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Proton affinities of methyl esters of *N*-acetylated amino acids

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Abstract

The proton affinities of the methyl esters of *N*-acetylated amino acids, Ac-Xxx-OMe, where Xxx is Gly, Leu, Phe, Pro, Glu and Arg have been calculated and characterized at B3LYP/6-31++G(d,p) and compared against experimentally measured values by the kinetic method. The proton is attached to the carbonyl group in five cases out of the six derivatized amino acids. Proton affinities of 15 derivatized amino acids have been measured experimentally. The average absolute deviation between theory and experiment is 1.2 kcal mol^{-1}. The proton affinities of all derivatized amino acids are higher than those of the corresponding underivatized parents, reflecting the ionic hydrogen bonding stabilization. A Bader analysis of the intramolecular hydrogen bonding within these protonated amides is presented. (Int J Mass Spectrom 219 (2002) 101–114)
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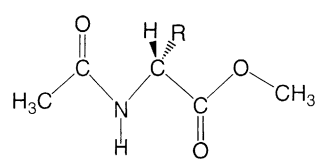
Keywords: *N*-Acetylated amino acids; Proton affinities; Methyl esters

1. Introduction

Amino acids are the basic structural units of peptides and proteins. Their structural and chemical properties have been heavily investigated by both theoretical and experimental techniques thereby providing valuable insights into the chemistries of peptides [1,2]. In a peptide, there are several functional groups that can interact intramolecularly after protonation via hydrogen bonds. Relatively little is known about the structural changes and the thermochemical differences that may occur as a result of protonation.

In this study, we calculate the proton affinities (PAs) of, and examine the ionic hydrogen bonding in, the methyl esters of *N*-acetylated amino acids, $CH_3CONHCHRCOOCH_3$, where R is the amino acid side chain.



The amino acid derivatives examined are Ac-Gly-OMe, Ac-Leu-OMe, Ac-Phe-OMe, Ac-Gln-OMe, Ac-Pro-OMe and Ac-Arg-OMe. Their calculated PAs will be compared with experimental PAs measured using Cooks' kinetic method [3–6]. These species serve as models for ionic hydrogen interactions of amide linkages in protonated peptides [7–10].

Complementary binding between functional groups and similarities between these groups are both key

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concepts in understanding molecular recognition in biochemical processes. However, calculating the chemical properties of large peptides is extremely time-consuming with current quantum chemical codes for all but the smallest systems. The practical approach is to study a small model system that contains the active site. In these biointeractions, the distribution of electron density ($\rho(r)$) is an important property describing the manner in which the electronic charge is distributed through real space and determining the appearance or shape of a molecule. The structure and bonding of molecules can thus be described in terms of topological characteristics of $\rho(r)$ and its Laplacian ($\nabla^2\rho$). This approach has been well documented by Bader [11]. Previously at the B3LYP/6-31++G(d,p) level of theory, we have found stabilizing 5- and 7-membered rings involving hydrogen bonds to stabilize protonated triglycine. The cyclization is a consequence of hydrogen bonding of the type O–H$\cdots$N and O–H$\cdots$O [12]. For protonated triglycine, calculating the location ($\boldsymbol{r}_b$) of the bond critical point (CP, defined by $\nabla\rho(\boldsymbol{r}_b) = 0$) of these hydrogen bonds, the value of $\rho(\boldsymbol{r}_b)$ and its Laplacian at this point, and the formation of ring critical points were found to be too computationally intensive. In this paper we are, however, able to implement these topological calculations on these smaller derivatized amino acid systems; this permits us to view the hydrogen bonding interactions using the Laplacian of the electron density.

The Cooks' kinetic method [3–6] is an effective method for measuring the relative proton affinities of nonvolatile bases, such as amino acids and peptides, as it does not involve generating a population of the neutral bases in the gas phase. The method is based on the dissociation of a proton-bound heterodimer of the bases, and the logarithm of the relative rate of dissociation of the two reaction channels is used to estimate the relative proton affinities of the two bases:

$$[B_i\cdots H\cdots B]^+ \rightarrow B + B_iH^+, \quad \text{rate constant} = k_i$$

$$[B_i\cdots H\cdots B]^+ \rightarrow B_i + BH^+, \quad \text{rate constant} = k$$

where B_i is a reference base whose proton affinity is known, and B is the unknown base whose proton affinity is being measured. From transition state theory and assuming that abundances reflect rate constants and that no reverse activation barriers exist [4] then

$$\ln\left(\frac{k_i}{k}\right) = \ln\left(\frac{[B_iH]^+}{[BH]^+}\right) = \ln\left(\frac{Q_i^*}{Q^*}\right) - \frac{PA}{RT_{eff}} + \frac{PA(i)}{RT_{eff}} \quad (1)$$

where $[B_iH]^+$ and $[BH]^+$ are the abundances of the protonated reference base and the unknown base, Q_i^* and Q^* are the partition functions of the activated complex of the reference base and that of the unknown base, PA(i) and PA are the respective proton affinities, R is the gas constant, and T_{eff} is a parameter in temperature units that reflects the internal energy of the dissociating heterodimer. For reference bases that are very similar in structure to the unknown base, it is frequently assumed that $Q_i^* = Q^*$ and the $\ln(Q_i^*/Q^*)$ term is zero. Thus, plotting $\ln([B_iH]^+/[BH]^+)$ versus PA(i) for a series of B_i's will yield a linear plot with the slope equal to $1/RT_{eff}$ and with the x-intercept equal to PA.

For cases in which B_i's are not structurally similar to B, but are structurally similar among themselves, plotting $\ln([B_iH]^+/[BH]^+)$ versus PA(i) for a series of B_i's will still yield a linear plot. However, the y-intercept is now $\ln(Q_i^*/Q^*) - PA/RT_{eff}$ but the slope is unchanged from $1/RT_{eff}$. Making the measurements at several collision energies (several T_{eff} values) enables several plots to be made. A new plot that charts the intercepts (i.e., $\ln(Q_i^*/Q^*) - PA/RT_{eff}$ values) versus the slopes (i.e., the $1/RT_{eff}$ values) will also be a straight line whose slope equals to the PA of the unknown base [5].

The aforementioned intercept-versus-slope plot is inherently straight as the two parameters are correlated [6]. This correlation is minimized if $\ln([B_iH]^+/[BH]^+)$ is charted against PA(i)–PA_{avg}, where PA_{avg} is the mean PA of the reference bases, instead of PA(i); the new slope in the intercept-versus-slope plot equals the difference between the PA of the unknown base and PA_{avg}. Removing the inherent correlation allows proper statistical evaluation of the quality of the data. The experimental

proton affinities reported in this paper have all been obtained using the above statistical treatment.

2. Computational methods

Each structure optimization exercise began with a conformational search using Monte Carlo simulations available in Spartan [13]. During the simulated annealing the molecule or ion was heated to 5000 K and subsequently cooled to 300 K. From each molecular mechanics conformational search, the relative energies were calculated, and those conformers whose energies were within 5 kcal mol^{-1} of the lowest energy structure were then optimized at higher, more reliable, levels of theory. The highest level of theory that we employed was the density functional theory (DFT) hybrid method, B3LYP, in conjunction with the 6-31++G(d,p) basis set [14–22] available in Gaussian 98 [23]. Table 1 shows the energetics of the lowest-energy neutral and protonated *N*-acetylated amino acid methyl esters calculated at this level of theory. All structures were characterized to be at minima via harmonic frequency calculations. Table 2 lists the proton affinities of the amino acid derivatives calculated from the electronic, rotational, vibrational and translational energies of the neutral and protonated molecules.

The gas-phase basicity (GB) of a base B is the free-energy change, $\Delta G^{\circ}_{r,298}$, whereas the proton affinity is the enthalpy change, $\Delta H^{\circ}_{r,298}$, of reaction 2.

$$BH^+ \rightarrow B^+H^+ \quad (2)$$

The GB and the PA of B are linked by

$$\Delta G^{\circ}_{r,298} = \Delta H^{\circ}_{r,298} - T\,\Delta S_{r,298} \quad (3)$$

$\Delta H^{\circ}_{r,298}$ may be calculated from results of molecular orbital calculations.

$$\Delta H^{\circ}_{r,298} = \Delta E_{elec} + \Delta E_{ZPVE}(0) + \Delta E_{int}(298) + \frac{5RT}{2} \quad (4)$$

where ΔE_{elec}, $\Delta E_{ZPVE}(0)$, and $\Delta E_{int}(298)$ refer to the changes in electronic energy, zero-point vibrational energy, and thermal energy required to calculate the enthalpy change of reaction 2 at 298.15 K, respectively. The constant $5RT/2$ is the classical estimation of the effect of gaining three translational degrees of

Table 1
Energetics of *N*-acetylated amino acid methyl esters

Molecule	Electronic energies (hartrees)	Thermal corrections[a]	$T\Delta S$ (kcal mol^{-1})
Acetamide	−209.23808	49.4	21.6
AcetamideH$^+$	−209.57970	57.9	20.7
Methyl acetate	−268.41003	60.1	23.3
Methyl acetateH$^+$	−268.73357	68.1	23.8
N-Methyl acetate	−248.54820	67.4	23.1
N-Methyl acetateH$^+$	−248.89973	76.6	23.7
Ac-Gly-OMe	−476.43689	97.7	30.8
Ac-Gly-OMeH$^+$	−476.79282	105.7	29.4
Ac-Gly-OMeH$^+$(a)	−476.77203	105.7	28.8
Ac-Leu-OMe	−633.70717	172.1	38.5
Ac-Leu-OMeH$^+$	−634.07073	180.1	37.5
Ac-Phe-OMe	−746.81914	170.2	40.2
Ac-Phe-OMeH$^+$	−747.18518	178.0	39.0
Ac-Phe-OMeH$^+$(a)	−747.17681	178.5	39.2
Ac-Pro-OMe	−593.16881	139.6	33.5
Ac-Pro-OMeH$^+$	−593.53907	147.7	32.5
Ac-Pro-OMeH$^+$(a)	−593.53274	148.0	33.8
Ac-Gln-OMe	−723.79261	154.5	38.6
Ac-Gln-OMeH$^+$	−724.16029	161.1	37.0
Ac-Gln-OMeH$^+$(a)	−724.16042	161.3	36.8
Ac-Arg-OMe	−798.57294	192.7	43.2
Ac-Arg-OMeH$^+$	−798.98591	201.1	42.6

[a] $(H_{298} - H_0) + \text{ZPE}$ in kcal mol^{-1}.

Table 2
Relative electronic energies, computed proton affinities and gas-phase basicities (all in kcal mol^{-1}) of *N*-acetylated amino acid methyl esters

Molecule	ΔE	PA	GB
Acetamide	214.4	207.3	198.6
Methyl acetate	203.0	196.5	189.2
N-Methyl acetate	220.6	213.0	205.7
Ac-Gly-OMe	223.3	216.9	207.7
Ac-Leu-OMe	228.1	221.7	212.9
Ac-Phe-OMe	229.7	223.4	214.3
Ac-Pro-OMe	232.3	225.8	217.0
Ac-Gln-OMe	230.7	225.6	216.2
Ac-Arg-OMe	259.1	252.2	243.8

freedom ($3RT/2$) for the proton plus RT, the PV work term for the proton. Furthermore,

$$T\,\Delta S_{r,298} = (298.15)[S(BH^+) - S(B)] - 7.8\,\text{kcal mol}^{-1} \quad (5)$$

where the constant $7.8\,\text{kcal mol}^{-1}$ is the entropy of the proton at 298.15 K. All entropies are taken from the vibrational frequency calculations computed from Gaussian 98. The basicity can then be determined by substituting Eqs. (4) and (5) into Eq. (3). Gas-phase basicities are also listed in Table 2.

The electron-density topology was determined using the AIMPAC series of programs, including the new AIM2000 software package [24]. The topological analysis invokes Bader's theory of "Atoms in Molecules" (AIMs) [11] to extract chemical information from wavefunctions. The AIM approach, analyzing the topological properties of charge density by determination of critical points, provides a method of viewing bonds and, in this study, is used to detect hydrogen bonds. The electron topology images were produced and viewed with the graphical user interface Molden [25].

3. Experimental

Experiments were conducted on an MDS SCIEX (Concord, Ont., Canada) triple–quadrupole mass spectrometer (TAGA 6000E). The electrospray probe was fabricated from an approximately 3-cm long, 33-gauge stainless steel tube (Hamilton, ca. 100 μm i.d.) that had been attached to a length of 1/16-in. o.d. stainless steel tube with epoxy glue. The probe tip was electropolished prior to use. Electrical biasing of the probe tip was achieved via a 50-MΩ current-limiting resistor in series with a high-voltage power supply (Tennelec, Model TC 950) set typically between 2.5 and 3.5 kV. The electrospray current was monitored via a custom-built microammeter that could be floated above the ground potential.

Gas-phase proton-bound heterodimers of the *N*-acetylated amino acid methyl esters were generated by means of electrospraying 50/50 water/methanol solutions containing 1 mM of the amino acid derivative and 1 mM of the reference base. Table 3 lists the reference bases used for each *N*-acetylated amino acid derivative. The amino acids were commercially available (Sigma, St. Louis); they were *N*-acetylated and *O*-methylated using standard procedures [26]. The abundances of the protonated bases were measured under essentially single-collision conditions with Ar as the neutral gas at four center-of-mass energies: 0.75, 1.25, 1.75 and 2.25 eV. For a given amino acid derivative, the $\ln([B_i H]^+/[BH]^+)$ values were plotted against $PA(i)$–PA_{avg} for the four collision energy conditions. The four intercepts were then charted against the four slopes to yield the PA of the *N*-acetylated amino acid methyl ester. Fig. 1 shows the results for Ac-Leu-OMe as an example.

Table 3
Reference bases and their proton affinity values in kcal mol^{-1}

Molecule	Proton affinity
Amides	
$CH_3CH_2CONH_2$	209.4
$HCON(CH_3)_2$	212.1
$CH_3CONHCH_3$	212.4
$CH_3CONHC_2H_5$	214.6
$CH_3CON(CH_3)_2$	217.0
Amines	
CH_3NH_2	214.9
$C_2H_5NH_2$	218.0
$C_3H_7NH_2$	219.4
$C_4H_9NH_2$	220.2
$C_5H_{11}NH_2$	220.7
$C_6H_{13}NH_2$	221.7
$C_8H_{17}NH_2$	222.0

4. Results and discussion

The amide group is the universal functional group in all proteins. The amino acid derivatives examined in this study have two types of carbonyl groups—the amide and the ester carbonyl groups. The intrinsic proton affinities of these two groups are revealed in an examination of the model amide, *N*-methyl acetamide, and the model ester, methyl acetate. The reference PAs [27] of the two are 212.4 and 196.4 kcal mol^{-1},

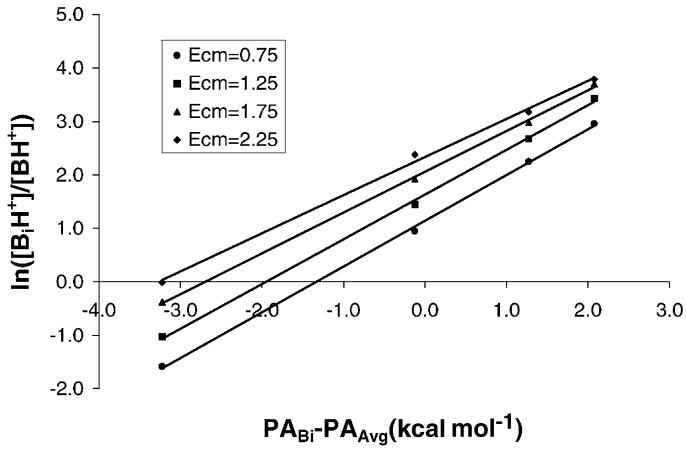
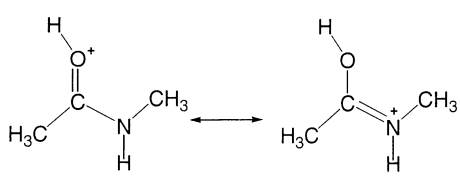


Fig. 1. Logarithms of the ratios of product ions against proton affinities of the reference bases at different collision energies for Ac-Leu-OMe.

respectively, the protonation site being the carbonyl oxygen in both instances. The higher proton affinity of *N*-methyl acetamide is due to resonance stabilization that places part of the positive charge on the nitrogen atom, to hyperconjugation from the C-methyl group, and to electron-donating effects of the *N*-methyl group.



These effects are less dramatic or absent in methyl acetate, thereby accounting for its much lower PA. From these data, it is apparent that protonation will likely occur on the amide carbonyl oxygen, rather than on the ester carbonyl oxygen of the amino acid derivative, provided that the side chain bears no functional group with a high proton affinity (for example, the guanidine group). Parenthetically, it may be debated that the *N*-acetylated amino acid amides would make better models of non-terminal residues in peptides and proteins than *N*-acetylated amino acid methyl esters. However, the protonation and bonding scenarios are comparable; the PA of *N*-methyl acetamide (the model of the N-terminal amide) at 212.4 kcal mol^{-1} is still considerably higher than that of acetamide (the model of the C-terminal amide) at 206.4 kcal mol^{-1}. Furthermore, derivatization to the amide is significantly more difficult than that to the ester.

4.1. *N*-Acetyl glycine methyl ester

Optimization of Ac-Gly-OMe at the B3LYP/6-31++G(d,p) level of theory yielded two lowest energy structures that are within 2.0 kcal mol^{-1} of each other in terms of free energies. The lower-energy conformer, Ac-Gly-OMe (see Fig. 2 for all optimized structures of neutral and protonated *N*-acetylated amino acid methyl esters), has its two carbonyl groups *trans* to each other, whereas the higher-energy conformer has theirs in the *cis* position. The lowest energy structure for protonated Ac-Gly-OMe is Ac-Gly-OMeH$^+$, in which the proton attaches to the amide carbonyl oxygen and hydrogen-bonds to the ester carbonyl oxygen, thus, resulting in a compact 7-membered ring that contains the O–H$\cdots$O interaction. The hydrogen bond has a length of 1.481 Å and the O–H$\cdots$O bond angle is 163.9°.

Contour line diagrams of the calculated Laplacian ($\nabla^2\rho$) concentration show that there are spheres of charge concentration and depletion surrounding each atom in a molecule. These spheres are distorted in unique ways that provide insight into electronic

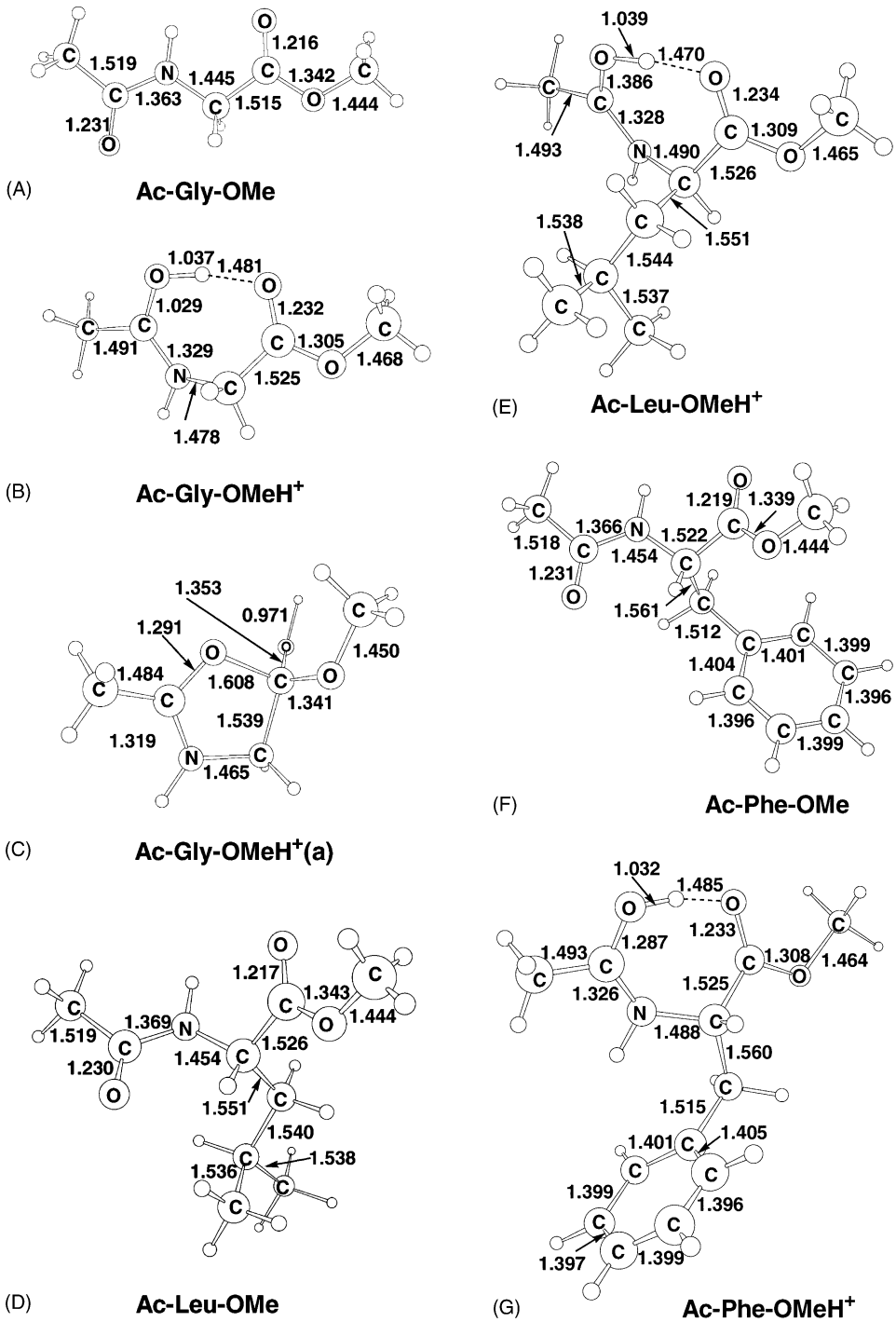
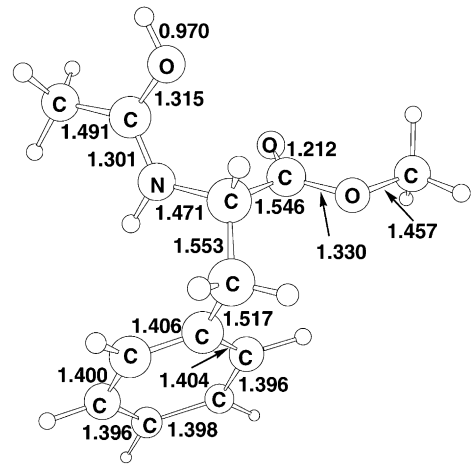
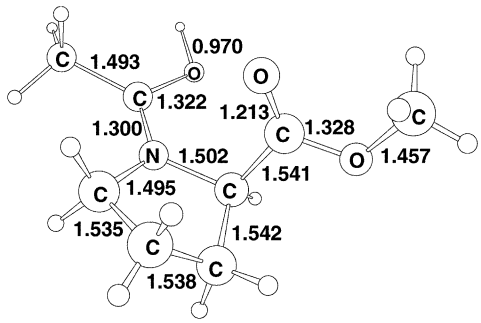


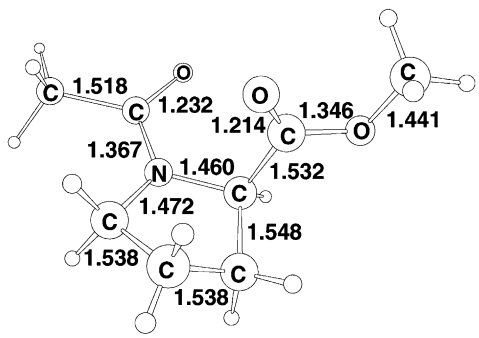
Fig. 2. Optimized structures for Ac-Xxx-OMe and Ac-Xxx-OMeH$^+$ at B3LYP/6-31++G(d,p) with bond lengths in Angstroms and bond angles in degrees.



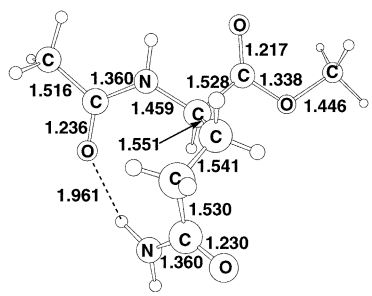
(H) **Ac-Phe-OMeH$^+$(a)**



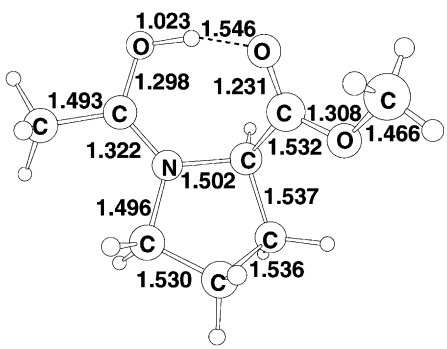
(K) **Ac-Pro-OMeH$^+$(a)**



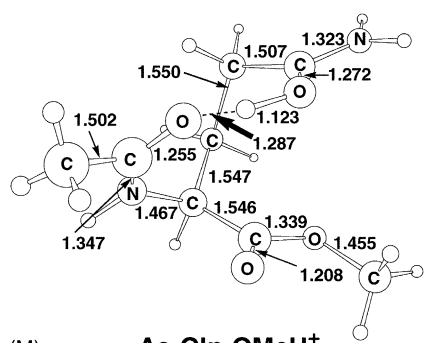
(I) **Ac-Pro-OMe**



(L) **Ac-Gln-OMe**

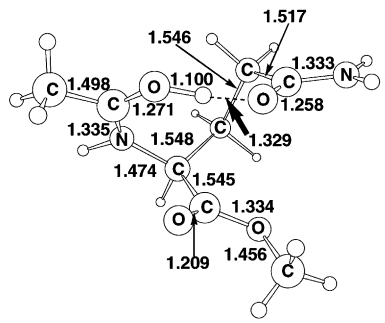


(J) **Ac-Pro-OMeH$^+$**

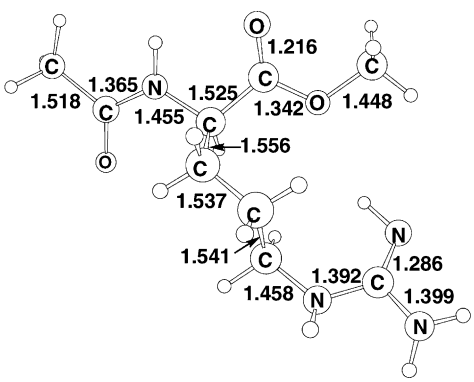


(M) **Ac-Gln-OMeH$^+$**

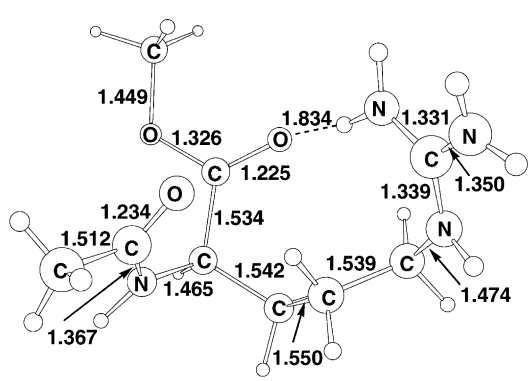
Fig. 2. (*Continued*).



(N) **Ac-Gln-OMeH$^+$(a)**



(O) **Ac-Arg-OMe**



(P) **Ac-Arg-OMeH$^+$**

Fig. 2. (*Continued*).

and bonding patterns and provide information on intramolecular hydrogen bonding. These types of interactions are dominated by the contraction of charge away from the interatomic surface towards each of the nuclei. Also the density $\rho(r)$ is relatively low in value and the Laplacian is positive. The sign of the Laplacian is determined by the positive curvature of ρ along the interaction line. If the nuclei are linked to form a ring then a ring critical point is found near the center. Bader analysis revealed that there is a ring critical point in this 7-membered ring (Fig. 3a) of Ac-Gly-OMeH$^+$ and an ionic bond within the O–H$\cdots$O interaction with a Laplacian of $+0.15$. In contrast, the O–H bond has a Laplacian of -1.44 and the electronic charge is concentrated in the internuclear region. These results thus confirm the existence of the internal H-bond in Ac-Gly-OMeH$^+$. Other conformers of protonated Ac-Gly-OMe, e.g., Ac-Gly-OMeH$^+$(a) which is an orthoester, contain 5-membered rings but with no hydrogen bonding. The computed proton affinity and basicity for Ac-Gly-OMe are 216.9 and 207.7 kcal mol^{-1}, respectively. The PA value agrees well with our experimental proton affinity of 216.3 ± 0.6 kcal mol^{-1}, and with the literature value of 217.2 ± 2.0 kcal mol^{-1} [27]. Density functional theory energetics deviate typically by ± 2.0 kcal mol^{-1} from the best experimental values [28,29].

4.2. *N*-Acetyl leucine methyl ester

Geometric optimization yielded two neutral conformers of Ac-Leu-OMe of comparable energy; the higher-energy structure is 0.8 kcal mol^{-1} above the global minimum, Ac-Leu-OMe. The structures of the two conformers are quite similar. In comparison with the glycine derivative, the leucine derivative possesses a bulkier alkyl side chain, which is more effective in spreading the proton's charge through its σ-system. As in Ac-Gly-OMeH$^+$, a 7-membered ring is formed when the proton attaches to the amide carbonyl oxygen and hydrogen-bonds to the ester carbonyl oxygen. However, in Ac-Leu-OMeH$^+$, a shorter hydrogen bond of 1.470 Å and a longer N–CR bond of 1.490 Å are formed. In a Bader plot (not shown here) a ring critical point is present. Four higher-energy, but structurally similar, protonated conformers are within 6.5 kcal mol^{-1} of the global minimum, in terms of

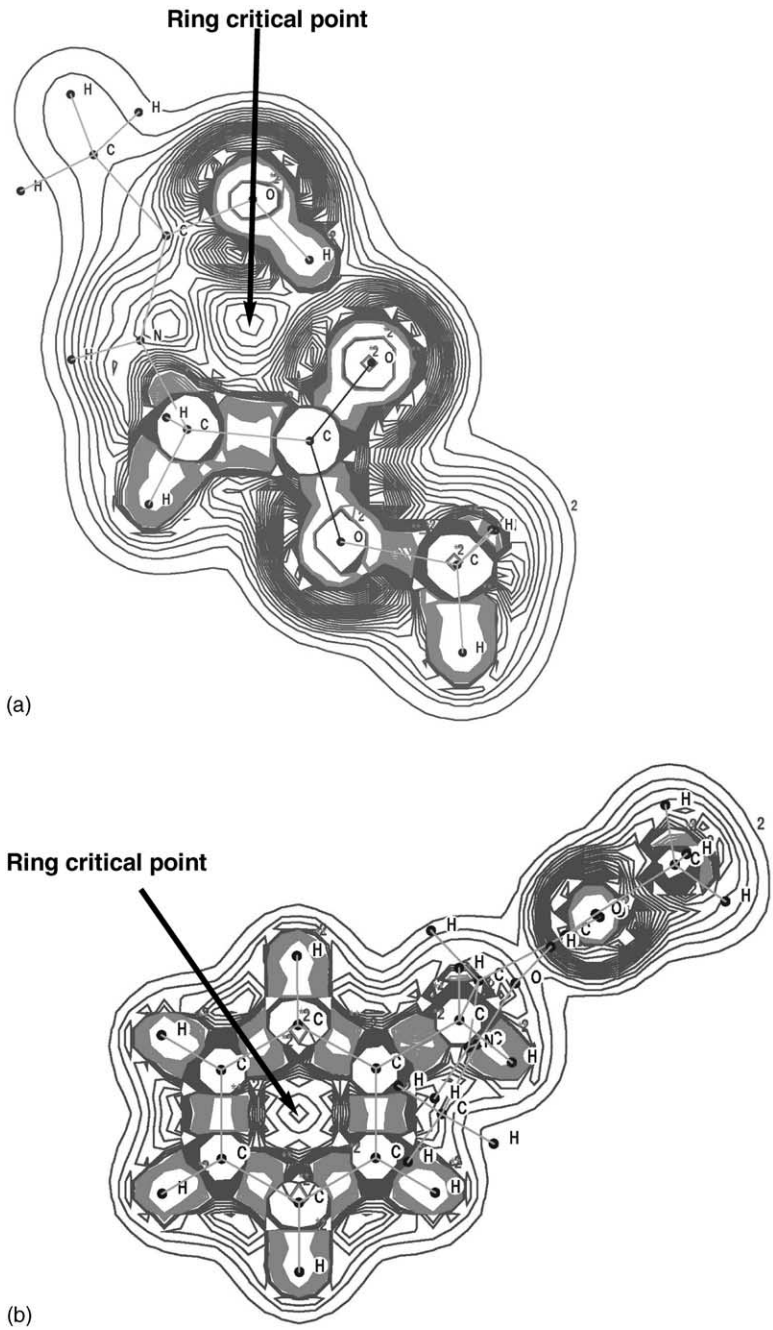
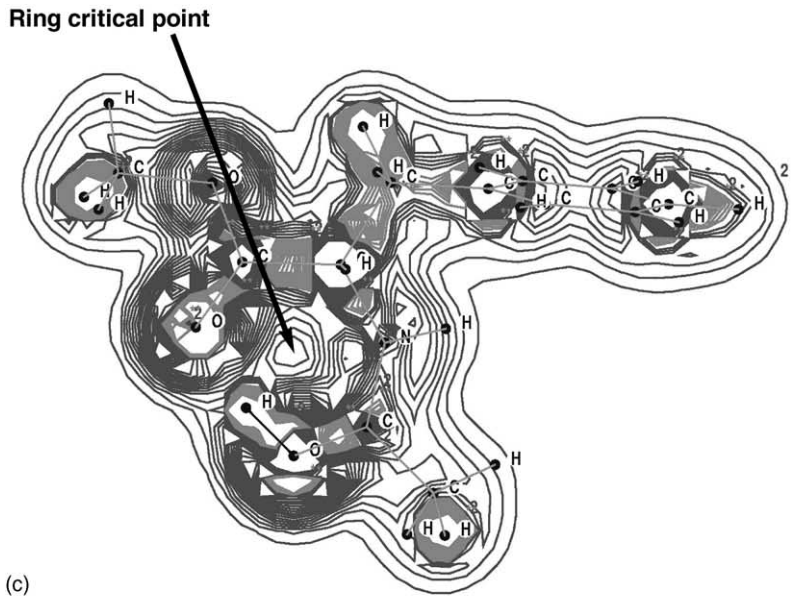
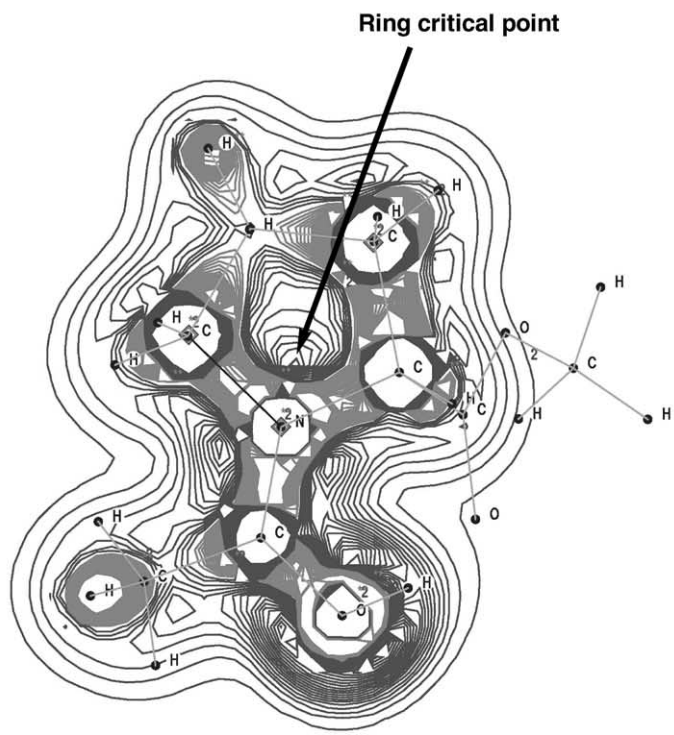


Fig. 3. Laplacian for (a) protonated Ac-Gly-OMe showing the ring critical point for the 7-membered ring; (b) protonated Ac-Phe-OMe showing the ring critical point for the 6-membered ring; (c) protonated Ac-Phe-OMe showing the ring critical point for the 7-membered ring; (d) protonated Ac-Pro-OMe showing the ring critical point for the 5-membered ring; (e) protonated Ac-Pro-OMe showing the ring critical point for the 7-membered ring.



(c)



(d)

Fig. 3. (*Continued*).

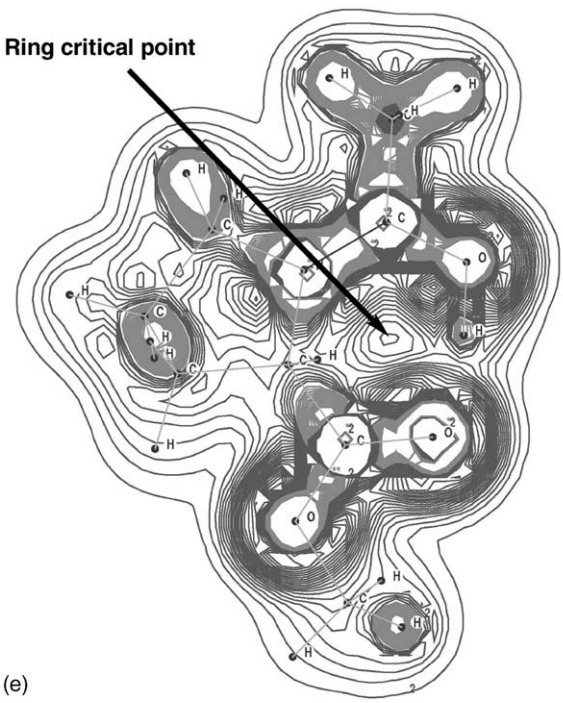


Fig. 3. (*Continued*).

free energies. The calculated proton affinity and basicity are 221.7 and 212.9 kcal mol^{-1}, respectively; these are 9.4 and 5.2 kcal mol^{-1} higher than those of Ac-Gly-OMe. In comparison, our experimental PA is 225.7 ± 1.6 kcal mol^{-1}.

4.3. N-Acetyl phenylalanine methyl ester

Two Ac-Phe-OMe conformers have been located that are within 0.8 and 0.4 kcal mol^{-1} of each other, in terms of enthalpies and free energies. The lower-energy conformer is structure Ac-Phe-OMe. As in the above two amino acid derivatives, the proton attaches to the amide carbonyl oxygen and hydrogen-bonds to the ester carbonyl oxygen, thereby producing a 7-membered ring, structure Ac-Phe-OMeH$^+$. This hydrogen bond is absent in a higher-energy conformer (Ac-Phe-OMeH$^+$(a)) and thus an estimate of the strength of the hydrogen bond can be obtained from the relative energies of these two structures. These are 6.4 kcal mol^{-1} in enthalpy and 5.7 kcal mol^{-1} in free energy. These energies are likely to have underestimated the hydrogen-bond energy as they also reflect the strain energies in the 7-membered ring. Bader analyses unambiguously established two ring critical points—one in the 6-membered phenyl ring (Fig. 3b) and the other in the 7-membered ring (Fig. 3c), as a result of the ionic hydrogen bond. The calculated proton affinity and basicity of Ac-Phe-OMe are 223.4 and 214.3 kcal mol^{-1}, respectively. The former compares well with the experimental proton affinity of 223.3 ± 0.2 kcal mol^{-1}.

4.4. N-Acetyl proline methyl ester

Proline has an unusual amino acid structure in that the amino nitrogen is in a 5-membered ring. Three neutral conformers of Ac-Pro-OMe were obtained in the conformational search and subsequent reoptimization, the highest-energy structure being 1.0 kcal mol^{-1} higher in free energy than that at the global minimum, Ac-Pro-OMe. Again, protonation results in the proton residing on the amide carbonyl oxygen and hydrogen-bonding to the ester carbonyl oxygen in the lowest energy structure, Ac-Pro-OMeH$^+$. The four lowest energy protonated conformers exist within a free-energy span of 16.3 kcal mol^{-1}. The energy differences between Ac-Pro-OMeH$^+$ and Ac-Pro-OMeH$^+$(a), in which the O–H···O hydrogen bond is absent, provide another estimate of the strength of hydrogen bonds in these structures. Here, values of 4.3 and 3.0 kcal mol^{-1} in terms of enthalpy and free energy are obtained. These estimates are lower than those in Ac-Phe-OMe, in accordance with the longer hydrogen bond, 1.546 Å, in Ac-Pro-OMeH$^+$ than the analogous bond, 1.485 Å, in Ac-Phe-OMeH$^+$. Bader analyses on the protonated proline derivative showed ring critical points in both the 5-membered proline ring (Fig. 3d) and the 7-membered, hydrogen-bonded ring (Fig. 3e). Calculated proton affinity and basicity are 225.8 and 217.0 kcal mol^{-1}, respectively. The former value is virtually identical to the experimental proton affinity of 225.9 ± 1.6 kcal mol^{-1}.

4.5. N-Acetyl glutamine methyl ester

The side chain of glutamine terminates in an amide group. Geometric optimization revealed three low lying Ac-Gln-OMe isomers that are within spans of 5.3 and 4.0 kcal mol^{-1} in terms of enthalpy and free energy. In Ac-Gln-OMe, the lowest energy structure, a 9-membered ring exists as a result of interaction of the type, N–H$\cdots$O, that involves the side chain amide N–H and the N-terminal carbonyl oxygen atom. In Ac-Gln-OMeH$^+$, the lowest energy conformer of protonated Ac-Gln-OMe, the proton resides on the carbonyl oxygen of the side chain amide group and hydrogen bonds to the N-terminal carbonyl oxygen. This isomer, however, is only lower in enthalpy by 0.1, and in free energy by 0.3 kcal mol^{-1}, than Ac-Gln-OMeH$^+$(a), an almost identical isomer in which the proton attaches to the carbonyl oxygen of the N-terminal amide group and hydrogen-bonds to the carbonyl oxygen of the side chain amide group. Both protonated Ac-Gln-OMe isomers, Ac-Gln-OMeH$^+$ and Ac-Gly-OMeH$^+$(a), contain a 9-membered ring as a consequence of hydrogen bonding. In Ac-Gln-OMeH$^+$, the hydrogen bond is quite short, 1.287 Å, and the O–H$\cdots$O bond angle is almost linear at 173.7°, both characteristics of a rather strong hydrogen bond. In Ac-Gly-OMeH$^+$(a), the hydrogen bond at 1.329 Å is considerably longer and weaker. The calculated proton affinity and basicity of Ac-Gln-OMe are 225.6 and 216.2 kcal mol^{-1}, respectively. The experimental proton affinity at 226.6 $\pm$ 2.9 kcal mol^{-1} is slightly higher but the theoretical PA is still within the experimental uncertainty.

4.6. N-Acetyl arginine methyl ester

Arginine's side chain terminates in a highly basic guanidine group. Four low energy conformers of neutral Ac-Arg-OMe were obtained. The lowest energy structure is Ac-Arg-OMe. Protonation of arginine results in attachment of the proton to the imino nitrogen of the guanidine group and hydrogen bonding to the ester carbonyl oxygen (Ac-Arg-OMeH$^+$) thereby forming a 10-membered ring with an N–H$\cdots$O type interaction. It is somewhat surprising that the guanidine proton prefers the ester carbonyl oxygen rather than the more basic amide carbonyl oxygen. This perhaps reflects a lower ring strain in the 10-membered ring as well as the strong resonance stabilization in the guanidine group. The hydrogen bond is relatively long at 1.834 Å with an N–H$\cdots$O bond angle of 171.5°. The proton affinity and basicity of Ac-Arg-OMe are 252.2 and 243.8 kcal mol^{-1}, respectively. There is no experimental proton affinity for Ac-Arg-OMe due to the lack of suitable reference bases of comparable proton affinities.

4.7. Proton attachment to N-acetylated amino acid methyl esters

In all six amino acid derivatives examined, proton attachment results in dicoordination of the proton. The proton attaches to the most basic site—in five cases out of six, a carbonyl oxygen atom—and then hydrogen bonds to a carbonyl oxygen atom. Thus, it is apparent that intramolecular ionic hydrogen bonds must be a regular feature in protonated peptides. However, in protonated oligopeptides the formation of proton bridges between adjacent residues is in competition with those between residues that are further away in the sequence, but may be nearby through space in a given conformation. We have not observed any conformers that have the proton in a coordination number larger than two in amino acid derivatives that contain more than two basic sites, e.g., Ac-Gln-OMe and Ac-Arg-OMe.

Of the five amino acid derivatives for which there are both DFT and kinetic method results, the average absolute deviation between the two sets is 1.2 kcal mol^{-1}. By comparison, the average uncertainty in the five experimental PA data is also 1.2 kcal mol^{-1}. Thus, the difference between the DFT and experimental results may be entirely due to experimental uncertainty; however, there are too few data points to draw definite conclusions. As pointed out earlier, density functional theory energetics deviate typically by no more than 2 kcal mol^{-1} from the best experimental values [28,29]. The deviations observed

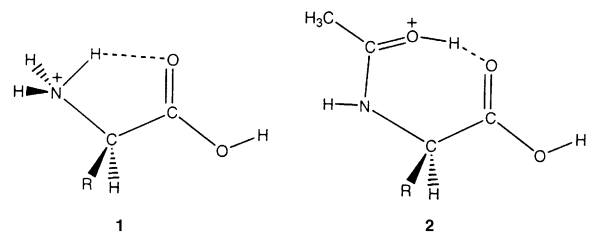
Table 4
Experimental proton affinities (kcal mol^{-1}) of Ac-Xxx-OMe as obtained by the kinetic method

Ac-Xxx-OMe	Proton affinity	Uncertainties	PA(Xxx)[a]
Ac-Gly-OMe	216.3	0.6	211.9
Ac-Ala-OMe	221.0	0.4	215.5
Ac-Val-OMe	223.3	1.7	217.6
Ac-Leu-OMe	225.7	1.6	218.6
Ac-Ile-OMe	225.3	0.8	219.3
Ac-Phe-OMe	223.3	0.2	220.6
Ac-Pro-OMe	225.9	0.7	220.0
Ac-Asn-OMe	230.5	1.0	222.0
Ac-Gln-OMe	226.6	2.9	224.1
Ac-Ser-OMe	223.6	1.5	218.6
Ac-Met-OMe	224.5	2.0	223.6
Ac-Cys-OMe	220.6	0.6	215.9
Ac-Thr-OMe	223.4	0.6	220.5
Ac-Trp-OMe	228.8	1.8	226.8
Ac-Tyr-OMe	224.4	0.6	221.0

[a] Values for amino acids taken from [27].

in this study are entirely consistent with this general trend. Table 4 lists the kinetic method results of the five aforementioned *N*-acetylated amino acid methyl esters plus those of another ten measured along with them. The reference PAs [27] of the unmodified amino acids are also listed for comparison. It is evident that the PAs of all amino acid derivatives are higher, by 0.9–8.5 kcal mol^{-1}, than those of their unmodified analogues, apparently reflecting the stabilizing effect of the ionic proton bridge and better resonance stabilization of the *N*-acetyl group. A few of the amino acid derivatives such as those of methionine and glutamine have proton affinities that are similar, 0.9 and 2.5 kcal mol^{-1}, to those of their parent amino acids. These derivatives have basic side chains that contain two or more methylene groups thereby providing the flexibility to create larger rings containing hydrogen bonds. These interactions then stabilize the protonated parent amino acids making their proton affinities comparable to the derivatized counterparts [30].

Primary amines and secondary amides have similar proton affinities (methylamine and *N*-methyl acetamide have values of 214.9 and 212.4 kcal mol^{-1}, respectively). The higher proton affinities of the methyl esters of *N*-acetylated amino acids (secondary amides) relative to those of the corresponding amino acids (amines) (Table 4), are attributable to the larger ring sizes of the internally hydrogen bonded cations formed by protonation of the amides. Glycine has a slightly lower proton affinity than methylamine (211.9 compared to 214.9 kcal mol^{-1}). This is due to the electron-withdrawing properties of the carboxylic acid group that is somewhat compensated for by the internal hydrogen bond in the cation (structure **1**, R=H).



The 5-membered ring in **1** provides far from optimum conditions for a hydrogen bond (N–H···O are not colinear). Esters of *N*-acetylated amino acids protonate on the oxygen of the amide group and the resulting cations are stabilized by hydrogen bonds to the carbonyl oxygen of the ester (structure **2**, R=H). In this structure, there is a 7-membered ring, and this provides a more optimal arrangement for the hydrogen bond. The result is a reversal of the relative proton affinities of the amine and the secondary amide, with glycine having a proton affinity 4.4 kcal mol^{-1} lower than that of the methyl ester of *N*-acetylated glycine. A similar situation occurs with leucine and proline, and their *N*-acetylated derivatives. In larger systems such as protonated triglycine both interactions **1** and **2** are evident in the lowest free energy isomers, but it is the hydrogen bonding of type **2** that results in the lowest free energy [12]. Although the hydrogen bond length for Ac-Gly-OMeH$^+$ is short (1.481 Å), the hydrogen bond is best visualized and characterized by the Laplacian of the density shown in Fig. 3a where the 7-membered ring with type **2** interaction is shown. Fig. 3c and e also show this type of interaction for Ac-Phe-OMeH$^+$ and Ac-Pro-OMeH$^+$, respectively.

Protonation of phenylalanine gives an NH_3^+ group that can be stabilized by two interactions simultaneously—one with the carbonyl oxygen as

in **1**, and the other with the π-system of the phenyl ring. This enhanced interaction results in a proton affinity close to that of the *N*-acetyl derivative, where stabilization of the cation occurs through only one interaction (see **2**). This is also the case for tryptophan and tyrosine.

Unlike in other amino acids examined here, in glutamine there are two sites of similar basicities (NH_2 and $CONH_2$) and the added proton will attach to one site and hydrogen bond to the other. For protonated glutamine, the resulting ring involving the hydrogen bond contains seven atoms, while for the corresponding acetyl derivative the ring is 9-membered. Rings of these sizes can both comfortably accommodate a hydrogen bond, so the compounds have similar proton affinities.

5. Conclusion

The proton affinities of fifteen methyl esters of *N*-acetylated amino acids measured by the kinetic method span a range of 216.3–230.5 kcal mol^{-1}. They are all larger than the proton affinities of the corresponding amino acids with the differences varying by 0.9–8.5 kcal mol^{-1}. The calculated structures of six of these derivatized amino acids indicate that the dominant interaction is a hydrogen bond between the two carbonyl groups forming a 7-membered ring. This hydrogen bond can be best visualized and characterized by Bader plots of the Laplacian of the electron density.

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