ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/6962851

Fragmentations of Protonated Arginine, Lysine and Their Methylated Derivatives: Concomitant Losses of Carbon Monoxide or Carbon Dioxide and an Amine †

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY A · AUGUST 2006

Impact Factor: 2.69 · DOI: 10.1021/jp055426k · Source: PubMed

CITATIONS
34

READS
65

5 AUTHORS, INCLUDING:

Yuyong ke
Endoceutics inc, Canada
18 PUBLICATIONS **296** CITATIONS

SEE PROFILE

Available from: Yuyong ke
Retrieved on: 25 January 2016

Fragmentations of Protonated Arginine, Lysine and Their Methylated Derivatives: Concomitant Losses of Carbon Monoxide or Carbon Dioxide and an Amine†

P. Y. Iris Shek, Junfang Zhao, Yuyong Ke, K. W. Michael Siu, and Alan C. Hopkinson*

Department of Chemistry and Centre for Research in Mass Spectrometry, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3

Received: September 23, 2005; In Final Form: November 8, 2005

The fragmentation pathways of protonated arginine, protonated N_α,N_α-dimethylarginine, the N_α,N_α,N_α-trimethylarginine ion, three protonated N_ϵ,N_ϵ-dimethyllysines, and three permanent lysine ions in which the charge is fixed by trimethylation are reported. Ion assignment was facilitated by ^{15}N-labeling and deuterium substitution. The chemistries are dominated by charge-induced elimination of the amino groups as neutrals, including dimethylamine, trimethylamine and guanidine. Competitive losses of the α-amino and side-chain amino groups were observed; these losses led to intermediates that had different structures and different subsequent dissociation reactions. Concomitant losses of CO or CO_2 with these amines were also commonly observed. However, the ionic products of amine losses did not subsequently lose CO or CO_2, suggesting strongly that in these concomitant eliminations, it is the CO or CO_2 that was first eliminated, followed immediately by the loss of the amine. Results of density functional theory calculations on protonated arginine and protonated N_α,N_α-dimethylarginine reveal that, in such concomitant eliminations, the dissociating complex is vibrationally hot and the intermediate ion formed by losing CO or CO_2 can immediately dissociate to eliminate the amine.

Introduction

The sequence of amino acids in a protein determines its biological function and, consequently, knowledge of this sequence is important. Mass spectrometry (MS)-based determinations of sequences of protonated peptides and proteins are extensively documented.[1] Many studies have shown that bond cleavage is typically charge proximal; i.e., the bond that breaks is adjacent to the atom that formally carries the positive charge; protonation of the amide nitrogen is believed to precede cleavage of the peptide bond, C(O)−NH. The proton added to a peptide is "mobile"; i.e., it migrates relatively freely between the terminal amino group and the carbonyl atoms of the amide groups along the peptide backbone.[2−15] After cleavage, if the charge remains on the N-terminal fragment, the products are usually b_n ions (oxazolones[16−18]); if the charge remains on the C-terminal fragment, then y_m ions (protonated truncated peptides or the protonated C-terminal amino acid) are formed.[19] In a peptide, the types of amino acid residues present dictate which bonds are cleaved and which fragment carries the charge; the type of residues also determines what fragments (if any) originate from the side chain.

Arginine and lysine residues are important in mass spectrometric examinations of enzymatically cleaved peptides; the most common protease, trypsin, cleaves peptide bonds C-terminal to arginine and lysine residues. Arginine and lysine have high proton affinities and the side chains of these amino acid residues compete effectively for the mobile proton. The arginine residue, the most basic of all residues, exerts a localization effect on the proton; i.e., the proton is less mobile and consequently comparatively fewer backbone fragmentations occur for singly protonated arginine-containing peptides.[20−22]

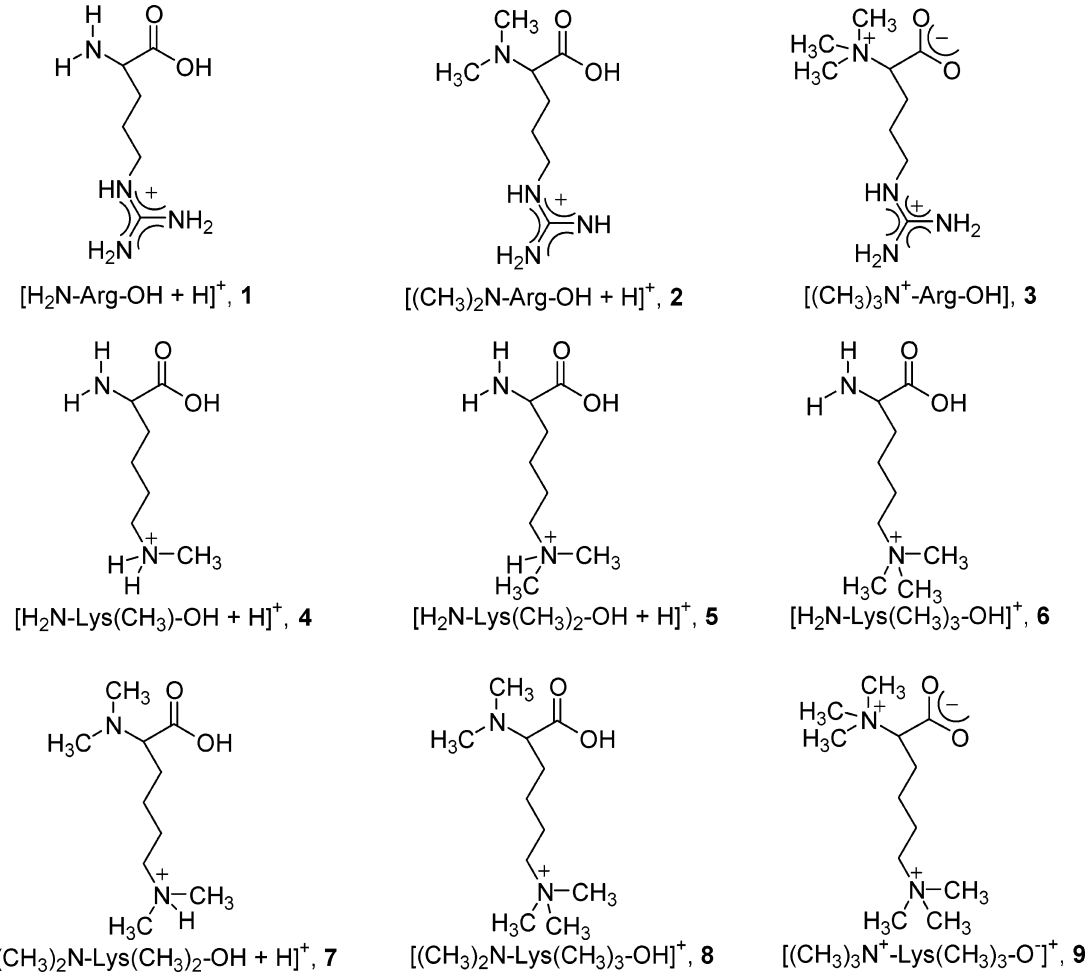
The fragmentation reactions of protonated lysine and arginine have been studied extensively.[23−27] The preferred protonation site of these two basic amino acids is on a nitrogen atom in the side chain and, not surprisingly, the major fragmentation reactions involve the charge site and result in loss of the functional group that is protonated. In the case of lysine, loss of NH_3 occurs, and experiments on ^{15}N-labeled lysine established that it is the amino group from the side chain that is lost;[23] the ionic product of this reaction is believed to be a cyclic species, protonated pipecolic acid.[24] Protonated arginine also loses NH_3 from the guanidinyl group on the side chain,[25] but this reaction is in competition with loss of the whole guanidinyl group, $HN{=}C(NH_2)_2$; in this latter reaction, another cyclic ion, protonated proline, is formed.[26] The loss of water is a minor channel in the fragmentations of both protonated arginine and lysine; unlike in the CID of other protonated amino acids, however, this reaction is not accompanied by concomitant loss of CO and a cyclic structure is believed to be formed after loss of water.[24] In addition, the structure of another fragment ion from protonated arginine, $[M + H - 45]^+$ (130 Th), has not been assigned. In one study, it was suggested that [C, H_3, N, O] is lost,[25] and in another that [C, O_2, H] is lost.[28]

Methylation of an amino group tends to increase the proton affinity and attracts the mobile proton.[29] Methylation may, therefore, alter ion structure and fragmentation chemistry. Methylation of lysine and arginine residues in histones is a key posttranslational modification that is evolutionarily conserved from yeast to mammals. Histone methylation regulates DNA-based functions, including gene transcription and DNA repair, through either alteration of chromatin structure or creating binding sites for regulatory proteins.[30] On the molecular level, a recent computational study shows that substitution of three hydrogens by methyl groups on the side chain nitrogens of the amino acid arginine stabilizes the zwitterionic structure to the

† Part of the "Chava Lifshitz Memorial Issue".

10.1021/jp055426k CCC: $33.50 © 2006 American Chemical Society
Published on Web 12/21/2005

SCHEME 1



extent that it becomes the lowest-energy structure.[31] The possibility of effecting different fragmentation reactions via methylation stimulated us to examine the fragmentation reactions of protonated lysines and arginines that have been methylated on one or more nitrogen atoms. In these ions, the charge is *potentially* localized on the methylated nitrogen. In addition, we also examined analogous ions having a trimethylated amino group and, therefore, a *formally* localized charge on the methylated nitrogen.

In the current study, we examine protonated arginine (**1**), protonated N_α,N_α-dimethylarginine (**2**), N_α,N_α,N_α-trimethylarginine ion (**3**), three protonated N_ϵ-methyl and N_ϵ,N_ϵ-dimethyllysines (ions **4**, **5**, and **7**), three lysine ions in which the charge is fixed by trimethylation (ions **6**, **8**, and **9** in Scheme 1). This collection of ions has enabled us to systematically study the effect on the fragmentation pathways of ions that have been methylated on the nitrogens of the side chain and also that of the α-amino group. An impetus that led to this investigation was our preliminary work on protonated arginine, where we found only *one* precursor, the parent ion at 175 Th, for the fragment ion at 130 Th. This implies that either CO plus NH_3 or COOH leaves in one step. Furthermore, in our initial investigation of the CID spectrum of the N_ϵ-trimethyllysine ion, **6**, we also observed a low-abundance ion at $[M + H - 45]^+$. In the course of this investigation, we observed an even more unusual fragmentation pathway in the CID spectra of some methylated lysines and methylated arginines, loss of the α-amine plus CO_2, or an amine from the side chain plus CO_2. Plausible mechanisms for some of these unusual reactions were probed using density functional theory (DFT) calculations.

Experimental Section

Materials. Methylated *N*-lysine derivatives were purchased from Bachem Biosciences (King of Prussia, PA). Arginine, lysine, formaldehyde, sodium cyanoborohydride, iodomethane, methanol-d_4 and deuterium oxide were available from Sigma-Aldrich Ltd. (Oakville, ON, Canada). α-^{15}N-labeled arginine and lysine were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA). All other chemicals and solvents were of analytical or HPLC grade.

Sample Preparation. *Dimethylation of Amino Acids.* Dimethylation of amino acids were performed according to a literature procedure.[32] Briefly, 10 molar equivalents of 37% formaldehyde was added to 0.5 mg of amino acid in 1 mL of 50 mM sodium acetate, pH 5.0. The solution was mixed and, immediately, 10 molar equivalents of sodium cyanoborohydride was added and mixed thoroughly. The resulting solution was allowed to stand overnight.

Trimethylation of Amino Acids. One milligram of an amino acid was dissolved in 1 mL of 90/10 methanol/water (v/v), and the pH was adjusted to 10.0 with NH_4OH. One hundred microliters of iodomethane was added. The mixture was heated to 60 °C and stirred for 3 h; thereafter, stirring continued at room-temperature overnight. The resulting solution was dried by Speed-Vac and the peptide was redissolved in 1 mL of 50/50 methanol/water (v/v) prior to MS analysis.

Deuterium Substitution (H/D Exchange). To a 150 μL solution of amino acid in 50/50 methanol/water (v/v) were added 50 μL of deuterium oxide and 150 μL of methanol-d_4. Acetic acid-d_4 was then added until the resulting solution contained 0.1% of the acid. The solution was allowed to stand for 1 day to ensure equilibrium was achieved.

Mass Spectrometry. MS/MS data were obtained on a prototype of the API 3000 electrospray/triple-quadrupole mass spectrometer (MDS SCIEX, Concord, Canada). The amino acid solutions were 100 μM in 50/50 methanol/water (v/v) containing 0.1% acetic acid, and the dimethylated amino acid solutions were 100 μM in sodium acetate. The samples were continuously introduced into the pneumatically assisted electrospray source at a flow rate of 2 μL/min by means of a syringe pump. Dry air was used as the nebulizing gas. Nitrogen was used as the curtain and collision gas. Mass spectra were acquired in the positive-ion detection mode with unit mass resolution.

Calculations. DFT calculations employing the hybrid B3LYP method (using Becke's three-parameter exchange functional[33] and the correlation functional of Lee, Yang, and Parr[34]) with the 6-31G* basis set[35] were used for calculation of the optimized geometries and energetics.[36] All critical points were characterized by harmonic frequency calculations; intrinsic reaction coordinate (IRC) calculations were employed to establish which two minima were associated with a given transition state.[37] Zero-point energies and thermal corrections obtained from the DFT calculations were used to determine relative enthalpies at 298 K. Finally, entropy terms were included to obtain free energies.

Results and Discussion

Mass Spectrometry. *Arginine and Its Derivatives.*

(a) Protonated Arginine, **1**.

Figure 1a shows the CID results of protonated arginine, **1**. Our results agree with previous observations:[24−26] At low collision energies, NH_3 is lost exclusively from the side chain to create an ion at 158 Th. At higher collision energies, nucleophilic attack by the α-NH_2 displaces guanidine from the side chain and results in *N*-protonated proline at 116 Th. The mechanisms by which these reactions occur have been the subject of a recent computational study.[38] In addition to the major products, there is also an ion at 130 Th, corresponding to loss of 45 Da. Formation of this ion has previously been attributed to the loss of [C, H_3, N, O][25] or [C, O_2, H].[28] Our results show that, after deuterium substitution, eight D atoms are incorporated into protonated arginine and the unlabeled ion originally at 130 Th is shifted to 135 Th (Figure S1); i.e., the neutral product that is lost contains *three* exchangeable hydrogen atoms. Precursor ion scans established that the only precursor of the product ion at 130 Th was protonated arginine at 175 Th (Figure S2); in addition, product ion scans of the ion at 158 Th (resulting from the loss of NH_3 from protonated arginine) established that the 158 Th ion does not fragment to give the 130 Th ion (Figure S3). These observations eliminate [C, O_2, H] as a viable neutral product that accompanies formation of the 130 Th ion. Furthermore, as the neutral products contain three exchangeable hydrogen atoms, the most plausible neutral combination is NH_3 and CO. The ionic product after losing NH_3 from protonated arginine (158 Th) does not, in turn, lose CO; this means that protonated arginine first loses CO, followed by the immediate loss of NH_3 (see later).

Incorporation of ^{15}N in the α-amino group established that, in forming the 130 Th ion, the NH_3 originates entirely from the α-amino group; by contrast, when only NH_3 is lost in creating the 158 Th ion, the loss is exclusively from the side chain (Figure 1b). In comparing Figure 1a,b, most peaks are shifted by 1 Da; only two peaks are at the same *m*/*z* values, those at 130 Th and 60 Th (the latter is assigned as protonated guanidine), indicating that these product ions do not contain the α-amino group. CID experiments on the ion at 130 Th established that this fragmentation product is quite stable toward further dissociation (abundant products were only observed ≥2.5 eV, center-of-mass frame). We postulate that the ion at 130 Th is an aldehyde, a derivative of 1-butanal with a guanidyl substituent on the δ-carbon (Scheme 2). Concomitant loss of NH_3 plus CO is observable at collision energies as low as ≤1 eV (center-of-mass frame), suggesting that the energy barrier against this dissociation reaction is low.

CID of the 130 Th ion gives abundant product ions at 71 and 70 Th, and ions of lower abundances at 60, 88, 43 and 112 Th (Figure 1c). Possible structures for these product ions, originating from the proposed aldehydic precursor ion and assigned on the basis of H/D exchange (Figure S4), are given in Scheme 2. We postulate that the most abundant ion at 71 Th is a cyclic oxonium ion that has *no* exchangeable hydrogen atoms and is formed by displacement of guanidine via nucleophilic attack by the carbonyl oxygen on the δ-carbon. The 60 Th ion is probably protonated guanidine; guanidine has a high proton affinity (236 kcal mol^{-1}).[29]

The second most abundant ion in the spectrum in Figure 1c, at 70 Th, corresponds to loss of H_2O followed by loss of carbodiimide and is assigned the protonated 1-pyrroline structure. Cleavage of the C−N bond to form 1-pyrroline initially produces protonated carbodiimide, but the low proton affinity of carbodiimide (196.6 kcal mol^{-1})[39] results in facile proton transfer from the nascent protonated carbodiimide to nascent 1-pyrroline. From the lack of an ion at 46 Th in the spectrum of the H/D exchanged ion, we conclude that the observed, low-abundance ion at 43 Th is not protonated carbodiimide but is the isopropyl cation. Protonated 1-pyrroline contains a single exchangeable hydrogen; incorporation of a single deuterium gives an ion at 71 Th, which is isobaric with the oxonium ion.

(b) Protonated N_α,N_α-Dimethylarginine, **2**.

The CID spectrum of protonated N_α,N_α-dimethylarginine (Figure 2) shows major differences from that of protonated arginine. Methylating the α-amino group increases the basicity of this nitrogen and presumably the probability of protonation and results in loss of dimethylamine, thereby producing the ion at 158 Th. By contrast, loss of individual NH_3 from the α-amino group of protonated arginine does not occur (see Figure 1b). Another difference is that loss of NH_3 from the guanidyl group of **2** does not occur, whereas this loss is apparent in the fragmentation of **1**. The most abundant product ion, 144 Th (Figure 2a), in the CID spectrum of protonated N_α,N_α-dimethylarginine is formed by loss of guanidine and, by analogy with that of protonated arginine, is assigned as protonated *N*,*N*-dimethylproline. This ion contains the α-nitrogen, in accordance with CID results on $^{15}N_\alpha$,$^{15}N_\alpha$-dimethylarginine, in which the most abundant product ion is at 145 Th and is assigned as ^{15}N,^{15}N-dimethylproline (Figure 2b).

As previously discussed, the 130 Th ion observed in the fragmentation of **1** is formed by concomitant loss of CO and the α-amino group as an ammonia. In the case of **2**, the analogous concomitant loss involves CO and dimethylamine. (Note that both parts a and b of Figure 2 display this 130 Th ion, consistent with interpretation of the loss of the α-nitrogen.) The 130 Th ions originating from **1** and **2** gave identical CID

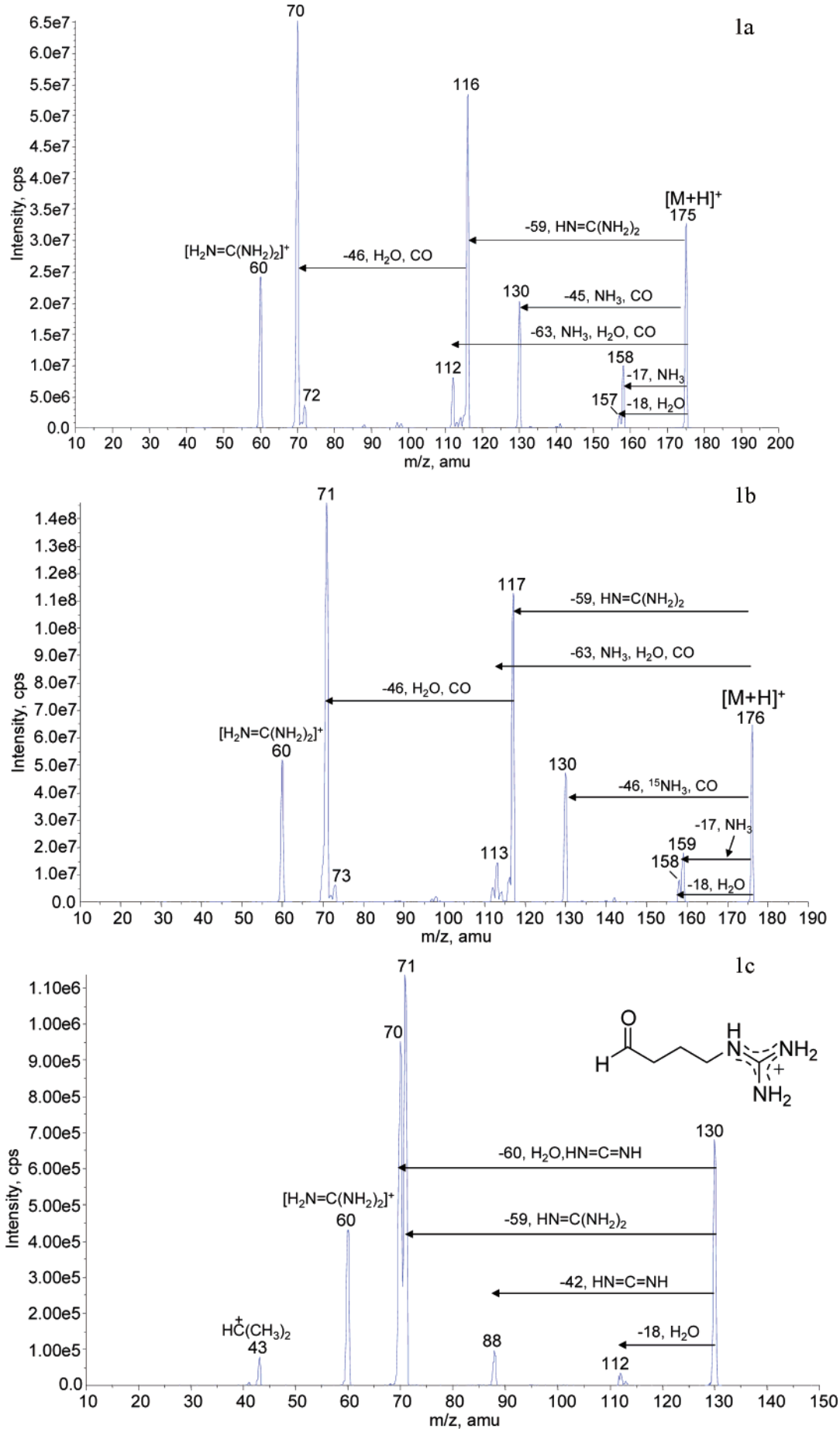
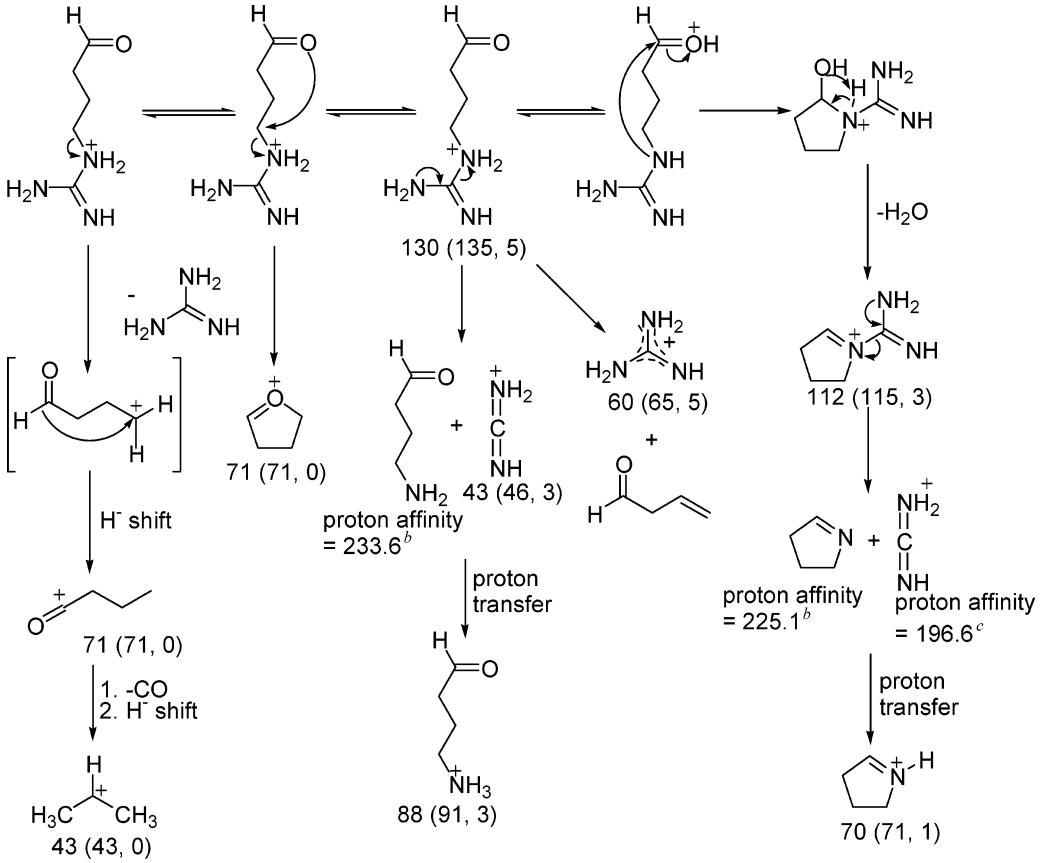


Figure 1. ESI/CID spectra of (a) protonated arginine, **1**, (b) protonated $^{15}N_{\alpha}$-labeled arginine, **1** and (c) 130 Th from protonated arginine, **1**, with $E_{CM} = 2.7$ eV.

SCHEME 2[a]



[a] The first and second values in parentheses are *m*/*z* values under H/D exchange conditions and the number of exchangeable protons present, respectively. All proton affinities are in kcal mol^{-1}. [b]The value is calculated by using B3LYP/6-31G* level. [c]The value is obtained from ref 29.

spectra, in accordance with our interpretation of their being identical ions.

The two abundant product ions at 100 and 114 Th (Figure 2a) are postulated to form via concomitant losses of, respectively, CO_2 plus $HN{=}C(NH_2)_2$ (103 Da) and CO_2 plus $(CH_3)_2$-NH (89 Da) from protonated N_α,N_α-dimethylarginine. Precursor ion analyses of these product ions showed only a single precursor, protonated N_α,N_α-dimethylarginine (Figure S5). The methyl ester of N_α,N_α-dimethylarginine did not display these neutral losses, indicating that loss of CO_2 requires the presence of a mobile hydrogen, as in COOH (Figure S6). It is noteworthy that loss of CO_2 was not observed in the CID of protonated arginine (see Figure 1) or lysine but was found to occur in the CID of protonated α-methylated lysines (see later). These findings suggest strongly that increasing the basicity of the α-amino group via methylation may lower the energy of the zwitterionic amino acid structure, which in turn facilitates the loss of CO_2. Methylating the COOH group, as in the methyl ester of N_α,N_α-dimethylarginine, eliminates the zwitterionic tautomer and blocks the two channels that provide concomitant losses of CO_2. Postulated fragmentation chemistries of N_α,N_α-dimethylarginine are summarized in Scheme 3. The energetics of some of these reactions have been calculated at B3LYP/6-31G* and the results will be presented in a later section.

(c) N_α,N_α,N_α-Trimethylarginine Ion, **3**.

The fragmentation of this ion is dominated by the fixed charge. The most abundant product ion, at 114 Th, is due to concomitant loss of $(CH_3)_3N$ plus CO_2 (103 Da); additionally, the second most abundant ion, at 158 Th, is due to loss of $(CH_3)_3N$ (59 Da) (Figure 3a). Loss of guanidine would also give an ion at 158 Th, but CID results on the $^{15}N_\alpha$-labeled N_α,N_α,N_α-trimethylarginine ion (Figure 3b) established that neutral loss can only be due to $(CH_3)_3N$. Additionally, two ions of lower abundance, at 130 and 116 Th, also do not contain ^{15}N. The former fragment ion is identical to the ion formed in the fragmentations of **1** and **2**, whereas the latter is protonated proline, formed by loss of $(CH_3)_3N$ and HN═C═NH.

Lysine and Its Derivatives.

(a) Protonated Lysine Derivatives with Methylation on Only the ϵ-Nitrogen.

Figure 4 shows the CID spectra of (a) protonated N_ϵ-methyllysine, **4**, (b) protonated N_ϵ,N_ϵ-dimethyllysine, **5**, and (c) the N_ϵ,N_ϵ,N_ϵ-trimethyllysine ion, **6**. The dissociations are dominated again by loss of the basic functional group—methylamine in **4**, dimethylamine in **5**, and trimethylamine in **6**—and forming the 130 Th ion. This product ion was postulated by Yalcin and Harrison[24] to be protonated pipecolic acid, formed via nucleophilic attack by the α-nitrogen on the ϵ-carbon. Elimination of the ϵ-amino group and cyclization result in protonated pipecolic acid. A second prominent product ion is at 84 Th, also observed by Yalcin and Harrison[24] and described as $[C_5H_{10}N]^+$. The corresponding neutral loss is 46 Da, CO plus H_2O, which is common in the fragmentation of protonated amino acids.[26] Pipecolic acid is similar to proline, differing by having an additional methylene group in the ring. Concomitant loss of CO plus H_2O results in the pyrrolinium ion.[23] All other fragment ions are <10% in abundance; two of these are due to losses of 17 and 45 Da, which have been seen and interpreted previously in the fragmentation of protonated arginine and its derivatives as loss of NH_3 and the concomitant loss of CO plus NH_3. The fragmentation chemistries and proposed structures are summarized in Scheme 4.

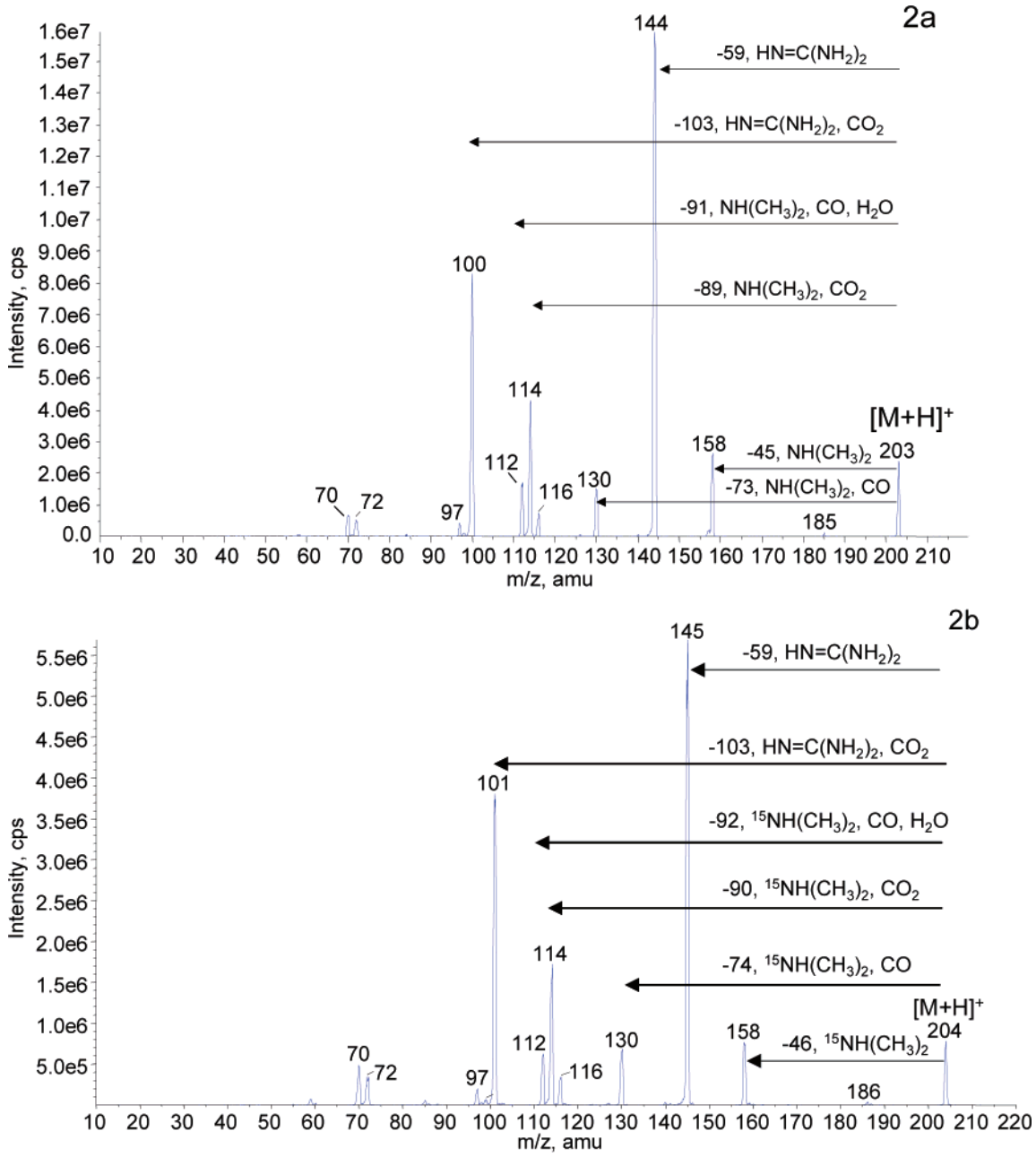


Figure 2. ESI/CID spectra of (a) protonated $(CH_3)_2N-Arg-OH$, **2**, and (b) protonated $^{15}N_\alpha$-labeled $(CH_3)_2N-Arg-OH$, **2**, with $E_{CM} = 2.4$ eV.

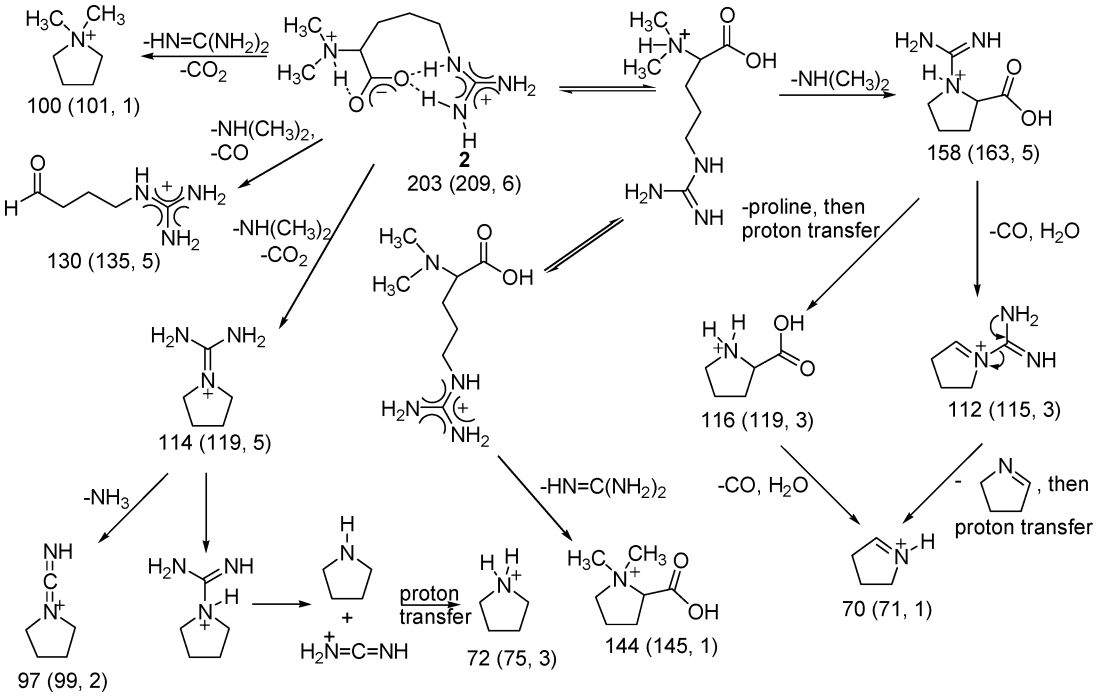
For the loss of NH_3, the resulting product ions are 144 Th in **4** and 158 Th in **5** (not observable in **6**), whereas for the concomitant loss, they are 116 Th in **4**, 130 Th in **5** (N.B. this is isobaric with protonated pipecolic acid), and 144 Th in **6**. As the protonated pipecolic acid ion at 130 Th is abundant in the fragmentation of all three precursor ions, and when the abundances of all three product ions due to the concomitant loss of CO plus NH_3 are compared, the 130 Th product ion seen in Figure 4b must be mostly protonated pipecolic acid. Additional proof comes from examining the CID of the d_4-derivative of **5** (179 Th), the two product ions in question were no longer isobaric: the much more abundant deuterated pipecolic acid was at 133 Th and the much less abundant ion due to concomitant loss of CO plus ND_3 was at 131 Th (see Figure S7). It is of note that the methyl esters of **4**, **5** and **6** did not show losses of CO plus NH_3 (see Figure S8 in contrast to Figure 4). As shown previously for protonated arginine and its derivatives, precursor ion scans showed that the loss of CO and NH_3 is concomitant with no measurable intermediate (see Figure S9).

(b) Protonated Lysine Derivatives with Methylation on Both α- and ϵ-Nitrogens.

As methylation of the α-amino group of arginine promotes loss of that functional group, and methylation of the ϵ-amino group of lysine also promotes loss of the side chain functional group, it was decided to examine possible competitive losses between α- and ϵ-amino groups in protonated $N_\alpha,N_\alpha,N_\epsilon,N_\epsilon$-tetramethyllysine, **7**, the $N_\alpha,N_\alpha,N_\epsilon,N_\epsilon,N_\epsilon$−pentamethyllysine ion, **8**, and the $N_\alpha,N_\alpha,N_\alpha,N_\epsilon,N_\epsilon,N_\epsilon$−hexamethyllysine ion, **9**.

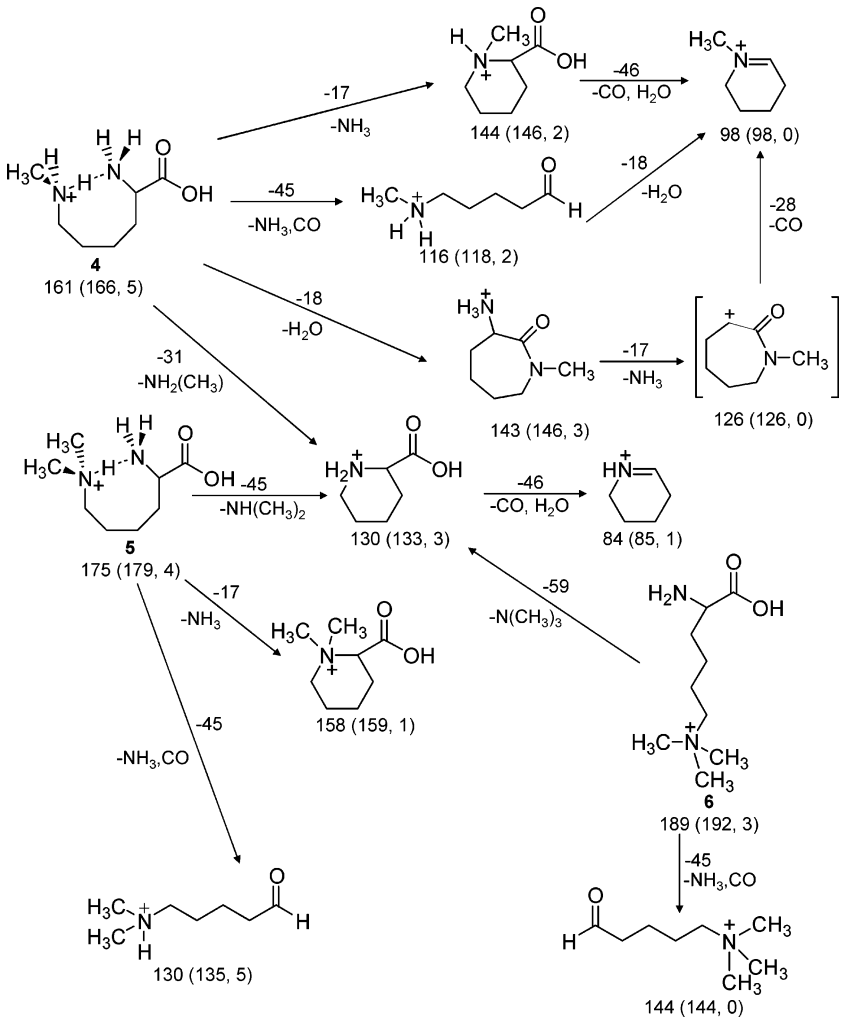
Figure 5 shows the CID spectra of (a) ion **7**, (b) $^{15}N_\alpha$-labeled ion **7**, and (c) d_2-labeled ion **7**. The most abundant product ion is formed by elimination of dimethylamine, resulting in the 158 Th ion in (a) from losing $NH(CH_3)_2$; the 158 and 159 Th ions in (b) from losing, respectively, $^{15}NH(CH_3)_2$ and $^{14}NH(CH_3)_2$; and the 159 Th ion in (c) from losing $ND(CH_3)_2$. From the ratio of the 158 to 159 Th ions in (b), at approximately 1:2, it is

SCHEME 3[a]



[a] The first and second values in parentheses are *m*/*z* values under H/D exchange conditions and the number of exchangeable protons present, respectively.

SCHEME 4[a]



[a] The numbers in parentheses are the *m*/*z* ratios from H/D exchange conditions and the numbers of exchangeable protons, respectively.

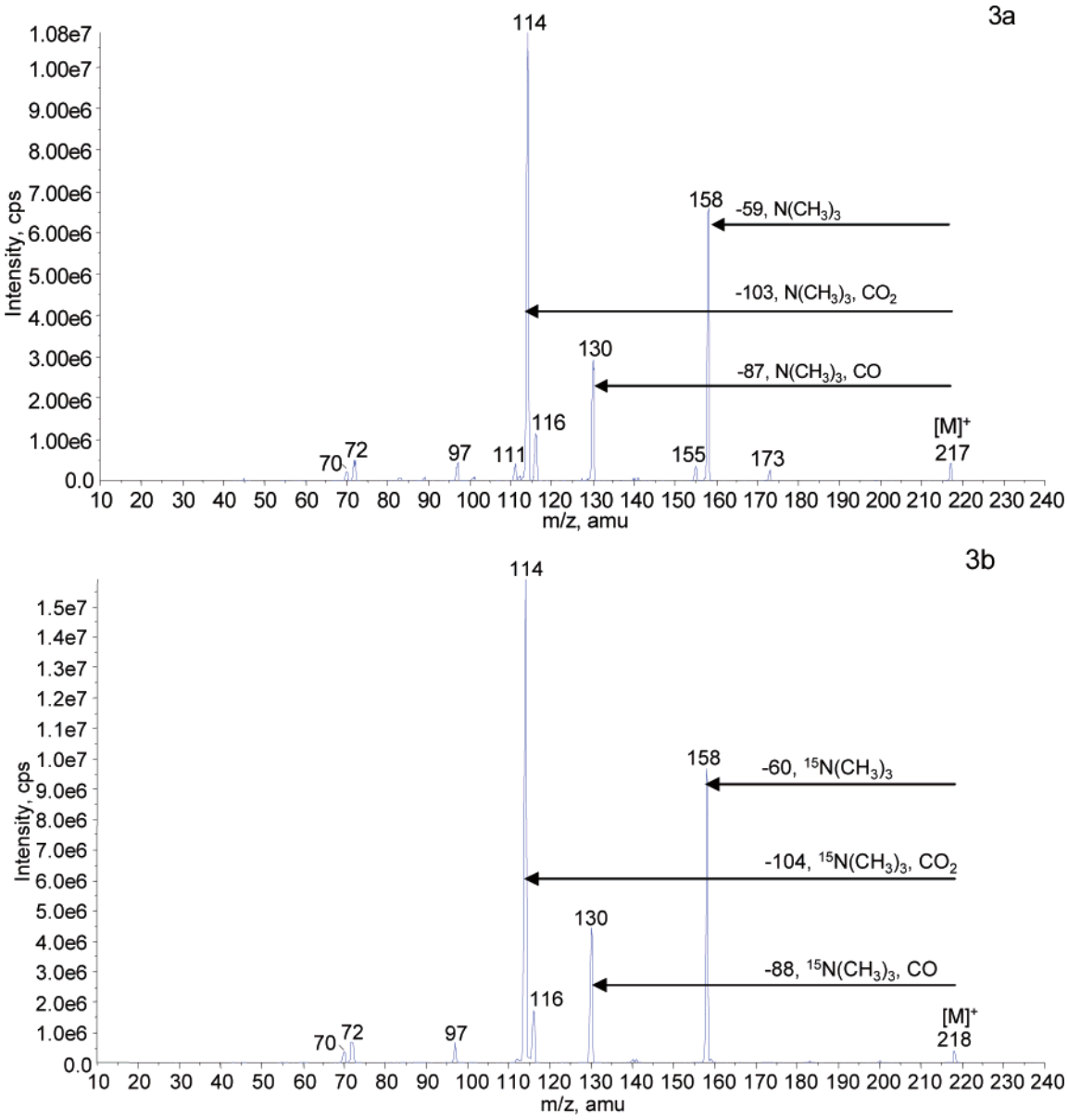


Figure 3. ESI/CID of (a) $[(CH_3)_3N^+-Arg-OH]$, **3**, and (b) $^{15}N_\alpha$-labeled $[(CH_3)_3N^+-Arg-OH]$, **3**, with $E_{CM} = 2.3$ eV.

evident that the preferred loss of dimethylamine occurs at the side chain; this probably reflects the higher proton affinity of the ϵ-dimethylamino group than that of the α-dimethylamino group. Protonation on the ϵ-nitrogen followed by nucleophilic displacement by the α-dimethylamino group results in the *N*,*N*-dimethylpipecolic acid ion, 158 Th in (a), which can then lose 46 Da, H_2O and CO concomitantly, to give the *N*,*N*-dimethyltetrahydropyridinyl ion, 112 Th in (a) (Scheme 5). The 158 Th ion in (b), resulting from loss of $^{15}NH(CH_3)_2$, is not an *N*,*N*-dimethylpipecolic acid ion, as its dissociation chemistry is different. The primary fragmentation product from the 158 Th ion is the 130 Th ion after eliminating a 28 Da neutral, probably CO. This fact was established by both CID of the 158 Th ion and precursor ion scan of the 130 Th ion (see Figure S10). The proposed fragmentation pathway is shown in Scheme 5: Protonation at the α-nitrogen, followed by loss of $^{15}NH(CH_3)_2$ formally produces a carbocation that is destabilized by the adjacent COOH group.[40] Such a destabilized ion is unlikely to be ever fully formed, even as a transient intermediate, in the presence of the adjacent nucleophile, the carbonyl oxygen. Thus the likely scenario is a nucleophilic displacement of $^{15}NH(CH_3)_2$ by the carbonyl oxygen, producing a three-membered lactone with a terminal protonated dimethylamino group and having an *m*/*z* value of 158 Th. Elimination of CO from this sterically strained ion produces an aldehyde at 130 Th. (N.B. this is isobaric with the aldehyde formed from protonated arginine). Results from d_2-labeled, protonated N_α,N_α,N_ϵ,N_ϵ-tetramethyllysine show that the aldehyde ion contains one exchangeable hydrogen, consistent with the above interpretation (Scheme 5).

The second most abundant product ion in Figure 5a is at 114 Th, a difference of 89 Da from ion **7**. The combination of CO_2 and $NH(CH_3)_2$ gives 89 Da, but a precursor ion scan of the 114 Th ion showed that the 158 Th ion does not fragment to give this product ion; i.e., ion **7** does not eliminate $NH(CH_3)_2$ and CO_2 in sequence. This dissociation chemistry is also evident in the CID of ion **8** and becomes prominent in that of ion **9**, the N_α,N_α,N_α,N_ϵ,N_ϵ,N_ϵ-hexamethyllysine ion. Figure 6 shows the CID of (a) ion **8**, (b) ion **9** and (c) the $^{15}N_\alpha$,$^{15}N_\alpha$,$^{15}N_\alpha$,N_ϵ,N_ϵ,N_ϵ-hexamethyllysine ion. The fragmentation chemistries parallel those of ions **6** and **7**, albeit with different emphasis. Ion **9** is permanently zwitterionic with two cationic trimethylamino groups and one carboxylate anion. The most abundant product

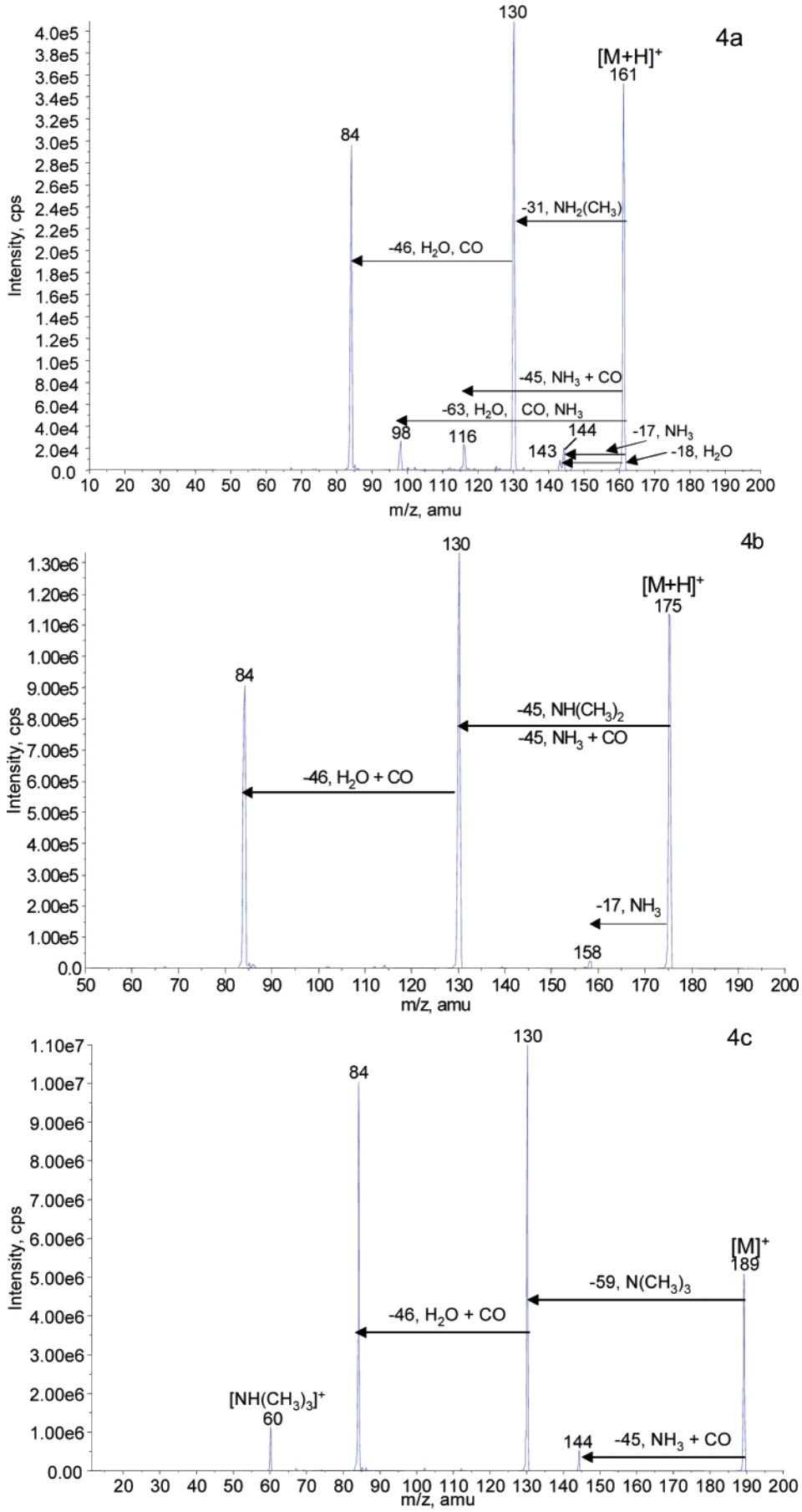


Figure 4. ESI/CID spectra of (a) protonated $H_2N-Lys(CH_3)-OH$, **4**, with $E_{CM} = 2.2$ eV, (b) protonated $H_2N-Lys(CH_3)_2-OH$, **5**, with $E_{CM} = 2.1$ eV, and (c) $[H_2N-Lys(CH_3)_3-OH]^+$, **6**, with $E_{CM} = 2.6$ eV.

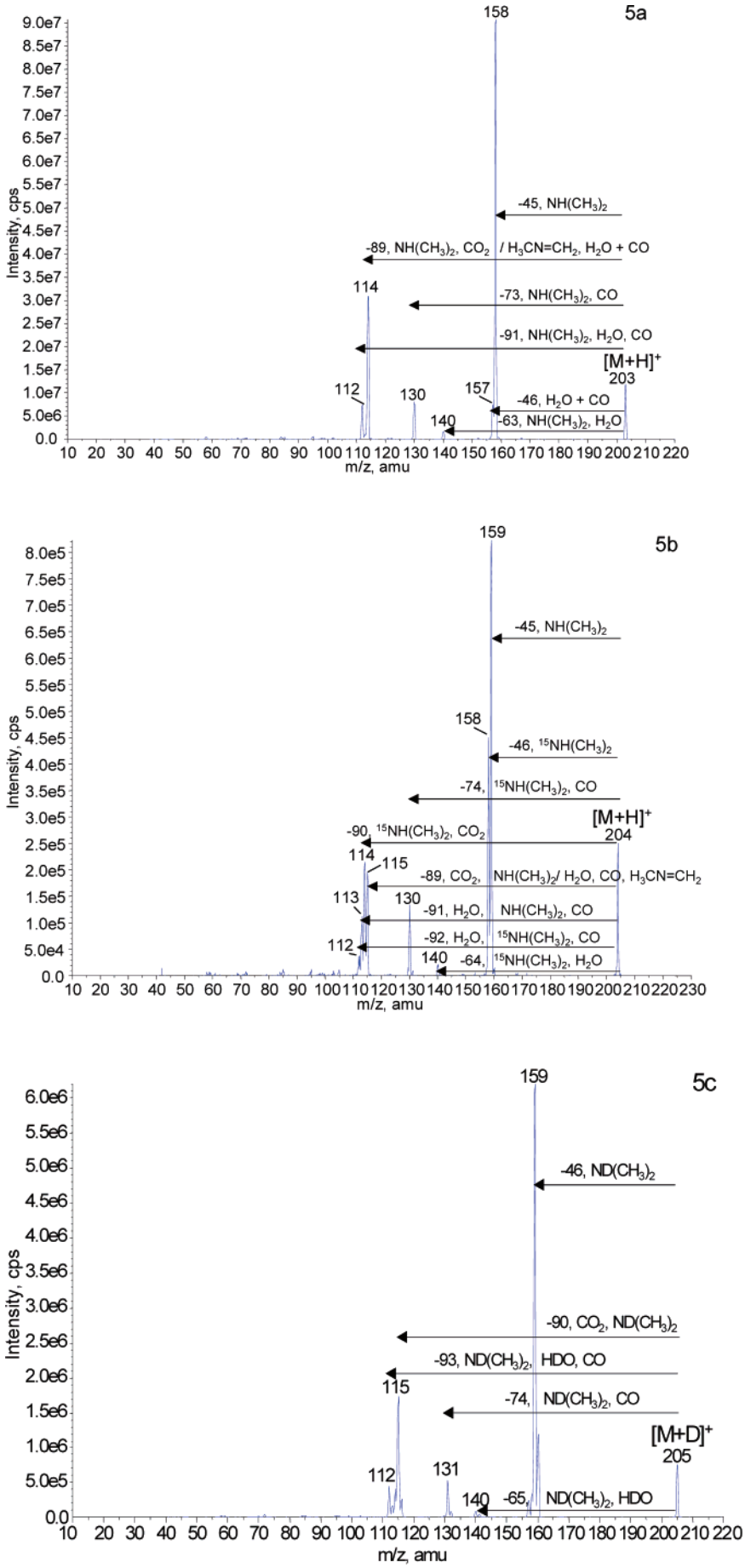
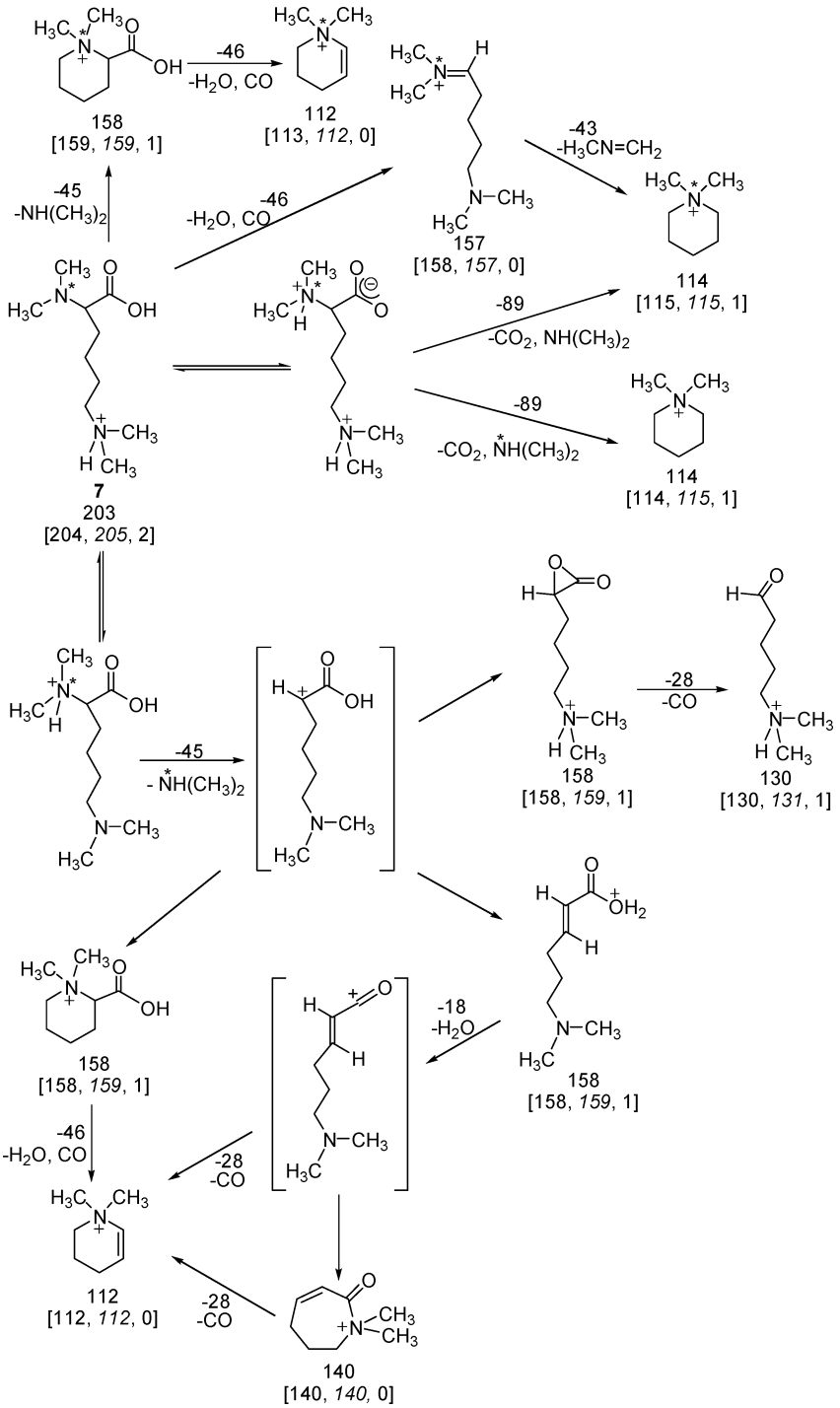


Figure 5. ESI/CID spectra of (a) protonated $(CH_3)_2N-Lys(CH_3)_2-OH$, **7**, (b) protonated $^{15}N_\alpha$-labeled $(CH_3)_2N-Lys(CH_3)_2-OH$, **7**, and (c) deuterated $(CH_3)_2N-Lys(CH_3)_2-OH$, **7** with $E_{CM} = 2.4$ eV.

SCHEME 5[a]



[a] The asterisk represents a ^{15}N-labeled nitrogen; the numbers in parentheses are the *m*/*z* ratios from ^{15}N-labeling, the H/D exchange value, and the number of exchangeable protons, respectively.

from ion **9** is at 128 Th, a direct product from ion **9**; the neutral loss is 103 Da, which equals the combination of CO_2 and $N(CH_3)_3$. Figure 6c shows that both the α- and the ϵ-nitrogens are lost at rates that are similar. From the $^{15}N_\alpha$,$^{15}N_\alpha$,$^{15}N_\alpha$,N_ϵ,N_ϵ,N_ϵ-hexamethyllysine ion, loss of $^{14}N(CH_3)_3$ from the side chain gives the 173 Th ion, whereas loss of $^{15}N(CH_3)_3$ from the α-position gives the 172 Th ion at comparable abundances. However, only the *latter* ion further eliminates CO to give the 144 Th ion. The 129 Th ion involves loss of $^{14}N(CH_3)_3$ from the side chain and the 128 Th ion loss of $^{15}N(CH_3)_3$ from the α-carbon, at a ratio of approximately 3:2. The proposed chemistries are summarized in Scheme 6. Loss of $^{14}N(CH_3)_3$ from the side chain probably involves nucleophilic attack by the COO^- group on the ϵ-carbon, thereby resulting in a 6-hexanolactone with a trimethylamino group in the *2*-position (173 Th). By contrast, loss of $^{15}N(CH_3)_3$ from the α-carbon involves nucleophilic attack by the COO^- group on the α-carbon and results in a propanolactone with a trimethylbutylamino group in the 2-position (172 Th); elimination of CO from the strained ring gives the 144 Th ion.

As the lactones formed by eliminating trimethylamines do not then lose CO_2, the concomitant loss of CO_2 plus trimethylamine must be induced first by the loss of CO_2. Formally, loss of CO_2 produces zwitterionic hexamethylpentadiamine with a

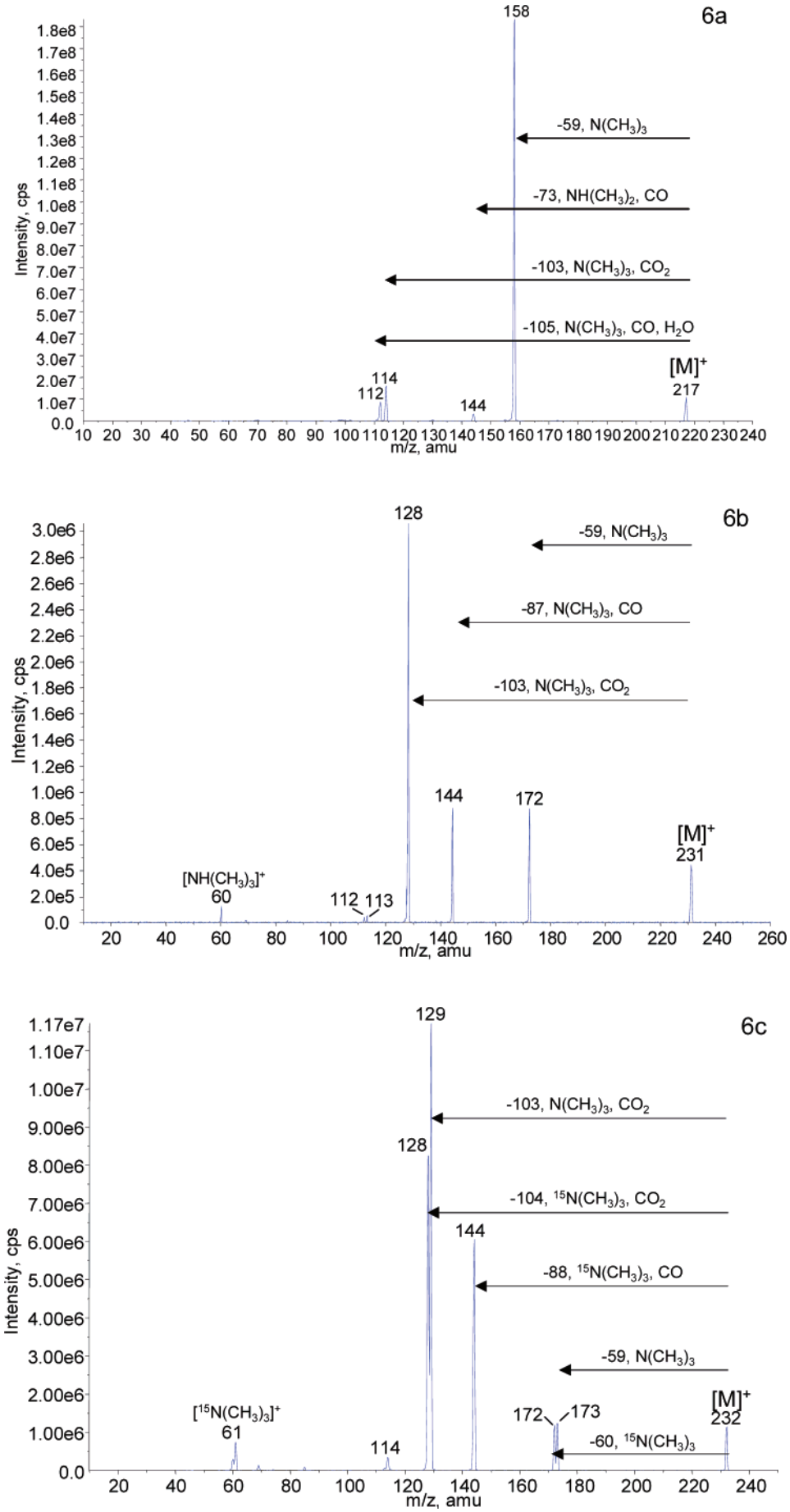
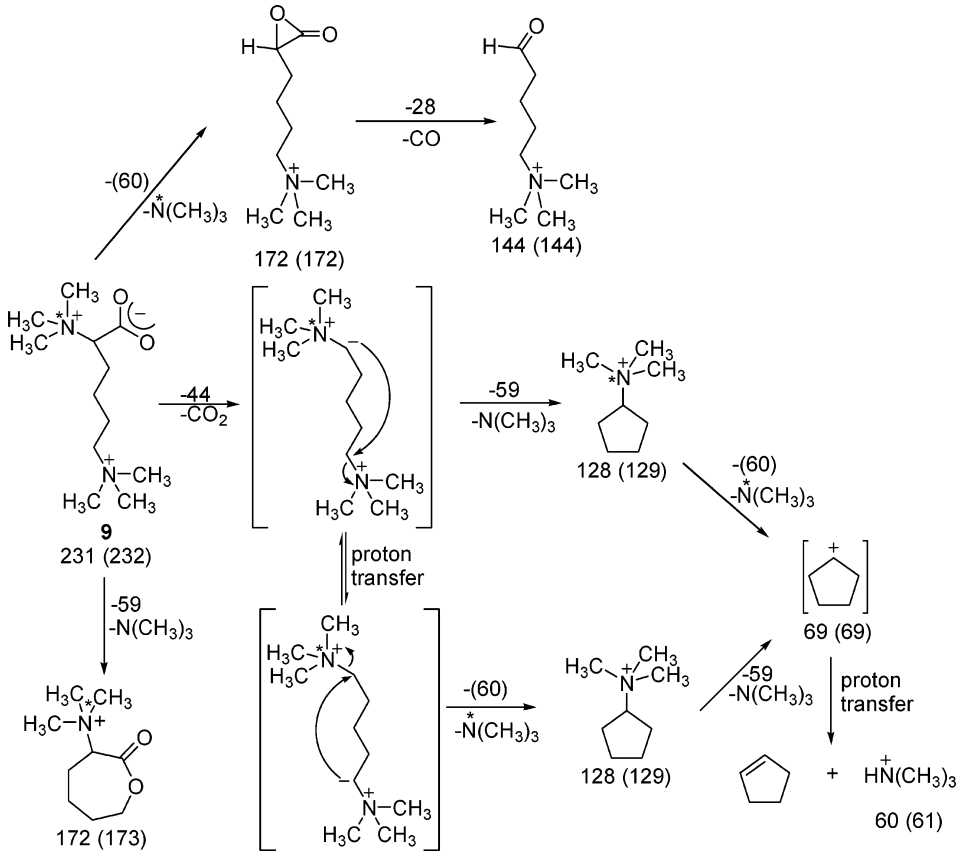


Figure 6. ESI/CID spectra of (a) $[(CH_3)_2N{-}Lys(CH_3)_3{-}OH]^+$, **8**, with $E_{CM} = 2.3$ eV, (b) $[(CH_3)_3N{-}Lys(CH_3)_3{-}O^-]^+$, **9**, and (c) $^{15}N_\alpha$-labeled $[(CH_3)_3N{-}Lys(CH_3)_3{-}O^-]^+$, **9**, with $E_{CM} = 2.2$ eV.

SCHEME 6[a]



[a] The asterisk represents a ^{15}N-labeled nitrogen, and the numbers in parentheses represent the *m/z* ratio from ^{15}N-labeling.

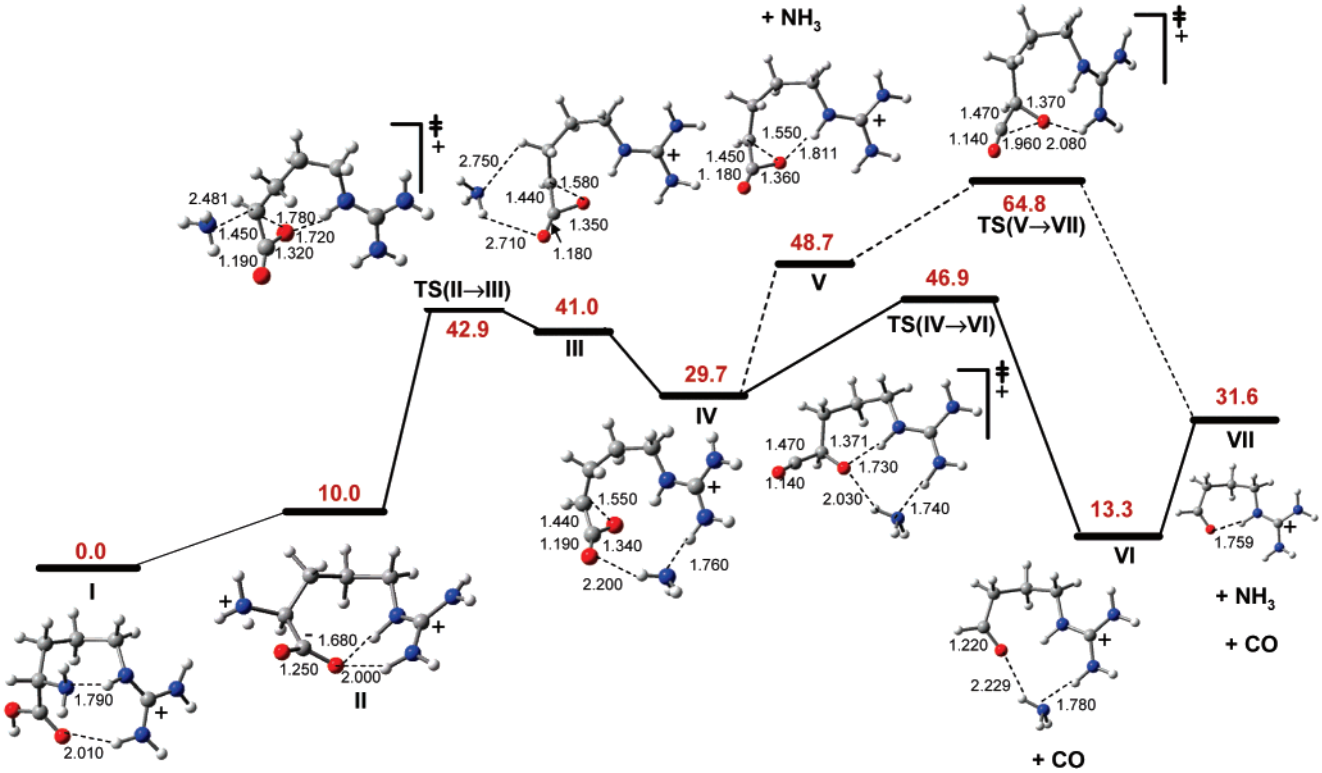


Figure 7. Potential energy hypersurface for the loss of (CO plus NH_3) starting from side chain protonated arginine, ion **1**. The numbers (in kcal/mol) are relative enthalpies at 0 K. All energies are relative to the global minimum of protonated arginine, **1**.

CH^- group on the α-carbon. Immediate nucleophilic attack on the ϵ-carbon eliminates the side chain trimethylamino group and results in cyclopentane with a trimethylamino substituent at 129 Th. Alternatively, the zwitterionic hexamethylpentadiamine tautomerizes by a 1,4-proton transfer to give a second zwitterionic hexamethylpentadiamine with a CH^- group on the

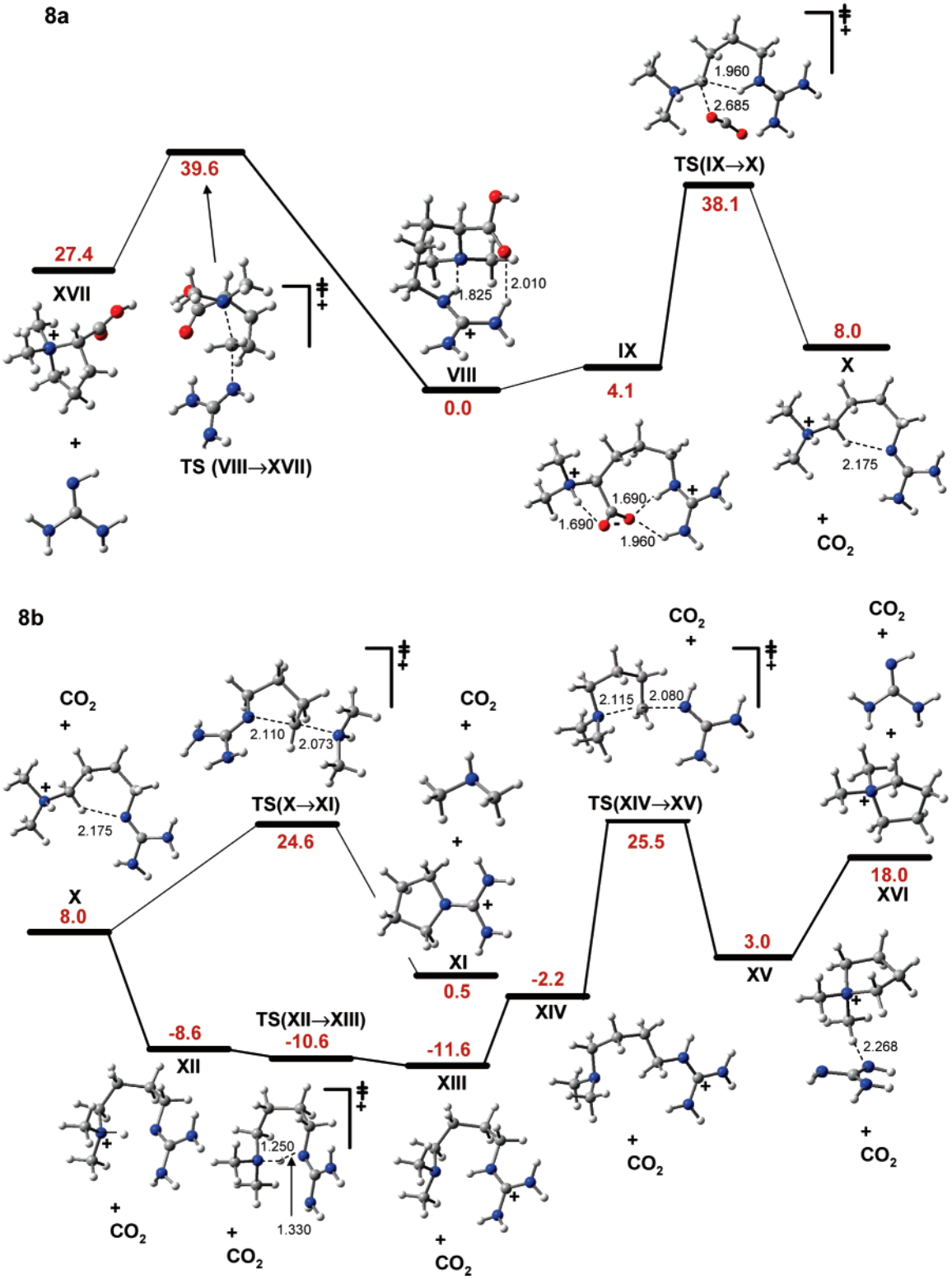


Figure 8. (a) and (b) Potential energy hypersurface for the loss of (CO_2 plus guanidine), (CO_2 plus $(CH_3)_2NH$), guanidine, and $(CH_3)_2NH$ from the protonated N_α,N_α-dimethylarginine, **2**. The numbers (in kcal/mol) are relative enthalpies at 0 K. All energies are relative to the global minimum of protonated N_α,N_α-dimethylarginine, **2**.

ϵ-carbon. Nucleophilic attack on the ϵ-carbon eliminates the α-trimethylamino group and results also in the *N*,*N*,*N*-trimethylcyclopentamine ion, but at 128 Th.

Computational Studies. Results of computational studies are consistent with the interpretations presented above. For tractability, we elected to calculate energy profiles on the dissociation of protonated arginine, **1**, and that of protonated N_α,N_α-dimethylarginine, **2**, as representatives of the class of molecules under investigation. In addition, as there was a recent computational study on protonated arginine also at the B3LYP/6-31G* level,[38] this provided a useful starting and calibrating point for our work.

Figure 7 shows the energy profile for protonated arginine leading to elimination of NH_3 and concomitantly CO plus NH_3. Consistent with the findings of Csonka et al.,[38] the canonical structure **I** of protonated arginine is the lowest energy isomer; the salt-bridged structure **II** is higher by 10.0 kcal mol^{-1}.

Nucleophilic attack by the COO^- group on the α-carbon and lengthening of the $N-C_\alpha$ bond result in structure **III**, an ion−molecule complex in which a lactone with a propylguanidinium ion side chain is solvated by NH_3. Migration of the solvating NH_3 to the guanidinium ion produces a lower-energy complex **IV**. The limiting barrier thus far is formation of **TS(II→III)** at 42.9 kcal mol^{-1}. Elimination of NH_3 from **IV** gives ion **V**; the overall endothermicity is 48.7 kcal mol^{-1}, which is higher than the barrier of 46.9 kcal mol^{-1} required for losing CO and NH_3 (**TS(IV→VI)**). Loss of CO requires first migration of an COO^- oxygen to the α-carbon, accompanied by lengthening of the $C_\alpha-CO$ bond. Ion **VI** and CO lie only 13.3 kcal mol^{-1} above **I**. Elimination of the solvating NH_3 in **VI** to form ion **VII** requires only additional 18.3 kcal mol^{-1}. As the combination of **VII** + CO + NH_3 lie below **TS(IV→VI)**, the loss of CO and NH_3 is expected to be experimentally concomitant. This conclusion is consistent with MS results shown earlier. Loss of CO from ion **V** has the highest barrier at 64.8 kcal mol^{-1} (**TS(V→VIII)**) and is noncompetitive. In agreement with this we found that the ion at 158 Th (protonated arginine$-NH_3$) does not lose CO to give an ion at 130 Th.

The lowest energy structure of protonated N_α,N_α-dimethylarginine is also canonical, **VIII**, and is structurally very similar to that of protonated arginine (Figure 8). Protonation on the guanidyl group results in effective charge delocalization within this group; in addition, the charge is further delocalized by hydrogen bonds from the NH of the guanidine to the dimethylamino group, and from one of the NH_2 groups to the carbonyl oxygen. Methylation renders the α-amino group more basic and stabilizes the salt-bridged structure, **IX**; as a consequence, in protonated N_α,N_α-dimethylarginine, **IX** lies only 4.1 kcal mol^{-1} above **VIII**. Loss of CO_2 has a barrier of 38.1 kcal mol^{-1} (**TS(IX→X)**). The initial product, ion **X**, is N_α,N_α-(dimethylamino)-δ-guanidylbutane. This ion, however, is not expected to be experimentally observable, as barriers against additional neutral losses, including dimethylamine (**TS(X→XI)** = 24.6 kcal mol^{-1}) and guanidine (**TS(XIV→XV)** = 25.5 kcal mol^{-1}) are all considerably lower than 38.1 kcal mol^{-1}. Again, these conclusions are consistent with MS results. Both ions **XI** and **XVI** are pyrrolidine derivatives; the former has a $C^+(NH_2)_2$ substituent on the ring nitrogen, and the latter has two CH_3 substituents on the ring nitrogen, which formally carries the positive charge. Loss of only guanidine from ion **VIII** to give ion **XVII**, *N*,*N*-dimethylproline has also a low barrier at 39.6 kcal mol^{-1} (**TS(VIII→XVII)**), consistent with experimental results.

Conclusions

The fragmentation chemistries of protonated arginine and its methyl derivatives share many similarities with those of protonated methylated lysines. Aside from elimination of the charged amino group as, for example, dimethylamine, trimethylamine and guanidine, losses of these neutrals concomitant with CO and CO_2 are also common. Both experiments and theory strongly suggest that, in these concomitant losses, the vibrationally hot dissociating complexes eliminate first the CO or CO_2 followed immediately by the amines, resulting in no observable intermediates.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada, MDS SCIEX and York University.

Supporting Information Available: Additional spectra are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Papayannopoulos, I. A. *Mass Spectrom. Rev.* **1995**, *14*, 49.
(2) Dongré, A. R.; Jones, J. L.; Somogi, Á.; Wysocki, V. H. *J. Am. Chem. Soc.* **1996**, *118*, 8365.
(3) McCormack, A. L.; Somogyi, Á.; Dongré, A. R.; Wysocki, V. H. *Anal. Chem.* **1993**, *65*, 2859.
(4) Jones, J. L.; Dongré, A. R.; Somogyi, Á.; Wysocki, V. H. *J. Am. Soc. Chem.* **1994**, *116*, 8368.
(5) Dongré, A. R.; Somogyi, Á.; Wysocki, V. H. *J. Mass Spectrom.* **1996**, *31*, 339.
(6) Gu, C.; Somogyi, Á.; Wysocki, V. H.; Medzihradszky, K. F. *Anal. Chim. Acta* **1999**, *397*, 247.
(7) Cox, K. A.; Gaskell, S. J.; Morris, M.; Whiting, A. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 522.
(8) Thorne, G. C.; Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 249.
(9) Ballard, K. D.; Gaskell, S. J. *Int. J. Mass Spectrom. Ion Processes* **1991**, *111*, 173.
(10) Burlet, O.; Yang, C. Y.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 337.
(11) Burlet, O.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 461.
(12) Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 477.
(13) Alexander, A. J.; Boyd, R. K. *Int. J. Mass Spectrom. Ion Processes* **1989**, *90*, 211.
(14) Alexander, A. J.; Thibault, P.; Boyd, R. K. *Int. J. Mass Spectrom. Ion Processes* **1990**, *98*, 107.
(15) Tang, X. J.; Thibault, P.; Boyd, R. K. *Anal. Chem.* **1993**, *65*, 2824.
(16) Yalcin, T.; Csizmadia, I. G.; Peterson, M. R.; Harrison, A. G. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 233.
(17) Yalcin, T.; Khouw, C.; Csizmadia, I. G.; Peterson, M. R.; Harrison, A. G. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 1165.
(18) Nold, M. J.; Wesdemiotis, C.; Yalcin, T.; Harrison, A. G. *Int. J. Mass Spectrom. Ion Processes* **1997**, *164*, 137.
(19) Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.
(20) Jones, J. L.; Dongàe A. R.; Somogyi, Á.; Wysocki, V. H. *J. Am. Chem. Soc*. **1994**, *116*, 8368.
(21) Summerfield, S. G.; Gaskell, S. J. *Int. J. Mass Spectrom.* **1997**, *165/166*, 509.
(22) Tang, X.; Boyd, R. K. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 651.
(23) Milne, G. W. A.; Axenrod, T.; Fales, H. M. *J. Am. Chem. Soc.* **1970**, *92*, 5170.
(24) Yalcin, T.; Harrison, A. G. *J. Mass Spectrom.* **1996**, *31*, 1237.
(25) Zwinselman, J. J.; Nibberring, N. M. M.; van der Greef, J.; Ten Noever de Brauw, M. C. *Org. Mass Spectrom.* **1983**, *18*, 525.
(26) Dookeran, N. N.; Yalcin, T.; Harrison, A. G. *J. Mass Spectrom.* **1996**, *31*, 500.
(27) Kulik, W.; Heerma, W. *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 9.
(28) Chait, B. T.; Agosta, W. C.; Field, F. H. *Int. J. Mass Spectrom. Ion Phys.* **1981**, *39*, 339.
(29) All proton affinities are obtained from the NIST chemistry webbook (http://webbook.nist.gov/chemistry/) except other sources are stated.
(30) Bannister, A. J.; Kouzarides, T. *Nature* **2005**, *436*, 1103.
(31) Julian, R. R.; Jarrold, M. F. *J. Phys. Chem. A* **2004**, *108*, 10861.
(32) Dearborn, N.; Jentoft, D. G. *Methods Enzymol.* **1983**, *91*, 570.
(33) Becke, A. D. *J. Chem. Phys*. **1988**, *88*, 1053.
(34) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev.* **1988**, *37*, 785.
(35) (a) Hariharan, P. C.; Pople, J. A. *Chem. Phys. Lett.* **1972**, *66*, 217. (b) Chandrasekhar, J.; Andrade, J. G.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1981**, *103*, 5609. (c) Chandrasekhar, J.; Spitznagel, G. W.; Schleyer, P. v. R. *J. Comput. Chem.* **1983**, *4*, 294.
(36) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Peterson A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.6; Gaussian, Inc.: Pittsburgh, PA, 1998.
(37) Gonzales, C.; Schlegel, H. B. *J. Chem. Phys*. **1989**, *90*, 2154.
(38) Csonka, I. P.; Paizs, B.; Suhai, S. *J. Mass Spectrom.* **2004**, *39*, 1025.
(39) Kallies, B.; Mitzner, R. *J. Phys. Chem. B* **1997**, *101*, 2959.
(40) Rodriquez, C. F.; Vuckovic, D. L.; Milburn, R. K.; Hopkinson, A. C. *J. Mol. Struct. (THEOCHEM)* **1997**, *401*, 117.