

# Bond dissociation of the dipeptide dialanine and its derivative alanine anhydride induced by low energy electrons

## Journal Item

# Bond dissociation of the dipeptide dialanine and its derivative alanine anhydride induced by low energy electrons

Elahe Alizadeh,[1,2] David Gschliesser,[1] Peter Bartl,[1] Michaela Hager,[1] Achim Edtbauer,[1] Violaine Vizcaino,[1] Andreas Mauracher,[1] Michael Probst,[1] Tilmann D. Märk,[1] Sylwia Ptasińska,[2] Nigel J. Mason,[2] Stephan Denifl,[1,a)] and Paul Scheier[1]
[1]*Institut für Ionenphysik and Angewandte Physik, and Center of Molecular Biosciences Innsbruck, Universität Innsbruck, Technikerstraße 25, A-6020 Innsbruck, Austria*
[2]*Department of Physics and Astronomy, The Open University, Milton Keynes, MK7 6AA, United Kingdom*

(Received 21 September 2010; accepted 30 December 2010; published online 1 February 2011)

Dissociative electron attachment to dialanine and alanine anhydride has been studied in the gas phase utilizing a double focusing two sector field mass spectrometer. We show that low-energy electrons (i.e., electrons with kinetic energies from near zero up to 13 eV) attach to these molecules and subsequently dissociate to form a number of anionic fragments. Anion efficiency curves are recorded for the most abundant anions by measuring the ion yield as a function of the incident electron energy. The present experiments show that as for single amino acids (M), e.g., glycine, alanine, valine, and proline, the dehydrogenated closed shell anion $(M–H)^-$ is the most dominant reaction product. The interpretation of the experiments is aided by quantum chemical calculations based on density functional theory, by which the electrostatic potential and molecular orbitals are calculated and the initial electron attachment process prior to dissociation is investigated. © *2011 American Institute of Physics*. [doi:10.1063/1.3544217]

## I. INTRODUCTION

Amino acids are among the most important building blocks of living systems. They play a central role both as subunits of proteins and as intermediates in metabolic processes. When the carboxylic acid group of one amino acid reacts with the amine group of another amino acid, the resulting OC–NH bond is called a peptide bond (*amide bond*). Thereby a dipeptide is formed, and during this intermolecular condensation reaction a water molecule is released.[1] Peptides, which are defined as chains up to about 100 amino acids, have received considerable attention[2] because of their relative simplicity and their important structural role in proteins. Peptide bonds in proteins are the primary basis for the structure of a number of hormones, antibiotics, antitumor agents, and neurotransmitters, and consequently for the development and continuation of life. Two examples of important biodipeptides are carnosine ($\beta$-alanyl-L-histidine), which is present in high concentrations in muscle and brain tissues, and anserine ($\beta$-alanyl-*N*-methylhistidine) found in the skeletal muscle and brain of the mammals.

Amino acids are also now believed to be formed in interstellar space. The next generation of telescopes (e.g., the Atacama Large Millimeter Array (ALMA)) will be used to search for such compounds and explore their formation mechanisms. Then it may be possible to determine whether such compounds are a natural consequence of stellar synthesis and therefore a natural product of star formation. In the latter case they may be present in any solar system, where they may be used as the "building blocks of life."[3,4] Hence, the peptide bonds are always a subject of intense investigation not only in biology, but also in (astro)chemistry.

Since glycine and alanine are the two simplest amino acids, the peptide bonds involving these molecules and investigations of the properties for the corresponding peptides are widely studied both by theoreticians and experimentalists.[5–9] These systems also allow high-accuracy *ab initio* calculations and are often considered as model systems for the study of more complex structures. In addition, the amino acid alanine has attracted attention due to its radiation dosimetric properties and has been formally accepted as a secondary standard for high-dose and transfer dosimetry.[10–13] When two molecules of alanine join covalently through the formation of a peptide bond, L-alanine–L-alanine (shortly dialanine) is formed. Some of the dialanine derivatives have recently been developed as water-soluble photosensitizers with the potential for application in photodynamic therapy and treatment of malignant tissues.[14]

In recent years there have been several investigations of the ionization and fragmentation of small and medium-size peptides and proteins using soft ionization techniques such as matrix-assisted laser desorption ionization (MALDI),[15–18] electrospray ionization (ESI),[19,20] and collision induced dissociation (CID).[21–26] Moreover, amino acid clustering and especially the role of chiral discrimination in the formation of such clusters has been studied by mass spectrometry.[27–31] However, to date only a few studies of the interaction of low energy electrons with small peptides isolated in the gas phase have been carried out. Negative ion mass spectra determining the fragmentation pattern at two different electron energies (~1–2 eV and ~5–6 eV) have been reported for alanine

a)Author to whom correspondence should be addressed. Electronic mail: Stephan.Denifl@uibk.ac.at.

 

di- and polypeptides[32] and for small peptides with cysteine residues at about 1 eV. (Ref. 33). However, to our knowledge no measurements of anion efficiency curves for dialanine have been reported so far, where the anion yield was measured as a function of the electron energy. Such curves allow determining resonance energies for the capture process and studying the mechanism of electron attachment and subsequent dissociation. The present paper therefore reports the anion efficiency curves of fragment anions formed upon dissociative electron attachment (DEA) to dialanine ($C_6H_{12}N_2O_3$) in the gas phase. This work is an extension of previous DEA studies for amino acids,[34–38] which explored the fragmentation of small amino acids in the gas phase.

In the course of the present experiments, we found that the dialanine sample was contaminated with alanine anhydride. Alanine anhydride is a simple cyclopeptide and belongs to the class of diketopiperazines (for more details on the biological activity of this class of molecules, see Ref. 39); it can be formed, when peptides such as dialanine undergo a loss of $H_2O$ as a result of thermal heating.[40] Therefore, in order to differentiate those anions formed by DEA to dialanine from those formed upon DEA to alanine anhydride, we have also investigated DEA to the commercially available alanine anhydride ($C_6H_{10}N_2O_2$).

## II. EXPERIMENTAL SETUP

All the experimental data reported in this paper were obtained using a double focusing two sector field mass spectrometer (VG-ZAB2) of reversed Nier–Johnson type BE geometry. This apparatus has been described elsewhere,[41] so only brief details will be given here. An electron beam derived from a tungsten/rhenium filament is guided by a homogeneous magnetic field of about 20 mT into the interaction region, where it intersects the neutral molecular beam at an angle of 64°. To achieve a good signal-to-noise ratio the lowest electron current used was 10 $\mu$A (at an energy of 2 eV) resulting in an electron energy resolution of approximately 1 eV (FWHM).

The anions formed in the ion source are extracted from the ion source housing by a weak electric field produced by a repeller plate and then accelerated to 8 keV toward the mass spectrometer entrance slit. After passing the first field free region, the ions are analyzed according to their momentum by a magnetic sector field B. After passing a 1.4 m long field-free region, they are finally analyzed in an 81° electric sector and are detected by a channel electron multiplier (purchased from Dr. Sjuts Optotechnik GmbH) operated in single pulse counting mode. The nominal maximum mass resolution of the mass spectrometer is 125 000 (10% valley definition). However, in the present experiment the slits were widened to gain higher sensitivity, which resulted in a mass resolution $m/\Delta m$ of a few hundred. In order to separate isobaric anions formed upon DEA $m/\Delta m$ was about 4000.

The present study was carried out using one of the two methodologies. In the first high resolution negative ion mass spectra were recorded at fixed electron energies in order to determine the absolute mass of anions (the calibration of the mass scale was done with known anions, for example, from $SF_6$ and $H_2O$) and in the second the mass spectrometer was preset to a certain mass and the corresponding ion yield was recorded as a function of the electron energy (in the range about 0–13 eV). The electron energy scale was calibrated using the well known electron attachment reactions to $SF_6$:

$$e^- + SF_6 \leftrightarrow (SF_6)^{*-}, \quad (1)$$

$$e^- + SF_6 \leftrightarrow (SF_6)^{*-} \rightarrow SF_5 + F^-. \quad (2)$$

The first process exhibits a narrow *s*-wave resonance at 0 eV and the second reaction ($F^-/SF_6$) has resonances at higher energies (5.5, 9, and 11.5 eV) (Ref. 42).

The dialanine sample was purchased from Sigma–Aldrich with a stated purity of 99% and was used without further purification. Dialanine is a powder under standard conditions (room temperature) and therefore had to be heated up to about 120°C in a home-built stainless steel oven in order to generate an effusive molecular beam of sufficient intensity. Although this temperature is well below the melting point, we observe thermal decomposition products of the molecules. In the course of the present experiments with the commercial dialanine powder we have noticed that the ratio of ion signals at certain masses are strongly temperature and time dependent. Such an effect may arise from two different processes, (i) contamination of the dialanine sample with substances that possess a different vapor pressure and (ii) thermal decomposition induced by heating in the oven. In a previous study on electron ionization mass spectra of dipeptides it was supposed that the recorded positive ion mass spectra were a superposition of signals from dipeptides and cyclopeptides formed by the heating process.[43] The cyclization process of dialanine (the parent cation can be found at *m/z* 160) led to an abundant peak at *m/z* 142 (parent mass of alanine anhydride) by the loss of $H_2O$.[43] In the present experiment we also observe indication of the presence of the cyclopetide alanine anhydride in the sample because the ratio between the ion signal at *m/z* 141 (which would correspond to the mass of the dehydrogenated parent anion of alanine anhydride) and the $(M–H)^-$ signal of dialanine (*m/z* 159) turned out to be strongly temperature dependent. When heating up a fresh sample, much higher ion yield can be observed for $(M–H)^-$ from alanine anhydride than from dialanine. After some days of heating at about 120 °C the $(M–H)^-$ signal of alanine anhydride decreased compared to dialanine and reached a rather low (stable) value. Under these conditions we have measured the anion efficiency curves of the most abundant anions of dialanine, which are shown in Figs. 2 and 3. When we raise the temperatures above 130 °C, we observe again an increase in the signal ascribed to alanine anhydride and, moreover, in the electron ionization mass spectra we find evidence of ion signal arising from the protonated parent ion. This indicates a transformation within the alanine anhydride sample into polymers and further thermal decomposition.

Thus in our opinion alanine anhydride was already present as impurity even in a fresh dialanine sample, but it is also formed as a thermal decomposition product by heating. However, by performing complementary DEA measurements with alanine anhydride using the same experimental arrangement (see the corresponding anion efficiency curves in

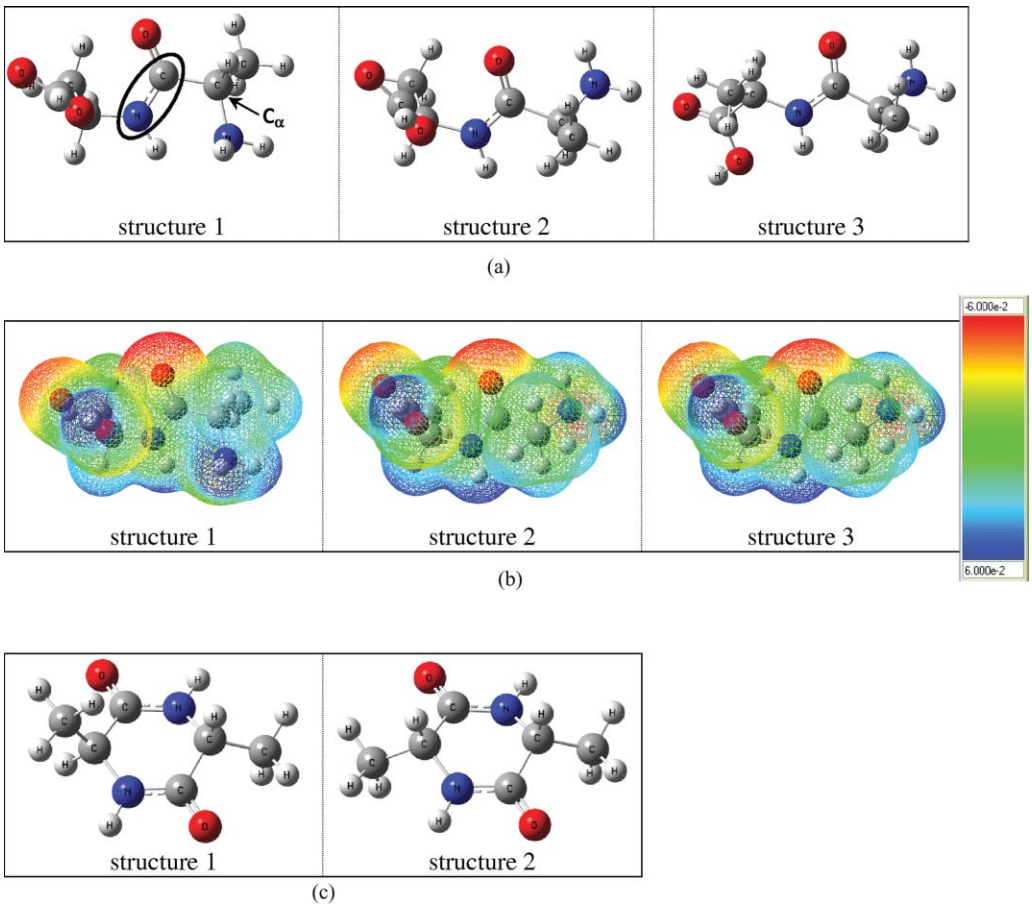


FIG. 1. (a) Optimized structures for dialanine obtained at B3LYP/6–311++G(d,p). In structure 1 the peptide bond and the $C_\alpha$ atom are indicated; (b) electrostatic potentials mapped on the isosurfaces of the total electron densities; (c) optimized structures for alanine anhydride obtained at B3LYP/6–311++G(d,p).

Figs. 4 and 5), we can identify those anions formed by DEA to alanine anhydride. For the commercial alanine anhydride sample an oven temperature of about 70°C is high enough to obtain a reasonable ion signal in our experiment since this compound has a substantial higher vapor pressure than the dipeptide.

## III. QUANTUM CHEMICAL CALCULATIONS

To both complement and help interpret our experimental results we also performed density functional theory (DFT) calculations with the B3LYP hybrid functional[44,45] and the basis set 6–311++G(*d*,*p*) (Ref. 46) using the GAUSSIAN 03 program package.[47] We searched the potential energy surface of the neutral dialanine and alanine anhydride systems for local energy minima. Three fully optimized structures for dialanine and two for alanine anhydride were found and are shown in Figs. 1(a) and 1(c), respectively. The lowest total energy for the dipeptide can be assigned to structure 1, whereas structure 2 is at 0.15 eV and structure 3 is at 0.07 eV and are thus less stable. However, these energy differences derived are within the uncertainty of the method and basis set used, which is approximately 0.25 eV. Therefore, all three structures might occur with the same probability. A similar situation is obtained for alanine anhydride. Structure 2 [see Fig. 1(c)] is only slightly more stable than structure 1 (by 0.01 eV) and thus both structures may be formed with same probability. We have also determined the possibility of stable zwitterion structures for dialanine. However, no stable structure could be found since the proton added to the amino group migrates back to carboxyl group. Moreover, we calculated the peptide binding energy by means of the G3(MP2) method.[48] This is an extrapolation method that uses the results from several quantum chemical calculations in order to extrapolate towards molecular energies that would be obtained if complete inclusion of correlation energy and an unlimited basis set was possible. In general, the accuracy of G3(MP2) energies is of the order of about ±0.15 eV (Ref. 48).

## IV. RESULTS AND DISCUSSION

### A. Fragment anions formed from DEA to dialanine

In our study we did not observe any signal of a stable parent anion at *m*/*z* 160. The absence of a parent molecular anion was confirmed by measuring the anion efficiency curves at *m*/*z* 160 and 161 (not shown), which show the same shape as the dehydrogenated parent anion. Thus the anion yields obtained at *m*/*z* 160 and 161 originate exclusively from dehydrogenated dialanine molecules containing the isotopes $^{13}C$, $^{17}O$, $^{18}O$, or $^{15}N$. The relative abundances of these three anions at *m*/*z* 159, 160, and 161 are 100:7.5:0.9 and are in excellent agreement with the calculated isotopic pattern for dehy-

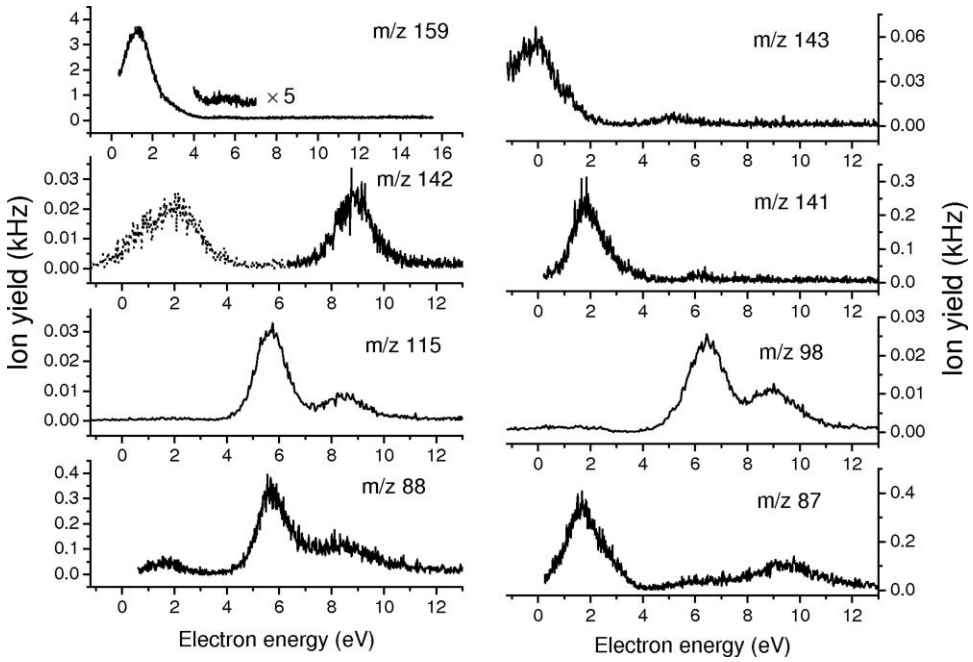


FIG. 2. Anion efficiency curves as a function of the incident electron energy for fragments between *m/z* 87 and *m/z* 159 formed via DEA to dialanine.

drogenated dialanine. The absence of parent anions is in line with all other small amino acids studied previously.[34–38]

In general, a negative ion is formed either due to a dipole bound state or due to electron attachment to one of the valence orbitals. The present calculations show that the adiabatic electron affinity (AEA) is negative for all three structures; the dipole moments are calculated to be 5.01 D, 4.47 D, and 4.17 D for structures 1–3. These dipole moments are high enough to allow formation of a dipole bound anion. To determine the favorable sites of electron attachment we plot for all three structures the electrostatic potential mapped on an isosurface of the total electron density [see Fig. 1(b)]. The isovalue of the electron density was 0.004 $e^2/au^3$. In addition, we also visualize the highest occupied molecular orbitals (HOMO, MO 43) and lowest unoccupied molecular orbitals (LUMO, MO 44), see Fig. 6.

One of the most abundant ions from DEA to dialanine is observed at *m/z* 159 (see Fig. 2). It can be assigned to the anion $(C_6H_{11}N_2O_3)^-$ or $(M–H)^-$ that is formed via loss of one hydrogen atom. The formation of this anion exhibits a maximum cross section for electrons with energies of about 1.2 eV (see also Table I). In many previous studies of DEA to amino acids (e.g., Ref. 34 and 35) the formation of $(M–H)^-$ at around 1 eV incident energy was assigned to electron attachment into the $\pi^*$ (C=O) orbital, which is coupled to the dissociative $\sigma^*$ (O–H) orbital of the carboxyl

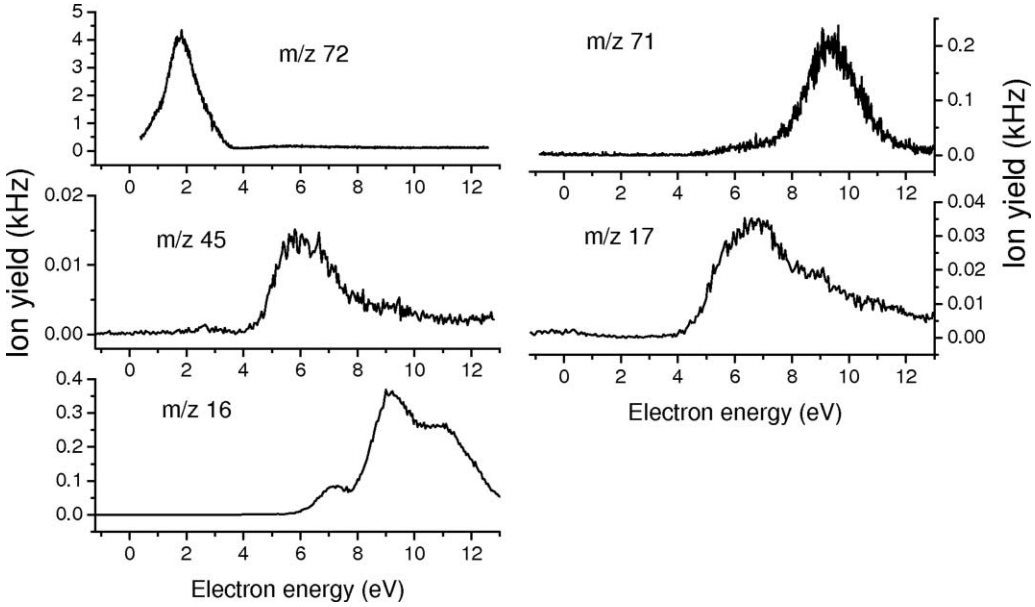


FIG. 3. Anion efficiency curves as a function of the incident electron energy for fragments between *m/z* 16 and *m/z* 72 formed via DEA to dialanine.

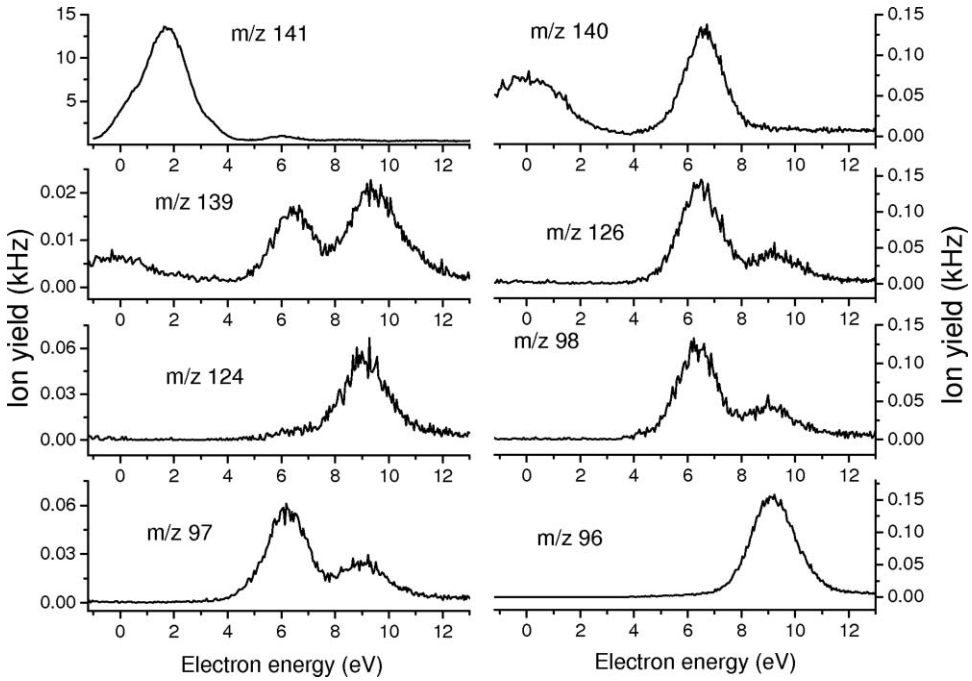


FIG. 4. Anion efficiency curves as a function of the incident electron energy for fragments between *m/z* 96 and *m/z* 141 formed via DEA to alanine anhydride.

group. However, the attachment energy for the $\pi^*$ orbital considered lies about 0.8 eV above the measured DEA peak positions and recently it was suggested that the excess electron goes directly into the $\sigma^*$ (O–H) orbital.[49] Although the latter is high in energy (5.3 eV), the resonance is very broad (5.8 eV) and Scheer *et al.*[50] therefore suggested that the resonance may contribute to DEA leading to $(M–H)^-$ already at low electron energies.

An alternative pathway arises from the decay of vibrational Feshbach resonances formed by dipole bound anion states[51,52] and thus the mechanism, by which $(M–H)^-$ anions are formed in DEA of amino acids, is still not resolved. However, to shed some more light on the case of dipeptides we may once again consider the electrostatic potential mapped on an isosurface of the total electron density shown in Fig. 1(b), where negative regions are colored red while positive regions are colored blue. Electrons will be attracted mainly around hydrogen atoms connected to the carboxyl group, amide group, and amino group. The strongest attractive potential may be expected to be near the carboxyl group. This is in agreement with the distribution of the LUMOs in Fig. 6(b) and with previous experimental data for amino acids, where the dehydrogenation is starting at the carboxylic group $COO^-$. However, the exact site of hydrogen loss cannot be confirmed by the present experiment and thus we cannot make a final assignment.

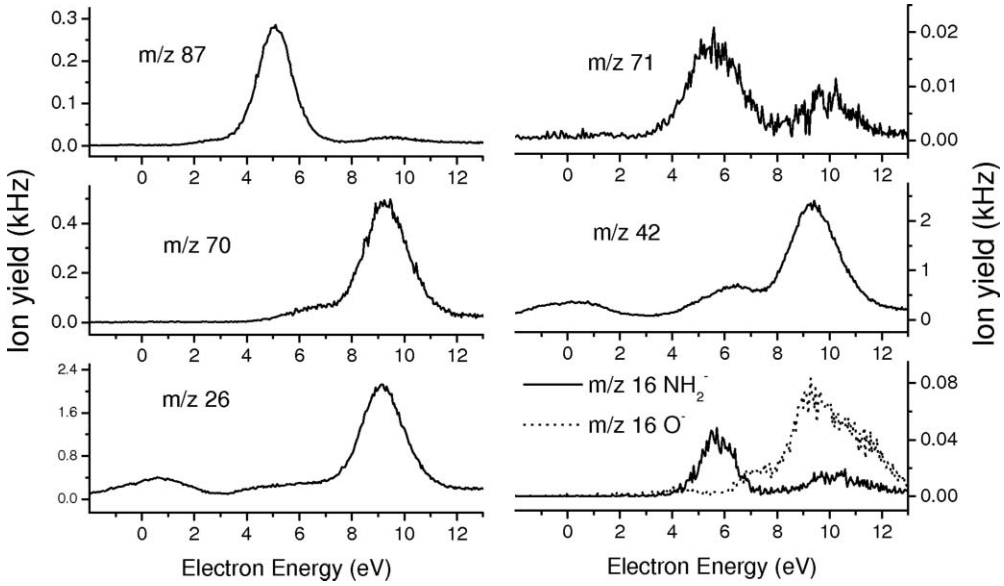


FIG. 5. Anion efficiency curves as a function of the incident electron energy for fragments between *m/z* 16 and *m/z* 87 formed via DEA to alanine anhydride.

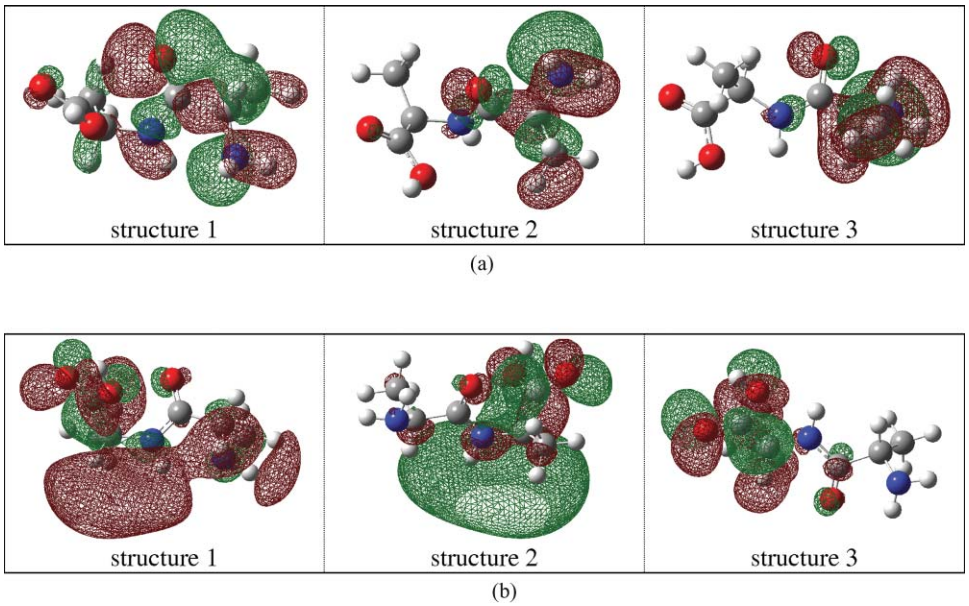


FIG. 6. (a) The highest occupied molecular orbitals (HOMO, MO 43); and (b) the lowest unoccupied molecular orbitals (LUMO, MO 44) for all three structures of dialanine.

In addition to the formation of the dehydrogenated dialanine anion, 12 other product anions were observed (within the present sensitivity of the instrument) as the result of DEA to gas phase dialanine. The possible chemical compositions of these fragments and the positions of their resonances are listed in Table I, and Figs. 2 and 3 show the corresponding anion efficiency curves.

As discussed above, the fragment ion at *m/z* 141 (see Fig. 2) may be formed via loss of a neutral water molecule ($H_2O$) from the dehydrogenated molecule upon DEA to dialanine. However, the ion yield at low electron energies (peaking at 1.85 eV) may be ascribed to the dehydrogentated parent anion $(M–H)^-$ of alanine anhydride rather than $(M–H–H_2O)^-$ formed upon DEA to dialanine, while the second weak resonance for the formation of this fragment observed at 6.2 eV may be energetically accessible.[32] We note that a comparison of $(M–H)^-$ formed from pure alanine anhydride (see Fig. 4) also shows a resonance at 6.2 eV, which thus also contributes to the present ion yield for dialanine.

TABLE I. Mass, chemical composition, and peak positions for all observed anions formed upon DEA to dialanine.

| *m/z* | Fragment anion formed upon DEA to dialanine | Resonance position (eV)[a] |
|---|---|---|
| 159 | $C_6H_{11}N_2O_3^-/(M–H)^-$ | 1.3, 5.5 |
| 143 | $(M–NH_3)^-/(M–OH)^-$ | (∼0), 5.1 |
| 142 | $(M–H_2O)^-$ | 8.9 |
| 141 | $(M–H–H_2O)^-$ | (1.9), 6.2 |
| 115 | $(M–COOH)^-$ | 1.9, 5.3, 8.4 |
| 98 | $C_5H_8NO^-$ | 6.5, 8.9 |
| 88 | $C_3H_6NO_2^-$ | 1.7, 5.5, 8.4 |
| 87 | $C_3H_7N_2O^-$ | 1.7, 6.1, 9.5 |
| 72 | $C_3H_4O_2^-$ | 2.0, 5.8 |
| 71 | $C_3H_5NO^-$ | 6.4, 9.4 |
| 45 | $HCOO^-$ | 2.5, 6.0, 9.3 |
| 17 | $OH^-$ | (∼0), 6.8, 9.1, 11.0 |
| 16 | $O^-$ | 6.8, 9.0, 10.8 |

[a]The values have been obtained by Gaussian-fits. Values in brackets are likely due to contaminations or thermal activation.

The anion yield at *m/z* 142 has a low energy resonance, which we ascribe to the isotope of $(M–H–H_2O)^-$ (shown as dotted line in Fig. 2), while the resonance at 9.8 eV can be ascribed to $(M–H_2O)^-$. We note that in a recent study[53] the site of dissociation for the anions from $(M–16)^-$ up to $(M–19)^-$ was determined for small amino acids by high mass-resolution experiments and it was concluded that $(M–18)^-$ ions are due to the loss of the $NH_2$ group and additional two hydrogen molecules. However, for peptides of alanine the loss of water was supposed to be more likely,[32] which we can confirm here by the present results.

In contrast to $(M–H_2O)^-$, *m/z* 142, the ion yield for *m/z* 143 has an abundant low energy resonance and only a weak resonance at about 5.1 eV. For single amino acids the latter was assigned to $(M–OH)^-$ and the first resonance (at about 1.8 eV) to $(M–NH_3)^-$;[53] moreover, anion yield at *m/z* 143 observed in the negative ion mass spectrum of alanine containing peptides at about 1–2 eV was ascribed exclusively to $(M–NH_3)^-$.[32] The present high resolution mass scans at the two resonance energies indeed show that also for dialanine the low energy resonance can be ascribed to $(M–NH_3)^-$ and the resonance at 5.1 eV to $(M–OH)^-$.

No signature for $(M–O)^-/(M–NH_2)^-$ was found in the present experiments but as reported in earlier work[32] another heavy-mass fragment anion formed upon free electron attachment to dialanine is found at *m/z* 115. The corresponding anion efficiency curve is shown in Fig. 2. This mass corresponds to loss of a neutral COOH. Three important anionic fragments are observed at *m/z* 72, 87, and 88 (see Figs. 2 and 3). The latter anion is formed by cleavage of the peptide bond while observations of anions at *m/z* 72 and 87 are indicative of the N–$C_\alpha$ bond being broken.[32] Sobcyk *et al.*[54] calculated the

vertical attachment energies for electron capture into various $\sigma^*$ and $\pi^*$ orbitals of the dialanine molecule and predicted an indirect mechanism for the N–$C_\alpha$ cleavage (formation of the central carbonyl C=O $\pi^*$ anion and electronic coupling to the dissociative $\sigma^*$ N–$C_\alpha$ bond), which makes the reaction channel accessible for electrons with energies already close to 2.5 eV. Indeed we can observe here the main resonances for the corresponding anions *m/z* 72 and *m/z* 87 at about 2 eV and 1.7 eV. Considering the expected overestimation[54] of the energies calculated by Sobcyk *et al.* using unrestricted Hartree–Fock level (and the lack of correlation there), the agreement between theory and experiment is good. In contrast, they predict a direct electron capture into any of the $\sigma^*$ orbitals only with an attachment energy of more than 6 eV. For example, this seems to be the case for the formation of the anion at *m/z* 71 (see Fig. 3), which has a weak resonance at 6.4 eV and a much stronger one at 9.4 eV. The resonance position of 5.5 eV observed for the anion at *m/z* 88 (cleavage of the peptide bond[32]) also indicates initial electron attachment to the corresponding $\sigma^*$ orbital (C–N bond).[54] As in Ref. 32 we observe a weak low energy contribution at about 1.7 eV, which was ascribed in Ref. 32 to an impurity by alanine monomers in the sample. This is further supported by the present *ab initio* calculations utilizing the G3(MP2) method, which predict a peptide bond dissociation energy of about 4.4 eV for dialanine. One can expect that the electron affinity of the fragment formed in the peptide bond dissociation (which corresponds to alanine minus a hydrogen from the amino group) is substantially lower and thus the energetic threshold is above this first resonance.

In DEA to dialanine we observed only a few fragment anions with masses below *m/z* 70, which cannot be ascribed to contaminations from alanine anhydride. For example, abundant anions at *m/z* 42 and *m/z* 26 are formed rather from alanine anhydride than from dialanine. This is in agreement with previous electron capture induced dissociation experiments with protonated dialanine,[55] where the $CN^-$ and $OCN^-$ anion yield resulting from two electron transfer collisions was exceedingly small. However, an anion formed upon DEA to dialanine can be found at *m/z* 45, i.e., likely $HCOO^-$, which is by about 1.6 eV more stable than $COOH^-$.[56] The corresponding anion yield (see Fig. 3) differs to that for alanine monomers and thus is not due a contamination. Further light anions are formed at *m/z* 16 and *m/z* 17. By calibrating the mass scale with $O^-$ and $OH^-$ peaks from $H_2O$ introduced into the ion source, we can ascribe the anions at *m/z* 16 and *m/z* 17 for dialanine to $O^-$ and $OH^-$, respectively. They are formed only in high energy resonances at around 7 eV, 9 eV, and 11 eV.

### B. Fragment anions formed from DEA to alanine anhydride

In the case of DEA to the cyclopeptide alanine anhydride the mass spectra also show a strong abundance of the dehydrogenated parent anion $(M–H)^-$. This anion is formed most efficiently through resonances at about 1.9 eV and more weakly at 6.2 and 8.2 eV (see Fig. 4). All the other detected fragment anions were formed with intensities at least a factor 6 lower at their resonance maxima of 6.3 eV and 9.2 eV (see Figs. 4 and 5 and Table II), which lie above the threshold for electronic excitation. We have also calculated the dipole moments for the structures shown in Fig. 1(c). They are 1.11 D and 1.19 D for structure 1 and 2, respectively. We note that for this molecule therefore no anion formation via dipole states is possible since these dipole moments are not high enough to allow formation of a dipole bound anion.

TABLE II. Mass, chemical composition, and peak positions for all observed anions formed upon DEA to alanine anhydride.

| *m/z* | Fragment anion formed upon DEA to alanine anhydride | Resonance position (eV)[a] |
|---|---|---|
| 141 | $C_6H_9N_2O_2^-$/$[M–H]^-$ | 1.9, 6.2, 8.2 |
| 140 | $[M–2H]^-$ | (∼0), 6.6 |
| 139 | $[M–3H]^-$ | (∼0), 6.5, 9.4 |
| 126 | $[M–CH_3–H]^-$ | 6.5, 9.2 |
| 124 | $[M–OH–H]^-$ | 6.3, 9.1 |
| 98 | [M–OCNH] | 6.3, 9.1 |
| 97 | [M–OCNH-H] | 6.2, 9.1 |
| 96 | $[M–OCNH-H-H]^-$ | 9.2 |
| 87 | $C_3H_7N_2O^-$ | 2.9, 5.1, 9.5 |
| 71 | $C_3H_5NO^-$/$[M/2]^-$ | 5.6, 9.9 |
| 70 | $C_3H_4NO^-$/$[M/2-H]^-$ | 6.3, 9.3 |
| 42 | $OCN^-$ | ∼0, 6.4, 9.4 |
| 26 | $CN^-$ | ∼0, 6.4, 9.2 |
| 16 | $O^-$/$NH_2^-$ | 4.4, 7.1, 9.2, 10.3/5.7,10.2 |

[a]The values have been obtained by Gaussian-fits. Values in brackets are likely due to contaminations or thermal activation.

Anion yields close to zero eV can be found for *m/z* 140 and *m/z* 139, which would nominally correspond to $(M–2H)^-$ and $(M–3H)^-$. One may speculate that $H_2$ formation may lower the threshold energy of the anion formation. However, no peaks corresponding to the H+H channel—which should be 4.5 eV higher (the bond strength of two hydrogen atoms[57])—were found and thus we ascribe those peaks rather to thermal activation of the sample in spite of the low vaporization temperature used. Interestingly, anions at *m/z* 42 and *m/z* 26 are also formed at very low electron energies. Generally both anions show a very similar shape in their anion efficiency curves (see Fig. 5) consisting of three peaks. For the anion at *m/z* 42 the maxima are located at about 0 eV, 6.3 eV, and 9.4 eV. The anions $C_2H_2O^-$ or $OCN^-$ would be possible fragments at this mass. We determined the chemical composition by recording negative ion mass spectra at the resonance energies and comparing the ratio between the ion signal of *m/z* 43 and *m/z* 42 with the calculated isotopic ratio. The latter is 1.49% for $OCN^-$ and 2.2% for $C_2H_2O^-$. The ratio of the ion yields calculated from the experimental data is 1.6% and thus lies only slightly above the ratio for $OCN^-$. Thus we ascribe the anion at *m/z* 42 to the cyanate $OCN^-$ with an additional very small contribution of $HNCO^-$ at *m/z* 43.

The anion efficiency curve of the fragment at *m/z* 26 may arise from either $CN^-$ or $C_2H_2^-$. The resonances are located at about 0 eV, 6.4 eV, and 9.2 eV. For $CN^-$ the relative abundance of the isotope at *m/z* 27 is 1.5% and for $C_2H_2^-$ is 2.2%. From the recorded mass spectra we deduce that the ratio of the ion yield of *m/z* 27 and 26 is about 1.5%. This matches

well with $CN^-$ and its first heavy isotope that consists of both $^{13}C^{14}N^-$ and $^{12}C^{15}N^-$. Thus we conclude that DEA to alanine anhydride predominantly leads to the formation of the cyanide anion $CN^-$.

Finally the question remains why we observe both the cyanate and cyanide anions at very low electron energies close to 0 eV. Both can be formed only by multiple cleavages of (ring) bonds, which will largely exceed the (although appreciable) high electron affinity of both compounds (about 3.8 eV (Ref. 58), i.e., exceeding even that of halogen atoms). As proposed in Ref. 56 for the amino acid valine we therefore suppose additional intra-molecular reactions after electron capture with formation of new molecules. Such reactions were also recently supposed to be operative in DEA to acetamide and other amide derivatives.[59] We also note that a further indication for rearrangement processes is the formation of the fragment anion at *m/z* 87 ($C_3H_7N_2O^-$) and *m/z* 16 ($NH_2^-$), which cannot be formed by simple bond cleavages. At the latter mass we were also able to determine the anion efficiency curve of the isobaric anion $O^-$, which is preferentially formed as in the case for dialanine only above 6 eV (see Fig. 5).

## V. CONCLUSIONS

The present work shows that low energy electron attachment is an effective fragmentation process for both the alanine dipeptide and its cyclic derivative, alanine anhydride. Anion efficiency curves for overall 28 negatively charged fragments have been measured for both samples over an extended electron energy range (from ~0 to 13 eV) with an energy resolution of ~1 eV. In both DEA experiments the abundant formation of the dehydrogenated parent anion can be observed below 2 eV like for other amino acids, i.e., for example, glycine, alanine, valine, and proline. The present calculations of the dipole moments indicate that $(M–H)^-$ may be formed via dipole bound states in the case of dialanine while for alanine anhydride the dipole moment is subcritical. For the DEA measurements with dialanine we observe good agreement between the measured resonance positions and previously predicted vertical attachment energies leading to anions formed by cleavage of the N–$C_\alpha$ as well the peptide bond.

## ACKNOWLEDGMENTS

This work was supported by the Fonds zur Förderung der wissenschaftlichen Forschung (FWF, P22665 and P19073), Wien, and Engineering and Physics Sciences Research Council EPSRC, UK. S.D. gratefully acknowledges an APART grant from the Austrian Academy of Sciences. E.A. gratefully acknowledges financial support of her stay at the Open University, Milton Keynes, UK, during a STSM supported by the COST CM0601 ECCL action (reference number CM0601-05307). Support by the RFBR-FWF (project 09-03-91001-a) and by the Austrian Ministry of Science (Infrastructure grant to the LFU scientific computing platform) is also acknowledged.

[1] A. R. Goldfarb, L. J. Saidel, E. Mosovich, J. Biol. Chem. **193**, 397 (1951).
[2] T. G. Oas and P. S. Kim, Nature (London) **336**, 42 (1988).
[3] P. Chaudhuri and S. Canuto, J. Mol. Struct.: THEOCHEM **577**, 267 (2002).
[4] A. G. Császár and A. Perczel, Prog. Biophys. Mol. Biol. **71**, 243 (1999).
[5] W. D. Cornell, I. R. Gould, and P. A. Kollman, J. Mol. Struct.: THEOCHEM **392**, 101 (1997).
[6] I. K. Roterman, M. H. Lambert, K. D. Gibson, and H. A. Scheraga, J. Biomol. Struct. Dyn. **7**, 421 (1989).
[7] B. M. Pettitt and M. J. Karplus, Chem. Phys. Lett. **121**, 194 (1985).
[8] A. Solov'yov, A. V. Yakubovich, and W. Greiner, J. Exp. Theor. Phys. **103**, 463 (2006).
[9] M. Kohtani, G. A. Breaux, and M. F. Jarrold, J. Am. Chem. Soc. **126**, 1206 (2004).
[10] D. F. Regulla and U. Deffner, Int. J. Appl. Radiat. Isot. **33**, 1101 (1982).
[11] M. Z. Heydari, E. Malinen, E. O. Hole, and E. Sagstuen, J. Phys. Chem. A **106**, 8971 (2002).
[12] V. Nagy, J. M. Puhl, and M. F. Desrosiers, Radiat. Phys. Chem. **57**, 1 (2000).
[13] S. Ebraheem, W. B. Beshira, S. Eid, R. Sobhy, and A. Kovacs, Radiat. Phys. Chem. **67**, 569 (2003).
[14] M. S. Walczak, K. Lawniczak-Jablonska, A. Sienkiewicz, M. Czuba, M. Klepka, and A. Graczyk, J. Phys.: Condens. Matter **19**, 285214 (2007).
[15] M. Karas and F. Hillenkamp, Anal. Chem. **60**, 2299 (1988).
[16] F. Hillenkamp and M. Karas, Int. J. Mass. Spectrom. **200**, 71 (2000).
[17] M. Karas, U. Bahr, I. Fournier, M. Gluckmann, and A. Pfenninger, Int. J. Mass. Spectrom. **226**, 239 (2003).
[18] M. Wind and W. Lehmann, J. Anal. At. Spectrom. **19**, 20 (2004).
[19] S. Brøndsted Nielsen, J. U. Andersen, P. Hvelplund, B. Liu, and S. Tomita, J. Phys. B: At. Mol. Opt. Phys. **37**, R25 (2004).
[20] J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, and C. M. Whitehouse, Science **246**, 64 (1989).
[21] B. Lucas, G. Grégoire, J. Lemaire, P. Maitre, J. M. Ortega, A. Rupenyan, B. Reimann, J. P. Schermann, and C. Desfrancois, Phys. Chem. Chem. Phys. **6**, 2659 (2004).
[22] J. Laskin, E. Denisov, and J. H. Futrell, J. Am. Chem. Soc. **122**, 9703 (2000).
[23] M. J. Polce, D. Ren, and C. Wesdemiotis, J. Mass Spectrom. **35**, 1391 (2000).
[24] F. Rogalewicz, Y. Hoppilliard, and G. Ohanessian, Int. J. Mass Spectrom. **195**, 565 (2000).
[25] J. Laskin and J. H. Futrell, J. Chem. Phys. **116**, 4302 (2002).
[26] J. Wang, S. O. Meroueh, Y. Wang, and W. L. Hase, Int. J. Mass Spectrom. **230**, 57 (2003).
[27] S. C. Nanita and R. G. Cooks, Angew. Chem., Int. Ed. **45**, 554 (2006).
[28] R. G. Cooks, D. X. Zhang, K. J. Koch, F. C. Gozzo, and M. N. Eberlin, Anal. Chem. **73**, 3546 (2001).
[29] F. Ferreira da Silva, P. Bartl, S. Denifl, T. D. Märk, A. M. Ellis, and P. Scheier, Chem. Phys. Chem. **11**, 90 (2010).
[30] F. Ferreira da Silva, S. Denifl, T. D. Märk, A. M. Ellis, and P. Scheier, J. Chem. Phys. **132**, 214306 (2010).
[31] S. Denifl, I. Mähr, F. Ferreira da Silva, F. Zappa, T. D. Märk, and P. Scheier, Eur. Phys. J. D **51**, 73 (2009).
[32] Y. V. Vasil'ev, B. J. Figard, J. Morré, and M. L. Deinzer, J. Chem. Phys. **131**, 044317 (2009).
[33] S. Geddes, J. Zahardis, J. Eisenhauer, and G. A. Petrucci, Int. J. Mass Spectrom. **282**, 13 (2009).
[34] S. Denifl, H. D. Flosadóttir, A. Edtbauer, O. Ingólfsson, T. D. Märk, and P. Scheier, Eur. Phys. J. D **60**, 37 (2010); P. Sulzer, E. Alizadeh, A. Mauracher, T. D. Märk, and P. Scheier, Int. J. Mass Spectrom. **277**, 274 (2008); S. Ptasińska, S. Denifl, P. Candori, S. Matejcik, P. Scheier, and T. D. Märk, Chem. Phys. Lett. **403**, 107 (2005); S. Ptasińska, S. Denifl, A. Abedi, P. Scheier, and T. D. Märk, Anal. Bioanal. Chem. **377**, 1115 (2003).
[35] S. Gohlke, A. Rosa, E. Illenberger, F. Brüning, and M. A. Huels, J. Chem. Phys. **116**, 10164 (2002); J. Kopyra and H. Abdoul-Carime, *ibid.* **132**, 204302 (2010); H. Abdoul-Carime, C. König-Lehmann, J. Kopyra, B. Farizon, M. Farizon, and E. Illenberger, Chem. Phys. Lett. **477**, 245 (2009).
[36] J. Kocicek, P. Papp, P. Mach, Y. V. Vasil'ev, M. L. Deinzer, and S. Matejcik, J. Phys. Chem. A **114**, 1677 (2010).
[37] Y. V. Vasil'ev, B. J. Figard, V. G. Voinov, D. F. Barofsky, and M. L. Deinzer, J. Am. Chem. Soc. **128**, 5506 (2006).
[38] H. D. Flosadóttir, S. Denifl, F. Zappa, N. Wendt, A. Mauracher, A. Bacher, H. Jónsson, T. D. Märk, P. Scheier, and O. Ingólfsson, Angew. Chem., Int. Ed. **46**, 8057 (2007).
[39] M. B. Martins and I. Carvalho, Tetrahedron **63**, 9923 (2007).

[40]A. D. Hendricker and K. J. Voorhees, J. Anal. Appl. Pyrolysis **36**, 51 (1996).
[41]D. Huber, M. Beikircher, S. Denifl, F. Zappa, S. Matejcik, A. Bacher, V. Grill, T. D. Märk, and P. Scheier, J. Chem. Phys. **125**, 084304 (2006).
[42]E. Illenberger and J. Momigny, Eds., Gaseous Molecular Ions, *An Introduction to Elementary Processes Induced by Ionization* (Steinkopff: Darmstadt/Springer: New York , 1992).
[43]H. J. Svec and G. A. Junk, J. Am. Chem. Soc. **86**, 2278 (1964).
[44]A. D. Becke, J. Chem. Phys. **98**, 5648 (1993).
[45]C. Lee, W. Yang, and R. G. Parr, Phys. Rev. B **37**, 785 (1988).
[46]K. Raghavachari, J. S. Binkley, R. Seeger, and J. A. Pople, J. Chem. Phys. **72**, 650 (1980).
[47]M. J. Frisch, G. W. Trucks, H B. Schlegel *et al.* GAUSSIAN 03*, Revision C.02*, Gaussian, Inc., Wallingford, CT, 2004.
[48]L. A. Curtiss, P. C. Redfern, K. Raghavachari, V. Rassolov, and J. A. Pople, J. Chem. Phys. **110**, 4703 (1999).
[49]G. A. Gallup, P. D. Burrow, and I. I. Fabrikant, Phys. Rev. A **79**, 042701 (2009).
[50]A. M. Scheer, P. Mozejko, G. A. Gallup, and P. D. Burrow, J. Chem. Phys **126**, 174301 (2007).
[51]R. Abouaf, Chem. Phys. Lett. **451**, 25 (2008).
[52]Y. V. Vasil'ev, B. J. Figard, D. F. Barofsky, and M. L. Deinzer, Int. J. Mass. Spectrom. **268**, 106 (2007).
[53]P. V. Shchukin, M. V. Muftakhov, J. Morre, M. L. Deinzer, and Y. V. Vasil'ev, J. Chem. Phys. **132**, 234306 (2010).
[54]M. Sobczyk, I. Anusiewicz, J. Berdys-Kochanska, A. Sawicka, P. Skurski, and J. Simons, J. Phys. Chem. A **109**, 250 (2005).
[55]P. Hvelplund, B. Liu, S. Brøndsted Nielsen, S. Panja, J. C. Poully, and K. Støchkel, Int. J. Mass Spectrom. **263**, 66 (2007).
[56]P. Papp, J. Urban, S. Matejcik, M. Stano, and O. Ingólfsson, J. Chem. Phys. **125**, 204301 (2006).
[57]CRC, *Handbook of Chemistry and Physics*, 83rd ed. CD-Rom.
[58]Nist Chemistry Webbook, available from http://www.webbook.nist.gov/chemistry/.
[59]C. Koenig-Lehmann, J. Kopyra, I. Dąbkowska, J. Kočišek, and E. Illenberger, Phys. Chem. Chem. Phys. **10**, 6954 (2008).