DFT Studies on Schiff Base Formation of Vitamin B6 Analogues. Reaction between a Pyridoxamine-Analogue and Carbonyl Compounds

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A comprehensive theoretical study based on density functional theory calculations (B3LYP and M06−2X functionals) of the formation of Schiff bases of pyridoxamine analogues with two different aldehydes was conducted. The reaction mechanism was found to involve two steps, namely: (1) formation of a carbinolamine and (2) dehydration of the carbinolamine to give the final imine. Also, consistent with available experimental evidence, the carbinolamine dehydration was the rate-determining step of the process determined by means of M06−2X functional. Using an appropriate solvation method and reactant conformation ensures that all proton transfers involved will be intramolecular, which substantially reduces energy barriers and facilitates reaction in all cases. The formation of a Schiff base between pyridoxal 5-phosphate (PLP) and an amine or amino acid requires the contribution of an external water molecule in order to facilitate proton transfers. On the other hand, the formation of a Schiff base between pyridoxamine 5-phosphate (PMP) and a carbonyl compound requires no external aid since the spatial arrangement of the functional groups in PMP ensures that all proton transfers will be intramolecular.

Introduction

Pyridoxal 5-phosphate (PLP) is one form of vitamin B_6 and the coenzyme for a large number of enzymes catalyzing a wide variety of amino acid metabolic reactions.[1,2] The first step in these processes is a transaldimination reaction by which PLP, initially in the form of a Schiff base with an ε-amino residue in protein lysine (an internal aldimine), forms a new imine (an external aldimine) with the amino group in the incoming amino acid substrate. The catalytic properties of the new compound arise from its ability to stabilize carbanionic intermediates by effect of their high molecular electronic resonance (an electron-sink effect). This stabilizing effect relies heavily on the protonation status of the imine nitrogen[3−5] involved in the enolimine−ketoamine tautomeric equilibrium and that of the pyridine nitrogen, which facilitates the formation of quinonoid forms.[6,7] A previous DFT study of atomic charge distribution conducted by our group[8] revealed that protonation of the pyridine nitrogen promotes enolimine−ketoenamine tautomerism (Scheme 1) and also that the electron-sink effect on the carbanionic intermediate depends markedly on the protonation status of the imine nitrogen. Thus, in the enolimine tautomers, where the imine nitrogen is deprotonated, as much as 70% of the electron charge is delocalized on the pyridine ring; in the ketoenamine tautomers, where the imine nitrogen is fully deprotonated, only 20% of the charge is delocalized on the ring, however.

In the PLP-catalyzed reaction of some PLP-dependent enzymes, such as dialkylglycine decarboxylase[5] and all transaminases,[9] the cofactor is converted into pyridoxamine 5-phosphate (PMP), which subsequently reacts with a second ketoacid substrate to form a new Schiff base—a ketimine in this case—and is eventually hydrolyzed back to PLP (see Scheme 2). Unlike PLP, PMP never bonds covalently to the protein polymer. Rather, it interacts with the phosphate group in it via electrostatic forces.[1,9] This is so even with glutamate-1-semialdehyde aminomutase, which is a structurally asymmetric α2-dimeric vitamin B_6-dependent enzyme catalyzing the transformation of glutamate-1-semialdehyde into 5-amino-levulinic acid.[10]

PMP is also the coenzyme for a group of enzymes including CDP-6-deoxy-L-threo-D-glycero-4-hexulose 3-dehydratase, which catalyzes C−O bond cleavage reactions in deoxyhexose bacterial biosynthesis.[11−13] The first step in this process is also the formation of a ketimine between the carbonyl group in the 4-ketoglucose derivative and the amino group in PMP, which is followed by various steps involving prototropic shifts and dehydration of the resulting adduct.[13]

Finally, pyridoxamine (PM) is especially important as an inhibitor of the nonenzymatic glycoxidation and lipoxidation of proteins, and also of the formation of advanced glycoxidation end-products (AGEs) and advanced lipoxidation end-products (ALEs).[14,15] Previous studies of our group on the reactivity of pyridoxamine with various sugars[16,17] and other glycating compounds[18] have shown that this process also involves the formation of an initial Schiff base that can undergo various transformations depending on the particular nature of the reactants and reaction medium. Pyridoxamine can also form Schiff bases with Amadori compounds;[19] however, its potential to inhibit the formation of AGEs is seemingly more closely related to its chelating metal ions than to its sequestering carbonyl groups ability.[19,20]

Notwithstanding its biological and biomedical significance, the formation of Schiff bases of PM and PMP has scarcely been studied in kinetic terms. Unlike the Schiff bases of PLP,[21−23] the absence of π-bond conjugation between the imino group and the pyridine ring, and the little displacement in the formation equilibria,[16−18,24] result in too small changes in the UV−vis spectrum for the initial chromophore to be readily detected.[25] On the basis of ^{1}H NMR and ^{13}C NMR data, however, the process clearly takes place via intermediate carbinolamines,

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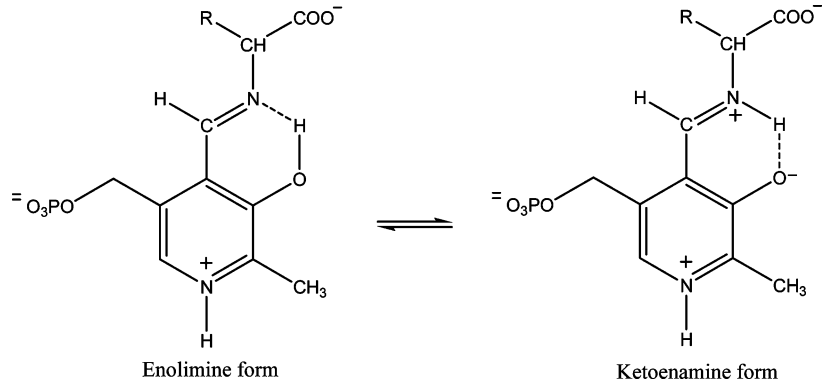
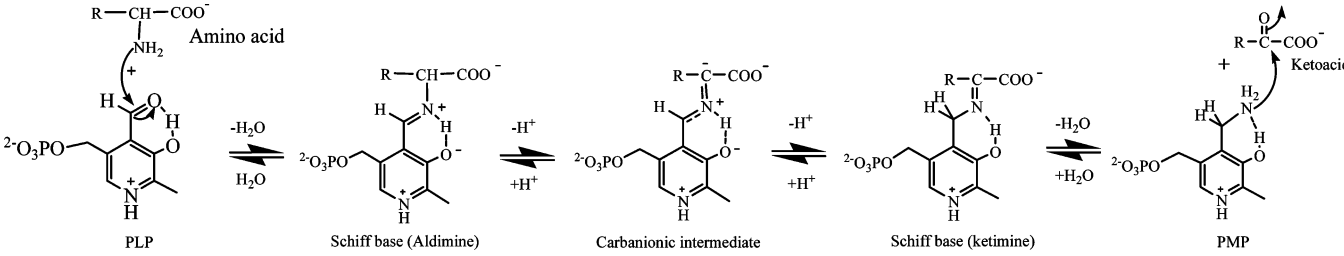
SCHEME 1: Enolimine−Ketoenamine Tautomerism.



SCHEME 2: PLP-PMP Interconversion.



some of which (e.g., that formed in the reaction of PM with formaldehyde)[26] are especially stable. This is a departure from the characteristics of the Schiff bases of PLP, where dehydration of an intermediate carbinolamine is also the rate-determining step, but the carbinolamine can rarely be detected.[23]

Quantum mechanics calculations for a reactive system can provide a detailed description of the intermediates and transition state geometries involved. Most reported theoretical mechanistic studies of the reactions between B_6 vitamers in their pyridoxal form have focused on the carbanionic intermediates formed,[27] the spectroscopic properties of their isomers[28] or the elucidation of the catalytic mechanisms of transamination,[29,30] decarboxylation,[3,4] and racemization,[31,32] among others.

In a previous work, we conducted a theoretical study based on DFT (B3LYP/6-31+G(d) calculations of the formation of Schiff bases by a PLP analogue that allowed the geometries of all intermediates and transition structures involved in the reaction pathway to be elucidated.[33] On the basis of the results, the carbinolamine was the main intermediate, and its dehydration was the rate-determining step of the process. Also, we exposed the major mechanistic role of an auxiliary water molecule in activating the reactants and in accepting and releasing key protons in the intermediate tautomers. Liao et al.[30] assigned such a role in the transamination of amino acids catalyzed by PMP analogues to the carboxyl group in the substrate.

This paper reports a comprehensive DFT study conducted at the B3LYP/6-31+G(d), B3LYP/6-31+G(d,p) and M06−2X/6-31+G(d,p) level of theory and continuum solvation method (CPCM) on the formation of a Schiff base between the PM analogue 3-hydroxy-4-aminomethylpyridine (**PM-a**) and two model carbonyl compounds [acetaldehyde (**Act**) and glycolaldehyde (**Gla**)] with a view to establishing the influence of the hydroxyl group on the reaction and determining the energies of its intermediates. No auxiliary water molecule was considered and the activation parameters thus obtained were within the typical range for this type of reaction. Also, the results underline the extreme significance of the proton exchange between the 3-hydroxy group and the imine nitrogen to the overall reaction mechanism.

Methodology

The present study was conducted on **PM-a**. This analogue has a protonated pyridine nitrogen since this is the prevalent form when the compound acts as a cofactor in an enzyme medium. The carbonyl compounds reacted with it were **Act** and **Gla** (see Scheme 3).

DFT calculations were performed with the Gaussian03 and 09 software packages.[34] All structures were fully optimized at the B3LYP level,[35] using the 6-31+G(d) basis set in gas phase. Also, the geometries of the reactants, products, intermediates, and transition states involved in the reactions were all fully optimized by using B3LYP/6-31+G(d), B3LYP/6-31+G(d,p), and M06−2X/6-31+G(d,p), the new hybrid meta exchange correlation functional proposed recently by Thrular,[36,37] with the Cosmo Polarizable Continuum Method (CPCM)[38,39] in order to mimic the water solvent effect.

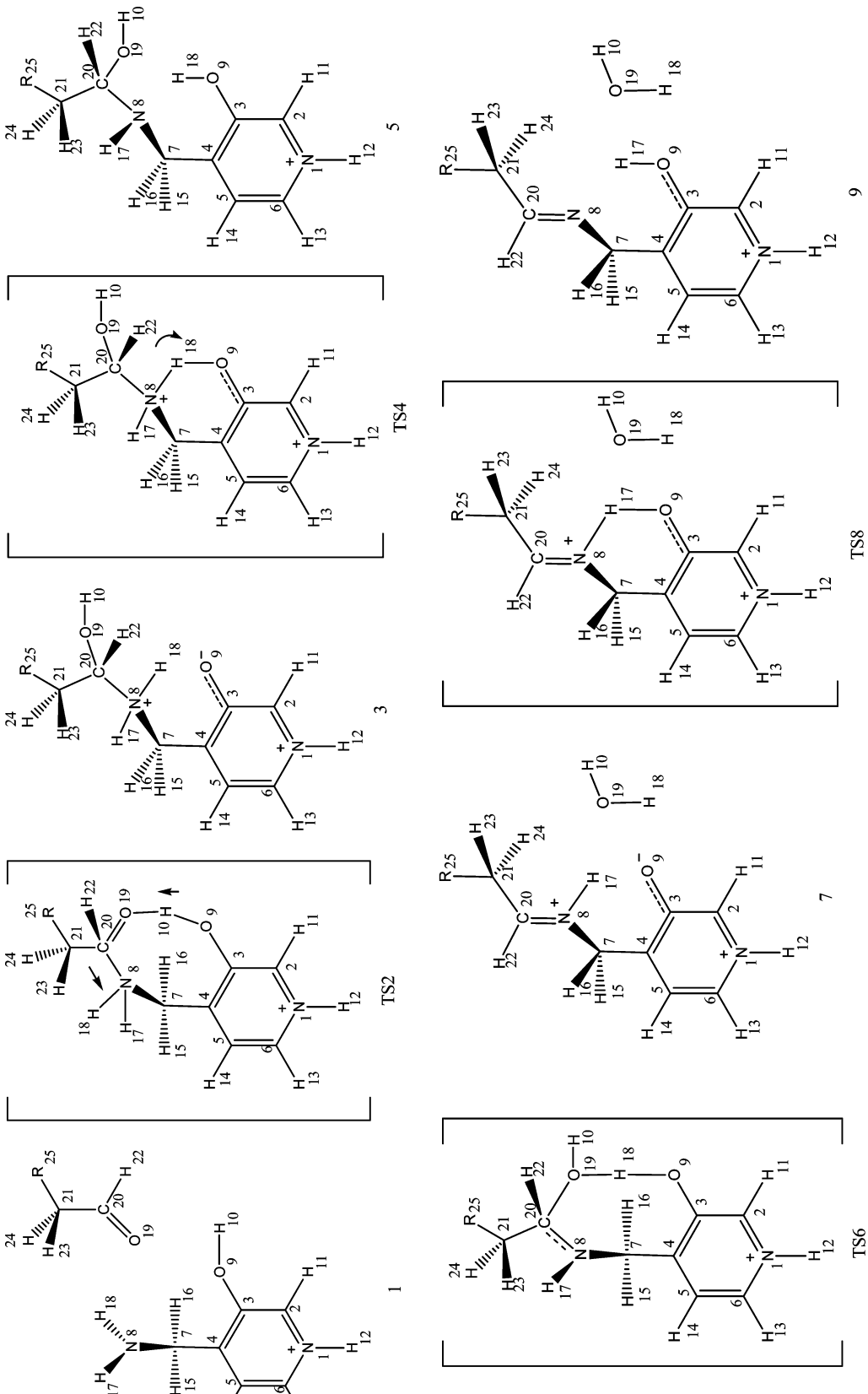
The structures thus obtained were subjected to vibrational analysis calculations toward their characterization as local minima (all positive force constants) or transition states (one imaginary force constant only). The standard state is 1 atm, which is the default in Gaussian calculations.

Results and Discussion

The mechanism of the Schiff base formation reactions of **PM-a** with **Act** and **Gla** involves two steps, namely: (1) formation of a carbinolamine (**1**−**5** in Scheme 3) and (2) dehydration of the carbinolamine to give the final imine (**5**−**9** in Scheme 3). Figures 1 and 2 show the energy profiles for the process in solvated phase with different methodologies. Tables 1 and 2 show relative energy, $\Delta H°$ and $\Delta G°$ data for the structures involved in the process.

The reaction is started by a nucleophilic attack of the amine nitrogen in **PM-a**, with its pyridine nitrogen and phenol oxygen both in protonated form, on the carbonyl group in the aldehyde (**Act** or **Gla**). This is a concerted step where the nucleophilic attack takes place simultaneously with transfer of the phenol proton to the aldehyde oxygen. The reaction occurs via a **TS2** clearly involving the formation of an N8−C20 bond and an

SCHEME 3: Mechanism of Schiff Base Formation between a Pyridoxamine Analog (PM-a) and Acetaldehyde (Act, R25 = H25) or Glycolaldehyde (Gla, R25 = O25H26).



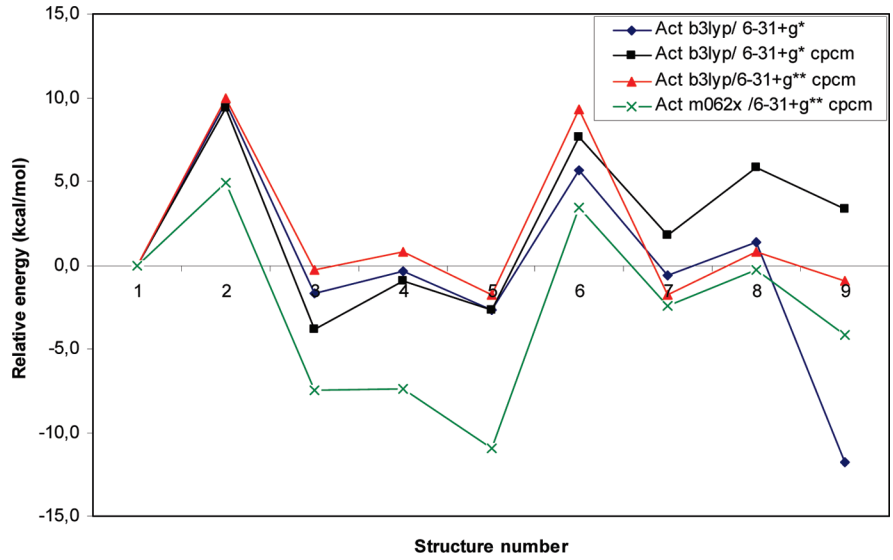


Figure 1. Energy profiles comparison for the reactions of **PM-a** with **Act** in the gas phase B3LYP/6-31+G* (blue line) and solvated phase with: B3LYP/6-31+G(d) (black line), B3LYP/6-31+G(d,p) (red line), and M06−2X/6-31+G(d,p) (green line).

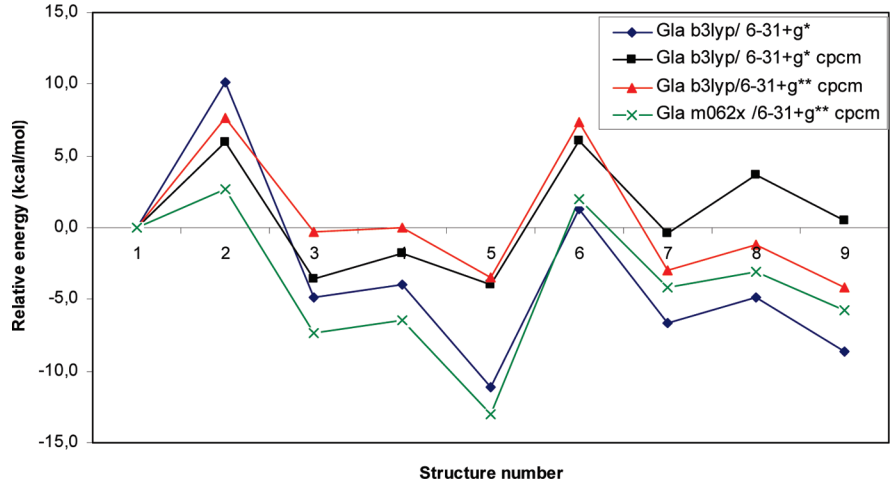


Figure 2. Energy profiles comparison for the reactions of **PM-a** with **Gla** in the gas phase B3LYP/6-31+G* (blue line) and solvated phase with: B3LYP/6-31+G(d) (black line), B3LYP/6-31+G(d,p) (red line), and M06−2X/6-31+G(d,p) (green line).

TABLE 1: Relative Energies (kcal/mol) for the Structures Involved in the Reaction Computed at the Level of Theory: B3LYP/6-31+G(d) in Gas Phase and B3LYP/6-31+G(d), B3LYP/6-31+G(d,p), and M06-2X/6-31+G(d,p) with CPCM Approach. Comparison with PM + Glyoxylic acid Reaction[30] and PL + Methylamine Reaction[33]

	PM-a + Act				PM-a + Gla				PM + glyoxylic acid[a]		PL + methylamine[b]
structure	gas phase[c]	CPCM[c]	CPCM[d]	CPCM[e]	gas phase[c]	CPCM[c]	CPCM[d]	CPCM[e]	gas phase	PCM	gas phase
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TS2	9.7	9.4	10.0	4.9	10.1	6.0	7.7	2.7	3.9	1.3	7.9
3	−1.7	−3.8	−0.3	−7.5	−4.9	−3.6	−0.3	−7.3	−2.2	−8.5	5.1
TS4	−0.4	−1.0	0.8	−7.4	−4.0	−1.8	0.0	−6.4	−3.1	−9.0	10.0
5	−2.7	−2.7	−1.8	−10.9	−11.1	−4.0	−3.5	−13.0	−10.3	−7.7	0.7
TS6	5.7	7.6	9.3	3.5	1.3	6.1	7.3	2.0	6.7	1.2	20.3
7	−0.6	1.8	−1.8	−2.4	−6.7	−0.4	−3.0	−4.2	4.3	−0.3	−6.6
TS8	1.3	5.9	0.8	−0.3	−4.9	3.7	−1.2	−3.1	8.6	8.9	−0.5
9	−11.8	3.4	−1.0	−4.2	−8.6	0.5	−4.2	−5.8	−11.9	−6.7	−3.5

[a] These structures stand for **Re**, **TS1**, **Int1**, **TS2**, **Int3**, **TS3**, **Int4**, **TS4**, and **Int5**. B3LYP/6-311++G(2df,2pd)//B3LYP/6-31G(d) calculations.[30] [b] These structures stand for **1**, **TS2**, **3**, **TS4, 5**, **TS6**, **9**, **TS10**, and **11**. B3LYP/6-31+G(d) calculations.[33] [c] B3LYP/6-31+G(d). [d] B3LYP/6-31+G(d,p). [e] M06−2X/6-31+G(d,p).

O19−H10 bond. The energy barriers for the reactions of **PM-a** with **Act** and **Gla** are 9.7 and 10.1 kcal/mol, respectively, in the gas phase. The solvation modified the energy barriers, especially in the case of the glycoaldehyde reaction. Inclusion of polarization function on the hydrogen practically has no effect on the barriers. On the other hand, M06−2X calculations predicts smaller barriers (4.9 and 2.7 kcal/mol respectively; see Table 1).

Downhill from these transition state structures, the system evolves to the adduct form **3** (Scheme 3) via the formation of

TABLE 2: $\Delta G°$ and $\Delta H°$ (in brackets) in kcal/mol at 298.15 K for the Structures Involved in the Reaction As Computed at the Level of Theory: B3LYP/6-31+G(d) in Gas Phase and B3LYP/6-31+G(d), B3LYP/6-31+G(d,p), and M06-2X/6-31+G(d,p) with CPCM Approach

	PM-a + Act				PM-a + Gla			
structure	gas phase[a]	CPCM[a]	CPCM[b]	CPCM[c]	gas phase[a]	CPCM[a]	CPCM[b]	CPCM[c]
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TS2	13.3 (9.2)	14.7 (11.3)	14.4 (11.4)	10.2 (5.5)	17.2 (9.8)	11.5 (8.3)	15.8 (8.4)	10.4 (2.4)
3	5.4 (1.6)	3.7 (0.1)	6.4 (3.5)	−0.3 (−5.0)	5.3 (−1.7)	1.2 (−0.2)	7.7 (2.5)	0.8 (−4.3)
TS4	4.3 (0.1)	3.8 (−0.5)	4.8 (1.7)	−1.5 (−6.8)	5.3 (−3.4)	1.2 (−1.7)	7.7 (0.5)	0.8 (−4.7)
5	4.0 (0.3)	4.0 (0.2)	4.0 (1.4)	−3.9 (−8.2)	−0.5 (−7.9)	−0.2 (−0.5)	4.4 (−0.7)	−5.7 (−11.0)
TS6	11.2 (6.7)	12.7 (8.2)	13.5 (10.0)	9.6 (4.2)	10.9 (2.7)	9.2 (7.6)	14.4 (8.2)	8.6 (2.3)
7	1.5 (0.4)	3.0 (3.9)	−2.0 (−0.1)	−0.1 (−1.3)	−2.2 (−5.4)	0.2 (1.7)	−0.4 (−1.9)	−1.9 (−3.6)
TS8	0.1 (−0.5)	6.4 (3.2)	−2.5 (−0.8)	−0.4 (−2.2)	−2.9 (−6.7)	0.9 (2.3)	−1.4 (−3.3)	−3.2 (−5.5)
9	−10.9 (−10.8)	5.6 (3.3)	−2.0 (0.0)	−2.7 (−3.7)	−4.6 (−7.7)	0.4 (2.6)	−2.3 (−3.7)	−4.9 (−5.7)

[a] B3LYP/6-31+G(d). [b] B3LYP/6-31+G(d,p). [c] M06−2X/6-31+G(d,p).

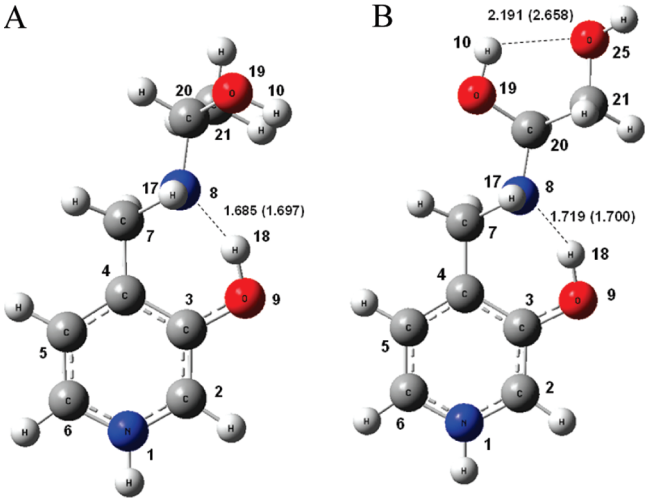


Figure 3. Structures of the carbinolamines formed by the pyridoxamine analogue with **Act** (A) and **Gla** (B) calculated by B3LYP/6-31+G*. Selected interatomic distances (Å) are given.

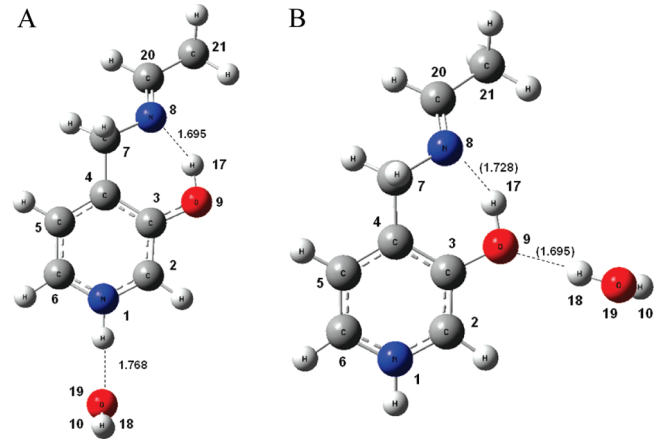


Figure 4. Structure **9** in the reaction between the pyridoxamine analogue and **Act** in the gas phase (A) and solvated phase (B) calculated by B3LYP/6-31+G(d). Selected interatomic distances (Å) are given.

an N8−C20 covalent bond and the complete transfer of the phenol proton to O19 to form a zwitterionic structure. In fact, the product is the zwitterionic tautomer of the carbinolamine.

The proton transfer from the amine nitrogen to the phenol oxygen gives the nonzwitterionic tautomer of the carbinolamine (**5**). The molecular geometry of intermediate **3** remains virtually unchanged all the way to the carbinolamine **5**. This process is subject to a very low energy barrier (ca. 1 kcal/mol) in the gas phase and in the solvated phase—solvation in a continuum hinders intramolecular transfers to some extent. The formation of this tautomer is essential since, as shown below, the phenol proton plays a key role in the carbinolamine dehydration.

As can been seen from Table 1, the carbinolamine formed between **PM-a** and **Gla** in the gas phase is substantially more stable (about 8 kcal/mol) than that formed with **Act**. This is a result of the O25−H10 intramolecular bond established in the former case, which is obviously impossible in the latter (Figure 3). The energy difference is much smaller in the solvated phase (Table 1) by effect of the previous hydrogen bond being considerably longer.

The next step in the reaction is the dehydration of the carbinolamine to give the corresponding Schiff base, which involves the concerted release of the O19−H10 hydroxyl group and the transfer of H18 from the phenol group to produce the leaving water molecule. This step additionally causes the formation of an imine double bond between C20 and N8—in fact, the distance between these two atoms decreases from 1.485/1.476 Å in **5** to 1.282/1.277 Å in 7 **PM-a** + **Act** with B3LYP/6-31+G(d,p)/M06−2X/6-31+G(d,p). Compound **7** is the zwitterionic tautomer of the Schiff base.

Finally, intermediate **7** is in tautomeric equilibrium with the enolomine form of the Schiff base (**9**). That formed in the reaction between **PM-a** and **Act** in the gas phase (point 9 in Table 1) is highly stabilized by the water molecule released by effect of the dehydration, which returns to its starting position and solvates the protonated pyridine nitrogen via a strong hydrogen bond (1.768 Å) (Figure 4). The reaction with **Gla** involves no migration of the water molecule; rather, such a molecule continues to solvate the phenol group, so no similar stabilizing effect is observed.

As a rule, solvation weakens hydrogen bonds in the system and restricts motion in atoms and molecules. This causes the water molecule formed in the solvated medium to remain virtually motionless near the phenol group and prevents the high stabilizing effect observed in the gaseous phase (Table 1).

If one considers the energy values (Table 1) calculations B3LYP/6-31+G(d,p), CPCM show that the initial attack of the aldehyde has an energy barrier of 10.0/7.7 kcal/mol for the reaction of **PM-a** with **Act/Gla**, whereas the potential barrier for dehydration of the carbinolamine takes the values of 11.1/10.8. This suggests that the initial attack is the rate-limiting step of the process. In the other hand, the M06−2X values are 4.9/2.7 and 14.1/15.0. These values clearly show that the limiting step is the dehydration of the carbinolamine, which is consistent with expeerimental results.[6]

If energy Gibbs is considered, B3LYP calculations show barriers of 14.4/15.9 for the initial attack and 9.5/10.0 for the

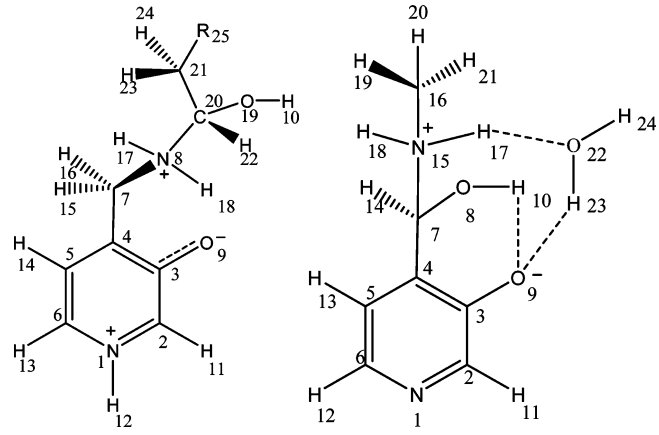
dehydration of the carbinolamine. For the same steps, the M06−2X functional predicts values of 10.2/10.4 and 13.5/14.3, corroborating the fact that the latter functional predicts that carbinolamine dehydration is the rate-determining step. These results corroborate the idea that M06−2X functional gives hight performance for barrier height calculations.[36,37]

In a recent experimental study of the formation of Schiff bases between pyridoxamine and various carbonyl compounds,[26] we found substrates with an OH group in α with respect to the C═O bond to give a Schiff base with a kinetic formation constant roughly 1 order of magnitude greater than those obtained in the absence of the hydroxyl group and ascribed the effect to the potential formation of an intramolecular hydrogen bond lowering the energy of the transition state for the rate-determining step. However, the results obtained in this work do not justify the difference in kinetic constant; in fact, the potential barrier for the rate-determining step was virtually the same in both cases, and this was a result of the energy difference between the two reactions falling within the error range for the measuring method used.

In a recent theoretical study of the transamination reaction between pyridoxamine and glyoxylic acid, Liao et al.[30] obtained a carbinolamine similar to our compound **5** that subsequently evolved to a Schiff base. However, they proposed another mechanism requiring three different proton transfers for the carbinolamine to form. In the first, the acid proton in glyoxylic acid is transferred to the aldehyde oxygen; in the second, the phenol proton is transferred to the acid group, and finally that in $-NH_2^+$ to the phenolate group. The dehydration of the carbinolamine also requires three proton transfers, namely: that of the acid proton to the OH group in the alcohol formed by the aldehyde, with release of a water molecule; that from the phenol to the deprotonated acid group; and that of $=NH^+-$ to the phenolate group. Obviously, our reaction cannot develop via this mechanism since our substrate contains no acid group. Also, their mechanism involves no zwitterionic species (our compound **3**), which would otherwise have been highly stabilized by forming a strong intramolecular bond—particularly in the solvated phase as shown above. Therefore, the mechanism depicted in Scheme 3 is much more simple and widely applicable than that of Liao et al.[30] These authors also found the carbinolamine dehydration to be the rate-determining step in the formation of the Schiff base, with an energy barrier of 17.0 kcal/mol in the gas phase, which is slightly higher that ours. In addition, they found a lower barrier in the solvated phase (8.9 kcal/mol), in addition to a new one of 9.2 kcal/mol due to the proton transfers from the phenol to the acid group and $=NH^+-$ groups to the phenolate. By contrast, we obtained a single barrier (ca. 10−15 kcal/mol), possibly because Liao et al. dealt with the water solvent effect via single-point calculations of the optimized gas-phase geometries, whereas we performed total geometry optimization in the solvated phase. For comparison, Table 1 lists the energy values reported by these authors.

Previously, we studied the formation of the Schiff base of the PLP analogue 3-hydroxy-4-pyridine-aldehyde with methylamine,[33] the explicit presence of a water molecule was thought to be essential in order to facilitate the nucleophilic attack of the amine on the carbonyl group and the proton transfers in the different intermediates. As shown in this work, the use of a continuum method in combination with an appropriate configuration allows all proton transfers in a Schiff base formation reaction between a pyridoxamine analogue and a carbonyl compound in a solvated medium to be assumed intramolecular;

SCHEME 4: Zwitterionic Forms of Carbinolamines



as a result, the energy barriers involved are considerably lower and all reaction steps more favorable. This could have interesting biological implications since the cofactor for some PLP-dependent enzymes becomes PMP during the catalytic process and this reacts with a second substrate to regenerate PLP, both processes involving the formation of a Schiff base.

On the basis of our theoretical calculations, the first step requires the contribution of some residue and/or water molecule at the active site. However, the functional groups in PMP allow the second step to occur with no external contribution. Scheme 4 shows the structures of the zwitterionic forms of the carbinolamines obtained in the two reactions. As can be seen, the presence of the O8−H10 bond in PLP hinders intramolecular proton transfer.

One other salient difference is that all intermediates formed in the reaction of the PL analogue are less stable than the starting reactants. On the other hand, all involved in the reaction with the PM analogue studied here are more stable than the reactants (see Figures 1-2) with the exception of the results obtained with B3LYP/6-31+G(d). Table 1 includes the energy values obtained with the PL analogue.

As noted earlier and can be seen from Figures 1 and 2, solvation has a strong stabilizing effect on the carbinolamine in both zwitterionic and nonzwitterionic forms, especially in the M06−2X calculations. Thus, its tautomers are much more stable than are the end-products, which may account for available experimental evidence that the reactions of formaldehyde with 4-aminomethylpyridine[26] and pyridoxamine[40] yield carbinolamine as end-product since this does not further evolve to a Schiff base.

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