Determination of Acidic Dissociation Constants of Glycine, Valine, Phenylalanine, Glycylvaline, and Glycylphenylalanine in Water Using ab Initio Methods

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In this study, pK_a values of glycine, valine, phenylalanine, glycylvaline, and glycylphenylalanine were determined in aqueous solution by an ab initio method. To explain the acidic dissociation constants obtained, we investigated the molecular conformations and solute−solvent interactions of the peptides and amino acid anions, using the density functional theory (DFT) method. Several ionization reactions and equilibria in protic solvents, which possess a high hydrogen-band-donor capability, are shown. The mentioned reactions and equilibria constitute the indispensable theoretical basis to calculate the acidity constants of glycine, valine, phenylalanine, glycylvaline, and glycylphenylalanine. Basis sets at the B3LYP/6-31+G(d) level of theory were used for calculations. Tomasi's method was used to analyze the formation of intermolecular hydrogen bonds between the existent species and water molecules. In this way, it was determined that in alkaline aqueous solutions the cation, anion, and neutral species of glycine, valine, phenylalanine, glycylvaline, and glycylphenylalanine are solvated with one, two, three, and four molecules of water, respectively. In this study, there is comparable agreement between the experimentally determined pK_a values for the acid−base reactions selected by potentiometric and those reported in the literature demonstrating the theoretically calculated pK_a values.

Introduction

Peptides are an amazing class of compounds. Although they are all constructed from relatively simple building blocks (the amino acids), they exhibit a remarkable range of biological properties: peptides can act as antibiotics, hormones, food additives, poisons, or pain-killers, and it is primarily because of their medicinal properties that the study of peptides has become one of the most active areas of current research. Glycine, valine, and phenylalanine are α-amino acids, which means that the amino and carboxylic acid groups are both attached to the same carbon atom. Therefore, they all possess the same generalized structure shown in Figure 1, and the only difference between them is the nature of the side chain. When two amino acids are covalently linked together by amide bonds, the resulting molecules are called dipeptides. With an amino and a carboxylic acid group being present in these molecules, there is both a basic and an acidic component in them. Both functional groups can be ionized. Although peptides are composed of amino acids, the amide bond itself shows neither the properties of the amino group nor those of the carboxylic acid group. In fact, the properties of the amide group are governed by the conjugation of the nitrogen lone pair with the carbonyl group—this is a mesomeric effect which can be expressed as a resonance between two canonical forms.[1]

Amino acids exist as zwitterions at the isoelectric point, in which both the amino and carboxylic acid groups are almost totally ionized; the physical properties of the amino acids are largely governed by the degree of ionization at different pHs. The side chain of an amino acid can alter its physical properties by modifying: (i) the net charge at a given pH, (ii) the relative affinity for water, and (iii) the pH at which there is no net charge (the isoelectric point). Of course, peptides are themselves composed of covalently bonded amino acids; their properties will therefore be dominated by the nature of the side chains on the constituent amino acids.[1]

Different experimental procedures are frequently used for the determination of acidity constants, for example, high-pressure liquid chromatography, liquid−liquid partitioning, and methods that involve potentiometric titrations or spectrophotometric determinations in water or in mixtures of solvents. The determination of the ionization constant by UV−vis spectrophotometry is more time-consuming than by potentiometry. However, spectrophotometry is an ideal method when a substance is too insoluble for potentiometry or when its pK_a value is particularly low or high.[2−8] On the other hand, during the last two decades there has been much interest in developing a methodology enabling theoretical prediction of pK_a values, employing various quantum theoretical techniques. As pK_a equals $\Delta G/2.303RT$, where ΔG is a free energy change of the dissociation reaction either in a gas or solution, acidity of a compound can be determined by the ΔG value.[9−13] Polarizable continuum models have been applied to calculate free energy differences for cations, neutral compounds, and their anions. On the basis of solvation free energies, the pK_a values were obtained for the compounds in question by using thermodynamic equations, involving the combined experimental and calculated data.[14]

This paper deals with the influence of factors such as the Self-Consistent Reaction Field (SCRF) model applied, choice of a particular thermodynamic equation, atomic radii used to build a cavity in the solvent (water), optimization of geometry in water, inclusion of electron correlation, and the dimension of the basis set on the solvation free energies and on the calculated pK_a values. In this study, pK_a values of glycine, valine, phenylalanine, glycylvaline, and glycylphenylalanine were determined in aqueous solution by an ab initio method

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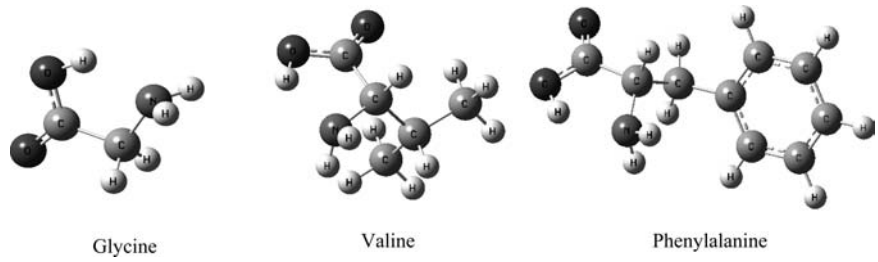

Figure 1. Optimized structures of glycine, valine, and phenylalanine for carrying out the calculations.

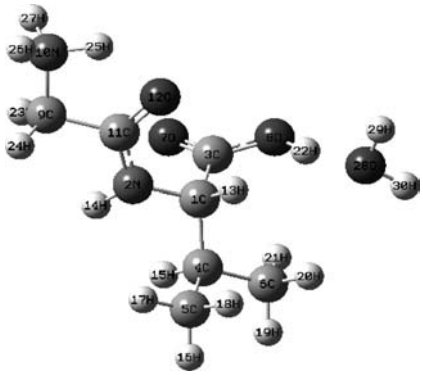

Figure 2. Optimized structure of the glycylvaline cation solvated with a water molecule and practical numbering system adopted for carrying out the calculations.

and at a temperature of 25 °C. To explain the acidic dissociation constants obtained, we investigated the molecular conformations and solute−solvent interactions of the cation, anion, and neutral species of glycine, valine, phenylalanine, glycylvaline, and glycylphenylalanine, using ab initio and density functional theory (DFT) methods.

Computational Method

In this study, the optimized geometries of the initial molecules and the practical numbering system adopted for performing the calculations by the semi empirical PM3 method were included in program CS Chem3D version 5.0.[15] All the geometries of the initial and solvated molecules in water, considering intermolecular hydrogen bonds of water with amino and carboxyl groups, were optimized with the Gaussian 98 program packages using the B3LYP/6-31+G(d) methods and the default convergence criteria[16] as shown in Figure 2 for the optimized structure of glycylvaline. Similar optimized structures (intermolecular hydrogen bonds of water molecules with the amino and carboxylic groups) for other molecules were also obtained by the calculated method mentioned above. To analyze the solvent effects on all species involved in the selected ionization reaction, the polarized continuum model (PCM) of Tomasi et al. was used.[17] Furthermore, to shed light on the experimental pK_a values of amino acids and dipeptides in water, several conformers were tested by the program, but some of the conformers were not considered further because the estimated error in its acidic dissociation constants was unacceptable. Finally, we selected the solvation of the species by means of intermolecular hydrogen bonds (IHBs) that involve one molecule of the mentioned species and some molecules of water (see Table 1).

Results and Discussion

The tendency of a molecule to lose its hydrogen atom as an acidic proton is quantified as pK_a. Fully protonated glycine, valine, phenylalanine, glycylphenylalanine, and glycylvaline have two acid groups: ammonium and carboxyl. A proton can be lost from either of the two groups to give different ionized species: the loss of a proton from the carboxyl group is most probable and from the ammonium group least probable. Therefore, this concept of microscopic ionization constants k_1 and k_2 may be applied, where k_1 involving the carboxyl proton is[18]

$$k_1 = \frac{[H^+][NH_3{}^+CHRCOO^-]}{[NH_3{}^+CHRCOOH]} \quad (1)$$

and k_2 involving the ammonium proton is

$$k_2 = \frac{[H^+][NH_2CHRCOOH]}{[NH_3{}^+CHRCOOH]} \quad (2)$$

where R is H, $CH(CH_3)_2$, $CH_2(C_6H_5)$, (HCO)(NH)(CH)-$(CH_2(C_6H_5))$, and (HCO)(NH)(CH)$(CH(CH_3))$ for glycine, valine, phenylalanine, glycylphenylalanine, and glycylvaline, respectively. It can be shown that for a dibasic acid the first ionization constant K_1 is the sum $k_1 + k_2$ and the second ionization constant K_2 is $(k_{12} \cdot k_{21})/(k_{12} + k_{21})$, where the subscript 12 denotes loss of proton 2 following loss of proton 1 and subscript 21 denotes loss of proton 1 following loss of proton 2.

The chemical interpretation of the changes is not straightforward, even though from model compounds the carboxyl proton is predicted to be the most acidic. Calculations involving the microscopic constants indicate that the first and second K correspond to removal of the carbonyl proton and from the ammonium almost, but not completely, exclusively. It can be determined by NMR spectroscopy exactly.[18−21] The different models of molecules (zwitterions and unzwitterions) were investigated by the G98 program. Considering eqs 1 and 2, different reactions including cationic, neutral, and anionic species were tested, but some of the reactions were not considered further because the estimated error in its acidic dissociation constants was unacceptable. The models finally chosen for the studied system and the calculated values of the acidic dissociation constants for different amino acids and peptides are listed in Table 2.

The acidic dissociation constants of amino acids and dipeptides have been extensively studied in different kinds of background electrolytes.[22−30] It is known that, in general, potentiometry and spectroscopic methods are highly sensitive and as such are suitable for studying chemical equilibria solutions. These methods involve the direct determinations of the mole ratio of acid−base conjugate pairs in a series of buffered solutions of known pH. If the components involved in the equilibrium can be obtained in pure form and if their spectral responses do not overlap, the analysis is very simple.[2,31]

The acidic dissociation constants of glycylvaline have been determined using the potentiometric technique. The method of determining acidic dissociation constants was previously described, and its values are used in this work.[2,40] These values are listed in Table 2 together with the calculated values using the Tomasi method at the B3LYP/6-31+G(d) level of theory.

Table 1. Calculated Total Energy Using the Tomasi Method at the B3LYP/6-31+G(d) Level of Theory for Cationic, Neutral, and Anionic Species of Glycine, Valine, Phenylalanine, Glycylphenylalanine, and Glycylvaline, at 298.15 K[a]

		G^o_{sol}	G^o_{sol}/molecule		G^o_{sol}	G^o_{sol}/molecule
N	solvated species	(Hartree)	(kJ·mol^{-1})	solvated species	(Hartree)	(kJ·mol^{-1})
		Glycine			Valine	
0	H_2L^+	$-2.8491\cdot10^2$	$-7.4803\cdot10^5$	H_2L^+	$-4.0284\cdot10^2$	$-1.0577\cdot10^6$
1	$H_2L^+(H_2O)$	$-3.6135\cdot10^2$	$-4.7437\cdot10^5$	$H_2L^+(H_2O)$	$-4.7929\cdot10^2$	$-6.2919\cdot10^5$
0	HL	$-2.8447\cdot10^2$	$-7.4687\cdot10^5$	HL	$-4.0221\cdot10^2$	$-1.0560\cdot10^6$
1	$HL(H_2O)$	$-3.6091\cdot10^2$	$-4.7377\cdot10^5$	$HL(H_2O)$	$-4.7883\cdot10^2$	$-6.2859\cdot10^5$
2	$HL(H_2O)_2$	$-4.3734\cdot10^2$	$-3.8274\cdot10^5$	$HL(H_2O)_2$	$-5.5529\cdot10^2$	$-4.8597\cdot10^5$
0	L^-	$-2.8401\cdot10^2$	$-7.4567\cdot10^5$	L^-	$-4.0195\cdot10^2$	$-1.0553\cdot10^6$
1	$L^-(H_2O)$	$-3.6045\cdot10^2$	$-4.7318\cdot10^5$	$L^-(H_2O)$	$-4.7838\cdot10^2$	$-6.2800\cdot10^5$
2	$L^-(H_2O)_2$	$-4.3689\cdot10^2$	$-3.8235\cdot10^5$	$L^-(H_2O)_2$	$-5.5483\cdot10^2$	$-4.8557\cdot10^5$
3	$L^-(H_2O)_3$	$-5.1330\cdot10^2$	$-3.3692\cdot10^5$	$L^-(H_2O)_3$	$-6.3126\cdot10^2$	$-4.1434\cdot10^5$
4	$L^-(H_2O)_4$	$-5.8980\cdot10^2$	$-3.0970\cdot10^5$	$L^-(H_2O)_4$	$-7.0769\cdot10^2$	$-3.7161\cdot10^5$
		Phenylalanine			Glycylvaline	
0	H_2L^+	$-5.5529\cdot10^2$	$-1.4579\cdot10^6$	H_2L^+	$-6.1088\cdot10^2$	$-1.6039\cdot10^6$
1	$H_2L^+(H_2O)$	$-6.3174\cdot10^2$	$-8.2931\cdot10^5$	$H_2L^+(H_2O)$	$-6.8731\cdot10^2$	$-9.0227\cdot10^5$
0	HL	$-5.5484\cdot10^2$	$-1.4567\cdot10^6$	HL	$-6.1042\cdot10^2$	$-1.6027\cdot10^6$
1	$HL(H_2O)$	$-6.3127\cdot10^2$	$-8.2870\cdot10^5$	$HL(H_2O)$	$-6.8685\cdot10^2$	$-9.0166\cdot10^5$
2	$HL(H_2O)_2$	$-7.0773\cdot10^2$	$-6.1938\cdot10^5$	$HL(H_2O)_2$	$-7.6330\cdot10^2$	$-6.6801\cdot10^5$
0	L^-	$-5.5439\cdot10^2$	$-1.4555\cdot10^6$	L^-	$-6.0998\cdot10^2$	$-1.6015\cdot10^6$
1	$L^-(H_2O)$	$-6.3082\cdot10^2$	$-8.2811\cdot10^5$	$L^-(H_2O)$	$-6.8642\cdot10^2$	$-9.0109\cdot10^5$
2	$L^-(H_2O)_2$	$-7.0726\cdot10^2$	$-6.1897\cdot10^5$	$L^-(H_2O)_2$	$-7.6285\cdot10^2$	$-6.6762\cdot10^5$
3	$L^-(H_2O)_3$	$-7.8370\cdot10^2$	$-5.1440\cdot10^5$	$L^-(H_2O)_3$	$-8.3929\cdot10^2$	$-5.5089\cdot10^5$
4	$L^-(H_2O)_4$	$-8.6011\cdot10^2$	$-4.5165\cdot10^5$	$L^-(H_2O)_4$	$-9.1572\cdot10^2$	$-4.8085\cdot10^5$
		Glycylphenylalanine			Water[14]	
0	H_2L^+	$-8.0005\cdot10^2$	$-2.1005\cdot10^6$	H_3O^+	$-7.6862\cdot10^1$	$-2.0180\cdot10^5$
1	$H_2L^+(H_2O)$	$-8.3975\cdot10^2$	$-1.1024\cdot10^6$	$H_3O^+(H2O)$	$-1.5330\cdot10^2$	$-2.0124\cdot10^5$
0	HL	$-7.6285\cdot10^2$	$-2.0029\cdot10^6$	$H_3O^+(H2O)_2$	$-2.2973\cdot10^2$	$-2.0105\cdot10^5$
1	$HL(H_2O)$	$-8.3929\cdot10^2$	$-1.1018\cdot10^6$	H_2O	$-7.6434\cdot10^1$	$-2.0068\cdot10^5$
2	$HL(H_2O)_2$	$-9.1574\cdot10^2$	$-8.0142\cdot10^5$	$(H_2O)_2$	$-1.5287\cdot10^2$	$-2.0068\cdot10^5$
0	L^-	$-7.6241\cdot10^2$	$-2.0017\cdot10^6$	OH^-	$-7.5952\cdot10^1$	$-1.9941\cdot10^5$
1	$L^-(H_2O)$	$-8.3885\cdot10^2$	$-1.1012\cdot10^6$	$OH^-(H_2O)$	$-1.5240\cdot10^2$	$-2.0006\cdot10^5$
2	$L^-(H_2O)_2$	$-9.1529\cdot10^2$	$-8.0103\cdot10^5$	$OH^-(H_2O)_2$	$-2.2885\cdot10^2$	$-2.0028\cdot10^5$
3	$L^-(H_2O)_3$	$-9.9170\cdot10^2$	$-6.5093\cdot10^5$	$OH^-(H_2O)_3$	$-3.0529\cdot10^2$	$-2.0039\cdot10^5$
4	$L^-(H_2O)_4$	$-1.0681\cdot10^3$	$-5.6088\cdot10^5$	$OH^-(H_2O)_4$	$-3.8173\cdot10^2$	$-2.0044\cdot10^5$

[a] *N*: total number of solvation water molecules; G^o_{sol}, total free energy in solution; G^o_{sol}/molecule, total energy of solvated species per water molecule; H_2L^+, cation species; HL, neutral; L^-, anion species.

Table 2. Values of pK_a for the Protonation of Glycine, Valine, Glycylvaline, Phenylalanine, and Glycylphenylalanine Obtained Using the Tomasi Method at the B3LYP/6-31+G(d) Level of Theory, at 298.15 K[a]

species	selected equations	pK_a (calculated)	pK_a (experimental)	ref
glycine	$H_2L^+(H_2O) + H_2O \rightleftarrows HL(H_2O) + H_3O^+$	2.2916	2.34 ($I = 0$)	40
	$HL(H_2O)_2 \rightleftarrows L^-(H_2O) + H_3O^+$	9.6432	9.60 ($I = 0$)	40
valine	$H_2L^+(H_2O) + H_2O \rightleftarrows HL(H_2O) + H_3O^+$	2.2003	2.30 ($I = 0$)	40
	$HL(H_2O)_2 \rightleftarrows L^-(H_2O) + H_3O^+$	9.0744	9.61 ($I = 0$)	40
phenylalanine	$H_2L^+(H_2O) \rightleftarrows HL + H_3O^+$	2.2799	2.20 ($I = 0$)	40
	$HL(H_2O) \rightleftarrows L^- + H_3O^+$	8.8079	9.31 ($I = 0$)	40
glycylvaline	$H_2L^+(H_2O) + 2H_2O \rightleftarrows HL(H_2O)_2 + H_3O^+$	2.5466	2.86 ($I = 0$)	40
	$HL(H_2O)_2 + H_2O \rightleftarrows L^-(H_2O)_2 + H_3O^+$	7.9583	8.18 ($I = 0$)	40
glycylphenyl alanine	$H_2L^+(H_2O) + 2H_2O \rightleftarrows HL(H_2O)_2 + H_3O^+$	3.9151	3.23 ($I = 0.1$, $NaClO_4$)	38
	$HL(H_2O)_2 + H_2O \rightleftarrows L^-(H_2O)_2 + H_3O^+$	7.2077	8.11 ($I = 0.1$, $NaClO_4$)	38

[a] *I*, ionic strength.

Solvent−Solute Interactions

Ionic Product of Water. It is well-known that all aqueous solutions contain hydrogen (H^+) and hydroxyl (OH^-) ions. In pure water these ions are derived completely from the ionization of the water molecules

$$H_2O \rightleftarrows H^+ + OH^- \quad (3)$$

Considering that the H^+ ion is hydrated, appearing predominantly as H_3O^+, the autoprotolysis of water is better represented by the reaction

$$2H_2O \rightleftarrows H_3O^+ + OH^- \quad (4)$$

Taking into account that water is only slightly dissociated and to simplify the discussion, we shall make the approximations of replacing the activities in acidity constants by the numerical values of the molar concentrations. Consequently

$$K_w = [H_3O^+][OH^-] \quad (5)$$

At 298.15 K, $K_w = 1.008\cdot10^{-14}$, showing that only a few of the water molecules are ionized.[32]

Conventionally, eqs 4 and 5 are those more used in studies of acid−base equilibria in aqueous media. On the other hand, the solvation of anions is effective in protic solvents where hydrogen bonds may be formed between the proton of the solvent and the lone pairs of electrons of the anion.[33] The total energies of the single and solvated OH^- ion have been calculated in water at the B3LYP/6-31+G(d) level of theory, using Tomasi's model. To illustrate, Figure 3 shows the structures of

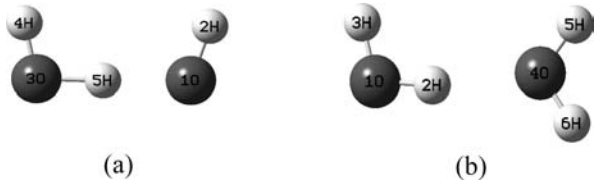

Figure 3. Optimized structure of the hydroxyl ion solvated with one water molecule (a) and two water molecules together (b).

the hydroxyl ion solvated with one water and also two water molecules together.

It can be observed that the distance between the O_1^- atom of the hydroxyl group and the H_5 atom of the water (Figure 3a) is equal to 1.557 Å, whereas the bond angle (A_{bond}) that forms the involved atoms ($O_3-H_5-O_1^-$) in the hydrogen-bond-donor (IHB) is 177.8°. Furthermore, Figure 3b shows that the distance and bond angle formed by the involved atoms (H_2O_4, $O_1H_2O_4$) in the IHB are 1.905 Å and 171.6°, respectively. The data allow the conclusion that the IHBs between the OH^- ion and the water molecules of solvation belong to the class of moderate or strong H bonds.[34] The calculated total energy values show a striking decrease of the total energy of the OH^- ion when its solvation increases. For each solvation water molecule, the OH^- ion decreases its relative energy by (200 and 222) kJ·mol^{-1}, respectively.[35]

Considering these facts and to provide a more satisfactory representation of the protolysis of water, the reaction has been shown as follows

$$3H_2O \rightleftarrows H_3O^+ + OH^-(H_2O) \qquad (6)$$

The selected reaction considers that both H^+ and OH^- ions are hydrated with one water molecule. Moreover, indicating with K_N the equilibrium constant of the reaction of eq 6 and taking into account eqs 4 and 5, it is inferred that[31]

$$K_w = K_N[H_2O]$$

where $[H_2O]$ is the molar concentration of water. Consequently, at 298.15 K, it was calculated that

$$K_N = \frac{K_w}{[H_2O]} = 1.831 \cdot 10^{-16} \qquad (7)$$

Similarly, the total energies of the single and solvated glycine, valine, phenylalanine, glycylphenylalanine, and glycyvaline species (cationic, neutral, and anionic) were calculated in water at the B3LYP/6-31+G(d) level of the theory, using Tomasi's model. Table 1 summarizes the variations of the total energy (kJ·mol^{-1}) of the species per water molecule as a function of the total number of solvation water molecules. Figures 4, and 5 and Table 1 show the marked increase of the total energies of ions when the solvation increases.

The data show that the water, exerting its hydrogen-bond-donor (HBD) capability, forms IHBs with the glycine, valine, phenylalanine, glycylphenylalanine, and glycylvaline anions.[36] These hydrogen bonds have been classified as strong, moderate, and weak, according to their lengths, angles, and energies.[34]

First Ionization Constant of Glycylvaline. It was selected that in alkaline solutions glycylvaline suffers a reaction of partial neutralization as follows

$$H_2L^+(H_2O) + OH^- \rightleftarrows HL(H_2O)_2 \qquad K_{C1} \qquad (8)$$

In this reaction, $H_2L^+(H_2O)$ is the glycylvaline cation solvated with one water molecule, and $HL(H_2O)_2$ represents glycylvaline

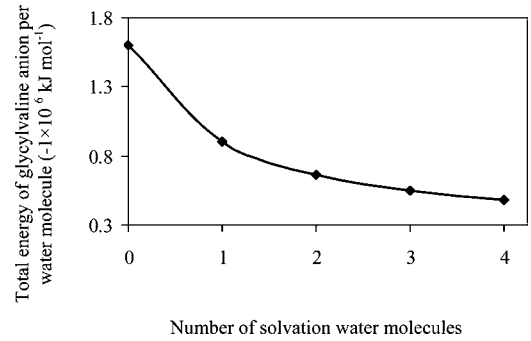

Figure 4. Plot of the total energy (kJ·mol^{-1}) of a solvated glycylvaline anion per water molecule against the total number of solvation water molecules.

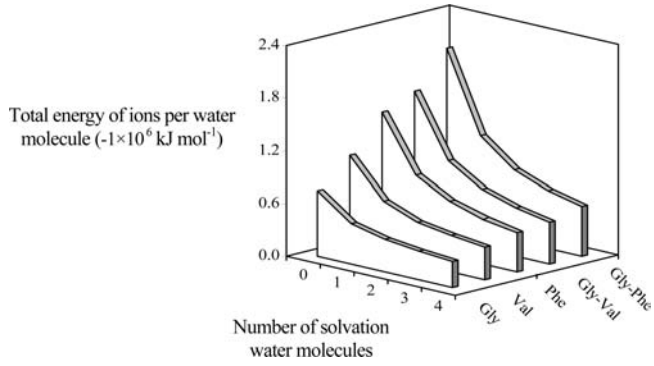

Figure 5. Plot of the total energy (kJ·mol^{-1}) of solvated glycylphenylalanine, glycylvaline, phenylalanine, valine, and glycine anions per water molecule against the total number of solvation water molecules.

solvated with two water molecules. The previous reaction is characterized by an equilibrium constant, K_{C1}, which was theoretically determined. Besides, water autopyrolysis also takes place

$$2H_2O \leftrightarrows H_3O^+ + OH^- \qquad (4)$$

By combining eqs 8 and 4, we obtain the reaction of eq 9, which defines the first ionization constant of glycylvaline (K_{a1}) and which considers the solvation of the neutral glycylvaline.

$$H_2L^+(H_2O) + 2H_2O \rightleftarrows HL(H_2O)_2 + H_3O^+ \qquad (9)$$

It is evident that the constants K_{C1}, K_w, and K_{a1} are related by

$$K_{a1} = K_{C1}K_w \qquad (10)$$

The above equation was used to determine theoretically the value of the first ionization constant of glycylvaline in water. Table 2 summarizes the optimized values of molecular properties of the $H_2L^+(H_2O)$ cation (Figure 2), OH^- ion, and $HL(H_2O)_2$ neutral molecule (Figure 6) obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method in water at 298.15 K. It must be noted that in the formation of the neutral glycylvaline solvated with two water molecules the neutral molecules practically do not have the structure that characterizes the solvated glycylvaline cation (Figure 2 and Table 3). Obviously, the formation of the neutral glycylvaline implies that the electronic density of the N_{10} atom increases notably (in absolute value) with respect to the N_{10} atom of the glycylvaline cation (Table 3).

Furthermore, the negative atomic charge of O_8 (qO_8) of the neutral glycylvaline decreases (in absolute value). These facts help to explain that the H_{22} atom of the carboxyl (O_8-H_{22}) group

Figure 6. Calculated structure for neutral glycylvaline solvated with two water molecules, at the B3LYP/6-31+G(d) level of theory and using the Tomasi's method in water at 298.15 K.

bound to C_3 of solvated neutral glycylvaline is more acidic than the H_{22} atom of the carboxyl (O_8-H_{22}) group bound to the C_1 atom of the solvated glycylvaline cation. Also, it should be remarked that the nucleophilic attack of the O^- atom of the OH^- ion (see eq 8) on the H_{25} atom of the NH_3 group bound to N_{10} of the glycylvaline cation produced the neutral glycylvaline and a molecule of water ($H_{32}O_{30}H_{31}$, Figure 6).

This molecule of water originated from the acid−base reaction, together with the hydration water molecule of the neutral glycylvaline, and these are the two molecules of water that interact with the N_{10} and O_7 of the neutral glycylvaline molecule by means of two IHBs. The distances and angles that characterize these IHBs (Table 3 and Figure 6) indicate that they belong to the class of weak closely to moderate and moderate IHB.[34] According to ref 34, the properties of the moderate hydrogen bonds have the following characterization: bond lengths of H···B are between (1.5 and 2.2) Å, and the bond angle is 130° to 180°. For weak hydrogen bonds, the bond length and angle are (2.2 to 3.2) Å and 90° to 150°, respectively, and for strong hydrogen bonds are (1.2 to 1.5) Å and 175° to 180°, respectively. The IHB of the cation and anion of glycylvaline belongs to the weak closely to moderate and moderate (see Table 3 and Figures 2 and 7). All other investigated molecules show results similar to glycylvaline (see Appendices A to D).

It is reasonable to observe that the molecular volume of the neutral glycylvaline molecule solvated with two water molecules is approximately the sum of the molecular volumes of the species that form it (i.e., neutral glycylvaline molecule solvated with one water and OH^-). On the other hand, the pK_{a1} value theoretically obtained ($pK_{a1} = 2.5466$) is relatively comparable with the experimental pK_{a1} value ($pK_{a1} = 2.86$).[40]

Second Ionization Constant of Glycylvaline. Here, it is selected that the neutral $HL(H_2O)_2$ suffers a total neutralization as follows

$$HL(H_2O)_2 + OH^-(H_2O) \rightleftarrows L^-(H_2O)_2 + 2H_2O \quad (11)$$

In the above reaction, $L^-(H_2O)_2$ represents the anion solvated with two water molecules. The reaction described in eq 11 is characterized by another equilibrium constant, K_{C2}, which was also theoretically determined. Combining eqs 6 and 11, the second ionization reaction of glycylvaline was obtained

$$HL(H_2O)_2 + (H_2O) \rightleftarrows L^-(H_2O)_2 + H_3O^+ \quad (12)$$

The equilibrium constant K_{a2} that characterizes the above reaction is linked with constants K_{C2} and K_N by eq 13

Table 3. Calculated Structural Magnitudes Using Tomasi's Method at the B3LYP/6-31+G(d) Level of Theory for the Cation, Neutral Molecule, and Anion of Glycylvaline at 298.15 K[a]

species	calculated magnitudes		
glycylvaline	$H_2L^+(H_2O)$	$HL(H_2O)_2$	$L^-(H_2O)_2$
K_{Cl}	$2.8182 \cdot 10^{+11}$	−	−
K_{C2}	$6.0115 \cdot 10^{+7}$	−	−
K_{a1}	$2.8408 \cdot 10^{-3}$	−	−
K_{a2}	$2.1007 \cdot 10^{-8}$	−	−
a_0	4.55	4.76	4.89
D-$O_8C_3C_1N_2$	149.396	−	−
D-$O_{26}H_{28}O_7C_3$	−	−	−0.50688
D-$O_{27}H_{28}O_7C_1$	−	−18.199	−
D-$O_{27}H_{29}N_{10}C_{11}$	−	115.467	−
D-$O_{28}H_{22}O_8C_3$	−140.969	−	−
D-$O_{29}H_{31}O_8C_3$	−	−	−94.221
D-$H_{28}O_7C_3C_1$	−	−	179.073
D-$H_{29}N_{10}C_{11}C_9$	−	−37.780	
D-$H_{29}O_{28}H_{22}O_8$	30.388		
D-$H_{31}N_{10}C_{11}C_9$	−	24.970	
D-$H_{31}O_8C_3C_1$	−	−	179.300
D-$N_2C_{11}C_9N_{10}$	−175.398	−	−
D-$C_9N_2C_3C_1$	−	−132.787	−
D-$C_3C_1N_2C_{11}$	−	−	−135.477
qO_7	−0.370	−0.498	−0.689
qO_8	−0.612	−0.523	−0.639
qO_{12}	−	−0.504	−
qO_{26}	−	−	−1.090
qO_{27}	−	−1.116	−
qO_{28}	−1.012	−	
qO_{29}	−	−	−1.145
qO_{30}	−	−1.077	−
qH_{22}	0.587	−	−
qH_{28}	−	0.569	0.579
qH_{29}	−	0.565	−
qH_{31}	−	0.572	0.613
qH_{32}	−	0.499	−
qN_2	−0.457	−0.332	−0.412
qN_{10}	−0.921	−1.088	−0.843
qC_9	−	0.588	−
$dO_{28}H_{22}$	1.7646	−	−
$dH_{28}O_7$	−	2.0310	1.914
$dH_{29}N_{10}$	−	2.010	−
$dH_{31}O_8$	−		1.731
$dH_{31}N_{10}$	−	2.949	−
A-$O_{26}H_{28}O_7$	−	−	169.538
A-$O_{27}H_{28}O_7$	−	159.382	−
A-$O_{27}H_{29}N_{10}$	−	150.777	−
A-$O_{28}H_{22}O_8$	172.723		−
A-$O_{29}H_{31}O_8$	−		174.274
A-$H_{28}O_7C_1$	−	141.417	−
A-$H_{28}O_7C_3$	−	−	125.855
A-$H_{29}N_{10}C_{11}$	−	128.880	−
A-$H_{29}O_{28}H_{22}$	114.294	−	−
A-$H_{30}O_{28}H_{22}$	115.347	−	−
A-$H_{31}N_{10}C_{11}$	−	135.934	−
A-$H_{31}O_8C_3$	−	−	122.832

[a] K_{C1} and K_{C2}, equilibrium constants of equations; K_{a1} and K_{a2}, first and second acidic dissociation constants of species in water; D, dihedral angle between the indicated atoms (Å); a_0, bohr radius (Å); q, total atomic charge (Muliken) (au); r, bond lengths between the indicated atoms; d, distance of the IHB between the indicated atoms (Å); A, H-bond angles (°).

$$K_{a2} = K_{C2} \cdot K_N \quad (13)$$

This equation is similar to eq 10, and it was used to obtain the value of the second ionization constant of glycylvaline in water. Table 3 gives the values of the molecular parameters and properties calculated for the $L^-(H_2O)_2$ anion, in water at 298.15 K, while Figure 7 shows the structure of this anion. The glycylvaline anion solvated with two water molecules possesses various structural characteristics that are different from those of the neutral glycylvaline molecule and the glycylvaline cation solvated with one and two water molecules, respectively.

Figure 7. Calculated structure for the glycylvaline anion solvated with two water molecules, at the B3LYP/6-31+G(d) level of theory and using Tomasi's method in water at 298.15 K.

Thus, the acid−base reactions and solvation of species change structure. From Figure 7 and Table 3, it can be observed that dihedral angles are practically different. Furthermore, the negative charges of the O_7^- and O_8^- atoms are high (in absolute value) and very similar to each other. This fact facilitates the interactions of these atoms with the molecules of water which have a moderate HBD ability[36] and originate in the formation of two moderate IHBs (Figure 7).

It must be noted that the pK_{a2} value theoretically calculated (pK_{a2} = 7.9583) is relatively comparable with the experimentally determined pK_a (pK_{a2} = 8.18).[40]

Similarly with glycylvaline, total energies and molecular parameters were obtained for glycine, valine, phenylalanine, and glycylphenylalanine systems, using Tomasi's method at the B3LYP/6-31+G(d) level of theory for the anion, cation, and neutral species at 298.15 K. The resulting values are shown in the Tables 1 and 2 and the Appendices. A lot of acid−base reactions were considered for the mentioned systems. The values of acidic dissociation constants for all of the reactions were calculated using a computer program, but the reactions were not further considered because the estimated error in their acidic dissociation constants was unacceptable. The models finally selected and their acidic dissociation constants are listed in Table 2.

Conclusions

In this paper, we showed the feasibility of a theoretical method that uses pH values to determine the ionization constants of glycine, phenylalanine, valine, phenylalanylglycine, and glycylvaline. Also, we have shown that these constants can be calculated with an acceptable degree of accuracy. With this purpose, we selected various acid−base reactions that take into account the solvation of the hydrogen, hydroxyl ions, and other cations or anions in protic solvents such as water, which possess a high hydrogen-bond-donor capability. We also observed that the nucleophilic attack on the hydrogen atoms of the COOH and NH_3^+ groups of glycine, valine, phenyalanine, glycylvaline, and glycylphenylalanine to form the corresponding species is produced by the OH^- and the OH^- ion hydrated with a water molecule. The calculations performed at the B3LYP/6-31+G(d) levels of theory using Tomasi's method allowed us to prove that cations, neutral molecules, and anions form IHBs with some molecules of water. The theoretical ionization constants show relatively suitable agreement with the acidity constants experimentally determined (Figures 8 and 9). The regression lines are also used for comparison of the different methods. Each point on the graph represents a single sample analyzed

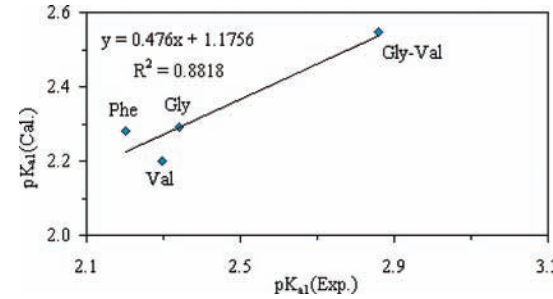

Figure 8. Comparison of calculated and experimental dissociation constants of step one (pK_{a1}) in zero ionic strength.

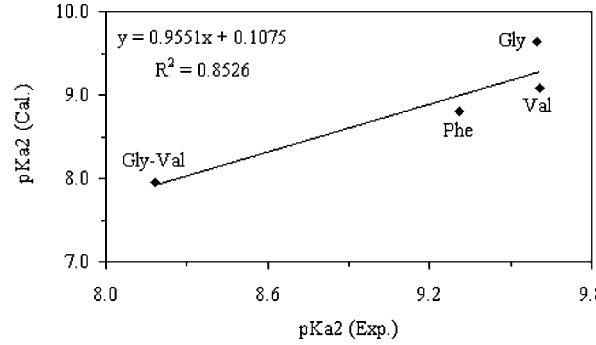

Figure 9. Comparison of calculated and experimental dissociation constants of step two (pK_{a2}) in zero ionic strength.

by two separate methods. Obviously, if each sample yields an identical result with both methods, the regression line will have zero intercept and a slope and a correlation coefficient of one. In practice, of course, this never occurs even if systematic and maybe random errors are entirely absent. In practice, the analyst most commonly wishes to test for an intercept differing significantly from zero and for a slope from one. Such tests are performed by determining the confidence limits for slope and intercept, generally at the 95 % significant level.[39] According to Figure 8 for pK_{a1}, the regression calculated results are as follows

$$Y = 0.476X + 1.1756$$

Further calculations show that

$$S_r = 0.0640,\ S_\alpha = 0.3027,\ S_\beta = 0.1249$$

where S_r, S_α, and S_β are standard deviation regression, intercept, and slope, respectively.

The appropriate *t*-value for 2 degrees of freedom is 4.30. It gives 95 % confidence limits for the intercept and slope as

$$\alpha = 1.1756 \pm 4.30 \cdot 0.3027 = 1.1756 \pm 1.3018$$

$$\beta = 0.476 \pm 4.30 \cdot 0.1249 = 0.476 \pm 0.5372$$

Since these 95 % confidence intervals clearly include one and zero, respectively, we must conclude that the regression line shows the experimental values are as close as the calculated data. For pK_{a2}, shown in Figure 9, the regression calculations are as follows with the same results.

$$\alpha = 0.1075 \pm 4.30 \cdot 2.5829 = 0.1075 \pm 11.1065$$

$$\beta = 0.9551 \pm 4.30 \cdot 0.2809 = 0.9551 \pm 1.2080$$

However, the differences are mostly due to the different techniques, various ionic strengths with different background electrolytes, and different temperatures that were used.[37] The observed differences that are especially shown in the pK_{a2} can

arise from a small change of pH in the more basic conditions at the second dissociation constant. Therefore, the pK_{a2} values are obtained with more standard error than pK_{a1} values. Also, the theoretical calculations have intrinsic errors due to the nonconsideration of some terms such as overlap integral, etc. On the other hand, in Gaussian calculations, ionic strength at zero is considered, and therefore it is better to compare the theoretical data with the experimental values at zero ionic strength (see Table 2).

Acknowledgment

Thanks are gratefully extended to the Faculty of Chemistry, University of Mazandaran, for its valuable help with this work.

Appendix A

Table A. Calculated Structural Magnitudes Using Tomasi's Method at the B3LYP/6-31+G(d) Level of Theory for the Cation, Neutral Molecule, and Anion of Glycine, Valine, Phenylalanine, and Glycylphenylalanine, at 298.15 K

species	calculated magnitudes			
glycine	$H_2L^+(H_2O)$	$HL(H_2O)$	$HL(H_2O)_2$	$L^-(H_2O)$
K_{C1}	$2.7907 \cdot 10^{+13}$	–	–	–
K_{C2}	$1.2418 \cdot 10^{+6}$	–	–	–
K_{a1}	$5.1098 \cdot 10^{-3}$	–	–	–
K_{a2}	$2.2738 \cdot 10^{-10}$	–	–	–
a_0	3.89	3.87	3.93	3.88
D-$O_{10}H_{11}O_5C_1$	–	–	–	0.357
D-$O_{10}H_{12}O_4C_1$	–	–	–	0.117
D-$O_{11}H_{12}O_4C_3$	–	64.222	–	–
D-$O_{11}H_{12}O_5C_1$	–	–	−42.372	–
D-$H_{13}O_{11}H_{10}N_2$	–	141.652	–	–
D-$H_{13}O_{12}H_{10}N_2$	−87.580	–	–	–
D-$H_{14}O_{12}H_{10}N_2$	110.612	–	–	–
D-$N_2C_1C_3O_4$	−174.829	6.484	–	–
qO_4	−0.501	−0.760	−0.589	−0.790
qO_5	−0.614	−0.678	−0.614	−0.774
qO_{11}	–	−1.087	−1.116	−1.044
qO_{14}	–	–	−1.083	–
qH_6	–	–	0.438	0.405
qH_7	–	–	0.440	0.427
qH_8	0.516	0.515	+0.295	–
qH_9	0.527	0.524	–	–
qH_{10}	0.589	0.534	0.536	–
qH_{11}	0.574	–	–	0.517
qH_{12}	−1.057	0.545	0.572	0.516
qH_{13}	–	0.551	0.531	–
qH_{16}	–	–	0.567	–
qN_2	−1.011	−0.966	−0.993	−0.939
$dO_{11}H_{10}$	–	1.948	–	–
$dO_{11}H_{16}$	–	–	1.813	–
$dO_{12}H_{10}$	1.723	–	–	–
dH_8O_4	2.024	–	–	–
$dH_{11}O_5$	–	–	–	1.994
$dH_{12}O_4$	–	1.882	–	2.027
$dH_{12}O_5$	–	–	1.826	–
A-$O_{11}H_{12}O_5$	–	–	168.062	–
A-$H_{11}O_5C_1$	–	–	–	104.697
A-$H_{12}O_4C_3$	–	106.933	–	
A-$H_{12}O_5C_1$	–	–	126.412	104.465
A-$H_{13}O_{11}H_{10}$	–	147.690	–	–
A-$H_{13}O_{12}H_{10}$	124.459	–	–	–
A-$H_{14}O_{12}H_{10}$	127.479	–	–	–

Appendix B

Table B

glycylphenylalanine	$H_2L^+(H_2O)$	$HL(H_2O)$	$HL(H_2O)_2$	$L^-(H_2O)_2$
K_{C1}	$1.2062 \cdot 10^{+10}$	–	–	–
K_{C2}	$3.3851 \cdot 10^{+8}$	–	–	–
K_{a1}	$1.2158 \cdot 10^{-4}$	–	–	–
K_{a2}	$6.1982 \cdot 10^{-8}$	–	–	–
a_0	5.02	5.18	5.17	5.16
D-$O_{11}C_3C_1N_2$	0.869	–	–	–
D-$O_{11}H_{29}N_{14}H_{30}$	–	−1.465	–	–
D-$O_{30}H_{31}O_{12}C_3$	–	–	–	−1.554
D-$O_{30}H_{32}O_{11}C_3$	–	–	–	−0.126
D-$O_{31}H_{33}O_{11}C_3$	–	−113.796	−121.230	–
D-$O_{32}H_{33}O_{11}C_3$	−26.973	–	–	–
D-$O_{34}H_{36}O_{11}C_3$	–	–	96.785	–
D-$H_{18}O_{33}H_{35}O_{12}$	–	–		−28.549
D-$H_{18}O_{34}H_{36}O_{11}$	–	–	−41.607	–
D-$H_{31}O_{12}C_3C_1$	–	–	–	−175.526
D-$H_{32}O_{11}C_3C_1$	–	–	–	175.849
D-$H_{33}O_{31}H_{30}N_{14}$	–	−112.307	–	–
D-$H_{33}O_{32}H_{31}N_{14}$	−42.137	–	–	–
D-$H_{34}O_{32}H_{31}N_{14}$	174.144	–	–	–
D-$C_3C_1N_2H_{18}$	–	–	–	33.695
qO_{11}	−0.565	−0.672	−0.877	−0.658
qO_{12}	−0.508	−0.483	−0.613	−0.774
qO_{30}	–	–	–	−1.020
qO_{31}	–	−1.077	–	–
qO_{32}	−1.108	–	–	–
qO_{33}	–	–	–	−1.105
qH_{18}	–	–	0.486	0.467
qH_{26}	–	–	0.296	–
qH_{28}		0.456	0.539	0.424
qH_{29}	0.524	0.585	0.569	0.432
qH_{30}	0.534	0.484	0.553	–
qH_{31}	0.578	–	−1.086	0.535
qH_{32}	–	0.540	–	0.517
qH_{33}	0.591	0.558	0.565	–
qH_{34}	0.562	–	−1.097	0.529
qH_{35}	–	–	–	0.583
qH_{36}	–	–	0.565	–
qN_{14}	−1.038	−1.074	−1.046	−0.040
$dH_{18}O_{33}$	–	–	–	2.026
$dH_{30}O_{31}$	–	2.074	−2.129	–
$dH_{31}O_{12}$	–	–	–	2.011
$dH_{32}O_{11}$	–	–	–	2.104
$dH_{33}O_{11}$	1.8194	2.1209	1.876	–
$dH_{29}N_{14}$	–	1.991	–	–
$dO_{11}H_{29}$	–	–	1.588	–
$dO_{11}H_{36}$	–	–	1.825	–
$dO_{12}H_{35}$	–	–	–	1.685
$dO_{32}H_{31}$	1.674	–	–	–
$dO_{34}H_{18}$	–	–	2.183	–
A-$O_{32}H_{31}N_{14}$	159.506	–	–	–
A-$H_{33}O_{11}C_3$	156.728	–	–	–
A-$H_{18}O_{33}H_{35}$	–	–	–	71.370
A-$H_{18}O_{34}H_{36}$	–	–	80.565	–
A-$H_{31}O_{12}C_3$	–	–	–	105.288
A-$H_{31}O_{32}H_{33}$	116.285	–	–	–
A-$H_{32}O_{11}C_3$	–	–	–	106.007
A-$H_{33}O_{11}C_3$	–	–	116.613	–
A-$H_{33}O_{11}H_{29}$	–	89.547	–	–
A-$H_{33}O_{31}H_{30}$	–	96.035	97.287	–
A-$H_{36}O_{11}H_{29}$	–	–	74.398	–
A-$H_{36}O_{34}H_{26}$	–	–	108.766	–
A-$H_{29}N_{14}H_{30}$	–	87.509	–	–

Appendix C

Table C

valine	$H_2L^+(H_2O)$	$HL(H_2O)$	$HL(H_2O)_2$	$L^-(H_2O)$
K_{C1}	$6.2554 \cdot 10^{+11}$	–	–	–
K_{C2}	$8.3586 \cdot 10^{+4}$	–	–	–
K_{a1}	$6.3055 \cdot 10^{-3}$	–	–	–
K_{a2}	$8.4254 \cdot 10^{-10}$	–	–	–
a_0	4.27	4.17	4.59	4.64
D-$O_7C_1O_8H_{19}$	–	−0.151	–	–
D-$O_7C_1C_3N_2$	−11.153	–	–	–
D-$O_{19}H_{20}O_7C_1$	–	–	–	−0.232
D-$O_{19}H_{21}O_8C_1$	–	–	–	−0.274
D-$O_{20}H_{21}O_7C_1$	–	−3.977	–	–
D-$O_{20}H_{22}O_7C_3$	–	–	−61.605	–
D-$O_{21}H_{10}N_2C_3$	−118.300	–	–	–
D-$O_{21}H_{23}O_7C_1$	28.697	–	–	–
D-$H_{12}O_{23}H_{25}O_{20}$	–	–	6.302	–
D-$H_{22}O_{20}H_{19}O_8$	–	−114.043	–	–
D-$H_{23}O_{21}H_{10}N_2$	62.446	–	–	–
D-$H_{24}O_{23}H_{12}N_2$	–	–	138.288	–
D-$H_{25}O_{23}H_{12}N_2$	–	–	11.292	–
D-$N_2C_1C_3O_8$	–	−176.106	164.177	–
D-$N_2C_3C_1O_7$	–	6.128	−15.088	–
D-$C_1C_3N_2H_{10}$	–	–	–	−78.593
qO_7	−0.475	−0.597	−0.767	−0.762
qO_8	−0.596	−0.647	−0.625	−0.737
qO_{19}	–	–	–	−1.036
qO_{20}	–	−1.070	−1.123	–
qO_{21}	−1.056	–	–	–
qO_{23}	–	–	−1.106	–
qH_9	0.536	0.417	–	0.415
qH_{10}	0.610	–	0.536	0.423
qH_{11}	0.558	–	0.538	–
qH_{12}	–	–	0.575	–
qH_{17}	–	0.214	–	–
qH_{19}	–	0.585	–	–
qH_{20}	0.583		–	0.523
qH_{21}	–	0.548	0.540	0.515
qH_{22}	0.567	0.543	0.587	–
qH_{23}	0.532	–	–	–
qH_{24}	–	–	0.544	–
qH_{25}	–	–	0.581	–
qN_2	−1.187	–	−1.211	−0.885
dO_7H_9	–	2.252	–	–
dO_7H_{21}	–	1.982	–	–
dO_8H_{15}	–	2.470	–	–
dO_8H_{17}	–	2.867	–	–
$dO_{20}H_{19}$	–	1.782	–	–
$dO_{20}H_{25}$	–	–	1.723	–
$dO_{21}H_{10}$	1.714	–	–	–
$dO_{23}H_{12}$	–	–	1.766	–
$dH_{10}O_7$	2.456	–	–	–
$dH_{11}O_7$	2.688	–	1.777	–
$dH_{20}O_7$	–	–	–	2.030
$dH_{21}O_8$	–	–	–	2.001
$dH_{22}O_7$	–	–	1.749	–
$dH_{23}O_7$	2.558	–	–	–
A-$O_{21}H_{10}N_2$	170.440	–	–	–
A-$H_9O_7C_1$	–	83.511	–	–
A-$H_{10}O_7H_{11}$	33.134	–	–	–
A-$H_{12}O_{23}H_{25}$	–	–	105.809	–
A-$H_{20}O_7C_1$	–	–	–	104.899
A-$H_{21}O_7C_1$	–	109.077	–	–
A-$H_{21}O_8C_1$	–	–	–	104.830
A-$H_{22}O_7C_3$	–	–	110.441	–
A-$H_{22}O_{20}H_{19}$	–	123.668	–	–
A-$H_{23}O_7C_1$	131.636	–	–	–

Appendix D

Table D

phenylalanine	$H_2L^+(H_2O)$	HL	$HL(H_2O)$	L^-
K_{C1}	$5.2071 \cdot 10^{+11}$	–	–	–
K_{C2}	$8.4994 \cdot 10^{+6}$	–	–	–
K_{a1}	$5.2488 \cdot 10^{-3}$	–	–	–
K_{a2}	$1.5562 \cdot 10^{-9}$	–	–	–
a_0	4.68	4.30	–	4.62
D-$H_{14}N_2C_1C_3$	–	–	–	−103.597
D-$H_{15}O_{12}C_3C_1$	–	–	–	2.525
D-$H_{15}N_2C_1C_3$	–	–	–	8.956
D-$H_{15}N_2C_1C_4$	–	–	−136.857	
D-$H_{16}O_{11}C_3C_1$	–	–	–	36.263
D-$H_{16}O_{12}C_3O_{11}$	–	−179.089	–	–
D-$H_{13}O_{11}C_3C_1$	–	−22.445	–	–
D-$H_{26}O_{11}C_3O_{12}$	–	–	2.713	–
D-$H_{26}O_{25}H_{14}N_2$	104.016	–	–	–
D-$H_{27}O_{25}H_{14}N_2$	−88.765	–	–	–
D-$O_{11}C_3C_1N_2$	–	171.325	–	–
D-$N_2C_1C_4C_5$	−55.221	−58.442	–	−69.016
D-$N_2C_1C_3O_{11}$	164.169	–	–	–
D-$N_2C_1C_3O_{12}$	−18.206	–	–	–
qO_{11}	−0.475	−0.672	−0.575	−0.731
qO_{12}	−0.645	−0.701	−0.619	0.748
qO_{24}	–	–	−1.047	–
qO_{25}	−1.060	–		–
qN_2	−1.165	−1.029	−1.050	−0.858
qC_3	–	0.425		0.449
qH_{14}	0.597	0.535	0.468	0.418
qH_{15}	0.522	0.507	0.547	0.396
qH_{16}	0.592	0.528	0.453	–
qH_{18}	–	–	–	0.208
qH_{24}	0.593	–	–	–
qH_{25}	–	–	0.501	–
qH_{26}	0.536	–	0.554	–
qH_{27}	0.545	–		–
$dO_{11}H_{26}$	–	–	1.992	–
$dO_{12}H_{16}$	2.250	–	–	–
$dO_{25}H_{14}$	1.748	–	–	–
$dH_{13}O_{11}$	–	2.637	–	–
$dH_{15}O_{12}$	–	–	–	2.044
$dH_{16}O_{11}$	–	–	–	2.543
$dH_{16}O_{12}$	–	1.729	–	–
$dH_{15}N_2$	–	–	1.888	–
A-$H_{13}O_{11}C_3$	–	55.526	–	–
A-$H_{15}O_{12}C_3$	–	–	–	88.380
A-$H_{16}O_{12}C_3$	90.2427	93.859	–	–
A-$H_{16}O_{12}H_{24}$	160.169	–	–	–
A-$H_{25}O_{12}H_{15}$	–	–	154.883	–
A-$H_{26}O_{11}C_3$	–	–	117.129	–
A-$H_{27}O_{25}H_{14}$	127.256	–		–
A-$O_{12}C_3O_{11}$	–	132.672	–	–

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