Structure of the [M + H − H 2 O] + Ion from Tetraglycine: A Revisit by Means of Density Functional Theory and Isotope Labeling

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY A · JANUARY 2011

Impact Factor: 2.69

CITATION
1

READS
8

7 AUTHORS, INCLUDING:

Michael J Van Stipdonk
Duquesne University
143 PUBLICATIONS **2,833** CITATIONS
SEE PROFILE

Benjamin J. Bythell
University of Missouri - St. Louis
36 PUBLICATIONS **241** CITATIONS
SEE PROFILE

THE JOURNAL OF PHYSICAL CHEMISTRY A

ARTICLE

pubs.acs.org/JPCA

Structure of the $[M + H - H_2O]^+$ Ion from Tetraglycine: A Revisit by Means of Density Functional Theory and Isotope Labeling

Udo H. Verkerk,[†] Junfang Zhao,[†] Michael J. Van Stipdonk,[‡] Benjamin J. Bythell,[§,#] Jos Oomens,[∥,⊥] Alan C. Hopkinson,[†] and K.W. Michael Siu*[,†]

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

[‡]Department of Chemistry, Wichita State University, Wichita, Kansas 67260-0051, United States

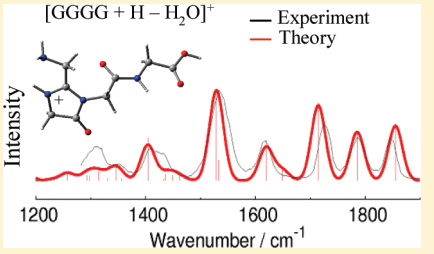
[§]Computational Proteomics Group, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, 69120, Heidelberg, Germany

[∥]FOM Institute for Plasma Physics, 3430 BE Nieuwegein, The Netherlands

[⊥]University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands

Supporting Information

ABSTRACT: Collision-induced dissociations of protonated ^{18}O-labeled tetraglycines labeled separately at either the first or the second amide bond established that water loss from the backbone occurs from the N-terminal residue. Density functional theory at B3LYP/6-311++G(d,p) predicted that the low-energy $[G_4 + H - H_2O]^+$ product ion is an N_1-protonated 3,5-dihydro-4*H*-imidazol-4-one. The ion at the lowest energy, **III**, is 24.8 kcal mol^{-1} lower than the protonated oxazole structure, **II**, proposed by Bythell et al.(*J. Phys. Chem A* **2010**, *114*, 5076−5082). In addition, structure **III** has a predicted IR spectrum that provides a better match with the published experimental IRMPD spectrum than that of structure **II**.



INTRODUCTION

Collision-induced dissociation (CID) of protonated peptides in the gas phase constitutes the basis for protein identification in proteomics.[1−3] The mass-to-charge (m/z) ratios of the fragment ions are matched to those of theoretical fragments predicted from known dissociation chemistries of protonated peptides, generated from in silico tryptic digestion of proteins in a database. The best match is considered to have identified the peptide/protein. Our understanding of the dissociation chemistries of protonated peptides has undergone a series of improvements and refinements in the last couple of decades. Tandem mass spectrometry (MS/MS) of isotopically labeled peptides coupled with that of purported intermediates has been instrumental in the development and refinement of these chemistries.[4−23] In addition, infrared multiple-photon dissociation (IRMPD) spectroscopy has evolved into an arbitrator for the structures of key intermediates and products in these reactions.[24−30]

In a recent article, Bythell et al.[31] reported results of an investigation on the structure of the $[M + H - H_2O]^+$ ion from tetraglycine (G_4) using MS/MS as well as IRMPD spectroscopy; in addition, they used density functional theory (DFT) to support their assignments. A key proposal from this work and its predecessors[32−34] was that $[G_4 + H - H_2O]^+$ does not have a protonated oxazolone structure, **I** (see Scheme 1), but instead a different, substituted oxazole structure situated near the N-terminus, structure **II**; this is despite the fact that **II** is higher in enthalpy at 0 K than **I** by 14.7 kcal mol^{-1}.[31] Such situations are not without precedent in mass spectrometry (e.g., protonated diketopiperazines are energetically more favorable than protonated oxazolones, yet the latter were experimentally observed).[9,16,17,24−26] Of the various substituted 5- and 6-membered ring candidate structures considered,[31−34] the most probable and best fit to experimental results was this oxazole structure, **II**. This was also the only structure considered that offered a reasonable explanation for the loss of 29 Da in the MS^3 experiment and the resulting IRMPD spectrum.[31] Despite this rationale, the data obtained were far from a perfect match and thus indicate the possibility of additional or alternative structures, which were not considered in that study[31] but could work better.

Stimulated by these observations, we pursued further examination of the dehydration product of protonated G_4 by means of DFT calculations and MS/MS of isotopically labeled protonated G_4; the latter experiments were designed to determine from which amide group the water is lost. Herein we report that these investigations have led us to postulate new structures for the $[M + H - H_2O]^+$ ion of G_4 that are much lower in energy than both **I** and **II**; in addition, the predicted IR spectrum of **III** matches the

Received: March 25, 2011
Revised: April 28, 2011

   dx.doi.org/10.1021/jp202820h | *J. Phys. Chem. A* XXXX, XXX, 000–000

Scheme 1

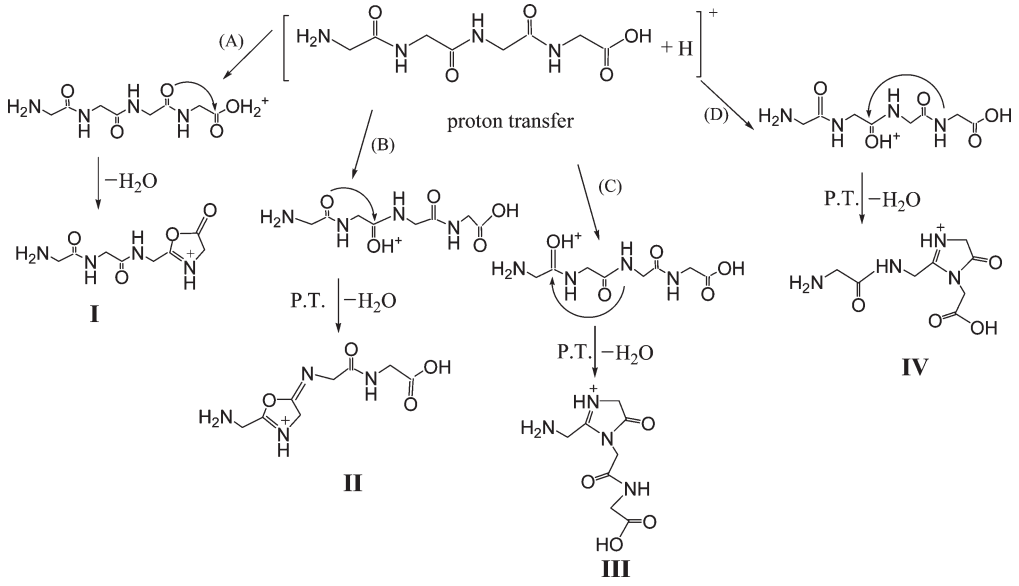


Table 1. Relative ($E_{relative}$) Enthalpies at 0 K of I−IV Shown in Scheme *1*

isomers	$E_{relative}$/kcal mol^{-1} (B3LYP/6-31+G(d,p)//(B3LYP/6-31G(d))[a]	$E_{relative}$/kcal mol^{-1} (B3LYP/6-311++G(d,p))
I	0.0	0.0
II	14.7	15.1
III	−9.4	−9.7
IV	−7.8	−8.0

[a] Reference 31.

experimental IRMPD spectrum more closely than that of the previously postulated **II**.[31] Similar results were also obtained for the $[M + H - H_2O - 29]^+$ ion.

EXPERIMENTAL SECTION

Peptide Synthesis. ^{18}O-Labeled peptides were synthesized using conventional solid-phase synthetic methods.[35] $H_2{}^{18}O$ was purchased from Medical Isotopes Inc. (Pelham, NH). Fmoc-glycine-loaded Wang resin and Fmoc-protected glycine (where Fmoc is 9-fluorenylmethyloxycarbonyl) were available from Advanced ChemTech (Louisville, KY). All other chemicals and reagents were available from Sigma-Aldrich (St. Louis, MO). Fmoc-protected ^{18}O-labeled glycine was synthesized according to Marecek et al.[36] Labeled peptides, once cleaved from the resin, were used without further purification. The peptide sequence and location of the ^{18}O label were verified by multiple-stage tandem mass spectrometry using an API 2000 linear ion trap prior to CID studies.

Mass Spectrometry. Experiments were performed on a quadrupole ion-trap mass spectrometer (LCQ, Finnigan-MAT, San Jose, CA) and a prototype of the API 2000 linear ion-trap mass spectrometer (MDS SCIEX), both equipped with an electrospray ionization source. Helium was used as the CID gas in the former, while nitrogen was used in the latter instrument.

DFT. Geometry optimizations and harmonic vibrational frequencies were calculated using the Gaussian 03 suite of programs[37] at the B3LYP/6-31G(d), B3LYP/6-31+G(d,p), and B3LYP/6-311++G(d,p) levels. Relative energies and IR spectra were predicted at the B3LYP/6-311++G(d,p) level. Wavenumbers of the calculated IR spectra were scaled by a factor of 0.984.

RESULTS AND DISCUSSION

A thorough search of the potential energy hypersurface of protonated G_4 using DFT at the B3LYP/6-31+G(d,p) level led to novel, low-energy product ions, **III** and **IV**, via the loss of water. Possible mechanisms that result in these products along with **I** and **II** are summarized in Scheme 1, and the optimized structures of **I**−**IV** are given in the Supporting Information, Figure S1. Both **III** and **IV** are N_1-protonated 3,5-dihydro-4*H*-imidazol-4-ones formed by eliminating water-containing oxygen that originated from a backbone amide; **III** is from the first peptide bond (the peptide bond that is closest to the N-terminus), while **IV** is from the second peptide bond. Earlier studies on loss of water from the backbone, which suggested a retro-Ritter reaction, concluded that the water loss occurred from the second peptide bond.[31−34] By contrast, **I** is a classical oxazolone formed by eliminating water from the carboxylic acid group, and **II** is an oxazole derivative formed by the loss of water postulated to be from the second peptide linkage.[31−34] (We found that structure **II** could also be formed by loss of water from the first peptide linkage, although this had not been postulated.[31−34]) Significantly, **III** and **IV** are considerably lower in energy than **I** and **II** (see Table 1).

Structure **III** is lower in energy than **I** and **II** by 9.4 and 24.1 kcal mol^{-1}, respectively, at the B3LYP/6-31+G(d,p) + ZPE (B3LYP/6-31G(d) level of theory; structure **IV** lies 1.6 kcal mol^{-1} above **III**. Increasing the level of theory to B3LYP/6-311++G(d,p) slightly increases the energy differences: structures **III** and **IV** are now 24.8 and 23.1 kcal mol^{-1} below **II**.

Figure 1 shows experimental support for the formation of **III** from the CID of protonated G_4. A comparison of the CID

dx.doi.org/10.1021/jp202820h |*J. Phys. Chem. A* XXXX, XXX, 000–000

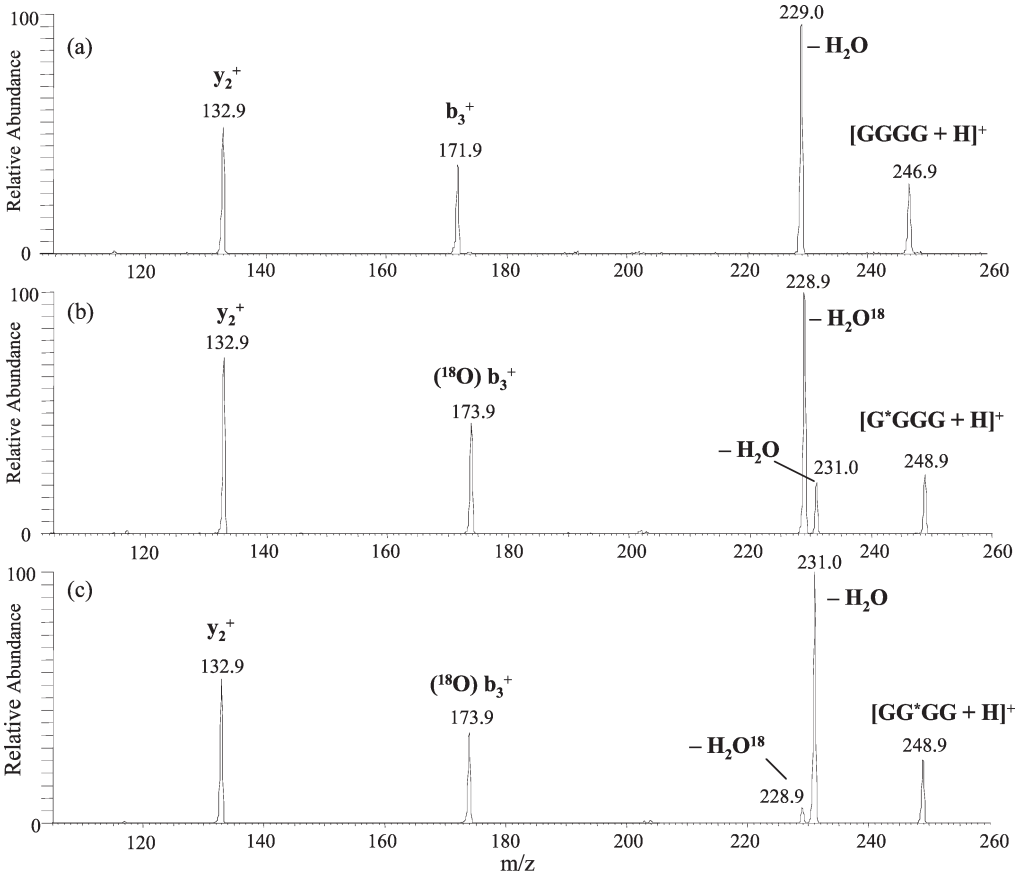


Figure 1. MS/MS spectra of protonated (a) GGGG, (b) G*GGG, and (c) GG*GG from the LCQ instrument (relative collision energy = 16%).

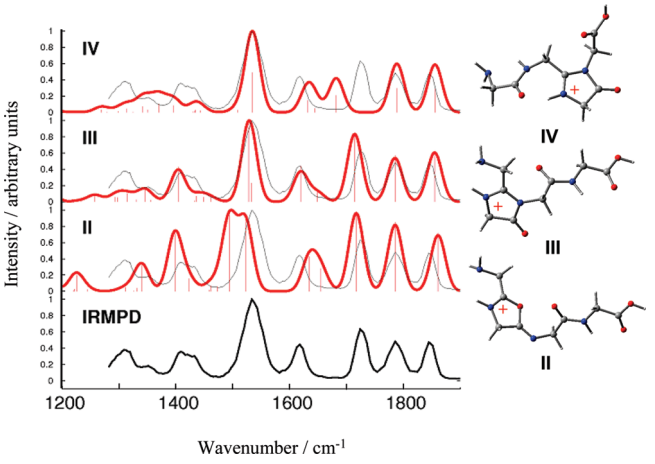


Figure 2. IRMPD spectrum[31] of the $[G_4 + H - H_2O]^+$ ion, and IR absorption spectra for **II**, **III**, and **IV** predicted at the B3LYP/6-311++G(d,p) level (fwhm = 30 cm^{-1}).

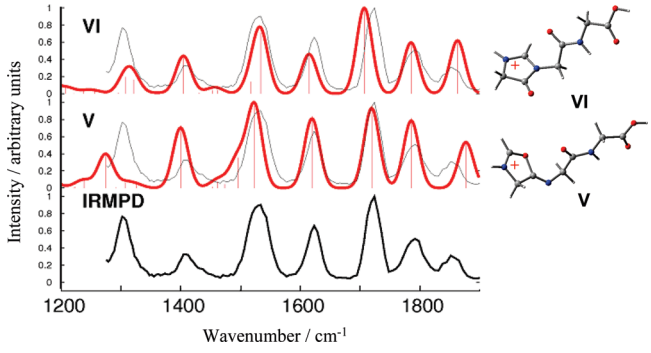


Figure 3. IRMPD spectrum[31] of the $[G_4 + H - H_2O - 29]^+$ ion, and IR absorption spectra for **V** and **VI** predicted at the B3LYP/6-311++G(d,p) level (fwhm = 30 cm^{-1}).

spectrum of G*GGG, or $(NH_2CH_2C^{18}O)$GGG (Figure 1b) and that of GG*GG or G$(NHCH_2C^{18}O)$GG (Figure 1c) clearly shows the loss of water involves elimination of the oxygen from the *first* peptide linkage. Elimination of this oxygen can result in formation of **III** or **II**. There is also evidence for some water loss predominately from the carboxy group (~20%).

Additional support that structure **III** is the predominant experimental product ion is provided by IRMPD spectroscopy. Figure 2 shows a comparison of predicted IR spectra of **III** and **IV** along with that of **II** and the experimental IRMPD spectrum.[31] It is apparent that the predicted IR spectrum of **III** produces the best match to the IRMPD experiment. The strong IR absorption at ~1855 cm^{-1} is due to stretching of the C=O bond exocyclic to the imidazolidinone ring in **III**, whereas it was originally assigned to that of the C=N bond exocyclic to the oxazole ring in **II**.[31] The two prominent bands between 1700 and 1800 cm^{-1} are attributable to amide and carboxylic C=O stretches, as in Bythell et al.[31] The strong band at ~1530 cm^{-1} is in good agreement with the C−N stretch in the imidazolidinone ring and the N−H wag of the amide group in structure **III**. It is of note that the IR

dx.doi.org/10.1021/jp202820h |J. Phys. Chem. A XXXX, XXX, 000–000

spectrum of structure **II** contains a strong, unresolved absorption band at ~1490 cm^{-1} that is absent in the IRMPD spectrum, while no such apparent disagreement exists for **III**. The small absorption band at ~1620 cm^{-1} is assignable to the C=N(H) stretch in the imidazolidinone of **III** coupled to the N−H scissor mode of the free amino group at the N-terminus; these assignments are analogous to those of Bythell et al.[31]

CID of the $[M + H - H_2O]^+$ ion of G_4 produced a major secondary product ion via the loss of 29 Da, assigned as methanimine, NH=CH_2.[31] The optimized structures, **V** and **VI**, of the $[M + H - H_2O - 29]^+$ ion, originating from the CID of **II** and **III**, respectively, are shown in the Supporting Information, Figure S1. These secondary product ions are formed via elimination of the N-terminus as methanimine, which preserves the oxazole structure of **II** in **V** and the imidazolidinone structure of **III** in **VI**. Structure **VI** is lower in energy than **V** by 28.9 kcal mol^{-1} at the B3LYP/6-311++G(d,p) level of theory. In addition, the predicted IR spectrum of **VI** provides a slightly better match to the IRMPD spectrum of the $[M + H - H_2O - 29]^+$ ion than that of **V** with the exception of the band at 1720 cm^{-1} (Figure 3).

CONCLUSION

All new evidence shown herein strongly suggest that the dehydration product of protonated G_4 during CID is formed predominantly by the loss of water from the N-terminal amide group. The resulting product is an N_1-protonated 3,5-dihydro-4*H*-imidazol-4-one, structure **III**, an ion that can eliminate methanimine from the N-terminus to give structure **VI**, thereby preserving the imidazolidinone structure. Both ions **III** and **VI** are the lowest energy structures on their potential energy surfaces.

ASSOCIATED CONTENT

Supporting Information. Optimized structures and tables of total energies and Cartesian coordinates of the structures, **I**−**VI**, determined at the B3LYP/6-311++G(d,p) level. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: (416)650-8021. Fax: (416)736-5936. E-mail: kwmsiu@yorku.ca.

Present Address

#Current address: Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310-4005, United States

ACKNOWLEDGMENT

This study was supported by the Natural Sciences and Engineering Research Council of Canada and made possible by the facilities of the Shared Hierarchical Academic Research Computing Network (http://www.sharcnet.ca) and the High Performance Computing Virtual Laboratory (http://www.hpcvl.org). M.J.V.S. acknowledges support by the U.S. National Science Foundation (grant CHE-0239800). B.J.B. thanks the DKFZ for a guest scientist fellowship.

REFERENCES

(1) Mann, M.; Hendrickson, R. C.; Pandey, A. *Annu. Rev. Biochem.* **2001**, *70*, 437–473.
(2) Aebersold, R.; Goodlett, D. R. *Chem. Rev.* **2001**, *101*, 269–295.
(3) Savitske, M. M.; Kjeldsen, F.; Nielsen, M. L.; Garbuzynskiy, S. O.; Galzitskaya, O. V.; Surin, A. K.; Zubarev, R. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 1481–1484.
(4) Cheng, C.; Gross, M. L. *Mass Spectrom. Rev.* **2000**, *19*, 398–420.
(5) Johnson, R. S.; Martin, S. A.; Biemann, K.; Stults, J. T.; Watson, J. T. *Anal. Chem.* **1987**, *59*, 2621–2625.
(6) Johnson, R. S.; Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1988**, *86*, 137–154.
(7) Wysocki, V. H.; Tsaprailis, G.; Smith, L. L.; Breci, L. A. *J. Mass Spectrom.* **2000**, *35*, 1399–1406.
(8) Wysocki, V. H.; Cheng, G.; Zhang, Q.; Hermann, K. A.; Beardsley, R. L.; Hilderbrand, A. E. In *Principles of Mass Spectrometry Applied to Biomolecules*; Laskin, J., Lifshitz, C., Eds.; John Wiley and Sons: Hoboken, NJ, 2006; Chapter VIII, pp 279−300.
(9) Paizs, B.; Suhai, S. *Mass Spectrom. Rev.* **2005**, *24*, 508–548.
(10) Harrison, A. G.; Young, A. B.; Bleiholder, C.; Suhai, S.; Paizs, B. *J. Am. Chem. Soc.* **2006**, *128*, 10364–10365.
(11) Garcia, I.; Giles, K.; Bateman, R. H.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 609–613.
(12) Harrison, A. G. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1776–1780.
(13) Kapp, E. A.; Schultz, F.; Reid, G. E.; Eddes, J. S.; Moritz, R. L.; O'Hair, R. A. J.; Speed, T. P.; Simpson, R. J. *Anal. Chem.* **2003**, *75*, 6251–6254.
(14) Huang, Y.; Triscari, J. M.; Tseng, G. C.; Pasa-Tolic, L.; Lipton, M. S.; Smith, R. D.; Wysocki, V. H. *Anal. Chem.* **2005**, *77*, 5800–5813.
(15) Somogyi, Á.; Wysocki, V. H.; Mayer, I. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 704–717.
(16) Rodriquez, C. F.; Cunje, A.; Shoeib, T.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. *J. Am. Chem. Soc.* **2001**, *123*, 3006–3012.
(17) El Aribi, H.; Rodriquez, C. F.; Almeida, D. R. P.; Ling, Y.; Mak, W. W. N.; Hopkinson, A. C.; Siu, K. W. M. *J. Am. Chem. Soc.* **2003**, *125*, 9229–9236.
(18) El Aribi, H.; Orlova, G.; Rodriquez, C. F.; Almeida, D. R. P.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2004**, *108*, 18743–18749.
(19) Bleiholder, C.; Osburn, S.; Williams, T. D.; Suhai, S.; Van Stipdonk, M.; Harrison, A. G.; Paizs, B. *J. Am. Chem. Soc.* **2008**, *130*, 17774–17789.
(20) Vachet, R. W.; Bishop, B. M.; Erickson, B. W.; Glish, G. L. *J. Am. Chem. Soc.* **1997**, *119*, 5481–5488.
(21) Mouls, L.; Aubagnac, J. L.; Martinez, J.; Enjalbal, C. *J. Proteome Res.* **2007**, *6*, 1378–1391.
(22) Yague, J.; Paradela, A.; Ramos, M.; Ogueta, S.; Marina, A.; Barabona, F.; de Castro, J. A.; Vazquez, J. *Anal. Chem.* **2003**, *75*, 1524–1535.
(23) Bythell, B. J.; Molesworth, S.; Osburn, S.; Cooper, T.; Paizs, B.; Van Stipdonk, M. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1788–1798.
(24) Yoon, S. H.; Chamot-Rooke, J.; Perkins, B. R.; Hilderbrand, A. E.; Poutsma, J. C.; Wysocki, V. H. *J. Am. Chem. Soc.* **2008**, *130*, 17644–17645.
(25) Oomens, J.; Young, S.; Molesworth, S.; Van Stipdonk, M. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 334–339.
(26) Polfer, N. C.; Oomens, J.; Suhai, S.; Paizs, B. *J. Am. Chem. Soc.* **2005**, *127*, 17154–17155.
(27) Perkins, B. R.; Chamot-Rooke, J.; Yoon, S. H.; Gucinski, A. C.; Somogyi, A.; Wysocki, V. H. *J. Am. Chem. Soc.* **2009**, *131*, 17528–17529.
(28) Erlekam, U.; Bythell, B. J.; Scuderi, D.; Van Stipdonk, M.; Paizs, B.; Maitre, P. *J. Am. Chem. Soc.* **2009**, *131*, 11503–11508.
(29) Verkerk, U. H.; Siu, C.-K.; Steill, J. D.; EI Aribi, H.; Zhao, J.; Rodriquez, C. F.; Oomens, J.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. Lett.* **2010**, *1*, 868–872.
(30) Bythell, B. J.; Maitre, P.; Paizs, B. *J. Am. Chem. Soc.* **2010**, *132*, 14766–14779.

(31) Bythell, B. J.; Dain, R. P.; Curtice, S. S.; Oomens, J.; Steill, J. D.; Groenewold, G. S.; Paizs, B.; Van Stipdonk, M. J. *J. Phys. Chem. A* **2010**, *114*, 5076–5082.
(32) Ballard, K .D.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 477–481.
(33) O'Hair, R. A. J.; Styles, M. L.; Reid, G. E. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 1275–1284.
(34) Reid, G. E.; Simpson, R. J.; O'Hair, R. A. *Int. J. Mass Spectrom.* **1999**, *190/191*, 209–230.
(35) Chan, W. C.; White, P. D. *Fmoc Solid phase peptide synthesis: a practical approach*; Oxford: New York, 2000.
(36) Marecek, J.; Song, B.; Brewer, S.; Belyea, J.; Dyer, R. B.; Raleigh, D. P. *Org. Lett.* **2007**, *24*, 4935–4937.
(37) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K. ; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T. ; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B. ; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision D.01; Gaussian, Inc.: Wallingford, CT, 2004.