1. Introduction

The 5- and 6-halouracils have been shown to exhibit antitumor and antiviral activities [1–18]. The ability of 5- and 6-halouracils to control damage to healthy tissues during radiation therapy has been evaluated [1]. 5-Chlorouracil and 5-bromouracil have been studied for their use in the treatment of inflammatory tissue [2]. 5-Fluorouracil is currently employed in colorectal, breast, and head and neck cancer treatments [3–5] due to its ability to inhibit thymine synthase, and thus prevent the conversion of uracil to thymine [4,5]. 5-Fluorouracil is incorporated into DNA by DNA polymerase and excised by DNA glycosylase leading to toxic abasic sites and subsequent DNA strand breaks [5]. Fluorine is similar in size to a hydrogen atom, and like uracil 5-fluorouracil is excised by DNA glycosylase, whereas chlorine and bromine are similar in size to a methyl group such that 5-chlorouracil and 5-bromouracil, like thymine, are not excised by DNA glycosylase. Similar mechanisms for 5-chlorouracil and 5-bromouracil utilizing thymine DNA glycosylase have been suggested [19]. However, the mechanisms of antitumor and antiviral actions of these nucleobases are not well understood. Thus, research into the structures and binding interactions of halouracils are of great importance in pharmaceutical research.
Sodium cations are essential to biological systems as they play vital roles in heart activity, blood and fluid regulation, nerve impulse transmission, and metabolic functions. One of the most recognized functions of sodium cations is their role in creating ion gradients with potassium ions. Secondary active transport utilizes a Na⁺/K⁺ ion gradient to transport amino acids, nucleotides, and other biological molecules across cell membranes. Voltage-gated ion channels found in neurons also utilize a Na⁺/K⁺ ion gradient to pass electrical signals from neuron to neuron [20]. Sodium cations are also involved in nucleic acid chemistry exhibiting charge neutralization and noncovalent interactions that lead to the stabilization of DNA and RNA [21,22]. The presence of metal cations influences the function, stability, and conformational behavior of surrounding molecules. Metal cations binding to the nucleobase can produce more significant effects on DNA and RNA conformation than those binding to the phosphate backbone [23]. The formation and stabilization of minor nucleobase tautomers can be caused by the presence of metal cations [24]. These nucleobase modifications can potentially lead to base mispairing and cause errors in the transfer of genetic information [25].

The properties and characteristics of protonated and sodium cationized uracil and modified uracils have been extensively studied [25-31]. Previous infrared multiple photon dissociation (IRMPD) action spectroscopy studies by Crampton et al. of protonated halouracils [26], Salpin et al. of uracil [27], and Nei et al. of uracil, thioketo-, and methylthioketo-uracils [28] found that protonation preferentially stabilizes minor noncanonical tautomers of all of the nucleobases examined thus far except 4-thiouracil. In contrast, previous CID and theoretical studies by Rodgers and Armentrout of sodium cationized uracil [29] and by Yang et al. of sodium cationized methyl- [25], thioketo- [30], and halo-substituted uracils, [31] suggest that the ground-state structures involve the canonical tautomers and the dominant CID processes observed arise from loss of the intact neutral nucleobase. Similarly, the IRMPD study by Nei et al. of sodium cationized uracil, thioketo- and methylthioketo-uracils found the ground-state structures to involve the canonical tautomer of the nucleobase except in complexes of 4-thiouracil and 2,4-dithiouracil, where minor tautomers are preferentially stabilized [32].

Structural information regarding the sodium cationized halouracils in the previous CID study was inferred by comparison of measured and theoretically estimated bond dissociation energies [31]. In the current study, IRMPD action spectroscopy techniques are used to probe the structures of sodium cationized halouracils. The influences of halogenation and noncovalent interactions with sodium cations on the gas-phase tautomeric conformations of the halouracils are also examined. The structures and relative stabilities at 298 K of all possible tautomers of the sodium cationized halouracils are calculated. The halouracils investigated in this study include: 5-fluouracil (5FU), 5-chlorouracil (5ClU), 5-bromouracil (5BrU), 5-iodouracil (5IU), and 6-chlorouracil (6ClU), allowing the influence of the size and position of the halogen substituent on the structure of sodium cationized halouracils to be elucidated. The canonical structures of neutral uracil and the halouracils investigated here are shown in Fig. 1. In order to definitively determine the ground-state and low-energy tautomeric conformations of the sodium cationized forms of these nucleobases, IRMPD action spectra of these complexes are measured and compared to theoretical linear IR spectra of the stable low-energy structures of the sodium cationized halouracil complexes derived from electronic structure calculations performed at the B3LYP/6-31G(d) level of theory.

### 2. Experimental and computational

#### 2.1. Mass spectrometry and photodissociation

Experimental studies were carried out at the FELIX facility using a 4.7 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) coupled to a free electron laser (FEL) [33–35]. IRMPD action spectra were obtained for five Na⁺(xU) complexes, where xU represents 5FU, 5ClU, 5BrU, 5IU, and 6ClU. 5FU and 5BrU were obtained from Sigma–Aldrich, whereas 5ClU, 5IU, and 6ClU were obtained from TCI Tokyo Kasei. Solution conditions employed for the samples examined were 1–2 mM nucleobase with 1–2 mM NaCl in 50%:50% to 80%:20% MeOH:H₂O solutions.

Sample solutions were delivered via a syringe pump to a Micromass z-spray electrospray ionization (ESI) source at a flow rate of 10 μL/min and a needle voltage of 3.2–3.4 kV. Ions emanating from the ESI source were accumulated in a hexapole ion trap for several seconds, and then pulse extracted and sent through a quadrupole bender into an octopole ion guide. Ions were slowed within the octopole ion guide by traveling up a potential difference created by a negative dc bias with relative ground potential at the ICR cell to allow for easy capture by gated ion trapping [33]. Once ions were trapped within the ICR cell, stored waveform inverse Fourier transform (SWIFT) excitation was used to isolate the desired sodium cationized halouracil complex from unwanted ions formed in the ionization process. As chlorine and bromine exist naturally in two isotopes, complexes with both isotopes were isolated and used to produce greater precursor ion intensity and to properly identify the desired precursor ion. Selected ions were irradiated by the FEL (entering from the back of the ICR cell) at pulse energies of ~40 mJ per macropulse of 5 μs duration for 3 s, corresponding to interaction with 15 macropulses over the wavelength range extending from 10.5 μm (950 cm⁻¹) to 5.3 μm (1900 cm⁻¹) to induce photodissociation. Dissociation occurs when the wavelength of the radiation corresponds to a resonant absorption mode of the precursor ion. Precursor ions absorb photons and distribute the absorbed energy throughout the

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**Fig. 1.** Structures of uracil (U) and the halouracils (xU), where xU = 5FU, 5ClU, 5BrU, 5IU and 6ClU.
internal modes of the ion by intramolecular vibrational redistribution until the dissociation threshold is reached. The absorption of tens to hundreds of photons is generally required for dissociation. The multiple pass arrangement allows for increased interaction with the FEL. The intensities of the precursor and photofragment ions were monitored as a function of the FEL wavelength. The IRMPD yield is calculated from the precursor ion intensity \( I_p \) along with the sum of the fragment ion intensities \( I_f \) at each frequency as shown in Eq. (1).

\[
\text{IRMPD yield} = \frac{\sum I_f}{I_p + \sum I_f}
\]  

The IRMPD yield was power corrected using linear normalization to account for changes in the power of the laser as a function of wavelength, i.e., the wavelength of the FEL. This total fragment yield was then plotted as a function of the wavelength, producing the IRMPD spectrum.

2.2. Computational details

Theoretical calculations were performed using the Gaussian 03 suite of programs [36] to determine all stable tautomeric conformations of the neutral and sodium cationized uracil and halouracils. Structures and relative stabilities of the 13 stable tautomeric conformations of each of the neutral halouracils can be found in Fig. S1 along with the 13 tautomers of uracil (U) previously reported [28]. The structures and relative stabilities of a total of 187 stable tautomeric conformations of the sodium cationized halouracils including: 35 of Na+(5FU), 36 Na+(5CIU), 34 Na+(5BrU), 31 Na+(5IU), 27 Na+(6CIU), and the 24 tautomers of sodium cationized uracil that were previously reported [28] can be found in Fig. S2. Optimized structures and harmonic vibrational frequencies were calculated at the B3LYP/6-31G(d) level of theory. Single point energies used to determine the relative enthalpies at 0 K for each conformer were calculated at the B3LYP/6-31+G(2d,2p) level of theory. Thermal corrections to convert 0 K enthalpies to enthalpies and Gibbs free energies at 298 K were determined using standard formulae (based on rigid rotor and harmonic oscillator approximations) and the rotational constants and vibrations frequencies determined at the B3LYP/6-31G(d) level of theory. In the case of 5-iodouracil an iodine effective core potential was used to decrease the computational effort by focusing on only valence electrons of the iodine atom, whereas the core electrons are represented by the LANL2DZ and modified LANL2DZ effective core potentials [37,38]. Theoretical linear IR spectra were generated using the harmonic vibrational frequencies and IR intensities. The vibrational frequencies are scaled by a factor of 0.96 as recommended by Foresman and Frisch [39]. Calculated vibrational frequencies are convoluted with a 20 cm\(^{-1}\) fwhm Gaussian line shape for comparison to the measured IRMPD spectra.

3. Results

3.1. IRMPD action spectroscopy

In all cases, IRMPD of the Na+(xU) complexes leads to loss of the intact nucleobase and detection of Na+(m/z = 23). IRMPD action spectra of the Na+(xU) complexes measured in this work along with the spectrum for the Na+(U) complex previously reported [32] are compared in Fig. 2. As can be seen in the figure, these complexes exhibit highly parallel spectral features. However, the spectra exhibit systematic shifting of the band positions of several features allowing facile differentiation of these complexes. The intense band at \(~1801\) cm\(^{-1}\) in the IRMPD spectrum of Na+(U) is slightly blue shifted to \(~1808\) cm\(^{-1}\) for all of the Na+(xU) complexes. The intense band observed at \(~1659\) cm\(^{-1}\) for Na+(U), is blue shifted by \(16\) cm\(^{-1}\) for Na+(5FU) to \(~1675\) cm\(^{-1}\). Relative to Na+(5FU), this band is increasingly red shifted for the other Na+(x5U) complexes as the size of the halogen substituent increases, and appears at the lowest frequency for the Na+(6CIU) complex. The red shifting of this intense band as well as the appearance of a new feature in the IRMPD spectra of the Na+(xU) complexes that also red shifts increasingly as the size of the halogen substituent increases, leads to narrowing of this broad feature and the appearance and eventual resolution of two bands in the IRMPD spectra of the Na+(xU) complexes. In the IRMPD spectrum of Na+(5FU), the new feature is masked by the intense band between \(~1648\) and \(~1675\) cm\(^{-1}\), whereas for Na+(5CIU) a shoulder to the red is observed at \(~1628\) cm\(^{-1}\). The corresponding band is further red shifted and observed as a separate band at \(~1608\) cm\(^{-1}\) in the IRMPD spectrum of Na+(5BrU), and is even further red shifted to \(~1602\) cm\(^{-1}\) for Na+(5IU) and to \(~1584\) cm\(^{-1}\) for Na+(6CIU). Three peaks of low intensity appear in the IRMPD spectra of Na+(U) and the Na+(5xU) complexes between \(~1295\) and \(~1496\) cm\(^{-1}\), with the band appearing at the lowest frequency being the weakest and that at the highest frequency the most intense. The weak band at \(~1485\) cm\(^{-1}\) for Na+(U) is blue shifted by \(11\) cm\(^{-1}\) to \(~1496\) cm\(^{-1}\) for Na+(FU). In contrast, this band is red shifted for the other halouracils, by \(10\) cm\(^{-1}\) to \(~1474\) cm\(^{-1}\) for Na+(5CIU) and Na+(6CIU), 18 cm\(^{-1}\) to \(~1468\) cm\(^{-1}\) for Na+(5BrU), and 21 cm\(^{-1}\) to \(~1464\) cm\(^{-1}\) for Na+(5IU). The band observed at \(~1417\) cm\(^{-1}\) in the IRMPD spectra of Na+(U) and Na+(5FU) is red shifted by \(14\) cm\(^{-1}\) to \(~1403\) cm\(^{-1}\) for Na+(5CIU), Na+(5BrU), and Na+(6CIU), and is further
red shifted by 19 cm$^{-1}$ to 1398 cm$^{-1}$ in the IRMPD spectrum of Na$^+$(5IU). The very weak band that appears at $\sim$1328 cm$^{-1}$ in the IRMPD spectrum of Na$^+$(U), is red shifted by 17 cm$^{-1}$ for Na$^+$(5FU) and Na$^+$(5CIU) to 1311 cm$^{-1}$, by 21 cm$^{-1}$ for Na$^+$(5BrU) and Na$^+$(5IU) to 1307 cm$^{-1}$, and by 33 cm$^{-1}$ to 1295 cm$^{-1}$ for Na$^+$(6CIU). It cannot be determined from the spectra alone which of the two bands between 997 and 1231 cm$^{-1}$ for each of the halouracils corresponds to the band exhibited at 1198 cm$^{-1}$ for Na$^+$(U). Both features observed for the Na$^+$(5xU) complexes are increasingly red shifted such that the frequency difference between these two peaks also increases as the size of the halogen substituent increases. The higher energy feature exhibits a greater intensity for all of the 5-halouracils, whereas it is of lower intensity for Na$^+$(6CIU). Na$^+$(6CIU) exhibits new features at $\sim$1350 and 1688 cm$^{-1}$ that are not observed in the spectra of the Na$^+$(5xU) complexes. It cannot be determined from the spectra alone which of the three bands at $\sim$1000, 1110, and 1150 cm$^{-1}$ in the IRMPD spectrum of Na$^+$(6CIU) correspond to the two bands observed for each of the 5-halouracils. The IRMPD yield is greater for Na$^+$(6CIU) than Na$^+$(5CIU), which may suggest an increased density of states, reactivity, or the presence of multiple conformers in the experiments.

The position of the halogen substituent, 5 versus 6, in Na$^+$(5CIU) and Na$^+$(6CIU) can be readily differentiated by their IR spectra. Distinguishing features between the spectra include red shifting of the shoulder at 1628 cm$^{-1}$ observed for Na$^+$(5CIU) by 44 cm$^{-1}$ to 1584 cm$^{-1}$ for Na$^+$(6CIU). The two spectra can also be distinguished by the presence of two bands at $\sim$1075 and 1175 cm$^{-1}$ for Na$^+$(5CIU), whereas Na$^+$(6CIU) exhibits a band at $\sim$1000 cm$^{-1}$ of similar intensity and two low intensity features at $\sim$1110 and 1150 cm$^{-1}$. Na$^+$(6CIU) also exhibits new features at $\sim$1350 and 1688 cm$^{-1}$ that are not observed in the IRMPD spectrum of Na$^+$(5CIU), which further aid in the differentiation of these species.

3.2. Theoretical results

The 13 most stable tautomeric conformations of uracil and the halouracils are shown in Fig. 3, whereas those of the Na$^+$(U) and Na$^+$(xU) complexes are shown in Fig. 4. The B3LYP/6-31G(d) optimized structures and B3LYP/6-31+G(2d,2p) relative Gibbs free energies at 298 K of the stable low-energy tautomers of the 5-halouracils are included in Fig. S1 of the Supplementary Information, whereas those for the Na$^+$(U) and Na$^+$(xU) complexes are shown in Fig. S2. Relative enthalpies and Gibbs free energies calculated at the B3LYP/6-311+G(2d,2p) level of theory at 298 K including zero point energy and thermal corrections for the 12 most stable tautomers of the Na$^+$(xU) complexes along with those for the Na$^+$(U) complex previously reported [32] are listed in Table 1. In all cases, the ground-state tautomeric conformations of the sodium cationized halouracils are the $A^2$ and $A^1$ conformers shown in Fig. 4. These conformers result from binding of the sodium cation to the O4 carbonyl oxygen atom of the canonical $a$ tautomers of the halouracils. In the case of the 5-halouracils, the sodium cation gains additional stabilization via chelation with the O4 carbonyl oxygen and the 5-halo-substituent, the $A^2$ conformer, resulting in a $\text{CO}_4\text{Na}$ bond angle of 118.9–138.9° for the 5-halouracils. In contrast, the $\text{CO}_4\text{Na}$ bond angle is nearly linear (173.5°) in the $A^1$ conformer of the Na$^+$(6CIU) complex.

3.2.1. Na$^+$(5xU)

As discussed above, the ground-state structures of the Na$^+$(5xU) complexes are the $A^2$ conformers shown in Fig. 4 resulting from sodium cationization of the $a$ conformers of 5xU (Fig. S1). In these structures protons are bound to the N1, N3, and C6 atoms with the sodium cation chelating between the O4 carbonyl oxygen atom and 5-halo-substituent. Sodium cationization of the $a$ conformers at the O2 position leads to the $D$ conformers. These conformers lie 24.6 kJ/mol for Na$^+$(5FU), 25.7 kJ/mol for Na$^+$(5CIU) and Na$^+$(5BrU), and 25.8 kJ/mol for Na$^+$(5IU) above the ground-state $A^2$ conformer. The first-excited conformers of the Na$^+$(5xU) complexes are the $C$ conformers, which lie 16.6 kJ/mol above the ground-state $A^2$ conformers for Na$^+$(5FU), 18.0 kJ/mol for Na$^+$(5CIU), 17.3 kJ/mol for Na$^+$(5BrU), and 19.1 kJ/mol for Na$^+$(5IU). These conformers result from the sodium cation chelating with the O2 and N3 atoms of the $c_2$ conformer. In these structures protons are bound to the N1, O4, and C6 atoms with the O4H atom oriented toward the 5-halo-substituent. These conformers could also be generated by binding of the sodium cation at the O2 position of the $a$ conformers and proton transfer from the N3 to O4 position. The $C$ conformers exemplify the stabilization of minor tautomers upon sodium cationization as the relative Gibbs free energies at 298 K decrease from 63.7 kJ/mol for neutral $c_2$ to 16.5 kJ/mol for sodium cationized C in Na$^+$(5FU) as compared to the $a$ and $A^2$ ground-state conformers of these species, respectively. Stabilization of the minor $c_2$ tautomer is also found for the other Na$^+$(5xU) complexes with the relative Gibbs free energy at 298 K decreasing from 61.8 kJ/mol for 5CIU to 18.0 kJ/mol for Na$^+$(5CIU), from 61.9 kJ/mol for 5BrU to 17.3 kJ/mol for Na$^+$(5BrU), and from 64.9 kJ/mol for 5IU to 19.1 kJ/mol for Na$^+$(5IU). In addition to these two low-energy conformers, 32 higher lying stable conformers were calculated for Na$^+$(5FU), 34 for Na$^+$(5CIU), 32 for Na$^+$(5BrU), and 29 for Na$^+$(5IU). The relative Gibbs free energies of these higher-energy conformers vary from 21.8 to
214.6 kJ/mol as compared to the ground-state $A^2$ conformer for Na+(U), 25.7–208.1 kJ/mol for Na+(5ClU), 25.7–200.1 kJ/mol for Na+(5BrU), and 25.8–205.6 kJ/mol for Na+(5IU) (see Fig. S2 of the Supplementary Information).

3.2.2. Na+(6ClU)

Similar to that found for the Na+(5xU) complexes, the ground-state structure of Na+(6ClU) is the $A^1$ conformer shown in Fig. 4 and Fig. S2 resulting from sodium binding at the O4 position to the a conformer of 6ClU. In this structure protons are bound to the N1, N3, and C5 atoms. The first-excited conformer of Na+(6ClU) is the $B$ conformer, which lies 14.7 kJ/mol above the ground-state conformation. This conformer results from the sodium cation chelating with the O2 and N3 atoms of the $c_1$ conformer with the protons bound to the N1 and O4 atoms oriented away from the N3 atom. The E conformer lies 16.1 kJ/mol above the ground-state $A^1$ conformer. This conformer involves chelation of the sodium cation with O2 and N1 of the $f_1$ conformer with the protons bound to the N3 and O4 atoms oriented away from the N3 atom. Rotation of the 4-hydroxyl group of the $B$ conformer away from the sodium cation results in the $C$ conformer, which lies 16.6 kJ/mol above the ground-state $A^1$ conformer. This conformer results from the sodium cation chelating with the O2 and N3 atoms of the $c_2$ conformer with the protons bound to the N1 and O4 atoms oriented away from the N3 atom.
D conformer, which lies 17.9 kJ/mol above the ground-state A^1 conformer, results from binding of the sodium cation at the O2 position of the a conformer. Sodium cationization of 6ClU stabilizes multiple minor tautomers to relative Gibbs free energies at 298 K below 20 kJ/mol. The relative Gibbs free energy at 298 K decreases from 44.9 kJ/mol for neutral c1 to 14.7 kJ/mol for sodium cationized B, from 64.2 kJ/mol for neutral f1 to 16.1 kJ/mol for sodium cationized E, and from 70.2 kJ/mol for neutral c2 to 16.6 kJ/mol for sodium cationized C. In addition to these low-energy conformers, 22 higher-energy conformers were calculated with relative Gibbs free energies varying from 25.7 to 208.6 kJ/mol as compared to the ground-state A^1 conformer (see Fig. S2 of the Supplementary Information).

4. Discussion

4.1. Comparison of experimental IRMPD and theoretical IR spectra of Na+(5ClU)

The experimentally measured IRMPD action spectrum and theoretical linear IR spectra, calculated at the B3LYP/6-31G* level of theory, for the three most stable tautomeric conformations of Na+(5ClU) are compared in Fig. 5. Also included in Fig. 5 are the structures and B3LYP/6-31G(2d,2p) relative Gibbs free energies of these species at 298 K. This comparison shows good agreement between the measured IRMPD action spectrum and the calculated linear IR spectra for the ground-state A^2 conformer. The intense band at 1808 cm\(^{-1}\) is blue shifted by 3 cm\(^{-1}\) to 1811 cm\(^{-1}\) in the theoretical IR spectrum, whereas all other features are red shifted by up to 35 cm\(^{-1}\) suggesting that theory may be underestimating the frequency of these spectral features. The theoretical linear IR spectrum of the first excited C conformer exhibits several features that allow for differentiation from the ground-state A^2 conformer. A new band is observed at \(-1528\) cm\(^{-1}\) and the intense feature observed near \(-1800\) cm\(^{-1}\) is absent for the C conformer. In addition, the intense peak at \(-1668\) cm\(^{-1}\) for the A^2 conformer is blue shifted by 23 cm\(^{-1}\). The blue shifting of this intense peak would cause further broadening of the corresponding feature in the measured IRMPD action spectrum if this conformer was accessed experimentally. In addition, the 1413 cm\(^{-1}\) band (blue shifted by \(-33\) cm\(^{-1}\) from 1380 cm\(^{-1}\) for the ground-state A^2 conformer) and the 1450 cm\(^{-1}\) band (red shifted by \(-23\) cm\(^{-1}\) from 1473 cm\(^{-1}\) for the ground-state) would appear as severe broadening or new features in the measured IRMPD action spectrum. These differences indicate that the C conformer does not contribute to the measured IRMPD action spectrum. The estimated Maxwell-Boltzmann population of conformer C at 298 K is only \(-0.1\%, providing further support for the conclusion that this conformer does not contribute measurably to the IRMPD action spectrum. The second excited D conformer of Na+(5ClU) lies 25.7 kJ/mol higher in free energy than the ground-state A^2 conformer, and involves binding of the sodium cation to the O2 carbonyl oxygen atom of the canonical a tautomer. As compared to the ground-state A^2 conformer, the intense band at 1808 cm\(^{-1}\) is red shifted by 67 cm\(^{-1}\), whereas the bands at \(-1668\) and 1602 cm\(^{-1}\) are blue shifted by 31 and 18 cm\(^{-1}\), respectively, for binding of sodium at O2 versus O4. These band shifts indicate that experimentally accessed structures bind more strongly to O4 than O2. In addition, these positions should be readily distinguished by their IRMPD spectra. Theoretical energetics verify that the ground-state A^2 conformer of Na+(5ClU) is the canonical a tautomer with Na^+ bound to the O4 carbonyl oxygen atom. As with the C conformer, these band shifts would appear as shoulders, severely broadened, or even new features, in the measured IRMPD action spectrum. The absence of new or broadened features indicates that the D conformer does not contribute measurably to IRMPD action spectrum.

4.2. Comparison of Na+(5ClU) to Na+(5FU), Na+(5BrU), and Na+(5IU)

The experimentally measured IRMPD action spectra and theoretical linear IR spectra, calculated at the B3LYP/6-31G* level of theory, for the two most stable tautomeric conformations A^2 and C, and the canonical O2 sodium cation binding D conformer of the Na+(5FU), Na+(5BrU), and Na+(5IU) complexes are included in Figs. S3–S5 of the Supplementary Information. Also included in Figs. S3–S5 are the structures and B3LYP/6-31+G(2d,2p) relative Gibbs free energies of these species at 298 K. Highly parallel behavior is found across these Na+(5xU) complexes and the experimental and theoretical results for Na+(5ClU). However, the relative stabilities of the second and third excited conformers, D and G^2, differ for Na+(5FU) as compared to the other Na+(5xU) complexes, where the G^2 conformer is 5–6 kJ/mol more stable for Na+(5FU). A new feature in the IR spectra of the C conformers appears between 1552 and 1518 cm\(^{-1}\) and decreases in frequency as the size of the halogen substituent increases. The bound carbonyl stretch of the C conformers is blue shifted, by between 18 and 29 cm\(^{-1}\), with the largest shifts observed for the largest halogen substituents. The band appearing between 1394 and 1374 cm\(^{-1}\) in the ground-state A^2 conformers, exhibits increased blue shifting in the C conformers from 19 to 36 cm\(^{-1}\) as the size of the halogen substituent increases. The band appearing between 1482 and 1468 cm\(^{-1}\) in the ground-state A^2 conformers, exhibits increased red shifting for the C conformers as the size of the halogen substituent increases. In the IR spectra of the D conformers the free carbonyl stretch is red shifted by 34–44 cm\(^{-1}\) as compared to the ground-state A^2 conformer. In the conformer, the free carbonyl stretch of conformer D is blue shifted by 24–39 cm\(^{-1}\) as compared to the ground-state A^2 conformer. In both cases, the magnitude of the frequency shifting increases with the size of the halogen substituent. The C=a stretch is red shifted by

![Fig. 5. Comparison of the measured IRMPD action spectrum of Na+(5ClU) and the linear IR spectra predicted for the three most stable conformers of Na+(5ClU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies at 298 K of each conformer are also shown. To facilitate comparison of the measured and computed spectra, the IRMPD spectrum is overlaid (in grey) with each of the computed spectra and scaled to match the intensity of the C=O4 carbonyl stretch. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
18 cm$^{-1}$ for both Na$^+$(5ClU) and Na$^+$(5FU), 20 cm$^{-1}$ for (Na$^+$(5IU), and 22 cm$^{-1}$ for Na$^+$(5FU).

As observed for Na$^+$(5ClU), there is a slight red shifting of all features except the free carbonyl stretch in the theoretical IR spectra for all of the Na$^+$(5xU) complexes. The red shifting, as observed in Na$^+$(5ClU), is less severe for the bound carbonyl stretch, 1181 in plane rocking, and the C6–N1 stretch as compared to other spectral features in all cases except Na$^+$(5FU). The free carbonyl stretch, as in the case of Na$^+$(5ClU), is blue shifted by 3 cm$^{-1}$ to 1811 cm$^{-1}$ in the theoretical IR spectra for the other Na$^+$(5xU) complexes.

4.3. Comparison of experimental IRMPD and theoretical IR spectra of Na$^+$(6ClU)

The experimentally measured IRMPD action spectrum and theoretical linear IR spectra, calculated at the B3LYP/6-31G* level of theory, for the five most stable tautomeric conformations of Na$^+$(6ClU) are compared in Fig. 6. Also included in Fig. 6 are the structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energies of these species at 298 K. This comparison shows very good agreement between the measured IRMPD action spectrum and the calculated linear IR spectrum for the ground-state A$^1$ conformer. Red shifting of all features by 2–33 cm$^{-1}$ except the free carbonyl stretch at ~1808 cm$^{-1}$ and the N3–H in-plane rocking at ~1350 cm$^{-1}$ is again observed. This suggests theory is underestimating the frequency of the red shifted spectral features. There are also additional features present in the measured IRMPD spectrum compared to the theoretically calculated IR spectrum for the ground-state A$^1$ conformer. Na$^+$(6ClU) exhibits five conformations below 20 kJ/mol, whereas the other Na$^+$(5xU) complexes exhibit only two. Thus, halogenation at the 6-position leads to increased stabilization of minor tautomers. All excited conformers exhibit blue shifting of the bound carbonyl stretch and red shifting of the C–X stretch. The first and third excited conformations, B and C, differ only by the rotation of the 4-hydroxyl group. Both conformations exhibit bands at ~1190 and ~1530 cm$^{-1}$ that are not observed in the measured IRMPD spectrum. The 298 K Maxwell–Boltzmann population calculated for the first excited state is ~0.26%. The second excited conformation exhibits new bands at ~1380 and ~1440 cm$^{-1}$, and blue shifting of the bound carbonyl from ~1650 to ~1675 cm$^{-1}$, which would appear as new features or broadening in the measured IRMPD action spectrum. The forth excited D conformer, which involves sodium cation binding to the O2 carbonyl oxygen atom of the canonical a tautomer, should be readily differentiated from the O4 binding ground-state A$^1$ conformer. The position of the free carbonyl stretch red shifts for O2 binding by 35 cm$^{-1}$ versus O4 binding. All excited conformers of Na$^+$(6ClU) exhibit bands at ~1380 and ~1440 cm$^{-1}$ that are not observed in the measured IRMPD spectrum. Severe broadening observed in the measured IRMPD spectrum suggests that excited conformers are indeed accessed and contribute to the measured IRMPD spectrum. This broadening in the measured IRMPD spectrum of Na$^+$(6ClU) is present at frequencies where the low-energy excited conformers exhibit high intensity bands, suggesting that the observed broadening of many spectral features is likely due to minor contributions of excited conformers accessed in the experiments, and thus theory may be underestimating the stabilities of these excited conformers for the Na$^+$(6ClU) complex.

Observed band positions (in cm$^{-1}$) of the vibrational modes of the Na$^+$(xU) complexes in the IR fingerprint region are compared in Table 2. Assignments are made based on comparison of the IRMPD action spectra and theoretical IR spectra of the ground-state canonical A$^2$ and A$^1$ conformers optimized at the B3LYP/631G(d) level of theory. Comparison of the IRMPD action spectrum to the theoretical IR spectrum of the ground-state A$^2$ and A$^1$ conformers of the Na$^+$(xU) complexes suggests that the absorption bands at ~1810 and ~1650 cm$^{-1}$ correspond to the C4=O and C2=O stretching, respectively. The small shoulder at ~1600 cm$^{-1}$ corresponds to C5=C6 stretching. The small feature at ~1475 cm$^{-1}$ corresponds to N1–H rocking. The small feature at ~1375 cm$^{-1}$ is composed of two weak absorption bands that correspond to N3–C4–C5 asymmetric stretching and N3–H rocking. The small feature at ~1300 cm$^{-1}$ corresponds to C6–H rocking of Na$^+$(5xU) complexes and N1–H and C5–H symmetric rocking of Na$^+$(6ClU). The feature at 1150 cm$^{-1}$ is composed of two weak absorption bands that correspond to N1–H and C6–H asymmetric rocking and C2–N3–C4 asymmetric stretching in Na$^+$(5FU) and Na$^+$(5IU). The feature at 1150 cm$^{-1}$ is composed of two weak absorption bands that correspond to N1–C6 stretching and C2–N3–C4 asymmetric stretching of Na$^+$(5ClU) and Na$^+$(5FU). The feature at 1092 cm$^{-1}$ in Na$^+$(6ClU) corresponds to N1–C6 stretching and C5–H rocking. The feature at 1279 cm$^{-1}$ in Na$^+$(6ClU) corresponds to N1–H and C5–H symmetric rocking. The feature at

Fig. 6. Comparison of the measured IRMPD action spectrum of Na$^+$(6ClU) and the theoretical IR spectra for the five most stable conformers of Na$^+$(6ClU) calculated at the B3LYP/6-31G(d) level of theory. The structures and B3LYP/6-311+G(2d,2p) relative Gibbs free energy at 298 K of each conformer are also shown. To facilitate comparison of the measured and computed spectra, the IRMPD spectrum is overlaid (in grey) with each of the computed spectra and scaled to match the intensity of the C=O4 carbonyl stretch. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
1203 cm$^{-1}$ in Na$^+(5$FU), 1048 cm$^{-1}$ in Na$^+(5$CIU), 1022 cm$^{-1}$ in Na$^+(5$BrU), and 1004 cm$^{-1}$ in Na$^+(5$IU), corresponds to C5–X stretching. The feature at 970 cm$^{-1}$ in Na$^+(5$FU) corresponds to C6–X stretching and N3–C4–C5 symmetric stretching.

### 4.4. Effect of halogenation on the IRMPD spectra

The effect of halogenation on the IRMPD spectra of sodium cationized uracil and the halouracils can be seen in the comparison of Fig. 2. The frequency of the free carbonyl (C2=O) stretch is preserved for all of the Na$^+(x$U) complexes as the halogen substituent clearly has no direct interaction with this carbonyl moiety. In contrast and not surprisingly, the bound carbonyl (C4=O) stretch exhibits systematic shifts that vary with the size of the halogen substituent. The C4=O stretch observed at ~1659 cm$^{-1}$ for Na$^+(U)$ is blue shifted to 1675 cm$^{-1}$ for Na$^+(5$FU), and red shifts by between 7 and 20 cm$^{-1}$ relative to Na$^+(5$FU) for the other Na$^+(5$xU) complexes, with the largest shifts observed for the largest halogen substituents. The C5=C6 stretch is not resolved from the C4=O stretch for Na$^+(U)$ and Na$^+(5$FU). The C5=C6 stretch observed at 1643 cm$^{-1}$ for Na$^+(5$IU), corresponds to C5–C6–X stretching and the largest shifts observed for the largest halogen substituents. The C5=C6 stretch is not resolved from the C4=O stretch for Na$^+(U)$ and Na$^+(5$FU).

#### Table 2

<table>
<thead>
<tr>
<th>Vibrational mode</th>
<th>Na$^+(5$FU)</th>
<th>Na$^+(5$CIU)</th>
<th>Na$^+(5$BrU)</th>
<th>Na$^+(5$IU)</th>
<th>Na$^+(6$CIU)</th>
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<td>C–X stretch</td>
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<td>1048</td>
<td>1022</td>
<td>1004</td>
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<td>N3–C4–C5 symmetric stretch</td>
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<td>986</td>
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<td>1158</td>
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<td>–</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>1279</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
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<td>1811</td>
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</table>

$^a$ Assignments based on comparison with the IRMPD action spectra of the ground-state canonical diketo A2$^*$ and A1$^*$ conformers optimized at the B3LYP/6–311+G(2d,2p)//B3LYP/6-31G(d) level of theory.

### 4.5. Effect of sodium cationization on the IR spectra

The IRMPD action spectra of the Na$^+(x$U) complexes measured in this work and the theoretical linear IR spectra, calculated at the B3LYP/6–31G(d) level of theory, for the ground-state tautomeric conformations of the neutral 5-halouracils and 6-chlorouracil are compared in Fig. S6. Also included in Fig. S6 are the neutral ground-state canonical tautomeric conformations.

Sodium cationization of the neutral halouracils causes shifting of several of the spectral features. Sodium cationization results in a blue shift of the O2 carbonyl stretch by 30–34 cm$^{-1}$ with the largest shifts observed for the smallest halogen substituents. Not surprisingly, sodium cationization results in a large red shift of the O4 carbonyl stretch (the binding site) by 67–81 cm$^{-1}$ with the largest shifts observed for the largest halogen substituents, and is red shifted the furthest by 88 cm$^{-1}$ for Na$^+(6$CIU). Sodium cationization results in a red shift of the C=C stretch by up to 8 cm$^{-1}$ with the largest shifts observed for the smallest halogen substituents, whereas a much larger shift of 20 cm$^{-1}$ occurs for Na$^+(6$IU). In the case of Na$^+(5$FU), the C=C stretch is not resolved from the O4 carbonyl stretch. Sodium cationization results in a blue shift of the peak at ~1475 cm$^{-1}$ by 25–45 cm$^{-1}$, with the largest shifts observed for the smallest halogen substituents. Sodium cationization results in a blue shift of the peak at ~1400 cm$^{-1}$ by 39 cm$^{-1}$ for Na$^+(5$FU), 47 cm$^{-1}$ for Na$^+(5$CIU), 49 cm$^{-1}$ for Na$^+(5$BrU), 46 cm$^{-1}$ for Na$^+(5$IU), and by 53 cm$^{-1}$ for Na$^+(6$CIU). Sodium cationization results in a red shift of the peak at ~1310 cm$^{-1}$ by 2–39 cm$^{-1}$, with the largest shifts observed for the smallest halogen substituents. Sodium cationization results in a blue shift of the peak at ~1150 cm$^{-1}$ by 7 cm$^{-1}$ for Na$^+(5$FU), 14 cm$^{-1}$ for Na$^+(5$CIU) and Na$^+(5$BrU), and 8 cm$^{-1}$ for Na$^+(5$IU). Sodium cationization results in a red shift of the C–X stretch by 4 cm$^{-1}$ for Na$^+(5$FU), and a blue shift of 45 cm$^{-1}$ for Na$^+(5$CIU), 44 cm$^{-1}$ for Na$^+(5$BrU), 46 cm$^{-1}$ for Na$^+(5$IU), and 143 cm$^{-1}$ for Na$^+(6$CIU). Clearly, sodium cationization has a significant impact on the bond positions of all features leading to facile differentiation from the neutral halouracils.

### 4.6. Comparison to H$^+$ (xU), H$^+$ (xSU), and Na$^+(x$SU) systems previously investigated

Previous studies of the protonated and sodium cationized forms of uracil and a variety of substituted uracils including thioketouracils and methylthioketouracils demonstrate stabilization of minor tautomers of these nucleobases as a result of protonation and sodium cationization [25,28–32]. In addition,
protonation of halouracils has similarly shown stabilization of minor tautomers [26]. The effects of protonation are more significant in the preferential stabilization of minor tautomers than that of sodium cationization. Sodium cationization typically favors canonical ground-state tautomers with few exceptions. The present theoretical results provide additional evidence for the preferential stabilization of minor tautomers due to sodium cationization of the halouracils. In comparison with protonation of halouracils the effects due to sodium cationization are again less dramatic. Protonation of the halouracils leads to a greater increase in the stability of minor tautomers than sodium cationization such that minor tautomers become the ground-state conformations, whereas the canonical tautomers remain the ground-state conformers of the sodium cationized halouracils.

4.7. Comparison with CID work

The results of this work are consistent with previous energy-resolved CID studies of alkali metal cationized uracil and methyl-, thioketone-, and halo-substituted uracils from guided ion beam tandem mass spectrometry studies [25,29–31]. The ground-state structures of uracil and the modified uracils almost exclusively involve Na⁺ binding to the O4 carbonyl oxygen atom of the canonical a tautomer. The Na⁺(4SU) complex is the only system examined thus far where this is not the case, and binding at O2 is preferred. Theoretical calculations using the MP2(full)/6-31G* optimized geometries of Na⁺(4SU) complexes performed in the previous CID study for the uracil and halouracil complexes similarly find the canonical a tautomer as the ground-state for these systems with binding at the O4 position. [31] The dissociation behavior observed upon IR irradiation in the current study, corresponding to loss of the intact neutral nucleobase, is also consistent with the dominant CID process for alkali metal cationized uracil and methyl-, thioke toto-, and halo-substituted uracils previously observed [25,29–31].

5. Conclusions

IRMPD action spectra of the sodium cationized halouracil complexes, Na⁺(5FU), Na⁺(5CU), Na⁺(5BrU), Na⁺(5IU), and Na⁺(6CIU), in the infrared fingerprint region extending from ~950 to 1900 cm⁻¹ have been obtained. The primary IRMPD pathways observed are consistent with those observed for uracil and thioke toto-uracils in which dissociation leads to detection of the sodium cation and loss of the intact neutral nucleobase [32]. These results are also consistent with energy-resolved CID data for uracil and methyl-, thioke toto-, and halo-substituted uracils from guided ion beam tandem mass spectrometry studies in our laboratory [25,29–31]. Comparison with linear IR spectra calculated at the B3LYP/6–31G(d) level of theory allow the experimentally accessed conformations to be identified. Based on the computed linear IR spectra, O2 versus O4 binding of the sodium cation should be easily differentiated. The band positions differ by at least 35 cm⁻¹ for the free carbonyl stretch, 25 cm⁻¹ for the sodium bound carbonyl stretch, and at least 19 cm⁻¹ for all C=C stretches. In all cases, the experimental IRMPD action spectra indicate preferential binding of the sodium cation to the O4 carbonyl oxygen atom of the canonical diketo a tautomer. The present results suggest that only a single conformer is accessed for all of the Na⁺(5xU) complexes, whereas multiple conformers are accessed for the Na⁺(6CIU) complex. Theoretical energetics verify that the ground-state structures of these complexes involve Na⁺ binding to the O4 carbonyl oxygen atom of the canonical tautomer, with chelation to the halogen substituent providing additional stabilization for all of the 5-halouracils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijms.2014.07.016.

References


