Gas Phase Protonation Thermochemistry of Phenylalanine and Tyrosine

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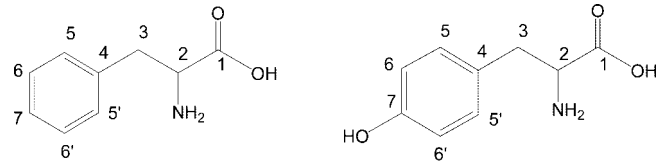
Gas phase basicities of phenylalanine and tyrosine, GB(Phe) = 892.0 ± 1.3(2.6) kJ·mol^{-1} and GB(Tyr) = 894.9 ± 2.8(5.9) kJ·mol^{-1} (uncertainties are standard deviation and, in parentheses, 95% confidence limit), have been experimentally determined by the extended kinetic method using ESI-TQ tandem mass spectrometry. Proton affinities deduced from these experiments, PA(Phe) = 931.3 ± 1.1(2.3) kJ·mol^{-1} and PA(Tyr) = 934.8 ± 2.5(5.2) kJ·mol^{-1}, are perfectly reproduced by theoretical calculations performed at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level. An entropy loss of approximately −25 J·mol^{-1}·K^{-1} occurs upon protonation of both Phe and Tyr. The origin of this entropy change is attributed (i) to the change in strength of the interaction between the amino group and the aromatic moiety in the neutral and protonated forms and (ii) to the larger entropy of mixing associated with the population of neutral conformers with respect to their protonated counterparts. Previous neglect of the protonation entropy term has led to underestimated tabulated PA values; the evaluated values proposed in the present study are PA(Phe) = 932 ± kJ·mol^{-1} and PA(Tyr) = 935 ± kJ·mol^{-1}.

1. Introduction

The intrinsic acid−bases properties of the 20 naturally occurring α-amino acids have attracted the interest of a number of researchers for several decades.[1−15] A critical examination of the available data reveals that the entropy change associated with the protonation process (quantified by the difference $\Delta_pS°$ = $S°(MH^+)$ − $S°(M)$, called the "protonation entropy") may be of importance for functionalized side chain amino acids.[1,2,5−10] Significant negative $\Delta_pS°$ values have been experimentally detected for methionine,[5] aspartic acid,[6] asparagine,[6] glutamic acid,[6] glutamine,[6] arginine,[7,8] histidine,[6,9] and lysine.[6,9,10] The main reason invoked to explain the existence of negative $\Delta_pS°$ values is the freezing of internal rotation in the protonated form MH^+ with respect to its neutral counterpart M. Accordingly, intramolecular stabilizing interactions, such as, in particular, hydrogen bonds, are dramatically enhanced in the MH^+ ion, thus reducing the $S°(MH^+)$ term.[16−19] From this point of view, it was of interest to investigate the protonation energetics of phenylalanine and tyrosine (Scheme 1), since the presence of an aromatic ring in the side chain may be at the origin of stabilizing interactions between polarized hydrogen atoms and the π-electron system both in the neutral and in the protonated forms.

Theoretical investigation of the benzene/NH_3 neutral system reveals that the most stable structure is a monodentate complex (one H of the ammonia molecule points toward the π-electrons system of the benzene ring) characterized by a binding energy of about 7−9 kJ·mol^{-1}.[20] This weak π−H interaction energy seems to be due to a subtle mixture of attractive (electrostatic, inductive, and dispersive) and repulsive exchange components. By contrast, the interaction energy between the NH_4^+ cation and aromatic molecules is 1 order of magnitude higher. For example, the 298 K enthalpy of complexation between benzene and NH_4^+, as determined from variable temperature equilibrium constant determinations,[21] is equal to 71 kJ·mol^{-1}. This is confirmed by quantum chemistry computations, since the interaction energy calculated between benzene and ammonium ion is predicted to be in the 60−75 kJ·mol^{-1} range.[22,23] The most stable structure of the NH_4^+/C_6H_6 complex involves interaction between two hydrogen atoms of the ammonium ion and the π-electrons system of the benzene molecule. The present theoretical picture describes the ammonium−cation π-electrons interaction as essentially governed by an electrostatic effect, with significant contribution from the polarization.[23] These elements lead to the expectation that the difference in NH_2 or $NH_3^+\cdots\pi$-electrons interactions that may occur in aromatic amino acids may be at the origin of significant effects on their protonation thermochemistry.

SCHEME 1



Phenylalanine (Phe) [F] Tyrosine (Tyr) [Y]

Experimental determinations of the gas phase basicities, GB, of phenylalanine and tyrosine have been envisaged in the 1980s. In the case of phenylalanine, measurement of gas phase proton transfer equilibrium constants has been done by Mautner et al.[11] in a high pressure ion source, using aniline and *n*-butylamine as reference bases. Considering these data and assuming a protonation entropy $\Delta_pS°$(Phe) of −5 J·mol^{-1}·K^{-1}, Hunter and Lias[24] proposed GB(Phe) values of 877.9 and 882.1 kJ·mol^{-1}. A second approach of GB(Phe) based on the measurement of proton transfer equilibrium constant was obtained using ion cyclotron resonance mass spectrometry.[12] These experiments lead Hunter and Lias to derive a GB(Phe) estimate of 895.4 kJ·mol^{-1}. It consequently emerges from experiments using the equilibrium method an average GB(Phe) of 885.1 kJ·mol^{-1} associated with a significantly large standard deviation of 9.1

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kJ·mol^{-1}. Concerning tyrosine, only one measurement originating from ion cyclotron resonance mass spectrometry has been reported.[12] The GB(Tyr) value deduced from these experiments by Hunter and Lias[24] is equal to 905.2 kJ·mol^{-1}, a value surprisingly larger than GB(Phe) by 10−20 kJ·mol^{-1}. In 1990−2000, several authors used the simple kinetic method with the aim of determining the proton affinity, PA, of phenylalanine and tyrosine.[13−15] The resulting values fall into the following ranges: PA(Phe) = 915−928 kJ·mol^{-1} and PA(Tyr) = 917−930 kJ·mol^{-1}. Here again, large uncertainties are attached to the measurements but, by contrast with GB, PA values of phenylalanine and tyrosine seem to be comparable. Finally, it may be emphasized that, to date, neither experimental nor theoretical information on the protonation entropy of these molecules are available. In line with these observations, it may be recalled that the simple kinetic method has been demonstrated to be erroneous when significant protonation entropy is involved.[25−31] If negative protonation entropy is associated with the protonation of the molecule of interest, the "apparent" proton affinity given by the simple kinetic method is lower than the true value. Similarly, if the protonation entropy is not negligible, it must be included in the temperature correction on the GB values obtained by the equilibrium method. It is consequently crucial to gain information on the possible $\Delta_pS°$(Phe) and $\Delta_pS°$(Tyr) terms and to gain other quantitative estimates of the proton affinities and gas phase basicities of phenylalanine and tyrosine.

The present study was undertaken with the goal to bring detailed information on the protonation thermochemistry of phenylalanine and tyrosine. For this purpose, experiments were done using a tandem quadrupole mass spectrometer equipped with an electrospray source. The data were treated by the extended kinetic method, which provided the protonation entropies, $\Delta_pS°$, and proton affinities, PA, of phenylalanine and tyrosine. Quantum chemical calculations, up to the B3LYP/6-31+G(3df,2p)//B3LYP/6-31+G(d,p) level, were used to identify the most probable conformers and to compute thermochemical quantities associated with reaction 1:

$$MH^+ \rightarrow M + H^+ \qquad (1)$$

i.e. proton affinity, PA(M) = $\Delta_1H°$, gas phase basicity, GB(M) = $\Delta_1G°$, and protonation entropy, $\Delta_pS°$ (i.e., the difference $S°(MH^+) - S°(M)$). Theoretical estimates of third law entropies including consideration of the hindered rotations contribution have been done in order to compute individual Gibbs free energies and protonation entropies.

2. Methods

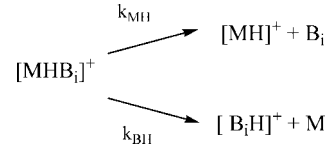
ESI-MS-MS experiments were carried out in a Waters Quattro II tandem quadrupole QhQ (where Q and h stand for quadrupole and hexapole, respectively) mass spectrometer. Cone voltage was set at 5 V while capillary voltage was varied between 3.0 and 3.7 kV to optimize the conditions for obtaining the maximum intensity of the protonated dimers. Typical values for the other source parameters were as follows: counter electrode, 0.5 kV; rf lens, 0.2 V; skimmer, 1.2 V; skimmer lens offset, 5 V; source temperature, 80 °C. CID-MS-MS spectra were obtained after selection of the ion of interest by the first quadrupole and activation of them in the rf-only hexapole using argon as the collision gas at a pressure of $\sim 2 \times 10^{-3}$ mbar. The ionic dissociation products were mass analyzed by scanning the second quadrupole of the QhQ device. Experimental data have been collected at several different collision energies in the laboratory frame, $E_{laboratory}$. Practically, the range of explored $E_{laboratory}$ values extends from 4 to 50 eV. For each ion of interest, the center of mass collision energy, E_{cm}, has been calculated by the usual conversion expression: $E_{cm} = E_{laboratory} m_{target}/(m_{target} + m_{ion})$. A scan rate of 1 s/scan was used for all experiments with a data acquisition duration of 40 s for each energy step. The acquired spectra were summed for interpretation. The collision induced dissociations of mass selected $[MHB_i]^+$ ions were examined by the kinetic method; that is, the natural logarithm of the fragment ions abundances $y_i = \ln([MH]^+/[B_iH]^+)$ has been correlated with the proton affinity of the reference base B_i, PA(B_i). The $[MH]^+$ and $[B_iH]^+$intensities were evaluated by summing the fragment ion abundances of each protonated species. The results discussed below correspond to y_i determined at E_{cm} values situated between 0.5 and 4.0 eV.

Sample solutions were prepared in a 50/50 methanol/water mixture acidified by 0.1% formic acid and dissolved to achieve typically a concentration of 10^{-4} M for both the amino acid and the reference bases. All solutions were infused at a flow rate of 10 μL·mn^{-1} with a CIL Cluzeau (Courbevoie, France) syringe. The samples, bases, and solvents of HPLC grade were purchased from Sigma-Aldrich (St. Quentin Fallavier, France) and used as received without any further purification.

Molecular orbital calculations have been conducted under the density functional theory frame using the GAUSSIAN suite of programs.[32] Geometries were optimized at the B3LYP/6-31+G(d,p) levels, which is one of the most reasonable approaches taking into account the electron correlation effects for systems containing more than ten heavy atoms, as considered here. Searches for local minima and rotational barriers have been conducted at the same level of theory using the relaxed rotation approach, i.e. by optimization of all geometrical parameters except the explored dihedral angle. Enthalpies at 298 K were calculated using thermal corrections obtained from unscaled B3LYP/6-31+G(d,p) vibrational frequencies and single point calculations using the 6-311++G(3df,2p) basis set. The performance of density functional theory at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level of theory in the computation of proton affinities is well documented.[16,33] However, we cautiously controlled the validity of the proton affinity calculation on two reference systems, namely isopropylamine, which mimics at best the protonation site of the investigated molecules, and *tert*-butylamine, which presents a PA value very close to those of Phe and Tyr. The calculated B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) PA values of isopropylamine and *tert*-butylamine at 298 K are equal to 922.9 and 934.0 kJ·mol^{-1}, respectively (see Table S1 of the Supporting Information for the relevant total energies and 298 K enthalpies), while the tabulated experimental values are 923.8 and 934.3.[24] The deviation between computed and experimental proton affinities is consequently less than 1.0 kJ·mol^{-1}.

3. Results

3.1. Extended Kinetic Method. The kinetic method[17,34] considers the competitive dissociations of a series of proton bound dimers $[MHB_i]^+$, involving the molecule of interest M and a reference base B_i:



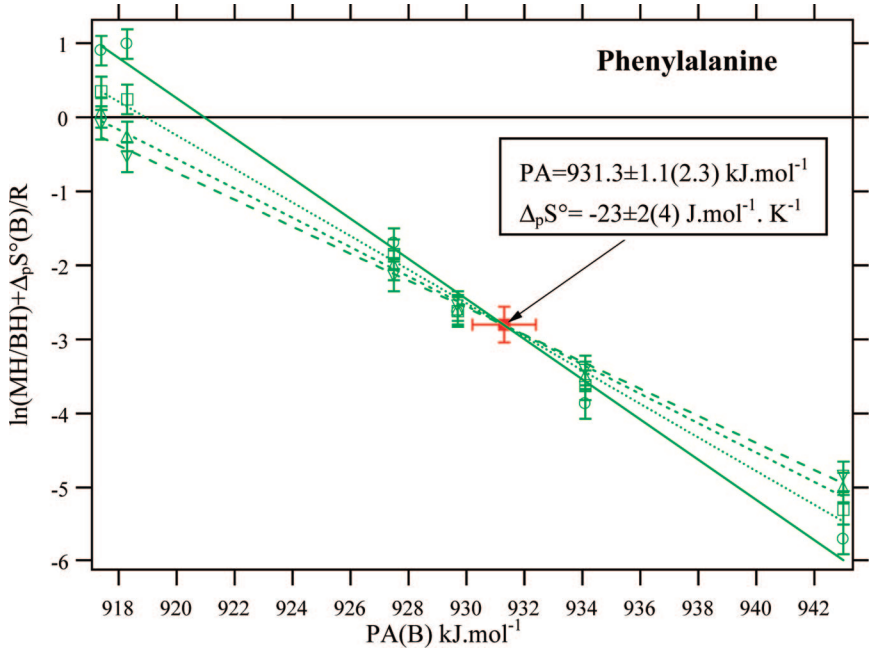


Figure 1. Kinetic method plot for phenylalanine.

The starting point of the method is to assume that the ratio of measured peak intensities $[MH]^+/[B_iH]^+$ is equal to the ratio of rate constants k_{MH}/k_{BH}. Then, using the canonical transition state theory to express k and considering several simplifying assumptions, the natural logarithm of the ratio of peak intensities may be expressed by

$$\begin{aligned} y_i &= \ln([MH]^+/[B_iH]^+) \sim \ln(k_{MH}/k_{BH}) \\ &= [G^\circ_T(M) + G^\circ_T(B_iH^+) - G^\circ_T(MH^+) - G^\circ_T(B_i)]/RT \\ &= [PA_{298}(M) - PA_{298}(B_i) + T\Delta S^\circ_1 + \Delta H^\circ_{298\_T} + \\ &\quad T\Delta S^\circ_{298\_T}]/RT \end{aligned} \tag{2}$$

where T is an "effective temperature" related to the excitation energy of the dissociating $[MHB_i]^+$ species, PA_{298} is the proton affinity at 298 K of the species of interest, and $\Delta S^\circ_i = \Delta_pS^\circ_{298}(M) - \Delta_pS^\circ_{298}(B_i)$. The terms $\Delta H^\circ_{298\_T}$ and $\Delta S^\circ_{298\_T}$ are thermal corrections for enthalpy and entropy, respectively, which, because of the structural similarities of $MH^+ + B_i$ on one hand and $M + B_iH^+$ on the other, is generally assumed to cancel to zero. In this hypothesis, eq 2 reduces to

$$y_i = [PA_{298}(M) - PA_{298}(B_i) + T\Delta S^\circ_i]/RT \tag{3}$$

and thus, for a series of experiments using several bases B_i at a temperature T, y_i vs $PA_{298}(B_i)$ follows a linear relationship characterized by a slope equal to $1/RT$ and an intercept with the PA_{298} scale given by $PA_{app} = PA_{298}(M) + T\langle\Delta S^\circ_i\rangle$ (where $\langle\Delta S^\circ_i\rangle$ is the mean value of the ΔS°_i terms). Alternatively, as suggested by Zheng and Cooks,[35] a variable change taking into account each individual $\Delta_pS^\circ(B_i)$ term,

$$y'_i = \ln([MH]^+/[B_iH]^+) + \Delta_pS^\circ(B_i)/R \tag{4}$$

may be used. Under these circumstances, the x axis intercept of the plot of y' vs $PA(B_i)$ becomes $PA'_{app} = PA_{298}(M) + T\Delta_pS^\circ(M)$.Whatever the use of eq 3 or 4, the "simple kinetic method" considers that the "apparent" proton affinities PA_{app} or PA'_{app} may be equated to $PA_{298}(M)$. This is obviously only possible if $\Delta_pS^\circ(M)$ is equal to zero. If it is not the case, to obtain both $PA_{298}(M)$ and $\Delta_pS^\circ(M)$, it is necessary to use several sets of experiments realized under different conditions of activation of the adduct ion and thus corresponding to different effective temperatures T_j (eq 5). This forms the basis of the "extended" kinetic method, which has been questioned and improved in recent years.[17,27−30] A straightforward method for extracting thermochemical information from the extended kinetic method consists in the use of the entire set of experimental observables y_{ij} obtained from n_j experiments differing in the adduct ion activation conditions and, for each j, from the n_i points corresponding to the number of reference bases B_i:[25−27]

$$y_{ij} = \Delta S^\circ_i/R + [PA_{298}(M) - PA_{298}(B_i)]/RT_j \tag{5}$$

Accordingly, the y_{ij} vs $PA_{298}(B_i)$ points may be fitted by a set of regression lines $(y_{ij})_{calc} = y_0 + b_j(x_0 - x_i)$ intersecting in a common point of coordinate $x_0 = PA_{iso}(M)$ and $y_0 = \Delta S^\circ_{iso}/R$, called the "isothermal"[25,26] or "isoequilibrium"[27] point. A statistical treatment of eq 4, leading to $PA_{iso}(M)$, $\Delta S^\circ_{iso}/R$, and the values of the n_j effective temperatures T_j, has been proposed recently by Ervin and Armentrout.[27] The method is based on the orthogonal distance regression (ODR) method,[36] a least-squares regression analysis which takes into account simultaneously all the $[n_i,n_j]$ data points. In the present study, the ODR method has been applied to several sets of y'_i (eq 4) values obtained at variable collision energies. The coordinates of the isothermal point are thus expected to provide $PA_{298}(M)$ and $\Delta_pS^\circ(M)/R$.

3.2. Phenylalanine. ***Experimental Protonation Thermochemistry of Phenylalanine.*** The experimental data obtained using the extended kinetic method with phenylalanine as unknown base M and a set of six reference bases B_i are summarized in Table S1 of the Supporting Information. The corresponding plot of y'_{ij} (eq 4) vs $PA(B_i)$ is presented in Figure 1. The correlation lines show a clear location of the isothermal

TABLE 1: Summary of the Experimental and Theoretical Protonation Thermochemistry of Phenylalanine (M) (in Bold: This Work)

M	method	GB(M) (kJ·mol^{-1})	PA(M) (kJ·mol^{-1})	$\Delta_pS°$(M) (J·K^{-1}·mol^{-1})
phenylalanine	equilibrium	887.4[a]/883.8[a]/895.4[b]		
	simple kinetic		915.2[c]/926.2[d]/927.9[e]	
	extended kinetic	**892.0 ± 1.3 (2.6)**	**931.3 ± 1.1 (2.3)**	**−23 ± 2 (4)**
	theoretical:			
	monoconformer	**894.7**	928.3[f]/924.9[g]/**927.7**	**−2**
	average	**890.9 (892.4)**[h]	**930.3 (931.1)**[h]	**−23 (−21)**[h]
	evaluated	888.9[i]	922.9[i]	−5[i]
		892	**932**	**−25**

[a] Equilibrium constant measurements at 570 K (high pressure mass spectrometry[11]) corrected to 298 K using $\Delta_pS°(M) = -25$ J·K^{-1}·mol^{-1}. [b] Equilibrium constant measurements (ion cyclotron resonance mass spectrometry[12]), as adapted by Hunter and Lias.[24] [c] Reference 13. Apparent proton affinities determined by the simple kinetic method. [d] Reference 14. Apparent proton affinities determined by the simple kinetic method, corrected to the Hunter and Lias scale.[24] [e] Reference 15. Apparent proton affinities determined by the simple kinetic method corrected to the Hunter and Lias scale.[24] [f] B3LYP/6-31+G(d,p)+ZPE(B3LYP/6-31G(d)) calculation corrected to 298 K. [g] *Ibid* but using the G2(MP2) calculation. [h] Calculated assuming a 29 8K Boltzmann (or, in parentheses, uniform) population of the eight most stable conformers of neutral phenylalanine and a 1/1 mixture of the two conformers **PheH** and **PheHb** of protonated phenylalanine. [i] Evaluated by E. P. Hunter and S. G. Lias;[24] note that the protonation entropy of −5 J ·K^{-1}·mol^{-1} has been assigned by comparison with methylamine.

point, leading to an accurate determination of both PA(Phe) and $\Delta_pS°$(Phe). The ODRFIT procedure[36] allows the assignment of the following values: PA(Phe) = 931.3 ± 1.1(2.3) kJ·mol^{-1} and $\Delta_pS°(Phe) = -23 \pm 2(4)$ J·mol^{-1}·K^{-1} (uncertainties are standard deviation and, in parentheses, 95% confidence limit). Combining these two terms, we deduce a gas phase basicity value, GB(Phe), equal to 892.0 ± 1.3(2.6) kJ·mol^{-1}. These results are indicated in bold in Table 1, which gathers also previous experimental and theoretical thermochemical data concerning protonation of phenylalanine.

As recalled in the Introduction, two estimates of the gas phase basicity of phenylalanine have been previously deduced from measurements of proton transfer equilibrium constants using either a high pressure mass spectrometry device[11] or an ion cyclotron resonance mass spectrometer.[12] Sufficient data are given in ref 11 to recalculate the corresponding GB(Phe), including thermal correction using a more correct protonation entropy. Using $\Delta_pS°(Phe) = -25$ J·mol^{-1}·K^{-1}, the GB(Phe) values become 883.8 and 887.4 kJ·mol^{-1} with aniline and *n*-butylamine as references bases, respectively. Unfortunately, the original data obtained by ion cyclotron resonance mass spectrometry were not published and a similar correction cannot be done on the GB(Phe) value of 895.4 kJ·mol^{-1} given in ref 12. These data lead to an average GB(Phe) value of 888.9 ± 5.9 kJ·mol^{-1}. Our experimental determination of 892.0 ± 1.3 kJ·mol^{-1} appears to fall into the range delimited by these previous estimates.

Another essential finding, evidenced here for the first time, concerns the occurrence of a large and negative protonation entropy. It should be emphasized that the value given by the extended kinetic method is generally an underestimate of the true $\Delta_pS°$.[17] This means that, in the present case, the absolute value of $\Delta_pS°$(Phe) is at least equal to 23 J·mol^{-1}K^{-1}. It is clear that the assignment of $\Delta_pS°(Phe) = -5$ J·mol^{-1}·K^{-1}, used by default until now,[24] should be corrected. Another consequence is that the PA(Phe) value tabulated is too low (by at least 5.7 kJ·mol^{-1}) because it was deduced from experimental GB values corrected by consideration of a too low $\Delta_pS°$(Phe) absolute value.

A comparison of the PA determined here with all the values derived from the use of the simple kinetic method is also done in Table 1.[13−15] As recalled in the preceding section, the simple kinetic method provides an apparent proton affinity given by $PA_{app} = PA_{298} + T\Delta S_i°$, where T is the effective temperature of the experiment. Since the term $\Delta S_i° = \Delta_pS°(M) - \Delta_pS°(B_i)$ is close to $\Delta_pS°$(M) if B_i are monofunctional reference bases, it results that $T\Delta S_i°$ is a negative term which may amount to several kJ·mol^{-1}. The apparent proton affinity deduced from the simple kinetic method is consequently an underestimate of the true PA_{298}. This is exactly what is observed in Table 1; the present PA value (931 kJ·mol^{-1}) is higher than any of the previous simple kinetic method estimates, which range from 915 to 928 kJ·mol^{-1}. Other arguments in favor of a PA close to 930 kJ·mol^{-1} and more details on the structural aspect of the protonation of Phe will be provided by quantum chemistry computation of the proton affinity of Phe as detailed below.

Conformational Analysis of Neutral Phenylalanine. Because of the presence of an aromatic chromophore, Phe has been extensively investigated by ultraviolet spectroscopy and combined laser-mass spectrometry techniques.[37−44] The present understanding of these experiments lies on large theoretical exploration of the conformational landscape of neutral phenylalanine.[38−41,45] Mass selected resonant two photon ionization on the jet-cooled amino acid and UV and IR ion depletion spectra were interpreted by the coexistence of the six conformers I−VI, according to the nomenclature introduced by Snoek et al.[38] (conformers will be denoted here **PheI**−**PheXIV**, where **I**−**XIV** is the numbering used in Snoek's nomenclature,[38] see Figure 2. This assignment has been further confirmed by similar two color experiments on monohydrated phenylalanine[39] and from vertical ionization energies of phenylalanine conformers.[40] Finally, interpretation of the partially resolved rotational bands of the resonant two photons ionization spectrum leads to a conformational reassignment, where **PheV** and **PheIV** are replaced by **PheVII** and **PheIX**, respectively.[41] These proposals provide also a basis for the interpretation of the resonance enhanced multiphoton ionization spectra of phenylalanine derivatives.[46] Theoretical conformational analysis of neutral phenylalanine has been conducted at various levels of theory: MP2/6-311G(d,p),[38] B3LYP/6-31+G(d),[38] MP2/6-311+G(d,p),[41] and B3LYP/6-311++G(d,p).[45] Depending upon the level used, the number of low lying conformers identified in a given energy range is variable; it is for example situated between 3[38] and 6[41,45] in the first 5 kJ·mol^{-1} energy gap. However, these studies generally agree with a comparable set of most stable conformers even though the predicted order of stability may be slightly different.

For the purpose of evaluating the protonation thermochemistry of phenylalanine, we re-examined at the uniform B3LYP/6-31+G(d,p) level the phenylalanine conformational landscape.

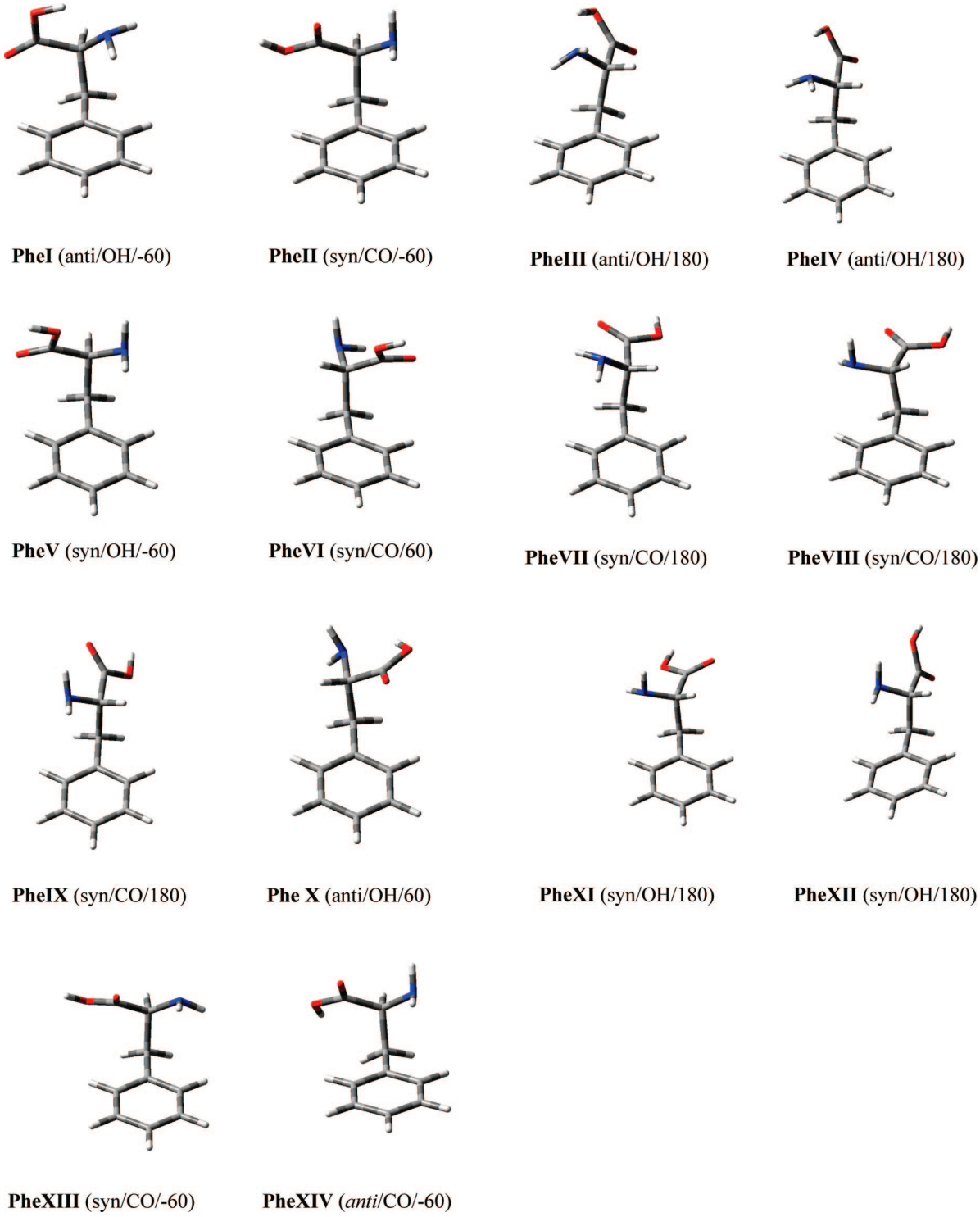


Figure 2. Most stable conformers of neutral phenylalanine (B3LYP/6-31+G(d,p) geometries).

In addition, we investigate the energy barriers separating the most stable conformers in order to estimate the entropies of hindered rotations. The essential results are summarized in Table 2 and illustrated by Figure 2 (the complete data sets are given in Tables S2 and S3 of the Supporting Information).

Before discussing the conformational aspect of neutral Phe, it is convenient to recall that neutral glycine exists essentially in three conformations at 298 K.[47−50] The most stable conformer is characterized by a *syn* HOCO arrangement and a bifurcated $NH_2\cdots O{=}C$ hydrogen bonding involving the carbonyl oxygen (denoted here *syn*/CO). The second conformer (less stable than the previous one by only 2−4 kJ·mol^{-1}) presents an *anti* HOCO arrangement and an $OH\cdots NH_2$ single hydrogen bond (denoted here *anti*/OH). The third conformer (situated ca. 7 kJ·mol^{-1} above the global minimum) is characterized by a *syn* HOCO arrangements and a bifurcated $NH_2\cdots OH$ hydrogen bond involving the hydroxylic oxygen (denoted here *syn*/OH).

Stable *syn*/CO, *anti*/OH, and *syn*/OH conformations were indeed identified for neutral phenylalanine. However, in addition to the *syn* or *anti* arrangement of the four atoms of the HOC(1)O acidic moiety and its H bonding with the amino group, the other structural features of phenylalanine are the dihedral angles of the aliphatic chain and possible hydrogen bonding between the amino acid part and the aromatic ring. A major result of the

TABLE 2: Summary of Relative Energies, Enthalpies, and Third Law Entropies Calculated for Neutral and Protonated Phenylalanine Conformers

	B3LYP/6-31+G(d,p)// B3LYP/6-31+G(d,p)			B3LYP/6-311++G(3df,2p)// B3LYP/6-31+G(d,p)		
species	$\Delta E_0^{\circ a,b}$	$\Delta H_{0\rightarrow 298}^{\circ c}$	$\Delta H_{298}^{\circ a}$	$\Delta E_0^{\circ a,b}$	$\Delta H_{298}^{\circ a}$	$S^{\circ d}$
PheI	0	0.201905	0	0	0	456.0
PheII	4.9	0.201714	4.4	4.6	4.1	474.6
PheIII	0.1	0.201927	0.1	−0.7	−0.7	465.3
PheIV	1.1	0.201775	0.8	0.6	0.3	465.1
PheV	7.8	0.201814	7.5	7.7	7.5	
PheVI	6.2	0.201683	5.6	5.0	4.4	477.7
PheVII	6.3	0.201529	5.3	4.6	3.6	479.1
PheVIII	6.6	0.201568	5.7	5.6	4.7	478.9
PheIX	7.2	0.201457	6.0	6.0	4.8	477.3
PheX	13.5	0.201700	12.9			
PheXI	11.9	0.201555	11.0			
PheXII	9.2	0.201449	8.0			
PheXIII	13.7	0.201461	12.5			
PheXIV	30.6	0.201377	29.2			
PheHa	0	0.215978	0	0	0	463.2
PheHb	2.9	0.215901	2.7	2.7	2.5	461.2
PheHc	23.8	0.215928	23.7			
PheHd	16.1	0.216000	16.2			
PheHe	17.7	0.215890	17.5			
PheHf	44.0	0.215872	43.7			
PheHg	37.1	0.215481	35.8			
PheHh	39.8	0.215286	38.0			

[a] Energies and enthalpies in kJ·mol^{-1}. [b] Total energies of **PheI** and **PheHa**$^+$ are −554.841530 hartree and −555.206690 hartree at the B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) levels and −554.999119 hartree and −555.364442 hartree at the B3LYP/6-311++G(3df,2p)// B3LYP/6-31+G(d,p) level, respectively (1 hartree = 2625.3 kJ·mol^{-1}). [c] B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) enthalpy correction at 298 K (in Hartree). [d] Total third law entropies in J·mol^{-1}·K^{-1} calculated after consideration of the hindered rotors approximation of Pitzer for internal rotations (see text and Table S2 for details).

previously published conformational studies,[38,41,45] confirmed here, is that all the most stable conformations of neutral Phe correspond to a perpendicular arrangement of the aliphatic chain with respect to the aromatic ring. This means that the C(2)C(3)C(4)C(5) dihedral angle is always close to 90°. Accordingly, for all the conformations examined here, this angle is situated between 80 and 105°. More dramatic is the incidence of the dihedral angle χ = C(1)C(2)C(3)C(4) on the conformational stabilities. Clearly, χ takes values close to −60°, 60°, and 180°, demonstrating the greater stability of the staggered conformations, as expected.

The most stable conformers in a 5 kJ·mol^{-1} range (B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level, including the 298 K enthalpy correction, Table 2) pertain (i) to the *anti*/OH group with $\chi \sim -60$ and 180° (structural features summarized by the notations *anti*/OH/−60 and *anti*/OH/180 for conformers **PheI**, **PheIII**, and **PheIV** in Figure 2) and (ii) to the *syn*/CO group with $\chi \sim -60$ and 180° (conformers **PheII**, **PheVI**, **PheVII**, and **PheIX**, Figure 2). Each set of conformers will be examined separately.

The three *anti*/OH conformers **PheI**, **PheIII**, and **PheIV** present comparable OH···NH$_2$ distances (1.886, 1.918, and 1.901 Å, respectively), pointing probably to similar H bond energies. A second interaction which may be involved in the stabilization of these three conformers occurs between one amino hydrogen and the π-electrons of the aromatic system. In these *anti*/OH conformers, the OH···NH$_2$ hydrogen bond probably leads to an increased polarization of the amino hydrogens, allowing them to enjoy an additional stabilizing interaction with the π-electrons of the aromatic ring. The fact that conformer **PheX**, characterized by a χ angle of ca. +60° and by a remoteness of the amino group from the aromatic ring (see Figure 2), is more than 10 kJ·mol^{-1} above **PheI** confirms the above proposal.

The *syn*-HOCO side of the conformational space of phenylalanine also presents interesting features. As shown in Table 2, the conformers **PheII** and **PheVI**−**PheIX** are very close in energy to the conformers **PheI**, **PheIII**, and **PheIV** described above. The most stable of the *syn*/CO conformers, species **PheII**, is the only structure which presents a pure bifurcated NH$_2$···OCOH hydrogen bonding with quasi identical C═O···HN distances of ca. 2.77 Å. It is noteworthy that, for conformer **PheVI**, no NH$_2$···π-electron is possible because the NH$_2$ group is *anti* with respect to the phenyl ring. Nevertheless, its enthalpy is comparable to that of **PheII** and also to those of **PheVII** and **PheIX**, which may exhibit a favorable interaction between one hydrogen atom of the amino group and the phenyl ring. The stabilization brought by the NH$_2$···π-electron seems consequently negligible for these conformers. A similar conclusion emerges from the comparable enthalpies of **PheVIII** and **PheVII** or **PheIX**. In fact, for these *syn*/CO conformers, the NH$_2$ acts as hydrogen bond donor with the more proximate oxygen of the carbonyl group thus reducing its ability to interact in a similar way with the phenyl ring.

To summarize, no less than eight conformers are predicted, at the B3LYP/6-311++G(3df,2p)// B3LYP/6-31+G(d,p) level, including the 298 K enthalpy correction, to be situated in the 5 kJ·mol^{-1} energy range above the global minimum of the neutral phenylalanine energy surface. The most stable conformers are **PheI**, **PheIII**, and **PheIV**, all pertaining to the *anti*/OH class. The five other conformers **PheII**, **PheVI**, **PheVII**, **PheVIII**, and **PheIX** are of *syn*/CO configuration and situated only 3−5 kJ·mol^{-1} above **PheI**. These results are in good agreement with previous computations[38,41,45] and with the assignment based on experimental data.[38−41]

Conformational Analysis of Protonated Phenylalanine. Protonated phenylalanine has been less studied by quantum chemical calculations than its neutral counterpart. A preferential protonation of the amino group and formation of structure relevant to the *syn*/CO have been described by several authors.[3,44,51,52] In the present study, we explored the conformational space of nitrogen protonated phenylalanine by a systematic examination of the internal rotations. Eight conformers originating from nitrogen protonation, **PheHa**−**PheHh**, were identified in the first 40 kJ·mol^{-1} energy range. Owing to the fact that, by analogy with glycine,[47,49,50] protonation at the carbonyl oxygen would lead to isomeric forms situated at least 100 kJ·mol^{-1} above **PheHa**, protonation on basic sites other than N has not been considered here.

In fact, three groups of conformers, denoted *syn*/CO, *syn*/OH, and *anti*/CO, may be defined for the eight identified conformers (Figure 3). Conformers of the *syn*/CO group, namely **PheHa**, **PheHb**, and **PheHc** (Figure 3), present a strong internal hydrogen bond between one hydrogen atom of the NH$_3^+$ group and the oxygen of the carbonyl, the second most basic site of the molecule. In addition to this internal hydrogen bonding, conformers **PheHa** and **PheHb** find an additional stability in a favorable interaction between the NH$_3^+$ group and the π-electrons of the aromatic ring. This stabilization is evident if we consider the conformer **PheHc**, where the NH$_3^+$ group is far away from the π-electron system and cannot play its part in the stabilization of this species, since it lies ∼20 kJ·mol^{-1} above **PheHa** and **PheHb**. The second set of conformers, **PheHd**, **PheHe**, and **PheHf**, is characterized by a *syn*/OH arrangement (Figure 3). Thus, the intramolecular hydrogen bond is now

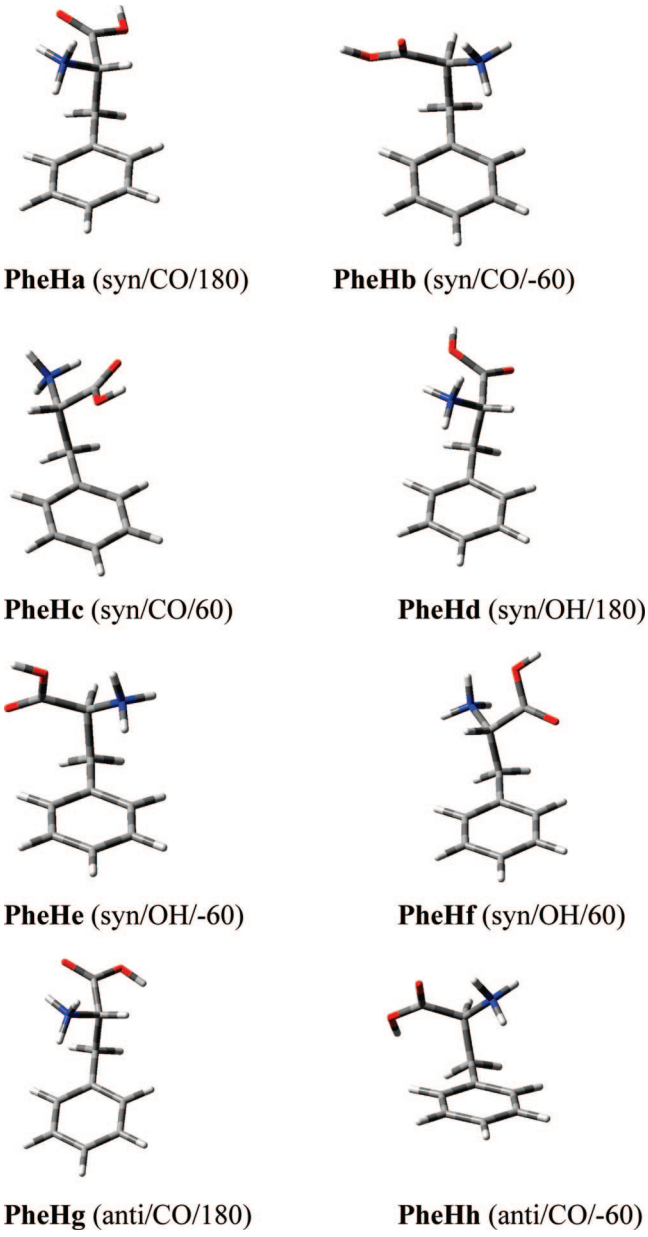


Figure 3. Most stable conformers of protonated phenylalanine (B3LYP/6-31+G(d,p) geometries).

assumed by the oxygen of the hydroxyl group, not by the carbonyl oxygen as above. Considering the fact that the oxygen of the carbonyl group of a carboxylic acid function is more basic than the hydroxylic oxygen, conformers *syn*/OH are consequently expected to be less stable than their *syn*/CO counterparts. Accordingly, **PheHd**, **PheHe**, and **PheHf** are situated between 16 and 20 kJ·mol^{-1} above their homologues in the first series, i.e. **PheHa**, **PheHb**, and **PheHc**. It is noteworthy that similar differences of ~20 kJ·mol^{-1} have been calculated between the *syn*/CO and *syn*/OH conformers for protonated glycine.[47,49] Again, the pronounced stability of conformers characterized by a NH_3 group close to the phenyl ring is attested by the fact that conformers **PheHd** and **PheHe** are situated ~28 kJ·mol^{-1} below **PheHf**. Finally, the two most stable conformers characterized by a *anti*/CO arrangement were considered. These conformers, **PheHg** and **PheHh**, are of poor stability since their energies, relative to **PheHa** and **PheHb**, are larger than 35 kJ·mol^{-1}. This observation is in keeping with results previously obtained for various amino acids. For example, the energy difference between the most stable *syn* and *anti* HOCO conformers of the N-protonated forms of glycine is in the range 34−38 kJ·mol^{-1}.[47,49]

It is clear that a considerable change in the number of low-lying conformers occurs upon protonation of phenylalanine. This may be briefly discussed, since it will have consequences on the thermochemical parameters relevant to the gas phase protonation of phenylalanine. Comparison between the neutral and protonated conformer landscape reveals the following:

- The three *anti*/OH conformers **PheI**, **PheIII**, and **PheIV** of neutral phenylalanine have no protonated counterparts because of the impossibility for the protonated nitrogen to act as a basic site.
- The *syn*/CO/−60 conformers **PheII** and **PheHb** may be considered as homologues, but for the *syn*/CO/180 conformers, the neutral C-NH_2 rotamers **PheVII**, **PheVIII**, and **PheIX** cannot be retrieved after protonation; they possess only **PheHa** as a protonated homologue.
- The energy gap between the *syn*/CO/±60 conformers **PheVI** and **PheII** is negligible because no significant interaction exists between NH_2 and the phenyl ring for these species. This is clearly not the case for their protonated counterparts **PheHc** and **PheHb**, where the later is more stable by 21 kJ·mol^{-1}, thus rendering improbable the participation of **PheHc** in the population of protonated phenylalanine experimentally sampled.
- The NH···OC interaction occurring in the *syn*/CO conformers is more pronounced than the NH···OH interaction present in the *syn*/OH conformers. This effect is however limited for the neutral species (the difference in enthalpy attains only 3 kJ·mol^{-1} between **PheXII** and **PheVII**) but is important in the protonated forms, thus rendering the presence of conformers such as **PheHd** and **PheHe** less probable than that of **PheHa** and **PheHb** (the difference in enthalpy is ca. 15 kJ·mol^{-1} in those cases).

The net results of these differences in conformer stabilities is that the number of conformers in the first 10 kJ·mol^{-1} range of enthalpy, is equal to nine for the neutral and to two for the protonated phenylalanine.

Third Law Entropies of Neutral and Protonated Phenylalanine. Computation of the protonation entropy, $\Delta_p S°(M) = S°(MH^+) - S°(M)$, obviously needs the precise knowledge of the individual third law entropies of M and MH^+. It is however well-known that the entropy values given by the Gaussian suite of programs are only approximate, particularly because the contributions of the low frequencies are estimated within the harmonic oscillator approximation. This limitation is particularly crucial for internal rotations. A means to more correctly estimate the entropy in such situation is to treat separately each internal rotation by using a hindered rotor model such as that developed by Pitzer.[53] This approach has been successfully applied to monofunctional molecules containing one, two, or three internal rotations[54] and to the protonation of several bifunctional bases.[55] Briefly, this procedure involves calculation of the rotational energy barrier, V_0, appearing in the variation of the potential energy with the dihedral angle ϕ, $V_0(\phi) = V_0/2(1 - \cos n\phi)$, where n is the symmetry of the rotation. The rotational energy levels are obtained by solving the corresponding Schrödinger equation. Then, the hindered rotor partition function is calculated and the corresponding thermochemical functions are deduced from the usual statistical thermodynamic relationships.[17] This procedure has been applied to the most stable neutral and protonated conformers of phenylalanine. Potential energy barriers associated with internal rotations in neutral and protonated

phenylalanine have been estimated at the B3LYP/6-31+G(d,p) level of theory. These data, reported in Table S2 of the Supporting Information, are the results of extensive conformational explorations involving conformers **PheI**−**PheXIV** and **PheHa**−**PheHb**. Details on these investigations are given in part S5 of the Supporting Information. Only the most relevant data, i.e. the resulting third law entropies, $S°$, are presented in the last column of Table 2. The uncertainty on the computed $S°$ values is essentially related to the uncertainty on the V_0 barriers. Assuming a relative error of 25% on the B3LYP/6-31+G(d,p) rotational barrier estimates, an error of 0.5−1.0 J·mol^{-1}·K^{-1} per hindered rotation results in the V_0 range explored here. The expected error on the computed $S°$ values reported in Table 2 is thus probably ∼4 J·mol^{-1}·K^{-1}.

As a general observation, the *syn*/CO and *anti*/OH structures present different entropy content. The sum of the hindered rotor contribution to entropy ($S°_{hind}$, Table S2) is equal to 101 J·mol^{-1}·K^{-1} for the *syn*/CO conformer **PheII** but is only 85 and 91 J·mol^{-1}·K^{-1} for the *anti*/OH conformers **PheI** and **PheIII**, respectively. As evidenced in Table S2, this difference in $S°_{hind}$ is mainly originating from the different energy barriers associated with the C(1)C(2) and C(2)NH$_2$ rotations, which are higher for the *anti*/OH conformers. A second finding is that the hindered rotor contribution is reduced, as expected, upon protonation. This is illustrated by the change of $S°_{hind}$ from 101 to 85 J·mol^{-1}·K^{-1} when passing from **PheII** to **PheHa**, with both conformers pertaining to the *syn*/CO class.

Computed Protonation Thermochemistry of Phenylalanine. Ideally, thermochemical quantities relevant to reaction 1 correspond to molar species in thermal equilibrium at a given temperature T, generally 298 K. Computation of these quantities obviously needs the knowledge of the MH$^+$ and M populations of conformers at this temperature.[17] Assuming a Boltzmann distribution of N distinguishable conformers, evaluation of the molar fractions x_i is possible using the relationship

$$x_i = \exp(-(G°_T)_i/RT) \sum_1^N \exp(-(G°_T)_i/RT) \qquad (6)$$

where $(G°_T)_i$ represents the Gibbs free energy of conformer i. Using the third law entropies and the enthalpy difference calculated at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level (Table 2), the Gibbs free energies $(G°_T)_i$ and molar fractions x_i may be calculated using eq 6 for neutral and protonated phenylalanine. The values of x_i are 0.05, 0.09, 0.20, 0.13, 0.14, 0.19, 0.12, and 0.08 for **PheI**, **PheII**, **PheIII**, **PheIV**, **PheVI**, **PheVII**, **PheVIII**, and **PheIX**, and they are 0.78 and 0.22 for **PheHa** and **PheHb**, respectively. Contributions of conformers with $(G°_T)_i$ larger than 7 kJ·mol^{-1} (i.e., **PheV** and **PheX** or higher) correspond to x_i less than 0.01 and have been consequently neglected.

In order to calculate an averaged proton affinity over N conformers of molar fractions x_i, the summed molar enthalpy, given by eq 7,

$$\langle H°_T \rangle = \sum_1^N x_i (H°_T)_i \qquad (7)$$

has been considered for both Phe and PheH. The resulting 298 K averaged proton affinity ⟨PA(Phe)⟩ is equal to 930.3 kJ·mol^{-1} (Table 1).

Following this reasoning, the populations of neutral and protonated conformers may also be considered to estimate the averaged entropy terms via eq 8:

$$\langle S°_T \rangle = \sum_1^N x_i (S°_T)_i - R \sum_1^N x_i \ln x_i \qquad (8)$$

where the second component corresponds to the entropy of mixing. Using eq 8, the averaged entropies, $\langle S°_{298}(\text{Phe}) \rangle = 489.3$ J·mol^{-1}·K^{-1} and $\langle S°_{298}(\text{PheH}^+) \rangle = 465.8$ J·mol^{-1}·K^{-1}, are calculated and a theoretical protonation entropy of $\langle \Delta_p S°(\text{Phe}) \rangle = -23.5$ J·mol^{-1}·K^{-1} may be deduced. It may be observed that $\langle \Delta_p S°(\text{Phe}) \rangle$ can be decomposed into two terms corresponding to the two components of eq 7: one due to the difference in averaged molar entropies (−9.8 J·mol^{-1}·K^{-1}) and the second due to the difference in entropy of mixing (−13.7 J·mol^{-1}·K^{-1}). The first contribution is due to the largest participation of the conformers of high entropies (i.e., *syn*/CO) in the mixture of neutral phenylalanine. The second is coming from the drastic change in conformer numbers in the same energy range from neutral to protonated forms. Finally, combining the averaged proton affinity and protonation entropy, we deduce an averaged gas phase basicity ⟨GB(Phe)⟩ of 890.9 kJ·mol^{-1} (Table 1).

It is evident that, following eq 6, the estimate of meaningful x_i necessitates an excellent precision on $(G°_T)_i$, i.e., on both the $(H°_T)_i$ and $(S°_T)_i$ terms for each conformer. Uncertainties probably less than 4 J·mol^{-1}·K^{-1} may be expected on the individual third law entropy estimates, as underlined in the preceding part. Considering the enthalpy term, the ability of B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) theory to reproduce relative energies of conformers may be discussed. Several reviews were recently devoted to the evaluation of the performance of density functional methods.[56−59] From the most comprehensive studies,[56,59] where hydrogen bonding interactions are explicitly considered, it emerges that a mean absolute deviation in the range 0.5−3 kJ·mol^{-1} may result in conformational energies when using the B3LYP hybrid functional with Pople-type polarized basis sets. It can be concluded that absolute deviation on $(G°_T)_i$ may attain 4 kJ·mol^{-1} at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level, thus leading to large deviation on the x_i distribution of conformers. Fortunately, average values of the thermochemical parameters are only marginally sensitive on the precise population of conformers. This may be seen for example by considering a uniform population of the eight most stable conformers of neutral phenylalanine and a 1/1 mixture of the two conformers of protonated phenylalanine. The resulting average entropies are $\langle \Delta_p S°(\text{Phe}) \rangle_{uni} = 489.1$ J·mol^{-1}·K^{-1} and $\langle S°_{298}(\text{PheH}^+) \rangle_{uni} = 468.0$ J·mol^{-1}·K^{-1}, thus leading to a protonation entropy of $\langle \Delta_p S°(\text{Phe}) \rangle_{uni} = -21$ J·mol^{-1}·K^{-1} (with obviously a large standard deviation of 8.6 J·mol^{-1}·K^{-1}). Similarly, the average proton affinity becomes $\langle \text{PA(Phe)} \rangle_{uni} = 931.1 \pm 2.4$ kJ·mol^{-1} and the gas phase basicity $\langle \text{GB(Phe)} \rangle_{uni} = 892.4 \pm 3.5$ kJ·mol^{-1}. These values are very close to those calculated using the precise computed molar fractions x_i and may constitute a rough approximation of the corresponding quantities.

Most of the time, the thermochemical parameters associated with reaction 1 are computed by considering only one conformer for both the neutral and the protonated molecule. This "monoconformer" procedure may be tested here and compared to the averaged method. The proton affinity calculated assuming that protonated and neutral phenylalanine are exclusively the pure structures **PheHa** and **PheIII**, i.e. the most stable species in

terms of enthalpy at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level, is equal to PA_{mono}(Phe) = 927.7 kJ·mol^{-1} (Tables 1 and S2). In their estimation, Bleiholder et al.[3] considered conformers **PheHa** and **PheI** (rather than **PheIII**, but the difference in enthalpy is less than 1 kJ·mol^{-1}; see Table 2), and they proposed a 0 K proton affinity of phenylalanine equal to 923.0 kJ·mol^{-1} based on B3LYP/6-31+G(d,p) calculations. A 298 K estimate based on the $\Delta H^{\circ}_{0\rightarrow 298K}$ correction presented in Table 2 leads to a PA_{298} value of 928.3 kJ·mol^{-1}, in correct agreement with our B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) calculation. In the present case, the "monoconformer" proton affinity is lower than the averaged value by 2.6 kJ·mol^{-1}. This is due to the fact that the mean excess enthalpy brought by the mixture of conformers is larger for the neutral than for protonated phenylalanine ($\langle\Delta H^{\circ}\rangle$ = 3.2 kJ·mol^{-1} with respect to **PheIII** and 0.6 kJ·mol^{-1} with respect to **PheHa**).

The protonation entropy calculated considering the most stable conformers **PheIII** and **PheHa** is equal to $\Delta_pS^{\circ}_{mono}$(M) = S°(**PheHa**) − S°(**PheIII**) = −2 J·mol^{-1}·K^{-1}, a value clearly lower than the average value of −23 J·mol^{-1}·K^{-1} (Table 1). This large difference is mainly due to the fact that the entropy of mixing is not considered in the "monoconformer" estimate.

The "monoconformer" gas phase basicity value corresponding to the **PheIII/PheHa** system is equal to GB_{mono}(Phe) = 894.7 kJ·mol^{-1}, thus demonstrating an excess of 3.8 kJ·mol^{-1} with respect to the average ⟨GB(Phe)⟩ value (Table 1).

Comparison with Experiments. When comparing theoretical and experimental thermochemical properties, it is essential to know which neutral and protonated species are accessible under the given experimental conditions. For proton transfer equilibrium constant methods using either high pressure or ion cyclotron resonance mass spectrometry, the number of collisions generally ensure that the thermal equilibrium is attained for reactants and products. The situation may be questioned for the kinetic method experiments where activated MHB^+ adducts are studied. To answer this question, two observations can be made. First, a comfortable stabilization energy is expected for the experimentally sampled adducts $PheHB^+$. Accordingly, a simple estimate obtained by using the empirical relationship proposed by Mautner[19] gives a dissociation enthalpy of 97 kJ·mol^{-1} for AHB^+ complexes involving nitrogen bases A and B. As far as amino acids are considered, binding energies of 100 and 110 kJ·mol^{-1} have been recently reported for complexes $GlyHNH_3^+$ and $(Gly)_2H^+$.[60,61] Second, calculation of rotational barriers on the Phe and PheH energy surfaces (see Supporting Information section S5) shows that the highest barriers correspond to the *syn* → *anti* isomerizations. In particular, interconnection between the *anti*/OH and *syn*/CO families is possible via the transition structures **PheI→PheV** and **PheII→PheXIV**, which lie ∼ 60 kJ·mol^{-1} above the global minimum **PheIII**. One can assume that these barriers are of the same size in the potential energy surface associated with the complexes $PheHB^+$ experimentally studied using the kinetic method. This proposal is corroborated by a recent computation on the *syn* → *anti* reaction in $GlyHNH_3^+$ complexes which reveals a similar barrier of 60 kJ·mol^{-1} at the B3LYP/6-311+G(d,p) level.[60] Concerning the two most stable protonated forms **PheHa** and **PheHb**, the energy barrier separating them is only 12 k J·mol^{-1} (Supporting Information section S5b). Given the fact that the endothermicities of the reactions $PheHB^+ \rightarrow Phe + BH^+$ or $PheHB^+ \rightarrow PheH^+ + B$ are, as recalled above, in the range 100−110 kJ·mol^{-1}, i.e. ca. 40 kJ·mol^{-1} greater than the highest *syn* → *anti* barrier, the entire surface can be fully sampled in the electrospray ion source before the activation/dissociation process. Therefore, a statistical distribution of conformers is presumably present in the sampled mixture of adducts under our experimental conditions and should be considered when compared with theory. In line with this conclusion, the following observations can be made:

- The proton affinity obtained using the extended kinetic method (931.3 kJ·mol^{-1}, Table 1) is very close to the theoretical value calculated by considering the equilibrium populations of neutral and protonated conformers of phenylalanine at 298 K, ⟨PA(Phe)⟩ = 930.3 kJ·mol^{-1}; the monoconformer approximation PA_{mono}(Phe) = 927.7 kJ·mol^{-1} offers an underestimate of the true proton affinity, as discussed above.
- It clearly appears that the theoretical averaged protonation entropy $\langle\Delta_pS^{\circ}(Phe)\rangle$ = −23.5 J·mol^{-1}·K^{-1} is in better agreement with the experimental value (−23 J·mol^{-1}·K^{-1}, Table 1) than the simple estimate based on the occurrence of only one conformer ($\Delta_pS^{\circ}_{mono}$(M) = −2 J·mol^{-1}·K^{-1}); the excellent agreement observed should be, however, pondered by the fact that (i) the experimentally determined Δ_pS°(Phe) value probably represents only a lower limit of the true protonation entropy and that (ii) the uncertainty on the averaged $\langle\Delta_pS^{\circ}(Phe)\rangle$ estimate is unknown but may probably attain 5−10 J·mol^{-1}·K^{-1}.
- Finally, an averaged gas phase basicity ⟨GB(Phe)⟩ of 890.9 kJ·mol^{-1} has been obtained; this theoretical value compares favorably with the value deduced from the extended kinetic method determination (892.0 kJ·mol^{-1}, Table 1) and also with the mean value determined from a proton transfer equilibrium constant determination of 888.3 ± 6.5 kJ·mol^{-1}.

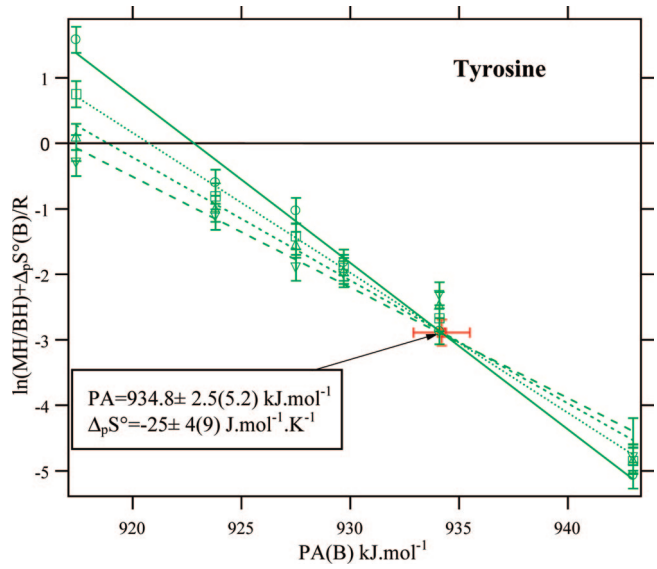


Figure 4. Kinetic method plot for tyrosine.

3.3. Tyrosine. ***Experimental Protonation Thermochemistry of Tyrosine.*** Determination of the proton affinity and protonation entropy of tyrosine has been done by the extended kinetic method using six reference bases B_i (Table S4 of the Supporting Information). The graph of y'_i (eq 4) versus the proton affinities of the reference bases is presented in Figure 4 for four center of mass collision energies (E_{coll} =1, 2, 3, and 4 eV). Using these data, the orthogonal distance regression method leads to PA(Tyr) = 934.8 ± 2.5(5.2) kJ·mol^{-1} and Δ_pS°(Tyr) = −25 ± 4(9) J·K^{-1}·mol^{-1}, which, combined, give GB(Tyr) = 894.9 ±

TABLE 3: Summary of the Experimental and Theoretical Protonation Thermochemistry of Tyrosine (M) (in Bold: This Work)

M	method	GB(M) (kJ·mol^{-1})	PA(M) (kJ·mol^{-1})	$\Delta_pS°$(M) (J·K^{-1}·mol^{-1})
tyrosine	equilibrium	905.4[a]		
	simple kinetic		917.0[b]/ 930.2[c]/ 929.8[d]	
	extended kinetic	**894.9 ± 2.8 (5.9)**	**934.8 ± 2.5 (5.2)**	**−25 ± 4 (9)**
	theoretical:			
	monoconformer	**901.2**	931.8[e]/927.6[f]/**931.5**	**7**
	average	**896.0 (895.1**[g]	**935.2 (934.7>**[g]	**−23 (−24)**[g]
	evaluated	892.1[h]	926.0[h]	−5[h]
		895	**935**	−25

[a] Equilibrium constant measurements (ion cyclotron resonance mass spectrometry[12]), as adapted by Hunter and Lias in ref 42. [b] Reference 13. Apparent proton affinities determined by the simple kinetic method. [c] Reference 14. Apparent proton affinities determined by the simple kinetic method, corrected to the Hunter and Lias scale.[24] [d] Reference 15. Apparent proton affinities determined by the simple kinetic method corrected to the Hunter and Lias scale.[24] [e] Reference 3. B3LYP/6-31+G(d,p)+ZPE(B3LYP/6-31G(d)) calculation corrected to 298 K. [f] *Ibid* but using G2(MP2) calculation. [g] Calculated assuming a 298 K Boltzmann (or, in parentheses, uniform) population of the sixteen most stable conformers of neutral tyrosine and of the four most stable conformers of protonated tyrosine. [h] Evaluated by E. P. Hunter and S. G. Lias;[24] note that the protonation entropy of −5 J·K^{-1}·mol^{-1} has been assigned by comparison with methylamine.

2.8(5.9) kJ·mol^{-1}. The values are reported in Table 3 beside previous experimental and theoretical estimates.

Only one gas phase basicity determination obtained from equilibrium constant measurement has been reported in the literature.[12] Surprisingly, the deduced value,[24] GB(Tyr) = 905.4 kJ·mol^{-1}, is ca. 10 kJ·mol^{-1} above the value derived from the extended kinetic method. One may underline however that no thermal correction can be done on the experimental data, since the necessary information is lacking; consequently, the uncertainty on this value is probably large. Moreover, it may be seen from examination of Table 3 that the gas phase basicity of phenylalanine, deduced from the data of ref 12 is 10 kJ·mol^{-1} above the average experimental PA(Phe) of 885.1 kJ·mol^{-1}. If a comparable deviation also affects the GB(Tyr) determination of 905.4 kJ·mol^{-1},[12] clearly, a more correct comparison is evident between the two experimental data.

The occurrence of a significant entropy loss upon protonation of tyrosine is a new and important finding. It is noteworthy that $\Delta_pS°$(Tyr) is practically equal to $\Delta_pS°$(Phe), in the experimental error limits. As will be shown below in the conformational analysis section, this observation is in keeping with the structural similarities of the two amino acids and the fact that the OH group does not play a significant role during the protonation process.

Apparent proton affinities given by the simple kinetic method are situated in the 917−930 kJ·mol^{-1} range, depending upon the experimental conditions.[13−15] According to the relationship $PA_{app} = PA_{298} + T\Delta S_i^\circ$ and owing to the fact that $\Delta_pS°$(Tyr) is negative, it is expected that PA_{app} should be lower than the true PA_{298}. The data reported in Table 3 confirm this expectation. It may also be observed that all experiments point to a slightly larger basicity and proton affinity of tyrosine with respect to phenylalanine (see Table 1). Using the extended kinetic results, the difference appears to be close to 3 kJ·mol^{-1}.

Conformational Analysis of Neutral and Protonated Tyrosine. Obviously, in many aspects, the behavior of tyrosine is comparable to that of phenylalanine, since both molecules differ only by the presence of the *para* hydroxy group in the former. This functional group is expected to play a modest role on the protonation thermochemistry, and we consequently restricted the theoretical investigation of neutral and protonated tyrosine to only the necessary information. Tyrosine conformers may be classified using the same nomenclature as for phenylalanine (i.e., the **TyrI**, **TyrII**,...). However, since the orientation of the phenolic hydroxyl group may take two distinguishable positions, the number of conformers is multiplied by two with respect to phenylalanine and the nomenclature used will be of the type **TyrI$_l$** and **TyrI$_r$** for the "left" and "right" orientations (see Figure 5).

A number of experimental and theoretical studies were devoted to the conformers of isolated neutral tyrosine.[37,62−65] Martinez et al.[37] have studied the electronic spectrum of tyrosine and interpreted their results by the existence of a mixing of 10 conformers (2 × 5 according to the "l" and "r" orientations). The resonance enhanced multiphoton ionization spectroscopy of tyrosine was interpreted by the occurrence of two types of conformations in which the carboxylic group is situated either *anti* or *gauche* with respect to the phenyl ring. A vibrational assignment of the bands of the resonant two photons ionization spectrum has been proposed later on based on HF/6-31G calculations.[63] In 2005, Ramaeker et al.[62] presented a Fourier transform infrared spectroscopic study of tyrosine combined with B3LYP/6-31++G(d,p) calculations. The authors identified 2 × 9 conformers in the first 14 kJ·mol^{-1} potential energy. The two most stable (called IIa and IIa$_r$ in ref 62) correspond to an *anti*/OH(−60) conformation, i.e. to **TyrI$_l$** and **TyrI$_r$** (Figure 5). The second conformer (IIb and IIb$_r$) is an *anti*/OH(180) species, which may be **TyrIII** or **TyrIV**. The most comprehensive conformational study of tyrosine is due to Zhang et al.,[64] who investigated 76 conformers at the B3LYP/6-311++G(d,p) and MP2/6-311G(2df,p)//B3LYP/6-311++G(d,p) levels. The number of conformers lying in the first 5 kJ·mol^{-1} is dependent on the method used (as also observed for phenylalanine). At the MP2 level, only three conformers, namely **TyrI**, **TyrIII**, and **Tyr II** were identified, whereas the B3LYP calculations give five structures in the following order: **TyrI**, **TyrIII**, **TyrIV**, **TyrII**, and **TyrVI**. A later computational study was limited to the 12 conformers **TyrI**−**TyrVI**,[65] optimized at the B3LYP/6-31+G(d) level. The energy ordering predicted at this level of theory is as follows: **TyrI**, **TyrIII**, **TyrII**, **TyrIV/VI**, and **TyrV**. Based on these computations, the authors were able to interpret the laser induced fluorescence spectrum of tyrosine.[65]

Our B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) calculations (Table 4 and Figure 5) confirm the earlier results of Zhang et al.[64] The order of increasing energy of the investigated conformers is **TyrI**, **TyrIII**, **TyrIV**, **TyrII** and, above the 5 kJ·mol^{-1} of relative enthalpy, **TyrVII**, **TyrVI**, **TyrVIII**, **TyrIX**. For the three most stable conformers, this energy ordering is not significantly changed when considering the single point energy calculations at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level (Table 4). Note that conformers **TyrV** and **TyrX**−**TyrXIV** have not been examined, since the correspond-

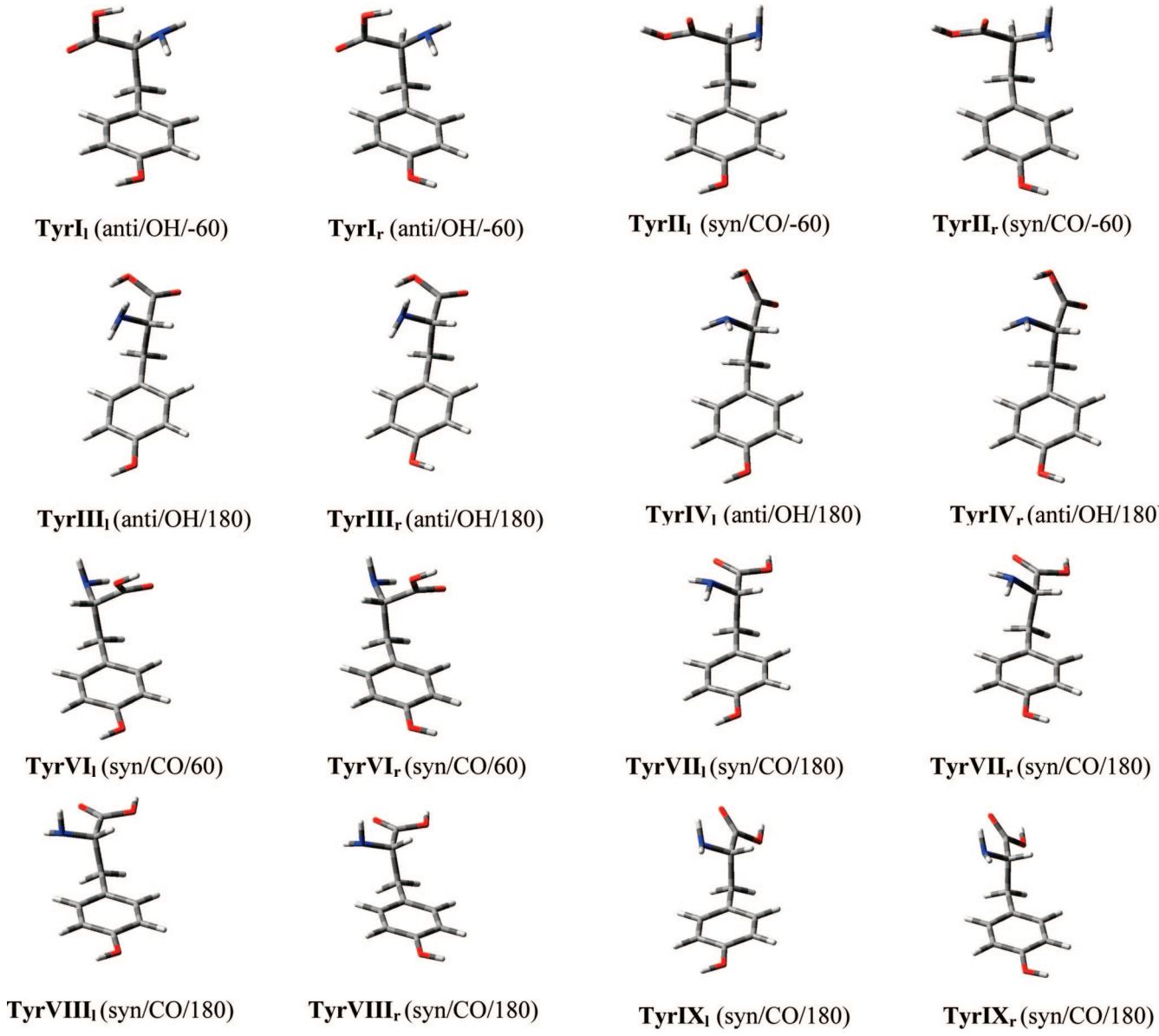


Figure 5. Most stable conformers of neutral tyrosine (B3LYP/6-31+G(d,p) geometries).

ing phenylalanine conformers are situated well above the low lying conformer **PheI**. It may also be observed that, as expected, the differences in energy between the two rotamers "l" and "r" are very small.

Protonated tyrosine has recently been characterized by its ultraviolet photofragmentation spectrum.[44,66] The vibrationally resolved band allowed the assignment of the observed signals to four conformers. Comparison of the measured infrared spectra to B3LYP/6-31++G(d,p) harmonic vibrational frequencies allows the assignment to conformers homologues of **PheHa** and **PheHb** examined above. These four conformers, namely **TyrHa$_l$** and **TyrHb$_l$** and their two OH rotamers "r", are presented in Figure 6. As expected from the results obtained with phenylalanine, these structures correspond to protonation of the amino group and present the *syn*/HOCO arrangement, allowing a single NH···CO hydrogen bond and a NH···π interaction.

Computed Protonation Thermochemistry of Tyrosine and Comparison with Experiments. The proton affinity calculated assuming only the most stable conformers **TyrI$_l$** and **TyrHa$_r$** is equal to 931.5 kJ·mol^{-1} at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level corrected to 298 K (see Table 4 and Table S3 of the Supporting Information). Applying the correction for enthalpy at 298 K given in Table 4, a comparable PA(Tyr) value of 931.8 kJ·mol^{-1} may be deduced from the B3LYP/6-31+G(d,p) calculations reported in ref 3. These theoretical, monoconformer estimates of the proton affinity are close to, although slightly lower than, the experimental value of 934.2 kJ·mol^{-1} given by the extended kinetic method. The monoconformer procedure applied to entropy leads to $\Delta_p S°$(Tyr) = 7 J·mol^{-1}·K^{-1}, a positive value clearly at variance from experiment. As a corollary, the monoconformer gas phase basicity, GB(Tyr) = 901.2 kJ·mol^{-1}, is significantly higher than the experimental value.

As developed in the preceding section for the case of phenylalanine, a correct interpretation of the experimental thermochemical data should consider the possible mixture of conformers of both the neutral and protonated forms of the considered species. Such an estimate can be done for tyrosine by taking into account the full set of conformers **TyrI**−**TyrIX** and **TyrHa**−**TryHb** presented in Table 4 and Figure 6. A simplifying assumption will be that the contributions to entropy of hindered rotations are similar from phenylalanine to tyrosine

TABLE 4: Summary of Relative Energies, Enthalpies, and Third Law Entropies Calculated for Neutral and Protonated Tyrosine Conformers

	B3LYP/6-31+G(d,p)// B3LYP/6-31+G(d,p)			B3LYP/6-311++G(3df,2p)// B3LYP/6-31+G(d,p)		
species	$\Delta E_0^{\circ a}$	$\Delta H^{\circ}_{0\rightarrow 298}{}^{b}$	ΔH°_{298}	$\Delta E^{\circ}_{298}{}^{a}$	ΔH°_{298}	$S^{\circ c,d}$
TyrI$_l$	0.0	0.207158	0.0	0.0	0.0	478.1
TyrI$_r$	1.5	0.207134	1.5	1.3	1.3	
TyrII$_l$	6.0	0.206885	5.3			500.5
TyrII$_r$	6.0	0.206902	5.3			
TyrIII$_l$	1.3	0.207161	1.4	0.7	0.7	489.2
TyrIII$_r$	1.2	0.207162	1.2	0.6	0.6	
TyrIV$_l$	2.8	0.207026	2.5	2.5	2.1	490.7
TyrIV$_r$	2.8	0.207039	2.3	2.3	2.0	
TyrVI$_l$	7.5	0.206926	6.9			500.9
TyrVI$_r$	7.3	0.206944	6.7			
TyrVII$_l$	7.6	0.206751	6.6			501.7
TyrVII$_r$	7.7	0.206744	6.6			
TyrVIII$_l$	7.4	0.206846	6.6			501.8
TyrVIII$_r$	7.9	0.206837	7.0			
TyrIX$_l$	8.4	0.206680	7.1			501.9
TyrIX$_r$	8.8	0.206666	7.5			
TyrHa$_l$	0.2	0.221185	0.2	0.2	0.2	485.0
TyrHa$_r$	0.0	0.221193	0.0	0.0	0.0	
TyrHb$_l$	2.7	0.221052	2.4	2.6	2.2	481.5
TyrHb$_r$	3.3	0.221046	2.9	3.1	2.7	

[a] Energies and enthalpies in kJ·mol^{-1}. [b] The total energies of **TyrI$_l$** and **TyrHa$_r$**$^+$ are −630.067325 hartree and −630.433429 hartree at the B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) level and −630.252432 hartree and −630.618843 hartree at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level, respectively (1 hartree = 2625.3 kJ·mol^{-1}). [c] Total third law entropies in J·mol^{-1}·K^{-1} calculated after consideration of the hindered rotors approximation of Pitzer for internal rotations (see text and Table S6 for details). [d] B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) enthalpy correction at 298 K (in Hartree).

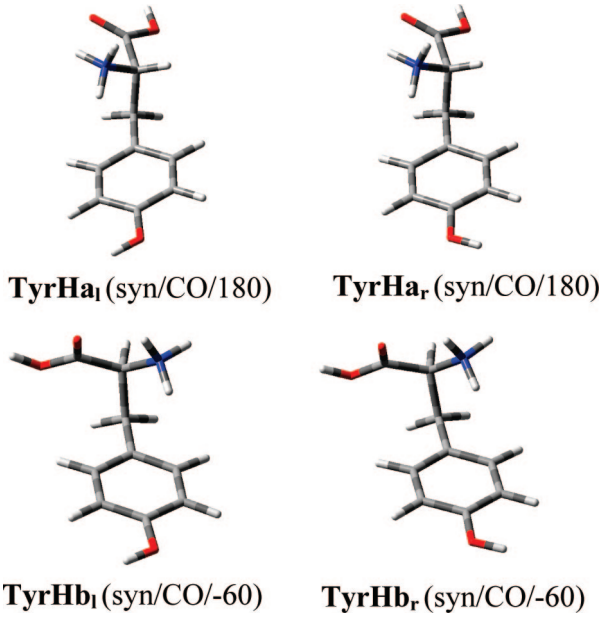


Figure 6. Most stable conformers of protonated tyrosine (B3LYP/6-31+G(d,p) geometries).

for a given type of conformers (see Table S6 of the Supporting Information). This approximation seems reasonable because the OH group in the *para* position is not expected to change the rotational barriers in the opposite part of the molecule. Under this assumption, the third law entropies of **TyrI**−**TyrIV** are equal to 478, 500, 489, and 491 J·mol^{-1}·K^{-1}, a single value of 502 J·mol^{-1}·K^{-1} applies for **TyrVI**−**TyrIX**, and the third law entropies of **TyrHa** and **TyrHb** are 485 and 481 J·mol^{-1}·K^{-1}, respectively (Table S6). Using these estimated entropies and the calculated enthalpy differences (Table 4), the corresponding relative Gibbs free energy and molar fractions x_i of neutral and protonated tyrosine may be calculated (eq 6). Using eq 8, the averaged entropies $\langle S^{\circ}_{298}(\mathrm{Tyr})\rangle$ = 494.5 J·mol^{-1}·K^{-1} and $\langle S^{\circ}_{298}(\mathrm{TyrH}^+)\rangle$ = 484.2 J·mol^{-1}·K^{-1} are calculated. These estimates lead to a theoretical protonation entropy of $\langle \Delta_p S^{\circ}_{298}(\mathrm{Tyr})\rangle$ = −22.7 J·mol^{-1}·K^{-1}. Again, the quantity $\langle \Delta_p S^{\circ}_{298}(\mathrm{Tyr})\rangle$ may be decomposed into two terms corresponding to (i) the difference in averaged molar entropies (−10.3 J·mol^{-1}·K^{-1}) and (ii) the difference in entropy of mixing (−12.4 J·mol^{-1}·K^{-1}). As observed with phenylalanine, the agreement between the averaged estimate of the protonation entropy, $\langle \Delta_p S^{\circ}_{298}(\mathrm{Tyr})\rangle$, and experiment (−25 J·mol^{-1}·K^{-1}, Table 3) is gratifying.

By considering these computed equilibrium populations of neutral and protonated conformers of tyrosine, an averaged proton affinity value ⟨PA(Tyr)⟩ may be calculated. The value, ⟨PA(Tyr)⟩ = 935.2 kJ·mol^{-1}, is also in excellent agreement with experiment (934.8 kJ·mol^{-1}, Table 3). Combining the calculated averaged proton affinity and protonation entropy, we calculate an averaged gas phase basicity ⟨GB(Tyr)⟩ of 896.0 kJ·mol^{-1}, which compares favorably with the value deduced from the extended kinetic method determination (894.9 kJ·mol^{-1}, Table 3).

It may be noted that a correct approximation of the averaged thermochemical parameters may also be obtained here by considering an identical amount of each conformer for the neutral and the protonated tyrosine. The results are as follows: $\langle \mathrm{PA(Tyr)}\rangle_{\mathrm{uni}}$ = 934.7 ± 3.1 kJ·mol^{-1}, $\langle \Delta_p S^{\circ}_{298}(\mathrm{Tyr})\rangle_{\mathrm{uni}}$ = −24.1 ± 9.0 J·mol^{-1}·K^{-1}, and $\langle \mathrm{GB(Tyr)}\rangle_{\mathrm{uni}}$ = 895.1 ± 3.1 kJ·mol^{-1} (in parentheses in Table 3).

4. Concluding Remarks

The present study provides new determinations of the thermochemical parameters associated with the gas phase protonation of phenylalanine and tyrosine. The experimental method used, namely the "extended kinetic method", allows the measurement of proton affinities and protonation entropies $\Delta_p S^{\circ}(\mathrm{M}) = S^{\circ}(\mathrm{MH}^+) - S^{\circ}(\mathrm{M})$ of the considered amino acids M. The resulting values are PA(Phe) = 931.3 ± 1.1 kJ·mol^{-1}, $\Delta_p S^{\circ}$(Phe) = −23 ± 2 J·mol^{-1}·K^{-1}, PA(Tyr) = 934.8 ± 2.5 kJ·mol^{-1}, and $\Delta_p S^{\circ}$(Tyr) = −25 ± 4 J·mol^{-1}·K^{-1}. Our experiments thus confirm the slightly better proton affinity of tyrosine with respect to phenylalanine, with the difference being ca. 3 kJ·mol^{-1}. An important finding is the negative and similar $\Delta_p S^{\circ}$ values for phenylalanine and tyrosine, a point evidenced here for the first time.

Quantum chemical investigation of a large set of conformers has been conducted at the B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) level. Proton affinities appear to be correctly reproduced using B3LYP/6-311++G(3df,2p)//B3LYP/6-31+G(d,p) computation including the 298 K enthalpy correction. An essential result, however, is the observation that a mixture of conformers should be considered when comparison with mass spectrometry experiment is done. Consideration of a Maxwell−Boltzmann population of conformers slightly increases the resulting computed proton affinity based on only one (the most stable) conformer for both the neutral and the protonated molecule by ca. 3 kJ·mol^{-1}. Averaged proton affinities of 930.3 kJ·mol^{-1} and 935.2 kJ·mol^{-1} are thus calculated for phenylalanine and tyrosine, respectively. Calculation of third law entropies has been done for a subset of conformers by including explicit treatment of hindered rotations. Using these data, and

considering the entropy of mixing, protonation entropies of −23 $J \cdot mol^{-1} \cdot K^{-1}$ were computed for both phenylalanine and tyrosine. This entropy loss has two origins: (i) the increase in strength of the interaction between the amino group and the aromatic moiety when passing from the neutral to the protonated forms in their *syn*/CO conformations and (ii) the larger entropy of mixing associated with the population of neutral conformers with respect to their protonated counterparts. This latter effect has two combined origins. First, protonation reduces the number of distinguishable conformers (**PheVII**, **PheVIII**, **PheIX** compared with **PheHa**, for example, in the phenylalanine series). Second, protonation increases the energy gap between the most stable conformer and the others because of the increased strength of the hydrogen bonds involving the hydrogen atoms of the NH_3^+ group and either the oxygen atoms or the π-electrons of the phenyl ring.

It emerges from these experimental and computational results that the following evaluated thermochemical parameters may be proposed: PA(Phe) = 932 $kJ \cdot mol^{-1}$, PA(Tyr) = 935 $kJ \cdot mol^{-1}$, $\Delta_p S°$(Phe) = $\Delta_p S°$(Tyr) = −25 $J \cdot mol^{-1} \cdot K^{-1}$ (with probable uncertainties of ±2 kJ/mol for the enthalpic quantities and ±10$J \cdot mol^{-1} \cdot K^{-1}$ for the entropies). From these figures, the corresponding gas phase basicities become GB(Phe) = 892 $kJ \cdot mol^{-1}$ and GB(Tyr) = 895 $kJ \cdot mol^{-1}$.

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Supporting Information Available: Experimental data points used in the extended kinetic method for phenylalanine and tyrosine are given in Tables S1 and Table S4. Total energies calculated at the B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) and B3LYP/6-31+G(3df,2p)//B3LYP/6-31+G(d,p) levels on neutral and protonated phenylalanine, tyrosine, isopropylamine, and terbutylamine are given in Table S3. Contributions to entropy of translation, rotation, and vibration as given from B3LYP/6-31+G(d,p) optimized structures and hindered rotor computations using the Pitzer model are given in Tables S2 and Tables S6 for phenylalanine and tyrosine, respectively. Rotational barriers associated with the conformational changes of neutral and protonated phenylalanine are presented in section S5. This information is available free of charge via the Internet at http://pubs.acs.org.

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