JOC Article

Tautomeric Equilibria of Pyridoxal-5′-phosphate (Vitamin B_6) and 3-Hydroxypyridine Derivatives: A Theoretical Study of Solvation Effects

G. S. M. Kiruba and Ming Wah Wong*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543

chmwmw@nus.edu.sg

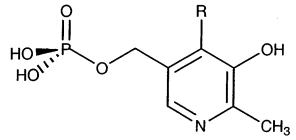
Received November 9, 2002

The tautomeric equilibria of a series of 3-hydroxypyridine derivatives including pyridoxal-5′-phosphate (PLP), the active form of vitamin B_6, have been studied using density functional calculations (B3LYP/6-311+G**//B3LYP/6-31G*) in the gas phase and in different solvents. Three different approaches, namely continuum, discrete, and hybrid (combined discrete/SCRF), were employed to investigate the effects of solvation on the tautomeric equilibria. In all cases, the neutral hydroxy form is significantly more stable than the zwitterionic oxo form (by 43−56 kJ mol^{-1}) in the gas phase. The tautomeric energies reduce substantially in the presence of a polarizable dielectric medium. However, the neutral form is calculated to be the dominant form in nonpolar and aprotic polar solvents. On the other hand, a reversal of tautomeric equilibrium, in favor of the zwitterionic form, is predicted in an aqueous medium. This study highlights the role of both water molecules and bulk solvent effect in influencing the tautomeric equilibria of the PLP related compounds. A combination of explicit microsolvation and continuum reaction field is required to account fully for the energetic effect of aqueous solvation. The tautomeric free energy differences (ΔG_{298}) of PLP in the gas phase and in aprotic polar ($\epsilon = 40$) and aqueous media are predicted to be 47, 22, and −29 kJ mol^{-1}, respectively.

Introduction

Vitamin B_6 plays a vital role in several enzymatic reactions. It acts as a coenzyme for numerous enzyme-catalyzed reactions such as transamination, α- and β-decarboxylations, β- and γ-eliminations, racemizations, and aldol reactions.[1] Vitamin B_6 exists in three different forms, namely the aldehydic (pyridoxal-5′-phosphate, PLP), alcoholic (pyridoxine-5′-phosphate, PNP), and amino forms (pyridoxamine-5′-phosphate, PMP).[2] The biologically active form of vitamin B_6 is the aldehydic form (PLP). The remarkably versatile chemistry of PLP is due to its ability to form Schiff base adducts with α-amino groups of amino acids and to act as an effective electron sink to stabilize reaction intermediates.[3] The activities of at least 60 enzymes involved in the metabolism of various amino acids depend on PLP.

The PLP molecule has several protonable groups. As a result, it can exist in many different tautomeric forms. Experimentally, tautomeric equilibrium constants of PLP are severely affected by solvent polarity.[4] The influence of solvent polarity on the physiochemical features of vitamin B_6 and its derivatives has been studied extensively by Llor and Cortijo et al.[5] Under physiological conditions, PLP exists in two tautomeric forms: neutral hydroxy and zwitterionic oxo forms. UV−visible spectroscopy studies of 3-hydroxypyridine and pyridoxine demonstrated that the neutral form is predominant in a nonpolar medium such as dioxane, while the zwitterionic form is favored in an aqueous solution.[6] The presence of the zwitterionic form in the neutral solution of pyridoxine was supported by ^{13}C NMR studies.[7]



R = CHO pyridoxal-5'-phosphate (PLP)
CH_2OH pyridoxine-5'-phosphate (PNP)
CH_2NH_2 pyridoxamine-5'-phosphate (PMP)

(1) (a) Marino, G.; Sannia, G.; Bossa, F., Eds. *Biochemistry of Vitamin B6 and PQQ*; Birkauser Verlag: Basel, Switzerland, 1994. (b) Evangelopoulos, A. E. *Chemical and Biological Aspects of Vitamin B6 Catalysis*; A. R. Liss: New York, 1984.

(2) Vogtle, F. *Fascinating Molecules in Organic Chemistry*; John Wiley & Sons: New York, 1992.

(3) (a) Makela, M. T.; Korpela, T. K. *Chem. Soc. Rev*. **1983**, *12*, 309. (b) Martell, A. E. *Acc. Chem. Res*. **1989**, *22*, 115.

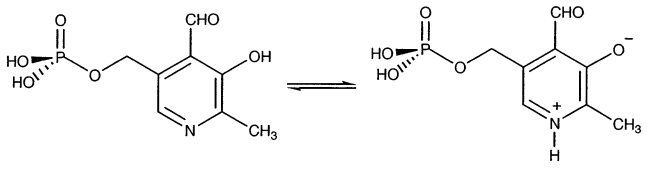
(4) Llor, J.; Sanchez-Ruiz, J. M.; Cortijo, M. *J. Chem. Soc., Perkin Trans. 2* **1988**, 951.

(5) (a) Llor, J.; Cortijo, M. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1111. (b) Llor, J.; Cortijo, M. *J. Chem. Soc., Perkin Trans. 2* **1978**, 409. (c) Sanchez-Ruiz, J. M.; Asensio, S.; Llor, J.; Cortijo, M. *J. Chem. Soc., Perkin Trans. 2* **1987**, 169. (d) Cortijo, M.; Jimenez, J. S.; Llor, J. *Biochem. J*. **1978**, *171*, 497. (e) Cortijo, M.; Llor, J.; Sanchez-Ruiz, J. M. *J. Biol. Chem*. **1988**, *263*, 17960.

(6) (a) Sanchez-Ruiz, J. M.; Llor, J.; Cortijo, M. *J. Chem. Soc., Perkin Trans. 2* **1984**, 2047. (b) Llor, J.; Asensio, S. B. *J. Soln. Chem*. **1995**, *24*, 1293.

(7) (a) Harruf, C.; Jenkin, W. T. *Org. Magn. Reson*. **1976**, *8*, 548. (b) Witherup, T. H.; Abbott, E. H. *J. Org. Chem*. **1975**, *40*, 2229. (c) Llor J.; Munoz, L. *J. Org. Chem*. **2000**, *65*, 2722.

10.1021/jo0266792 CCC: $25.00 © 2003 American Chemical Society
Published on Web 03/04/2003



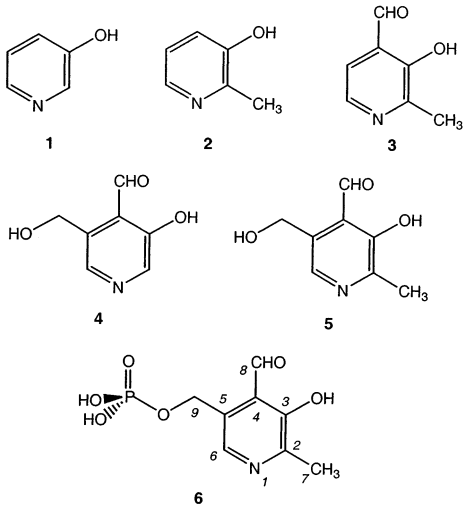
The simplest moiety common to all vitamin B_6 related compounds is the 3-hydroxypyridine unit, which serves as a good model system for the present theoretical study. Due to their biological importance, the tautomeric equilibria in PLP and in a variety of model systems such as 3-hydroxypyridine, 3-hydroxypyridine-4-carbaldehyde, pyridoxal, pyridoxine, etc. have been studied both by experiments, based on various spectroscopic techniques such as infrared,[8,9] Raman,[9] NMR,[7,10] fluorescence,[11] UV,[6,12] photoelectron[13] and mass[14] spectroscopies, and by theoretical calculations.[15]

Since the PLP-dependent enzymes in the human body are present in an aqueous solution, it is of immense importance to investigate the influence of a solvent medium on the biological activity of PLP. In fact, there is strong evidence that the PLP site of PLP-dependent enzymes is less polar than water.[16] Thus, it is necessary that we first gain sufficient insight into the physical, chemical, and biological aspects of PLP activity to better understand the mechanism of action of PLP-dependent enzymes. To complement the experimental investigations of the tautomeric equilibrium of PLP, we have embarked on a computational study of the tautomeric equilibria of a series of 3-hydroxypyridine derivatives, including PLP (**1**−**6**, Chart 1) with special emphasis on the effects of solvation. Three different models of solvation were employed to shed light on the effects of bulk solvation and specific solute−solvent interaction and to assess the performance of various theoretical models of incorporating solvent effects.

Computational Methods

The neutral (**N**) and zwitterionic (**Z**) forms of 3-hydroxypyridine derivatives **1**−**6** were fully optimized using the B3-LYP[17] formulation of density functional theory, i.e., Becke's 3-parameter exchange functional[17a] and Lee−Yang−Parr correctional functional,[17b] together with the 6-31G* basis set. Vibrational frequency calculations were performed at the B3LYP/6-31G* level to confirm the nature of all optimized stationary points as minima. These calculations were also used to compute zero-point energies and vibrational and rotational contributions to the enthalpy and free energy, using the standard rigid-rotator harmonic-oscillator statistical mechanical approximations. The DFT calculated zero-point vibrational energies were scaled by a factor of 0.9804.[18] Higher level relative energies were obtained through B3LYP single-point energy calculations, using a larger 6-311+G** basis set. Unless otherwise noted, all relative energies (ΔE_0) reported in the text correspond to the B3LYP/6-311+G**//B3LYP/6-31G* + ZPE level.

CHART 1. 3-Hydroxypyridine Derivatives (1−6)[a]



[a] Atom labels are given in italics.

The solvation effects on the tautomeric equilibria of the 3-hydroxypyridine systems were studied by three different approaches: (1) a continuum model that takes into account the nonspecific solute−solvent interaction, using the reaction field formalism, (2) a discrete model that accounts for the strong and specific solute−solvent interaction, by explicitly incorporating solvent molecules in the first solvation shell, and (3) a hybrid model where the explicit solvent and continuum models are combined. In the continuum approach, the solvent is considered as a polarizable continuous dielectric medium, characterized by a dielectric constant ϵ, and the solute is imbedded inside a cavity in the continuum. In this study, the self-consistent reaction field (SCRF)[19] method based on Onsager's reaction field[20] theory and the self-consistent isodensity surface polarized continuum model (SCIPCM)[21] were employed for the continuum-based calculations. For the SCRF calculations, the cavity radii were derived from the molar volume based on a quantum mechanical approach.[22] An isodensity surface cutoff of 0.0004 au was used for the SCIPCM calcula-

(8) (a) Person, W. B.; Del Bene, J. E.; Szajda, W.; Szczepaniak, K.; Szczesniak, M. *J. Phys. Chem.* **1991**, *95*, 2770. (b) Buyl, F.; Smets, J.; Maes, G.; Adamowicz, L. *J. Phys. Chem.* **1995**, *99*, 14967. (c) Anderson, F. J.; Martell, A. E. *J. Am. Chem. Soc.* **1964**, *86*, 715. (d) Bartl, F.; Urjasz, H.; Brzezinski, B. *J. Mol. Struct.* **1998**, *441*, 77.

(9) Mehdi, K. C. *Indian J. Phys.* **1984**, *58B*, 328.

(10) (a) Llor, J.; Lopez-Mayorga, O.; Munoz, L. *Magn. Reson. Chem.* **1993**, *31*, 552. (b) Stefaniak, L. *Tetrahedron*, **1976**, *32*, 1065. (c) Korytnyk, W.; Singh, R. P. *J. Am. Chem. Soc.* **1963**, *85*, 2813. (d) Jo, B. H.; Nair, V.; Davis, L. *J. Am. Chem. Soc.* **1977**, *99*, 4467.

(11) Bridges, J. W.; Creaven, P. J.; Davis, D. S.; Williams, *Biochem. J.* **1963**, *88*, 65.

(12) (a) Metzler, D. E.; Harris, C. M.; Johnson, R. L.; Siano, C. B.; Thomson, J. A. *Biochemistry* **1973**, *12*, 5377. (b) Metzler, D. E.; Snell, E. E. *J. Am. Chem. Soc.* **1955**, *77*, 2431. (c) Savin, F. A.; Morozov, Y. V. *Mol. Biol.* **1975**, *9*, 238. (d) Metzler, C, M.; Cahill, A.; Metzler, D. E. *J. Am. Chem. Soc.* **1980**, *102*, 6075. (e) Harris, C. M.; Johnson, R. J.; Metzler, D. E. *Biochim. Biophys. Acta* **1976**, *421*, 181.

(13) Cook, M. J.; El-Abbady, S.; Katritzky, A. R.; Guimon, C.; Pfister-Guillouzo, G. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1652.

(14) Baldwin, M. A.; Langley, G. *J. Chem. Soc., Perkin Trans. 2* **1988**, 347.

(15) (a) Wang, J.; Boyd, R. J. *J. Phys. Chem.* **1996**, *100*, 16141. (b) Karelson, M. M.; Katritzky, A. R.; Szafran, M.; Zerner, M. C. *J. Org. Chem.* **1989**, *54*, 6030. (c) Fabian, W. M. F. *J. Comput. Chem.* **1991**, *12*, 17. (d) Scalan, M. J.; Hillier, I. H.; MacDowell, A. A. *J. Am. Chem. Soc.* **1983**, *105*, 3568.

(16) Yang, I. Y.; Harris, C. M.; Metzler, D. E.; Korytnyk, W.; Lachmann, B.; Potti, P. P. G. *J. Biol. Chem.* **1975**, *250*, 2947.

(17) (a) Becke, D. A. *J. Chem. Phys.* **1993**, *98*, 5648. (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(18) (a) Wong, M. W. *Chem. Phys. Lett.* **1996**, *256*, 391. (b) Scott, A. P.; Radom, L. *J. Phys. Chem.* **1996**, *100*, 16502.

(19) (a) Wong, M. W.; Frisch, M. J.; Wiberg, K. B. *J. Am. Chem. Soc.* **1991**, *113*, 4776. (b) Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Chem. Phys.* **1991**, *95*, 8991. (c) Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 1645.

(20) Onsager, L. *J. Am. Chem. Soc.* **1936**, 58, 1486-1493.

(21) (a) Wiberg, K. B.; Castejon, H.; Keith, T. A. *J. Comput. Chem.* **1996**, *17*, 185. (b) Wiberg, K. B.; Keith, T. A.; Frisch, M. J.; Murcko, M. *J. Phys. Chem.* **1995**, *99*, 7702.

(22) Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Comput. Chem.* **1995**, *16*, 385.

tions. In the discrete approach, a number of solvent molecules were incorporated explicitly to interact with the solute molecules (in the so-called solvation sites). The resulting supermolecules were fully optimized at the B3LYP/6-31G* level. The hybrid approach improves part of the continuum deficiency by adding explicit solvent molecules to interact with the solute. This supermolecule is then imbedded in the dielectric medium. In other words, the combined effect of microscopic and macroscopic solvation is incorporated in this hybrid method. Such an approach has been used successfully to investigate chemical reactions in aqueous medium.[23] In this study, we investigate the solvation effects on the tautomeric equilibria of **1**−**6** in three different solvent media: nonpolar ($\epsilon = 2.0$), polar aprotic ($\epsilon = 40.0$), and aqueous ($\epsilon = 78.4$) solutions.

A systematic conformer search at the AM1 level was performed initially with use of the SPARTAN[24] program on all 3-hydroxypyridine derivatives with and without explicit water molecules to locate the most stable conformation for each tautomer. All ab initio and density functional calculations were performed with the Gaussian 98 series of programs.[25]

Results and Discussion

Benchmark Calculations. It is instructive to examine first the effects of correlation and basis set, and the choice of continuum solvation model on our calculated tautomeric energies. To this end, we have performed a series of benchmark calculations on a representative system, namely 3-hydroxy-5-hydroxymethylpyridine-4-carbaldehyde (**4**).

(a) Effects of Correlation and Basis Set. The energy differences between the neutral and zwitterionic forms of **4** were examined by three different theoretical methods, HF, B3LYP, and MP2, together with a hierarchy of three basis sets, 6-31G*, 6-311+G**, and 6-311+G(3df,2p) basis sets (Table 1). The tautomeric energies computed at the HF level are found to be significantly higher than those calculated with the DFT and MP2 methods. It is clear that the HF theory overestimates the tautomeric energies. The calculated B3LYP and MP2 tautomeric energies are comparable and their energies converge as the size of the basis set increases. In fact, the tautomeric energy converges satisfactorily at the 6-311+G** basis set. The B3LYP and MP2 values are in close agreement with those computed at the higher QCISD(T)/6-31G* and G3(MP2)[26] levels (Table 1), which are known to have a well-established accuracy for a wide variety of chemical problems. These benchmark calculations lend us confidence that the B3LYP/6-311+G** level employed in this study will provide satisfactory predictions of the tautomeric energies of **1**−**6**.

TABLE 1. Calculated Tautomeric Energies (ΔE, kJ mol^{-1})[a,b] of 3-Hydroxy-5-hydroxymethylpyridine-4-carbaldehyde (4) in the Gas Phase ($\epsilon = 1.0$) and in a Dielectric Medium of $\epsilon = 40.0$

		$\epsilon = 40.0$	
level	$\epsilon = 1.0$	SCRF[c]	SCIPCM[c]
HF/6-31G*	88.8	60.8	47.4
HF/6-311+G**	90.6	61.2	49.2
HF/6-311+G(3df,2p)	90.1	61.9	50.0
B3LYP/6-31G*	56.6	30.3	23.3
B3LYP/6-311+G**	56.0	27.9	24.6
B3LYP/6-311+G(3df,2p)	57.8	28.0	25.1
MP2/6-31G*	57.5	31.7	
MP2/6-311+G**	63.1	28.2	
MP2/6-311+G(3df,2p)	60.3		
QCISD(T)/6-31G*	61.4		
G3(MP2)	63.0		

[a] Based on the B3LYP/6-31G* optimized geometry, including ZPE correction. [b] Relative energy between the zwitterionic and neutral forms. [c] Based on the SCRF-B3LYP/6-31G* optimized geometry.

(b) Effect of the Continuum Solvation Model. We have examined the bulk solvent effect using two reaction field models, namely SCRF and SCIPCM models. The SCIPCM method provides a more realistic estimation of the shape of the cavity, based on an isodensity surface. It is clear from Table 1 that the computed solvent effect on the tautomeric energy of **4** is rather independent of the choice of the continuum solvation model. In particular, the DFT tautomeric energies predicted by both methods are very close as the size of basis set increases. As a result, all subsequent continuum-based calculations in this paper were carried out with the SCRF model, which is computationally less demanding than the SCIPCM method. As with the gas-phase benchmark calculations, our calculations confirm that the B3LYP/6-311+G** level is also an appropriate choice for the solvent-effect calculations.

(c) Effect of Dipole Moment. It is well established that solvents with high dielectric constants favor the more polar conformation. In other words, a change of preferred conformation is possible on going from the gas phase to solution, particularly for the dipolar species. Thus, it is important to establish the most stable conformation of both tautomers for **1**−**6** in different environments. We have considered several plausible conformations, particularly those with a large dipole moment, of both the neutral and zwitterionic tautomers in each case. The geometries of these conformations are fully optimized in the gas phase, and in nonpolar ($\epsilon = 2.0$) and polar ($\epsilon = 40.0$) media, using the SCRF method. Our calculations verify that the most stable conformer of both tautomers in the gas phase is also the most stable conformer in solution.

Tautomeric Equilibria in the Gas Phase and Substituent Effects. Experimental studies (IR,[8a,b,9] Raman,[9] NMR,[10] UV,[6,12a] photoelectron,[13] and mass[14] spectroscopies) have established unambiguously that 3-hydroxypyridine (**1**) exists as the neutral hydroxy form in the isolated, liquid, and solid states. The tautomeric equilibrium of the parent compound (**1N**/**1Z**) has been examined previously by various theoretical methods.[15] Our calculations confirm that the neutral hydroxy form (**1N**) is strongly preferred in the isolated state (by 52 kJ

(23) (a) Aleman, C. *Chem. Phys.* **2000**, *253*, 13. (b) Aleman, C. *Chem. Phys. Lett.* **1999**, *302*, 461.

(24) SPARTAN 5.0, Wavefunction Inc.: Irvine, CA, 1997.

(25) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98* (Revision A.2); Gaussian, Inc.: Pittsburgh, PA, 1998.

(26) Curtiss, L. A.; Redfern, P. C.; Raghavachari, K.; Pople, J. A. *J. Chem. Phys.* **2000**, *114*, 108.

TABLE 2. Calculated Relative Energies (ΔE_0, ΔH_{298}, and ΔG_{298}, kJ mol^{-1})[a] and Dipole Moments (D) of Various 3-Hydroxypyridine Derivatives in the Gas Phase and Solutions[b]

	dipole moment				
solute	**N**	**Z**	ΔE_0	ΔH_{298}	ΔG_{298}
$\epsilon = 1.0$					
1	1.26	6.61	52.2	52.1	52.0
2	1.17	6.03	43.7	43.7	43.0
3	1.83	7.92	56.2	57.4	54.1
4	2.25	7.52	56.0	55.9	55.9
5	1.78	7.84	47.7	47.6	47.2
6	2.89	6.73	49.1	49.7	47.4
$\epsilon = 2.0$					
1	1.38	7.26	40.7	40.5	40.6
2	1.27	6.75	35.9	35.8	35.2
3	2.02	8.75	45.5	46.6	43.4
4	2.55	8.37	47.1	46.9	47.2
5	2.01	8.82	38.0	37.8	37.6
6	3.29	8.59	42.8	43.7	40.0
$\epsilon = 40.0$					
1	1.59	8.45	18.4	18.0	18.5
2	1.47	7.71	21.6	21.4	20.7
3	2.35	10.29	25.0	26.0	23.0
4	3.16	9.97	29.8	29.3	30.1
5	2.45	10.76	18.1	17.7	18.0
6	4.23	13.04	22.6	23.3	21.8
$\epsilon = 78.4$					
1	1.60	8.62	17.5	17.2	17.6
2	1.46	7.73	21.1	20.9	20.1
3	2.38	10.61	20.7	21.7	18.7
4	3.16	10.10	29.1	28.6	29.4
5	2.46	10.84	17.3	16.9	17.2
6	4.25	13.12	21.8	22.4	20.9

[a] B3LYP/6-311+G**//B3LYP/6-31G* level. $\Delta E = E$(zwitterionic) $- E$(neutral). [b] The SCRF method was employed for the solvent-effect calculations.

mol^{-1}). As with the parent compound, the neutral form is significantly more stable than the zwitterionic form (by 44−56 kJ mol^{-1}, Table 2) for all other 3-hydroxypyridine derivatives (**2**−**6**). The free energy difference (ΔG_{298}) for PLP (**6**) in the gas phase is predicted to be 47 kJ mol^{-1}. Here, the free energy difference is computed from the equation $\Delta G_T = \Delta H_T - T\Delta S$, where H, T, and S are enthalpy, temperature, and entropy, respectively. For all the species examined in this paper, the entropies and temperature corrections ($H_T - H_0$) of both tautomeric forms are fairly close, leading to almost identical ΔE, ΔH, and ΔG values (Table 2).

The optimized B3LYP/6-31G* geometries of **1**−**6** are shown in Figure 1. The most stable conformation of both tautomers is mainly influenced by intramolecular hydrogen bonds and to a certain extent even the CH···O interactions. In particular, there is a relatively strong intramolecular hydrogen bonding interaction between the carbonyl oxygen and the hydroxyl hydrogen in the neutral form of **3**−**6**. In addition, we note that the orientation of the aldehyde group (at C_4) in the neutral tautomer is different from that in the dipolar form for species **3**−**6**.

To investigate the effects of substituent on the tautomeric equilibrium of PLP, we have introduced a series of substituents, namely, methyl, formyl, hydroxymethyl, and phosphate, systematically to the parent compound. Comparing the tautomeric energies of **1** with **2** and **4** with **5** shows that the introduction of a methyl group reduces the tautomeric energy by ∼9 kJ mol^{-1} (Table 2) and leads

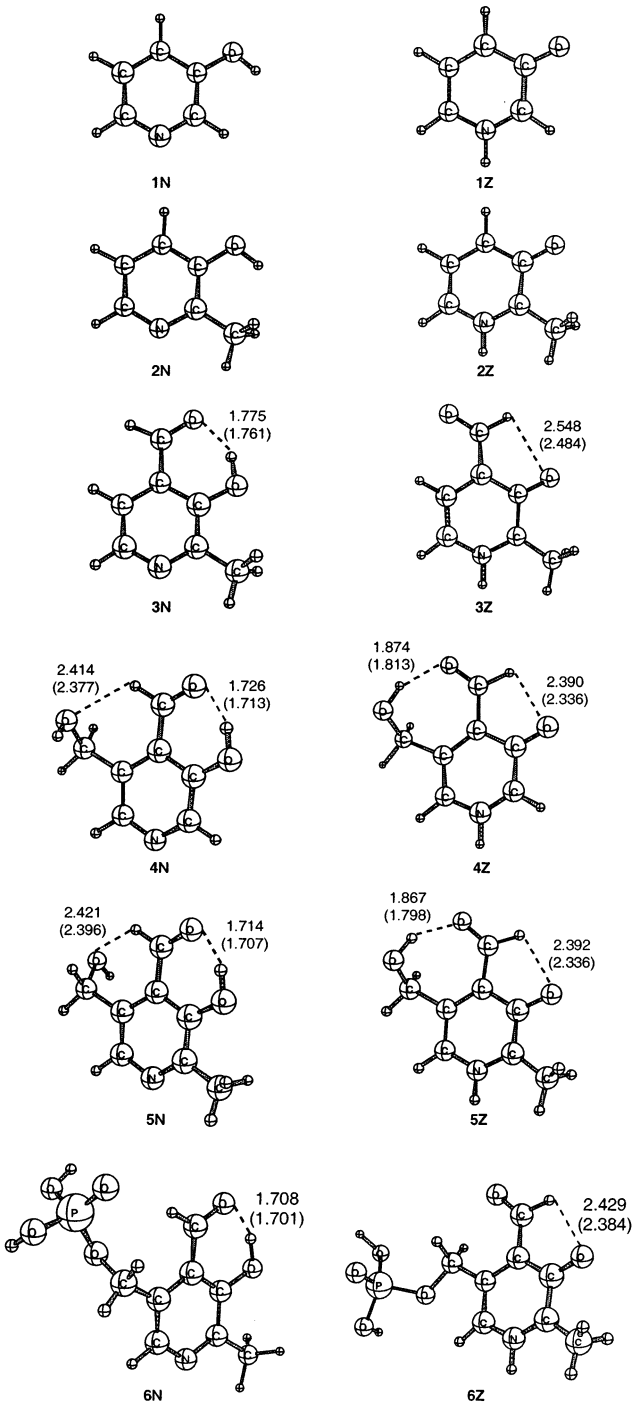


FIGURE 1. Optimized (B3LYP/6-31G*) geometries of the neutral (**N**) and zwitterionic (**Z**) forms for **1**−**6** (bond lengths are given in Å). The SCRF values ($\epsilon = 40.0$) are in parentheses.

to a greater stabilization of the zwitterionic form. On the other hand, introduction of a formyl group to **2** increases the tautomeric energy by 13 kJ mol^{-1}. The stabilization of the neutral form in **3** may be explained in terms of the intramolecular hydrogen bond between the hydroxyl hydrogen and the aldehydic oxygen (Figure 1). The hydroxymethyl group has a stabilizing effect on the zwitterionic tautomer as reflected in the reduction of tautomeric energy on going from **3** to **5** (by 9 kJ mol^{-1}). Comparing the tautomeric energies of **5** and **6** indicates

that the phosphate substituent has a small influence on the tautomeric equilibrium.

Tautomeric Equilibria in Condensed Phases. The zwitterionic forms (**1Z**−**6Z**), with a large degree of charge separation, are predicted to have a significantly larger dipole moment than the corresponding neutral form, by 4−5 D (Table 2). Thus, one would expect the polar zwitterionic form to be stabilized differentially in a dielectric medium. Indeed, experimental studies have shown that the tautomeric equilibrium in PLP is severely affected by solvent polarity.[4] UV−visible and NMR spectroscopic studies of 3-hydroxypyridine indicate that the neutral form is the preferred tautomer in nonaqueous solutions while the zwitterionic form is more stable in an aqueous medium.[6,10a] To better understand the influence of solvent on the tautomeric equilibrium of the biologically active form of vitamin B_6, we have studied the tautomeric equilibria of **1**−**6** using three different approaches: (a) continuum, (b) discrete, and (c) hybrid models.

(a) Continuum Model. First, we examine the effect of nonspecific solute−solvent interaction using the SCRF continuum solvation models. The reaction field model is expected to yield reliable results for a nonpolar or aprotic polar solution, where specific solute−solvent interaction is not important. We have computed the tautomeric energies of **1**−**6** in a dielectric medium of $\epsilon = 2.0$ (representing a nonpolar medium), 40.0 (a polar aprotic medium), and 78.4 (an aqueous solution). The calculated tautomeric energies and dipole moments obtained with the SCRF calculations are given in Table 2.

As expected, polarizable dielectric media stabilize preferentially the dipolar tautomers. The energy differences between the neutral and zwitterionic forms of all species decrease with increasing dielectric constant. For instance, the ΔG_{298} value in PLP (**6**) reduces by 7, 26, and 30 kJ mol^{-1} in a dielectric medium of $\epsilon = 2.0$, 40.0, and 78.4, respectively. In other words, the tautomeric equilibrium shifts in the direction of the zwitterionic tautomer when the dielectric constant of the solvent increases. Our calculated solvent dependence of the tautomeric equilibrium of 3-hydropyridine (**1**) agrees well with that reported previously by Wang and Boyd.[15a] Although there is a substantial reduction in the tautomeric energy on going from the gas phase to a polar medium, it is not sufficient to reverse the tautomeric equilibrium. The predicted stability order of **1** in nonaqueous medium ($\epsilon = 2.0$ or 40.0) by the reaction field model is consistent with the experimental findings.[6,10a,b] However, the calculated SCRF tautomeric energies in an aqueous medium ($\epsilon = 78.4$) do not follow the experimental findings. It thus appears that incorporating the nonspecific solute−solvent interaction alone cannot account for the observed tautomeric equilibria in water solution.

In a medium of high dielectric constant, the permanent dipoles of the zwitterionic forms increase substantially (Table 2). For instance, the calculated dipole moment of **6Z** with $\epsilon = 40.0$ is almost twice of that in the gas phase. It is worth noting that the calculated tautomeric energies with $\epsilon = 40.0$ and 78.4 are fairly close for all cases.

(b) Discrete Model. In the discrete model, the solvent molecules around the solute are treated as individual entities. Generally, in this microscopic representation of the solvent, only the solvent molecules near the solute are retained. In both the neutral and zwitterionic forms of **1**−**6**, there are several protonable sites which are capable of forming a hydrogen bond with a protic solvent such as water. Thus, we investigated the effect of specific solute−solvent interaction by explicitly including two water molecules to all the solutes considered. The resulting supermolecules, i.e., 1:2 solute−water complexes, were fully optimized at the B3LYP/6-31G* level. The optimized structures of the solute−water complexes are depicted in Figure 2. In all cases, the water solvent molecules are strongly coordinated to the solute via hydrogen bonds, with the nitrogen and hydroxyl hydrogen in the neutral forms (**1N**−**6N**) and the pyridine hydrogen and carbonyl oxygen in the zwitterions (**1Z**−**6Z**). The stronger solvent stabilization of the zwitterionic forms is readily reflected in the hydrogen-bonding distances. Comparing the tautomeric energies in an isolated state and in the solute−water supermolecule (Tables 2 and 3) shows that inclusion of specific solute−solvent interaction also leads to a substantial reduction of the tautomeric energy, by 15−40 kJ mol^{-1}. The greater stabilization of the zwitterionic form is mainly attributed to the fact that the NH−OH_2 hydrogen bond in the zwitterionic form is stronger than the corresponding

TABLE 3. Calculated Relative Energies (ΔE_0, ΔH_{298}, and ΔG_{298}, kJ mol^{-1})[a] and Dipole Moments (D) of the 1:2 Solute−H_2O Complexes (Solute = 1−6) in the Gas Phase and Solutions[b]

	dipole moment				
solute	N	Z	ΔE_0	ΔH_{298}	ΔG_{298}
$\epsilon = 1.0$					
1[c]	4.55	6.74	44.8	42.6	50.3
1	6.70	5.95	34.9	33.8	37.2
2	3.86	8.39	28.5	27.9	31.3
3	5.96	10.01	22.1	22.9	21.0
4	3.62	7.88	16.4	15.5	17.2
5	3.14	9.84	13.9	13.4	15.0
6	3.98	11.00	20.5	21.5	18.9
6[d]	3.58	6.80	10.9	11.7	8.8
$\epsilon = 2.0$					
1[c]	5.06	7.91	36.7	34.7	40.6
1	7.64	6.88	33.3	32.9	33.7
2	6.70	11.93	13.4	13.8	13.2
3	6.53	12.38	5.4	7.3	1.5
4	4.14	12.19	4.0	3.6	3.5
5	3.73	12.13	−1.5	−1.3	−2.0
6	5.02	13.79	1.6	3.8	−9.4
$\epsilon = 40.0$					
1[c]	5.99	12.68	3.6	0.8	6.4
1	9.14	17.31	−28.7	−28.3	−32.2
2	8.52	16.10	−18.1	−20.6	−13.0
3	7.54	18.69	−42.7	−42.1	−43.0
4	5.44	13.14	−18.5	−19.4	−17.1
5	5.07	18.85	−50.9	−52.2	−47.0
6	7.42	19.24	−32.9	−34.3	−30.8
$\epsilon = 78.4$					
1[c]	6.05	13.14	−1.3	−1.7	−3.6
1	9.22	17.56	−30.7	−30.4	−33.9
2	8.88	15.73	−15.6	−15.8	−16.0
3	7.58	18.81	−44.6	−43.9	−44.8
4	5.46	13.82	−19.6	−20.5	−18.3
5	5.14	18.98	−52.6	−54.0	−48.6
6	9.69	19.12	−27.6	−27.0	−29.4
6[d]	6.91	24.09	−24.0	−23.1	−27.0

[a] B3LYP/6-311+G**// B3LYP/6-31G* level. $\Delta E = E$(zwitterionic) − E(neutral). [b] The SCRF method were employed for the solvent-effect calculations. [c] 1:1 3-hydroxypyridine−water complex. [d] 1:4 **6**−water complex.

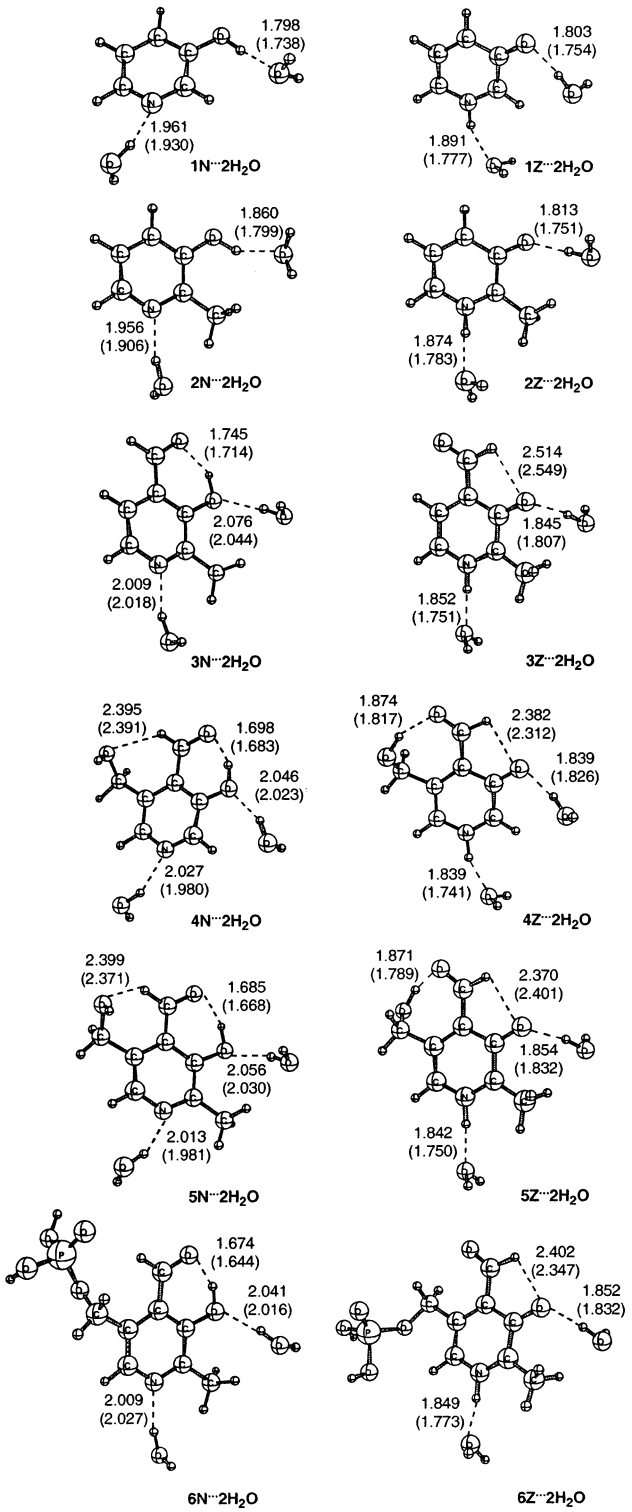


FIGURE 2. Optimized (B3LYP/6-31G*) geometries of the neutral (**N**) and zwitterionic (**Z**) forms for the 1:2 solute−water complexes (solute = **1**−**6**) (bond lengths are given in Å). The SCRF values ($\epsilon = 40.0$) are in parentheses.

N−H_2O interaction in the neutral tautomer. This differential stabilization effect is particularly large for compounds **4**−**6**. As with the continuum-based calculations, the discrete model is unable to predict the correct stability order in an aqueous medium. For instance, the dipolar form of PLP is 21 kJ mol^{-1} less stable than the neutral tautomer based on this discrete solvation model. Hence, we may conclude that neither specific nor nonspecific solute−solvent interaction alone can correctly predict the stability order in an aqueous environment.

It is important to note that there is a significant increase in the dipole moments of both the neutral and zwitterionic forms on going from the isolated state (Table 2) to the 1:2 solute−water complex (Table 3). This indicates that these supermolecules may be further stabilized in a polarizable dielectric medium via the bulk solvation effect.

The optimized geometries of the solute−water complexes together with their hydrogen-bonding distances are given in Figure 2. There is no significant change in the geometry of the solute on going from the isolated state to the solvated complex. In all the neutral forms of solute−water complexes, the nitrogen atom acts as a hydrogen bond acceptor and the hydroxyl oxygen acts as a hydrogen bond donor. In sharp contrast, the nitrogen atom serves as a hydrogen bond donor and the carbonyl oxygen acts as a hydrogen bond acceptor in the zwitterionic forms.

(c) Hybrid Model. Finally, we have employed a hybrid approach to investigate the combined effect of specific and nonspecific solute−solvent interactions. To this end, the SCRF calculations were performed for all the 1:2 solute−water complexes. In sharp contrast to the continuum and discrete models, the hybrid model (with $\epsilon = 78.4$) predicts a preference of the zwitterionic oxo form in all cases (Table 3). These results are in excellent accord with the experimental findings.[6] This readily demonstrates that it is important to include both the microscopic and macroscopic representations in the theoretical treatment of aqueous solvation for the 3-hydroxypyridine derivatives. With respect to the geometry changes of the microsolvated structures that take place upon relaxing the geometries in solution, there is a systematic tendency of all hydrogen bonds to decrease in bond distance (Figure 2). In some cases, the hydrogen-bonding distance is reduced significantly by 0.011−0.114 Å.

In addition to the 1:2 solute−water complexes of various 3-hydroxypyridine derivatives, we also have performed the hydrid-based calculations on the 1:1 complexes of **1N** and **1Z**. As seen in Table 3, the tautomeric energies of the 1:1 solute−water complexes are significantly higher than those of the 1:2 complexes. This result indicates that it is necessary to have a minimum of two explicit water molecules for a balanced description of the specific-solute interaction.

The aldehyde, hydroxymethyl, and phosphate groups in **3**−**6** may act as a hydrogen donor or acceptor in specific solvation with water molecules. Therefore, it is instructive to examine also other solute−solvent complexes involving more than 2 explicit water molecules. To this end, we have examined a 1:4 solute−water complex of PLP (**6**). The optimized geometries (Supporting Information) indicate that the interaction of the water molecules with pyridine N and hydroxyl O in **6N**, and with NH and carbonyl O in **6Z**, are essentially the same as those in the corresponding 1:2 solute−water complexes. The computed tautomeric free energy in aqueous solution employing the hybrid model is 27 kJ mol^{-1} (Table 3), close to that predicted with the 1:2 solute−solvent supermolecule, 29 kJ mol^{-1}. This result lends us confi-

dence that our choice of two explicit water molecules is sufficient for a balanced description of the specific solvent effect for both the neutral and zwitterionic forms.

In summary, we found that both specific and nonspecific solvent−solute effects play an equally important role in determining the tautomeric equilibria of **1**−**6** in water solution. The hybrid approach provides a significant improvement over the pure continuum and discrete methods in predicting the solvation effects in aqueous medium.

Infrared Spectra and ^{13}C NMR Chemical Shifts. The calculated B3LYP/6-31G* harmonic vibrational frequencies and infrared intensities of the neutral and zwitterionic forms of 3-hydroxypyridine (**1**) in the gas phase and solutions are tabulated in Tables S1 and S2, respectively. The computed frequencies were scaled by a factor of 0.9614.[18] The characteristic C═O, N−H, and O−H stretching frequencies can be used to differentiate between the two tautomeric forms. Infrared and Raman studies of 3-hydroxypyridine in the gas phase[8a] and in the solid and pure liquid states[9] showed the presence of the neutral hydroxy form only. The observed infrared and Raman spectra of 3-hydroxypyridine do not show any infrared absorption in the 1650−1740 cm^{-1} region, which has the characteristic C═O stretching vibration of the zwitterionic form (**1Z**). The vibrational spectrum of **1N** has been studied by Person et al. in argon matrix.[8a] The observed absorption bands were assigned based on the calculated HF/6-31G* frequencies. However, we found that there are significant differences between the HF and B3LYP frequencies. We believe that the assignment of the ν_{15}−ν_{20} bands is probably incorrect. Thus, we have reassigned these absorption peaks (see Table S1) based on the more accurate B3LYP/6-31G* IR spectrum, together with the vibrational spectra in the liquid state.[9] Our calculated and observed frequencies are in very good agreement, with a root-mean-square error of 18 cm^{-1}. As with the calculated frequencies, the calculated intensity values too correlate well with the observed intensities (Table S1).

The calculated gas-phase C═O stretching frequencies of zwitterions **1Z**−**6Z** are 1653, 1635, 1631, 1637, 1623, and 1625 cm^{-1}, respectively. The carbonyl stretching frequency decreases steadily on introducing substituents to 3-hydroxypyridine. The calculated C═O stretching frequency is the highest in **1** and the lowest in **5**. As with other carbonyl compounds,[19b] a significant red shift is predicted for the C═O stretching frequency for all zwitterions. The predicted C═O stretching frequencies of **1Z**−**6Z** in a polar medium (ϵ = 40.0) are 1622, 1617, 1622, 1624, 1618, and 1621 cm^{-1}, respectively. The calculated red shift is significant for 3-hydroxypyridine (31 cm^{-1}) but relatively smaller for PLP (4 cm^{-1}). As expected, the changes in frequencies and intensities in the presence of a solvent reaction field are larger for the zwitterionic form than the less polar neutral form (Tables S1 and S2).

NMR spectroscopy provides a valuable tool to investigate the tautomerism of chemical and biological molecules.[27] To facilitate future experimental studies on the tautomerization of **1**−**6** via NMR spectroscopy, we have calculated their NMR chemical shifts using the "gauche-including" atomic orbital (GIAO)[28] method. The ^{13}C chemical shifts, using TMS as a reference compound, of both the neutral and zwitterionic forms of **1**−**6** were computed at the B3LYP/6-311G** level, based on the B3LYP/6-31G* optimized geometry (Table 4). We have shown in previous studies that this level of theory provides good agreement with experimental results.[29] For all the 3-hydroxypyridine derivatives examined in this paper, the chemical shifts of the neutral and zwitterionic forms, particularly those at C_4 and C_6, are sufficiently different that the NMR technique can be used to characterize the tautomeric equilibrium. The temperature dependence of the ^{13}C chemical shifts of 3-hydroxypyridine (**1**) in D_2O has been studied by Llor and coworkers.[10a] Since this system has a rapid tautomeric equilibrium, the observed NMR chemical shifts correspond to the weighed average of the contributing tautomeric forms. Hence, they employed a two-state model to determine the chemical shifts of the pure forms of **1N** and **1Z**. For **1N**, the calculated chemical shifts are in excellent accord with the experimental values[10a,b] (Table 4). The agreement between theory and experiment for **1Z** is less satisfactory. This may be due to the solvent effect on chemical shifts or the deficiency of the two-state model.

TABLE 4. Calculated ^{13}C NMR Chemical Shifts[a] (ppm, Relative to TMS) for the Neutral and Zwitterionic Forms of 1−6

atom[b]	neutral	zwitterionic	atom[b]	neutral	zwitterionic
1[c]			**2**		
C_2	144.5 [138.7][d]	137.8	C_2	147.0	150.3
C_3	160.1 (153.9)	170.3 (162.7)	C_3	159.1	170.6
C_4	122.7 (124.0)	146.3 (133.6)	C_4	125.5	138.5
C_5	128.2 [125.0][d]	131.2	C_5	128.6	130.7
C_6	148.0 (142.0)	114.1 (128.1)	C_6	147.0	111.8
			C_7	20.3	22.4
3			**4**		
C_2	159.2	162.9	C_2	152.1	147.7
C_3	162.5	171.6	C_3	165.2	174.0
C_4	125.9	137.3	C_4	125.6	143.3
C_5	126.1	130.1	C_5	140.2	154.9
C_6	145.5	113.3	C_6	144.9	116.5
C_7	23.2	22.5	C_8	205.9	204.1
C_8	203.2	194.8	C_9	67.9	68.0
5			**6**		
C_2	161.8	161.6	C_2	163.1	160.6
C_3	163.8	174.4	C_3	163.5	172.8
C_4	124.5	136.9	C_4	123.9	133.2
C_5	138.5	153.0	C_5	133.5	147.0
C_6	143.7	113.7	C_6	145.8	114.8
C_7	23.4	22.3	C_7	23.5	22.0
C_8	206.0	203.3	C_8	206.1	201.5
C_9	67.9	68.2	C_9	68.0	75.2

[a] GIAO calculations at the B3LYP/6-311G**//B3LYP/6-31G* level. [b] Atom labels are illustrated in Chart 1. [c] Experimental chemical shifts of **1N** and **1Z** were taken from ref 10a. [d] From ref 10b.

Charge Distributions. We have examined the charge distributions of **1N** and **1Z** both in the gas phase and in

(27) Koch, A.; Kleinpeter, E. *J. Mol. Model.* **1997**, *3*, 375.

(28) (a) Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, M. J. *J. Chem. Phys.* **1996**, *104*, 5497. (b) Ditchfield, R. *Mol. Phys.* **1974**, *27*, 789.

(29) (a) Kollenz, G.; Holzer, S.; Kappe, C. O.; Dalvi, T. S.; Fabian, W. M. F.; Sterk, H.; Wong, M. W.; Wentrup, C. *Eur. J. Org. Chem.* **2001**, 1315. (b) Plug, C.; Wallfisch, B.; Andersen, H. G.; Bernhardt, P. V.; Baker, L. J.; Clark, G. R.; Wong, M. W.; Wentrup, C. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2096. (c) Chen. C. J. M.Sc. Thesis, National University of Singapore, 2001. (d) Barone, G.; Gomez-Paloma, L.; Duca, D.; Silvestri, A.; Riccio, R.; Bifulco, G. *Eur. J. Chem.* **2002**, *8*, 3223.

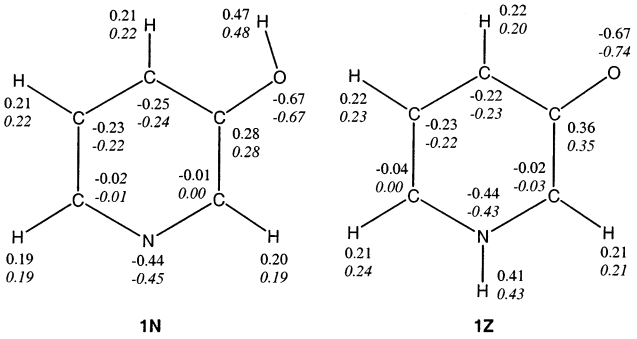


FIGURE 3. Calculated (B3LYP/6-311+G**//B3LYP/6-31G*) NBO atomic charges of **1N** and **1Z**.

TABLE 5. Calculated Relative Energies (ΔE_0, ΔH_{298}, and ΔG_{298}, kJ mol^{-1})[a] and Dipole Moments (D) of the Keto and Enol Forms of Various 3-Hydroxypyridine Derivatives[c] in the Gas Phase and Solutions[b]

	dipole moment				
solute	enol	keto	ΔE_0	ΔH_{298}	ΔG_{298}
$\epsilon = 1.0$					
3	5.34	1.83	116.9	118.1	115.1
4	4.76	2.25	113.3	114.3	112.1
5	3.99	1.78	110.0	110.9	108.8
6	4.75	2.89	104.6	105.2	104.9
$\epsilon = 2.0$					
3	6.01	2.02	112.4	113.6	110.8
4	5.35	2.55	110.4	111.3	109.2
5	4.54	2.01	108.3	109.2	107.1
6	5.69	3.29	103.3	104.1	102.5
$\epsilon = 40.0$					
3	7.31	2.35	103.3	104.3	101.8
4	6.53	3.17	104.5	105.4	103.4
5	5.67	2.45	104.4	105.2	103.3
6	7.62	4.23	99.2	100.2	97.5

[a] B3LYP/6-311+G**// B3LYP/6-31G* level. $\Delta E = E$(enol) $- E$(keto). [b] The SCRF method was employed for the solvent-effect calculations.

a dielectric medium of $\epsilon = 40.0$ using the natural bond orbital (NBO) analysis,[30] based on the B3LYP/6-311+G** wave function. A recent study on the experimental charge density of N_2O_4 has shown that the NBO atomic charges compare well with the experimentally derived charges.[31] As expected, the hydroxyl hydrogen in **1N** and the pyridine hydrogen in **1Z** have strong positive charge (Figure 3). Accordingly, the oxygen and nitrogen in **1N** and **1Z** bear strong negative charge. In the presence of a solvent reaction field, there is a significantly larger degree of charge separation in the zwitterion **1Z** but relatively unperturbed in the neutral form. The atomic charges of the oxygen and NH hydrogen in **1Z** increase on going from the gas phase to solution. Unexpectedly, there is also a significant increase in the atomic charge at the carbon and hydrogen atoms adjacent to the NH group.

Keto−Enol Tautomerism. For compounds **3**−**6** with an aldehyde group, there exists an additional tautomeric form, namely the enol tautomer. The corresponding keto form is the neutral aldehyde form discussed in previous sections. The calculated enol/keto tautomeric energies in the gas phase and solutions are given in Table 5. In all cases, the enol tautomer is substantially higher in energy than the keto form, by 100−120 kJ mol^{-1}. The energy difference reduces slightly in an aprotic solvent (Table 5). This result indicates that the high-energy enol tautomer plays a less important role in the tautomerization of PLP.

Conclusions

We have examined the tautomeric equilibria of the biologically active form of vitamin B_6, namely, pyridoxal-5′-phosphate (PLP), and a series of 3-hydroxypyridine derivatives in the gas phase and in nonaqueous and aqueous solvents. In the isolated state, the neutral form is the preferred tautomer for all the 3-hydroxypyridine derivatives studied (**1**−**6**). Incorporation of solvent effects reduces substantially the tautomeric energies. For all cases, a reversal of equilibrium, in favor of the zwitterionic oxo form, is predicted in an aqueous medium. A hybrid solvation model including both the macroscopic and microscopic solvent effects is necessary to predict correctly the tautomeric equilibria in aqueous environment. Thus, our calculations confirm that the zwitterionic tautomer is the predominant form of PLP in physiological conditions. The calculated vibrational frequencies and ^{13}C NMR chemical shifts of 3-hydroxypyridine correlate well with the experimental results.

Acknowledgment. This research was supported by the National University of Singapore (Grant No. R-143-000-079-112). We are grateful for the invaluable preliminary calculations carried out by S. Abirami.

Supporting Information Available: Tables S1 and S2, calculated B3LYP/6-31G* vibrational frequencies and infrared intensities of **1N** and **1Z**, respectively; Table S3, Cartesian coordinates and absolute energies of all calculated compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0266792

(30) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899.
(31) Messerschmidt, M.; Wagner, A.; Wong, M. W.; Luger, P. *J. Am. Chem. Soc.* **2002**, *124*, 732.