International Journal of Mass Spectrometry 303 (2011) 129–136

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

UV-photodissociation of non-cyclic and cyclic mononucleotides

Jesse C. Marcum, Sydney H. Kaufman, J. Mathias Weber*

JILA, NIST, Department of Chemistry and Biochemistry, University of Colorado, 440 UCB, Boulder, CO 80309-0440, USA

ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form 18 January 2011
Accepted 18 January 2011
Available online 2 February 2011

Keywords:
Nucleotide
Mononucleotide
Photodissociation
Fragmentation

ABSTRACT

Irradiation of nucleotides in the gas phase with ultraviolet light can lead to their fragmentation. We present a comparison of UV photofragmentation data on ribo-, deoxyribo- and cyclic nucleotides with guanine and adenine as nucleobases. The envelope of the UV photofragment spectra does not depend significantly on the detailed structure of the sugar–phosphate backbone. The fragment channels observed in the photofragmentation of ribonucleotides are very similar to those of deoxyribonucleotides with small differences in the relative abundances of the product ions. In fragmentation of cyclic nucleotides, the deprotonated base anions are the most abundant fragments, in contrast to the non-cyclic nucleotides, where this channel is significantly weaker. We discuss the abundances in the context of possible fragmentation mechanisms and structural differences of the parent molecules.

1. Introduction

The study of UV-induced photofragmentation of gas-phase nucleotides provides a basis for the description of the intrinsic photophysical and photochemical properties of nucleotides. While nucleotides in the absence of solvent can be expected to behave differently from physiological conditions, this comparison may aid in a better molecular-level understanding of possible processes involved in UV photodamage to DNA and the role of the solvent in condensed phase DNA photochemistry [1]. In addition, gas-phase experiments provide insight into the fragmentation processes that occur following the activation of oligonucleotides in mass spectrometry studies.

In order to elucidate the complete process of nucleotide photofragmentation it is natural to start with mononucleotides. The primary UV chromophores in nucleotides are the nucleobases [2]. There is a great deal of spectroscopic information pertaining to the individual nucleobases which forms the basis of knowledge for all studies on higher-order DNA/RNA systems [1,3]. In aqueous environments, all of the nucleobases show broad absorption features in the general range of 4–6 eV. Absorption is expected to continue further into the UV, but absorption features of the solvent, and possibly bands involving charge transfer from the nucleobases to the solvent, make analysis more difficult. Numerous theoretical studies (see e.g., [3–6] and references therein) attribute the features in the range of 4–6 eV mainly to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. The dipole-forbidden $n \rightarrow \pi^*$ transitions have much weaker oscillator strengths when compared to the $\pi \rightarrow \pi^*$ transitions [1] and, as a result, contributions due to $\pi \rightarrow \pi^*$ transitions are expected to dominate the spectra. Similar behavior has been observed in gas-phase nucleobases (see, e.g., [7,8]).

Gas-phase spectroscopic studies on nucleosides and nucleotides are far less available. Some work has been performed by Nir et al. on jet-cooled guanosine as well as several substituted guanosines [7,8]. Marcum et al. [9] used UV photodissociation action spectroscopy to probe the electronic transitions of the four deprotonated 2′-deoxynucleoside-5′-monophosphate mononucleotides. Comparison of the gas-phase spectra with absorption spectra of aqueous solutions of the mononucleotide sodium salts revealed solvatochromic shifts similar to those of the individual nucleobases [3,9]. The onset of spectral features for the gas-phase mononucleotides correspond remarkably well with the origin peaks for the gas-phase nucleobases [3,9]. This suggests that the presence of the charged phosphate group does not cause a dramatic shift in excitation energies.

Following the absorption of ultraviolet radiation, nucleobases have been shown to undergo internal conversion from the initially excited electronic state back into a vibrationally "hot" electronic ground state on a time scale of up to a few picoseconds [3]. Further work compared the excited state lifetimes of nucleosides to nucleotides and individual nucleobases [10–12]. While the presence of sugar and/or phosphate subgroups was found to have an effect on the excited state lifetimes, the differences were not substantial and the underlying photophysical process of rapid internal conversion can be expected to take place for all systems. Later work on jet-cooled gas-phase nucleobases found a strong excitation wavelength dependence on the relaxation lifetimes of the isolated bases [13]. Excitation at the S_1 origin bands resulted in considerably longer decay lifetimes, presumably due to the lack of available vibrational energy to facilitate internal conversion. Excitation into

* Corresponding author. Tel.: +1 303 492 7841; fax: +1 303 492 5235.
E-mail address: weberjm@jila.colorado.edu (J.M. Weber).

1387-3806/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2011.01.021

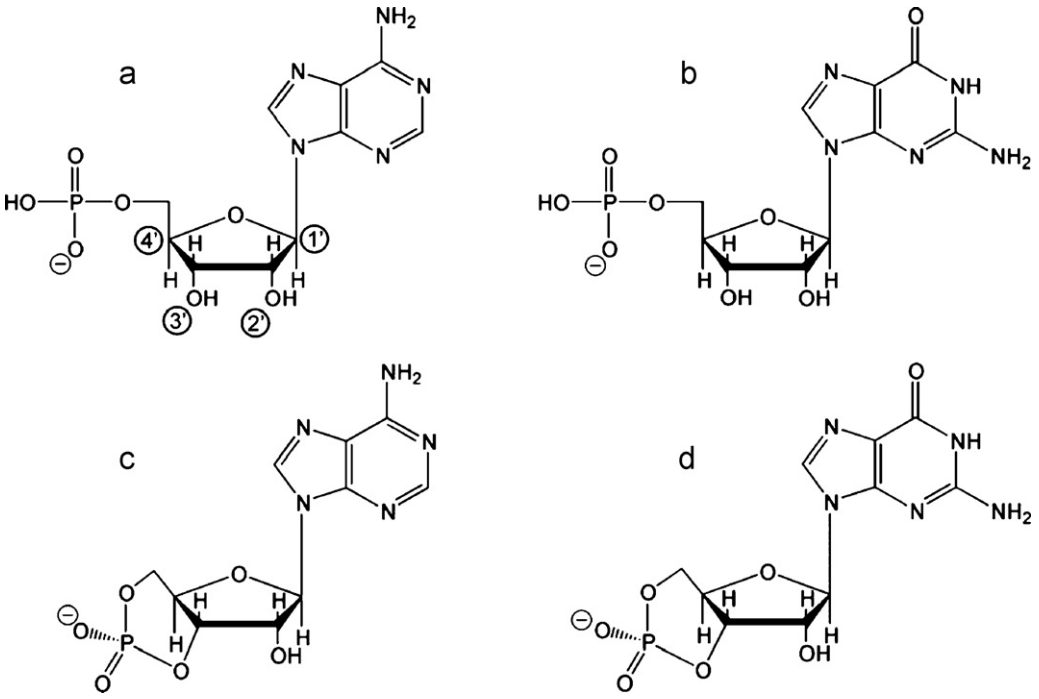

Fig. 1. Structures of the four deprotonated nucleotides under study; (a) adenosine-5′-monophosphate, (b) guanosine-5′-monophosphate; the carbon atoms of the sugar moiety have been labeled with numbers in circles, (c) adenosine cyclic-3′,5′-monophosphate, (d) guanosine cyclic-3′,5′-monophosphate.

states with considerable vibrational energy led to lifetimes on the order of 1 ps [14,15], which were much closer to aqueous results. The short excited-state lifetimes of nucleobases are thought to be made possible by conical intersections that enable efficient coupling of ground and excited electronic surfaces [3,15–19]. It has been proposed that relaxation can proceed directly from the initially excited $\pi\pi^*$ states [20] as well as *via* $n\pi^*$ [18] or $\pi\sigma^*$ [21–23] states. The very similar electronic relaxation times of free and solvated nucleobases suggest that solvation of nucleobases does not dramatically change the *electronic* relaxation mechanism, despite the presence of H-bonding interactions between the base and the solvent. On the other hand, the subsequent fragmentation is certainly modified by the presence of solvent. The rapid coupling of vibrational energy into the solvent should occur on a time scale on the order of tens of picoseconds, making fragmentation energetically inaccessible [24]. In the gas phase, there is no such fast relaxation into the solvent, and fragmentation occurs on a multi-nanosecond [25] to microsecond [26] time scale.

Overall, little work has been done on the UV photofragmentation of gas-phase mononucleotides thus far. Two fixed frequency studies on AMP have been published [25,26], and a recent study from our laboratory [9] investigated the photofragmentation spectra of the 2′-deoxynucleoside-5′-monophosphate mononucleotides. The studies by Nielsen et al. [26] and by Andersen and coworkers [25] focused on the dissociation time scales of electrosprayed deprotonated AMP anions after irradiation by 266 nm radiation. Andersen and coworkers also investigated the photofragments of deprotonated AMP anions and found analogous fragments to those found in UV photodissociation of dAMP [9].

In the current article, we present a study of UV photofragmentation of anionic, deprotonated ribonucleoside-5′-monophosphates and the ribonucleoside-3′,5′-cyclic-monophosphate mononucleotides, in both cases with guanine and adenine as nucleobases. We compare these chemically modified species with the previously published data on the corresponding DNA nucleotides [9]. We discuss the results on cyclic and non-cyclic mononucleotides in terms of fragmentation mechanisms and offer some explanation of the differences between the fragmentation patterns of the different variants.

2. Experimental

Our experimental setup has previously been described elsewhere [9]. Briefly, it consists of an electrospray ionization (ESI) source coupled to a reflectron time-of-flight mass spectrometer (RETOF) and a UV/Vis optical parametric converter. The deprotonated nucleotide ions under study (see Fig. 1) are produced from electrospray using an ∼5 mM solution of the nucleotide of interest, namely adenosine-3′,5′-cyclic-monophosphate sodium salt, adenosine-5′-monophosphate disodium salt, guanosine-5′-monophosphate disodium salt hexahydrate (MP Biomedicals) or guanosine-3′,5′-cyclic-monophosphate sodium salt (Sigma–Aldrich) in a mixture of ∼1:1 methanol/water. Upon spraying and desolvation, the ions are accumulated in a hexapole ion trap. After an accumulation time of ∼25 ms, the ions are injected into the acceleration region of a Wiley–McLaren RETOF. At the first space focus of the mass spectrometer, ions are mass selected using a home-built mass gate and are irradiated by the output of an optical parametric converter, tunable from 220 to 2500 nm. In a second mass spectrometry step, photofragment ions are separated from any remaining undissociated parent ions using a two-stage reflectron. Ions are then detected at the second space focus of the mass spectrometer. Photodissociation action spectra are obtained by scaling the reflectron to the appropriate mass-to-charge ratio and monitoring the photofragment ions as a function of photon energy and correcting for laser fluence. The mass spectrometer is operated at a repetition rate of 40 Hz and the laser system at 20 Hz so that the ions are irradiated only during alternating mass spectra. This allows us to obtain and correct for signal due to the unimolecular decay of metastable parent ions. Branching ratios for the various fragment channels were calculated using peak areas from Gaussian fits of the parent and fragment ion mass spectra and were normalized to laser power. Laser fluence was varied in the peak of the fragment action spectrum for each fragment

Table 1
Observed fragment masses and assignments (see text).

Parent [M−H]⁻	M = AMP		M = GMP		M = cAMP		M = cGMP	
Fragments	*m/z*	Assignment	*m/z*	Assignment	*m/z*	Assignment	*m/z*	Assignment
	79	PO_3^-	79	PO_3^-	79	PO_3^-	79	PO_3^-
	97	$H_2PO_4^-$	97	$H_2PO_4^-$ [a]	–	–	97	$H_2PO_4^-$
	134	A^-	150	G^- [a]	134	A^-	150	G^-
	139	N/A [a]	–	–	–	–	–	–
	151	N/A [b]	151	N/A [a]	–	–	–	–
	193	$[M-AH-H_2O]^-$	193	$[M-GH-H_2O]^-$ [a]	175	$[M-AH-H_2O]^-$	175	$[M-GH-H_2O]^-$ [a]
	211	$[M-AH]^-$	211	$[M-GH]^-$	193	$[M-AH]^-$	193	$[M-GH]^-$

[a] Fragment mass peaks were observed, but were too weak to obtain fragment action spectra.
[b] A tentative assignment is discussed in the text (Section 3.1).

to test for multiphoton absorption. No multiphoton effects were found.

3. Results and discussion

3.1. Fragment channels

Upon absorption of ultraviolet radiation, all four of the deprotonated nucleotides under study, abbreviated $[M-H]^-$, undergo fragmentation into the same general classes of fragments that were observed in previous experiments [9,25,27–29]. The observed fragment masses and assignments are collected in Table 1. The first class of fragments involves breaking of the CN glycosidic bond leading to formation of the deprotonated base, B^- (here, B = A and G), as well as an ion of the form $[M-H-BH]^-$ that corresponds to the loss of neutral, protonated base (BH) from the parent ion. Also in this class of fragments is the species $[M-H-BH-H_2O]^-$, which is presumably formed *via* the loss of water from the fragment ion $[M-H-BH]^-$ [28]. Another fragment channel in this class, which is not observed for all parent ions, has *m/z* = 151. This fragment has been observed before [29] but it has not been identified. A fragment ion that presumably had *m/z* = 152 was recently observed by Aravind et al. following UVPD of $[AMP-H]^-$ and was assigned as $B^-{\cdot}H_2O$ [25]. While this could be a possibility, the fact that we only observe *m/z* = 151 and the mass to charge ratio of the peak does not depend on the identity of the attached nucleobase suggests that the fragment involves the sugar–phosphate group that remains after glycosidic bond cleavage and not the nucleobase itself. One possibility for the identity of this ion is the subsequent loss of C_2H_2 (involving the 2′ carbon) from the deoxyribonucleotide fragment $[dBMP-H-BH-H_2O]^-$ to give the ion $[dBMP-H-BH-H_2O-C_2H_2]^-$ which has *m/z* = 151. If a ribonucleotide is used as the parent ion instead, subsequent loss of C_2H_2O from the ribonucleotide fragment $[BMP-H-BH-H_2O]^-$ will give the ion $[BMP-H-BH-H_2O-C_2H_2O]^-$, which will also have *m/z* = 151. Due to the successive loss of nucleobase and water, the fragment ion $[M-H-BH-H_2O]^-$ should consist of a substituted furan ring. Furan has previously been shown to lose C_2H_2 as a result of thermal decomposition [30]. However, the presence of the charged phosphate group, as well as an extra hydroxyl group at the 2′ position in the case of the ribonucleotides, will likely lead to a considerably different mechanism.

The second class of fragments consists of the phosphate-based products PO_3^- and $H_2PO_4^-$ which are formed by cleaving phosphate–sugar bonds. Finally, a very weak fragment ion, which we only observe for the parent ion $[AMP-H]^-$, has *m/z* = 139. Interestingly, this fragment seems to appear in the fragment ion mass spectrum of Aravind et al., although they did not explicitly label it [25]. We do not believe that peak assignment is appropriate at this time without further study, but we mention its observation for completeness.

An interesting point to note is that most of the fragment ions that we observe are the result of a considerable amount of nuclear rearrangement. All fragment channels have been previously seen following the activation of gas-phase nucleotides and oligonucleotides using a number of methods including collision induced dissociation (CID), infrared multiphoton dissociation (IRMPD), post-source decay (PSD) and blackbody induced radiative dissociation (BIRD) [27,31–34]. Many of these methods involve the thermal excitation of molecular vibrations, leading to an increase in total energy in the molecule which can undergo a number of rearrangement reactions that lead to the observed products. The parallels

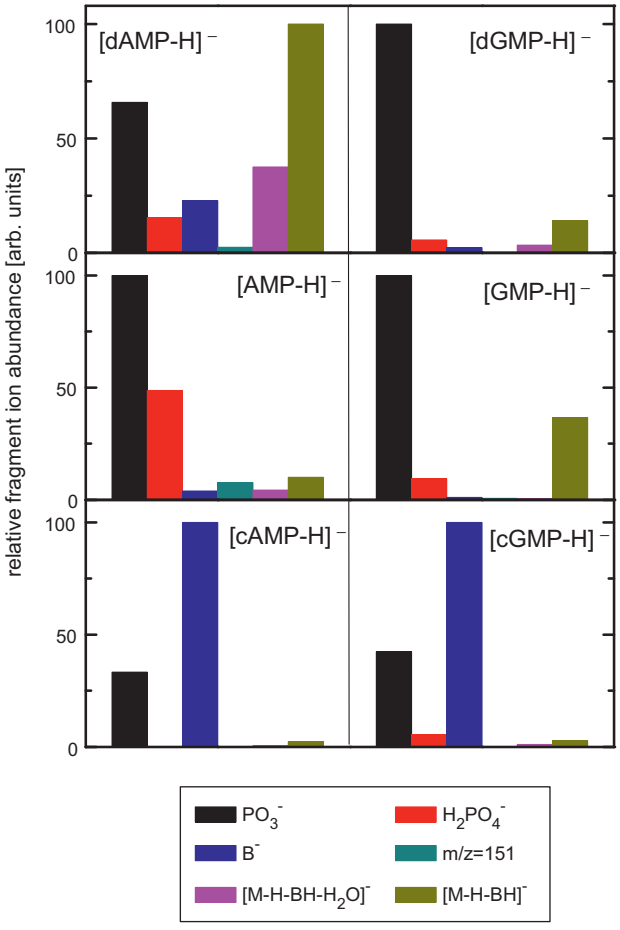

Fig. 2. Branching ratios for the different fragment channels for the 2′-deoxymononucleotides obtained during our previous study [9] (top) along with branching ratios obtained during the current study (center and bottom). Branching ratios are grouped according to parent ions $[XMP-H]^-$, where X = dA, dG, A, G, cA, and cG. All values have been scaled so that the fragment ion with the highest recorded intensity has a branching ratio of 100 arbitrary units.

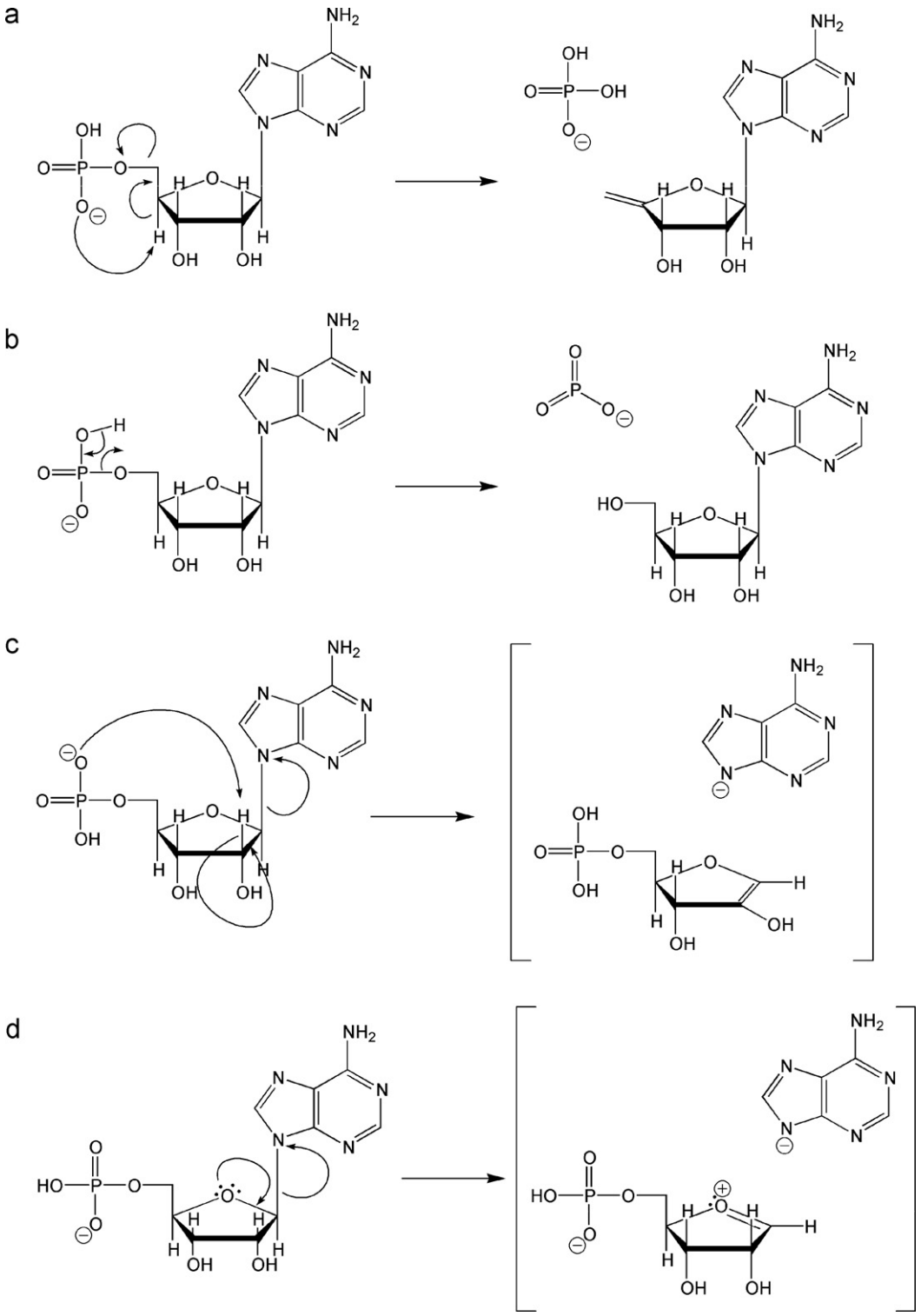

Fig. 3. Schemes for formation of observed fragments (see Section 3.2); (a) $H_2PO_4^-$; (b) PO_3^-; (c) deprotonated base ion by intramolecular E2 reaction; (d) deprotonated base ion by heterolytic cleavage of CN glycosidic bond.

to these other activation mechanisms suggest that UV-excited mononucleotides fragment due to a large amount of vibrational energy. This is consistent with previous work which indicated that nucleobases and nucleotides may undergo fast internal conversion through conical intersections to reach a vibrationally excited ground electronic state [3,26]. The electronic energy that is converted into vibrational energy in the molecule (up to 5.8 eV in our experiment) can then be used to induce unimolecular fragmentation. Only one fragment channel, B^-, could in principle result from direct dissociation on a repulsive curve. While this is generally viewed as an unlikely possibility, there are currently no experimental data suited to completely exclude this process.

All observed fragment ions correspond in principle to loss of genetic information; loss of the base in the case of CN glycosidic bond cleavage and strand-breaking in the case of the phosphate-based products. However, the long decay times observed previously

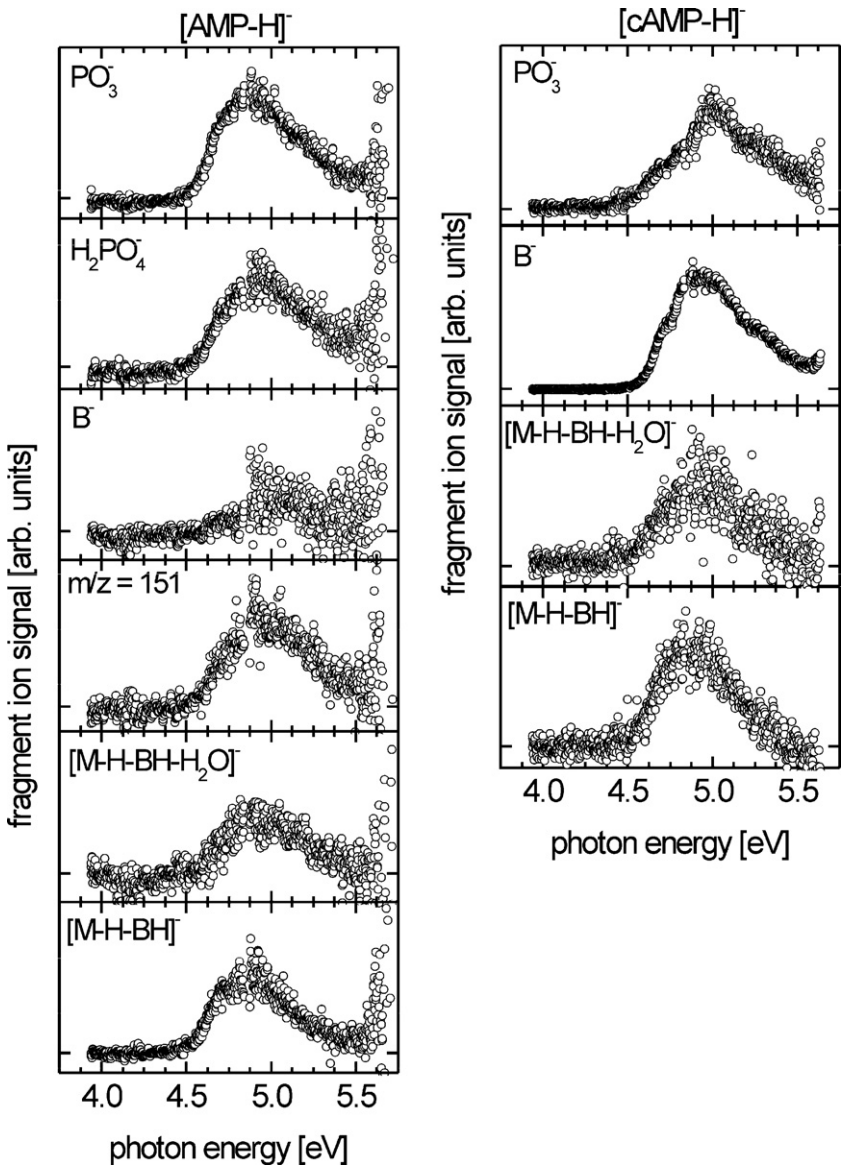

Fig. 4. Photodissociation action spectra for adenosine species; *left*: fragment action spectra from $[AMP–H]^-$ parent ions; *right*: fragment action spectra from $[cAMP–H]^-$ parent ions.

[25,26] render similar dissociation events in the condensed phase very rare events at best.

3.2. Relative abundance of fragments

Fig. 2 shows the relative abundances of fragment ions for the different parent ions $[M–H]^-$, where M = dAMP, dGMP, AMP, GMP, cAMP and cGMP close to the peak maxima of their respective photofragmentation cross sections (see Section 3.3). As reported previously [9], the dominant fragmentions for the 2′-deoxyribonucleotides are PO_3^- and the phosphate–sugar fragment remaining after the loss of the neutral base $[M–H–BH]^-$. Replacing the hydrogen at the 2′ carbon with an OH group changes the fragmentation patterns, but the changes are not uniform for AMP and GMP with respect to their 2′-deoxy analogs. In the case of AMP, the phosphate based fragments gain in abundance relative to the fragmentation of dAMP, while the relative importance of $[M–H–AH]^-$ decreases. In contrast, $[M–H–GH]^-$ abundance increases for the fragmentation of GMP vs. dGMP, while PO_3^- decreases.

The mechanisms that lead to these types of fragments are not well understood. Ho and Kebarle [27] utilized CID under single-collision conditions to activate parent ions. They extracted threshold enthalpies for the individual fragment channels. Comparison of their experimental data with theoretical threshold enthalpies obtained using semi-empirical calculations and transition state theory led them to propose a set of fragmentation mechanisms. The formation of phosphate-based products from the 5′-monophosphates was proposed to occur by two separate mechanisms. In the first mechanism (see Fig. 3a), the phosphate group abstracts a proton from the 4′ carbon of the sugar in an E2-type elimination, resulting in the formation of $H_2PO_4^-$. In the second mechanism (see Fig. 3b), transfer of a proton from a phosphate OH group to the phosphoric acid ester oxygen, with concomitant PO bond cleavage, results in PO_3^- fragment ions. These mechanisms are fairly straightforward and are consistent with many of the mechanisms that have been proposed for nucleotide backbone cleavage [35]. While Ho and Kebarle did not investigate the fragmentation energetics of cAMP, GMP or cGMP, activation energies for different 5′-mononucleotides ranged from 120 to 150 kJ/mol for

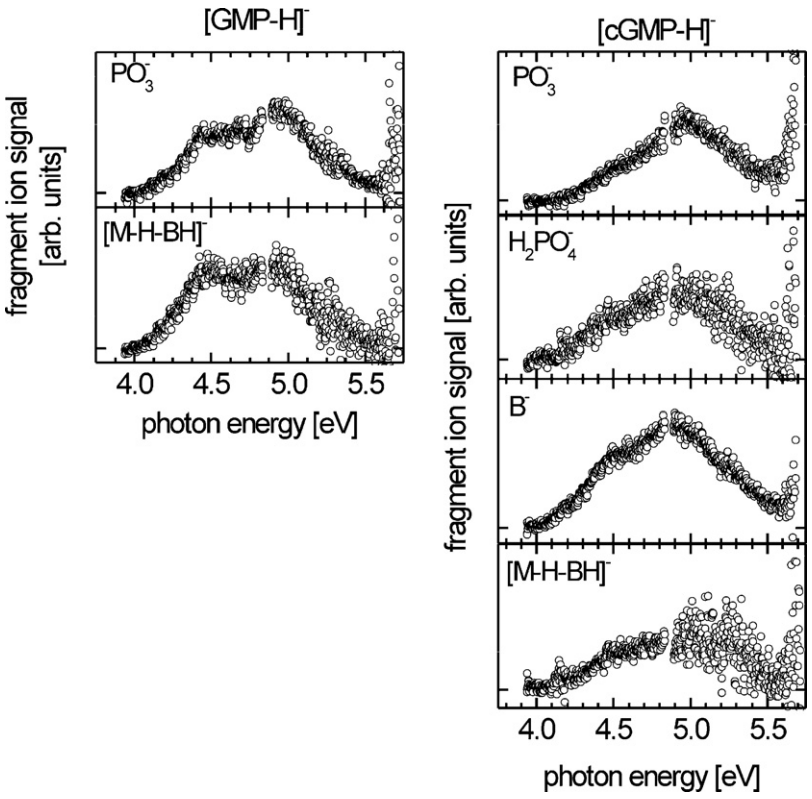

Fig. 5. Photodissociation action spectra for guanosine species; *left*: fragment action spectra from $[GMP–H]^-$ parent ions; *right*: fragment action spectra from $[cGMP–H]^-$ parent ions.

observation of PO_3^- and from 170 to 200 kJ/mol for observation of $H_2PO_4^-$ [27].

The situation is far less clear for mechanisms involving glycosidic bond cleavage. The mechanism proposed by Ho and Kebarle [27] (see Fig. 3c) based upon earlier work on dinucleotides by Rodgers et al. [36], involved proton transfer from the 2′ carbon of the sugar to the phosphate group. This proton transfer is accompanied by loss of the nucleobase in an E2-type elimination process. The B^- ion that is formed remains attached to the remainder of the molecule in a weakly bound encounter complex. This encounter complex can then go on to dissociate, resulting in B^- fragment ions. Alternatively, a second proton transfer step may occur prior to dissociation of the complex, resulting in BH fragments and $[M–H–BH]^-$ fragment ions.

The mechanisms proposed for the fragmentation of ribonucleoside-5′-monophosphates and their 2′-deoxy analogs cannot explain the differences in the trends for the guanine and adenine-based ions. Aravind et al. [25] pointed out the differences between the fragment branching ratios of $[dAMP–H]^-$ reported by Marcum et al. [9] and their data on $[AMP–H]^-$. The ribonucleotide branching ratios in the present work are consistent with those by Aravind et al., suggesting that the observed changes in the fragmentation patterns are due to a significant change in the chemistry when an OH group is present at the 2′ carbon. Activation energies for various non-cyclic 5′-mononucleotides were found by Ho and Kebarle [27] to be in the range of 150–170 kJ/mol for observation of deprotonated base anions and 140–155 kJ/mol for observation of the loss of neutral base.

A much more pronounced difference in fragmentation patterns is observed in the case of the 3′,5′-cyclic ribonucleotides cAMP and cGMP, where the phosphate group is tethered to the sugar moiety by two covalent bonds (to the 5′ carbon and the 3′ carbon). In both cases, the anionic base fragments A^- and B^- are the dominant fragments, with small abundances of PO_3^- and nearly negligible contributions from other channels (see Fig. 2). Chiavarino et al. [37] performed infrared multiphoton dissociation (IRMPD) experiments on $[cAMP–H]^-$ and observed A^- as their only fragment channel. This difference in fragment ion abundance, when compared to the results of the present study, can be explained by considering the way in which energy is deposited into the molecule. In IRMPD, the target molecules are heated slowly by sequential absorption of infrared photons. If a hot molecule fragments on a time scale that is fast compared to the heating process, one can expect that only the lowest energy fragment channel will be observed. The IRMPD work by Chiavarino et al. [37] therefore suggests that A^- is the lowest energy fragment channel for $[cAMP–H]^-$. In contrast to IRMPD, UV activation deposits a large amount of energy in a single event (fast heating). While one might expect the lowest energy channel to dominate, higher energy channels should occur as well, which is consistent with our observations. This is also compatible with the expectation that the generation of PO_3^- should be suppressed because two covalent bonds have to be cleaved to produce this fragment, while only a single bond must be cleaved to result in the formation of B^-.

The sequence of events leading to the observed fragments is unclear. According to Ho and Kebarle [27], cleavage of a PO bond along the lines of Fig. 3b is the lowest energy channel for the non-cyclic nucleotides. This reaction, however, cannot be active in cyclic nucleotides, because the hydrogen that is transferred in Fig. 3b is missing. In principle, a process similar to Fig. 3c could be responsible for B^- production, but a PO or CO bond would have to be cleaved first in this case since the phosphate group is otherwise not free to perform an attack on the β-face of the sugar moiety. While the presence of phosphate-based fragments indicates that this can indeed happen on the time scale of our experiment, it seems appropriate to search for an alternative reaction that could produce B^- in the case of the cyclic nucleotides. We caution, however, that we cannot unambiguously rule out or confirm one particular reaction.

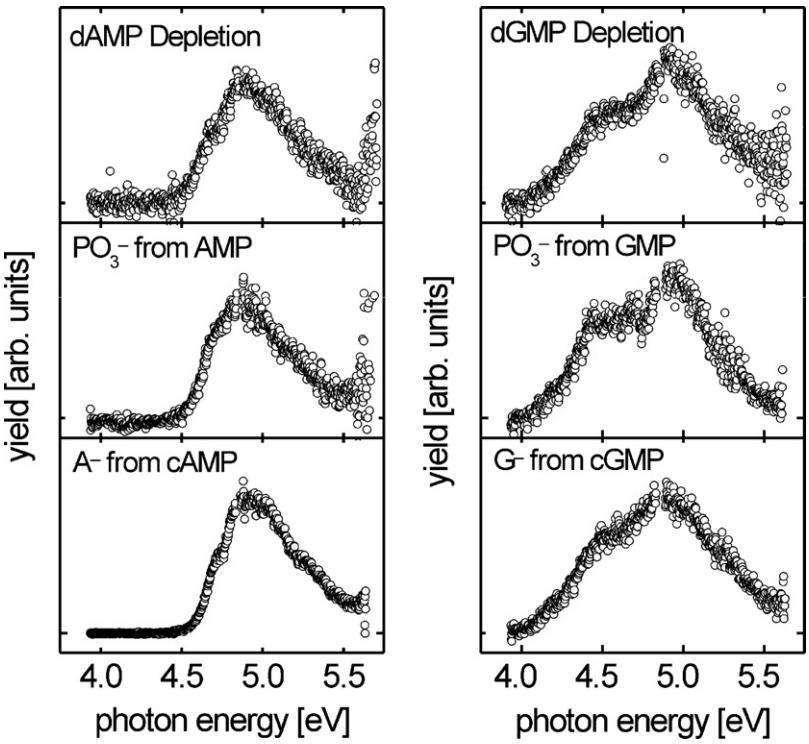

Fig. 6. Comparison of the photodissociation spectra across the various chemical modifications; *left*: comparison of parent ion depletion [9] of $[dAMP–H]^-$ with the most abundant fragment channels from $[AMP–H]^-$ and $[cAMP–H]^-$; *right*: comparison of parent ion depletion [9] of $[dGMP–H]^-$ with the most abundant fragment channels from $[GMP–H]^-$ and [cGMP–H].

A possible mechanism involving glycosidic bond cleavage without the aid of the phosphate group is a 1,2-elimination of the nucleobase and has been discussed earlier [38]. However, the initial products formed during this mechanism are the neutral protonated base, BH, and the corresponding anion $[M–H–BH]^-$, not the deprotonated base, B^-. While this mechanism can explain the small amount of $[M–H–BH]^-$ fragment ions that we observe following irradiation of the cyclic mononucleotides (see Fig. 2), it cannot explain the dominance of B^- fragments. A mechanism that leads to the observed B^- products is depicted in Fig. 3d. This mechanism, which is similar to some that have been proposed before for both nucleotide anions [33] and cations [31], involves a direct heterolytic bond cleavage of the CN glycosidic bond leading to B^- and a zwitterionic fragment that is resonance stabilized due to the sugar oxygen [39].

3.3. Photofragment action spectra

Fig. 4 shows the UV photodissociation action spectra for the most abundant fragment channels of $[AMP–H]^-$ and $[cAMP–H]^-$, respectively. The spectra show broad unresolved structures with a peak at ca. 4.9 eV, a shoulder at ca. 4.7 eV and a long high energy tail that decreases until a minimum is reached at ca. 5.5 eV. For some of the fragment channels, the lowest energy features are suppressed. A similar behavior has been observed in our previous study on the 2′-deoxyribonucleotides [9], which could be traced to the fact that the effective appearance energies for some of the fragment ions (i.e., the energies to observe fragments *within our window of observation*) were within the energy range of the lowest energy features in the spectra. We assume that this effect is active in the spectra shown in Fig. 4 as well. In particular, we observe that the lower energy feature in the fragment action spectrum of PO_3^- from $[cAMP–H]^-$ is strongly suppressed compared to A^-. This is compatible with the fact that two bonds have to be broken in order to observe PO_3^- from a cyclic nucleotide while the observation of A^- in principle only needs cleavage of one bond. Our observation is also in line with the fact that IRMPD experiments on $[cAMP–H]^-$ showed only A^- which can therefore be assumed to be the lowest energy fragment (see also Section 3.2). The most abundant fragment channels in $[GMP–H]^-$ and $[cGMP–H]^-$ are shown in Fig. 5. All spectra show two broad unresolved peaks at ca. 4.5 eV and ca. 4.95 eV. At higher energies, a minimum on the photofragment action cross sections is observed at ca. 5.6 eV, followed by a steep rise, which is probably due to higher electronic transitions. We observe suppression of the phosphate based fragments from $[cGMP–H]^-$ at low energies, in particular in the PO_3^- channel, in analogy to the effects observed for $[cAMP–H]^-$ parent ions.

In order to study the effects of changes in the sugar–phosphate "backbone" structure on the electronic spectra of mononucleotides, we can compare the electronic spectra of deoxyribonucleotides [9] with those of the species in the present study. The left column of Fig. 6 shows $[dAMP–H]^-$ parent ion depletion [9], together with the most abundant fragment ions from $[AMP–H]^-$ and $[cAMP–H]^-$, respectively. The spectra of all three species are very similar. Comparison of $[dGMP–H]^-$ depletion [9] and the most abundant fragments from $[GMP–H]^-$ and $[cGMP–H]^-$ (the right column of Fig. 6) shows that all spectra of the guanine based species are also very similar. The independence of the envelope of the UV photofragmentation spectra of the details in the phosphate–sugar structures suggests that the UV chromophore is indeed the base without significant contributions from the rest of the molecule, as has been commonly assumed. We note that fluence dependence measurements for the photofragment channels exhibited no signs of multiphoton characteristics.

4. Summary

We have performed a series of experiments to probe the gas-phase ultraviolet photodissociation of mononucleotides. For these molecules, the photodissociation process involves a series of steps

that begins with absorption of an ultraviolet photon to induce $\pi \rightarrow \pi^*$ transitions, is followed by fast internal conversion to a vibrationally hot electronic ground state, and ends with fragmentation that can occur *via* a number of thermal fragmentation mechanisms. Fragmentation of cyclic mononucleotides shows a strong preference for loss of anionic base (B^-). This behavior can be explained by considering that the phosphate is tethered to the sugar at two points and that there are fewer acidic protons available for a number of fragmentation mechanisms that involve proton transfer. While it is possible that similar mechanisms as those proposed for the non-cyclic nucleotides are also responsible for fragmentation of their cyclic counterparts, it is necessary to consider new fragmentation pathways. In particular, mechanisms that involve direct bond cleavage of the CN glycosidic bond may be of significance.

The different geometries of the phosphate–sugar "backbone" part of cyclic and non-cyclic nucleotides do not affect the envelope of the electronic spectra of adenosine and guanosine-based nucleotides, implying that the UV chromophore is localized on the base without significant contributions from the phosphate or sugar parts of the molecule.

Acknowledgements

The authors would like to thank Professors Veronica Bierbaum and Chuck DePuy for useful discussions on properties of ion-molecule complexes. The authors gratefully acknowledge the National Science Foundation for funding through the JILA Atomic, Molecular and Optical Physics Frontier Center (Grant No. PHY-0551010) and through Grant No. CHE-0845618.

References

[1] S.Y. Wang, Photochemistry and Photobiology of Nucleic Acids, Academic Press, New York, San Francisco, London, 1976.
[2] J. Eisinger, R.G. Shulman, Excited electronic states of DNA, Science 161 (1968) 1311–1319.
[3] C.E. Crespo-Hernandez, B. Cohen, P.M. Hare, B. Kohler, Ultrafast excited-state dynamics in nucleic acids, Chem. Rev. 104 (2004) 1977–2019.
[4] B. Kohler, Nonradiative decay mechanisms in DNA model systems, J. Phys. Chem. Lett. 1 (2010) 2047–2053.
[5] T. Gustavsson, R. Improta, D. Markovitsi, DNA/RNA: building blocks of life under UV irradiation, J. Phys. Chem. Lett. 1 (2010) 2025–2030.
[6] R. Improta, V. Barone, The excited states of adenine and thymine nucleoside and nucleotide in aqueous solution: a comparative study by time-dependent DFT calculations, Theor. Chem. Acc. 120 (2008) 491–497.
[7] E. Nir, I. Hunig, K. Kleinermanns, M.S. de Vries, Conformers of guanosines and their vibrations in the electronic ground and excited states, as revealed by double-resonance spectroscopy and ab initio calculations, ChemPhysChem 5 (2004) 131–137.
[8] E Nir, P. Imhof, K. Kleinermanns, M.S. de Vries, REMPI spectroscopy of laser desorbed guanosines, J. Am. Chem. Soc. 122 (2000) 8091–8092.
[9] J.C. Marcum, A. Halevi, J.M. Weber, Photodamage to isolated mononucleotides-photodissociation spectra and fragment channels, Phys. Chem. Chem. Phys. 11 (2009) 1740–1751.
[10] J. Peon, A.H. Zewail, DNA/RNA nucleotides and nucleosides: direct measurement of excited-state lifetimes by femtosecond fluorescence up-conversion, Chem. Phys. Lett. 348 (2001) 255–262.
[11] T. Gustavsson, A. Sharonov, D. Markovitsi, Thymine, thymidine and thymidine 5′-monophosphate studied by femtosecond fluorescence upconversion spectroscopy, Chem. Phys. Lett. 351 (2002) 195–200.
[12] T. Gustavsson, A. Sharonov, D. Onidas, D. Markovitsi, Adenine, deoxyadenosine and deoxyadenosine 5′-monophosphate studied by femtosecond fluorescence upconversion spectroscopy, Chem. Phys. Lett. 356 (2002) 49–54.
[13] F. Piuzzi, M. Mons, I. Dimicoli, B. Tardivel, Q. Zhao, Ultraviolet spectroscopy and tautomerism of the DNA base guanine and its hydrate formed in a supersonic jet, Chem. Phys. 270 (2001) 205–214.
[14] H. Kang, K.T. Lee, B. Jung, Y.J. Ko, S.K. Kim, Intrinsic lifetimes of the excited state of DNA and RNA bases, J. Am. Chem. Soc. 124 (2002) 12958–12959.
[15] H. Kang, B. Jung, S.K. Kim, Mechanism for ultrafast internal conversion of adenine, J. Chem. Phys. 118 (2003) 6717–6719.
[16] C.T. Middleton, K. de La Harpe, C. Su, Y.K. Law, C.E. Crespo-Hernandez, B. Kohler, DNA excited-state dynamics: from single bases to the double helix, Annu. Rev. Phys. Chem. 60 (2009) 217–239.
[17] J.M.L. Pecourt, J. Peon, B. Kohler, Ultrafast internal conversion of electronically excited RNA and DNA nucleosides in water, J. Am. Chem. Soc. 122 (2000) 9348–9349.
[18] N. Ismail, L. Blancafort, M. Olivucci, B. Kohler, M.A. Robb, Ultrafast decay of electronically excited singlet cytosine via π,π^* to n_0,π^* state switch, J. Am. Chem. Soc. 124 (2002) 6818–6819.
[19] S.B. Nielsen, T.I. Sølling, Are conical intersections responsible for the ultrafast processes of adenine, protonated adenine, and the corresponding nucleosides? ChemPhysChem 6 (2005) 1276–1281.
[20] M Merchan, L. Serrano-Andres, Ultrafast internal conversion of excited cytosine via the lowest $\pi\pi^*$ electronic singlet state, J. Am. Chem. Soc. 125 (2003) 8108–8109.
[21] A.L. Sobolewski, W. Domcke, On the mechanism of nonradiative decay of DNA bases: ab initio and TDDFT results for the excited states of 9H-adenine, Eur. Phys. J. D 20 (2002) 369–374.
[22] A.L. Sobolewski, W. Domcke, C. Dedonder-Lardeux, C. Jouvet, Excited-state hydrogen detachment and hydrogen transfer driven by repulsive $^1\pi\sigma^*$ states: a new paradigm for nonradiative decay in aromatic biomolecules, Phys. Chem. Chem. Phys. 4 (2002) 1093–1100.
[23] H. Satzger, D. Townsend, M.Z. Zgierski, S. Patchkovskii, S. Ullrich, A. Stolow, Primary processes underlying the photostability of isolated DNA bases: adenine, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 10196–10201.
[24] B. Liu, N. Haag, H. Johansson, H.T. Schmidt, H. Cederquist, S.B. Nielsen, H. Zettergren, P. Hvelplund, B. Manil, B.A. Huber, Electron capture induced dissociation of nucleotide anions in water nanodroplets, J. Chem. Phys. 128 (2008).
[25] G. Aravind, R. Antoine, B. Klaerke, J. Lemoine, A. Racaud, D.B. Rahbek, J. Rajput, P. Dugourd, L.H. Andersen, Sub-microsecond photodissociation pathways of gas phase adenosine 5′-monophosphate nucleotide ions, Phys. Chem. Chem. Phys. 12 (2010) 3486–3490.
[26] S.B. Nielsen, J.U. Andersen, J.S. Forster, P. Hvelplund, B. Liu, U.V. Pedersen, S. Tomita, Photodestruction of adenosine 5′-monophosphate (AMP) nucleotide ions in vacuo: statistical versus nonstatistical processes, Phys. Rev. Lett. 91 (2003) 048302.
[27] Y.H. Ho, P. Kebarle, Studies of the dissociation mechanisms of deprotonated mononucleotides by energy resolved collision-induced dissociation, Int. J. Mass Spectrom. Ion Proc. 165 (1997) 433–455.
[28] S. Habibigoudarzi, S.A. McLuckey, Ion-trap collisional activation of the deprotonated deoxymononucleoside and deoxydinucleoside monophosphates, J. Am. Soc. Mass Spectrom. 6 (1995) 102–113.
[29] R.L. Cerny, M.L. Gross, L. Grotjahn, Fast-atom-bombardment combined with tandem mass-spectrometry for the study of dinucleotides, Anal. Biochem. 156 (1986) 424–435.
[30] A. Vasiliou, M.R. Nimlos, J.W. Daily, G.B. Ellison, Thermal decomposition of furan generates propargyl radicals, J. Phys. Chem. A 113 (2009) 8540–8547.
[31] D.R. Phillips, J.A. McCloskey, A Comprehensive study of the low-energy collision-induced dissociation of dinucleoside monophosphates, Int. J. Mass Spectrom. Ion Proc. 128 (1993) 61–82.
[32] J. Yang, K. Hakansson, Characterization of oligodeoxynucleotide fragmentation pathways in infrared multiphoton dissociation and electron detachment dissociation by Fourier transform ion cyclotron double resonance, Eur. J. Mass Spectrom. 15 (2009) 293–304.
[33] K.X. Wan, M.L. Gross, Fragmentation mechanisms of oligodeoxynucleotides: effects of replacing phosphates with methylphosphonates and thymines with other bases in T-rich sequences, J. Am. Soc. Mass Spectrom. 12 (2001) 580–589.
[34] J.S. Klassen, P.D. Schnier, E.R. Williams, Blackbody infrared radiative dissociation of oligonucleotide anions, J. Am. Soc. Mass Spectrom. 9 (1998) 1117–1124.
[35] J. Wu, S.A. McLuckey, Gas-phase fragmentation of oligonucleotide ions, Int. J. Mass Spectrom. 237 (2004) 197–241.
[36] M.T. Rodgers, S. Campbell, E.M. Marzluff, J.L. Beauchamp, Low-energy collision-induced dissociation of deprotonated dinucleotides – determination of the energetically favored dissociation pathways and the relative acidities of the nucleic-acid bases, Int. J. Mass Spectrom. Ion Proc. 137 (1994) 121–149.
[37] B. Chiavarino, M.E. Crestoni, S. Fornarini, F. Lanucara, J. Lemaire, P. Maitre, D. Scuderib, Infrared spectroscopy of isolated nucleotides: 1. The cyclic 3′,5′-adenosine monophosphate anion, Int. J. Mass Spectrom. 270 (2008) 111–117.
[38] S.A. McLuckey, S. Habibigoudarzi, Decompositions of multiply-charged oligonucleotide anions, J. Am. Chem. Soc. 115 (1993) 12085–12095.
[39] M. Jones Jr., Organic Chemistry, 2nd ed., W.W. Norton & Company, Inc., New York, NY, 2000.