Density Functional Theory Studies on Transimination of Vitamin B6 Analogues through Geminal Diamine Formation

Antoni Salvà, Josefa Donoso,* Juan Frau, and Francisco Muñoz*

Institut Universitari d'Investigació en Ciències de la Salut (IUNICS), and Departament de Química, Universitat de les Illes Balears, 07122 Palma de Mallorca, Spain

Received: June 23, 2004; In Final Form: September 27, 2004

A theoretical study on the transimination reaction in the gas phase of a molecular complex that consists of a Schiff base formed from a vitamin B6 model compound, methylamine, and one water molecule is reported. In this nonenzymatic environment, DFT calculations (B3LYP/6-31+G*) with full optimization of each intermediate and transistion state structure and a further study of the topology of the charge density function according to Bader's AIM theory are carried out. This work has allowed the detailed outline of the energy profile of the reaction leading to the geminal diamine formation and the description of the geometries of all the structures involved. The reaction determining step is the addition of the incoming amine onto the Schiff base and the main intermediate of the overall reaction is a geminal diamine structure. The mechanism also points out the reactive role of one water molecule by allowing the proton transfer between distant sites of the molecular complex by means of the formation of a concerted transition state.

Introduction

Pyridoxal phosphate (PLP) serves as a prosthetic group for enzymes that catalyze reactions of amino acids such as transamination, racemization, decarboxylation, aldol cleavage, elimination, and replacement.[1] In enzymes, PLP is bound covalently by means of an imine bond to the ϵ-amino group of an active-site lysine residue (internal aldimine). An incoming substrate amino acid can react with this Schiff base producing a newly formed imine (external aldimine) and the displacement of the amino group of lysine. This reaction, known as transimination or transaldiminization, is symmetrical and in the opposite way converts external aldimines into internal aldimines. The external aldimines can be released to different active sites of the protein. This reaction is a very general step for metabolic reactions involved with amino acids and is of greatest importance in cellular reactions.[2]

From a mechanistic point of view, Snell and Jenkins[3] first postulated that transimination proceeds through the formation of a geminal diamine as the main intermediate. Further experimental work[4−8] gave evidence for the existence of such intermediate. However, other authors have proposed alternative pathways, like a 2-fold addition−elimination mechanism that involves two different carbinolamines.[9,10] The high rate and complexity of enzymatic transimination have made very difficult the study of this reaction from a chemical point of view. Whereas Toney and Kirsch[11] produced a direct transimination scheme with no intermediate in the replacement of Lys258 by alanine in wild-type aspartate aminotransferase, Drewe and Dunn[12,13] and Miles[14] showed that a geminal diamine is an intermediate in the second step in the PLP-requiring tryptophan synthase. UV and NMR spectroscopic studies of nonenzymatic transimination rely on the formation of geminal diamines as intermediate compounds.[15−23] This experimental work has yielded the kinetic and equilibrium description of various tautomerization and cyclization reactions that involve the formation of such intermediate. The influence of general acid base catalysis on the proceeding of these reactions and solvent effects regarding the proton transfer have also been studied experimentally.[2,8,24,25]

Quantum mechanics calculations on model compounds that keep the chemical functionality of the bulk molecular systems can provide a detailed description of each one of the intermediate and transition state structures that play a part in a reaction. This description involves the geometry and energy. Further properties such as the topology of the charge density function[26] can also be obtained. These results allow the modelization of the mechanism of a reaction, which gives complementary information to the experimental evidence.

The extended conjugated π-electron system of the pyridine ring of PLP has been the object of theoretical studies on transamination intermediates[27] and on the ability of forming carbanion substrates.[28] Bach et al.[29] studied the role of pyridoxyl ring in PLP-mediated amino acid decarboxylation by means of ab initio calculations on PLP model compounds. One of the conclusions of this work is that the formation of a neutral but zwitterionic tautomer of the substrate as a consequence of general acid catalysis lowers the energy gap between the ground state and the transition structure. Toney[30] noted the catalytic role of the 3′-hydroxyl group of PLP in Schiff base formation in a PM3 study regarding nonenzymatic and enzymatic pyridoxal phosphate catalyzed decarboxylation of 2-aminoisobutyrate.

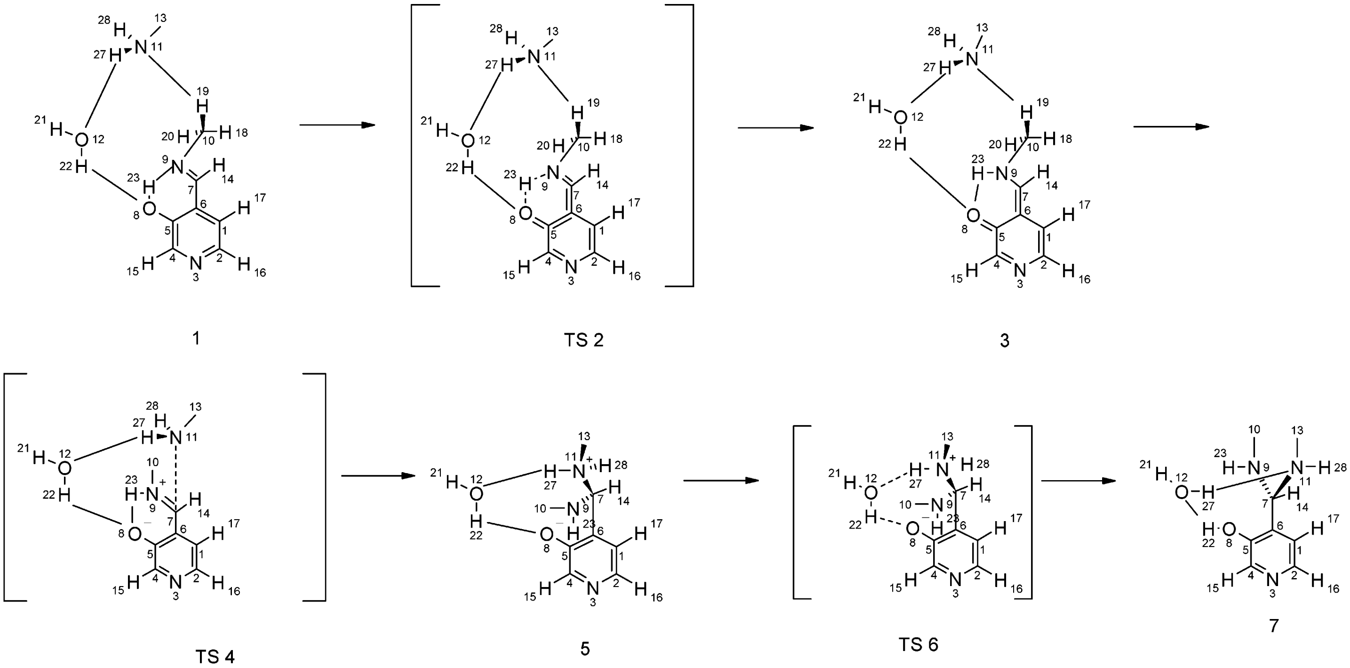
We have reported a theoretical study using a semiempirical PM3 Hamiltonian on the transimination of molecular complex consisting of a Schiff base of a PLP model compound and methylamine, another molecule of methylamine, and two water molecules.[31] This modelization in the gas phase and using two different solvation methods gave results that allowed the description of a transimination mechanism based on the existence of a geminal diamine intermediate and a concerted

---

* Corresponding author. E-mail: dqufmi0@uib.es.

10.1021/jp047258m CCC: $27.50

SCHEME 1: Scheme of the Geminal Diamine Formation[a]



[a] Hydrogen bonds are represented as bold dotted lines whereas in transition state structures thin dashed lines represent the bonds connecting nuclei that take part significantly in the transition state vector regarding the imaginary vibration.

transition state intermediate in which one water molecule acts as a reactive by exchanging one of its hydrogen atoms with another hydrogen atom of the incoming amine and the phenolic group of the pyridine ring. At a more reliable level of theory, a DFT study regarding the formation of a Schiff base from the same PLP analogue, methylamine, and one water molecule also produced such a concerted transition state among the mechanism of the reaction which evolved to a carbinolamine intermediate.[32]

In this work the mechanism of the transimination reaction of a PLP model compound and methylamine, another molecule of methylamine and one water molecule in the gas phase is described by means of DFT calculations on the intermediate transition state structures. The results are discussed in contrast with the PM3 study and the experimental evidence.

Methodology

The model compound used was the Schiff base formed from 4-pyridinaldehyde and methylamine, plus one molecule of water (**1** in Scheme 1). Though the Schiff base stands for the aldimine, the methylamine is the analogue for the amino acid with the free amino group. The water molecule is included in the molecular complex not only because of its consideration as a reactive species for the proceeding of the reaction but also for the need of consideration of the solvent to avoid the lack of potential barrier in reactions involving a nucleophilic approach, as reported by several theoretical studies.[33−35]

DFT calculations were performed using the Gaussian94 program package[36] running in a Silicon Graphics Octane computer. All structures were fully optimized at the B3LYP level of theory.[37,38] Density functional theory methods with the exact exchange in hybrid form, in particular B3LYP, offer geometrical parameters, also for hydrogen bonds, in better accordance with MP2 data than results of HF calculations.[39,40] The 6-31+G* basis set has been used in this study. Polarized and diffuse basis functions for heavy atoms were included to ensure reliable results for small charge-localized charged atoms.

Reaction coordinates were used with full optimization of every parameter until the desired steady-state point was reached. Further vibrational analyses were performed on all optimized structures to characterize them as minima or saddle points in the energy hypersurface. All minima showed all the force constants positive, whereas saddle points had one, and only one, negative force constant. For the latter structures, IRC calculations were performed along the transition vector defined by the vibration mode of this imaginary frequency to assess that the saddle point structure connected downhill the corresponding forward and backward ground-state structures. This methodology allowed the identification of reaction intermediates and transition state structures along the reaction path.

Further, using the electron-correlated (B3LYP/6-31+G*) wave function of each intermediate and transition state structure, a topological study of the density charge function was performed according to Bader's AIM theory[26] using the AIM2000 program[41] running on a PC. Once bond and ring critical points were located, molecular paths connecting bond critical points (BCP), and bond and ring critical points (RCP) were also computed for each of the structures along the reaction path found. When indicated, atomic charges are the result of integration of the charge density function in the region closed by the zero flux surface around the atom (atomic basin). Bond orders of C−C bonds have been calculated according to an expression found by Bader and co-workers that fits for the bond order the value of the charge density obtained by using the 6-31G* basis at the bond critical point of the C−C bond.[42]

Results and Discussion

The detailed mechanism for the formation of the geminal diamine in the transimination reaction using the sequence of ground and transition state structures for the molecular complex described above is shown in Scheme 1. The energies for each of the structures appear in Table 1. The main geometrical values for each of those structures are listed in Table 2. The energy profile for the geminal diamine formation (first half-reaction of the overall symmetrical process) is outlined in Figure 1.

The beginning of the mechanism is structure **1**, which is displayed in a perspective view in Figure 2. In the aldimine

TABLE 1: Energies (RB+HF-LYP) and Free Energies (Sum of Electronic and Thermal Free Energies) for Each of the Structures of the Reaction Path from the Standard Thermochemistry Output of a Frequency Calculation[a]

structure	*E*(RB−HF-LYP)	$\epsilon_o + G_{corr}$
1	−628.595751	−628.406542
TS**2**	−628.587901	−628.404716
3	−628.595595	−628.408745
TS**4**	−628.577133	−628.381402
5	−628.579829	−628.381259
TS**6**	−628.574815	−628.381121
7	−628.591218	−628.393194

[a] ϵ_o Is the total electronic energy and G_{corr} is the thermal correction to the Gibbs free energy. All energies are in au.

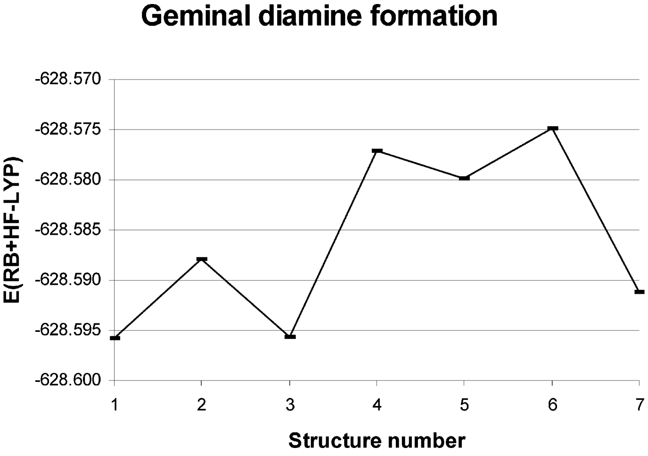


Figure 1. Energy profile for the reaction. Energy is in au.

molecule, an intramolecular hydrogen bond is found between H23 and N9 (1.692 Å). The water molecule is doubly hydrogen bonded to the phenolic oxygen (distance O8H22 is 1.925 Å) and to one of the hydrogen atoms (O12H27 is 2.130 Å) of the incoming amine. These hydrogen-bonding interactions are confirmed by means of the finding of paths of maximum electron density from one nucleus to the other (bond paths) through BCPs (BCPs 1, 2, and 3 in Figure 3, respectively). The incoming amine lies far apart from C7 (distance N11C7 is 4.692 Å) with no interaction of this amine to the target of the nucleophilic attack the aldimine, C7, as is revealed by the lack of a BCP between both nuclei.

The analysis of the topology of the charge density function also provides the location of ring critical points (RCPs). Paths from the BCPs between the nuclei that form such a ring to the corresponding RCP are found. As can be seen from Figure 3, not only the expected RCP is found in the middle of the pyridine ring (RCP1), but also two more RCPs (RCP2 and RCP3) can be found. Whereas RCP2 is associated with the intramolecular hydrogen bond (H23N9) and the bonding of atoms O8, C5, C6, and C7; RCP3 reveals a cyclic structure perpendicular to the pyridine plane formed by the nuclei participating in the hydrogen bonding network of the incoming amine, the water molecule, and the aldimine. In this latter ring structure, even a weak hydrogen bonding interaction between H19 and N11 at a distance of 2.501 Å is found. This is the only preliminary interaction of the incoming amine with the aldimine−water molecular complex in this initial structure.

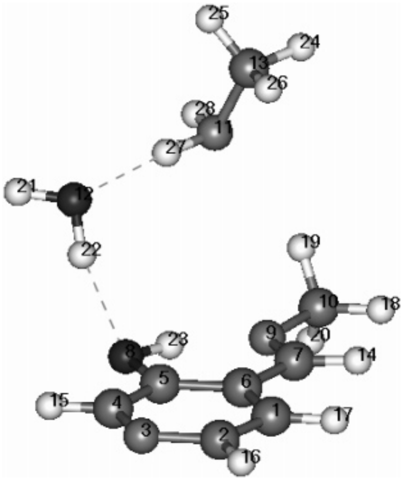


Figure 2. Perspective view of **1**, the starting structure for the reaction path.

The nucleophilic attack of the incoming amine to C7 is not performed directly from structure **1**. Before that, the internal proton transfer of H23 to N9 is realized through the surmounting of the transition state structure TS**2** with an energy barrier of 0.00482063 au (3.02 kcal/mol). The hydrogen of the phenolic group is transferred to the imine nitrogen N9 with no significant change in the structure of the external hydrogen bonding network or in the geometry of the aldimine molecule (see Table 2). The ionization of the phenolic group of the pyridine ring by means of this internal proton transfer was also found in the first step of the DFT study of the formation of a Schiff base from 4-pyridinaldehyde and methylamine[32] and in the PM3 modelization of the transimination reaction.[31] This result is in accordance with Toney's suggestion from his computational studies[30] that the phenolic hydrogen plays an important catalytic role in Schiff base formation.[43]

TABLE 2: Main Geometrical Data of the Structures (Distances in Å and Angles in Degrees)

	structure	**1**	TS**2**	**3**	TS**4**	**5**	TS**6**	**7**
distance	C1−C2	1.392	1.389	1.374	1.389	1.391	1.393	1.395
	C2−N3	1.344	1.347	1.367	1.350	1.348	1.343	1.339
	N3−C4	1.335	1.331	1.313	1.327	1.328	1.333	1.336
	C4−C5	1.408	1.410	1.448	1.434	1.432	1.413	1.403
	C5−C6	1.413	1.420	1.447	1.431	1.430	1.413	1.410
	C6−C1	1.404	1.404	1.422	1.405	1.402	1.403	1.401
	C7−N9	1.281	1.292	1.313	1.397	1.446	1.452	1.457
	C5−O8	1.355	1.323	1.277	1.301	1.302	1.336	1.371
	O8−H23	1.011	1.222	1.724	1.925	2.039	2.176	2.229
	C6−C7	1.465	1.447	1.416	1.500	1.507	1.523	1.532
	C7−N11	4.692	4.795	4.798	1.734	1.553	1.522	1.484
	O12−H27	2.130	2.086	3.068	1.779	1.686	1.414	1.003
	O8−H22	1.925	1.844	1.798	1.633	1.586	1.331	0.996
	N9−H23	1.692	1.269	1.036	1.025	1.026	1.022	1.019
dihedral angle	C1−C6−C7−N9	71.1	178.7	179.9				
	C6−C5−O8−H23	0.0	0.7	−1.3	23.1	27.9		
	C1−C2−N3−C4	0.1	0.0	0.1	0.3	0.2	−0.6	−0.6

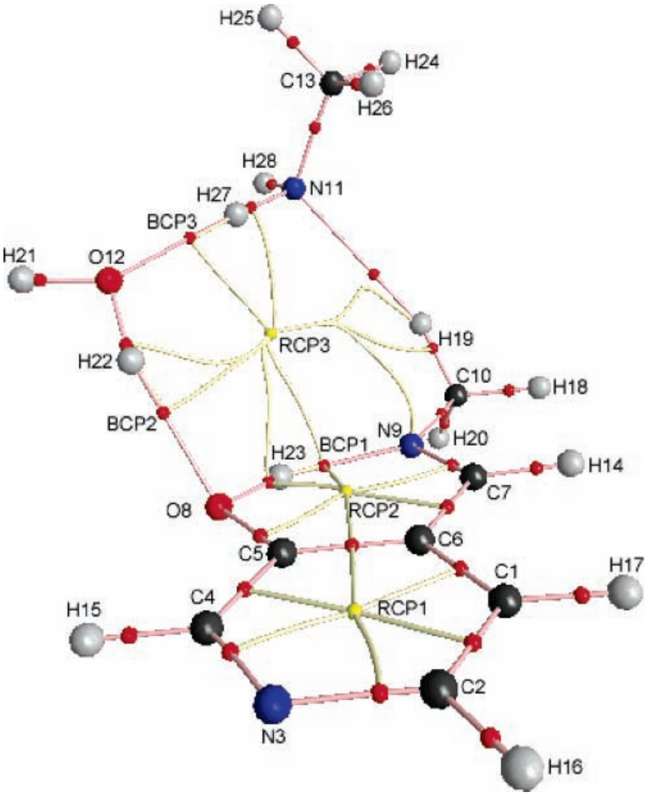


Figure 3. Molecular graph of the starting point structure **1**, showing the bond critical points, ring critical points, and paths connecting them.

Downhill TS**2** the intermediate **3** is formed. This structure can be formally written both as a quinonoid-like and a zwitterionic structure. The increase of the ellipticity value of the BCP in C5−O8 from intermediate **1** to **3** ($\epsilon = 0.009$ in **1** $\epsilon = 0.019$ in **3**) and the shortening of this distance (from 1.355 Å in **1** to 1.277 Å in **3**) reveals an increase of a double bond character. Accordingly, the imine bond C7−N9 lowers its π population along this bond ($\epsilon = 0.223$ in **1** to $\epsilon = 0.129$ in **3**) whereas this distance changes from 1.281 to 1.313 Å. In regard to the C6C7 bond, the bond order changes from 1.19 in **1** to 1.38 in **3** and the ellipticity of the BCP from $\epsilon = 0.107$ in **1** to $\epsilon = 0.183$ in **3**. Thus, the quinonoid-like structure of **3** should prevail over the zwitterionic one.

One of the consequences of the increase of the π character of the pyridine−imine system from **1** to **3** is the delocalization of the charge across the nuclei of the planar structure. Thus, it is found that the net charge on the C7 atomic basin in intermediate **1** is larger (+0.72) than in **3** (+0.63). However, near C7 in intermediate **3**, two (3, +1) critical points of the Laplacian of the charge density function ($\nabla^2\rho$) can be located, which are most susceptible to nucleophilic attack. These sites correspond with the “holes” for C7 in **3** in the relief map of $-\nabla^2\rho$ in the plane containing the C6C7 axis perpendicular to that of the pyridine ring−imine bonding planar system. Such sites are not found in the same relief map for structure **1** (Figure 4).

From structure **3**, the nucleophilic attack of the incoming amine is performed via the transition state structure TS**4** (Figure 5). The approaching path of the amine is almost perpendicular to the plane formed by the pyridine ring (dihedral angle N11C7C6C1 is 96.5°) at a N11C7 distance of 1.734 Å. This transition state structure keeps the hydrogen bonding network of the previous intermediate **3**. As seen in Figure 1 the energy gap from intermediate **3** to TS**4** (0.01846184 au or 11.58 kcal/mol) is far the highest in the geminal diamine formation profile and in the overall transimination process. This result is in accordance with experimental evidence that locates the addition−elimination as the rate-determining step of the transimination process.[2,8] This energy gap is quite similar to that found in a DFT theoretical study of Schiff base formation for the nucleophilic attack of methylamine on the carbonyl atom (10.06 kcal/mol).[32]

From this transition state structure, intermediate **5** is formed. This is an adduct structure with an increase in zwitterionic character. The C5O8 bond appears with a decrease in π character with respect to intermediate **3** ($\epsilon = 0.005$) and the distance increases to 1.302 Å. The net charge on the O8 atomic basin is

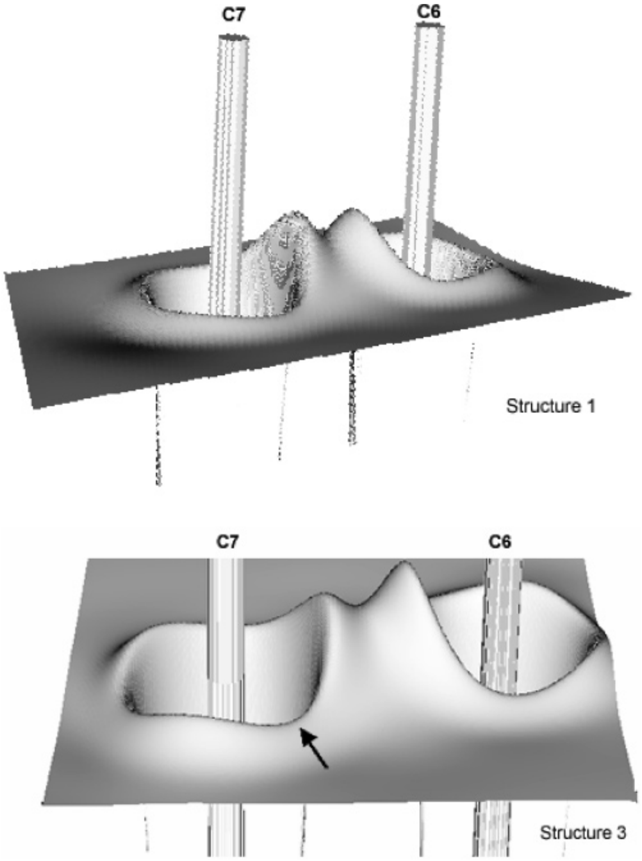


Figure 4. Displays of $-\nabla^2\rho$ for intermediates **1** (top) and **3** (bottom) in a perpendicular plane to the pyridine-imine planar π system containing the C6C7 axis. The arrow points to the (3, +1) critical point near C7 in intermediate **3**, which is most susceptible to nucleophilic attack.

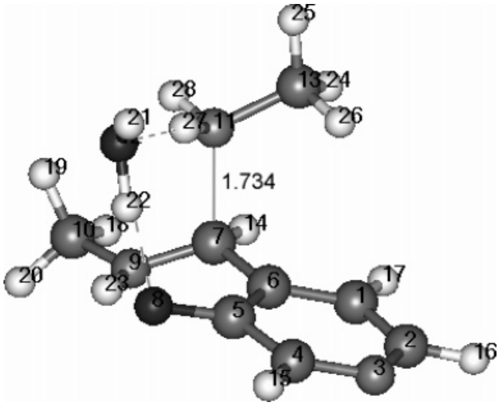


Figure 5. Perspective view of TS**4**, the concerted transition state structure corresponding to the addition of the incoming amine. The energy gap of the achievement of this structure is the rate-determining step for the reaction.

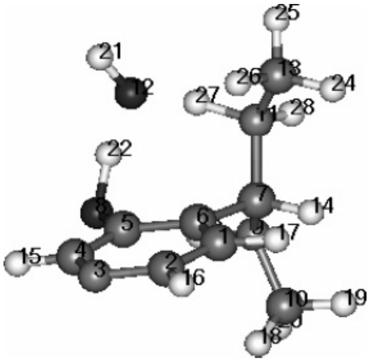


Figure 6. Perspective view of TS**6**, the concerted transition state structure that evolves to the geminal diamine structure. The water molecule exchanges one hydrogen atom (H22) for one hydrogen (H27) of the just attached amine nitrogen N11. H22 of the water molecule is transferred to the phenolate oxygen O8.

−0.25 e. From **3** to **5** a change in the bond order of C6−C7 is also clearly appreciated (1.37 to 1.05) and C7 acquires a tetrahedral geometry.

The formation of the geminal diamine needs the deprotonation of N11 and the regeneration of the phenol group in O8. Provided a water molecule is present in the model molecular complex, this cannot be achieved by means of a direct proton transfer because of the distance (H27O8 is 2.738 Å) and the relative orientation of the H27N11 bond with respect to O8 (angle N11H27O8 is 113.5°).

It is in this point of the mechanism where the water molecule, which maintains the double hydrogen bond with H27 and O8, takes an active role as a reactive species by exchanging the hydrogen atom H22 pointing to the phenolate oxygen O8 by the hydrogen atom H27 bound to the just attached amine nitrogen N11. The concerted transition state structure thus formed is TS**6** (Figure 6). As in the first proton transfer in TS**2**, the value of the energy barrier to achieve this structure from **5** is low (0.00501342 au or 3.15 kcal·mol^{-1}) with respect to the one of the previous transition state structure. A similar concerted transition state involving a simultaneous double hydrogen transfer mediated by a water molecule has been reported in a DFT theoretical description of the formation of a Schiff base[32] and in a semiempirical PM3 modelization of the transimination reaction.[31]

From TS**6**, the geminal diamine **7** intermediate is formed. In the structure of **7** two additional RCPs (RCP2 and RCP3 in Figure 7) to that of the expected pyridine ring RCP1 are found. This reveals a complex hydrogen bonding ring-like network for each of the amines attached to C7. The hydrogen of the phenolic group H22 points to the water molecule and H27 of the water molecule to the nitrogen of the previous incoming amine N11 (H22O12 is 1.700 Å and H27N11 is 1.780 Å), whereas H23 of the nitrogen of the former imine group N9 forms a hydrogen bond to O8 (H23O8 is 2.230 Å). As can be seen in the reaction profile (Figure 1), the geminal diamine is not the most stable intermediate of the overall process. However, it is far more stable (0.01138956 au or 7.15 kcal·mol^{-1}) than the zwitterionic adduct **5** once the nucleophilic attack of the incoming amine has been realized on the quinonoid-like Schiff base tautomer **3**.

Once the water molecule is released from the hydrogen bonding to N11 and O8, the single bond character of C6C7 (bond order is 0.99) allows the original nitrogen (N9) of the aldimine **1** to rotate upward and, then, be released as a free amine in the reverse reaction of the one described that has formed the geminal diamine, which requires a reallocation of a water molecule.

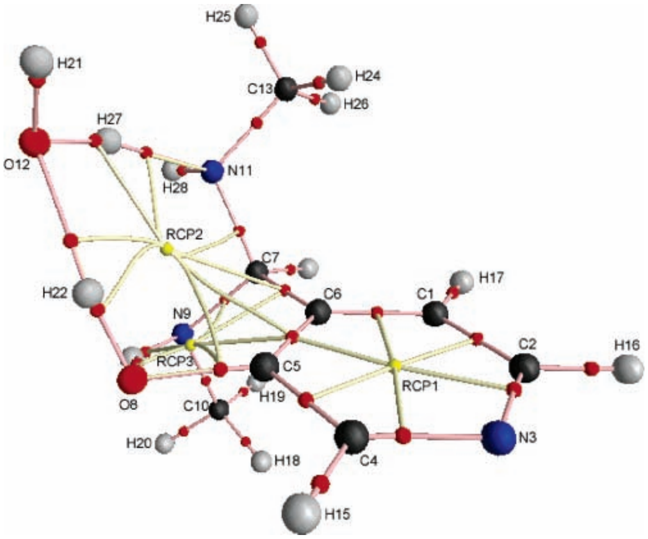


Figure 7. Molecular graph of the geminal diamine structure, showing the bond critical points, ring critical points and paths connecting them. Two ring systems, corresponding to ring critical points RCP2 and RCP3, are associated to both methylamines bound to C7.

Structural studies on the reaction catalyzed by serine hydroxymethyltransferase suggested that the conversion from the geminal diamine to a external aldimine occurs via proton transfer from a substrate amino group nearby.[44] For the nonenzymatic model described here, as reported in a previous semiempirical theoretical study,[31] this conversion takes place by means of the achieving of the water-mediated concerted transition state TS**6** from **7**. This energy barrier is the highest one of the second half reaction (0.016402975 au or 10.29 kcal·mol^{-1}) and the second one of the overall reaction. A slow exchange of enzyme-bound ^{2}H for the ^{1}H of solvent water is observed in enzymatic catalysis studies related to such bonds, as has been reported in the first stage of the biosynthesis of L-Trp catalyzed by $\alpha_2\beta_2$ tryptophan synthase complex.[45] In that context, this has been understood as indicative of a special environment where access of solvent water to the catalytic site is highly restricted. In the nonenzymatic mechanism of the transimination reaction reported in this present work, the results suggest that such slow hydrogen−deuterium exchange would also be expected. However, contrary to the enzymatic studies conclusions, it is not the restriction of water availability but the presence of one water molecule in the hydrogen bonding network in the geminal diamine intermediate that appears to be critical to trigger the proton transfer from the phenolic group to the amine that will be released through the achievement of TS**6** in the **7**−TS**6**−**5** step. That reverse sequence will lead to the release of an amine and the formation of a new aldimine.

A previous PM3 study of the transimination reaction that used the same molecular complex model reported that the nucleophilic attack of the incoming amine onto C7 of the pyridoxal structure is performed once the hydrogen of the phenolate group has been internally transferred to the imine nitrogen. This internal proton transfer and the presence of a water molecule forming a complex network of hydrogen bonds in the phenol group region of the pyridoxal group appears to be a requirement for the nucleophilic attack on C7 to occur, as suggested by the results of this study and of the one on Schiff base formation previously reported.[32] In this DFT study, an insight in the properties derived from the study of the topology of the charge density function of intermediate **3** supports its description as a quinonoid-like structure. Also, according to that previous semiempirical study on transimination,[31] the geminal diamine is the main intermediate of the reaction and one water molecule has a key role by allowing the proton transfer from the phenolic group to the incoming amine through the formation of a concerted transition state. The main energy gap for geminal diamine formation (from **1** to **6**) is 13.14 kcal/mol (0.020936 au).

The present DFT study on transimination has reached similar conclusions and has provided the detailed outline of the geminal diamine formation by means of the description of a simpler mechanism at a more reliable level of theory (B3LYP/6-31+G*), with 13 structures for the global transimination reaction, than the one suggested by the former PM3 study (17 structures).

The fact that transimination through geminal diamine formation is a general step in vitamin B6-dependent catalysis and the known general presence of water molecules in the active sites of the enzymes involved suggest that water molecules should be taken into account beyond water's consideration as a solvent, specifically when proton transfer processes are involved.

Acknowledgment. This work has been possible thanks to the grant from the Spanish Government (CTQ2004-02434).

References and Notes

(1) Metzler, D. E. *Biochemistry*; Harcourt-Academic Press: San Diego, 2001.
(2) Mäkelä, M.; Korpela, T. *Chem. Soc. Rev.* **1983**, *12*, 309.
(3) Snell, E. E.; Jenkins, W. T. *J. Cell. Comput. Physiol. 1* **1959**, *54*, 161.
(4) Tobias, P. S.; Kallen, R. G. *J. Am. Chem. Soc.* **1975**, *97*, 6530.
(5) Fischer, H.; De Candis, F. X.; Ogden, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 1340.
(6) Schirch, L. *J. Biol. Chem.* **1975**, *250*, 1939.
(7) Ulévitch, R. J.; Kallen, R. G. *Biochemistry* **1977**, *16*, 5355.
(8) Vázquez, M. A.; Muñoz, F.; Donoso, J. *J. Phys. Org. Chem.* **1992**, *5*, 142.
(9) Hershey, S.; Leussing, D. L. *J. Am. Chem. Soc.* **1977**, *99*, 1992.
(10) Jo Byeong, H.; Nair, V.; Davis, L. *J. Am. Chem. Soc.* **1977**, *99*, 4467.
(11) Toney, M. D.; Kirsch, J. F. *Biochemistry* **1993**, *32*, 1471.
(12) Drewe, W. F., Jr.; Dunn, M. F. *Biochemistry* **1985**, *24*, 3977.
(13) Drewe, W. F., Jr.; Dunn, M. F. *Biochemistry* **1986**, *25*, 2494.
(14) Miles, E. W. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1991**, *64*, 93.
(15) Korpela, T.; Mäkelä, M.; Löhnberg, H. *Arch. Biochem. Biophys.* **1981**, *212*, 581.
(16) Abbott, E. M.; Martell, A. E. *J. Am. Chem. Soc.* **1970**, *92*, 1754.
(17) Abbott, E. M.; Martell, A. E. *J. Am. Chem. Soc.* **1971**, *93*, 5852.
(18) O'Learly, M. H.; Payne, J. R. *J. Biol. Chem.* **1976**, *251*, 2248.
(19) Lapper, R. D.; Mansch, H. H.; Smith, I. C. P. *Can. J. Chem.* **1975**, *53*, 2406.
(20) Hogg, J. L.; Jencks, D. A.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 4772.
(21) Metzler, C. M.; Cahill, A.; Metzler, D. E. *J. Am. Chem. Soc.* **1980**, *102*, 6075.
(22) Robitaille, P. M.; Scott, R. D.; Wanb, J.; Metzler, D. E. *J. Am. Chem. Soc.* **1989**, *111*, 3034.
(23) Kresge, A. *J. Pure Appl. Chem.* **1981**, *53*, 189.
(24) Ritchie, C. D.; Minasz, R. J.; Kamego, A. A.; Sawada, M. J. *J. Am. Chem. Soc.* **1977**, *93*, 3747.
(25) Lehtokari, M.; Puisto, J.; Raunio, T.; Korpela, T. *Arch. Biochem. Biophys.* **1980**, *202*, 533.
(26) Bader, R. F. W. *Atoms and Molecules: A Quantum Theory*; Clarendon Press: Oxford, 1990.
(27) Nero, T. L.; Iskander, M. N.; Wong, M. G. *J. Chem. Soc., Perkin Trans. 2*, **1993**, 431.
(28) Alagona, G.; Ghio, C.; Agresti, A. *Comput. Chem. (Oxford)* **2000**, *24*, 311.
(29) Bach, R. D.; Canepa, C.; Glukhovstev, M. N. *J. Am. Chem. Soc.* **1999**, *121*, 6542.
(30) Toney, M. D. *Biochemistry* **2001**, *40*, 1378.
(31) Salvà, A.; Donoso, J.; Frau, J.; Muñoz, F. *Int. J. Quantum Chem.* **2002**, *89*, 48.
(32) Salvà, A.; Donoso, J.; Frau, J.; Muñoz, F. *J. Phys. Chem. A* **2003**, *107*, 9409.
(33) Weiner, S. J.; Singh, U. C.; Kollman, P. A. *J. Am. Chem. Soc.* **1985**, *97*, 2219.
(34) Frau, J.; Donoso, J.; Muñoz, F.; García-Blanco, F. *J. Comput. Chem.* **1992**, *13*, 681.
(35) Frau, J.; Donoso, J.; Vilanova, B.; Muñoz, F.; García-Blanco, F. *Theor. Chim. Acta* **1993**, *86*, 229.
(36) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, revision D.4; Gaussian, Inc.: Pittsburgh, PA, 1995.
(37) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785
(38) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5468.
(39) Lozynshi, M.; Rusinska-Roszak, D.; Mack, H. G. *J. Chem. Phys.* **1997**, *101*, 1542.
(40) Kim, K.; Jordan, K. D. *J. Chem. Phys.* **1994**, *98*, 10089.
(41) Biegler-König, F.; Schönbohm, J.; Bayles, D. *J. Comput. Chem.* **2001**, *22*, 545.
(42) Wiberg, K. B.; Bader, R. F. W.; Lau, C. D. H. *J. Am. Chem. Soc.* **1987**, *109*, 985.
(43) French, T. C.; Auld, D. S.; Bruice, T. C. *Biochemistry* **1965**, *4*, 77.
(44) Krishna Rao, J. V.; Prakash, V.; Appaji Rao, N.; Savithri, H. S. *Eur. J. Biochem.* **2000**, *267*, 5967.
(45) Hur, O.; Leja, C.; Dunn, M. F. *Biochemistry* **1996**, *35*, 7378.