Structural and Energetic Effects of 2′-Ribose Methylation of Protonated Purine Nucleosides

C. C. He,[a] L. A. Hamlow,[a] Zachary J. Devereaux,[a] Y. Zhu,[a] Y.-w. Nei,[a] L. Fan,[a] C. P. McNary,[b] P. Maitre,[c] V. Steinmetz,[c] B. Schindler,[d] I. Compagnon,[d,e] P. B. Armentrout,[b] and M. T. Rodgers[a,*]

[a]*Department of Chemistry, Wayne State University, Detroit, Michigan 48202, United States*
[b]*Department of Chemistry, University of Utah, Salt Lake City, UT 84112, United States*
[c]*Laboratoire de Chimie Physique, Université Paris Sud, UMR8000 CNRS, Faculté des Sciences, 91405 Orsay Cedex, France*
[d] *Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F-69622 Villeurbanne, France*

ABSTRACT

The chemical difference between DNA and RNA nucleosides is their 2′-hydrogen versus 2′-hydroxyl substituents. Modification of the ribosyl moiety at the 2′-position, and 2′-O-methylation in particular, is common among natural posttranscriptional modifications of RNA. 2′-Modification may alter the electronic properties and hydrogen-bonding characteristics of the nucleoside, and thus may lead to enhanced stabilization or malfunction. The structures and relative glycosidic bond stabilities of the protonated forms of the 2′-O-methylated purine nucleosides, 2′-O-methyladenosine and 2′-O-methylguanosine, were examined using two complementary tandem mass spectrometry approaches, infrared multiple photon dissociation (IRMPD) action spectroscopy and energy-resolved collision-induced dissociation (ER-CID). Theoretical calculations were also performed to predict the structures and relative stabilities of stable low-energy conformations of the protonated forms of the 2′-O-methylated purine nucleosides and their infrared spectra in the gas phase. Low-energy conformations highly parallel to those found for the protonated forms of the canonical DNA and RNA purine nucleosides are also found for the protonated 2′-O-methylated purine nucleosides. Importantly, the preferred site of protonation, nucleobase orientation, and sugar puckering are preserved among the DNA, RNA and 2′-O-methylated variants of the protonated purine nucleosides. The 2′-substituent does however influence hydrogen-bond stabilization as the 2′-O-methyl and 2′-hydroxyl substituents enable a hydrogen-bonding interaction between the 2′- and 3′-substituents, whereas a 2′-hydrogen atom does not. 2′-O-Methylation does however reduce the number of stable low-energy hydrogen-bonded conformations possible, and importantly inverts the preferred polarity of this interaction, versus that of the RNA analogs. Trends in the $CID_{50\%}$ values extracted from survival yield analyses of the 2′-O-methylated and canonical DNA and RNA forms of the protonated purine nucleosides are employed to elucidate their relative glycosidic bond stabilities, which are found to follow the order DNA < 2′-O-methylated < RNA. The glycosidic bond stability of the protonated purine nucleosides thus appears to be correlated with the hydrogen-bond stabilization of the sugar moiety.

Keywords: Electronic Structure Theory, Energy-Resolved Collision-Induced Dissociation (ER-CID), Glycosidic Bond Stability, Hydrogen-Bonding Interactions, Infrared Multiple Photon Dissociation (IRMPD) Action Spectroscopy, Nucleobase Orientation, 2′-O-Methyladenosine, 2′-O-Methylguanosine, 2′-O-Methylation, Posttranscriptional Modification, Protonation, Sugar Puckering

INTRODUCTION

Posttranscriptional modifications have been found in many types of RNA across archaea, bacteria, and eukarya.[1-3] Each of the canonical RNA nucleobases, adenine, cytosine, guanine, and uracil, can undergo modification at several sites; however, modification of the ribosyl moiety has only been found to occur naturally at the 2′-position.[1-2] The major chemical difference between DNA and RNA is their 2′-hydrogen versus 2′-hydroxyl substituents. This seemingly minor chemical change however greatly impacts the structures, stability, and functions of DNA and RNA nucleic acids. DNA typically adopts B-form double-stranded structures with C2′-endo sugar puckering,[4] whereas RNA typically exists as A-form single-stranded structures that exhibit C3′-endo sugar puckering.[3] But both DNA and RNA can exist in a variety of other structures. Modifications at the 2′-position of the ribose moieties may influence the overall structure and behavior of RNA. Although the known modifications of RNA nucleosides include a large diversity of chemical functionalities, methylation is involved in about two thirds of the modified nucleosides.[5-7] As a result, 2′-O-methylated nucleosides are being investigated in a variety of biological systems.[2]

2′-O-Methylated nucleosides are present in all major RNAs, and have also been discovered in small non-coding RNAs, such as spliceosomal RNAs (snRNAs).[2, 4-6] In ribosomal RNAs (rRNAs), the majority of these methylation sites are highly conserved among all living organisms.[8] Methylation on the ribose increases the stability of DNA:RNA and RNA:RNA duplexes.[9-13] In particular, during a study of thermophilic archaea by McCloskey and coworkers,[14] they found that the level of 2′-O-methyladenosine in 16S rRNA as well as the levels of 2′-O-methylcytidine and 2′-O-methylguanosine in 23S rRNA significantly increased when cultured at an increased temperature. These results indicate that both purine nucleosides, adenosine and guanosine, are influenced notably by temperature, and that 2′-O-methylation may lead to the increased stability of the rRNA of thermophilic archaea. 2′-O-Methylated purine nucleosides are also known to participate in the functions of transfer RNA (tRNA).[7,8, 15-17] For example, a single 2′-O-methylguanosine controls the activation or inhibition of Toll-like receptor 7, which is one of the

mediators of tRNA.[18] Furthermore, it has been discovered that the 5′-guanosine cap of messenger RNA (mRNA) of higher eukaryotes is 2′-O-methylated, and many viruses mimic this modification on their mRNA to avoid the self and non-self discrimination of mRNA from the host.[19-21] Thus, the structures of 2′-O-methylated purine nucleosides are worthy of study and important to compare to the canonical DNA and RNA purine nucleosides to elucidate the influence of this important and ubiquitous modification.

Methylation of the ribosyl moiety at the 2′-postion leads to changes in the electronic and hydrogen-bonding characteristics.[3] Compared to DNA nucleosides, the 2′-hydroxyl substituents in RNA nucleosides increases the number of favorable rotational orientations of the 2′- and 3′-hydroxyl substituents and the potential for hydrogen-bonding interactions.[3] The 2′-O-methylated substituent exhibits hydrogen-bonding characteristics that are intermediate between that of DNA and RNA, as it can act as a hydrogen-bond acceptor but not a hydrogen-bond donor, which limits the nature of the hydrogen-bonding interactions possible between the 2′- and 3′-hydroxyl substituents. Furthermore, the presence of the 2′-hydroxyl substituent also leads to a slight contraction of the glycosidic bond, suggesting that it increases the stability of the glycosidic bond of RNA nucleosides compared to the corresponding DNA nucleoside. Crystal structures have been reported for 2′-O-methyladenosine and 2′-O-methylguanosine.[22-23] Mass spectrometry has contributed significantly to the study of RNA modifications,[24-25] and in particular, in the work of McCloskey and coworkers.[13-14,26] Modified nucleosides are typically examined in their protonated forms in the mass spectrometer. The structures of protonated 2′-O-methylated purine nucleosides in the gas phase have not yet been reported. Establishing the structural similarities and differences of protonated 2′-O-methylated purine nucleosides in the gas and condensed phases can further validate the detection method of modified nucleosides and provide a better understanding of the ion behavior in mass spectrometry. The gas-phase structures of the neutral and protonated and sodium cationized forms of the canonical DNA and RNA nucleosides.[27-40] and deprotonated, neutral and protonated forms of the canonical DNA and RNA mononucleotides have been thoroughly investigated using IRMPD action spectroscopy[39, 41-50] and ion mobility mass

spectrometry approaches.[51] Comparison of results for 2′-O-methylated nucleosides to the analogous canonical nucleosides enables the influence of the 2′-O-methyl substituent on the structure and glycosidic bond stability of the nucleoside to be elucidated. With the understanding of their structural and conformational differences, nature's reason(s) for 2′-O-methylation may be revealed.

In the present work, the protonated forms of 2′-O-methyladenosine and 2′-O-methylguanosine, $[Adom+H]^+$ and $[Guom+H]^+$, are examined by infrared multiple photon dissociation (IRMPD) action spectroscopy[52-54] in the IR fingerprint region and hydrogen-stretching region.[55-56] Candidate structures are built, subjected to simulated annealing procedures, and their electronic structures are computed to determine the stable low-energy conformers and their relative stabilities. Linear IR spectra for the stable low-energy conformers are compared to the measured IR spectra to determine the conformers present in the experiments. Energy-resolved collision-induced dissociation (ER-CID) experiments are also performed to examine the relative glycosidic bond stabilities of $[Adom+H]^+$ and $[Guom+H]^+$ with respect to the canonical DNA and RNA purine nucleosides. Comparisons of $[Adom+H]^+$ and $[Guom+H]^+$ to their canonical DNA and RNA analogs elucidates the influence of the 2′-O-methyl substituent on the structures and glycosidic bond stability of the protonated purine nucleosides.

EXPERIMENTAL AND COMPUTATIONAL METHODS

Materials. 2′-O-methyladnosine (Adom) and 2′-O-methylguanosine (Guom) were purchased from Alfa Aesar (Haverhill, MA, USA). The HPLC grade methanol, water, and formic acid used for IRMPD experiments region were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France). The HPLC grade methanol used for the ER-CID measurements was purchased from Fischer Scientific (Waltham, MA, USA), and the HPLC grade water and formic acid used in these experiments were purchased from Sigma Aldrich (St. Louis, MO, USA).

IRMPD Action Spectroscopy Experiments. For the experiments in the IR fingerprint region, the analytes were dissolved and diluted to 10 μM in a MeOH:H_2O (50:50 v/v) mixture to

which 0.1% formic acid was added. The IRMPD spectra of the protonated forms of the 2′-O-methylated purine nucleosides, $[Adom+H]^+$ and $[Guom+H]^+$, were measured using the free electron laser (FEL) at the CLIO user facility, which is coupled to a 7 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS, Apex Qe, Bruker Daltonics). These instrumental setups have been described in detail elsewhere.[57-59] Briefly, ions were generated via an electrospray ionization (ESI) source. Ions were accumulated in the hexapole ion trap, extracted, and mass selected in a quadrupole mass filter prior to being transmitted to the ICR cell for trapping and photodissociation. The trapped $[Adom+H]^+$ or $[Guom+H]^+$ ions of interest were irradiated by the free electron laser for 280 ms at 5-10 dB attenuation over the range of ~900-1900 cm^{-1}. For the experiments in the hydrogen-stretching region, Adom and Guom were dissolved and diluted to 250 μM in a MeOH:H_2O (50:50 v/v) mixture with 0.1% formic acid added. The IRMPD spectra of $[Adom+H]^+$ and $[Guom+H]^+$ were collected on a modified ion trap mass spectrometer with an ESI source (LCQ classic, Thermo Finnigan) at the University of Lyon.[60-62] Ions were irradiated by a YAG-pumped tunable IR OPO/OPA (optical parametric oscillator/amplifier(laser system (LaserVision, Belleview, WA, USA) in the ion trap for 0.4−0.9 s in order to achieve visible IR bands between 2850 cm^{-1} and 3700 cm^{-1}. The IRMPD yield was plotted against the laser wavelength, and the yield was calculated as the ratio of the total fragment ion intensity versus the total ion intensity using eq 1,

$$\text{IRMPD yield} = \sum_i I_{f_i} / (\sum_i I_{f_i} + I_p) \quad (1)$$

where I_{f_i} and I_p are the ion intensities of the fragment and precursor ions, respectively. Each wavelength was power corrected for the frequency dependent variations in laser output.

ER-CID Experiments. Energy-resolved collision-induced dissociation (ER-CID) experiments for the protonated forms of the 2′-O-methylated purine nucleosides, $[Adom+H]^+$ and $[Guom+H]^+$ as well as the protonated forms of the analogous DNA and RNA nucleosides, $[dAdo+H]^+$, $[Ado+H]^+$, $[dGuo+H]^+$, and $[Guo+H]^+$, were performed on a quadrupole ion trap mass spectrometer (QIT MS, amaZon ETD, Bruker Daltonics, Billerica, MA, USA) in our laboratory at Wayne State University. The experimental protocal described in detail in previous work was again

employed for the experiments performed here.[32, 34] Ions were generated by ESI from solutions containing 10 μM Adom or Guom in a MeOH:H_2O (50:50 v/v) mixture with 1% (v/v) acetic acid infused at a rate of 3μL/min. Then ions were guided into in the ion trap, and the protonated nucleoside of interest was mass selected and subjected to CID. Helium buffer gas (~1 mtorr) was introduced into the ion trap for efficient trapping and cooling of the ions, but it also served as the collision gas for the CID experiments. The q_z value of ER-CID experiments was set to 0.25, which corresponds to a low mass cutoff at 27% of the precursor ion m/z. The rf excitation amplitude was increased from 0 V to the rf excitation amplitude required to produce complete dissociation of the precursor ion at a step size of 0.01 V. Experiments were performed in triplicate in order to access reproducibility. The data were processed using DataAnalysis 4.0 (Bruker Daltonics, Bremen, Germany).

Survival Yield Analyses. The intensities of the precursor and fragment ions were extracted from the ER-CID measurements. The survival yield was computed as the ratio of the precursor ion intensity to the total ion intensity using eq 2,

$$\text{Survival yield} = I_p/(\sum_i I_{f_i} + I_p) \quad (2)$$

where I_{f_i} and I_p are again the ion intensities of the fragment and precursor ions, respectively. The survival yield was plotted as a function of the rf excitation amplitude to produce the survival yield curve. The rf excitation amplitude required to produce 50% dissociation ($CID_{50\%}$) was extracted from the data by fitting the survival yield curve using a four parameter logistic dynamic algorithm, as shown in eq 3.

$$\text{Survival yield} = \min + (\max - \min)/[1 + (\text{rf}_{\text{EA}}/\text{CID}_{50\%}]^{\text{CIDslope}} \quad (3)$$

In this equation, max and min are the maximum and minimum values (1 and 0, respectively) of the survival yield, rf_{EA} is the rf excitation amplitude applied to induce fragmentation, and CIDslope is the slope of the declining region of the survival yield curve. Because the only CID pathway observed in the experiments was glycosidic bond cleavage with the proton retained by the nucleobase, the $CID_{50\%}$ values of the protonated canonical and 2′-O-methylated purine nucleosides were compared to elucidate their relative glycosidic bond stabilities. Data analyses were performed

using SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA, USA) and custom software developed in our laboratory.

Computational Details. The chemical structures of the canonical and 2′-O-methylated purine nucleosides are shown in Figure 1. The most favorable protonation sites of each 2′-O-methylated purine nucleoside were investigated, and include the N1, N3, and N7 positions of Adom and the N3, O6, and N7 positions of Guom. Simulated annealing procedures[63] were performed using HyperChem[64] software with the AMBER 3 force field to generated candidate structures for more accurate quantum mechanical calculations. The initial structure for the N1, N3, and N7 protonated forms of Adom and the N3, O6, and N7 protonated forms of Guom were built by modifying the most stable conformers determined for the analogous protonated forms of Ado and Guo found in previous work by simply replacing the 2′-hydroxyl hydrogen atom by a methyl group.[27-28] Each initial structure was subjected to 0.3 ps of thermal heating elevating the temperature from 0 to 1000 K, the temperature was held at 1000 K for 0.2 ps to allow sampling of conformational space, after which the system was cooled down to 0 K over a period of 0.3 ps. The resulting structure was optimized to a local minimum using the AMBER 3 force field. Molecular mechanics calculations were performed on the annealing structures every 1 fs of each cycle, and a snapshot of the lowest energy structure at the end of each cycle was captured and used as the initial structure for the subsequent cycle. This process was repeated for 300 cycles for each protonated form of each nucleoside, N1, N3, and N7 protonated Adom and N3, O6, and N7 protonated Guom, providing 900 structures for each protonated nucleoside. The 30 lowest-energy structures based on the molecular mechanical calculations were chosen for higher level quantum mechanical calculations. Additional structures were also built to ensure comprehensive sampling of the structure pool, with these additional conformers choices biased by results found for the analogous protonated forms of Ado and Guo.[27-28] Geometry optimizations and harmonic vibrational frequency analyses were performed using the Gaussian 09 suite of programs.[65] The B3LYP/6-311+G(d,p) level of theory was employed for geometry optimizations and frequency analyses to provide results directly comparable to those of previous investigations of the protonated forms of

Ado and Guo.[27-28] The frequencies were scaled by a factor of 0.980 in the fingerprint region and 0.954 in the hydrogen-stretching region to help correct for limitations in the theory caused by the harmonic approximation, use of finite basis sets, and approximate treatment of electron correlation. In order to reproduce the observed experimental broadening, the computed IR spectra were convoluted with a Gaussian line shape of 20 cm^{-1} fwhm (full width at half maximum) in the fingerprint region and 10 cm^{-1} fwhm in the hydrogen-stretching region. In the previous studies of the protonated forms of Ado and Guo, energetics based on both MP2 and B3LYP theories were examined. Comparisons of the measured IRMPD spectra and the predicted IR spectra and relative stabilities of the conformers populated in the experiments suggested that B3LYP predicted the energetics of these systems slightly better than MP2 theory. Therefore, energetic calculations performed here were limited to B3LYP with a 6-311+G(2d,2p) basis set to again provide results directly comparable to those of previous investigations of the protonated forms of Ado and Guo.[27-28] To help explain the possible presence of higher-energy conformers of $[Adom+H]^+$ and $[Guom+H]^+$ in the experiments, the effects of solvation on the relative stabilities of ground and the relevant excited conformers of the these species were also examined using the same theoretical approaches, but in a polarizable continuum model corresponding to water.

RESULTS

IRMPD Action Spectroscopy. Only a single photodissociation pathway is observed for $[Adom+H]^+$ and $[Guom+H]^+$ in both the fingerprint and hydrogen-stretching regions. For both systems, photodissociation results in glycosidic bond cleavage with the excess proton retained by the nucleobase, resulting in elimination of the neutral O2′-methylated sugar moiety as summarized in reactions 4 and 5.

$$[Adom+H]^+ + n\,h\nu \rightarrow [Ade+H]^+ + (Adom\text{-}Ade) \quad (4)$$

$$[Guom+H]^+ + n\,h\nu \rightarrow [Gua+H]^+ + (Guom\text{-}Gua) \quad (5)$$

These results exactly parallel those found for the protonated canonical DNA and RNA purine nucleosides, $[dAdo+H]^+$, $[Ado+H]^+$, $[dGuo+H]^+$, and $[Guo+H]^+$.[27-28]

The IRMPD spectrum measured here for $[Adom+H]^+$ is compared with those measured for the corresponding canonical DNA and RNA analogs, $[dAdo+H]^+$ and $[Ado+H]^+$ in Figure 2. As can be seen in the figure, the IRMPD spectra of these protonated nucleoside analogs are highly parallel. Except for the broadening and splitting of the intense feature spanning the region from ~1070-1150 cm^{-1} and the minor splitting of the feature at ~1660 cm^{-1} in the spectrum of $[Adom+H]^+$, only modest shifts in the band positions are observed in both the fingerprint and hydrogen-stretching regions, indicating a high degree of structural similarity among the conformers populated for these protonated nucleosides. In contrast, significant differences in the IRMPD yields are observed. Spectral features observed at ~1100, 1130, 1210, 1650 and 3430 cm^{-1} in the spectrum of $[Adom+H]^+$ are much more intense than the corresponding features in the spectra of $[dAdo+H]^+$ and $[Ado+H]^+$, whereas virtually all other features become much less intense and may only be discernible from baseline with amplification (see Figure 2). The differences in the IRMPD yields may arise from the variation in the sugar moiety across these systems; however, discrimination resulting from differences in the experimental set-ups employed in the work performed here versus that used for the canonical nucleosides, which were measured at the FELIX facility, cannot be ruled out. It should be noted that although the overall IRMPD yields for $[dAdo+H]^+$ and $[Ado+H]^+$ exhibit a greater degree of similarity, the features that do exhibit the largest variation in yield in the spectrum of $[Adom+H]^+$ also exhibit sizeable differences in yield in the spectra of the canonical analogs.

The IRMPD spectrum measured here for $[Guom+H]^+$ is compared with those measured for the corresponding canonical DNA and RNA analogs, $[dGuo+H]^+$ and $[Guo+H]^+$ in Figure 3. Similar to that found for the protonated adenine nucleosides, the IRMPD spectra of the protonated guanine nucleosides are also highly parallel. Except for the disappearance of the shoulder to the red of the intense feature observed at ~1100 cm^{-1} in the spectrum of $[Guom+H]^+$, only modest shifts in the band positions are again observed in both the fingerprint and hydrogen-stretching

regions. These spectral similarities are clear indicators of a high degree of structural similarity among the conformers populated for these protonated guanine nucleoside analogs. Significant differences in the IRMPD yields of several features are also again observed, with features at ~1110 and 3470 cm^{-1} in the spectrum of $[Guom+H]^+$ being much more intense than the corresponding features in the spectra of $[dGuo+H]^+$ and $[Guo+H]^+$. Other spectral features of $[Guom+H]^+$ are much less intense than those in the spectra of the canonical analogs, particularly in the range between ~1160 and 1550 cm^{-1} (see Figure 3).

Energy-Resolved Collision-Induced Dissociation. As found in the photodissociation experiments, CID of $[Adom+H]^+$ and $[Guom+H]^+$ proceeds via a single fragmentation pathway involving glycosidic bond cleavage with the excess proton retained by the nucleobase, resulting in elimination of the neutral O2′-methylated sugar moiety as summarized in reactions 6 and 7.

$$[Adom+H]^+ + n\ He \rightarrow [Ade+H]^+ + (Adom\text{-}Ade) \quad (6)$$

$$[Guom+H]^+ + n\ He \rightarrow [Gua+H]^+ + (Guom\text{-}Gua) \quad (7)$$

These results exactly parallel those found for the protonated canonical DNA and RNA purine nucleosides, $[dAdo+H]^+$, $[Ado+H]^+$, $[dGuo+H]^+$, and $[Guo+H]^+$. Representative CID mass spectra of the protonated forms of all six of these nucleosides are shown in Figure 4 at rf excitation amplitudes that produce slightly less than 50% fragmentation. The parallel dissociation behavior observed in the IRMPD and CID experiments indicates that activation via multiple low-energy collisions or multiple IR photon absorption results in the same unimolecular dissociation pathways for these protonated nucleosides.

Survival Yield Analyses. The survival yield curves measured for the protonated 2′-O-methylated purine nucleosides, $[Adom+H]^+$ and $[Guom+H]^+$, are compared to those measured for their canonical DNA and RNA analogs in Figure 5. Most notably, the declining region of the survival yield curves for the protonated adenine nucleosides are all shifted to significantly higher rf excitation amplitudes than the protonated guanine nucleosides, indicating that the glycosidic bonds of the protonated adenine nucleosides are stronger than those of the protonated guanine nucleosides. This observation is consistent with activation energies (AEs) measured for glycosidic

bond cleavage of the protonated forms of the canonical DNA and RNA nucleosides of adenine and guanine.[66-67] $CID_{50\%}$ values and estimates for the uncertainties in these values extracted from fitting the survival yield data using eq 3 are also given in Figure 5. Trends in the $CID_{50\%}$ values follow the order $[dAdo+H]^+ < [Ado+H]^+ < [Adom+H]^+$ for the protonated adenine nucleosides, suggesting that 2′-O-methylation strengthens the glycosidic bond relative to the canonical DNA and RNA nucleosides. In contrast, the $CID_{50\%}$ values follow the order $[dGuo+H]^+ < [Guom+H]^+ < [Guo+H]^+$ for the protonated guanine nucleosides, which suggest that 2′-O-methylation slightly weakens the glycosidic bond relative to the RNA form, but is still stronger than the DNA form. Clearly accurate thermodynamic measurements of the AEs for glycosidic bond cleavage of the protonated 2′-O-methylated purine nucleosides are of interest and would help clarify the differences that this modification produces to the stability of the protonated purine nucleosides.

Theoretical Results. The stable conformations of $[Adom+H]^+$ and $[Guom+H]^+$ were comprehensively sampled as described in the Computational Details section. Each of the stable B3LYP/6-311+G(d,p) conformers computed are classified based on the site of protonation, nucleobase orientation, sugar puckering, and relative B3LYP/6-311+G(2d,2p) Gibbs free energy at 298 K. The classifications employed for the nucleobase orientation and sugar puckering are described in Figure S1 of the Supporting Information.[68-69] All stable B3LYP/6-311+G(d,p) conformers computed for $[Adom+H]^+$ are shown in Figure S2, whereas those computed for $[Guom+H]^+$ are displayed in Figure S3. The nomenclature employed for each conformer is based on the site of protonation, N3, N1 or N7 for $[Adom+H]^+$ and N7, O6, and N3 for $[Guom+H]^+$, followed by a letter that indicates the order of relative stability among all conformers protonated at that site, where A is used for the most stable conformer protonated at a given site, B for the next most stable, etc.

N3, N1, and N7 protonation of $[Adom+H]^+$. The stable conformers calculated for $[Adom+H]^+$ are highly parallel to those found for $[dAdo+H]^+$ and $[Ado+H]^+$.[27] In particular, the most stable structure computed for each site of protonation of Adom is very similar to those of found for dAdo and Ado. A detailed comparison of the geometric parameters of the most stable

N3, N1 and N7 protonated forms of Adom, **N3A**, **N1A** and **N7A**, to those of dAdo and Ado is provided in Table 1. The ground N3 protonated conformer of Adom is favored over the most stable N1 and N7 protonated conformers by 25.9 and 33.2 kJ/mol. These stability differences are within 1 and 4 kJ/mol of that found for $[dAdo+H]^+$ and $[Ado+H]^+$, respectively. The preference for N3 protonation results in a *syn* nucleobase orientation stabilized by a strong $N3H^+\cdots O5'$ hydrogen-bonding interaction and C2′-endo puckering in the ground conformer. In contrast, the N1 and N7 protonated structures prefer an *anti* nucleobase orientation as no such hydrogen-bonding interaction is possible. Changes in the sugar puckering, nucleobase orientation, and the hydrogen-bonding interaction between the 2′- and 3′-substituents, are less destabilizing than changes in the site of protonation such that the stable conformers computed exhibit a wide variety of sugar puckerings and both *syn* and *anti* nucleobase orientations. The most common C2′-endo and C3′-endo puckering modes are favored over all others, with C3′-endo preferred over C2′-endo for the N3 and N1 protonated species, and C2′-endo favored over C3′-endo for the N7 protonated conformers.

N7, O6, and N3 protonation of $[Guom+H]^+$. As found for $[Adom+H]^+$, the stable conformers calculated for $[Guom+H]^+$ are also highly parallel to those found for $[dGuo+H]^+$ and $[Guo+H]^+$.[28] The geometric parameters of the most stable N7 and O6, and second most stable N3 protonated conformers, **N7A**, **O6A**, and **N3B** are compared in Table 2. The ground N7 protonated conformer of Guom is favored over the most stable O6 and N3 conformers by 37.4 and 47.6 kJ/mol (or 48.4 kJ/mol for **N3B**). These stability differences are within 4.0 and 6.8 kJ/mol of that found for $[dGuo+H]^+$ and $[Guo+H]^+$, respectively. The N7 and O6 protonated conformers exhibit a preference for an *anti* nucleobase orientation, whereas similar to that found for Adom, the N3 protonated conformers are stabilized by a strong $N3H^+\cdots O5'$ hydrogen-bonding interaction such that *syn* is preferred over *anti* for these conformers. Changes in the sugar puckering, nucleobase orientation, and the hydrogen-bonding interaction between the 2′- and 3′-substituents, are less destabilizing than changes in the site of protonation such that the stable conformers computed also exhibit a wide variety of sugar puckerings and both *syn* and *anti* nucleobase orientations. The most

common C2′-endo and C3′-endo puckering modes are again favored over others, with C3′-endo preferred over C2′-endo for the N7 protonated species, and C2′-endo favored over C3′-endo for the O6 and N7 protonated conformers.

The relative enthalpies and Gibbs free energies at 0 and 298 K of the most stable N3, N1, and N7 conformers of $[Adom+H]^+$ and the most stable N7, O6, and N3 conformers of $[Guom+H]^+$ (along with two other N7 protonated conformers that are populated in the experiments) and their nucleobase orientations, pseudorotation phase angles (P), and sugar puckerings are compared in Table 3. For more comprehensive comparisons of the stable conformations of the protonated forms of the DNA, RNA, and O2′-methylated purine nucleosides, the interested reader is directed to the Figures in the Supporting Information associated with this manuscript as well as the Figures in the Supporting Information of references 27 and 28.

DISCUSSION

Conformers of $[Adom+H]^+$ Populated by ESI in the Gas Phase. The computed IR spectra for the stable conformations of $[Adom+H]^+$ were compared with the experimental IRMPD spectrum to elucidate the structures populated in the experiments. The predicted IR signatures vary with the site of protonation, and both N3 and N1 protonated structures provide IR features that match the experimental spectrum well. The calculated IR spectra predicted for the **N3A** and **N1A** conformers are compared with the IRMPD spectrum of $[Adom+H]^+$ in Figure 6. Both conformers provide a very good match, and combined provide an excellent match, to the measured IRMPD spectrum. This is particularly apparent in the broadening of the feature observed at ~1660 cm^{-1}, and suggests that both conformers contribute, possibly nearly equally given the splitting and peak shape of this feature, to the experimental population. Similar spectral comparisons of other low-energy N3 protonated conformers exhibiting a variety of nucleobase orientations and sugar puckerings are shown in Figure S4. Based on spectral misalignments indicated with red highlighting, none of these conformers were significantly populated in the experiments. Similar spectral comparisons of N3 protonated *syn* C2′-endo conformers are shown in Figure S5. Spectral

misalignments highlighted in red again eliminate these conformers from contributing significantly to the experimental population. Figures S6 and S7 provide similar spectral comparisons for a variety of N1 protonated conformers, and indicate that N1 conformers with different sugar puckering, nucleobase orientation, or hydrogen-bonding interactions than **N1A** are not accessed in the experiments. Obvious spectral misalignments are found for all N7 protonated conformers, eliminating them as significant contributors to the experimental population as well; several example comparisons are shown in Figure S8.

Although **N1A** is predicted to be 25.9 kJ/mol less stable (298 K Gibbs free energy) than **N3A**, the broadening and splitting of the band observed at ~1660 cm^{-1} suggests that both of these conformers contribute to that spectral feature. This IR feature arises from C6–N6 stretching and is sensitive to the site of protonation of the nucleobase. In general, N3 protonated conformers exhibit this IR feature at ~1650 cm^{-1}, whereas N1 protonated conformers exhibit this feature at ~1670 cm^{-1}. In order to understand the presence of **N1A** in the experiments given its relatively high free energy versus that of **N3A**, these conformers were also computed in a polarizable continuum model corresponding to water. The order of relative stability of these two conformers was maintained in the polarizable continuum, but their energy difference reduced from 25.9 to 9.3 kJ/mol. More accurate calculations, possibly involving a few explicit water molecules might further shift the relative stabilities of these conformers in solution and thus explain their seemingly roughly equal populations in the experiments. Vibrational band assignments of the features observed in the IRMPD spectrum of $[Adom+H]^+$ are provided in Table 4 and based on vibrational mode analyses of the **N3A** and **N1A** conformers, which appear to contribute nearly equally to the experimental population accessed by ESI.

Conformers of $[Guom+H]^+$ Populated by ESI in the Gas Phase. The computed IR spectra for the stable conformations of $[Guom+H]^+$ were compared with the experimental IRMPD spectrum to elucidate the structures populated in the experiments. The predicted IR signatures again vary with the site of protonation, and only N7 protonated conformers are found to exhibit features that match the experimental spectrum well. The calculated IR spectra predicted for the

N7A, **N7B**, and **N7H** conformers are compared with the measured IRMPD spectrum of $[Guom+H]^+$ in Figure 7. Although the ground **N7A** conformer might be expected to be the dominant contributor to the experiments, the spectral shift of the intense feature near 1100 cm^{-1} suggests that may not be the case, and that there may be other conformers that are also important contributors to the measured spectrum. Conformer **N7B** provides good agreement with the experimental spectrum except that the minor feature observed at ~3550 cm^{-1} is predicted at a slightly higher vibrational frequency in the theoretical spectrum. However, this feature is associated with the C8H···O5′ noncanonical hydrogen bond, and the vibrational frequency of such hydrogen-bonded modes are often overestimated in harmonic calculations.[70-73] Therefore, the **N7B** conformer probably contributes to the measured spectrum as well. A very minor peak is observed at ~3520 cm^{-1} that cannot be explained by the IR signatures of either the **N7A** or **N7B** conformers. A stretch is predicted at this vibrational frequency for the **N7H** conformer, a *syn*, C2′-endo structure predicted to be less stable than the ground **N7A** conformer by 23.3 kJ/mol. The low intensity of this IRMPD feature suggests a minor presence of the **N7H** conformer in the experiments, which is consistent with its high relative Gibbs free energy. Interestingly, the **N7H** conformer probably contributes to the broad band at ~1100 cm^{-1} and provides better spectral alignment with the peak at ~3450 cm^{-1} as well, suggesting that its population may not be quite so minor. In order to understand the presence of **N7H** in the experiments given its relatively high free energy versus that of **N7A**, these conformers were also computed in a polarizable continuum model corresponding to water. The order of relative stability of these two conformers was also maintained in the polarizable continuum, but their energy difference reduced from 23.3 to 10.9 kJ/mol. Again, more accurate calculations might further shift the relative stabilities of these conformers in solution and thus explain the presence of **N7H** in the experiments. Detailed comparisons of other N7 protonated conformers that exhibit different combinations of nucleobase orientation and sugar puckering with the experimental IRMPD spectrum are shown in Figure S9. Similar comparisons for other N7 protonated conformers that adopt *anti* and C3′-endo, *anti* and C2′-endo, and *syn* and C2′-endo conformations are shown in Figures S10-12, respectively. Spectral mismatches for each

conformer are again highlighted in red and eliminate these conformers as important contributors to the experimental population. Three other N7 protonated conformers besides those shown in Figure 6 exhibit relatively good agreement with the experimental IRMPD spectrum, and include **N7I** (Figure S10), **N7D** (Figure S11), and **N7O** (Figure S12). **N7D** and **N7B** differ in the orientation of the 3′-hydroxyl substituent, **N7D** is computed to be 8.5 kJ/mol higher in free energy and thus is likely present in minor abundance in the experiments as changes in the orientation of the 3′-hydroxyl substituent should be readily achieved in solution such that both conformers are accessed by the ESI process. Similarly, the **N7I** and **N7O** conformers are 3′-hydroxyl rotamers of the **N7A** and **N7H** conformers, respectively, and their predicted IR spectra do not provide unique features that enable them to be eliminated from the experimental population. However, the higher Gibbs free energy of the **N7D**, **N7I**, and **N7O** conformers suggest that they are less important contributors to the experiments than **N7A**, **N7B**, and **N7H**. Comparison of IR spectra predicted for stable O6 protonated conformers to the measured IRMPD spectrum are shown in Figure S13. All O6 protonated structures exhibit a strong IR feature at ~1690 cm^{-1} associated with C5−C6 stretching. The lack of this feature in the experimental spectrum indicates that O6 structures are not present in measureable abundance in the experiments. This conclusion is further supported by the mismatch in vibrational frequency of the O6–H stretch, which is predicted at ~3580 cm^{-1} some 20 cm^{-1} below the frequency of the feature observed in this region. Comparisons of IR spectra predicted for stable N3 protonated conformers to the measured IRMPD spectrum are shown in Figure S14. The free C=O stretch of these conformers is predicted to occur at ~1820 cm^{-1}, whereas the vibrational frequency of the nearest spectral feature observed is ~1670 cm^{-1} indicating that it is instead most likely associated with the C2−N2 stretching mode of N7 protonated structures. Further, the asymmetric NH_2 stretching mode is predicted at ~3540 cm^{-1}, some 30 cm^{-1} to the red of the nearest feature in the IRMPD spectrum. These spectral mismatches clearly establish the absence of N3 protonated conformers in the experimental population. Thus, only N7 protonated conformers of $[Guom+H]^+$ are accessed in the experiments, with the **N7A**, **N7B**, and **N7H** conformers present in largest abundance, and **N7D**, **N7I**, and **N7O** conformers possibly present in

lower abundance. Vibrational band assignments of the features observed in the IRMPD spectrum of $[Gdom+H]^+$ are provided in Table 5 and based on vibrational mode analyses of the **N7A** and **N7H** conformers as the frequencies of the vibrational modes of the other N7 protonated conformers are nearly identical to those of these two conformers.

Structural Effects of the 2′-Substituent on the Stable Conformations of Protonated Ado and Guo Nucleosides. The ground **N3A** conformer of $[Adom+H]^+$ is compared to the ground conformations previously reported for $[dAdo+H]^+$ and $[Ado+H]^+$ in Figure 1. The geometric properties of the ground **N3A** conformers as well as those of the most stable N1 and N7 protonated conformers, **N1A** and **N7A**, of all three nucleosides are listed in Table 1.[27] Variation in the 2′-substituent has very little impact on the preferred conformation of these adenine nucleosides as the site of protonation (N3), nucleobase orientation (*syn*), and sugar puckering (C2′-endo) are preserved for dAdo, Ado, and Adom. The 2′-substituent does however influence hydrogen-bond stabilization as the 2′-O-methyl and 2′-hydroxyl substituents enable a hydrogen-bonding interaction between the 2′- and 3′-substituents, whereas a 2′-hydrogen atom does not. The conformational diversity enabled by the hydrogen-bonding interaction between the 2′- and 3′-substituents however differs, as Ado can form either O2′H···O3′ or O2′···HO3′ hydrogen-bonding interactions, whereas Adom can only form O2′···HO3′ hydrogen-bonding interactions. As a result, 2′-O-methylation reduces the number of very favorable low-energy hydrogen-bonding orientations possible, and importantly inverts the preferred polarity of this interaction in $[Adom+H]^+$ versus that of $[Ado+H]^+$. The 2′-O-methyl substituent leads to an enhancement in the IRMPD yield of several features of $[Adom+H]^+$ providing a stronger spectroscopic handle for elucidation of the conformers present in the experiments than the 2′-hydrogen and 2′-hydroxyl substituents of the canonical analogs. For $[dAdo+H]^+$, five conformers exhibited predicted IR spectra that suggested that they may be populated in the experiments, with **N3A** (*syn*, C2′-endo), **N3B** (*syn*, C3′-endo), **N3C** (*syn*, C2′-endo), and **N1A** (*anti*, C2′-endo) dominant in the population, and a minor presence of **N3D** (*anti*, C2′-endo). For $[Ado+H]^+$, only three conformers were appreciably populated by ESI, **N3A** (*syn*, C2′-endo, O2′H···O3′), **N3C** (*syn*, C3′-endo, O2′H···O3′), and **N1A** (*anti*, C2′-endo,

O2′H···O3′). For $[Adom+H]^+$, only two conformers were populated by ESI, **N3A** (*syn*, C2′-endo, O2′···HO3′) and **N1A** (*anti*, C2′-endo, O2′···HO3′). Overall, the conformations of these protonated adenine nucleosides populated by ESI are all very similar; the 2′-substituent does not alter the preferred site of protonation or sugar puckering, but does influence intramolecular hydrogen-bond stabilization. The structural effects of O2′-methylation thus elucidated for the protonated adenine nucleosides are somewhat limited, however the bulky methyl group would alter hydrogen-bonding interactions with the sugar and sterics within the minor groove of double helical structures with O2′-methylated nucleosides.[74]

The ground **N7A** conformer of $[Guom+H]^+$ is compared to the ground conformations previously reported for $[dGuo+H]^+$ and $[Guo+H]^+$ in Figure 1. Geometric properties of the ground **N7A** conformers as well as those of the most stable O6 and N3 protonated conformers, **O6A** and **N3B**, are compared in Table 2. As found for the adenine nucleosides, variation of the 2′-substituent has very little impact on the preferred conformation of these guanine nucleosides as the preferred site of protonation (N7), nucleobase orientation (*anti*), and sugar puckering (C3′-endo) are preserved among dGuo, Guo, and Guom. For $[dGuo+H]^+$, only two conformers, **N7A** (*anti*, C3′-endo) and **N7B** (*anti*, C2′-endo) were populated in the experiments. The diversity of conformations populated is enhanced by intramolecular hydrogen-bonding interactions for $[Guo+H]^+$ with **N7A** (*anti*, C3′-endo , O2′···HO3′), **N7B** (*anti*, C2′-endo, O2′···HO3′), **N7C** (*anti*, C3′-endo, O2′H···O3′), and **N7D** (*anti*, C2′-endo, O2′H···O3′) present along with a minor population of **N7E** (*syn* C2′-endo, O2′···HO3′). Only two conformers of $[Guom+H]^+$ are appreciably populated, **N7A** (*anti*, C3′-endo , O2′H···O3′), **N7B** (*anti*, C2′-endo , O2′H···O3′), and minor populations of **N7H** (*syn*, C2′-endo), and less stable 3′-hydroxyl rotamers of these conformers, **N7D**, **N7I**, and **N7O** . The structures of these protonated guanine nucleosides populated by ESI are very similar again indicating that the 2′-substituent does not alter the preferred site of protonation or sugar puckering, but does influence intramolecular hydrogen-bond stabilization. The structural effects of O2′-methylation thus elucidated for the protonated guanine nucleosides are also somewhat limited such

that the influence of this substituent is likely exhibited in the way it alters interactions with other molecules.

Influence of the 2′-Substituent on the Glycosidic Bond Stabilities of Protonated Ado and Guo Nucleosides. The $CID_{50\%}$ values extracted from survival yield analyses of the ER-CID data suggest that the glycosidic bond stabilities of the protonated adenine nucleosides exceed those of the protonated guanine nucleosides. This conclusion is consistent with threshold collision-induced dissociation (TCID) studies performed using guided ion beam tandem mass spectrometry approaches. The measured the AEs for glycosidic bond cleavage of of $[dGuo+H]^+$ and $[Guo+H]^+$ were measured as 93.6±2.9 kJ/mol and 114.8±2.9 kJ/mol, respectively. The AEs for glycosidic bond cleavage of $[dAdo+H]^+$ and $[Ado+H]^+$ are roughly 50 kJ/mol larger, 147.6±4.8 kJ/mol and 164.0±4.8 kJ/mol, respectively.[66-67] As concluded in the TCID work, base rotation induced by N3 protonation of the adenine residue and leading to formation of a strong $N3H^+\cdots O5'$ hydrogen-bonding interaction of the protonated adenine nucleosides plays a pivotal role in the intrinsic mechanisms for their glycosidic bond cleavage and is the primary reason for the large increase in their AEs for glycosidic bond cleavage versus the analogous guanine nucleosides.

The $CID_{50\%}$ values for the guanine series follow the order: $[dGuo+H]^+ < [Guom+H]^+ < [Guo+H]^+$. The $CID_{50\%}$ values, and therefore the relative glycosidic bond stabilities, exhibit an inverse correlation with the C1′–N9 glycosidic bond lengths of these species (see Table 2) as expected. However, the C1′–N9 glycosidic bond lengths of the protonated adenine nucleosides also follow the same trend as the protonated guanine nucleosides (see Table 1). We interpret the change in the relative $CID_{50\%}$ values, and thus relative glycosidic bond stabilities of the protonated adenine nucleosides, as resulting from the strong $N3H^+\cdots O5'$ hydrogen bonding interaction that stabilizes these nucleosides. In addition, the O2′···HO3′ hydrogen-bonding interaction of Adom should further constrain the transition state versus the slightly weaker O2′H···O3′ hydrogen-bonding interaction of Ado. The inversion of the O2'-O3' hydrogen bonding interactions between the 2'- and 3'-hydroxyl substituents does not appear as limited in the absence of the strong ionic hydrogen-bonding interactions. Clearly, mapping of the potential energy surfaces for glycosidic

bond cleavage of $[Adom+H]^+$ and $[Guom+H]^+$ along with accurate thermodynamic measurements of their AEs for glycosidic bond cleavage would definitively resolve remaining questions regarding the mechanistic and energetic effects of 2′-O-methylation of the protonated purine nucleosides.

CONCLUSIONS

The structural and energetic effects of 2′-O-methylation of the protonated purine nucleosides, $[Adom+H]^+$ and $[Guom+H]^+$, were investigated experimentally by IRMPD action spectroscopy and ER-CID and theoretically via electronic structure calculations. The calculations find that the stable low-energy conformations of the protonated 2′-O-methylated purine nucleosides are highly parallel to those found for the protonated forms of the canonical purine nucleosides. The preferred site of protonation (N3 for Adom and N7 for Guom), nucleobase orientation (*syn* for $[Adom+H]^+$ and *anti* for $[Guom+H]^+$), and sugar puckering (C2′-endo for $[Adom+H]^+$ and C3′-endo for $[Guom+H]^+$), are preserved among the DNA, RNA and 2′-O-methylated variants of the protonated purine nucleosides. The 2′-substituent does influence intramolecular hydrogen-bond stabilization, and inverts the preferred polarity from O2′···HO3′ for the RNA nucleoside to O2′H···O3′ for the 2′-O-methylated nucleoside. Thus, not surprisingly, the IRMPD spectra of the protonated purine nucleosides are also highly parallel. Only minor splitting or shifts in the band positions are observed across the DNA, RNA, 2′-O-methylated series for the protonated adenine and guanine nucleosides. However, significant differences are seen in the IRMPD yields. Interpretation of the IRMPD spectra also finds that the conformers of $[Adom+H]^+$ and $[Guom+H]^+$ populated in the experiments by ESI are also quite parallel to those found for the canonical DNA and RNA purine nucleosides. Both N3 and N1 protonated conformers of $[Adom+H]^+$ contribute, whereas only N7 protonated conformers of $[Guom+H]^+$ are present in the experiments. The conformations populated in the experiments by ESI generally involve the ground and very low-lying conformations, but excited conformers also contribute and their presence is explained based on preferential stabilization of these excited conformers in solution. Trends in the

$CID_{50\%}$ values extracted from survival yield analyses of the 2′-O-methylated and canonical DNA and RNA forms of the protonated purine nucleosides indicate that its influence is base dependent. 2′-O-methylation slightly increases the glycosidic bond stability of $[Adom+H]^+$, but leads to a slight weakening of the glycosidic bond of $[Guom|H]^+$. The differential influence of 2′-O-methylation likely arises due to the strong $N3H^+\cdots O5'$ hydrogen bonding interaction at play in $[Adom+H]^+$. Overall, the structural effects of 2′-O-methylation of the protonated purine nucleosides are modest, lead to inversion of the hydrogen-bonding interaction between the 2′- and 3′-substituents, and indeed may primarily involve changes in the way in which the nucleoside interacts (via hydrogen-bonding interactions) with other molecules. Likewise, the energetic effects of 2′-O-methylation of the protonated purine nucleosides are modest as well, and appear to be influenced by differences in intramolecular hydrogen-bond stabilization within these systems.

SUPPORTING INFORMATION

Complete citations for references 64 and 65. Figures demonstrating the values of the ∠C4N1C1′O4′ dihedral angle that define the nucleobase orientation as syn or anti and the nomenclature employed to describe the sugar puckering, showing all stable B3LYP/6-311+G(d,p) conformers computed for $[Adom+H]^+$ and $[Guom+H]^+$ including the conformer designation, the nucleobase orientation, sugar puckering conformation, and relative B3LYP/6-311+G(2d,2p) Gibbs free energies at 298 K, comparing the IRMPD measured spectra of $[Adom+H]^+$ and $[Guom+H]^+$ with the IR spectra predicted for various stable conformers of these species that exhibit spectral signatures that preclude their importance in the experiments.

ACKNOWLEDGMENTS

This work is financially supported by the National Science Foundation, under Grants OISE-0730072 and OISE-1357787 (for international travel expenses), DBI-0922819 (for the Bruker amaZon ETD QITMS employed in this work), and CHE-1709789 (for other research costs). C.C.H., L.A.H., Y.Z., and Y.-w.N. gratefully acknowledge support from Wayne State University Thomas C. Rumble Graduate Fellowships and Summer Dissertation Fellowships. We thank

Wayne State University C&IT for excellent computational resources and support. The skillful assistance of the CLIO staff is gratefully acknowledged.

REFERENCES

1. Limbach, P. A.; Crain, P. F.; Mccloskey, J. A. Summary: The Modified Nucleosides of RNA. *Nucleic Acids Res* **1994**, *22*, 2183-2196.
2. Cantara, W. A.; Crain, P. F.; Rozenski, J.; McCloskey, J. A.; Harris, K. A.; Zhang, X. N.; Vendeix, F. A. P.; Fabris, D.; Agris, P. F. The RNA Modification Database, RNAMDB: 2011 Update. *Nucleic Acids Res* **2011**, *39*, D195-D201.
3. Rozenski, J.; Crain, P. F.; McCloskey, J. A. The RNA Modification Database: 1999 Update. *Nucleic Acids Res* **1999**, *27*, 196-197.
4. Nelson, D. L.; Cox, M. M., *Lehninger Principles of Biochemistry*, Seventh ed.; W. H. Freeman and Company: New York, NY, 2017.
5. Dunin-Horkawicz, S.; Czerwoniec, A.; Gajda, M. J.; Feder, M.; Grosjean, H.; Bujnicki, J. M. MODOMICS: A Database of RNA Modification Pathways. *Nucleic Acids Res* **2006**, *34*, D145-D149.
6. Czerwoniec, A.; Dunin-Horkawicz, S.; Purta, E.; Kaminska, K. H.; Kasprzak, J. M.; Bujnicki, J. M.; Grosjean, H.; Rother, K. MODOMICS: A Database of RNA Modification Pathways. 2008 Update. *Nucleic Acids Res* **2009**, *37*, D118-D121.
7. Machnicka, M. A.; Milanowska, K.; Oglou, O. O.; Purta, E.; Kurkowska, M.; Olchowik, A.; Januszewski, W.; Kalinowski, S.; Dunin-Horkawicz, S.; Rother, K. M.; Helm, M.; Bujnicki, J. M.; Grosjean, H. MODOMICS: A Database of RNA Modification Pathways-2013 Update. *Nucleic Acids Res* **2013**, *41*, D262-D267.
8. Motorin, Y.; Helm, M. RNA Nucleotide Methylation. *Wires RNA* **2011**, *2*, 611-631.
9. Majlessi, M.; Nelson, N. C.; Becker, M. M. Advantages of 2'-O-Methyl Oligoribonucleotide Probes for Detecting RNA targets. *Nucleic Acids Res* **1998**, *26*, 2224-2229.
10. Lesnik, E. A.; Guinosso, C. J.; Kawasaki, A. M.; Sasmor, H.; Zounes, M.; Cummins, L. L.; Ecker, D. J.; Cook, P. D.; Freier, S. M. Oligodeoxynucleotides Containing 2'-O-Modified Adenosine - Synthesis and Effects on Stability of DNA-RNA Duplexes. *Biochemistry-Us* **1993**, *32*, 7832-7838.
11. Egli, M. Structural Aspects of Nucleic Acid Analogs and Antisense Oligonucleotides. *Angew Chem Int Edit* **1996**, *35*, 1895-1910.
12. Lesnik, E. A.; Freier, S. M. What Affects the Effect of 2'-Alkoxy Modifications? 1. Stabilization Effect of 2'-Methoxy Substitutions in Uniformly Modified DNA Oligonucleotides. *Biochemistry-Us* **1998**, *37*, 6991-6997.
13. Lubini, P.; Zurcher, W.; Egli, M. Stabilizing Effects of the RNA 2'-Substituent: Crystal Structure of an Oligodeoxynucleotide Duplex Containing 2'-O-Methylated Adenosines. *Chem Biol* **1994**, *1*, 39-45.
14. Noon, K. R.; Bruenger, E.; McCloskey, J. A. Posttranscriptional Modifications in 16S and 23S rRNAs of the Archaeal Hyperthermophile *Sulfolobus Solfataricus*. *J Bacteriol* **1998**, *180*, 2883-2888.
15. Edmonds, C. G.; Crain, P. F.; Gupta, R.; Hashizume, T.; Hocart, C. H.; Kowalak, J. A.; Pomerantz, S. C.; Stetter, K. O.; Mccloskey, J. A. Posttranscriptional Modification of Transfer-RNA in Thermophilic Archaea (Archaebacteria). *J Bacteriol* **1991**, *173*, 3138-3148.

16. Singh, S. K.; Gurha, P.; Tran, E. J.; Maxwell, E. S.; Gupta, R. Sequential 2'-O-Methylation of Archaeal Pre-tRNA^{Trp} Nucleotides Is Guided by the Intron-encoded but trans-Acting Box C/D Ribonucleoprotein of Pre-tRNA. *J Biol Chem* **2004**, *279*, 47661-47671.
17. Bjork, G. R.; Ericson, J. U.; Gustafsson, C. E. D.; Hagervall, T. G.; Jonsson, Y. H.; Wikstrom, P. M. Transfer-RNA Modification. *Annu Rev Biochem* **1987**, *56*, 263-287.
18. Jockel, S.; Nees, G.; Sommer, R.; Zhao, Y.; Cherkasov, D.; Hori, H.; Ehm, G.; Schnare, M.; Nain, M.; Kaufmann, A.; Bauer, S. The 2'-O-Methylation Status of a Single Guanosine Controls Transfer RNA-Mediated Toll-Like Receptor 7 Activation or Inhibition. *J Exp Med* **2012**, *209*, 235-241.
19. Daffis, S.; Szretter, K. J.; Schriewer, J.; Li, J. Q.; Youn, S.; Errett, J.; Lin, T. Y.; Schneller, S.; Zust, R.; Dong, H. P.; Thiel, V.; Sen, G. C.; Fensterl, V.; Klimstra, W. B.; Pierson, T. C.; Buller, R. M.; Gale, M.; Shi, P. Y.; Diamond, M. S. 2'-O Methylation of the Viral mRNA Cap Evades Host Restriction by IFIT Family Members. *Nature* **2010**, *468*, 452-456.
20. Zust, R.; Cervantes-Barragan, L.; Habjan, M.; Maier, R.; Neuman, B. W.; Ziebuhr, J.; Szretter, K. J.; Baker, S. C.; Barchet, W.; Diamond, M. S.; Siddell, S. G.; Ludewig, B.; Thiel, V. Ribose 2'-O-Methylation Provides a Molecular Signature for the Distinction of Self and Non-Self mRNA Dependent on the RNA Sensor Mda5. *Nat Immunol* **2011**, *12*, 137-U146.
21. Coutard, B.; Barral, K.; Lichiere, J.; Selisko, B.; Martin, B.; Aouadi, W.; Lombardia, M. O.; Debart, F.; Vasseur, J. J.; Guillemot, J. C.; Canard, B.; Decroly, E. Zika Virus Methyltransferase: Structure and Functions for Drug Design Perspectives. *J Virol* **2017**, *91*.
22. Prusiner, P.; Sundaralingam, M. Molecular and Crystal Structure of the Modified Nucleoside 2'-O-Methyladenosine. A Novel 2'-Exo-3'-Endo $_2T^3$ Sugar Pucker. *Acta Crystallogr B* **1976**, *32*, 161-169.
23. Chow, S.; Wen, K.; Sanghvi, Y. S.; Theodorakis, E. A. Novel Synthesis of 2'-O-Methylguanosine. *Bioorg Med Chem Lett* **2003**, *13*, 1631-1634.
24. Helm, M.; Motorin, Y. Detecting RNA Modifications in the Epitranscriptome: Predict and Validate. *Nat Rev Genet* **2017**, *18*, 275-291.
25. Dudley, E.; Bond, L. Mass Spectrometry Analysis of Nucleosides and Nucleotides. *Mass Spectrom Rev* **2014**, *33*, 302-331.
26. Guymon, R.; Pomerantz, S. C.; Crain, P. F.; McCloskey, J. A. Influence of Phylogeny on Posttranscriptional Modification of rRNA in Thermophilic Prokaryotes: The Complete Modification Map of 16S rRNA of Thermus thermophilus. *Biochemistry-Us* **2006**, *45*, 4888-4899.
27. Wu, R. R.; Yang, B.; Berden, G.; Oomens, J.; Rodgers, M. T. Gas-Phase Conformations and Energetics of Protonated 2'-Deoxyadenosine and Adenosine: IRMPD Action Spectroscopy and Theoretical Studies. *J Phys Chem B* **2015**, *119*, 2795-2805.
28. Wu, R. R.; Yang, B.; Berden, G.; Oomens, J.; Rodgers, M. T. Gas-Phase Conformations and Energetics of Protonated 2'-Deoxyguanosine and Guanosine: IRMPD Action Spectroscopy and Theoretical Studies. *J Phys Chem B* **2014**, *118*, 14774-14784.
29. Wu, R. R.; Yang, B.; Frieler, C. E.; Berden, G.; Oomens, J.; Rodgers, M. T. N3 and O2 Protonated Tautomeric Conformations of 2'-Deoxycytidine and Cytidine Coexist in the Gas Phase. *J Phys Chem B* **2015**, *119*, 5773-5784.
30. Wu, R. R.; Yang, B.; Frieler, C. E.; Berden, G.; Oomens, J.; Rodgers, M. T. Diverse Mixtures of 2,4-Dihydroxy Tautomers and O4 Protonated Conformers of Uridine and 2'-Deoxyuridine Coexist in the Gas Phase. *Phys Chem Chem Phys* **2015**, *17*, 25978-25988.

31. Wu, R. R.; Yang, B.; Frieler, C. E.; Berden, G.; Oomens, J.; Rodgers, M. T. 2,4-Dihydroxy and O2 Protonated Tautomers of dThd and Thd Coexist in the Gas Phase: Methylation Alters Protonation Preferences versus dUrd and Urd. *J Am Soc Mass Spectr* **2016**, *27*, 410-421.
32. Zhu, Y.; Hamlow, L. A.; He, C. C.; Lee, J. K.; Gao, J.; Berden, G.; Oomens, J.; Rodgers, M. T. Gas-Phase Conformations and N-Glycosidic Bond Stabilities of Sodium Cationized 2'-Deoxyguanosine and Guanosine: Sodium Cations Preferentially Bind to the Guanine Residue. *J Phys Chem B* **2017**, *121*, 4048-4060.
33. Zhu, Y.; Hamlow, L. A.; He, C. C.; Roy, H. A.; Cunningham, N. A.; Munshi, M.; Berden, G.; Oomens, J.; Rodgers, M. T. Conformations and N-glycosidic bond stabilities of sodium cationized 2-deoxycytidine and cytidine: Solution conformation of $[Cyd + Na]^+$ is Preserved upon ESI. *Int J Mass Spectrom* **2018**, *429*, 18-27.
34. Zhu, Y.; Hamlow, L. A.; He, C. C.; Strobehn, S. F.; Lee, J. K.; Gao, J.; Berden, G.; Oomens, J.; Rodgers, M. T. Influence of Sodium Cationization versus Protonation on the Gas-Phase Conformations and Glycosidic Bond Stabilities of 2'-Deoxyadenosine and Adenosine. *J Phys Chem B* **2016**, *120*, 8892-8904.
35. Zhu, Y.; Roy, H. A.; Cunningham, N. A.; Strobehn, S. F.; Gao, J.; Munshi, M. U.; Berden, G.; Oomens, J.; Rodgers, M. T. IRMPD Action Spectroscopy, ER-CID Experiments, and Theoretical Studies of Sodium Cationized Thymidine and 5-Methyluridine: Kinetic Trapping During the ESI Desolvation Process Preserves the Solution Structure of $[Thd+Na]^+$. *J Am Soc Mass Spectr* **2017**, *28*, 2423-2437.
36. Zhu, Y.; Roy, H. A.; Cunningham, N. A.; Strobehn, S. F.; Gao, J.; Munshi, M. U.; Berden, G.; Oomens, J.; Rodgers, M. T. Effects of Sodium Cationization versus Protonation on the Conformations and N-Glycosidic Bond Stabilities of Sodium Cationized Urd and dUrd: Solution Conformation of $[Urd+Na]^+$ is Preserved upon ESI. *Phys Chem Chem Phys* **2017**, *19*, 17637-17652.
37. Salpin, J. Y.; Scuderi, D. Structure of Protonated Thymidine Characterized by Infrared Multiple Photon Dissociation and Quantum Calculations. *Rapid Commun Mass Sp* **2015**, *29*, 1898-1904.
38. Ung, H. U.; Huynh, K. T.; Poutsma, J. C.; Oomens, J.; Berden, G.; Morton, T. H. Investigation of Proton Affinities and Gas Phase Vibrational Spectra of Protonated Nucleosides, Deoxynucleosides, and Their Analogs. *Int J Mass Spectrom* **2015**, *378*, 294-302.
39. Salpin, J. Y.; MacAleese, L.; Chirot, F.; Dugourd, P. Structure of the Pb^{2+}-Deprotonated dGMP Complex in the Gas Phase: a Combined MS-MS/IRMPD Spectroscopy/Ion Mobility Study. *Phys Chem Chem Phys* **2014**, *16*, 14127-14138.
40. Abo-Riziq, A.; Crews, B. O.; Compagnon, I.; Oomens, J.; Meijer, G.; von Helden, G.; Kabelac, M.; Hobza, P.; de Vries, M. S. The Mid-IR Spectra of 9-Ethyl Guanine, Guanosine, and 2'-Deoxyguanosine. *J Phys Chem A* **2007**, *111*, 7529-7536.
41. Nei, Y.-w.; Crampton, K. T.; Berden, G.; Oomens, J.; Rodgers, M. T. Infrared Multiple Photon Dissociation Action Spectroscopy of Deprotonated RNA Mononucleotides: Gas-Phase Conformations and Energetics. *J Phys Chem A* **2013**, *117*, 10634-10649.
42. Nei, Y.-w.; Hallowita, N.; Steill, J. D.; Oomens, J.; Rodgers, M. T. Infrared Multiple Photon Dissociation Action Spectroscopy of Deprotonated DNA Mononucleotides: Gas-Phase Conformations and Energetics. *J Phys Chem A* **2013**, *117*, 1319-1335.
43. Wu, R. R.; Hamlow, L. A.; He, C. C.; Nei, Y. W.; Berden, G.; Oomens, J.; Rodgers, M. T. The Intrinsic Basicity of the Phosphate Backbone Exceeds that of Uracil and Thymine Residues: Protonation of the Phosphate Moiety is Preferred over the Nucleobase for pdThd and pUrd. *Phys Chem Chem Phys* **2017**, *19*, 30351-30361.

44. Wu, R. R.; Hamlow, L. A.; He, C. C.; Nei, Y. W.; Berden, G.; Oomens, J.; Rodgers, M. T. N3 and O2 Protonated Conformers of the Cytosine Mononucleotides Coexist in the Gas Phase. *J Am Soc Mass Spectr* **2017**, *28*, 1638-1646.
45. Wu, R. R.; He, C. C.; Hamlow, L. A.; Nei, Y.-w.; Berden, G.; Oomens, J.; Rodgers, M. T. Protonation Induces Base Rotation of Purine Nucleotides pdGuo and pGuo. *Phys Chem Chem Phys* **2016**, *18*, 15081-15090.
46. Wu, R. R.; He, C. C.; Hamlow, L. A.; Nei, Y.-w.; Berden, G.; Oomens, J.; Rodgers, M. T. N3 Protonation Induces Base Rotation of 2'-Deoxyadenosine-5'-Monophosphate and Adenosine-5'-Monophosphate. *J Phys Chem B* **2016**, *120*, 4616-4624.
47. Chiavarino, B.; Crestoni, M. E.; Fornarini, S.; Lanucara, F.; Lemaire, J.; Maitre, P.; Scuderib, D. Infrared Spectroscopy of Isolated Nucleotides 1. The cyclic 3',5'-Adenosine Monophosphate Anion. *Int J Mass Spectrom* **2008**, *270*, 111-117.
48. Lanucara, F.; Crestoni, M. E.; Chiavarino, B.; Fornarini, S.; Hernandez, O.; Scuderi, D.; Maitre, P. Infrared Spectroscopy of Nucleotides in the Gas Phase 2. The Protonated Cyclic 3',5'-Adenosine Monophosphate. *Rsc Adv* **2013**, *3*, 12711-12720.
49. Ligare, M. R.; Rijs, A. M.; Berden, G.; Kabelac, M.; Nachtigallova, D.; Oomens, J.; de Vries, M. S. Resonant Infrared Multiple Photon Dissociation Spectroscopy of Anionic Nucleotide Monophosphate Clusters. *J Phys Chem B* **2015**, *119*, 7894-7901.
50. Pedersen, S. O.; Stochkel, K.; Byskov, C. S.; Baggesen, L. M.; Nielsen, S. B. Gas-Phase Spectroscopy of Protonated Adenine, Adenosine 5'-Monophosphate and Monohydrated Ions. *Phys Chem Chem Phys* **2013**, *15*, 19748-19752.
51. Gidden, J.; Bowers, M. T. Gas-Phase Conformations of Deprotonated and Protonated Mononucleotides Determined by Ion Mobility and Theoretical Modeling. *J Phys Chem B* **2003**, *107*, 12829-12837.
52. Valle, J. J.; Eyler, J. R.; Oomens, J.; Moore, D. T.; van der Meer, A. F. G.; von Helden, G.; Meijer, G.; Hendrickson, C. L.; Marshall, A. G.; Blakney, G. T. Free Electron Laser-Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Facility for Obtaining Infrared Multiphoton Dissociation Spectra of Gaseous Ions. *Rev Sci Instrum* **2005**, *76*.
53. Polfer, N. C.; Oomens, J.; Moore, D. T.; von Helden, G.; Meijer, G.; Dunbar, R. C. Infrared Spectroscopy of Phenylalanine Ag(I) and Zn(II) Complexes in the Gas Phase. *J Am Chem Soc* **2006**, *128*, 517-525.
54. Polfer, N. C.; Oomens, J. Reaction Products in Mass Spectrometry Elucidated with Infrared Spectroscopy. *Phys Chem Chem Phys* **2007**, *9*, 3804-3817.
55. Stuart, B. H., *Infrared Spectroscopy: Fundamentals and Applications*; John Wiley&Sons, Ltd: Chichester, UK, 2004.
56. Rijs, A.; Oomens, J., *Gas-Phase IR Spectroscopy and Structure of Biological Molecules*, 2015; Vol. 364, p 1-406.
57. Prazeres, R.; Glotin, F.; Insa, C.; Jaroszynski, D. A.; Ortega, J. M. Two-Colour Operation of a Free-Electron Laser and Applications in the Mid-Infrared. *Eur Phys J D* **1998**, *3*, 87-93.
58. Bakker, J. M.; Besson, T.; Lemaire, J.; Scuderi, D.; Maitre, P. Gas-Phase Structure of a π-Allyl-Palladium Complex: Efficient Infrared Spectroscopy in a 7 T Fourier Transform Mass Spectrometer. *J Phys Chem A* **2007**, *111*, 13415-13424.
59. Bakker, J. M.; Sinha, R. K.; Besson, T.; Brugnara, M.; Tosi, P.; Salpin, J. Y.; Maitre, P. Tautomerism of Uracil Probed via Infrared Spectroscopy of Singly Hydrated Protonated Uracil. *J Phys Chem A* **2008**, *112*, 12393-12400.

60. Schindler, B.; Joshi, J.; Allouche, A. R.; Simon, D.; Chambert, S.; Brites, V.; Gaigeot, M. P.; Compagnon, I. Distinguishing Isobaric Phosphated and Sulfated Carbohydrates by Coupling of Mass Spectrometry with Gas Phase Vibrational Spectroscopy. *Phys Chem Chem Phys* **2014**, *16*, 22131-22138.
61. Schindler, B.; Renois-Predelus, G.; Bagdadi, N.; Melizi, S.; Barnes, L.; Chambert, S.; Allouche, A. R.; Compagnon, I. MS/IR, a New MS-Based Hyphenated Method for Analysis of Hexuronic Acid Epimers in Glycosaminoglycans. *Glycoconj J* **2017**, *34*, 421-425.
62. Schindler, B.; Barnes, L.; Gray, C. J.; Chambert, S.; Flitsch, S. L.; Oomens, J.; Daniel, R.; Allouche, A. R.; Compagnon, I. IRMPD Spectroscopy Sheds New (Infrared) Light on the Sulfate Pattern of Carbohydrates. *J Phys Chem A* **2017**, *121*, 2114-2120.
63. Bertsimas, D.; Tsitsiklis, J. Simulated Annealing. *Stat Sci* **1993**, *8*, 10-15.
64. Wolinski, K.; Hinton, J. F.; Wishart, D. S.; Sykes, B. D.; Richards, F. M.; Pastone, A.; Saudek, V.; Ellis, P. D.; Maciel, G. E.; McIver Jr., J. W., et al. *HyperChem Computational Chemistry Software Package, version 7.0*, Hypercube, Inc.: Gainsville, FL, 2001.
65. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A., et al. *Gaussian 09*, Gaussian, Inc.: Wallingford, CT, USA, 2009.
66. Wu, R. R.; Rodgers, M. T. Mechanisms and Energetics for N-Glycosidic Bond Cleavage of Protonated Adenine Nucleosides: N3 Protonation Induces Base Rotation and Enhances N-Glycosidic Bond Stability. *Phys Chem Chem Phys* **2016**, *18*, 16021-16032.
67. Wu, R. R.; Chen, Y.; Rodgers, M. T. Mechanisms and Energetics for N-Glycosidic Bond Cleavage of Protonated 2'-Deoxyguanosine and Guanosine. *Phys Chem Chem Phys* **2016**, *18*, 2968-2980.
68. Altona, C.; Sundaralingam, M. Conformational Analysis of Sugar Ring in Nucleosides and Nucleotides. A New Description Using Concept of Pseudorotation. *J Am Chem Soc* **1972**, *94*, 8205-8212.
69. Saenger, W. Principles of Nucleic Acid Structure. Springer-Verlag: New York, 1984; pp 16-104.
70. Moore, D. T.; Oomens, J.; van der Meer, L.; von Helden, G.; Meijer, G.; Valle, J.; Marshall, A. G.; Eyler, J. R. Probing the Vibrations of Shared, OH^+O-Bound Protons in the Gas Phase. *Chemphyschem* **2004**, *5*, 740-743.
71. Heine, N.; Fagiani, M. R.; Rossi, M.; Wende, T.; Berden, G.; Blum, V.; Asmis, K. R. Isomer-Selective Detection of Hydrogen-Bond Vibrations in the Protonated Water Hexamer. *J Am Chem Soc* **2013**, *135*, 8266-8273.
72. Cooper, T. E.; O'Brien, J. T.; Williams, E. R.; Armentrout, P. B. Zn^{2+} Has a Primary Hydration Sphere of Five: IR Action Spectroscopy and Theoretical Studies of Hydrated Zn^{2+} Complexes in the Gas Phase. *J Phys Chem A* **2010**, *114*, 12646-12655.
73. Contreras, C. S.; Polfer, N. C.; Oomens, J.; Steill, J. D.; Bendiak, B.; Eyler, J. R. On the Path to Glycan Conformer Identification: Gas-Phase Study of the Anomers of Methyl Glycosides of N-Acetyl-D-Glucosamine and N-Acetyl-D-Galactosamine. *Int J Mass Spectrom* **2012**, *330*, 285-294.
74. Lubini, P.; Zurcher, W.; Egli, M. Stabilizing effects of the RNA 2 '-substituent: crystal structure of an oligodeoxynucleotide duplex containing 2 '-O-methylated adenosines. *Chem Biol* **1994**, *1*, 39-45.

Table 1. Geometric Parameters of the Most Stable N3, N1 and N7 Protonated Conformers of Adom and the Canonical DNA and RNA Nucleosides of Adenosine, dAdo and Ado .[a]

Conformer	Properties	$[Adom+H]^+$	$[dAdo+H]^+$ [b]	$[Ado+H]^+$ [b]
N3A	$H^+\cdots N3$	1.035 Å	1.035 Å	1.033 Å
	C1′···N9	1.461 Å	1.465 Å	1.459 Å
	∠C4N9C1′O4′	43.4°	45.1°	45.4°
	∠O5′C5′C4′O4′	-59.7°	-60.4°	-60.3°
	∠C2′C3′O3′H	40.7°	79.3°	152.6°
	∠C1′C2′O2′(H/C)	156.1°	–	-87.4°
	Relative ΔG_{298}	0.0 kJ/mol	0.0 kJ/mol	0.0 kJ/mol
N1A	$H^+\cdots N1$	1.013 Å	1.013 Å	1.013 Å
	C1′···N9	1.473 Å	1.479 Å	1.470 Å
	∠C4N9C1′O4′	238.5°	239.2°	232.4°
	∠O5′C5′C4′O4′	-65.3°	-66.1°	-65.5°
	∠C2′C3′O3′H	37.4°	77.7°	157.2°
	∠C1′C2′O2′(H/C)	159.1°	–	-84.7°
	Relative ΔG_{298}	25.9 kJ/mol	25.3 kJ/mol	21.8 kJ/mol
N7A	$H^+\cdots N7$	1.010 Å	1.010 Å	1.010 Å
	C1′···N9	1.503 Å	1.512 Å	1.502 Å
	∠C4N9C1′O4′	202.2°	199.8°	202.2°
	∠O5′C5′C4′O4′	-61.2°	-61.7°	-62.5°
	∠C2′C3′O3′H	-37.8°	84.7°	101.6°
	∠C1′C2′O2′(H/C)	91.4°	–	-146.6°
	Relative ΔG_{298}	33.2 kJ/mol	33.6 kJ/mol	37.2 kJ/mol

[a] Structures optimized at the B3LYP/6-311+G(d,p) level of theory, whereas relative Gibbs free energies at 298 K are determined at the B3LYP/6-311+G(2d,2p) level of theory.
[b] Values for the protonated canonical nucleosides are taken from references 21 and 22.

Table 2. Geometric Parameters of the Most Stable N7, O6 and N3 Protonated Conformers of Guom and the Canonical DNA and RNA Nucleosides of Guanosine, dGuo and Guo .[a]

Conformer	Properties	$[Guom+H]^+$	$[dGuo+H]^+$[a]	$[Guo+H]^+$ [b]
N7A	$H^+\cdots N7$	1.013 Å	1.012 Å	1.012 Å
	C1′···N9	1.501 Å	1.508 Å	1.498 Å
	∠C4N9C1′O4′	204.9°	203.0°	203.8°
	∠O5′C5′C4′O4′	-62.2°	-62.6°	-63.0°
	∠C2′C3′O3′H	-38.9°	88.0°	103.6°
	∠C1′C2′O2′(H/C)	90.6°	–	-145.0
	Relative ΔG_{298}	0.0 kJ/mol	0.0 kJ/mol	0.0 kJ/mol
O6A	$H^+\cdots O6$	0.971 Å	0.971 Å	0.971 Å
	C1′···N9	1.470 Å	1.475 Å	1.468 Å
	∠C4N9C1′O4′	239.4°	240.1°	233.9°
	∠O5′C5′C4′O4′	-65.4°	-66.2°	-65.5°
	∠C2′C3′O3′H	38.5°	78.8°	157.8°
	∠C1′C2′O2′(H/C)	158.6	–	-85.5°
	Relative ΔG_{298}	37.4 kJ/mol	39.0 kJ/mol	35.0 kJ/mol
N3B	$H^+\cdots N3$	1.028 Å	1.027 Å	1.026 Å
	C1′···N9	1.475 Å	1.478 Å	1.472 Å
	∠C4N9C1′O4′	21.8°	22.1°	19.4°
	∠O5′C5′C4′O4′	-60.4°	-59.0°	-57.4°
	∠C2′C3′O3′H	-34.3°	74.5°	95.8°
	∠C1′C2′O2′(H/C)	89.6°	–	-148.5°
	Relative ΔG_{298}	48.4 kJ/mol	52.4 kJ/mol	41.2 kJ/mol

[a] The N3B conformer is consistent with the lowest N3 conformers of $[dGuo+H]^+$ and $[Guo+H]^+$, and its Gibbs free energy is only less than 1 kJ/mol higher than the **N3A** conformer.
[b] Values are taken from Ranran's work in ref 27 and 28.

Table 3. Relative Enthalpies and Free Energies of Select Low-Energy Conformers of $[Adom+H]^+$ and $[Guom+H]^+$ at 0 and 298 K in kJ/mol.

Species	Conformer	ΔH_0	ΔH_{298}	ΔG_{298}	Nucleobase Orientation	P	Sugar Puckering
$[Adom+H]^+$	**N3A**	0.0	0.0	0.0	*syn*	153.9	C2′-endo ($^2T_1$)
	N1A	29.6	29.6	25.9	*anti*	174.6	C2′-endo ($^2T_3$)
	N7A	38.3	38.3	33.2	*anti*	8.8	C3′-endo ($^3T_2$)
$[Guom+H]^+$	**N7A**	0.0	0.0	0.0	*anti*	8.9	C3′-endo ($^3T_2$)
	N7B	2.5	2.5	2.0	*anti*	176.7	C2′-endo ($^2T_3$)
	N7H	20.1	20.1	23.3	*syn*	160.6	C2′-endo ($^2T_1$)
	O6A	38.0	38.0	37.4	*anti*	174.5	C2′-endo ($^2T_3$)
	N3A	45.6	45.6	47.6	*syn*	149.2	C2′-endo ($^2T_1$)

Table 4. Vibrational Band Assignments of $[Adom+H]^+$ Based on the **N3A** and **N1A** Conformers.

Experimental Band (cm^{-1})	**N3A**	**N1A**
1089	C3′ wagging/C1′−O4′ stretch	Sugar C−H twisting
1103	−	Sugar C−H rocking
1132	(O2′)CH_3 twisting/C2′ stretch	−
1210	Sugar C−H rocking	C8−H/O5′−H scissoring
1320	Sugar twisting/scissoring	C4′−H/O3′−H scissoring
1445	C6−N1 stretch/C1′ scissoring	Base C−N stretch
1492	−	C4−C5 stretch
1584	N6−H_2 scissoring	N6−H_2/N1−H scissoring
1654	C6−N6 stretch	−
1665	−	C6−N6 stretch
3431	N6−H_2 symmetric stretch	N6−H_2 symmetric/N1−H stretch
3554	N6−H_2 asymmetric stretch	N6−H_2 asymmetric stretch
3613	O3′−H stretch	O3′−H stretch
3675	O5′−H stretch	O5′−H stretch

Table 5. Vibrational Band Assignments of $[Adom+H]^+$ Based on **N7A** and **N7H** Conformers.

Experimental Band (cm^{-1})	**N7A**	**N7H**
1110	Sugar stretch/scissoring	Sugar twisting/scissoring
1232	C5′−H twisting/N7−H scissoring	C5′−H twisting/ N7−H/C8−H /O−H/(O2′)CH_3 wagging
1311	C2′−H/N1−H scissoring	−
1365	C4′−H/C8−H/N7−H scissoring	N1−H scissoring/sugar twisting
1469	(O2′)CH_3/C5′−H scissoring	C5′−H/(O2′)CH_3 scissoring
1577	N2−H_2/N1−H scissoring	N2−H_2/N1−H scissoring
1605	C4−C5/C4−N3 stretch	C4−C5/C4−N3 stretch
1628	C2−N2 stretch	C2−N2 stretch
1772	C6=O6 stretch	C6=O6 stretch
3424	N1−H stretch	N2−H_2 symmetric/N1−H stretch
3464	N2−H_2 symmetric/N7−H stretch	N7−H/O5′−H stretch
3544	−	N2−H_2 asymmetric stretch
3572	N2−H_2 asymmetric stretch	−
3602	O3′−H stretch	−
3675	O5′−H stretch	O3′−H stretch

Figure Captions

Figure 1. Chemical structures of the 2′-O-methyadenosine (Adom) and 2′-O-methyguanosine (Guom) nucleosides and their canonical DNA (dAdo and dGuo) and RNA (Ado and Guo) counterparts, where X = OCH_3, H, OH, respectively. B3LYP/6-311+G(d,p) optimized geometries of the ground conformers of the protonated forms of the Adom and Guom versus similar conformers determined for their canonical DNA and RNA counterparts. The site of protonation site, nucleobase orientation, and the sugar puckering are given for each conformer. Structures for the canonical DNA and RNA nucleosides are taken from references 27 and 28.

Figure 2. Comparison of the measured IRMPD spectra of $[Adom+H]^+$ to those of its canonical DNA and RNA nucleoside counterparts over the fingerprint region and hydrogen-stretching regions. IRMPD spectra previously reported for $[dAdo+H]^+$ and $[Ado+H]^+$ are taken from reference 27.

Figure 3. Comparison of the measured IRMPD spectra of $[Guom+H]^+$ to those of its canonical DNA and RNA nucleoside counterparts over the fingerprint region and hydrogen-stretching regions. IRMPD spectra previously reported for $[dGuo+H]^+$ and $[Guo+H]^+$ are taken from references 28.

Figure 4. CID mass spectra of $[Adom+H]^+$ and $[Guom+H]^+$ at an rf excitation amplitude (rf_{EA}) that results in ~50% dissociation.

Figure 5. Comparison of the survival yield curves of $[Adom+H]^+$ and $[Guom+H]^+$ to those of their canonical DNA and RNA nucleoside counterparts. $CID_{50\%}$ values and estimated errors in these values determined from survival yield analyses are also given.

Figure 6. Comparison of the experimental IRMPD spectrum of $[Adom+H]^+$ with IR spectra predicted at the B3LYP/6-311+G(d,p) level of theory for the **N3A** and **N1A** conformers. The B3LYP/6-311+G(2d,2p) relative Gibbs free energies at 298 K are also listed.

Figure 7. Comparison of the experimental IRMPD spectrum of $[Guom+H]^+$ with IR spectra predicted at the B3LYP/6-311+G(d,p) level of theory for the **N7A** and **N7B, and N7H** conformers. The B3LYP/6-311+G(2d,2p) relative Gibbs free energies at 298 K are also listed.