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Molecular Radical Cations of Oligopeptides

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An unprecedented method of producing molecular radical cations of oligopeptides in the gas phase has been discovered. Electrospraying a methanolic mixture of a Cu(II)−amine complex, e.g., $Cu^{II}(dien)(NO_3)_2$ (where dien = diethylenetriamine), and an oligopeptide (M) yields the $[Cu^{II}(dien)M]^{\bullet 2+}$ ion, whose collision-induced dissociation (CID) produces $[Cu^{I}(dien)]^+$ and $M^{\bullet +}$, the molecular cation of the oligopeptide. Abundant $M^{\bullet +}$ is apparent when the oligopeptide contains both a tyrosyl and a basic residue—arginyl, lysyl, or histidyl. These structural requirements are similar to those in the metalloradical enzyme process in photosystem II. Tandem mass spectrometry of $M^{\bullet +}$ produces fragment ions that are both common to and also different from $[M + H]^+$. The fragmentation chemistry of $M^{\bullet +}$ and of its products appear to be radical driven.

Protein radicals have generated a lot of recent interest because of their unusual role in catalyzing a number of important reactions,[1] including the oxidation of water to oxygen for use in a photosynthesis system in plants and algae.[1a,b] A common theme in these protein radicals is that they are synthesized post-translationally and their formation involves metallo cofactors located either adjacent to the amino acid residue being oxidized or on a second subunit or activating enzyme that participates in the oxidation.[1] Frequent radical sites are located on the glycyl, tyrosyl, and tryptophanyl residues; the structure of the glycyl radical in pyruvate formate lyase has been found to be planar and maintain a gas-phase-like structure, despite being embedded in the protein.[1d]

Here we report results of a serendipitous discovery of an unprecedented route for generating molecular radical cations of oligopeptides in the gas phase. Some of the conditions under which these oligopeptide radical cations are generated bear a resemblance to those in vivo for protein radicals. It is noteworthy that this discovery centers on molecular radical cations as opposed to the more frequently encountered radical ions produced from protonated peptides capturing an electron[2] and metal-bearing peptide ternary complexes.[3] Although electron ionization (EI) is the most widely used method in mass spectrometry to generate radical cations, it is not amenable to oligopeptides because of their low volatility. Very few dipeptides and, as far as we know, only one tripeptide have been successfully ionized in this manner; their mass spectra were rich and contained a wealth of sequencing information.[4] We are reporting herein that electrospraying a methanolic mixture of a Cu(II)−amine complex, e.g., $Cu^{II}(dien)(NO_3)_2$ (where dien = diethylenetriamine), and an oligopeptide (M) yields the $[Cu^{II}(dien)M]^{\bullet 2+}$ ion, whose collision-induced dissociation (CID) produces $[Cu^{I}(dien)]^+$ and $M^{\bullet +}$, the odd-electron (OE) molecular cation of the oligopeptide. Abundant $M^{\bullet +}$ is apparent only when the oligopeptide contains a tyrosyl and a basic residue—arginyl, lysyl, or histidyl.

Figure 1 shows the product ion spectra of the $[Cu(dien)M]^{\bullet 2+}$ complex of leucine enkephalin−Arg, YGGFLR;[5] the precursor ions, m/z = 438.4 and 439.9, contain respectively and principally ^{63}Cu and ^{65}Cu. The CID is straightforward and results in $[Cu(dien)]^+$ (m/z = 166.2 and 168.1 for ^{63}Cu and ^{65}Cu, respectively) and $M^{\bullet +}$ (m/z = 711.5), which cleaves facilely to the ion at m/z = 605.6; the 106 Da neutral product that is eliminated is likely to be *p*-quinomethide from the tyrosyl side chain. The presence of the basic arginine residue is crucial to the formation of $M^{\bullet +}$. The CID of the $[^{63}Cu(dien)M]^{\bullet 2+}$ ion of leucine enkephalin, YGGFL, under similar conditions yielded $[Cu(M - H)]^+$ (m/z = 617.3), $[M + H]^+$ (m/z = 556.1) and $[M + H - 108]^+$ (m/z = 448.5); the 108 Da neutral fragment eliminated from protonated YGGFL is likely to be *p*-cresol. Replacing the tyrosyl residue in $[Cu(dien)M]^{\bullet 2+}$ of YGGFLR with the tryptophanyl residue, i.e., M = WGGFLR, and performing MS/MS resulted in mostly $[Cu(M - H)]^+$, $[M + H]^+$, and some $M^{\bullet +}$ (less than 10% of the $[M + H]^+$ abundance). A similar replacement with the methionyl residue, i.e., M = Met−GGFLR, however, produced only $[Cu(M - H)]^+$ and $[M + H]^+$, and no $M^{\bullet +}$. The presence of Cu^{2+} is also essential—replacing Cu^{2+} in $[Cu(dien)M]^{\bullet 2+}$ of YGGFLR with Ni^{2+} yielded only $[M + H]^+$. The nature of the nitrogen ligand chelating to the Cu^{2+}, however, is less crucial; CID of both $[Cu(Me_5dien)M]^{\bullet 2+}$ (where Me_5-dien = *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine) and $[Cu(terpy)M]^{\bullet 2+}$ (terpy = 2,2′:6′,2″-terpyridine) of YGGFLR resulted in a high abundance of $M^{\bullet +}$.

Copper(II) complexes containing amine and pyridine ligands are typically five-coordinate with a distorted square pyramidal or trigonal bipyramidal geometry.[6] It is likely that in $[Cu(dien)M]^{\bullet 2+}$, the dien coordinates to Cu^{II} in a meridonal fashion and that the oligopeptide acts as a bidendate ligand. We are postulating that the $[Cu(dien)M]^{\bullet 2+}$ precursor ion structure that subsequently fragments to yield $[Cu(dien)]^+$ and $M^{\bullet +}$ has the phenolic oxygen of the tyrosyl side chain ligating to the Cu^{2+}; the other ligating atom can, for example, be the N-terminal

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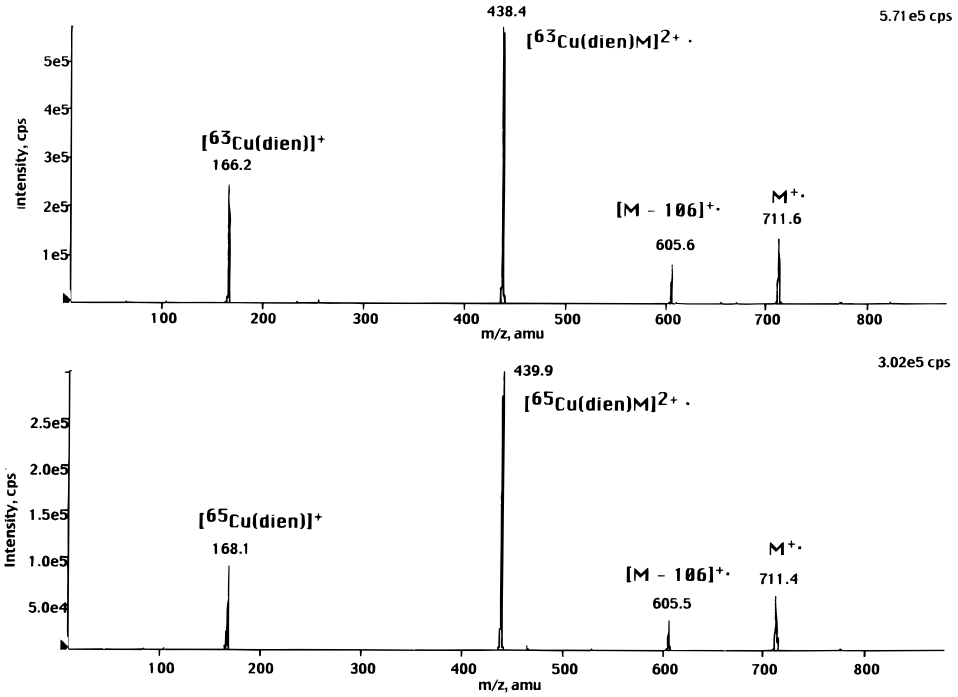


Figure 1. Product ion spectra of (a) $[^{63}Cu(dien)M]^{2+}$ and (b) $[^{65}Cu(dien)M]^{2+}$, where M = YGGFLR, center-of-mass energy (E_{cm}) = 1.3 eV.

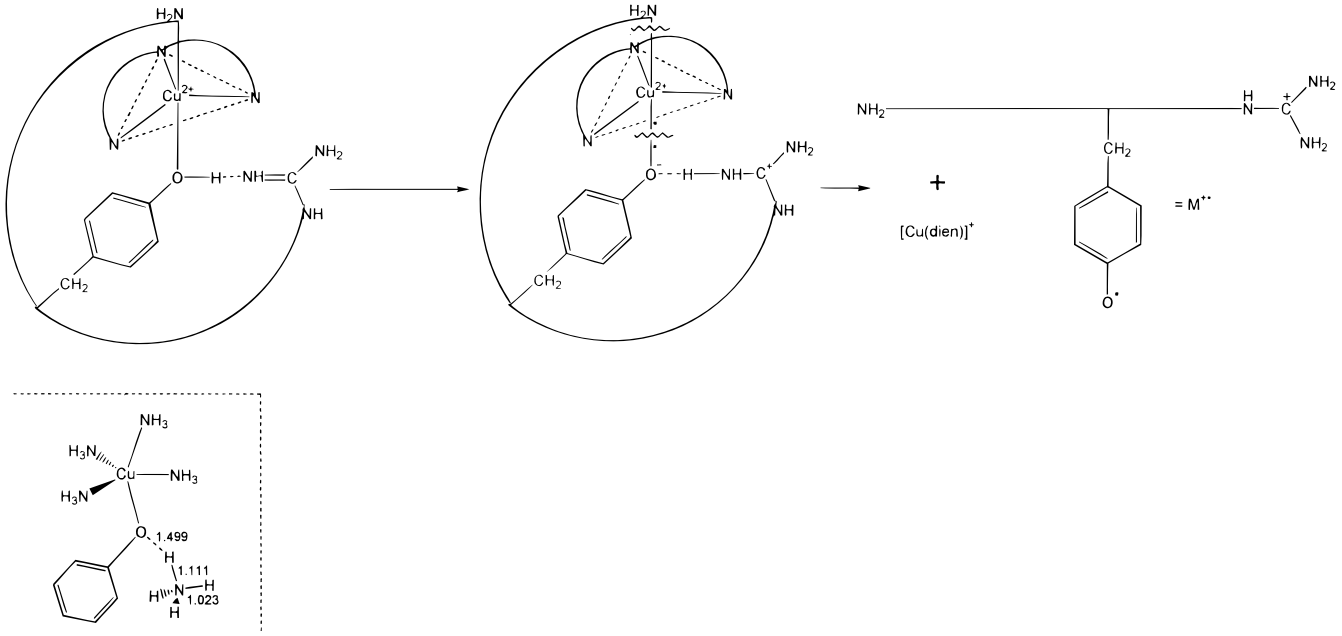


Figure 2. Proposed mechanism for the formation of $M^{\bullet+}$. The lower left corner shows the DFT-optimized geometry of a model complex (see text for details); bond distances are in ångstroms.

nitrogen or a carbonyl oxygen,[3] with the imino nitrogen on the side chain of the basic residue hydrogen-bonded to the phenolic hydrogen atom (Figure 2). Collisional activation results in proton transfer from the phenolic oxygen to the imino nitrogen of the arginine side chain. Homolytic cleavage of the formally $Cu^{2+}-O^{-}$ bond along with dissociation of the $Cu^{2+}\leftarrow N$ bond at the N-terminus produces $[Cu(dien)]^{+}$ and $M^{\bullet+}$. Analogous reduction of Ru^{III} by a tyrosyl radical with a neighboring strong base has been observed to take place in solution.[7] The deprotonation, reduction, and formation of the tyrosyl radical parallel the reduction of the chlorophyll complex, P680.[1b]

Support for this mechanism is provided by molecular orbital calculations. Geometric optimization using density functional theory (DFT) at the UB3LYP/6-31++G(d,p) level of theory on a model complex between trigonal bipyramidal $Cu^{2+}(NH_3)_4(C_6H_5OH)$, in which Cu^{2+} is bound to the four ammonia nitrogen atoms and the phenolic oxygen atom, and a fifth ammonia molecule is hydrogen-bonded to the phenolic hydrogen atom, results in a minimal energy structure wherein the phenolic hydrogen has jumped to the fifth ammonia nitrogen to produce an ammonium ion while remaining hydrogen-bonded to the oxygen atom[8] (see inset in Figure 2).

The product ion spectra of $M^{\bullet+}$ are very different from those of corresponding $[M + H]^{+}$ and display rich fragmentation chemistry. Figure 3 shows a comparison of the product ion spectra of these two ions of YAGFLR. It is apparent that CID of the $[M + H]^{+}$ leads to conventional b_n, a_n, and y_n'' ions,[9] whereas that of the $M^{\bullet+}$ results in facile cleavage of *p*-

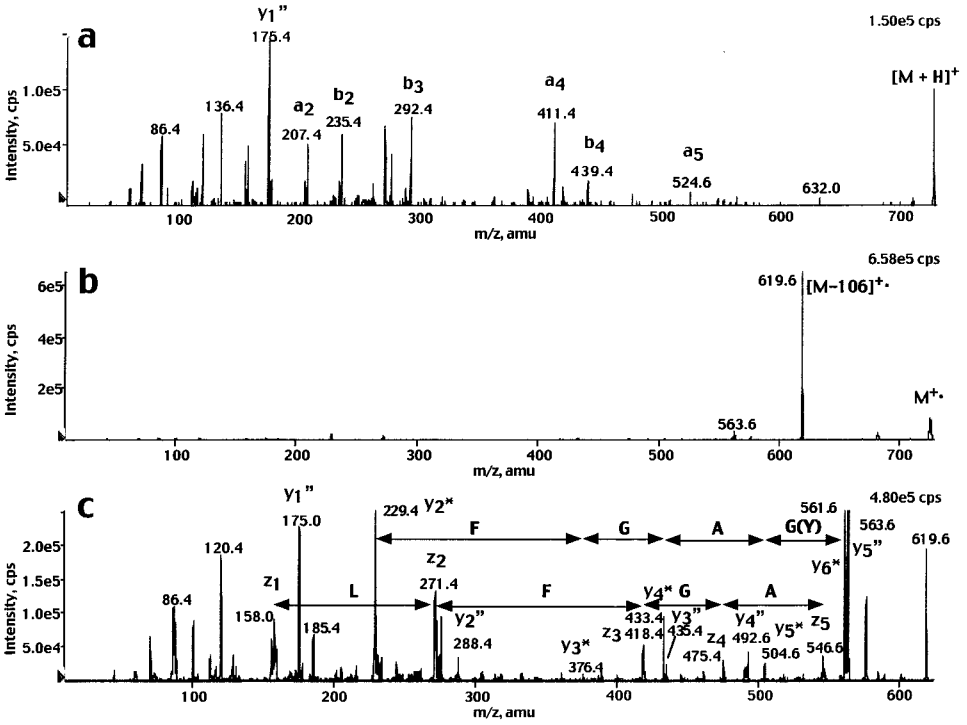
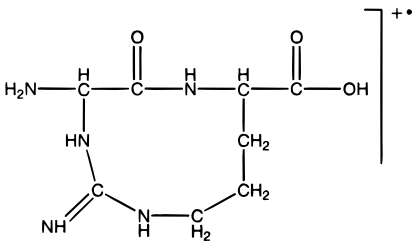


Figure 3. Product ion spectra of M = YAGFLR: (a) $[M + H]^+$, $E_{cm} = 1.3$ eV; (b) $M^{\bullet+}$, $E_{cm} = 1.0$ eV; (c) $[M - 106]^{\bullet+}$, $E_{cm} = 1.5$ eV. The y_2* ion is likely to be



The cleavage of the tyrosyl residue that has lost *p*-quinomethide results in a *m*/*z* reduction that is identical to the cleavage of a glycyl residue.

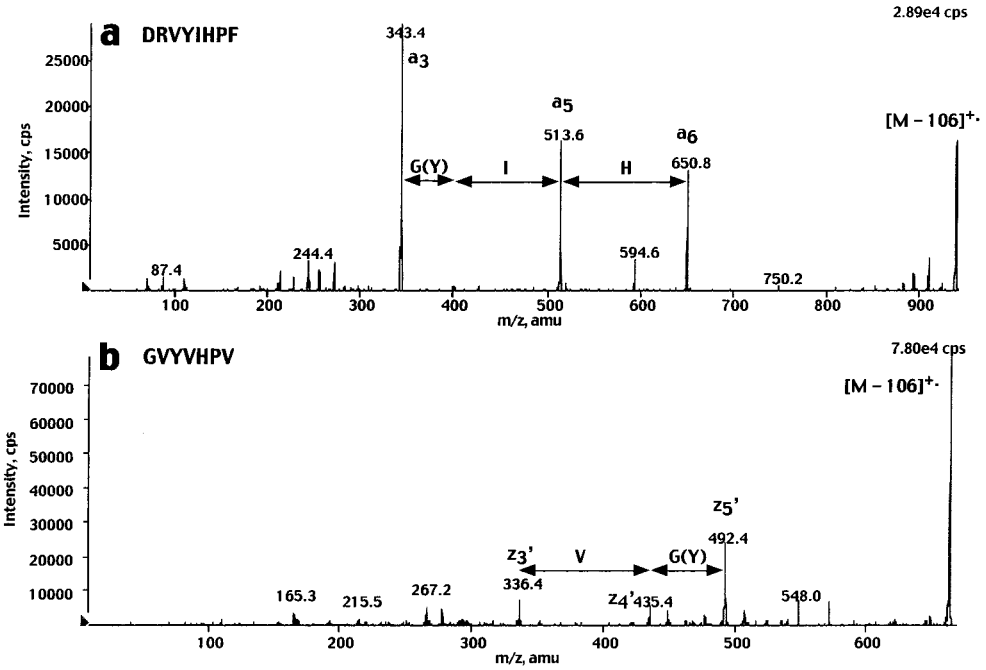
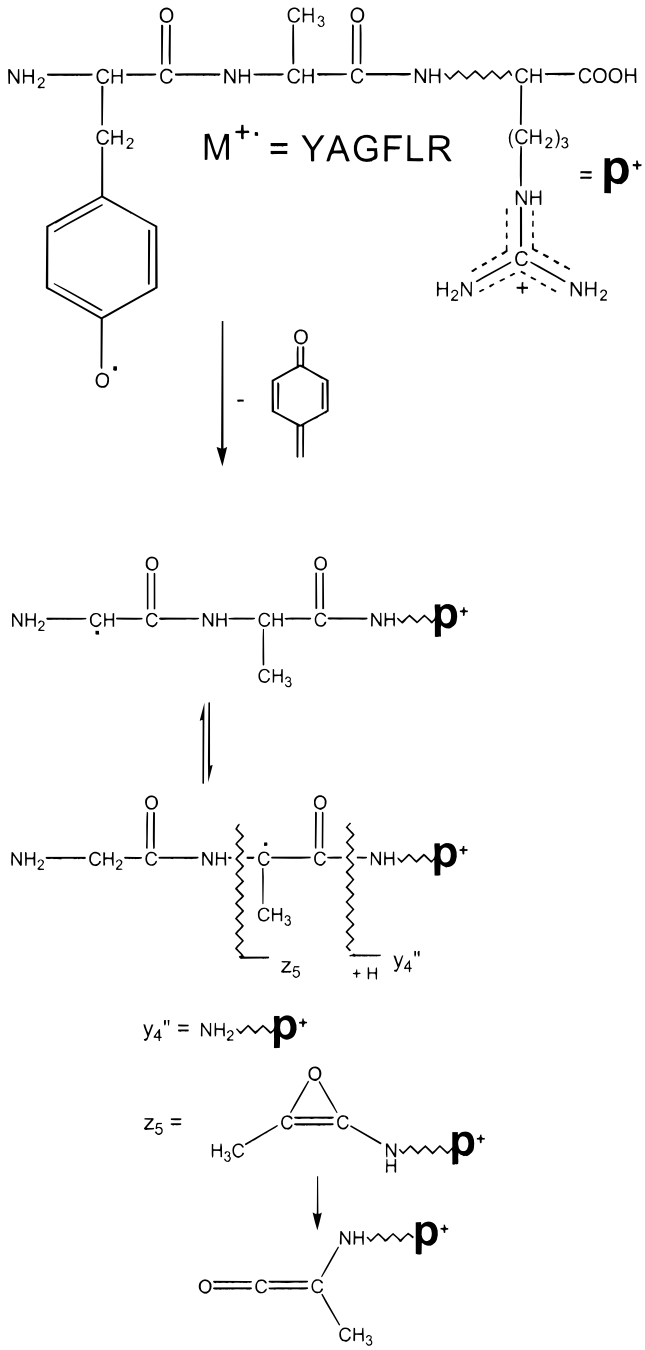


Figure 4. Product ion spectra of the $[M - 106]^{\bullet+}$ ions of (a) angiotensin II, DRVYIHPF, $E_{cm} = 0.75$ eV, and (b) angiotensin III anti-peptide, GVYVHPV, $E_{cm} = 1.1$ eV. The cleavage of the tyrosyl residue that has lost *p*-quinomethide results in a *m*/*z* reduction that is identical to the cleavage of a glycyl residue.

SCHEME 1



quinomethide to form the product ion at *m*/*z* 619.6 (Figure 3b). This product ion fragments to form other product ions at higher collision energies, and these are more apparent in the product ion spectrum of the $[M - 106]^{\bullet+}$ ion at *m*/*z* 619.6 (Figure 3c). Three series of product ions are evident, the even electron (EE) y_n'', the EE z_n, and the OE y_n*. The rich and diverse fragmentation chemistry of the peptide radical cations is emphasized in Figure 4, where the product ion spectra of the $[M - 106]^{\bullet+}$ ions of (a) angiotensin II (DRVYIHPF) and (b) angiotensin III anti-peptide (GVYVHPV) are displayed. In the former, the EE a_n are the abundant fragment ions, whereas in the latter, the OE z_n' ions predominate. All fragment ions contain the basic residue, in accordance with the expectation that its side chain functional group is protonated to form $M^{\bullet+}$. CID results indicate that the fragmenting $[M - 106]^{\bullet+}$ ion is likely to have a protonated basic residue, which is in agreement with the expectation that a highly basic residue, such as arginine, sequesters the proton.[10] Furthermore, hydrogen atom transfer from the protonated amino site to the radical site is not expected to be energetically favorable ($\Sigma\Delta_f H°(NH_4^+,CH_3^{\bullet}) = 150.7 + 34.8 = 185.5$ kcal/mol; $\Sigma\Delta_f H°(NH_3^{\bullet+},CH_4) = 223.2 - 17.8 = 205.4$ kcal/mol)[11] and is apparently only observed in the y_n* series of YGGFLR and YAGFLR. Nevertheless, even in the y_n* ions, the charge is localized on the arginyl side chain. (For the model peptide backbone, $[NH_2CHCONH_2]^{\bullet}$, DFT calculations at the UB3LYP/6-31++G(d,p) level of theory show that $NH_2C^{\bullet}HCONH_2$ is 17.0 kcal/mol ($\Delta E_{electronic}$) more stable than $NH_2CHC(OH)N^{\bullet}H$, 18.3 kcal/mol more stable than $^{\bullet}NHCH_2CONH_2$, and 33.2 kcal/mol more stable than $NH_2CH_2CONH^{\bullet}$.)

It appears that the rich fragmentation chemistry is induced by the radical, which is mobile and may be passed from one peptide linkage to the next. Subsequent rearrangement reactions proximal to the radical lead to y_n'' and z_n ions. The former are common fragment ions from $[M + H]^+$ but are of low abundance from peptides that have an arginyl side chain,[9,10] while the latter are rarely observed in the fragmentation of $[M + H]^+$ under collision energies <100 eV. The formation of the ketene structure of the z_n ion via the oxirene intermediate is the analogue of the gas-phase Wolff rearrangement for ketocarbenes.[12]

Although we are only witnessing the first few examples of the gas-phase fragmentation chemistry of the $M^{\bullet+}$ of oligopeptides, the glimpses that we have suggest a picture of fascinating and rich radical-induced chemistry. The availability of fragmentation channels that are different from and complementary to those of protonated peptides will provide extra sequence information to that available today in MS/MS of protonated peptides.

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References and Notes

(1) (a) Stubbe, J.; van der Donk, W. A. *Chem. Rev.* **1998**, *98*, 705−762. (b) Tommos, C.; Babcock, G. T. *Acc. Chem. Res.* **1998**, *31*, 18−25. (c) Knappe, J.; Neugebauer, F. A.; Blaschkowski, H. P.; Gänzler, M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1332−1335. (d) Himo, F.; Eriksson, L. A. *J. Chem. Soc., Perkin Trans.* **1998**, 305−308.

(2) (a) Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *J. Am. Chem. Soc.* **1998**, *120*, 3265−3266. (b) Kruger, N. A.; Zubarev, R. A.; Horn, D. M.; McLafferty, F. W. *Int. J. Mass Spectrom.* **1999**, *185/186/187*, 787−793.

(3) (a) Gatlin, C. L.; Rao, R. D.; Tureček, F.; Vaisar, T. *Anal. Chem.* **1996**, *68*, 263−270. (b) Vaisar, T.; Gatlin, C. L.; Tureček, F. *J. Am. Chem. Soc.* **1996**, *118*, 5314−5315.

(4) (a) Biemann, K. *Mass Spectrometry Organic Chemical Applications* McGraw-Hill: New York, 1962; pp 294−296. (b) Biemann, K.; McCloskey, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 3192−3193.

(5) Experiments were performed on a PE-SCIEX API 365 and API III. Pseudo MS^3 experiments were performed by inducing fragmentation in the source, isolating the fragment ion of interest in the first quadrupole, inducing further fragmentation in the second quadrupole, and mass-analyzing in the third quadrupole.

(6) Henke, W.; Kremer, S.; Reinen, D. *Inorg. Chem.* **1983**, *22*, 2858−2863.

(7) Sun, L.; Burkitt, M.; Tamm, M.; Raymond, M. K.; Abrahamsson, M.; LeGourriérec, D.; Frapart, Y.; Magnuson, A.; Kenéz, P. H.; Brandt, P.; Tran, A.; Hammarström, L.; Styring, S.; Åkermark, B. *J. Am. Chem. Soc.* **1999**, *121*, 6834−6842.

(8) DFT calculations were performed using Gaussian 98, Revision A.5: 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., Jr.; 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.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J.

V.; Stefarnov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian Inc.: Pittsburgh, PA, 1998.

(9) Papayannopoulos, I. A. *Mass Spectrom. Rev.* **1995**, *14*, 49−73.

(10) Cox, K. A.; Gaskell, S. J.; Morris, M.; Whiting, A. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 522−531.

(11) Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. 1.

(12) (a) Csizmadia, I. G.; Font, J.; Strausz, O. P. *J. Am. Chem. Soc.* **1968**, *90*, 7360−7361. (b) Hopkinson, A. C.; Lien, M.; Yates, K.; Csizmadia, I. G. A Non-Empirical Molecular Orbital Study of Oxirene and its Valence Tautomers. In *Applications of MO Theory in Organic Chemistry*; Csizmadia, I. G., Ed.; Progress in Theoretical Organic Chemistry, Vol. 2; Elsevier: Amsterdam, 1977; pp 230−247.