0.91° en el conformero B) y C4-N9-C8=N7 (0.95°, A3). La correlación entre los ángulos torsionales de la base heterocíclica es de tal forma que su suma algebraica es 0°, con una desviación standard de 0.4° y un valor absoluto no superior a 1.0°.

Tabla 7.3.7. Longitudes de enlace (Å) y ángulos de enlace (°) calculados de la base de purina en la molécula A1 del monómero, dímero, trímero y pentámero de INO (molécula A en los tres casos), y los valores experimentales en el cristal^{37,38}. Entre paréntesis vienen las desviaciones standard. Valores de HX-19 calculados en B3LYP.

Parámetros	Monómero	Dímero	Trímero	Pentámero	Experimental	HX-19 aislada
N(1) – C(2)	1.358	1.358	1.363	1.356	1.355 (.003)	1.365
C(2) – N(3)	1.305	1.305	1.305	1.311	1.308 (.003)	1.303
N(3) – C(4)	1.369	1.369	1.366	1.365	1.365 (.004)	1.362
C(4) – C(5)	1.395	1.396	1.395	1.390	1.374 (.021)	1.396
C(5) – C(6)	1.446	1.446	1.440	1.438	1.433 (.013)	1.447
C(6) – N(1)	1.441	1.440	1.403	1.419	1.397 (.044)	1.436
C(6) – O(6)	1.215	1.215	1.223	1.231	1.233 (.018)	1.217
C(5) – N(7)	1.373	1.373	1.375	1.376	1.371 (.002)	1.378
N(7) – C(8)	1.313	1.312	1.317	1.318	1.307 (.006)	1.311
C(8) – N(9)	1.384	1.385	1.371	1.375	1.372 (.012)	1.379
N(9) – C(4)	1.374	1.375	1.370	1.374	1.372 (.002)	1.371
N(9) – C(1')	1.468	2.643	1.471	1.470	1.477 (.009)	
N(1) – C(2) – N(3)	124.1	124.1	124.1	125.0	124.6 (0.5)	124.6
C(2) – N(3) – C(4)	112.9	113.1	112.7	112.3	111.9 (1.0)	112.0
N(3) – C(4) – C(5)	127.9	127.7	128.1	127.7	128.5 (0.6)	128.9
C(4) – C(5) – C(6)	119.4	119.5	119.1	119.3	118.3 (1.1)	119.1
C(5) – C(6) – N(1)	109.0	109.0	109.4	110.3	111.0 (2.0)	108.9
C(6) – N(1) – C(2)	126.7	126.6	126.5	125.4	125.4 (1.3)	126.6
C(5) – C(6) – O(6)	131.2	131.2	130.6	128.8	128.4 (2.8)	
N(1) – C(6) – O(6)	119.8	119.8	120.0	120.9	120.6 (0.8)	
C(6) – C(5) – N(7)	129.8	129.7	130.6	130.4	130.1 (0.3)	
C(4) – C(5) – N(7)	110.8	110.8	110.2	110.3	111.6 (0.8)	111.0
C(5) – N(7) – C(8)	104.5	104.4	104.7	105.1	103.8 (0.7)	104.5
N(7) – C(8) – N(9)	113.4	113.5	113.0	112.5	113.6 (0.2)	113.0
C(8) – N(9) – C(4)	105.6	105.5	106.2	106.4	105.7 (0.1)	106.6
C(8) – N(9) – C(1')	127.0	133.8	127.5	129.2	128.1 (1.1)	
N(3) – C(4) – N(9)	126.4	126.6	126.1	126.6	126.1 (0.3)	
N(9) – C(4) – C(5)	105.7	105.7	105.7	105.7	105.4 (0.3)	105.0
C(4) – N(9) – C(1')	127.2	94.7	126.3	124.3	126.0 (1.2)	

La Tabla 7.3.7. presenta las longitudes de enlace de la base de purina en el conformero A1 de INO (además la molécula A del dímero, trímero y pentámero, con el que se ha identificado el sólido) comparadas con los valores experimentales obtenidos por rayos-X

en el cristal^{37,38}. Aquí nos ceñiremos al monómero y en los siguientes puntos desarrollaremos los demás valores. Las longitudes de los enlaces simples C-N y C-C en el confórmero A1 son intermedias entre las de los correspondientes enlaces aromáticos y los saturados, lo cual indica cierto carácter aromático en la estructura del anillo. Las mayores diferencias respecto del experimental corresponden a C6–N1 (0,044 Å) y C4–C5 (0,021Å), con una desviación standard de 0.013 Å. Los ángulos presentan similar concordancia. Las mayores diferencias corresponden a C5–C6–O6 (2,8°) y C5–C6–N1 (2,0°), con una desviación standard de 0.9°. Respecto de la molécula HX-19 (en B3LYP) las diferencias del monómero no son significativas.

7.3.3. Anillo de la furanosa en la inosina

Los impedimentos espaciales en la furanosa determinan probablemente cambios en el plegado del azúcar. Debido a la hibridación del átomo C1', la furanosa se sitúa fuera del plano del anillo de HX, con ángulo torsional N9-C1'-C2'-C3' de 155.2° en el confórmero A1. Debido a ello se deforman todos los ángulos vecinos aunque los valores son similares a los encontrados en nucleósidos análogos. El ángulo C4-N9-C8 (105.6° en el confórmero A1) está de acuerdo con el indicado en el cristal, 105.7°. También los ángulos de la furanosa son similares a los del cristal, siendo la mayor desviación de 6.1° que corresponde a C(2')–C(3')–O(3'), y 5.7° correspondiente a O(3')–C(3')–C(4'), y con una desviación standard de 2.078°.

Tabla 7.3.8. Comparación entre las longitudes de enlace (Å) y los ángulos (°) del anillo de la furanosa en la molécula A1 del monómero de la inosina y en el dímero, trímero y pentámero (molécula A), y los valores experimentales en el cristal⁵⁵. En paréntesis vienen las desviaciones standard.

Párametros	Monómero	Dímero	Trímero	Pentámero	Experimental
C(1')–C(2')	1.545	1.548	1.546	1.549	1.530 (.015)
C(2')–C(3')	1.536	1.535	1.535	1.530	1.525 (.011)
C(3')–C(4')	1.536	1.531	1.535	1.530	1.522 (.014)
C(4')–O(4')	1.459	1.461	1.456	1.465	1.459 (.0)
C(4')–C(5')	1.524	1.519	1.526	1.531	1.506 (.018)
C(5')–O(5')	1.419	1.423	1.421	1.414	1.428 (.009)
C(2')–O(2')	1.404	1.408	1.409	1.410	1.420 (.016)
C(3')–O(3')	1.417	1.417	1.416	1.427	1.413 (.004)
O(4')–C(1')	1.409	1.408	1.402	1.406	1.417 (.008)
C(1')–C(2')–C(3')	101.4	101.5	101.1	101.6	100.6 (0.8)
C(2')–C(3')–C(4')	103.5	103.2	102.9	103.2	101.5 (2.0)
C(3')–C(4')–O(4')	106.1	105.5	105.7	104.5	104.0 (2.1)
C(4')–O(4')–C(1')	109.8	111.0	110.5	110.5	109.6 (0.2)
O(4')–C(4')–C(5')	105.8	109.2	109.0	107.8	107.3 (1.5)
C(1')–C(2')–O(2')	114.8	113.8	113.8	113.2	109.6 (4.8)
O(2')–C(2')–C(3')	109.4	109.3	109.9	109.7	107.5 (1.9)
O(3')–C(3')–C(4')	108.5	108.6	108.4	109.2	114.2 (5.7)
C(3')–C(4')–C(5')	115.6	115.9	115.8	115.4	114.1 (1.5)
C(4')–C(5')–O(5')	112.3	109.2	113.0	111.9	112.0 (0.3)
C(8)–N(9)–C(1')	128.1	125.2	127.5	129.6	128.1 (0.0)
N(9)–C(1')–O(4')	108.4	109.1	108.0	110.4	108.4 (0.0)
C(2')–C(3')–O(3')	109.8	109.9	109.9	109.1	115.9 (6.1)
O(4')–C(4')–C(5')	107.3	109.2	109.0	110.1	110.0 (2.7)

Se observa el acortamiento del enlace O4'-C1' (1.409 Å calculado vs 1.417 Å en el cristal), también C2'-O2 (1.404 Å vs 1.420 Å en el cristal) y C5'-O5' (1.419 Å vs 1.428 Å). Algunos enlaces se alargan, el más notable corresponde a C1'-C2' (0.015 Å), siendo la desviación standard 0.011 Å.

La furanosa se caracteriza por tres parámetros estructurales³⁹: (i) Los ángulos de torsión endocíclicos $\nu_0 - \nu_4$, que presentan un gran rango de variabilidad. La suma algebraica de los ángulos diedros ν_i (para $i = 0, 1, \dots, 4$) es 0 para todos los conformeros y cae dentro de un rango de -1.0° a 1.5° , su valor promedio es igual a 0.8° . (ii) El ángulo P, cuyos valores de éste tienen preferentemente una distribución trimodal cubriendo entre los conformeros A, B y C. (iii) El valor de ν_{\max} , en el que se tiene un amplio rango de conformeros A. El valor de ν_{\max} representa el radio del ciclo pseudorrotacional y por ello tiene un impacto alto como parámetro estructural.

7.3.4. Cargas atómicas y enlaces de hidrógeno intramoleculares

En el conformero A1 los átomos O3', O2' y O5' tienen valores de la carga negativa más alta, $-0.8 e$; H2' del grupo OH2' tiene la carga positiva más alta ($0.519 e$), lo cual determina que sea el más reactivo. Los átomos tienen las cargas H3' ($0.502 e$) y H1 en el anillo de HX ($0.451 e$). Los restantes átomos de H tienen una carga mucho menor $0.3 - 0.2 e$, y serán menos reactivos. La carga positiva más alta corresponde al átomo C6 ($0.650 e$), en concordancia con la alta carga negativa sobre el átomo O6. La carga en N7 ($-0.446 e$) determina el enlace con el átomo de Pt en las formas neutras de las drogas de Pt-INO⁴⁰.

Diferentes investigadores han estudiado los enlaces de H intramoleculares en los nucleósidos^{41,42}, pero nosotros hemos considerado la clasificación según Desiraju et al⁴³. Estos enlaces tienen lugar preferentemente entre los H2' del anillo de la furanosa con los átomos N3 de HX, también entre H3' y O2'. Ambos tienen una fortaleza media y dan estabilidad a la molécula.

7.3.5. Estado sólido

Experimentalmente los cristales de INO se han estudiada en estado sólido mediante rayos-X^{37,38}. INO se ha obtenido cristalizado en forma rómbica y monoclinica, Munns³⁷ ha estudiado la forma monoclinica y ha determinado su estructura correspondiente a la forma A1. Nosotros hemos simulado la célula unidad del cristal mediante las formas del

dímero, trímero y pentámero de este confórmero. En el estudio llevado a cabo para la publicación nº1, los valores calculados del ángulo χ están en forma *anti* como se encuentra en la determinación por rayos X^{38} . El pentámero muestra un gran valor del ángulo χ correspondiente a ${}_3E$.

Las Tablas 7.3.7. y 7.3.8. establecen la comparación entre las longitudes de enlace y ángulos del confórmero A1, monómero, dímero, trímero y pentámero y los valores experimentales. Los valores de las longitudes de enlace del anillo de hipoxantina en esta molécula son similares a los valores experimentales en estado sólido. No obstante, el pentámero presenta una mayor concordancia con los valores experimentales. Los enlaces que presentan una diferencia apreciable son: C4–C5, 0,016 Å, C6–N1, 0,022 Å y N7–C8, 0,011 Å. Los ángulos de enlace no presentan diferencias significativas.

En la Tabla 7.3.8. se comparan las longitudes de enlace de la furanosa en el confórmero A1 de las tres formas que simulan el sólido. Los valores estructurales entre las tres formas no varían significativamente, dados los datos que tenemos en cálculos anteriores (publicación nº 1) y lo obtenido en la Tabla 7.3.7. nos centramos en el pentámero al comparar con los valores experimentales, las mayores diferencias son: C4'–C5' (0.025 Å), C1'–C2' (0,019 Å), C5'–O5' (0.014 Å), C3'–O3' (0.014 Å) y O4'–C1' (0,011 Å). Los ángulos tienen la mayor variación (1.3°) en C3'–C4'–C5'. Al analizar las estructuras del dímero, trímero y pentámero (publicación nº 1) se tienen los valores del pentámero más concordantes con los experimentales. En la Tabla 7.3.8. se establece la comparación entre el anillo de furanosa en el pentámero y el experimental, observándose que los valores experimentales de los enlaces presentan una diferencia O4'–C1' (0.011 Å), C2'–O2' y C5'–O5' (0.009 Å), en otros casos hay una diferencia negativa: C'–C2' (0.019 Å). Los ángulos también concuerdan observándose las mayores desviaciones en los ángulos C2'–C3'–O2' (3.2°) y O3'–C3'–C4' (5.0°).

7.3.6. Espectro IR de la inosina

La Tabla 7.3.9. muestra el espectro del confórmero A1 de INO. Las frecuencias que presenta fueron identificadas mediante el programa GaussView. En la siguiente Tabla 7.3.10 se tienen las frecuencias de A1 que pueden compararse con las correspondientes de los tautómeros HX-17. Lógicamente son pocas las frecuencias que pueden compararse con la de este tautómero dado que la estructura de A1 es más próxima a HX-19.

Tabla 7.3.9. Frecuencias IR (cm^{-1}) de HX-19 (experimental)^{44,45} y calculadas del conformero A1 de INO.

Experimental				
HX-19	Intensidad absoluta	Intensidad relativa	Asignación	Calculadas A1
542/534	5/5	10/10	Rings vib.	547
640	3	6	(N1-H)/rings vib. (omp)	651
691	3	6	Rings vib.	672
725	11	12	(N1-H)/ring vib.(omp)	737
860	4	8	(C2-H)/pirimidinic ring vib.(omp)	856
			(C2-H)/ ring vib.	
1234	11	22	Rings/(N9-H)/(C8-H)	1346
1534	9	8	Rings/(C2-H)/(C=O)v.	1543
1555	11	22	vC=O)/(N1-H)/rings	1571/1646
1753	52	100	v(N1-H)	1839
				3604

Tabla 7.3.10. Frecuencias IR (cm^{-1}) de HX-17 (experimental)^{44,45} y calculadas del conformero A1 de INO.

HX-17	Intensidad absoluta	Intensidad relativa	Asignación	Calculadas A1
657	10	9	Rings vib./ (N1-H)(omp)	650
1735	110	100	N(C=O)/ring	1839
3464	35	32	N(N7-H)	3294

7.3.7. Resumen y conclusiones

El resumen y las principales consecuencias de lo expuesto en los párrafos anteriores son las siguientes:

1. Se han identificado cinco tautómeros de INO, obteniendo sus valores optimizados principalmente en los niveles de cálculo MP2 y B3LYP/6-31G/d,p).
2. De éstos, el tautómero N1 es el más estable, obteniéndose por rotación de los ángulos exocíclicos χ , γ , β , ε y ε' 69 conformeros energéticamente estables (B3LYP) de los cuales 3 *anty* y 2 *syn* tienen una energía relativa entre 0-2 kcal/mol y 31 *anty* y 10 *syn* en el rango 2-8 kcal/mol.
3. Generalmente los conformeros A son más estables que los B y C. El conformero A1 es el más estable en el nivel B3LYP, sin embargo en el nivel MP2 es B1.
4. Los conformeros con alta estabilidad de la forma *high-anty* tiene los valores de ε comprendidos entre 149.7° y 151.2° y la forma *syn* entre -85.5° y 149.2°. Los valores de β entre -178.5° y 172.9° ó 65.8° y 66.9°, los valores de χ entre los rangos -172.6° y 167.4°

y 54.1° y 54.9° y, por último ϵ' entre 52.8 y 59.4° y -86.0° y 151.5°. Los valores anteriores indican que se trata de una molécula de alta flexibilidad.

5. Los valores de las longitudes de enlace no presentan grandes diferencias respecto de los valores experimentales y, en el sólido simulado mediante el pentámero, los ángulos torsionales concuerdan con los valores experimentales.
6. El espectro IR calculado del conformero A1 presenta las principales vibraciones correspondientes a los valores experimentales de HX.
7. Los datos calculados en el estado sólido, representado por el pentámero, presentan buena concordancia con los valores experimentales.

7.3.8. Referencias

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7.4. Aciclovir

El aciclovir (ACV), (Zovirax) o 9-2(hidroxi-etoximetil) guanina es un derivado de la guanina, que posee actividad antiviral, y es usado generalmente en el tratamiento del herpes. En la figura 7.4.1. se observa claramente la estructura ACV con la base guanina unida a una cadena que recuerda la estructura de la furanosa. La numeración de la base es análoga a la de HX, por tanto la cadena lateral se une en N9. La cadena lateral se numera según indica la figura.

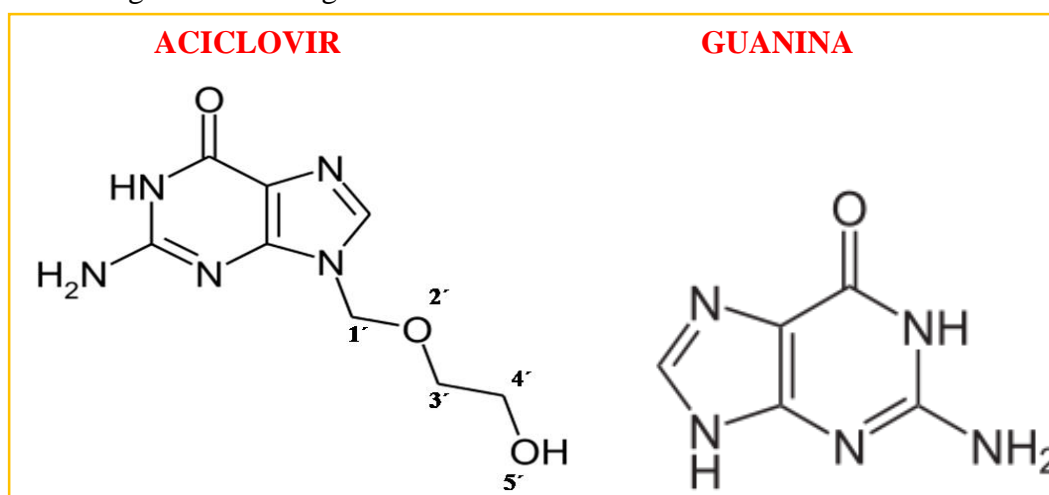


Figura 7.4.1. Estructura de la molécula de aciclovir y de guanina¹.

Se obtiene a partir de la guanina, calentando durante dos días, a reflujo, una guanina parcialmente sililada (introducción del grupo R_3Si en la molécula) con 1,3-dioxano en presencia de un selectivo catalizador de alquilación y hidrolizando el producto resultante^{2a}. Debido a que este método tiene un tiempo de reacción muy largo se usa un método que utiliza microondas en tres pasos^{2b}. Así se mejora el rendimiento y se reduce el tiempo de reacción. También se obtiene a partir de N-Ac-guanina usando fosfato natural, especialmente tratado, como catalizador ácido de Lewis³.

ACV es un potente agente antiviral que se usa principalmente como inhibidor del virus del herpes (HSV) de tipos 1 y 2^{2a,4-7}. Se ha evaluado la actividad contra el virus 1 y 2 del herpes simple de una serie de guaninas modificadas, tricíclicas, análogas del aciclovir (ACV, 1) (y ganciclovir), mostrando similar actividad que ACV y ganciclovir⁸. Se ha encontrado que la actividad contra el herpes depende de la naturaleza y de los efectos estéricos de los sustituyentes en las posiciones 6 y/o 7⁹.

La mayoría de las personas que están infectadas con el virus humano de inmunodeficiencia tipo (HIV-1) también suelen estar infectadas con el herpes simple tipo

2 (HSV-2), el cual está asociado a un aumento de los niveles de HIV-1 genital. Se tiene información de que ACV inhibe la co-infección de HIV sobre el virus de herpes humano (HHV) en los tejidos cultivados. Se encontró que esta actividad estaba relacionada con la fosforilación de la droga a la forma de monofosfato¹⁰. Recientes estudios indican que ACV disminuye el HIV-1 RN y suprime ambos virus en los tejidos coinfectados¹¹. Sobre este tema hay numerosos estudios¹²⁻¹⁵, también sobre el impacto de ACV sobre los pacientes seropositivos HIV y HSV-2^{16,17} y sobre el tratamiento de ACV sobre las infecciones resistentes^{7,18}. También se han realizado pruebas para liberar de drogas antivirales en los tratamientos orales por largos períodos de tiempo¹⁹.

Con lo dicho anteriormente se deduce que ACV se ha estudiado extensamente desde los puntos de vista médicos y farmacéuticos, sin embargo, sobre su estructura molecular hay pocos estudios realizados, y solamente estudios de bajo nivel computacional²⁰ y, según hemos encontrado en la bibliografía, no se tienen datos sobre su conformación (DFT) o mediante los métodos ab initio de la química cuántica. Por ello en este trabajo nos hemos propuesto desarrollar este su estudio estructural. Se tienen datos sobre las conformaciones de algunos derivados de ACV con actividad biológica²¹ y en complejos de ACV con varios metales^{22,23}.

El estudio desde el punto de vista tautomérico de los componentes estructurales del DNA tiene un gran interés actualmente y se tienen numerosas publicaciones sobre este tema²⁴⁻²⁸. El equilibrio de los tautómeros de ACV entre la forma *ceto* y la forma *enol* se ha estudiado mediante los espectros UV/VIS²⁹. Este equilibrio depende de la polaridad del disolvente y, por tanto, en disolución acuosa prevalece la forma *ceto* mientras que en cloruro de metileno prevalece la forma *enol*. Por ello, uno de nuestros objetivos es el estudio de los posibles tautómeros de ACV y la obtención de su población a temperatura ambiente y a 273.15 K, así como estudiar su estabilidad y su dependencia de los diferentes parámetros. Además, en este trabajo nos hemos propuesto obtener las diferentes conformaciones posibles de ACV comparándolas con el nucleósido deoxiguanosina (dG). El estudio de los diferentes conformeros de ACV, su distribución de carga, interacciones moleculares, su estructura en el estado sólido y flexibilidad de la molécula son interesantes para nuevas investigaciones sobre drogas y antivirales. Por ello hemos intentado determinar si las diferentes formas geométricas de ACV se relacionan o interactúan entre ellas. Hemos intentado explicar si los diferentes enlaces de H tienen importancia en el comportamiento de los conformeros de ACV.

7.4.1. Cálculos realizados

Éstos se han realizado principalmente mediante los métodos computacionales DFT que dan valores con buena concordancia respecto de MP2²²⁻²⁴, método que como sabemos es computacionalmente más caro y, además, debido al tamaño de la molécula solamente podrían usarse las bases 6-31G(d). Las bases que hemos empleado en la determinación de la energía fueron 6-31G(d,p). Los 3D PES de la molécula se determinaron por rotación de de los ángulos endocíclicos y exocíclicos, que son:

φ_1 C4-N9-C1'-O2' (equivalente a χ en los nucleósidos)³⁰ que determina la orientación de la base respecto de la cadena abierta.

φ_2 N9-C1'-O2'-C3'

φ_3 C1'-O2'-C3'-C4' determina los plegamientos de la cadena.

φ_4 O2'-C3'-C4'-O5' determina la orientación de O5'H.

φ_5 C3'-C4'-O5'-H5' define la orientación de H5'.

La rotación de estos ángulos se realizó en pasos de 60° entre 0° y 360°. A partir de la estructura obtenida inicialmente se fueron rotando los valores para los diferentes ángulos según se ha indicado, logrando 78 figuras geométricas optimizadas estables. Se aplicó la optimización de Berni con el criterio TIGHT y se determinaron las cargas mediante el procedimiento Natural NBO³¹. Se ha considerado la estructura de la cadena en ACV como análoga a la del azúcar en los nucleósidos y además se han definido otros parámetros en concordancia con otros trabajos previos³²:

- i) El vector R (N9...O5') que determina la distancia del grupo OH respecto de la base.
- ii) El ángulo β (C4-N9...O5') que define el ángulo del grupo OH respecto del plano de la base.
- iii) El ángulo Φ (C1'-N9...O5') que determina la posición del grupo OH. Tiene similitudes con el ángulo β de los nucleósidos³⁰.

Las frecuencias se obtuvieron en el mismo nivel que en la optimización, y evaluando la segunda derivada de la energía respecto del desplazamiento nuclear. Estos cálculos se realizaron sobre los conformeros optimizados para confirmar que corresponden al de un mínimo local. Todas las estructuras optimizadas presentan solamente frecuencias positivas, que corresponden a mínimo local de la energía. La energía calculada incluye ZPE. ΔG se evaluó como suma de la energía electrónica+ZPE y la contribución térmica. El equilibrio conformacional a 298.15 K se evaluó mediante la ecuación de Boltzman. En la Figura 7.4.2. se tienen las estructuras correspondientes al tautómero N1 y la

desoxiguanosina en sus dos formas *cis* y *trans*. Se observa que la figura del aciclovir concuerda con la forma *trans*, teniéndose un enlace de H intra-molecular análogo $H5' \cdots N3$.

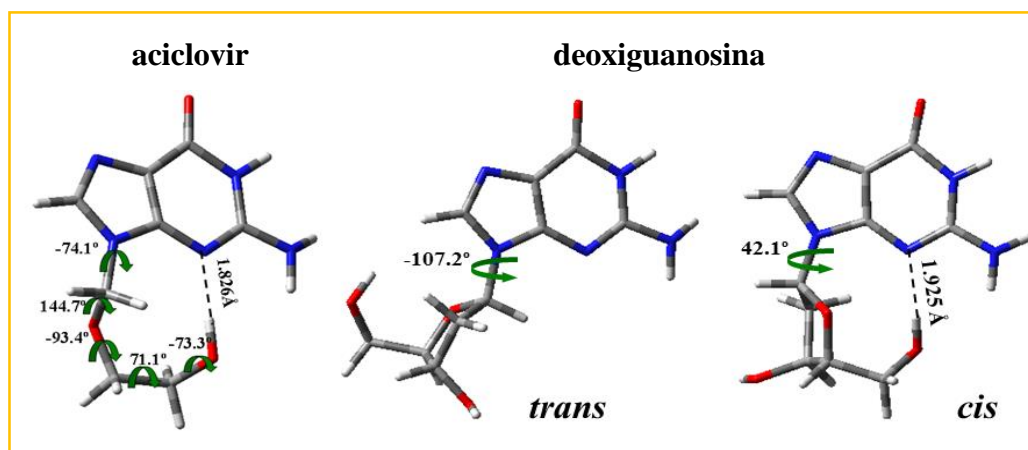


Figura 7.4.2. Estructura de las moléculas de aciclovir y deoxiguanosina.

7.4.2. Tautómeros de ACV

ACV tiene cinco posibles tautómeros, tres formas ceto N1, N3 y N7 y dos formas enol OHC (*cis*) y OHT (*trans*) según la posición del H de la base. En la Tabla 7.4.1. se muestran las cinco formas junto con ΔE en MP2 y en B3LYP/6-31G(d,p) ($\Delta E + ZPE$). El más estable, como puede comprobarse, es N1 y por ello hemos centrado este estudio en este tautómero. En los cálculos realizados en la publicación n°2, N1 es el más abundante, 48.1% a 25° C, seguido de OHC con 37.7% a la misma temperatura. El siguiente es OHT, 14.3% a 25°C. Las formas más inestables y con muy baja población, son las restantes dos formas cetónicas N3 y N7.

Se ha realizado un amplio estudio conformacional del tautómero N1 por rotación de los torsionales ángulos exocíclicos φ_1 , φ_2 , φ_3 , φ_4 y φ_5 . Los conformeros se clasifican según el valor del ángulo φ_1 : conformeros A con el valor de φ_1 negativo y conformeros B con φ_1 positivo. Se obtuvieron 78 conformeros de los cuales 47 son A y los restantes B. La inspección de estas tablas indica que los conformeros A1-A2 y B1-B2 son los únicos que tienen importancia ya que los demás tienen una población muy baja $< 1\%$. Según este método se determinaron las energía relativas en nucleósidos análogos³⁴⁻³⁷ y el mínimo global calculado en estas moléculas en MP2/6-31G(d,p) está en concordancia con el calculado por B3LYP/6-31G(d,p).

La diferencia de energía de los conformeros es muy pequeña. Los 78 conformeros optimizados caen dentro de un rango de la energía electrónica $\Delta E = 0-8.5 \text{ kcal}\cdot\text{mol}^{-1}$ y la energía de Gibbs $\Delta G = 0-6.6 \text{ kcal}\cdot\text{mol}^{-1}$. Este rango de ΔG es menor que el calculado en otras moléculas como dU³⁸ ($0-9 \text{ kcal}\cdot\text{mol}^{-1}$), dT³⁹ ($0-7 \text{ kcal}\cdot\text{mol}^{-1}$).

Tabla 7.4.1. ΔE en MP2 (en negrita) y en B3LYP/6-31G(d,p) de los cinco tautómeros de ACV.

Tautómero	ΔE (a.u.)
N1	-808.643998 -810.723396
OHC	-808.643037 -810.723165
OHT	-808.642196 -810.722251
N7	-808.613237 -810.695482
N3	-808.603429 -810.682713

En la Tabla 7.4.2. se tienen los conformeros más estables dentro de un rango de energía de 1.0-3.0 kcal/mol, 9 conformeros, de los cuales cuatro son *anti* ($\varphi_1 \sim -100^\circ$) y los restantes *syn* ($\varphi_1 \sim 90^\circ$). Las estructuras *anti* forman los nucleótidos y polinucleótidos en los sistemas biológicos^{35-37,40}. En la tabla se tienen todos los parámetros que definen a cada conformero, así se observa que, para estos conformeros más estables, el valor de R, se encuentra por MP2 entre los valores 3.673 Å (conformero B4) y 5.007 Å (conformero B3). Los mayores valores de μ por MP2 aparecen en los conformeros A4 (8.026 D) y B5 (8.520 D), que origina su mayor solubilidad en los disolventes polares. ΔG tiene el máximo valor por B3LYP/6-31G(d,p) en el conformero B5, 1.463 kcal/mol y el menor en A1. Los conformeros A1 y B1 son los más abundantes 41.5% y 36.1% respectivamente a la temperatura de 298.15 K.

En nuestro trabajo publicado se muestran los enlaces de H intramoleculares, que estabilizan la molécula y que son más fuertes en los conformeros más estables. Se ha realizado también el estudio de los ángulos exocíclicos y de los demás parámetros R, β

y Φ , así como de su relación respecto del ángulo φ_1 , lo cual nos llevará a las conclusiones que expondremos. En este punto nos ceñiremos al estudio de la parte de la molécula correspondiente a la base guanina y al estudio de la cadena lateral.

7.4.3. Estudio de la base guanina en el aciclovir

La Tabla 7.4.3. presenta las longitudes de enlace (\AA) en la parte G de la molécula, en los cinco tautómeros de la molécula de ACV, calculados al nivel B3LYP/6-31G(d,p). Los valores de las longitudes de enlace en los tautómeros ceto indican un alargamiento de los enlaces entre los átomos en los que se sitúa H, (N1, N3 y N7), y un alargamiento del enlace C-O en los tautómeros enol, dado que el doble enlace C=O se transforma en enlace simple y, por tanto, se alarga.

La Tabla 7.4.4. muestra las longitudes de enlace y los ángulos de la base guanina en ACV (confórmero A1) calculados en el nivel B3LYP/6-31G(d,p) y los valores de la molécula de guanina observadas⁴¹ y de la guanosina experimental (rayos X)⁴². Las diferencias más significativas corresponden a la longitud de enlace C6-N1, que respecto a G presenta un alargamiento de 0.054 \AA y de 0.047 \AA respecto de la guanosina. El enlace C5-C6 presenta también un alargamiento de 0.026 \AA y 0.023 \AA respecto de G y guanosina. C6-O6 se acorta en 0.023 \AA respecto de G y algo menor 0.014 \AA respecto de guanosina. El mayor cambio corresponde a los enlaces correspondientes a C6, posiblemente relacionados con los enlaces intramoleculares entre O6 y H5'. También el enlace C8-N9 sufre un alargamiento $\sim 0.020 \text{\AA}$. El ángulo que presenta una mayor variación (+3.3°) es C5-C6-O6 respecto de G.

Tabla 7.4.2. Los 9 conformeros óptimos más estables del tautómero N1 de la molécula de ACV en los niveles de cálculo: **B3LYP/6-311++G(3df,pd)** (en negrita); **MP2/6-31G(d)** (entre llaves),; **B3LYP/6-31G(d,p)** (valores tipo normal); **O3LYP/6-31G(d,p)** (entre corchetes); **B3PW91/6-31G(d,p)** (entre paréntesis); **B3LYP/cc-pVDZ** (entre comillas) y **B972/6-31G(d,p)** (en cursiva). La distancia *R* en Å, los ángulos torsionales en grados, el momento dipolar en debyes, los incrementos de energía en kcal·mol⁻¹ y la población (%) a 298.15 K (*P*_{298.15}) y 273.15 K (*P*_{273.15}).

Conformeros	R	β^a	Φ^a	φ₁	φ₂	φ₃	φ₄	φ₅	μ	ΔE	ΔG	P_{298.15}	P_{273.15}
A1	3.963	72.5	54.3	-75.3	146.4	-88.5	69.8	-69.3	5.739	0^b		41.5	41.5
	{2.859}	{73.8}	{50.9}	{-71.8}	{151.8}	{-96.3}	{68.8}	{-74.2}	{6.182}	{0.064}			
	3.925	72.3	53.7	-74.1	144.7	-93.4	71.1	-73.3	5.375	0 ^c	0 ^d		
	[3.956]	[72.7]	[53.9]	[-75.4]	[144.5]	[-94.0]	[71.5]	[-72.1]	[5.369]	[0] ^e	[0.638]		
	(3.891)	(72.7)	(53.2)	(-75.1)	(145.2)	(-92.3)	(70.3)	(-74.1)	(5.492)	(0) ^f	(0) ^g		
	``3.922``	``72.0``	``54.0``	``-73.9``	``143.2``	``-92.0``	``72.3``	``-74.4``	``5.343``	``0`` ^h	``0`` ⁱ		
3.913	72.8	53.3	-75.0	145.3	-93.4	70.8	-73.6	5.502	0 ^j	0 ^k			
A2	3.774	76.8	52.5	-97.7	65.9	74.6	-67.0	-34.1	5.367	1.258		8.7	8.7
	{3.727}	{77.7}	{50.7}	{-96.9}	{66.0}	{76.2}	{-65.5}	{-42.7}	{5.776}	{0.095}			
	2.892	77.3	51.5	-97.0	67.7	72.4	-65.1	-39.3	5.382	0.928	1.324		
	[3.779]	[77.9]	[51.3]	[-98.8]	[68.1]	[72.8]	[-65.5]	[-38.3]	[5.361]	[1.189]	[2.009]		
	(3.715)	(77.0)	(51.6)	(-97.4)	(67.4)	(72.8)	(-65.4)	(-38.6)	(5.436)	(0.655)	(1.034)		
	``3.730``	``77.1``	``51.6``	``-96.6``	``67.6``	``72.4``	``-64.9``	``-38.4``	``5.385``	``1.000``	``1.370``		
3.736	77.4	51.5	-97.9	67.4	73.2	-65.5	-38.7	5.438	1.000	1.018			
A3	5.155	118.5	37.5	-107.7	71.0	171.1	-63.7	57.6	5.762	1.202		0.9	0.9
	{5.004}	{111.4}	{37.6}	{-101.9}	{64.7}	{164.9}	{-61.1}	{53.9}	{5.586}	{2.240}			
	5.104	118.6	36.4	-108.5	69.9	168.3	-61.1	53.4	5.594	2.303	1.014		
	[5.149]	[121.2]	[36.1]	[-112.1]	[71.9]	[168.7]	[-63.0]	[55.9]	[5.653]	[0.585]	[0.073]		
	(5.079)	(119.1)	(36.8)	(-110.2)	(69.3)	(168.7)	(-60.9)	(52.4)	(5.702)	(1.795)	(0.553)		
	``5.105``	``120.0``	``36.7``	``-109.9``	``71.0``	``169.9``	``-60.7``	``52.5``	``5.610``	``2.864``	``1.513``		
5.094	119.1	36.7	-110.0	69.6	168.6	-61.9	54.8	5.673	2.864	0.095			
A4	4.592	116.2	64.7	-106.0	73.2	178.6	65.0	-56.8	7.630	1.726		0.4	0.4
	{4.487}	{113.4}	{64.9}	{-100.7}	{67.1}	{178.0}	{61.3}	{-52.8}	{8.026}	{2.887}			
	4.517	117.5	65.1	-106.7	71.7	-179.5	62.2	-52.9	7.390	2.765	1.457		
	[4.552]	[119.3]	[65.0]	[-109.9]	[73.7]	[-179.3]	[64.3]	[-55.3]	[7.309]	[1.030]	[0.432]		
	(4.500)	(118.5)	(65.1)	(-107.9)	(71.0)	(-179.6)	(62.0)	(-52.8)	(7.480)	(2.309)	(1.009)		
	``4.500``	``117.5``	``65.1``	``-107``	``71.0``	``-179.6``	``62.0``	``-52.8``	``7.480``	``3.256``	``1.871``		
4.515	118.4	65.1	-108.2	71.4	-179.7	62.9	-54.0	7.467	3.256	0.503			
B1	3.928	73.4	53.4	75.1	-146.7	95.5	-68.8	72.8	5.812	0.072		36.1	36.1
	{3.885}	{74.5}	{50.3}	{70.1}	{-151.5}	{94.2}	{-68.1}	{76.8}	{6.204}	{0.056}			
	3.880	73.4	52.8	73.5	-145.4	90.9	-69.3	76.7	6.746	0.083	0.151		
	[3.927]	[73.6]	[53.1]	[74.4]	[-145.4]	[92.6]	[-69.8]	[74.6]	[5.485]	[0.124]	[0.822]		

	(3.855) ``3.870`` 3.882	(73.5) ``73.3`` 73.7	(55.6) ``51.1`` 52.7	(74.2) ``73.1`` 74.0	(-145.7) ``-144.2`` -145.8	(90.5) ``89.6`` 91.5	(-68.9) ``-70.0`` -69.3	(76.7) ``77.7`` 76.0	(5.595) ``5.374`` 5.601	(0.061) ``0.099`` 0.099	(0.108) ``0.195`` 0.090		
B2	3.770 {3.726} 2.905 [3.784] (3.713) ``3.727`` 3.736	77.2 {17.9} 77.6 [78] (77.3) ``77.5`` 77.8	52.0 {50.4} 51.2 [50.8] (51.2) ``51.2`` 51.1	97.0 {95.6} 96.4 [98.5] (96.8) ``96.2`` 97.3	-66.7 {-66.8} -68.5 [-68.9] (-68.2) ``-68.4`` -68.2	-74.1 {-75.8} -72.0 [-72.6] (-72.4) ``-72.9`` -72.8	66.6 {65.5} 65.1 [65.4] (65.4) ``64.0`` 65.4	36.1 {44.0} 40.6 [40.4] (39.9) ``39.8`` 40.3	5.348 {5.664} 5.340 [5.321] (5.404) ``5.250`` 5.398	1.216 {0} ^l 0.87 [1.118] (0.612) ``0.968`` 0.968	1.251 [1.900] (0.975) ``1.316`` 0.941	9.6	9.6
B3	5.157 {5.007} 5.104 [5.149] (5.080) ``5.106`` 5.094	117.3 {110.9} 117.9 [120.9] (118.1) ``119.5`` 118.6	37.3 {37.4} 36.2 [35.9] (36.5) ``36.5`` 36.6	106.1 {100.4} 107.1 [111.5] (108.2) ``108.6`` 108.9	-71.7 {-65.2} -70.3 [-72.1] (-69.7) ``-71.3`` -69.9	-171.1 {-165.1} -168.1 [-168.6] (-168.2) ``-169.7`` -168.4	63.5 {60.9} 60.9 [62.9] (60.8) ``60.6`` 61.7	-57.6 {-53.8} -53.3 [-55.9] (-53.3) ``-52.4`` -54.7	5.951 {5.998} 5.839 [5.872] (5.921) ``5.814`` 5.907	1.157 {2.179} 2.257 [0.542] (1.748) ``2.819`` 2.819	0.903 [0] ^m (0.412) ``1.394`` 0.001	0.9	0.9
B4	4.529 {3.673} 3.817 [4.300] (3.814) ``3.839`` 3.858	95.5 {66.1} 68.7 [85.9] (68.9) ``68.8`` 70.3	65.4 {78.5} 79.7 [70.0] (79.4) ``79.2`` 78.6	78.3 {56.6} 56.7 [69.9] (56.4) ``55.9`` 58.0	70.8 {63.8} 64.6 [70.9] (64.5) ``65.4`` 64.6	-178.7 {-147.2} -149.1 [-168.2] (-150.0) ``-150.2`` -151.4	63.6 {63.0} 63.8 [63.2] (63.4) ``63.6`` 63.4	-54.4 {-52.4} -50.2 [-52.1] (-48.6) ``-48.6`` -49.6	6.470 {6.649} 5.870 [6.144] (5.913) ``5.680`` 5.949	2.031 {1.707} 2.722 [1.331] (2.523) ``3.179`` 3.179	2.366 [0.867] (2.080) ``2.791`` 1.557	0.4	0.4
B5	4.598 {4.507} 4.526 [4.559] (4.510) ``4.524`` 4.524	115.9 {112.9} 117.5 [119.2] (118.4) ``117.6`` 118.3	64.6 {64.3} 64.8 [64.9] (64.8) ``64.7`` 64.8	105.4 {99.6} 106.4 [109.6] (107.6) ``106.6`` 107.6	-72.8 {-66.5} -71.4 [-73.3] (-70.7) ``-72.3`` -71.1	-178.2 {-177.0} 180.0 [179.7] (-61.8) ``-179.9`` -179.8	-64.9 {-61.1} -62.0 [-64.2] (52.7) ``-61.7`` -62.7	56.7 {52.8} 52.8 [55.2] (52.7) ``52.1`` 53.8	7.911 {8.520} 7.735 [7.629] (7.826) ``7.517`` 7.793	1.693 {2.832} 2.757 [1.012] (2.289) ``3.262`` 3.262	1.463 [0.420] (0.978) ``1.920`` 0.470	0.4	0.4

^aNotación empleada ref. [33]. ^b $\Delta E = 0 = -811.214731$ a.u. en el nivel B3LYP/6-311++G(3df, pd); ^c $\Delta E = 0 = -810.723396$ a.u. en B3LYP/6-31G(d, p); ^d $\Delta G = 0 = -810.764701$ a.u.; ^e $\Delta E = 0 = -810.449499$ a.u. en el nivel O3LYP-31G(d,p); ^f $\Delta E = 0 = -810.419555$ a.u. en el nivel B3LYP/6/cc-pVDZ; ^g $\Delta G = 0 = -810.460766$ a.u.; ^h $\Delta E = 0 = -810.760402$ a.u. en el nivel B3PW91/6-31G(d,p); ⁱ $\Delta G = 0 = -810.801662$ a.u.; ^j $\Delta E = 0 = -810.444324$ a.u. en el nivel B972/6-31G(d,p); ^k $\Delta G = 0 = -810.485454$ a.u.; ^l $\Delta E = 0 = -808.643998$ a.u. en el nivel MP2/6-31G(d); ^m $\Delta G = 0 = -810.491798$ a.u.

Tabla 7.4.3. Longitudes de enlace (Å) en G de ACV en los 5 tautómeros en el nivel de cálculo B3LYP/6-31G(d,p).

Longitudes de enlace (Å)	N1	N3	N7	OHT	OHC
N1-C2	1.367	1.290	1.331	1.354	1.353
C2-N3	1.316	1.409	1.377	1.350	1.345
N3-C4	1.358	1.385	1.330	1.337	1.337
C4-C5	1.391	1.373	1.382	1.396	1.402
C5-C6	1.441	1.469	1.459	1.405	1.405
C6-N1	1.447	1.422	1.380	1.224	1.331
C5-N7	1.383	1.385	1.379	1.386	1.388
N7-C8	1.307	1.305	1.333	1.308	1.305
C8-N9	1.290	1.395	1.355	1.391	1.392
N9-C4	1.372	1.369	1.400	1.377	1.374
C6-O6	1.216	1.217	1.233	1.342	1.338

Tabla 7.4.4. Longitudes de enlace (Å) y ángulos de enlace calculadas de G en el tautómero N1 de ACV en el nivel de cálculo B3LYP/6-31G(d,p), observadas de guanina⁴¹ y experimentales de guanosina⁴².

Longitudes de enlace (Å)	ACV conf. A1 (B3LYP)	Guanina (observadas)	Guanosina Rayos X
N1-C2	1.367	1.375	1.368
C2-N3	1.316	1.327	1.327
N3-C4	1.358	1.355	1.349
C4-C5	1.391	1.377	1.378
C5-C6	1.441	1.415	1.418
C6-N1	1.447	1.393	1.400
C5-N7	1.383	1.389	1.390
N7-C8	1.307	1.304	1.300
C8-N9	1.290	1.374	1.370
N9-C4	1.372	1.377	1.376
C6-O6	1.216	1.239	1.230
Ángulos(°)			
C6-N1-C2	126.8	124.9	
N1-C2-N3	122.6	124.0	125.4
C2-N3-C4	113.6	111.8	123.8
N3-C4-C5	128.8	128.4	111.8
C4-C5-C6	118.7	119.1	128.9
C5-C6-N1	109.5	111.7	111.1
C4-C5-N7	110.6	110.8	110.5
C5-N7-C8	104.4	104.2	104.5
N7-C8-C9	113.5	113.5	113.1
C8-N9-C4	105.5	106.0	106.4
N9-C4-C5	106.0	105.6	105.4
N3-C4-N9	125.3	126.0	
C6-C5-N7	130.7	130.1	
N1-C2-N2	118.4	116.3	
N3-C2-N2	119.0	119.7	
N1-C6-O6	118.9	120.0	119.9
C5-C6-O6	131.6	128.3	

7.4.4. En el estado sólido

Experimentalmente se determinó la estructura de ACV en estado sólido mediante difracción de rayos-X^{43,44}. Se obtuvieron cristales monoclínicos con tres moléculas independientes A, B y C^{33,44} junto con dos moléculas de agua. Las moléculas A y B tienen conformaciones análogas pero la molécula C es diferente. Para el estudio teórico del estado sólido, éste se simuló mediante las estructuras del dímero y el tetrámero (figura 7.4.3.). En la Tabla 7.4.5. se tienen los parámetros más importantes de ambas estructuras.

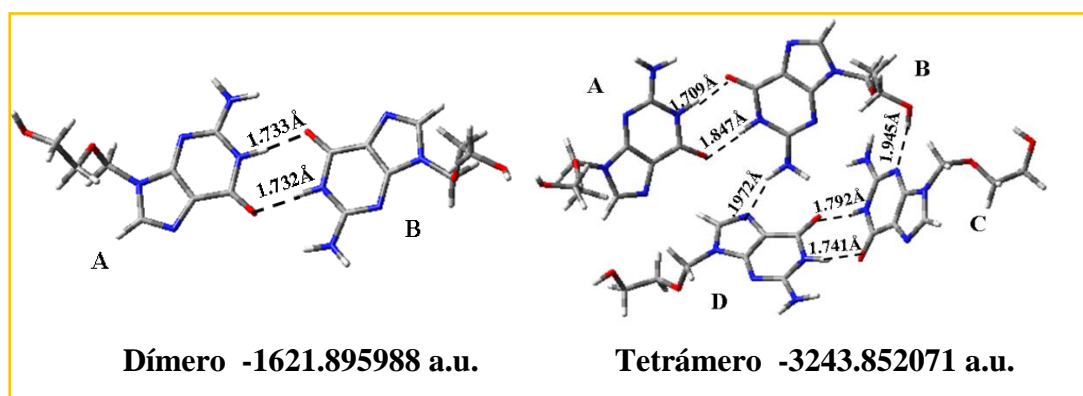


Figura 7.4.3. Las formas del dímero y del tetrámero del conformero A1 de ACV en el nivel de cálculo B3LYP/6-31G(d,p). Al pie de la figura E(RB+HF-LYP) en a. u.

En la figura 7.4.3. se representan el dímero y el tetrámero de ACV, con los enlaces de H intermoleculares que en general son enlaces fuertes. Se tienen cuatro puntos donores H1_(N1), H2_(N2), H2'_(N2) y H5' y cinco aceptores O6, N3, N7, O2' y O5'. En la Tabla 7.4.5. se tienen las longitudes de enlace de las moléculas A y B del dímero, comparadas con los valores experimentales⁴⁴, las diferencias no son grandes: C4-C5, aumenta en 0.025 Å, C2-N2' en 0.023 Å y C3'-C4' y N1-C6 en 0.016 Å., los demás enlaces prácticamente coinciden.

En la siguiente Tabla 7.4.6. se tienen las longitudes de enlace de las moléculas A y B del tetrámero y los valores experimentales. No aparecen grandes diferencias entre los valores de A calculados y los experimentales, las más relevantes son C4-C5 (+0.024 Å), C3'-C4' (+0.022 Å), N1-C6 (+0.017 Å) y C2-N2' (+0.014 Å).

La Tabla 7.4.7. muestra los ángulos de enlace de la molécula A y B del dímero y la molécula A del tetrámero, junto con los valores experimentales. Se observa en general una buena concordancia.

Tabla 7.4.5. Longitudes de enlace en las moléculas A y B del dímero de ACV y los datos experimentales.

Longitudes de enlace	Dímero: molécula A	Dímero: molécula B	Ref. 44. moléc. A
N1-C2	1.371	1.371	1.368
N1-C6	1.404	1.404	1.388
C2-N2'	1.359	1.358	1.336
C2-N3	1.321	1.321	1.328
N3-C4	1.350	1.350	1.348
C4-N5	1.400	1.400	1.375
C5-C6	1.429	1.429	1.419
C6-O6	1.245	1.245	1.245
C4-N9	1.377	1.377	1.376
C5-N7	1.385	1.385	1.385
N7-C8	1.305	1.305	1.304
C8-N9	1.392	1.392	1.375
N9-C1'	1.457	1.457	1.462
C1'-O2'	1.399'	1.399	1.408
O2'-C3	1.423	1.423	1.436
C3'-C4'	1.515	1.515	1.499
C4'-O5'	1.419	1.419	1.421

Mediante rayos X se han determinado otras formas de ACV anhidro con diferentes valores de los enlaces de H entre las moléculas⁴⁵⁻⁴⁷. Estudios de ACV en disolución indican que ACV se encuentra también en formas polimórficas y pseudo polimórficas de solvatos⁴⁷.

Tabla 7.4.6. Longitudes de enlace en las moléculas A y B del tetrámero de ACV y los datos experimentales.

Longitudes de enlace	Tetrámero: molécula A	Tetrámero: molécula B	Ref. 44.: estruct. de la moléc. A
N1-C2	1.373	1.373	1.368
N1-C6	1.405	1.397	1.388
C2-N2'	1.350	1.360	1.336
C2-N3	1.329	1.321	1.328
N3-C4	1.353	1.350	1.348
C4-C5	1.399	1.398	1.375
C4-N9	1377	1.379	1.376
C5-C6	1.430	1.425	1.419
C6-O6	1.244	1.252	1.245
C5-N7	1.384	1.384	1.385
N7-C8	1.305	1.308	1.304
C8-N9	1.383	1.388	1.375
N9-C1'	1.458	1.462	1.462
C1'-O2'	1.403	1.396	1.408
O2'-C3'	1.429	1.426	1.436
C3'-C4'	1.521	1.514	1.499
C4'-O5'	1.415	1.420	1.421

Tabla 7.4.7. Ángulos de enlace de la molécula A del dímero y del tetrámero de ACV / la molécula A experimental.

Ángulos	Dímero: molécula A	Dímero: molécula B	tetrámero: molécula A	Ref. 44.: struct. molécula A
C2-N1-C6	125.7	125.7	125.6	125.6
N1-C2-N2	115.7	115.7	115.6	117.1
N1-C2-N3	123.9	123.9	124.0	123.2
N2-C2-N3	120.4	120.4	120.3	119.7
C2-N3-C4	112.1	112.1	112.1	112.3
N3-C4-C5	129.0	129.0	128.8	128.7
N3-C4-N9	125.7	125.7	126.0	125.8
C5-C4-N9	105.3	105.3	105.2	105.5
C4-C5-C6	118.0	118.0	118.2	118.6
C4-C5-N7	111.0	111.0	110.9	110.9
C6-C5-N7	131.0	131.0	130.8	130.5
N1-C6-C5	111.3	111.3	111.2	111.7
N1-C6-O6	119.6	119.6	120.0	120.1
C5-C6-O6	129.1	129.1	128.8	128.3
C5-N7-C8	104.2	104.2	104.6	104.3
N7-C8-N9	113.7	105.8	113.2	113.1
C4-N9-C8	105.8	105.8	106.1	106.2
C4-N9-C1'	126.2	126.2	126.1	125.7
C8-N9-C1'	128.0	128.0	127.6	128.0
N9-C1'-O2'	114.3	114.3	114.2	111.5
C1'-O2'-C3'	114.9	115.0	115.0	113.5
O2'-C3'-C4'	108.8	108.8	108.8	109.4
C3'-C4'-O5'	108.6	108.6	108.5	113.2

7.4.5. Breve estudio del espectro del confórmero A1

Tabla 7.4.8. Comparación de las frecuencias IR de la molécula de Guanina (ν , experimental) con las calculadas (ω) y escaladas (ν) al nivel B3LYP/6-31G(d,p) para la estructura de la Guanina del confórmero A1 en la molécula de ACV.

Guanina ⁴⁸				ACV		
Modo	ν (cm ⁻¹)	I ^a	Asignación	ω (cm ⁻¹)	ν (cm ⁻¹)	A ^b
v3	3439	64	ν (N1)H	3604	3439	46
v4	3.428	31	ν_{sym} NH ₂	3575	3412	97
v6	1749	270	ν (C=O)	1836	1769	671
v7	1.629	375	NH ₂ , ring	1626	1571	221
v9	1588	140	NH ₂	1578	1525	85
v10	1547	48	Ring	1534	1449	3
v13	1361	16	δ N(9)H, ring	1394	1352	88
v14	1329	21	Ring, δ N(1)H	1343	1303	32
v16	1271	20	Ring, δ C(8)H	1251	1216	24
v17	1140	40	δ ring, δ C(8)H	1244	1210	28
v18	1104	24	Ring, NH ₂ ro	1150	1121	121
v19	1052	10	Ring, NH ₂ ro	1141	1113	72
v21	1010	30	Ring, NH ₂ ro	1109	1082	27
v23	932	5	γ C(8)H	822	811	7
v35	482	41	δ ring	495	502	7
v38	309	86	NH ₂ inv	340	356	68

^a Intensidad (unidades arbitrarias); ^b Intensidad IR calculada (km·mol⁻¹).

El espectro IR obtenido en B3LYP/6-31G(d,p) (75 frecuencias) se ha comparado con el espectro experimental IR de la guanina en el tautómero oxo-amino⁴⁸, obteniéndose un conjunto de frecuencias análogas que se han identificado mediante el espectro obtenido en GaussView, y escalando estos valores según ($\nu=34.6 + 0.9447\omega$) y estas frecuencias (de A1 escaladas) se han comparado con las obtenidas experimentalmente²⁹. Se han obtenido las Tablas 7.4.8 y 7.4.9. Entre las frecuencias correspondientes a G se tienen una buena concordancia, ACV tiene un espectro con mayor número de frecuencias, dada la mayor complejidad de la molécula.

Se han considerado las frecuencias calculadas de ACV que presentaban las vibraciones análogas a las obtenidas experimentalmente; en la Tabla 7.4.8. se tienen las que presenta el conformero A1 observándose cierta diferencia en las intensidades, la más acusada se tiene en la frecuencia 1749 cm^{-1} (experimental) respecto de 1836 cm^{-1} (calculada en el conformero A1) que vale 400.6 unidades. Las demás presentan valores de la intensidad menos dispares que van desde ~ 150 unidades hasta 2.3 unidades.

Tabla 7.4.9. Asignación de las frecuencias (ν , cm^{-1}) experimentales en KBr^{29} en la molécula de ACV mediante las frecuencias escaladas al nivel B3LYP/6-31G(d,p).

ν (experimentales)	ω (calculadas)	ν (escaladas)	Asignación
1696 s	1680	1622	βNH_2
1663 w			
1629 s	1625	1570	βNH_2 , $\beta\text{C}2\text{'H}_2$, $\beta\text{C}8\text{-H}$, $\beta\text{N}1\text{-H}$
1602 w			
1574 m	1579	1526	$\delta\text{C}1\text{'H}_2$
1539 m	1534	1482	$\beta\text{O}5\text{'-H}$
1479 m	1479	1432	$\delta\text{C}4\text{'-H}_2$

β : Flexión simétrica en el plano; δ : Flexión simétrica fuera del plano.

7.4.6. Resumen y conclusiones

De lo escrito en los puntos anteriores y en las publicaciones anexas se deduce:

1. De ACV se optimizaron cinco tautómeros de los cuales N1 es el más abundante a temperatura ambiente, también tienen una proporción importante OHC y OHT. La energía está relacionada con el momento dipolar y con el ángulo de torsión ϕ_1 .
2. Se ha estudiado el tautómero N1 en los niveles de cálculo B3LYP y MP2 del que se han logrado 78 conformeros estables con energías relativas comprendidas entre 0.095 y 8.451.

3. Las longitudes de enlace de ACV que presentan mayores diferencias respecto a G y guanosina son los correspondientes a C6, dada la formación de enlaces de H con C=O.
4. El conformero A1 es el más estable en DFT y, en MP2, es B2. Se ha establecido la distribución de todos los conformeros según rangos de estabilidad de los ángulos torsionales. Se han obtenido unos valores que indican la flexibilidad de ACV.
5. En los principales conformeros se tienen solamente dos enlaces de H intramoleculares, lo cual da lugar a que la molécula sea flexible. Comparado con dG, que presenta seis, se observa que es más flexible.
6. Las cargas negativas en los átomos de N y O2' y O5' son más altas que en dG lo cual motiva su mayor reactividad.
7. La mayor estabilidad se observa cuando la cadena lateral está cerca de la base con unos valores de R comprendidos entre 3.925-2.892 Å y el ángulo Φ cerca de 54°.
8. El estado sólido, simulado mediante la estructura del dímero y del tetrámero, da unos valores que concuerdan con los obtenidos con rayos X.
9. El espectro de ACV presenta algunas concordancias con el espectro experimental de G.

7.4.7. Referencias

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8. Estudio inicial de las moléculas 6-clorouracilo y Nikavir

Tal como se indica en la *Introducción* de este documento, nuestro interés se centra en el estudio de la estructura, propiedades y aplicaciones de aquellos fármacos directamente relacionados con enfermedades graves como tumores, infecciones virales y una serie de afecciones citadas en puntos anteriores.

Dentro de este grupo de moléculas se encuentran 6-clorouracilo y Nikavir que, aunque no están relacionadas directamente con HX, ya que son derivados de la pirimidina, sin embargo si pertenecen a un grupo de fármacos derivados de bases nucleicas que tienen aplicación en medicina, en los casos citados en puntos anteriores. Así, tienen especial interés los derivados en que el átomo de halógeno se sitúa en posición 5 del anillo de U, que guarda similitud estructural con **T** por sustitución del grupo metilo por halógeno, pudiendo sustituir esta base en el DNA *in vivo*. Estos compuestos presentan propiedades antitumorales y antivirales²⁻⁴.

8.1. 6-clorouracilo

Los halouracilos, compuestos en los que se ha sustituido un átomo de H del uracilo (U) por halógeno, tienen importancia en medicina principalmente porque inhiben el crecimiento tumoral. Se aplican en el control de los daños en los tejidos enfermos en las terapias de radiación¹.

En la figura 8.1. se tiene la fórmula y la numeración del 6-clorouracilo, una de las sustancias que han demostrado su actividad antitumor^{1,2}. Forma complejos con iones de metales (II) como cobalto, níquel, cobre, cinc, paladio, rutenio, manganeso y rodio que tienen actividad antitumor⁴.

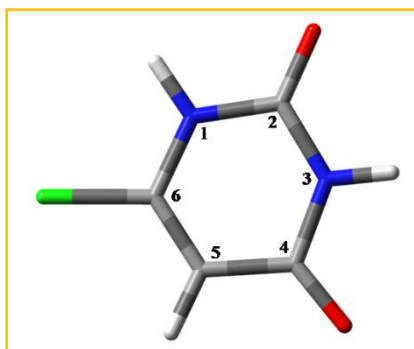


Figura 8.1. Estructura y numeración de la molécula de 6-clorouracilo.

8.1.1. Objetivos

Estos fueron los principales objetivos en el estudio de esta molécula:

- i) Estudio de la molécula 6-clorouracilo en estado sólido mediante la espectroscopía Raman e IR.
- ii) Obtención de los tautómeros más estables y sus energías mediante métodos teóricos.
- iii) Simulación del estado sólido y estudio de los números de onda de la molécula aislada y en estado sólido mediante métodos DFT.
- iv) Obtener los valores escalados de los números de onda en el estado aislado y en estado sólido, y compararlos con los valores experimentales.
- v) Estudio de las cargas atómicas.
- vi) Determinar la diferencia de energías de los orbitales HOMO y LUMO.

8.1.2. Tautomerismo

El tautomerismo en las bases de los ácidos nucleicos tiene especial importancia porque los tautómeros provocan alteraciones en el apareamiento de las bases dando lugar a mutaciones en las hélices del DNA o RNA⁵⁻⁷. Respecto del tautomerismo de las bases nitrogenadas se han realizado trabajos teóricos^{8-10, 11-13} y aproximaciones experimentales^{14,15}. Entre los posibles tautómeros de 6-ClU los más importantes y los más estudiados son seis nombrados T1-T6 en la figura 8.2. Los cálculos realizados confirman que el más estables es T1, que está de acuerdo con estudios previos¹⁶.

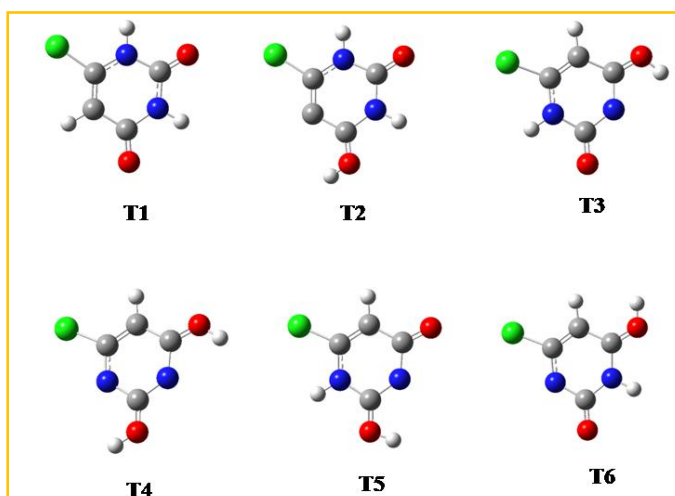


Figura 8.2. Tautómeros del 6-clorouracilo: (T1) 2,4-dioxo-6-clorouracilo, (T2) 2-hidroxi-4-oxo-6-clorouracilo, (T3) 2-oxo-4-hidroxi-6-clorouracilo, (T4) 2,4-dihidroxi-6-clorouracilo, (T5) 1H-2hidroxi-4-oxo-6-clorouracilo, (T6) 3H-2oxo-4-hidroxi-6-clorouracilo.

Las formas enol y dienol son mucho menos estables que las formas ceto. T2 y T4 son las formas enol más estables.

Tabla 8.1. Energía relativa y ΔG de los tautómeros de 6-clorouracilo en el nivel de cálculo B3LYP/6-311++G(3df,pd) (kJ/mol).

$\Delta E+ZPE$	T1	T2	T3	T4	T5	T6
	0	35.61	46.11	40.34	78.21	65.67
ΔG	0	36.28	46.52	41.51	78.08	65.79

8.1.3. Estado sólido

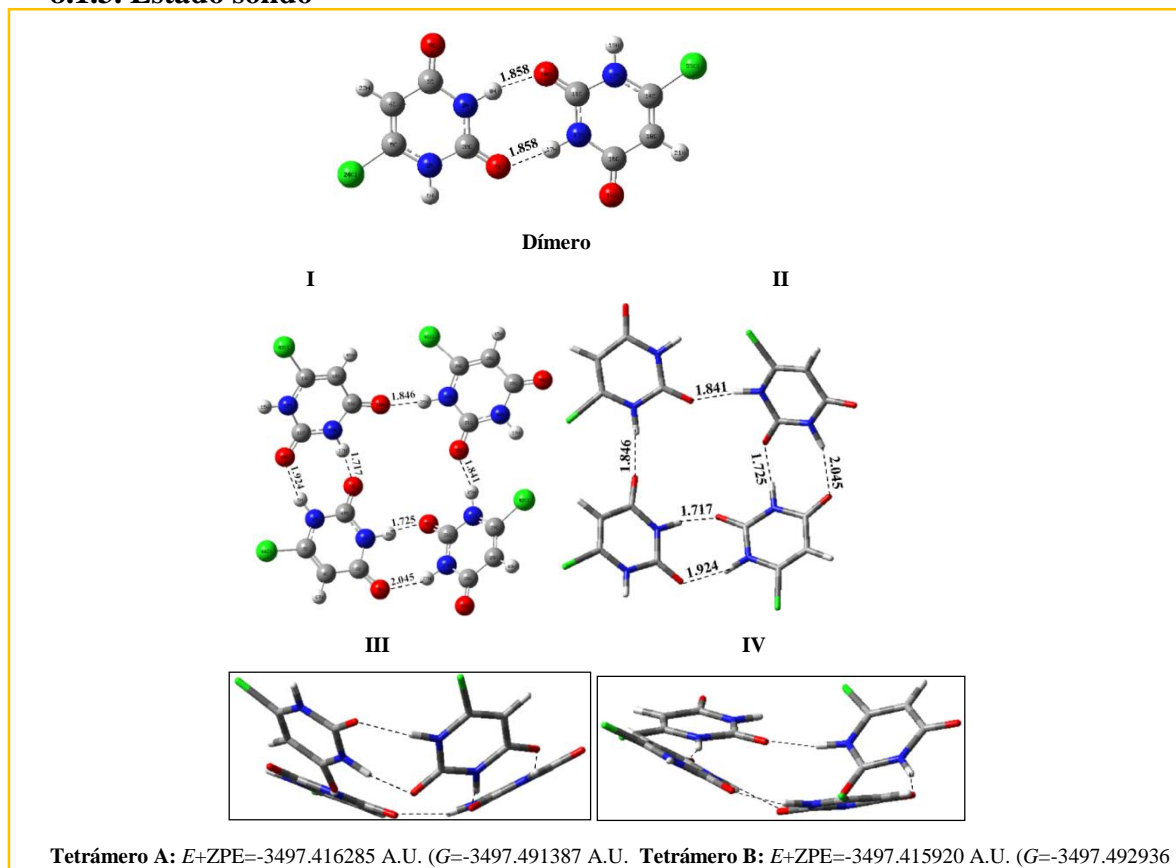


Figura 8.3. Estado sólido del 6-clorouracilo: Dímero y formas A y B del tetrámero. $E+ZPP$ en U.A.

El estado sólido se ha representado mediante el dímero (figura 8.3.) y mediante el tetrámero (figura 8.4.). En el dímero la estructura optimizada es plana lo cual concuerda con lo determinado en la molécula de U. No se cuenta con los datos experimentales obtenidos con rayos X, pero si con los de 5-CIU y otros 5-halouracilos^{17,18}. En estas estructuras las moléculas aparecen asociadas mediante enlaces de H entre N1H y N3H de una molécula y el O del grupo carbonilo de otra.

En el tetrámero se simularon las formas A y B, parecidas a lo esperado en el cristal, y las formas C y D no planares. Estas últimas no se presentan en el cristal. Los enlaces de H estabilizan la estructura, como en el dímero entre N1-H y N3-H y el grupo carbonilo

de otra molécula, lo cual altera la longitud de los enlaces vecinos. Una vez calculadas las frecuencias indicadas se llega a las siguientes conclusiones:

8.1.4. Conclusiones

1. Se tienen 6 tautómeros más estables de 6-CIU siendo T2 y T4 los que presentan mayor estabilidad. Todos los tautómeros de 6-CIU son más estables que los de la molécula de U.
2. La halogenación produce un efecto mayor en los derivados de 6-CIU que en 5- CIU.
3. Los cálculos realizados con el método B3LYP son útiles para interpretar los espectros IR y Raman cuando se aplican con ecuaciones o factores de escalado.
4. La concordancia entre los valores escalados y los experimentales es aceptable.
5. La simulación del estado sólido mediante el tetrámero es adecuada y el error cuadrático medio es reducido.
6. Al determinar las interacciones entre moléculas se observa una mínima interacción del Cl con los átomos de las moléculas próximas.
7. La presencia del halógeno en la molécula de 6-CIU altera algunas propiedades respecto de la molécula del uracilo, pero estos efectos son menores que en los derivados halogenados en la posición 5.
8. El decrecimiento del ``gap'' entre la energía HOMO y LUMO facilita la transferencia de carga intramolecular, lo cual hace que la molécula de 6-CIU sea un material NLO activo.

8.2. Nikavir

La figura 8.4. muestra la estructura de este nucleótido y los ángulos endocíclicos y exocíclicos. Es una molécula similar a AZTM que presenta una gran actividad frente al HIV¹⁹. Dadas las características de su actividad antiviral es importante el estudio de esta droga.

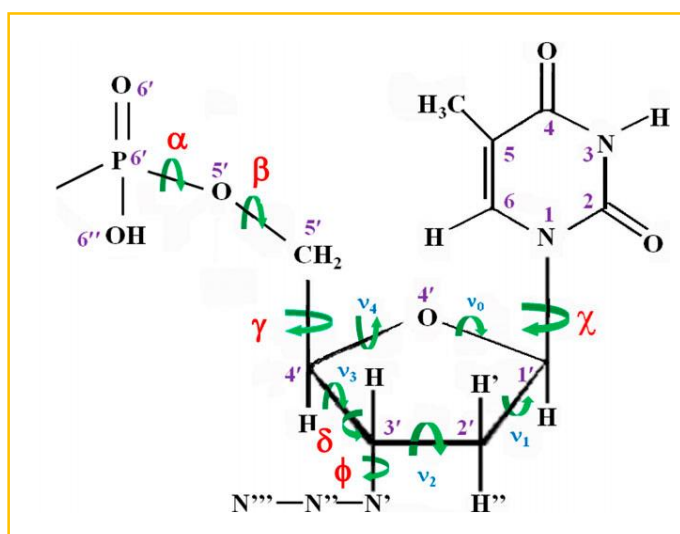


Figura 8.4. Estructura del Nikavir y sus correspondientes ángulos endocíclicos y exocíclicos.

8.2.1. Objetivos

Los objetivos propuestos en este estudio son:

- i) Establecer relaciones entre la estructura, características conformacionales y las propiedades físicoquímicas de este compuesto.
- ii) Comparar los resultados con el nucleósido AZT y con el nucleósido natural dT.
- iii) Definir los ángulos conformacionales de la molécula.
- iv) Calcular los parámetros estructurales de la molécula.
- v) Obtener los confórmeros, la energía y los parámetros conformacionales más importantes de los mismos.
- vi) Estudio de las cargas de la molécula en el confórmero más estable.
- vii) Breve estudio de las bajas frecuencias y la hidratación del confórmero más estable.
- viii) Breve estudio de la estructura y actividad de los derivados de NK.

8.2.2. Confórmeros

En la figura 8.5. se tienen los tres tipos de conforméromos, al nivel B3LYP/6-31G(d,p), y según rotación del ángulo χ , con valores de los ángulos β y γ aproximadamente de 60° . Por rotación de los ángulos χ , α , β , γ , δ y φ con valores de 60° se obtienen 67 conforméromos favorables energéticamente.

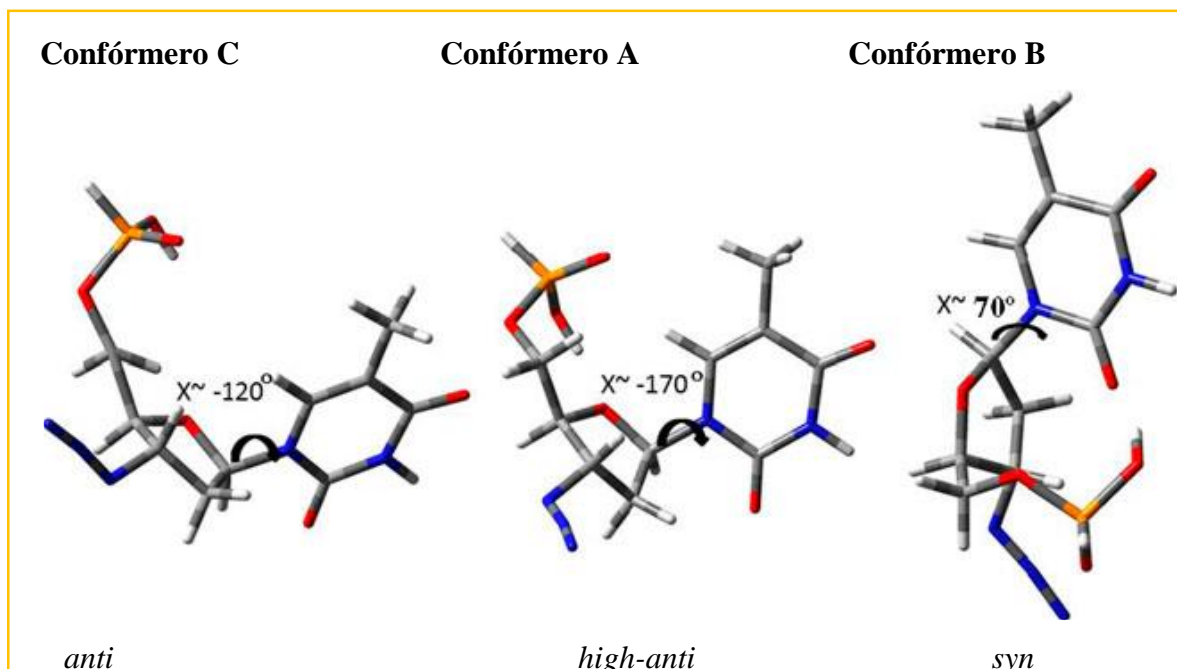


Figura 8.5. Conforméromos de NK obtenidos por rotación del ángulo χ con β y $\gamma \sim 60^\circ$.

Completados los estudios propuestos en el apartado anterior se establecen las conclusiones siguientes:

8.2.3. Conclusiones

1. De los 67 conforméromos obtenidos en el nivel de cálculo B3LYP/6-31G(d,p) favorables energéticamente hay tres *anti* y dos *syn* que tienen una energía comprendida entre 0-2 kcal/mol (E+ZPE), y 30 *anti* y 17 *syn* en el intervalo 2-8 kcal/mol.
2. Los conforméromos más estables están en la forma *anti*. Los enlaces de H intermoleculares favorecen la estabilidad.
3. Se estableció la distribución de los conforméromos según los rangos de estabilidad de los ángulos torsionales, y se observa la flexibilidad de NK mayor que AZT.
4. Las cargas sobre los átomos son análogas a las obtenidas en AZT. Los átomos de O en el grupo fosfato tienen la carga negativa más alta.

5. La carga negativa del grupo fosfato incrementa el valor de P y v_{\max} respecto de AZT lo cual da lugar a un incremento de la flexibilidad de la molécula.
6. Los enlaces de H del grupo fosfato dan lugar a un acortamiento del enlace N1-C1' y a un alargamiento de 5'...O2 y O5'...O4 comparado con dT.
7. No se encontraron correlaciones entre v_{\max} y el ángulo γ . Los ángulos χ , α , β , γ , δ y φ no muestran correlaciones, como tampoco v_0 , v_1 , v_2 , v_3 y v_4 y los anteriores ángulos.
8. Se realizó un estudio de las vibraciones bajas del conformero C1 y se comparó con las obtenidas en dT. El número vibraciones bajas indican la alta flexibilidad de NK.
9. El hidrato más estable del conformero C1 corresponde a 20 moléculas de agua, en donde se aumenta las distancias P...N1 y O''...N1 y se rompe en enlace de H O6''-H...O4'.

8.3. Referencias

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9. Estudio comparativo de las moléculas HX, ALO, INO y ACV

Al comprar los resultados obtenidos en los cálculos realizados en las moléculas bajo estudio se obtuvo la Tabla 9.1., en la cual se muestra el número de tautómeros de cada molécula, considerando los más estables y, en su caso, el número de confórmeros y el más estable.

Tabla 9.1. Tautómeros de los compuestos HX, ALO, INO y ACV.

Compuesto	HX	ALO	INO	ACV
Nº de tautómeros	14 determinados: 6 más estables HX-17, HX-19, HX-37, HX-70, HX-90 _{cis} , HX- 90 _{trans} .	11 determinados Más estable: ALO-15.	N1, N3, N7, OH _{cis} , OH _{trans} Más estable: N1.	N1 , N3, N7, OH _{cis} , OH _{trans} Más estable: N1 .
Confórmeros	-	-	69 confórmeros: A1 (B2) los más estables.	78 confórmeros: A1 (más estable).

Tabla 9.2. Planaridad de las moléculas.

Molécula (B3LYP/6- 31G(d,p))	HX-19	HX-17	ALO-15	INO	ACV
Planaridad	Totalmente plana.	Totalmente plana.	Molécula plana.	Pequeña no planaridad en la parte HX.	Pequeña no planaridad en la parte G
Estabilidad	El tautómero que se tiene en los valores experimentales.	El más estable y abundante.	El tautómero más estable.	El confórmero A1 el más estable (en el tautómero N1).	El confórmero A1 el más estable (en el tautómero N1).

La planaridad de la base sufre pequeñas fluctuaciones en la molécula más compleja. En la Tabla 9.2. se observa que las moléculas totalmente planas corresponden a los tautómeros de la hipoxantina HX-19 y HX-17 y también a ALO-15, las moléculas de INO y ACV presentan una pequeña no planaridad. La mayor estabilidad en cada molécula corresponde a HX-17, en la hipoxantina; ALO-15, en alopurinol y, en cada caso, al confórmero A1 en INO y ACV.

La estructura de las moléculas estudiadas, con formaciones análogas a HX o sus derivados, permite comparar sus estructuras. En general los ángulos calculados no presentan diferencias notables respecto de los valores experimentales. En las longitudes de enlace se aprecian algunas variaciones significativas.

El enlace O-H se incrementa en todos los enol tautómeros de HX respecto de la forma ceto, debido a la formación de un enlace simple en C6-O frente al doble enlace de las formas cetónicas. Los valores más altos de los ángulos de HX corresponden a los cálculos en MP2/6-31G(d,p).

Tabla 9.3. Principales características de las moléculas estudiadas.

Molécula	HX-19	HX-17	ALO-15	INO conf. A1 (derivado de HX)	ACV conf. A1 (derivado de G)
Longitudes de enlace	Valores muy similares a los experimentales. Los mayores valores corresponden al nivel B3LY/6-31G(d,p).	El desplazamiento de H de N9 a N7 aumenta la longitud de los enlaces vecinos.	Presenta un cambio del enlace C-O versus los datos experimentales.	Las diferencias respecto de HX-19 no son significativas.	Las diferencias respecto de G presentan valores significativos.
Ángulos	Similares a los experimentales	Similares a los experimentales	Similares a los experimentales	Similares a los experimentales	Presentan diferencias respecto a los datos experimentales.

Tabla 9.4. Cargas atómicas de las moléculas y enlaces intramoleculares.

Molécula	HX-19	HX-17	ALO-15	INO conf. A1 (derivado de HX)	ACV conf. A1 (derivado de G)
Cargas atómicas	La mayor carga negativa sobre O6 y N1 y la mayor positiva sobre C6.	La mayor carga negativa sobre O6 y N1 y la mayor positiva sobre C6.	La mayor carga negativa sobre N5 y O y la mayor positiva sobre los átomos de H.	La mayor carga negativa sobre O2' y O3' y la positiva sobre C6.	La mayor carga negativa sobre N2 y O5' (en MP2) y la positiva sobre C6 y C2.
Enlaces de H intramoleculares	-	-	-	Por orden de fuerza: N3...H2 _(O2') N3...H5 _(O5') O2'...H3 _(O3') O3'...H2 _(O2') O5'...H3 _(O3')	Por orden de fuerza (fuerza de valor medio) H5' _(O5') ...N3 H5' _(O5') ...O2'

En la Tabla 9.4. se observa que en las tres bases la mayor carga negativa se tiene sobre O6 (O5 en ALO) y N1 (N5 en ALO) y la mayor carga positiva sobre C6. En ALO esta carga se tiene sobre los átomos de H. En el conformero A1 de INO las mayores cargas negativas se tienen sobre los átomos de O laterales: O2' y O3', y la mayor positiva sobre

C6. En el conformero A1 de ACV (en el nivel de cálculo MP2) la carga negativa más alta sobre N2 y O5' y la positiva sobre los átomos C6 y C2. En general se observa que la carga positiva más alta se sitúa sobre C6, aunque las negativas no se den en los átomos vecinos a éste.

INO y ACV tienen una estructura que permite la formación de enlaces de H dentro de la molécula. En INO los enlaces se forman preferentemente entre los átomos de H unidos a O2' y O3' y los átomos N3 y O (del azúcar).

En ACV las fuerzas no tienen valores altos, se dan entre H5' y N3 y O2'. En el estado sólido las moléculas se enlazan preferentemente entre N1 y O6 con enlaces fuertes.

Las Tablas 9.5.1. y 9.5.2. muestran los valores más significativos de la hidratación de las bases. En HX se han obtenido los monohidratos de todos los tautómeros considerados. La hidratación en los dos tautómeros HX-19 y HX-17 se realizó al nivel de cálculo MP2 hasta obtener 8-9 moléculas de agua sumadas, considerando la más estable (la de menor energía) y a ésta sumando la siguiente molécula de agua en las tres posiciones óptimas y siguiendo en B3LYP hasta obtener 30 moléculas de agua. Los valores de de la energía vienen indicados para 20 y 10 moléculas de agua añadidas.

En ALO se tienen cuatro posiciones óptimas de las moléculas H₂O añadidas y

Tabla 9.5.1. Hidratación de los tautómeros HX-19, HX-17 y ALO-15.

Moléculas	HX-19	HX-17	ALO-15
Hidratos: se sigue en todos los casos el método E.M.	Presentan 3 posiciones posibles de la molécula de agua: A, B, C. En B3LYP se obtuvieron: 1/30 moléc de agua. En MP2: 1/8 moléc. de agua.	Presentan 3 posiciones posibles de la molécula de agua: A, B, C. En B3LYP se obtuvieron: 1/30 moléc de agua. En MP2: 1/8 moléc. de agua.	Presentan 4 posiciones posibles de la molécula de agua: A, B, C, D. En B3LYP se obtuvieron: 1/30 moléc de agua. En MP2: 1/8 moléc. de agua.

Tabla 9.5.2. Hidratación de los tautómeros HX-19, HX-17 y ALO-15 (continuación)

	+20 H ₂ O	+10 H ₂ O	+20 H ₂ O	10 H ₂ O	+20 H ₂ O	+10 H ₂ O
$\Delta E_{B-W_n}^{CP}$ (en kcal/mol)	-88.228	-10.428	2.719	-68.493	-67.381	-74.878
$\Delta E_{W_n}^{CP}$ (en kcal/mol)	-198.835	-129.049	-285.974	-68.568	-227.418	-51.180
$\Delta E_{B(W_n)}^{CP}$ (en kcal/mol)	-287.063	-139.477	-283.255	-133.207	-254.799	-126.058

siguiendo el mismo procedimiento tanto en MP2 como en B3LYP, se obtuvieron los monohidratos de todos los tautómeros, pero solamente se desarrolló la hidratación de ALO-15 por la pequeña población de los demás.

Tabla 9.6. Estado sólido de las moléculas.

Molécula	HX-19	ALO-15	INO conf. A1	ACV conf. A1
Estructura sólida más próxima a la experimental	Tetrámero	Heptámero	Pentámero	Tetrámero

Se ha simulado el sólido de cada molécula mediante diferentes agrupaciones: dímero, trímero....., las que presentan una mayor concordancia con los valores experimentales vienen indicadas en el Tabla 9.6. Las estructuras que concuerdan más con los valores experimentales son las correspondientes a agrupaciones de mayor número de moléculas.

Tabla 9.7. Algunas relaciones espectrales de los tautómeros (confórmeros) más estables.

Moléculas	HX-19	HX-17	INO conf. A1	ACV conf A1
B3LYP	Buena concordancia del espectro calculado con los valores experimentales.	Las frecuencias calculadas presentan buena concordancia con las experimentales.	Presenta frecuencias calculadas características de HX (experimental).	Presenta frecuencias calculadas características de G.

Los espectros obtenidos presentan unos valores esperados respecto de los datos experimentales. En el capítulo 7.1. se han estudiado los espectros de los tautómeros de la hipoxantina, los espectros estudiados corresponden a HX-17 y HX-19 que son los tautómeros más estables y abundantes y HX-70 como forma enol más abundante. Los cálculos se han realizado en los niveles de cálculo B3LY/6-31G(d,p), B3LYP/6-311++G(3df,pd) y MP2, que presentan buena concordancia con los valores experimentales.

En el capítulo 7.3. se tiene una breve exposición de las frecuencias del confórmero A1 de INO concuerda con las frecuencias experimentales correspondientes a HX-19, estructura presente en la molécula.

En el capítulo 7.4. se comparan las frecuencias de ACV calculadas en B3LYP/6-31G(d,p) con las obtenidas experimentalmente en la guanina, se observa buena concordancia en las frecuencias pero la intensidad presenta valores con ciertas diferencias.

10. Resumen

Se parte de una introducción inicial de los componentes de los ácidos nucleicos y su caracterización, y de una introducción al estudio de la química computacional, destacando la optimización de las estructuras moleculares sobre las que se trabaja, criterios de convergencia y cálculo de las frecuencias. Para ello, se realiza un resumen de los métodos cuánticos más generalizados centrado principalmente en los métodos basados en la Teoría del Funcional de la Densidad y en MP2. Para la descripción de la hidratación de las moléculas, se empleó el modelo explícito con la corrección, en los cálculos de la energía, por BSSE (Basis Set Superposition Error) usando el procedimiento CP (Counterpoise Procedure).

1. Estudio de las moléculas hipoxantina y alopurinol

En los trabajos realizados sobre las dos bases HX y ALO, en primer lugar, se obtuvieron en ambos casos los tautómeros más estables y se estudió su geometría y su energía a diferentes niveles de cálculo, partiendo de MP2 como referencia. En HX los tautómeros más estables son HX-19 y HX-17, y en éstos se calculó la hidratación determinando las posiciones más favorables para las moléculas de agua (tres en total en ambos).

En ALO el principal tautómero es ALO-15 seguido, muy alejado el ALO-25. Las posiciones favorables son cuatro. En todos los tautómeros este cálculo se realizó desde una molécula de H₂O hasta 30, obteniéndose una primera esfera de hidratación con 13 H₂O para HX y 12 para ALO-15, que es el tautómero más estable.

Los cálculos realizados en los monohidratos de HX-19, HX-17, ALO-15 y ALO-25 fueron con la corrección de energía (CP). Se determinaron las energías de deformación de la molécula, y de la primera molécula de agua en cada una de las posiciones estudiadas, así como la fuerza de los enlaces de H, que nos indica la posición del monohidrato más estable en B3LYP y MP2. De la misma forma se calcularon la energía de deformación, de interacción y la energía de formación en los complejos de HX-19 y HX-17 y ALO-15 con 10 y 20 moléculas de agua.

Se realizó el estudio de los momentos dipolares en la molécula aislada y en los hidratos más estables en B3LYP y MP2 (en este nivel se obtuvieron solamente los valores de los hidratos hasta 8 moléculas de agua por la dificultad de los cálculos en MP2).

Se realizó un estudio de las longitudes de enlace de los hidratos en relación con el número de moléculas de agua añadidas, observando que éstas se estabilizan cuando el número de H₂O es superior a 10.

La simulación del estado sólido se realizó preferentemente en el tautómero HX-19, siguiendo la información obtenida de la bibliografía, y mediante las estructuras del dímero, trímero, tetrámero y pentámero, con sus enlaces de H correspondientes. Se obtuvo el tetrámero como forma más próxima a la experimental. En ALO-15 el sólido se simuló mediante la estructura del heptámero observándose una gran similitud con el experimental.

En HX se estudió el espectro comparando los valores teóricos de los números de onda de los tautómeros más estables y demás datos, con los valores experimentales obtenidos en la bibliografía.

2. Estudio de las moléculas inosina y aciclovir

Se han estudiado estas dos moléculas, ya que la primera trata de un nucleósido derivado de HX. La segunda, porque a pesar de su estructura próxima a dG, ésta presenta también bastantes coincidencias respecto a INO (la cadena lateral se considera análoga al azúcar).

Un estudio de los tautómeros de la INO indica que de los cinco posibles, el más estable es N1. Se han estudiado 69 conformeros de este tautómero obtenidos por rotación de sus ángulos exocíclicos χ , γ , β , ε y ε' . Se obtuvieron tres tipos de conformeros según el valor del ángulo χ con valores de β y $\gamma \sim 60^\circ$, A, B y C. El de menor energía es A1, y respecto de éste se han calculado las demás energías. Se obtienen 7 conformeros cuya energía es inferior a 3 kcal/mol: A1, A2, A3, A4, A5 y B1 y B2. ΔG presentan una variación análoga. Al realizar los cálculos "single point" en MP2 el conformero de menor energía es B1.

En ACV se han caracterizado también cinco tautómeros y el más estable y abundante es N1. El análisis de las energías de este conformero indica su dependencia del momento dipolar y del ángulo torsional φ_1 . Del tautómero N1 se obtienen 78 conformeros, de los que se ha realizado un amplio estudio por rotación de los ángulos torsionales exocíclicos φ_1 , φ_2 , φ_3 , φ_4 y φ_5 . Con el fin de determinar la posición de la cadena lateral se ha definido el vector R (N9...O5'), el ángulo β (C4-N9...O5') y el ángulo Φ (C1'-N9...O5').

Los conformeros se clasificaron según el valor del ángulo φ_1 : conformeros A si su valor es negativo y B si es positivo. Los 78 conformeros optimizados caen dentro de un rango de la energía electrónica $\Delta E = 0-8.5$ kcal·mol⁻¹ y la energía de Gibbs $\Delta G = 0-6.6$ kcal·mol⁻¹. Del estudio realizado solamente cuatro conformeros tienen una energía

relativa menor que 1kcal/mol A1, A2, B1 y B2, que son también los más abundantes y tienen los enlaces intramoleculares fuertes.

En INO se ha estudiado de forma especial la parte de la molécula correspondiente a HX, analizando su planaridad y su estructura (longitudes de enlace y ángulos de enlace), y comparándola con la de los valores obtenidos experimentalmente. Se ha observado un cierto carácter aromático en la molécula. El anillo de furanosa, se sitúa fuera del plano del anillo de HX, con ángulo torsional N9-C1'-C2'-C3' de 155.2° en el conformero A1.

Las mayores cargas negativas corresponden a O2', O3' y O6 y la carga positiva más alta corresponde al átomo C6.

En los seis conformeros más estables y en la parte de la molécula correspondiente a guanina en ACV se ha determinado una pequeña no planaridad < de 1°. La mayor no planaridad corresponde al conformero A2. Se han comparado las longitudes de enlace con las de la guanina observándose ciertas diferencias, siendo la mayor en C6-N1 (0.051 Å). Estas variaciones son posiblemente debidas a la presencia de la cadena lateral. Los átomos con mayor carga negativa son N2 y O5' con cargas de -0.90 y -0.84 *e* respectivamente en el nivel MP2, y el siguiente es N1. La mayor carga positiva en C6 en el nivel MP2.

Respecto de la cadena lateral se han definido además de los ángulos exocíclicos φ_1/φ_5 , el vector R (N9...O5') que determina la distancia del grupo OH de la base, el ángulo β (C4-N9...O5') que define el ángulo del grupo OH respecto del plano de la base, y el ángulo Φ (C1'-N9...O5') que determina la posición del grupo OH.

El estado sólido de INO se ha representado mediante el dímero, trímero y pentámero, siendo esta última estructura la que presenta una mayor concordancia con los valores experimentales. En ACV el estado sólido se ha representado mediante el dímero y el tetramero siendo esta última estructura la más concordante con los valores experimentales

11. Conclusiones generales

1. Se han identificado los tautómeros más estables por B3LYP y MP2, en la molécula de **HX**: HX-17 (88.8% de población) y HX-19 (19.1%), **ALO**: ALO-15 (98.9%), en **INO**: (N1) y en **ACV** (N1).
2. Al comparar las moléculas optimizadas de HX-19 con los valores experimentales se observan ciertas diferencias, con un alargamiento de C5-C6 y C6-N1 y un acortamiento de C=O.
3. HX-17 presenta algunas diferencias estructurales significativas respecto de HX-19, debido al cambio de un H de N9 a N7.
4. Las mayores cargas negativas se sitúan en O6 y N1 en todos los tautómeros y a todos los niveles de cálculo mientras que la carga positiva aparece en C6 y C2.
5. HX-19 y HX-17 presentan tres posiciones favorables a la hidratación. La primera esfera de hidratación se completa con 13 moléculas de agua alterándose la carga sobre O6, N1 y C5. La segunda esfera prácticamente no altera las cargas. Las longitudes de enlace se alargan al aumentar el número de moléculas de agua pero a partir de 15 se estabilizan.
6. ALO -15 completa la primera esfera de hidratación con 12 moléculas de agua. Las longitudes de enlace presentan una variación similar a HX.
7. Se han identificado 69 conformeros en la molécula de INO y en su tautómero más estable A1. Éstos se clasificaron según el valor del ángulo χ : **A** ($\chi \sim 175^\circ$) (*high-anti*), son los más estables, **B** ($\chi \sim 70^\circ$) (*syn*), y **C** ($\chi \sim 120^\circ$) (*anti*). Todos aparecieron dentro de un rango de energía relativa de 0-10.749 kcal/mol.
8. Se obtuvieron 78 conformeros estables del tautómero N1 de ACV, clasificados según el valor del ángulo φ_1 en conformeros A y B, y dentro de un rango de energía 0-8.451 kcal/mol. El conformero más estable fue A1 ($\varphi_1 = -75.3^\circ$).
9. El estado sólido se simuló en HX principalmente mediante el pentámero que es el tautómero identificado experimentalmente en estado sólido. ALO-15 se representó mediante el heptámero que tiene una estructura análoga a la obtenida mediante rayos X.
10. En INO se representó mediante la figura del pentámero, que concuerda con el experimental y en ACV se simuló mediante el dímero y el tetrámero que presentan una buena concordancia con los valores experimentales.

12. Lista de abreviaturas y acrónimos

5-CIU	5-clorouracilo
6-CIU	6-Clorouracilo
A	Adenina
ACV	Aciclovir
AIDS	Acquired immunodeficiency syndrome
AM1	Austin Model 1
ATP	Trifosfato 5' de adenina
ALO	Alopurinol
a.u.	atomic units
AZT	Acidotimidina
B3LYP	Becke 3 parameter hybrids & Lee-Yang-Parr correlation
BDZ	Benzodiazepina
BSSE	Basis Set Superposition Error
C	Citosina
CLOA	Combinación Lineal de Orbitales Atómicos o aproximación
CP	Counterpoise procedure
D4T	Stavudine
DFT	Density Functional Theory (Método del funcional de la densidad)
dG	Deoxiguanosina
DNA	Ácido desoxirribonucleico
dT	Desoxitimidina
dU	Desoxiuridina
DZ	Double-Zeta basis set
E	Energía
E_{elec}	Energía electrónica ϕ
E_{nuc}	Energía nuclear
E_{tot}	Energía total
$E(RB+HF-LYP)$	
EM	Explicit model
FF	force field
ϕ	función base
Ψ	Funcion de ondas
Ψ_{elec}	Función de ondas electrónica
G	Guanina
\mathbf{H}	Operador Hamiltoniano
H_{elec}	Hamiltoniano electrónico
H	Entalpía
H-bond	hydrogen bonding
HF	Método de Hartree Fock
HOMO	Highest Occupied Molecular Orbital
HSV-2	Herpes genital
HIV	Virus de inmunodeficiencia humano
HX	Hipoxantina
INO	Inosina
IR	Infra rojos
LCAO	Linear Combination Atomic Orbitals

IPCM	Isodensity Surface Polarized Continuum Model
IMP	Inosina monofosfato
LDA	Local Density Approximation
LUMO	Lowest Unoccupied Molecular Orbital
MC	Monte Carlo
MM	Molecular Mechanics; Major Major groove
MO	Molecular Orbital
MP	Møller-Plesset perturbation theory
MSM	Modified Scheme of Monosolvation
MST	Miertus-Scrocco-Tomasi method
NBO	Natural Bond Orbital
NLO material	Non linear optical material
NPA	Natural Population Analysis
OA	Orbitales Atómicos
PCM	Polarized Continuum Model
PES	Superficie de energía Potencial
pol	polarización
pot	potencial
QM	Química cuántica
RHF	Restricted Hartree-Fock method
RMSd (o r.m.s.d)	Root Mean Square deviation
RNA	Ácido ribonucleico
S	Entropía
SCF	Self Consistent Field
SCRF	Self-consistent Reaction Field
STO	Slater Type Orbital
T	Timina
T	Temperatura
TZ	Triple-Zeta basis set
U	Uracilo
UHF	Unrestricted Hartree-Fock method
UV	Ultravioleta
VIS	Visible
XR	Rayos X
ZDO	Zero differential overlap
ZPE	Zero Point Energy

13. Anexos

Tabla 13.7.1.8.

Distancias	B3LYP/6-31G(d, p)	B3LYP/6-311++G(3df, pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
N1 – C2	1.355	1.349	1.365	1.363
C2 – N3	1.362	1.320	1.329	1.326
N3 – C4	1.346	1.339	1.351	1.347
C4 – C5	1.410	1.404	1.408	1.406
C5 – C6	1.394	1.389	1.398	1.395
C6 – N1	1.324	1.317	1.321	1.317
C6 – O	1.348	1.346	1.354	1.352
C2 – H	1.087	1.084	1.082	1.085
C5 – N7	1.379	1.375	1.367	1.366
O - H	0.972	0.969	0.971	0.972
N7 – C8	1.374	1.969	1.377	1.377
C8 – N9	1.312	1.307	1.319	1.317
N9 – C4	1.382	1.377	1.382	1.381
N7 – H	1.008	1.007	1.008	1.011
C8 – H	1.082	1.079	1.078	1.081

Tabla 13. 7.1.9: Ángulos (en grados) del tautómero HX-70 en todos los niveles de cálculo.

Ángulos	B3LYP/6-31G(d, p)	B3LYP/ 6-311++G(3df,pd)	MP2/6-31G(d, p)	MP2/6-311++G(2d,p)
N-C2-N	128.1	127.6	128.2	127.8
C-N3-C	113.6	114.0	113.4	113.7
N3-C4-C5	122.9	122.8	123.0	122.9
C4-C5-C6	117.9	117.9	118.2	118.1
C5-C6-N1	119.5	119.4	119.4	119.5
C6-N1-C	117.9	118,3	117.8	118.1
C5-N7-C8	105.7	105.8	106.0	105.9
N7-C8-N	114.1	113.9	113.9	113.7
C8-N9-C	104.5	104.8	104.0	104.4
N9-C4-C	110.1	109.8	110.3	110.0

Tabla 13.7.1.10: Parámetros geométricos del tautómero HX-90 cis de la hipoxantina, longitudes de enlace (Å).

Distancias	B3LYP/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
N1 – C2	1.346	1.341	1.355	1.353
C2 – N3	1.334	1.327	1.335	1.332
N3 – C4	1.337	1.331	1.342	1.338
C4 – C5	1.407	1.402	1.406	1.404
C5 – C6	1.404	1.398	1.405	1.402
C6 – N1	1.333	1.326	1.330	1.327
C6 – O	1.339	1.335	1.345	1.342
C2 – H	1.087	1.084	1.082	1.085
O - H	0.972	0.969	0.971	0.972
C5 – N7	1.385	1.381	1.380	1.379
N7 – C8	1.309	1.303	1.324	1.323
C8 – N9	1.382	1.377	1.373	1.372
N9 – C4	1.375	1.371	1.374	1.374
N9 – H	1.009	1.007	1.008	1.011
C8 – H	1.082	1.079	1.078	1.081

Tabla 13.7.1.11: Ángulos (en grados) del tautómero HX-90 cis en todos los niveles de cálculo.

N-C2-N	127.9	127.3	127.9	127.5
C-N3-C	111.7	112.2	111.3	111.7
N3-C4-C5	127.2	126.9	127.5	127.2
C4-C5-C6	114.5	114.7	114.6	114.7
C5-C6-N1	120.1	119.9	120.2	120.0
C6-N1-C	118.6	119.1	118.6	118.9
C5-N7-C8	103.9	104.3	103.2	103.5
N7-C8-N	113.8	113.5	113.9	113.7
C8-N9-C	106.6	106.6	106.7	106.7
N9-C4-C	104.5	104.7	104.4	104.6
C4-C5-N7	111.2	110.9	111.8	111.5

Tabla 13. 7.1.12: Parámetros geométricos del tautómero HX-90-trans de la hipoxantina, longitudes de enlace (Å).

Distancias	B3LYP/6-31G(d, p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d, p)	MP2/6-311++G(2d,p)
N1 – C2	1.346	1.341	1.355	1.353
C2 – N3	1.338	1.331	1.340	1.337
N3 – C4	1.335	1.329	1.341	1.337
C4 – C5	1.401	1.396	1.400	1.398
C5 – C6	1.404	1.398	1.403	1.401
C6 – N1	1.328	1.321	1.327	1.322
C6 – O	1.343	1.339	1.348	1.345
C2 – H	1.087	1.084	1.082	1.085
°O - H	0.971	0.968	0.970	0.972
C5 – N7	1.384	1.381	1.380	1.379
N7 – C8	1.311	1.305	1.326	1.325
C8 – N9	1.381	1.376	1.372	1.372
N9 – C4	1.378	1.374	1.377	1.377
N9 – H	1.009	1.008	1.008	1.011
C8 – H	1.082	1.079	1.078	1.081

Tabla 13.7.1.13: Ángulos (en grados) del tautómero HX-90 trans en todos los niveles de cálculo.

Ángulos	B3LYP/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d, p)	MP2/6-311++G(2d,p)
N-C2-N	128.6	128.0	128.8	128.3
C-N3-C	111.5	112.0	111.0	111.5
N3-C4-C5	126.4	126.1	126.6	126.4
C4-C5-C6	115.7	115.8	115.8	115.9
C5-C6-N1	119.8	119.6	119.9	119.7
C6-N1-C	118.0	118.5	117.9	118.2
C5-N7-C8	103.8	104.2	103.1	103.4
N7-C8-N	113.6	113.3	113.7	113.5
C8-N9-C	106.7	106.8	106.9	106.8
N9-C4-C	104.3	104.5	104.1	104.4

Tabla 13.1.30. Número de ondas (ω cm^{-1}), intensidad IR absoluta (I), intensidad IR relativa (Ir) calculadas en el tautómero HX-17 y experimentales.

B3LYP/6-311++G (3df, pd)				IR Experimental, ref. 6, 55				
ω	I	Ir	Asignaciones teóricas Freq=intmodes	ν	I	Ir	Asignaciones ⁶	
1	159.3	14	2	9% $\delta(\text{C8N3C5C6})+11\%$ D(C4C5C6O)+9% $\delta(\text{C4C5C6N1})+8\%$ $\delta(\text{C6C5C4N9})+7\%$				
2	195.4	11	1	$\delta(\text{C6C5C4N3})+7\%$ $\delta(\text{C5C6N1C2})+9\%$ $\delta(\text{OC6N1C2})$ omp.				
3	276.2	5	1	9% $\delta(\text{C8N7C5C6})+9\%$ $\delta(\text{N7C5C6O})+10\%$ $\delta(\text{N7C5C6N1})+7\%$ $\delta(\text{C6C5C4N9})+9\%$				
4	307.2	16	2	$\delta(\text{C2N3C4N9})$ omp.				
5	516.0	3	0	8% $\delta(\text{C8N9C4N3})+9\%$ $\delta(\text{N7C5C4N3})+7\%$ $\delta(\text{C5C6N1C2})+8\%$ $\delta(\text{OC6N1C2})+9\%$				
6	523.7	79	10	$\delta(\text{C6N1C2N3})+8\%$ $\delta(\text{C6N1C2H})$ (omp).	524	17	15	Imidazolic ring
7	550.7	2	0	11% $\delta(\text{N3C5C6})+11\%$ $\delta(\text{C5C6O})+10\%$ $\delta(\text{OC6N1})+7\%$ $\delta(\text{N9C4N3})$	542/534	5/17	5/15	vib+(N7-H)vib (omp)
8	564.1	26	3	11% $\delta(\text{C5C6N1})+7\%$ $\delta(\text{C6N1C2})+7\%$ $\delta(\text{HN1C2})+10\%$ $\delta(\text{C2N3C4})$	558/553	17/10	15/9	rings vib.
9	613.7	7	1	15% $\delta(\text{N9C8N7H})+16\%$ $\delta(\text{HC8N7H})+17\%$ D(HN7C5C6)+15% D(HN7C5C4)(omp)	598	3	3	rings vib.
10	635.6	29	4	7% $\delta(\text{N7C5C6})+8\%$ $\delta(\text{C6C5C4})+9\%$ $\delta(\text{C5C6O})+8\%$ $\delta(\text{N3C4N9})/8\%$ $\delta(\text{C5C4N3})$	602	5	5	rings vib.
11	672.1	24	3	7% $\delta(\text{N9C8N7H})+9\%$ $\delta(\text{C5C6N1H})+9\%$ $\delta(\text{HN1C2N3})+9\%$ $\delta(\text{HN1C2H})$ omp	626/622	3/8	3/7	rings vib.+(N-H) vib.
12	710.1	3	0	11% $\delta(\text{C5C6N1H})+10\%$ $\delta(\text{OC6N1H})+12\%$ D $\delta(\text{HN1C2N3})+11\%$ $\delta(\text{HN1C2H})$ (omp)	657	10	9	(omp)
13	741.8	23	3	10% $\nu(\text{C6N1})+11\%$ $\nu(\text{N3C4})$	691	3	3	rings vib.+(N1-H)
14	807.0	10	1	8% $\delta(\text{N7C5C6O})+12\%$ $\delta(\text{OC6N1H})+7\%$ $\delta(\text{HN1C2N3})$ (omp)	725	11	10	vib. (omp)
15	874.2	12	2	7% $\delta(\text{N7C5C6O})+9\%$ $\delta(\text{C6C5C4N3})$ (omp)	775			rings vib.
16	902.0	3	0	20% $\delta(\text{C4N9C8H})+18\%$ $\delta(\text{HC8N7C5})+22\%$ $\delta(\text{HC8N7H})$ (omp)	860	4	4	rings vib.+(N-H) vib.
17	939.7	4	1	11% $\delta(\text{N1C2N3})+9\%$ $\delta(\text{C2N3C4})$				(omp)
18	960.8	1	0	17% D(C6N1C2H)+21% D(HN1C2H)+19% D(HC2N3C4)/omp				rings vib.+(N1-H)
19	1077.8	14	2	8% $\delta(\text{C8N9C4})+12\%$ $\delta(\text{N9C8N7})+9\%$ $\delta(\text{C8N7C5})+10\%$ $\delta(\text{C5N7H})$	1054	6	5	vib. (omp)
20	1096.7	40	5	9% $\nu(\text{N1C6})+8\%$ $\delta(\text{C8N7H})$	1062	8	7	rings vib.
21	1121.9	7	1	13% $\nu(\text{N7C8})+8\%$ $\delta(\text{HC8N7})+14\%$ $\delta(\text{C8N4H})+12\%$ $\delta(\text{C5N7H})$	1084	8	7	(N1-H)vib.+ rings vib.(omp)
22	1203.3	77	10	13% $\nu(\text{N1C2})+10\%$ $\delta(\text{C6N1C2})+11\%$ $\delta(\text{HN1C2})$				(C8-H) vib.+rings vib.(omp)
23	1290.1	1	0	8% $\delta(\text{N9C8H})+7\%$ $\delta(\text{HC8N7})$				(C2-H)vib.+pyrimidinic ring
24	1345.6	35	4	8% $\nu(\text{C8N9})+7\%$ $\nu(\text{N9C4})+13\%$ $\delta(\text{N9C8H})+11\%$ $\delta(\text{HC8N7})$	1324	11	10	vib.(omp)
25	1397.1	84	11	9% $\nu(\text{C4N9})+7\%$ $\nu(\text{C5N7})+7\%$ $\nu(\text{N3C4})+10\%$ $\delta(\text{N1C2H})+11\%$ $\delta(\text{N3C2H})$	1371	33	30	rings vib.+(C2-H)vib. +(N7-H)
26	1410.1	24	3	13% $\delta(\text{C8N7H})$	1384, 1381	18/18	16/16	vib.+v(C=O)
27	1433.6	23	3	13% $\delta(\text{N1C2H})+15\%$ $\delta(\text{N3C2H})$	1433	4	4	rings vib.+(N-H)vib. +v(C=O)
				8% $\delta(\text{C8N7H})+10\%$ $\delta(\text{C5N7H})+11\%$ $\delta(\text{C6N1H})+11\%$ $\delta(\text{HN1C2})$				2modes rings vib.+(NH)vib.+
								v(C=O)
								rings vib.+(C8-H) vib.+v(C=O)

28	1461.1	30	4	9% $\nu(\text{N9C8})+9\% \delta(\text{N9C8H})+9\% \delta(\text{N9C8H})+9\% \delta(\text{HC8N7})$					
29	1539.2	18	2	10% $\nu(\text{C4C5})+10\% \nu(\text{N3C4})$					
30	1551.1	25	3	8% $\delta(\text{C8N7H})+8\% \delta(\text{C5N7H})$					
31	1633.9	69	9	11% $\nu(\text{C2N3})+9\% \delta(\text{N1C2H})+7\% \delta(\text{N3C2H})$	1595	22	20	rings vib.+(C2-H)vib. + $\delta(\text{N1-H})$ vib.+ $\nu(\text{C=O})$	
32	1765.6	781	100	12% $\nu(\text{C5C6})+16\% \nu(\text{C=O})+10\% \delta(\text{C6N1H})+7\% \delta(\text{C5C6O})$	1735	110	100	$\nu(\text{C=O})$ +rings vib.+(N1-H)vib.	
33	3176.8	5	1	76% $\nu(\text{C2-H})$					
34	3248.1	1	0	76% $\nu(\text{C8-H})$					
35	3595.0	67	9	78% $\nu(\text{N1H})$	3428	30	27	$\nu(\text{N1-H})$	
36	3650.2	97	12	77% $\nu(\text{N7-H})$	3464*	35	32	$\nu(\text{N7-H})$	

Las frecuencias experimentales de HX (aislada)^{6,55} a temperatura T = 11K.

ν = tension; δ = Flexión simétrica fuera del plano τ = torsion; γ = movimiento fuera del plano; (omp) = fuera del plano molecular. * se sitúa también en el tautómero HX-19⁵⁷.

Tabla 13.1.31. Número de ondas del tautómero HX-70 (cm^{-1}) en B3LYP y experimental

BLYP/6-31G(d,p)				Experimental			
ω	I	Ir	Asignaciones teóricas Freq=Intmodes	ν^{55}	I	Ir	Asignaciones ⁵⁵
1	170.2	4	3	10% D(C8,N7,C5,C6)+12% D(C4,C5,C6,O10)+9% D(C4,C5,C6,N1)+8% D(C6,C5,C4,N9)+8% DC6,C5,C4,N3) (omp)			
2	217.3	0	0	10% D(C8,N7,C5,C6)+10% (N7,C5,C6,O10)+11% D(N7,C5,C6,N1)+9% D(C2,N3,C4,N9) (omp)			
3	291.4	0	0	10% A(N7,C5,C6)+13% A(C5,C6,O10)+ 12% A(O10,C6,N1)			
4	309.2	4	3	9% D(C8,N9,C4,N3)+9% D(N7,C5,C4,N3)+9% D(N7,C6,N1,C2) (omp)			
5	461.5	0	0	16% D(N9,C8,N7,H)+17% D(H,C8,N7,H)+20% D(H,N7,C5,C6)+ 17% D(H,N7,C5,C4) (omp)			
6	528.3	2	2	8% D(H,N7,C5,C6)+27% D(C5,C6,N7,H)+25% D(N1,C6,O10,H)(omp)			
7	536.9	130	100	9% A(6,5,4)+9% A(C5,C6,O10)+13% A(5,6,1)/9%A(2,3,4)			
8	545.9	1	1	8% A(7,5,6)+9% A(5,6,10)+9% A(10,6,1)+11% A(9,4,3)+ 9% A(5,4,3)			
9	582.7	1	1	8% D(7,5,6,10)+8% D(6,1,2,3)+10% D(1,2,3,4) (omp)			
10	618.3	1	1	7% R(5,6)+7% A(7,5,4)	626		ρOH
11	641.7	1	1	9% D(9,8,7,H)+8% D(H,8,7,1H) (omp)			
12	695.2	2	2	8% D(7,5,6,10)+8% D(1,6,10,H) (omp)			
13	729.3	2	2	9% R(3,4)			
14	801.4	4	3	8% D(6,5,4,3) (omp)			
15	880.6	1	1	9% A(6,1,2)+12% A(1,2,3)+10% A(2,3,4)			
16	890.7	4	3	20% D(4,9,8,H)+18% D(H,8,7,5)+21% D(H,8,7,H) (omp)			
17	948.6	4	3	8% R(5,4)+8%A)8,9,4)+12% A(9,8,7)+9% A(8,7,5)+ 10% A(5,4,13)			
18	968.1	1	1	9% D(C6,N1,C2,N3)+24% D(C6,N8,C2,H)+8% D(N1,C2,N3,C4)+25% D(H,C2,N3,C4) (omp)			
19	1078.2	2	2	10% ν A(C8,N7,H)			

20	1109.5	2	2	13% R(C8,N7)+11% A(C8,N7,H)+8% A(C6,O10,H)		
21	1157.5	3	2	11% A(C6,O10,H)	1168	δ C-OH
22	1269.6	55	42	12% A(N9,C8,H)+11% A(H,C8,N7)+A(C6,O10,H)		
23	1300.1	5		12% R(N1,C2)		
24	1337.4	3		8% (R(N7,C5)+10% R(C2,N3)+13% A(C6,O10,H)		
25	1369.9	2		10% A(C6,O10,H)+14 %A(N1,C2,H)+14% A(N3,C2,H)	1381	vC-OH
26	1395.3	7		9% R(N9,C4)+8% R(C5,C4)	1384	vC-OH
27	1411.2	5		8% R(N7,C5)		
28	1434.7	3		9% R(C8, N7)+8% A(N9,C8,H)+13% A(C8,N7,H)+15% A(C5,N7,H)		
29	1524.1	4		13% A(N1,C2,H)+12% A(N3,C2,H)		
30	1541.4	5		11% R(N9,C8)+9% A(N9,C8,H)+12% A(H,C8,N7)+9% A(C8,N7,H)		
31	1605.8	16		9% R(C5,C4)+8% R(C6N1)+10% R(N3,C4)		
32	1702.7	13		12% R(C5,C6)		
33	3189.7	7		79% R(C2,H)		
34	3256.6	7		76% v(C8,H)		
35	3672.4	9		78% v(N7,H)		
36	3756.9	9		93% v(O10,H)	3575	v-OH

v = tensión; δ = flexión simétrica fuera del plano; τ = torsion; ρ = flexion asimétrica en el plano; γ = movimiento fuera del plano; (omp) = fuera del plano molecular; ms = medio fuerte, s= fuerte y vw = muy déb

Tabla 13.1.32. Número de ondas IR escaladas (cm^{-1}) de HX-70 y las asignaciones teóricas.

	B3LYP/ 6-31G(d,p)				B3LYP/6-311++G(3df,pd)				MP2 6-31G(d,p)			$\omega^{v(3)}$ (cm^{-1})	Asignaciones teóricas (Freq=intmodes*)
	ω (cm^{-1})	ω^a	$\omega^{v(1)}$ (cm^{-1})	$\omega^{v(1)}$ (cm^{-1})	ω (cm^{-1})	ω^a	$\omega^{v(2)}$ (cm^{-1})	ω^I (cm^{-1})	ω (cm^{-1})	ω^a	$\omega^{v(3)}$ (cm^{-1})		
1	170.2	169.1	195.4	163.6	168.3	167.3	192.0	162.9	158.8	157.8	183.3	149.7	10%D(C8,N7,C5,C6)+12% D(C4,C5,C6,O10)+9%D(C4,C5, C6,N1)+8%D(C6,C5,C4,N9)+8%DC6,C5,C4,N3) (omp)
2	217.3	216.0	239.9	208.9	215.8	214.5	237.2	208.9	207.9	206.6	229.3	196.0	10%D(C8,N7,C5,C6)+10%(N7,C5,C6,O10)+11% D(N7,C5,C6,N1)+9% D(C2,N3,C4,N9) (omp)
3	291.4	289.6	309.9	280.1	294.1	292.3	311.6	284.7	285.9	284.1	302.4	269.5	10%A(N7,C5,C6)+13%A(C5,C6,O10)12%A(O10, C6,N1)
4	309.2	307.3	326.7	297.2	305.9	304.0	322.9	296.1	301.7	299.8	317.3	284.4	9%D(C8, N9,C4,N3)+9%D(N7,C5,C4,N3)/ 9%D(N7,C6,N1,C2) (omp)

5	461.5	458.6	470.6	443.6	455.1	452.3	464.8	440.5	461.4	458.5	466.9	435.0	16%D(N9, C8,N7,H)+17%D(H,C8,N7,H)+20%D(H,N7,C5,C 6) 17%D(H,N7,C5,C4) (omp)+
6	528.3	525.0	533.7	507.9	519.5	516.3	526.0	502.8	516.6	513.4	518.7	487.0	8%D(H, N7,C5,C6)+27%D(C5,C6,N7,H)+25%D(N1,C6,O 10,H)(omp)
7	536.9	533.6	541.8	516.1	531.2	527.9	537.2	514.1	526.5	523.2	527.9	496.3	9%A(6,5,4)+9%A(C5,C6,O10)+13%A(5,6,1)+9% A(2,3,4)
8	545.9	542.5	550.3	524.8	548.2	544.8	553.3	530.6	540.3	537.0	540.9	509.3	8%A(7,5,6)+9%A(5,6,10)+9%A(10,6,1)/11%A(9,4 ,3)+9%A(5,4,3)
9	582.7	579.1	585.1	560.1	588.1	584.5	591.3	569.2	551.4	548.0	551.3	519.8	8%D(7,5,6,10)+8%D(6,1,2,3)+10%D(1,2,3,4) (omp)
10	618.3	614.5	618.7	594.4	619.3	615.5	621.0	599.4	617.0	613.2	612.8	581.6	7%R(5,6)+7%A(7,5,4)
11	641.7	637.7	640.8	616.9	640.5	636.5	641.1	619.9	622.6	618.7	618.0	586.9	9%D(9,8,7,H)+8%D(H,8,7,1H) (omp)
12	695.2	690.9	691.6	668.3	706.2	701.8	703.6	683.5	654.2	650.1	647.6	616.7	8%D(7,5,6,10)+8%D(1,6,10,H) (omp)
13	729.3	724.8	723.6	701.1	727.6	723.1	724.0	704.2	693.8	689.5	684.7	654.0	9%R(3,4)
14	801.4	796.4	791.7	770.4	823.9	818.8	815.6	797.5	729.3	724.8	718.0	687.5	8%D(6,5,4,3) (omp)
15	880.6	875.1	866.5	846.5	890.5	885.0	878.9	861.9	842.7	837.5	824.3	794.4	9%A(6,1,2)+12%A(1,2,3)+10%A (2,3,4) +20%D(4,9,8,H)+18%D(H,8,7,5)+
16	890.7	885.2	876.0	856.2	892.4	886.9	880.8	863.8	892.5	887.0	871.0	841.4	21%D(H,8,7,H) (omp)
17	948.6	942.7	930.7	911.9	950.8	944.9	936.3	920.3	939.1	933.3	914.6	885.3	8%R(5,4)+8%A(8,9,4)+12%A(9,8,7)+9%A(8,7,5) +0%A(5,4,13)
18	968.1	962.1	949.2	930.6	976.9	970.8	961.1	945.5	953.3	947.4	927.9	898.7	9%D(C6, N1,C2,N3)+24%D(C6,8N1C2,H)+8%D(N1,C2,N3, C4)+25%D(H,C2,N3,C4) (omp)
19	1078.2	1071.5	1053.2	1036.5	1072.7	1066.0	1052.3	1038.3	1080.3	1073.6	1047.0	1018.4	10%A(C8,N7,H)
20	1109.5	1102.6	1051.0	1066.6	1102.3	1095.5	1080.4	1066.9	1123.2	1116.2	1087.2	1058.8	13%R(C8,N7)+11%A(C8,N7,H)+8%A (C6,O10,H)
21	1157.5	1150.3	1128.1	1112.7	1150.9	1143.8	1126.6	1114.0	1167.3	1160.1	1128.5	1100.4	11%A(C6,O10,H)
22	1269.6	1261.7	1234.0	1220.5	1260.8	1253.0	1231.2	1220.3	1286.8	1278.8	1240.5	1213.1	12%A(N9,C8,H)+11%A(H,C8,N7)+A(C6,O10,H)
23	1300.1	1292.0	1262.8	1249.8	1285.3	1277.3	1254.5	1244.0	1313.3	1305.2	1265.3	1238.0	12%R(N1,C2)
24	1337.4	1329.1	1298.0	1285.6	1322.2	1314.0	1290.2	1279.8	1345.8	1337.5	1295.8	1268.7	8%(R(N7,C5)+10%R(C2,N3)+13%A(C6,O10,H)
25	1369.9	1361.4	1328.7	1316.9	1357.4	1349.0	1323.1	1313.8	1388.7	1380.1	1336.0	1309.1	10%A(C6,O10,H)+14%A(N1,C2,H)+14%A(N3,C 2,H)
26	1395.3	1386.6	1352.7	1341.3	1378.4	1369.9	1343.0	1334.2	1436.2	1427.3	1380.5	1353.9	9%R(N9,C4)+8%R(C5,C4)
27	1411.2	1402.5	1367.8	1356.6	1399.2	1390.5	1362.8	1354.3	1444.3	1435.3	1388.1	1361.5	8%R(N7,C5)

28	1434.7	1425.8	1390.0	1379.2	1419.5	1410.7	1382.1	1373.9	1475.2	1466.1	1417.1	1390.7	9%R(C8,N7)+8%A(N9,C8,H)+ 13%A(C8,N7,H)+15%A(C5,N7,H)
29	1524.1	1514.7	1474.4	1465.1	1509.4	1500.0	1467.6	1460.9	1534.2	1524.7	1472.4	1446.3	13%A(N1,C2,H)+12%A(N3,C2,H)
30	1541.4	1531.8	1490.8	1481.7	1528.6	1519.1	1485.9	1479.5	1554.0	1544.4	1490.9	1465.0	11%R(N9,C8)+9%A(N9,C8,H)+12%A(H,C8,N7) +9%A(C8,N7,H)
31	1605.8	1595.8	1551.6	1543.7	1589.1	1579.2	1552.3	1538.1	1632.7	1622.6	1564.7	1539.1	9%R(C5,C4)+8%R(C6N1)+10%R(N3,C4)
32	1702.7	1692.1	1643.1	1636.8	1685.5	1675.0	1635.1	1631.4	1736.0	1725.2	1661.5	1636.5	12%R(C5,C6)
33	3189.7	3169.9	3047.9	3066.3	3179.4	3159.7	3056.1	3077.3	3275.1	3254.8	3103.9	3087.4	79%R(C2,H)
34	3256.6	3236.4	3111.1	3130.6	3241.8	3221.7	3115.5	3137.7	3331.7	3311.0	3157.0	3140.8	76%v(C8,H)
35	3672.4	3649.6	3503.9	3530.3	3660.3	3637.6	3513.6	3542.8	3731.0	3707.9	3531.2	3517.2	78%v(N7,H)
36	3756.9	3733.6	3583.7	3611.5	3753.3	3730.0	3602.0	3632.8	3813.7	3790.1	3608.7	3595.2	93%v(O10,H)

V= escalado (Palafox, M. A.; Iza, N. y Gil, M.) [$v^{(1)} = 34.6 + 0.9447*v$] y [$v^{(2)} = 31.9 + 0.9512*v$] y [$v^{(3)} = 34.5 + 0.9372*v$]
v = forma escalada (Gaussian 2003) $v^{(1)} = 0.9613*v$ y [$v^{(3)} = 0.9427*v$]
vI = escalado (Gaussian 2003) *Int. J Mol. Sci.* 2007.8. 760 – 776
a = escalado (*J.Phys. Chem. A* 1997 (101) p. 8309 – 8318)

Tabla 13.1.33 . Número de ondas (cm^{-1}) calculadas en B3LYP/6-311++G(3df,pd). $T = 11K$ del tautómero HX-37.

BLYP6/311++G(3df, pd)

ω (cm^{-1})		IR Intensidad absoluta	IR Intensidad relativa	Asignaciones teóricas Freq=intmodes
133.4	1	5	1	12% D(C4C5C6O)+11% D(C4C5C6N1)+8% D(C5C6N1C2)+9% D(OC6N1C2)(omp)
179.9	2	0	0	10% D(C8N7C5C6)+8% D(HN7C5C6)/+ % D(N7C5C6O)+9% D(N7C5C6N1)+8%D(C6C5C4N9)+9% D(C2N3C4N9)/(omp)
302.1	3	16	3	11% δ (N7C5C6)+9% δ (C5C6O)+8% δ (OC6N1)+9% δ (N9C4N3)
303.8	4	21	3	10%D (C8N9C4N3)+10% D(N7C5C4N3)+8% D(OC6N1C2)+7% D(C6N1C2N3)(omp)
467.5	5	38	6	10% D(N1C2N3H)+11% D(HC2N3H)+14% D(HN3C4N9)+12% D(HN3C4C5)(omp)
508.3	6	6	1	11% δ (C5C6N1)+10% δ (C6N1C2)+9% C2N3C4)+% δ (N9C4N3)+8% δ (C5C4N3)
535.6	7	3	0	8% δ (C6C5C4)/+ 0% δ (C5C6O)
562.9	8	127	20.	13% D(N9C8N7H)+13% D(HC8N7H)+16% D(HN7C5C6)+13% D(HN7C5C4)/(omp)
580.6	9	17	3	13% D(N1C2N3H)+12% D(HC2N3H)+10% D(HN3C4N9)+10% D(HN3C4C5)(omp)

631.9	10	1	0	8% δ (OC6N1)
658.4	11	7	1	11% D(N9C8N7H)+10% D(HC8N7H)+10% D(HN7C5C4)/(omp)
715.1	12	5	1	7% v(C5C6)+12% v(C6N1)+9% v(N3C4)
717.4	13	2	0	7% D(HN3C4N9)(omp)
810.5	14	8	1	9% D(N7C5C6O)+8% D(C4C5C6N1)+7% D(C6C5C4N3)+7% D(C5C6N1C2)/(omp)
858.5	15	14	2	19% D(C4N9C8H)+17% D(HC8N7C5)+ 22% D(HC8N7H)/(omp)
907.2	16	2	0	8% δ (C6N1C2)+11% δ (N1C2N3)+9% δ (C2N3C4)
959.2	17	5	1	19% D(C6N1C2H)+18% D(HC2N3C4)+22% D(HC2N3H)
965.7	18	6	1	8% δ (C8N9C4)+12% δ (N9C8N7)+10% δ (C8N7C5)+9% δ (C5N7H)
1037.1	19	53	8	13% v(C6N1)+8% δ (C5C6O)+8% δ (OC6N1)
1097.2	20	13	2	8%v(C8N7)+8% δ (HC8N7)+13% δ (C8N7H)+10% δ (C5N7H)/
1128.3	21	26	4	7% v(C8N7)+8% v(C2N3)+7% δ (C8N3H)+8% δ (C5N7H)+10% δ (C2N3H)+7% δ (C4N3H)
1209.1	22	30	5	11% δ (N9C8H)+9% δ (HC8N7)
1289.2	23	18	3	8% v(N9C8)+10% δ (N9C8H)+9% δ (HC8N7)+7% δ (C4N3H)
1337.8	24	4	1	11% v(N9C4)+8% v(N7C5)+9% δ (C2N3H)
1377.6	25	16	3	7% δ (C8N7H)+7% δ (C2N3H)
1415.6	26	36	6	7% (C6N1)+20% δ (N1C2H)+17% δ (N3C2H)/
1449.9	27	77	12	7% v(C8N7)+12% δ (C8N3H)+13% δ (C5N7H)/+ % δ (C4N3H)
1473.2	28	66	11	10%v(N9C8)+12% δ (N9C8H)+14% δ (HC8N7)+7% δ (C2N3H)+7% δ (C4N3H)
1551.0	29	97	16	9% v(C4C5)+9% v(N1C2)+9% v(N3C4)
1604.8	30	59	9	9% δ (C2N3H)+10% δ (C4N3H)
1654.1	31	158	25	9% v(C5C4)+10% v(N1C2)+7% v(N3C4)+/9% δ (N3C2H)
1733.2	32	624	100	13% v(C5C6)+24% v(C6O)+10% δ (C5C6N1)
3151.9	33	15	2	76% v(C2-H)
3256.5	34	2	0	75% v(C8-H)
3627.8	35	93	15	78% v(N3-H)
3646.2	36	105	17	77% v(N7-H)

Tabla 13.1.34. Números de onda obtenidos con el método B3LYP en el anillo de HX-37 (cm^{-1})
 6-31G(d,p) 6-311++G(3df,pd)

Calc.		Calc		IR int.		Raman Int.		Dep.	Dep	Red..	Force
ω	ω^v	ω	ω^v	Abs. ^a	Rel. ^b	Abs. ^c	Rel. ^d	ratios(P) ^e	ratios(U)	mass ^f	const. ^g
136.4	163.5	133.4	158.8	5	1	0	0	0.75	0.86	8.7	0.1
179.4	204.1	179.9	203.0	0	0	0	0	0.75	0.86	6.6	0.1
295.9	314.1	302.1	319.3	16	3	1		0.49	0.66	7.1	0.4
							1				
306.7	324.3	303.8	320.9	21	3	0	0	0.75	0.86	5.0	0.3
467.2	476.0	467.5	476.6	38	6	1	1	0.75	0.86	2.0	0.3
504.6	511.3	508.3	515.4	6	1	3	2	0.75	0.86	9.8	1.5
532.1	537.3	535.6	541.4	3	0	3	2	0.39	0.56	7.4	1.3
565.1	568.4	562.9	567.3	127	20	0	0	0.75	0.86	1.6	0.3
578.0	580.6	580.6	584.2	17	3	0	0	0.75	0.86	1.6	0.3
629.2	629.0	631.9	633.0	1	0	2	1	0.58	0.74	7.7	1.8
659.4	657.5	658.4	658.2	7	1	0	0	0.75	0.86	2.0	0.5
710.3	705.6	715.1	712.1	5	1	24	16	0.06	0.11	8.7	2.6
715.6	710.6	717.4	714.3	2	0	0	0	0.75	0.86	7.4	2.2
775.1	766.8	810.5	802.8	8	1	0	0	0.75	0.86	9.1	3.5
838.8	827.0	858.5	848.5	14	2	1	1	0.75	0.86	1.5	0.6
904.1	888.7	907.2	894.8	2	0	3	2	0.20	0.34	7.1	3.4
947.1	929.3	959.2	944.3	5	1	0	0	0.75	0.86	1.4	0.8
963.0	944.3	965.7	950.5	6	1	4	3	0.25	0.40	7.4	4.1
1039.7	1016.8	1037.1	1018.4	53	8	2	1	0.52	0.68	5.7	3.6
1098.2	1072.1	1097.2	1075.6	13	2	4	3	0.12	0.21	1.7	1.2
1131.1	1103.6	1128.3	1105.1	26	4	18	12	0.18	0.31	2.1	1.6
1212.3	1179.9	1209.1	1182.0	30	5	1	1	0.48	0.65	2.3	1.9
1298.7	1261.5	1289.2	1258.2	18	3	10	7	0.28	0.44	2.6	2.5
1348.6	1308.6	1337.8	1304.4	4	1	16	10	0.49	0.65	3.9	4.2
1384.5	1342.5	1377.6	1342.3	16	3	53	35	0.16	0.28	4.2	4.7
1423.2	1379.1	1415.6	1378.4	36	6	14	9	0.13	0.23	1.6	1.9
1461.5	1415.3	1449.9	1411.0	77	12	10	7	0.41	0.58	2.5	3.1
1487.4	1439.7	1473.2	1433.2	66	11	18	112	0.56	0.72	3.0	3.9
1568.4	1516.3	1551.0	1507.2	97	16	23	15	0.34	0.51	9.8	13.8
1616.8	1562.0	1604.8	1558.4	59	9	6	4	0.27	0.42	3.6	5.5
1669.4	1611.7	1654.1	1605.3	158	25	53	35	0.28	0.44	7.3	11.7
1775.7	1712.1	1733.2	1680.5	624	100	71	46	0.25	0.40	12.8	22.6
3272.0	3125.7	3256.5	3129.5	2	0	112	73	0.28	0.44	1.1	6.9
3639.8	3473.1	3627.8	3482.7	93	15	141	92	0.21	0.34	1.1	8.4
3661.3	3493.4	3646.2	3500.2	105	17	87	57	0.10	0.33	1.1	8.5

^v forma escalada, ^a Intensidade absoluta de IR. Km/Mol, ^b Intensidad relativa de IR %, ^c Intensidad absolute Raman. Å⁴/AMU, ^d Intensidades Relativas Raman. %
^e Raman depolarization ratios, ^f Masa reducida. AMU, ^g Constantes de fuerzas. mDina/Å, ^h Calculado en el nivel B3LYP/6-311G

Tabla 13.1.35. Número de ondas (cm⁻¹) escaladas del tautómero HX-37.

	3LYP /6-31G(d,p)				B3LYP/6-311++G(3df,pd)				MP2/6-31G(d,p)			
	ω	ω ^a	ω ^{v(1)}	ω ^{v(1)}	ω	ω ^a	ω ^{v(2)}	ω ^{vI}	ω	ω ^a	ω ^{v(3)}	ω ^{v(3)}
1	136.4	135.6	163.5	131.1	133.4	132.6	158.8	129.1	113.1	112.4	140.5	106.6
2	179.4	178.3	204.1	172.5	179.9	178.8	203.0	174.1	164.8	163.8	189.0	155.4
3	295.9	294.1	314.1	284.4	302.1	300.2	319.3	292.4	293.7	291.9	309.8	276.9
4	306.7	304.8	324.3	294.8	303.8	301.9	320.9	294.0	294.0	292.2	310.0	277.2
5	467.2	464.3	476.0	449.1	467.5	464.6	476.6	452.5	434.5	431.8	441.7	409.6
6	504.6	501.5	511.3	485.1	508.3	505.1	515.4	492.0	505.4	502.3	508.2	476.4
7	532.1	528.8	537.3	511.5	535.6	532.3	541.4	518.4	531.0	527.7	532.2	500.6
8	565.1	561.6	568.4	543.2	562.9	559.4	567.3	544.8	543.4	540.0	543.8	512.3
9	578.0	574.4	580.6	555.6	580.6	577.0	584.2	562.0	575.0	571.4	573.4	542.1
10	629.2	625.3	629.0	604.8	631.9	628.0	633.0	611.6	615.1	611.3	611.0	579.9
11	659.4	655.3	657.5	633.9	658.4	654.3	658.2	637.3	628.4	624.5	623.4	592.4
12	710.3	705.9	705.6	682.8	715.1	710.7	712.1	692.1	682.5	678.3	674.1	643.4
13	715.6	711.2	710.6	687.9	717.4	713.0	714.3	694.4	689.5	685.2	680.7	650.0
14	775.1	770.3	766.8	745.1	810.5	805.5	802.8	784.5	719.6	715.1	708.9	678.4
15	838.8	833.6	827.0	806.3	858.5	853.2	848.5	830.9	796.0	791.1	780.5	750.4
16	904.1	898.5	888.7	869.1	907.2	901.6	894.8	878.1	905.1	899.5	882.8	853.2
17	947.1	941.2	929.3	869.1	959.2	953.3	944.3	928.4	936.3	930.5	912.0	882.7
18	963.0	957.0	944.3	910.4	965.7	959.7	950.5	934.7	961.8	955.8	936.0	906.7
19	1039.7	1033.3	1016.8	925.7	1037.1	1030.7	1018.4	1003.8	1057.8	1051.2	1025.9	997.2
20	1098.2	1091.4	1072.1	999.5	1097.2	1090.4	1075.6	1062.0	1110.6	1103.7	1075.4	1047.0
21	1131.1	1124.1	1103.2	1055.7	1128.3	1121.3	1105.1	1092.1	1151.1	1144.0	1113.3	1085.1
22	1212.3	1204.8	1179.9	1087.3	1209.1	1201.6	1182.0	1170.3	1234.2	1226.5	1191.2	1163.5
23	1298.7	1290.6	1261.5	1165.4	1289.2	1281.2	1258.2	1247.8	1324.7	1316.5	1276.0	1248.8
24	1348.6	1340.2	1308.6	1248.4	1337.8	1329.5	1304.4	1294.9	1383.3	1374.7	1330.9	1304.0

25	1384.5	1375.9	1342.5	1296.4	1377.6	1369.1	1342.3	1333.4	1401.8	1393.1	1348.3	1321.5
26	1423.2	1414.4	1379.1	1330.9	1415.6	1406.8	1378.4	1370.2	1445.7	1436.7	1389.4	1362.9
27	1461.5	1452.4	1415.3	1368.1	1449.9	1440.9	1411.0	1403.6	1509.6	1500.2	1449.3	1423.1
28	1487.4	1478.2	1439.7	1404.9	1473.2	1464.1	1433.2	1425.9	1520.3	1510.9	1459.3	1433.2
29	1568.4	1558.7	1516.3	1429.8	1551.0	1541.4	1507.2	1501.2	1600.9	1591.0	1534.9	1509.2
30	1616.7	1606.7	1561.9	1507.7	1604.8	1594.9	1558.4	1553.3	1641.1	1630.9	1572.5	1547.1
31	1669.4	1659.0	1611.7	1554.1	1654.1	1643.8	1605.3	1601.0	1671.9	1661.5	1601.4	1576.1
32	1775.7	1764.7	1712.1	1604.8	1733.2	1722.5	1680.5	1677.6	1792.6	1781.5	1714.5	1689.9
33	3165.5	3145.9	3025.0	1707.0	3151.9	3132.4	3030.0	3050.7	3247.1	3227.0	3077.7	3061.0
34	3272.0	3251.7	3125.7	3043.0	3256.5	3236.3	3129.5	3152.0	3345.8	3325.1	3170.2	3154.1
35	3639.8	3617.2	3473.1	3145.4	3627.8	3605.3	3482.7	3511.3	3709.2	3686.2	3510.8	3496.7
36	3661.3	3638.6	3493.4	3498.9	3646.2	3623.6	3500.2	3529.2	3719.9	3696.8	3520.8	3506.7

v= escalado (Palafox, M. A.; Iza, N. y Gil, M.) [$v^{(1)} = 34,6 + 0,9447*v$] y [$v^{(2)} = 31,9 + 0,9512*v$] y [$v^{(3)} = 34,5 + 0,9372*v$]

v = Forma escaleda (Gaussian 2003) $v^{(1)} = 0,9613*v$ y [$v^{(3)} = 0,9427*v$]

vI =Escalado (Gaussian 2003) *Int. J Mol. Sci.* 2007,8, 760 - 776[*factor = 0.9679.*]

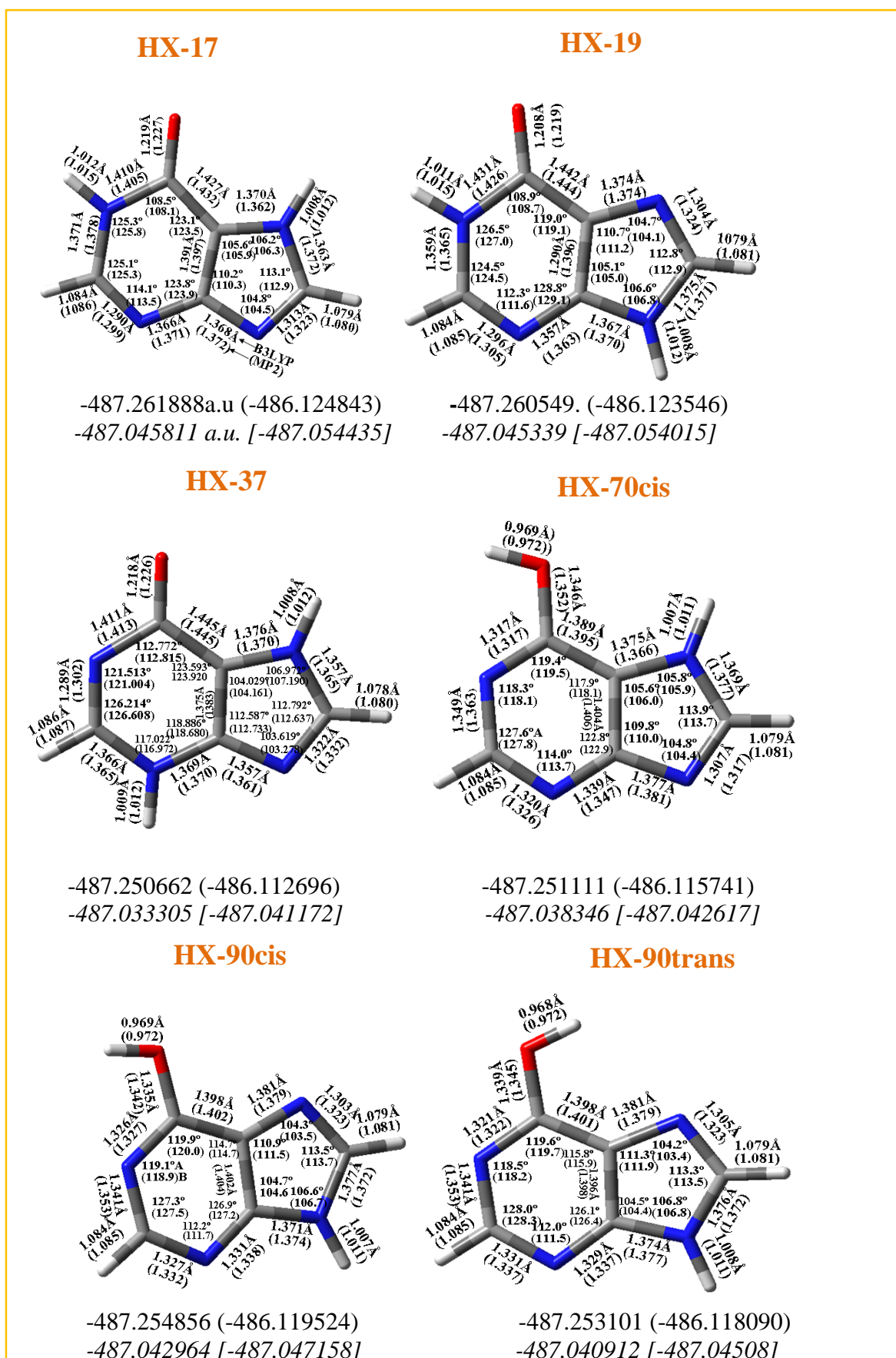


Figura 13.1. Anexo. Longitudes de enlace y ángulos de los seis tautómeros de HX.

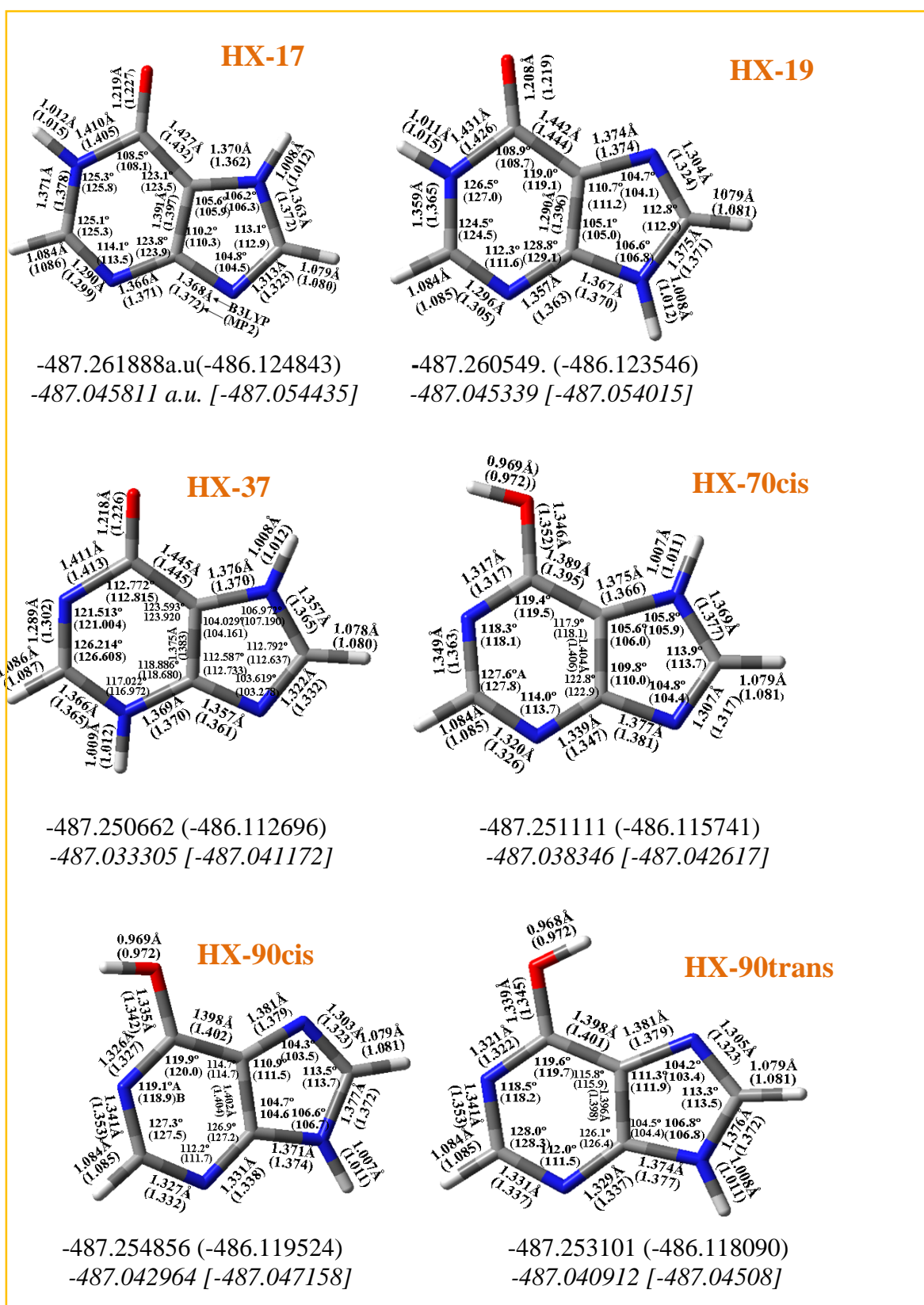


Figura13.1. Anexo. Longitudes de enlace y ángulos de los seis tautómeros de HX.

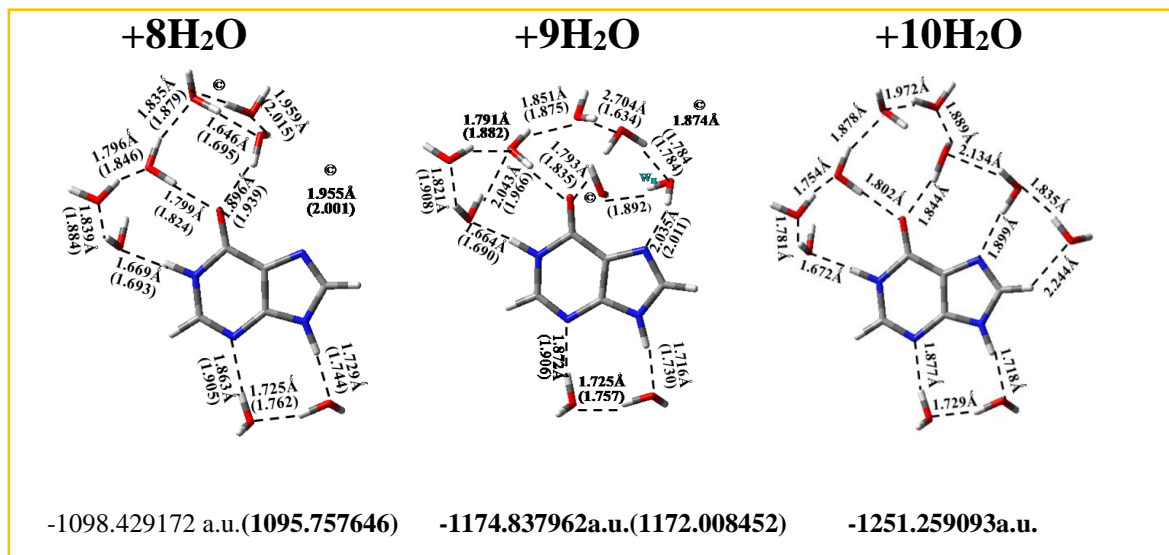


Figura 13.2. Anexo. Hidratos de HX-19 con 8/10 moléculas de agua.

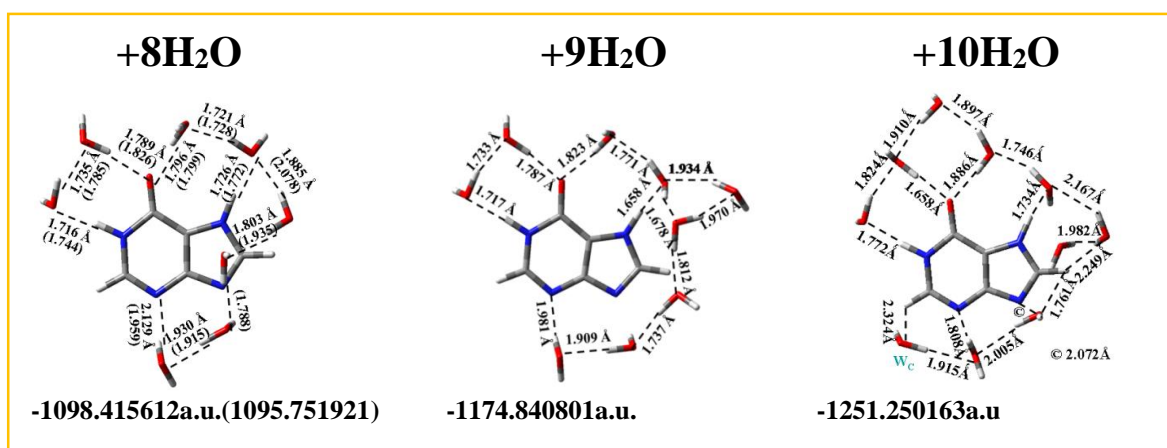


Figura 13.3. Anexo. Hidratos de HX-17 con 8/10 moléculas de agua.

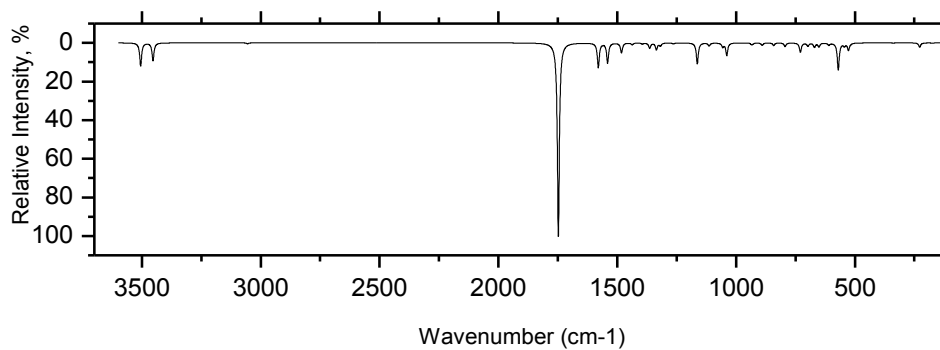


Figura 13.4. Espectro del tautómero HX-19 calculado en B3LYP/6-311++G(3df,pd).

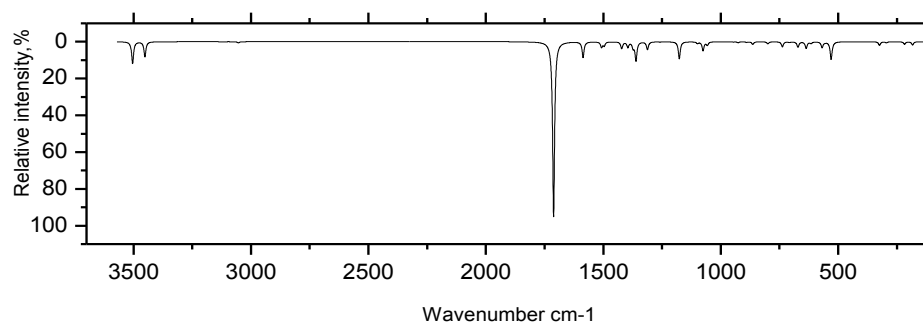


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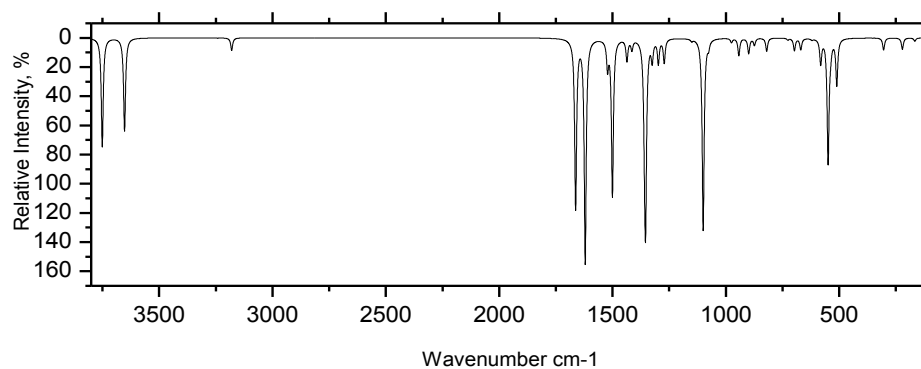


Figura 13.6. Espectro del tautómero HX-90C calculado en B3LYP/6-311++G(3df,pd).

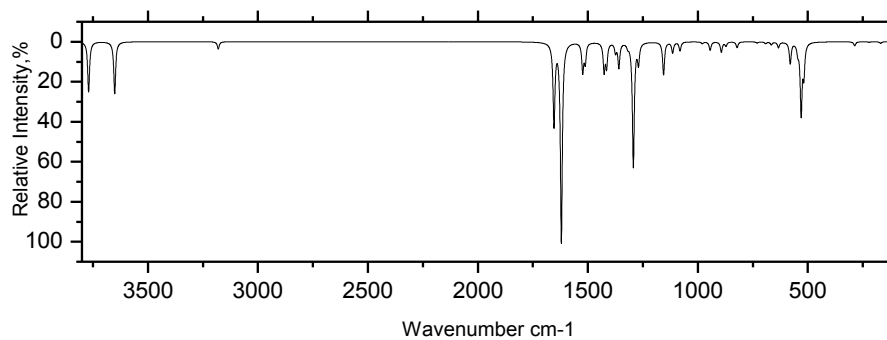


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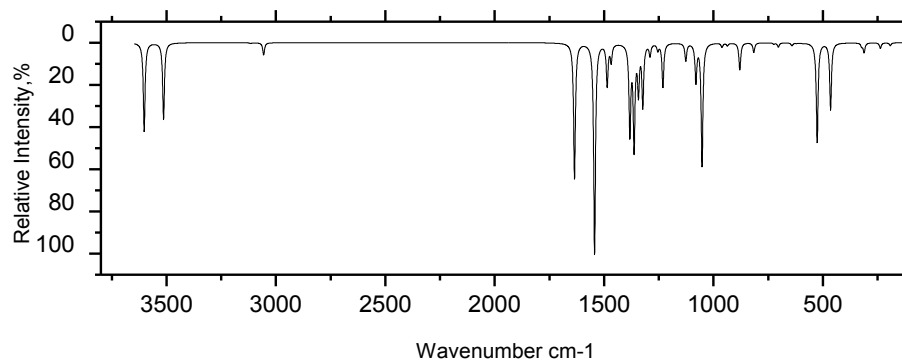


Figura 13.8. Espectro del tautómero HX-70 calculado en B3LYP/6-311++G(3df,pd).

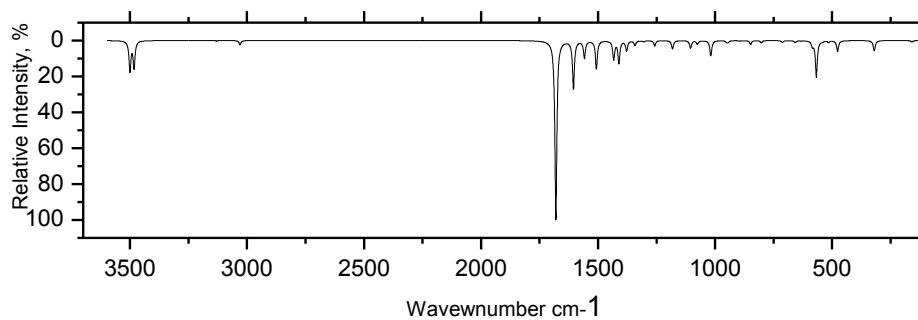


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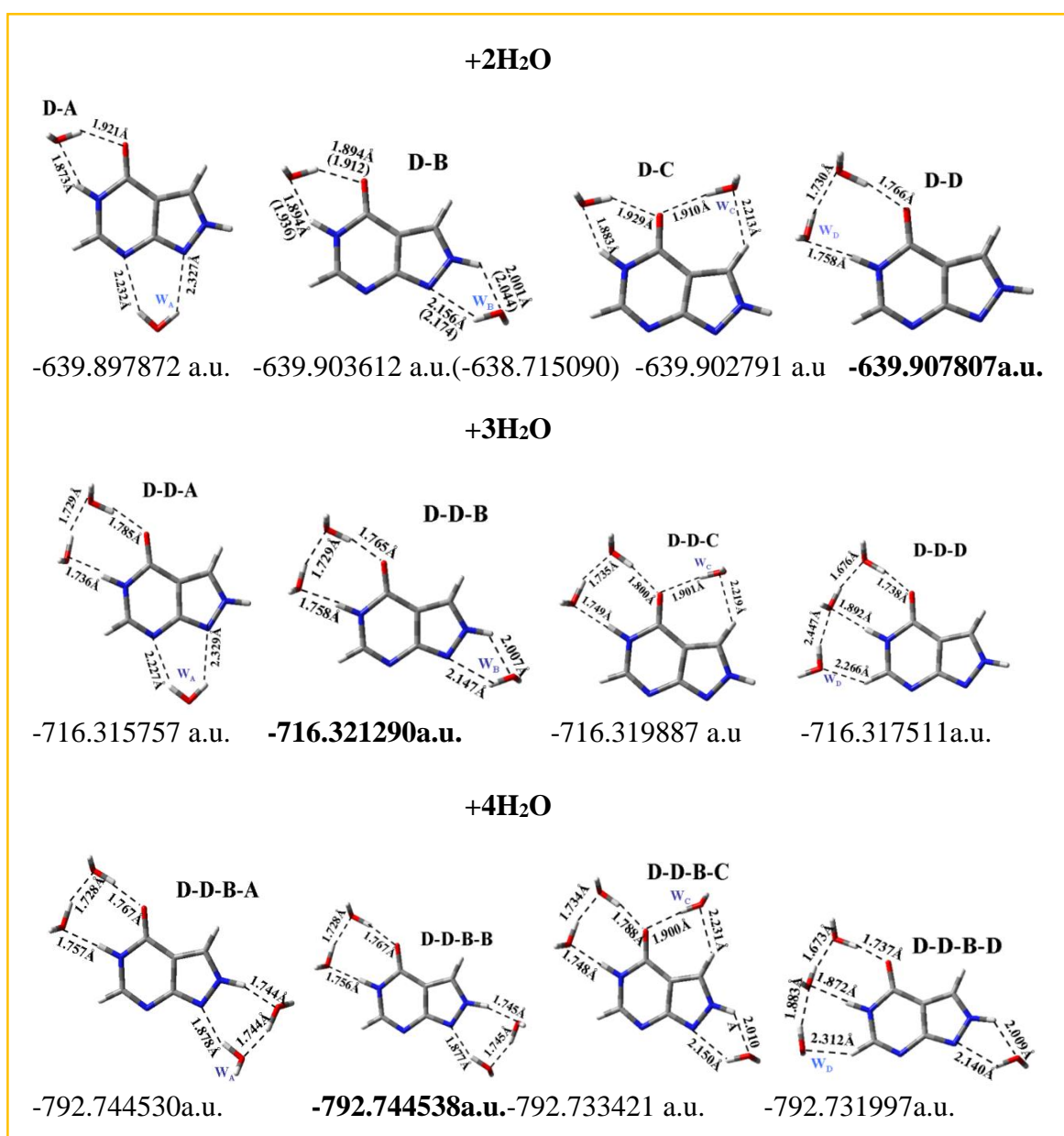


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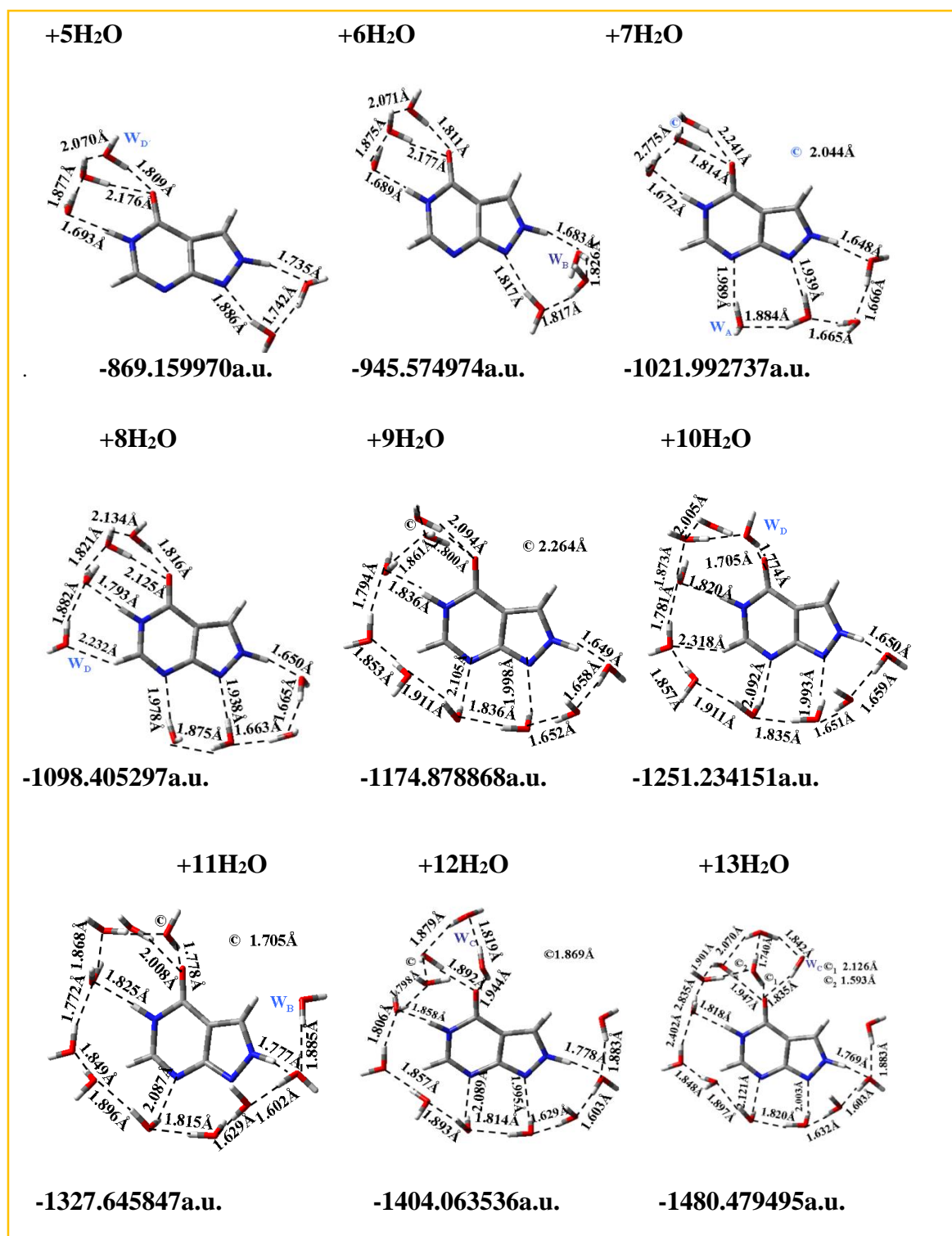


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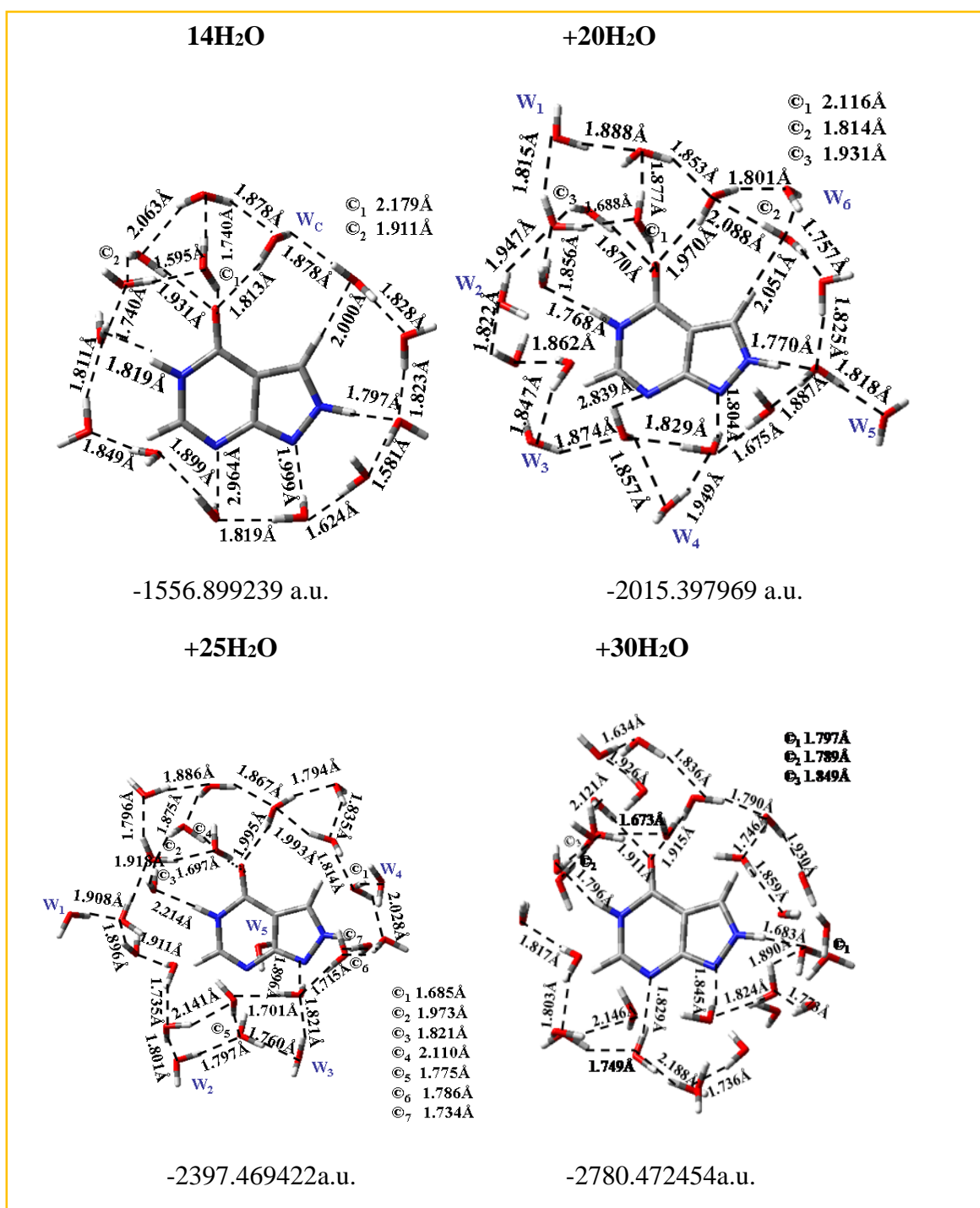


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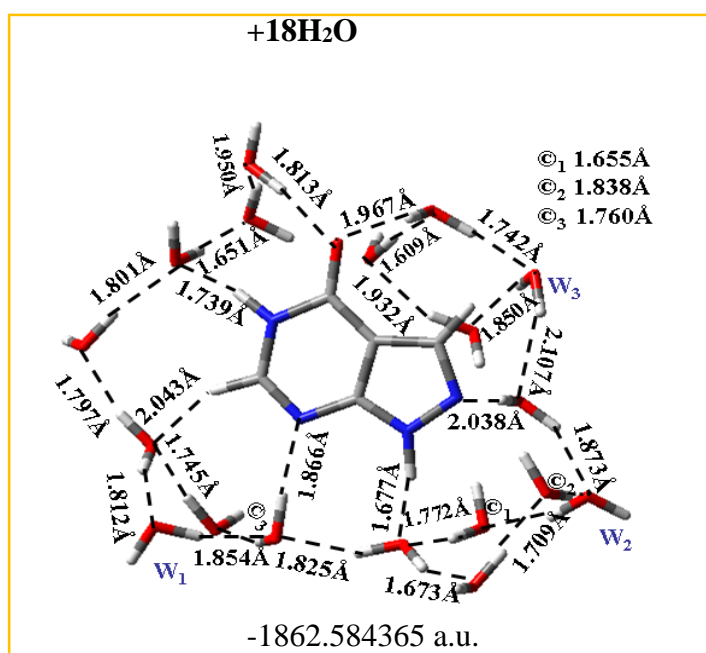


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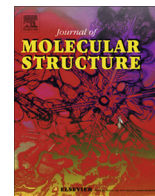
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Molecular structure of the nucleoside analogue inosine using DFT methods: Conformational analysis, crystal simulations and possible behaviour

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HIGHLIGHTS

- The five tautomers of the nucleoside inosine were determined and optimized.
- In the most stable tautomer N1 were calculated and optimized 69 stable structures.
- The lack of the NH₂ group of guanosine reduces the negative charge on N3 and N1 atoms.
- X-ray crystal unit cell state was simulated through a pentamer form.

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ABSTRACT

Five tautomers of the nucleoside inosine were determined and optimized at the MP2 and B3LYP levels of theory. Several correlations were identified. A comprehensive conformational analysis was carried out on the most stable tautomer N1, and the whole conformational parameters ($\chi, \beta, \gamma, \delta, \epsilon, \epsilon', P, \nu_{\max}$) were studied as well as the NBO Natural atomic charges. The calculations were carried out with full relaxation of all geometrical parameters. The search located at least 69 stable structures, 3 of which are within a 1 kcal/mol electronic energy range of the global minimum, and 4 conformers are within a 1 kcal/mol Gibbs energy range. A lower reactivity in inosine than in the natural nucleoside guanosine appears in the N1 and N3 nitrogen atoms. The solid state was simulated through a pentamer form and the structural parameters were compared with the X-ray crystal data available. Several general conclusions were emphasized.

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1. Introduction

Inosine (INO) and its derivatives are purine nucleosides of considerable interest because they are one of the components of nucleic acid's building blocks [1]. INO is formed when hypoxanthine is attached to a ribose ring (ribofuranose) via a β -N₉-glycosidic bond. It can be obtained from deamination of adenine by RNA editing [2]. As compared with guanosine, INO lacks the NH₂ group at the C2 position.

INO, whose structure appears in Scheme 1, is biologically found in tRNA. It is an adenosine deamination product in DNA, which must be repaired to maintain genomic fidelity [3]. It is observed in the first anti-codon position, which pairs with the third codon position on mRNA [1]. It is also an endogenous BDZ-receptor ligand. Administration of INO is very useful for its neuroprotective properties and, as other nucleosides, it plays a role in the

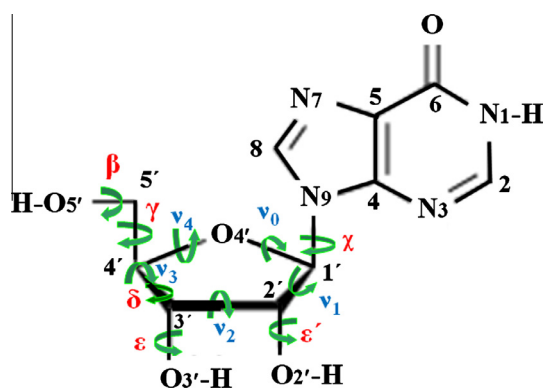
pathophysiology of some neurodegeneratives and neuro psychiatric diseases [4]. It increases myocardial gross energy improving cardiac performance and preserving ATP ischemia [5–7]. It has been proposed for spinal cord injury [8] and it stimulates significant axonal reorganization after strokes, which leads to improve performance on several sensorimotor tasks [9].

INO's ability to act as an 'universal pairing base' was recognized soon after the sequences of many tRNA's became available [3,10]. Therefore, base pairs formed by INO play an important role in many physiological processes as well as in various DNA technologies. Examples include INO containing primers for the detection, isolation, and sequencing of genes and its use in DNA micro-array hybridization [11]. Thus, INO may be used to detect and to analyze the target of DNA strand containing ambiguities through binding with weak H-bond to any of the four natural DNA bases.

With the development of nucleoside chemistry, a number of INO analogues have been synthesized and their structural properties, stabilities, potential antitumor and antiviral activities have been studied [12–14]. One of these derivatives, 2'-deoxyinosine (2-dI), enhances the antitumoral activity of 5-fluorouracil (5-FU)

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Scheme 1. Molecular structure and definition of the exocyclic and endocyclic torsional angles in tautomer N1 of inosine.

when it is combined with 5-FU in human xerografts [1]. Another derivative, Inosine 5'-Monophosphate Dehydrogenase (IMPDH) is involved in the GMP nucleotide biosynthesis pathway and its isoform type II is produced in a large amount by the cancer and tumour cells [15].

The conformational isomerism of DNA structural components has an intense interest today and many theoretical researches have been reported [16]. However, few studies appear on INO. As in other nucleosides, several tautomeric forms are possible in INO, although the vibrational IR and Raman spectra indicate that INO and its methylated analogues occur predominantly in the *keto* form in neutral aqueous solution [17]. The *enol* tautomer, if reasonably stable, could form base pairs of the Watson-Crick type with uridine [17]. Thus, spontaneous mutations may arise from mispairing due to the appearance of rare tautomers.

One of our aims is to study the possible tautomers of INO (Fig. 1), and focusing the attention on the most stable one, in the calculation of their different conformers. From our understanding would be interesting to analyze the different conformational possibilities of INO and its charge distribution, and to compare the results with the natural nucleoside guanosine (G). An accurate knowledge of the flexibility and conformer properties of a nucleoside is an important help for the interpretation of their interactions. For this reason, different authors have analyzed previously the conformers of several natural and analogues nucleosides

[16,18–24]. Now, an extensive theoretical study of the conformational preferences and intramolecular interactions in INO has been carried out with full relaxation of all geometric parameters. We will attempt to determine herein, if the various geometric features in INO are correlated or interact with one another. We are also interested in whether alternative forms of hydrogen bonding make significant contributions to the conformational behaviour of INO.

2. Computational details

Calculations were carried out by using the Becke exchange functional (B) [25a], Becke's three-parameter exchange functional (B3) [25b], Handy's OPTX modification of Becke's exchange functional (O) [25c,25d] and the extended (X3) [25e], in combination with the correlation functionals of Lee, Yang, and Parr (LYP) [25f], and Perdew and Wang's 1991 (PW91) [25g]. The Handy, Tozer and coworkers modification (B972) was also used [25h]. B3LYP Density Functional method (DFT) is the most used today, and for this reason the majority of the calculations were carried out with it.

All the methods appear implemented in the GAUSSIAN 03 program package [26]. The UNIX version with standard parameters of this package was used in the alpha computer of the Computational Centre from University Complutense of Madrid, in which all quantum chemical computations were performed. DFT methods provide adequate compromise between the desired accuracy and the heavy demands put on computer time and power. Different studies have shown that the data obtained with DFT methods are in good agreement with those obtained by expensive computational methods as MP2 [27]. Moreover, they have been used satisfactory in many studies on nucleosides and on drug design [19,23–25,28–32], and they predict vibrational frequencies of DNA bases better than HF and MP2 methods [33–37]. Several basis set were selected but the 6-31G(d,p) represents a compromise between accuracy and computational cost and thus it was used in the majority of the calculations.

The 3D Potential Energy Surface (PES) of this molecule was determined by rotation of the exocyclic and endocyclic torsional angles χ (glycosidic bond), γ , β , ϵ and ϵ' . These dihedral angles were simultaneously hold fixed at values varying between 0° and 360° in steps of 60° in a first study. All other geometrical parameters were relaxed during these optimisations. 69 optimized geometries were obtained in this step by minimizing the energy with respect to all

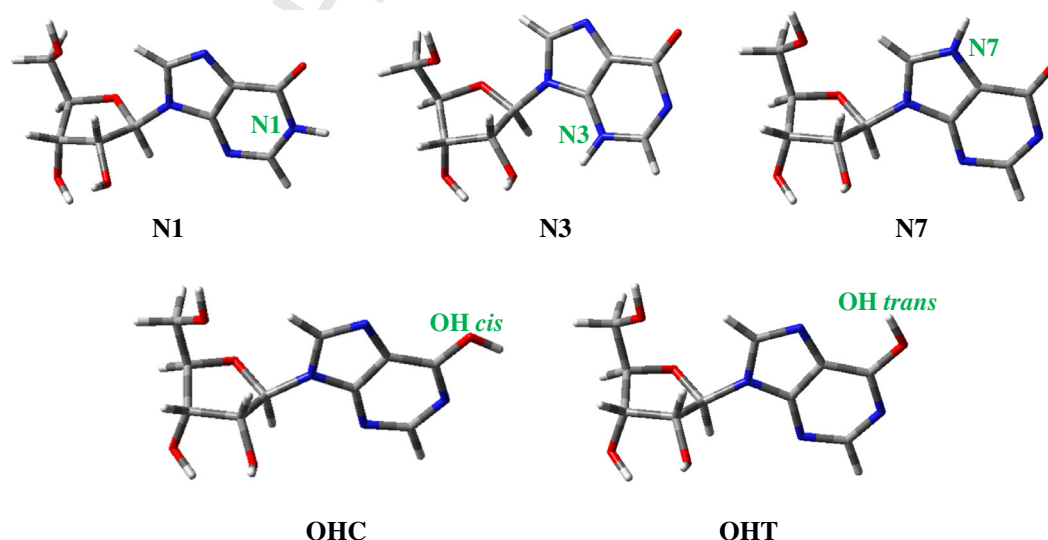


Fig. 1. Main tautomers of inosine molecule.

Table 1
The optimum stable tautomers calculated in inosine molecule, with endocyclic and exocyclic torsional angles in degrees, pseudorotational angle P in degrees and energy increments in (kcal mol⁻¹). The values are listed successively at the MP2/6-31G(d,p) level (values in bold), B3LYP/6-31G(d,p) level + ZPE (values in parentheses), and PCM at the B3LYP/6-31G(d,p) level (values in quotation marks).

Tautomer	χ	β	γ	δ	ϵ	ϵ'	ν_0	ν_1	ν_2	ν_3	ν_4	P^a	S^b	ν_{\max}^c	ΔE	ΔG	μ
N1	166.5	66.6	58.0	153.5	152.5	65.2	-17.5	33.3	-35.7	27.0	-6.0	170.8	² E	36.5	0	(0)*	4.858
	(167.4)	(65.0)	(57.1)	(145.3)	(150.7)	(59.4)	(-25.9)	(37.4)	(-34.1)	(20.5)	(3.3)	(156.3)	(² E)	(37.4)	(0)	(3.859)	(3.859)
N3	"163.9"	"79.6"	"50.8"	"142.8"	"167.2"	"62.1"	"-26.4"	"38.2"	"-34.9"	"20.9"	"3.3"	"156.4"	" ² E"	"38.1"	"0"	"(0)"	"13.132"
	(-175.1)	(162.4)	(50.6)	(156.3)	(147.6)	(158.1)	(-6.9)	(26.6)	(-34.8)	(31.9)	(-16.0)	(187.7)	(² T)	(35.1)	(24)	(-21.985)	(12.483)
N7	164.5	64.8	56.1	149.9	153.1	64.2	-25.2	38.0	-35.8	22.8	1.3	159.3	² E	38.3	21.060	(-19.045)	9.485
	(165.6)	(64.5)	(55.6)	(144.2)	(151.0)	(60.5)	(-28.6)	(39.1)	(-34.2)	(19.4)	(5.6)	(152.9)	(² T)	(38.4)	(18.874)	(8.151)	(8.151)
OHT	161.1	63.8	57.8	150.4	153.8	68.0	-24.0	37.3	-35.8	23.3	0.4	160.8	² E	37.9	3.117	(-4.464)	2.774
	(163.1)	(63.3)	(57.2)	(143.8)	(151.4)	(63.0)	(-28.7)	(38.9)	(-33.9)	(18.8)	(6.1)	(152.2)	(² T)	(38.3)	(3.309)	(2.331)	(2.331)
OHC	-125.8	174.8	49.6	151.3	146.0	152.1	-18.0	33.6	-35.7	26.3	-5.4	169.7	² E	36.3	8.819	(-7.837)	5.655
	(-123.3)	(174.6)	(50.1)	(148.3)	(143.1)	(151.6)	(-14.6)	(30.2)	(-33.3)	(25.9)	(-7.3)	(165.7)	(² E)	(34.4)	(8.017)	(4.987)	(4.987)

^a Definition: $\text{tg}P = \frac{(\nu_4 + \nu_1) - (\nu_3 + \nu_0)}{2\nu_2 \sin(\frac{\nu_3 + \nu_0}{2}) + \sin(\frac{\nu_4 + \nu_1}{2})}$. When ν_2 is negative, 180° is added to the calculated value of P . Ref. [24d].

^b Notation used from Ref. [40].

^c $\nu_2/\cos P$.

^d $\chi_{\text{OHC}} = 0 = -983.184779$ a.u. at the B3LYP/6-31G(d,p) level.
 $\chi_{\text{OHT}} = 0 = -983.228991$ a.u.

geometrical parameters without imposing molecular symmetry constraints. Bery optimisations under the μ criterion were used. Atomic charges were determined with the Natural NBO procedure [38,39].

Harmonic wavenumber computations were carried out at the same level of the respective optimization process and by the analytic evaluation of the second derivative of the energy with respect to nuclear displacement. Vibrational frequencies calculations were performed on all **optimized** conformers to confirm that they corresponded to local minima. All **optimized** structures showed only positive harmonic vibrations (local energy minima). Relative energies were obtained by adding zero-point vibrational energies (ZPEs) to the total energy. For the calculation of the ZPEs, the frequencies were retained unscaled. The ΔG values were sums of electronic and thermal Free Energies.

3. Results and discussion

INO has five tautomers, Fig. 1. All them were fully **optimized** at different levels of computation, Table 1. The most stable one corresponds to N1, and we focus the study only in this tautomer. The remaining forms were left for futures researches.

An analysis of the relative energies of these tautomers shows that they can be related to the dipole moment (μ), and a relationship/tendency can be established, Fig. 2. It indicates that the least stable tautomer in the isolated state has the highest μ , i.e. it is the most stable in water solution. Other tendencies can be observed between ΔE and the exocyclic torsional angle γ , or through the furanose pucker P , although in this case the deviations are very large.

3.1. Definition of the conformational angles

Following the Saenger's notation [40], the atomic description of tautomer N1 of INO, as well as the most important exocyclic and endocyclic torsional angles, is defined in Scheme 1. The conformation can be characterized by the following seven important structural parameters: (i) the glycosylic torsional angle, $\chi(C4'-N9-C1'-O4')$, which determines the two orientations of the base relative to the furanose ring, denoted as the *anti* and *syn* forms. (ii) The exocyclic torsional angle $\beta(H5'-O5'-C5'-C4')$ describing the orientation of the hydroxyl hydrogen H5'. (iii) The exocyclic torsional angle $\gamma(O5'-C5'-C4'-C3')$ which shows the orientation of the OH group relative to the furanose ring. This ring is twisted out-of-plane in order to minimize non-bonded interactions between their substituents. (iv) The torsional angle $\epsilon(C2'-C3'-O3'-H3')$ which determines the orientation of hydroxy group O3'H. This orientation has been also defined by other authors [40] through the $C4'-C3'-O3'-H3'$ angle. (v) The torsional angle $\epsilon'(C1'-C2'-O2'-H2')$ describing the orientation of the hydroxy group O2'H, (vi) the torsional angle $\delta(C5'-C4'-C3'-O3')$ defining the orientation of the O2'H relative to the CH₂OH group, and finally, (vii) the furanose pucker P defined in the bottom of Table 1.

3.2. Conformers and energetics

An extensive conformational study of tautomer N1 was carried out through a rotation of the exocyclic χ , γ , β , ϵ , ϵ' , δ torsional angles. A detailed collection of the most important conformational parameters of these **optimized** forms is included in Table 2. The conformers were classified according to the three ranges of rotation of χ , Fig. 3:

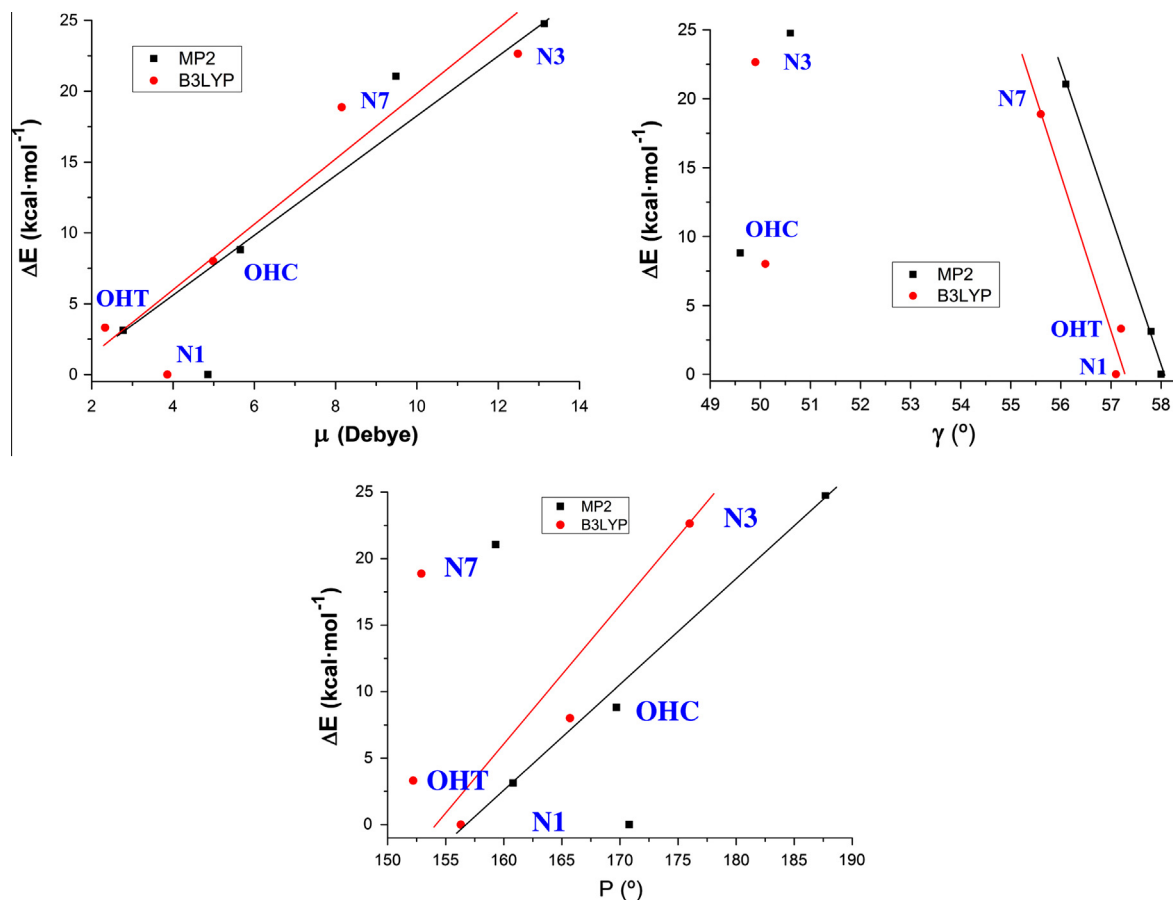


Fig. 2. Relationship/tendency observed between the relative energy ΔE of the different tautomers versus the dipole moment (μ) and the exocyclic torsional angle γ .

- Conformers A (χ ca. 175°) (*high-anti*) are the most stable and also the conformers found in the crystal.
- Conformers B (χ ca. 70°) (*syn*) are the non-active forms [41,42,31,43].
- Conformers C (χ ca. -120°) (*anti*) are the possible biological active forms.

Two energy criteria were considered for each conformer: the electronic energy $\Delta E + \text{ZPE}$ correction, and the Gibbs energy ΔG , the last two columns of Table 2. For the numbering on the conformers in each range of rotation of χ , Table 2, was followed the $\Delta E + \text{ZPE}$ criterion. Calculations at different levels, as well as single point calculations at the MP2/6-31G(d,p)//B3LYP/6-31G(d,p) level were carried out to confirm the stability of the main conformers, Tables 2 and 3-Sup. In general, the stability order remains, although small changes are observed. In all the cases, conformer A1 appears as the most stable one, with the exception of the result at the B3LP/6311++G(3df,pd) level, in which conformer B1 appears as the global minimum.

By the same methodology, the relative energies of the different conformers have been determined in related nucleosides [24b,41b]. The global minimum calculated in these nucleosides by MP2/6-31G(d,p) was in accordance to that found by B3LYP/6-31G(d,p). Thus, our results by B3LYP can be considered acceptable.

The conformers differ in general very little in energy. Thus, in our calculations 69 optimized conformers were found within the electronic energy range $\Delta E = 0-10.7$ kcal/mol, and Gibbs energy range $\Delta G = 0-9.6$ kcal/mol with respect to the global minimum. This range of values of ΔG is noticeable larger than that reported in dT [18], $0-7.5$ kcal/mol.

Only three conformers are found within the electronic energy range $\Delta E = 0-1.0$ kcal/mol (by criterion of $\Delta E + \text{ZPE}$), with χ (167.4° *high-anti*, and $54.1-54.9^\circ$ *syn*), with S-type furan puckering, and with similar values for β and γ angles in the *g₊* form. Among these conformers, B2 with the highest dipole moment 6.709 D (in A1 is 3.859 D) is favoured in a polarisable environment with water. Another two conformers appear within the electronic energy range $\Delta E = 1.0-2.0$ kcal/mol, with χ (175.1° and 169.1°) *high-anti*. It is noted that *anti* structures are the expected forms for the natural nucleosides that form the nucleotides and polynucleotides in biological systems [44]. The ratio *anti/syn* is slightly decreased from 4.5 in the low-energy group (<4 kcal/mol) to 3.1 in the 4-10 kcal/mol energy group.

The global minimum by B3LYP corresponds to the *high-anti-gg-gg* form with respect to χ , γ and β torsional angles, respectively. This conformer is denoted as A1 (Figs. 4 and 5) and it appears stabilized by two intramolecular H-bonds. The optimized bond lengths and natural NBO atomic charges on this conformer are collected in Fig. 4. This global minimum by criterion of $\Delta E + \text{ZPE}$ agree well to that obtained by criterion of ΔG , but differs to that obtained by MP2, a *syn* form, Table 3-sup. It is because of the small difference in energy between both forms, A1 and B1. This global minimum in the *syn* form by MP2 is in accordance to that obtained in other nucleosides [24a-c], but differs of the *anti* form expected for the natural nucleosides and nucleotides in biological systems [40].

The second most stable conformer is B1, also 2E , with values of $\chi = 54.1^\circ$, $\beta = 66.9^\circ$ and $\gamma = 48.2^\circ$. Its O3'H group is similar to A1, *trans* ($\epsilon = 149.2^\circ$). The third best optimum conformer corresponds to B2, also 2E , with values $\chi = 54.9^\circ$, $\beta = 65.8^\circ$ and $\gamma = 46.6^\circ$. These

Table 2 (continued)

Conf.	χ	β	γ	δ	ε	ε'	v_0	v_1	v_2	v_3	v_4	P^a	S^b	v_{\max}^c	μ	ΔE	ΔG
B11	70.0	-70.9	-63.9	150.0	32.0	-91.1	-8.5	26.4	-33.3	29.6	-13.4	184.5	$\frac{3}{2}T$	33.4	5.950	7.442	7.107
B12	66.5	73.5	-74.0	150.7	147.4	147.5	-19.4	33.4	-34.0	24.0	-3.1	166.2	$\frac{3}{2}E$	35.0	6.710	7.637	6.994
B13	79.1	-87.9	46.9	148.1	151.4	133.3	-30.8	40.3	-34.2	17.6	8.1	149.5	$\frac{3}{2}T$	39.7	7.701	8.512	8.580
B14	66.8	58.4	178.2	140.7	147.0	148.4	-17.0	32.0	-33.9	25.3	-5.5	170.1	$\frac{3}{2}E$	34.4	7.172	8.545	7.963
B15	67.0	58.3	178.1	147.7	-81.0	-82.8	-14.2	30.3	-33.9	27.1	-8.2	175.0	$\frac{3}{2}T$	34.0	9.011	9.034	8.513
B16	73.2	62.2	177.5	152.8	31.3	-89.1	-3.6	23.6	-33.5	32.7	-18.5	193.1	$\frac{3}{2}E$	34.4	6.914	9.696	9.310
C1	-132.9	176.1	-58.9	152.0	-47.4	-146.1	-9.7	-16.0	33.2	-40.3	31.8	212.8	$\frac{3}{2}T$	39.5	5.354	4.372	3.139
C2	-123.4	173.6	49.7	81.8	143.7	151.3	-14.7	30.3	-33.5	26.0	-7.4	173.6	$\frac{3}{2}T$	33.7	5.371	4.418	4.838
C3	-148.8	164.9	47.9	148.5	-154.9	-142.3	3.8	-24.2	33.9	-32.7	18.5	192.5	$\frac{3}{2}E$	34.7	5.675	4.684	4.296
C4	-117.6	60.6	57.1	87.3	-153.6	-82.6	-41.0	32.4	-13.0	-10.2	32.2	109.1	$\frac{3}{2}T$	39.8	6.556	5.271	3.903
C5	-108.8	69.2	62.9	113.5	146.5	151.5	-26.5	37.4	-33.7	19.4	4.3	154.8	$\frac{3}{2}E$	37.2	4.666	5.411	4.581
C6	-123.0	172.0	49.2	144.8	-83.1	-80.7	-14.8	30.1	-33.2	25.9	-7.1	173.4	$\frac{3}{2}T$	33.4	8.379	5.416	4.949
C7	-117.7	175.5	47.7	151.5	35.5	-88.8	-7.8	25.9	-33.3	29.9	-14.0	185.7	$\frac{3}{2}T$	33.5	6.569	6.040	5.838
C8	-145.2	-56.4	178.6	148.7	-158.4	-141.2	-5.1	-18.5	33.2	-37.0	26.9	206.3	$\frac{3}{2}E$	37.0	4.910	6.086	5.113
C9	-125.3	-51.8	172.8	85.3	-78.0	-81.4	-27.6	38.0	-33.8	18.9	5.4	153.4	$\frac{3}{2}T$	37.8	7.440	6.301	5.048
C10	-129.2	-54.7	178.1	148.0	-159.9	-75.1	-40.7	34.1	-15.8	-7.2	30.3	113.4	$\frac{3}{2}T$	39.8	6.126	6.550	5.264
C11	-107.9	66.4	58.0	116.3	35.5	-95.4	-33.2	38.4	-29.4	11.2	13.7	140.7	$\frac{3}{2}T$	38.0	6.852	5.655	5.425
C12	-102.5	70.6	63.8	133.5	34.3	-90.8	-19.7	32.6	-32.8	22.5	-1.8	164.5	$\frac{3}{2}E$	34.0	6.815	6.659	5.789
C13	-126.3	-50.7	172.5	144.5	147.0	150.9	-24.8	37.2	-35.0	22.0	1.6	158.9	$\frac{3}{2}E$	37.5	5.683	6.684	5.633
C14	-129.5	-179.3	-67.1	144.0	-81.0	-83.9	-31.5	39.1	-31.7	14.7	10.6	145.7	$\frac{3}{2}T$	38.4	7.216	6.732	5.622
C15	-124.3	-89.4	52.7	142.4	-73.9	-78.6	-14.6	30.6	-34.2	27.0	-7.9	174.4	$\frac{3}{2}T$	34.4	9.260	6.812	6.349
C16	-129.3	-77.6	-66.4	153.4	-89.9	-83.6	-31.1	39.1	-32.1	15.3	9.9	146.7	$\frac{3}{2}T$	38.4	8.069	6.998	5.886
C17	-120.7	-52.6	172.3	142.6	29.3	-88.6	-22.6	34.9	-33.7	21.7	0.4	198.0	$\frac{3}{2}E$	35.4	6.057	7.204	5.729
C18	-118.7	-90.0	50.3	144.6	33.2	-86.8	-7.4	26.2	-34.1	30.9	-14.9	186.6	$\frac{3}{2}T$	34.3	7.439	7.231	7.046
C19	-129.1	-67.8	-64.5	150.5	147.1	152.9	-28.4	39.1	-34.4	19.0	5.7	152.9	$\frac{3}{2}T$	38.6	5.060	7.595	6.648
C20	-128.7	-174.3	-67.4	144.0	147.0	154.2	-27.3	38.7	-34.9	20.2	4.2	155.0	$\frac{3}{2}T$	38.5	4.460	8.237	7.184
C21	-121.3	-72.8	-64.5	146.0	34.0	-89.8	-22.6	35.3	-34.2	22.4	0.0	161.4	$\frac{3}{2}E$	36.1	6.852	8.338	7.068
C22	-122.1	-178.6	-68.0	144.6	28.1	-88.1	-22.6	35.2	-34.1	22.3	0.1	161.3	$\frac{3}{2}E$	36.0	5.368	8.345	6.962
C23	-130.7	68.9	-75.2	145.1	146.5	149.3	-30.7	39.6	-33.3	16.6	8.7	148.7	$\frac{3}{2}T$	39.0	5.829	8.747	7.742
C24	-126.2	76.9	-74.1	141.0	24.8	-87.3	-29.1	38.1	-32.7	17.1	7.5	150.2	$\frac{3}{2}T$	37.7	5.974	9.088	7.902
C25	-136.0	57.5	173.8	139.0	145.8	149.2	-31.3	39.4	-32.3	15.3	9.9	178.4	$\frac{3}{2}T$	32.3	6.686	9.291	7.804
C26	-134.2	-168.5	-174.7	138.2	-76.2	-80.8	-32.1	39.7	-32.2	14.9	10.8	145.6	$\frac{3}{2}T$	39.0	8.000	9.563	8.000
C27	-148.8	45.8	160.3	141.3	33.5	-86.6	3.6	19.8	-34.2	37.4	-26.1	204.2	$\frac{3}{2}E$	37.5	7.268	9.629	8.985
C28	-134.2	-174.6	-176.4	135.5	145.1	149.5	-33.5	39.9	-31.0	12.7	13.0	142.3	$\frac{3}{2}T$	39.2	5.958	9.908	8.453
C29	-129.8	-170.4	-174.5	125.6	31.9	-94.5	-36.9	39.4	-27.3	7.0	18.7	133.7	$\frac{3}{2}E$	39.5	6.531	10.350	8.996
C30	-108.8	69.2	180.0	144.8	146.5	151.5	-26.5	37.4	-33.7	19.4	4.3	154.8	$\frac{3}{2}E$	37.2	6.583	10.749	9.100

^a Definition: $tgP = \frac{(v_4+v_1)-(v_3+v_0)}{2v_2(\sin(36)+\sin(72))}$. When v_2 is negative, 180° is added to the calculated value of P . Ref. [24d].

^b Notation used from Ref. [40].

^c $v_{\max} = v_2 / \cos P$.

^d $\Delta E = 0 = -983.184779$ a.u. at the B3LYP/6-31G(d,p) level.

^e $\Delta G = 0 = -983.228991$ a.u.

^f $\Delta E = 0 = -983.766372$ a.u. at the B3LYP/6-311++G(3df,2pd) level.

^g $\Delta E = 0 = -983.852341$ a.u. at the O3LYP-31G(d,p) level.

^h $\Delta G = 0 = -982.896698$ a.u.

ⁱ $\Delta E = 0 = -983.234338$ a.u. at the B3LYP/6/cc-pVDZ level.

^j $\Delta G = 0 = -983.278235$ a.u.

^k $\Delta E = 0 = -982.819307$ a.u. at the B3PW91/6-31G(d,p) level.

^l $\Delta G = 0 = -982.863160$ a.u.

^m $\Delta E = 0 = -982.850820$ a.u. at the B972/6-31G(d,p) level.

ⁿ $\Delta G = 0 = -982.894695$ a.u.

values are similar to B1 but its O3'H group is g_- ($\varepsilon = -85.5$) as well as its O2'H group.

Fig. 5 shows the 12 best optimum conformers selected in each range of χ : six are A (A1–A6), three are B (B1–B3), and three are C (C1–C3). The values of the intramolecular H-bonds and the most important structural angles of each conformer are included in the figure. Figs. 6–9 (and Figs. 1 and 2 Sup.) show the distribution of the 69 optimized conformers according to their energies, exocyclic torsional angles, and values of P and v_{\max} . The 12 best conformers selected are pointed in these figures.

3.3. Conformational angle analysis

An overall examination of the six exocyclic torsional angles, defining the conformational space in tautomer N1 of INO, leads to conclude the following:

(i) The interring dihedral angle χ presents a trimodal distribution, (Figs. 6 and 7 and Fig. 1-Sup.): $-148.8^\circ \leq \chi_t \leq -102.5^\circ$ (conformers C), $39.4^\circ \leq \chi_{g^+} \leq 79.1^\circ$ (conformer B), and $-155.7^\circ \leq \chi_t \leq 161.6^\circ$ (conformers A). *Anti* forms (A and C, 53 conformers) prevail in number over *syn* ones (B, 16 conformers) and cover a wider range of χ values. This fact has been interpreted [18] by the less sterical restricted by noncovalent interactions between the base and the sugar residue in these *anti* forms. This *anti* orientation has been found in the crystal of several nucleosides [45–49], and it is the form for biological activity [44]. Although, in many nucleoside analogues *syn* and *anti* forms have similar energy, however, the global minimum corresponds in general to the *syn* form, as in AZT [24c]. In purine nucleosides there is relatively little restraint to rotation about the glycosidic bond [50].

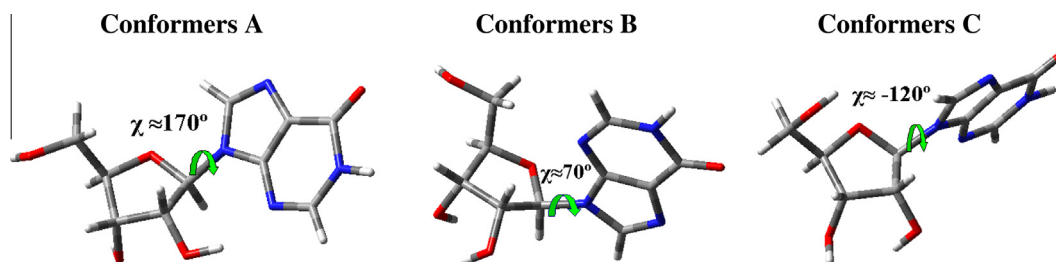


Fig. 3. Three types of conformers determined in tautomer N1 of inosine corresponding to the three ranges of rotation of χ with β and $\gamma \sim 60^\circ$.

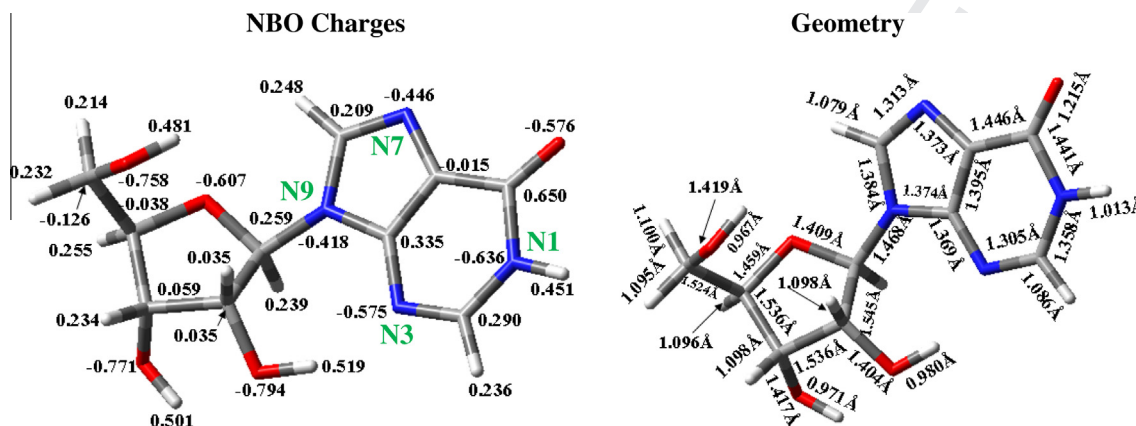


Fig. 4. Natural atomic charges and optimum bond lengths in conformer A1 of tautomer N1 of inosine at the B3LYP/6-31G(d,p) level.

- 293 (ii) The β angle has a clear trimodal distribution: $44.3^\circ \leq \beta_{g^+} < 76.9^\circ$ (22 conformers), $-168.5^\circ \leq \beta_t \leq 164.9^\circ$ (23 conformers), and $-90.0^\circ \leq \beta_{g^-} < -50.7^\circ$ (24 conformers),
294 Fig. 7. The value of this angle has little influence (less than 2 kcal/mol) in the conformational energy of the molecule.
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296 (iii) The γ angle has a trimodal distribution: the main group cover the $-171.9^\circ \leq \gamma_t \leq 160.3^\circ$ (28 conformers), $46.6^\circ \leq \gamma_{g^+} \leq 63.8^\circ$ (20 conformers), and $-75.5^\circ \leq \gamma_{g^-} < -58.9^\circ$ (21 conformers). Conformers with γ in *trans* are the least stables. Correlation of γ value with χ , ε and δ angle values was not observed.
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298 (iv) The angle δ mainly cover the $-167.5^\circ \leq \delta_t \leq 160.3^\circ$ range (62 conformers), with few ones in the $78.7^\circ \leq \delta_{g^+} \leq 88.1^\circ$ (5 conformers) and $-88.3^\circ \leq \delta_{g^-} < -78.9^\circ$ (2 conformers) ranges.
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300 (v) The ε angle has a tetramodal distribution: $24.8^\circ \leq \varepsilon_{g^+} < 94.2^\circ$ (16 conformers), $143.7^\circ \leq \varepsilon_{g^+} \leq 151.4^\circ$ (25 conformers), $-89.9^\circ \leq \varepsilon_{g^-} < -47.4^\circ$ (23 conformers) and $-160.6^\circ \leq \varepsilon_t \leq -153.6^\circ$ (5 conformers). In the second range appears the conformers with the highest stability. Variations in the ε angle have little influence in the energy of the molecule.
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302 (vi) The ε' angle has mainly a tetramodal distribution with thin ranges: $-98.4^\circ \leq \varepsilon'_{g^+} < -59.8^\circ$ (23 conformers), $133.3^\circ \leq \varepsilon'_{g^+} \leq 154.2^\circ$ (25 conformers), $52.8^\circ \leq \varepsilon'_{g^+} \leq 86.5^\circ$ (16 conformers) and $-151.2^\circ \leq \varepsilon'_t < -141.2^\circ$ (5 conformers).
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320 Correlations among the exocyclic torsional angles β , γ , δ , ε , ε' ,
321 and χ were not found, and an almost regular distribution of the conformers can be observed. It can be explained by the high flexibility of the structure, which permits many value combinations of the exocyclic torsional angles. The stability areas of the conformers were established. The largest ranges where conformers were not determined correspond to: $-102.5^\circ \leq \chi \leq 39.4^\circ$, $-50.7^\circ \leq \beta \leq 44.3^\circ$, $-58.9^\circ \leq \gamma \leq 46.6^\circ$ and $-59.8^\circ \leq \varepsilon' \leq 52.8^\circ$.
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3.4. Hypoxanthine moiety

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329 In the analysis of the six most stable conformers, the base heterocycle has a very small non-planarity, in general with torsional angles lower than 1° . Among the dihedral angles describing the base heterocycle *non-planarity*, those involving N3 and N9 atoms are the most dependent on the nucleoside conformation. The highest deviations correspond to $C4-N9-C8=N7$ (-0.94° , conformer A1), $C2=N3-C4=C5$ and $N3-C4=C5-C6$ (-0.95° and -0.91° , conformer B2) and $C4-N9-C8=N7$ (0.95° , A3). The torsional angles of the base heterocycle correlate with each other in such a way that their algebraic sum is close to 0° , with standard deviation of 0.4° and absolute value no greater than 1.0° .
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3.5. Furanose moiety

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341 The sugar is usually characterized by three structural parameters [40]: (i) the endocyclic torsion angles $\nu_0-\nu_4$; (ii) the pseudorotation phase angle P , Table 2; and (iii) the maximum torsional angle (degree of pucker), ν_{\max} . Fig. 6 shows three diagrams with the distribution of the 69 calculated conformers according to the χ angle *versus* the ΔE , ΔG energies and ν_{\max} . The following was observed in these figures:
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- 348 (i) The endocyclic torsional angles have a large range of variability: $-41.0^\circ \leq \nu_0 \leq 19.6^\circ$, $-26.0^\circ \leq \nu_1 \leq 40.3^\circ$, $-38.3^\circ \leq \nu_2 \leq 36.0^\circ$, $-40.3^\circ \leq \nu_3 \leq 38.6^\circ$, and $-36.5^\circ \leq \nu_4 \leq 45.9^\circ$. The algebraic sum of ν_i dihedral angles [40] ($i = 0, 1, \dots, 4$) is close to 0 for all conformers and it fails into the -1.0° to 1.5° range, its average is equal to 0.8° . These values are larger than those calculated in dT.
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355 (ii) The P angle has mainly a trimodal distribution covering the ranges: $140.7^\circ \leq P \leq 176.1^\circ$ (C_2' -endo, S-type) (42 conformers), and $177.2^\circ \leq P \leq 212.8^\circ$ (C_3' -exo, S-type) (21 conformers), with 6 conformers out of these ranges. Conformers with P values of
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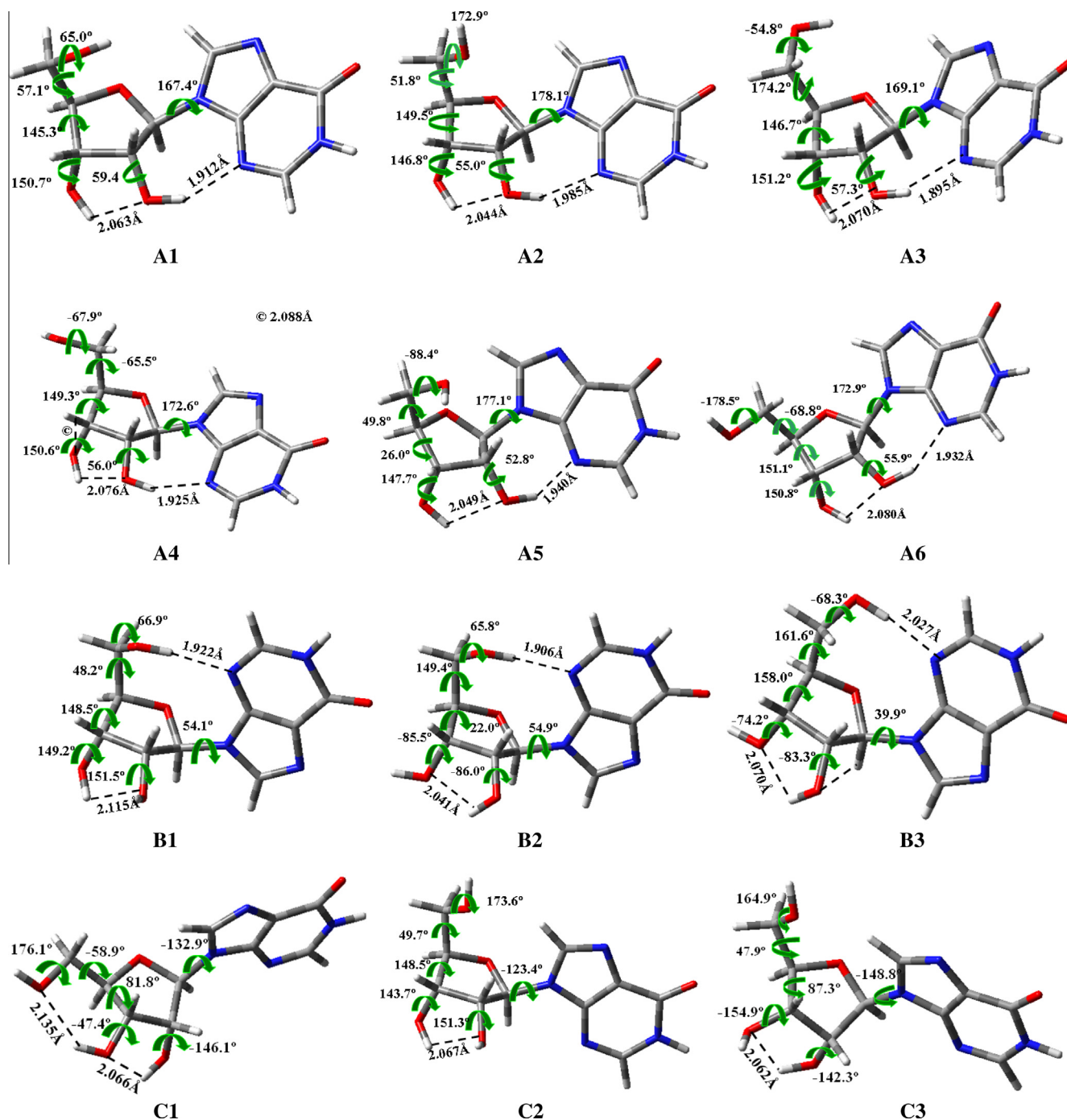


Fig. 5. Geometry of the twelve most optimum conformers selected for each rotation angle χ determined in tautomer N1 of inosine at B3LYP/6-31G(d,p) level. The values of the strongest intramolecular H-bonds are also included.

18.0° ± 90.0°, 216.0° ± 36.0°, 253.0° ± 36.0° and 288.0° ± 36.0° ranges were not determined. Approximately, conformers A appear in the 156.3° to 208.0° range, conformers B in the 162.3° to 187.0° range and conformers C in the 109.1° to 212.8° range.

The P angle covers all range of variation for S-type conformers (69 conformers) and averaging 171.4°. The relative energies do not vary in a regular manner with respect to the values of P, although the most stable ones correspond to conformers A. Thus, values of P of S-type furan pucker and ca. 160° lead to the most stable conformers.

(iii) v_{\max} appears in conformers A in the large range 33.9° < v_{\max} < 45.3°, while in conformers B it is slight shorter (33.2° → 39.7°), as well as in conformers C (32.3° → 39.8°). The conformers with high stability appear in general with a value of v_{\max} -ca. 37°. In particular, the ten most stable conformers have a v_{\max} in the range 33.9-37.4° (8 conformers A and 2 conformers B). Taking into account that the value of v_{\max} represents the radius of the pseudorotational cycle, its impact as a structural parameter is quite high. An increment in the ring pucker produces an increase in the flexibility of the molecule, which could make easier to adapt to the active site. The

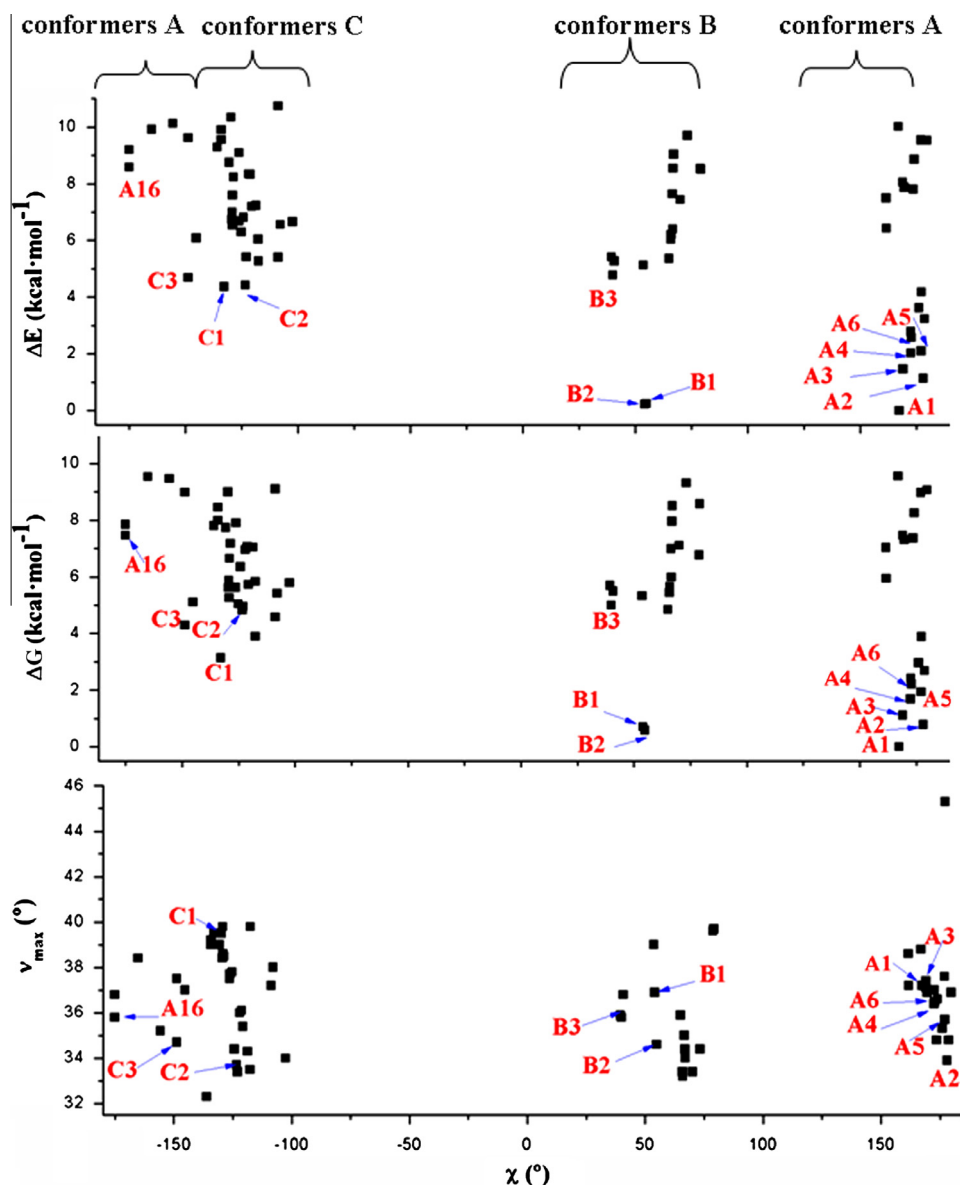


Fig. 6. Distribution of the 69 optimum stable calculated conformers in tautomer N1 of inosine according to their exocyclic torsional angle χ and their: (a) relative electronic energy $\Delta E + \text{ZPE}$ correction; (b) relative Gibbs energy ΔG ; and (c) puckering amplitude ν_{max} . The most stable conformers of each type are pointed.

381 values of ν_{max} appear independent of the value of χ and ϵ'
382 parameters, Fig. 9.

384 3.6. Intramolecular H-bonds

385 *Syn* and *anti* forms of the hypoxanthine moiety relative to the
386 furan ring provide opportunities for H-bonds involving the **O2'H**
387 and **O5'H** hydroxyl moiety with hypoxanthine's nitrogen atom at
388 position 3. Several authors have studied the intramolecular
389 H-bonds in related nucleosides, in special using AIM method
390 [51–53]. In the present manuscript, we have considered the classi-
391 fication of the H-bonds according to Desiraju et al. [54,55]. Five
392 intramolecular H-bonds may be observed in the conformers of
393 the main tautomer N1 of INO molecule, Fig. 5: (i) $\text{O2}' \cdots \text{H3}'_{(03')}$,
394 (ii) $\text{O3}' \cdots \text{H}_{(02')}$, (iii) $\text{N3} \cdots \text{H2}'_{(02')}$, (iv) $\text{N3} \cdots \text{H5}'_{(05')}$ and (v)
395 $\text{O5}' \cdots \text{H3}'_{(03')}$.

396 Mainly, conformers A involve the (i) and (iii) H-bonds, con-
397 formers B involve (i) and (iv), and conformers C (ii) and (v). In
398 general, the H-bonds are of medium-strong strength. H-bonds (i)
399 and (iii) give a great stability to the structure and they lead to

the best conformers. The medium-strong strength of the H-bonds
may be responsible of the high flexibility of the structure, with
high values of ν_{max} . Conformers *syn* have similar flexibility that
anti and *high-anti*. H-bond (iii) is the strongest ones in INO. The
stability in decreasing order is: (iii) > (iv) > (i) \approx (ii) > (v). In con-
formers A an increment in the strength of (iii) leads to high ν_{max} .
Similarly, in conformers C an increment in the strength of (v)
increases ν_{max} .

397 3.7. Natural NBO atomic charges

398 The calculated values in conformer A1 appear collected in Fig. 4.
399 Each of the electron-rich sites of the hypoxanthine residue is in-
400 volved in H-bonds, with N1, N3, N7 and O6 acting as single accep-
401 tors. The largest negative charge corresponds to **O2'**, **O3'** and
402 **O5'** atoms, ca. $-0.8 e$ (where e is the charge of an electron). The next
403 atom with large negative charge is N1 and **O4'** (ca. $-0.6 e$). The
404 value of this charge on N1 is the same that found in HX-19 tautomer
405 of hypoxanthine, and slightly lower than in guanosine (G),
406 $-0.69 e$. The main effect of the NH_2 group of G is a remarkable
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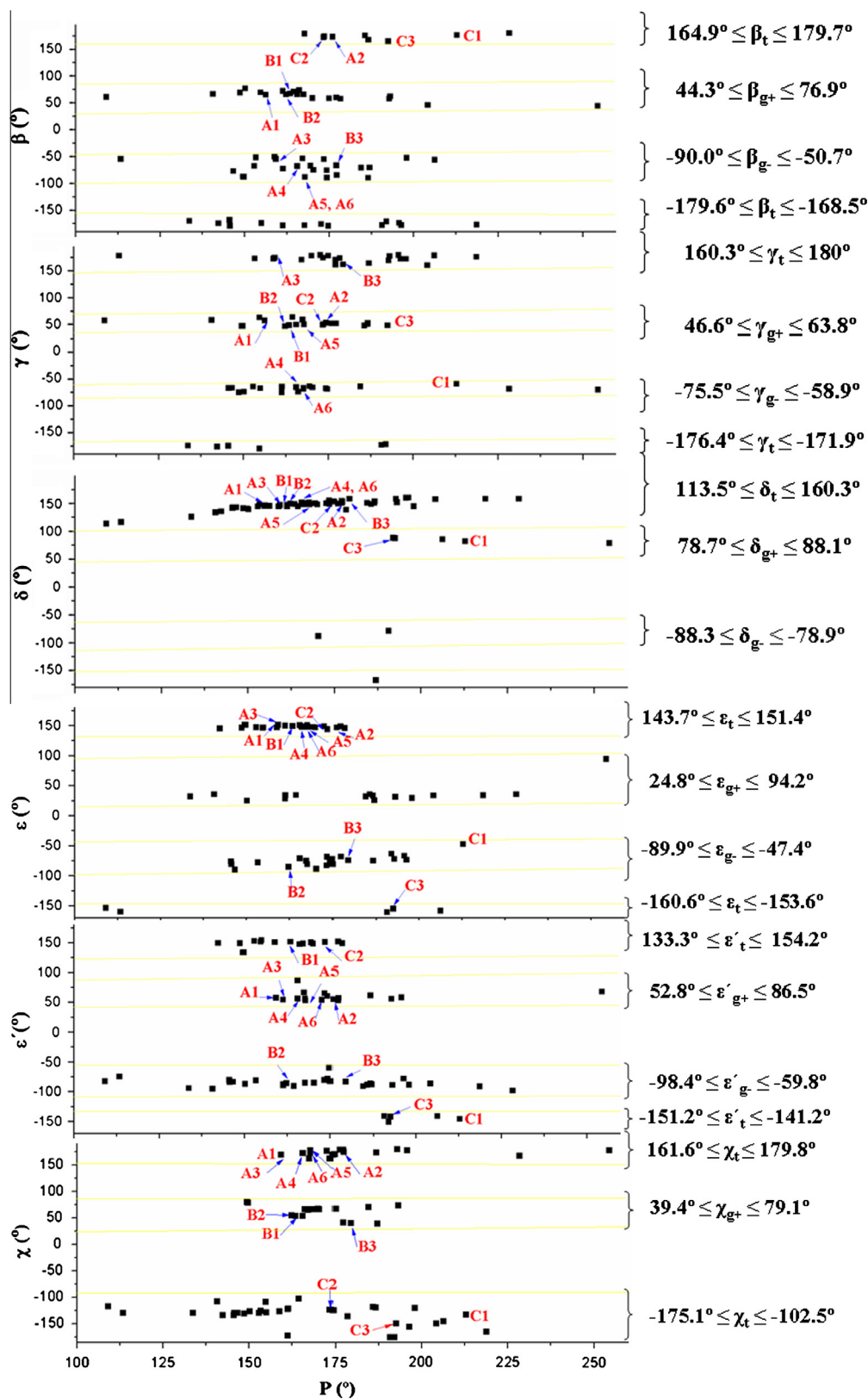


Fig. 7. Distribution of the 69 optimum stable calculated conformers in tautomer N1 of inosine according to the values of the exocyclic torsional angles: χ , ϵ , ϵ' , β , δ and γ versus the pseudorotational phase angle P .

418 increment in the positive charge on C2, 0.74 e , because of the high
 419 negative charge on the amino nitrogen, $-0.85 e$. Consequently, a
 420 noticeable increase of the negative charge on N3 is observed,
 421 $-0.70 e$ ($-0.58 e$ in INO).

In N9 the negative charge ($-0.418 e$) is lower than on N1, N3
 422 and N7, and also slightly lower than in G, $-0.48 e$. However, in
 423 HX-19 is $-0.6 e$. It is because in INO the bonding to the furanose
 424 ring reduces the negative charge on N9.
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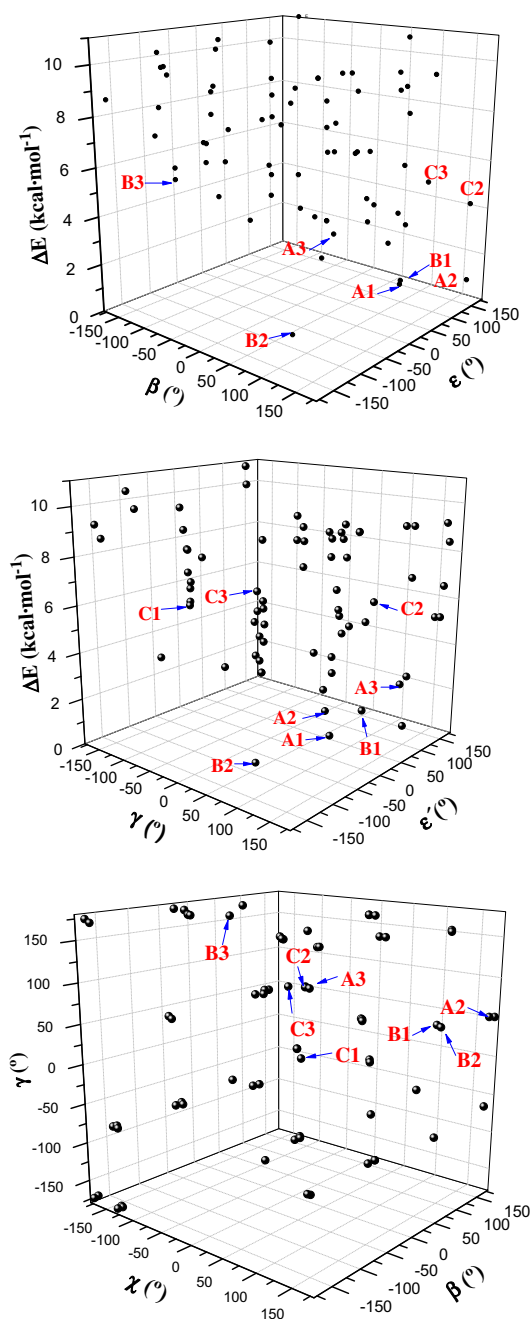


Fig. 8. 3D plots with the relative energies of the 69 optimum stable conformers according to the values of the exocyclic torsional angles: β , γ , ε and ε' . In the bottom is shown the distribution of the conformers according to the values of the exocyclic torsional angles γ , χ and β .

The value of the charge on N7 is important because in the neutral form of anti-tumour platinum drugs, the platinum atom has a strong preference for nitrogen N7 rather than oxygen atoms of the base for its coordination [56]. Also, the N7 position in DNA is the most open to attack. N1 position is also important because when deprotonation of the weakly acid INO occurs, the metal binding site changes to N1, which is the formally deprotonated site [57]. Raman spectrum of INO in H₂O indicates that the site of deprotonation in basic solutions is N1, while the site of protonation in acidic solutions is N3 and N7, the same sites that in the nucleotide INO-5'-P. These results are useful for identification and characterization of its structure in natural occurring biopolymers [58].

H2'(O2') has the highest positive charge (0.519 e), i.e. it is the most reactive. With slightly lower values appear H3', H5'(O5')

and H1(N1). The remaining hydrogen atoms have much less positive charge, ca. 0.3–0.2 e, i.e. they are less reactive. All the ribose acts as donor in H-bonds, with the exception of the ribose ring O atom, which is an acceptor of H-bond. The highest positive charge appears on C6 atom, 0.650 e, in concordance to the high negative charge on O6.

3.8. In the solid state

Experimentally, INO has been studied in the solid state by means of X-ray [51,59,60]. It has been crystallized in three different crystal forms [60]. Two of them occur in the monoclinic system and one in the orthorhombic form. In this last form, the two crystallographically independent molecules are essentially identical with regard to bond lengths, bond angles and conformational

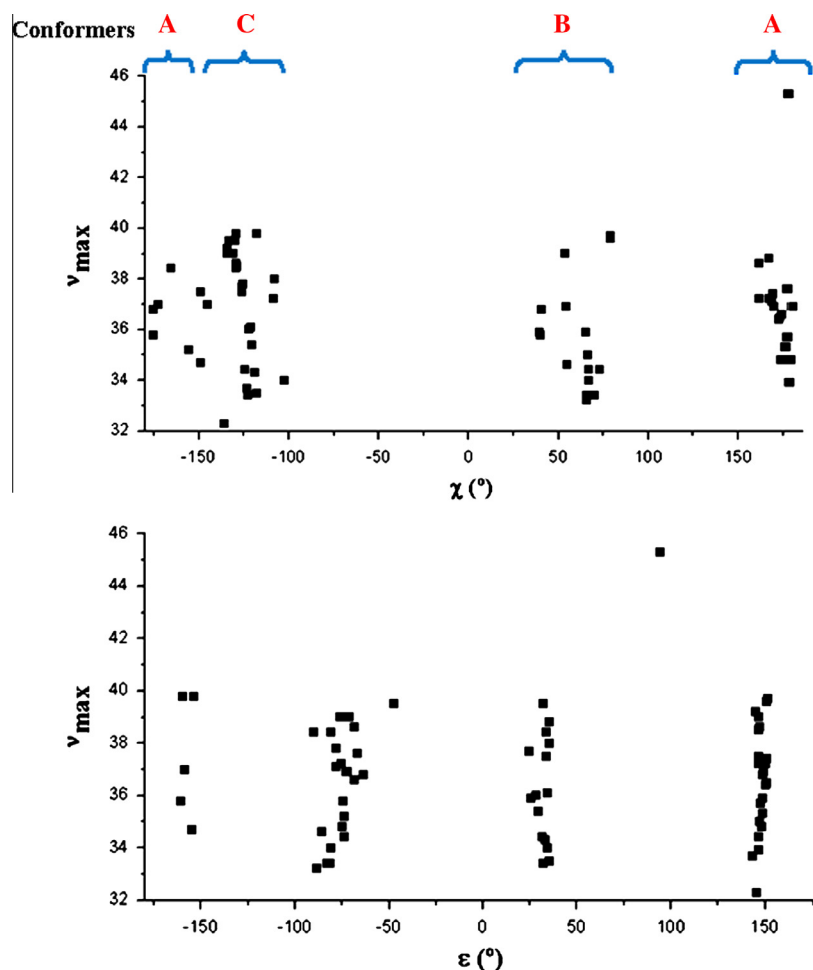


Fig. 9. Distribution of the 69 optimum stable conformers according to the values of the exocyclic torsional angles χ and ϵ versus v_{\max} .

parameters. The location in the crystal of a proton on N1 confirms the tautomeric form as *keto* for both molecules. The *syn* orientation is not an active form, and thus we have not further considered these structures.

The crystals with monoclinic symmetry [59] show also two molecules in the unit cell and a special arrangement of intermolecular H-bonds. We have tried to simulate this arrangement through a dimer, trimer and pentamer forms, Fig. 10. The calculated value of the inter- and intramolecular H-bonds, as well as the total energy of the structure is included in the Figure. All possible H-bonds are formed and there are a number of close intermolecular contacts, some involving H atoms attached to C atoms of the purine residue. This structure is tightly bonded although not so tight as that reported in the crystal. There are four H donor sites: N1, H2'(O2'), H3'(O3') and H5'(O5'), and six possible acceptor sites: N3, N7, O6, O2', O3' and O5'.

Dimer, trimer and pentamer structures are built with the most stable conformer A1. In the dimer there is only one intermolecular H-bond between the two molecules, which come from the disappearance of H3'...O2'. In the trimer appears five intramolecular H-bonds and four inter-, while in the pentamer is six intra- and five inter-. However, in the crystal appear more H-bonds than in our simulation. It seems probable that strong intermolecular packing forces of the crystal cause the differences observed with our simulated model.

The steric hindrance in furanose moiety probably determines changes in the puckering. Because of the sp^3 hybridization of the

C1' atom, the furanose moiety appears out-of the hypoxanthine ring plane, with a calculated N9-C1'-C2'-C3' torsional angle of 155.2° in molecule A of the pentamer form. The bulky of this substitution deforms all the neighbour angles, but the values are similar to those of the crystal [59]. The endocyclic and exocyclic torsional angles, as well as the pseudorotational angle P of our simulated structures are shown in Table 3. The calculated values of χ angle are in *anti* form, as it is found by X-ray. The pentamer form shows a large value of P , corresponding to ${}_3E$. Unfortunately, few values of these angles appear in Ref. [59], but they can be complemented with those of Ref. [50].

Tables 1-Sup. and 2-Sup. show a comparison of the calculated bond lengths and angles in the monomer and pentamer forms with the experimental ones [59]. In the pentamer form, the computed bond lengths in the hypoxanthine moiety are similar to those in the crystal, and only three bonds show a noticeable difference: C4-C5 (0.016 Å), C6-N1 (0.022 Å) and N7-C8 (0.011 Å). The agreement is also observed in the angles, with small discrepancies in our model as compared to the crystal. In Table 2-Sup. is collected the computed bond lengths in the furanose moiety of molecule A and the experimental values. A small shortening in our calculated values is observed in O4'-C1' (0.011 Å), C2'-O2' (0.016 Å) and C5'-O5' (0.009 Å), while other bonds are lengthened, the largest one corresponds to C1'-C2' (0.019 Å).

A general agreement was also obtained in the angles. Thus, the calculated *ipso* angle C4-N9-C8 (106.4°) is in accordance to that reported in the crystal, 105.7°. The angles of the furanose moiety

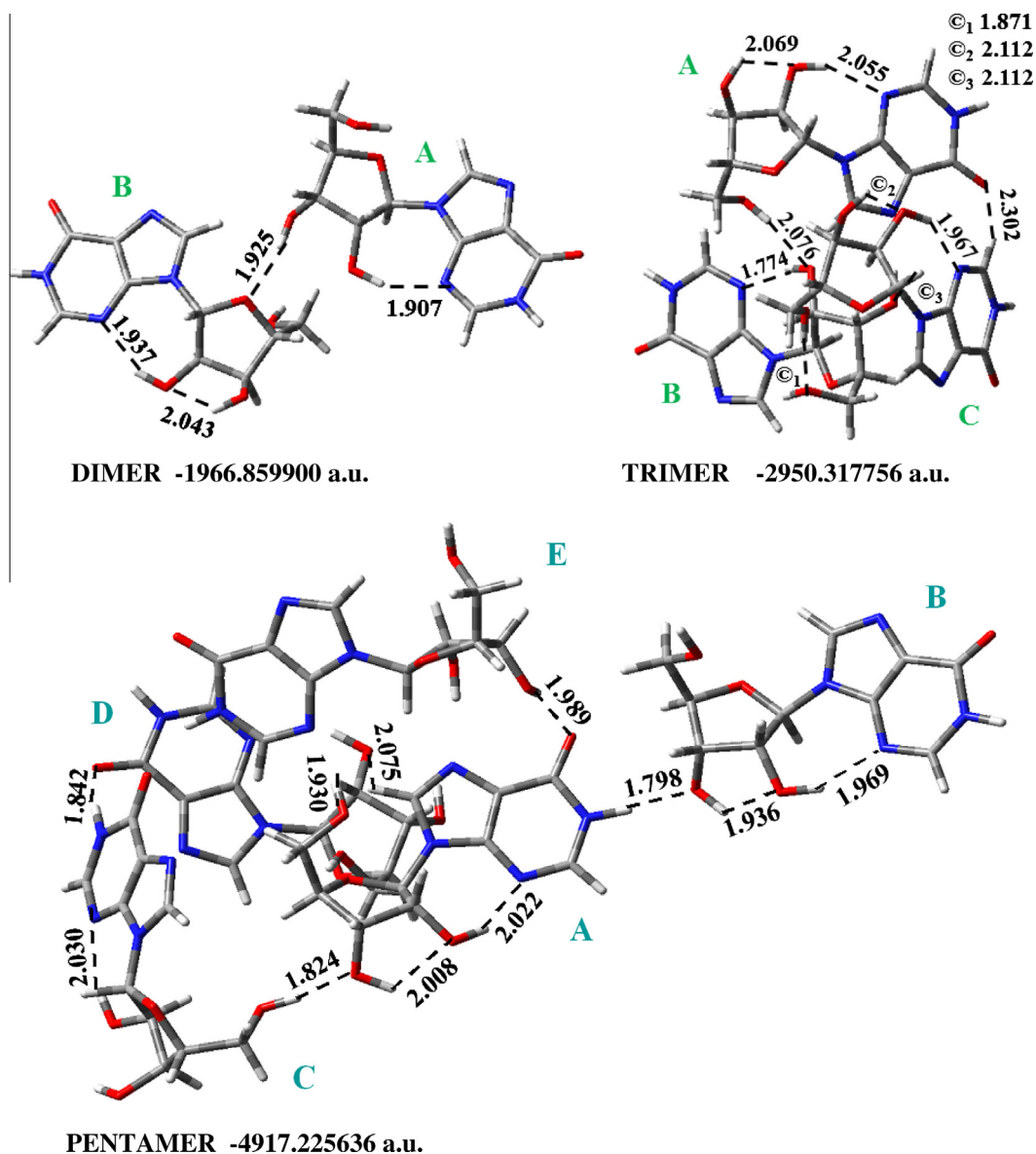


Fig. 10. The optimized dimer, trimer and pentamer forms in conformer A1 of inosine at the B3LYP/6-31G(d,p) level. The H-bonds observed are in Å and the E(RB + HF-LYP) in a.u.

Table 3

A comparison of the most important structural parameters in the dimer, trimer and pentamer forms calculated at the B3LYP/6-31G(d,p) level in inosine molecule with those in the crystal. The endocyclic and exocyclic torsional angles and the pseudorotational angle *P* are in degrees.

Solid form	χ	β	γ	δ	ϵ	ϵ'	ν_0	ν_1	ν_2	ν_3	ν_4	<i>P</i>	<i>S</i>	V_{\max}
<i>Dimer</i>														
Molecule A	162.9	64.9	56.7	141.6	179.9	65.1	-26.6	36.7	-32.3	18.2	5.2	153.2	1^2T	36.2
Molecule B	175.0	161.1	50.0	149.8	148.1	57.4	-8.5	27.3	-34.2	30.2	-13.9	184.5	3^2T	34.3
<i>Trimer</i>														
Molecule A	179.8	91.4	55.0	150.6	148.6	54.2	-9.2	27.8	-34.2	30.2	-13.	183.6	3^2T	34.3
Molecule B	163.3	61.7	51.5	138.9	-170.8	47.8	-33.5	42.2	-35.5	16.5	10.5	147.5	1^2T	42.1
Molecule C	157.8	135.3	47.0	152.6	148.9	68.0	-9.0	28.2	-35.3	31.3	-14.1	184.3	3^2T	35.4
<i>Pentamer</i>														
Molecule A	179.9	118.9	66.6	155.7	145.3	55.0	-4.3	24.4	-34.0	32.6	-17.9	191.8	$3E$	34.7
<i>X-ray data</i>														
Ref. [60]	147	74.7	169.0											
Molecule A ^a			64.0				-22.5	36.9	-36.6	24.4	-1.3	163.3	$2E$	38.2
Molecule B ^a			47.0				-31.2	41.2	-34.7	17.8	8.0	149.6	1^2T	40.2

^a Inosine dihydrate. Ref. [51].

are also similar to those of the crystal. The largest deviation is 3.2° corresponding to $C2'-C3'-O3'$, and 5.0° corresponding to $O3'-C3'-C4'$.

Crystal structure of INO dihydrate [50] and sodium salt of N1-deprotonated INO with 2.5 H₂O [57] have been also reported. In the first case, the monoclinic crystals show two nucleoside molecules and four water molecules per asymmetric unit. The torsion angles reported in molecule A and B of the crystal are also collected in Table 3. Some similarities appear with our calculated data in the central molecule A of the pentamer form. In the second case, the N1-deprotonated INO has a *syn* conformation, which is stabilized by intramolecular $O5'-H \cdots N3$ hydrogen bonds, our B1 conformer of Fig. 5.

4. Summary and conclusions

In the present work we have shown a comprehensive compendium of the possible conformers of the N1 tautomer of INO. The geometries and values of the properties presented here appear to be the most accurate to date. The most important findings of the present work are the following:

- (1) Five tautomers of INO were identified and fully optimized. The relative energies of these tautomers appear related to the dipole moment (μ), and to the exocyclic torsional angle γ . Thus, the least stable tautomer (N7) in the isolated state has the highest μ , i.e. it is the most favored in a polarisable environment with water.
- (2) In the isolated state, the most stable tautomer is N1 by both, B3LYP and MP2 methods. In this tautomer, and through a rotation of $\chi, \beta, \gamma, \delta, \epsilon$ and ϵ' angles, the 69 most energetically favourable conformers were identified at the B3LYP level, 3 *anti* and 2 *syn* falling into the 0–2 kcal/mol $\Delta E + ZPE$ energy range and 31 *anti*, 10 *syn* into the 2–8 kcal/mol energy range.
- (3) Conformer A1 appeared to be energetically the most favourable by B3LYP, but by MP2 was found conformers B1. In general, conformers A are more stable than B and C.
- (4) The distribution of all the conformers according to the ranges of stability of the characteristic torsional angles was established. The values obtained indicate the flexible nature of INO. For a high stability the conformers should have a high-*anti* form, a value of ϵ in the $149.7^\circ \rightarrow 151.2^\circ$ range and of ϵ' in the $52.8^\circ \rightarrow 59.4^\circ$ range.
- (5) In INO the lack of the NH₂ group of guanosine remarkably reduces the positive charge on C2, and consequently, a noticeable decrease of the negative charge on N3 is observed, i.e. it is less reactive. Similarly, the negative charge on N1 is also reduced although in less amount.
- (6) The intermolecular H-bonds of the solid state were well simulated through a pentamer form. The structure was tightly bonded and the values of the torsional angles appear in agreement with those found in the crystal. The differences observed can be attributed to the packing forces of the crystal.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molstruc.2013.05.035>.

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Article

Conformational Analysis, Molecular Structure and Solid State Simulation of the Antiviral Drug Acyclovir (Zovirax) Using Density Functional Theory Methods

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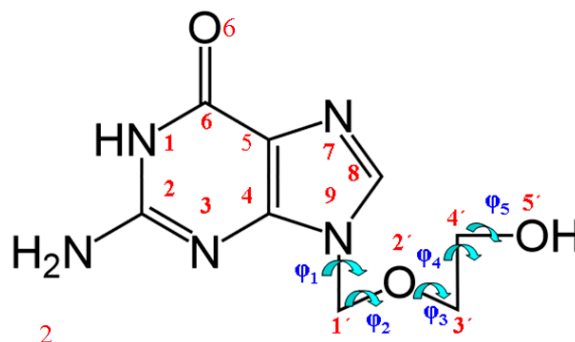
Abstract: The five tautomers of the drug acyclovir (ACV) were determined and optimised at the MP2 and B3LYP quantum chemical levels of theory. The stability of the tautomers was correlated with different parameters. On the most stable tautomer N1 was carried out a comprehensive conformational analysis, and the whole conformational parameters (R , β , Φ , φ_1 , φ_2 , φ_3 , φ_4 , φ_5) were studied as well as the NBO Natural atomic charges. The calculations were carried out with full relaxation of all geometrical parameters. The search located at least 78 stable structures within 8.5 kcal/mol electronic energy range of the global minimum, and classified in two groups according to the positive or negative value of the torsional angle φ_1 . In the nitrogen atoms and in the O2' and O5' oxygen atoms of the most stable conformer appear a higher reactivity than in the natural nucleoside deoxyguanosine. The solid state was simulated through a dimer and tetramer forms and the structural parameters were compared with the X-ray crystal data available. Several general conclusions were emphasized.

Keywords: acyclovir; 9-(2-hydroxyethoxymethyl) guanine; tautomer; antiviral drug; conformational analysis; Zovirax

1. Introduction

Acyclovir (ACV, 9-(2-hydroxyethoxymethyl) guanine, Zovirax [1,2], Figure 1), is a guanine derivative possessing antiviral activity and commonly used in the treatment of herpes. It is a potent antiviral agent that is used as a highly specific inhibitor of herpes viruses (HSV) types 1 and 2 [3–7]. A series of new guanine base modified tricyclic analogues of ACV and ganciclovir were evaluated for activity against herpes simplex virus type 1 and 2, showing similar antiherpetic potency as the parent compounds ACV and ganciclovir [8]. The antiherpetic activity was found to be strongly dependent on the nature and esteric demands of the substituents in the 6 and/or 7 positions [9].

Figure 1. Molecular structure and definition of the torsional angles in tautomer N1 of acyclovir.



Most persons who are infected with human immunodeficiency virus type (HIV-1) are also infected with herpes simplex type2 (HSV-2), which is associated with increased plasma and genital levels of HIV-1. Thus, it has been reported that ACV inhibits HIV upon human herpes virus (HHV) coinfection in tissue cultures. This activity was found to be correlated with the phosphorylation of the parent drug to the monophosphate form mediated by HHV-encoded kinase [10]. Recent studies show that ACV decreases the HIV-1 RN and suppress both viruses in coinfecting tissues [11], with great impact on HIV and HSV-2 seropositive patients [12,13]. The treatment of ACV-resistant HSV infections [7,14] and tests for release it at oral therapeutic levels have been also reported [15].

As it is shown above, ACV has been extensively studied from the pharmaceutical and medical point of view. However, few studies appear on its molecular structure, only at low level [16], and to our knowledge, there is no data on its conformational characteristics by Density Functional Methods (DFT) or *ab initio* quantum chemical methods. Thus, it is one of the tasks of the present manuscript. Conformations of some derivatives of ACV with biological activities have been reported [17], as well as studies of complexes of ACV with several metals [18,19].

The tautomeric study of DNA structural components has a great interest today with numerous research publications [20,21]. The tautomeric equilibrium in ACV between the keto and enol forms has been observed from the UV/VIS spectra [22]. This equilibrium depends on the polarity of the solvent, and therefore, in water solution the keto form prevails, while in methylene chlorides it is the enol one. Thus, another goal of the present manuscript is to study the possible tautomers of ACV, and the determination of their % populations at room temperature and at 273.15 K. The stability of these tautomers and its dependence of different parameters is another point analyzed here.

Finally, from our understanding, would be interesting to calculate the different conformational possibilities of ACV and compare the results with the natural nucleoside deoxyguanosine (dG). This is the last goal of the present manuscript. An accurate knowledge of the different conformers of ACV, its charge distribution, inter- and intra-molecular interactions, solid structure, and flexibility would be an important help for the interpretation of drug-target interactions, as well as to design new antiviral.

For this reason, the conformers of natural and analogues nucleosides have been analyzed by different authors. Now, an extensive theoretical study of the conformational preferences in ACV has been carried out with full relaxation of all geometric parameters, in an attempt to gain insights into molecular features responsible for activity. We will attempt to determine herein, if the various geometric features in ACV are correlated or interact with one another. We are also interested in whether alternative forms of hydrogen bonding make significant contributions to the conformational behavior of ACV.

2. Computational Details

Calculations were carried out by using the Becke exchange functional (B) [23], Becke's three-parameter exchange functional (B3) [24], Handy's OPTX modification of Becke's exchange functional (O) [25,26] and the extended (X3) [27], in combination with the correlation functionals of Lee, Yang, and Parr (LYP) [28], and Perdew and Wang's 1991 (PW91) [29]. B3LYP Density Functional method (DFT) is the most used today, and for this reason the majority of the calculations were carried out with it.

All the methods appear implemented in the GAUSSIAN 03 program package [30]. The UNIX version with standard parameters of this package was used in the alpha computer of the Computational Centre from University Complutense of Madrid, in which all quantum chemical computations were performed. DFT methods provide adequate compromise between the desired accuracy and the heavy demands put on computer time and power. Different studies have shown that the data obtained with DFT methods are in good agreement with those obtained by expensive computational methods as MP2 [31–35]. Also they give good results in many studies on nucleosides and on drug design [36,37] and they predict vibrational frequencies of DNA bases better than HF and MP2 [38–42]. Moreover, because of the high size of ACV, MP2 calculations were only possible with the 6–31G(d) basis set for memory computer problems. However, we used the 6–31G(d,p) basis to calculate single point energies.

The 3D Potential Energy Surface (PES) of this molecule was determined by rotation of the exocyclic and endocyclic torsional angles ϕ_1 (equivalent to χ angle in nucleosides [43]), ϕ_2 , ϕ_3 , ϕ_4 and ϕ_5 . These dihedral angles were simultaneously hold fixed at values varying between 0° and 360° in steps of 60° in a first study. All other geometrical parameters were relaxed during these optimizations. 78 optimized geometries were obtained in this step by minimizing the energy with respect to all geometrical parameters without imposing molecular symmetry constraints. Berny optimizations under the TIGHT criterion were used. Atomic charges were determined with the Natural NBO procedure [44].

Harmonic wavenumber computations were carried out at the same level of the respective optimization process and by the analytic evaluation of the second derivative of the energy with respect to nuclear displacement. Vibrational frequencies calculations were performed on all optimized

conformers to confirm that they corresponded to local minima. All optimized structures showed only positive harmonic vibrations (local energy minima). Relative energies were obtained by adding zero-point vibrational energies (ZPEs) to the total energy. For the calculation of the ZPEs, the frequencies were retained unscaled. The ΔG values were sums of electronic and thermal Free Energies. The conformational equilibrium at 298.15 K was evaluated by means of the Boltzmann distribution formula $\exp(-\Delta G/kT)$, where ΔG is the relative Gibbs energy.

3. Results and Discussion

ACV has five possible tautomers (Figure 2), that were fully optimized at different levels of computation, Table 1. The most stable one corresponds to N1, and thus we have focused the study only in this tautomer. The remaining forms were left for future research. In the last two column of this Table is shown the % population of the different tautomers at 298.15 K and 273.15 K. At room temperature the largest population corresponds to tautomer N1 (48.1%). The second population is due to OHC tautomer (37.7%) and the third one is to OHT (14.3%). Tautomers N3 and N7 have very little population, less than 0.05%.

Figure 2. Five tautomers described in acyclovir.

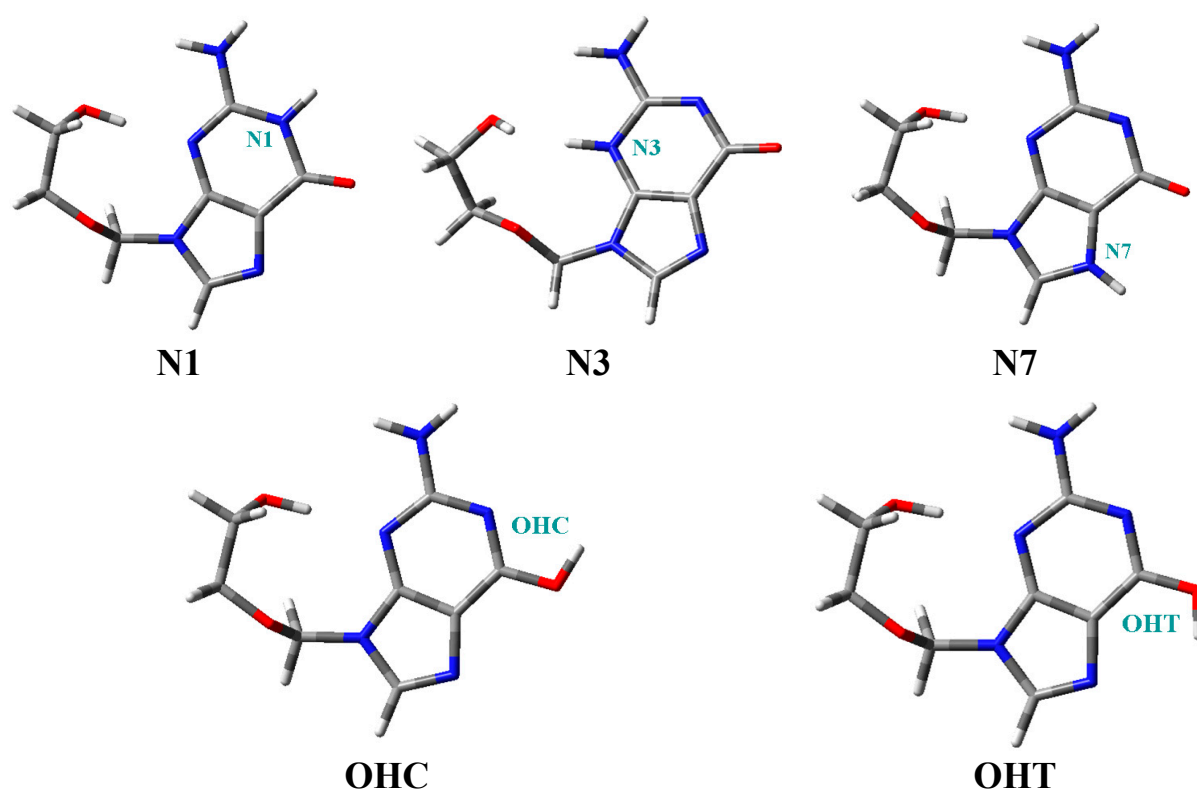


Table 1. The optimum stable tautomers calculated in acyclovir molecule at the levels: B3LYP/6-311++G(3df,pd) (values in bold); MP2/6-31G(d,p) (values in key); B3LYP/6-31G(d,p) (values in normal); O3LYP/6-31G(d,p) (values in brackets); B3PW91/6-31G(d,p) (values in parenthesis); B3LYP/cc-pVDZ (values in quotation marks) and B972/6-31G(d,p) (values in italic type). Torsional angles in degrees, dipole moments in debyes, distance R in Å, energy increments in kcal·mol⁻¹ and population (%) at 298.15 K (P_{298.15}) and at 273.15 K (P_{273.15}).

Tautomers	R	β	Φ	φ_1	φ_2	φ_3	φ_4	φ_5	μ	ΔE	ΔG	P _{298.15}	P _{273.15}
N1	3.963	72.5	54.3	-75.3	146.4	-88.5	69.8	-69.3	5.739	0 ^a			
	{3.892}	{73.5}	{51.3}	{-71.9}	{150.3}	{-94.6}	{69.1}	{-75.4}	{6.207}	{0}			
	3.925	72.3	53.7	-74.1	144.7	-93.4	71.1	-73.3	5.375	0	0	48.1	49.2
	[3.956]	[72.7]	[53.9]	[-75.4]	[144.5]	[-94.0]	[71.5]	[-72.1]	[5.369]	[0]	[0.638]		
	(3.891)	(72.7)	(53.2)	(-75.1)	(145.2)	(-92.3)	(70.3)	(-74.1)	(5.492)	(0)	(0)		
	“3.922”	“72.0”	“54.0”	“-73.9”	“143.2”	“-92.0”	“72.3”	“-74.4”	“5.34”	“0”	“0”		
3.913	72.8	53.3	-75.0	145.3	-93.4	70.8	-73.6	5.502	0	0			
N3	{3.672}	{77.0}	{53.5}	{-45.9}	{149.1}	{-92.5}	{60.2}	{-92.6}	{12.220}	{25.457}			
	3.877	80.5	49.5	-48.4	155.0	-93.9	61.1	-88.	11.183	25.529	24.811	<0.05	<0.05
N7	{3.741}	{73.0}	{52.0}	{-76.4}	{140.5}	{-80.8}	{72.4}	{-92.7}	{9.841}	{19.302}			
	3.745	72.8	53.3	-79.9	136.9	-78.5	71.8	-91.5	7.785	17.516	17.689	<0.05	<0.05
OHC	{3.826}	{73.5}	{51.7}	{-72.3}	{146.5}	{-89.2}	{70.4}	{-82.6}	{2.471}	{0.603}			
	3.839	72.9	53.4	-75.9	141.9	-87.0	71.1	-81.5	2.100	0.145	0.284	37.7	37.7
OHT	{3.792}	{73.5}	{52.0}	{-72.4}	{145.0}	{-87.2}	{70.6}	{-86.1}	{3.437}	{1.131}			
	3.806	72.9	53.6	-76.2	140.7	-85.0	71.0	-84.9	2.105	0.719	0.830	14.3	13.1

^a $\Delta E = 0 = -811.214731$ a.u. at B3LYP/6-311++G(3df,pd) level; ^b $\Delta E = 0 = -808.643998$ a.u. at MP2/6-31G(d,p) level; ^c $\Delta E = 0 = -810.723396$ a.u. at B3LYP/6-31G(d,p) level;

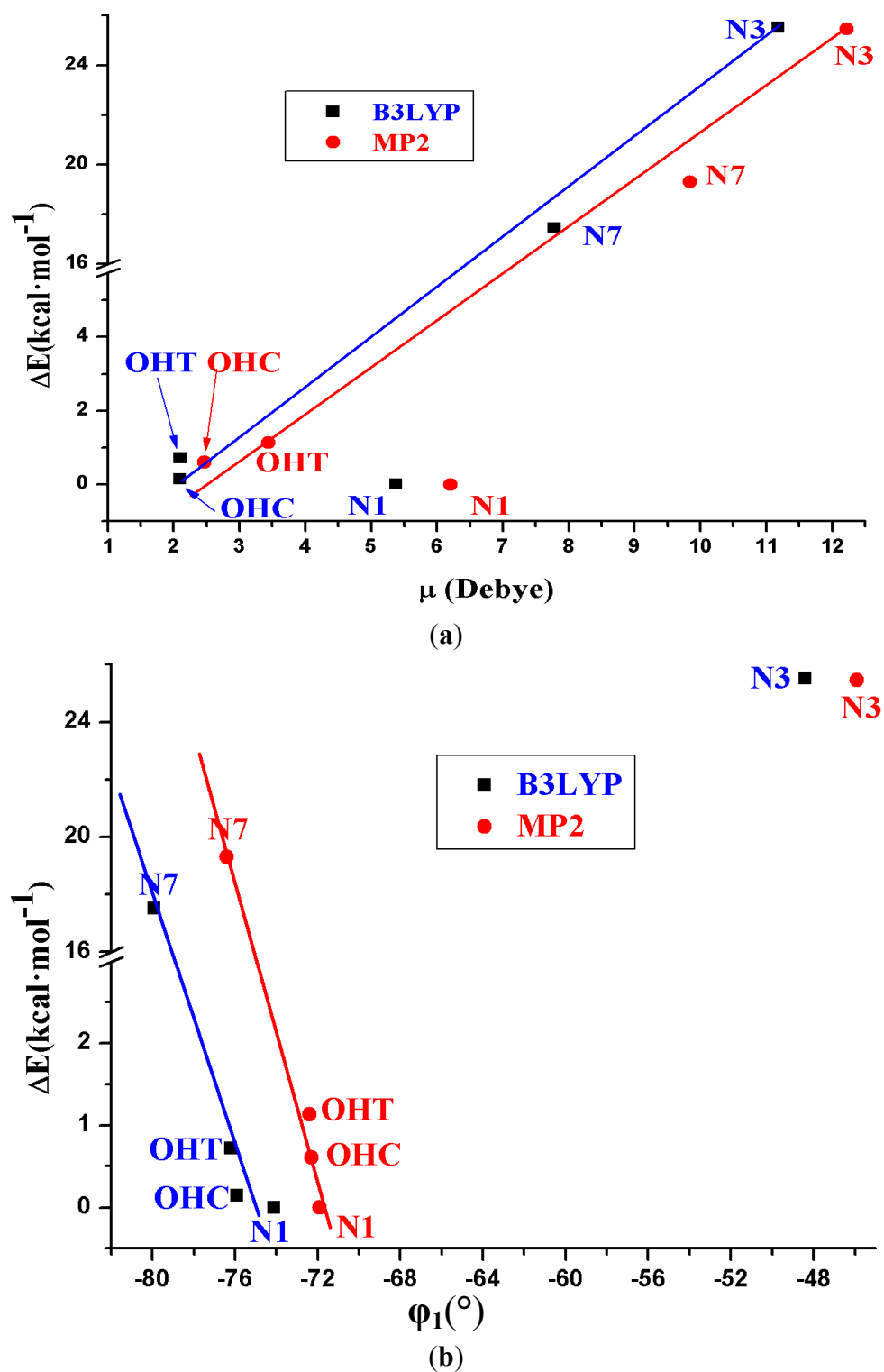
^d $\Delta G = 0 = -810.764701$ a.u.; ^e $\Delta E = 0 = -810.449499$ a.u. at O3LYP/6-31G(d,p) level; ^f $\Delta G = 0 = -810.490782$ a.u.; ^g $\Delta E = 0 = -810.419555$ a.u. at B3PW91/6-31G(d,p) level;

^h $\Delta G = 0 = -810.460766$ a.u.; ⁱ $\Delta E = 0 = -810.760402$ a.u. at B3LYP/cc-pVDZ level; ^j $\Delta G = 0 = -810.801662$ a.u.; ^k $\Delta E = 0 = -810.444324$ a.u. at B972/6-31G(d,p) level;

^l $\Delta G = 0 = -810.485454$ a.u.

An analysis of the relative energies of these tautomers shows that they can be related to the dipole moment (μ) and to the torsional angle φ_1 , Figure 3. Thus, the least stable tautomer in the isolated state (N7) has the highest μ , *i.e.*, it is the most stable in water solution. Other relations can be observed between ΔE and φ_1 . It is noted that tautomer N7 has the highest negative value of φ_1 angle.

Figure 3. Relationship/tendency observed between the relative electronic energy $\Delta E + \text{ZPE}$ correction of the different tautomers vs. (a) the dipole moment μ . and (b) the exocyclic torsional angle φ_1 .



3.1. Definition of the Conformational Angles

The atomic description of tautomer N1 of ACV, as well as the most important exocyclic and endocyclic torsional angles, is defined in Figure 1. The conformation can be characterized by the following five torsional angles: (i) the torsional angle, φ_1 (C4-N9-C1'-O2'), which determines the two orientations of the base relative to the opened chain, denoted in the present manuscript as conformers A and B; (ii) The exocyclic torsional angle φ_2 (N9-C1'-O2'-C3'); (iii) φ_3 (C1'-O2'-C3'-C4') that determine the different folds of the chain; (iv) φ_4 (O2'-C3'-C4'-O5') describing the O5'H orientation; and (v) the torsional angle φ_5 (C3'-C4'-O5'-H5') that define the orientation of the hydroxyl hydrogen H5'. This φ_5 angle has some similarities with the β angle of the nucleosides [43].

Considering the structure of the chain, that likes the structure of the sugar in the nucleosides, and in accordance to previous works [45], other three structural parameters were defined to fix the chain position respect to the plane of the nucleobase: (vi) The vector R (N9 \cdots O5') which determines the distance of the OH group relative to the base; (vii) The angle β (C4-N9 \cdots O5') which defines the angle of the OH group relative to the base plane; and (viii) the angle Φ (C1'-N9 \cdots O5') which also determines the position of the OH group.

3.2. Conformers and Energetics

An extensive conformational study of tautomer N1 was carried out through a rotation of the exocyclic φ_1 , φ_2 , φ_3 , φ_4 and φ_5 torsional angles. A detailed collection of the most important conformational parameters of these optimized forms is included in Table 2. The conformers were classified according to the two ranges of rotation of φ_1 : conformers A with the φ_1 values negative and conformers B with φ_1 positive.

Two energy criteria were considered for each conformer: the electronic energy $\Delta E + \text{ZPE}$ correction, and the Gibbs energy ΔG . For the numbering of the conformers in each range of rotation of φ_1 was followed the $\Delta E + \text{ZPE}$ criterion. Calculations at different levels, as well as single point calculations at the MP2/6-31G(d,p)//B3LYP/6-31G(d,p) level were carried out to confirm the stability of the main conformers, Table 2 and Table 3. In general, the stability order remains, although several changes are observed. Thus, conformer B2 appears as the most stable one instead of A1 predicted by the DFT methods. The % population of the different conformers at 298.15 K ($P_{298.15}$) and at 273.15 K ($P_{273.15}$) was calculated. It indicates that only conformers A1 (41.5%), B1 (36.1%), B2 (9.6%) and A2 (8.7%) have importance. The population of the remaining conformers is lower than 1% and thus they are not of interest. The temperature effect is not significant.

By the same methodology, the relative energies of the different conformers have been determined in related nucleosides [34,46–48]. The global minimum calculated in these molecules by MP2/6-31G(d,p) was in accordance to that found by B3LYP/6-31G(d,p). Thus, our results by B3LYP can be considered acceptable.

Table 2. The 78 optimum stable conformers calculated in tautomer N1 of acyclovir molecule at the levels: B3LYP/6-311++G(3df,pd) (values in bold); MP2/6-31G(d) (values in keys); B3LYP/6-31G(d,p) (values in normal type); O3LYP/6-31G(d,p) (values in brackets); B3PW91/6-31G(d,p) (values in parenthesis); B3LYP/cc-pVDZ (values in quotation marks) and B972/6-31G(d,p) (values in italic type) level. Distance R in Å, torsional angles in degrees, dipole moments in debyes, and energy increments in kcal·mol⁻¹.

Conformers	R	β^a	Φ^a	φ_1	φ_2	φ_3	φ_4	φ_5	μ	ΔE	ΔG
A1	3.963	72.5	54.3	-75.3	146.4	-88.5	69.8	-69.3	5.739	0^b	
	{2.859}	{73.8}	{50.9}	{-71.8}	{151.8}	{-96.3}	{68.8}	{-74.2}	{6.182}	{0.064}	
	3.925	72.3	53.7	-74.1	144.7	-93.4	71.1	-73.3	5.375	0 ^c	0 ^d
	[3.956]	[72.7]	[53.9]	[-75.4]	[144.5]	[-94.0]	[71.5]	[-72.1]	[5.369]	[0] ^e	[0.638]
	(3.891)	(72.7)	(53.2)	(-75.1)	(145.2)	(-92.3)	(70.3)	(-74.1)	(5.492)	(0) ^f	(0) ^g
	“3.922”	“72.0”	“54.0”	“-73.9”	“143.2”	“-92.0”	“72.3”	“-74.4”	“5.343”	“0” ^h	“0” ⁱ
<i>3.913</i>	<i>72.8</i>	<i>53.3</i>	<i>-75.0</i>	<i>145.3</i>	<i>-93.4</i>	<i>70.8</i>	<i>-73.6</i>	<i>5.502</i>	<i>0^j</i>	<i>0^k</i>	
A2	3.774	76.8	52.5	-97.7	65.9	74.6	-67.0	-34.1	5.367	1.258	
	{3.727}	{77.7}	{50.7}	{-96.9}	{66.0}	{76.2}	{-65.5}	{-42.7}	{5.776}	{0.095}	
	2.892	77.3	51.5	-97.0	67.7	72.4	-65.1	-39.3	5.382	0.928	1.324
	[3.779]	[77.9]	[51.3]	[-98.8]	[68.1]	[72.8]	[-65.5]	[-38.3]	[5.361]	[1.189]	[2.009]
	(3.715)	(77.0)	(51.6)	(-97.4)	(67.4)	(72.8)	(-65.4)	(-38.6)	(5.436)	(0.655)	(1.034)
	“3.730”	“77.1”	“51.6”	“-96.6”	“67.6”	“72.4”	“-64.9”	“-38.4”	“5.385”	“1.000”	“1.370”
<i>3.736</i>	<i>77.4</i>	<i>51.5</i>	<i>-97.9</i>	<i>67.4</i>	<i>73.2</i>	<i>-65.5</i>	<i>-38.7</i>	<i>5.438</i>	<i>1.000</i>	<i>1.018</i>	
A3	5.155	118.5	37.5	-107.7	71.0	171.1	-63.7	57.6	5.762	1.202	
	{5.004}	{111.4}	{37.6}	{-101.9}	{64.7}	{164.9}	{-61.1}	{53.9}	{5.586}	{2.240}	
	5.104	118.6	36.4	-108.5	69.9	168.3	-61.1	53.4	5.594	2.303	1.014
	[5.149]	[121.2]	[36.1]	[-112.1]	[71.9]	[168.7]	[-63.0]	[55.9]	[5.653]	[0.585]	[0.073]
	(5.079)	(119.1)	(36.8)	(-110.2)	(69.3)	(168.7)	(-60.9)	(52.4)	(5.702)	(1.795)	(0.553)
	“5.105”	“120.0”	“36.7”	“-109.9”	“71.0”	“169.9”	“-60.7”	“52.5”	“5.610”	“2.864”	“1.513”
<i>5.094</i>	<i>119.1</i>	<i>36.7</i>	<i>-110.0</i>	<i>69.6</i>	<i>168.6</i>	<i>-61.9</i>	<i>54.8</i>	<i>5.673</i>	<i>2.864</i>	<i>0.095</i>	

Table 2. Cont.

Conformers	R	β^a	Φ^a	φ_1	φ_2	φ_3	φ_4	φ_5	μ	ΔE	ΔG
A4	4.592	116.2	64.7	-106.0	73.2	178.6	65.0	-56.8	7.630	1.726	
	{4.487}	{113.4}	{64.9}	{-100.7}	{67.1}	{178.0}	{61.3}	{-52.8}	{8.026}	{2.887}	
	4.517	117.5	65.1	-106.7	71.7	-179.5	62.2	-52.9	7.390	2.765	1.457
	[4.552]	[119.3]	[65.0]	[-109.9]	[73.7]	[-179.3]	[64.3]	[-55.3]	[7.309]	[1.030]	[0.432]
	(4.500)	(118.5)	(65.1)	(-107.9)	(71.0)	(-179.6)	(62.0)	(-52.8)	(7.480)	(2.309)	(1.009)
	“4.500”	“117.5”	“65.1”	“-107”	“71.0”	“-179.6”	“62.0”	“-52.8”	“7.480”	“3.256”	“1.871”
	<i>4.515</i>	<i>118.4</i>	<i>65.1</i>	<i>-108.2</i>	<i>71.4</i>	<i>-179.7</i>	<i>62.9</i>	<i>-54.0</i>	<i>7.467</i>	<i>3.256</i>	<i>0.503</i>
A5	5.119	123.3	36.2	-81.4	-71.2	-170.1	60.7	-53.6	5.166	2.952	1.976
A6	4.839	130.3	50.2	-93.9	-178.3	174.3	-60.6	54.4	7.387	3.870	2.472
A7	4.342	110.7	69.0	-100.5	73.3	-114.2	-57.0	53.3	4.342	4.088	2.821
A8	5.314	99.1	61.8	-108.8	70.2	174.4	179.8	123.1	5.490	4.529	3.020
A9	4.316	72.3	29.9	-100.9	86.9	94.8	-69.7	165.3	8.401	4.540	3.284
A10	4.317	98.8	29.8	-100.4	87.1	62.4	118.4	-119.6	8.402	4.541	3.295
A11	4.316	98.8	29.8	-100.5	87.1	94.7	-69.8	165.6	8.403	4.541	3.293
A12	5.248	97.3	61.6	-107.1	71.8	176.2	-179.4	177.3	6.700	4.778	3.132
A13	5.137	115.6	37.5	-109.6	69.9	168.6	-72.0	169.7	6.500	4.912	3.329
A14	3.804	130.6	83.0	-137.1	68.2	-147.3	63.6	64.2	7.916	4.944	4.017
A15	3.851	140.4	75.7	-114.8	-115.6	140.7	-61.6	63.4	7.669	5.082	3.917
A16	4.561	120.1	66.8	-112.4	70.5	-178.3	63.9	60.5	7.527	5.103	3.411
A17	4.400	99.7	34.4	-112.0	67.2	57.8	44.9	48.3	6.225	5.139	4.217
A18	5.204	119.3	39.3	-110.7	69.8	173.8	-64.3	-63.3	7.512	5.216	3.730
A19	3.411	68.6	64.3	-92.1	98.3	-114.1	64.4	39.1	7.760	5.354	4.890
A20	4.387	136.6	10.3	-88.2	158.8	79.4	-71.0	172.6	8.042	5.378	3.969
A21	3.562	167.7	60.8	-94.2	-126.1	83.2	-74.5	178.0	6.300	5.384	4.494
A22	4.486	118.5	67.8	-112.8	70.8	-179.7	71.7	-171.9	8.081	5.432	3.764
A23	5.209	88.5	51.3	-104.5	77.3	91.3	180.0	-69.9	6.218	5.476	3.817
A24	4.381	115.3	11.5	-102.7	-162.7	-77.3	73.0	-170.7	8.685	5.514	4.179
A25	4.948	80.5	59.5	-102.5	67.2	74.6	176.3	-68.2	6.425	5.588	4.277
A26	4.655	80.0	78.1	-99.5	74.6	-104.0	-175.2	72.4	7.000	5.633	4.093

Table 2. Cont.

Conformers	R	β^a	Φ^a	φ_1	φ_2	φ_3	φ_4	φ_5	μ	ΔE	ΔG
A27	4.609	83.1	78.2	-102.2	71.5	-107.6	-174.8	179.6	5.473	5.662	4.371
A28	5.146	128.0	36.4	-81.4	-70.5	-167.5	71.8	-170.7	6.229	5.675	4.396
A29	4.653	84.8	51.8	-103.7	70.9	-109.1	-174.5	-74.1	6.731	5.845	4.325
A30	5.235	90.1	49.7	-102.9	80.0	96.4	179.4	-173.8	7.848	5.850	4.085
A31	4.400	99.7	34.4	-112.0	67.2	57.8	44.9	48.3	6.225	5.890	4.508
A32	5.220	123.3	38.2	-81.4	-70.2	-172.6	64.6	64.8	4.345	5.909	4.708
A33	4.415	114.1	12.6	-104.1	-163.1	-74.6	69.3	69.4	7.384	5.917	4.782
A34	5.955	104.4	31.3	-95.4	179.0	-178.1	179.2	72.9	6.080	6.174	4.503
A35	5.898	124.0	30.3	-96.6	179.3	179.3	179.9	179.6	6.676	6.195	4.544
A36	5.956	124.3	31.3	-96.4	179.5	178.1	-179.0	-72.9	7.716	6.254	4.762
A37	4.565	102.4	34.8	-112.6	66.4	60.5	56.3	-178.8	7.684	6.290	5.085
A38	4.273	110.2	72.9	-105.8	71.1	-103.2	-66.6	167.8	6.458	6.432	4.855
A39	4.970	138.6	46.2	-100.0	-179.8	172.1	-72.6	166.4	8.071	6.610	4.755
A40	5.011	104.7	46.2	-97.2	-179.7	-176.1	72.9	-166.1	7.023	6.779	5.097
A41	5.030	104.4	48.4	-95.2	179.6	-177.9	64.3	61.5	8.220	7.100	5.519
A42	5.722	115.4	19.7	-99.3	-176.9	-84.1	-179.2	70.4	6.405	7.372	5.289
A43	5.350	87.6	45.5	-90.3	138.5	-88.9	-177.6	-70.3	7.007	7.483	7.510
A44	5.386	92.4	40.2	-91.6	146.1	-91.1	178.5	173.8	7.133	7.627	5.567
A45	5.699	118.8	18.3	-101.1	-173.8	-84.4	-178.6	176.1	7.994	7.665	5.672
A46	5.765	120.0	17.5	-101.1	-170.2	-80.2	-173.9	-69.9	6.680	7.684	5.636
A47	4.832	106.9	33.0	-107.0	-164.9	-72.0	-62.7	178.1	8.554	8.451	6.636
B1	3.928	73.4	53.4	75.1	-146.7	95.5	-68.8	72.8	5.812	0.072	
	{3.885}	{74.5}	{50.3}	{70.1}	{-151.5}	{94.2}	{-68.1}	{76.8}	{6.204}	{0.056}	
	3.880	73.4	52.8	73.5	-145.4	90.9	-69.3	76.7	6.746	0.083	0.151
	[3.927]	[73.6]	[53.1]	[74.4]	[-145.4]	[92.6]	[-69.8]	[74.6]	[5.485]	[0.124]	[0.822]
	(3.855)	(73.5)	(55.6)	(74.2)	(-145.7)	(90.5)	(-68.9)	(76.7)	(5.595)	(0.061)	(0.108)
“3.870”	“73.3”	“51.1”	“73.1”	“-144.2”	“89.6”	“-70.0”	“77.7”	“5.374”	“0.099”	“0.195”	
3.882	73.7	52.7	74.0	-145.8	91.5	-69.3	76.0	5.601	0.099	0.090	

Table 2. Cont.

Conformers	R	β^a	Φ^a	φ_1	φ_2	φ_3	φ_4	φ_5	μ	ΔE	ΔG
B2	3.770	77.2	52.0	97.0	-66.7	-74.1	66.6	36.1	5.348	1.216	
	{3.726}	{17.9}	{50.4}	{95.6}	{-66.8}	{-75.8}	{65.5}	{44.0}	{5.664}	{0} ¹	
	2.905	77.6	51.2	96.4	-68.5	-72.0	65.1	40.6	5.340	0.87	1.251
	[3.784]	[78.0]	[50.8]	[98.5]	[-68.9]	[-72.6]	[65.4]	[40.4]	[5.321]	[1.118]	[1.900]
	(3.713)	(77.3)	(51.2)	(96.8)	(-68.2)	(-72.4)	(65.4)	(39.9)	(5.404)	(0.612)	(0.975)
	“3.727”	“77.5”	“51.2”	“96.2”	“-68.4”	“-72.9”	“64.0”	“39.8”	“5.250”	“0.968”	“1.316”
3.736	77.8	51.1	97.3	-68.2	-72.8	65.4	40.3	5.398	0.968	0.941	
B3	5.157	117.3	37.3	106.1	-71.7	-171.1	63.5	-57.6	5.951	1.157	
	{5.007}	{110.9}	{37.4}	{100.4}	{-65.2}	{-165.1}	{60.9}	{-53.8}	{5.998}	{2.179}	
	5.104	117.9	36.2	107.1	-70.3	-168.1	60.9	-53.3	5.839	2.257	0.903
	[5.149]	[120.9]	[35.9]	[111.5]	[-72.1]	[-168.6]	[62.9]	[-55.9]	[5.872]	[0.542]	[0] ^m
	(5.080)	(118.1)	(36.5)	(108.2)	(-69.7)	(-168.2)	(60.8)	(-53.3)	(5.921)	(1.748)	(0.412)
	“5.106”	“119.5”	“36.5”	“108.6”	“-71.3”	“-169.7”	“60.6”	“-52.4”	“5.814”	“2.819”	“1.394”
5.094	118.6	36.6	108.9	-69.9	-168.4	61.7	-54.7	5.907	2.819	0.001	
B4	4.529	95.5	65.4	78.3	70.8	-178.7	63.6	-54.4	6.470	2.031	
	{3.673}	{66.1}	{78.5}	{56.6}	{63.8}	{-147.2}	{63.0}	{-52.4}	{6.649}	{1.707}	
	3.817	68.7	79.7	56.7	64.6	-149.1	63.8	-50.2	5.870	2.722	2.366
	[4.300]	[85.9]	[70.0]	[69.9]	[70.9]	[-168.2]	[63.2]	[-52.1]	[6.144]	[1.331]	[0.867]
	(3.814)	(68.9)	(79.4)	(56.4)	(64.5)	(-150.0)	(63.4)	(-48.6)	(5.913)	(2.523)	(2.080)
	“3.839”	“68.8”	“79.2”	“55.9”	“65.4”	“-150.2”	“63.6”	“-48.6”	“5.680”	“3.179”	“2.791”
3.858	70.3	78.6	58.0	64.6	-151.4	63.4	-49.6	5.949	3.179	1.557	
B5	4.598	115.9	64.6	105.4	-72.8	-178.2	-64.9	56.7	7.911	1.693	
	{4.507}	{112.9}	{64.3}	{99.6}	{-66.5}	{-177.0}	{-61.1}	{52.8}	{8.520}	{2.832}	
	4.526	117.5	64.8	106.4	-71.4	180.0	-62.0	52.8	7.735	2.757	1.463
	[4.559]	[119.2]	[64.9]	[109.6]	[-73.3]	[179.7]	[-64.2]	[55.2]	[7.629]	[1.012]	[0.420]
	(4.510)	(118.4)	(64.8)	(107.6)	(-70.7)	-179.9	(-61.8)	(52.7)	(7.826)	(2.289)	(0.978)
	“4.524”	“117.6”	“64.7”	“106.6”	“-72.3”	“-179.9”	“-61.7”	“52.1”	“7.517”	“3.262”	“1.920”
4.524	118.3	64.8	107.6	-71.1	-179.8	-62.7	53.8	7.793	3.262	0.470	
B6	5.119	123.1	36.3	81.0	70.9	170.1	-60.7	54.1	5.460	2.993	2.035
B7	4.991	113.3	29.3	107.2	-74.7	-87.7	-56.9	46.5	6.027	3.636	2.427

Table 2. Cont.

Conformers	R	β^a	Φ^a	φ_1	φ_2	φ_3	φ_4	φ_5	μ	ΔE	ΔG
B8	3.582	165.9	56.3	82.7	65.4	73.0	-75.2	178.4	6.463	4.240	3.708
B9	3.793	70.4	79.1	57.2	68.0	-93.9	-49.8	60.8	5.069	4.263	3.900
B10	4.325	99.2	29.1	98.3	-88.0	-94.7	69.5	-166.2	8.268	4.496	3.334
B11	5.318	99.2	61.7	108.3	-70.8	-175.3	179.6	71.1	5.640	4.508	3.006
B12	5.002	132.0	29.1	77.5	74.7	88.4	57.0	-46.2	5.222	4.791	3.824
B13	5.138	115.0	37.3	108.2	-70.2	-168.4	71.8	-170.5	6.133	4.845	3.212
B14	5.246	98.4	61.7	108.6	-71.5	-176.6	179.2	177.1	6.553	4.852	3.244
B15	5.312	99.2	62.1	108.6	-70.7	-175.8	-178.5	-70.6	7.964	4.893	3.413
B16	4.407	99.6	33.7	110.2	-68.1	-57.9	-44.6	-48.7	5.939	5.042	4.065
B17	5.205	118.2	39.0	109.3	-69.6	-172.9	64.6	63.9	7.429	5.101	3.551
B18	3.562	167.3	60.9	93.8	125.8	-83.0	74.6	-178.0	6.069	5.456	4.582
B19	5.277	89.9	49.5	103.3	-79.4	-94.8	-179.7	69.2	6.054	5.577	3.960
B20	4.876	78.3	61.1	101.0	-65.9	-75.2	179.3	-69.0	6.102	5.694	4.494
B21	5.253	91.0	49.4	103.8	-80.2	-97.6	-178.6	174.8	8.034	5.950	3.922
B22	4.534	101.8	35.0	112.6	-66.2	-59.3	-55.7	179.6	7.559	6.169	5.032
B23	3.146	83.0	95.1	73.1	67.3	-116.9	63.5	64.3	5.334	6.305	6.199
B24	4.610	77.9	77.5	79.0	72.8	-102.2	-173.9	-175.2	5.317	6.617	5.315
B25	4.365	150.0	33.5	76.4	67.0	59.5	40.5	41.6	5.882	6.677	5.427
B26	4.618	108.0	52.5	77.2	68.5	-105.1	-173.1	-79.3	5.308	6.689	5.500
B27	5.184	143.6	51.8	78.9	76.5	89.5	-178.9	-69.4	6.563	6.718	5.374
B28	4.635	114.4	78.3	79.8	73.8	-101.1	-174.8	72.3	7.031	6.831	5.483
B29	5.194	141.1	52.7	77.5	74.5	87.3	175.3	68.0	5.203	6.997	5.596
B30	5.129	142.9	52.3	79.1	76.5	90.0	-179.5	-175.2	7.031	7.015	5.609
B31	4.964	136.4	32.2	77.2	71.0	78.8	64.1	-178.9	5.033	7.508	6.051

^a Notation used from ref. [49]; ^b $\Delta E = 0 = -811.214731$ a.u. at B3LYP/6-311++G(3df,pd) level; ^c $\Delta E = 0 = -810.723396$ a.u. at B3LYP/6-31G(d,p) level; ^d $\Delta G = 0 = -810.764701$ a.u.; ^e $\Delta E = 0 = -810.449499$ a.u. at O3LYP/6-31G(d,p) level; ^f $\Delta E = 0 = -810.419555$ a.u. at B3LYP/cc-pVDZ level; ^g $\Delta G = 0 = -810.460766$ a.u.; ^h $\Delta E = 0 = -810.760402$ a.u. at B3PW91/6-31G(d,p) level; ⁱ $\Delta G = 0 = -810.801662$ a.u.; ^j $\Delta E = 0 = -810.444324$ a.u. at B972/6-31G(d,p) level; ^k $\Delta G = 0 = -810.485454$ a.u.; ^l $\Delta E = 0 = -808.643998$ a.u. at MP2/6-31G(d) level; ^m $\Delta G = 0 = -810.491798$ a.u.

Table 3. Single point calculations at the MP2/6-31G(d,p)//B3LYP/6-31G(d,p) level.

Conformer	$\Delta E/\text{kcal}\cdot\text{mol}^{-1}$
A1	0.143
A2	0.082
A3	2.553
A4	3.112
B1	0.122
B2	0
B3	2.491
B4	1.962
B5	3.071

The conformers differ in general very little in energy. Thus, in our calculations 78 optimized conformers were found within the electronic energy range $\Delta E = 0\text{--}8.5$ kcal/mol, and Gibbs energy range $\Delta G = 0\text{--}6.6$ kcal/mol with respect to the global minimum. This range of values of ΔG is smaller than that calculated in dG, in dT [50], $0\text{--}7$ kcal/mol and in dU [31], $0\text{--}9$ kcal/mol.

Only four conformers are found within the electronic energy range $\Delta E = 0\text{--}1.0$ kcal/mol (by criterium of $\Delta E + \text{ZPE}$), Table 2, with $\varphi_1 -72^\circ\text{--}97^\circ$ as g^- and $70^\circ/96^\circ$ as g^+ by MP2. Among these conformers, B1 has the highest dipole moment 6.20 D although very close to that in A1, 6.18 D. These conformers are slightly favored in a polarizable environment with water.

Another seven conformers appear within the electronic energy range $\Delta E = 1.0\text{--}3.0$ kcal/mol, three values were *anti* (φ_1 ca. -100°) and four *syn* (φ_1 ca. 90°). The *anti* structures are the expected forms in the natural nucleosides that form the nucleotides and polynucleotides in biological systems [33,35,46–48]. The ratio *anti/syn* in conformers A is 0.8 in the low-energy group (<3 kcal/mol) but it increases up to 1.7 in the $3.0\text{--}8.5$ kcal/mol range.

The global minimum by DFT methods corresponds to the conformer denoted as A1 (Figure 4) and it appears stabilized by an intramolecular H-bond. The optimised bond lengths and natural NBO atomic charges on this conformer are collected in Figure 5. This global minimum by criterium of $\Delta E + \text{ZPE}$ agree well to that obtained by criterium of ΔG , but differs of that obtained by MP2/6-31G(d) and MP2/6-31G(d,p)//B3LYP/6-31G(d,p), Table 3 and Figure S3. It is because of the small difference in energy between both forms A1 and B2. This global minimum in the *syn* form by MP2 is in accordance to that obtained in other nucleosides [33–35] but differs of the *anti* form expected for the natural nucleosides and nucleotides in biological systems [43]. The second most stable conformer is B1 with values of $\varphi_1 = 70^\circ$, $\varphi_2 = -152^\circ$ and $\varphi_3 = 94^\circ$ by MP2.

Figure 4 shows the six best optimum conformers selected in the two ranges of φ_1 : three are **A** (A1–A3), and three are **B** (B1–B3). The values of the intramolecular H-bonds and the most important structural angles of each conformer are also included. Figures 6–9 show the distribution of the 78 optimised conformers according to their energies, exocyclic torsional angles $\varphi_1\text{--}\varphi_5$, the angles β , Φ , and the vector R. The best significant conformers are pointed in these figures.

Figure 4. Geometry of the six most optimum conformers selected for each rotation angle ϕ_1 determined in A1 conformer of N1 tautomer of acyclovir at B3LYP/6-31G(d,p) level. The values of the strongest intramolecular H-bonds are also included.

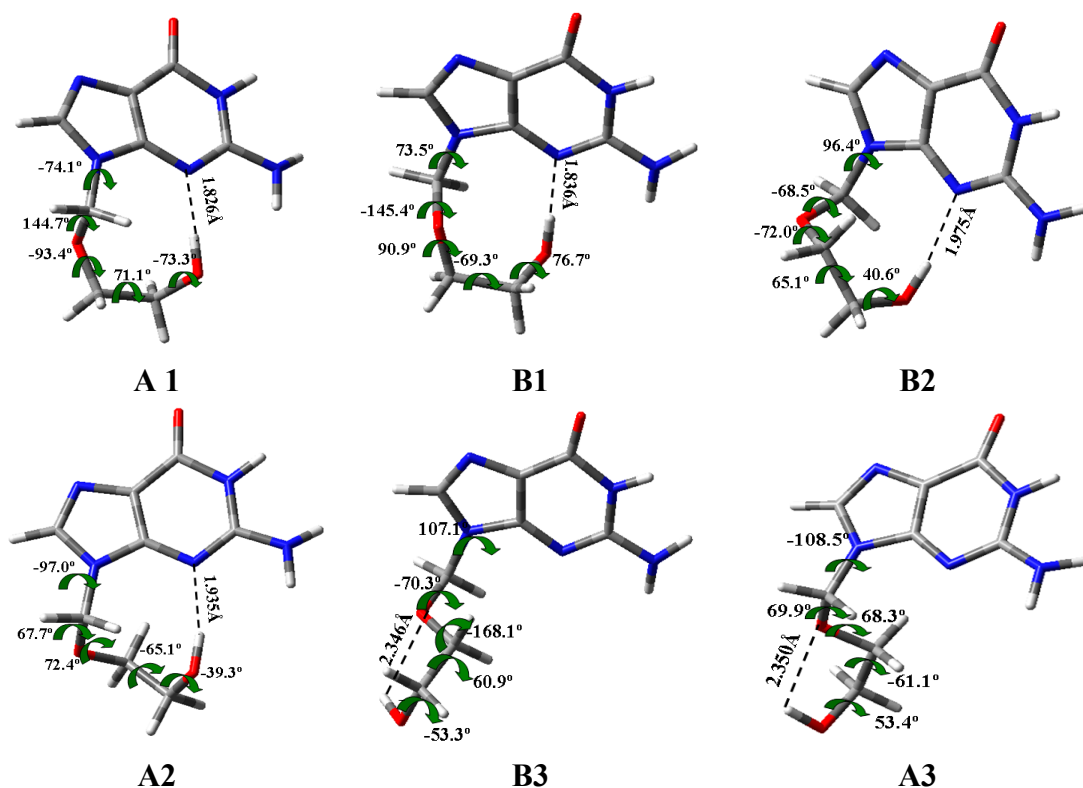


Figure 5. Natural atomic charges and optimum bond lengths in conformer B2 of tautomer N1 of acyclovir at B3LYP/6-31G(d,p) and MP2/6-31G(d,p) levels, in fuchsia and green colours, respectively.

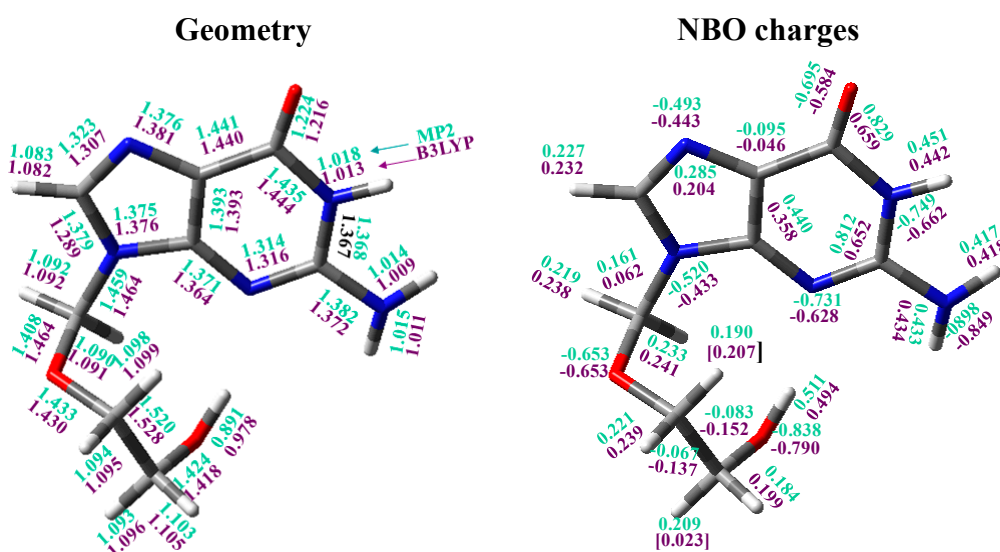


Figure 6. Distribution of the 78 optimum stable calculated conformers at the B3LYP/6-31G(d,p) level in tautomer N1 of acyclovir according to their exocyclic torsional angle ϕ_1 and their: (a) relative electronic energy $\Delta E + \text{ZPE}$ correction; (b) relative Gibbs energy ΔG ; and (c) the distance R. The most stable conformers of each type are pointed.

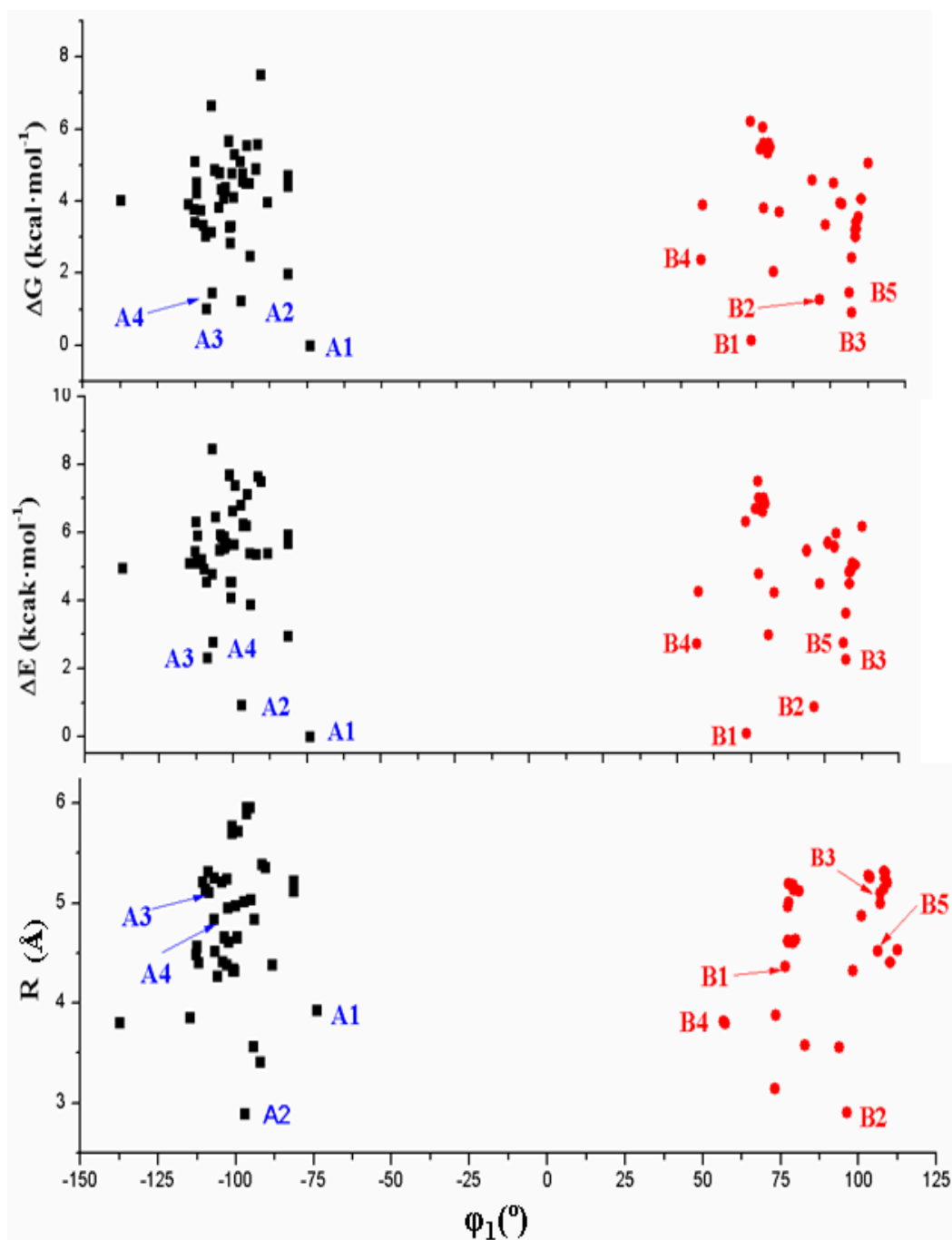


Figure 7. Distribution of the 78 optimum stable calculated conformers in tautomer N1 of acyclovir, according to the values of the exocyclic torsional angles: ϕ_2 , ϕ_3 , ϕ_4 and ϕ_5 versus the angle ϕ_1 .

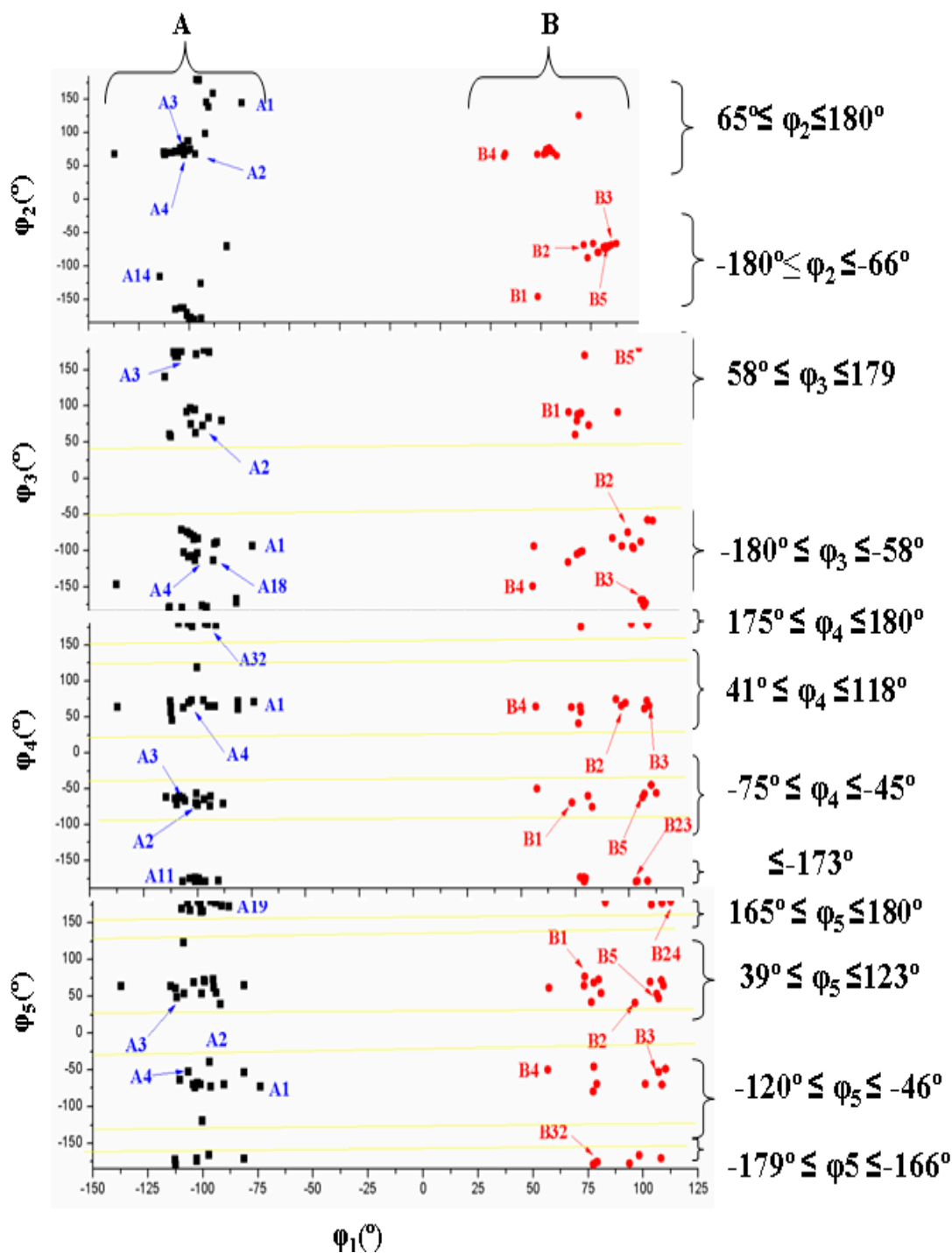


Figure 8. Distribution of the 78 optimum stable calculated conformers in tautomer N1 of acyclovir according to the values of the angles Φ and β , and the dipole moment μ versus the angle ϕ_1 .

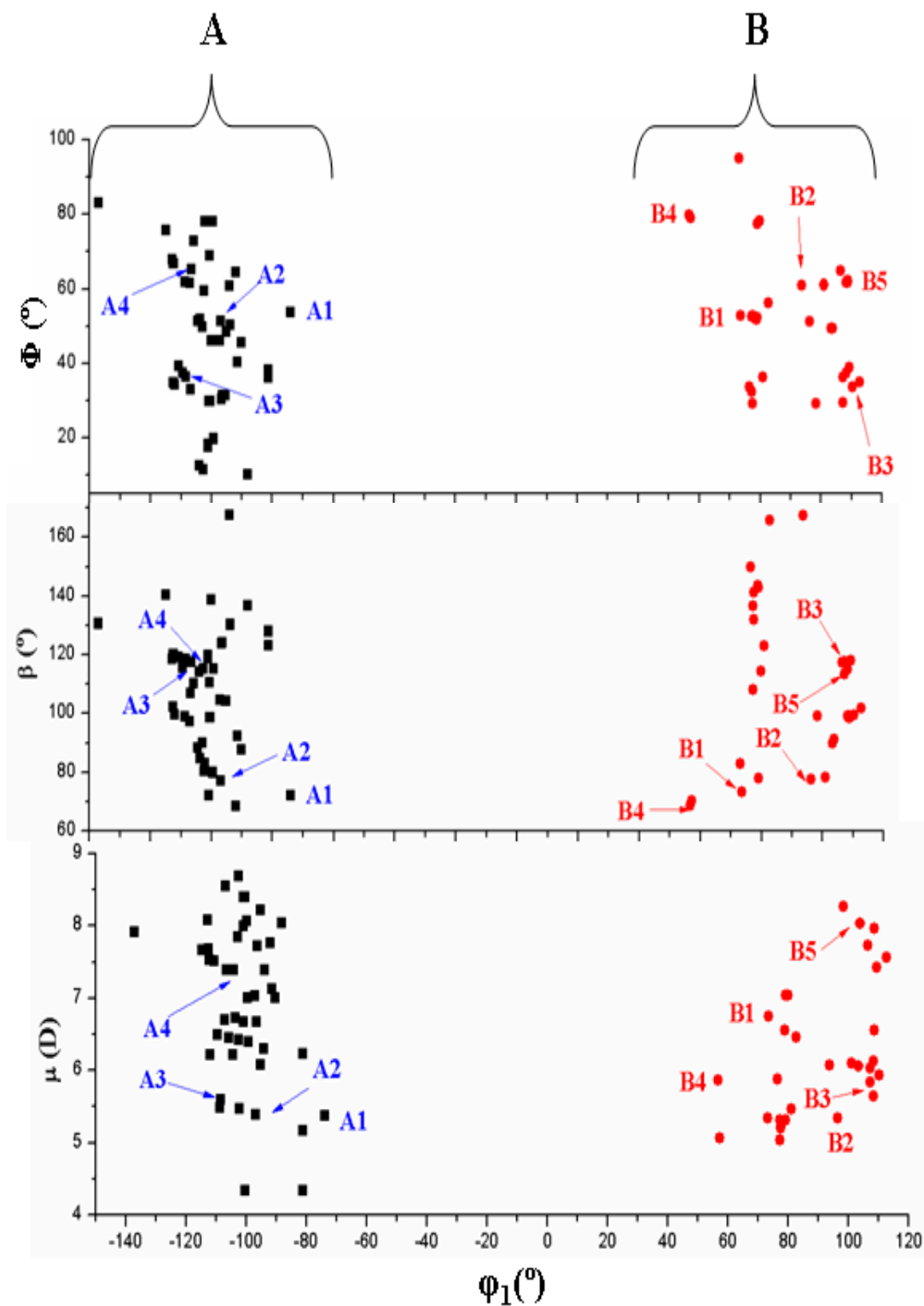
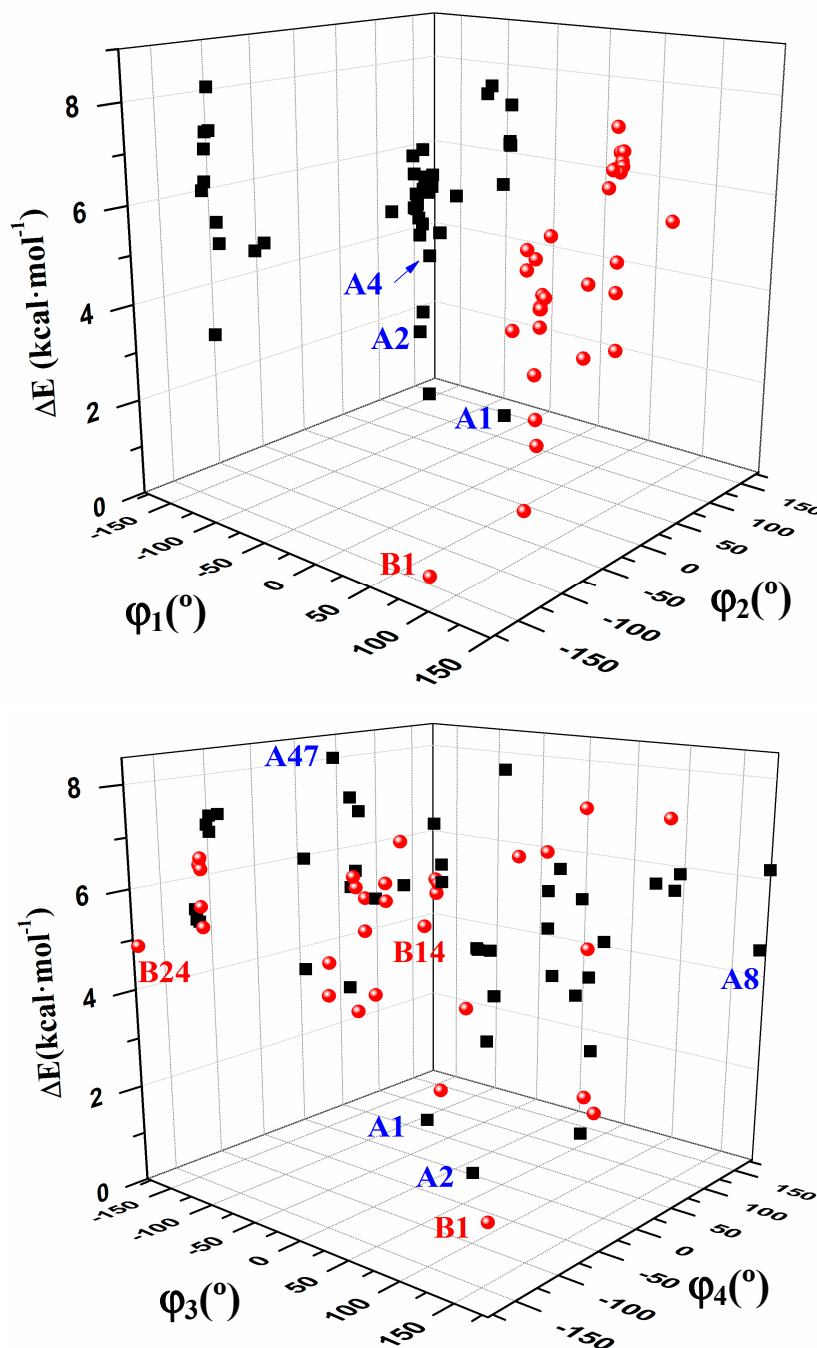


Figure 9. 3D plots with the relative energies of the 78 optimum stable conformers according to the values of the exocyclic torsional angles: φ_1 , φ_2 , φ_3 and φ_4 versus relative electronic energy $\Delta E + \text{ZPE}$.



3.3. Conformational Angle Analysis

An overall examination of the five exocyclic torsional angles and two bond angles, defining the conformational space in tautomer N1 of ACV, leads to conclude the following, Figures 7–9:

- (i) The dihedral angle φ_1 presents a bimodal distribution: $(-137^\circ \leq -74^\circ)$ 47 conformers **A** (*anti*) and $(57^\circ \leq 113^\circ)$ 31 conformers **B** (*syn*). *Anti* forms prevail in number over *syn* ones and cover a slight wider range of φ_1 values. This *anti* orientation has been found in the crystal of several

nucleosides [51–53], and it is the form for biological activity [54]. Although, in many nucleoside analogues *syn* and *anti* forms have similar energy, however, the global minimum corresponds in general to the *syn* form, as in AZT [35]. In purine nucleosides there is relatively little restraint to rotation about the N9-C1' bond [55]. In ACV the high population of the *anti* forms and the wider range of φ_1 values are factors that facilitate its antiviral activity. The sugar ring of the natural nucleoside dG can be mimics by the similar exocyclic angles of ACV.

- (ii) The torsional φ_2 angle has a clear bimodal distribution: $65^\circ \leq \varphi_2 \leq 180^\circ$ (49 conformers, 34 are A and 15 are B) and $-180^\circ \leq \varphi_2 \leq -66^\circ$ (29 conformers, 13 are A and 16 are B), Figure 7.
- (iii) The φ_3 angle also has a bimodal conformation: $58^\circ \leq \varphi_3 \leq 180^\circ$ (32 conformers, 22 are A and 10 are B) and $-180^\circ \leq \varphi_3 \leq -58^\circ$ (46 conformers, 25 are A and 21 are B).
- (iv) The φ_4 angle has a trimodal distribution: $-173^\circ \leq \varphi_4 \leq 175^\circ$ (28 conformers, 16 are A and 12 are B), $41^\circ \leq \varphi_4 \leq 118^\circ$ (28 conformers, 17 are A and 11 are B), and $-75^\circ \leq \varphi_4 \leq -45^\circ$ (22 conformers, 14 are A and 8 are B).
- (v) The torsional φ_5 angle also has a trimodal distribution: $-166^\circ \leq \varphi_5 \leq 165^\circ$ (29 conformers, 19 A and 10 B), $39^\circ \leq \varphi_5 \leq 123^\circ$ range (29 conformers, 16 A and 13 B), and $-120^\circ \leq \varphi_5 \leq -46^\circ$ (20 conformers, 12 A and 8 B).
- (vi) In the bond angle β has been observed a regular distribution in the $69^\circ \leq \beta \leq 168^\circ$ range, Figure 8. This large range indicates the high flexibility of the chain with multiple O5'H orientations. The most stable conformers with $\Delta E < 1 \text{ kcal}\cdot\text{mol}^{-1}$ have the β angle with values between 72° and 78° , while the conformers with higher ΔE have some values larger than 140° .
- (vii) The bond Φ angle also shows a regular distribution in the $10^\circ \leq \Phi \leq 83^\circ$ range for conformers A, and $29^\circ \leq \Phi \leq 95^\circ$ for conformers B. The value of this angle appears between 51° and 54° for the four best conformers with $\Delta E < 1 \text{ kcal/mol}^{-1}$. In general, the most stable conformers do not have large values of Φ , neither low values.

Finally, the R vector has a large range of values, between 2.905 Å and 5.956 Å, and it determines the distance of the OH end of the chain from the base plane.

3.4. Guanine Moiety

In the analysis of the six most stable conformers, the base heterocycle has a very small non-planarity, in general with torsional angles lower than 1° , with the exception of those involving N3 and N9 atoms with a value higher than 2° . It is due to the strong intramolecular H5'··N3 hydrogen bond which produces a ring deformation around N3. Thus, among the dihedral angles describing the base heterocycle nonplanarity, we have defined: ν_0 (N1-C2=N3-C4), ν_1 (C2=N3-C4=C5), ν_2 (N3-C4=C5-C6), ν_3 (N7=C8-N9-C4) and ν_4 (C6-N1-C2=N3).

An analysis of the six most stable conformers (Figure 4) shows that the highest base heterocycle nonplanarity corresponds to conformer A2 with the torsional angles ν_1 (2.8°), ν_2 (-2.3°) and ν_0 (-1.3°). The other conformers show deviations higher than 1° in the following torsional angles: Conformer A1 in ν_2 (-1.2°) and ν_3 (-1.1°), conformer A3 in ν_1 (1.5°) and ν_2 (-1.3°), conformer B1 in ν_0 (1.3°) and ν_1 (1.1°), and conformer B2 in ν_4 (1.2°).

Comparing the bond lengths of ACV (conformer A1) with those of dG, it is observed that ACV has several bonds of the guanine moiety larger than the corresponding bonds in dG, while in the remaining bonds are smaller. The largest difference appears in C4-N3.

3.5. The Side Chain

The most important characteristic of the structure of ACV is the conformation of the side chain that is attached to N9, which is characterized by the exocyclic torsional angles φ_1 to φ_5 , by the bond angles β and Φ that determine the position of the chain from the base, and by the distance R. The bonds along this chain can be in *trans* respect to each other and thus it gives an extended zig-zag structure or, by contrast, one or several alternate bonds can be in *gauche* in such a way that it resembles at least partially a portion of the furanose moiety of dG [49,56]. In this last case, it is capable of adopting conformations resembling a portion of the pentose ring, a factor which undoubtedly plays an important role in their biological activities [57]. Thus, three types of structures appear: two that have the side chain partially folded (conformers A and B), and one structure which has all bonds in *trans* orientation and leading to an almost planar zig-zag arrangement [49].

3.6. Intramolecular H-Bonds

Several authors have studied the intramolecular H-bonds in related nucleosides, in special using AIM method [58,59]. However, the absence of the furanose ring in ACV reduces largely the number of possible H-bonds. Therefore, only two intramolecular H-bonds may be observed in the main conformers of ACV: (i) The hydroxyl hydrogen H5'(O5') and the guanine's position 3 nitrogen atom, H5'...N3, and (ii) The hydroxyl hydrogen H5'(O5') and the guanine's position 2 oxygen atom, H5'...O2'.

Figure 4 shows these types of H-bonds in the six most stable conformers. H-bond (i) appears in conformers A1, B1, B2 and A2; while (ii) is observed in conformers B3 and A3 with values of 2.346 Å and 2.350 Å, respectively. H-bond (i) is stronger than (ii), and it gives a great stability to the structure. Thus, the conformers with the H-bond (i) are the most stable, $\Delta E < 1 \text{ kcal}\cdot\text{mol}^{-1}$, while the conformers with the H-bond (ii) have higher energy, $\Delta E > 2 \text{ kcal}\cdot\text{mol}^{-1}$.

3.7. Natural NBO Atomic Charges

The calculated values in conformer A1 appear collected in Figure 5. The largest negative charge corresponds to N2 and O5' atoms, -0.90 and $-0.84 e$, respectively by MP2 (where e is the charge of an electron). The next atom with large negative charge is N1 (*ca.* $-0.7 e$). The value of this charge is slightly higher than in dG, $-0.69 e$. The main effect of the NH₂ group is a remarkable increment in the positive charge on C2, $0.81 e$, because of the high negative charge on the amino nitrogen, $-0.90 e$. Consequently, a noticeable increase of the negative charge on N3 is observed, $-0.73 e$ ($-0.70 e$ in dG). The electron-rich sites of the guanine moiety are involved in H-bonds, with N3 and O2' acting as single acceptors.

In the nitrogen N9 the negative charge ($-0.52 e$) is lower than on N1, N3 and N7, but slightly higher than in dG. It is because in ACV the bonding to the chain increases the negative charge on N9.

The value of the charge on N7 is important because in the neutral form of anti-tumour platinum drugs, the platinum atom has a strong preference for nitrogen N7 rather than for oxygen atoms of the base for its coordination [51]. Also, the N7 position in DNA is the most open to attack. N1 position is also important because when deprotonation of the weakly acid ACV occurs, the metal binding site changes to N1, which is the formally deprotonated site [60]. Raman spectra of related nucleosides in H₂O indicate that the site of deprotonation in basic solutions is N1, while the site of protonation in acidic solutions is N3 and N7, the same sites that in its phosphorylated form. These results are useful for identification and characterization of its structure in natural occurring biopolymers [61].

The negative charge on the oxygen atoms is high, $-0.65 e$ on O2' and $-0.69 e$ on O6. The values in O2' and O5' are slightly higher than in dG, by B3LYP $-0.603 e$ and $-0.761 e$, respectively. By contrast, in O6 the negative charge is lower than in dG, $-0.592 e$.

C6 is the atom with the highest positive charge, $0.829 e$ by MP2, in concordance to the high negative charge on O6, *i.e.*, it is the most reactive. With a slightly lower value appears C2 ($0.812 e$), and with much lower values H5'(O5'), H1(N1), H2(N2) and H2'(N2). The remaining hydrogen atoms have much less positive charge and they are less reactive.

3.8. Solid State Simulation

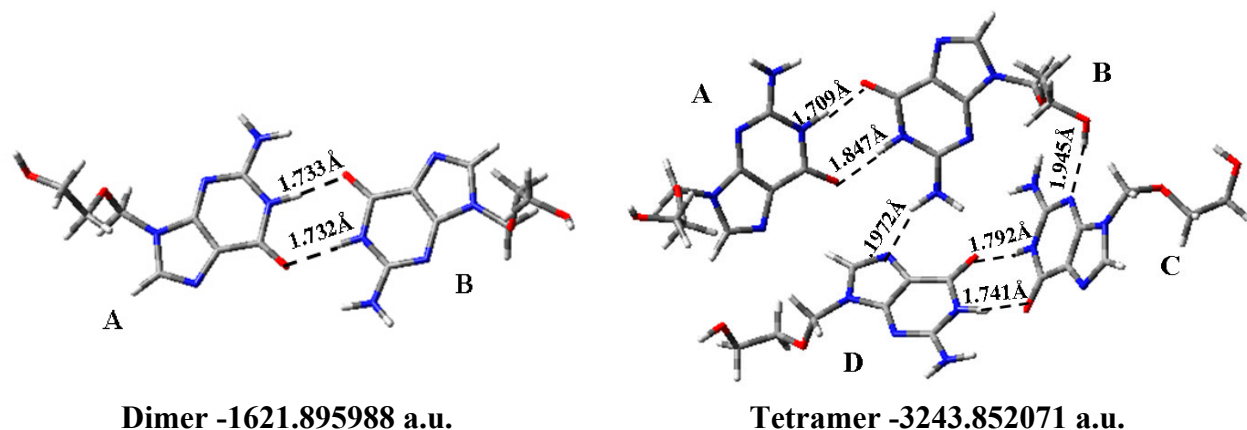
The structure of ACV (CSD code CEHTAK) in the solid state was determined by X-ray diffraction [53,60–63]. Birnbaum *et al.* [49,62] obtained the crystals that belong to monoclinic space with three independent molecules (A, B and C), together with two water molecules [49]. Molecules A and B have similar conformation while that of molecule C is different, Table 4. In molecules A and B the angle φ_1 and φ_2 are in the preferred g^- form, but in molecule C φ_2 is in *trans*. It means that molecules A and B showed a partially folded conformation of the side chain, while molecule C appears extended [62]. Molecules A and B correspond to conformer A5 in the isolated state, while molecule C is represented by conformer A34. Molecules A and B are *ca.* $3 \text{ kcal}\cdot\text{mol}^{-1}$ more stable than molecule C. The difference in energy between molecule A and B is small and it is due to the flexibility of the side chains.

Table 4. A comparison of the most important structural parameters in the dimer and tetramer forms calculated at the B3LYP/6-31G(d,p) level in acyclovir molecule with those in the crystal. The torsional angles are in degrees and R in Å.

Solid form	R	β	Φ	φ_1	φ_2	φ_3	φ_4	φ_5
Dimer								
Molecule A	5.150	127.9	-82.1	-82.1	-70.4	-168.4	71.9	-168.8
Molecule B	5.148	127.9	-88.8	-88.8	-70.3	-168.1	71.8	-168.9
Tetramer								
Molecule A	5.148	128.6	-82.3	-82.3	-70.4	-167.3	72.5	-167.4
Molecule B	5.212	117.2	-80.1	-80.1	-76.3	176.8	64.2	-90.8
Molecule C	5.110	122.0	158.6	158.6	-71.7	-50.5	60.1	-53.8
Molecule D	4.313	150.6	-95.3	-95.3	-93.1	-100.2	70.6	-160.4
X-Ray [62]								
Molecule A				-76.5	-76.9	173.2	60.6	
Molecule B				-74.4	-66.3	-176.2	73.5	
Molecule C				-90.5	-173.3	-171.9	-174.4	

We have simulated this arrangement through a dimer and tetramer forms, Figure 10. The calculated value of the inter- and intramolecular H-bonds distances and the total energy is included in the Figure. The calculated bond lengths, bond angles and torsional angles are collected in Tables S1 and S2. The simulated structure is tightly bonded but not so tight as it the reported in the crystal. There are four donor sites: H1(N1), H2(N2), H2'(N2) and H5', and five possible acceptors: O6, N3, N7, O2' and O5'.

Figure 10. The optimized dimer and tetramer forms in conformer A1 of acyclovir at the B3LYP/6-31G(d,p) level. The H-bonds observed are in Å and the E (RB+HF-LYP) in a. u.



The dimer, and tetramer forms were built with the most stable conformer A1. In the dimer there are two intermolecular H-bonds between the two molecules: N1 {in molecule A} and O6 {in molecule B} 1.733 Å, and O6 {in A} and N1 {in B} 1.732 Å. The tetramer shows seven H-bonds: (i) two between molecule A and B: H_(N1) {in molecule A} and O6 {in molecule B} 1.709 Å, and O6 {in molecule A} and H_(N1) {in molecule B} 1.847 Å; (ii) There is only a H-bond between molecule B and C: H_(O5') {in B} and N3 {in C}, 1.945 Å; (iii) There are two H-bonds between molecule C and D: H_(N1) {in molecule C} and O6 {in molecule D}, 1.741 Å, and O6 {in C} and H_(N1) {in D}, 1.792 Å. (iv) At list there are two H-bonds between molecule D and B: N7 {in molecule D} and H_(N2') {in B}, 1.972 Å.

Comparing the calculated bond lengths and angles of molecule A in the dimer with the X-ray data [62], Tables S2 and S3, it is observed the good agreement in the values of our simulated structure. The greater differences appear in C4=C5 (0.025 Å), C2-N2 (0.023 Å), C3'-C4' and N1-C6 (0.016 Å), and the difference is almost null in C6=O, C5-N7, N7=C8 and C4-N9. A slight larger error is obtained when the comparison theory-experiment is carried out with the molecule B of the dimer. This error is slight reduced when the comparison is carried out with the simulated molecule A of the tetramer. In the angles the difference is almost null, with similar values of all the angles. Only several angles in the side chain show some noticeable differences, *i.e.*, C3'-C4'-O5', 4.7°.

Other four anhydrous forms of ACV and a new hydrate have been characterized by X-ray diffraction, with significant differences in the intermolecular H-bonding networks among the ACV forms [63–65]. A study of the solution forms of ACV shows that ACV can exist as polymorphic and pseudopolymorphic solvates [66]. We have simulated other dimer, trimer and pentamer forms with different conformers and collected in Figure S4.

4. Conclusions

In the present work we have shown a comprehensive compendium of the possible conformers in tautomer N1 of ACV. The geometries and values of the properties presented here appear to be the most accurate to date. The most important findings of the present work are the following:

- (1) Five tautomers of ACV were identified and fully optimized. At room temperature only tautomer N1 (48.1%), OHC (37.7%) and OHT (14.3%) have a noticeable population. It is very small in tautomers N3 and N7, less than 0.05%.
- (2) The relative energies of the five tautomers appear related to the dipole moment and to the torsional angle φ_1 . The least stable tautomer N3 in the isolated state has the highest μ and thus, it is the most favoured in a polarisable environment with water.
- (3) In the isolated state the most stable tautomer is N1 by both B3LYP and MP2 methods. In this tautomer, and through a rotation of φ_1 , φ_2 , φ_3 , φ_4 and φ_5 exocyclic torsional angles, 78 optimized stable conformers were identified, two *syn* and two *anti* falling into the 0–1 kcal·mol⁻¹ $\Delta E + \text{ZPE}$ energy range.
- (4) The calculated most stable conformer by all DFT levels corresponds to A1, while by MP2 is B2. In the nitrogen atoms and in the O2' and O5' oxygen atoms of conformer A1 appear a higher reactivity than in the corresponding natural nucleoside deoxyguanosine.
- (5) The distribution of all the conformers according to the ranges of stability of the characteristic torsional angles was established. The values obtained indicate the flexible nature of ACV, which is higher than dG. An increase of the stability appears when the side chain is near to the purine base, with a value of R that falls into 3.925–2.892 Å range, and an angle Φ close to 54°.
- (6) Only two intramolecular H-bonds may be observed in the main conformers of ACV, in contrast to the six H-bond types calculated in dG. It leads to a flexibility higher in ACV than in dG.
- (7) The solid state was simulated through a dimer and tetramer forms. An excellent agreement with the X-ray crystal data was obtained, which indicates the good accuracy of the theoretical methods used.

Author Contributions

This research corresponds to the PhD Thesis of M.C. Alvarez-Ros, in which M. Alcolea Palafox is the director.

Conflicts of Interest

The authors declare no conflict of interest.

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Effects of the hydration on the tautomers of hypoxanthine by means of MP2 and DFT methods

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Abstract

A comparative theoretical analysis on the effect of the solvent in the molecular structure and energetics on the two most stable tautomers HX-17 and HX-19 of the purine base hypoxanthine (HX) was carried out. Solvent effects were considered using the Tomasi's Polarized Continuum Model (PCM) and the Discrete model including a variable number up to 30 of explicit water molecules surrounding HX in order to simulate the first and second hydration shell. More than two hundred cluster structures with water were analysed by B3LYP and MP2 quantum chemical methods. In the isolated state tautomer HX-17 appears as the most stable and followed by HX-19. However, with 4 to 20 explicit water molecules HX-19 is the most stable. With the further hydration HX-17 is the most stable. The CP-corrected formation and interaction energies for HX and water molecules were determined. The effect of the hydration on the geometry, total atomic charges and thermodynamics parameters was discussed. The crystal unit cell of HX-19 tautomer in the solid state was simulated with a tetramer form. Several general conclusions on hydrogen bonds network and involved interaction energies were underlined.

Keywords: hypoxanthine, 1,7-dihydro-6H-purin-6-one, tautomers, HX-17, HX-19, hydration, solid state, DFT, MP2

1. Introduction

Hypoxanthine (1,7-dihydro-6H-purin-6-one), in short HX (or Hyp [1,2], I [3]) is a mutagenic purine base that occasionally occurs as constituent of nucleic acids structure [4], where it is present in the first anticodon position in various tRNA sequences [1,6], in the form of its nucleoside inosine. It is an intermediate product of purine metabolism and component of the cellular nucleotide pool [5,7]. It is a mutation that occurs in DNA when adenine undergoes oxidative deamination, and as such it is a damaged base in DNA and a mutagenic agent. Damaged bases are associated with carcinogenesis and cell death [8]. The mutagenicity of HX lies from the fact that unlike adenine, which H-bonds to thymine in the double helix, HX prefers to H-bond with cytosine, i.e. it causes an A·T to G·C transition. HX and guanine are continually made from nucleic acids degradation, in which HX is oxidized to xanthine and further to uric acid in human beings. Two inborn errors of metabolism are characterized by elevated excretion of xanthine and HX. These defects in purine nucleotide metabolism cause the incorporation of HX and xanthine into DNA and RNA [3].

HX is an important molecule in the research of medicine [9-13], nucleic acids [16], biology [17] and of some drugs [18]. It is a necessary additive in certain cell, bacteria, and parasite cultures as a substrate and nitrogen source. E.g., it is commonly a required reagent in malaria parasite cultures, since *Plasmodium falciparum* requires a source of HX for nucleic acid synthesis and energy metabolism [19,20]. Moreover, it is noteworthy that recent NASA meteorite studies [21] revealed some DNA and RNA nucleobases, among which there are adenine, guanine, xanthine, hypoxanthine and purine capable to form canonical base pairs, may have been formed extraterrestrially in outer space. Due to the importance of HX, we have studied it in the present manuscript.

HX is often referred to as a “universal” nucleobase [1] that binds without discrimination to any of the natural bases and results in duplexes with similar stability as the corresponding duplexes composed of all natural nucleobases. Indeed, HX can adopt *anti* and *syn* orientations when bonded to a deoxyribose sugar, which provide two H-bonding faces for interactions with the four natural nucleobases [1]. Thus, it has potential used in many applications of molecular biology [6].

It is well-known that enzymes attack purines at preferred positions, and therefore, their tautomerism is crucial in biochemical and pharmacological research. They have been the subject of extensive theoretical [22-33] and experimental studies in nucleic acid bases and in HX, which is important in the DNA polymerism [18]. Thus, geometry of the different tautomers, relative stabilities, vibrational wavenumbers [1,2,34-41] and polarizabilities of HX and other similar purines have been reported [42], including density functional studies on the monocationic tautomers of HX, mainly on HX-19 and HX-17 [43], and on dicationics [44]. The anionic tautomers of HX-19 and HX-17 [45], and their Pt complexes have been also studied [46,47]. The survey of computational studies suggest that in the gas phase HX exists mainly in the two keto prototropic tautomeric forms, HX-17 and HX-19, with the former being the dominant species [18,48]. This predominance of HX-17 is in good agreement with the experimental evidence obtained from UV photoelectron spectra [49] and the IR spectra [50].

However, in the crystal HX occurs as HX-19 tautomeric form only, **and there are not publications that explain this feature, neither** its crystal unit cell has been simulated theoretically. Thus, this is one of the goal of the present manuscript.

Under aqueous solvation the theoretical calculations using continuum or discrete models have shown that the enol HX-90 is largely destabilized, and the *keto* HX-19 form is more favored than HX-17 [48,51,52]. Also, in neutral aqueous medium the UV [38,48,53] and $^1\text{H}/^{13}\text{C}$ NMR [54] spectroscopic studies indicate the dominance of the HX-19 form over HX-17. However, other theoretical calculations [18,55,56] and the data of HX tautomerism obtained by FTIR spectroscopy in aqueous medium [38] and Raman spectroscopy in solution with physiological pH [57] suggest that HX-17 and HX-19 tautomers coexist in equilibrium. By SERS [58] the HX-19 form exists at acidic and normal pH, but the enol HX-90 form at alkaline pH. We focus the attention on this subject with the simulation of the first and second hydration shell of HX-17 and HX-19. This is the main goal of the present manuscript, and for this purpose the hydration of the two most stable tautomers of HX was studied with different number of water molecules up to 30, which cover the first and second hydration shell.

2. Calculations

Calculations were carried out by using the second-order Møller-Plesset (MP2) ab initio quantum chemical method, and the density functional theory (DFT) including the Becke's three parameter and the Lee-Yang-Parr correlation functional (B3LYP). These methods appear implemented in the GAUSSIAN 09 program package [59] using the Quipu computer of the Computational Center from University Complutense of Madrid. Standard parameters of this package were used under the UNIX version. DFT methods are the most adequate because they provide a compromise between the desired chemical accuracy and heavy demands put on computer time and power. Also they provide reasonable results for hydrogen bonded complexes. Moreover, DFT methods have been used satisfactory in many studies of drug design [60-63]. The B3LYP method was chosen because different studies have shown that the data obtained with this level of theory are in good agreement with those obtained by other more computationally costly methods such as MP2, and it predicts vibrational wavenumbers of DNA bases better than HF and MP2 methods [64-68]. Also the energy and intermolecular distances obtained from B3LYP calculations in different nucleosides and nucleic basis have been compared with those obtained at the MP2 level [69,87,108-110].

The new M05-2X [71] and M06-L [72] DFT methods were also used. They are members of the M05 and M06 families of density functionals developed by Zhao, Truhlar and Schultz, which were designed to yield broad applicability in chemistry [73]. M05-2X, a meta-hybrid functional, has been shown to yield good results for dispersion-dominated interactions [74-76] Also M06-L, a local (non-hybrid) meta functional, generally yields good results for a broad range of interactions, including non-covalent interactions [74,76]. As M06-L is a local functional, it has much reduced cost compared to other meta functionals, and its performance has been tested in the conformation of a peptide [77]. In all the structures, when computer requirements allowed it, MP2 computations were also performed to confirm or correct the DFT results.

Several basis set were used starting from 6-31G(d) to 6-311++G(3df,pd), but the 6-31G(d,p) represents a compromise between accuracy and computational cost, and thus it was the most used for the calculations. The Tomasi's Polarized Continuum Model (PCM) [78-79] was performed by default integral equation formalism model, IEF-PCM [82,84]. The optimum geometry was determined by minimizing the energy with respect to all geometrical parameters without imposing molecular symmetry constraints. Berny optimisation under the TIGHT convergence criterion was used. The tautomeric equilibrium at 298.15 K

was evaluated by means of the Boltzmann distribution. Atomic charges were determined with the Natural NBO [85,86] procedure.

The harmonic wavenumber calculations were carried out at the same level of the respective optimization process and by the analytic evaluation of the second derivative of the energy with respect to the nuclear displacement. When it was possible, the wavenumber calculations were performed in all the optimised conformers to assess that they correspond to real minimum. All the optimized structures only had positive harmonic vibrations, true energy minimum. Relative energies were obtained by including zero-point vibrational energies (ZPE). For the calculation of ZPE, the wavenumbers were retained unscaled.

The scaling equation used for the vibrational frequencies was [65]: $v_{\text{scaled}} = 34.6 + 0.9447 v_{\text{calculated}}$

2.1. Interaction energies in the tetramer

The interaction energies obtained at the B3LYP/6-31G(d,p) level in the tetramer molecules were corrected for basis set superposition error (BSSE) using the standard counterpoise (CP) procedure [70] as follows:

The total CP corrected interaction energy, $\Delta E_{A_n}^{CP}$, between the four molecules of HX-19 in the tetramer is calculated according to:

$$\Delta E_{A_n}^{CP} = E_{A_n}^{(A_n)} - \sum_{i=1}^n E_{A_i}^{(A_n)} + \sum_{i=1}^n E_{A_i}^{def}(A_n)$$

and where $E_{A_n}^{(A_n)}$ stands for the electronic energy at the optimized geometry of the whole tetramer system, and $E_{A_i}^{(A_n)}$ is the electronic energy of the isolated molecule A_i in the entire system A_n .

The deformation energy $E_X^{def}(A_n)$ of monomer X ($\equiv A_i$) is defined as:

$$E_X^{def}(A_n) = E_X^X(A_n) - E_X^X(X)$$

Here the subscripts denote the molecular system and the superscripts indicate whether the calculation is done with the basis set of the entire system (A_n), or the basis set of the monomer (X). The parentheses indicate whether the calculation is done at the optimised geometry of the entire system (A_n), or at the monomer optimised geometry (X).

2.2. Hydration effects

Three procedures have been suggested [89] to simulate theoretically the hydration: (i) the empirical scaling of the quantum mechanical force constants of the isolated molecule, (ii) the use of a continuum model, and (iii) the use of the discrete model (DM) by including sufficient numbers of explicit water molecules.

The continuum model procedure is used mostly today, owing to its simplicity and the low computational time required. In the present paper it was carried out with the Tomasi's Polarized Continuum Model (PCM) [78]. However, DM model is preferred since it gives account of hydrogen bonds explicitly. In DM the water molecules provide a description of both the microscopic structure of the solvent and specific solute-solvent interactions. A selected number of solvent configurations are included in the quantum-mechanical description of the system. Unfortunately, both the large number of solvent molecules required to mimic a dilute solution and the computational cost [90] limit the applicability of this method to small-medium size systems. Thus, in the present work only up to 30 explicit water molecules surrounding HX was simulated, which covers the first and second hydration shells. The methodology used, the so-called Modified Scheme of Monosolvation (MSM) [91], consists of the following steps [92,93]: the first water molecule was placed in all the possible positions around HX, and of all these possible monohydrated complexes is chosen that one with the lowest energy. Next, a second water molecule was added on this

monohydrated complex chosen, and the hydrated complex with the lowest energy is selected. Successively, this process is repeated for incorporating the remaining water molecules in the selected complex with the lowest energy until the hydration completed.

2.2.1 Interaction energies with the discrete model

In the hydration of HX with explicit water molecules the energies obtained were corrected for basis set superposition error (BSSE) using the counterpoise (CP) procedure from Danilov *et al.* [92,101]. The CP-corrected B-(H₂O)_n formation energy of the BW_n complex, where B ≡ HX and W_n ≡ water nmer, is calculated as sum of two contributions, the base-water interaction $\Delta E_{B-(W_n)}^{CP}$, and water-water interaction

$$\Delta E_{(W_n)}^{CP} : \quad \Delta E_{B-(W_n)}^{CP} = \Delta E_{B-(W_n)}^{CP} + \Delta E_{(W_n)}^{CP} \quad (1)$$

where the CP corrected interaction energy between the base (B ≡ HX) and the water nmer (W_n) is calculated according to:

$$\Delta E_{B-(W_n)}^{CP} = E_{BW_n}^{(BW_n)}(BW_n) - E_B^{(BW_n)}(BW_n) - E_{(W_n)}^{(BW_n)}(BW_n) + E_B^{(def)}(BW_n) \quad (2)$$

and where the CP corrected water-water interaction energy, corresponding to the formation energy of a water n-mer (in the presence of the base), at the geometry it adopts in the BW_n complex is defined as:

$$\Delta E_{(W_n)}^{CP} = E_{(W_n)}^{(BW_n)}(BW_n) - \sum_{i=1}^n E_{W_i}^{(BW_n)}(BW_n) + \sum_{i=1}^n E_{W_i}^{def}(BW_n) \quad (3)$$

The deformation energy of monomer X (X ≡ HX or W) is:

$$E_X^{(def)}(BW_n) = E_X^{(X)}(BW_n) - E_X^{(X)}(X)$$

Here the subscripts denote the molecular system and the superscripts indicate whether the calculation is done with the basis set of HX, the basis set of a water molecule, (W), or the basis set of the entire system, (BW_n). The contents of the baseline parentheses indicate whether the calculation is done at the optimized geometry of the entire system, (BW_n), or at the monomer optimized geometry (X)

3. RESULTS AND DISCUSSION

3.1. In the isolated state

3.1.1 Geometry optimisation

HX can exist in several tautomeric forms differing mainly by the position of all four hydrogen atoms, which may be bound to either the ring nitrogen and carbon atoms or to the exocyclic oxygen [1]. The six most stable ones are represented in the optimized form in Fig. 1 at the B3LYP and MP2 levels and in Table 1 are their relative energies at different computational levels. They are chosen among the fourteen possible ones [35]. [The notation used for the tautomers was from ref. \[xx\].](#) With the exception of M052X and M06L, the order of stability of all the tautomers is the same for all the methods. Scheme 1 shows the labelling of the atoms in HX. The optimised bond lengths and angles of these six main tautomers of HX using DFT and MP2 methods are shown in Tables 2 and 3, and in Tables 1-4 SUP (Supplementary material section) for HX-17, HX-19, HX-37, HX-70, XH-90cis and HX-90trans tautomers, respectively. These sets of values are the most accurate today.

The global minimum at all the levels of calculation corresponds to HX-17, and the second one to HX-19. Both are *keto* with the C=O group, as well as HX-37, while HX-70 and HX-90 with two isomers *cis* and *trans* are in the *enol* form. Brovarets and Hovorun [1] have expressed the hypothesis that the *keto* tautomer of HX is a mutagenic compound, while the *enol* form with

hydroxyl group in *cis*-orientation does not possess mutagenic properties. The HX-19 tautomer is the “canonical” structure and the most relevant biologically, while HX-17 is of importance intrinsically due to its high stability.

HX-17 and HX-19 tautomers have the largest population 64.7% (HX-17) and 35.1% (HX-19) at MP2/6-31G(d,p) level and 298.15 K. The population of the other tautomers is too small for consideration: in HX-90C (0.23%), HX-90t (0.03%), and in HX-37 and HX-70 (< 0.005). These results are in accordance to the 5% of the enol form found in the IR spectroscopic study of HX in Ar matrix [50]. The calculated population reported [1] at MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level is HX-17 (81.01%) and HX-19 (18.28%), close to our results. Studies by UV photoelectron spectra in the gas phase [36,49] and FTIR spectroscopy in Ar matrix [39,51,95] also indicate the stability of HX-17 over HX-19. By X-ray spectroscopy [94] is reported a ratio of ca. 1:2 between both tautomers. The calculated ΔG difference between HX-17 and HX-19 is 1.40 kJ mol⁻¹ at MP2/6-31G(d,p) level, and 3.44 kJ mol⁻¹ at B3LYP/6-311++G(3df,pd), close to 2.38 kJ mol⁻¹ reported [94]. Due to the low population of HX-90, HX-37 and HX-70 tautomers, the present work was only carried out on HX-17 and HX-19.

Under physiological conditions the content of HX enolic form may be neglected compared to the content of guanine enolic form [2]. Thus, the possibility of formation of mismatched pairs HX:U and thereby possible mistakes of codon-anticodon recognition of replacement of guanine by HX in anticodon triplets of tRNAs is very small [2,50].

Table 3 collects the calculated bond lengths (Å) and angles (degrees) of the HX-19 tautomer at different levels, together with the experimental ones. Fig. 2 shows the bond lengths and angles of HX-17 and HX-19 in the isolated state at B3LYP/6-31G(d,p) and MP2/6-31G(d,p) levels. The values of the bond lengths are similar at both levels of calculation, the largest differences in HX-19 are on N7-C8 (0.015 Å), C6-N1 (0.009 Å) and C=O, (0.008 Å), and in HX-17 on C5-N7 (0.011 Å) and C6-N1 (0.010 Å). The C5-N7 bond has similar value at all the computational levels used. The angles are almost the same between both levels.

In M052X and M06L the largest differences in the values of the bond lengths correspond to N7-C8 and C8-N9 (0.049 Å and 0.062 Å by M052X and 0.054 Å and 0.059 Å by M06L) and C4-C5, C5-C6, C6-N1 that are longer than the experimental. In HX-17 only an angle C2 agree to molecule B. Angles at M052X and M06L levels are very similar to the experimental values, the difference is about 1°.

Tautomer HX-17 appears with the bond lengths N1-C2, N3-C4, C6-O, N7-C8 longer than those in HX-19, but shorter C2-N3, C5-C6, C6-N1, C5-N7, C8-N9 and N9-C4 at every level of calculation. This feature indicates that the change of the proton from N9 to N7 involves a change in the imidazolic ring: the N9-C4 and C8-N9 bonds become shorter when N9 loss its proton and the N7-C8 bond becomes longer and the C5-N7 bond shorter when a proton is bonded on N7. **The changes on the imidazolic ring have little influence in the pyrimidinic ring.**

The shift of the proton from N1 (HX-17) to N3 (HX-37) leads to a lengthening of C2-N3 and N3-C4 bonds and shortening N1-C2 and C4-C5, Table 1-SUP. The imidazolic ring changes little related to HX-17.

The new O-H bond of HX-70 (Table 2-SUP) as compared to HX-17 increases the C6-O bond length and decreases the two neighbour bonds C6-N1 and C5-C6. Tautomers HX-70 and HX-90cis (Table 3-SUP) have almost the same pyrimidinic ring, but the shift of the proton from N7 to N9

increases the length of the C8-N9 and N9-C4 bonds related to HX-19. The C2-N3, C6-O, C5-N7, C8-N9 and N9-C4 bonds in HX-90 (Table 4-SUP) are longer than in HX-19. The proton of the OH group has similar influence in both HX-90 tautomers (*cis* and *trans*).

The dipole moments of all the tautomers are shown in Table 5b-SUP (*hacer*). It is noteworthy that the most stable tautomer HX-17 is the least polar. This feature is in accordance to that reported by AM1 method [2].

3.1.1 Wavenumber calculations

One of the goals of the present manuscript is to distinguish HX-17 of HX-19 in the isolated state, solid state and water solution. For this purpose, in the isolated state we have plotted in Fig. 3 several characteristic wavenumbers of the ring structures of both tautomers. They were selected because a noticeable contribution from the N7/N7-H or N9/N9-H groups. The wavenumbers of several normal modes appear very close between HX-17 and HX-19 and thus they cannot be used for differentiation purposes. E.g. mode 23 is calculated at 1301 cm^{-1} in HX-17 and at 1302 in HX-19, and in both cases with very low or almost null IR intensity. In other modes the IR intensity is too low to be distinguished in the spectra, e.g. modes 9 and 12 at 611 and 712 cm^{-1} , respectively in HX-17. Also there are other modes that are out of the IR spectrophotometer possibilities, e.g. modes 1 to 4. Thus, in Table 4 we have collected the main normal modes that can be used to distinguish between HX-17 and HX-19. For simplicity, some of them are not plotted in Fig. 3, but in others its wavenumber appears underlined.

The calculated wavenumbers were scaled using a linear scaling equation. This procedure leads to lower errors than when it is used an overall scale factor or few of them [64-68]. Thus, our scaled values are better than those reported by Ramaekers et al [39] and collected for comparison purposes in the columns 3rd and 5th, for HX-17 and HX-19 tautomers, respectively. Due to this improvement in the scaled values and the close proximity of many experimental bands, we have suggested the reassignment of several of these bands, which were included in red colour.

We have also change the characterization of several of these bands. Thus, the experimental band at 1062 cm^{-1} and related to the scaled value [39] of 1075 cm^{-1} in HX-17 has been assigned [39] as $52\% \nu(\text{N7-C8}) + 22\% \delta(\text{N7-H})$. However, the reduced mass of the calculated wavenumber is only 1.96 AMU, which doesn't correspond with this assignment reported. In Table 4 appears the adequate assignment for this band. Another clear example correspond to the scaled wavenumber [39] at 1056 cm^{-1} in HX-19 and assigned as $58\% \nu(\text{C8-N9}) + 29\% \delta(\text{N9-H})$, which is in clear contradiction with a reduced mass of this vibration of only 1.68 AMU. The corrected assignment mainly as $\delta(\text{N9-H}) + \delta(\text{C8-H})$ is shown in Table 4.

3.2. In the solid state.

3.2.1 Geometry optimisation

The X-ray values exhibits two crystallographically independent molecules (A and B) [7], and it is reported that both molecules of the crystal occurs as HX-19 tautomeric form only. To know the reason, we have simulated first the crystal unit cell of the solid state through a tetramer form with HX-19, Fig. 4. The donor-acceptor distances at the B3LYP/6-31G(d,p) level are included in the figure. Each molecule is involved in four H-bonds with the neighbour ones, of the type C-H...O and C-H...N that are very weak, and N-H...N and N-H...O that are medium and strong,

respectively. The calculated interaction energy in this tetramer is -53.7580 kcal/mol, and the highest deformation energy corresponds to molecule C (according to the notation used in Fig. 3), and the lowest to molecule A. The theoretical bond lengths of the four molecules of the system are in general in accordance to that reported by X-ray, but with values slight longer (ca. 0.02 Å) than those corresponding to the experimental molecules A and B, Table 3. In the angles the largest difference as compared to the experimental values corresponds to C6-N1-C and C-N3-C.

We have calculated the corresponding tetramer form with tautomer HX-17, Fig. 3, and it is 5.066 kcal/mol more stable than with HX-19. Moreover, its interaction energy is -57.3375 kcal/mol, higher than that with HX-19. To confirm this feature, we have carried out calculations at the B3LYP/6-311++G(3df,pd) and M06L/6-31G(d,p) levels, and in both case the results are similar, with tetramer HX-17 more stable than HX-19, 4.20 and xxx kcal/mol, respectively. Moreover, we have carried out single point calculations at the MP2/6-311++G(2d,p)//B3LYP/6-31G(d,p) level and gives similar result, 5.76 kcal/mol. So, the question is: why in the solid state appears only HX-19?

To resolve it, we have analysed in detail the X-ray crystal data [7]. The protonation at N7 or N9 increases by about 2° the angle, C5-N7-C8 and C4-N9-C8, respectively. Our calculated C4-N9-C8 angle in molecule C (the highest deformed) of tetramer with HX-19 found the value of 107.0°, the same value that in molecule A of the crystal, while this angle in tetramer with HX-17 is 103.9°. Our calculated C5-N7-C8 angle in tetramer with HX-19 is 105.0°, more close to the crystal value (103.7° in molecule A, and 104.8° in molecule B) than in the calculated tetramer with HX-17, 105.6°. The N7-C8 bond length is 1.313 Å in HX-19, more close to the X-ray values (1.321 and 1.311 Å) than that in HX-17, 1.360 Å. Also, the C8-N9 bond length in HX-19 (1.373 Å) is more close to the experimental value (1.348, and 1.375 Å) than that in HX-17 (1.324 Å). All these features demonstrate that the structure reported by X-ray [7] is really HX-19. However, the crystals were grown [7] from a 0.25 M H₂SO₄ solution at 313 K, and in our calculations the dipole moment of the tetramer with HX-19 is remarkably higher than that in HX-17, Fig. 3. Thus, in water solution HX-19 will be the stable tautomer, instead of HX-17.

N9 in HX-17 has slightly higher negative charge than N7 in HX-19. Thus, in acid medium N9 of HX-17 accept better a proton than N7 of HX-19, e.g. in acid medium the HX-19 tautomer is favoured. For these two features, the structure reported in solid state by X-ray corresponds to HX-19. If the crystals are obtained in other conditions, we think that perhaps the tautomer will not be HX-19.

3.2.2 Wavenumber calculations

To elucidate the tautomer form in the solid state, a detailed analysis of the vibrational bands calculated in the tetramer forms of HX-17 and HX-19, together with a comparison with the IR and Raman spectra reported for the solid state [38,40] was carried out. Based in the selected ring normal modes of Table 3, we have obtained the results shown in Table 5.

The HX tautomers are quasi-rigid structures [2], which is evidenced by the presence of low-frequency (<150 cm⁻¹) fundamental vibrations in their calculated spectra. Thus, for example, the frequencies of the lowest energy nonplanar vibrations for HX-17 and HX-19 are 134 and 145 cm⁻¹, respectively, corresponding to 141 and 148 cm⁻¹ [35]. Ver low fre

3.3. Charges

The natural NBO atomic charges on the atoms appear collected in Fig. 2. The largest negative charge in both tautomers is on O6 and N1 atoms, while the highest positive charge corresponds to C6, in concordance to the high negative charge on O6. HX-19 has several heteroatoms that could accept a proton. The most basic site is N7 (PA of 219.6 kcal mol⁻¹), and the next one is O6 (213.9 kcal mol⁻¹) [8]. In general MP2 predicts a larger negative charge on the atoms than B3LYP.

H9 is the hydrogen atom with the highest positive charge in HX-19, while H7 is the most positive in HX-17, i.e. they are the most reactive. Closely is H1 in both tautomers. The remaining hydrogens have much lower positive charge, ca. 0.23e. These results are in accordance to the fact that the most acidic site of HX-19 is on N9-H (330.5 kcal mol⁻¹ [8]), while N1-H is slightly less acidic (337.0 kcal mol⁻¹). The C-H protons are the least acidic. HX-17 is also quite acidic, with N7-H of 331.3 kcal mol⁻¹ (similar to that in N9-H of HX-19) and N1-H of 334.7 kcal mol⁻¹ [8]. N9-H is more acidic than that of adenine and guanine (*comprobar*), and this could be a path by which AAG discriminates damaged bases from normal bases [8].

3.4. Hydration

The effect of the water molecules on the molecular structure is also of special biological interest [57]. Hydration of DNA takes place through both hydrophilic and hydrophobic sites forming two hydration shells, clearly described in the bibliography. It has been established for some time that there is a shell of tightly bound water molecules at the surface of DNA with significantly different properties from those of bulk water [96]. Experimental results in gas phase reported [97] in uracils and thymines reveal an important function of water in protecting our genetic code from photodamage. Several attempts have been also made to decipher the effect of hydration on the photophysics of nucleic acid bases, including UV spectroscopy of hydrated guanine formed in a supersonic jet [98], and femtosecond pump-probe ionisation mass spectroscopy of hydrated adenine clusters [99]. A consistent number of theoretical studies have focused on the microhydration of uracil [93,100], thymine [92], and other nucleic acid bases [91-93,101,102]. Despite these efforts, however, the effect of hydration on the geometrical parameters and vibrational spectra remains unclear in HX. A previous work has been reported with the hydration of HX but only with two explicit water molecules [48,107]. In the present work we studied the clusters of HX-19 and HX-17 tautomers up to 30 water molecules for the first time, which cover the first and second hydration shell.

In the nucleoside inosine, the molecule of HX is bonded to the deoxyribose moiety through N9. Therefore HX-19 tautomer is the biologically relevant one. Our calculations indicate that in HX-19 the first hydration shell is more or less completed with 12 water molecules and in HX-17 with 11 water molecules. This feature is in accordance to that estimated experimentally by densimetric and ultrasonic measurements [104,105], in the range of 12-20 water molecules of hydration per nucleoside pair, and to that reported [106] in guanine in the first hydration shell, 7-13 water molecules.

The hydration was only studied in HX-17 and HX-19 due to their similar stability and the large difference with the other tautomers. The effect of the hydration on the bond lengths and angles is included in the Tables 6-SUP to 9-SUP at the B3LYP and MP2 levels.

3.4.1. Monohydration: HX-17(H₂O) and HX-19(H₂O)

Three regions in HX appear favorable for water molecules, labelled as positions A, B and C, Scheme 2. Water molecules are denoted as W_A to W_C, in reference to its position, when they can be clearly identified. Under hydration, the carbonyl oxygen and the amino nitrogen are involved in the H-bonds, and they slightly changes their geometry and charge distribution. As expected, the C-H bonds are not sensitive to water. If the water molecule acts both as proton acceptor and proton donor, the relevant structure is energetically favoured over the alternative double donor or double acceptor hydrogen bonding. These structures are cooperative and the binding energies with water are expected to be larger than the energies of open structures [100].

Fig. 4 plots the possible positions (A, B, C) of the water molecule in all the tautomers. In HX-17 the most stable is with the water molecule in B position, while in HX-19 is in position A. It is due to in these positions the water molecule appear stronger H-bonded (shorter intermolecular H-bond) than in the other positions. Some selected optimised bond lengths and angles in HX-17 and HX-19 at the B3LYP and MP2 levels are plotted in Fig. 2-SUP. The change in the bond lengths with the hydration is in general small: a shortening of N1-C6 and lengthening of C=O. The changes in the charges are also small, only an increment in O6 0.048e and in N3 0.026e. In position C the changes are larger, mainly on N3 and N9. The C=O...H_w H-bond distance is slightly longer than N-H...O_w.

Table 5 shows the CP-corrected complex formation Energy in the monohydrated HX-17 and HX-19 at the B3LYP and MP2 levels. They were calculated from Table 10-SUP. The most negative corresponds to position B in HX-17 and to position A in HX-19, Fig. 5a, in accordance to their highest stability. The maximum deformation energy ΔE_{HX-19}^{def} appears in position A in HX-19 (Fig. 5b) and in position B in HX-17 (Fig. 5c) at MP2 level. The lowest deformation in HX-19 is in position B, while in HX-17 is in C position. In both cases the lowest deformation is due to the largest H-bonds.

In the monohydrated form the energy gaps between the most stable forms of HX-17 and HX-19 increases up to 2.74 kcal mol⁻¹ at the MP2/6-31G(d,p) level (0.36 kcal mol⁻¹ is in the isolated state). The energy gaps of the other tautomers are also increased. I.e. in HX-90cis is 5.91 kcal mol⁻¹ vs 3.341 in the isolated state. This stronger H-bonding of the water molecules in HX-17 than in HX-19 is responsible for the increment of the energy gap between both tautomers.

HX(H₂O)₂ A second water molecule was added in the most stable cluster calculated in the monohydrated form, in HX-17 is the position B while in HX-19 is the position A. Calculations at the MP2 level confirm the B3LYP results that B-A cluster is the most stable in HX-17 and A-A in HX-19, Fig. 6. In general, the bond lengths and angles obtained by B3LYP are close to those by MP2. However, the calculated H-bonds by DFT methods are somewhat too short, as a rule as compared to MP2, in agreement to that reported in other molecules [97]. Thus, the analysis of the H-bonds was carried out mainly on the MP2 values.

In the dihydrated forms the energy gap between HX-17 and HX-19 is reduced up to 1.76 kcal mol⁻¹. This reduction is due to the H-bonds are stronger in HX-19 than in HX-17. However, this reduction is decreased because in HX-17 there are four H-bonds vs. three in HX-19.

HX(H₂O)₃₋₁₀ Following the methodology before, a third water molecule was added in the most stable dihydrated cluster. The results obtained are shown in Fig. 7. B3LYP reproduces well the stability trend as compared to MP2. The addition of a third water molecule has a relatively very small effect in the energy gap between HX-17 and HX-19, however, with four water molecules, HX-19 is 0.77 kcal mol⁻¹ more stable than HX-17.

Successively, the same methodology has been applied for incorporating the remaining water molecules up to the first hydration shell. Fig. 7 collects the optimum clusters with 4 water molecules, while Fig. 4-SUP shows the order of filling from 5 to 10 water molecules on HX-17 and HX-19. For simplicity, weak H-bonds are not shown in these figures. Clusters with more than 9 water molecules were not possible to be calculated by MP2 due to computer memory problems.

The effect of the water molecules on the N1-H, C=O and N1-C2 bond lengths is shown in Fig. 10. The first five water molecules largely affect these bond lengths, with lengthening of N1-H and C=O bonds involved in H-bonds, and shortening of N1-C2. However, the effects are lower with the further hydration. This feature is in accordance to experimental studies in methyl-substituted uracils and thymines indicating that when more than four water molecules are attached, the photophysics properties of these hydrated clusters should rapidly approach to that in the condensed phase [97].

3.4.2. First hydration shell

In the clusters of HX-17 and HX-19 with 11 and 12 water molecules, respectively, all the intermolecular H-bonds between HX and the water molecules appear established. Thus, they can be considered as the first hydration shell. Moreover, with the progress of the hydration does't appear new H-bonds between HX and the water molecules. Fig. 8 shows the cluster corresponding to HX-17, and Fig. 9 that to HX-19.

In this first hydration shell, the main effects of the water molecules is a lengthening of the C=O (0.032 Å) and N7-C8 (0.010 Å) bonds, a shortening of N1-C6 and C5-C6 bonds, 0.037 and 0.021 Å, respectively, and an opening of the angles on C6 and N3, 2.6° and 1.3°, respectively. A small shortening of C2-N3, C8-N9 and N9-C4 bonds, and closing of N3-C4-C5 (2.2°) and C2-N1-C6 (1.2°) are also abserved.

The hydration leads to a general enhancement of the charges, mainly on the oxygen atoms, Fig. 2. E.g., the charge on O6 increases 0.122e, N7 (0.088e), C8 (0.040e), N3 (0.051e), C4 (0.027e) and C6 (0.011e), and decrease on N1 (0.023e) and C5 (0.015e). This increment of the charges enhances the reactivity of the molecule and it is accompanied by an increase in the energies of HOMO and LUMO.

3.4.3. The second hydration shell

It is more or less completed with 30 water molecules. Several optimum clusters up to 30 water molecules are shown in Figs. 8 and 9, corresponding to HX-17 and HX-19, respectively. Fig. 5-

SUP also includes other two optimized clusters. The analysis of these Figs. reveals the following information:

- (i) The clusters with all the water molecules appearing H-bonded at the same face of the HX molecule are preferred that those with the water molecules distributed in both faces of HX. It is due to the H-bonds between water molecules are stronger than between a water molecule and HX. We have tried to find clusters with the water molecules distributed in both faces of HX, due to with them the effects of the water molecules on the molecular structure of HX are better represented.
- (ii) The optimum distribution of the water molecules binding to the most polar groups is in general in accordance with the previous hydrated structure found in uracil [68] and related molecules [108-110].
- (iii) The water molecules little affect the planarity of the purine ring, with change in its torsional angles of less than 2°. **check**
- (iv) After the first hydration shell the C=O and N1-H bond lengths are little changed with the progress of the hydration, Fig. 10. They tend to be established. Significant differences are not observed in the bond lengths and angles. The largest differences compared to the cluster of the first hydration shell are in C=O (0.011 Å), N1-C6 (-0.012 Å) and C8-N9 (-0.017 Å).
- (v) In the atomic charges, the cluster with 30 water (HX-17 or 19) molecules only show noticeable differences in N7 (0.064e), O (0.036e), N3 (-0.032e), N1 (-0.028e) and C2 (0.025e).
- (vi) In clusters with large number of water molecules, as in the 2nd hydration shell, HX-17 is remarkably more stable than HX-19, i.e. tautomerisation is reduced. This feature is in accordance to the large dipole moment of HX-17 in the cluster with 30 water molecules.
- (vii) Table 6 shows the CP-corrected interaction energy $\Delta E_{B-(W_n)}^{CP}$ between HX-19 and the water molecules (B-W_n) with W_n= 20, and the CP-interaction energy water-water $\Delta E_{(W_n)}^{CP}$. The value obtained for $\Delta E_{B-(W_n)}^{CP}$ is more negative than that found in the nucleoside analogue D4T(H₂O)₁₃, ca. -180 kcal/mol [108], but lower than in 5-fluorouracil (H₂O)₁₀, -274 kcal/mol [69].

3.4.4. PCM.

The values of the bond lengths obtained with PCM in HX-17 and HX-19 are between those of the isolated state and those in the cluster with 20 water molecules, Fig. 2. The largest differences appear in the atoms involved in H-bonds. The hydration effect of the PCM model on the geometry structure is lower than that obtained using explicit water molecules. As expected, the largest difference obtained in HX-17 as compared to the isolated state appears in C=O and N1-C6 with a lengthening of 0.008 Å. In HX-19 the differences are slightly larger. In the angles the differences are very small. The effect of the hydration with PCM is also small in the calculation of the atomic charges (Fig. 3) and in the of the HOMO and LUMO energies. With PCM, as well as with explicit water molecules, the largest negative charge is on O6 and N1, while on the carbon atoms the greater positive charge is on C6 in both tautomers.

Calcular la poblacion de HX-17 y HX-19 por PCM y el ΔE entre ambos.

In a continuum model the calculated population reported [1] at MP2/6-311++G(2df,pd)/B3LYP/6-311++G(d,p) level is HX-17 (49.26%) and HX-19 (50.72%). By AM1 and the COSMO solvation model, HX-19 was 1.06 kcal mol⁻¹ more stable than HX-17 [86].

3.4.5. Dipole moment (μ).

The dipole moments in HX-19 and HX-17 from the isolated state up to 30 water molecules are collected in Table 4. Noticeable differences are observed between HX-17 and HX-19, and between B3LYP and MP2 values. In the isolated state or with few water molecules HX-17 has lower μ than HX-19. However, with more than 5 water molecules HX-17 has larger μ than HX-19, increasing the difference with the progress of the hydration specially with 25 and 30 water molecules. I.e. the population of HX-17 increases with the hydration. The difference in the calculated solvation ΔG° energy ($-77.4 \text{ kJ mol}^{-1}$ in HX-17 and $-82.4 \text{ kJ mol}^{-1}$ in HX-19 [55]) is in accordance to this feature.

HX-17 increases μ with the progress of the hydration up to 6 water molecules ($\mu=9.66 \text{ D}$), decrease from 6 to 15 water molecules, and remarkably increases again up to the value of 15.7 D with 30 water molecules. By contrast, HX-19 has an irregular behaviour in its μ .

HX-37 keto tautomer is also stabilized upon solvation, but it is still disfavored by more than $3.0 \text{ kcal mol}^{-1}$ with respect to HX-19. The enol tautomers are destabilized by at least 11 kcal mol^{-1} . I.e. the stabilization of the keto forms over the enol ones is greater under solvation because of electrostatic effects [52].

It has been reported that leaving group ability of certain damaged bases is enhanced in a nonpolar environment, relative to in aqueous solution [6], and that Nature could take advantage of this enhancement by providing a nonpolar enzyme environment to help discriminate damaged from normal bases for cleavage. Comparar con el momento dipolar de la adenine al nivel MP2 (aislada y con 30 H₂O) The human genome is protected by an enzyme, alkyladenine DNA glycosylase (AAG). It provides a nonpolar environment that aids in the discrimination of damaged from normal nucleobases [6] from DNA.

3.4.6. Frequencies

The results of calculations of molecular structures of nucleic acid bases in polar environment using Polarized Continuum Model of solvent combined with the MP2/cc-pvdz level of ab initio theory demonstrate considerable polarization of thymine, cytosine, and guanine. This phenomenon can be related to considerable contribution of zwitter-ionic resonant forms into total structure of the studied species. It leads to significant increase (about 30%) of frequencies of the out-of-plane pyrimidine ring vibrations and is related to considerable decrease of conformational flexibility of heterocycle due to smaller out-of-plane deformability of pyrimidine ring in zwitter-ionic resonant forms. In the case of adenine, the presence of a polar environment results in an increase of conformational flexibility of pyrimidine ring. [111]. Barrier \uparrow , $\gamma\uparrow$, Flexibility \downarrow , polarization \uparrow .

4. Summary and Conclusions

In the present work, we have studied the tautomerism of hypoxanthine and the influence of the hydration in the stability of these tautomers. A good agreement is obtained, whenever available, with analogous theoretical studies, supporting the quality of our results derived from computations. The most important findings are the following:

- (1) The geometric parameters obtained at different DFT and MP2 levels of theory for the six most stable tautomers of HX are the most complete set of values reported today.

- (2) The proton transfer from N7 to N9 leads to a slight change in the imidazolic ring, and almost none in the pyrimidinic ring. HX-70cis, HX-90cis and HX-90trans show a lengthening of the C-O bond and shortening of N1-C6, C5-C6 and C8-N9 related to HX-17 and HX-19 tautomers.
- (3) O6 and N1 atoms have the largest negative charge in all the tautomers and at all levels of calculation, while C6 and C2 atoms have the largest positive charge. H9 of HX-19 and H7 of HX-17 are the most positive H atoms, i.e. they are the most reactive.
- (4) The crystal unit cell of the solid state with HX-19 was simulated with a tetramer form. The main differences compared to the experimental values correspond to C5-C6 and C6-N1 bonds that are lengthened, and C=O bond which is shortened.
- (5) The hydration of HX-17 and HX-19 tautomers up to 30 water molecules were calculated for the first time at the B3LYP and MP2 (up to 8 water molecules) quantum chemical levels. More than 200 cluster-optimized structures were determined.
- (6) Three different positions A, B and C are possible for the hydration of HX. In the monohydrated HX-17 the B position is the most stable, while in HX-19 it corresponds to the A position. The B position is the most unstable with the smallest CP-corrected complex formation energy. The main effect produced by the water molecules is a lengthening of the C=O bond and shortening of N1-C6. The C=O and N1-H bond lengths increase with the increment of the number of water molecules, but when the number of them is more than 15 the lengthening is reduced. The variation of the angles is very small.
- (7) The monohydration increases the energy gaps between HX-17 and HX-19, and with the other tautomers, but it is reduced with two water molecules. However, with four to 20 water molecules HX-19 is more stable than HX-17, but with larger number of water molecules HX-17 is remarkably more stable than HX-19, i.e. tautomerisation is reduced. This feature is in accordance to the large dipole moment of HX-17 in the cluster with 30 water molecules.
- (8) The reversion of the stability between HX-17 and HX-19 upon solvation with few water molecules can be explained by the larger dipole moment of HX-19 than HX-17 in the isolated state. With more than 5 water molecules HX-17 has larger μ than HX-19, increasing the difference with the progress of the hydration specially with 25 and 30 water molecules. I.e. the population of HX-17 increases with the hydration.
- (9) The first hydration shell is completed with 11 water molecules in HX-17 and 12 water molecules in HX-19. The effect of the second hydration shell on the bond lengths is very small, but the charges shows a remarkable increase. The main effect observed with the hydration, by both continuum and discrete methods is a general enhancement of the positive and negative charges on the atoms, that is, an increase of their reactivities, mainly on O6, N1 and C5 atoms.
- (10) Closer structures are more stable than structures with the water molecules distributed, and it can be observed with the successive hydration.
- (11) The hydration effect with PCM in the geometry of HX-17 and HX-19 is smaller than with 20 explicit water molecules. I.e. the PCM method does not reproduce well the hydration pattern of HX.

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Supplementary Material

Table 1-SUP. Geometrical parameters optimised in HX-37 tautomer of hypoxanthine. Bond lengths (Å) and angles (degrees).

Parameters	B3LYP/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
Bond lengths				
N1-C2	1.294	1.289	1.304	1.302
C2-N3	1.373	1.366	1.368	1.365
N3-C4	1.375	1.369	1.374	1.370
C4-C5	1.380	1.375	1.384	1.383
C5-C6	1.450	1.445	1.448	1.448
C6-N1	1.418	1.411	1.416	1.413
C6-O	1.224	1.218	1.231	1.226
C5-N7	1.380	1.376	1.370	1.370
N7-C8	1.361	1.357	1.366	1.365
C8-N9	1.328	1.322	1.334	1.332
N9-C4	1.361	1.357	1.362	1.361
Bond angles				
N-C2-N	126.5	126.2	126.8	126.6
C-N3-C	117.0	117.0	117.0	117.0
N3-C4-C5	118.6	118.9	118.3	118.7
C4-C5-C6	123.9	123.6	124.4	123.9
C5-C6-N1	112.7	112.8	112.6	112.8
C6-N-C	121.3	121.5	120.9	121.0
C5-N7-C8	107.0	107.0	107.3	107.2
N7-C8-N	113.0	112.8	112.8	112.6
C8-N9-C	103.3	103.6	102.9	103.3
N9-C4-C	112.9	112.6	113.1	112.7
C4-C5-N7	103.9	104.0	103.9	104.2

Table 2-SUP. Geometrical parameters optimised in HX-70 tautomer of hypoxanthine. Bond lengths (Å) and angles (degrees).

Parameters	B3LYP/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
Bond lengths				
N1-C2	1.355	1.349	1.365	1.363
C2-N3	1.362	1.320	1.329	1.326
N3-C4	1.346	1.339	1.351	1.347
C4-C5	1.410	1.404	1.408	1.406
C5-C6	1.394	1.389	1.398	1.395
C6-N1	1.324	1.317	1.321	1.317
C6-O	1.348	1.346	1.354	1.352
C5-N7	1.379	1.375	1.367	1.366
O-H	0.972	0.969	0.971	0.972
N7-C8	1.374	1.969	1.377	1.377
C8-N9	1.312	1.307	1.319	1.317
N9-C4	1.382	1.377	1.382	1.381
Bond angles				
N-C2-N	128.1	127.6	128.2	127.8
C-N3-C	113.6	114.0	113.4	113.7
N3-C4-C5	122.9	122.8	123.0	122.9
C4-C5-C6	117.9	117.9	118.2	118.1
C5-C6-N1	119.5	119.4	119.4	119.5
C6-N1-C	117.9	118.3	117.8	118.1
C5-N7-C8	105.7	105.8	106.0	105.9
N7-C8-N	114.1	113.9	113.9	113.7
C8-N9-C	104.5	104.8	104.0	104.4
N9-C4-C	110.1	109.8	110.3	110.0
C4-C5-N7	105.6	105.6	105.8	106.0

Table 3-SUP. Geometrical parameters optimised in HX-90-cis tautomer of hypoxanthine. Bond lengths (Å) and angles (degrees).

Parameters	B3LYP/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
Bond lengths				
N1-C2	1.346	1.341	1.355	1.353
C2-N3	1.334	1.327	1.335	1.332
N3-C4	1.337	1.331	1.342	1.338
C4-C5	1.407	1.402	1.406	1.404
C5-C6	1.404	1.398	1.405	1.402
C6-N1	1.333	1.326	1.330	1.327
C6-O	1.339	1.335	1.345	1.342
O-H	0.972	0.969	0.971	0.972
C5-N7	1.385	1.381	1.380	1.379
N7-C8	1.309	1.303	1.324	1.323
C8-N9	1.382	1.377	1.373	1.372
N9-C4	1.375	1.371	1.374	1.374
Bond angles				
C-N3-C	111.7	112.2	111.3	111.7
N3-C4-C5	127.2	126.9	127.5	127.2
C4-C5-C6	114.5	114.7	114.6	114.7
C5-C6-N1	120.1	119.9	120.2	120.0
C6-N1-C	118.6	119.1	118.6	118.9
C5-N7-C8	103.9	104.3	103.2	103.5
N7-C8-N	113.8	113.5	113.9	113.7
C8-N9-C	106.6	106.6	106.7	106.7
N9-C4-C	104.5	104.7	104.4	104.6
C4-C5-N7	111.2	110.9	111.8	111.5
C-N3-C	111.7	112.2	111.3	111.7

Table 4-SUP. Geometrical parameters optimised in HX-90-trans tautomer of hypoxanthine. Bond lengths (Å) and angles (degrees).

Parameters	B3LYP/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	MP2/6-31G(d,p)	MP2/6-311++G(2d,p)
Bond lengths				
N1-C2	1.346	1.341	1.355	1.353
C2-N3	1.338	1.331	1.340	1.337
N3-C4	1.335	1.329	1.341	1.337
C4-C5	1.401	1.396	1.400	1.398
C5-C6	1.404	1.398	1.403	1.401
C6-N1	1.328	1.321	1.327	1.322
C6-O	1.343	1.339	1.348	1.345
O-H	0.971	0.968	0.970	0.972
C5-N7	1.384	1.381	1.380	1.379
N7-C8	1.311	1.305	1.326	1.325
C8-N9	1.381	1.376	1.372	1.372
N9-C4	1.378	1.374	1.377	1.377
Bond angles				
N-C2-N	128.6	128.0	128.8	128.3
C-N3-C	111.5	112.0	111.0	111.5
N3-C4-C5	126.4	126.1	126.6	126.4
C4-C5-C6	115.7	115.8	115.8	115.9
C5-C6-N1	119.8	119.6	119.9	119.7
C6-N1-C	118.0	118.5	117.9	118.2
C5-N7-C8	103.8	104.2	103.1	103.4
N7-C8-N	113.6	113.3	113.7	113.5
C8-N9-C	106.7	106.8	106.9	106.8
N9-C4-C	104.3	104.5	104.1	104.4
N-C2-N	128.6	128.0	128.8	128.3

Table 5-SUP. Relative energies (in kcal/mol) of six main tautomers of HX at B3LYP level, and population (in %) at 273.15 and 298.15 K.

Tautomer	6-31G(d,p) ^a	6-311++G(3df,pd) ^b	% (273.15K)	% (298.15K)
HX-17	0 ^a	0 ^b	82.8	80.8
HX-19	0.385	0.840	17.2	19.1
HX-37	7.690	7.044	< 0.005	< 0.005
HX-70	6.342	6.763	< 0.005	< 0.005
HX-90cis	3.687	4.413	0.02	0.04
HX-90trans	5.103	5.514	< 0.005	0.01

^a -305657.1229 kcal/mol

^b -305761.4106 kcal/mol

Table 5b-SUP. Calculated dipole moments (in Debyes) of the six main tautomers of HX at the MP2 level and different basis set.

Tautomer	6-31G(d,p)	6-311++G(2d,p) ^b	cc-pvtz
HX-17	1.425	1.242	1.301
HX-19	5.891	5.982	5.871
HX-37	5.446	5.596	5.469
HX-70	5.134	5.146	5.130
HX-90cis	2.801	2.796	2.735
HX-90trans	5.099	5.018	4.921

Table 6-SUP. Effect of the hydration on the bond lengths (Å) and angles (°) of the HX-17 molecule calculated at the B3LYP/6-31G(d,p) level.

Bond lengths	isolate d	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O	+9H ₂ O	+10H ₂ O	+11H ₂ O	+12 H ₂ O	+13H ₂ O
C=O	1.225	1.240	1.255	1.259	1.259	1.256	1.255	1.254	1.256	1.253	1.254	1.254	1.256	1.256
C6-N1	1.416	1.404	1.393	1.387	1.387	1.390	1.390	1.391	1.390	1.395	1.393	1.394	1.392	1.392
N1-C2	1.376	1.372	1.374	1.374	1.372	1.369	1.367	1.367	1.368	1.364	1.369	1.365	1.370	1.370
C2-N3	1.297	1.300	1.299	1.300	1.300	1.301	1.302	1.302	1.303	1.305	1.306	1.305	1.306	1.306
N3-C4	1.372	1.369	1.368	1.366	1.366	1.366	1.365	1.365	1.365	1.368	1.367	1.367	1.366	1.366
C4-C5	1.396	1.398	1.403	1.404	1.409	1.405	1.402	1.404	1.403	1.403	1.404	1.405	1.405	1.405
C5-C6	1.433	1.427	1.421	1.420	1.420	1.421	1.423	1.423	1.421	1.422	1.420	1.420	1.418	1.418
C5-N7	1.374	1.374	1.373	1.374	1.376	1.376	1.377	1.376	1.374	1.375	1.376	1.374	1.375	1.375
N7-C8	1.368	1.368	1.362	1.362	1.361	1.358	1.356	1.354	1.356	1.353	1.353	1.352	1.352	1.352
C8-N9	1.318	1.319	1.324	1.323	1.323	1.325	1.326	1.328	1.228	1.329	1.329	1.331	1.301	1.331
N9-C4	1.373	1.374	1.369	1.369	1.367	1.367	1.366	1.366	1.367	1.365	1.366	1.365	1.365	1.365
Bond angles														
N1-C6-C5	108.2	109.2	110.3	110.0	111.0	111.0	110.9	110.9	110.9	110.8	110.7	110.8	110.7	110.7
C6-C5-C4	123.4	122.8	122.2	122.6	121.5	121.3	121.3	121.3	121.7	121.5	121.4	121.6	121.5	121.5
C5-C4-N3	123.8	123.9	123.6	123.6	123.9	124.1	124.1	124.1	123.6	123.9	124.1	123.7	123.8	123.8
C4-N3-C2	113.8	114.0	113.8	113.1	113.7	113.8	114.1	113.9	113.9	113.9	114.2	114.0	114.4	114.4
N3-C2-N1	125.3	125.2	125.6	125.9	125.7	125.5	125.4	125.4	125.5	125.4	124.5	125.4	124.5	124.5
C2-N1-C6	125.5	125.2	124.4	124.3	124.2	124.4	124.4	124.4	124.3	124.4	125.1	124.3	125.0	125.0
C5-N7-C8	106.1	105.7	105.6	105.9	105.5	105.7	105.8	106.6	105.9	105.7	105.7	105.9	105.9	105.9
N7-C8-N9	113.4	114.5	115.1	113.8	114.5	114.2	114.0	114.2	113.9	114.1	114.1	113.9	113.9	113.9
C8-N9-C4	104.5	104.2	104.2	103.9	103.9	104.1	104.3	104.2	104.0	104.1	104.2	104.2	104.3	104.3
N9-C4-C5	110.5	110.3	110.2	110.5	110.5	110.4	110.2	110.2	110.5	110.3	110.2	110.2	110.1	110.1
C4-C5-N7	105.5	105.9	105.9	105.9	105.6	105.6	105.7	105.9	105.7	105.8	105.8	105.7	105.8	105.8

Table 6-SUP (continued)

Bond lengths	+15H ₂ O	+20H ₂ O	+21H ₂ O	+25H ₂ O	+30H ₂ O
C=O	1.259	1.260	2.258	1.250	1.265
C6-N1	1.384	1.385	1.390	1.393	1.384
N1-C2	1.361	1.360	1.363	1.360	1.363
C2-N3	1.304	1.309	1.310	1.306	1.307
N3-C4	1.361	1.366	1.368	1.363	1.363
C4-C5	1.395	1.403	1.402	1.396	1.398
C5-C6	1.415	1.412	1.415	1.422	1.407
C5-N7	1.374	1.372	1.374	1.375	1.372
N7-C8	1.348	1.357	1.357	1.351	1.351
C8-N9	1.337	1.333	1.332	1.337	1.330
N9-C4	1.373	1.364	1.365	1.370	1.365

Table 7-SUP. Effect of the hydration on the bond lengths (Å) and angles (°) of the HX-17 molecule calculated at the MP2/6-31G(d,p) level.

Bond lengths	isolated	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O
C=O	1.232	1.429	1.258	1.261	1.261	1.259	1.257	1.257	1.259
C6-N1	1.406	1.395	1.386	1.380	1.380	1.382	1.382	1.385	1.385
N1-C2	1.381	1.377	1.378	1.378	1.377	1.374	1.373	1.372	1.369
C2-N3	1.302	1.305	1.305	1.306	1.305	1.306	1.307	1.307	1.309
N3-C4	1.376	1.373	1.371	1.369	1.369	1.369	1.368	1.369	1.370
C4-C5	1.397	1.298	1.404	1.404	1.423	1.405	1.404	1.404	1.404
C5-C6	1.435	1.429	1.425	1.425	1.423	1.425	1.426	1.425	1.423
C5-N7	1.363	1.364	1.363	1.363	1.364	1.364	1.365	1.365	1.362
N7-C8	1.372	1.373	1.368	1.368	1.367	1.365	1.363	1.363	1.363
C8-N9	1.325	1.324	1.328	1.328	1.328	1.329	1.330	1.332	1.332
N9-C4	1.373	1.374	1.370	1.371	1.369	1.369	1.369	1.364	1.367
Bond angles									
N1-C6-N5	107.7	108.6	109.7	110.0	110.4	110.3	110.3	110.3	110.4
C6-C5-C4	123.9	123.1	122.6	122.6	122.0	121.9	121.8	121.6	121.9
C5-C4-N3	123.8	123.9	123.8	123.6	123.9	124.1	124.1	124.4	123.7
C4-N3-C2	113.2	113.4	113.3	113.1	113.2	113.3	113.4	113.3	113.7
N3-C2-N1	125.5	125.2	125.5	125.9	126.7	125.4	125.3	125.2	125.1
C2-N1-C6	126.0	125.9	125.1	124.7	124.8	125.0	125.1	125.2	125.0
C5-N7-C8	106.4	106.0	105.9	105.9	105.7	105.9	106.0	106.4	106.4
N7-C8-N9	113.1	113.7	113.8	113.8	114.2	112.9	113.6	112.9	113.4
C8-N9-C4	104.8	103.9	103.9	103.9	103.7	103.9	104.1	104.7	104.0
N9-C4-C5	110.7	110.5	110.5	110.5	110.6	110.5	110.3	110.2	110.5
C4-C5-N7	105.7	106.0	106.0	105.9	105.9	105.9	106.0	105.8	105.7

Table 8-SUP. Effect of the hydration on the bond lengths (Å) and angles (°) of the HX-19 molecule calculated at the B3LYP/6-31G(d,p) level.

Bond lengths	isolated	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O	+9H ₂ O	+10H ₂ O	+12H ₂ O	+13H ₂ O
C=O	1.217	1.232	1.235	1.234	1.234	1.238	1.241	1.241	1.243	1.243	1.246	1.249	1.249
C6-N1	1.436	1.420	1.412	1.417	1.417	1.411	1.406	1.405	1.404	1.402	1.400	1.399	1.400
N1-C2	1.365	1.361	1.362	1.358	1.355	1.356	1.354	1.354	1.355	1.355	1.356	1.361	1.358
C2-N3	1.303	1.306	1.307	1.309	1.310	1.310	1.311	1.312	1.311	1.312	1.312	1.312	1.310
N3-C4	1.362	1.359	1.357	1.364	1.362	1.365	1.364	1.364	1.364	1.364	1.364	1.363	1.358
C4-C5	1.396	1.397	1.397	1.396	1.399	1.399	1.399	1.397	1.400	1.398	1.397	1.400	1.399
C5-C6	1.447	1.449	1.439	1.438	1.437	1.436	1.434	1.433	1.430	1.429	1.427	1.426	1.428
C5-N7	1.378	1.379	1.380	1.378	1.376	1.377	1.377	1.379	1.376	1.375	1.373	1.377	1.378
N7-C8	1.311	1.321	1.310	1.314	1.315	1.316	1.316	1.317	1.315	1.318	1.319	1.321	1.322
C8-N9	1.379	1.380	1.381	1.376	1.375	1.372	1.373	1.372	1.376	1.372	1.372	1.372	1.374
N9-C4	1.371	1.371	1.371	1.365	1.365	1.365	1.365	1.366	1.364	1.366	1.367	1.365	1.363
Bond angles													
N1-C6-C5	108.9	110.2	110.5	110.6	110.6	110.8	111.2	111.3	111.5	111.6	111.7	111.5	111.6
C6-C5-C4	119.4	118.5	118.7	118.7	119.2	119.2	119.0	119.1	118.9	119.2	119.4	118.9	118.7
C5-C4-N3	128.9	128.6	128.4	128.1	127.1	126.9	126.9	126.7	126.8	126.5	126.3	126.7	126.8
C4-N3-C2	112.0	111.7	111.7	112.1	112.7	112.7	112.7	112.7	112.7	112.8	112.9	113.3	113.5
N3-C2-N1	124.6	125.1	125.6	125.2	125.2	125.2	125.2	125.3	125.3	125.3	125.3	124.1	124.0
C2-N1-C6	126.6	125.6	125.1	125.4	125.2	125.1	125.0	124.7	124.8	124.6	124.5	125.4	125.4
C5-N7-C8	104.5	104.4	104.4	104.2	104.0	104.2	104.2	104.1	103.8	104.3	104.8	104.8	104.9
N7-C8-N9	113.0	113.1	113.1	113.4	113.9	113.7	113.7	113.7	113.9	113.4	112.9	112.7	112.6
C8-N9-C4	106.6	106.6	106.6	106.1	105.7	105.9	105.9	106.0	105.8	106.1	106.4	106.7	106.6
N9-C4-C5	105.0	104.9	105.0	105.7	105.9	105.9	105.9	105.8	105.7	105.8	105.8	105.7	106.0
C4-C5-N7	111.0	111.0	111.0	110.5	110.5	110.2	110.3	110.4	110.7	110.4	110.2	110.1	109.8

Table 8-SUP (continued)

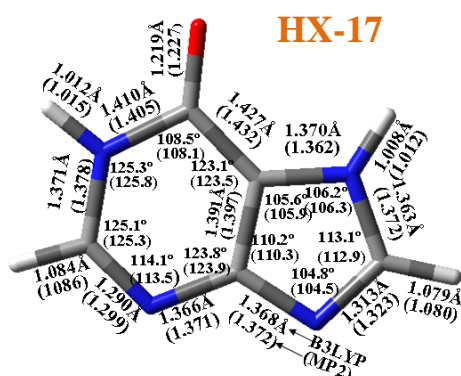
Bond lengths	+14 H ₂ O	+15H ₂ O	+20H ₂ O	+25H ₂ O	+30H ₂ O
C=O	1.259	1.250	1.244	1.250	1.262
C6-N1	1.399	1.400	1.402	1.395	1.378
N1-C2	1.360	1.359	1.351	1.353	1.356
C2-N3	1.309	1.309	1.317	1.317	1.307
N3-C4	1.357	1.357	1.363	1.360	1.351
C4-C5	1.299	1.399	1.394	1.397	1.399
C5-C6	1.428	1.429	1.434	1.425	1.413
C5-N7	1.381	1.381	1.381	1.380	1.379
N7-C8	1.323	1.323	1.320	1.318	1.326
C8-N9	1.365	1.365	1.374	1.371	1.358
N9-C4	1.362	1.361	1.368	1.366	1.360

Table 9-SUP. Effect of the hydration on the bond lengths (Å) and angles (°) of the HX-19 molecule calculated at the MP2/6-31G(d,p) level.

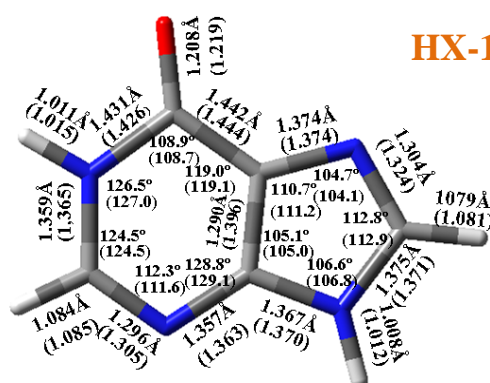
Bond lengths	isolated	+1H ₂ O	+2H ₂ O	+3H ₂ O	+4H ₂ O	+5H ₂ O	+6H ₂ O	+7H ₂ O	+8H ₂ O
C=O	1.225	1.237	1.239	1.238	1.239	1.243	1.246	1.246	1.247
C6-N1	1.427	1.413	1.406	1.410	1.410	1.404	1.400	1.400	1.398
N1-C2	1.368	1.365	1.365	1.362	1.360	1.360	1.359	1.359	1.360
C2-N3	1.307	1.310	1.311	1.312	1.313	1.313	1.314	1.314	1.314
N3-C4	1.367	1.363	1.362	1.367	1.369	1.367	1.367	1.367	1.367
C4-C5	1.396	1.397	1.397	1.392	1.400	1.400	1.400	1.400	1.399
C5-C6	1.336	1.440	1.439	1.438	1.437	1.436	1.435	1.435	1.430
C5-N7	1.374	1.375	1.376	1.374	1.372	1.372	1.373	1.373	1.371
N7-C8	1.326	1.325	1.325	1.329	1.329	1.330	1.330	1.330	1.330
C8-N9	1.371	1.372	1.372	1.368	1.369	1.365	1.366	1.366	1.368
N9-C4	1.371	1.371	1.371	1.367	1.366	1.366	1.366	1.366	1.366
Bond angles									
N1-C6-C5	108.5	109.7	110.0	110.1	110.1	110.3	110.7	110.7	111.0
C6-C5-C4	119.3	118.8	119.0	119.4	119.4	119.4	119.2	119.3	119.3
C5-C4-N3	129.2	129.0	128.3	127.4	127.4	127.3	127.3	127.1	127.0
C4-N3-C2	111.2	111.1	111.4	112.0	112.0	112.0	112.0	112.1	112.1
N3-C2-N1	124.7	125.2	125.2	125.1	125.1	125.1	125.1	125.2	125.2
C2-N1-C6	127.1	126.2	126.1	125.9	125.9	125.8	125.7	125.5	125.3
C5-N7-C8	103.8	103.7	103.6	103.3	103.3	103.7	103.6	103.6	103.2
N7-C8-N9	113.1	113.2	113.5	114.0	114.0	113.7	113.7	113.7	114.0
C8-N9-C4	106.9	106.8	106.4	106.0	106.0	106.2	106.2	106.3	106.1
N9-C4-C5	104.8	104.7	105.4	105.6	105.6	105.7	105.6	105.6	105.4
C4-C5-N7	111.5	111.5	111.1	111.1	111.1	110.8	110.8	110.9	111.4

Table 10-SUP. $\Delta E_{BW_1}^{(BW_1)}$ (BW₁) energy (in a.u.) in the three positions (A, B and C) of the water molecule in the monohydrated HX-17 and HX-19 tautomers, $\Delta E_B^{(BW_1)}$ (BW₁) energy of HX in the monohydrate, and $\Delta E_{W_1}^{(BW_1)}$ (BW₁) energy of the water molecule in the monohydrate.

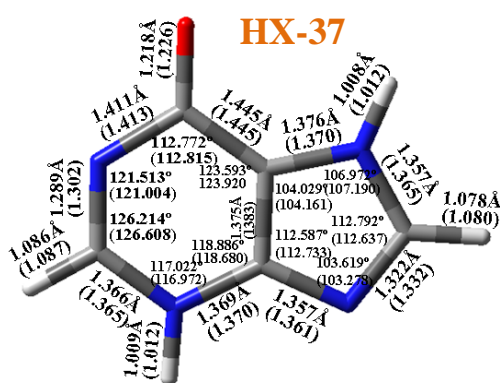
tautomer	energy	B3LYP/6-31G(d,p)			MP2/6-31G(d,p)		
		A	B	C	A	B	C
HX-17	$\Delta E_{BW_1}^{(BW_1)}$ (BW ₁)	-563.6403	-563.6447	-563.6288	-562.0691	-562.0737	-562.0609
	$\Delta E_B^{(BW_1)}$ (BW ₁)	-487.1953	-487.1948	-487.1961	-485.8263	-485.8259	-485.8268
	$\Delta E_{W_1}^{(BW_1)}$ (BW ₁)	-76.4195	-76.4194	-76.4195	-76.2196	-76.2195	-76.2196
HX-19	$\Delta E_{BW_1}^{(BW_1)}$ (BW ₁)	-563.6404	-563.6305	-563.6370	-562.0693	-562.0613	-562.0674
	$\Delta E_B^{(BW_1)}$ (BW ₁)	-487.1946	-487.1954	-487.1946	-485.8257	-485.8262	-485.8257
	$\Delta E_{W_1}^{(BW_1)}$ (BW ₁)	-76.4194	-76.4195	-76.4196	-76.2195	-76.2196	-76.2196



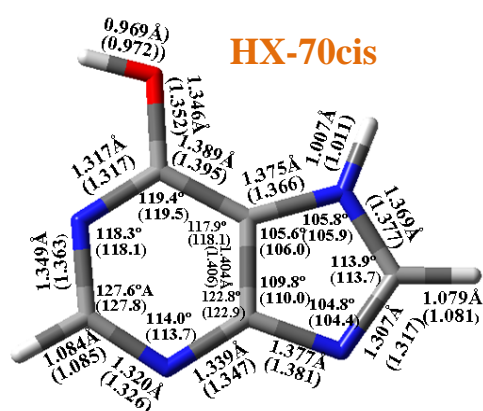
-487.261888 a.u. (-486.124843)
 -487.045811 a.u. [-487.054435]



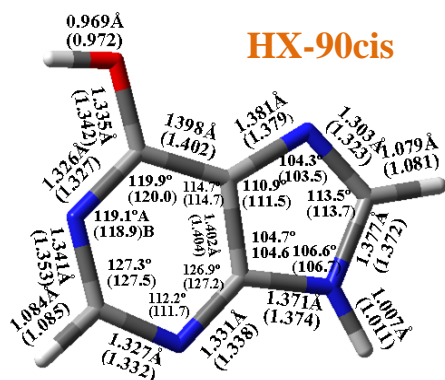
-487.260549. (-486.123546)
 -487.045339 [-487.054015]



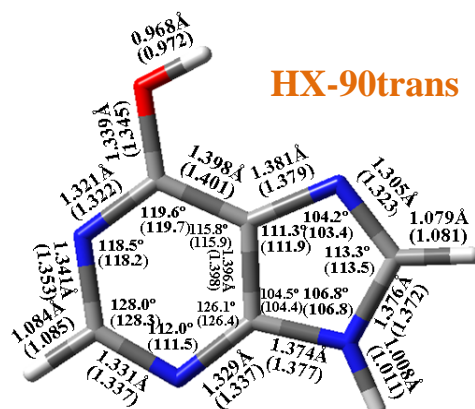
-487.250662 (-486.112696)
 -487.033305 [-487.041172]



- 487.251111 (-486.115741)
 -487.038346 [-487.042617]

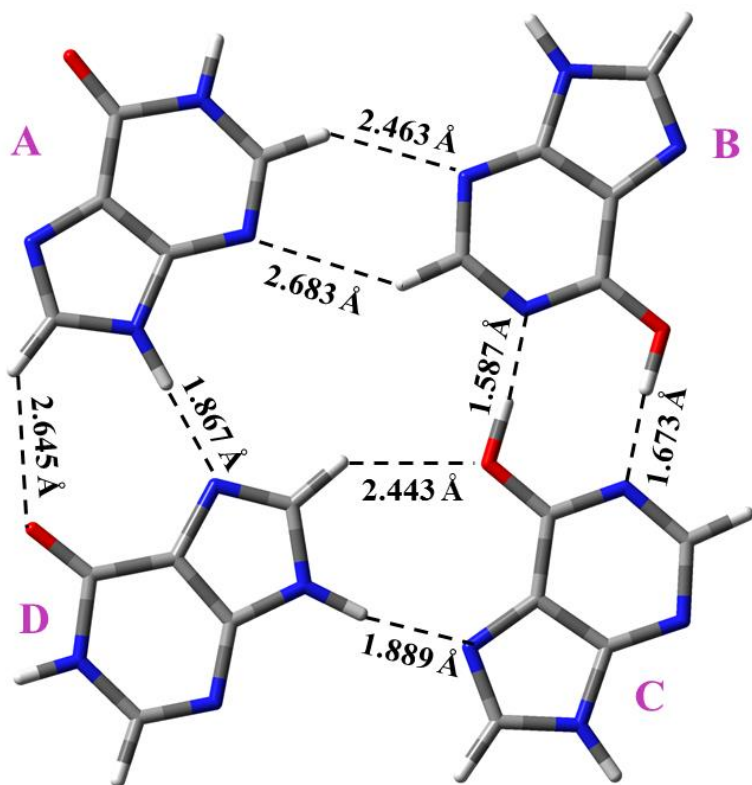


-487.254856 (-486.119524)
 -487.042964 [-487.047158]

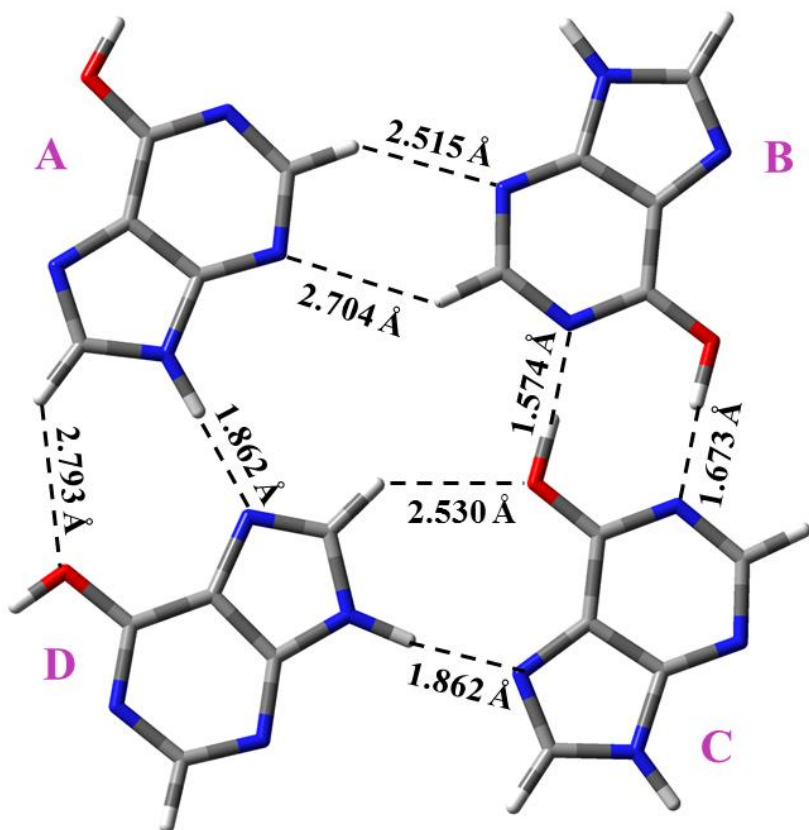


-487.253101 (-486.118090)
 -487.040912 [-487.04508]

(Fig. 1-Sup). Optimized bond lengths, bond angles and energies in the six main tautomers of hypoxanthine at B3LYP/6-311++G(3df,pd) and MP2/6-311++G(2d,p) levels (in parenthesis). The energy at M052X/6-31G(d,p) level is in italic type and at M06L/6-31G(d,p) level is in italic type and in brackets.



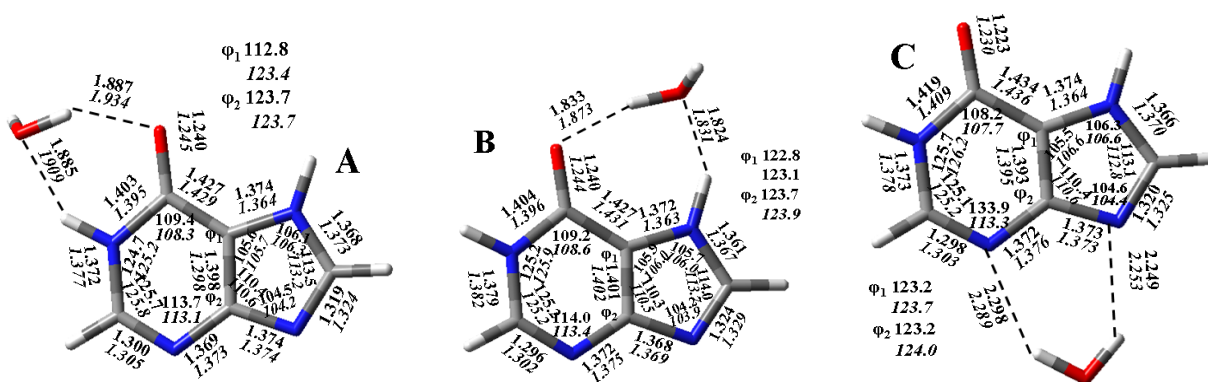
$E = -1948.445906$ (-1948.517142) $\mu = 14.669$ D



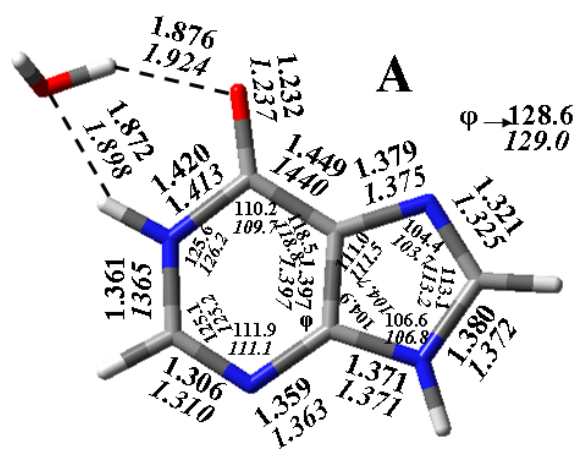
$E = -1948.433491$ (-1948.505027) $\mu = 8.306$ D

(Fig. 2-Sup). Optimized tetramer forms with the HX-90cis enol tautomeric form.

HX-17 + 1 H₂O

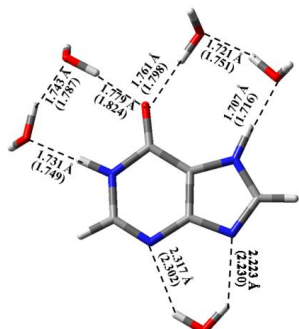


HX-19 + 1 H₂O



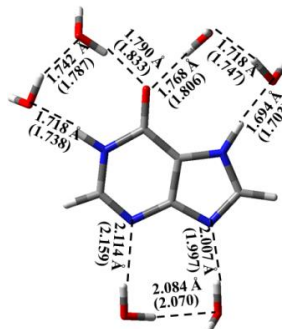
(Fig. 3-Sup). Geometry of the monohydrated HX-17 and HX-19 tautomers. The calculated intermolecular H-bonds correspond to B3LYP/6-31G(d,p) and MP2/6-31G(d,p) (in italic type). The bond lengths are in Å and angles in degrees.

a) HX-17 +5H₂O



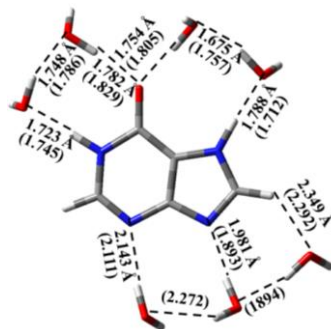
-869.175290 a.u. (-867.030794)

+6H₂O



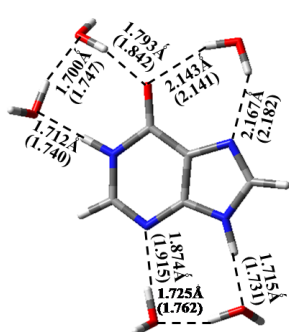
-945.587987 a.u. (-943.268199)

+7H₂O

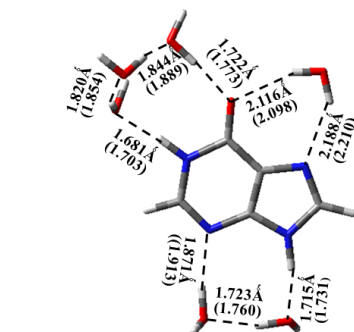


-1022.008564 a.u. (-1019.508735)

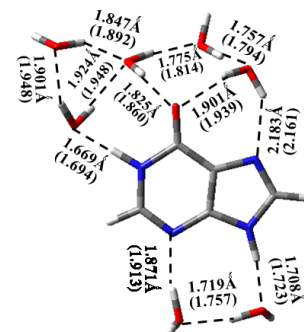
HX-19



-869.178656 a.u. (-867.033729)

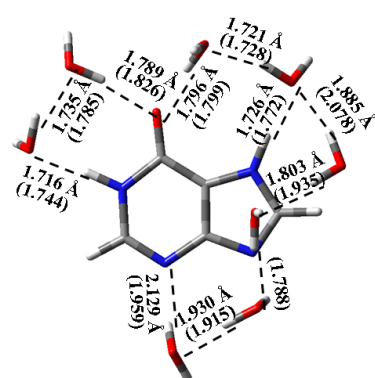


-945.593754 a.u. (-943.273761)



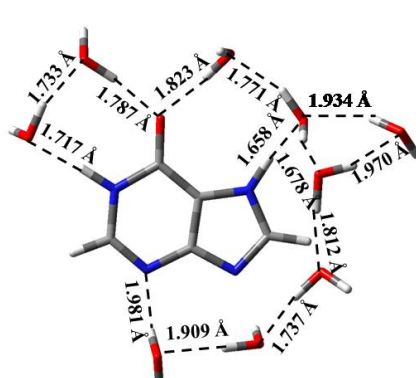
-1022.018278 a.u. (-1019.523893)

b) HX-17 +8H₂O



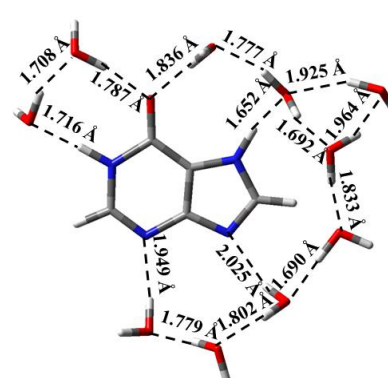
-1098.415612 a.u. (1095.751921)

+9H₂O



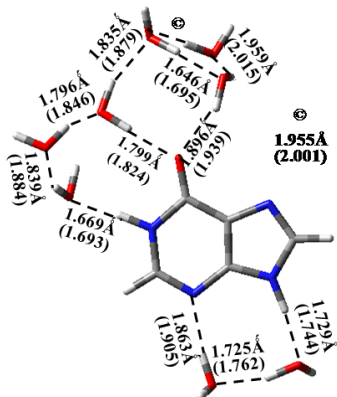
-1174.840801 a.u.

+10H₂O

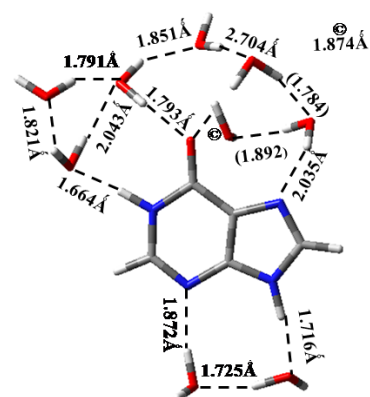


-1251.611981 a.u.

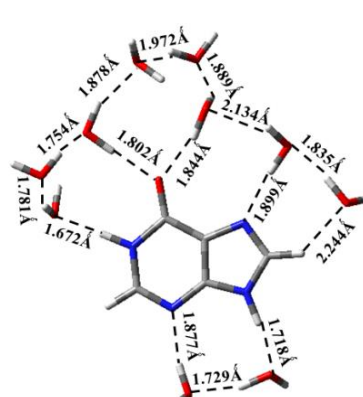
HX-19



-1098.429172 a.u. (-1095.757646)

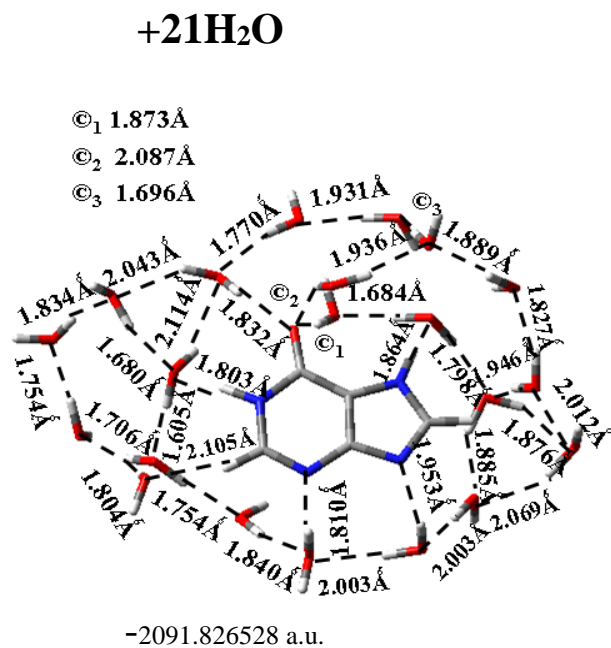
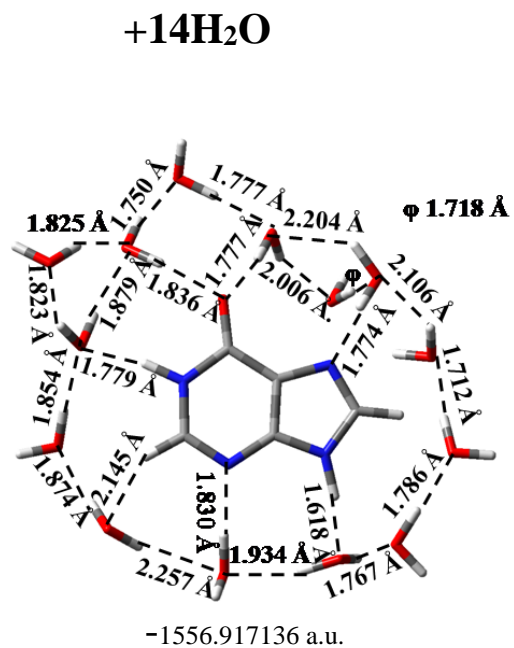


-1174.837962 a.u. (-1172.008452)



-1251.259093 a.u.

(Fig. 4-Sup.) Optimum clusters of HX-17 and HX-19 from 5 to 10 water molecules. The total energy+ZPE appears in the bottom of each figure at the B3LYP/6-31G(d,p) and MP2/6-31G(d,p) (in parenthesis) levels.



(Fig. 5-Sup.) Optimum clusters with 14 and 21 water molecules of tautomer HX-19. The total energy+ZPE at the B3LYP/6-31G(d,p) level appears in the bottom of each figure.

Simulation of the solid state and the First and second Hydration Shell of the xanthine oxidase inhibitor allopurinol: Structures Obtained Using DFT and MP2 Methods

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Abstract

Allopurinol (AL) was investigated in the isolated state, solid state, and in the hydrated form through an exhaustive quantum-chemical analysis. Full geometry optimization and energy calculations of the 11 most stable tautomers in the isolated state were performed. The crystal unit cell of the solid state was simulated by a heptamer form. The hydration of the two most stable tautomers: ALO-15 and ALO-25 was carried out. Solvent effects were considered using the Tomasi's polarized continuum model (PCM) and by the explicit model including a variable number of water molecules surrounding the base to simulate the first and second hydration shells. The deformation and interaction energies were corrected by the CP procedure.

Keywords: allopurinol, zloprim, tautomers, hydration, DFT

1. Introduction

Analogues of purine bases are highly relevant in the biological context and have been implicated as drug molecules for therapy against a number of diseases. Additionally, these molecules have been implicated to have a role in the prebiotic RNA world [1]. ZYLOPRIM (allopurinol, AL), known chemically as 1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one, is a purine hypoxanthine-based structural analogue and a well-known inhibitor of xanthine oxidase (XO), enzyme which catalyzes the conversion of hypoxanthine into xanthine and this one into uric acid. Overproduction of uric acid forms crystals into the blood and the tissues of the human body, causing gout attacks and others, and AL is used as a potent drug against it [2,3]. Thus, AL is used as long-term therapy to reduce the occurrence of gout flares [4-6], in clinical trials of patients with cardiovascular and chronic kidney disease [7], and in patients with classic Lesch-Nyhan disease/syndrome [8]. The long-term effective control of serum uric acid by AL may improve insulin resistance, decrease the serum levels of hs-CRP, reduce the carotid IMT, delay the development of atherosclerosis and improves renal function in patients with type 2 diabetes (T2DM) and asymptomatic hyperuricemia (HUA) [9,10].

Moreover, AL reduces oxidative stress, improves vascular function in several cardio metabolic diseases, prolongs exercise time in angina, alleviates hypertension and proteinuria [11], and improves the efficiency of cardiac contractility in chronic heart failure (CHF) [12] by its cardioprotective effect [13-18], and it has a protective effects [19,20] on hepatic [21] and renal [22] ischemia-reperfusion injury [23]. Also it has been used to treat different types of tissue and vascular injuries, and inflammatory diseases [24-29], and it is a chemoprotector during anticancer cytotoxic therapies [30,31]. Important is their applications on sarcopenia [32], in treatment of schizophrenia [33,34] and on bipolar mania [35]. AL is a good scavenger of OH radical and produced beneficial effects to living organism [36]. AL gel application can mitigate inflammation reactions associated with radiation-induced oral mucositis and dermatitis [37].

AL reduces severity of delayed neurologic sequelae in patients that survive of severe carbon monoxide (CO) poisoning by the generation of reactive oxygen species (ROS) [38]. XO also plays an important role in free radical generation during skeletal muscle contraction and thus, it has been related to the muscle damage associated to exhaustive exercise. I.e. it decreases transient skeletal muscle damage when occurring in top-level athletes, when administered before a competition or a particularly high-intensity training session [39,40].

AL has a metal co-ordination capability due to both, the existence of several electron donor atoms and their disposition in the framework. Metal drug complexes have increased interest by its enhanced therapeutic effect in combination with decreased toxicity. Many metal complexes involving AL as an uncharged (neutral) ligand and metals, such as Zn(II), Co(II), Ni(II), etc. have been reported [41]. AL has a good chelating ability to form monomeric complexes with Cu(II) interacting the metal with one N atom of the pyrazole ring and C=O [36, 42].

However, AL is the most commonly used urate-lowering therapy, has rare but potentially fatal adverse effects [43]. Thus, the use of AL has been associated with a risk of osteoporotic fracture [44,45], hypersensitivity reactions [46], and severe cutaneous adverse reactions [47].

Countless are the advantages for AL, and therefore the importance to study this molecule in the present work. From the theoretical point of view, AL has been studied [48-50] with DFT methods on neutral [51], anionic [52], deprotonated [53] and monoprotinated tautomers in gas phase [49]. However, its structure in aqueous solution is lacking. Thus, it is the aim of the present work, to analyse at high level of calculation the hydration effect on the geometries and charges.

2. Calculations

Geometry optimizations and total molecular energy calculations of the 11 most stable tautomers of AL were carried out by Quantum chemical calculations using the MP2 ab initio method and B3LYP DFT method. These two methods appear implemented in the GAUSSIAN 09 program package [54] that was running on the alpha computer of the Computational Center at University Complutense of Madrid. For the optimization process were used 6-31G(d,p) and 6-31G++(3df,pd) basis set. Wavenumber calculations were carried out to assess that all the geometries correspond to stationary points and to predict the IR and Raman theoretical spectra. Atomic charges were determined with the NBO (natural bonding orbital) procedure [55].

DFT methods provide an adequate compromise between the desired chemical accuracy and the demands on computer time and power. The B3LYP method was selected due to the data obtained with this level of theory are in good agreement with those obtained by other more computationally expensive methods [56-58]. When computer requirements allowed it, MP2 computations were also performed in all the structures to confirm or correct the B3LYP results.

The calculated harmonic wavenumbers were carried out at the same level of the respective optimization process and by the analytic evaluation of the second derivative of the energy with respect to the nuclear displacement. When it was possible, the wavenumber calculations were performed in all the optimised conformers to assess that they correspond to real minimum. All the optimized structures only showed positive harmonic vibrations (true energy minimum).

Relative energies were obtained by including zero-point vibrational energies (ZPE). For the calculation of the ZPE, the wavenumbers were retained unscaled, ΔG was obtained by correcting $\Delta E + ZPE$ with the thermal energies at 298.15 K.

2.1. Hydration methods

Three procedures have been reported to simulate the hydration [59-61]: a) the continuum model, b) the discrete model, and c) the explicit model (EM) by including enough number of explicit water molecules [62,63].

The EM is the preferred method in the present research, due to it gives a good description of the water molecule net and specific solute-solvent interactions, and therefore the first and second hydration shell can be good reproduced [64]. The effect of water on the AL tautomers was estimated by explicit number of water molecules surrounding the nucleobase up to 30. The methodology used, the so-called Modified Scheme of Monosolvation (MSM) consists of the following steps [65]: the first water molecule was placed in all the possible positions around AL. Thus, the structure of all possible monohydrated complexes is determined and the complex with the lowest energy is chosen. Next, a second water molecule was added and the hydrated complex with the lowest energy is located. Successively, this process is repeated for incorporating the remaining water molecules until the hydration wished, in the present work, the 2nd hydration shell.

2.2. Interaction energies with the discrete method

In the hydration AL with explicit water molecules the energies obtained were corrected for basis set superposition error (BSSE) using the counterpoise (CP) procedure from Danilov *et al.* [63]. The CP-corrected B-(H₂O)_n formation energy of the BW_n complex, where B ≡ AL and W_n ≡ water nmer, is calculated as sum of two contributions, the base-water interaction $\Delta E_{B-(W_n)}^{CP}$, and water-water interaction: $\Delta E_{(W_n)}^{CP}$

$$\Delta E_{B-(W_n)}^{CP} = \Delta E_{B-(W_n)}^{CP} + \Delta E_{(W_n)}^{CP} \quad (1)$$

where the CP corrected interaction energy between the base (B ≡ AL) and the water nmer (W_n) is calculated according to:

$$\Delta E_{B-(W_n)}^{CP} = E_{BW_n}^{(BW_n)} - E_B^{(BW_n)} - E_{(W_n)}^{(BW_n)} + E_B^{(def)}(BW_n) \quad (2)$$

and where the CP corrected water-water interaction energy, corresponding to the formation energy of a water n-mer (in the presence of the base), at the geometry it adopts in the BW_n complex is defined as:

$$E_{(W_n)}^{CP} = E_{(W_n)}^{(BW_n)} - \sum_{i=1}^n E_{W_i}^{(BW_n)} + \sum_{i=1}^n E_{W_i}^{def}(BW_n) \quad (3)$$

The deformation energy of monomer X (X ≡ AL or W) is:

$$E_X^{(def)}(BW_n) = E_X^{(X)}(BW_n) - E_X^{(X)}(X)$$

Here the subscripts denote the molecular system and the superscripts indicate whether the calculation is done with the basis set of AL, the basis set of a water molecule (W), or the basis set of the entire system, (ALW_n). The contents of the baseline parentheses indicate whether the calculation is done at the optimized geometry of the entire system, (BW_n), or at the monomer optimized geometry (X).

3. Results and discussion

The molecule of AL is a structural analogue to molecule of hypoxanthine, only changes the position of N3 (in hypoxanthine) to N2 in AL, and the corresponding changes of the C-H atoms, Scheme 1. Unfortunately, two notations have been used for AL: that shown in Scheme 1, reported by different authors [49,52,66,67] and selected by us, and that followed by other authors [2,3,36,68] according to that reported in hypoxanthine molecule [67].

3.1 Tautomers

The importance of tautomerism for reconnaissance the molecules is crucial in biochemistry and pharmacological research. Thus, there are many studies on the tautomerism of nucleic bases [69,70] and their derivatives [64,71-74]. The tautomerism in AL is determined by the positions of two labile hydrogen atoms in the molecule. Theoretically, the tautomerism of AL was studied with semiempirical, *ab initio* and density functional (DFT) quantum chemical methods. The analysis in AL reveals that there are 14 possible neutral tautomers [75] and 9 with canonical structures [67]. Among these 14 possible, the eleven most stable were selected and shown in Table 1, with their relative energies calculated at different MP2 and DFT levels of computation. All these calculations confirm that the oxo tautomer AL-15 with one of the labile hydrogens attached to N1 and the other to N5 nitrogen atoms is the most stable, Fig. 1. The second one most stable is AL-25. The relative energy (ΔE) order of stability is the same as the relative free energy (ΔG) order, although it changes a little with the hydration.

At the M052X/6-31G(d,p) level the stability of AL-25 is similar to AL-17, in contrast to that found at the other levels with the stability of AL-17 almost twice lower than AL-25. The stability order of the remaining tautomers and their relative energies appear analogous by the different methods. Previous theoretical calculations of relative energies of AL tautomers, carried out at a lower level, led to similar predictions [67,75].

Table 1 also includes the percentage of population of every tautomer. A large difference appears between the most stable with 99.9% and the 2nd one AL-25 with 0.1%. Little change of this population is observed with the increase of the temperature. The thermal population of any of the hydroxyl tautomers should be so low that these forms would not be detectable. These features have been confirmed in the matrix-isolated IR study [67], although changes induced by UV irradiation decrease the populations of oxo tautomers and increase the populations of hydroxyl tautomers.

Table 2 collects the bond lengths of the eleven tautomers of AL at B3LYP/6-31G(d,p), MP2/6-31G(d,p) and MP2/6-311++G(2d,p) levels, while the angles are included in Table 3. A graphical representation of all these values is shown in Fig. 1-Sup. (Supplementary Material section). The present manuscript is focused in the most stable AL-15 tautomer, and the second one AL-25. Figs. 1 and 2 show only the values in these two most stable tautomers.

The bond distances and angles in the pyrimidine portion of the AL ring are generally similar to the values found for hypoxanthine [82]. The five-membered ring portion of the base

has two neighbouring nitrogen atoms at positions N1 and N2 forming a diazole ring. All the diazole ring systems examined tend to exhibit considerable distortions of bond angles when compared to imidazole rings. Specifically, AL exhibits an increase in the endocyclic angles N2-C3-C9 and C8-N1-N2, which is accompanied by a decrease in the N1-N2-C3 angle.

Comparing the bond lengths of AL-15 and AL-25 the largest differences are on C8-C9, N2-C3 and N1-C8 bonds, as it is expected, due to the proton transfer from N1 in AL-15 to N2 in AL-25 mainly affects the bonds of the diazole ring.

The bond length N1-N2 has the largest values in the AL-76C and AL-56C tautomers at both B3LYP and MP2 levels of computation. It is due to only in both tautomers there is not hydrogen bonded to N1 or N2 atoms. The nitrogen atom withdraws negative charge from its bonded hydrogen atom, and thus its negative charge increases in ca. 0.05-0.09 e and give rises at the MP2/6-311++G(2d,p) level to the values of -0.403 e in N1 of AL-15 (vs. -0.343 e in AL-25), and -0.353 e in N2 of AL-25 (vs. -0.309 e in AL-15), Fig. 2. In AL-76C and AL-56C tautomers the negative charge of both N1 and N2 atoms is low, which forces to N1 atom to withdraw negative charge to C8, and leading to a remarkable shortening of the N1-C8 bond and consequently a lengthening of N1-N2, avoiding therefore a higher distortion of the ring angles.

The N5-C6 bond shows the longest value in AL-56C which can be due to the shortening of the C4-N5 bond in all the enol tautomers plus the shortest C6-N7 and the larger negative charge on N5 due to its bonded hydrogen atom. The angles are similar to the experimental values, the largest differences are ca. 1°. All the enol forms have the C4-C5-C6 and C8-C9-C4 angles closer than in the ceto forms.

3.1.1. Charges

Fig. 2 shows the values of the natural atomic charges (NBO) in the isolated state of AL-15 and AL-25 at B3LYP and MP2 levels of calculation. In both tautomers the largest negative charge is located to N5 and O. The next site with very large negative value corresponding to N7 and it would be also one of the preferred binding site for electrophiles. The negative charge on N7 is slightly larger in AL-15 than in AL-25, and it also larger than in the enol forms. I.e. this site in AL-15 may bind with electrophiles more strongly than the corresponding site of AL-25, and than of the enol forms. This N7 site of neutral of AL

appears to be one of the main binding site with the enzyme xanthine oxidase. The N2 site may also participate in binding with the receptor, although its charge is twice lower.

The largest positive charge is on C4, while on H atoms it corresponds to H_(N5) and H_(N1) in AL-15 and H_(N5), and H_(N2) in AL-25. It is due to the values of the negative charges on N5, N1 and N2 are the largest.

3.2. Solid state simulation

3.2.1. Geometry optimisation

The molecular structure of AL in the solid state has been previously determined by X-ray diffraction [76], in which only the oxo form appears. The Raman and IR spectra have been also recorded for crystalline AL [36], indicating the presence of only the oxo tautomers in the solid state. Thus, in the present paper we have simulated by B3LYP the crystal unit cell of AL-15 reported by X-ray as a heptamer form, Fig. 3. Its final structure optimized is similar to the experimental [76]. The intermolecular H-bonds are between O \cdots H_C, O \cdots H_N, N7 \cdots H_N and N2 \cdots H_N. The strongest are between molecules A and G [O \cdots H_(N1)], molecules F and G [H_(N5) \cdots N7], molecules E and F [H_(N5) \cdots N2], and molecules D and E [H_(N1) \cdots N2]. The remaining H-bonds are weaker and they were not considered. The experimental structure indicates that solid AL consists of stacks of parallel H-bonded sheets of bases with partial overlap of the rings.

In the Table 1-Sup are collected the bond length of every molecule of the heptamer, labeled as A, B, C up to G, Fig. 3. The molecules B and F are in the centrum of the structure and each one appears intermolecular H-bonded with three neighbor molecules, while the remaining molecules are less H-bonded, only with two neighbor molecules. Therefore, the geometric parameters of these molecules B and F represent better the values reported in the crystal unit cell than the other molecules, and they were included in Tables 2 and 3, together with the experimental values [76]. Molecule F appears strongly intermolecular H-bonded with the neighbor molecules than molecule B.

Comparing these molecules B and F of the heptamer with the experimental values, the largest differences in the bond lengths are in the N1-C8 and C4-C9 bonds, 0.04 and 0.045 Å, respectively. Our heptamer form reproduces well the interactions involved in these bonds, but their values little differ of those in the isolated state, i.e. they are little affected by the crystal forces. The error in the calculated bond lengths can be attributed to the basis set used, due to

by MP2/6-311++G(2d,p) the values are closer to the experimental, but by MP2/6-31G(d,p) the values are similar to B3LYP/6-31G(d,p).

The differences in the calculated N1-N2, C4-N5, C6-N7 and N7-C8 bonds of the molecules B and F of the heptamer are small (ca. 0.01 Å) as compared with the experimental ones. The best reproduced are the C3-C9, N5-C6 and C4=O bonds with a difference < 0.005 Å. In general, the values calculated in the heptamer form are better agreement with the experimental ones than those determined in the isolated state.

3.2.2 Intermolecular hydrogen-bonds

The intermolecular H-bonds in the heptamer system mainly lead to a shortening of C4-N5 (ca. 0.02 Å) and N5-C6 (0.01 Å) bonds due to the small lengthening (0.01 Å) of C4=O and N5-H bonds. The N5-C4-C9 angle is opening (1°) while C4-N5-C6 is closing (1°). The intermolecular H-bond through N7 leads to small lengthening (0.008 Å) of C6-N7 bond, while N7-C8 remains unaffected. The C6-N7-C8 angle is opening 1° and N7-C8-C9 is closing 2°.

The effect of the H-bond through N1-H is small in the bond lengths, only a slight shortening of N1-C8 (0.005 Å), while C8-C9 and N1-N2 remains almost unaffected. In the bond angles a closing of N2-N1-C (2°) and opening of N1-C8-C9 (1°) is observed. Also the effect of the H-bonds through N2 is very small on the bond lengths, only a slight lengthening of N2-C3 of 0.004 Å, but in the angles is larger with an opening of N1-N2-C3 (1°) and closing of N2-C3-C9 (4°).

In general, although intermolecular forces in solid AL exist, its global network features are successfully reproduced by the theoretical calculations done considering full geometry optimization of the heptamer form.

3.3. Hydration

The hydration of DNA takes place through both hydrophobic and hydrophilic sites providing a general stability to the helix. In addition, studies in uracils and thymines show [77] the significant function of water in protecting our genetic code from photodamage. Other studies also reveal the importance of the hydration in the DNA. In the surface of the DNA there is a shell of tightly bound water molecules with considerably distinct properties from those of bulk water [78]. Thus, we are interested in this shell bound water molecules, i.e. in the first and second hydration shells. Several authors have analysed this microhydration in

uracil [58], halouracils [64,65,79,80], and in thymine [63] with different number of explicit water molecules. In these clusters those with the water molecules bonded among them are energetically preferred over those with water molecules distributed around the nucleobase [63,80,64].

Other experimental [83,84] and theoretical [85] studies have been reported to interpret the effect of hydration on the photophysics of nucleic acid bases. However, in despite of these studies, many features, especially on the effect of hydration on the geometrical parameters and vibrational spectra have not been analysed in detail yet, which is one of the task of the present manuscript with AL.

Electronic spectra and others techniques have been used in solution of AL at different pH, showing that near to the physiological neutral pH, AL appears only as tautomeric oxo forms [2]. However, by ultraviolet resonance Raman spectra has been found that AL exists as a mixture of the keto AL-15 and AL-25 tautomers in solution [1]. Also by ^{13}C NMR spectroscopic measurements have been also suggested the coexistence of AL-15 and AL-25 tautomers in DMSO solution [81]. Due to these doubts in the present work the first and second hydration shells of AL-15 and AL-25 tautomers were determined at B3LYP level for the first time. These clusters appear more stable than the AL-16C and AL-16t enol forms.

3.3.1. Hydration of the different tautomers

All the tautomers considered in the present work on AL were hydrated with different number of water molecules, Figs. 4-9 and Figs. 2-Sup to 7-Sup. However, the attention was focused in AL-15, which is the most stable tautomer, and AL-25, the second most stable one. Only in these tautomers the hydration was carried out up to the first and second hydration shells, Figs. 7 and 9.

Monohydration

There are only four possible regions in the structure of all the tautomers in which the water molecules can be H-bonded, labeled as A, B, C and D (Scheme 1). These water molecules are denoted as W_A , W_B , etc, in reference to its position. The monohydration of AL-15 and AL-25 is shown in Fig. 4, while in the remaining tautomers it is collected in Fig. 2-Sup. In the bottom of each cluster appears the total energy+ZPE corresponding to B3LYP, and in parentheses the value at MP2 level. The energy of the most stable monohydrated

cluster is shown in bold type. Comparing the energies of these structures, it is observed that the stability order of the tautomers is not affected by the monohydration.

In AL-15 and AL-25 the calculated intermolecular H-bonds correspond to B3LYP/6-31G(d,p) and MP2/6-31G(d,p) (in parenthesis) levels. According to the position of the water molecule, the stability order is: **D>A>B>C** in AL-15 and **D>B>A>C** in AL-25 at both B3LYP and MP2 levels of computation. In both tautomers the position D of the water molecule leads to the most stable monohydrated form, while the least stable appears in position C. It can be explained because in position D the two intermolecular H-bonds are $O \cdots H_w$ and $NH \cdots O_w$, that are stronger than $NH \cdots O_w$ and $N \cdots H_w$ in positions A and B. The least stable in position C is due to the weak $CH \cdots O_w$ H-bond. In general, this feature has been also observed in the other tautomers, Fig. 2-Sup.

One water molecule slightly modifies the bond lengths and angles of the AL-15 structure, as compared to the isolated state. The largest differences at the MP2 level are in the bond lengths N2-C3 (+0.033 Å), C8-C9 (+0.028 Å), N1-N2 (-0.026 Å), C6-N7 (-0.023 Å), C3-C9 (+0.021 Å), as well as in several C-H and N-H bonds, such as C6-H +0.109 Å (in the isolated molecule is 1.083 Å), and in N5-H (+0.025 Å). At B3LYP level the differences are small, only in C=O (+0.015 Å) and C9-C4 (0.034 Å) the values are significant. The shifts in the angles are small, only N2-C3-C9, N1-N2-C3 and C8-N1-N2, at MP2 level have a difference about 7°-9° related to the isolated state.

In AL-25 few shifts are observed related to the isolated state. At the MP2 level are in C=O (0.013 Å), C4-C5 (-0.011 Å), and N5-H (0.013 Å), while at B3LYP level only are significant in C=O (0.015 Å), C4-N5 (-0.014 Å), and N5-H (+0.018 Å).

The calculated total energies in the monohydrated AL-15 and AL-25 are collected in Table 1-Sup. $\Delta E_{ALO15W1}^{ALO15W1}$, and $\Delta E_{ALO25W1}^{ALO25W1}$, together with the energies of AL-15 and AL-25 in the complex $\Delta E_{ALO15/ALO15W1-W1}^{ALO15W1}$ and $\Delta E_{ALO25/ALO25W1-W1}^{ALO25W1}$, and the energy of the water molecule in every complex $\Delta E_{W1/ALO15W1-ALO15}^{ALO15W1}$ and $\Delta E_{W1/ALO25W1-ALO25}^{ALO25W1}$ at B3LYP/6-31G(d,p) and MP2/6-311++G(2d,p) levels. The maximum CP-corrected complex formation energy (ΔE_{ALO-15}^{CP}) at the B3LYP and MP2 levels corresponds to D position, the most stable form, Fig. 5. The CP-corrected energies of AL-25 were very similar to those determined in AL-15, with the maximum value in position D.

The maximum deformation energy of AL-15, E_{ALO-15}^{def} , and water molecule, E_{W1}^{def} , at B3LYP and MP2 levels are also in D position, Fig. 5B. Similar result was obtained in AL-25, Fig. 5C.

AL-(H₂O)_n, n=2. Dihydrated forms

The second watermolecule was added to the monohydrated form in position D, following the MSM methodology, as described in Section 2.1. Also four positions appear for this 2nd water molecule. In AL-15 the stability order by MP2 is D-D >D-A >D-B >D-C, which is the same that by B3LYP, with the exception of the changes in C and A, Fig. 6. Weak intermolecular H-bonds were omitted in this Fig. 6. The best cluster is D-D due to it has the strongest H-bonds. Also in AL-25, the best cluster is D-D, Fig. 5-Sup.

With the dihydration, the ring is slightly modified, the largest differences observed at MP2 level in bond lengths were in C6-H (0.109 Å), N2-C3 (0.032 Å), C8-C9 (0.028 Å), N1-N2 (-0.026 Å), C6-N7 (-0.022 Å), and in C3-C9 (0.021 Å). In the angles the largest differences are in C3-N2-N1 which closeners 9.6⁰, as well as in C8-C9-C3 (-5.0⁰), and opener in N2-N1-C8 (8.3⁰), and C9-C3-N2 (7.0⁰).

AL-15 +3H₂O: The trihydration of AL-15 is shown in Fig. 6, while in AL-25 appears in Fig. 5-Sup. The stability order by MP2 is D-D-A > D-D-B > D-D-D >D-D-C. The most stable is D-D-A by both, MP2 and B3LYP methods.

The largest shifts in bond length appear in N2-C3 (0.034 Å), C9-C4 (0.030 Å), C8-C9 (0.029 Å), N1-N2 (-0.028 Å), C6-N7 (-0.021 Å) and C3-C9 (0.020 Å). These values are similar to those obtained in the mono- and di-hydrated forms. Each molecule that it is added its effect on the molecule structure is lower. The bond C6-H increases 0.109 Å, and C3-H and N5-H also increases 0.077 and 0.035 Å, respectively. The angles with largest shifts are in C3-N2-N1 (-8.6⁰), N2-N1-C8 (7.8⁰), C9-C3-N2 (6.9⁰) and C8-C9-C3 (-5.4⁰).

3.3.2 AL-15 +nH₂O, n= 4-12. The first hydration shell

Following the same methodology the 4th to 12th water molecules were added, Fig. 4-Sup. The first hydration shell of AL-15 is estimate to be completed with 12 water molecules, Fig. 7. This number of water molecules is in accordance to that found in related molecules. Thus, the number of water molecules in the first hydration shell of nucleotides has been estimated by experimental measurements between the ranges of 12 and 20 per nucleotide pair [86,87].

In studies on D4T and thymidine nucleosides this hydration shell is completed with 13 water molecules [59], while in 5-FU and BrU is with 10 water molecules [80,64].

Structures with a clustering and distributing the water molecules were determined. Although clustered structures were more stable than structures with the water molecules distributed around AL, we preferred this last one, because they show larger number of water-AL interactions, and it represents better the hydration pattern of AL.

In the first hydration shell of this distributed cluster structure the longest difference in the bond lengths, as compared to the isolated state, appears in C=O (0.037Å) and in C4-N5 (0.035 Å). The angles practically do not change. The effect of the first hydration shell on the bond angles is small, with changes in general lower than 2°.

The CP corrected formation and interaction base-water (B-W) energies in the cluster of AL-15+(H₂O)₁₀ are shown in Table 4, together with the deformation energies. These values appear lower than those found in the cluster of 5-fluorouracil (5-FU) with 10 water molecules [64]. It can be explained due to in tautomer T1 of 5-FU the water molecules appear out of the molecular ring plane of 5-FU, while in AL they are in the ring plane. This feature deforms remarkably more the ring ($E_B^{(def)}$) in 5-FU (0.183 a.u.) than in AL (0.008 a.u.), as well as the deformation of the water molecules $\Sigma E_{wi}^{(def)}$ in 5-FU is 0.040 a.u. while in AL is 0.006 a.u. The water molecules appear closer among them in 5-FU than in AL, with remarkably higher ΔE_{wn}^{CP} in 5-FU is -204.756 kcal/mol while in AL is only -51.180 kcal/mol. The interaction energies ΔE_{B-Wn}^{CP} is -78.313 a.u in 5-FU while in AL it is slightly lower -74.878 a.u.

3.3.3. The second shell of hydration

The optimized clusters at the B3LYP/6-31G(d,p) level with 20, 25 and 30 water molecules is shown in Fig. 7. In last one with (H₂O)₃₀ there is a second shell of water molecules around AL-15. Two views (general and lateral) of this optimized cluster with the second hydration shell is plotted in Fig. 7. The C=O and N1-N2 bond lengths remain almost constants from 15 to 30 water molecules, Fig. 8. In general in these three clusters the bond lengths and angles are very similar, i.e the second hydration shell little change the molecular structure of AL-15. The same feature is observed in the progress of the hydration of AL-25 up to 30 water molecules, Fig. 9.

In a detailed analysis of the change in the C=O and N1-N2 bond length with the progress of the hydration is noted that with more than 4 water molecules the N1-N2 bond little change

its value. However, in the C=O bond, the values change noticeable up to the 1st hydration shell.

3.3.4. PCM

The effect of the hydration with the continuum PCM model was also determined. Figs.1 and 2 show the change in the bond lengths and angles in AL-15 and AL-25 at the B3LYP level, as compared with those obtained in the cluster with (H₂O)₃₀ and with those in the isolated state. In general, the changes observed in the simulation of the hydration with explicit water molecules (EM) are remarkably larger than with the PCM model. I.e. EM model reproduces better the hydration than with PCM.

Fig 2 lists the NBO charges in the hydrated form of AL-15 and AL-25 with 30 water molecules and with PCM. Usually the charges in the hydrated molecule and PCM are larger than with the B3LYP method.

The main effect of PCM in the molecular structure of AL-15 are in the C4-N5 and N5-H bonds lengths with changes of 0.017 and -0.017 Å, respectively, as compared to the isolated state, and in the C=O, C3-N2, N2-N1 and N1-H bonds, between 0.012 to 0.015 Å. In AL-25 noticeable differences were only observed in N5-H (0.028 Å) and N2-H (0.018 Å).

3.3.5. Dipole moments

The dipole moments in the most stable clusters of AL-15 at B3LYP and MP2 levels is shown in Table 2-Sup. A very large fluctuation is observed in its value with the progress of the hydration. Thus, by MP2 begin at 4.462 D in the isolated, 3.312 D in the monohydrated form, 4.268 D in cluster with (H₂O)₄, 3.199 with (H₂O)₆ and it increases up to 6.479 D in the cluster with (H₂O)₉. By B3LYP the fluctuation is remarkably larger, from 1.421 D in the cluster with (H₂O)₈, up to 13.225 D in the cluster with (H₂O)₃₀. This last value is the largest and it can be interpreted by the remarkable enhanced of the atomic charges, Fig. 3.

4. Resume and Conclusions

A detailed study of the molecular structure of the different tautomers of AL was carried out. The effect in its structure of the intermolecular H-bonds of the solid state, and the water molecules was analyzed

(1) At all the levels of computation, the most stable tautomer was AL-15 with the large population of 99.8%. The next one was AL-25 with about 1% population.

- (2) In the isolated state the largest negative charges are on O and N5, at all the levels of calculation.
- (3) The crystal unit cell of the solid state of AL-15 was simulated by means of a heptamer form, which is the tautomer reported in the solid state. The H-bonds between the $O\cdots H_{N1}$, $N7\cdots H_{N5}$ and $N2\cdots H_{N7}$ are the strongest. Our geometry parameters calculated in this heptamer form agrees well with the experimental values reported by X-ray.
- (4) The monohydration leads to a moderate change in the molecular structure and energy, but it is not enough to change the stability order of the tautomers.
- (5) The first and second hydration shell of AL was 13 and 30 explicit water molecules was determined for the first time at the B3LYP level. More than 150 hydrated clusters with AL were optimized.
- (6) Four possible positions for the water molecules around the base appear in AL-15 and AL-25. The D position is the most stable one. In AL-15 and AL-25 the maximum CP-corrected complex formation energy (ΔE_{ALO-15}^{CP}) is in D position, at all the levels of calculation, and this is the most stable monohydrated. It is due to the intermolecular H-bonds are the strongest among the other clusters. The angles are little affected by the hydration.
- (7) The hydration produces a lengthening of the N-H and C=O bonds and thus a shortening of the neighboring bond lengths, and an opening of the ipso angles involved in H-bonds.
- (8) The first hydration shell was reached with about 12 water molecules, in accordance with that obtained in related molecules. With the progress of the hydration, the variations of the N1-N2 and C=O bond lengths are reduced and they are almost constant with more than 12 water molecules. Similar behavior was obtained in AL-25. The effect of the hydration on the angles is in general very small, with shifts lower than 2°.
- (9) The strongest effect with the hydration is observed with the introduction of the first water molecule (monohydration). Every additional water molecule introduced in the cluster the effect is reduced up to the first hydration shell. The molecular structure remains almost unchanged with the addition of the 2nd hydration shell.
- (10) The PCM little change the molecular structure of AL. The EM model reproduces better the hydration than the continuum model.

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Table 1. Relative energy ($\Delta E + ZPE$, in $\text{kcal}\cdot\text{mol}^{-1}$) of the eleven most stable tautomers of allopurinol at different levels of computation.

Tautomers	Notation [48]	B3LYP/6-31G(d,p)	(ΔG) B3LYP/6-31G(d,p)	MP2/6-311++G(2d,p)	M052X/6-31G(d,p)	M06L/6-31G(d,p)	MP2/cc-pvtz	% 273.15K
1,5-ceto (AL-15)	1H, 9H-AL	0 ^a	0 ^b	0 ^c	0 ^d	0 ^e	0 ^f	99.9
2,5-ceto (AL-25)	1H, 8H-AL	3.694	3.717	2.709	3.845	3.620	2.716	0.1
1-enol cis (AL-16C)	9H cis enol-AL	5.370	5.436	4.759	3.834	6.291	4.255	<0.005
1-enol trans (AL-16t)	9H trans enol-AL	10.98	10.798	10.065	9.547	6.291	9.393	<0.005
2-enol cis (AL-26C)	8H cis enol-AL	12.600	12.671	10.048	11.600	13.126	9.6303	<0.0005
2,7-ceto (AL-27)	3H, 8H-AL	12.901	12.764	12.789	13.308	13.225	13.094	<0.005
1,7-ceto (AL-17)	3H, 9H-AL	17.698	17.423	18.220	18.401	18.121	18.540	<0.005
2-enol (AL-26t)	8H trans enol-AL	19.344	19.485	16.313	18.671	19.790	15.698	<0.005
7-enol cis (AL-76C)	3H cis enol-AL	25.162	25.188	23.802	25.229	25.638	24.452	<0.005
1,2-ceto (AL-12)	8H, 9H-AL	34.112	33.815	50.528	35.795	34.403	39.829	<0.005
5-enol cis (AL-56C)	1H cis enol-AL	40.004	39.619	39.047	40.867	40.934	38.871	<0.005

^a-305646.695 $\text{kcal}\cdot\text{mol}^{-1}$

^b-305666.438 $\text{kcal}\cdot\text{mol}^{-1}$ (ΔG , Gibbsenergy)

^c-305035.56 $\text{kcal}\cdot\text{mol}^{-1}$

^d-305614.829 $\text{kcal}\cdot\text{mol}^{-1}$

^e-305620.917 $\text{kcal}\cdot\text{mol}^{-1}$

^f-305151.458 $\text{kcal}\cdot\text{mol}^{-1}$

Table 2. Optimized bond lengths in Å of the eleven most stable tautomers of allopurinol at the B3LYP/6-31G(d,p) (normal style), MP2/6-31G(d,p) (in parenthesis) and MP2/6-311++G(2d,p) (in italic) levels, together with the calculated values in molecules B and F of the heptamer form and the experimental ones [76].

Bonds	AL-15	AL-25	AL-16C	AL-16t	AL-26C	AL-27	AL-17	AL-26t	AL-76C	AL-12	AL-56C	Heptamer AL-15		Experimental
												B	F	
N1-N2	1.363 (1.351) <i>1.380</i>	1.356 (1.343) <i>1.346</i>	1.365 (1.352)	1.362 (1.350)	1.352 (1.337)	1.365 (1.352)	1.375 (1.363)	1.349 (1.334)	1.392 (1.384)	1.377 (1.360)	1.383 (1.378)	1.366	1.363	1.374
N2-C3	1.327 (1.343) <i>1.310</i>	1.352 (1.358) <i>1.359</i>	1.321 (1.339)	1.321 (1.339)	1.350 (1.361)	1.351 (1.355)	1.324 (1.337)	1.351 (1.362)	1.327 (1.347)	1.354 (1.350)	1.327 (1.347)	1.331	1.327	1.325.
C3-C9	1.417 (1.410) <i>1.390</i>	1.385 (1.387) <i>1.387</i>	1.425 (1.417)	1.428 (1.419)	1.392 (1.393)	1.388 (1.391)	1.421 (1.416)	1.395 (1.397)	1.422 (1.411)	1.377 ^o (1.383)	1.418 (1.405)	1.416	1.411	1.414
C4-N5	1.431 (1.419) <i>1.400</i>	1.414 (1.405) <i>1.407</i>	1.328 (1.320)	1.323 (1.316)	1.313 (1.309)	1.412 (1.419)	1.433 (1.429)	1.309 (1.306)	1.360 (1.350)	1.398 (1.395)	1.371 (1.358)	1.426	1.416	1.399
N5-C6	1.367 (1.369) <i>1.380</i>	1.380 (1.380) <i>1.381</i>	1.352 (1.359)	1.349 (1.357)	1.372 (1.374)	1.291 (1.297)	1.284 (1.291)	1.368 (1.370)	1.315 (1.322).	1.313 (1.320)	1.400 (1.395)	1.363	1.357	1.364
C6-N7	1.301 (1.301) <i>1.330</i>	1.292 (1.295) <i>1.298</i>	1.328 (1.326)	1.331 (1.328)	1.312 (1.316)	1.374 (1.367)	1.387 (1.378)	1.314 (1.314)	1.348 (1.341)	1.374 (1.369)	1.287 (1.295)	1.307	1.309	1.292
N7-C8	1.366 (1.368) <i>1.360</i>	1.380. (1.378) <i>1.382</i>	1.344 (1.345)	1.341 (1.342)	1.362 (1.357)	1.378 (1.374).	1.371 (1.370)	1.359 (1.354)	1.379 (1.369)	1.316 (1.321)	1.373 (1.366)	1.368	1.368	1.379
C8-C9	1.402 (1.398) <i>1.370</i>	1.429 (1.417) <i>1.418</i>	1.412 (1.345)	1.413 (1.407)	1.435 (1.422)	1.414 (1.403)	1.388 (1.385)	1.436 (1.424)	1.435 (1.428)	1.427 (1.416)	1.457 (1.450)	1.402	1.405	1.395
N1-C8	1.353 (1.355) <i>1.373</i>	1.336 (1.350) <i>1.351</i>	1.357 (1.359)	1.358 (1.360)	1.343 (1.359)	1.327 (1.340).	1.351 (1.353)	1.344 (1.360)	1.313 (1.325)	1.370 (1.368)	1.320 (1.333)	1.348	1.351	1.388
C4-O	1.219 (1.221) <i>1.230</i>	1.221 (1.223) <i>1.228</i>	1.340 (1.345)	1.347 (1.351)	1.343 (1.348)	1.220 (1.221)	1.218 (1.219)	1.351 (1.355)	1.336 (1.337)	1.226 (1.228)	1.342 (1.343)	1.224	1.228	1.228
C4-C9	1.442 (1.442) <i>1.420</i>	1.448 (1.446) <i>1.448</i>	1.405 (1.404)	1.411 (1.410)	1.415 (1.410)	1.467 (1.463)	1.461 (1.458)	1.423 (1.416)	1.385 (1.387)	1.476 (1.468)	1.368 (1.372)	1.440	1.441	1.395

Table 3. Optimized bond angles(in degrees) of of the eleven most stable tautomers of allopurinol at the B3LYP/6-31G(d,p) (normal style), MP2/6-31G(d,p) (in parenthesis), MP2/6-311++G(2d,p)(in italic) levels, together with the calculated values in molecules B and F of the heptamer form and the experimental ones[76].

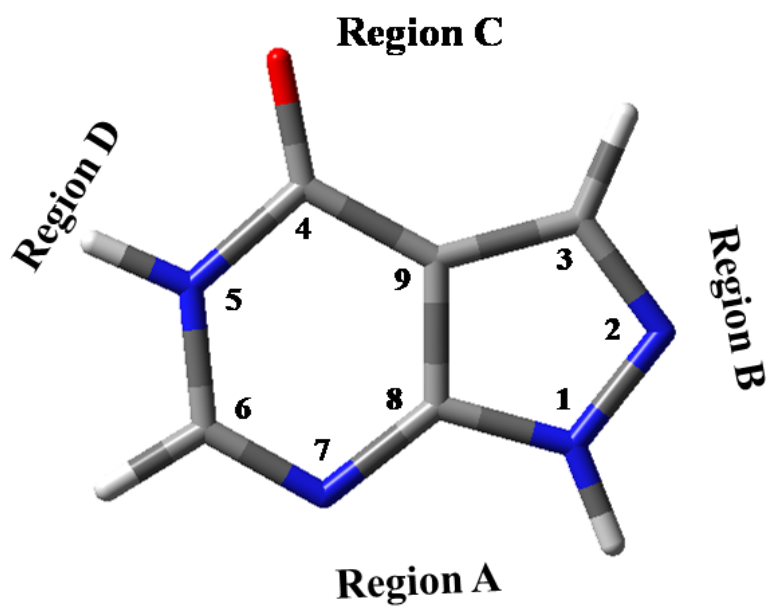
Angles	AL-15	AL-25	AL-16C	AL-16t	AL-26C	AL-27	AL-17	AL-26t	AL-76C	AL-12	AL-56C	Heptamer AL-15		Experim ental
												B	F	
N1-N2-C3	105.3 (105.4) <i>114.0</i>	115.1 (115.5) <i>115.7</i>	102.0 (106.2)	106.6 (106.1)	115.9 (116.4)	114.5 (114.9)	105.0 (105.2)	115.9 (116.3)	110.4 (110.4)	109.1 (109.6)	110.7 (110.6)	105.9	106.6	106.4
N2-C3-C9	111.3 (110.6) <i>103.9</i>	105.1 (104.5) <i>104.5</i>	110.9 (110.2)	111.1 (110.4)	104.6 (103.9)	106.0 (105.4)	112.1 (111.5)	104.8 (104.1)	109.9 (109.4)	108.6 (107.9)	109.3 (108.8)	106.9	110.6	110.4
C3-C9-C8	104.8 (105.2) <i>110.3</i>	105.0 (105.5) <i>105.5</i>	104.9 (105.3)	104.6 (105.0)	105.1 (105.7)	103.5 (104.0)	103.7 (104.1)	104.8 (104.8)	100.9 (101.7)	107.2 (107.1)	102.3 (101.3)	105.1	104.7	104.6
C9-C8-N1	106.0 (106.0) <i>107.2</i>	111.7 (111.5) <i>111.9</i>	105.6 (105.8)	105.8 (105.9)	111.4 (111.3)	114.1 (113.8)	107.8 (107.7)	111.5 (111.4)	113.4 (113.2)	106.6 (106.6)	106.1 (110.3)	106.8	107.0	107.8
C9-C4-N5	109.6 (109.7) <i>111.4</i>	109.6 (109.6) <i>109.3</i>	121.1 (121.1)	120.8 (120.8)	120.9 (121.0)	114.4 (114.5)	114.4 (114.6)	120.6 (120.7)	122.1 (122.3)	113.6 (113.4)	117.0 (117.0)	110.4	110.5	111.1
C4-N5-C6	125.8 (126.2) <i>125.2</i>	125.3 (125.6) <i>125.8</i>	117.9 (118.1)	117.9 (118.2)	117.6 (117.8)	120.7 (120.4)	121.1 (120.8)	117.8 (118.0)	118.6 (118.6)	120.1 (120.1)	121.8 (122.3)	125.0	125.4	125.6
N5-C6-N7	125.0 (124.9) <i>123.3</i>	125.9 (125.7) <i>125.8</i>	128.1 (127.6)	128.5 (128.0)	128.7 (128.0)	127.1 (127.1)	126.4 (126.5)	129.0 (128.4)	124.2 (123.8)	131.0 (131.0)	124.3 (124.0)	125.5	125.0	125.0
C6-N7-C8	112.3 (111.9) <i>112.2</i>	113.7 (113.1) <i>112.8</i>	112.2 (112.2)	112.0 (112.0)	113.6 (113.4)	117.3 (117.0)	116.1 (115.9)	113.4 (113.1)	119.4 (119.6)	110.4 (109.9)	115.8 (115.4)	112.5	113.3	112.4
N7-C8-C9	128.0 (128.4) <i>128.6--</i>	124.5 (125.3) <i>125.3</i>	126.5 (126.6)	126.4 (126.5)	123.4 (124.1)	119.1 (119.8)	121.6 (121.9)	123.4 (124.1)	118.5 (118.9)	126.9 (127.3)	123.1 (123.8)	127.3	126.3	127.3
C8-N1-N2	112.7 (112.7) <i>104.7</i>	103.1 (101.0) <i>102.6</i>	112.5 (112.1)	112.6 (112.1)	102.9 (102.7)	101.9 (101.9)	111.4 (111.5)	103.0 (102.8)	105.4 (105.2)	108.5 (108.7)	107.0 (106.5)	110.9	111.1	110.7
C8-C9-C4	119.2 (119.0) <i>119.3</i>	120.9 (120.7) <i>121.0</i>	114.3 (114.3)	114.4 (114.5)	115.8 (115.7)	121.4 (121.2)	119.2 (121.9)	115.8 (115.8)	117.2 (116.8)	118.0 (118.3)	117.9 (117.5)	119.3	119.5	118.6

Table 4. The formation, interaction base - water (B-W) and deformation energies of the most stable cluster of AL-15 with 10 waters molecules at B3LYP/6-31G(d,p) level.

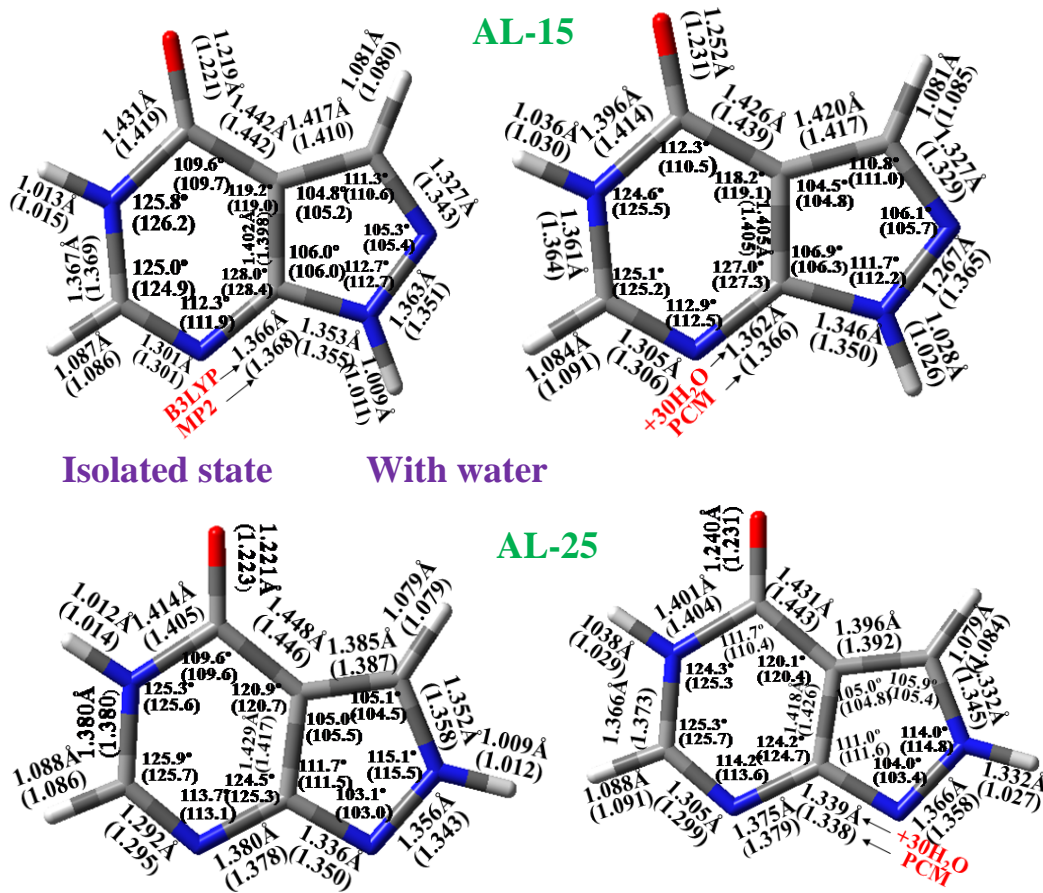
$E_{(BW_n)}^{(BW_n)}$ (BWn) in a.u.	-1251.578	$\Sigma E_{W_i}^{(def)}$ (BWn) (en a.u.)	0.00568
$E_{(B)}^{(BW_n)}$ (BWn) in a.u.	-487.171	$\Delta E_{B-W_n}^{CP}$ (en kcal/mol)	-74.878
$E_{(W_n)}^{(BW_n)}$ (BWn) in a.u.	-764.279	$\Delta E_{W_n}^{CP}$ (en kcal/mol)	-51.180
$E_B^{(def)}$ (BWn) in a.u.	0.00819	$\Delta E_{B(W_n)}^{CP}$ (en kcal/mol)	-126.058

Legend of figures

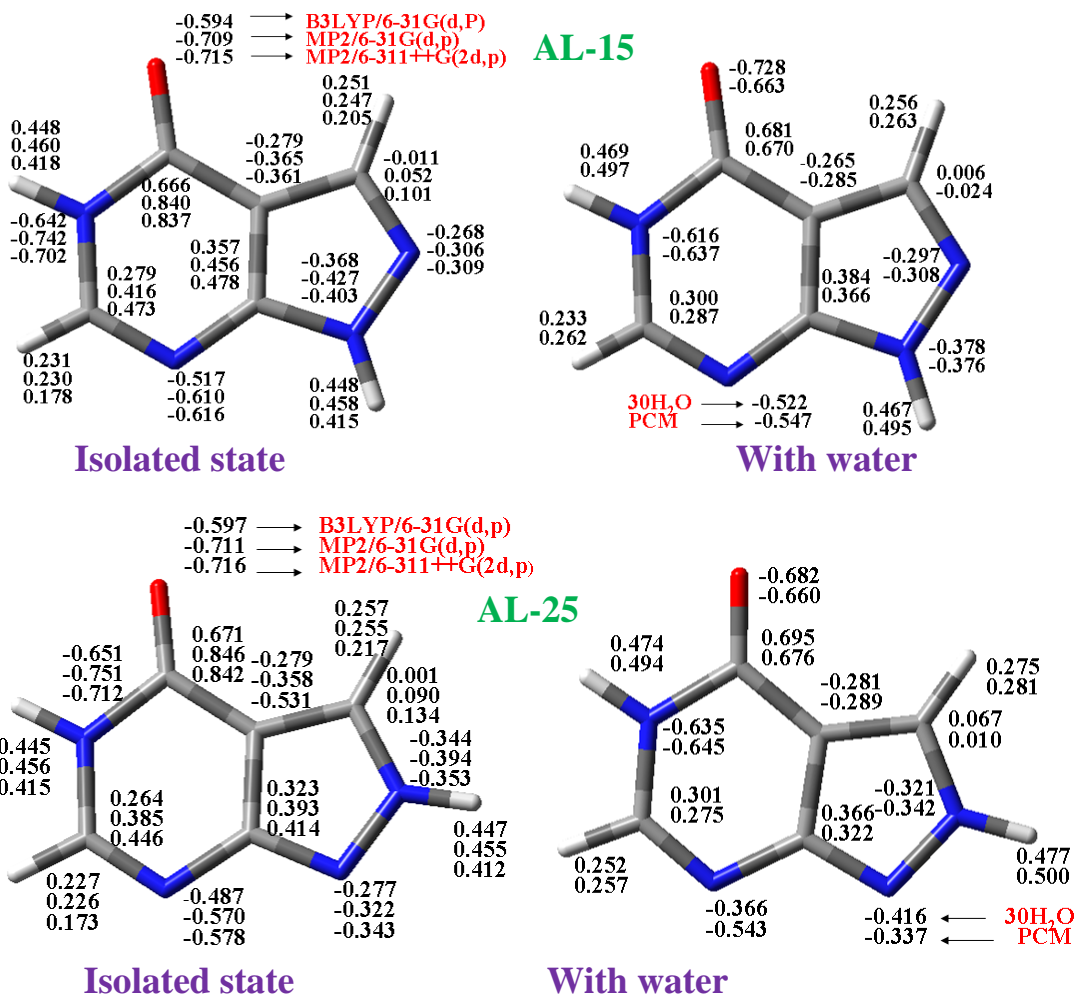
- Figure 1.** Optimized bonds lengths and angles in the isolated state and in hydrated form of AL-15 and AL-25 at B3LYP/6-31G(d,p) and MP2/6-31G(d,p) (in parentheses) levels.
- Figure 2.** Optimized Natural atomic charges (NBO) of AL-15 and AL-25 at B3LYP/6-31G(d,p) and MP2/6-31G(d,p) and MP2/6-311++G(2d,p) levels, in the isolated state and in hydrated form.
- Figure 3.** Simulated crystal unit cell of AL-15 by a heptamer form at the B3LYP/6-31G(d,p) level.
- Figure 4.** Optimized monohydrated structures of AL-15 and AL-25 tautomers in the four possible positions of one water molecule. The calculated intermolecular H-bonds correspond to B3LYP/6-31G(d,p) and MP2/6-31G(d,p) (in parentheses) levels. In the bottom of each cluster appears the total energy + ZPE corresponding to B3LYP/6-31G(d,p) in a.u. and to MP2 (in parentheses). The monohydrated cluster with minimum energy is shown in bold type.
- Figure 5.** Calculated relative energies at the B3LYP and MP2 levels in the monohydration of AL with the water molecule in the four possible positions: (i) $\Delta E_{ALO-15W1}^{CP}$, $\Delta E_{ALO-25W1}^{CP}$; (ii) ΔE_{W1}^{def} , ΔE_{ALO-15}^{def} , ΔE_{Tot}^{def} , and (iii) ΔE_{W1}^{def} , ΔE_{ALO-25}^{def} , ΔE_{Tot}^{def} .
- Figure 6.** Optimized dehydrated and trihydrated clusters of AL-15 in the four possible positions of one water molecule. The calculated intermolecular H-bonds correspond to B3LYP/6-31G(d,p) and MP2/6-31G(d,p) (in parentheses) levels. In the bottom of each cluster appears the total energy + ZPE corresponding to B3LYP/6-31G(d,p) in a.u. and to MP2 (in parentheses). The cluster with minimum energy is shown in bold type.
- Figure 7.** Optimized clusters in the first hydration shell (+ 11 H₂O) and second hydration shell (+ 30 H₂O) of AL-15 at the B3LYP/6-31G(d,p) level.
- Figure 8.** Variation of the N1-N2 and C2=O bond lengths in AL-15 with the hydration up to 30 water molecules.
- Figure 9.** Optimized clusters in the first hydration shell (+ 11 H₂O) and second hydration shell (+ 30 H₂O) of AL-25 at the B3LYP/6-31G(d,p) level.



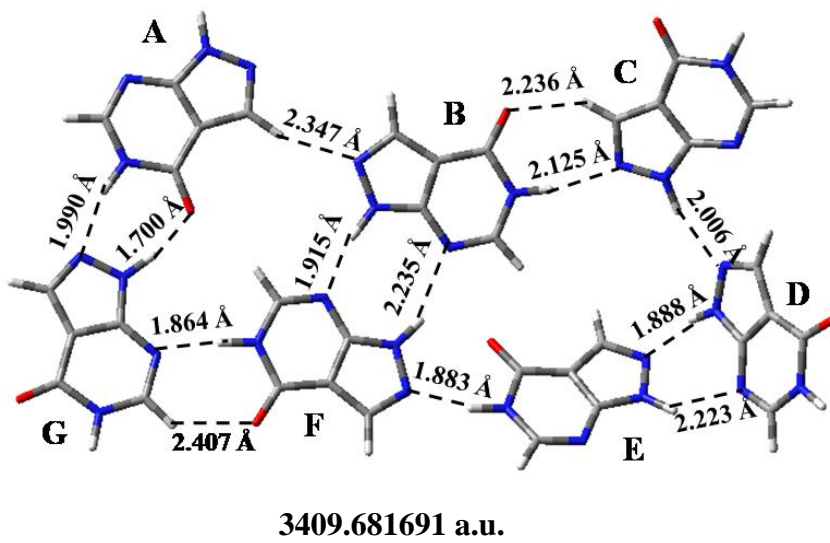
Scheme 1. Labelling of the atoms in allopurinol (tautomer AL-15), according to ref. [67], with their hydration regions.



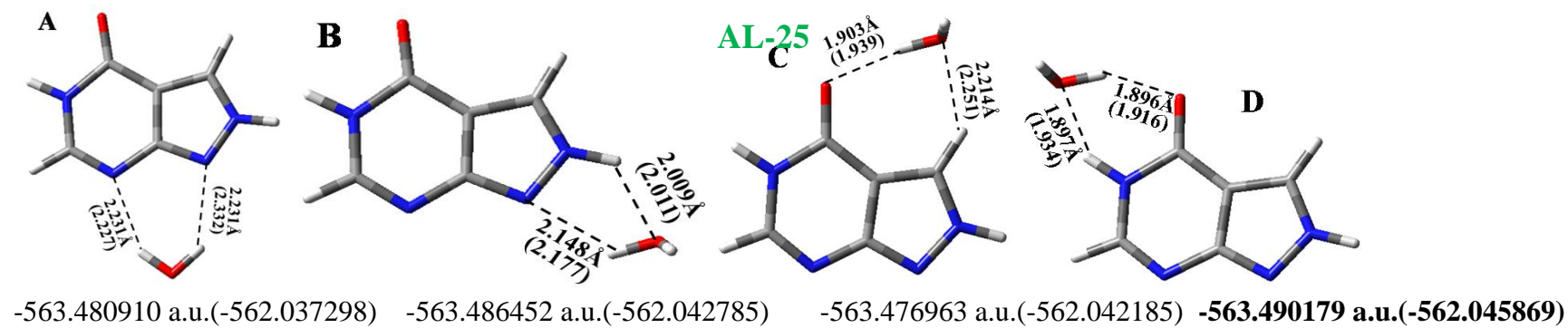
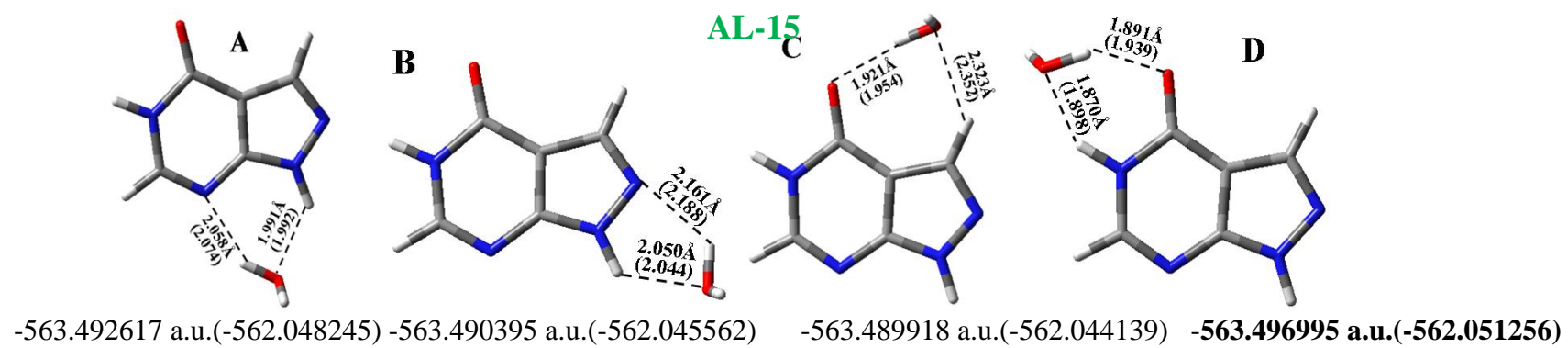
(Fig. 1)



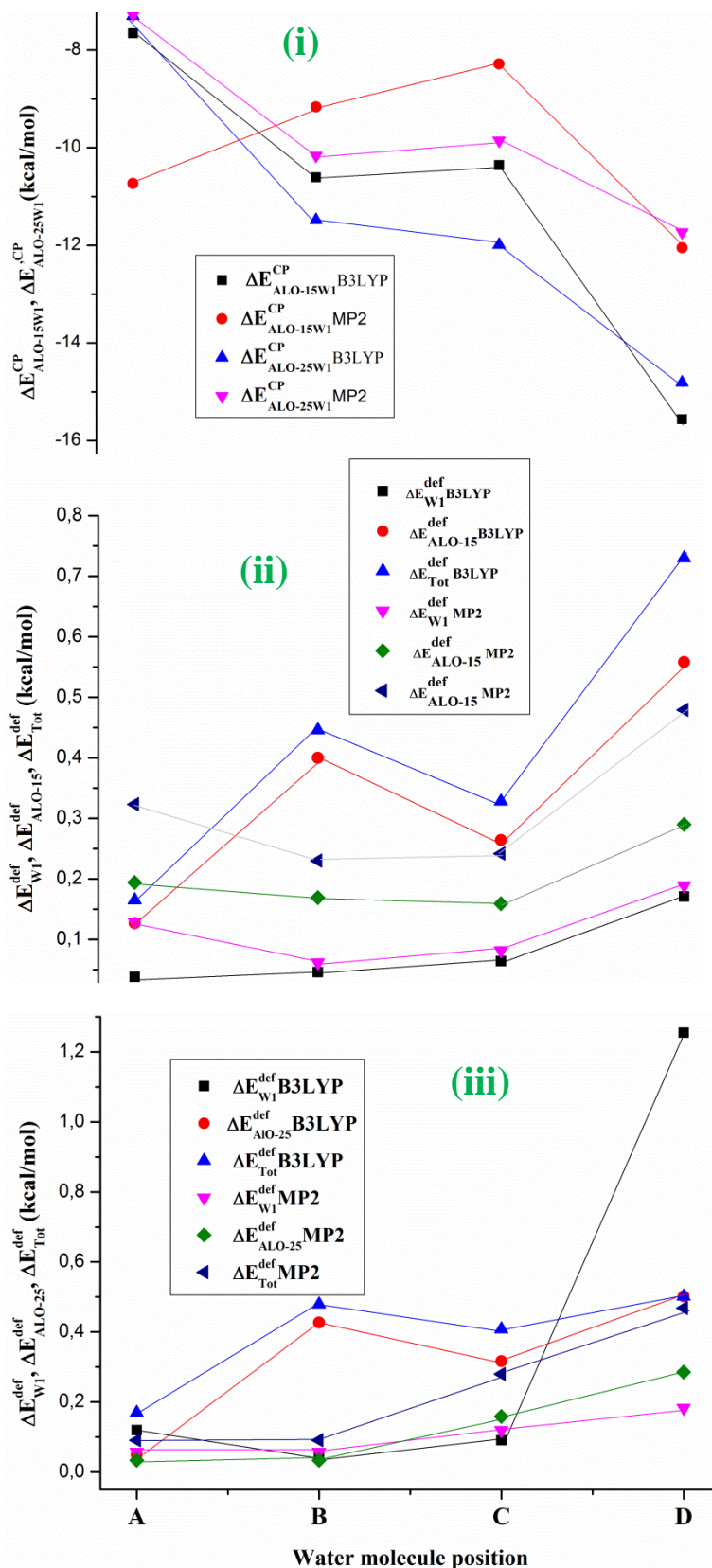
(Fig. 2)



(Fig. 3)

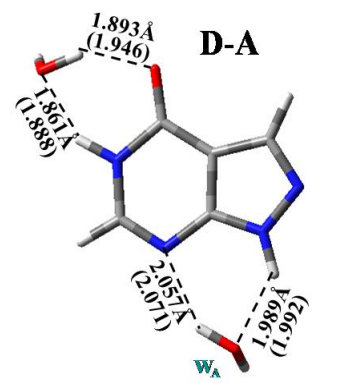


(Fig. 4)

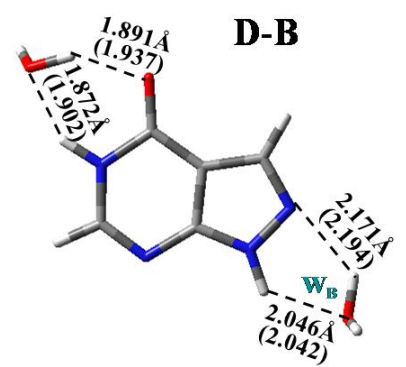


(Fig. 5)

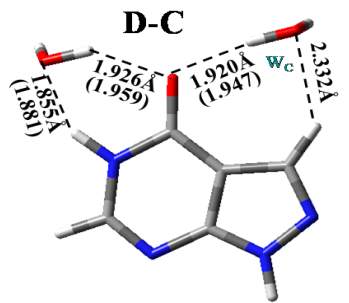
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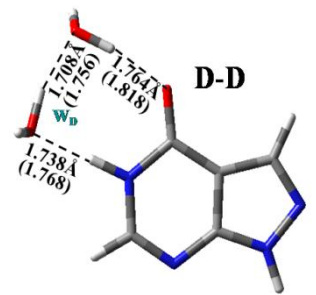
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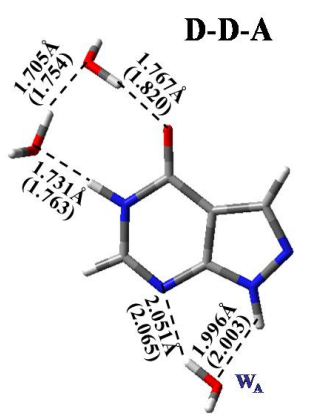
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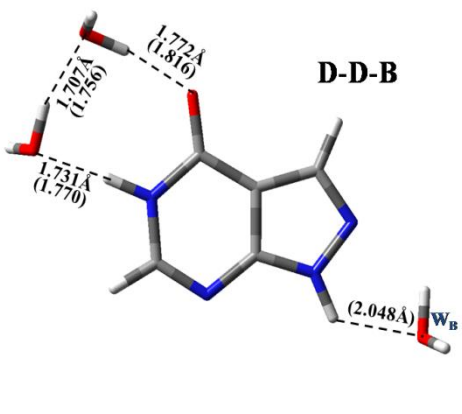
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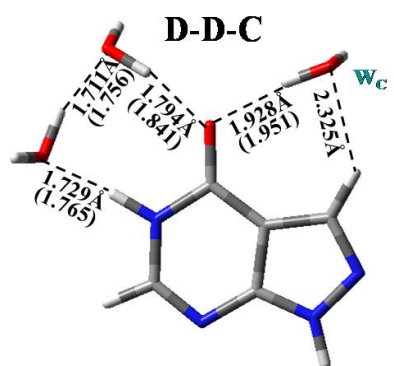
+3H₂O



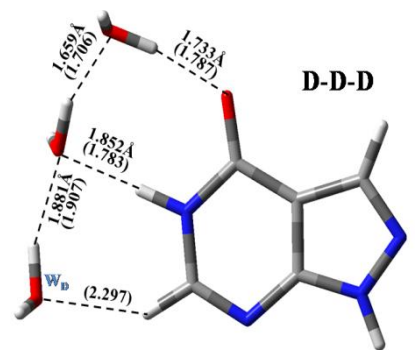
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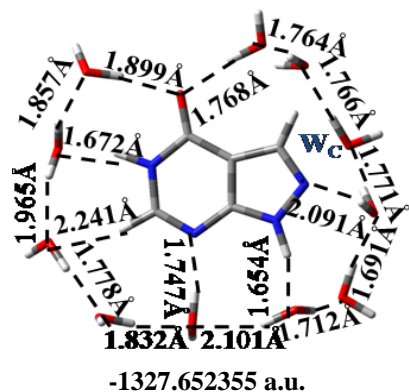


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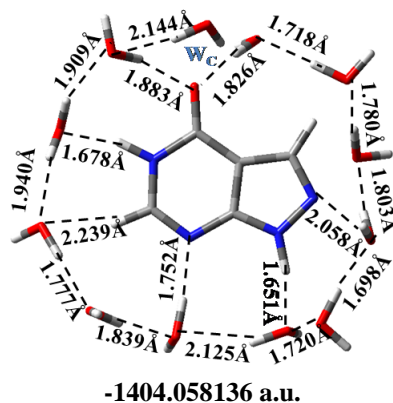


(Fig. 6)

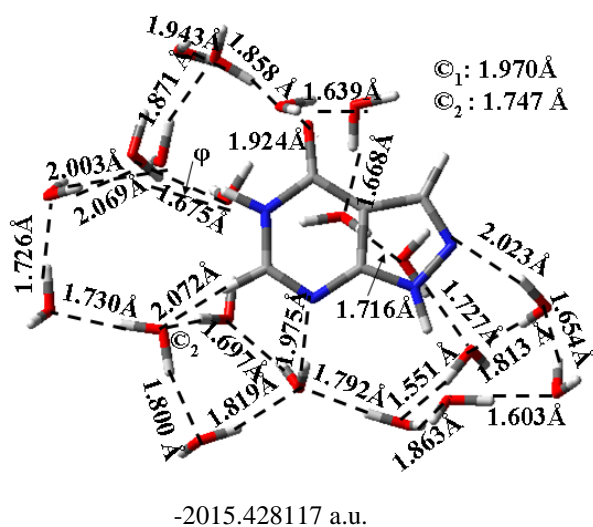
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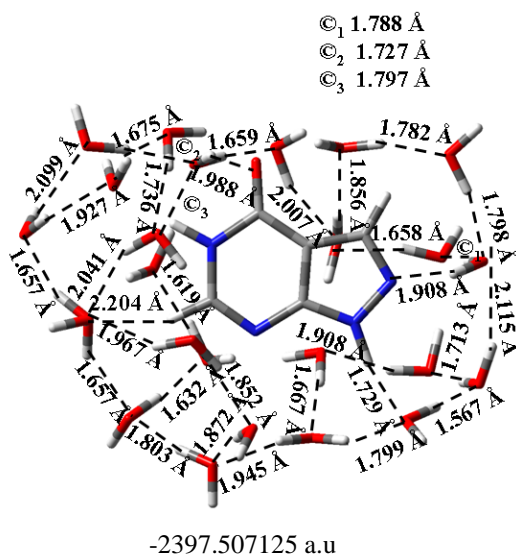
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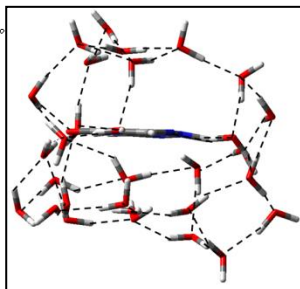
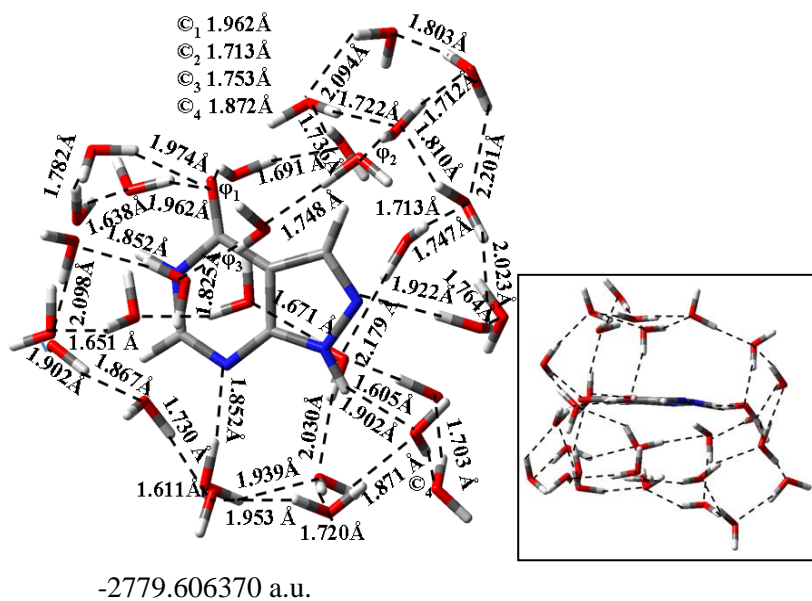
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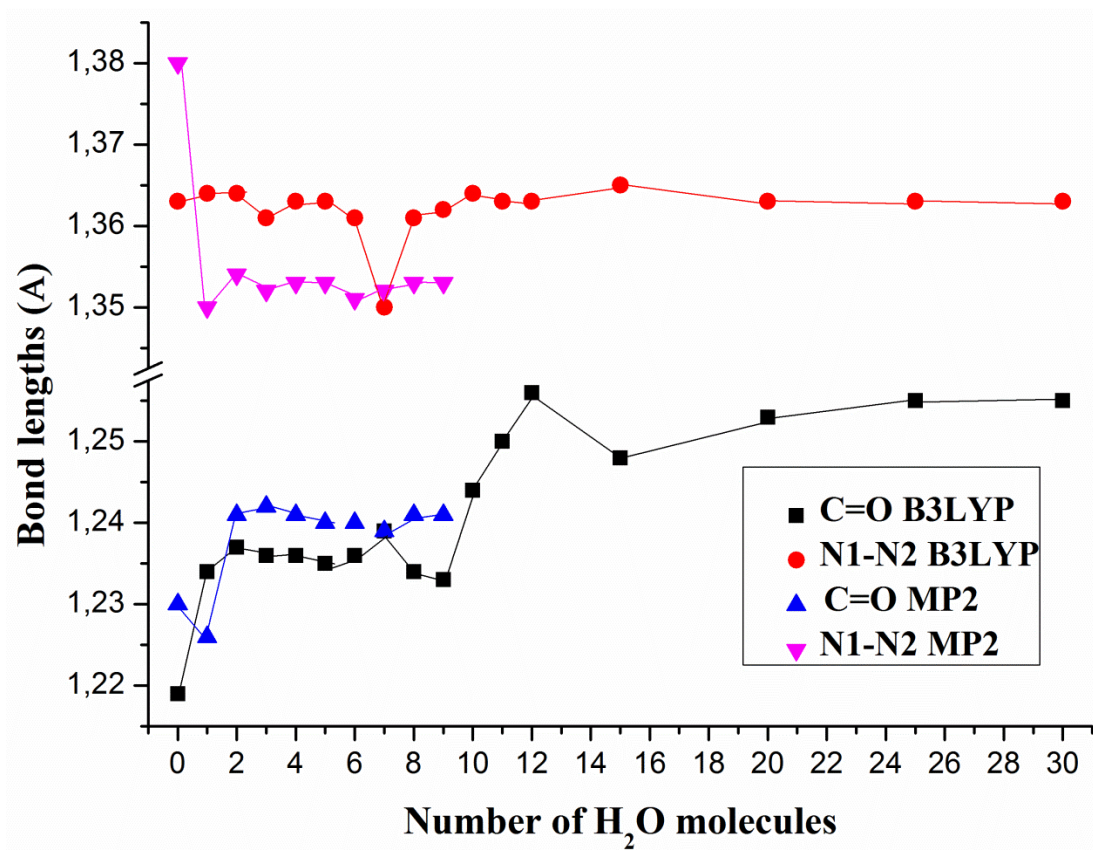
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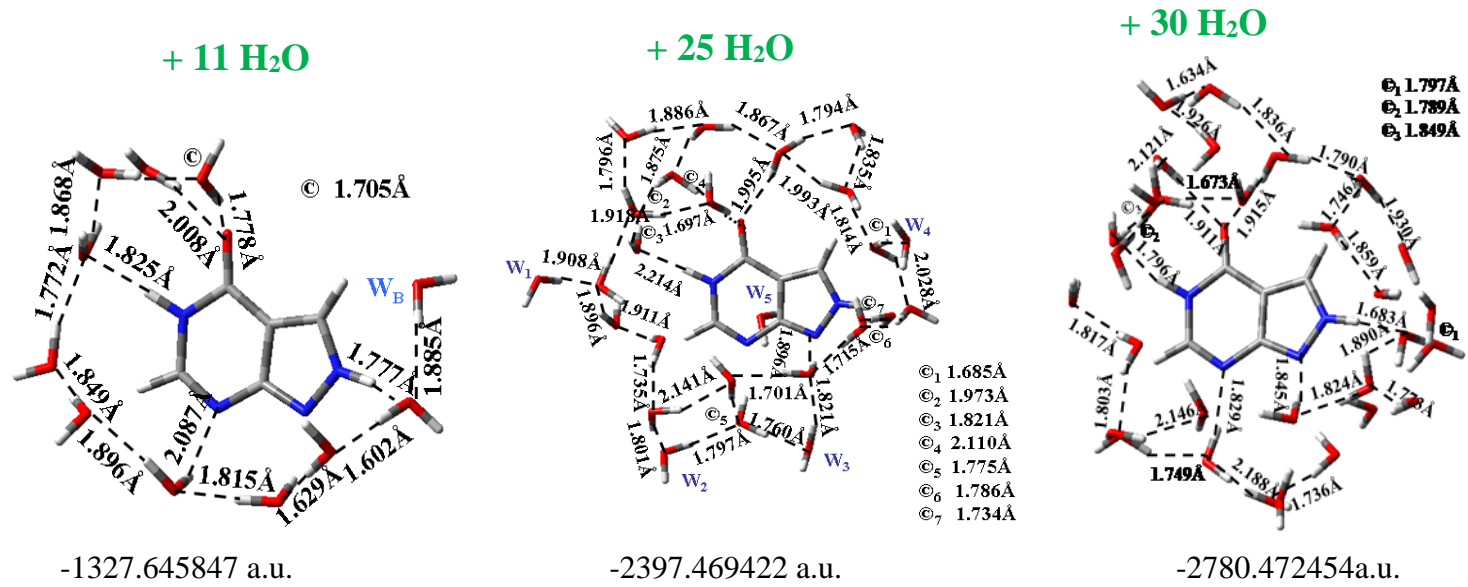
+30H₂O



(Fig. 7)



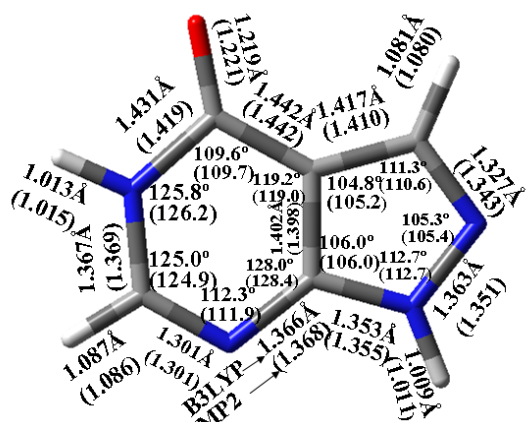
(Fig.8)



(Fig.9)

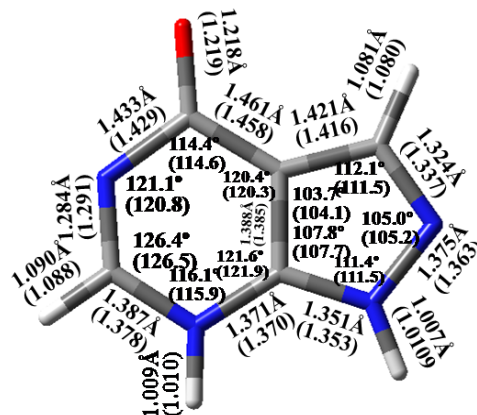
Supplementary material

AL-15



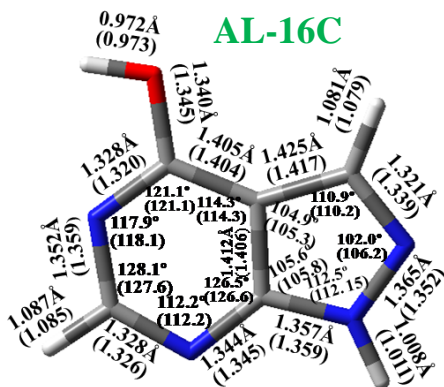
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AL-17



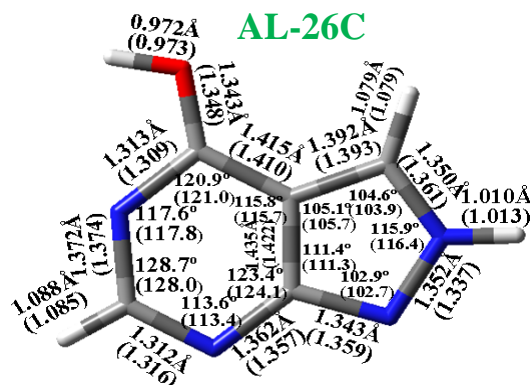
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AL-16C



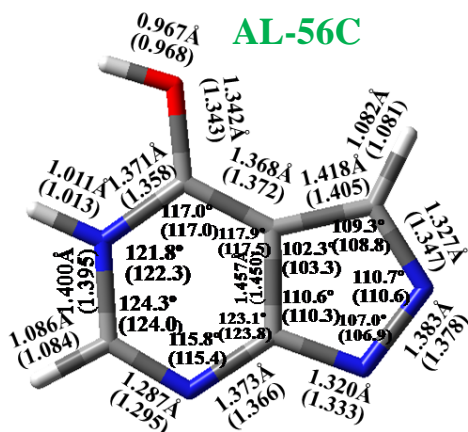
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AL-26C



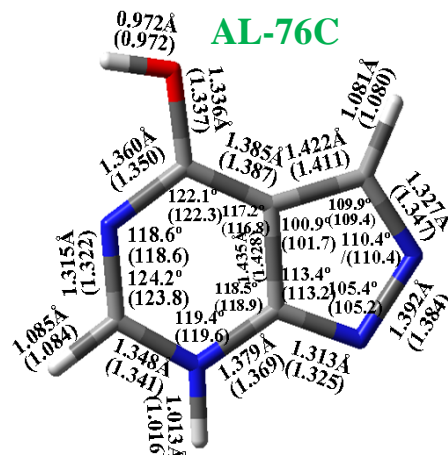
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AL-56C



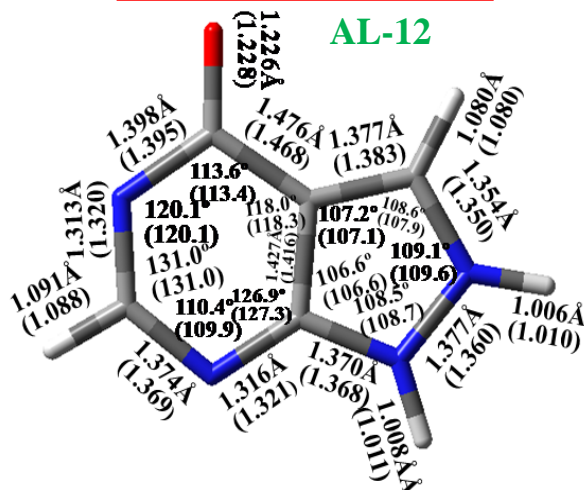
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AL-76C

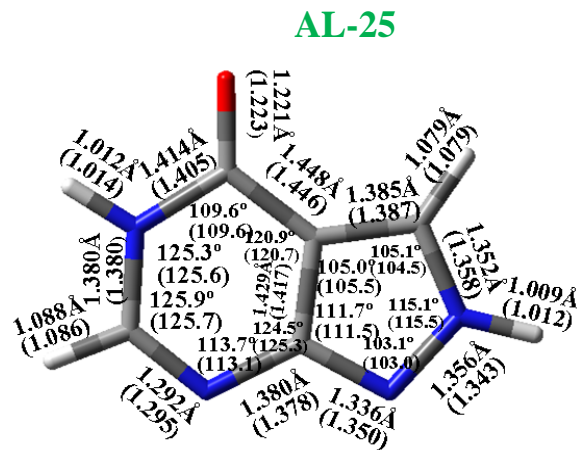


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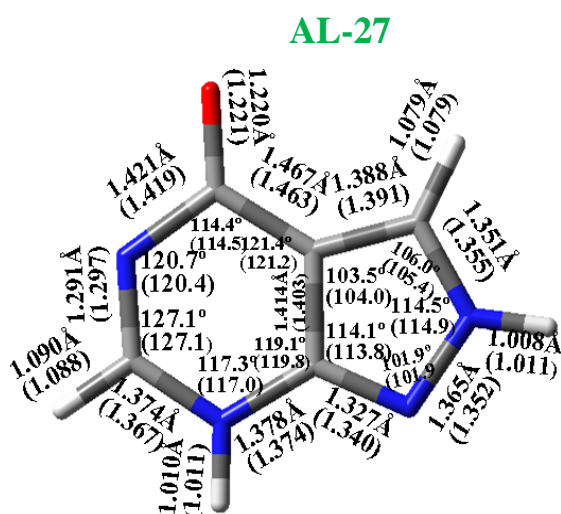
Viene en Wikipedia



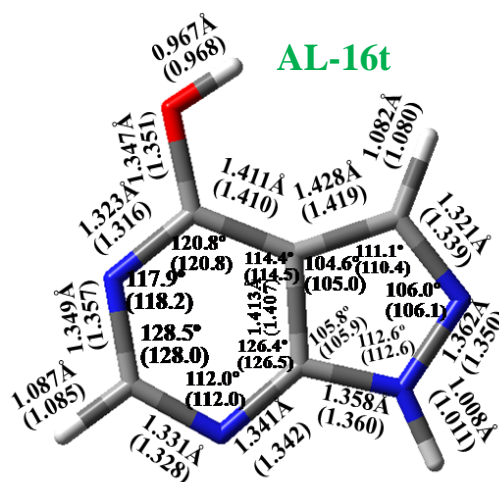
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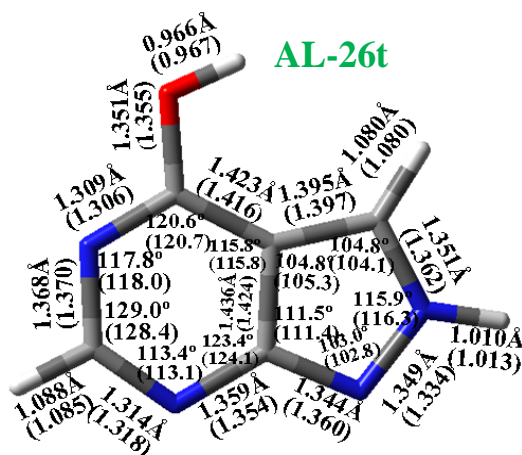
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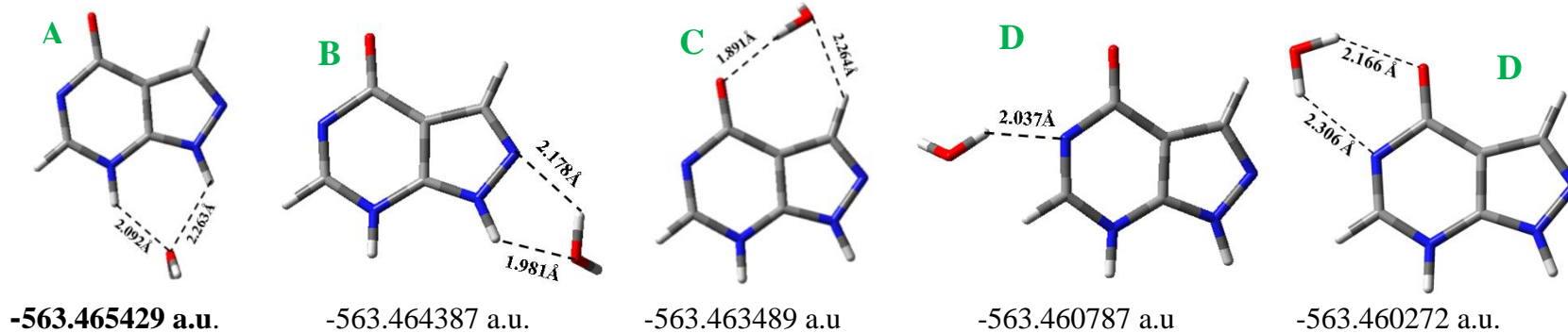
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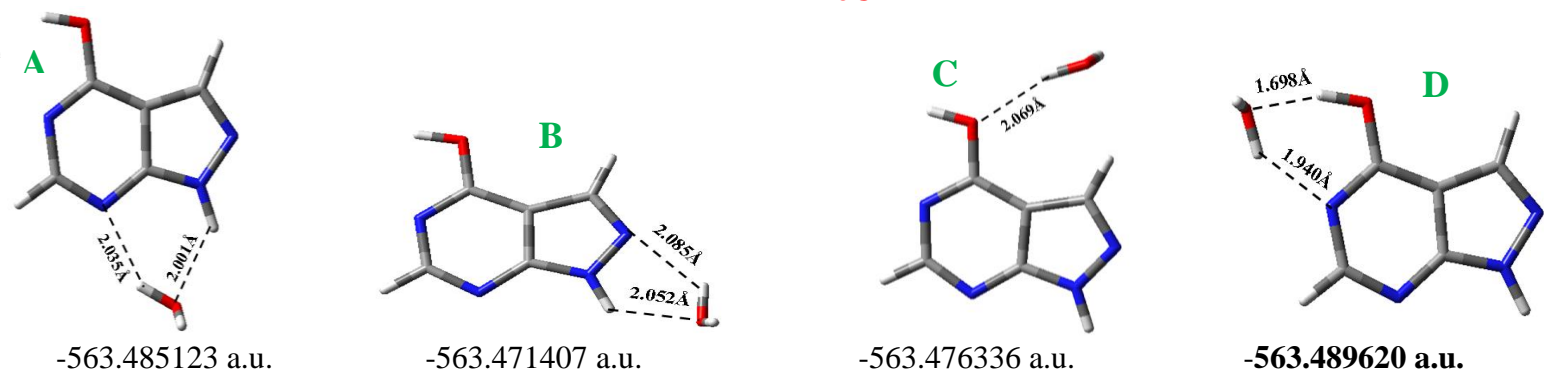
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Fig. 1-Sup. Tautomers of allopurinol [24] at the B3LYP/6-31G(d,p) and MP2/6-31++G(2d,p) (values in parenthesis) levels of calculation: bond lengths (Å), angles (degrees) and energy (a.u.).

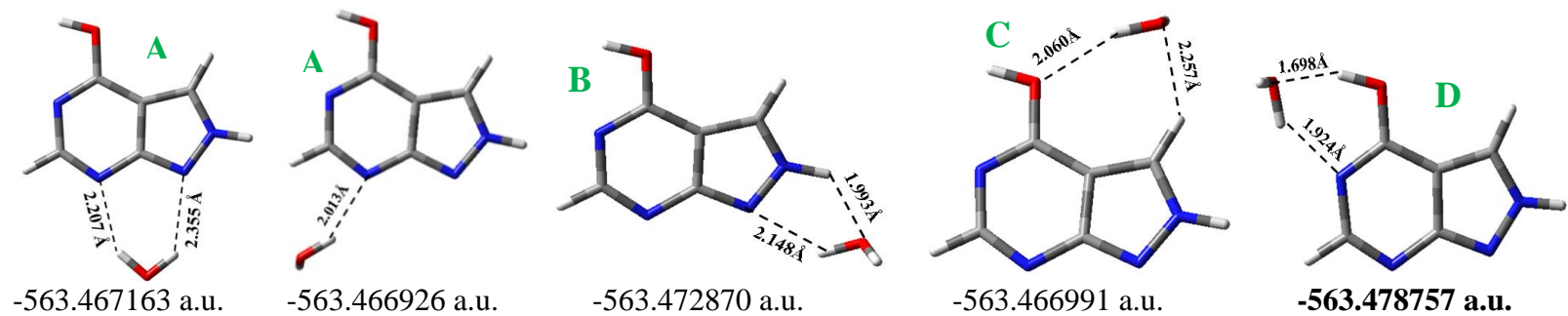
AL-17



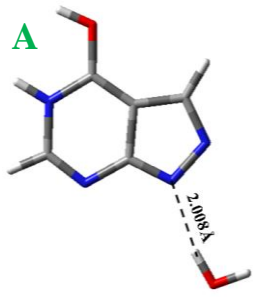
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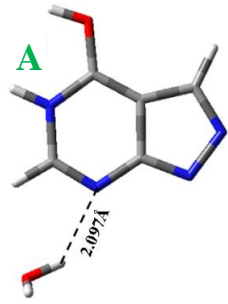
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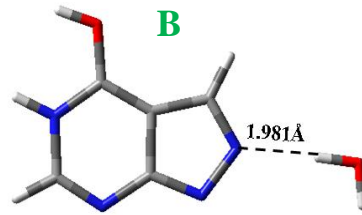
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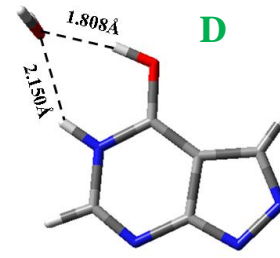
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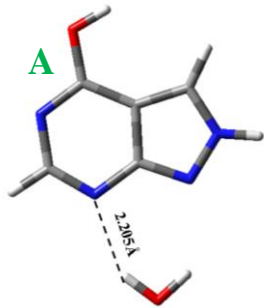


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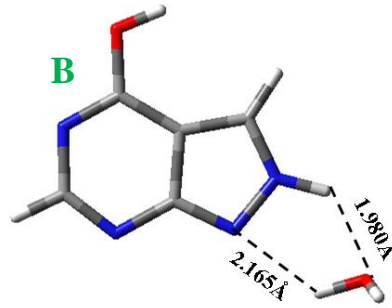


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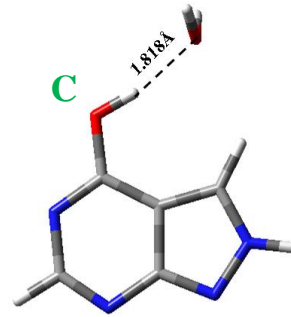
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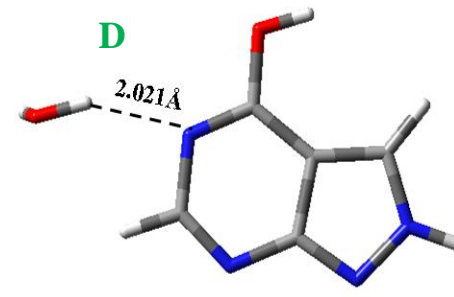
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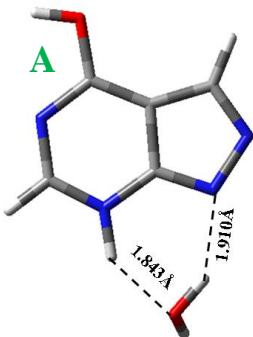


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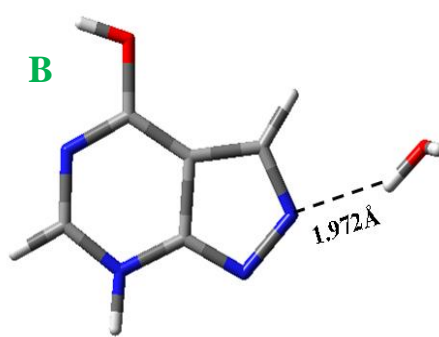


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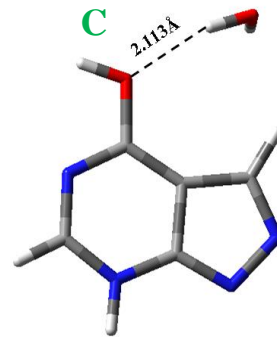
AL-76C



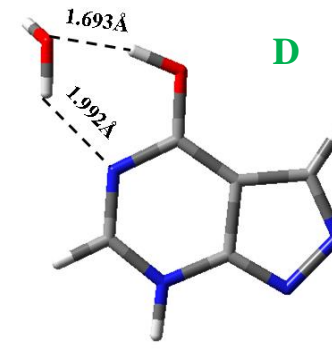
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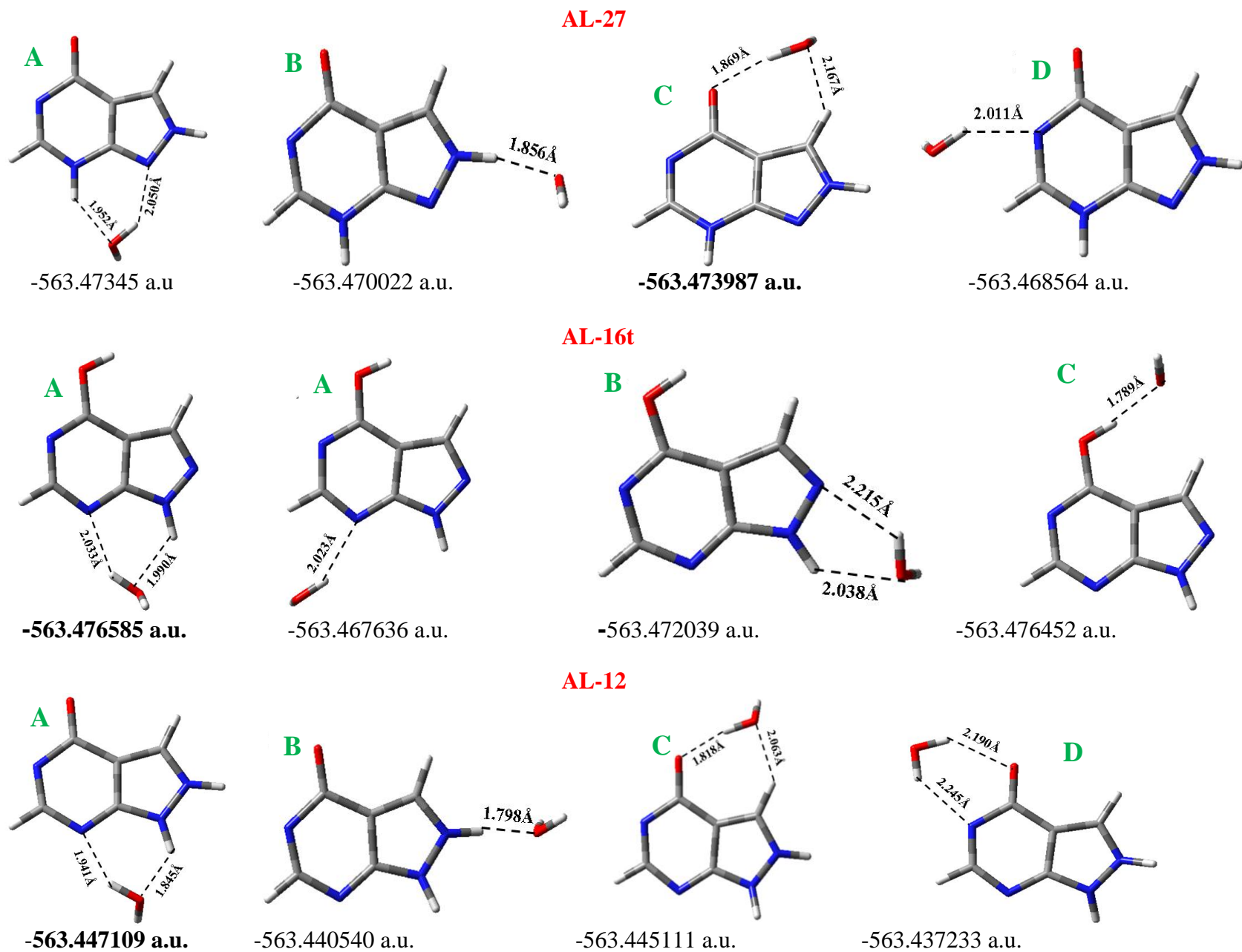


Fig. 2-Sup. Monohydration of allopurinol tautomers at the B3LYP/6-31G(d,p) level. In the bottom of each cluster appears the total energy + ZPE in a.u.. The cluster with minimum energy is shown in bold type.

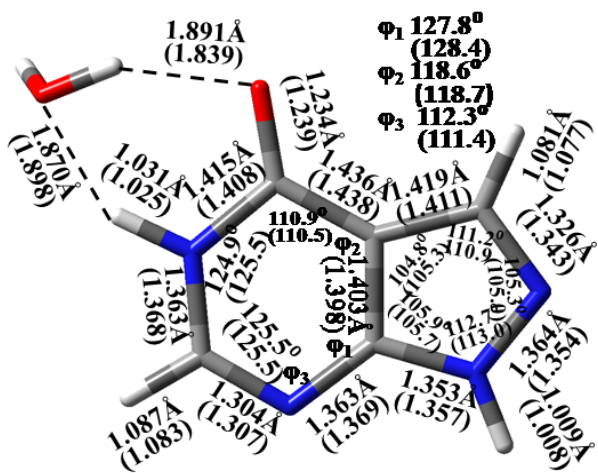
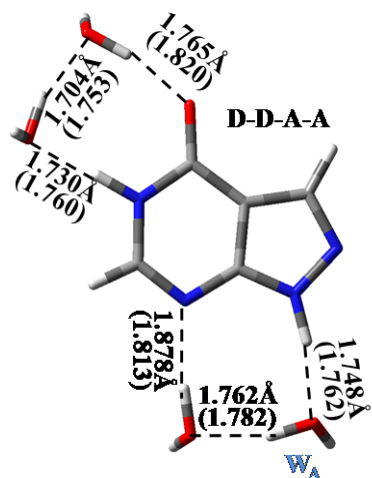
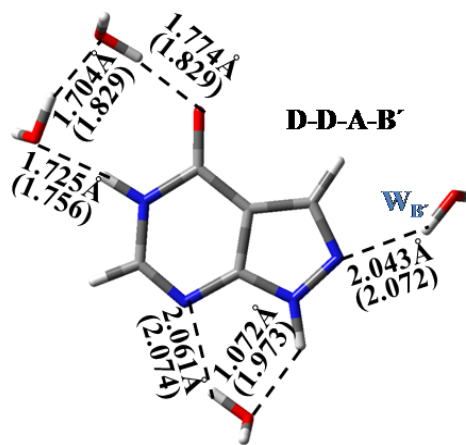


Fig. 3-Sup: Geometry of monohydrated cluster of AL-15.

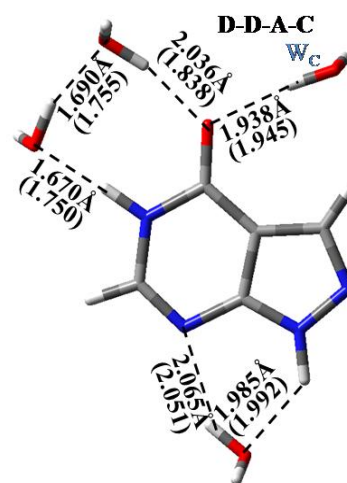
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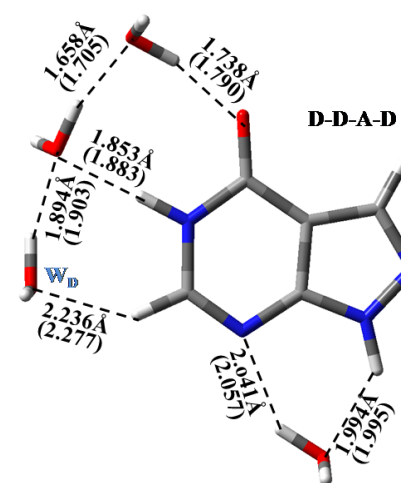
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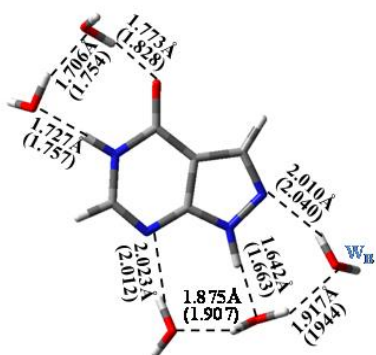


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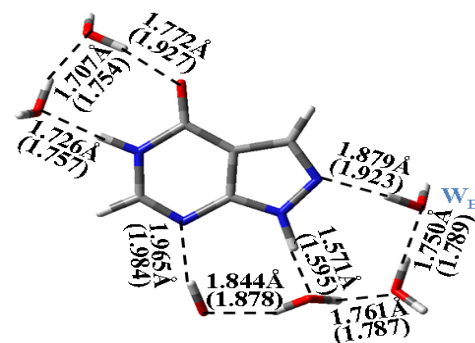
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+5H₂O



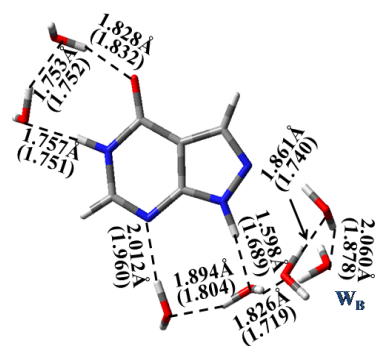
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+6H₂O



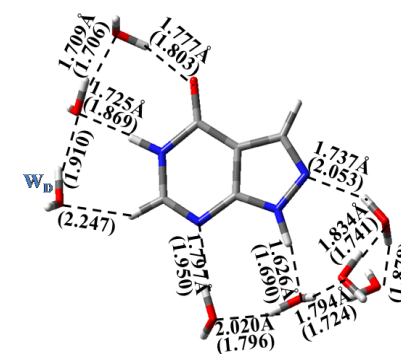
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+7H₂O



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+8H₂O



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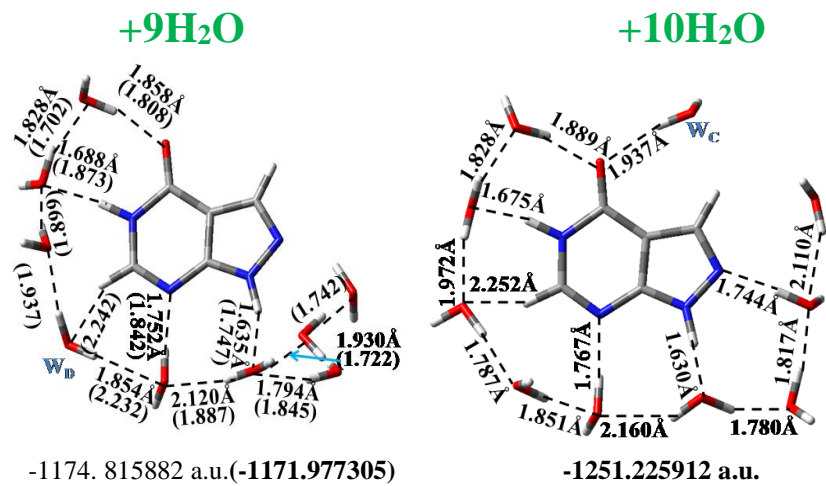


Fig. 4-Sup. Optimum forms from 5 water molecules to the complete hydrated shell in simulation of the first hydration shell of AL-15.

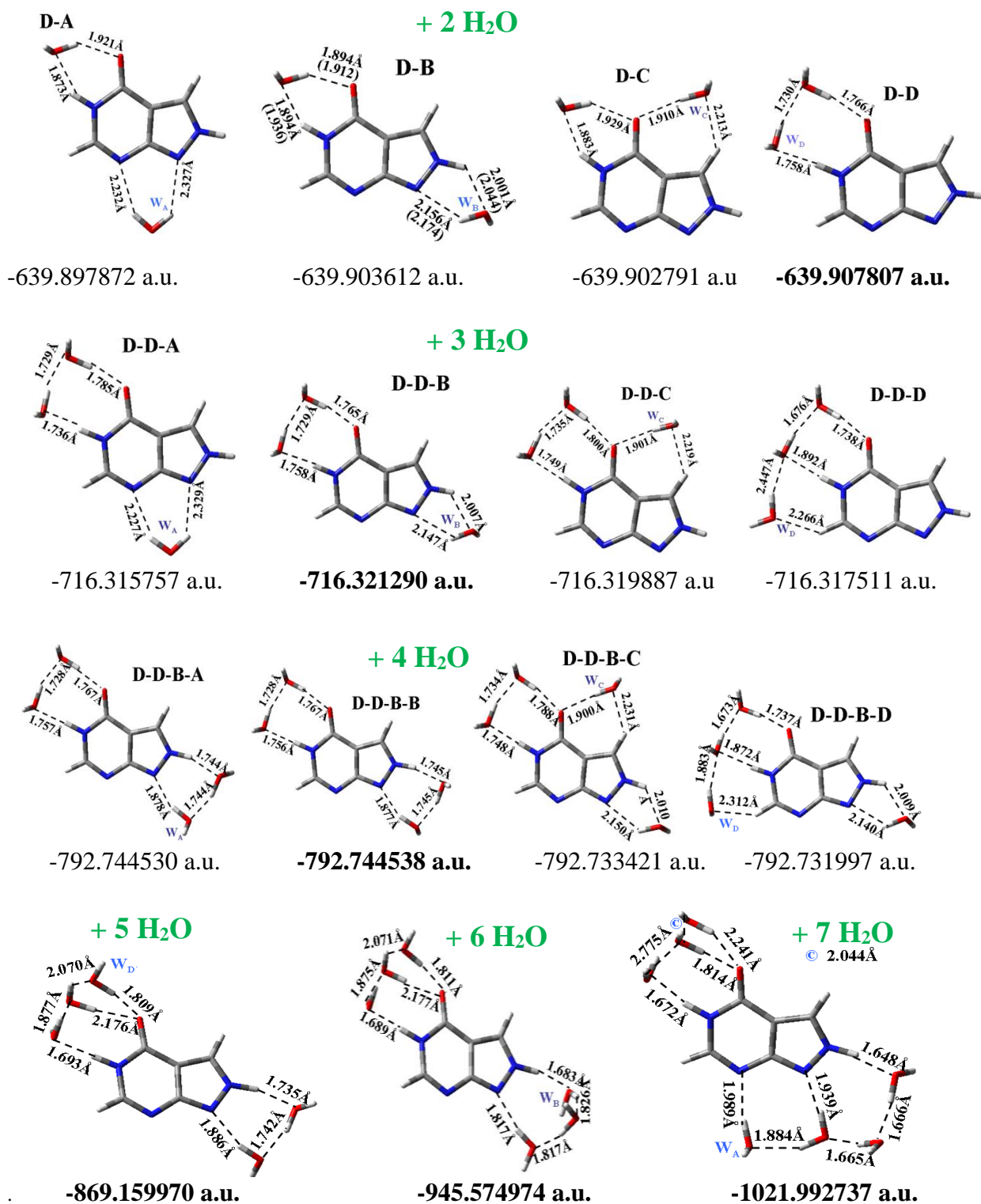


Fig. 5-Sup. Optimized clusters at the B3LYP/6-31G(d,p) level of tautomer AL-25 until the first hydration shell.

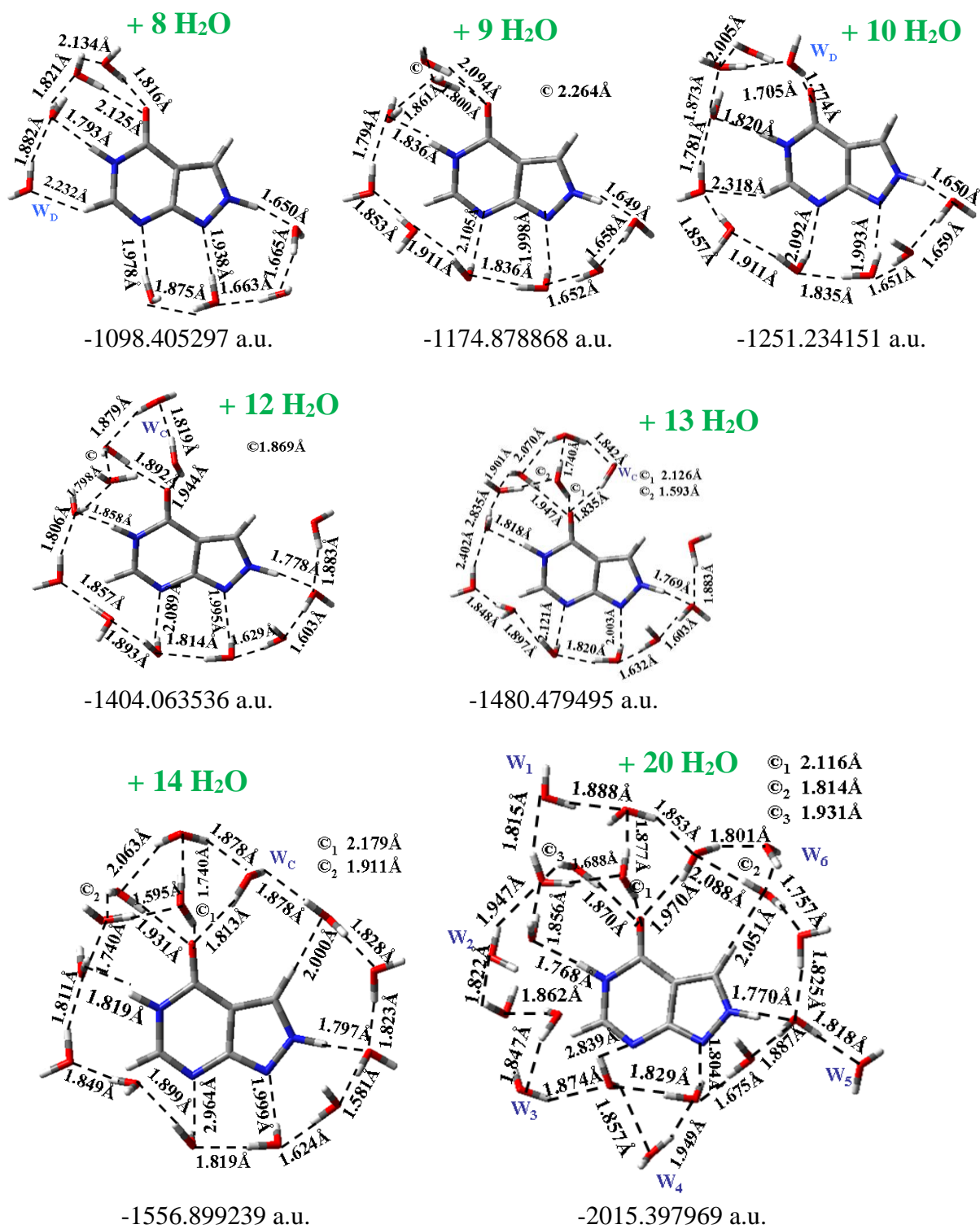


Fig. 6-Sup. Optimized clusters at the B3LYP/6-31G(d,p) level of tautomer AL-25 until the second hydration shell.

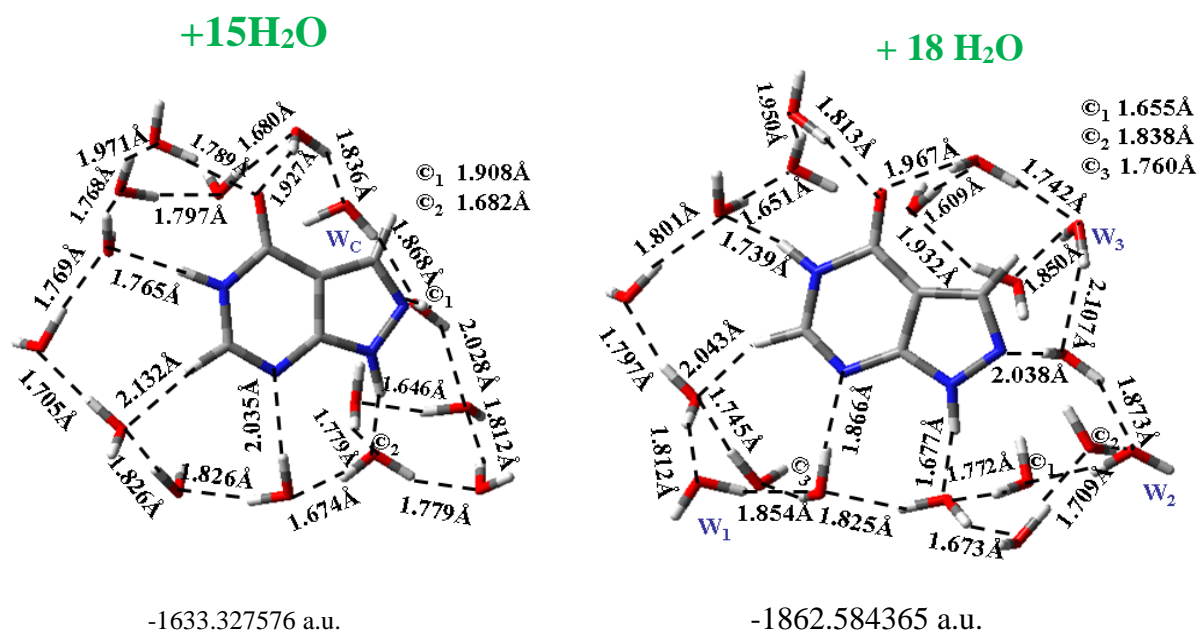


Fig. 7-Sup. Clusters of AL-15 with 15 and 18 water molecules.

Table 1-Sup. Calculated bond-lengths (in Å) of AL-15 in the simulated heptamer form of the crystal unit cell.

Bond-lengths	Heptamer							Experimental [21c]
	A	B	C	D	E	F	G	
N1-N2	1.366	1.366	1.366	1.359	1.366	1.363	1.361	1.374
N2-C3	1.325	1.331	1.331	1.330	1.328	1.327	1.330	1.325
C3-C9	1.423	1.416	1.415	1.416	1.411	1.411	1.416	1.414
C4-N5	1.409	1.426	1.433	1.435	1.413	1.416	1.436	1.399
N5-C6	1.368	1.363	1.365	1.363	1.361	1.357	1.361	1.364
C6-N7	1.302	1.307	1.301	1.303	1.307	1.309	1.305	1.292
N7-C8	1.362	1.368	1.370	1.373	1.364	1.368	1.366	1.379
C8-C9	1.406	1.402	1.403	1.404	1.404	1.405	1.402	1.395
C8-N1	1.350	1.348	1.349	1.346	1.353	1.351	1.366	1.388
C4-O	1.244	1.224	1.219	1.217	1.228	1.228	1.217	1.228
C9-C4	1.430	1.440	1.443	1.442	1.440	1.441	1.440	1.395

Table 2-Sup. Calculated bond angles (°) of AL-15 in the simulated heptamer form of the crystal unit cell.

Angles	Heptamer							Experimental [21c]
	A	B	C	D	E	F	G	
N1-N2-C3	106.8	105.9	106.1	106.3	106.2	106.6	106.3	106.4
N2-C3-C9	110.6	106.9	110.6	110.5	110.7	110.6	110.6	110.4
C3-C9-C8	105.1	105.1	104.8	104.7	105.2	104.7	104.4	104.6
C9-C8-N1	106.9	106.8	106.8	106.8	106.3	107.0	107.2	107.8
C9-C4-N5	111.6	110.4	109.6	109.6	110.8	110.5	109.8	111.1
C4-N5-C6	124.5	125.0	125.8	126.0	124.6	125.4	126.3	125.6
N5-C6-N7	125.5	125.5	125.1	124.8	126.0	125.0	123.8	125.0
C6-N7-C8	112.4	112.5	112.5	112.5	112.2	113.3	113.7	112.4
N7-C8-C9	127.7	127.3	127.6	127.7	127.1	126.3	127.3	127.3
C8-N1-N2	112.6	110.9	111.6	111.7	112.2	111.1	111.5	110.7
C8-C9-C4	118.4	119.3	119.4	119.2	119.2	119.5	119.1	118.6

Table 3-Sup. Energies (in a.u) of the monohydrated clusters of AL-15 and AL-25 $\Delta E_{AL15W1}^{AL15W1}$, $\Delta E_{AL25W1}^{AL25W1}$; Energies of AL-15 and AL-25 molecules in the clusters $\Delta E_{AL15/AL15W1-W1}^{AL15W1}$, $\Delta E_{AL25/AL25W1-W1}^{AL25W1}$; and energies of the water molecule in the clusters $\Delta E_{W1/AL15W1-AL15}^{AL15W1}$, $\Delta E_{W1/AL25W1-AL25}^{AL25W1}$, calculated at the B3LYP/6-31G(d,p) and MP2/6-311++G(2d,p) levels.

B3LYP				MP2		
Cluster AL-15 W1	$\Delta E_{AL15W1}^{AL15W1}$	$\Delta E_{AL15/AL15W1-W1}^{AL15W1}$	$\Delta E_{W1/AL15W1-AL15}^{AL15W1}$	$\Delta E_{AL15W1}^{AL15W1}$	$\Delta E_{AL15/AL15W1-W1}^{AL15W1}$	$\Delta E_{W1/AL15W1-AL15}^{AL15W1}$
A	-563.6113	-487.1794	-76.4197	-562.4119	-486.1049	-76.2899
B	-563.6159	-487.1790	-76.4200	-562.4095	-486.1049	-76.2900
C	-563.6153	-487.1792	-76.4196	-562.4081	-486.1049	-76.2900
D	-563.6231	-487.1788	-76.4195	-562.4139	-486.1047	-76.2900
Cluster AL-25 W1	$\Delta E_{AL25W1}^{AL25W1}$	$\Delta E_{AL25/AL25W1-W1}^{AL25W1}$	$\Delta E_{W1/AL25W1-AL25}^{AL25W1}$	$\Delta E_{AL25W1}^{AL25W1}$	$\Delta E_{AL25/AL25W1-W1}^{AL25W1}$	$\Delta E_{W1/AL25W1-AL25}^{AL25W1}$
A	563.6057	-487.1737	-76.4200	-562.4024	-486.1008	-76.2900
B	563.6120	-487.1737	-76.4200	-562.4070	-486.1008	-76.2900
C	-563.6123	-487.1732	-76.4200	-562.4062	-486.1006	-76.2899
D	-563.6163	-487.1730	-76.4197	-562.4089	-486.1004	-76.2898

Table 4-Sup. Calculated dipole moments of AL-15 tautomer (Debyes).

Number of water molecules	B3LYP/ 6-31G(d,p)	MP2/ 6-31G(d,p)
0	3.633	4.462
1	2.705	3.312
2	1.906	2.554
3	3.405	4.253
4	2.698	4.268
5	3.063	3.336
6	3.695	3.199
7	5.119	6.368
8	1.421	6.380
9	4.674	6.479
10	5.484	
11	4.379	
12	4.620	
15	3.414	
20	7.756	
25	4.296	
30	13.225	

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Conformational analysis of the anti-HIV Nikavir prodrug: comparisons with AZT and Thymidine, and establishment of structure–activity relationships/tendencies in other 6'-derivatives

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A comprehensive theoretical conformational analysis of the anti-HIV Nikavir prodrug was carried out; this prodrug has noticeable advantage over the approved drug AZT. The whole conformational parameters (χ , α , β , γ , δ , ϕ , P and v_{\max}) were analysed as well as the NBO natural atomic charges. The calculations were carried out by means of DFT/B3LYP and *ab initio* MP2 methods with full relaxation of all geometrical parameters. The search located at least 67 stable structures, 4 of which were within a 1 kcal/mol electronic energy range of the global minimum. By MP2 it corresponds to the calculated values of the exocyclic torsional angles $\chi = -108.0^\circ$, $\beta = 14.5^\circ$, $\gamma = 76.7^\circ$ and $\varepsilon = 71.5^\circ$. The results obtained are in accordance to those found in related anti-HIV nucleoside analogues. Comparisons of the conformers with those determined in the common anti-HIV drug AZT were carried out. A detailed analysis of the lowest vibrations ($<200 \text{ cm}^{-1}$) in the best conformer of Nikavir was carried out. The most stable hydrated cluster of this conformer with 20 explicit water molecules was determined.

Calculations in five of its 6'-derivatives were performed to identify structural trends that might correlate with the anti-HIV activity of these compounds. Ten structure–activity relationships/tendencies were established that can help for the design of new drugs. Several recommendations for this design were expressed.

Keywords: Nikavir; phosphazide; AZT; anti-HIV; AIDS; antiviral agents; reverse transcriptase inhibitors; DFT and MP2 methods

1. Introduction

Newly emerging viral infections represent a major threat to human health (Burke & Fish, 2009). Thus, infectious viral diseases are widely recognized as an important medical problem. In HIV infections the progress toward the treatment has steadily increased in the past two decades (Khandazhinskaya et al., 2009; Kukhanova, 2012). Many different strategies have been developed in the search for therapeutic agents against AIDS. Actually, nucleoside analogues play a crucial role in the current treatment of cancer and viral infections as the primary components of highly active anti-retroviral therapy. Currently, more than 20 drugs have been approved for it (Taft & Paula da Silva, 2006). Despite significant progress in the design of anti-HIV drugs, many problems remain. As a result, there is a critical need for more effective and less toxic therapeutics.

To be active, the nucleoside analogues must be phosphorylated by cellular kinase enzymes to give successively the corresponding nucleoside 5'-mono-, di- and

triphosphates (Hsu et al., 2007). The effectiveness of this process is extremely low, for example, .3% for AZT (Shirokova, Jasko et al., 2004). Therefore, many efforts have been made to improve the therapeutic properties by shortening this cascade and bypassing at least the first phosphorylation step (Piperno, Chiacchio, Iannazzo, & Romeo, 2006). Moreover, usually the first phosphorylation step, that is, the synthesis of nucleoside 5'-monophosphates (NMP), is the crucial step for the nucleoside triphosphate (NTP) formation. Because phosphorylation is indispensable for biological activity, nucleoside analogues that are poor substrate for phosphorylating enzymes are usually inactive (Hao et al., 1990). To bypass this enzymatic monophosphorylation step, efforts were focused on delivery into the cell 5'-monophosphates of nucleoside analogues (Parang, Wiebe, & Knaus, 2000; Hecker & Erion, 2008; Khandazhinskaya, Matyugina, & Shirokova, 2010). Unfortunately, under physiological conditions, they exist as dianions and cannot cross negatively charged cell membranes. Hence, it

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was assumed that if a phosphate moiety of mononucleotide is properly masked and became neutral, this should facilitate cell membrane penetration and increase concentration of drug inside the cell. This idea, called pronucleotide approach, triggered studies on various types of nucleotide derivatives, whose intracellular conversion to the desired NMP would occur via chemical and/or enzymatic hydrolysis of the phosphate masking groups. Therefore, phosphate-containing drugs appear as important agents for anticancer and antiviral therapy (Hecker & Erion, 2008; Schultz, 2003).

The most effective alternative substrates of the reverse transcriptase enzyme of HIV virus correspond to compounds containing unsaturated ribose ring structure, with lack 2'- and 3'-hydroxyl groups. The most common anti-HIV drug, 3'-azido-2',3'-dideoxythymidine (zidovudine, AZT) has the disadvantage of its toxicity and a short half-life in the organism. These limitations have prompted the development of strategies for designing prodrugs of AZT, and thus, a variety of 5'-O-substituted prodrugs of AZT have been explored (Parang et al., 2000; Troev, Mitova, & Ivanov, 2010). Other approaches encompass 5-halo-6-alkoxy-5,6-dihydro derivatives of AZT and 5'-ether prodrugs of AZT.

Ester prodrugs of AZT can be structurally categorized into two main groups: (A) 5'-O-carboxylic ester AZT prodrugs and (B) masked AZT 5'-monophosphate (AZTMP) prodrugs. The introduction of a H-phosphate group into the AZT 5' position results in significant improvement of its therapeutic properties (high anti-HIV activity and low toxicity) and it allows a new group of anti-HIV drugs, AZT 5'-monophosphates. Synthesis and biological activity of these compounds have been studied in recent years (Ivanova, Shmagel, & Vorobyeva, 2010; Jasko et al., 2006; Khandazhinskaya et al., 2009; Mackman et al., 2007) and they have been subject of several reviews (Khandazhinskaya et al., 2010; Piperno et al., 2006; Wagner, Iyer, & McIntee, 2000). One molecule in this group is AZT 5'-H-phosphonate (Nikavir[®], phosphazide), which has been approved in 1999 by the Russian Ministry of Health for the treatment of HIV-infected patients (Khandazhinskaya et al., 2010; Kukhanova & Shirokova, 2005) as a monotherapy or as a component of combined therapy. This molecule is ca. four times less toxic than AZT, but one order of magnitude less effectively than AZT. The effect of Nikavir is based on its ability to release AZT which, after intracellular transformation to AZT-5'-triphosphate (AZTTP) inhibits the replication of HIV. According to pharmacokinetic research data, clinical advantages of Nikavir are due to slower and more gradual increase of AZT concentration in the blood than in case of administration of proper AZT. Thus, due to the recently great interest on this compound, it is studied in the present work. For simplicity and avoid confusions it is called here as NK.

NK has structural similarities with AZTMP, but unlike AZTMP, it might be resistant to extra and/or intracellular dephosphorylation and it is able to penetrate through cellular membranes because of its low polar nature (Piperno et al., 2006). As compared to azidothymidine, NK is characterized by high anti-HIV activity, low cytotoxicity, longer half-life in organisms and a less frequent induction of HIV-1 variants. However, to our knowledge, there is no data on the conformational characteristics of NK, neither DFT nor *ab initio* studies. Thus, it is one of the tasks of the present manuscript.

Because the toxicity of NK remains rather high, and also it has the disadvantage in the development of resistance, it is necessary to design new anti-virus. Thus, another goal of the present manuscript is to establish relationships between structure, conformational features or physicochemical properties and activity of the nucleoside analogues that can help in the design of new anti-virus. It has been reported that anti-HIV-1 activity depends on ribose conformation, and differences in the ribose lead to appreciable changes in positions of the thymine ring and the C5'-OH group (Yates & Kirby, 1993).

From our understanding it would be interesting to analyse first the different conformational possibilities for NK, and compare the results with the single AZT nucleoside analogue and with the nucleoside natural deoxythymidine (dT). An accurate knowledge of the flexibility and conformer properties of a nucleoside would be an important help for the interpretation of drug-target interactions. For this reason, the conformers of natural and analogues nucleosides have been analysed by different authors (Alcolea Palafox & Iza, 2012; Alvarez-Ros & Alcolea Palafox, 2013; Baumgartner, Motura, Contreras, Pierini, & Briñón, 2003; Ponomareva, Yurenko, Zhurakivsky, van Mourik, & Hovorun, 2013, 2014; Saran & Ojha, 1993; Tamara & Alcolea Palafox, 2011; Yekeler, 2004; Yurenko, Zhurakivsky, Ghomi, Samijlenko, & Hovorun, 2007a, 2007b, 2008). Now, an extensive theoretical study of the conformational preferences in NK has been carried out with full relaxation of all geometric parameters, in an attempt to gain insights into molecular features responsible for activity. We will attempt to determine herein, if the various geometric features in NK are correlated or interact with one another. We are also interested in whether alternative forms of hydrogen bonding make significant contributions to the conformational behaviour of NK. Opportunities appear for hydrogen bonding involving the thymine moiety with the oxygen atoms of the phosphate chain. By the novelty of this drug, structural studies have not been reported yet.

2. Computational details

Calculations were carried out using the B3LYP density functional method (DFT), implemented in the

GAUSSIAN 03 program package (Frisch et al., 2003). The UNIX version with standard parameters of this package was used. DFT methods provide adequate compromise between the desired chemical accuracy and heavy demands put on computer time and power. Moreover, DFT calculations on 2'-deoxyribonucleosides provide results which are quantitatively in good agreement with those obtained at the MP2 level for the same compounds (Alcolea Palafox & Iza, 2010; Brovarets' & Hovorun, 2013; Danilov, Anisimov, Kurita, & Hovorun, 2005; Danilov et al., 2009; Hocquet, Leulliot, & Ghomi, 2000), and it predicts vibrational wavenumbers of DNA bases better than HF and MP2 methods (Alcolea Palafox, Iza, & Gil, 2002; Alcolea Palafox, Nielsen, Lang, Garg, & Rastogi, 2004; Alcolea Palafox & Rastogi, 2002; Ortiz, Alcolea Palafox, Rastogi, & Tomer, 2012; Rastogi & Alcolea Palafox, 2011). Therefore, DFT methods and in special B3LYP have been used satisfactory in many studies of 2'-deoxyribonucleosides (Ponomareva et al. 2012, 2013, 2014; Shen, Wang, & Xia, 2013; Shishkin, Pelmenschikov, Hovorun, & Leszczynski, 2000a; Shishkin, Gorg, Zhikol, & Leszczynski, 2004a, 2004b; Yurenko et al., 2007a, 2008), of 2'-deoxyribonucleotides (Palamarchuk, Shishkin, Gorb, & Leszczynski, 2009, 2013; Shishkin, Palamarchuk, Gorb, & Leszczynski, 2008), of DNA (Brovarets' & Hovorun, 2014) and of drug design (Alcolea Palafox, 2014b; Alcolea Palafox et al., 2011; Hoffmann & Rychlewski, 2002). The 6-31G** basis set represents a compromise between accuracy and computational cost, and thus it was the basis set selected as reference for all of the calculations. Because of the big size of NK, MP2 calculations were carried out with the 6-31G* basis set. All quantum mechanical computations were performed in the alpha computer of the Computational Centre from University Complutense of Madrid.

The 3D potential energy surface (PES) of this molecule was determined by rotation of the torsional angles χ (glycosidic bond), α (O6''-P-O5'-C5'), β (P-O5'-C5'-C4'), γ (O5'-C5'-C4'-C3') and ϕ (C2'-C3'-N'-N''). These dihedral angles were simultaneously held fixed at values varying between 0° and 360° in steps of 60° in a first study, as we have carried out on d4T molecule (Alcolea Palafox & Iza, 2012). All other geometrical parameters were relaxed during these optimizations. Sixty seven optimized geometries were obtained in this step by minimizing the energy with respect to all geometrical parameters without imposing molecular symmetry constraints. Berny optimization under the TIGHT convergence criterion was used. The conformational equilibrium at 298.15 K was evaluated by means of the Boltzmann distribution formula $\exp(-\Delta G/kT)$, where ΔG is the relative Gibbs energy.

Atomic charges were determined with the natural NBO procedure (Carpenter & Weinhold, 1988; Reed,

Curtiss, & Weinhold, 1988). Harmonic wavenumber computations were carried out at the same level of the respective optimization process and by the analytic evaluation of the second derivative of the energy with respect to nuclear displacement. Wavenumber calculations were performed on all optimized conformers to confirm that they corresponded to local minima. All optimized structures showed only positive harmonic vibrations (local energy minima). Relative energies were obtained by adding zero-point vibrational energies (ZPEs) to the total energy. For the calculation of the ZPEs, the wavenumbers were retained unscaled. The ΔG values were sums of electronic and thermal free energies and it was calculated at 298.15 K.

The intramolecular OH...O H-bonds in NK conformers were determined using geometrical and IR spectral criteria (Yurenko et al., 2007b). H-bond energies were evaluated by the empirical logansen's formula (Logansen, 1981):

$$-\Delta H = .33 (\Delta\nu - 40)^{\frac{1}{2}}$$

where ΔH is the H-bond energy in kcal/mol and $\Delta\nu$ is the frequency shift of a H-bonded stretching mode, $\nu(\text{OH})$, in cm^{-1} . The shifts of stretching modes were calculated as difference between the average frequencies of all conformers without relevant H-bonds and the frequencies upon H-bonding.

The calculated B3LYP/6-31G** frequencies were scaled with the scale equation obtained from the uracil molecule at this level (Alcolea Palafox, 1998; Alcolea Palafox, Iza, & Gil, 2002; Alcolea Palafox & Rastogi, 2002):

$$\nu^{\text{scaled}} = 34.6 + .9447 \cdot \omega^{\text{calculated}}$$

3. Results and discussion

The structures of 2'-deoxyribonucleosides (DNs) have been extensively studied by experimental methods (Saenger, 1984): X-ray, neutron diffraction and NMR spectroscopy. The results of these investigations can be basically rationalized in terms of the most preferable ranges for the characteristic conformational angles describing the primary features of the DN's geometry. However, the structures of 2'-deoxyribonucleotides (DNt) have been less studied.

3.1. Definition of the characteristic conformational angles in NK

Several parameters characterize the conformations of NK, namely the orientation of the base unit (BU) with respect to sugar unit (SU), the conformation of SU, and the orientation of the neutral phosphate unit (PU) with respect to SU. The atomic description of this molecule as well as the most important exocyclic and endocyclic

torsional angles is defined in Scheme 1, according to Saenger's notation (Saenger, 1984). The characteristic parameters can be briefly summarized as follows:

- (1) The glycosidic torsional angle $\chi(O4'-C1'-N1-C2)$ determines the two orientations of the base relative to the furanose ring, and denoted as the *anti* and *syn* forms. *Syn* is when the C2 carbonyl of pyrimidine lies over the furanose ring, and *anti* when this group is oriented in the opposite direction. The pyrimidine ring and the sugar residue are nearly perpendicular to each other in the *anti* forms. In these conformations an H-bond/interaction $C-H\cdots O$ between C6-H of thymine ring and oxygen atom in hydroxymethyl group is formed in DN_s, and between C6-H and O6' in DN_t.
- (2) The torsional angle $\alpha(O6''-P-O5'-C5')$ defines the orientation of the hydroxyl oxygen O6'' in the phosphate moiety.
- (3) The exocyclic torsional angle $\beta(P-O5'-C5'-C4')$ shows the orientation of the 5'-phosphate group relative to the furanose ring. This ring is twisted out-of-plane in order to minimize non-bonded interactions between their substituents.
- (4) The torsional angle $\gamma(O5'-C5'-C4'-C3')$ describes the orientation of the O5' atom relative to the furanose ring.
- (5) The exocyclic torsional angle $\delta(C5'-C4'-C3'-N')$ specifies the orientation of the substituents in C4' and C3'.
- (6) The torsional angle $\phi(C2'-C3'-N'-N'')$ determines the orientation of the azide moiety relative to the furanose ring. Finally, (vii) the furanose pucker P characterizes the sugar ring, and it is defined in the bottom of Table 1.

3.2. Conformers and energetics

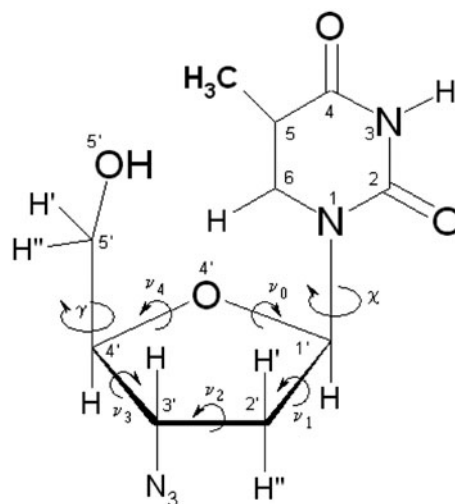
An extensive conformational study of the isolated NK molecule was carried out through a rotation of the exocyclic χ , α , β , γ and ϕ torsional angles. A detailed collection of the most important conformational parameters of these optimized forms is included in Table 1. The conformers were classified according to the three ranges of χ rotation: conformers C (*anti*) with χ : $-125 \pm 12^\circ$, conformers A (*high-anti*) with χ : $-169 \pm 6^\circ$ and conformers B (*syn*) with χ : $73 \pm 15^\circ$, Figure 1. The large range of χ values in *syn* conformers of NK, as compared to those of AZT and other nucleosides is because in NK the stabilization of the *syn* forms is through an H-bond of O2 with the O6''-H group.

Two energy criteria were considered for each conformer: the electronic energy $\Delta E + ZPE$ correction, and

the Gibbs energy ΔG , the last two columns of Table 1. The conformers were numbered according to the ΔE energy criterion and in the following order: firstly, the most stable and also possible biological active conformers C; Secondly, by analogy to AZT (Alcolea Palafox & Talaya, 2010) and related nucleosides (Alcolea Palafox, 2014b; Alcolea Palafox & Iza, 2012, 2013; Shishkin, Gorb, & Leszczynski, 2000; Shishkin, Gorb, Hobza, & Leszczynski, 2000), those conformers expected to be found in the crystal, conformers A and finally the least expected active biological conformers B. Simulations in the solid state of the nucleoside analogue d4T confirm that conformers A are presented in the crystal, and the high value of χ is due to the crystal forces (Alcolea Palafox & Iza, 2012). The *syn* forms are not common for A and B DNA strands, but they can be encountered in purine nucleotides involved in left handed (Z-form) helices of RNA and DNA.

To confirm the stability of the main stable conformers optimized at the B3LYP/6-31G** level, MP2/6-31G* calculations were carried out with full geometry optimization, Table 1. Moreover, single point computations at the MP2/6-31G**//B3LYP/6-31G** level were also performed, Table 1-Sup (Supplementary Material Section). In general, the stability order of the most stable conformers remains.

The conformers differ in general very little in energy. Thus, in our calculations 67 optimized conformers were found within the electronic energy range $\Delta E = 0-11.5$ kcal/mol, and Gibbs energy range $\Delta G = 0-11.0$ kcal/mol related to the global minimum. This range of values of ΔG is larger than that reported in the natural nucleoside dT (Yurenko et al., 2007b), 0-7.49 kcal/mol. Other conformers appear far of this range but for simplicity they were omitted in Table 1.



Scheme 1. Structure and definition of the exocyclic and endocyclic angles in AZT.

Table 1. The 67 optimum stable conformers calculated in NK molecule at the B3LYP/6-31G** and MP2/6-31G* (values in brackets) levels. Endocyclic and exocyclic torsional angles in degrees, pseudorotational angle P in degrees and energy increments in kcal mol⁻¹.

Conf	χ	α	β	γ	δ	ϕ	ν_0	ν_1	ν_2	ν_3	ν_4	P^a	S^b	ν_{\max}^c	ΔE	ΔG
C1	-115.3 [-114.0]	28.2 [31.4]	64.2 [61.8]	46.6 [43.9]	87.1 [85.6]	95.4 [85.5]	-35.1 [-37.0]	15.2 [16.0]	8.2 [9.0]	-29.2 [-31.4]	40.7 [43.0]	78.1 [77.7]	0T_4	39.9 [42.4]	.601 [.971]	0 ^c
C2	-112.5 [-108.0]	25.2 [14.5]	67.7 [76.7]	49.4 [48.5]	105.9 [121.7]	84.3 [71.5]	-42.3 [-44.6]	33.8 [43.7]	-13.9 [-27.0]	-10.7 [1.3]	33.1 [26.7]	109.6 [179.2]	0T_3	41.4 [36.9]	.636 [0] ^d	.984
C3	-115.3 [-110.3]	28.8 [26.3]	64.0 [67.4]	47.4 [46.7]	82.9 [104.8]	-46.9 [-51.1]	-36.5 [-44.8]	17.4 [37.4]	6.0 [-16.9]	-27.7 [-9.3]	40.5 [33.6]	81.4 [112.6]	0E_1	40.1 [44.0]	2.214 [1.265]	3.963
C4	-136.2 [-132.1]	-154.7 [-156.2]	-88.3 [-88.4]	175.1 [173.1]	135.0 [141.0]	72.3 [66.9]	-30.2 [-32.4]	37.6 [41.7]	-30.7 [-34.9]	13.9 [16.7]	10.2 [9.7]	145.8 [148.0]	2T_1	37.0 [41.1]	3.932 [5.234]	3.774
C5	-115.3 [-114.6]	-91.2 [-96.7]	101.5 [102.1]	57.7 [58.9]	80.2 [78.5]	96.3 [82.9]	4.2 [-20.0]	19.2 [-6.0]	-36.6 [27.8]	41.2 [-40.3]	-28.4 [38.2]	205.9 [47.2]	3E_1	40.7 [40.9]	4.248 5.556	3.239
C6	-128.4 [-131.1]	-162.5 [-166.5]	114.6 [113.1]	-68.7 [-72.7]	111.2 [118.0]	148.1 [169.9]	-39.1 [-42.4]	29.6 [36.4]	-10.2 [-17.4]	-12.3 [-7.1]	32.4 [30.8]	105.5 [114.6]	0T_1	38.2 [41.7]	4.565 [6.488]	4.269
C7	-136.8 [-133.0]	115.3 [163.6]	-74.0 [-91.6]	-52.8 [-58.9]	106.5 [113.7]	57.3 [62.9]	-41.1 [-40.3]	31.7 [32.2]	-11.6 [-13.1]	-12.0 [-9.9]	33.5 [31.6]	106.8 [109.5]	0T_1	40.1 [39.3]	5.441 5.630	4.556
C8	-125.0 [-122.7]	160.0 [74.8]	-115.9 [163.6]	55.6 [-62.6]	143.1 [93.1]	171.2 [152.8]	-22.2 [-27.0]	33.6 [5.7]	-31.6 [15.3]	19.6 [-31.6]	1.5 [37.3]	158.8 [65.1]	2E_1	33.9 [36.4]	5.706 6.757	4.256
C9	-122.7 [-132.3]	74.8 [97.9]	163.6 [-84.9]	-62.6 [-179.4]	93.1 [85.4]	152.8 [78.4]	-27.0 [11.9]	5.7 [-32.2]	15.3 [39.5]	-31.6 [-33.1]	37.3 [13.6]	65.1 [1.2]	0T_1	36.4 [39.5]	6.757 [1.514]	5.974
C10	-132.3 [-124.8]	-73.1 [-41.8]	142.4 [-50.9]	-72.0 [-162.8]	138.3 [81.4]	169.0 [89.3]	37.9 [-26.6]	-31.2 [1.4]	37.9 [21.8]	-29.8 [-37.7]	12.5 [41.0]	143.3 [57.8]	2T_1	38.0 [40.9]	7.007 7.072	4.382
C11	-125.2 [-119.1]	169.9 [113.4]	-120.8 [102.9]	57.3 [-62.8]	130.8 [83.7]	-59.1 [-53.8]	-19.6 [-10.8]	29.3 [-10.9]	-27.2 [26.4]	16.5 [-33.3]	1.8 [28.3]	157.8 [37.1]	2E_1	29.4 [33.1]	7.326 7.906	5.512
C12	-132.8 [-130.2]	75.1 [62.0]	174.9 [-93.8]	-172.7 [-57.6]	82.9 [129.3]	89.8 [175.1]	-27.1 [-35.4]	3.4 [36.6]	18.9 [-24.3]	-35.4 [4.4]	39.8 [19.5]	61.2 [130.6]	4E_1	39.2 [37.3]	7.931 8.166	4.714
C13	-170.3 [-172.0]	97.5 [97.9]	-84.9 [-85.8]	-179.4 [-178.3]	85.9 [84.5]	88.7 [78.4]	7.3 [11.9]	-27.0 [-32.2]	35.2 [39.5]	-31.4 [-33.1]	15.5 [13.6]	6.6 [1.2]	2T_1	35.4 [39.5]	1.336 [1.514]	1.875
A1	-169.7 [-172.0]	97.3 [97.9]	-84.9 [-85.8]	-179.3 [-178.1]	80.6 [79.9]	-48.7 [-50.6]	6.9 [11.9]	-31.1 [-32.3]	34.7 [39.5]	15.5 [-33.1]	7.0 [13.5]	7.0 [1.1]	3T_1	34.7 [39.5]	2.450 [1.922]	3.297
A2	-170.5 [-172.1]	-153.9 [-157.1]	-89.2 [-87.7]	179.7 [179.6]	85.5 [83.9]	86.9 [77.1]	4.7 [9.4]	-25.1 [-30.8]	34.6 [39.3]	-32.6 [-34.7]	18.0 [16.2]	11.1 [5.1]	3E_1	35.2 [39.5]	3.668 [5.079]	3.358
A3	-166.6 [-165.4]	-162.5 [70.6]	147.9 [-176.4]	-68.4 [52.9]	90.7 [82.9]	150.3 [94.6]	2.1 [1.9]	-23.1 [-23.3]	33.5 [34.0]	-33.0 [-33.8]	19.8 [20.5]	14.9 [15.5]	3E_1	34.7 [35.3]	5.012 5.242	4.823
A4	-162.2 [-170.7]	-145.2 [-102.6]	128.4 [52.7]	47.0 [158.7]	134.3 [88.7]	-51.7 [89.1]	-13.7 [10.2]	26.8 [-28.2]	-28.7 [34.5]	21.5 [-29.1]	-5.2 [12.3]	171.1 [1.6]	3T_1	29.0 [34.5]	5.795 6.849	5.976
A5	-170.7 [-170.7]	7.1 [52.6]	52.6 [89.1]	158.7 [158.7]	88.6 [89.1]	89.1 [89.1]	10.0 [10.0]	-28.1 [-28.1]	34.5 [34.5]	-29.2 [-29.2]	12.4 [12.4]	1.9 [1.9]	3T_1	34.5 [34.5]	6.850 6.850	6.303
A6	-168.3 [-173.8]	-70.8 [65.7]	177.2 [-158.3]	-64.3 [179.6]	92.3 [89.2]	153.4 [87.5]	3.5 [10.8]	-23.5 [-28.0]	32.8 [33.4]	-31.5 [-27.9]	18.0 [11.1]	12.6 [1.1]	3E_1	33.6 [33.4]	6.989 7.018	5.375
A7	-166.1 [-168.7]	-75.8 [85.5]	-169.7 [-98.8]	-68.6 [-61.5]	146.5 [93.7]	73.7 [156.3]	-19.3 [3.4]	33.2 [-23.0]	-33.6 [32.1]	23.5 [-30.8]	-2.9 [17.6]	165.8 [12.5]	2E_1	34.6 [32.9]	7.080 7.406	4.968
A8	-169.9 [-170.2]	68.5 [80.4]	80.4 [80.4]	179.5 [179.5]	83.1 [85.8]	85.8 [85.8]	.6 [.6]	-22.8 [-22.8]	34.5 [34.5]	-35.0 [-35.0]	22.1 [22.1]	17.6 [17.6]	3E_1	36.2 [36.2]	7.481 7.481	5.888
A9	-163.9 [-163.9]	-80.5 [147.3]	-133.5 [52.1]	-177.7 [159.1]	84.2 [83.2]	89.1 [87.2]	2.3 [2.0]	-23.5 [-23.4]	34.1 [34.3]	-33.6 [-33.9]	20.2 [20.5]	14.9 [15.4]	3E_1	35.3 [35.6]	7.649 7.844	5.863
A10	-165.9 [-169.7]	147.3 [-102.8]	-133.5 [52.1]	-64.7 [159.1]	143.8 [83.2]	74.0 [-50.0]	-22.6 [10.8]	34.8 [-28.6]	-33.1 [34.5]	21.0 [-28.7]	.8 [11.6]	159.9 [5.5]	2E_1	35.2 [34.5]	7.662 7.748	5.039
A11	-169.7 [-169.7]	-28.7 [-86.5]	-86.5 [128.4]	-176.1 [-71.8]	84.1 [90.4]	87.2 [101.8]	2.0 [3.9]	-23.4 [-23.8]	34.3 [33.1]	-33.9 [-31.5]	20.5 [17.8]	15.4 [11.9]	3E_1	35.6 [33.8]	7.844 7.932	7.161
A12	-168.4 [-168.4]	-67.7 [128.4]	128.4 [128.4]	-71.8 [128.4]	90.4 [90.4]	101.8 [101.8]	3.9 [3.9]	-23.8 [-23.8]	33.1 [33.1]	-31.5 [-31.5]	17.8 [17.8]	11.9 [11.9]	3E_1	33.8 [33.8]	7.932 7.932	6.857

(Continued)

Table 1. (Continued).

Conf	χ	α	β	γ	δ	ϕ	ν_0	ν_1	ν_2	ν_3	ν_4	P^a	S^b	v_{max}^c	ΔE	ΔG
A19	-169.1	75.5	85.5	171.5	150.0	69.9	-4.5	23.2	-31.6	29.8	-16.3	190.7	3E	32.2	8.087	6.179
A20	-166.1	147.8	77.4	166.5	149.4	71.0	-5.0	23.7	-31.8	29.8	-15.9	189.8	3E	32.3	8.296	6.375
A21	-174.9	-77.5	-166.5	-67.4	148.1	-49.2	3.4	17.0	-29.1	31.7	-22.6	204.4	3E	31.9	8.683	6.347
A22	-166.2	149.6	-100.9	68.4	133.6	-49.9	-7.9	21.2	-25.3	21.4	-8.7	180.7	3T	25.3	9.170	8.289
A23	-170.6	136.0	83.0	169.5	146.4	-51.8	6.9	14.1	-27.8	32.3	-25.2	210.4	3T	32.2	9.307	9.064
A24	-164.1	31.3	65.9	163.6	148.4	69.9	-7.0	25.0	-32.0	28.8	-14.0	186.3	3T	32.2	10.425	8.766
A25	-172.4	27.3	65.2	168.9	87.5	87.1	8.8	-27.3	33.9	-29.7	13.5	3.9	3T	33.9	10.484	7.552
A26	-172.8	30.1	65.3	168.8	88.2	87.1	9.8	-27.7	33.8	-29.0	12.5	2.2	3T	33.8	10.511	8.101
A27	-169.4	-86.0	-72.8	-55.8	83.9	-48.6	2.5	-23.1	33.2	-32.4	19.3	14.3	3E	34.3	11.347	10.275
B1	69.9	145.6	-98.6	66.7	89.5	115.5	-8.7	-10.1	23.1	-28.1	24.0	35.5	3T	28.4	0 ^c	0.937
B2	[68.0]	[148.7]	[-100.5]	[64.9]	[87.7]	[117.5]	[-10.4]	[-10.4]	[25.4]	[-32.4]	[27.2]	[37.3]	4E	[31.9]	[1.331]	-
B3	[65.2]	[-139.3]	[103.2]	[43.5]	[82.7]	[89.4]	[-20.6]	[-3.1]	[23.6]	[-36.5]	[36.1]	[51.1]	4E	[37.6]	[1.935]	-
B4	[80.0]	[103.8]	[-86.2]	[174.3]	[115.8]	[78.2]	[21.5]	[-22.2]	[15.2]	[-2.8]	[-11.8]	[311.6]	3E	[22.9]	[1.190]	-
B5	67.4	115.7	-73.9	-58.0	95.9	72.8	-2.9	-13.2	22.8	-24.9	17.9	24.9	3E	25.1	4.001	3.963
B6	66.8	-84.8	128.9	-40.2	111.3	149.8	11.5	-18.4	17.8	-11.5	.1	341.5	3E	18.8	4.061	4.339
B7	76.6	-155.8	-83.7	176.3	111.5	93.5	16.5	-20.3	16.6	-7.4	-5.7	325.5	3T	20.1	4.778	4.899
B8	88.4	2.9	83.9	97.8	84.4	59.9	-7	-20.8	33.0	-33.8	22.1	19.4	3E	34.9	4.903	5.415
B9	67.3	-94.5	43.1	168.7	96.3	100.7	-1.5	-13.9	22.8	-23.9	16.4	21.7	3E	24.5	5.685	5.079
B10	68.6	64.3	114.4	-68.2	113.9	154.0	4.5	-11.1	12.8	-10.5	3.9	358.3	3T	12.8	5.884	5.340
B11	81.6	49.0	96.8	52.0	86.9	97.5	-2.5	-17.4	29.0	-31.2	21.6	23.0	3E	31.5	5.970	5.885
B12	68.7	-33.8	-59.5	-164.8	80.0	-51.3	-14.4	-8.1	25.3	-34.0	30.8	42.4	3T	34.2	6.247	5.804
B13	57.9	-30.5	-92.5	155.0	96.3	93.6	-7.3	-7.6	18.0	-22.6	19.2	36.8	3T	22.5	6.253	6.505
B14	66.6	73.4	179.8	-172.6	95.3	96.8	-1.3	-14.1	22.6	-23.9	16.3	21.5	3E	24.3	6.309	4.400
B15	67.3	-73.9	-155.8	-171.9	97.3	96.8	1.2	-15.2	22.0	-21.9	13.3	15.5	3E	22.8	6.415	4.591
B16	73.8	73.4	-125.6	56.1	85.5	119.5	-9.9	-11.8	26.9	-33.5	27.7	35.6	3T	33.1	6.548	5.828
B17	70.1	40.0	98.2	49.8	83.6	100.3	-15.8	-6.9	24.7	-34.7	32.2	44.9	3T	34.9	7.546	6.631
B18	65.1	76.4	107.6	-79.0	151.1	80.3	-11.3	28.4	-33.5	28.0	-10.8	179.5	3T	33.5	7.624	6.920
B19	68.5	-160.2	-44.7	-166.8	85.9	95.0	-15.2	-7.9	25.8	-35.2	32.3	43.3	3T	35.4	7.965	5.244
B20	66.3	50.7	-178.7	-171.6	92.8	95.4	-4.2	-12.9	23.4	-26.5	19.7	27.5	3T	26.4	8.149	6.641
B21	66.2	80.9	109.9	-79.1	144.1	-54.1	-6.9	24.0	-30.6	27.5	-13.3	186.0	3T	30.8	9.271	8.434
B22	75.6	40.2	-147.4	41.5	78.4	143.3	-15.2	-10.6	29.8	-39.7	34.7	40.7	3T	39.3	9.274	9.334
B23	68.4	102.6	85.0	179.5	152.6	172.5	-9.4	26.9	-32.8	28.4	-12.1	182.4	3T	32.8	10.154	10.107
B24	[65.9]	[98.0]	[85.9]	[179.0]	[158.2]	[-175.0]	[-12.7]	[31.4]	[-37.0]	[30.9]	[-11.5]	[179.1]	3T	[37.0]	[13.222]	-
B24	69.0	-56.3	60.1	159.3	150.7	174.1	-6.2	23.2	-30.3	27.3	-13.4	186.9	3T	30.5	11.491	11.016

^aDefinition $tgP = \frac{(v_4+v_1)-(v_3+w)}{2v_2(\sin(3\phi)+\sin(7\phi))}$

When ν_2 is negative, 180° is added to the calculated value of P (Baumgartner et al., 2003).

^bNotation used (Saenger, 1984).

^c $v_{max} = \frac{av}{\cos P}$

^d $\Delta E = 0 = -1452.2892764$ a.u. at MP2/6-31G* level.

^e $\Delta G = 0 = -1455.780592$ a.u. at B3LY/6-31G** level.

^f $\Delta E = 0 = -1455.728209$ a.u. at B3LY/6-31G** level.

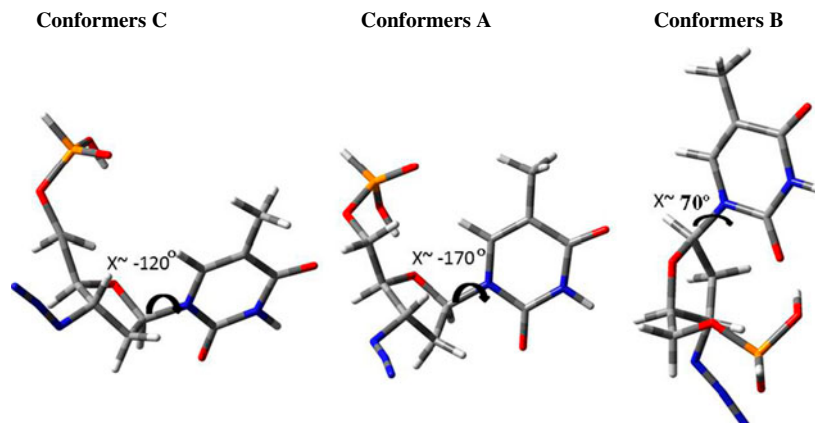


Figure 1. Three types of conformers determined in NK corresponding to the three ranges of rotation of χ , with β and $\gamma \sim 60^\circ$.

Only four conformers are found within the electronic energy range $\Delta E = 0\text{--}1.0$ kcal/mol, two *anti* and two *syn*. Of these conformers three are N-type furan puckering with great variation for the exocyclic torsional angles. According to the Boltzmann distribution, the total population of isolated NK almost correspond to conformers C1–C2, B1–B2. Only one conformer appears within the electronic energy range $\Delta E = 1.0\text{--}2.0$ kcal/mol, and two in the 2.0–3.0 range. *Anti* structures are the expected forms for the natural nucleosides that form the nucleotide and polynucleotides in biological systems (Painter, Aulabaugh, & Andrews, 1993; Painter, Andrews, & ZFurman, 2000; Saenger, 1984). The ratio of the number of *anti* to *syn* structures is slightly decreased from 2.3 in the low-energy group (<4 kcal/mol) to 1.8 in the 4–11.5 kcal/mol high-energy group. These features are close to that reported in the natural nucleosides and they confirm that the value of χ ca. -120° leads to the most stable structures. The small difference in the relative energy

between MP2 and B3LYP methods confirms the accuracy of our B3LYP calculations.

The global minimum by MP2/6-31G* corresponds to the *anti-gg-gg* form with respect to χ , γ and β torsional angles, respectively. This conformer is denoted as C2 (Figures 2–3) and it appears stabilized by two intramolecular H-bonds. The optimized bond lengths and natural NBO atomic charges on this conformer are collected in Figure 2. This global minimum differs to that calculated by B3LYP, conformer B1; although the difference of energy between both conformers is small, .601 kcal/mol. Conformer C2 appears through a rotation of the endocyclic torsional angles in C1. The sugar puckering in C1 is symmetrical twisted with C4' atom in *exo* position (0_4T). The second most stable conformer in the *syn* form is B2, with a molecular structure similar to B1 but with a rotation of α and β angles.

In AZT the global minimum by B3LYP and MP2 corresponds to the *syn* form B1, Table 2. However, in

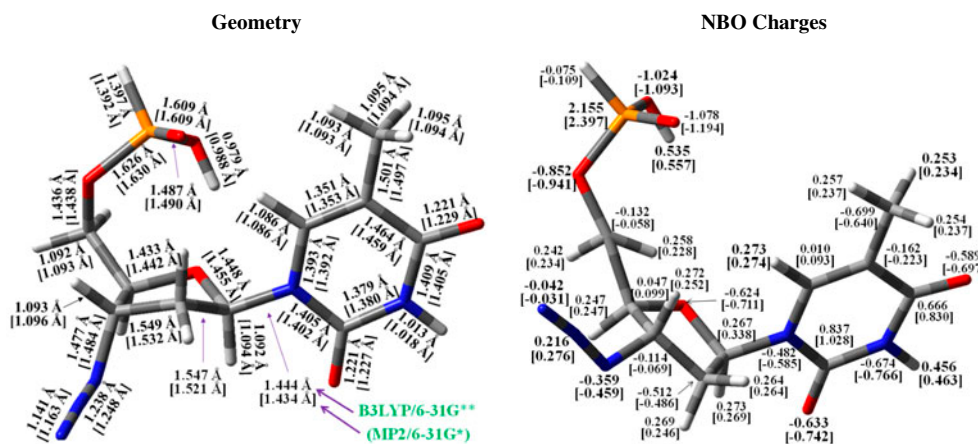


Figure 2. Calculated natural atomic charges on the atoms and optimum bond lengths in conformer C1 at the B3LYP/6-31G** level, and conformer C2 at the MP2/6-31G* (values in brackets) level.

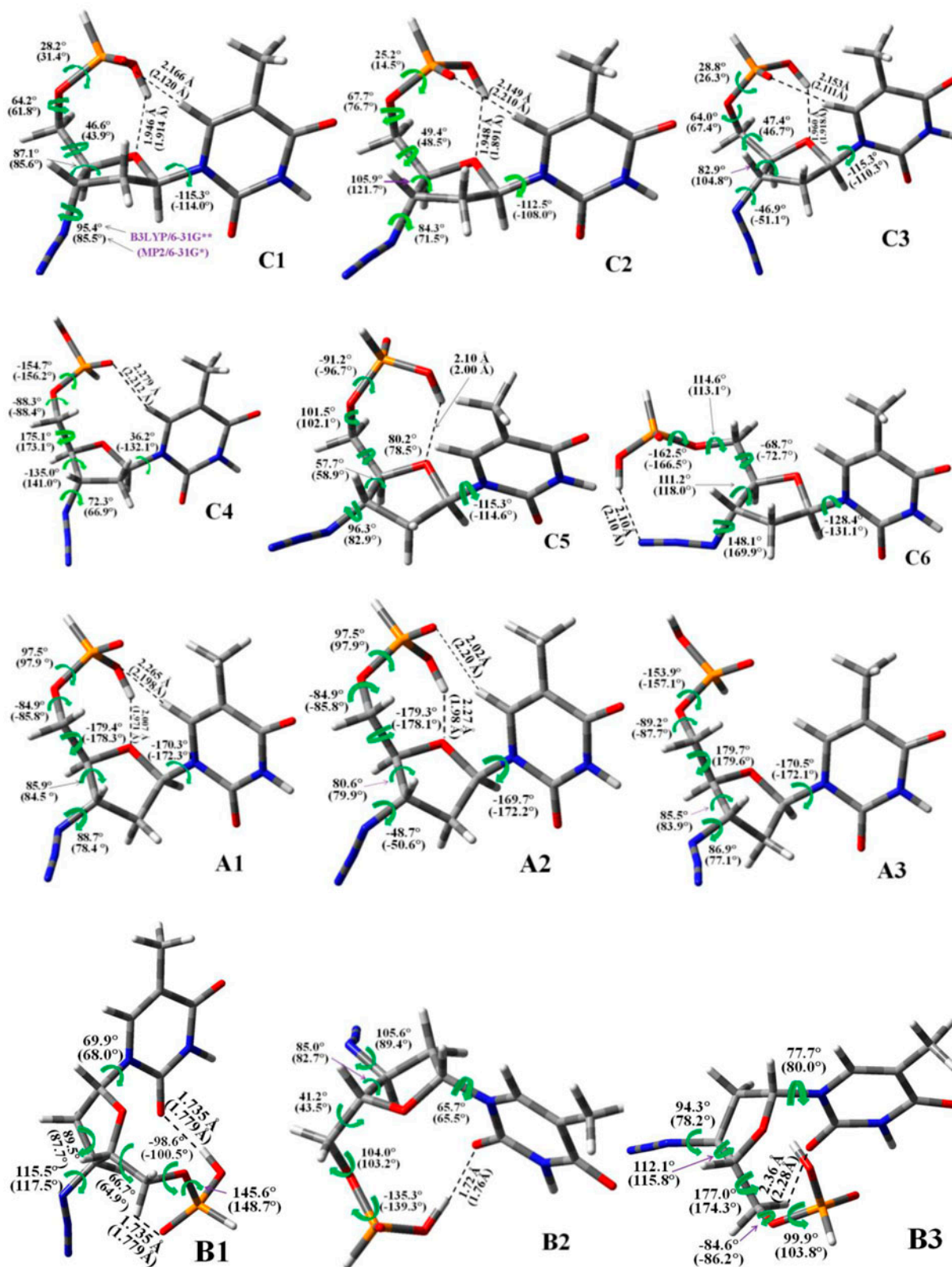


Figure 3. Geometry of the 12 most optimum conformers selected for each rotation angle χ determined in NK at the B3LYP/6-31G** and MP2/6-31G* level (in brackets) levels. The values of the strongest intramolecular H-bonds are also included.

Table 2. Comparison of the four most optimum conformers of dT, AZT and NK with full geometry optimization at the MP2/6-31G** level.

	Conformer	χ	β	γ	P	ν_{\max}	μ	ΔE
dT	C1	-128.9	176.1	50.1	163.3	37.0	7.765	0
	B1	61.4	40.1	46.4	44.5	31.1	4.697	.132
	B2	62.4	39.6	48.7	46.9	30.4	4.710	.221
	C2	-125.1	174.9	49.3	165.8	36.9	8.855	.642
AZT	B1	62.3	41.6	47.1	45.8	31.1	5.64	0
	B2	61.9	41.9	45.8	46.3	31.2	5.60	.047
	C1	-126.9	176.4	49.1	162.6	35.8	7.79	.627
	B3	62.3	41.5	47.5	47.0	31.2	5.61	.686
NK ^a	C2	-108.0	76.7	121.7	179.2	36.9	3.86	0
	C1	-114.0	61.8	43.9	77.7	42.4	4.735	.971
	B3	80.0	-86.2	174.3	311.6	22.9	4.705	1.190
	B1	68.0	-100.5	64.9	37.3	31.9	6.345	1.331

^aAt the MP2/6-31G* level.

NK the large negative charge of the phosphate group oxygens favoured the *anti* structure as the global minimum. It is due to a strengthening of the intramolecular H-bonds O6''-H...O4' and O6'...H-C6, which stabilizes the *anti* form. The phosphate group also increases the distance between the centres O5'...O2 and O5'...O4 as compared to dT. The optimized values in conformers C1-C3 of NK are in accordance to NMR experiments (Painter et al., 1993) for AZTTP with a value of $\chi = -120 \pm 12^\circ$, with γ_{g+} and $P = 60 \pm 10^\circ$, results that are adequate for the interaction of HIV-1 RT with AZTTP.

Figure 3 shows 12 optimum conformers selected in each range of the rotational angle χ : Six of them correspond to conformers C (C1-C6), three to conformers A (A1-A3), and three to conformers B (B1-B3). The values of the most important structural angles of each conformer are also included in the figure. The calculated low-energy B3LYP conformers (including the global minimum) are similar to those determined in AZT (Alcolea Palafox & Talaya, 2010). Figures 4-7 and Figure 1-Sup. to Figure 3-Sup. of the Supplementary Material Section show the distribution of the 67 optimized conformers according to their energies, exocyclic torsional angles and values of P and ν_{\max} . The 12 optimum conformers selected are pointed in these figures.

Among the most stable conformers, B1-B3 *syn* forms have a higher dipole moment than the *anti* ones C1-C3. It is in contrast to that observed in dT conformers with a higher dipole moment in the *anti* forms, i.e. they are favoured in a polarizable environment with water. It is noted that by B3LYP the dipole moment is in general underestimated, ca. .2 D, as compared to the calculated values by MP2.

3.3. Conformational angles analysis

An overall examination of the six exocyclic torsional angles, defining the conformational space of the NK prodrug, leads to conclude the following:

- (1) The interring dihedral angle χ presents a trimodal distribution, conformers A (from -175° to -162°), B (from 58° to 88°) and C (from -137° to -112°), Table 1, Figure 5 and Figure 1-Sup (a). Conformers C are the most stable in accordance to the results in dT (Yurenko et al., 2007b) and to the mean value of -135° reported in a crystal database of uridine analogues (Allen et al., 1979). *Anti* forms (conformers A and C, 43 conformers) prevail in number over *syn* ones (conformers B, 24 conformers) and cover a wider range of χ values. Also they slight dominate in the low energy range < 2 kcal/mol, *anti/syn* = 60/40%, similar to that found in AZT, 70/30%. This fact has been interpreted (Yurenko et al., 2007b) by the less sterical restricted by noncovalent interactions between BU and SU in the *anti* forms. X-ray data of AZT (Birnbbaum, Giziewicz, Gabe, Lin, & Prussoff, 1987; Carerman, Mastropaolo, & Carerman, 1987; Dyer, Low, Tollin, Wilson, & Howie, 1988; Parthasara, 1988) and related nucleosides show the χ angle in *anti* orientation, which is the preferred for biological activity (Painter et al., 2000). However, calculations in AZT (Alcolea Palafox & Talaya, 2010) reveal that the global minimum appears in the *syn* form and many conformers in the *syn* and *anti* forms have similar energy. In DN's this χ torsion angle is reported to have a calculated value by RFC (Relaxed force constants) within

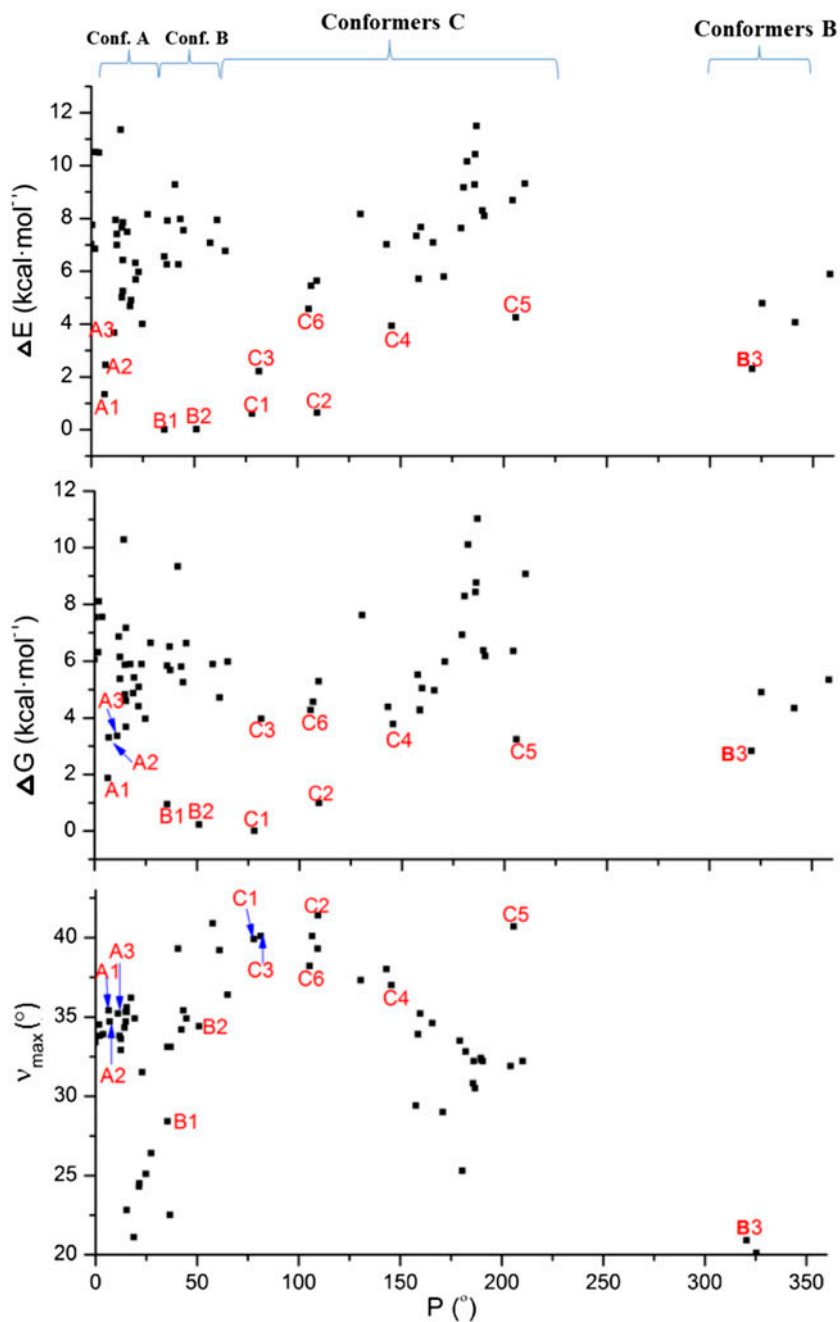


Figure 4. Distribution of the 86 optimum stable calculated conformers in NK at the B3LYP/6-31G** level, according to their phase angle of pseudorotation P and their: (a) relative electronic energy $\Delta E + \text{ZPE}$ correction; (b) relative Gibbs energy ΔG and (c) pucker amplitude, ν_{max} . The most stable conformers of each type are pointed: C1–C6, A1–A3 and B1–B3.

5–10 kcal·mol⁻¹·rad⁻² depending on the furanose conformation (Nikolaienko, Bulavin, & Hovorun, 2012a).

- (2) The α angle assumes values within the following ranges: $-162^\circ \leq \alpha_t \leq -135^\circ$ (8 conformers), $136^\circ \leq \alpha_t \leq 170^\circ$ (10 conformers), $-110^\circ \leq \alpha_{g^-} \leq -29^\circ$ (18 conformers) and $3^\circ \leq \alpha_{g^+} \leq 116^\circ$ (31 conformers), Figure 5. Those conformers in the last

range are the most stable, Figure 1-Sup (c). Correlation of α with χ , β and δ angles was not observed, Figure 2-Sup. and Figure 3-Sup.

- (3) In AZT and dT the β angle has a clear trimodal distribution with short range of values. However, in NK the definition of this angle is different and the ranges appear much larger: $43^\circ \leq \beta_{g^+} \leq 148^\circ$ (33 conformers), $164^\circ \leq \beta_t \leq 180^\circ$ (4 conformers),

$-179^\circ \leq \beta_t \leq -156^\circ$ (6 conformers) and $-147^\circ \leq \beta_{g-} \leq -45^\circ$ (24 conformers), Figure 5. In the β_t range the conformers are the least stable, more than 4 kcal/mol, Figure 6 and Figure 1-Sup. (d). In DNs this torsional angle is reported to be as soft as the pseudorotation phase P with a calculated value by RFC within $1\text{--}10 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{rad}^{-2}$ depending on the conformation (Nikolaienko et al., 2012a).

- (4) The γ angle has a trimodal distribution: the main group cover the $155^\circ \leq \gamma_t \leq 180^\circ$ and $-179^\circ \leq \gamma_t \leq -163^\circ$ ranges (29 conformers), and smaller ones at $41^\circ \leq \gamma_{g+} \leq 98^\circ$ (18 conformers) and at $-86^\circ \leq \gamma_{g-} \leq -40^\circ$ (20 conformers). Conformers with γ_{g+} are the most stables, Figure 6

and Figure 1-Sup. (f). Correlation of γ with the χ , ϕ and δ angles was not observed. In DNs this torsion angle is reported to be the most rigid one with a calculated value by RFC of $15\text{--}30 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{rad}^{-2}$ (Nikolaienko et al., 2012a).

- (5) The δ angle has a trimodal distribution: δ_{g+} around $112^\circ \pm 2^\circ$ (6 conformers), $78^\circ \leq \delta_{g-} \leq 106^\circ$ (41 conformers) and $129^\circ \leq \delta_t \leq 153^\circ$ (20 conformers). Some correlation/tendency is observed between δ and P angles, Figure 5. In conformers C an increment in δ produces an increase in P , while in conformers A and B the relation is not clear. The distribution of the conformers according to their energies and δ angle is plotted in Figure 6 and Figure 1-Sup. (b).

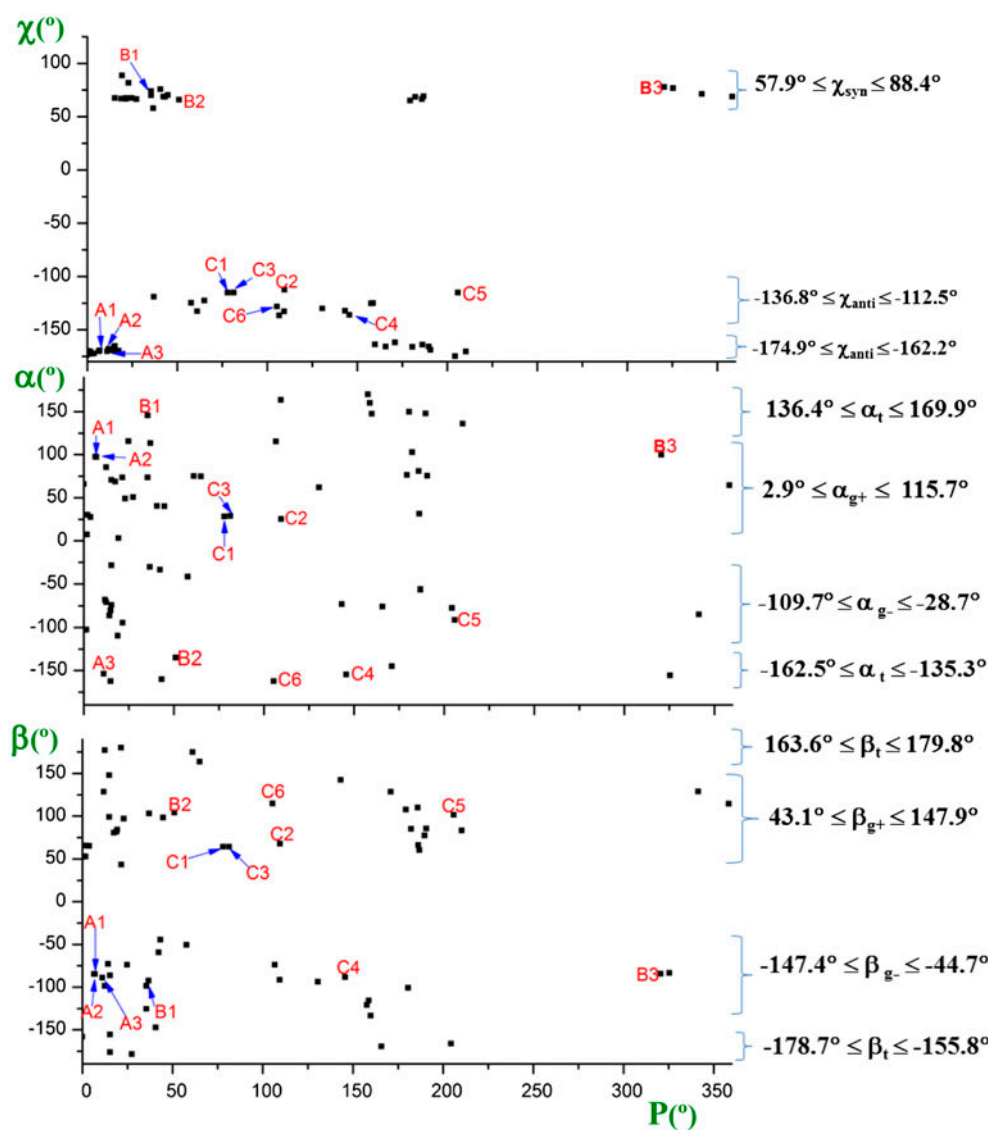


Figure 5. Distribution of the 86 optimum stable calculated conformers in NK, according to the values of the six exocyclic torsional angles: χ , ϕ , γ , α , β and δ , vs. the pseudorotational phase angle P .

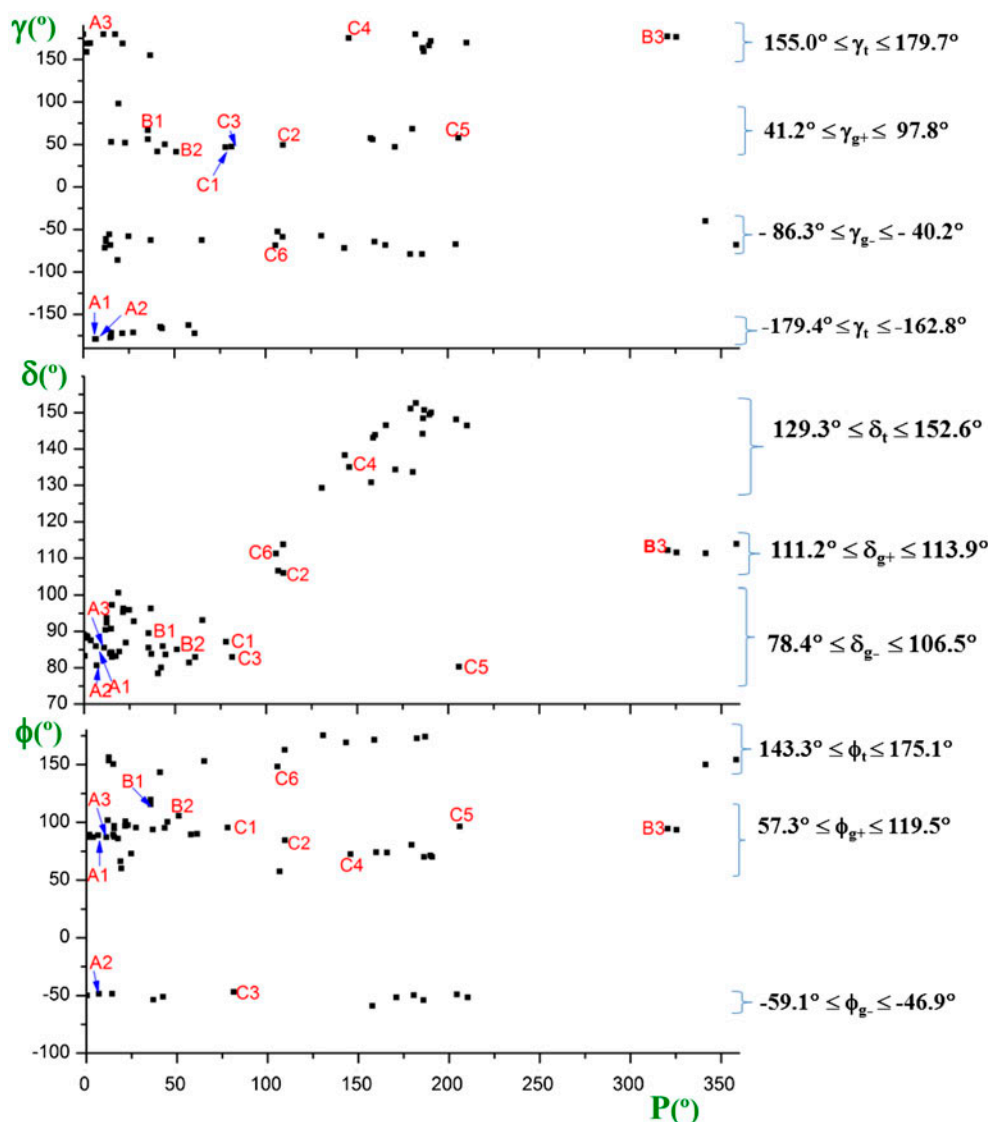


Figure 5. (Continued)

(6) A trimodal distribution has been obtained for the ϕ angle, Figure 5 and Figure 1-Sup. (e): $57^\circ \leq \phi_{g+} \leq 119^\circ$ (40 conformers), $143^\circ \leq \phi_t \leq 175^\circ$ (15 conformers) and $-59^\circ \leq \phi_{g-} \leq -47^\circ$ (12 conformers). In the ϕ_{g+} range appears the conformers with the highest stability, while in the other ranges the conformers are less stable, in general more than 4 kcal/mol, Figure 1-Sup. (e). Variations in the ϕ angle do not produce significant changes in the conformation adopted by the sugar (Figure 5 and 7(b)) or in the parameters of the thymine moiety. The rotations of the azide group to produce g^- and t azides states result in energy differences on the order of H-bonding strength. Crystallographic intermolecular forces (including H-bonding) may prevent in AZT a ϕ_{g-} azide orientation in the solid

state (Sabio & Topiol, 1992). The azide group is nonlinear with the $N3'-N3''-N3'''$ angle in conformer C1 of 173° .

Correlations among the exocyclic torsional angles α , γ , χ , ϕ and β were not found, and an almost regular distribution of the conformers can be observed, Figure 3-Sup. It can be explained by the high flexibility of the structure, which permits many value combinations of the exocyclic torsional angles. The flexibility of DNA constituents have been analysed by several authors (Nikolaienko et al., 2011). The stability areas of the conformers were established, and thus the largest ranges where there are not conformers correspond to: $-112^\circ \leq \chi \leq 58^\circ$, $-47^\circ \leq \phi \leq 57^\circ$, $-40^\circ \leq \gamma \leq 41^\circ$ and $-45^\circ \leq \beta \leq 43^\circ$.

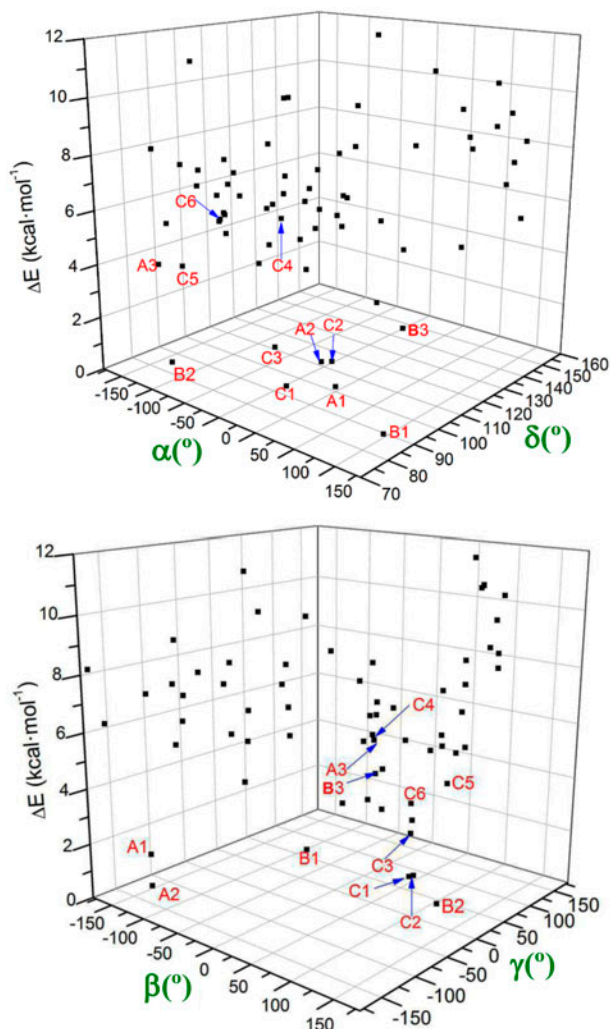


Figure 6. Relative energies of the 86 optimum stable conformers in NK, according to the values of the exocyclic torsional angles: γ , α , β and δ .

3.4. Thymine moiety

The pyrimidine ring in the uracil molecule shows a significant conformational flexibility (Isayev, Furmanchuk, Shishkin, Gorb, & Leszczynski, 2007; Shishkin, Gorb, & Leszczynski, 2000; Shishkin et al., 2000). High conformational flexibility of pyrimidine rings in nucleobases represents important source of relaxation in the molecular geometry for various inter- and intramolecular interactions (Shishkin, Gorb, & Leszczynski, 2009). According to several authors (Shishkin, Pelmenschikov et al., 2000a) the geometry of a nucleobase is almost unchanged when it is incorporated into the nucleosides, and it maintains a non-planar and non-rigid conformation. In the analysis of the six most stable conformers in NK, the base heterocycle also appears to be with a small nonplanarity, in general with torsional angles lower than 2° . It is arising due to the structural variability of the

bases (Hovorun, Gorb, & Leszczynski, 1999), the anisotropy influence of the sugar residue on the base, and the weak interactions or H-bonds of the thymine base with the phosphate moiety. Twice higher nonplanarity is calculated with the MP2 method. It is because DFT methods underestimate the conformational flexibility of the pyrimidine ring (Shishkin et al., 2003).

Among six dihedral angles describing the base heterocycle nonplanarity, $C5=C6-N1-C2$, $C6-N1-C2-N3$, $N1-C2-N3-C4$, $C2-N3-C4-C5$, $N3-C4-C5=C6$ and $C4-C5=C6-N1$, the three first are the most dependent on the nucleoside conformation (Yurenko et al., 2007b), and they fall into the largest ranges of values, while the remaining three angles cover rather narrower ranges and values lower than 1° . The six concerned torsional angles correlate with each other in such a way that their algebraic sum is close to 0° . A similar nonplanarity has been observed in dT, in this case due to interactions with the $-CH_2-O5'H$ moiety. Analogous results were obtained in related nucleosides (Alcolea Palafox & Iza, 2012; Alcolea Palafox & Talaya, 2010; Alvarez-Ros & Alcolea Palafox, 2013).

The bond lengths and angles do not differ significantly of resembling nucleosides. The $C2=O$ and $C4=O4$ bond lengths have similar values as in AZT (Alcolea Palafox & Talaya, 2010). The $C6-N1-C1'$ angle is slight more open, ca. 3° , than $C2-N1-C1'$, because of the weak $O2 \cdots H1'$ interaction. The sum of the dihedral angle on N1 is ca. 360° .

The methyl group orientation remains almost unchanged in many conformers. It corresponds to a gauche position of two of the methyl hydrogen atoms respect to O4, and it was denoted as conformation 2 (the only stable one) in the thymine molecule (Rastogi, Singh, Jain, & Alcolea Palafox, 2000). In conformer C1 of NK, the methyl group appears very little rotated with a $C4-C5-C7-H_{methyl}$ torsional angle of 176° , similar to that calculated in conformer I of AZT (Alcolea Palafox & Talaya, 2010), and with the same values of the bond lengths and angles. These features have been observed in other conformers, indicating that the phosphate moiety and the $-CH_2-O5'H$ moiety of AZT have in general little influence on the methyl group parameters.

3.5. Furanose moiety

The nucleosides are very flexible molecules, specially its sugar part. This inherent property of the nucleoside molecules manifests itself by their strong conformational inhomogeneity in the crystals and solutions (Jeffrey & Saenger, 1994; Saenger, 1984). The flexibility of a nucleoside depends on the furanose ring conformation, and this conformation of the furanose ring strongly depends on the nature and orientation of the nucleobase. All nucleosides possess different conformations of this ring,

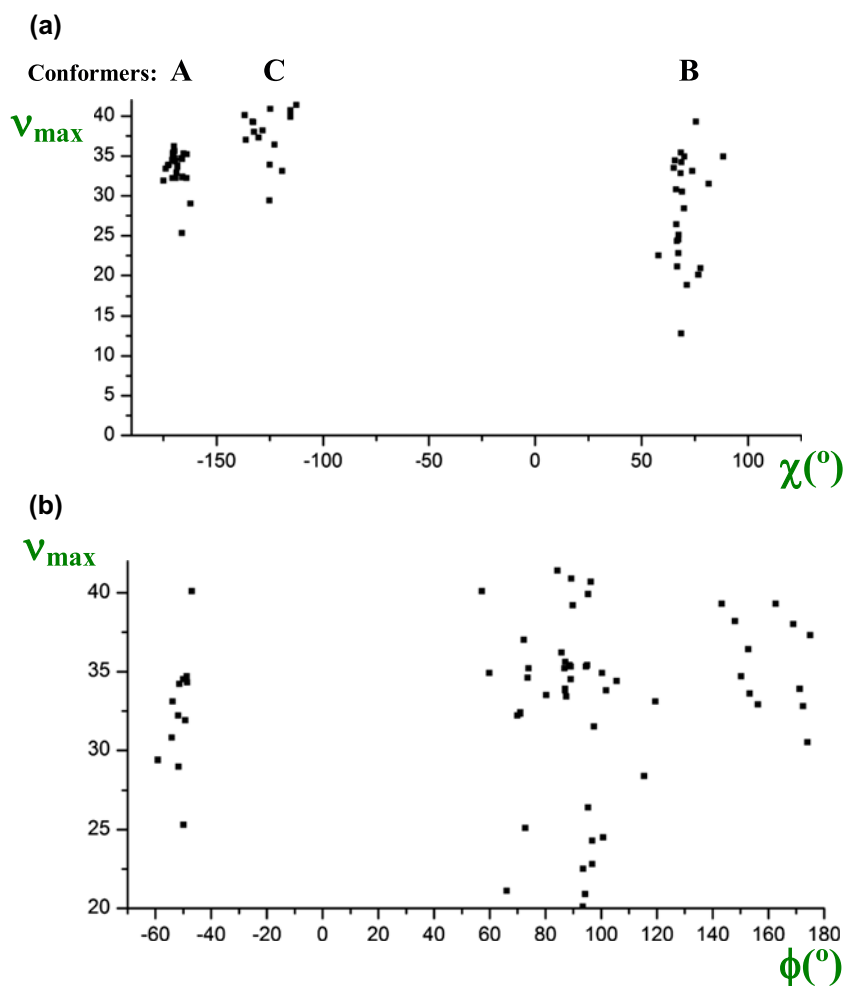


Figure 7. Distribution of the 86 optimum stable conformers in NK according to the values of the exocyclic torsional angles χ and ϕ vs. v_{\max} .

and significant influence of the steric repulsion between the nucleobase and the sugar ring give raises to changes in its puckering (Shishkin, Pelmenschikov et al., 2000a).

In NK the calculated N1–C1'–C2'–C3' torsional angle is 136° in conformer C1, ca. 20° lower than in C1 of AZT. The *ipso* angle C2–N1–C6, 122° , is the same that in AZT (Alcolea Palafox & Talaya, 2010) and in the natural nucleoside dT (Alcolea Palafox, Iza, de la Fuente, & Navarro, 2009). The C4'–O4' bond length is also the same in NK and AZT, 1.433 Å, while it is 1.439 Å in dT. However, C1'–O4' is lengthened, 1.448 vs 1.428 Å in AZT and 1.429 Å in dT. Both C–O4' bond lengths in the furanose ring have different value. It has been explained by the anomeric effect within the O4'–C1'–N fragment because of the interaction of a lone electron pair of the O4' atom and the antibonding orbital of the C1'–N bond (Jalluri, Yuh, & Taylor, 1993; Saenger, 1984). The values of the C–C bond lengths are

not equal within the furanose ring. They essentially depend on the conformation of the sugar ring, while the C–O4' bonds do not show such dependence (Shishkin, Pelmenschikov et al., 2000a).

A change in the puckering mode results in a considerable deformation of the endocyclic bond angles. However, in general, the values of these angles follow the order C–O–C > C–C–O > C–C–C.

The sugar is usually characterized by three structural parameters (Saenger, 1984): (i) the endocyclic torsional angles v_0 – v_4 ; (ii) the pseudorotation phase angle P , Table 1 and (iii) the maximum torsional angle (degree of pucker), v_{\max} . Whereas P and v_{\max} are biologically important parameters since their values are used to distinguish A- and B-conformations of the DNA and DNAs (Saenger, 1984), the values of v_0 – v_4 torsions present null interest for molecular biologists (Nikolaienko et al., 2012a).

Figure 4 shows three diagrams with the distribution of the 67 calculated conformers according to the P angle vs. the ΔE , ΔG energies and ν_{\max} .

(i) The endocyclic torsional angles have a large range of variability, in NK: $-42^\circ \leq \nu_0 \leq 18^\circ$, $-31^\circ \leq \nu_1 \leq 38^\circ$, $-37^\circ \leq \nu_2 \leq 38^\circ$, $-40^\circ \leq \nu_3 \leq 41^\circ$ and $-28^\circ \leq \nu_4 \leq 41^\circ$. The values of ν_0 to ν_3 angles have in general different sign in conformers A and C. This fact leads to values of the pseudorotation phase angle P as S-type in many conformers C and N-type in many conformers A. The algebraic sum of ν_i dihedral angles ($i=0, 1, \dots, 4$) is close to 0 for all conformers. These values are in accordance to those reported in dT (Yurenko et al., 2007b), and to those found in AZT (Alcolea Palafox & Talaya, 2010).

(ii) Six ranges of P are preferred for the different conformers: $0^\circ \leq P \leq 27^\circ$ (C_3 -endo, C_2 -exo, N-type) (26 conformers), $35^\circ \leq P \leq 65^\circ$ (C_3 -endo, C_4 -exo, N-type) (12 conformers), $78^\circ \leq P \leq 81^\circ$ (O_4 -endo, C_4 -exo, symmetrical twist N-type) (2 conformers), $105^\circ \leq P \leq 110^\circ$ (O_4 -endo, C_1 -exo, symmetrical twist S-type) (4 conformers), $131^\circ \leq P \leq 210^\circ$ (C_2 -endo, C_1 -exo, C_3 -exo, S-type) (21 conformers) and $321^\circ \leq P \leq 358^\circ$ (C_1 -endo, C_3 -endo, C_2 -exo, N-type) (4 conformers). The terms *endo* and *exo* refer to the displacement of an atom above or below the mean plane of the ring, respectively. Conformers with P values of $93 \pm 12^\circ$, $120 \pm 10^\circ$ and $265 \pm 55^\circ$ were not determined. The P angle covers a wider range of variation in NK than in AZT.

The number of N-type conformers (44 conformers) is almost twice that S-type (23 conformers). In AZT is also N-type but in dT is slightly S-type or almost the same (Yurenko et al., 2007b). S-conformations rather than the more usual N-conformations favour axially oriented 5'-substituents, i.e. the 5'-phosphorylation and anti-HIV activity. This feature is in accordance to our most stable conformer C2 by MP2.

(iii) ν_{\max} has a large range of values $13^\circ < \nu_{\max} < 41^\circ$. This range is slightly larger than in AZT. In conformers A and C the range is shorter than in conformers B, Figure 7(a). Also the values in A and C are in general higher than in B. In the *anti* orientation the conformers with high stability appear in general with large ν_{\max} , Figure 4. Taking into account that the value of ν_{\max} represents the radius of the pseudorotational cycle, its impact as structural parameters is quite high. An increase in the ring puckering produces an increment in the flexibility of the molecule, which could make easier to adapt to the active site. In d4T a ca. 10-fold difference in ν_{\max} between d4T and N-MCd4T is responsible for the 4–10-fold difference in potency (Choi, George et al., 2003).

The azide group in position t only gives rises to high values of ν_{\max} , Figure 7(b), while in positions g^- and g^+ the azide group has a large range of ν_{\max} values. Correlation between ν_{\max} and γ has not been observed. NK

has much higher ν_{\max} than those reported in the X-ray data of related NRTIs and collected in the Cambridge database (Choi, George et al., 2003; Choi, Moon, Yoshimura, & Marquez, 2003). Moreover, the most stable conformers in NK have in general a P and ν_{\max} higher than the corresponding ones of AZT. Perhaps it is due to in NK appears more intramolecular H-bonds and stronger than in AZT, and these H-bonds increase the ring puckering.

3.6. Intramolecular H-bonds

The *syn* and *anti* conformations of the thymine moiety relative to the furan ring provide opportunities for H-bonds involving the O6''H of the phosphate moiety with either thymine's carbonyl oxygen atom at position 2 (*syn* forms) or the furanose oxygen atom at position 4', respectively. Several authors have studied the intramolecular H-bonds in related nucleosides, in special using AIM method (Brovarets' & Hovorun, 2010; Fidanza, Sosa, Lobayan, & Peruchena, 2005; Nikolaienko et al., 2012b; Yurenko, Zhurakivsky, Samijlenko, Ghomi, & Hovorun, 2007; Yurenko, Zhurakivsky, Samijlenko, & Hovorun, 2011). According to the classification of the H-bonds (Desiraju & Steiner, 1999; Panigrahi & Desiraju, 2007), six intramolecular H-bonds may be observed in the main conformers of NK, Figure 3: (i) hydroxyl hydrogen H6''(O6'') and oxygen O4', O6''-H6'' \cdots O4', (ii) thymine's position six hydrogen atom and carbonyl oxygen O6', C6-H6 \cdots O6', (iii) thymine's position six hydrogen atom and hydroxyl oxygen O6'', C6-H6 \cdots O6'', (iv) thymine's carbonyl oxygen atom at position 2 and hydroxyl hydrogen H6'', O6''-H6'' \cdots O2, (v) C5'-H \cdots O6'' and (vi) C2'-H2'' \cdots O2.

Mainly, conformers C (*anti*) involve the H-bonds (i) to (iii), and conformers A (high-*anti*) the H-bonds (i) to (iii). By contrast, conformers B which is achieved by a *syn* relationship of the thymine and furan rings, involve the H-bonds (iv) and (v). Only in these conformers B the H-bonds appear through O2 and H5'(C5'). The characteristic of the C-H \cdots O hydrogen bonds in DNt clearly revealed their dependence on charge of the phosphate group (Shishkin et al., 2008). These C-H \cdots O intermolecular H-bonds in pair of natural nucleobases have been well analysed (Brovarets', Yurenko, & Hovorun, 2014).

H-bond (i) appears to give a great stability to the structure. Thus, it is observed in conformers C1-C3, C5 and in A1-A2, Figure 3. This H-bond (i) is stronger than (ii) and (iii), but slight weaker than (iv). In conformer C1 the value of H6'' \cdots O4' (1.891 Å) and the O4' \cdots H6''-O6'' angle (140°) confirms that H-bond (i) is relatively strong. In conformer C2 a value of this H-bond of 1.948 Å leads to the highest ν_{\max} , 41°. With longer H-bond, > 2.0 Å, the value of ν_{\max} remarkably decreases. In conformers C the rotation of

the base around the glycosyl bond χ toward higher angles (conformers A) leads to a decrease in the strengthening of this H-bond.

The strongest H-bond (iv) may be responsible of the low flexibility of conformers B, with the lowest ν_{\max} . This fact, together with a slight H-bond (v) in several conformers B may be responsible of a lower flexibility of these conformers than conformers A and C. Note that of the 12 conformers of Figure 3, only C1, A1, A2 and B1 are stabilized by two H-bonds. Conformers C2, C3, C5, A1 and A2 are stabilized by the same (i) conventional H-bond. The low stability of conformer A3 can be explained by the absence of intramolecular H-bonds.

As in related nucleosides, other weak H-bonds (not plotted in Figure 3) can appear in NK, such as (vi) $O2 \cdots H2'$, or $O4' \cdots H6$, but the values obtained indicate a weak or an almost non-existent H-bond. Several authors have reported (Yurenko et al., 2007) 13 types of intramolecular H-bonds in dT and related DN's. In the present work we have omitted those that are very weak, and thus only six types are shown in NK. However, neither of these H-bonds in NK can be compared with those found in AZT (Alcolea Palafox & Talaya, 2010), dT (Alcolea Palafox et al., 2009; Yurenko et al., 2007b) and d4T (Alcolea Palafox & Iza, 2012, 2013).

3.7. Low-frequency vibrations

Low-lying molecular vibrations have been studied by different authors (Hovorun et al., 2002; Martel, Hennion, Durand, & Calmettes, 1994; Pelmenchikov, Hovorun, Shishkin, & Leszczynski, 2000; Shishkin, Pelmenchikov, Hovorun & Leszczynski, 2000b) and their presence indicates a high flexibility in the molecular structure

that may be caused by various factors. This kind of vibrations may play an important role in some biological functions of DNA, such as the melting of the double helix, transcription and replication, specific interactions with proteins and drugs and gene expression (Shishkin et al., 2000b). Because the very large size of DNA systems, primary efforts have been focused on its small constituents, namely sugars (Krasnokutski, Ivanov, Izvekov, Sheina, & Blagoi, 1998; Zhbankov et al., 1998) and bases (Nowak, Lapinski, Kwiatkowski, & Leszczynski, 1997). DN's molecules possess high conformational flexibility and they are characterized by several vibrational modes below 200 cm^{-1} . These modes may be important for local recognition of every type of nucleoside. Moreover, the pattern of individual vibrations for every DN's also reflects the details of the conformation of the SU. Taking account the high deformability of SU, these low frequencies should be very sensitive to changes in the local environment, such as hydration and interactions with other biomolecules.

Thus, in the present work a theoretical analysis of the low-lying vibrations of NK with frequencies below 200 cm^{-1} was carried out. The results are shown in Table 3. We have considered only the most stable conformer C1 calculated in the *anti* form by B3LYP. In the last column of this Table is shown the characterization of every computed frequency, including the %PED. Contributions lower than 10% were not considered. Fourteen frequencies were found below 200 cm^{-1} , that are described in graphical form in Figure 4-Sup. These frequencies can be compared to the nine values calculated in dT at the B3LYP/6-31G** level and in the most optimum conformer C1, a *C2'-endo* ²E form. (Shishkin et al., 2000b) reported the values in dT at the B3LYP/6-31G* level, while all our values in dT and NK were at B3LYP/6-31G** level. The number and shape of these

Table 3. Calculated harmonic wavenumbers (ω , cm^{-1}), scaled vibrational values (ν , cm^{-1}), infrared intensities (A , KM/Mole), reduced masses (μ , AMU), force constants (f , mDyne/\AA) and characterization obtained in the far-IR region ($<200 \text{ cm}^{-1}$) in the isolated state of conformer C1 of NK at the B3LYP/6-31G** level.

ω	ν	A	μ	f	Characterization
7	41	1.4	5.7	.0	42% $\gamma(\text{NNN}) + 23\% \delta, \gamma(\text{TU}) + 14\% \delta, \gamma(\text{SU}) + 10\% \delta(\text{PO}_3\text{H})$
22	55	.5	11.3	.0	50% $\delta(\text{NNN}) + 26\% \delta(\text{PO}_3\text{H}) + 15\% \delta, \gamma(\text{TU})$
35	68	1.8	10.0	.01	48% $\gamma(\text{PO}_3\text{H}) + 25\% \gamma(\text{NNN}) + 14\% \delta(\text{CH}_2) + 10\% \delta, \gamma(\text{SU})$
40	72	.6	8.1	.01	44% $\delta, \gamma(\text{TU}) + 11\% \delta(\text{NNN}) + 30\% \delta(\text{PO}_3\text{H}) + 10\% \delta, \gamma(\text{SU})$
46	78	2.5	5.8	.01	45% $\tau(\text{TU}) + 26\% \gamma(\text{PO}_3\text{H}) + 11\% \gamma(\text{CH}_3) + 10\% \delta, \gamma(\text{SU})$
83	113	1.5	6.8	.03	41% puckering $O4 + 28\% \gamma(\text{PO}_3\text{H}) + 11\% \tau(\text{SU}) + 10\% \gamma(\text{CH}_3)$
108	137	5.1	7.0	.05	48% $\gamma(\text{PO}_3\text{H}) + 30\%$ puckering $N1 \cdots O4$ (TU) + 13% $\delta(\text{SU})$
120	148	2.7	5.8	.05	41% $\Gamma(\text{PO}_3\text{H}) + 19\%$ puckering $O2 + 18\% \gamma(\text{CH}_3) + 11\% \tau(\text{SU})$
136	163	2.1	6.5	.07	34% $\gamma(\text{NNN}) + 33\% \Gamma(\text{PO}_3\text{H}) + 13\% \tau(\text{SU}) + 11\% \Gamma(\text{CH}_3)$
146	173	5.2	3.1	.04	32% $\Gamma(\text{PO}_3\text{H}) + 29\% \Gamma(\text{CH}_3) + 24\% \tau(\text{SU}) + 10\% \delta(\text{TU})$
154	180	.7	1.2	.02	92% $\Gamma(\text{CH}_3)$
161	187	3.4	9.9	.15	56% Puckering $N3 + 23\% \gamma(\text{NNN}) + 10\% \Gamma(\text{PO}_3\text{H})$
166	191	2.0	6.5	.11	31% $\Gamma(\text{PO}_3\text{H}) + 27\% \gamma(\text{NNN}) + 18\% \tau(\text{SU}) + 12\% \Gamma(\text{TU}) + 10\% \Gamma(\text{CH}_3)$
193	217	.6	7.3	.16	26% $\Gamma(\text{PO}_3\text{H}) + 20\% \gamma(\text{NNN}) + 20\% \gamma(\text{C-O4'-C}) + 18\% \tau(\text{TU}) + 11\% \gamma(\text{SU})$

vibrations agree well with the high flexibility of the NK structure.

The first detail observed in Table 3 is the strong coupling of the azide (NNN) and phosphate (PO₃H) groups with thymine unit (TU) and SU. Moreover, the motions of these groups remarkably prevail over those of SU and TU. However, due to the importance of the SU and TU vibrations, we have analysed them in detail and compared the results with those reported by (Shishkin et al., 2000b). Every DN possesses a set of individual vibrations, whose frequencies and character strongly depend on the nature of the BU, that in the present case is TU, and on the SU conformation. An analysis of the shape of these vibrations in NK reveals that all modes corresponding to TU and SU can be divided into two groups, as it has been found in dT (Shishkin et al., 2000b).

The first group includes in dT the three lowest vibrations that have the same origin in all DNs. They represent the motion of BU and SU with respect to each other as almost rigid subsystems. The first vibration, the rotation around the glycosyl bond N1-C1' and notated as ν_1 (Shishkin et al., 2000b) is calculated at 37 cm⁻¹ in dT but in NK it is identified at 46 cm⁻¹. The rotation around N1-C1' does not involve any intramolecular deformation of both units, as it was found in dT. These units move relative to each other as rigid bodies. The calculated frequency at 7 cm⁻¹ can be also considered as ν_1 because it has a noticeable contribution of TU and SU.

The second vibration ν_2 is calculated in dT at 53 cm⁻¹, close to the value of 40 cm⁻¹ identified in NK. This vibration involves a strong deformation of TU, with an in-plane and out-of-plane motion of the atoms, at difference of the relative small deformation of both SU and TU units observed in dT. The motion of the whole structure seems as a γ (butterfly).

The third vibration ν_3 is calculated in dT at 57 cm⁻¹. In NK it is identified with some doubts at 83 cm⁻¹, because the motion of the atoms in SU matches to that observed in ν_4 . In NK the main contribution corresponds to a puckering of O4. This puckering is observed in dT but with strong contributions of O5'-H and O3'-H. Intramolecular deformation of both units is not observed in NK, and in dT this deformation appears only in TU. The motion of the whole structure seems as a γ (tilting "slanting").

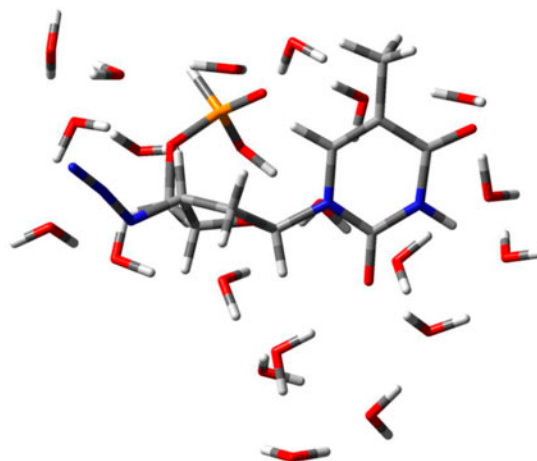
The second group of vibrations involve in dT intramolecular deformation of both TU and SU. The character and frequency of these vibrations strongly depend on the nature of TU, the conformation of SU and the mutual orientation of TU and SU (Shishkin et al., 2000b). The vibrations ν_4 and ν_8 in dT have a strong contribution corresponding to O3'-H and O5'-H groups. NK does not have these groups and thus these vibrations were not clearly identified.

The vibration ν_5 is calculated in dT at 107 cm⁻¹, very close to that at 108 cm⁻¹ in NK. This vibration can be described in SU as the rotation around the C2'-C3' bond and an in-plane deformation of C1'-O4'-C4'-C5', and in TU as a puckering on N1...O4 corresponding to normal mode No. 2 in uracil molecule (Alcolea Palafox, Iza, & Gil, 2002).

The vibration ν_6 in dT has a strong contribution due to the -C5'H₂-O5'H moiety. NK does not have this moiety and thus this vibration was not clearly identified. In dT the contribution of TU is very small while the contribution of the CH₃ group is very high. In NK the most similar vibration is that calculated at 154 cm⁻¹, although the contribution of TU and SU is almost null.

The vibration ν_7 can be described in SU as the rotation around the C1'...C4' axis, and in TU as a puckering on O2. It is calculated in dT at 142 cm⁻¹, while in NK is at 120 cm⁻¹. This difference can be due to the strong coupling in dT with the C5'H₂-O5'H in-plane rotation.

In dT the vibration ν_9 can be described in SU as the rotation around the C1'...C4' axis, but in NK it appears as the rotation around the C2'-C3' bond and an in-plane deformation of C1'-O4'-C4'-C5'. However, ν_9 is mainly represented as a puckering on N3 and characterized as normal mode No. 1 in uracil molecule. It is calculated in dT at 165 cm⁻¹, close to that in NK at 161 cm⁻¹. The small difference can be due to the coupling in dT with C5'H₂-O5'H in-plane rotation and with -O3'H deformation.



En.: -2984.065386 AU (-2984.173836); P = 67.12°, ν_{\max} = 39.51°

Figure 8. The most stable hydrated cluster of NikaVir with 20 explicit water molecules calculated at the B3LYP/6-31G** level. The furanose pucker P and maximum degree of pucker ν_{\max} are in degrees, and $E + ZPE$ and Gibbs energy (in parentheses) in AU.

3.8. Natural NBO Atomic Charges

The calculated values at the B3LYP and MP2 levels in conformer C1 appear collected in Figure 2. The largest negative charge is on O6' and O6'' atoms, ca. $-1 e$ (where e is the charge of an electron). The next atom with large negative charge is O5', ca. $-.8 e$. The values of the negative charges on these atoms of the phosphate moiety are much higher than any atom in AZT. Phosphate group charges represent an important role in stabilization of DNt (Gorb, Shishkin, & Leszczynski, 2005), and the incorporation of nucleotides to A-DNA macromolecules requires the minimum amount of deformation energy (Palamarchuk et al., 2009).

The charge on O2 is slight higher ca. $.03 e$ than on O4' at MP2 level, which it is only $.01 e$ higher than O4. In NK the charge on O4' is higher than in AZT, but the values on O2 and O4 are similar to those calculated in AZT. In the nitrogen atoms the negative charge on N3, $-.67 e$, is larger than on N1 atom (ca. $-.48 e$), as it was found in AZT (Alcolea Palafox & Talaya, 2010). H6'' hydroxylic hydrogen is the atom with the highest positive charge, $.557 e$ at the MP2 level, i.e. it is the most reactive. The next atom is H3, $.456 e$, with the same value that in AZT. The remaining hydrogen atoms have much less positive charge, ca. $.2 e$, i.e. they are less reactive. These features indicate that the phosphate group little affect the charge distribution on the thymine moiety.

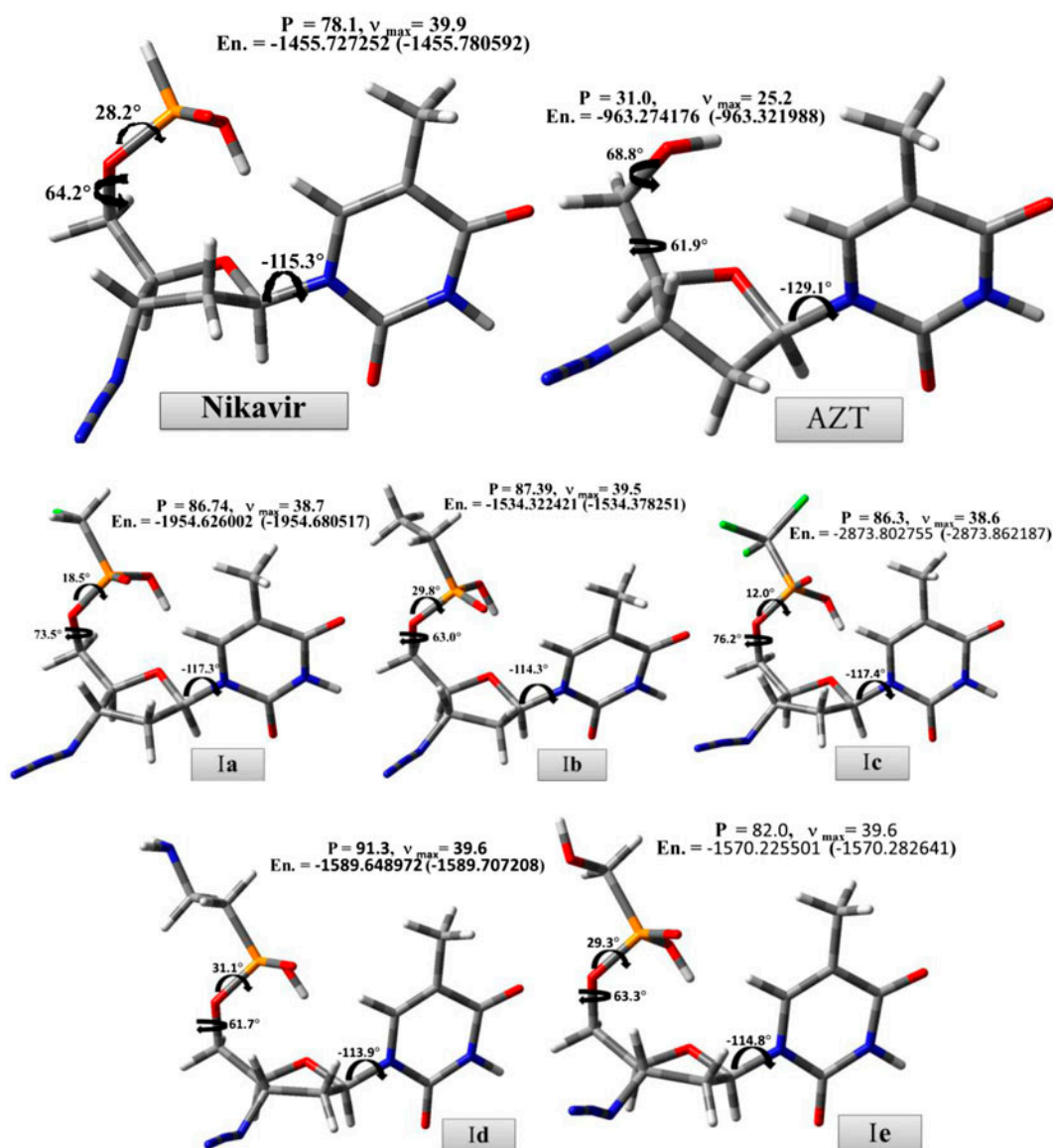


Figure 9. Optimized molecular structure at the B3LYP/6-31G** level of five NK derivatives. Furanose pucker P, and maximum degree of pucker v_{max} in degrees, and $E + ZPE$ and Gibbs energy (in parentheses) in AU.

In the carbon atoms, the highest positive charge is on C2 and C4 atoms, in concordance to the high negative charge on the O2 and O4 atoms, respectively. Because O2 has slight higher negative charge than O4, C2 has higher positive charge than C4.

The charges on the azide group could be of interest because it has been pointed the possibility that this group may not always be able to function as a terminal group, leading to one of the possible mechanisms for resistant mutations. Thus, nitrogen atoms with appropriate charges may participate in H-bonds with residues of the reverse transcriptase enzyme (RT), leading to a possible route of resistant mutations (Arissawa, Taft, & Felcman, 2003). The values obtained in NK indicate that the charge of N3''' is very low and almost zero, so bonding is not possible through this atom, although this possibility appears through N3' with a negative value of $-0.459 e$ by MP2.

3.9. Hydration of NikaVir

In previous publications, we have analysed the hydration effect on the most stable conformers in AZT (Alcolea Palafox & Talaya, 2010), dT, d4T (Alcolea Palafox et al., 2009; Alcolea Palafox & Iza, 2010) as well as in IUdR (Alcolea Palafox, 2014a). The hydration of nucleic acids with explicit water molecules by DFT and MP2 methods have been also reported by different authors (Danilov, Mourik, & Poltev, 2006; Danilov et al., 2009). In the present work the most stable cluster of the most stable conformer C1 in the *anti* form of NK with 20 explicit water molecules is shown in Figure 8.

The hydration was carried out following the so-called modified scheme of monosolvation, consists of the following steps (Danilov et al., 2006): first the structure of all possible monohydrated complexes is determined and the complex with the lowest energy is chosen; next, a

Table 4. Comparison with dT ($r_X - r_{dT}$, where $X = AZT, NK, Ia, Ib, Ic, Id, Ie$) of the geometrical values in the *anti* form of the most optimum conformer of several AZT derivatives at the B3LYP/6-31G** level.

Parameters	dT	$\Delta(r_X - r_{dT})$						
		$X = AZT$	NK	Ia	Ib	Ic	Id	Ie
C=O2	1.222	-.002	-.001	-.002	-.001	-.002	-.001	-.001
C=O4	1.223	-.010	-.010	-.010	-.010	-.011	-.010	-.010
C2-N3	1.384	-.001	-.005	-.005	-.004	-.006	-.005	-.004
N3-C4	1.406	0	.003	.003	.002	.003	.002	.003
N1-C6	1.385	.001	.008	.009	.008	.009	.008	.008
N1-C1'	1.464	.004	-.020	-.020	-.019	-.022	-.019	-.019
C1'-O4'	1.425	.003	.023	.022	.021	.024	.021	.022
C1'-C2'	1.535	.012	.012	.010	.007	.009	.006	.010
C2'-C3'	1.530	.008	.019	.019	.019	.019	.019	.019
O5'...O2	6.116	.146	.637	.580	.650	.544	.644	.648
O5'...O4	6.897	-.090	1.316	1.214	1.377	1.157	1.406	1.349
C2-N1-C1'	118.2	-.60	-.65	-.66	-.69	-.79	-.78	-.65
C3'-C4'-C5'	114.9	.12	1.33	.59	1.09	.51	1.19	1.24

Table 5. Comparison with dT ($q_X - q_{dT}$, where $X = AZT, NK, Ia, Ib, Ic, Id, Ie$) of the NBO atomic charges in the *anti* form of the most optimum conformer of several AZT derivatives at the B3LYP/6-31G** level.

Parameters	dT	$\Delta(q_X - q_{dT})$						
		$X = AZT$	NK	Ia	Ib	Ic	Id	Ie
O2	-.636	.004	.003	.004	.003	.005	.003	.003
O4	-.602	.010	.013	.014	.011	.017	.011	.011
N1	-.474	-.006	-.008	-.008	-.006	-.009	-.006	-.007
N3	-.674	0	0	0	0	0	0	0
C5	-.178	.012	.016	.017	.012	.021	.012	.013
C6	.032	-.013	-.02	-.022	-.020	-.023	-.020	-.020
H(C6)	.262	-.019	.011	.010	.015	.006	.014	.014
C3'	.070	-.185	-.184	-.183	-.183	-.183	-.183	-.184
C4'	.044	.011	.003	.007	.003	.008	.003	.003
O4'	-.593	-.005	-.031	-.032	-.028	-.034	-.028	-.029
O5'	-.778	.017	-.074	-.080	-.083	-.060	-.082	-.074

second water molecule is added and the hydrated complex with the lowest energy is located; this process is repeated until the water molecules form a closed chain around the NK structure. With this procedure, hydrated complexes were obtained that contain the water molecules distributed around the structure.

The hydration stabilizes a closed NK structure with a low value of χ up to -85° . This low value moves away the phosphate and the TU moieties, with a $P \cdots N1$ and $O6'' \cdots N1$ distances of ca. 5 Å. This increment in the distances and the broke of the $O6''-H \cdots O4'$ intramolecular H-bond perhaps facilitates the binding through $O6''H$ to

the ATP in the 2nd phosphorylation step, which should be in a perpendicular orientation to the TU ring. We are preparing a work with this simulation.

4. Five derivatives of Nikavir: structure–activity relationships

Another of the objectives of the present work was the possibility to establish general structure–activity relationships/tendencies with other antiviral drugs derivatives of NK, in special 6'-compounds. Thus, following the same methodology described above in NK, the five derivatives

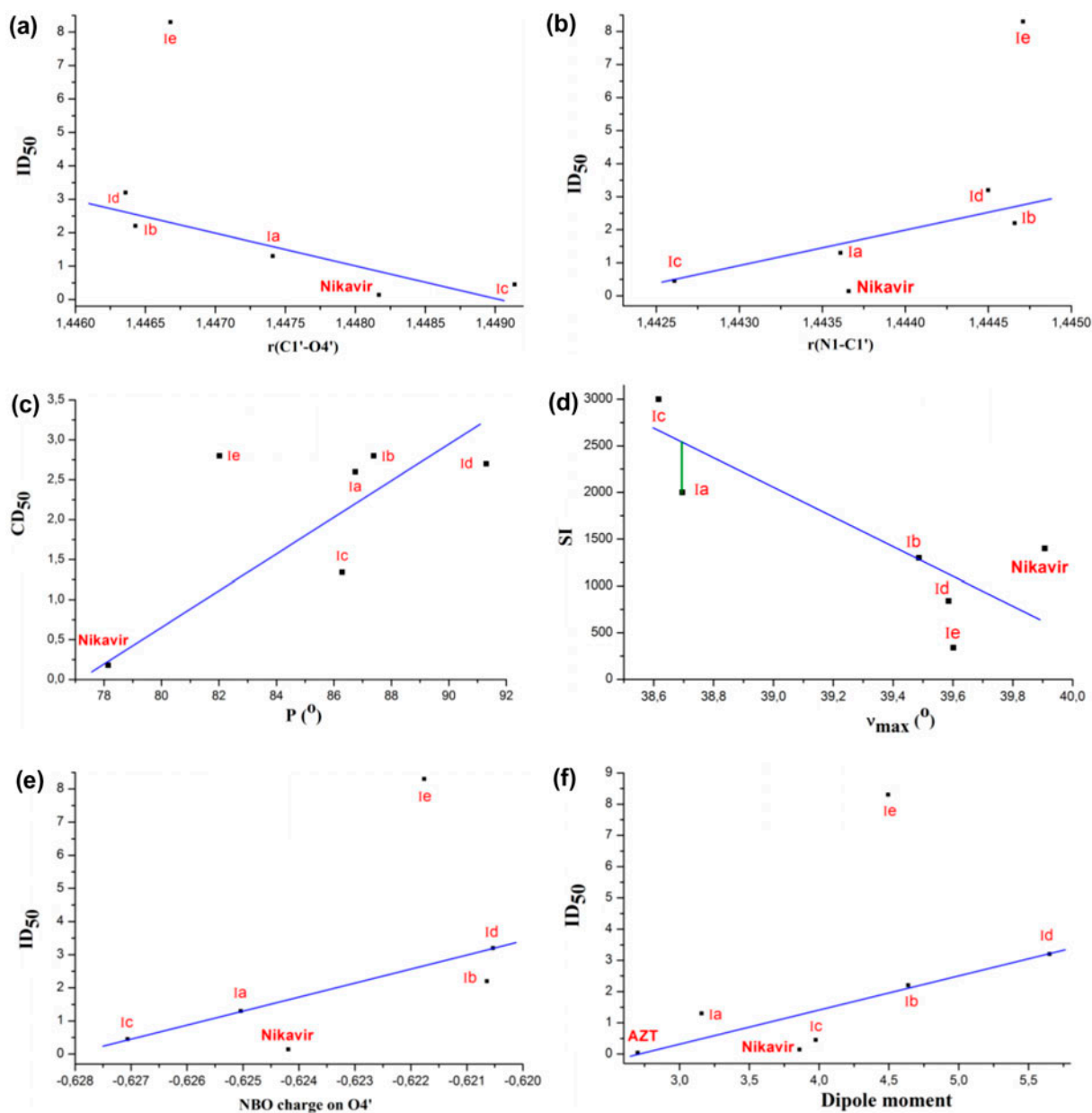


Figure 10. Structure–activity relationships/tendencies found in NK and five of its derivatives.

of NK with antiviral activity reported (Jasko et al., 2006) were studied, Scheme 2, and their molecular structures optimized, Figure 9. Frequency calculations were performed in all the structures to assess that they correspond to the global minimum. The global minimum in the *anti* form corresponds in all these derivatives to the C1 form. Considering this conformer, the main geometrical parameters and energies are summarized in Figure 9 as well as in Table 4. In this last case the values appear as difference related to dT. The different electronegativity of the substituents on P6' mainly produces a change in the NBO atomic charge on C3' and O5', Table 5, while the changes on O4, C6 and O4' can be considered small. The effect on O2, N3 and C4' is almost null. This feature gives rise to a small change in the dipole moment μ , as well as in the intramolecular distances, i.e. between the oxygen atoms O5'...O2 and O5'...O4.

The antiviral activities of the five 6'-NK derivatives selected, as well as those of AZT and NK are shown in Table 2-Sup (of Supplementary Material Section), which are expressed as CD₅₀, ID₅₀ and SI. It is noted that AZT is the compound with the lowest ID₅₀, i.e. with the highest antiviral activity. However, AZT has a high toxicity with strong secondary effects. NK is the 2nd compound with the highest activity but also with a noticeable toxicity, although much lower than AZT. The other 6'-derivatives under study have lower activity but also lower toxicity. With these derivatives several structure-activity relationships/tendencies can be observed, some of them are shown in Figure 10 as well as in Figure 5-Sup. of the Supplementary Material. In these figures the only compound far away corresponds to 1e. It has a very high ID₅₀ and very low SI index, i.e. very low activity that does not correspond according to its optimized structure in the isolated state. This compound has as substituent the HOCH₂ group and, perhaps the oxygen of this group (the other compounds do not have this oxygen) forms a strong H-bond net with the water molecules that difficult the bonding through the O'H group to ATP in the 2nd phosphorylation step, i.e. a low activity.

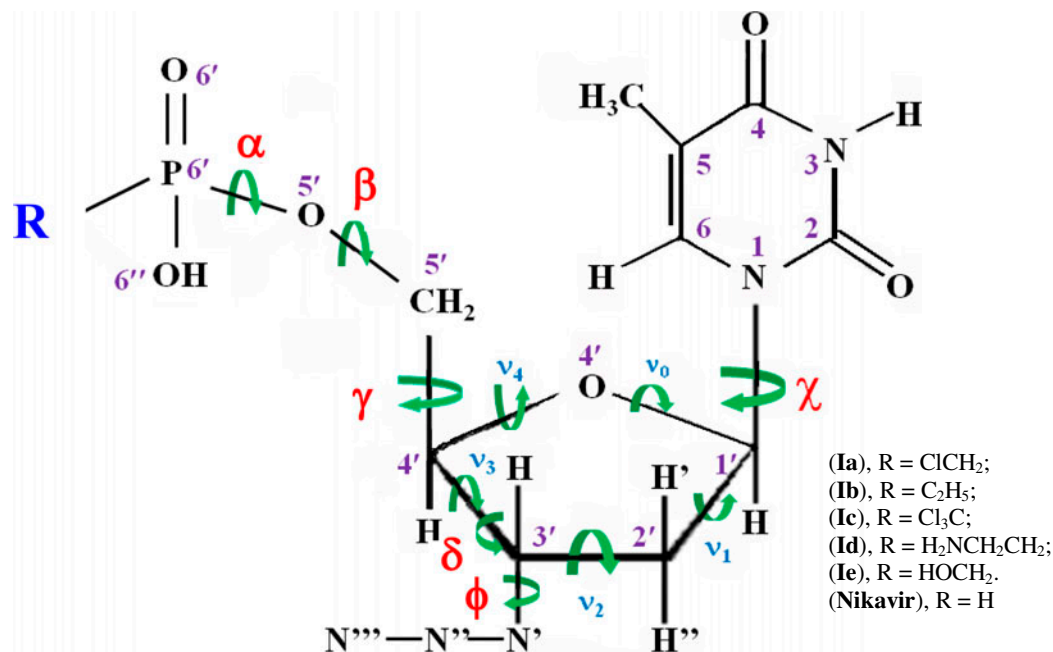
Avoiding this compound 1e, and analysing the different relations the following was noted:

- (1) A slight increase in the C1'-O4' bond length or decrease in N1-C1' reduces the value of ID₅₀, i.e. increases the activity, Figure 10(a) and (b), respectively. The relationships are almost linear. An increment in the C1'-O4' bond length also appears related to a decrease in CD₅₀, although in NK it has a very low value, Figure 5-Sup. (a).
- (2) A lengthening in the O5'...O2 intramolecular distance appears to reduce the value of ID₅₀, Figure 5-Sup. (b). Similar effect can be noted with the decrease of v_{\max} , Figure 5-Sup. (c). This decrease in v_{\max} improves the SI index, Figure 10(d).
- (3) Low concentration of CD₅₀ is required in compounds with small furanose pucker P, Figure 10(c). Correlations were not observed with the exocyclic torsional angles α , β and γ .
- (4) All the substituents on P6' that produce an increase in the NBO negative charge on O4', Figure 10(e), and decrease the dipole moment, Figure 10(f), increment the activity of the drug. Correlations were not observed with O5', neither with the other atoms. A decrease in the dipole moment also improves the SI index, Figure 5-Sup. (d).

5. Summary and Conclusions

A comprehensive compendium of the possible conformers of NK prodrug is presented and analysed. The geometries and values shown here appear to be the most accurate to date. Comparisons with AZT and dT provide support for the quality of our results. The most important findings of the present manuscript are the following:

- (1) Through a rotation of χ , α , β , γ , δ and ϕ angles in NK, the 67 most energetically favourable conformers were identified at the B3LYP/6-31G** level. Three *anti* and two *syn* falling into the 0–2 kcal/mol $\Delta E + \text{ZPE}$ energy range and 30 *anti*, 17 *syn* into the 2–8 kcal/mol energy range. MP2 computations were performed to confirm the stability order of the conformers.
- (2) Conformers in *anti* form are in general more stable than in high-*anti* and in *syn*. H-bond of H6'' with O4' is observed in the low-energy *anti* and high-*anti* conformations, while H6'...O2 is in the *syn* forms. These H-bonds appear to give a great stability to the structure.
- (3) The distribution of all the conformers according to the ranges of stability of the characteristic torsional angles was established. The values obtained indicate the flexible nature of NK, which is slightly higher than in AZT.
- (4) The phosphate group affects little the charge distribution on the thymine moiety, and the NBO charges on the atoms are almost the same that those calculated in AZT. The oxygen atoms of the phosphate moiety have the highest negative charge in AZT.
- (5) The negative charge of the phosphate group oxygens mainly leads to a remarkable increase in P and v_{\max} , as compared to AZT. This



Scheme 2. Molecular structure and definition of the exocyclic and endocyclic angles in Nikavir and several of its derivative prodrugs.

- increment in the ring puckering produces a higher flexibility of the molecule than AZT. Perhaps, it is due to in NK appears more intramolecular H-bonds and stronger than in AZT, and these H-bonds increase the ring puckering. These H-bonds of the phosphate group also give rise to a slight shortening of the N1-C1' bond length and an increment in the distances O5'...O2 and O5'...O4 as compared to dT.
- (6) Although in NK the number of N-type conformers is almost twice that S-type, the most stable conformer C1 by MP2 is S-type which is thought to facilitate the phosphorylation. The high flexibility of NK permits the subsequently switch to a N-type for a better interaction with RT.
 - (7) The value of v_{\max} has not correlation with the γ angle value. The angles χ , α , β , γ , δ and ϕ have not shown mutual correlation, as well as the angles χ , α , β , γ , δ , ϕ and v_0 , v_1 , v_2 , v_3 and v_4 .
 - (8) A detailed analysis of the lowest vibrations in conformer C1 has been carried out, and the results compared with those obtained in dT. The different vibrational modes were analysed. The presence of low-lying vibrational modes is indicative of the high conformational flexibility of NK.
 - (9) The most stable hydrated cluster of conformer C1 with 20 explicit water molecules was

determined. The hydration increments the P...N1 and O6''...N1 distances and it broke the O6''-H...O4' intramolecular H-bond, which perhaps facilitates the binding through O6''H to the ATP in the 2nd phosphorylation step.

- (10) All the NK derivatives with substituents on P6' that give rise to a lengthening in the C1'-O4' bond and in the O5'...O2 intramolecular distance, to an increase in the NBO negative charge on O4', to a decrease in N1-C1', v_{\max} , or in the dipole moment, increase the activity of the compound. The structure-activity relationships observed can help for the design of new anti-HIV drugs.

Our results indicate that NK possesses remarkably similar conformational profiles to those of its parent natural nucleoside dT. Due to this similarity NK mimics dT in biological systems and becomes incorporated into viral DNA and thereby brings about the termination of DNA synthesis. Good comprehension of the parameters investigated here could be essential for developing drugs with high anti-HIV activity and low toxicity.

Supplementary material

The supplementary material for this paper is available online at <http://dx.doi.10.1080/07391102.2014.909743>.

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Title: FT-IR AND FT-RAMAN SPECTRA OF 6-CHLOROURACIL: MOLECULAR STRUCTURE,
TAUTOMERISM AND SOLID STATE SIMULATION. A COMPARISON BETWEEN 5-CHLOROURACIL AND
6-CHOROURACIL

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Keywords: 6-Chlorouracil, 5-Chlorouracil, Raman spectra, IR spectra, Tetramer simulation, 6-
halouracil derivatives, tautomerism, mutagenicity

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Balachandran; D. K. Sharma

Abstract: A Raman and IR study of the biomolecule 6-chlorouracil was carried out in the solid state. The unit cell found in the crystal was simulated as a tetramer form by density functional calculations. Specific scale factors and scaling equations deduced from uracil molecule were employed in the predicted wavenumbers of 6-chlorouracil. The scaled wavenumbers were used in the reassignment of the IR and Raman experimental bands. Good reproduction of the experimental wavenumbers is obtained and the % error is very small in the majority of cases. A comparison between the molecular structure and charge distribution of 6-chlorouracil and 5-chlorouracil molecules was presented. The effect of the hydration with the PCM model in the molecular structure and charges was discussed. The optimum tautomers of 6-chlorouracil were optimized and analyzed. Six of them were related to those of uracil molecule. The effect of the halogen substitution in the sixth position of the pyrimidine ring in the stability of the different tautomers was evaluated. HOMO and LUMO orbital energy analysis were carried out.

Madrid February 4th 2014

SPECTROCHIMICA ACTA

Dear sir,

I submit to you, enclosed, the manuscript on " FT-IR AND FT-RAMAN SPECTRA OF 6-CHLOROURACIL: MOLECULAR STRUCTURE, TAUTOMERISM AND SOLID STATE SIMULATION. A COMPARISON BETWEEN 5-CHLOROURACIL AND 6-CHOROURACIL" by S. Ortiz, M.C. Alvarez-Ros, M. Alcolea Palafox, V.K. Rastogi, V. Balachandran, and D.K. Sharma to be published if suitable, in Spectrochimica Acta, part A.

Acknowledging your consideration, I remain.

Yours faithfully,

Dr. Mauricio Alcolea Palafox

Answer to the referee comments

Reviewer #1:

1.) There are some ambiguities and grammatical errors throughout the manuscript. That is the English in the whole the manuscript should be checked and corrected. Below, you can see a few examples.

➤ 3rd page 5th row: "Among these studies IT can be mentioned..."

➤ 3rd page, computational methods, 7th row: "... were selectED as more appropriated.

➤ 12th page, 3rd row: "The calculated wavenumber of mode (c2=O) remainS almost constant..."

➤ 15th page, 2nd paragraph, 3rd row: "This effect HAS been observed...."

Thanks for the comments. We have considered all the suggestions of the referee, and we have carried out the corresponding modifications.

2.) Several thermodynamic parameters were calculated and they are given in a table, however authors haven't explained them in the manuscript. There is just raw data, nothing about 6-chlorouracil and its tautomers?

We have added several lines to explain this Table. Also we have added several lines about the dipole moments in the tautomers.

3.) There are lots of things (such as depolarization ratios, etc.) in Table 1. In its current state, it is difficult to follow all the data, is it possible to simplify it?.

We suppose that the reviewer refers to Table 3. We have removed two columns in this Table 3.

4.) In Figure 7, in the experimental IR spectrum of 6-CIU, you can give the spectrum in the range between 3600-2000 cm^{-1} . So that, the noise between 4000 and 3600 cm^{-1} will be removed.

We have modified the spectra to the 3600-2000 cm^{-1} range.

Reviewer #2:

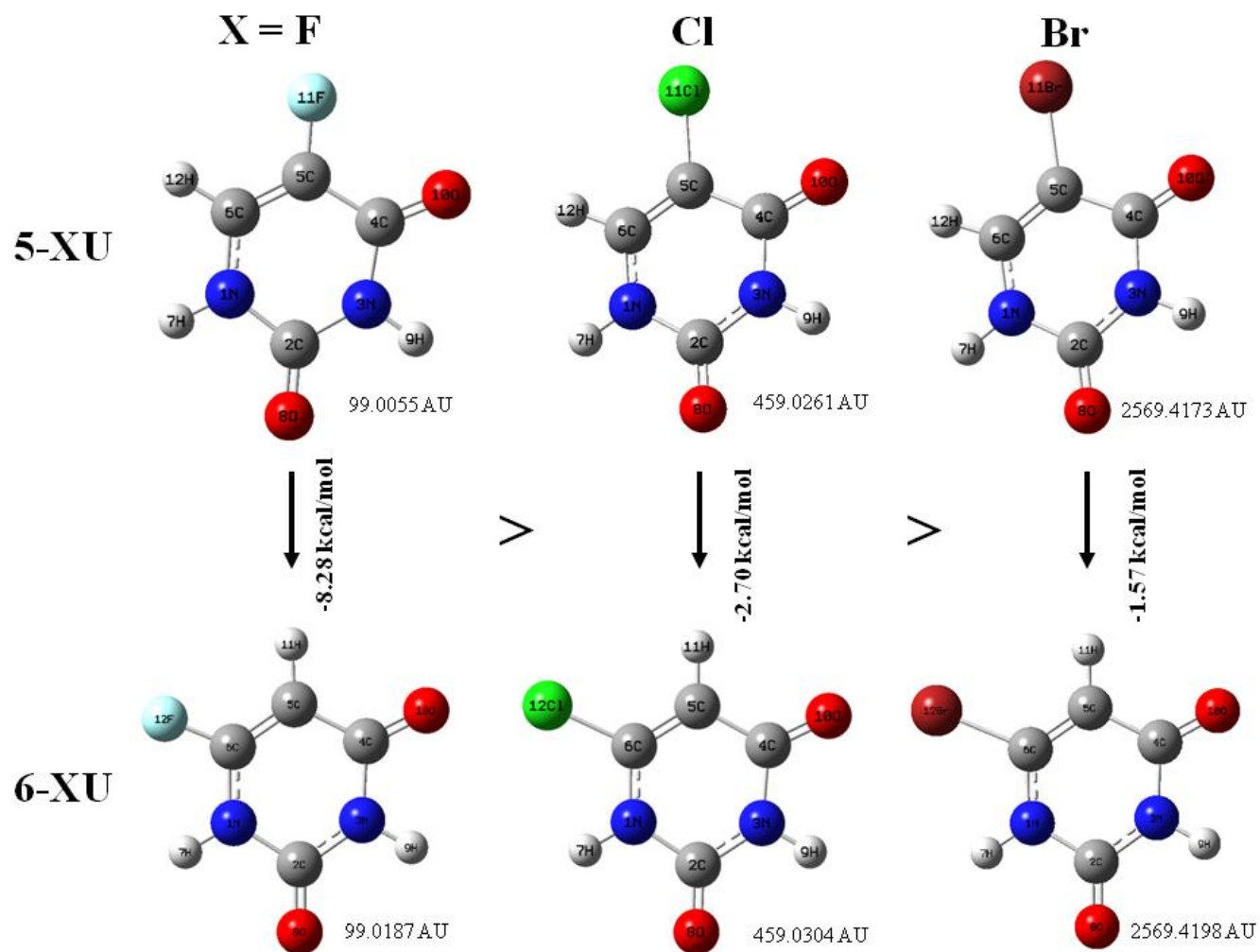
This is a profound paper in which the Raman and IR spectra of 6-chlorouracil in the solid state (a sample of great interest in pharmaceutical and clinical research) were simulated and interpreted. The authors have extended experience of this kind of studies of halouracils in the theoretical and vibrational point of view applying high level in the quantum chemical calculations. There are not many such studies performed particularly for solids and the present paper is a good demonstration for this and should be published essentially as is.

Thanks for these nice comments.

In order to be conform with SI units, I suggest to replace the units Kcal/mol by KJ/mol throughout manuscript including figures

We have changed them

HALOGENATION EFFECT IN THE ENERGY OF 5- AND 6- URACIL DERIVATIVES



Highlights

- All the tautomers of 6-chlorouracil are more stable than those in uracil molecule.
- The Raman and IR spectra of 6-ClU in the solid state were simulated and interpreted.
- The solid state simulation with scaled values was carried out by a tetramer form.
- Halogenation produces a smaller effect for 5-XU than for 6-XU derivatives.
- The low energy gap HOMO-LUMO facilitates the intra molecular charge transfer.

Spectrochim. Acta

FT-IR AND FT-RAMAN SPECTRA OF 6-CHLOROURACIL: MOLECULAR STRUCTURE, TAUTOMERISM AND SOLID STATE SIMULATION. A COMPARISON BETWEEN 5-CHLOROURACIL AND 6-CHOROURACIL

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ABSTRACT

A Raman and IR study of the biomolecule 6-chlorouracil was carried out in the solid state. The unit cell found in the crystal was simulated as a tetramer form by density functional calculations. Specific scale factors and scaling equations deduced from uracil molecule were employed in the predicted wavenumbers of 6-chlorouracil. The scaled wavenumbers were used in the reassignment of the IR and Raman experimental bands. Good reproduction of the experimental wavenumbers is obtained and the % error is very small in the majority of cases. A comparison between the molecular structure and charge distribution of 6-chlorouracil and 5-chlorouracil molecules was presented. The effect of the hydration with the PCM model in the molecular structure and charges was discussed. The optimum tautomers of 6-chlorouracil were optimized and analyzed. Six of them were related to those of uracil molecule. The effect of the halogen substitution in the sixth position of the pyrimidine ring in the stability of the different tautomers was evaluated. HOMO and LUMO orbital energy analysis were carried out.

Keywords: 6-Chlorouracil, 5-Chlorouracil, Raman spectra, IR spectra, Tetramer simulation, 6-halouracil derivatives, tautomerism, mutagenicity

1. Introduction

Substituted uracils have demonstrated today many biological properties, including those as inhibitors of nucleic acid metabolism and as clinical radiosensitizers of DNA in tumor cells. One possible class of radio-sensitizers is halouracils (5- and 6-XU) (X = F, Cl, Br and I), which belong to the group of substituted pyrimidines, and used to control damage to healthy tissues in radiation therapy [1]. When positive and negative ion formation is considered, the increased radiosensitivity very likely will be due to the enhanced dissociative electron attachment cross section for the halouracils leading to the destruction of the molecules and formation of genotoxic radicals [1]. Among these uracil derivatives, the halogenated forms were synthesized in the 1950s as potential antitumor agents after the discovery that certain tumors preferentially incorporated uracil rather than thymine into the DNA [2]. Thus, the substitution of uracil (U) or thymine (T) in RNA or DNA by XU (X = halogen) perturbs the replication of nucleic acids.

Halogenated pyrimidines are also chelating agents in metal complexes analogous to those where the uracil molecule plays a ligand role. After the successful identification of *cis*-platin as an anti-tumor agent, a new area of research has been opened up in the field of metal-complexes to search new anti-tumor agents. It is now well established that besides neutral platinum complexes other metal complexes also exhibit antitumor activity.

Special interest appears when in these derivatives the halogen atom is in position 5 on the uracil ring. This is because its structure is similar to that of thymine, for which the methyl group is exchanged by the halogen atom. Thus, they may easily replace T in DNA *in vivo*. These compounds have demonstrated antitumor and antiviral properties [3-5]. e.g., 5-fluorouracil (5-FU) has been the most active agent employed in the chemotherapy of colorectal cancer [6]. 5-Chlorouracil (5-CIU) and 5-bromouracil (5-BrU) have been used to treat inflammatory tissues [4,5], and complexes of 5-iodouracil (5-IU) with a variety of transition metal ions have shown antitumor activity [7].

Derivatives with the halogen atom in position 6- have been, however, much less studied [8]. In both isomers (5-chlorouracil and 6-chlorouracil) the halogen is bound to a carbon atom, but within a different environment, and this difference leads to remarkably strong effects in electron attachment. Although the mechanisms of the antitumor and antiviral action of these complexes are not well understood, it is likely that they inhibit the proper replication of DNA in tumor and infected cells. 6-uracil substituted derivatives are the most active as potential inhibitors of thymidine phosphorylase (TP) [9]. Thus the properties and characteristics of halouracils appear of great interest in pharmaceutical and clinical research.

Under the importance of this kind of compounds, previously we have studied the 5-halouracils [10,11], 5-FU [12,13], 5-BrU [14-16] and 5-IU [17,18]. Now the present manuscript is centered on 6-chlorouracil (6-CIU). It has antitumor activity in the complexes

with some metal (II) ions [19], as cobalt (II), nickel (II), copper (II), zinc (II), palladium (II), ruthenium (II), Mn (II) and rhodium (II). Considering the importance of this derivative of uracil in medicinal chemistry, however, theoretical studies are scarce and in general referred to the calculation of several properties. Also, their vibrational spectra have been relatively little looked into. Among these studies it can be mentioned a preliminary infrared and Raman assignment by Rastogi *et al* [19] but without any concrete theoretical support. Also, several authors have investigated the FTIR and Raman spectra of the ligand (6-CIU) and complex with Mn in the solid state. However, the present research analyzes accurately for the first time the 6-CIU molecule from the theoretical and vibrational point of view, using a high level in the quantum chemical calculations, and also two accurate procedures for scaling the wavenumbers.

2. Experimental

6-chlorouracil (solid state) of spectral grade was purchased from M/s Aldrich Chemical Co, USA and used as such without any further purification. The mid FTIR spectrum of the compound from 400-4000 cm^{-1} range was recorded with a Perkin Elmer FT-IR Model 1700 X instrument using the KBr technique with 1 mg sample per 300 mg KBr. For the spectrum acquisition, four scans were collected.

FT-Raman spectrum was recorded on a Bruker IFS 66 Optical Bench with a FRA 106 Raman module attachment, in the region 50-4000 cm^{-1} with a resolution of the order of 0.1 cm^{-1} . The Raman spectra were excited at 1064 nm using a Nd: YAG laser. The laser power was set at 100 mW and the spectrum was recorded over 500 scans at a fixed temperature. The sample was mounted in the sample illuminator using special mount.

3. Computational Methods

Ab initio geometry optimizations and wavenumber calculations were performed using MP2 [20] and density functional theory (DFT) [21] methods, along with the 6-31G(d,p) and 6-311++G(3df,pd) basis set. The presence of both polarization and diffuse functions in the basis set were necessary for a proper theoretical description of the molecular parameters. These procedures are implemented in the GAUSSIAN 03 [22] program package, which was utilized in the UNIX version with standard parameters. In DFT calculations the Becke's three-parameter exchange functional (B3) [23] with the non-local correlation functionals of Lee, Yang and Parr (LYP) [24] were selected as more appropriated [25]. The Perdew and Wang 91 (PW91) [26] correlation functional was also used and it leads to similar results.

The optimum geometry was determined by minimizing the energy with respect to all geometrical parameters without imposing molecular symmetry constraints. Berny optimization under the TIGHT convergence criteria was used. Calculation with Tomasi's polarized continuum model (PCM) [27] was used as implemented in Gaussian 03 by default using the integral equation formalism model, IEF-PCM.

Harmonic wavenumbers were calculated with the keyword `FREQ` at the same level of the respective optimization process, to characterize the stationary points and to evaluate the wavenumbers shifts due to complex formation with water. The keyword `RAMAN` was employed to obtain Raman intensities. All the optimized structures showed positive harmonic vibrations only (true energy minimum). For the calculations of the zero-point vibrational energy (ZPE), the wavenumbers were retained unscaled. The natural NBO atomic charges [28] were computed because they appear as the most accurate today to correlate properties. They were determined with the `POP = NPA` keyword.

4. RESULTS AND DISCUSSION

4.1 Geometry optimization

In Fig. 1 is plotted the molecule of 6-ClU with the labeling of atoms. The optimized bond lengths and angles obtained with MP2, B3LYP and B3PW91 methods, and with different basis sets are given in Table 1. The optimized ring structure of the molecule is planar, in agreement with those determined in uracil molecule (U).

It is noted in Table 1 that the length of the C-N and C-C single bonds are intermediate between the corresponding aromatic and the saturated bonds. Thus some aromatic character is on the ring structure. Comparing the different methods it is only observed appreciable differences (ca. 0.2 Å) in the N1-C2, C2-N3, C=O lengths, analogously as in uracil molecule.

With the 6-substitution on the uracil ring, several key effects and certainly inter-related, can be underlined that produces the marked differences in the biological and pharmacological activities of the nucleobases:

- (i) The first effect and the most important, is the drastic change of their functional ones expressed in terms of intermolecular interactions. This effect is especially remarkable with Cl. The higher electronegativity of the halogen atom compared to hydrogen atom produces a perturbation in the electronic environment of the heterocyclic ring. The halogen substitution also increases the polarizability of the nucleobase.
- (ii) The second effect is the distortion in the bond lengths and bond angles near the 6-position. The bond lengths are distorted in an alternating fashion: the N1-C2, N3-C4 and C4=O bonds are longer in 6-ClU, while the C2-N3, C4-C5, C5=C6 and C6-N1 bonds are shorter. The C2=O bond remains almost unchanged. In general, this distortion is very small; e.g. calculations at B3LYP/6-311++G(3df,pd) level gives for the C=C and C-C= bonds the values of 1.343 Å and 1.455 Å, respectively, very close to those determined in uracil molecule [29], 1.344 and 1.455 Å (1.350 Å and 1.460 Å by ED [30]). This fact produces a slightly opening of the *ipso* angle C4-C5=C6, $\sim 1-1.5^\circ$, and a slight closing of N3-C3-C5, while the N1-C2-N3 angle remains almost unchanged.

The hydration effect on the geometrical parameters of 6-ClU with the simplified PCM model is shown in the 13th column of Table 1. As expected, a noticeable shortening of the N-C and C-C bond lengths, and a lengthening of N-H and C=O is observed. An opening of ca. 1° in the bond angles N-C2-N, N-C4-C, C2-N1-H and C4-N3-H, and closing of the remaining bond angles is also determined.

4.2 Comparison of the energy and geometric parameters with other 5- and 6-halouracil derivatives

5-ClU and its isomer 6-ClU only differ in the position of the halogen atom. This

opens the interesting possibility to investigate the effect of a small structural difference on their different behavior. In both isomers the halogen is bound to a carbon atom, however with a different environment. As will be demonstrated this difference leads to remarkably strong effects in electron attachment. The tendency observed in the bond lengths can be explained by the change in the charge on the atoms. The following remarks could be noted in 6-XU derivatives as compared to 5-XU:

- (i) The C2=O bond length in 6-XU derivatives are always shorter than those of 5-XU. However, the reverse effect is noted in C4=O bond lengths, with longer value in 6-XU than in 5-XU.
- (ii) The C5=C6 and C4-C5 bond lengths of 6-halouracils are shorter than those of 5-halouracils, especially the C4-C5 distance is ca. 0.1 Å shorter than that of 5-XU.
- (iii) The halogen atom has little influence in the C5=C6 bond of 6-ClU and 6-BrU, but in 6-FU is slightly shorter, ca. 0.005 Å. Similar effect is observed in 5-XU derivatives, with very small change in 5-ClU and 5-BrU, and slightly shorter in 5-FU. However, the effect of the halogen atom on the C4-C5 bond is meaningful in 5-XU derivatives, as expected, with a lengthening of ca. 0.01 Å, and it is almost null in 6-XU.
- (iv) The C-X bond lengths of 6-XU (X= Cl, Br) derivatives are consistently longer than the corresponding values of 5-XU. However, C-F bond length in 6-FU is shorter than in 5-FU.
- (v) The N1-C2 and N3-C4 bond lengths appear longer in 6-XU than in 5-XU. However, in N1-C6 and C2-N3 is observed a reverse tendency, with shorter values in 6-XU than in 5-XU.
- (vi) The difference $\Delta(r_{6-XU} - r_{5-XU})$ in the bond lengths N1-C2 and N3-C4 are decreased according to the order FU > ClU = BrU.
- (vii) The C4-C5 bond lengths are increased according to 5(6)-FU < 5(6)-ClU = 5(6)-BrU.
- (viii) 6-XU derivatives show a higher stability, ranging from 6 to 35 kJ/mol, than the 5-XU counterpart. Our calculations at the B3LYP/6-311++G(3df,pd) level show that 6-ClU is 13.4 kJ/mol more stable than 5-ClU (11.3 kJ/mol at the MP2/6-31G(d,p) level). The difference $\Delta(E_{5-XU} - E_{6-XU})$ in the stability order is FU (32.6 kJ/mol) > ClU (13.4) > BrU (10.4). Fig. 2 shows this stability order with computations at the MP2/6-31G(d,p) level. Experimentally [31], 6-ClU is thermodynamically more stable than 5-ClU, by -31.0 kJ·mol⁻¹, which is three times higher than in our calculations. This fact has been also observed in other XU derivatives (X = NH₂, CH₃). Experimentally, the stability order has been reported to decrease from F to Br, in accordance to our computations. This is probably because of the increase of the steric hindrance due to the fact that the volume of the halogen atom increases from F to Br, and to the decrease of the electronegativities of the halogen atoms.

4.3 Tautomerisms

Since the 1950s, the tautomerism of nitrogenous bases has been intensively researched. A large amount of work has been performed using both theoretical [14-16,32-35] and experimental approaches [36-37]. Much of the interest is due to the fact that tautomers induce alterations in the normal base pairing, leading to the possibility of spontaneous mutations in the DNA or RNA helices. Tautomerism in nucleic acid bases and their derivatives has a role in mutagenesis of DNA [38-40].

Of all the possible combinations, six uracil tautomers, named T1-T6, are the most important and studied forms. The most stable one, the dioxo form **T1**, is the only one for which experimental information is available. Our calculated values in uracil molecule confirm the stability of **T1**, in agreement with previous DFT calculations [29]. T1 is predicted to be the only tautomer present in the vapour. Generally, the *keto* form of uracil exists as the main form in the double helix. The formation of specific AU Watson-Crick hydrogen bonds is responsible for the maintenance of the genetic code. If uracil is replaced by another type of base, it may lead to the introduction of a wrong genetic code. Although it is very difficult, however, mutations in the genetic code are observed, and one of the mechanisms of this mutation is the tautomerism.

Following the same methodology that in U and with the notation used in 5-FU [12] and 5-CIU [41] molecules, in 6-CIU was determined, in principle, eight tautomers, Fig. 3. Tautomers T1-T6 correspond to those of uracil molecule while T9 is only characteristic of 5-XU derivatives, and thus it was not observed in 6-CIU. The gas-phase relative energies of 5-CIU and 6-CIU tautomers are shown in Table 2, which can be compared with those corresponding to U. The relative stabilities of the tautomers have been found to be somewhat sensitive to both, the basis set and the theoretical method used, showing variations within 6.3 kJ/mol. The zero-point energy (ZPE) contribution also shows this sensitivity, with variations 0.8 – 2.9 kJ/mol (2-3%). Our previous results in U using the 6-311++G(3df,pd) basis set were in accordance to those reported at the sophisticated CCSD/6-31G(d) level [42], with differences in general lower than 4 kJ/mol [18]. It permits us to assume that the calculated values in 6-CIU are satisfactory.

As in U, the dioxo form **T1** of 6-CIU is the most stable one. All *enol* and *dienol* forms are energetically much less stable than the canonical *keto* tautomer. The relative energy of the *enol* tautomers is enough large to guarantee that T1 is the only important form in the isolated state of 6-CIU and in the others halo derivatives. Although, the presence of the halogen atom at position 6 increases the stability of all the *enol* tautomers as compared to U, ca. 12 kJ/mol more stable in T2 and T4, and ca. 21 kJ/mol in T6. Also, all the tautomers in 6-CIU are more stable than 5-CIU, with the exception of T5. Tautomers T2 and T4 appear as the most stable *enol* forms.

4.4 Tetramer simulation of the crystal structure

In Fig. 4 is plotted the molecule of 6-CIU in the dimer form with the labeling of its atoms. The optimized ring structure is planar, in agreement with those determined in U molecule. X-ray crystal data on 6-CIU has not been reported yet, although on 5-CIU and other 5-halouracil derivatives have been published [43,44]. In these studies the molecules appear associated in the crystal structure to form ribbons stabilized by N-H...O hydrogen bonds that involve N1H and N3H groups and the carbonyl oxygen atoms. As a simplification of this system, for the simulation of the unit cell in solid state of 6-CIU were considered only 4 molecules (tetramer), Figs. 5-6. Four tetramer forms were considered and simulated in 6-CIU: tetramers A and B correspond to those expected in the crystal (Fig. 5), and tetramers C and D corresponds to a non-planar structure, and thus, they are not believed to be in the crystal (Fig. 6). Tetramer C is the most stable one. However, the perpendicular orientation of two molecules in this structure is suppressed by the packing forces of the crystal, thus this tetramer as well as tetramer D are not expected in the crystal. Only tetramers A and B are possible.

All the tetramers appear stabilized by medium-strong H-bonds through the C=O and N1-H/ N3-H bonds of the neighboring molecules that leads to a slight planar form, although the packing crystal forces will press the structure to be almost full planar. These H-bonds give rise to a noticeable lengthening of the N-H and C=O bonds, and consequently, the neighboring N1-C2 and C2-N3 bonds appear shortened ca. 0.02 Å, as well as C4-C5.

Due to the orientation of the chlorine atoms in our tetramer simulation, they have no interaction with neighboring molecules, and thus their calculated bond lengths and angles are almost the same as that in the isolated state. I.e. in the solid state their vibrational modes can be expected to appear at similar wavenumbers as that in the isolated state.

4.5 Vibrational wavenumbers

4.5.1 Procedures to improve the wavenumbers: The scaling

The computation of the vibrational spectrum of a polyatomic molecule of even modest size is very lengthy. In spite of the tremendous advances made both in theoretical methods, in special with DFT methods and computer hardware, one may be forced to work at a low level, and consequently, one must expect a large overestimation of the calculated vibrational wavenumbers. This overestimation (which may be due to many different factors that are usually not even considered in the theory, such as anharmonicity, errors in the computed geometry, Fermi resonance, solvent effects, etc) can be remarkably reduced with the use of transferable empirical parameters for the force fields, or for the calculated wavenumbers. The scale factor is therefore designed to correct the calculated harmonic wavenumbers to be compared with the anharmonic values found by the experiment. The scale factor is a consequence of the deficiency of the theoretical approach and potentially allows vibrational

wavenumbers (and thermochemical information) of useful accuracy to be obtained from procedures of modest computational cost only. Widespread applications to molecules of moderate size are then possible [49].

To scale the wavenumbers, the simplest procedure is using an overall scale factor [45], which is the procedure generally applied in the bibliography, but it leads to a high error in the scaled values of several modes and it impedes a clear and accurate assignment of various modes. Thus, to reduce this error and get a trustworthy assignment can be employed two accurate procedures: the linear scaling equation and the specific scale factors for each mode. Both require the values of the wavenumbers determined at the same level in a simpler molecule, which corresponds in the present manuscript to the U molecule [29]. For simplicity and ease to be used, the results with the scaling equation procedure are preferred to be used here. This procedure was applied to the calculated wavenumbers given in Table 3, given rise to the scaled values of Table 4, and the corresponding IR and Raman predicted spectra, Figs. 7-11. The wavenumbers of these spectra can be directly compared with the experimental infrared and Raman bands, Fig. 1-Sup. and Fig. 2-Sup. of Supplementary Material. However, due to the intermolecular H-bonds, several bands appear remarkably changed from the isolated state spectra to its tetramer simulation. Tables 3-4 collect these differences in the C=O and N-H groups involved in H-bonds, and they show clearly why it is necessary the simulation of the crystal unit cell to avoid mislay in the assignment, and how the building of a simple tetramer form can noticeable improves the results.

Although with these scaling procedures the error continues being also large for several vibrations, in general a good correlation theory/experiment is established. The rms errors obtained are very close to other uracil derivatives studied earlier by us [17,46-48]. Larger basis set represent an excessive increase of the computational cost for a very slight improvement. Calculated anharmonic wavenumbers require also too much computational cost for a smaller improvement in the calculated values than with the simple use of the specific scale equation procedure, as it is shown in the present manuscript.

4.5.2 Calculated wavenumbers of 6-CIU

The computed vibrational data obtained with two of the three theoretical methods used and with the 6-311++G(3df,pd) basis set are shown in Table 3. The columns 2-4th correspond to B3PW91 values, while the columns 5-10th to B3LYP. MP2 values were not included due to they reproduce the wavenumbers poorer than B3LYP [25,49]. The first column lists for 6-CIU the numbers of ring normal modes according to the notation used in uracil structure [29]. The second and fifth columns show the calculated wavenumbers with the B3PW91 and B3LYP DFT methods, respectively. Small differences were found between both. The calculated absolute infrared intensities in km/mol by B3LYP appear in the 6th column, and their relative values in the 7th column. They were obtained by normalizing the

computed values to the intensity of the strongest one, mode no. 26. The calculated Raman intensities were normalized to mode no. 30, 8th column. Raman depolarization ratios for plane and unpolarized incident light of each vibration are shown in 9th-10th columns, respectively. The B3LYP results are slightly better than with B3PW91, and thus the discussion below mainly refers to it.

The dimer results are shown in 11-13th columns. Due to the symmetry of the dimer, for each vibration appears two wavenumbers, with very close values in the majority of the cases. Of these two wavenumbers, which one with the highest IR intensity is shown in bold type, while that with the highest Raman intensity is shown in italic form. It is noted that the highest IR and Raman intensities appear in mode 29, while in the isolated state they were calculated in modes 26 and 30. The tetramer values are collected in 14th-16th columns. In this case, four wavenumbers appear for each vibration. Also the wavenumber with the highest IR intensity is shown in bold type, and that with the highest Raman intensity in italic form. The discussion is mainly centred in these wavenumbers.

In the last column of this Table appears the characterization established by B3LYP for each calculated wavenumber, with the % PED of contribution of the different modes to a computed wavenumber. Contributions lower than 10% were not considered. Comparing to B3PW91, slight differences in the % of contribution of the different modes were found, but for simplicity they were not included in the Table. Only the displacements vectors higher than 0.07 for the carbon and nitrogen atoms, 0.05 for the oxygen atoms, 0.15 for the hydrogen atoms, and 0.04 for the chlorine atoms were described.

Table 4 collects the experimental values obtained in the solid state in KBr pellets as well as the Raman data. The absence of a $\nu(\text{OH})$ band in the 3500-3700 cm^{-1} range and the appearance of $\nu(\text{C}=\text{O})$ modes as strong bands indicate that in the solid state this compound appears only in the *keto* form. Comparing the spectrum with uracil molecule, it is noted that most of the uracil fundamentals absorb in relatively narrow wavenumber ranges. The strong coupling of the group modes in the uracil spectrum remains highly constant for its 6-CIU derivative, as well as in the other derivatives, and its variation is directly related to the electron donating or withdrawing effects of the substituent.

Differences appear in the wavenumbers if the experimental values are compared with the calculated ones of Table 3. An improvement can be carried out on the computed wavenumbers if it is used scaling procedures. For convenience, the fifth column collects the specific scale factors at B3LYP/6-311++G(3df,pd) level obtained in uracil. With it and with the corresponding scaling equations [29] were determined the scaled wavenumbers of columns 3rd, 4th and 6th. The scaled dimer and tetramer wavenumbers are collected in 9-10th columns. The tetramer results can be directly compared to the experimental infrared and Raman bands, 7-8th columns. The assignments of the bands were carried out according to these scaled values of the tetramer. The predicted scaled IR and Raman spectra are shown in

Figs. 7-11. Although with these scaling methods the error continues being also large for several vibrations, in general a good correlation theory/experiment is established. The rms errors obtained are very close to other uracil derivatives studied by us earlier [13,46-49].

4.3.3 Description of the different modes

N-H vibrational modes

In crystals with differently bound multiple NH- groups the bands due to the N-H stretchings and bendings are usually broad and extensively overlapped. In our computations on 6-CIU, the N-H stretching modes are essentially pure group modes, as in U and other derivatives, and as consequence the calculated and experimental wavenumbers appear little affected by the substituent [52-53] and with similar values that in U [29]. $\nu(\text{N1-H})$ is calculated at 3635 cm^{-1} in 6-CIU vs. 3642 cm^{-1} in U, and $\nu(\text{N3-H})$ at 3600 cm^{-1} in 6-CIU vs. 3598 cm^{-1} in U. In the tetramer form of 6-CIU the calculations predicted the two stretching N-H bands to occur within the $3500\text{-}3000\text{ cm}^{-1}$ range and the N-H oscillators to be uncoupled.

The $\nu(\text{N1-H})$ vibration (mode 30 according to the uracil ring notation [29]) is estimated to occur at higher wavenumbers than the $\nu(\text{N3-H})$ vibration (mode 29 in the U ring notation), in agreement to that observed in other U derivatives [17, 46-48], although of the four scaled wavenumbers for the $\nu(\text{N1-H})$ mode, that one with the highest IR intensity appears at higher wavenumber (at 3235 cm^{-1}) than that of the $\nu(\text{N3-H})$ mode (at 3248 cm^{-1}). The IR intensity of the N1-H stretching band is calculated in the isolated state to be by ca. 1.5 higher than that of the N3-H band. However, in the dimer and tetramer simulation it is the reverse, with the $\nu(\text{N3-H})$ intensity higher than $\nu(\text{N1-H})$. Thus, the very strong band observed at 3094 cm^{-1} was assigned to the $\nu(\text{N3-H})$ mode. In the Raman spectra they are predicted with very strong Raman intensity, and that for $\nu(\text{N3-H})$ is the highest in the tetramer form. Thus, the very strong Raman band observed at 3090.4 cm^{-1} was assigned to this mode beyond any doubt.

The N-H in-plane bends, $\delta(\text{N-H})$, are highly coupled to other groups or ring modes and the influence of substitution is not straight forward. $\delta(\text{N1-H})$ is calculated in the isolated state at 1486 cm^{-1} in 6-CIU vs. 1499 cm^{-1} in U, and $\delta(\text{N3-H})$ at 1403 cm^{-1} in 6-CIU vs. 1382 cm^{-1} in U. There are many vibrations (nos. 17, 22, 24-26) to which the $\delta(\text{N-H})$ in-plane bending modes contribute significantly. The $\delta(\text{N1-H})$ and $\delta(\text{N3-H})$ bendings correspond to modes 23 and 20, respectively. In the isolated state by B3PW91 and in the dimer and tetramer forms by B3LYP the IR and Raman intensities of the $\delta(\text{N1-H})$ bending mode are much higher than those of $\delta(\text{N3-H})$. Thus, the experimental IR band observed at 1494 cm^{-1} in KBr pellet was correlated to $\delta(\text{N1-H})$ mode, in accordance to its scaled wavenumber in the tetramer at 1453 cm^{-1} , while the IR band at 1439 cm^{-1} corresponds to $\delta(\text{N3-H})$ mode, good scaled at 1441 cm^{-1} . The Raman activity of the $\delta(\text{N1-H})$ mode is greater than that of the

$\delta(\text{N3-H})$ mode, in accordance to the Raman band with medium intensity observed at 1488.8 cm^{-1} , $\delta(\text{N1-H})$ mode, and to the weak band at 1436.9 cm^{-1} corresponding to $\delta(\text{N3-H})$. The scaled values at 1475 and 1441 cm^{-1} , N1-H and N3-H respectively, confirm the assignment beyond any doubt. In 5-CIU the experimental Raman bands were observed at 1473 and 1410 cm^{-1} , corresponding to N1-H and N3-H, respectively [52].

The $\gamma(\text{N1-H})$ and $\gamma(\text{N3-H})$ out-of-plane bending vibrations correspond to modes 8 and 9, respectively. These modes are calculated with a PED of 60-70%. The vibrations nos. 10 and 4 also contribute significantly to the $\gamma(\text{N-H})$ out-of-plane bending modes. They are predicted in the tetramer form with weak IR intensity at 756 and 934 cm^{-1} , N1-H and N3-H respectively, and with very weak or almost null Raman activity at 771 and 875 cm^{-1} , in good accordance with the experimental IR and Raman bands. In 5-CIU these vibrations were detected with strong intensity in the Raman spectrum at 830 and 875 cm^{-1} , very close to our results in 6-CIU.

C=O vibrational modes

The carbonyl stretching motions in 6-CIU couple significantly with the N-H bending motions, as in other uracil derivatives. In the isolated state the calculated $\nu(\text{C2=O})$ wavenumber is little affected by the different substituents [48]. The difference is in general less than 10 cm^{-1} . e.g. the calculated value at the B3LYP/6-311++G(3df,pd) level in 6-CIU is 1793.2 cm^{-1} , very close to those of U at 1791 cm^{-1} . Similar behavior appears with the $\nu(\text{C4=O})$ wavenumber, 1763 cm^{-1} in 6-CIU close to 1757 cm^{-1} of U. However, the values differ remarkably of those observed in the solid state. It is due to the strong intermolecular bonds formed in the solid state through the C=O bonds. In the simulated tetramer form the scaled wavenumbers for the $\nu(\text{C2=O})$ (no. 26) and $\nu(\text{C4=O})$ (no. 25) modes by IR at 1723 and 1712 cm^{-1} , respectively, agrees well with the experimental bands.

In the isolated state the IR band intensity of $\nu(\text{C4=O})$ is predicted by B3LYP remarkably higher than $\nu(\text{C2=O})$, and as a consequence, its intensity appears as the strongest one in the spectra. Analogously, in the tetramer form mode $\nu(\text{C4=O})$ is predicted with the highest IR intensity. Experimentally, both modes appear with similar IR intensity in the spectrum.

The $\delta(\text{C=O})$ in-plane bending vibrations appear spread out mainly in modes nos. 3, 5, 6 and 7. Mode 3 has the highest contribution and it is calculated with medium IR intensity and very weak Raman activity. It is scaled in the tetramer form at 436 (IR) and 424 (Raman) cm^{-1} , in accordance with the strong IR band at 451 cm^{-1} and with the weak Raman band detected at 420 cm^{-1} . It is predicted to remain almost unaffected under C6 substitution, in the IR spectrum of KBr at 451 cm^{-1} of 6-CIU vs. 446 cm^{-1} of 5-CIU [52].

The $\gamma(\text{C2=O})$ out-of-plane mode (no. 11) appears calculated at a higher wavenumber than

mode $\gamma(\text{C4}=\text{O})$ (no. 10), while in 5-halouracil derivatives the case is the reverse [52]. The IR intensity of mode $\gamma(\text{C4}=\text{O})$ is always lower than $\gamma(\text{C2}=\text{O})$, in accordance with the experimental. The calculated wavenumber of mode $\gamma(\text{C2}=\text{O})$ remains almost constant with the substitution, 765 cm^{-1} in U, and 757 cm^{-1} in 6-CIU. The fact that this mode remains approximately constant might be taken as evidence for a similar coupling ($\text{C2}=\text{O} + \text{NCN}$) in U molecule. By contrast a remarkably decrease of $40\text{-}50\text{ cm}^{-1}$ appears with the 6-substitution in the $\gamma(\text{C4}=\text{O})$ mode. e.g. in the isolated state of 5-CIU [52] it is scaled at 775 cm^{-1} vs. 722 cm^{-1} of 6-CIU and 726 cm^{-1} in U. It is due to in 5-CIU this mode $\gamma(\text{C4}=\text{O})$ appears coupled with $\gamma(\text{NCC}=\text{C})$ but in 6-CIU and in U it is very strongly coupled with the $\gamma(\text{C5-H})$ mode which decrease the wavenumber.

C6-X modes.

The $\nu(\text{C-Cl})$ stretching mode no. 27 is scaled in the tetramer and in Raman at 393 cm^{-1} , which agrees well with the experimental band at 384.6 cm^{-1} . In uracil derivatives, its wavenumber is decreased as the bond length C6-X is lengthened.

The theoretical wavenumber of $\delta(\text{C-C})$ in-plane bending vibration (no. 19) is scaled at 268 cm^{-1} in Raman, well correlated to the experimental band at 267.6 cm^{-1} . Obviously, the analogous C-H bending vibration band of the U molecule is calculated far away at 1227 cm^{-1} . In 6-CIU a high contribution of the $\delta(\text{C6-X})$ appears in the mode no. 7 computed at 600 cm^{-1} .

The $\gamma(\text{C-Cl})$ out-of-plane bending (no. 15) appears scaled at 198 cm^{-1} in Raman, and well related to the experimental Raman line at 186.5 cm^{-1} . In 5-CIU the $\gamma(\text{C-Cl})$ mode was identified in the Raman spectrum at 125 cm^{-1} [52].

C5-H modes

The C5-H stretching is a pure mode in 6-CIU, no. 28, however, it appears slightly coupled in uracil molecule with the $\nu(\text{C6-H})$ vibration. Due to this coupling, it is calculated with a lower wavenumber in U than in 6-CIU, 3200 cm^{-1} in U vs. 3260 cm^{-1} in 6-CIU. The very weak intensity calculated for this mode, made it absent in the experimental spectra. However, in KBr pellets due to the interaction of the solid state, two shoulders have been detected at 3175 and 3144 cm^{-1} .

The in-plane bending vibration, $\delta(\text{C-H})$, appears strongly coupled and distributed in modes nos. 22, 18 and 17. The mainly contribution corresponds to mode no. 17, which is scaled by IR in the tetramer at 1088 cm^{-1} with weak IR and Raman intensity.

The out-of-plane vibration, $\gamma(\text{C-H})$, (no. 13) is scaled in the tetramer at 815 cm^{-1} with medium IR intensity, in accordance to the strong band detected at 841 cm^{-1} . The influence of the substitution is almost null, e.g. in U it is scaled at 812 cm^{-1} . The Raman activity at 814 cm^{-1} is weak in accordance with the very weak band detected at 842.5 cm^{-1} .

Ring vibrations

The C-C and C-N stretching vibrations are identified in the modes 21, 18, 16, 14 and 12. They are strongly coupled with C-H, N-H and C=O modes. Mode 21 is scaled in the tetramer with strong IR intensity at 1383 cm^{-1} in accordance to the very strong band observed in the IR spectrum at 1414 cm^{-1} . In 5-CIU it is detected as very strong band in the IR spectrum (Nujol) at 1450 cm^{-1} .

Mode 18 is mainly characterized as C-N and it is predicted in the tetramer with very weak IR intensity at 1221 cm^{-1} and with medium Raman value at 1203 cm^{-1} , in accordance to the experimental IR band with very weak intensity at 1233 cm^{-1} and to the Raman line with very strong intensity at 1234.9 cm^{-1} . This mode is little affected by substitution at the C6 atom. E.g. it is observed in the IR spectrum of 5-CIU at 1220 cm^{-1} [52].

Ring mode no. 16 appears scaled in the IR spectrum of the tetramer at 980 cm^{-1} in accordance to the IR band observed at 1000 cm^{-1} . In U it is scaled at 978 cm^{-1} .

Mode no. 14 is defined as antisymmetric stretching of the opposite C4-C5 and N1-C2 bonds, and it is scaled at 999 cm^{-1} in agreement with the experimental IR band at 1031 cm^{-1} . The calculated IR intensity of this mode is medium in accordance to the experimental IR band, and with very weak Raman intensity in close agreement to the very weak Raman band at 1032.2 cm^{-1} . This fact confirms the adequacy of the tetramer simulation and the B3LYP results. In 5-CIU it appears scaled in the tetramer at 973 cm^{-1} and in the experimental IR spectrum at 1005 cm^{-1} [52].

The stretching of the C5=C6 bond appear identified in mode no. 24. It is scaled at 1608 cm^{-1} with strong IR intensity and medium Raman value, in accordance to the strong band observed at 1599 cm^{-1} in the IR spectrum and with the medium activity of the Raman line at 1630 cm^{-1} . In U it is scaled at 1623 cm^{-1} , indicating the weak effect of the chlorine substituent on the C=C bond.

Mode 5 appears strongly coupled with the C=O bending. The common notation used for the authors to call this mode is antisymmetric bending $\delta(\text{N3-C4-C5}) + \delta(\text{N1-C2-N3})$. It has been scaled at 541 cm^{-1} in the tetramer, and good related to the experimental IR band at 549 cm^{-1} , while in U it is scaled at 529 cm^{-1} .

Mode 6 appears scaled in the tetramer at 604 cm^{-1} with very weak IR and Raman intensities, in accordance to the no-detection of this mode in the experimental spectra. In 5-CIU was predicted at 614 cm^{-1} in accordance to our results. Mode 6 and 4 appear predicted in the tetramer at the same wavenumber and with the same very low IR and Raman intensities. Thus, we have doubts in the assignment of the experimental bands at 610 cm^{-1} (IR) and 602.9 cm^{-1} (Raman).

Of the three out-of-plane ring bending modes (nos. 1, 2 and 4), we have doubts in the

identification of mode 4 in the calculated band at 594 cm^{-1} in the isolated state. In this mode the displacement vectors determined little correspond to those characteristics of this mode [29]. However, modes 1 and 2 appear well identified in the calculated bands at 132 and 158 cm^{-1} , respectively, and coupled somewhat with the $\gamma(\text{C-Cl})$ mode. In the tetramer they were scaled at 228 and 199 cm^{-1} in concordance with the Raman lines at 150.8 and 298.4 cm^{-1} .

Overtone and combination bands

The bands observed with strong intensity in the IR spectrum at 2906 and 2807 cm^{-1} , and those at 2843 and 2772 cm^{-1} by its position can be assigned to overtones and combination bands. Similarly, the Raman bands with weak intensity detected at 2924 , 2896.5 , 2840.8 and 2805.1 cm^{-1} can be considered as overtones or combination bands.

Lattice modes

The low vibrational modes calculated in the dimer and tetramer forms are collected in Table 6. The notation used in the characterization of the modes is in accordance to that reported in other similar molecules [50,51]. For each vibration of the tetramer, that with the highest Raman intensity appears printed in italic type.

5. Other Molecular Properties

With the theoretical methods used, the natural NBO total atomic charges on the atoms were determined, which are collected in Table 5. Atomic charges have a significant importance in the application of quantum chemical calculations for molecular systems, because they are related to dipole moments, electronic structure, molecular polarizability and other various properties of the molecules. The last two columns list the electronic density values calculated on the atoms. For comparison purposes the computed values in uracil and 5-XU derivatives are included, as well as 6-FU and 6-BrU. Large differences appear between MP2 and B3LYP, and small differences between the DFT methods B3LYP and B3PW91.

In general, significant changes among 6-CIU, 5-CIU and U molecules were not observed, except in the atomic charges around C5 and C6. The chlorine atom withdraws electrons from C6, and it changes from a small positive value in U, $0.08 e$, to a value of $0.21 e$ in 6-CIU at the B3LYP/6-311++G(3df,pd) level. Consequently, the negative charge on C5 and N1 is slightly increased in 6-CIU. Among all carbon atoms in the ring, the C6 atom has the least positive charge value, while in C5 it is negative. The value in C5 and C6 decreases from 6-FU to 6-BrU. In 5-XU derivatives the positive charge on C5 in 5-FU decreases to the negative value of $-0.20 e$ in 5-CIU and to $-0.27 e$ in 5-BrU.

The charges on N1 and N3 have almost the same value in all 6-XU derivatives, while in 5-XU the charge on N3 is higher than in N1. An increase in the positive charge on the X

atom from –F to –Br has not effect in the negative charge on O8 and O10 atoms in 5-XU and 6-XU derivatives.

Halogenation alters many properties of uracil, e.g., dipole moment, polarizability, acidity, basicity. It alters the magnitude and direction of the dipole moment as well as the polarizability of U. These changes might induce minor conformational changes in DNA and alter the strength of the stacking interactions, both of which are likely to produce additional effects upon their functions as suggested by previous studies. Due to the importance of the dipole moments, they were calculated in U, 5-XU and 6-XU nucleobases. Because the halogens have much larger masses and they are more electronegative than hydrogen, the centre-of-mass and the electron density distribution of U are altered upon halo-substitution. In all cases, the centre of the dipole moment moves toward the halo-substituent. The orientation and electron withdrawing character of the halo-substituent result in a modest decrease (~10%) in the magnitude of the dipole moment for the 5-XU nucleobases and a much larger decrease (~35%) for 6-XU. Thus, the dipole moment is slightly lower than in U, e.g. at the B3LYP/6-311++G(3df,pd) level it is 4.481 D in U, while in 5-ClU is 4.041 D, and in 6-ClU is 3.271 D. In 6-FU is 3.034 D (3.326 D by MP2), in 6-BrU is 3.363 D (3.763 D by MP2). The dipole moment slightly increases from –F to –Br.

The simulated hydrated structure with the PCM model give rises to a noticeable increase of the NBO atomic charges in the atoms involved in H-bonds, i.e. H7, O8, H9 and O10, Table 5. This effect has been observed in other nucleobases [16,41] and it increases the reactivity of the molecule. The dipole moment is also remarkably increased, up to the value of 4.928 D, Table 7. A noticeable change is observed in all the values with the tetramer form, especially in the dipole moment, raising the value of 8.917 D.

The dipole moments remarkable diverge in the tautomers, from the lowest value of 0.647 D in T4 to the highest of 7.025 D in T6. In water solution, the biological medium, the tautomers with high dipole moment appear stabilized versus those with low value. Thus tautomer T4 will be difficult to appear in water solution, while T6 will have some probability, although the ΔE is high. The remaining tautomers have similar dipole moment that T1 (3.271 D). E.g. in T2 is 3.370 D, in T3 is 3.933 D, in T4b is 2.662 D and in T4c is 3.398 D. In T5 the value is something high, 5.588 D.

Several thermodynamic parameters and rotational constants were also computed and collected in Table 7. The values of the total energy include the ZPE (zero-point vibrational energies) correction. In the isolated state the values of the rotational constants are almost the same at the different levels used, even to the MP2 method. This fact confirms again the accuracy of the DFT methods applied. In the tetramer form the rotational constants are remarkable reduced to a very small value and almost zero.

The entropies were calculated without scaling. In the isolated state they also show the same value for the different levels used. The vibrational component is remarkable increased

in the tetramer to be the main one for the total value.

6. HOMO, LUMO energy gap

The frontier molecular orbital's, HOMO, LUMO and frontier orbital gap helps to exemplify the chemical reactivity and kinetic stability of the molecules and are important parameters for quantum chemistry [54]. The HOMO is the orbital that primarily acts as an electron donor and the LUMO is the orbital that largely acts as the electron acceptor. In order to evaluate the energetic behavior of the title compound, we carried out calculations in gas phase. HOMO and LUMO energies of LUMO+1, LUMO (first excited state), HOMO (ground state), HOMO-1 and their orbital energy gaps calculated by DFT/B3LYP/6-31+G(d,p). The 3D plots of the frontier orbitals namely ground state (HOMO), HOMO-1, HOMO-2 and first excited state (LUMO), LUMO+1, LUMO+2 are shown in Fig. 12. The positive phase is red and the negative one is green. It can be seen from the plots that the HOMO levels are spread over the entire molecule except the functional group oxygen and chlorine in ground state. The LUMO of first excited state is almost uniformly distributed over the entire molecule. The energy gap of HOMO-LUMO explains the eventual charge transfer interaction within the molecule, which influences the biological activity of the molecule. The decrease in energy gap between HOMO and LUMO facilitates intra molecular charge transfer which makes the material to be NLO active.

6. Summary and Conclusions

- i) The equilibrium geometries and harmonic wavenumbers of 6-CIU molecule were calculated for the first time using quantum chemical methods. The geometries and values of the properties presented appear to be the most accurate to date. DFT calculations were used as a tool in the identification of the bands presented in the solid state of 6-CIU.
- ii) Calculations with the B3LYP method and with the 6-31G(d,p) and 6-311++G(3df,pd) basis set, appear in general to be useful, when combining with a scaling equation procedure or with specific scale factors, for interpretation of the general features of the IR and Raman spectra.
- iii) To improve the calculated wavenumbers, the linear scaling equation procedure gives rise to a noticeable improvement in the predicted values and it is the best for this purpose. The agreement between the final scaled wavenumbers and the experimental values seems reasonable, with errors in general lower than 5%. Thus, the assignment of most of the fundamentals provided in this work is believed to be unambiguous.
- iv) Our tetramer simulation of the solid state is adequate. The shift in the wavenumbers of the C=O and N-H groups involved in H-bonds are well reproduced, and thus the RMS error is

remarkable reduced. This manuscript shows clearly why it is necessary the simulation of the crystal unit cell to avoid mislays in the assignment, and how a simple tetramer form can improve the results noticeably. This methodology is recommended here for the assignment of the vibrational spectra with the sample in the solid state.

- v) The effects of the intermolecular H-bonds in the vibrational wavenumbers of the C=O and NH groups were analyzed. A weak or almost null interaction is observed for the chlorine atoms with the neighbor molecules. The $\nu(\text{C}=\text{O})$ band position is predicted to remain little affected by changes in the molecular structure under C6 substitution.
- vi) The tautomeric forms of uracil and 6-CIU molecules were determined and optimized. Substitution of uracil by a chlorine atom at the 6-position changes the order and stability of the tautomers. All the tautomers in 6-CIU are more stable than in U. Tautomers T2 and T4 appear as the most stable. The relative stabilities of the tautomers have been found to be somewhat sensitive to both basis sets and theoretical methods used, showing variation within 6.3 kJ/mol. The relative energy order of 6-CIU tautomers is the same as the relative free energy (ΔG) order.
- vii) Halogenation alters many properties of uracil, including the magnitude and direction of the dipole moment. These changes might induce minor conformational changes in DNA and alter the strength of the stacking interactions. These effects were calculated to be somewhat smaller for 5-XU than for 6-XU derivatives.
- viii) The decrease in energy gap between HOMO and LUMO facilitates intra molecular charge transfer which makes 6-CIU to be NLO active.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at <http://>

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Table 1. Geometrical parameters optimized, bond lengths in Å and bond angles in degrees, in uracil (X=H) and halouracil derivatives (X=F, Cl, Br) with the halogen atom in position 5 and 6.

Parameters	X=H		5-XU				6-XU							
	(a)	(b)	F	Cl		Br	F		Cl				Br	
			(a)	(a)	(b)	(a)	(a)	(b)	(a)	(b)	(c)	(d)	(a)	(b)
<u>Bond lengths</u>														
N1-C2	1.3899	1.3896	1.385	1.3870	1.3877	1.3873	1.396	1.396	1.394	1.3951	1.3900	1.382	1.395	1.395
C2-N3	1.3790	1.3845	1.385	1.3811	1.3871	1.3812	1.376	1.381	1.376	1.3818	1.3726	1.373	1.377	1.383
N3-C4	1.4084	1.4080	1.402	1.4043	1.4034	1.4050	1.412	1.413	1.407	1.4076	1.4022	1.394	1.406	1.408
C4-C5	1.4546	1.4568	1.461	1.4663	1.4675	1.4656	1.452	1.454	1.455	1.4561	1.4516	1.444	1.456	1.458
C5=C6	1.3438	1.3513	1.340	1.3450	1.3532	1.3449	1.339	1.346	1.343	1.3513	1.3423	1.348	1.342	1.351
N1-C6	1.3700	1.3758	1.375	1.3704	1.3757	1.3702	1.362	1.367	1.369	1.3739	1.3646	1.364	1.368	1.373
N1-H	1.0081	1.0078	1.008	1.0083	1.0083	1.0086	1.010	1.009	1.009	1.0013	1.0084	1.029	1.009	1.009
C2=O	1.2101	1.2236	1.210	1.2092	1.2225	1.2090	1.208	1.221	1.208	1.2218	1.2065	1.219	1.208	1.222
C4=O	1.2127	1.2270	1.208	1.2078	1.2234	1.2079	1.212	1.226	1.212	1.2265	1.2102	1.226	1.212	1.226
C-X	-	-	1.334	1.7211	1.7183	1.8853	1.326	1.338	1.724	1.7217	1.7137	1.720	1.892	1.886
<u>Bond angles</u>														
N-C2-N	113.04	112.41	113.1	112.83	112.22	112.82	112.89	112.19	113.2	112.54	113.18	114.22	113.22	112.49
C-N3-C	127.99	128.86	128.6	128.68	129.52	128.69	128.02	128.88	127.7	128.58	127.84	126.86	127.70	128.68
N-C4-C	113.61	113.16	112.2	112.82	112.37	112.78	114.21	113.76	114.2	113.70	114.13	114.93	114.17	113.72
C-C5=C	119.81	119.94	121.6	120.12	120.36	120.19	118.26	118.34	119.2	119.38	119.10	118.81	119.16	119.08
C2-N1-H7	115.15	114.91	115.4	115.40	115.15	115.39	117.02	116.96	116.0	115.63	116.05	116.76	115.82	115.81
N3-C2=O	124.27	124.41	123.8	124.15	124.27	124.18	124.91	125.09	124.6	124.85	124.64	123.51	124.60	124.80
C4-N3-H9	116.27	115.88	115.9	115.70	115.37	115.69	116.06	115.69	116.4	115.96	116.31	117.06	116.38	115.91
N3-C4=O	120.20	120.56	121.8	120.86	120.29	120.68	119.98	120.21	120.3	120.64	120.35	120.06	120.38	120.66
C4-C5-X	118.27	118.47	117.4	118.23	118.01	118.29	-	-	-	-	-	-	-	-
N1-C6=C5	122.02	121.76	120.8	121.72	121.36	121.69	124.06	123.90	122.5	122.24	122.55	122.55	122.61	122.66
C5=C6-X	122.17	122.52	-	-	-	-	123.10	123.43	122.9	122.99	122.85	122.33	122.69	122.81

(a) At the B3LYP/6-311++G(3df,pd) level. (b) At the MP2/6-31G(d,p) level. (c) At the B3PW91/6-311++G(3df,pd) level. (d) At the PCM B3LYP/6-311++G(3df,pd) level

Table 2. Calculated gas-phase relative energies (kJ/mol) of uracil and 5-CIU and 6-CIU tautomers.

Method	$\Delta E + ZPE$									ΔG								
	T1	T2	T3	T4	T5	T6	T9	T4b	T4c	T1	T2	T3	T4	T5	T6	T9	T4b	T4c
Uracil:																		
B3LYP/6-311++G(3df,pd)	0 ^a	46.48	48.20	53.88	78.96	85.02	-	73.82	74.11	0 ^g	47.02	48.53	54.84	78.83	84.98	-	74.61	74.82
B3LYP/DGDZVP	0 ^b	48.82	53.34	60.48	84.10	90.41	-			0 ^h	49.45	53.80	61.61	84.10	90.41	-		
MP2/6-31G(d,p)	0 ^c	45.48	51.54	47.74	80.34	90.25	-			0 ⁱ	46.23	52.08	49.03	80.26	90.37	-		
5-CIU																		
B3LYP/6-311++G(3df,pd)	0 ^d	40.25	50.16	49.16	71.73	74.65	396.3	57.47	57.73	0 ^j	40.84	50.41	50.20	71.73	74.70	394.4	58.52	58.81
B3LYP/DGDZVP	0 ^e	42.34	54.93	55.34	77.29	80.59	425.1			0 ^k	43.01	55.30	56.51	77.33	80.67	423.0		
6-CIU																		
B3LYP/6-311++G(3df,pd)	0 ^f	35.61	46.11	40.34	78.21	65.67	-	59.69	59.44	0 ^l	36.28	46.52	41.51	78.08	65.79	-	60.74	60.44

^aTotal energy (+ZPE) -414.888816 AU (atomic units). ^b-414.790386 AU. ^c-413.578145 AU. ^d-874.517858 AU. ^e-874.353991 AU. ^f-874.522963 AU. ^g-414.919244 AU.

^h-414.820894 AU. ⁱ-512.615586 AU. ^j-874.550489 AU. ^k-874.386729 AU. ^l-874.555641 AU. [§]AU = 2622.9897 kJ/mol.

Table 3. Comparison of the calculated harmonic wavenumbers (ω , cm^{-1}), absolute (Abs, KmMole) and relative (A, %) infrared intensities, relative Raman scattering activities (S, %), Raman depolarization ratios for plane (ρ) and unpolarized (UP) incident light, and characterization obtained for 6-CIU with the B3PW91 and B3LYP methods and the 6-311++G(3df,pd) basis set.

Ring No.	Isolated state									Dimer			Tetramer			characterization
	B3PW91			B3LYP						B3LYP ^a			B3LYP ^a			
	ω	A	S	ω	ab.	A	S	ρ	UP	ω	A	S	ω	A	S	
1	132	0	0.3	132	2	0	0.3	0.75	0.86	147,144	0	0	204,194,191,190	0	0	40%, $\gamma(\text{C}=\text{O}) + 36\%$, $\gamma(\text{ring}, \text{N3}) + 24\%$, $\gamma(\text{C-CL})$
2	157	0	0.1	158	1	0	0.0	0.75	0.86	167,160	0	0	188,184,178,174	0	0	100%, puckering (N1, O10)
15 ^b	174	0	1.3	175	0	0	0.8	0.75	0.86	188,183	0	0	173,160, 154,149	0	0	65%, $\gamma(\text{ring}, \text{CL}) + 35\%$, $\gamma(\text{ring})$
19 ^b	247	0	0.1	246	3	0	0.0	0.74	0.85	255,252	0	1	261, 259,256,247	1	1	64%, $\delta(\text{C-CL}) + 36\%$, $\delta(\text{ring})$
27 ^b	367	1	2.0	364	2	1	2.1	0.46	0.63	372,370	0	2	379,377,372, 367	0	2	53%, $\nu(\text{C-CL}) + 37\%$, $\delta(\text{C}=\text{O}) + 10\%$, $\delta(\text{ring})$
3	425	3	1.3	425	35	3	0.5	0.09	0.16	437,425	7	2	446, 436,429,424	10	2	100%, $\delta(\text{OCNCO}, \text{ring})$
8	498	2	4.0	495	51	2	4.0	0.75	0.86	494,493	6	1	780, 763,514,504	4	1	68%, $\gamma(\text{N1-H}) + 17\%$, $\gamma(\text{ring}) + 15\%$, $\gamma(\text{C-H})$
5	522	1	4.0	523	19	1	4.2	0.60	0.75	531, 531	2	5	536, 536,531,529	4	2	100%, $\delta(\text{C}=\text{O}, \text{ring})$
6	586	8	0.7	585	6	7	0.1	0.08	0.16	588,586	2	6	603, 596,589,589	1	2	100%, $\delta(\text{ring})$
4?	595	0	6.0	594	0	0	5.4	0.75	0.86	597,596	1	0	603, 601, 585,583	2	1	36%, $\gamma(\text{C}=\text{C}) + 26\%$, $\gamma(\text{ring}) + 23\%$, $\gamma(\text{N3-H}) + 15\%$, $\gamma(\text{ring})$
7	603	6	1.7	600	0	6	2.6	0.08	0.14	610, 606	0	2	616,615,613,608	2	2	100%, $\delta(\text{ring})$
9	677	9	2.2	674	79	10	0.8	0.75	0.86	912, 881	1	0	952,932,922,890,852	1	0	60%, $\gamma(\text{N3-H}) + 15\%$, $\gamma(\text{N1-H}) + 15\%$, $\gamma(\text{ring}) + 10\%$, $\gamma(\text{C5-H})$
10	727	4	0.2	726	5	5	0.0	0.75	0.86	721, 721	2	0	732,722,691, 691	9	2	33%, $\gamma(\text{C5-H}) + 23\%$, $\gamma(\text{C4}=\text{O}) + 20\%$, $\gamma(\text{ring}) + 13\%$, $\gamma(\text{C2}=\text{O}) + 11\%$, $\gamma(\text{N1-H})$
11	764	1	18.0	762	42	1	18.4	0.75	0.86	740, 738	3	0	745, 743, 742,740	10	1	55%, $\gamma(\text{C2}=\text{O}) + 26\%$, $\gamma(\text{ring}) + 19\%$, $\gamma(\text{NCN})$
13	830	9	0.5	836	44	4	0.2	0.75	0.86	829,829	0	0	874, 826,825,821	8	4	53%, $\gamma(\text{C5-H}) + 29\%$, $\gamma(\text{C4-C5}) + 18\%$, $\gamma(\text{ring})$
12	906	2	1.5	892	9	2	1.2	0.04	0.07	898, 894	9	2	906,901, 898	13	2	100%, $\nu(\text{ring})$
14	1002	2	2.5	995	32	3	2.7	0.49	0.66	1028, 1027	11	1	1041,1039,1030, 1021	7	1	97%, $\nu(\text{ring})$
16	1009	10	0.3	1005	54	10	0.5	0.08	0.16	997,994	1	1	1010, 1001,999,996	1	2	95%, $\nu(\text{N-CC=}, \text{ring})$
17	1115	5	1.9	1106	23	2	2.2	0.22	0.36	1113,1112	3	1	1128,1125,1118, 1115	2	3	48%, $\nu(\text{ring}) + 29\%$, $\delta(\text{C-H}) + 23\%$, $\delta(\text{N1-H})$
18	1223	13	1.8	1205	26	16	1.3	0.28	0.44	1228,1228	2	4	1255,1250,1237,1224	1	7	48%, $\delta(\text{C-N-C}) + 29\%$, $\delta(\text{C-H}) + 23\%$, $\delta(\text{ring})$
22	1313	1	21.9	1316	9	2	26.5	0.45	0.62	1315,1315	2	1	1346,1342,1324^c,1318^c	2	1	46%, $\delta(\text{N3-H}) + 38\%$, $\delta(\text{N1-H}) + 10\%$, $\delta(\text{C-H})$
21	1400	6	1.1	1394	205	9	1.6	0.22	0.36	1427,1420	22	0	1436,1430,1427,1413	21	1	100%, $\nu(\text{C-N}, \text{ring})$
20	1411	9	2.0	1403	21	7	2.9	0.62	0.77	1468,1467	0	4	1489,1470,1461,1457	6	4	70%, $\delta(\text{N3-H}) + 17\%$, $\delta(\text{N1-H}) + 13\%$, $\delta(\text{ring})$
23	1496	11	11.0	1486	179	7	13.7	0.41	0.58	1494,1492	22	4	1525,1525,1502,1498	17	6	67%, $\delta(\text{N1-H}) + 33\%$, $\nu(\text{ring})$
24	1664	9	46.4	1650	229	11	46.9	0.08	0.15	1674,1674	16	6	1671, 1666,1663,1660	18	6	58%, $\nu(\text{C5}=\text{C6}) + 13\%$, $\delta(\text{N1-H}) + 12\%$, $\delta(\text{NCN}) + 10\%$, $\nu(\text{C4}=\text{O})$
25	1786	73	30.2	1766	780	100	39.5	0.28	0.44	1798,1796	84	7	1808, 1776,1768,1757	100	38	58%, $\nu(\text{C4}=\text{O}) + 17\%$, $\delta(\text{N3-H}) + 12\%$, $\nu(\text{C2}=\text{O}) + 11\%$, $\delta(\text{C5-H})$
26	1826	100	14.6	1805	636	83	31.0	0.11	0.21	1821,1811	33	15	1830,1813, 1797,1787	55	8	60%, $\nu(\text{C2}=\text{O}) + 14\%$, $\delta(\text{N1-H}) + 14\%$, $\nu(\text{C4}=\text{O}) + 11\%$, $\delta(\text{N3-H})$
28	3269	0	77.9	3260	8	0	75.6	0.24	0.38	3278,3278	1	25	3281,3278,3277,3250	1	46	100%, $\nu(\text{C5-H})$
29	3622	11	78.4	3600	81	11	74.5	0.22	0.36	3349,3314	100	100	3402,3266,3235,3179	68	100	99%, $\nu(\text{N3-H})$
30	3654	17	100	3635	116	16	100.0	0.17	0.29	3650,3649	5	8	3648,3645,3412, 3388	65	40	100%, $\nu(\text{N1-H})$

^aWith the 6-31G(d,p) basis set. ^b(C-Cl) substituent mode. ^cMainly $\delta(\text{N1-H}) + \delta(\text{C-H})$.

Table 4. Scaled and experimental vibrational wavenumbers in solid state of 6-CIU.

No.	Isolated state				Experimental		Dimer ^a	Tetramer ^a	characterization
	B3PW91	B3LYP			KBr	Raman	(d)	(d)	
	(a)	(b)	factor	(c)					
1	159	157				150.8 m	173, 171	227, 218, 215, 211	$\gamma(\text{C}=\text{O}) + \gamma(\text{ring})$
2	183	182	1.1198	177		208.4 w	212, 208	212, 209, 203, 199	puckering (N1, O10)
15 ^b	199	198	0.9982	175		186.5 w	192, 186	198, 186, 180, 175	$\gamma(\text{ring}) + \gamma(\text{ring})$
19 ^b	268	266	0.9989	246		267.6 m	276, 273	281, 280, 276, 268	$\delta(\text{C}-\text{Cl}) + \delta(\text{ring})$
27 ^b	381	378	0.9604	350		384.6 w	386, 385	393, 391, 386, 381	$\nu(\text{C}-\text{Cl}) + \delta(\text{C}=\text{O})$
3	436	436	0.9609	408	451 s	443 vw, 432.4 w, 420.0 w	448, 436	456, 447, 436, 435	$\delta(\text{OCNCO}, \text{ring})$
8	505	503	0.9788	484		757.8 vvw	501, 501	771, 756, 520, 510	$\gamma(\text{N1}-\text{H}) + \gamma(\text{ring})$
5	528	529	0.9791	512	549 s	539.4 w, 530.5 w	536, 536	541, 541, 536, 534	$\delta(\text{C}=\text{O}, \text{ring})$
6	588	588	0.9849	576			590, 588	604, 598, 591, 591	$\delta(\text{ring})$
4?	597	597	0.9917	589	610 w		602.9 vs	599, 598	$\gamma(\text{C}=\text{C}) + \gamma(\text{ring})$
7	604	603	1.0481	629	620 w		617.2 vs	611, 607	$\delta(\text{ring})$
9	674	673	0.9706	654	918 w		918.5 m	896, 867	$\gamma(\text{N3}-\text{H}) + \gamma(\text{N1}-\text{H})$
10	721	722	0.9830	714				716, 715	$\gamma(\text{C5}-\text{H}) + \gamma(\text{C4}=\text{O})$
11	756	757	0.9864	752	758 m		723.3 vvw	733, 732	$\gamma(\text{C2}=\text{O}) + \gamma(\text{ring})$
13	819	827	0.9779	818	849 sh, 841 s		842.5 vw	818, 818	$\gamma(\text{C5}-\text{H}) + \gamma(\text{C4}-\text{C5})$
12	890	880	1.0153	906	875 m			883, 880	$\nu(\text{ring})$
14	981	978	0.9875	983	1031 m		1032.2 vw	1006, 1005	$\nu(\text{ring})$
16	989	988	0.9958	1001	1000 m		994.2 w	977, 974	$\nu(\text{N}-\text{CC}=\text{O}, \text{ring})$
17	1088	1084	0.9943	1100	1131 s		1128.5 w	1086, 1085	$\nu(\text{ring}) + \delta(\text{C}-\text{H})$
18	1190	1178	0.9833	1185	1233 vw, 1203 vw		1234.9vs, 1206vw, 1181.7vw	1195, 1194	$\delta(\text{C}-\text{N}-\text{C}) + \delta(\text{C}-\text{H})$
22	1275	1284	0.9842	1295	1337 w		1334.8 vw	1277, 1277	$\delta(\text{N3}-\text{H}) + \delta(\text{N1}-\text{H})$
21	1357	1358	0.9875	1377	1414 vs		1406.6 s	1383, 1376	$\nu(\text{C}-\text{N}, \text{ring})$
20	1368	1366	0.9810	1376	1439 s		1436.9 w	1421, 1420	$\delta(\text{N3}-\text{H}) + \delta(\text{N1}-\text{H})$
23	1448	1445	0.9749	1449	1518 w, 1494 m		1516.4 vw, 1488.8 m	1446, 1444	$\delta(\text{N1}-\text{H}) + \nu(\text{ring})$
24	1607	1601	0.9813	1619	1654 s, 1599 s		1646.4 m, 1630 m	1616, 1616	$\nu(\text{C5}=\text{C6}) + \delta(\text{N1}-\text{H})$
25	1722	1712	0.9550	1687	1676 vs		1715.1 m	1734, 1731	$\nu(\text{C4}=\text{O}) + \delta(\text{N3}-\text{H})$
26	1760	1749	0.9752	1760	1763 sh, 1734 vs			1755, 1746	$\nu(\text{C2}=\text{O}) + \delta(\text{N1}-\text{H})$
28	3123	3133	0.9617	3135	3175 sh, 3144 sh			3131, 3131	$\nu(\text{C5}-\text{H})$
29	3456	3456	0.9551	3438	3094 vs, 3000 s		3090.4 vs, 2995 w	3199, 3166	$\nu(\text{N3}-\text{H})$
30	3487	3490	0.9567	3478	3439 vw			3482, 3482	$\nu(\text{N1}-\text{H})$

(a) With the scale equation obtained from uracil molecule: $\nu_{\text{sca.}} = 34.9 + 0.9393 \cdot \omega_{\text{cal. B3PW91}}$. (b) With the scale equation: $\nu_{\text{sca.}} = 31.9 + 0.9512 \cdot \omega_{\text{cal. B3LYP/6-311++G(3df, pd)}}$. (c) With the specific scale factors of the previous column obtained from uracil molecule at the B3LYP/6-311++G(3df, pd). (d) With the scale equation: $\nu_{\text{sca.}} = 34.6 + 0.9447 \cdot \omega_{\text{cal. B3LYP/6-31G(d, p)}}$. ^aWith the 6-31G(d, p) basis set. ^b(C-Cl) substituent mode.

Table 5. Total natural NBO atomic charges calculated in uracil (X=H) and halouracil derivatives (X=F, Cl, Br) with the halogen atom in position 5 and 6. The electronic density is only shown in uracil and 6-ClU.

Atom	Charge										Elect. density	
	X=H	5-XU			6-XU						6-XU	
		F	Cl	Br	F	Cl			Br	H	Cl	
	(a)	(a)	(a)	(a)	(a)	(a)	(b)	(c)	(d)	(a)	(a)	(a)
N1	-0.61	-0.603	-0.60	-0.60	-0.64	-0.63	-0.63	-0.75	-0.62	-0.63	7.98	8.08
C2	0.82	0.820	0.82	0.82	0.83	0.83	0.82	1.02	0.84	0.83	4.51	4.61
N3	-0.64	-0.639	-0.64	-0.64	-0.65	-0.64	-0.64	-0.78	-0.63	-0.64	8.20	8.22
C4	0.65	0.615	0.64	0.64	0.66	0.66	0.65	0.82	0.67	0.66	5.55	5.65
C5	-0.36	0.276	-0.20	-0.27	-0.43	-0.38	-0.39	-0.45	-0.39	-0.38	7.15	8.14
C6	0.08	-0.003	0.05	0.05	0.67	0.21	0.20	0.27	0.23	0.15	6.34	7.85
H7	0.42	0.420	0.42	0.42	0.43	0.43	0.43	0.48	0.48	0.43	0.36	0.36
O8	-0.63	-0.627	-0.62	-0.62	-0.62	-0.62	-0.61	-0.72	-0.69	-0.62	9.00	9.00
H9	0.42	0.423	0.42	0.42	0.42	0.42	0.43	0.47	0.46	0.42	0.36	0.35
O10	-0.60	-0.578	-0.58	-0.58	-0.60	-0.59	-0.59	-0.69	-0.68	-0.59	8.99	9.03
H	0.21	0.224	0.22	0.22	0.25	0.25	0.25	0.28	0.26	0.25	0.57	0.66
X	0.24	-0.325	0.06	0.13	-0.32	0.07	0.07	0.05	0.08	0.13	0.66	17.42

(a) At the B3LYP/6-311++G(3df,pd) level. (b) At the B3PW91/6-311++G(3df,pd) level. (c) At the MP2/6-31G(d,p) level. (d) At the PCM B3LYP/6-311++G(3df,pd) level

Table 6. Calculated low vibrational modes at the B3LYP/6-31G(d,p) level in the dimer and tetramer forms of 6-CIU, and comparison with the experimental Raman bands.

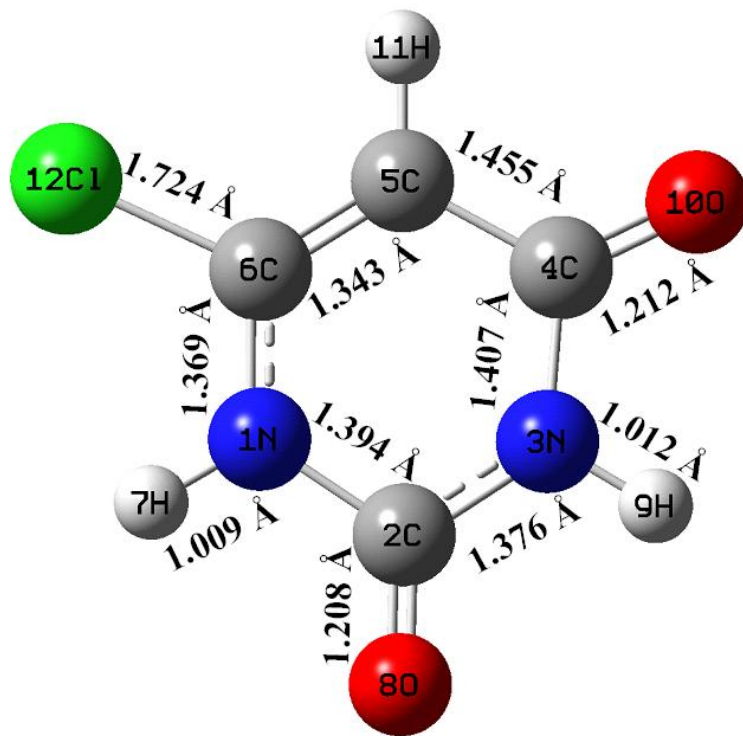
Characterization	dimer	tetramer	experimental
δ (H-bond stretching)	104	128, 113, 108	124 m, 115 m, 104.0 vs
δ (H-bond shearing)	73	91, 77, 70	95.0 vs, 81.4 s
γ (tilting “slanting”)	56	63, 56, 53	58.2 s
δ (cogwheel)	53	49, 45, 37	
γ (torsion “twist”)	21	28, 25, 23	
γ (butterfly)	13	17, 17, 7	

Table 7. Theoretical computed total energies (A.U.), rotational constants (GHz), entropies ($\text{J mol}^{-1} \text{K}^{-1}$) and dipole moments (Debyes) in 6-CIU.

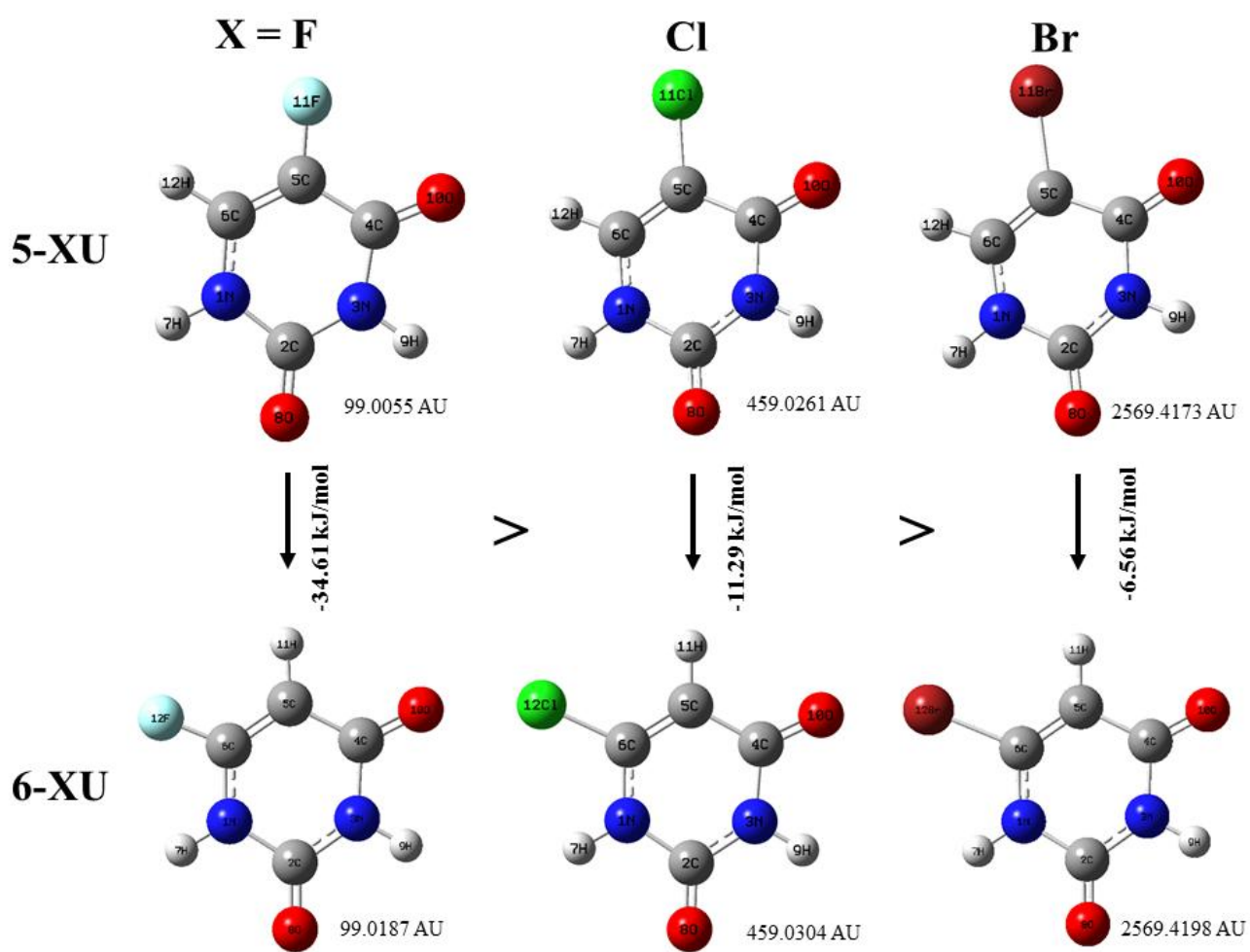
Parameters	isolated state				tetramer
	MP2/6-31G(d,p)	B3LYP/6-311++G(3df,pd)	B3LYP(PCM)/6-311++G(3df,pd)	B3PW91/6-311++G(3df,pd)	B3LYP/6-31G(d,p)
Total energy +ZPE	-872.608527	-874.522963	-874.554124	-874.305141	-3497.416285
Rotational constants	2.005 1.136 0.725	2.031 1.141 0.731	2.041 1.145 0.733	2.040 1.149 0.735	0.074 0.060 0.035
Entropy					
Total	363.49	360.7	358.6	360.7	973.5
Translational	170.75	170.5	170.5	170.5	188.1
Rotational	123.94	123.7	123.7	123.7	162.6
Vibrational	68.80	66.0	64.4	66.0	623.2
Dipole moment	3.554	3.271	4.928	3.269	8.917

LEGENDS TO FIGURES

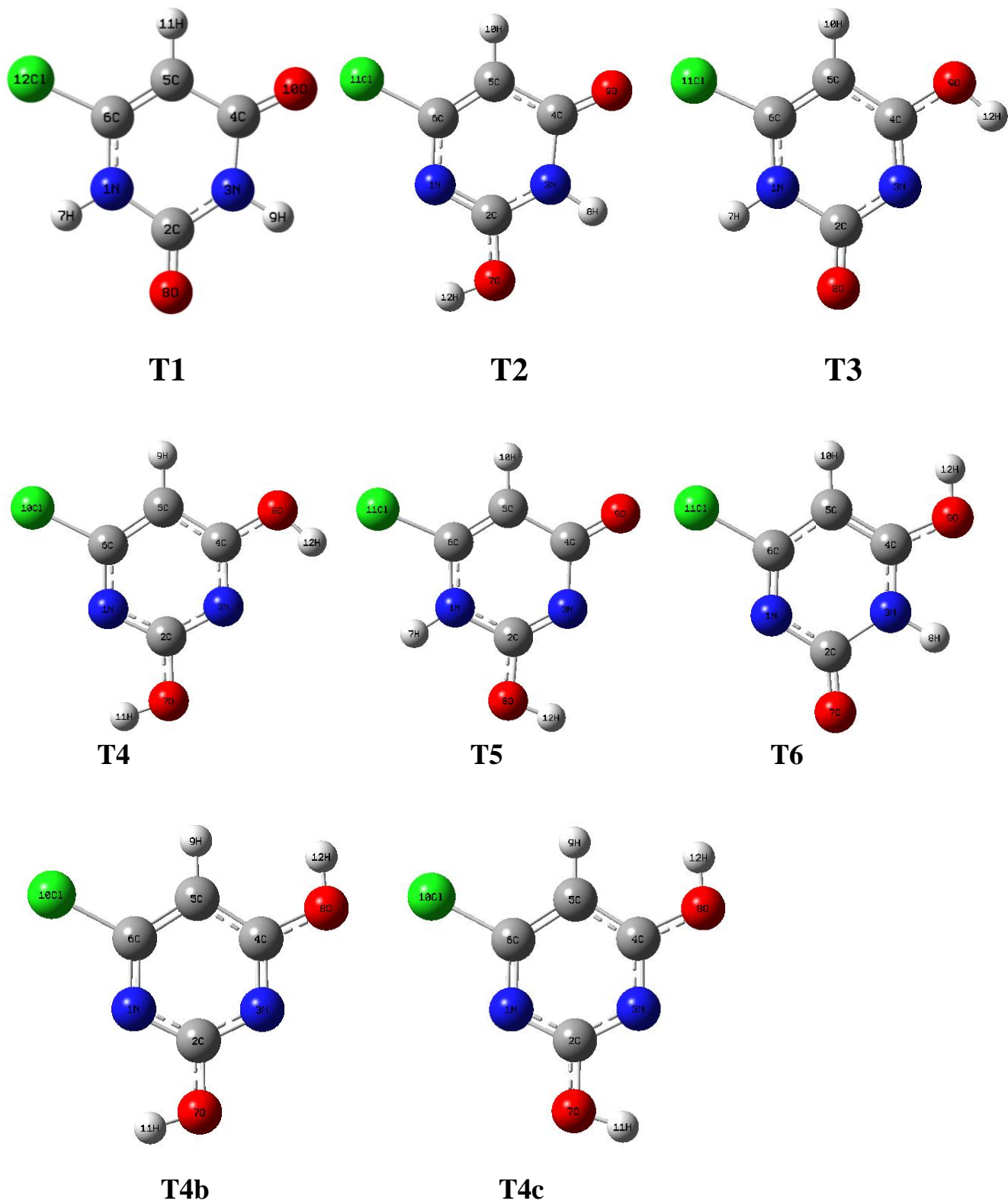
- Fig. 1.** Bond lengths (Å) and labeling of the atoms in 6-chlorouracil at the B3LYP/6-311++G(3df,pd) level.
- Fig. 2.** Energy increments $\Delta(E+ZPE_{\text{uracil}} - E+ZPE_{5(6)\text{-XU}})$ in AU calculated at the MP2/6-31G(d,p) level due to the introduction of -F, -Cl and -Br atoms in position (5) and (6) of the uracil molecule. $E+ZPE_{\text{uracil}} = -413.578145$ AU.
- Fig. 3.** Structures and labeling of the atoms in the neutral form of 6-chlorouracil tautomers. (T1) 2,4-dioxo 6-chlorouracil, (T2) 2-hydroxy-4-oxo 6-chlorouracil, (T3) 2-oxo-4-hydroxy 6-chlorouracil, (T4) 2,4-dihydroxy 6-chlorouracil, (T5) 1H-2-hydroxy-4-oxo 6-chlorouracil, (T6) 3H-2-oxo-4-hydroxy 6-chlorouracil, (T9) 3H-2,4-hydroxy 6-chloro (1H)uracil,
- Fig. 4.** Simulation of the solid state: Optimized dimer form of 6-CIU at the B3LYP/6-31G(d,p) level.
- Fig. 5.** Two views of the optimized planar forms of 6-CIU at the B3LYP/6-31G(d,p) level, tetramers A and B.
- Fig. 6.** Two views of the optimized non-planar forms of 6-CIU at the B3LYP/6-31G(d,p) level, tetramers C and D.
- Fig. 7.** Theoretical scaled IR spectrum in the 3800-2000 cm^{-1} range of 6-CIU using the scale equation procedure in the monomer and tetramer simulations, and comparison with the experimental ones.
- Fig. 8.** Theoretical scaled IR spectrum in the 2000-400 cm^{-1} range of 6-CIU using the scale equation procedure in the monomer and tetramer simulations, and comparison with the experimental ones.
- Fig. 9.** Theoretical scaled Raman spectrum in the 3550-2300 cm^{-1} range of 6-CIU using the scale equation procedure in the monomer and tetramer simulations, and comparison with the experimental ones.
- Fig. 10.** Theoretical scaled Raman spectrum in the 1800-800 cm^{-1} range of 6-CIU using the scale equation procedure in the monomer and tetramer simulations, and comparison with the experimental ones.
- Fig. 11.** Theoretical scaled Raman spectrum in the 800-50 cm^{-1} range of 6-CIU using the scale equation procedure in the monomer and tetramer simulations, and comparison with the experimental ones.
- Fig. 12.** The atomic orbital composition of the frontier molecular orbitals for 6-CIU.



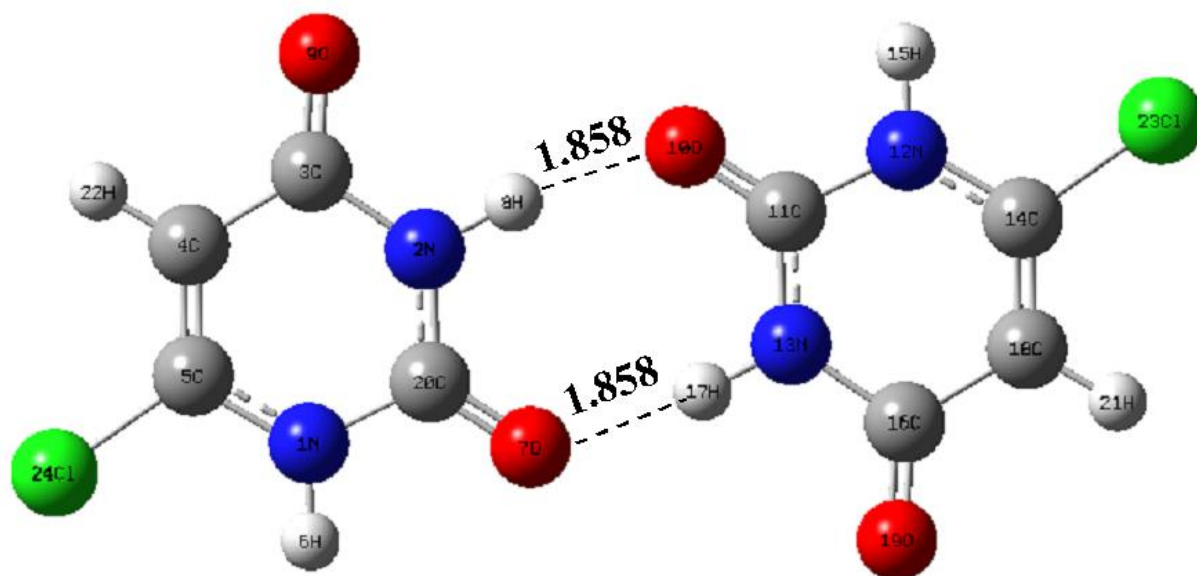
(Fig. 1)



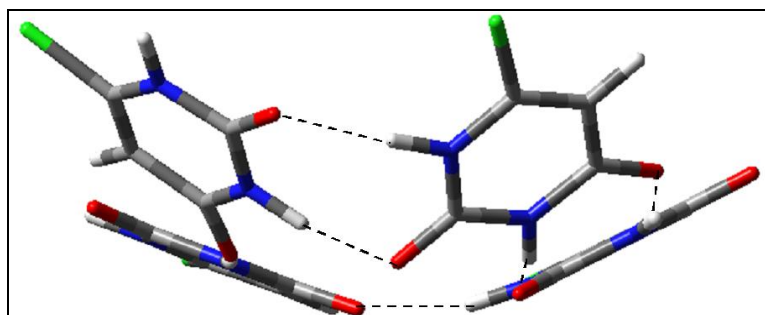
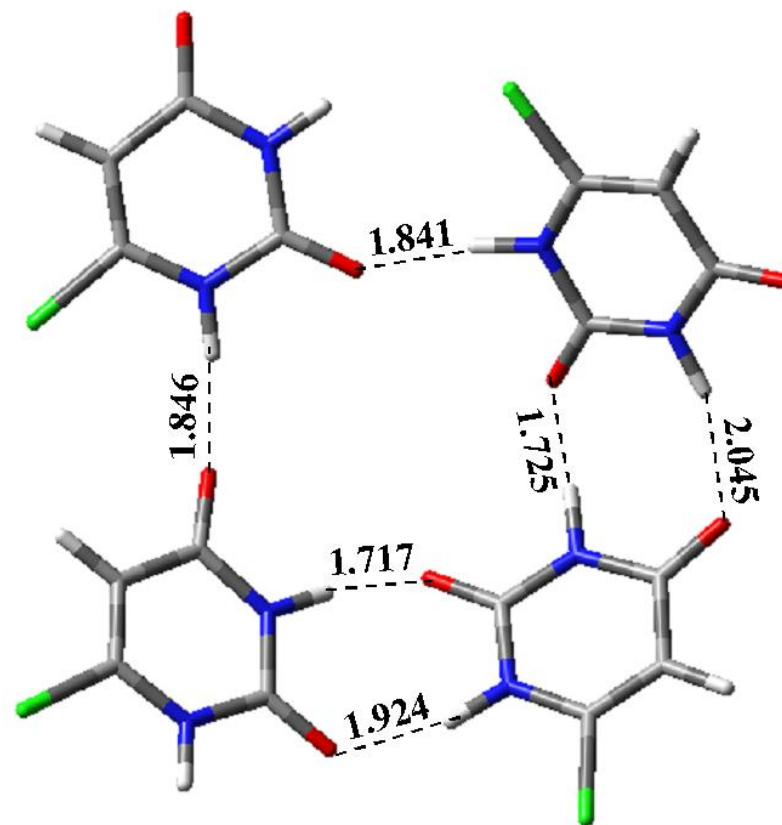
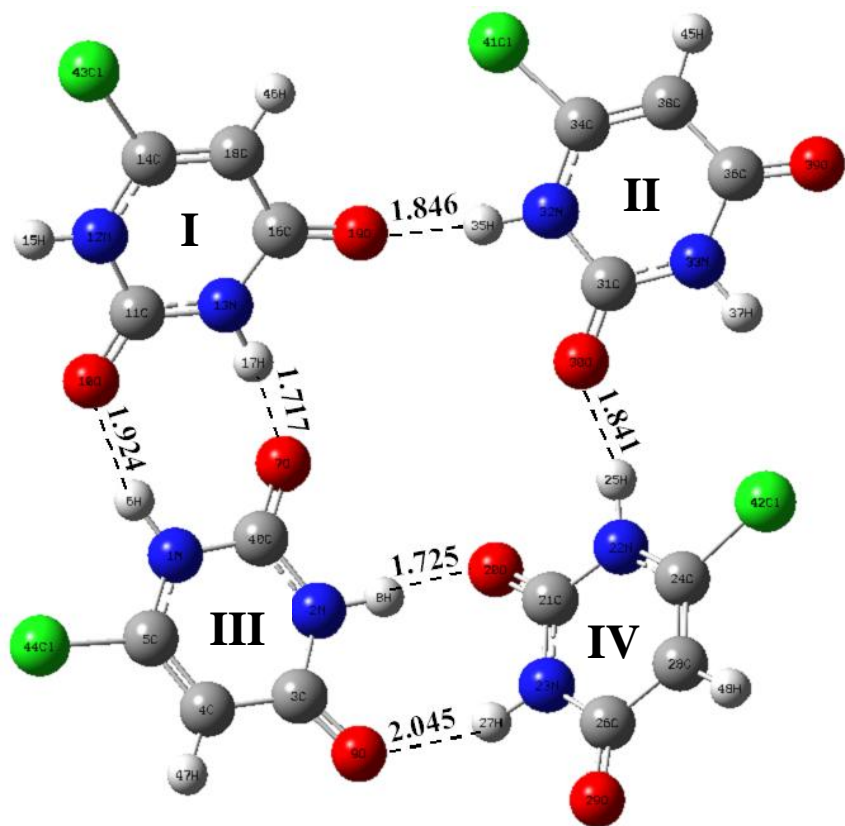
(Fig. 2)



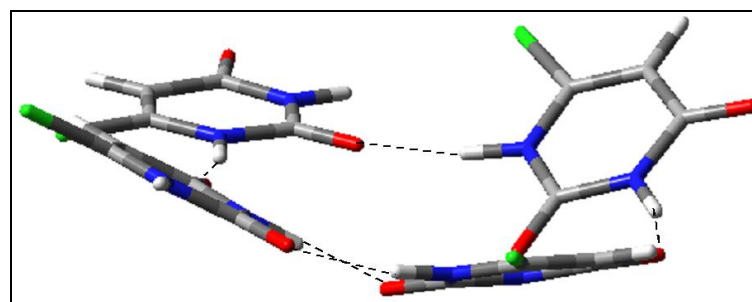
(Fig. 3)



(Fig. 4)

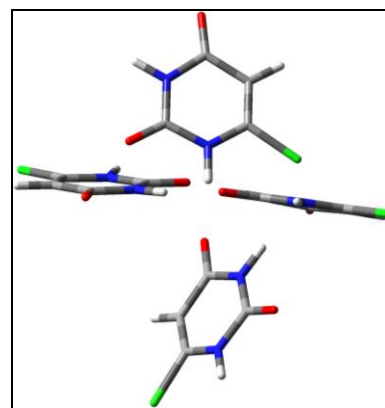
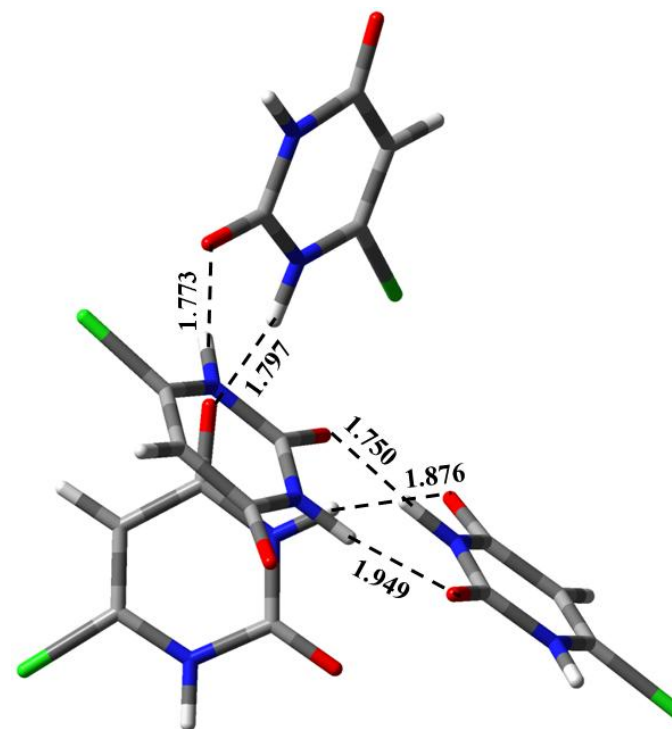
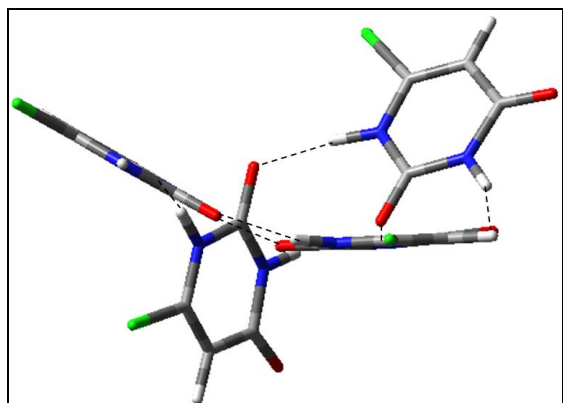
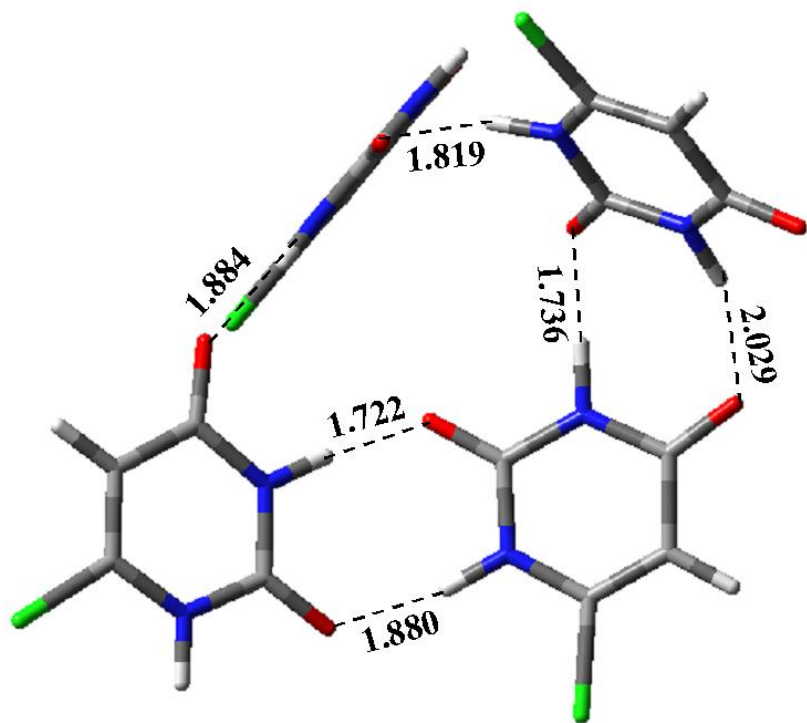


Tetramer A: $E+ZPE = -3497.416285$ AU ($G = -3497.491387$ AU)



Tetramer B: $E+ZPE = -3497.415920$ AU ($G = -3497.492936$ AU)

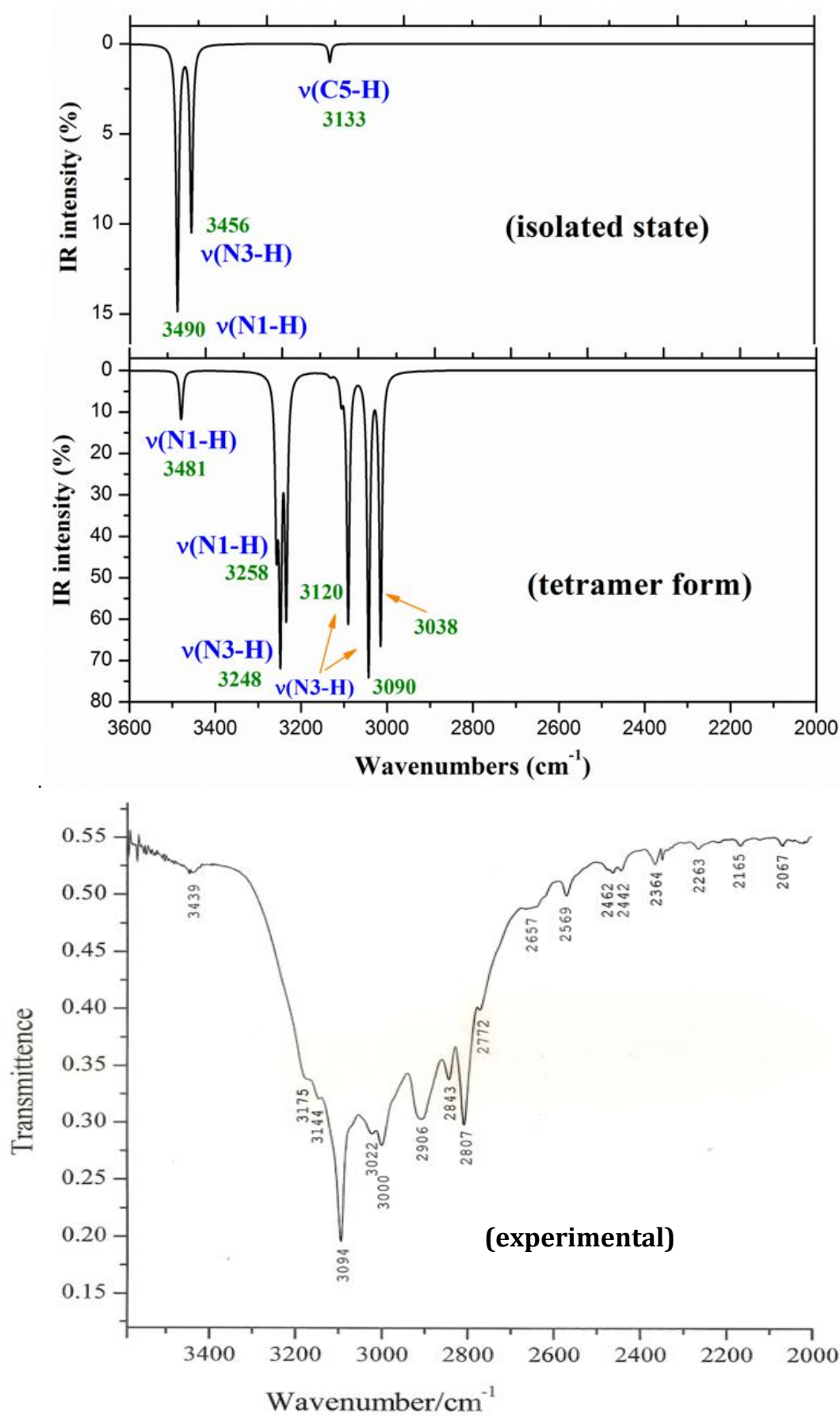
(Fig. 5)



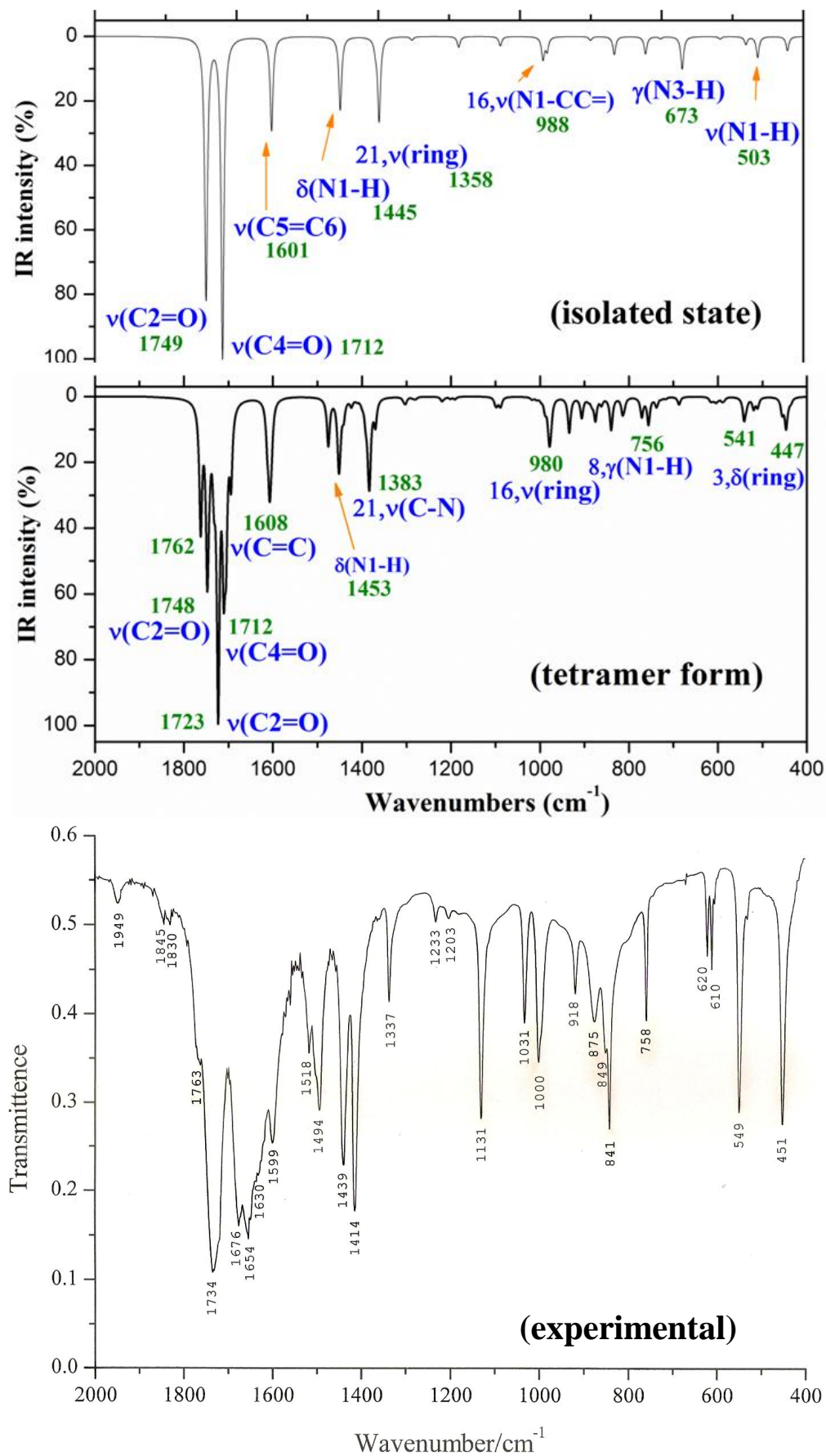
Tetramer C: $E+ZPE = -3497.419200$ AU ($G = -3497.493566$ AU)

Tetramer D: $E+ZPE = -3497.413196$ AU ($G = -3497.489949$ AU)

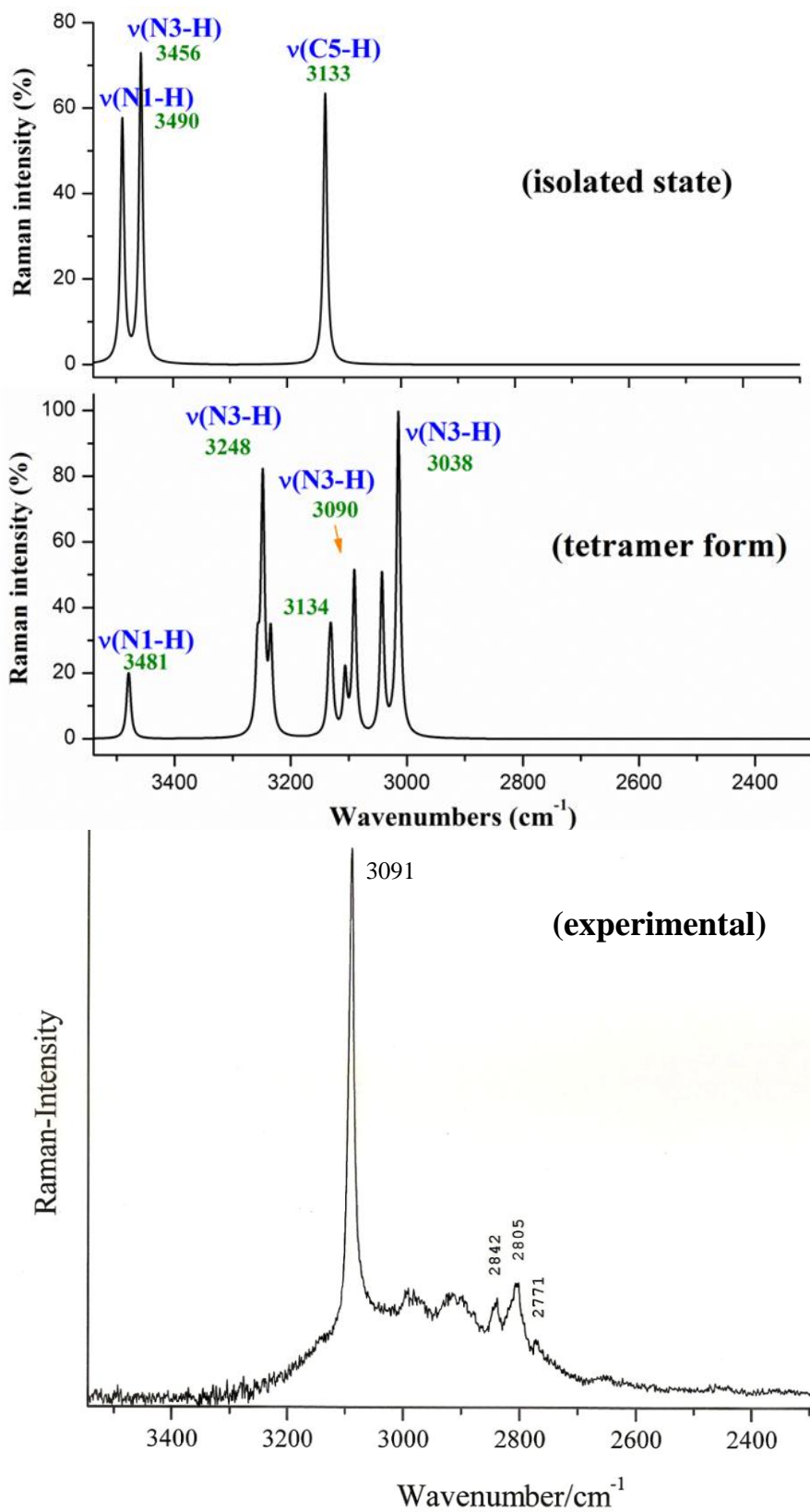
(Fig. 6)



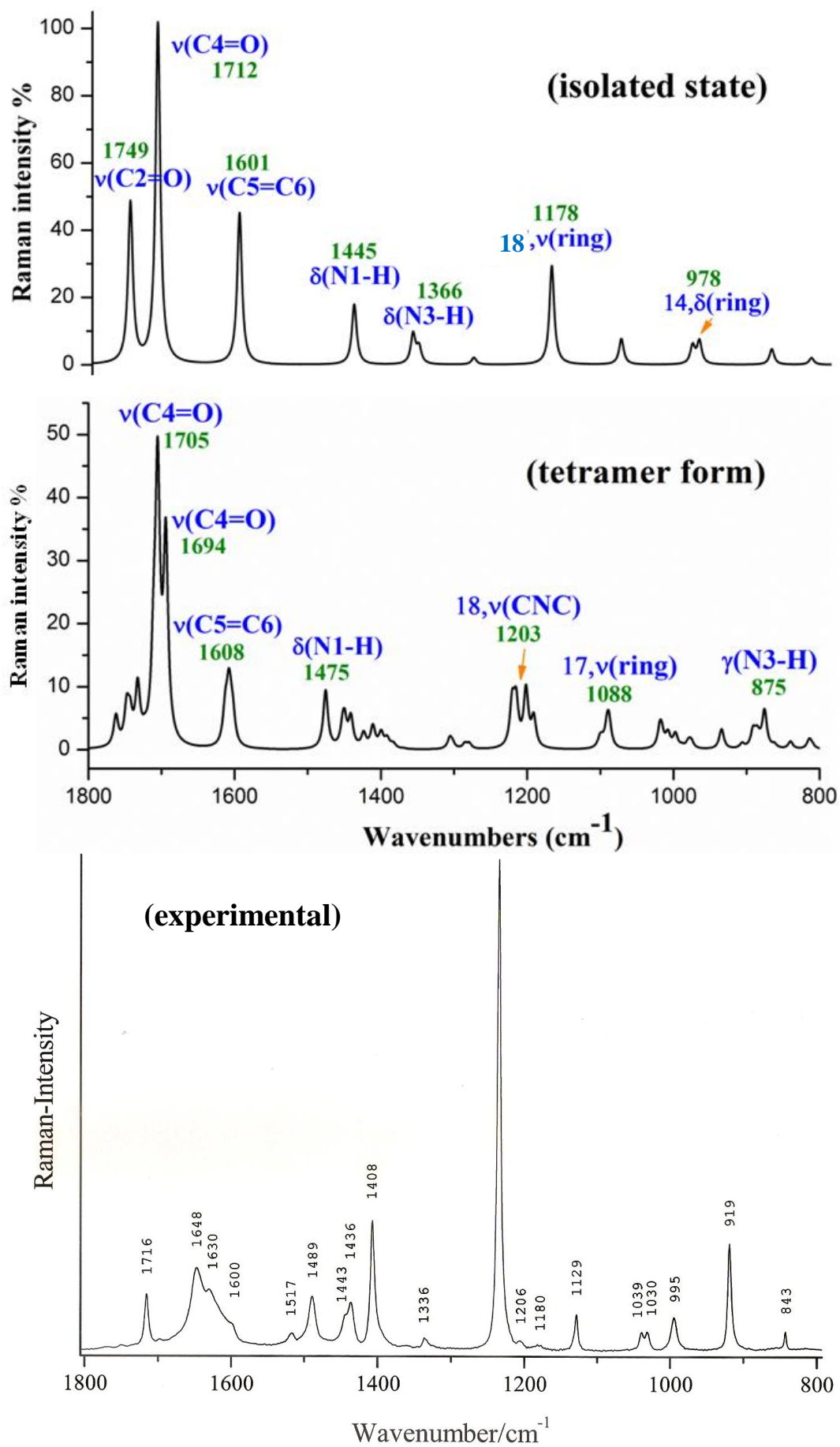
(Fig. 7)



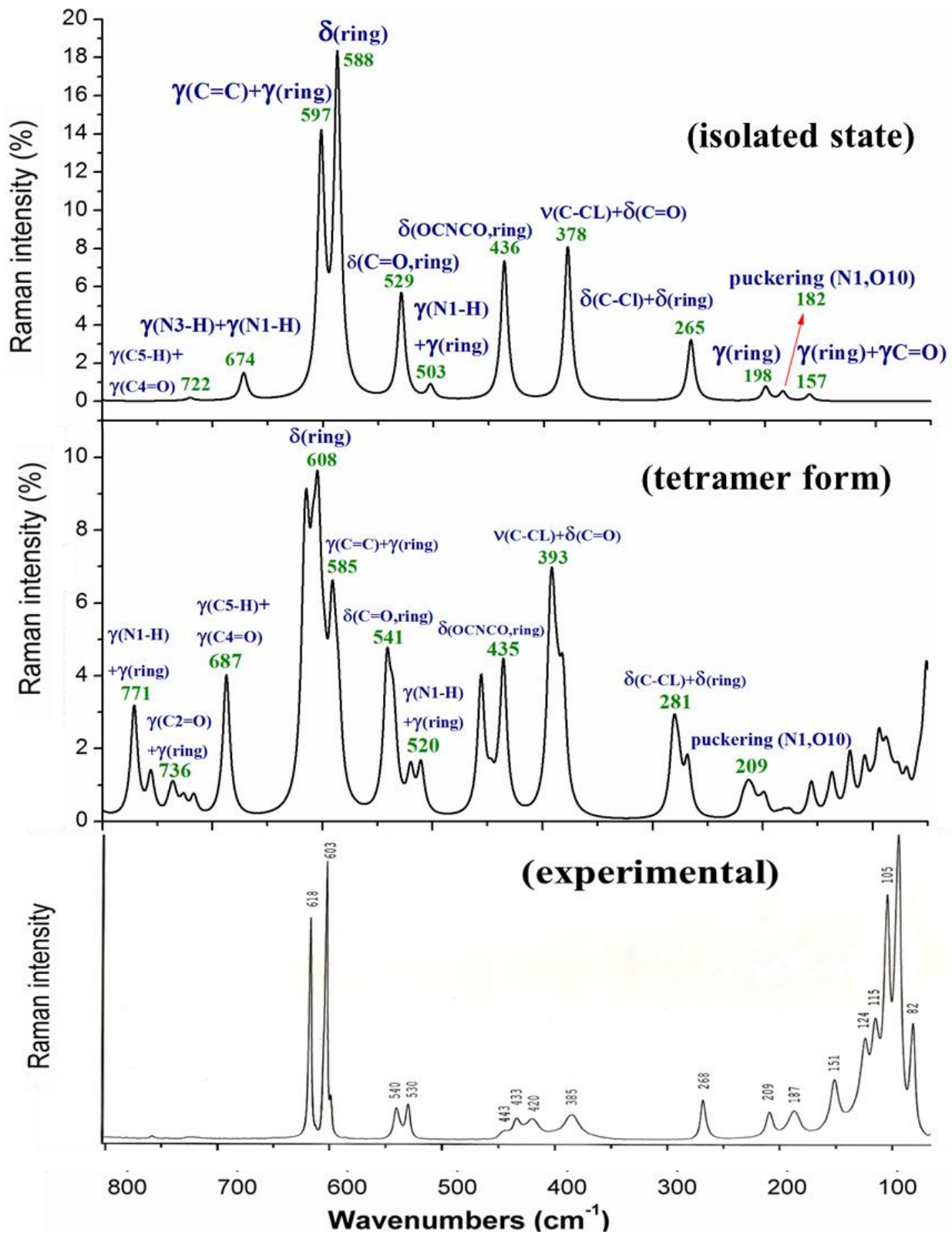
(Fig. 8)



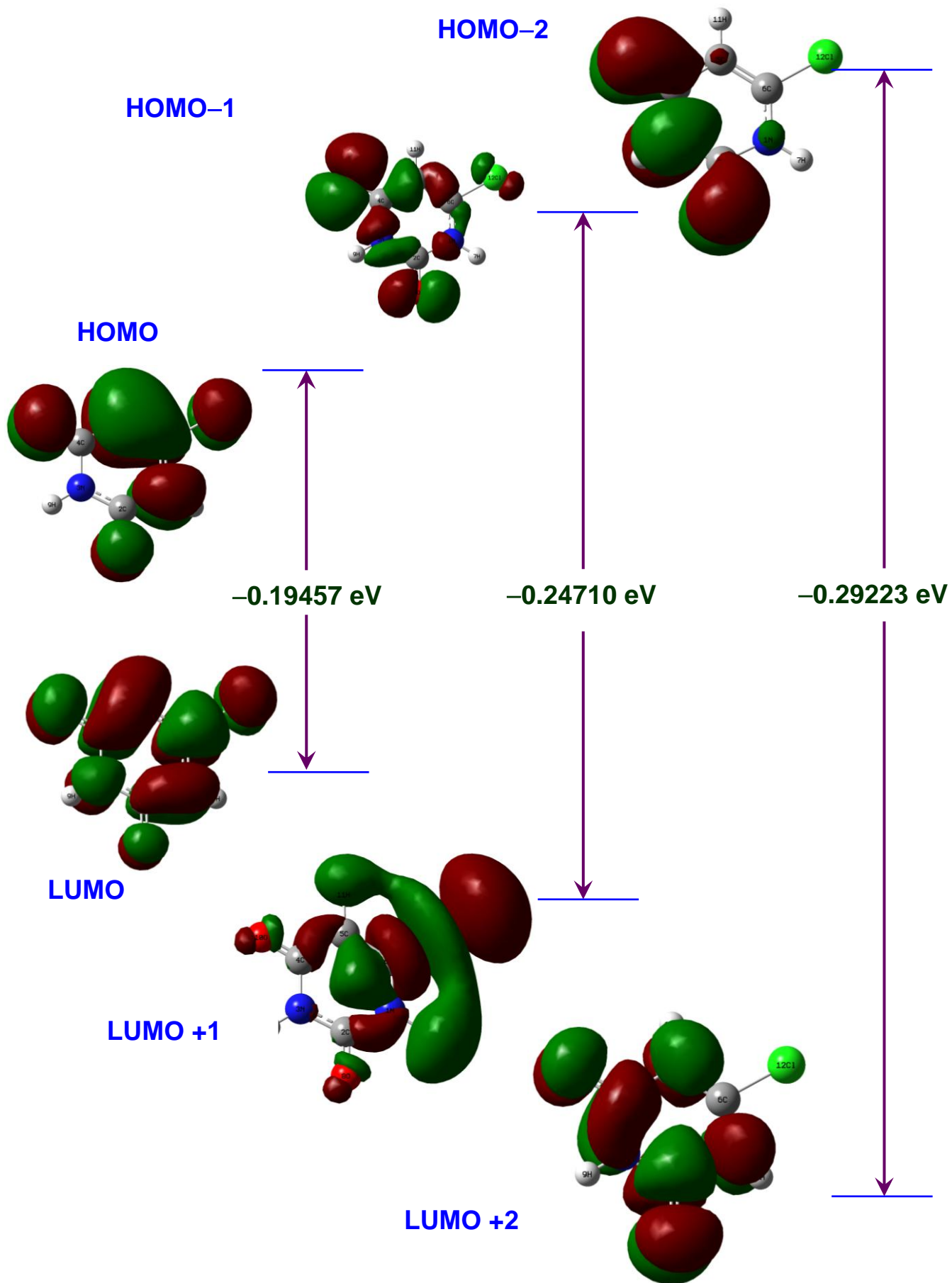
(Fig. 9)



(Fig. 10)



(Fig. 11)



(Fig. 12)

SUPPLEMENTARY MATERIAL

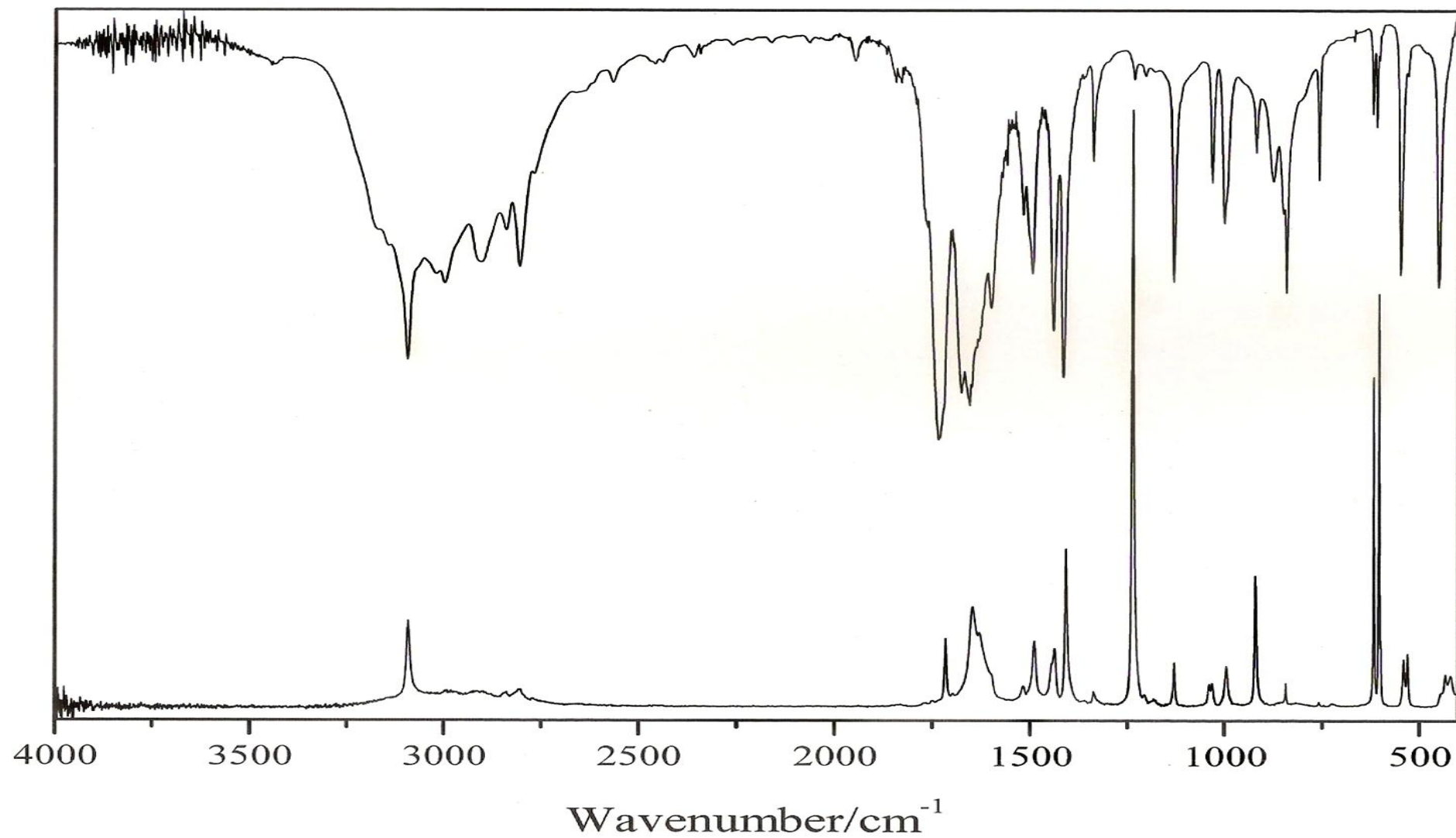


Fig. 1-Sup. Comparison of the IR and Raman spectra of 6-CIU.

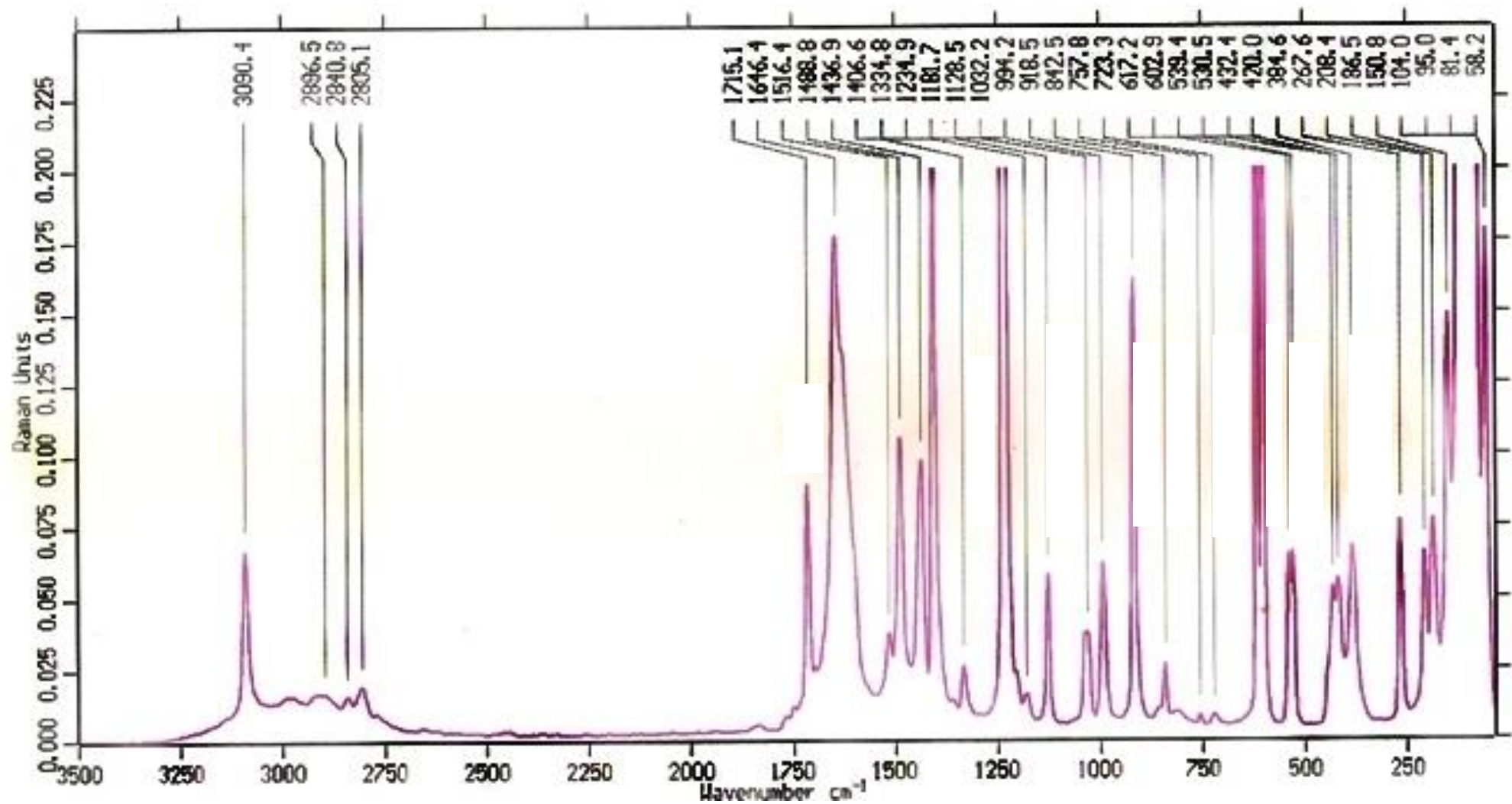


Fig. 2-Sup. Raman spectrum of 6-CIU with the wavenumber of the bands observed.