# Proton Affinity of Uracil. A Computational Study of Protonation Sites

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Relative stabilities of uracil tautomers and cations formed by gas-phase protonation were studied computationally with the B3LYP, MP2, QCISD, and QCISD(T) methods and with basis sets expanding from 6-31G(*d*,*p*) to 6-311+G(3*df*,2*p*). In accordance with a previous density functional theory study, the dioxo tautomer **1a** was the most stable uracil isomer in the gas phase. Gibbs free energy calculations using effective QCISD(T)/6-311+G(3*df*,2*p*) energies suggested >99.9% of **1a** at equilibrium at 523 K. The most stable ion isomer corresponded to *N*-1 protonated 2,4-dihydroxypyrimidine, which however is not formed by direct protonation of **1a**. The topical proton affinities in **1a** followed the order O-8 > O-7 > C-5 > N-3 > N-1. The thermodynamic proton affinity of **1a** was calculated as 858 kJ $mol^{-1}$ at 298 K. A revision is suggested for the current estimate included in the ion thermochemistry database. (J Am Soc Mass Spectrom 2000, 11, 1065–1071) © 2000 American Society for Mass Spectrometry

The thermochemistry of gas-phase ions derived from biologically important molecules has been studied extensively, in particular following the advent of soft ionization methods such as electrospray [1] and matrix-assisted laser desorption [2] that allow for facile formation of large ions in the gas phase. With ions formed by protonation, the gas-phase basicity and proton affinity are the two most important energy values that link the ion thermochemistry to that of neutral molecules. Unfortunately, proton affinities of polar multifunctional molecules, an attribute common to most biologically interesting molecules such as amino acids, peptides, nucleobases, nucleosides, etc., are difficult to measure by reliable experimental methods that are based on low pressure equilibria in ion-cyclotron resonance mass spectrometers [3], or high-pressure equilibria in pulse ionization methods [4, 5]. Most experimental data produced so far originated from competitive dissociations of proton-bound dimers (the kinetic method) [6]. In addition, experimental methods based on either equilibrium or kinetic measurements probe the most stable ion structures corresponding to protonation at the most basic sites in the molecule. Hence, topical proton affinities of less basic sites in multifunctional molecules are difficult to obtain by experimental methods.

Our interest in topical proton affinities is motivated by the use of ion–molecule reactions to generate well defined ion structures that can be used as precursors for the generation of transient radicals by fast electron transfer [7]. In this context, ab initio and density functional theory have been used previously to obtain topical proton affinities for several systems [8–11]. In the present work we report calculations investigating the gas-phase protonation of the RNA nucleobase uracil **(1)**. Uracil is an interesting model in that it can exist in several tautomers each providing several potential protonation sites leading to a multitude of ion isomers. The goal of this work was to determine the structures and energetics of protonated uracil isomers and draw conclusions on the formation of gas-phase ions by protonation of uracil. Topical proton affinities in the most stable uracil tautomer have been addressed previously at various levels of theory [12–14]. The present work provides a comprehensive view of protonated uracil isomers. It is the first study to address the stabilities of both neutral and cation tautomers at a high level of theory. Very recent MP4(SDTQ)/6-31+G(d,p) calculations reported the proton affinity of uracil as 857 kJ $mol^{-1}$ in agreement with our results [15].

## Calculations

Standard calculations were performed using the Gaussian 94 [16] and Gaussian 98 [17] suites of programs. Geometries were optimized with HF/6-31G(*d*,*p*) to obtain local energy minima that were characterized by harmonic frequency analyses (all frequencies real). HF frequencies were scaled by 0.893 [18] and used to calculate zero-point energy and 298 K enthalpy corrections. Selected structures were also optimized with density functional theory [19, 20] calculations using Becke's hybrid functional (B3LYP) [21, 22] and the 6-31+G(*d*,*p*) basis set including vibrational analysis.

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The B3LYP harmonic frequencies were scaled by 0.963 [23]. Vibrational enthalpies were treated within the rigid-rotor-harmonic oscillator approximation. Spin restricted calculations were used for all closed-shell systems. Improved energies were obtained from single-point calculations at several levels of theory. Moller–Plesset perturbational theory [24] truncated at second order, MP2(frozen core), was used with the 6-311G(2*d*,*p*), 6-311+G(2*d*,*p*), 6-311+G(2*df*,*p*), and 6-311+G(3*df*,2*p*) basis sets to account for correlation energy effects. In addition, two composite procedures were adopted that consisted of quadratic configuration interaction calculations [25], QCISD/6-31G(*d*,*p*) and QCISD(T)/6-31G(*d*,*p*), and basis set expansions through MP2 calculations to an effective QCISD/6-311+G(2*d*,*p*), QCISD(T)/6-311+G(2*df*,*p*), and QCISD(T)/6-311+G(3*df*,2*p*) levels of theory according to

$$\text{QCISD/6-311+G(2}d,p\text{)} \approx \text{QCISD/6-31 G(}d,p\text{)} + \text{MP2/6-311+G(2}d,p\text{)} - \text{MP2/6-31G(}d,p\text{)} \quad (1)$$

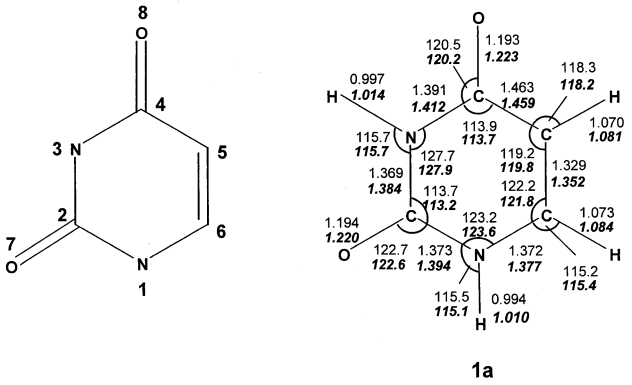
$$\text{QCISD(T)/6-311+G(2}df,p\text{)} \approx \text{QCISD(T)/6-31G(}d,p\text{)} + \text{MP2/6-311+G(2}df,p\text{)} - \text{MP2/6-31G(}d,p\text{)} \quad (2)$$

$$\text{QCISD(T)/6-311+G(3}df,2p\text{)} \approx \text{QCISD(T)/6-31 G(}d,p\text{)} + \text{MP2/6-311+G(3}df,2p\text{)} - \text{MP2/6-31G(}d,p\text{)} \quad (3)$$

Similar effective levels of theory, that are analogous to the Gaussian 2(MP2) [26] and (MP2,SVP) [27] schemes, were used previously for treating the energetics of several heterocyclic molecules, ions, and radicals [7, 28–32]. Density functional theory calculations were also performed using Becke's hybrid functional (B3LYP) and the 6-311G(2*d*,*p*), 6-311+G(2*d*,*p*), and 6-311+G(2*df*,*p*) basis sets. We showed previously that simple averaging of MP2 and B3LYP relative energies provided an improved fit to experimental data for several radical and cationic systems [11, 28–32] and the method (denoted B3-MP2) has recently been used successfully to calculate gas-phase acidities and anion relative energies [33].

## Results and Discussion

The two carbonyl groups in uracil allow the existence of several tautomers that have been studied previously by theory, as summarized recently [34]. The HF/6-31G(*d*,*p*) optimized structures of the six lowest-energy tautomers **1a–1f** were unexceptional and showed standard bond lengths and angles in line with previous calculations. A similar conclusion followed from the comparison of the HF and B3LYP-optimized structures for **1a** that showed only minor differences (Figure 1). Previous calculations and experimental studies [35–48] agreed on structure **1a** as the most stable uracil tautomer. The present calculations identified **1a** (Figure 1) and the next five most stable uracil tautomers as shown in Figure 2. The dioxo tautomer **1a** was the most stable structure at all present levels of theory. The energy differences discussed in the text refer to 298 K relative enthalpies from effective QCISD/6-311+G(2*d*,*p*) calculations. The relative enthalpies from the other calculations are summarized in Table 1. Enolization of the N-1–C-2–O-7 system led to the second most stable tautomer **1b**, which was 46 kJ mol$^{-1}$ less stable than **1a**. Interestingly, enolization of the N-3–C-4–O-8 system was more destabilizing compared with that of the N-1–C-2–O-7 system and resulted in tautomer **1c**, which was 50 kJ mol$^{-1}$ less stable than **1a**. Double enolization had a weak additional destabilizing effect, as shown by the 2,4-dihydroxypyrimidine structure **1d** which was 52 kJ mol$^{-1}$ less stable than **1a**. The two least stable structures corresponded to the enaminoimide structure **1e** formed by enolization of the O-7–C-2–N-3 system, and the iminodienol structure **1f** formed by homoenolization of the O-8–C-4–C-5–C-6–N-1 system, which, respectively, were 77 and 103 kJ mol$^{-1}$ less stable than **1a**.




**1a**

**Figure 1.** Optimized geometry of **1a**. Bond lengths in angstroms, bond and dihedral angles in degrees. Roman numerals: HF/6-31G(*d*,*p*) parameters. Bold italics: B3LYP/6-31+G(*d*,*p*) parameters.

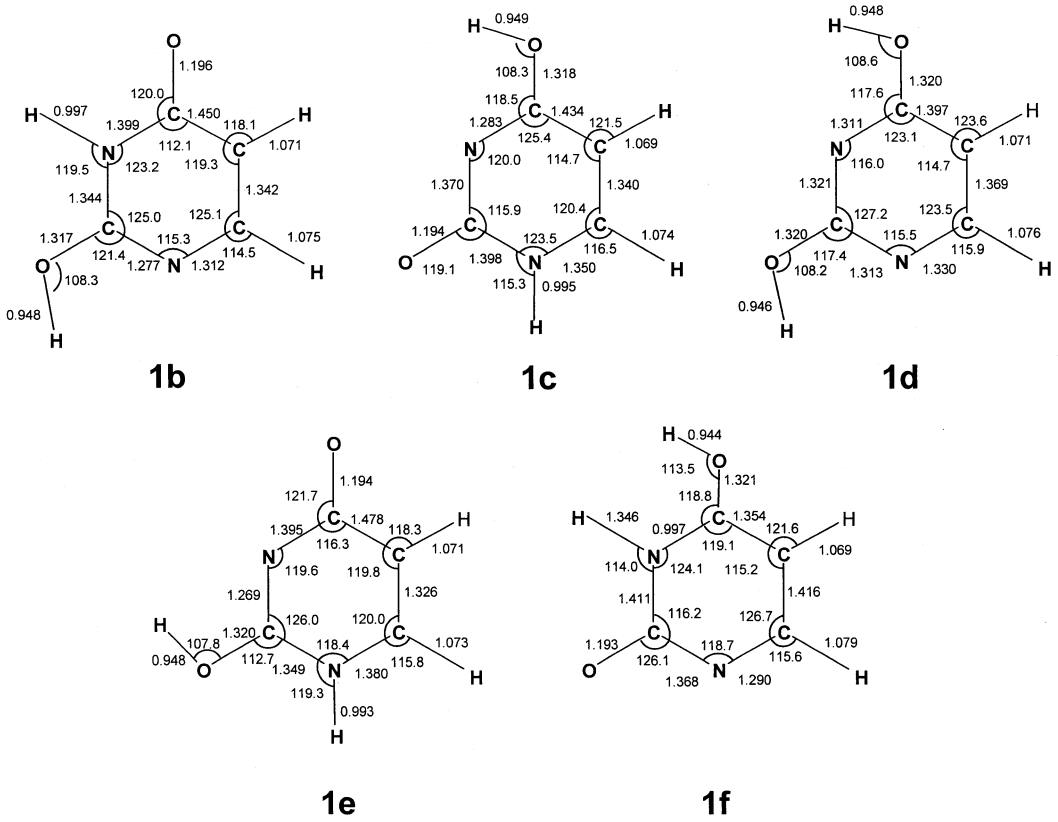



**Figure 2.** HF/6-31G(*d*,*p*) optimized geometries of **1b–1f**. Bond lengths in angstroms, bond and dihedral angles in degrees.

B3LYP, and QCISD relative energies were very similar, e.g., within 9 kJ mol$^{-1}$ absolute deviations and 4.9 and 2.6 kJ mol$^{-1}$ root-mean-square deviations for comparing the MP2 and B3LYP data, respectively, with those from the presumably "best" effective QCISD calculations. Considering that the above procedures use very different formalisms to account for electron correlation, the close consistency of the relative energies lends credit to the results.

The relative enthalpies together with the calculated entropies ($S_T$) were used to calculate the relative free energies and hence the equilibrium composition of the tautomer mixture in the gas phase. This was calculated for the typical temperature of a chemical ionization ion source used for uracil protonation (~523 K). Because the $S_{523}$ values were very similar for **1a–1f** (404–411 J mol$^{-1}$ K$^{-1}$), the equilibria were largely determined by the differences in the tautomer energies. For example, the free energy difference $\Delta G_{523}$(**1a**→**1b**) = 47.3 kJ mol$^{-1}$ indicates that there should be 99.99% of the more stable tautomer **1a** at gas phase equilibrium at 523 K. Hence, gas-phase protonation of uracil is predicted to occur exclusively in the most stable tautomer **1a**.

**Table 1.** Relative energies of uracil tautomers **1a–1f**

| | Relative energy[a] | | | | | |
|---|---|---|---|---|---|---|
| Method | **1a** | **1b** | **1c** | **1d** | **1e** | **1f** |
| MP2/6-311G(*d*,*p*) | 0 | 43 | 47 | 43 | 78 | 100 |
| MP2/6-311+G(2*d*,*p*) | 0 | 43 | 47 | 43 | 75 | 99 |
| B3LYP/6-311G(*d*,*p*) | 0 | 47 | 49 | 52 | 82 | 99 |
| B3LYP/6-311+G(2*d*,*p*) | 0 | 46 | 49 | 52 | 80 | 98 |
| QCISD/6-31G(*d*,*p*) | 0 | 48 | 55 | 48 | 83 | 108 |
| QCISD/6-311+G(2*d*,*p*) | 0 | 46 | 50 | 52 | 77 | 103 |

[a]In units of kJ mol$^{-1}$ at 298 K.

## *Uracil Cations*

Protonation of uracil tautomers can, in principle, take place at any C, N, or O atom to form tautomeric ions. The 18 most stable cation tautomers are shown in Figure 3. In addition, some ions existed as *syn* and *anti* rotamers differing in the dihedral angles of the O–H bonds. Only the more stable rotamers are listed here. The ion tautomers are marked according to the proton attachment sites, e.g., **178**$^+$ for the N-1 protonated dihydroxypyrimidine that was the most stable ion (Table 2). Note that ion **178**$^+$ can be formed convergently by protonating the N-1 position in **1d**, the O-7 position in **1c**, and the O-8 position in **1e**, but not by protonation of the two most stable uracil tautomers **1a** and **1b**. The protonation exothermicities are expressed as the topical proton affinities of the uracil tautomers and summarized in Table 3. The second most stable ion,

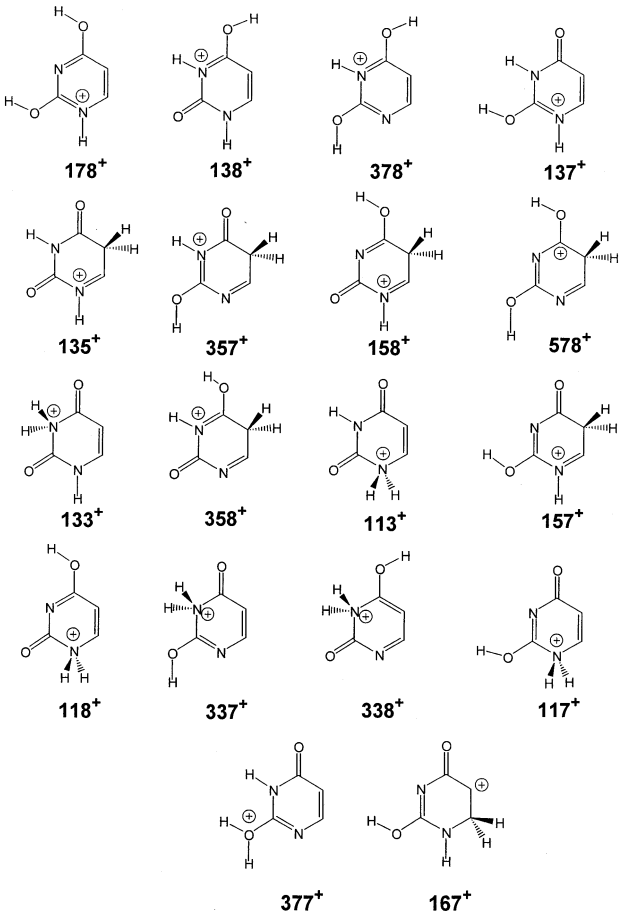



**Figure 3.** Structures of uracil cations.

$\mathbf{138}^+$, was only 5–10 kJ mol$^{-1}$ less stable than isomer $\mathbf{178}^+$ at several levels of theory (Table 2). Ion $\mathbf{138}^+$ can also be formed by protonation of O-8 in **1a**, N-3 in **1c**, or N-1 in **1f**. Protonation at O-7 in **1a** was 30–35 kJ mol$^{-1}$ less exothermic than at O-8 so that ion $\mathbf{137}^+$ was the fourth most stable isomer. Note that ion $\mathbf{137}^+$ can also be formed by protonation at N-1 in **1b**. Protonation at C-5 in **1a–1d** was generally less favorable than at the heteroatoms. For example, isomer $\mathbf{135}^+$ formed by protonation of **1a** at C-5 was 72 kJ mol$^{-1}$ less stable than the most stable isomer $\mathbf{178}^+$.

Protonation at C-5 can be accommodated by the electronic system of the pertinent uracil tautomer to form ions that can be expressed by conjugated canonical structures, e.g., $\mathbf{135}^+$, $\mathbf{357}^+$, $\mathbf{158}^+$, $\mathbf{578}^+$, $\mathbf{358}^+$, and $\mathbf{157}^+$ that all represent stable ion structures. By contrast, protonation at C-6 forms an $\alpha$-carbonyl carbocation moiety at C-5 that is known to be strongly destabilizing in aliphatic [49] and heterocyclic cations [29]. Consequently, starting structures in which a proton was attached to C-6 often underwent spontaneous isomerization by proton migration to C-5 or N-1. The only stable C-6- protonated ion structure found by the present study was that for ion $\mathbf{167}^+$, which was a high-energy isomer destabilized against $\mathbf{178}^+$ by >350 kJ mol$^{-1}$ (Table 2) and thus of little practical importance.

Protonation at the hydroxyl groups and amide nitrogen atoms was also less favorable. The O-7-H group in **1b** was the most basic one of those studied, and its protonation formed the high-energy ion $\mathbf{377}^+$. The N-3

**Table 2.** Relative energies of uracil cations

| | Relative energy[a,b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cation | B3LYP/ 6-311G(2*d*,*p*) | MP2/ 6-311G(2*d*,*p*) | | B3LYP/[c] 6- 311+G(2*df*,*p*) | MP2/[c] 6-311+G(2*df*,*p*) | | QCISD/[b] 6-311+G(2*d*,*p*) | QCISD(T)/[c] 6-311+G(2*df*,*p*) | QCISD(T)/[c] 6-311+G(3*df*,2*p*) |
| $\mathbf{178}^+$ | 0 | 0[d] | 0 | 0 | 0[d] | 0 | 0 | 0 | 0 |
| $\mathbf{138}^+$ | 5 (5)[e] | 7 | 9 (9)[e] | 4 | 7 | 10 (9)[f] | 7 | 10 | 9 |
| $\mathbf{378}^+$ | 31 (30)[e] | 32 | 32 (32)[e] | 31 | 32 | 32 (32)[f] | 33 | 32 | 32 |
| $\mathbf{137}^+$ | 45 (45)[e] | 44 | 44 (44)[e] | 43 | 43 | 44 (42)[f] | 38 | 42 | 40 |
| $\mathbf{135}^+$ | 86 (86)[e] | 84 | 83 (83)[e] | 87 | 87 | 88 (87)[f] | 72 | 83 | 82 |
| $\mathbf{357}^+$ | 100 | 101 | 102 | | | | | | |
| $\mathbf{158}^+$ | 120 | 119 | 118 | | | | | | |
| $\mathbf{578}^+$ | 122 | 126 | 130 | | | | | | |
| $\mathbf{133}^+$ | 130 (131) | 128 | 125 (125) | | | | 124 | | |
| $\mathbf{358}^+$ | 142 | 140 | 139 | | | | | | |
| $\mathbf{113}^+$ | 150 (149) | 144 | 139 (144) | | | | 132 | | |
| $\mathbf{157}^+$ | 168 | 168 | 168 | | | | | | |
| $\mathbf{118}^+$ | 172 | 170 | 168 | | | | | | |
| $\mathbf{337}^+$ | 199 | 196 | 194 | | | | | | |
| $\mathbf{338}^+$ | 221 | 221 | 221 | | | | | | |
| $\mathbf{117}^+$ | 236 | 231 | 226 | | | | | | |
| $\mathbf{377}^+$ | 340 | 337 | 335 | | | | | | |
| $\mathbf{167}^+$ | 334 | 352 | 369 | | | | | | |

[a]In units of kJ mol$^{-1}$ at 298 K.
[b]From single-point calculations on HF/6-31G(*d*,*p*) optimized structures and using HF/6-31G(*d*,*p*) zero-point energies and 298 K enthalpies.
[c]From single-point calculations on B3LYP/6-31+G(*d*,*p*) optimized structures and using B3LYP/6-31+G(*d*,*p*) zero-point energies and 298 K enthalpies.
[d]Averaged B3-MP2 relative energies.
[e]Calculated with the 6-311+G(2*d*,*p*) basis set.
[f]Calculated with the 6-311+G(3*df*,2*p*) basis set.

**Table 3.** Topical proton affinities in **1a–1f**[a]

| Tautomer | Method | Position | | | | |
|---|---|---|---|---|---|---|
| | | N-1 | N-3 | C-5 | O-7 | O-8 |
| **1a** | MP2/6-311G(2*d*,*p*) | 735 | 750 | 792 | 831 | 865 |
| | B3LYP/6-311G(2*d*,*p*) | 734 | 753 | 797 | 838 | 878 |
| | MP2/6-311+G(2*d*,*p*) | 718 | 732 | 775 | 813 | 848 |
| | B3LYP/6-311+G(2*d*,*p*) | 718 | 737 | 782 | 822 | 863 |
| | MP2/6-311+G(2*df*,*p*) | | | 767 | 812 | 845 |
| | MP2/6-311+G(3*df*,2*p*) | | | 769 | 815 | 848 |
| | B3LYP/6-311+G(2*df*,*p*) | | | 780 | 824 | 863 |
| | B3-MP2/6-311+G(2*df*,*p*) | | | 773 | 818 | 854 |
| | QCISD/6-311+G(2*d*,*p*) | 731 | 738 | 792 | 825 | 857 |
| | QCISD(T)/6-311+G(2*df*,*p*) | | | 782 | 824 | 855 |
| | QCISD(T)/6-311+G(3*df*,2*p*) | | | 784 | 826 | 858 |
| **1b** | MP2/6-311G(2*d*,*p*) | 917 | 886 | 787 | | |
| | B3LYP/6-311G(2*d*,*p*) | 935 | 899 | 813 | | |
| | MP2/6-311+G(2*d*,*p*) | 901 | 869 | | | |
| | B3LYP/6-311+G(2*d*,*p*) | 920 | 890 | | | |
| | QCISD/6-311+G(2*d*,*p*) | 916 | 883 | | | |
| **1c** | MP2/6-311G(2*d*,*p*) | 754 | 854 | 804 | 917 | |
| | B3LYP/6-311G(2*d*,*p*) | 760 | 867 | 812 | 935 | |
| | MP2/6-311+G(2*d*,*p*) | | 836 | | 901 | |
| | B3LYP/6-311+G(2*d*,*p*) | | 851 | | 920 | |
| | QCISD/6-311+G(2*d*,*p*) | | 897 | | 916 | |
| **1d** | MP2/6-311G(2*d*,*p*) | 917 | 886 | 788 | | |
| | B3LYP/6-311G(2*d*,*p*) | 935 | 899 | 813 | | |
| | MP2/6-311+G(2*d*,*p*) | 901 | 869 | | | |
| | B3LYP/6-311+G(2*d*,*p*) | 920 | 890 | | | |
| | QCISD/6-311+G(2*d*,*p*) | 916 | 884 | | | |
| **1e** | MP2/6-311G(2*d*,*p*) | 726 | 874 | 784 | 583 | 952 |
| | B3LYP/6-311G(2*d*,*p*) | 729 | 884 | 797 | 631 | 965 |
| | MP2/6-311+G(2*d*,*p*) | | 889 | | | 933 |
| | B3LYP/6-311+G(2*d*,*p*) | | 902 | | | 947 |
| | QCISD/6-311+G(2*d*,*p*) | | 902 | | | 941 |
| **1f** | MP2/6-311G(2*d*,*p*) | 953 | 754 | 835 | 925 | 904 |
| | B3LYP/6-311G(2*d*,*p*) | 965 | 761 | 839 | 934 | 914 |
| | MP2/6-311+G(2*d*,*p*) | 935 | | | 907 | |
| | B3LYP/6-311+G(2*d*,*p*) | 949 | | | 917 | |
| | QCISD/6-311+G(2*d*,*p*) | 948 | | | 917 | |

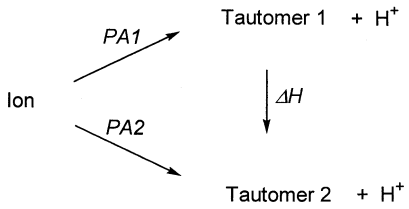
[a]In units of kJ mol$^{-1}$ at 298 K.

site in **1a** was the most basic amide nitrogen that upon protonation formed stable ion **133**$^+$.

The effects of basis set expansion and improved levels of correlation energy treatment were investigated for the five most stable ion isomers **178**$^+$, **138**$^+$, **378**$^+$, **137**$^+$, and **135**$^+$ (Table 2). In general, the MP2 and B3LYP relative energies calculated with the 6-311G(2*d*,*p*) and 6-311+G(2*d*,*p*) basis sets showed very good agreement, which was within 3 kJ mol$^{-1}$ absolute deviation. Averaging the relative energies by B3-MP2 therefore did not result in significant improvement. Further expansion of the basis set up to 6-311+G(3*df*,2*p*) had a negligible effect on the ion relative energies. Including quadratic configuration interaction also had a small effect on the ion relative energies. For example the B3-MP2 relative energies were within 3–4 kJ mol$^{-1}$ absolute of the extrapolated QCISD(T)/6-311+G(3*df*,2*p*) values (Table 2). It appears that reliable B3-MP2 relative energies can be obtained for protonated uracil tautomers with the economical 6-311G(2*d*,*p*) basis set. This is promising for investigations of larger systems for which higher-level QCISD or QCISD(T) single-point calculations are still prohibitively expensive in terms of computational time and memory requirements.

## *Topical and Thermodynamic Proton Affinities*

Combining the uracil tautomer relative enthalpies with those of the ions allows one to calculate the 298 K proton affinities and rank the neutral tautomers by their basicity in the gas phase (Table 3). Note that not all topical proton affinities are listed explicitly in Table 3. The PA values not listed can be obtained readily from




thermochemical cycles using the relative energies of uracil tautomers from Table 1 (Scheme 1). For example, the topical proton affinity of **1b** at O-8 is equal to the proton affinity of tautomer **1f** at O-7 plus the energy difference $\Delta H$(**1b**) − $\Delta H$(**1f**) (Table 1). Because only the most stable uracil tautomer **1a** exists in the gas-phase at thermal equilibrium, only the topical proton affinities in **1a** are of practical importance as far as direct uracil protonation is concerned, and therefore are discussed here. Table 3 shows the calculated proton affinities of the potential protonation sites in **1a**, which decrease in the order O-8 > O-7 > C-5 > N-3 > N-1. The proton affinity of the most basic site was studied at several levels of theory including basis set expansions for the B3LYP, MP2, and effective QCISD and QCISD(T) schemes. The energies calculated with the hybrid B3LYP method were insensitive to basis set expansion that added valence shells and polarization functions, e.g., from 6-31+G(*d*,*p*) to 6-311+G(2*df*,*p*). The energies calculated with the MP2(frozen core) showed a decrease upon adding valence shell, polarization, and diffuse functions [e.g., from 6-31G(*d*,*p*) to 6-311+G(2*df*,*p*)], followed by a small increase upon further basis set expansion. Treating **1a** and **138**$^+$ with QCISD(T) further stabilized the larger electronic system of the ion leading to an increased proton affinity. At the highest level of theory, which in this work was effective QCISD(T)/6-311+G(3*df*,2*p*), we obtain PA(uracil) = 858 kJ mol$^{-1}$ (Table 3). This value is very well reproduced by the B3-MP2 datum (854 kJ mol$^{-1}$) and the B3LYP values (861–863 kJ mol$^{-1}$). Previous calculations using B3LYP and Moller–Plesset theory with relatively small basis sets of split-valence double-$\zeta$ quality reported PA(uracil) = 860–870 kJ mol$^{-1}$ [13–15].

What is the best value for the thermodynamic proton affinity of uracil? The experimental PA of uracil is given as 872.7 kJ mol$^{-1}$ in recent compilations [50, 51], which might indicate high accuracy. It is instructive to follow the evolution of this figure in the literature. The experimental grounds for the proton affinity of uracil were provided by bracketing measurements of Wilson and McCloskey who found that uracil was protonated by $NH_4^+$ but not $CH_3NH_3^+$ in a conventional CI source of a sector mass spectrometer [52]. Wilson and McCloskey estimated the PA of uracil between 207 and 216.3 kcal mol$^{-1}$ (866 and 905 kJ mol$^{-1}$, respectively) [52] on the then valid PA scale, which corresponds to 853–899 kJ mol$^{-1}$ on the current proton affinity scale [50, 51]. Lias and Levine narrowed these limits to the middle of the range and estimated PA (**1a**) as 870 kJ mol$^{-1}$ [53], a value that was also used in the more recent complications of ion energy data, e.g., in 1988 [54], and furnished with additional significant figures in 1998 [49] and 1999 [51]. To the best of our knowledge, no recent experimental measurements have been reported that would support or justify the value and accuracy of the 872.7 kJ mol$^{-1}$ datum. As the closest analogy, the proton affinity of thymine was measured as 874 kJ mol$^{-1}$ by using the kinetic method [55]. If the effect on proton affinity of the methyl group in the uracil-thymine system is similar to that in the pyridine-picoline systems (($\Delta PA$ = 13–19 kJ mol$^{-1}$) [51], the PA of uracil can be estimated at 855–861 kJ mol$^{-1}$. Considering the above experimental uncertainties, the proton affinities calculated by B3LYP, B3-MP2, and effective QCISD(T) methods are in agreement with experiment. In addition, PA = 858 kJ mol$^{-1}$ is the current benchmark for the most accurate estimate judged by the known performance of the computational methods and the levels of theory used [56].

## Conclusions

Computational methods ranging from density-functional theory through Moller–Plesset theory up to quadratic configuration interaction identified structure **1a** as the most stable uracil tautomer, so it should predominate in the gas phase. The calculated topical proton affinities of uracil tautomers pointed to N-1- protonated 2,4-dihydroxypyrimidine as the most stable ion structure. The thermodynamic proton affinity of uracil, which is amenable to experimental measurements, was calculated as 858 kJ mol$^{-1}$ at the highest level of theory used in this work. This agrees with the results of the original bracketing measurements. We suggest that the PA value that was tabulated in recent compilations of thermochemical data should be remeasured to provide a reliable experimental datum to be compared with the calculated value.

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