Title: Electron detachment dissociation (EDD) pathways in oligonucleotides

Authors: Catherine Kinet, Valérie Gabelica, Dorothée Balbeur, Edwin De Pauw

PII: S1387-3806(09)00124-9
DOI: doi:10.1016/j.ijms.2009.03.012
Reference: MASPEC 14000

To appear in: *International Journal of Mass Spectrometry*

Received date: 28-11-2008
Revised date: 6-3-2009
Accepted date: 26-3-2009

Please cite this article as: C. Kinet, V. Gabelica, D. Balbeur, E. De Pauw, Electron detachment dissociation (EDD) pathways in oligonucleotides, *International Journal of Mass Spectrometry* (2008), doi:10.1016/j.ijms.2009.03.012

Electron detachment dissociation (EDD) pathways in oligonucleotides

Catherine Kinet, Valérie Gabelica*, Dorothée Balbeur, Edwin De Pauw

(1) Laboratoire de Spectrométrie de Masse, Université de Liège, Institut de Chimie, Bat B6c, B-4000 Liège, Belgium,

* Corresponding author: v.gabelica@ulg.ac.be

Abstract

Electron detachment dissociation (EDD) and electron photodetachment dissociation (EPD) are two novel fragmentation methods yielding radicals from negatively charged ions. With the goal of comparing EDD, EPD and the more traditional Collision-Induced Dissociation (CID) and Infrared Multiphoton Disscociation (IRMPD) fragmentation processes in oligonucleotides, we studied here the EDD fragmentation pathways of oligonucleotides of varying length. We chose polythymine oligonucleotides because these are the least prone to secondary structure formation, and found complete sequence coverage by EDD for up to dT_{20}. We also found that the fragmentation pathways change with oligonucleotide length: electron detachment is a mandatory step in the fragmentation of larger sequences, while shorter oligonucleotides can also fragment via direct electronic or vibrational excitation by the electrons. This is supported by (1) the fact that continuous ejection of the charge reduced species does not totally prevent fragmentation of short oligonucleotides dT_5 and dT_6, (2) the fact that CID and EDD fragments are more similar for small oligonucleotides (although double resonance experiments show that they are not all issued from the same mechanisms), and (3) the fact that electron-induced dissociation (EID) of singly charged dT_3 and dT_4 gives similar fragments as EDD of doubly charged dT_5 and dT_6. Finally, the detachment efficiency as a function of the nature of the nucleobase was studied. The effect of base on electron detachment in EDD (G > T > A > C) is different than in EPD (G > A > C > T), indicating different electron loss mechanisms.

Keywords : Electron detachment dissociation ; EDD ; Fourier transform ion cyclotron resonance ; FTICR ; Oligonucleotides ; mass spectrometry ; double resonance ; DNA.

1. Introduction

Mass spectrometry is widely used for the characterization of macromolecules of biological importance including nucleic acids. Electrospray Ionization (ESI) [1-3] and Matrix Assisted Laser Desorption/Ionization (MALDI) [4-6] are the ionization methods of choice for observing large biomolecules in the gas phase such as oligonucleotides. Numerous reports [7-12] discuss the application of tandem mass spectrometry for the sequence characterization of oligonucleotides and nucleic acids. Traditional MS/MS experiments employing vibrational excitation such as Collision-Induced Dissociation (CID) [13] or Infrared Multiphoton Dissociation (IRMPD) [14] cause base loss and *w* and *(a-BH)* ion formation, according to McLuckey's nomenclature [12]. Several fragmentation mechanisms involving a fragmentation initiated by base loss [15, 16] were proposed to explain *w* and *(a-BH)* ions formation. A disadvantage of these techniques is the formation of internal products (double fragmentation of the parent ion) which complicates the spectra.

More recently, numerous fragmentation methods involving electron-ion interaction (ECD, EDD, EID and EPD) were also studied. In Electron Capture Dissociation (ECD) [17-20], a multiply protonated molecule captures a thermal electron (<1eV) to form a radical ion that rapidly undergoes covalent bond cleavage. This fragmentation method generates different and often complementary fragmentation patterns when compared with CID and IRMPD. ECD has been successfully applied to peptides and proteins [21, 22], polymers [23], oligonucleotides [24, 25], peptide nucleic acids [26] and for the determination of post-translational modifications such as for glycosylation [27]. Moreover, non-covalent bonds can remain intact during ECD [22].

Another novel fragmentation method involving radical species is Electron Photodetachment Dissociation (EPD) introduced by Gabelica and co-workers [28, 29]. The UV irradiation of

oligonucleotides causes the detachment of electrons, and the resulting radical ions can be fragmented. As in ECD, no internal products are found. The efficiency of electron photodetachment is nucleobase dependent, with G >> A > C > T.

The method coined Electron Detachment Dissociation (EDD), first introduced by Zubarev for polypeptide di-anions [30], has also been used for oligonucleotide fragmentation [31-35]. In peptides, bombardment of multiply charged anions by electron (>10 eV) causes the loss of an electron followed by N–C_α and C_α–C bond cleavage. Zubarev and coworkers proposed that the N–C_α cleavage originates from an electron-hole recombination phenomenon. Anusiewicz and co-workers [36] performed ab-initio calculations to analyze backbone and side-chain cleavage. They showed that although the fragmentation of the nitrogen-centered radical formed might evolve through two different fragmentation channels, one is favored because of its smaller energy barrier. Hakansson and co-workers [31-35] described in details the EDD of small oligonucleotides. In their seminal paper [31], they reported that EDD fragmentation of hexamer oligonucleotides suggested that EDD offers complementary fragmentation pattern compared to CID, and complete sequencing was obtained. Also, secondary fragmentation is reduced and non-covalent interactions were conserved. They later showed that in the case of longer sequences, sequence coverage was lower than for the short hexamers, and this was attributed to residual secondary structure [32].

Finally, another method involving formation of even- and odd-electron products is the Electron Induced Dissociation (EID) [37], in which singly charged ion are irradiated by electrons (>10 eV). To our knowledge, EID has not yet been applied to oligonucleotide fragmentation.

Our goal was to study the EDD fragmentation pathways by applying FTICR double resonance ejection (DR) [38] during electron bombardment, in order to compare EDD to CID (in terms of sequence coverage) and to EPD (in terms of electron detachment mechanisms). While this

paper was in preparation, a study of EDD pathways by double resonance on short hexamers was published [35], which showed (1) that the charge-reduced species resulting from electron loss is a key intermediate in the fragmentation process, and (2) that *a/z•* radical ions are precursors of their corresponding *(a/z-T)* ions. We therefore focus the present article on the following novel aspects: the base-dependence of electron detachment yield in EDD (for comparison with EPD) and the study of polythymines of varying length.

2. Experimental section

2.1. Sample preparation

All oligonucleotides (dT_2, dT_3, dT_4, dT_5, dT_6, dT_{10}, dT_{15}, dT_{20}, dT_{30}, dA_6, dC_6, dG_6) were synthesized by Eurogentec (Liege, Belgium). Stock solutions were prepared in water. The final injected solution has a concentration of 10 µM of oligonucleotides in 50 percent methanol and in 50 mM ammonium acetate except for dG_6 where no ammonium acetate was added.

2.2. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

All experiments were performed on a 9.4 Tesla Apex-Qe FTICR mass spectrometer (Bruker Daltonics, Billerica,MA). The oligonucleotide solutions were infused via an external Apollo electrospray ion source at a flow rate of 120 µL/h with the assistance of N_2 nebulizing gas. The off axis sprayer was grounded, the end-plate was set to 3 kV and the inlet capillary was set to 3.5 kV for the generation of oligonucleotides anions. Heated N_2 drying gas (250 °C) was applied to assist desolvation of ESI droplets. Ions were accumulated in the first hexapole

for 1 s, transferred through the mass-selective quadrupole (5-10 Da isolation window) and mass selectively accumulated in the second hexapole for 1 to 3 s. The ions were transferred through high-voltage ion optics and captured by static trapping in an ICR cell. All mass spectra were acquired with XMASS (version 7.0.8, Bruker Daltonics) in broadband mode with 512 k data points and summed over 100 scans. A mass list, in which *m/z* values and peak heights are recorded, was created using DataAnalysis^{TM} (version 3.4, Bruker Daltonics).

2.3. Electron Detachment Dissociation (EDD) and double resonance

For EDD, the electrons are emitted by a cylindrical indirectly heated hollow dispenser cathode. A heating current of 1.9 A is applied to a heater element located behind the cathode. A lens of 6 mm diameter located in front of the cathode ensures the focalization of the electron beam (lens voltage = -18.8 V) the electrons were accelerated using a bias voltage of -18.2 V. The ions trapped in the ICR cell were subjected to 1s irradiation by the electron beam. For double resonance experiments, the *m/z* ratio of the ion to be ejected from the ICR-cell was converted in its cyclotron frequency by the software and the excitation voltage (200 V_{p-p}) was attenuated by 20 dB. Continuous ejection is conducted during the whole EDD irradiation time.

Because the absolute intensities of products are influenced by the double resonance event (absolute intensity of some products increase upon DR), the following procedure was used to assess whether the abundance of a product significantly decreased upon double resonance. First, several (at least three) EDD spectra without double resonance were acquired. The peak intensities were then normalized relative to several reference products that are not affected by the double resonance event. The chosen reference products varied for each double resonance experiment and depended of the *m/z* of the ejected ion. They must have a sufficiently large

intensity and their *m/z* must not be close to that of the ejected ion. Then, we have considered that a particular product was affected by DR event only if a significant decrease of its normalized intensity relative to each reference ion was observed.

2.4. Collision-Induced Dissociation (CID) and Infrared Multiphoton Dissociation (IRMPD)

CID fragmentation was performed in the collision hexapole of the Apex-Qe by increasing the potential at the collision cell entrance to 20-30V, depending on the oligonucleotide. IRMPD was performed using a 25 W CO_2 laser (Synrad, Mukilteo, WA) with a wavelength of 10.6 μm. The laser beam passes through the centre of the hollow dispenser cathode. Ions were irradiated for 100 ms at 50% laser power.

3. Results and discussion:

3.1. dT_2 to dT_{30}: influence of the oligonucleotide length on EID/EDD fragmentation

The objective of studying the effect of oligonucleotide length is to classify fragmentation pathways into two categories: (1) ergodic fragmentation channels with a relatively high threshold will be less favored as the number of degrees of freedom (hence the length) of the oligonucleotide increases, whereas (2) non-ergodic fragmentation channels and ergodic fragmentation channels with a relatively low threshold (as can happen in the fragmentation of radicals [39]) will remain observable as the length increases.

Hakansson et al. reported complete sequence coverage for 6-mer oligonucleotides upon EDD [31], but have shown that complete sequence coverage was difficult to obtain on 15-mers with mixed sequence due to secondary structure formation (hairpins) [32]. Here, in order to avoid sequence-related conformational effects and to study the fragmentation pathways and sequence coverage as a function of the oligonucleotide length, experiments were performed on oligonucleotides containing thymines exclusively. Polythymine sequences are the least prone to form secondary structures because the T-T base pair is the weakest of all natural and unnatural base pairs [40].

We studied the nature of the obtained products upon electron bombardment as a function of the oligonucleotide length, with no other activation than that imparted by the electron bombardment (as opposed to activated-ion EDD reported in [32]). Some experimental limitations were encountered: to observe the complete fragmentation pattern from the dissociation event, it was important to have a sufficient signal. In fact, the ratio between the intensities of the products (event- and odd-electron products) and the precursor ion was very small (a few percent) upon electron bombardment. Consequently, even if product ions were not detected during the experiment, some products could be present in the noise of the spectrum. Therefore, the charge state for all oligonucleotides was selected based on the peak intensity ($> 2 \times 10^6$), to ensure that most products are detected. Due to the palindromic nature of the sequence, ions tagged *a* can also be *z* ions, ions tagged *w* can also be *d* ions, ions tagged *c* can also be *x* ions and ions tagged *y* can also be *b* ions. Furthermore, due to the fact that the sequence is homogeneous, products identified as *(c/x-TH)* ions series could also be internal products resulting from double fragmentation: *w*-type cleavage at the 5' side and by a (*a-Base)*-type cleavage at the 3' side of the oligonucleotide (Figure 1a).

Figure 2 shows all fragments and the charge states that have been detected. Note that for dT_3 and dT_4, experiments were performed only on the singly charged precursor ion due to the insufficient intensity of the doubly charged species. Therefore, their charged product ions must be due to electron-induced dissociation (EID) and not electron *detachment* dissociation (EDD). For dT_3 to dT_{10}, we detected many *a/z*, *(a/z-TH)*, *c/x*, *(c/x-TH)*, *w/d* ions (closed shell species) and many *a/z•* and *c/x•* radical ions. These radical ions are identified by calculating their exact mass (which is equal to the one of the analogous closed shell species minus one hydrogen atom) and by checking their isotopic distribution. The first peak of the isotopic distribution is identified, and the theoretical isotopic distribution is overlapped to check for the potential contribution of another species containing additional hydrogens. Neutral losses (thymine, H_2O, etc) were also observed from the precursor ion and from the charge-reduced species. A few *y/b* and *(w/d-TH)* ions were also detected. Novel internal products are also observed, although in low abundance. These resulted from a *w*-type cleavage at the 5' side and by a *d*-type cleavage at the 3' side of the oligonucleotide (Figure 1b). For $dT_{n>10}$, *w/d* ions and *a/z•* radical ions dominate the spectra, in addition to neutral loss.

To determine which of these products are peculiar to electronic excitation (EID or EDD as the charge state of the precursor ion), we performed CID on the same dT_n sequences. The observed products are summarized in Figure 3. Based on the literature [12, 15, 16] about oligonucleotide fragmentation by vibrational activation, base loss, *w* and *(a-TH)* ions are expected. Even if thymine has the lowest proton affinity (G> C ≈ A >> T) [41], *(a-TH)* fragment ions are detected in T-rich oligonucleotides [16]. Other products like *y/b*, *a/z*, and *c/x* ions are also observed in CID for dT_5, dT_6, dT_{10}, dT_{15}, dT_{20},. Radical ions are observed only upon electron bombardment as is the case with *a/z•*, *c/x•* and *w/d•* radical ions and neutral loss from charge reduced species.

The comparison between CID and EDD is easier for longer oligonucleotides: the 5' fragments are the same (closed shell *w* ions) in CID and EDD, while the 3' fragments differ (closed shell *a/z* and *(a/z-TH)* in CID, radical ion *a/z•* in EDD). An important result is that complete sequence coverage is obtained in EDD for up to dT_{20}, with no other activation than that imparted by the electrons. This behavior contrasts with EPD. In EPD, laser irradiation at 260 nm caused only electron detachment [28] but in EDD and in EID, numerous even- and odd-electron product ions are detected along with electron detachment. This behavior is also different from the EDD results described by Hakansson [32] on the 15-mers with mixed-base sequences. Our results with the presumably unstructured polythymines confirm that incomplete sequence coverage can be due to residual intramolecular folding.

Regarding the influence of length on EDD fragmentation pathways, *w/d* and *a/z•* product ions remain observed whatever the oligonucleotide length, whereas *(a/z-TH)*, *c/x* and *c/x•*, *(w/d-TH)* and *y/b* ions disappear as the length increases. This suggests that product ions from the latter have a higher formation threshold than *w/d* and *a/z•* product ions. In CID, *(y/b-TH)* and *(w/d-TH)* are only observed for the smallest sequences, *y/b* ions disappear between dT_{15} and dT_{20}, and *c/x* ions persist even for the longest sequences.

3.2. Double-resonance (DR) EDD experiments on polythymines of varying length

In a double resonance (DR) experiment, an ion that is suspected of being the precursor of other product(s) is continuously ejected from the ICR cell during the whole MS/MS event (in EDD, during the whole electron bombardment event). This ejection is obtained by resonant excitation of the ion at its cyclotron frequency [42]. The disappearance of other products

indicates that they were issued from the ejected ion, and therefore related via a fragmentation pathway. A decrease in product intensity suggests part of their population was formed from the ejected ion.

In their recent report on DR-EDD of hexamer anions [35] Hakansson et al. reported for dT_6 that the whole *(a/z-TH)* ions series originated from secondary fragmentation of the corresponding *a/z•* radical ions, that the charge-reduced species was an intermediate in the EDD fragmentation process, and that the parent ion with a base loss $(M\text{-}TH)^{n-}$ was not. Here we extend the study to polythymine oligonucleotides of varying length, from dT_4, to dT_{15}. Additionally, ejection of *c/x* ions was also investigated.

3.2.1. *a/z•* radical ion ejection

In the EID spectrum of dT_3 and dT_4, even electron products like *a/z, (a/z-TH), c/x, (c/x-TH), y/b, w/d* and *(w/d-TH)* ions, and only one radical ion, a_3/z_3•, were detected. Upon ejection of this radical ion, supposed to be the precursor ion of $(a_3/z_3\text{-}TH)^-$ ion, no change was observed: DR-EID and EID spectra were similar. For dT_5, DR-EDD was performed on the doubly charged ion. Each detected *a/z* radical ions was ejected in a separate DR-EDD experiment. When a_2/z_2• radical ion was ejected during EDD event, no variation was observed. In contrast, significant abundance decrease (> 30%) was observed for $(a_3/z_3\text{-}TH)^-$ and $(a_4/z_4\text{-}TH)^-$ ions when a_3/z_3• and a_4/z_4• radical ions were ejected, respectively. dT_6 yielded results similar to those of dT_5 and to those recently reported by Hakansson [35]. For dT_{10}, DR-EDD was performed on the triply charged precursor ion. All *a/z•* radical ions were ejected in separate DR-EDD experiments, but due to the low abundance of these *(a/z-TH)* ions their abundance variation cannot be considered significant.

In conclusion, some of *(a/z-TH)* ions clearly originate from the decomposition of the corresponding *a/z•* radical ions, but other do not. This is not length-dependent, as both smaller and larger oligonucleotides than dT_6 fail to reveal a significant linkage between (*a/z-TH*) and *a/z•* ions. Furthermore, the observation that *(a/z-TH)* fragment ions are reduced but do not totally disappear when the corresponding *a/z•* radical ion is resonantly ejected can be interpreted in two ways. Either several formation channels coexist and their relative contribution is a function of the length of the oligonucleotide and of the *a/z•* radical ion that is ejected, or all *(a/z-TH)* ions derive from *a/z•* radical ions but the reaction kinetics changes as a function of the length of the oligonucleotide and of the *a/z•* radical ion that is ejected.

A limitation of the double resonance method is that the time it takes to eject an ion by resonance ejection must be much faster than the time required for the consecutive products to form from the ejected product. Products may be formed and detected before ejection is complete [38]. For the DR-EDD experiment on dT_5, a_4/z_4• radical ion ejection resulted in a decrease of *(a_4/z_4-TH)* ion. Therefore the *(a_4/z_4-TH)* ion results at least partially, if not completely from dissociation of the a_4/z_4• radical ion.

3.2.2. *c/x* ion ejection

In order to gain supplementary information on the EDD pathways, we performed DR-EDD experiments with ejection of the even electron *c/x* ions. According to the spectral analysis, no relationship between *c/x* and *(c/x-TH)* ions can be evidenced. As discussed previously, *(c/x-TH)* ions may also be internal products (structure in Figure 1a). The fact that they are not affected by the ejection of the *c/x* ions supports this hypothesis.

3.2.3. $(M\text{-}nH\text{-}TH)^{n-}$ ion ejection.

To test alternative formation pathways for *(a/z-TH)* ions observed in EDD, we checked whether they could come from the parent ion that has lost one base but no electron(s), like in vibrational activation methods (CID or IRMPD). If so, it would mean that electronic excitation due to collision with energetic electron (10 eV) can also be redistributed on vibrational normal modes. Figure 4 shows IRMPD spectra of dT_5 without (a) and with (b) continuous ejection of $(M\text{-}2H\text{-}TH)^{2-}$. Similar results were found with dT_6^{2-}, contrasting with the recent results reported by Hakansson et al [35], who did not observe any product intensity decrease upon ejection of base loss ion in IRMPD. Our results are in better agreement with the fragmentation mechanism at stake in vibrational excitation [16]. These spectra are compared to EDD spectra of dT_5 (a) without and (b) with continuous ejection of $(M\text{-}2H\text{-}TH)^{2-}$ (Figure 5). It is clear from Figure 5 that, on the contrary to IRMPD where neutral base loss is the first step of the fragmentation process, products of dT_5 upon EDD do not originate from this fragmentation channel, now in agreement with previous reports [35].

3.2.4. Ejection of the charge-reduced species

As suggested by its name, EDD fragmentation supposedly happens through further decomposition of the charge-reduced species. However, as shown above, electron-induced fragmentation of singly charged short oligonucleotides results in similar fragments as the doubly charged pentamers and hexamers. Furthermore, some doubly charged EDD fragments of the hexamers were also proposed to be issued from EID-like processes. We therefore

investigated whether the oligonucleotide length influenced the extent to which charge reduction is essential to fragmentation.

DR-EDD experiments on charge-reduced species were performed on dT_5, dT_6, dT_{10} and dT_{15}. For dT_5^{2-}, when $(M-2H)^{-\bullet}$ radical ion is ejected, a weak decrease of product abundance was observed, but a majority of products were still detected, including all *a/z•* radical ions. As odd-electron product formation from even-electron species is very unlikely, we conclude that the fragmentation of $dT_5^{\bullet-}$ occurs on a similar time scale as the DR time scale. Similar observations were made for the ejection of $(M-2H)^{-\bullet}$ radical ion from the 6-mer.

For dT_{10}, continuous ejection of species having lost one and two electron was performed. The EDD spectra (a) without DR, (b) with DR on $(M-3H)^{2-\bullet}$ radical ion and (c) with DR on $(M-3H)^{-\bullet\bullet}$ radical ion are shown in the Figure 6. A decrease of most product intensities was observed when $(M-3H)^{2-\bullet}$ radical ion was ejected (Figure 6b). However, no product abundance variation was observed when $(M-3H)^{-\bullet\bullet}$ radical ion was ejected (Figure 6c). The spectrum acquired by performing $(M-3H)^{-\bullet\bullet}$ radical ion ejection is similar to the one acquired without double resonance event. Consequently, no product ion clearly results from a subsequent decomposition of this radical species. The radical species that has lost one base, $(M-3H-TH)^{2-\bullet}$, was also ejected and no product abundance decreasing was observed. For dT_{15}^{4-} a strong decrease of product abundance was observed when $(M-4H)^{3-\bullet}$ radical ion was ejected like for dT_{10}. A few products pertaining to the *w* ions series were detected, such as w_8^{2-}, w_9^{2-}, w_{14}^{2-}, w_{14}^{3-}, w_{14}^{4-}. No significant product abundance variation was observed when the charge-reduced species resulting from two electron loss of the parent ion was ejected.

In summary, the dissociation pathways change with the oligonucleotide length. Fragments of short oligonucleotides can be formed even with ejection of the charge reduced species, but for long oligonucleotides the charge reduced intermediate becomes crucial for fragmentation.

3.3. Base influence on the detachment efficiency

Finally, in order to compare electron detachment in EPD and EDD, we studied quantitatively the detachment efficiency as a function of the nature of the nucleobases. The fraction of charge-reduced species was determined by adding all radical products to the charge-reduced species, because radical ions are originated from subsequent decomposition of $dB_6^{-\bullet}$ (section 3.2.4). Figure 7 shows the electron detachment efficiency normalized to that of dC_6. In EDD, the fraction $dB_6^{-\bullet}$ relative to the parent ion evolves as follows: $dG_6^{2-} > dT_6^{2-} > dA_6^{2-} > dC_6^{2-}$. This figure shows that electron detachment in EDD is nucleobase-dependent.

This electron detachment tendency is different from the one established for the electron detachment by absorption of a photon (EPD) [29] ($dG_6 > dA_6 > dC_6 > dT_6$), and different from the electron thermal autodetachment observed by Danell and Parks ($dT_7 > dC_7 > dA_7$)[43]. This observation therefore suggests that the mechanism of electron loss in bombardment by > 10 eV electrons (EDD) is different than in irradiation with 4.77 eV photons (EPD, where electron loss from the base was proposed [29] and from autodetachment from the phosphates.

4. Conclusion

The outcomes of this study are summarized as follows:

1. The complete sequencing of unstructured oligonucleotides containing up to 20 thymine nucleobases, without pre- or post-activation, is shown for the first time. This further

confirms the proposal by Hakansson et al. [32] that incomplete sequence coverage can be due to gas-phase intramolecular folding.

2. Comparison between electronic and vibrational excitation experiments for dT_n showed that many fragments were shared by the two dissociation methods, except for the presence of *a/z•* and *c/x•* radical ions and some neutral loss. However, the fragments that are common to EDD and CID are not produced via the same intermediates. The first step in CID fragmentation is the neutral base loss, whereas the first step in EDD fragmentation is the loss of one electron and consequently the formation of the charge reduced species.
3. A study of the electron detachment efficiency in EDD as a function of the nature of the nucleobases showed the following trend: $dG_6 > dT_6 > dA_6 > dC_6$. The mechanism of electron detachment in EDD and its comparison with EPD and autodetachment clearly warrant further investigation.
4. From the double resonance experiments on dT_n in which *a/z•* ions were ejected, we found that at least some *(a/z-TH)* ions originate from secondary decomposition of the corresponding *a/z•* ions. Therefore, in EDD, neutral base loss follows fragmentation, whereas in IRMPD the backbone fragmentation follows base loss.
5. The dissociation pathways change with the oligonucleotide length. For long oligonucleotides, electron detachment is mandatory, and leads predominantly to the formation of *w*/d and *a/z•* product ions. For shorter sequences, fragmentation does not necessarily proceed via electron detachment, and fragmentation of singly charged precursor ions is even possible (EID process), leading to the same kind of fragments as EDD on doubly charged dT_5 and dT_6 (including *c/x* and *(w-TH)* ions). In conclusion, the inelastic collisions of > 10 eV electrons with oligonucleotide anions result in ion activation that can have two kinds of outcomes: electron detachment followed by

dissociation (EDD), and/or energy redistribution and fragmentation (EID). The present results suggest that the "and" prevails for short doubly charged sequences.

Acknowledgement

We acknowledge financial support from the Walloon Regi$_o$n (Proj$_e$t FEDER FTIC*R)* and t*he* *FRS*-FNRS (Fonds de la Recherche Scientifique - FNRS) for funding. VG is a FNRS Research Associate and DB is a FNRS Doctoral Fellow.

References

Reference List

[1] P.A.Limbach, P.F.Crain and J.A.McCloskey, Curr. Opin. Biotechnol., 6 (1995) 96.

[2] P.F.Crain and J.A.McCloskey, Curr. Opin. Biotechnol., 9 (1998) 25.

[3] J.$_W$u and S.A.McLuckey, Int. J. Mass Spectrom., 237 (2004) 197.

[4] B.Spengler, Y.Pan, R.J.Cotter and L.S.Kan, Rapid Commun. Mass Spectrom., 4 (1990) 99.

[5] C.M.Bentzley, M.V.Johnston, B.S.Larsen and S.Gutteridge, Anal. Chem., 68 (1996) 2141.

[6] Y.Li, K.Tang, D.P.Little, H.Koster, R.L.Hunter and R.T.McIver, Jr., Anal. Chem., 68 (1996) 2090.

[7] S.A.McLuckey, G.J.Vanberkel and G.L.Glish, J. Am. Soc. Mass Spectrom., 3 (1992) 60.

[8] J.Ni, C.Pomerantz, J.Rozenski, Y.Zhang and J.A.McCloskey, Anal. Chem., 68 (1996) 1989.

[9] J.Ni and K.Chan, Rapid Commun. Mass Spectrom., 15 (2001) 1600.

[10] J.H.Banoub, R.P.Newton, E.Esmans, D.F.Ewing and G.Mackenzie, Chem. Rev., 105 (2005) 1869.

[11] K.M.Keller and J.S.Brodbelt, Anal. Biochem., 326 (2004) 200.

[12] K.X.Wan and M.L.Gross, J. Am. Soc. Mass Spectrom., 12 (2001) 580.

[13] S.A.McLuckey, J. Am. Soc. Mass Spectrom., 3 (1992) 599.

[14] D.P.Little, J.P.Speir, M.W.Senko, P.B.O'Connor and F.W.McLafferty, Anal. Chem., 66 (1994) 2809.

[15] S.A.McLuckey and S.Habibigoudarzi, J. Am. Chem. Soc., 115 (1993) 12085.

[16] Z.Wang, K.X.Wan, R.Ramanathan, J.S.Taylor and M.L.Gross, J. Am. Soc. Mass Spectrom., 9 (1998) 683.

[17] R.A.Zubarev, Mass Spectrom. Rev., 22 (2003) 57.

[18] R.A.Zubarev, D.M.Horn, E.K.Fridriksson, N.L.Kelleher, N.A.Kruger, M.A.Lewis, B.K.Carpenter and F.W.McLafferty, Anal. Chem., 72 (2000) 563.

[19] F.W.McLafferty, D.M.Horn, K.Breuker, Y.Ge, M.A.Lewis, B.Cerda, R.A.Zubarev and B.K.Carpenter, J. Am. Soc. Mass Spectrom., 12 (2001) 245.

[20] R.A.Zubarev, Curr. Opin. Biotechnol., 15 (2004) 12.

[21] R.A.Zubarev, N.L.Kelleher and F.W.McLafferty, J. Am. Chem. Soc., 120 (1998) 3265.

[22] D.M.Horn, Y.Ge and F.W.McLafferty, Anal. Chem., 72 (2000) 4778.

[23] B.A.Cerda, D.M.Horn, K.Breuker and F.W.McLafferty, J. Am. Chem. Soc., 124 (2002) 9287.

[24] K.N.Schultz and K.Hakansson, Int. J. Mass Spectrom., 234 (2004) 123.

[25] K.Hakansson, R.R.Hudgins, A.G.Marshall and R.A.O'Hair, J. Am. Soc. Mass Spectrom., 14 (2003) 23.

[26] J.V.Olsen, K.F.Haselmann, M.L.Nielsen, B.A.Budnik, P.E.Nielsen and R.A.Zubarev, Rapid Commun. Mass Spectrom., 15 (2001) 969.

[27] J.T.Adamson and K.Hakansson, J. Proteome Res., 5 (2006) 493.

[28] V.Gabelica, T.Tabarin, R.Antoine, F.Rosu, I.Compagnon, M.Broyer, E.De Pauw and P.Dugourd, Anal. Chem., 78 (2006) 6564.

[29] V.Gabelica, F.Rosu, T.Tabarin, C.Kinet, R.Antoine, M.Broyer, E.De Pauw and P.Dugourd, J. Am. Chem. Soc., 129 (2007) 4706.

[30] B.A.Budnik, K.F.Haselmann and R.A.Zubarev, Chem. Phys. Lett., 342 (2001) 299.

[31] J.Yang, J.J.Mo, J.T.Adamson and K.Hakansson, Anal. Chem., 77 (2005) 1876.

[32] J.Mo and K.Hakansson, Anal. Bioanal. Chem., 386 (2006) 675.

[33] J.Yang and K.Hakansson, J. Am. Soc. Mass Spectrom., 17 (2006) 1369.

[34] J.Yang and K.Hakansson, Int. J. Mass Spectrom., 276 (2008) 144.

[35] J.Yang and K.Hakansson, Eur. J. Mass Spectrom., 15 (2009) 10.1255/ejms.966.

[36] I.Anusiewicz, M.Jasionowski, P.Skurski and J.Simons, J. Phys. Chem., 109 (2005) 11332.

[37] J.J.Wolff, T.N.Laremore, H.Aslam, R.J.Linhardt and I.J.Amster, J. Am. Soc. Mass Spectrom., 19 (2008) 1449.

[38] C.Lin, J.J.Cournoyer and P.B.O'Connor, J. Am. Soc. Mass Spectrom., 17 (2006) 1605.

[39] F.Turecek, Journal of the American Chemical Society, 125 (2003) 5954.

[40] P.Hozba and J.Sponer, Chem. Rev., 99 (1999) 3247.

[41] F.Greco, A.Liguori, G.Sindona and N.Uccella, J. Am. Chem. Soc., 112 (1990) 9092.

[42] I.J.Amster, J. Mass Spectrom., 31 (1996) 1325.

[43] A.S.Danell and J.H.Parks, J. Am. Soc. Mass Spectrom., 14 (2003) 1330.

Figure captions

Figure 1: Detailed structures of the classical internal product (a) and of the novel internal product (b), illustrated for dT_4^-. Product (a) results from a *w*-type cleavage at the 5' side and by a *(a-Base)*-type cleavage at the 3' side. Product (b) results from a *w*-type cleavage at the 5' side and by a *d*-type cleavage at the 3' side.

Figure 2: Observed fragments and their charge states upon electron bombardment for (a) dT_3^-, (b) dT_4^-, (c) dT_6^{2-}, (d) dT_5^{2-}, (e) dT_{10}^{3-}, (f) dT_{15}^{4-}, (g) dT_{20}^{5-} respectively. Ions tagged *a* can also be *z* ions, ions tagged *w* can also be *d* ions, ions tagged *c* can also be *x* ions and ions tagged *y* can also be *b* ions. Some fragments were observed at more than one charge state. These different charge states are separated by a "/" symbol.

Figure 3: Observed CID fragments and their charge states for (a) dT_5^{2-}, (b) dT_6^{2-}, (c) dT_{10}^{3-}, (d) dT_{15}^{4-}, (e) dT_{20}^{4-} respectively. Ions tagged *a* can also be *z* ions, ions tagged *w* can also be *d* ions, ions tagged *c* can also be *x* ions and ions tagged *y* can also be *b* ions. Some fragments were observed at more than one charge state. These different charge states are separated by a "/" symbol.

Figure 4: IRMPD spectra of dT_5 (a) without DR and (b) with DR on $(M\text{-}nH\text{-}TH)^{n-}$ ion (spectra on same scale). When DR was applied on $(M\text{-}nH\text{-}TH)^{n-}$ ion, a disappearance of all the fragments is detected. (noise peaks are identified by an asterisk)

Figure 5: DR-EDD spectra of dT_5 (a) without DR and (b) with DR on $(M\text{-}nH\text{-}TH)^{n-}$ ion (spectra on same scale). As showed in the spectrum coupled to DR, no DR effect was observed. (Noise peaks are identified by an asterisk)

Figure 6: EDD spectra of dT10 (a) without DR, (b) with DR on $(M\text{-}3H)^{2-\bullet}$ radical ion and (c) with DR on $(M\text{-}3H)^{-\bullet\bullet}$ radical ion. Spectra are displayed on the same scale. (Noise peaks are identified by an asterisk).

Figure 7: Normalized electron detachment efficiency as a function of the nature of the nucleobase. The standard deviation was calculated from 4 experiments.

Graphical Abstract

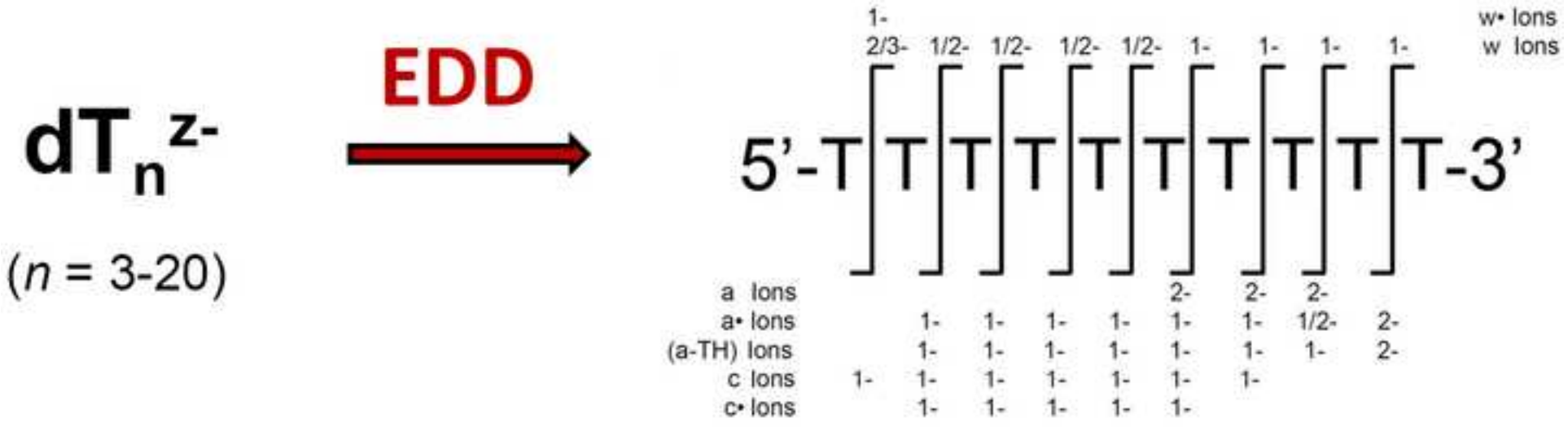
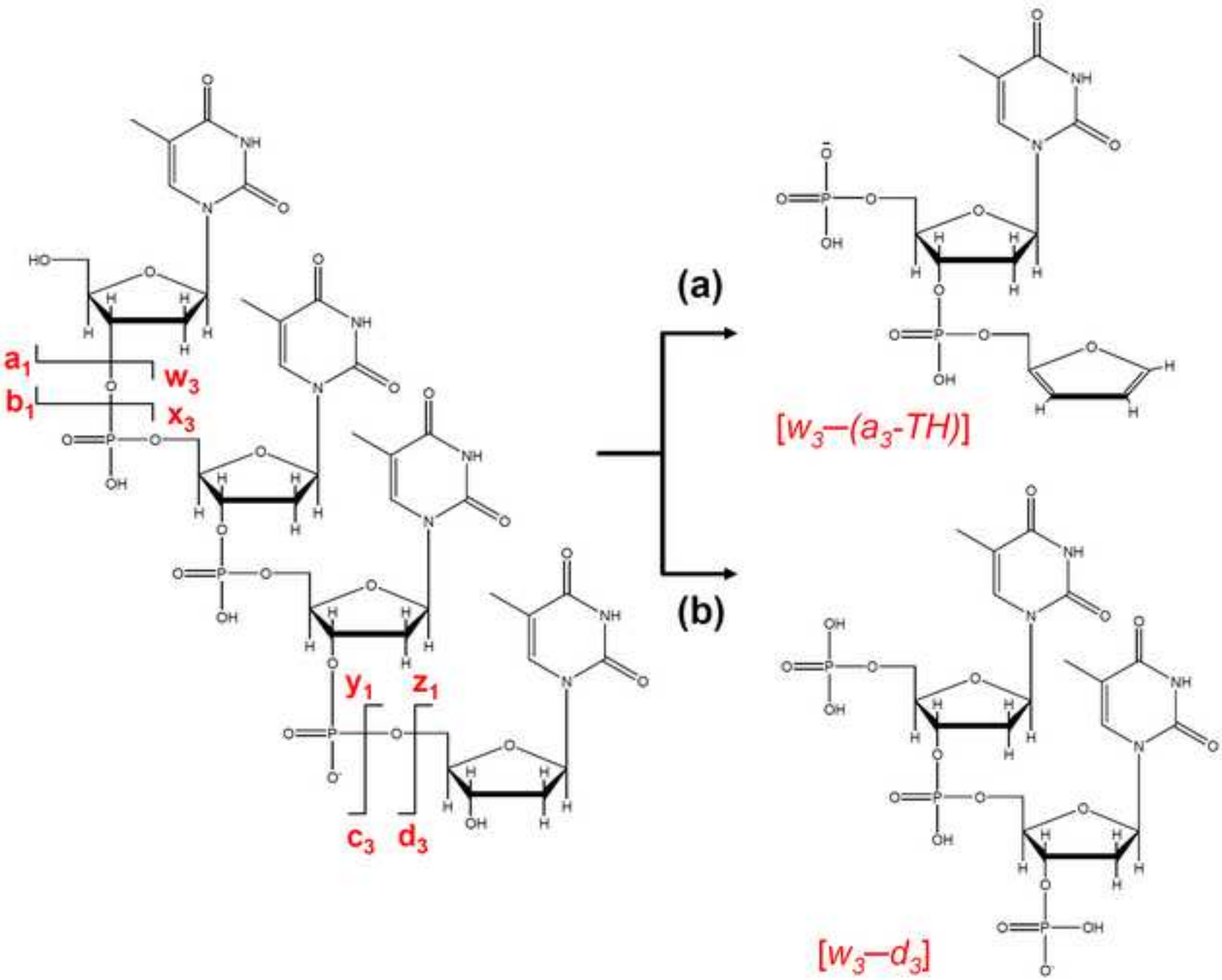
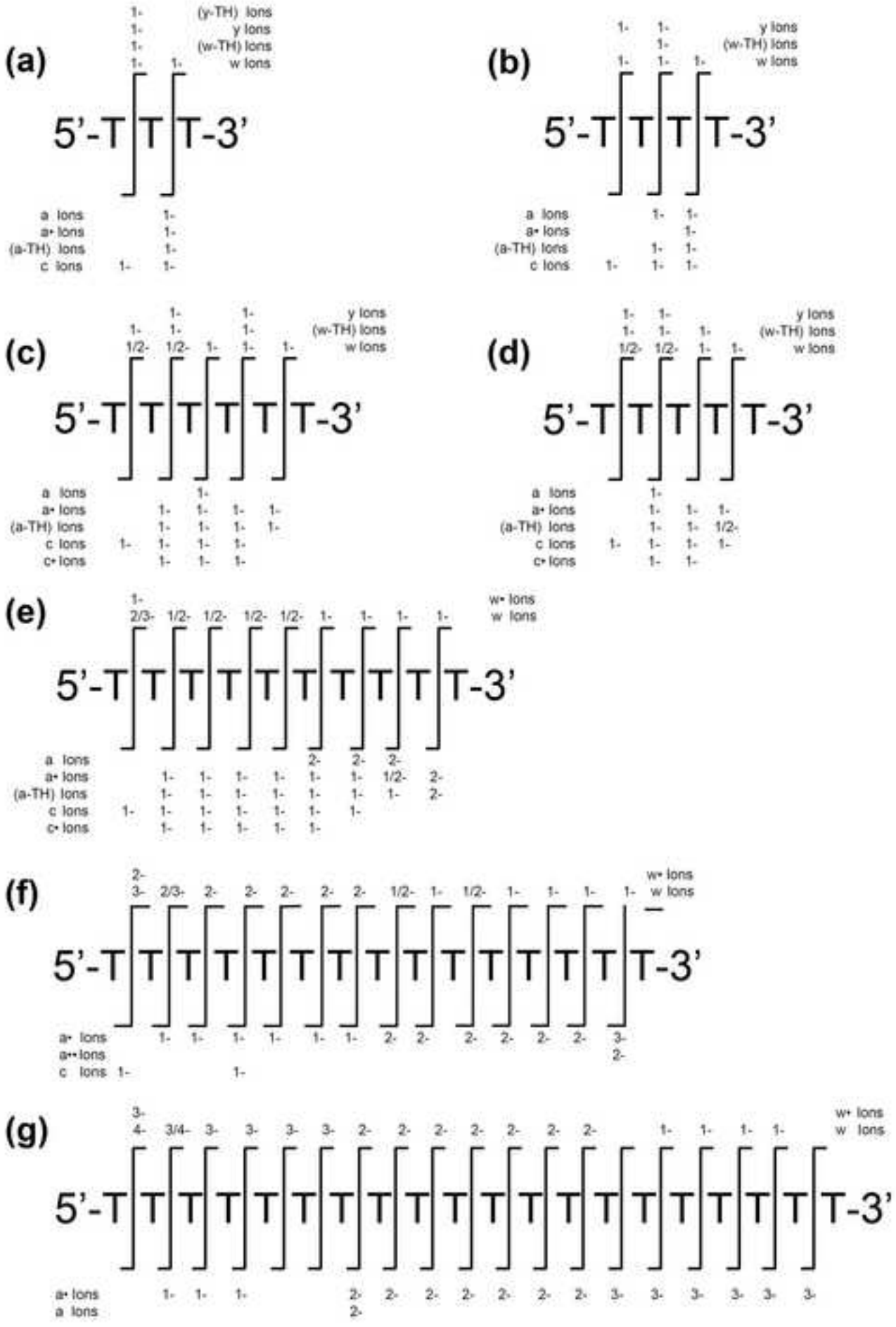
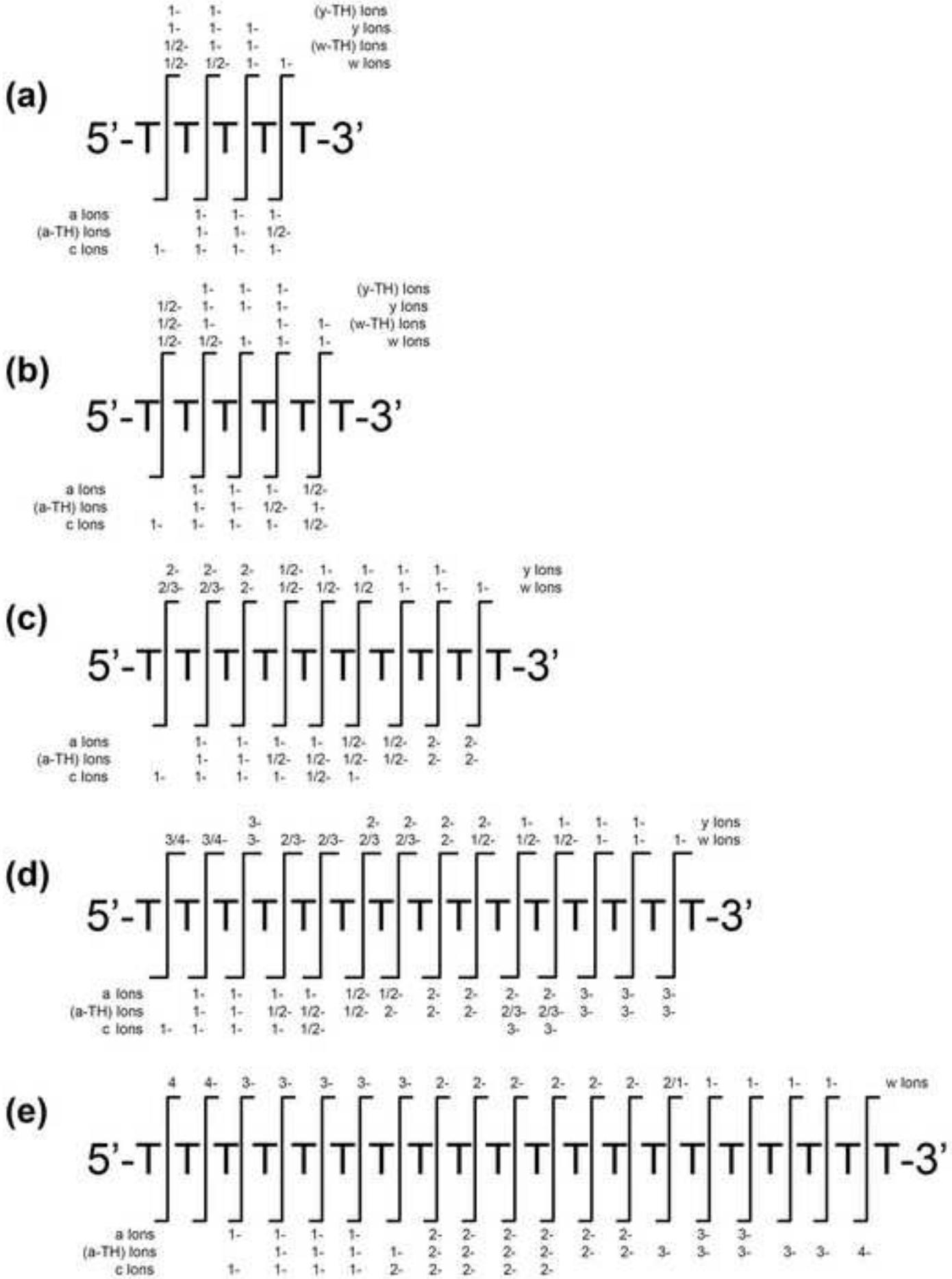
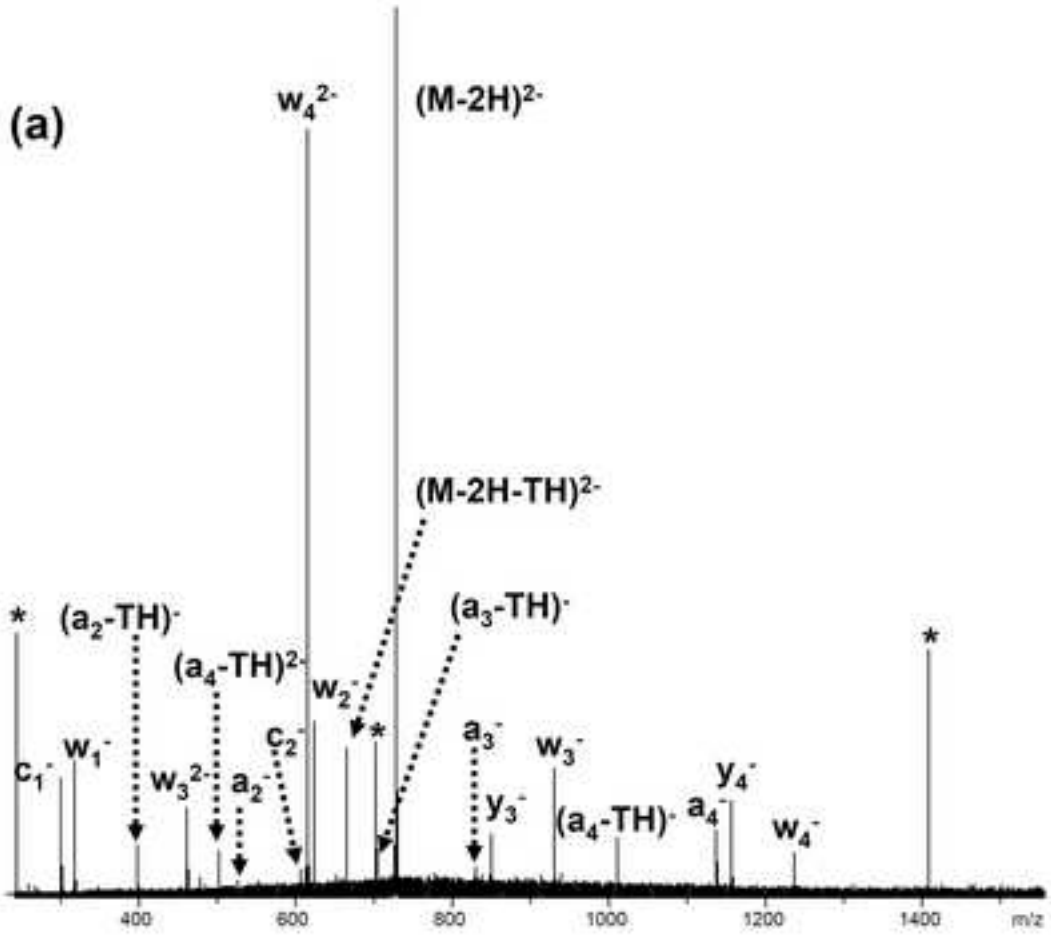


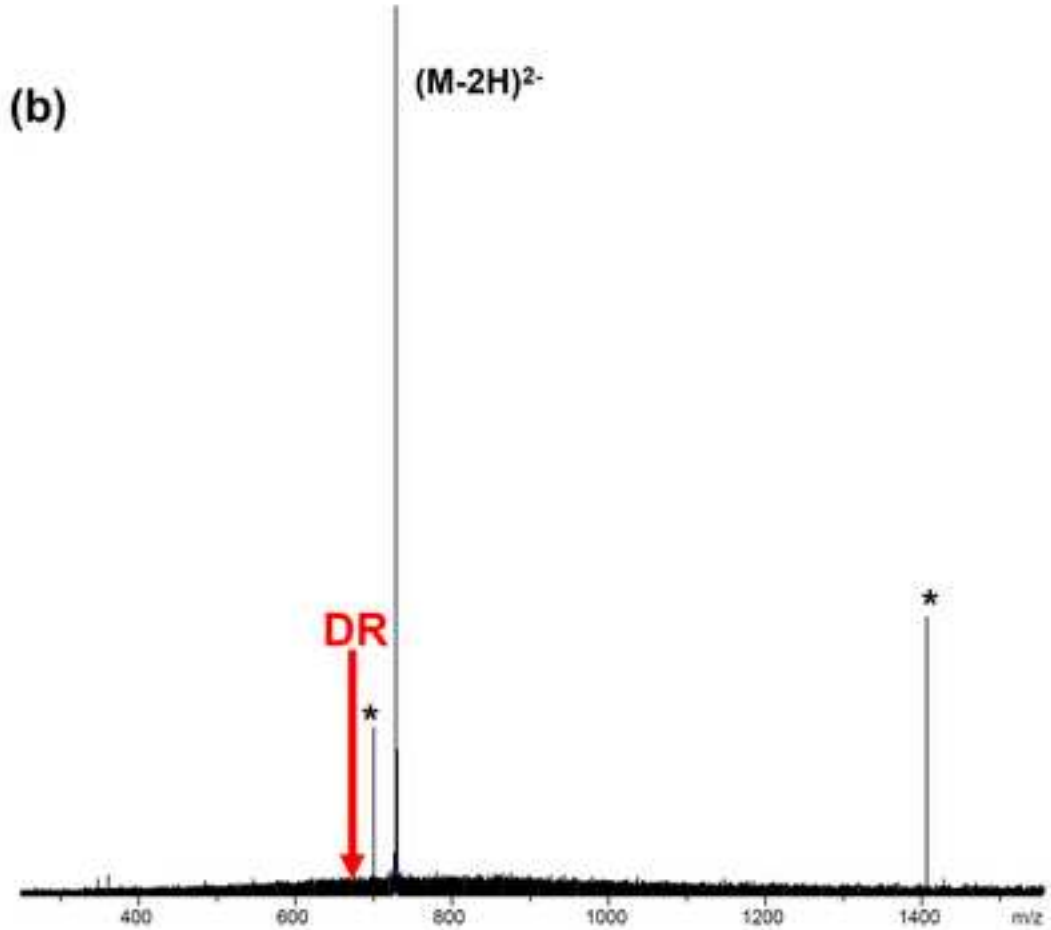
Figure 1

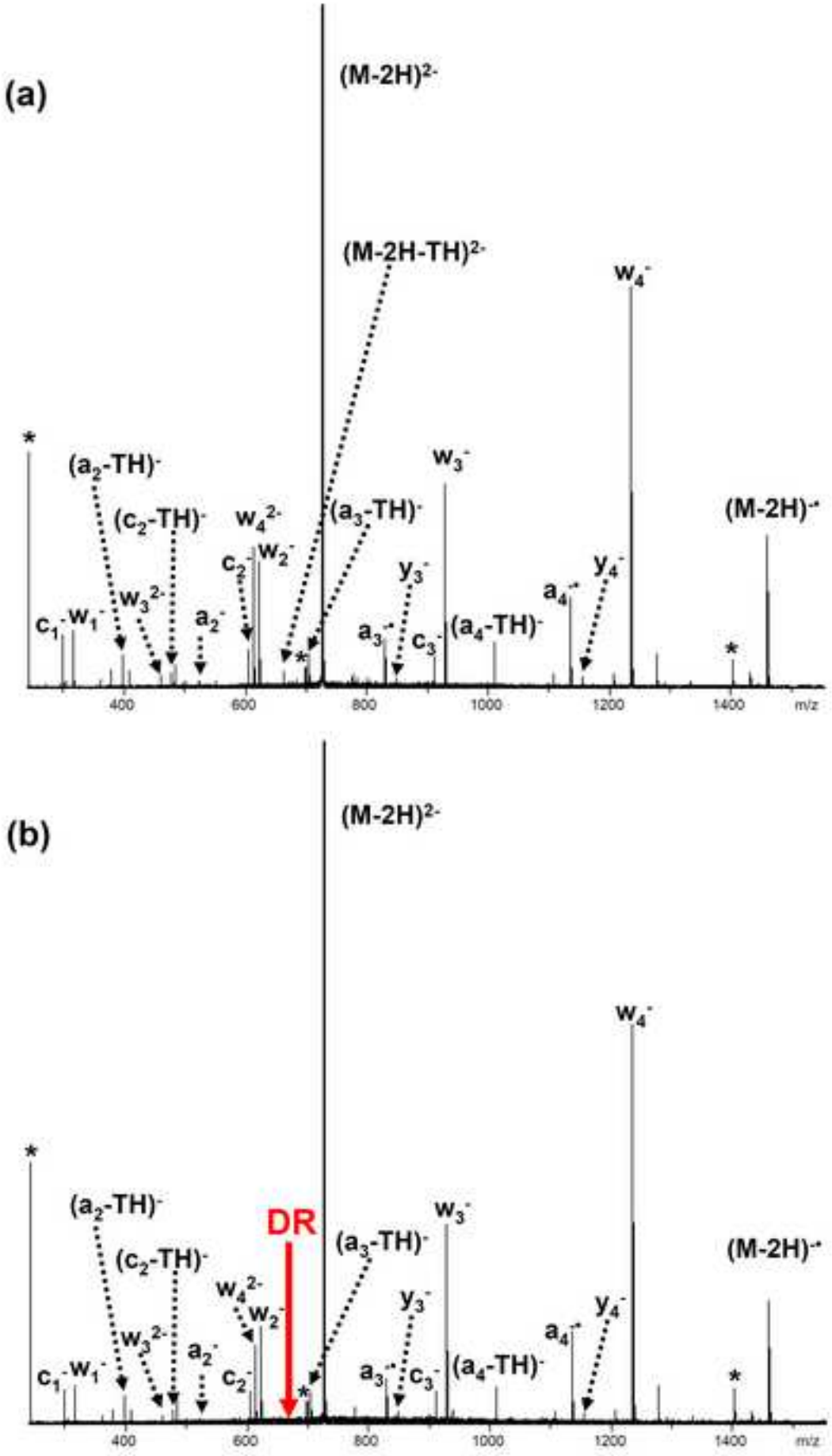












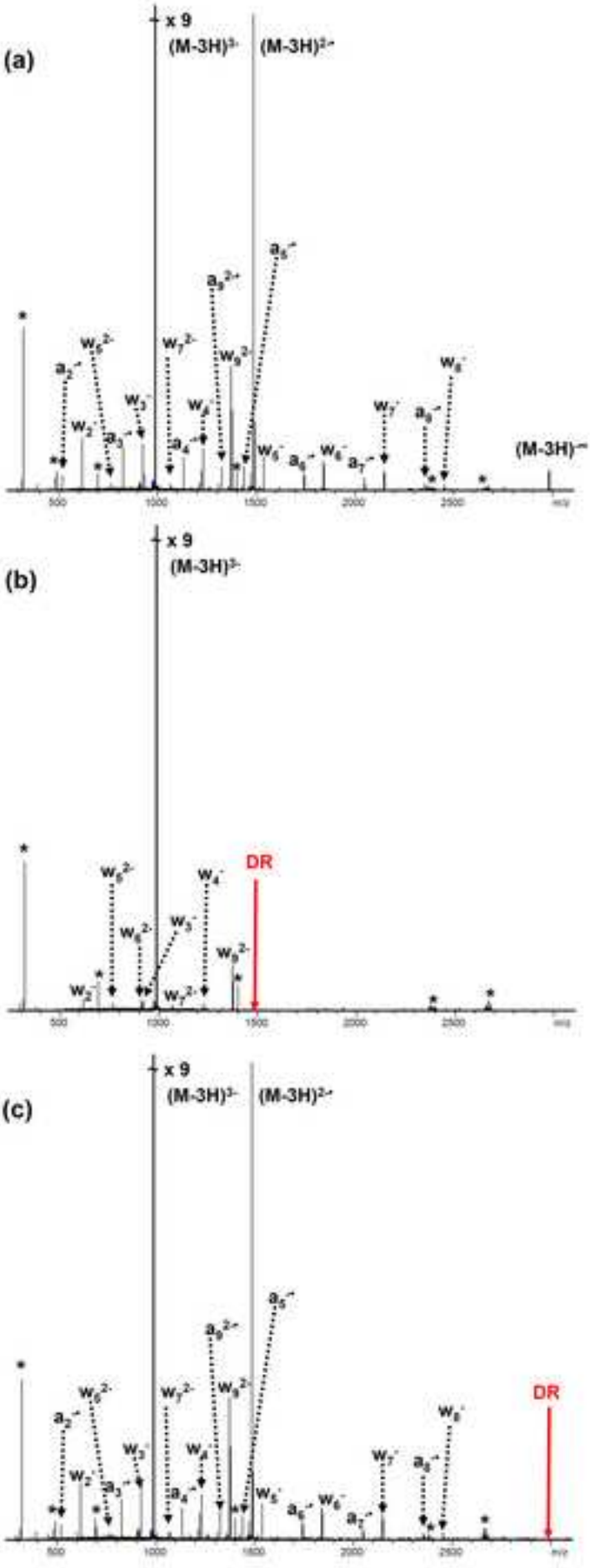
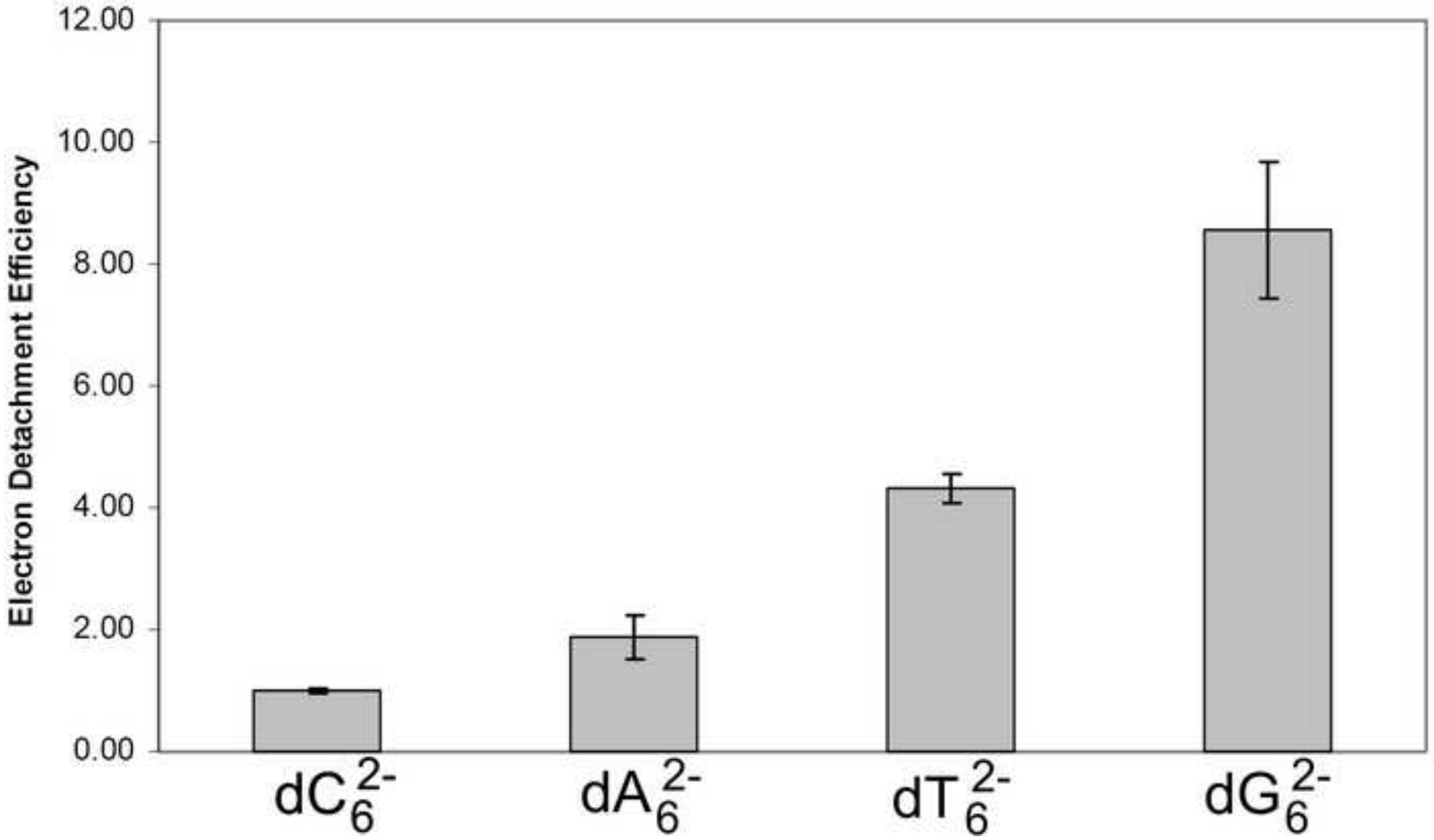


Figure 7


Electron Detachment Efficiency
12.00
10.00
8.00
6.00
4.00
2.00
0.00
dC6 2-
dA6 2-
dT6 2-
dG6 2-