



# Peptide Bond Formation in the Protonated Serine Dimer Following Vacuum UV Photon-Induced Excitation

Ori Licht, Darío Barreiro-Lage, Patrick Rousseau, Alexandre Giuliani, Aleksandar R. Milosavljević, Avinoam Isaak, Yitzhak Mastai, Amnon Albeck, Raj Singh, Vy T. T. Nguyen, Laurent Nahon, Lara Martínez-Fernández, Sergio Díaz-Tendero, and Yoni Toker\*

**Abstract:** Possible routes for intra-cluster bond formation (ICBF) in protonated serine dimers have been studied. We found no evidence of ICBF following low energy collision-induced dissociation (in correspondence with previous works), however, we do observe clear evidence for ICBF following photon absorption in the 4.6–14 eV range. Moreover, the comparison of photon-induced dissociation measurements of the protonated serine dimer to those of a protonated serine dipeptide provides evidence that ICBF, in this case, involves peptide bond formation (PBF). The experimental results are supported by ab initio molecular dynamics and exploration of several excited state potential energy surfaces, unraveling a pathway for PBF following photon absorption. The combination of experiments and theory provides insight into the PBF mechanisms in clusters of amino acids, and reveals the importance of electronic excited states reached upon UV/VUV light excitation.

Although mostly empty and cold the interstellar medium (ISM) is beaming with molecules. More than 200 molecular species have already been identified,<sup>[1]</sup> including many complex organic molecules. Moreover, analysis of meteorite samples show that amino acid and nucleic acid components are also present in space,<sup>[2–6]</sup> leading to the possible exogenic delivery of some of the basic building blocks of life to prebiotic earth.<sup>[7,8]</sup> There are two main approaches for understanding the origin of complex organic molecules in the ISM: the bottom-up approach which asserts that complex molecules are formed in molecular reactions between smaller species,<sup>[9]</sup> and the top-down approach which posits that large molecules are formed in the outflow of stars and then following excitation fragment to form smaller molecules.<sup>[10,11]</sup> Indeed molecules in space are exposed to

different types of energetic beams (photons, electrons, ions) leading to their ionization and fragmentation. An intermediate approach which is gaining attraction in recent years<sup>[12–17]</sup> is reactions occurring inside molecular clusters, which will refer to as intra-cluster bond formation (ICBF), induced by energetic processing. Molecular clusters are aggregates containing two or more entities which are held together non-covalently, via weakly-bonding interactions such as hydrogen bonds. In many cases the geometry of molecular clusters, i.e. their conformational landscape, appears to be conducive to the formation of molecular bonds. A classic example is clusters of amino acids, where the orientation of the OH...NH mode of hydrogen bonding is found, in some cases, to be suitable, upon the deposition of energy, for peptide bond formation (PBF).<sup>[18]</sup>

[\*] O. Licht, Y. Toker

Physics Department and Institute for Nanotechnology and Advanced Materials, Bar-Ilan University  
Ramat-Gan 5290002 (Israel)  
E-mail: yonitoker@gmail.com

D. Barreiro-Lage, L. Martínez-Fernández, S. Díaz-Tendero  
Departamento de Química, Universidad Autónoma de Madrid  
28049 Madrid (Spain)

P. Rousseau, R. Singh, V. T. T. Nguyen  
Normandie Univ, ENSICAEN, UNICAEN, CEA, CNRS, CIMAP  
14000 Caen (France)

A. Giuliani, A. R. Milosavljević, L. Nahon  
Synchrotron SOLEIL L'Orme des Merisiers Départementale 128  
91190 Saint-Aubin (France)

A. Giuliani  
INRAE, UAR1008, Transform Department, Rue de la Géraudière  
BP 71627, 44316 Nantes (France)

A. Isaak, Y. Mastai, A. Albeck

Chemistry Department and Institute for Nanotechnology and Advanced Materials, Bar-Ilan University  
Ramat-Gan 5290002 (Israel)

L. Martínez-Fernández, S. Díaz-Tendero  
Institute for Advanced Research in Chemistry (IAdChem),  
Universidad Autónoma de Madrid  
28049 Madrid (Spain)

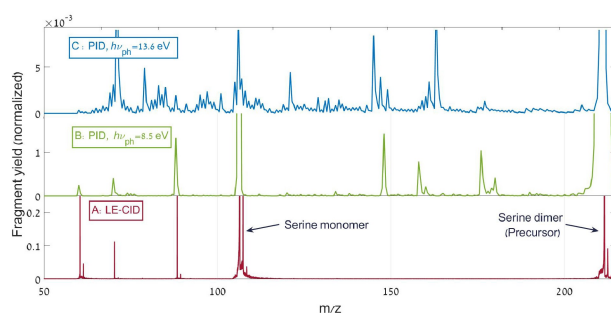
S. Díaz-Tendero  
Condensed Matter Physics Center (IFIMAC),  
Universidad Autónoma de Madrid  
28049 Madrid (Spain)

© 2023 The Authors. Angewandte Chemie International Edition published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

In studying ICBF the excitation method is of crucial importance, as different excitation sources can lead to different dynamics. For example, several recent works have shown that high-energy collision-induced dissociation (HE-CID, in which the collision energy is in the keV energy range or higher) can lead to knock-out mechanisms followed by the fusing together of molecules.<sup>[12]</sup> Recently, ICBF has been observed in HE-CID studies of  $\beta$ -alanine clusters, and quantum chemical calculations of the process have pointed to protonation as an important intermediate stage en-route to PBF.<sup>[15]</sup> ICBF following low-energy collision-induced dissociation (LE-CID) is less likely. This is because LE-CID results in the heating of the clusters leading to statistical products, with cluster evaporation usually being more likely than bond formation. Indeed, there are very little evidence of ICBF following LE-CID. These include observations of PBF following LE-CID of several protonated amino acids dimers<sup>[19]</sup> and a recent study of PBF in clusters of protonated serine dipeptides.<sup>[20]</sup> In both works PBF was not observed in protonated serine clusters following LE-CID. In contrast, photon absorption leads to excited state dynamics which can possibly result in different, non-statistical products. Indeed, a study has shown PBF in many proton-bounded peptide dimers following irradiation by 157 nm VUV light.<sup>[21]</sup> A further advantage of using photons is the ability to deposit a well defined amount of energy into the cluster. When a tunable photon source is available, scanning the energy of the incoming photon allows selection of the excited states which are populated. This can potentially allow control over processes such as PBF. The most widely studied amino acid clusters are those of serine, due to their remarkable preference for forming homochiral clusters of size  $N=8$ ,<sup>[22,23]</sup> leading to discussions of their role in homochirality (i.e. in the evolution of chirality in living systems).<sup>[24–27]</sup> Despite extensive LE-CID measurements by our group and others, ICBF has not been observed so far in protonated  $Ser_NH^+$  clusters, although it has been seen for radical  $Ser_N^+$  clusters in helium nano-droplet measurements.<sup>[28]</sup>

In this work we study photon-induced dissociation (PID) of protonated serine dimers, using photon energies in the UV/VUV range of 4.6–14 eV, and find clear evidence of ICBF, which is not seen in LE-CID measurements. The experimental results are supported by quantum chemistry calculations, which include simulations based on *ab initio* molecular dynamics and exploration of several potential energy surfaces of electronic excited states.

Figure 1A shows a typical LE-CID spectrum of the protonated serine dimer. The main fragmentation channel is monomer evaporation,  $Ser_2H^+ \rightarrow SerH^+ + Ser$ , resulting in a fragment of  $m/z$  106. Also visible are monomer fragments. These are the same fragments which we observe in LE-CID, and in PID ( $h\nu \leq 9.5$  eV) of the protonated serine monomer and correspond to loss of  $H_2O + CO$  (resulting in a fragment of mass 60 amu),  $H_2O$  loss (88 amu), and a minor peak corresponding to the loss of two  $H_2O$  molecules (70 amu). These fragmentation channels have been observed and discussed in previous works.<sup>[28–33]</sup> Importantly, in CID measurements of the protonated serine dimer we do not observe fragments whose mass is in between the masses of

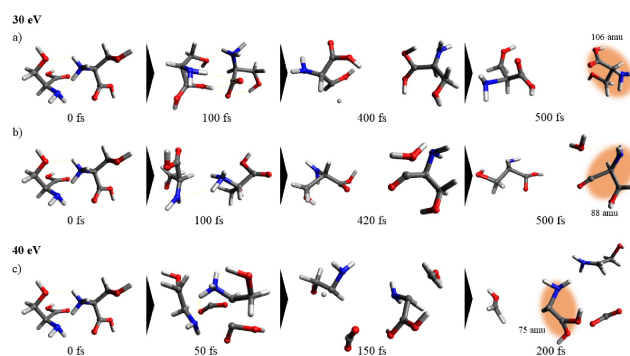


**Figure 1.** Comparison of CID with PID for the protonated serine dimer. A) LE-CID measurement. B) PID,  $\epsilon_{ph}=8.5$  eV. C) PID,  $\epsilon_{ph}=13.6$  eV. The fragment yield is normalized by the precursor intensity ( $m/z$  211).

the monomer and the dimer, nor were they seen in previous works.<sup>[20,28]</sup>

The experimental results were complemented by molecular dynamics (MD) simulations aimed at understanding the evolution of the protonated dimer under thermal excitation, thus mimicking the experimental conditions in LE-CID measurements. Figure 2 shows three examples of MD trajectories: Panel (a) shows the most probable fragmentation channel, where the protonated dimer is split into  $Ser + SerH^+$ , i.e. into both monomers, one of them keeping the proton. This so-called evaporation process results in cooling down thermal excitation of molecular clusters; Panels (b) and (c) show how one of the emitted monomers can further dissociate producing smaller fragments. In channel (b) a neutral  $H_2O$  molecule is released resulting in the fragment with  $m/z$  88. On the other hand, in panel (c) neutral fragment  $CO_2$  is released leading to the fragment with  $m/z$  75.

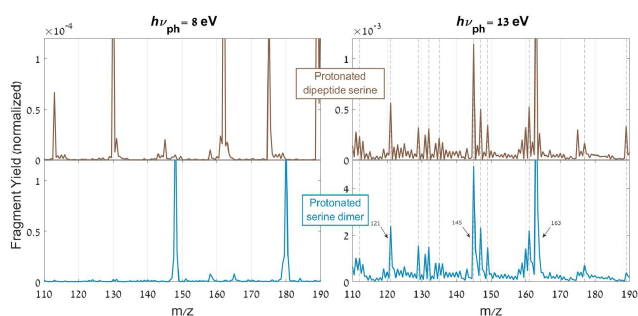
The MD simulations, thus confirm that thermal excitation (resulting from LE-CID) of the protonated serine dimer, in the electronic ground state, produces serine monomers and smaller fragments without ICBF. The lack of



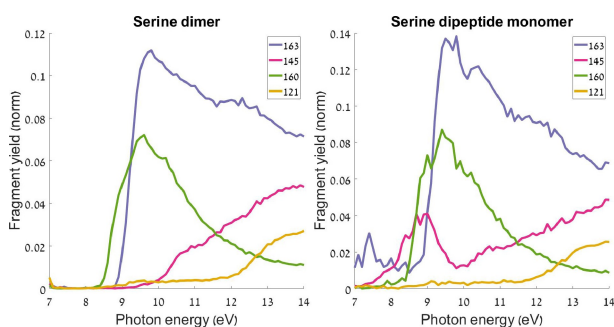
**Figure 2.** Three examples of trajectories obtained with molecular dynamics on the protonated serine dimers at internal excitation energies 30 and 40 eV, mimicking LE-CID conditions, with different fragmentation products. (a)  $Ser + SerH^+$ , (b)  $H_2O$  release, (c)  $CO_2$  release. All trajectories have been carried out using the M06-2X functional with the 6-31++G(d,p) basis set. Highlighted fragments are those carrying the positive charge, thus detectable in the experiment.

ICBF in this case is understandable as the dissociation into both monomers  $Ser + SerH^+$  corresponds to a process with a dissociation energy of  $DE = 1.58$  eV, while our calculations, discussed below, point to an energetic barrier of 2.48 eV for PBF. Thus, the thermal excitation in CID does not produce the peptide bond because energetically, kinetically and entropically it is more favourable to produce the separation into monomers.

In contrast in PID measurements, shown in Figure 1B and 1C, fragments whose mass is between that of the monomer and dimer are clearly visible. Such fragments can indicate ICBF, however another possibility is that they arise from fragmentation of one of the monomers while keeping the cluster intact. To rule out the second possibility we performed  $MS^3$  experiments. Here the protonated dimers are irradiated by light, subsequently the fragments of interest are mass selected and their CID spectrum is measured. In case these fragments correspond to a non-covalently bonded complexes, we expect that when heated the complex will evaporate, leading to fragments that are equal or smaller in mass than the monomer. Nevertheless, the mass of the prominent fragments observed in all the cases we studied (shown in the Supporting Information) are larger than the monomer (indeed, we saw no  $MS^3$  fragments corresponding to the monomer mass), providing proof of ICBF.



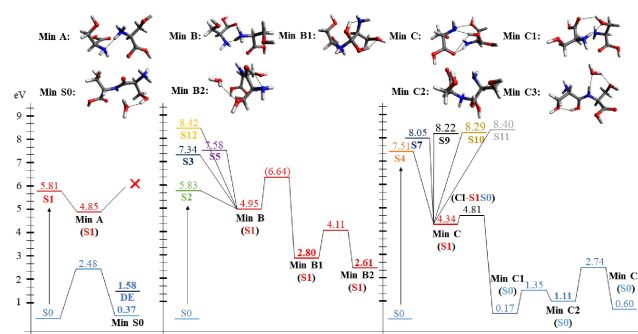
**Figure 3.** MS comparison between the protonated serine dimer (bottom) and the protonated dipeptide serine (top), for two different photon energies (left: 8 eV; right: 13 eV).



**Figure 4.** A comparison of the fragment yield (for the main fragmentation channels) as a function of photon energy between the protonated serine dimer (left) and the protonated serine dipeptide monomer (right). The fragment yield is normalized by the total fragment yield and given in different colors.

We now turn to the question of whether the ICBF we observe involves PBF? PBF is accompanied by  $H_2O$  loss and should therefore lead to a fragment of mass 193 amu. Such a fragment is observed at low photon energies ( $\leq 7$  eV), however, its yield is low (less than 1 % of the total ion yield, which is dominated by monomer evaporation, as shown in the Supporting Information). Nevertheless, there is reason to conclude that PBF is an intermediary stage in the formation of smaller fragments, with  $m/z$  that lie between that of the monomer and of the dimer. Figure 3 shows a comparison of PID of the protonated serine dimer to that of a protonated serine dipeptide for two representative photon energies. For low photon energies the two spectra look different. However for large photon energies ( $h\nu > 12.5$  eV) the PID spectra are almost identical. Figure 4 shows a comparison of the photon energy dependence of the yield of the most prominent fragmentation channels observed in the PID of the protonated serine dimer and that of the protonated serine dipeptide. Clearly, for photon energies of  $> 10$  eV the fragmentation pattern is very similar. Below this photon energy, the spectra are similar except for a resonance of the  $m/z$  145 fragment at 9 eV seen for the dipeptide but not for the protonated serine dimer, as well as a channel for formation of the  $m/z$  163 fragment below 8.5 eV only seen in the dipeptide. A possible explanation of such similarities is that the fragmentation of the protonated serine dimer is a two-step process, in which PBF occurs first, followed by fragmentation of the dipeptide. An alternative explanation is that an initial rapid fragmentation is followed by PBF.

To understand these results we performed calculations including electronic excited states. The Supporting Information includes the calculated electronic absorption spectrum as well as a characterization of the states. For all considered electronic excited states, we assume a direct excitation in the Franck–Condon region,  $S_0 \rightarrow S_n$  from the most stable conformer in the ground state and we allow relaxation from this point. In all cases, the evolution in the minimum energy path brings the system to a stable minimum in the first electronic excited state. Figure 5 depicts schematically the results for the first five electronic excited states as well as for the brightest,  $S_{12}$ , state. We find that excitation to  $S_1$  produces MinA; evolution from  $S_2$ ,  $S_3$ ,  $S_5$  and  $S_{12}$  leads to MinB; and from  $S_4$  to MinC (the fate of other electronic states are discussed in the Supporting Information). Notice that MinC is the most stable among those found in the relaxation from the Franck–Condon excitation. We performed further exploration of the corresponding potential energy surface from these minima towards the formation of the peptide bond. From MinA PBF is sterically hindered. In contrast, from MinB a path through a barrier, located at 6.64 eV with respect to the ground state  $S_0$ , leads to MinB1 where the peptide bond is formed; a second barrier (4.11 eV) leads to the  $H_2O$  release. Finally, a conical intersection ( $CI-S_1S_0$ ) very close in energy to most stable MinC, directly connects with a new minimum MinC1, located in the electronic ground state  $S_0$ , and from there small energy barriers lead to PBF and the release of  $H_2O$ . We thus confirm that electronic excitation to  $S_2$ ,  $S_3$ ,  $S_4$ ,  $S_5$



**Figure 5.** Energy Scheme showing the optimizations of the different electronic excited states evolving towards three minima: MinA, MinB and MinC. These minima are followed by the corresponding reaction paths leading to PBF. Each electronic excited state is represented with a different color. Particularly, critical points in the potential energy surface (PES) of the electronic ground state ( $S_0$ ) are represented in blue and those in the PES of the first excited state ( $S_1$ ) are represented in red. The dissociation energy (DE) of the dimer in the  $S_0$  is also presented. Each minima, for each pathway has been labeled with a letter and a number, i.e. A1, and its molecular structure is represented above each mechanism. The transition states connecting the minima are also given. Relative energies in the scheme are given in eV and are referred to the most stable dimer in the electronic ground state  $S_0$ .

and  $S_{12}$  relaxes into stable minima that could further evolve towards PBF. In contrast, the mechanism for PBF in the ground state  $S_0$  implies an energetic barrier of 2.48 eV, compared to  $\approx 0.5$ –1.6 eV from the excited states.

In summary, we have shown that in the case of protonated serine dimers, no ICBF is observed following LE-CID. This is in correspondence with the calculated energy for monomer evaporation being almost 1 eV lower than the barrier energy for PBF. In contrast, measurements show ICBF following VUV photon absorption (above 10 eV). This shows that excited state dynamics can lead to non-statistical pathways not accessible in LE-CID. Furthermore, the comparison of the PID for the protonated serine dimer to that of the protonated serine dipeptide suggests that PBF is an intermediate step in the ICBF process. These results are complemented by calculation showing a pathway for peptide bond formation from several (but not all) of the electronic excited states. Our findings are of particular interest in the astrophysical context: most parts of the ISM are cold enough for the formation of dimers (and larger clusters), and are dominated by Lyman- $\alpha$  radiation at 10.2 eV.<sup>[34,35]</sup> Our results show that photon-absorption in this VUV range, causes ICBF, and more precisely PBF, and is therefore an efficient mechanism for growth towards biomolecular complexity in cold gas phase environments and possibly at the surface of icy grains where amino-acids are thought to be forming.

## Acknowledgements

This article is based upon work from COST action CA18212—Molecular Dynamics in the GAS phase (MD-GAS), supported by COST (European Cooperation in Science and

Technology). The authors acknowledge the generous allocation of computer time at the Centro de Computación Científica at the Universidad Autónoma de Madrid (CCC-UAM). This work was partially supported by MICINN (Spanish Ministry of Science and Innovation) project PID2019-110091GB-I00 funded by MCIN/AEI/10.13039/501100011033, the “María de Maeztu” (CEX2018-000805-M) Program for Centers of Excellence in RD. D.B.-L. acknowledges the FPI grant associated with MICINN project CTQ2016-76061-P. R.S. acknowledges the Normandy Region and the European Union in the frame of operational program FEDER/FSE 2014-2020 (RIN MAG-IC) and the support received by the French ANR agency (ANR-18-CE30-0021, ANR FRAPA). V.T.T.N. acknowledges NucPhys Master Degree and EACEA (project number: 610575-EPP-1-2019-1-ES-EPPKA1-JMD-MOB, contract: 2019-2130) for support. We are grateful to the general staff of SOLEIL for smoothly running the facility and for providing beamtime under project #20210498, and to J.-F. Gil for his help in installing the ion trap set-up on the DESIRS beamline. This work was supported by the Agence Nationale de la Recherche, France, under project ANR-08-BLAN-0065.

## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Keywords:** Cluster Compounds • Gas-Phase Reactions • Mass Spectrometry • Quantum Chemistry • UV/Vis Spectroscopy

- [1] B. A. McGuire, *Astrophys. J. Suppl. Ser.* **2022**, 259, 30.
- [2] K. Kenvolden, J. Lawless, K. Pering, E. Peterson, J. Flores, C. Ponnampuram, I. R. Kaplan, C. Moore, *Nature* **1970**, 228, 923.
- [3] J. Oró, J. Gibert, H. Lichtenstein, S. Wikstrom, D. Flory, *Nature* **1971**, 230, 105.
- [4] J. E. Elsila, J. C. Aponte, D. G. Blackmond, A. S. Burton, J. P. Dworkin, D. P. Glavin, *ACS Cent. Sci.* **2016**, 2, 370.
- [5] A. Burton, J. Stern, J. Elsila, D. Glavin, J. Dworkin, *Chem. Soc. Rev.* **2012**, 41, 5459.
- [6] M. H. Engel, B. S. Nagy, *Nature* **1982**, 296, 837.
- [7] J. Oró, *Nature* **1961**, 190, 389.
- [8] U. Meierhenrich, *Comets and Their Origin*, Wiley-VCH, Weinheim, **2014**.
- [9] A. G. G. M. Tielens, *Rev. Mod. Phys.* **2013**, 85, 1021.
- [10] O. Berné, J. Montillaud, C. Joblin, *Astron. Astrophys.* **2015**, 577, A133.
- [11] A. Chuvilin, U. Kaiser, E. Bichoutskaia, N. A. Besley, A. N. Khlobystov, *Nat. Chem.* **2010**, 2, 450–453.
- [12] M. Gatchell, H. Zettergren, *J. Phys. B* **2016**, 49, 162001.
- [13] J. Chen, T. Chen, A. G. G. M. Tielens, *Astrophys. J.* **2018**, 863, 128.



- [14] S. A. Sandford, M. Nuevo, M. Nuevo, P. P. Bera, P. P. Bera, T. J. Lee, *Chem. Rev.* **2020**, *120*, 4616.
- [15] P. Rousseau, D. G. Piekarski, M. Capron, A. Domaracka, L. Adoui, F. Martín, M. Alcamí, S. Díaz-Tendero, B. A. Huber, *Nat. Commun.* **2020**, *11*, 3818.
- [16] T. Stein, J. Jose, *Isr. J. Chem.* **2020**, *60*, 842.
- [17] J. Jose, A. Zamir, T. Stein, *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2101371118.
- [18] E. J. P. Malar, P. Divya, *J. Phys. Chem. B* **2018**, *122*, 6462.
- [19] A. Singh, S. Kaur, J. Kaur, P. Singh, *Rapid Commun. Mass Spectrom.* **2014**, *28*, 2019.
- [20] M. Nihamkin, A. Isaak, A. Albeck, Y. Mastai, Y. Toker, *J. Phys. Chem. Lett.* **2020**, *11*, 10100–10105.
- [21] S. Lee, S. J. Valentine, J. P. Reilly, D. E. Clemmer, *J. Am. Chem. Soc.* **2011**, *133*, 15834.
- [22] J. Seo, S. Warnke, K. Pagel, M. T. Bowers, G. von Helden, *Nat. Chem.* **2017**, *9*, 1263.
- [23] V. Scutelnic, M. A. S. Perez, M. M. et al, *J. Am. Chem. Soc.* **2018**, *140*, 7554.
- [24] P. Yang, R. Xu, S. C. Nanita, R. G. Cooks, *J. Am. Chem. Soc.* **2006**, *128*, 17074.
- [25] C. A. Schalley, P. Weis, *Int. J. Mass Spectrom.* **2002**, *221*, 9.
- [26] S. C. Nanita, R. G. Cooks, *Angew. Chem. Int. Ed.* **2006**, *45*, 554; *Angew. Chem.* **2006**, *118*, 568.
- [27] K. J. Koch, F. C. Gozzo, S. C. Nanita, Z. Takats, M. N. Eberlin, R. G. Cooks, *Angew. Chem. Int. Ed.* **2002**, *41*, 1721; *Angew. Chem.* **2002**, *114*, 1797.
- [28] L. Tiefenthaler, J. Kočišek, P. Scheier, *Eur. Phys. J. D* **2020**, *74*, 85.
- [29] G. E. Reid, R. J. Simpson, R. A. J. O'hair, *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 1047.
- [30] P. Zhang, W. Chan, K. M. K. Lei, T. C. W. Poon, *Sci. Rep.* **2019**, *9*, 6453.
- [31] K. Lucas, G. L. Barnes, *J. Am. Soc. Mass Spectrom.* **2020**, *31*, 1114.
- [32] S. Hartweg, G. A. Garcia, D. K. Božanić, L. Nahon, *J. Phys. Chem. Lett.* **2021**, *12*, 2385.
- [33] F. Rogalewicz, Y. Hoppilliard, G. Ohanessian, *J. Am. Soc. Mass Spectrom.* **2000**, *195–196*, 565.
- [34] T. P. Robitaille, B. A. Whitney, R. Indebetouw, K. Wood, P. Denzmore, *Astrophys. J. Suppl. Ser.* **2006**, *167*, 256.
- [35] P. Modica, C. Meinert, P. de Marcellus, L. Nahon, U. J. Meierhenrich, L. L. S. d'Hendecourt, *Astrophys. J.* **2014**, *788*, 79.

Manuscript received: December 20, 2022

Accepted manuscript online: February 15, 2023

Version of record online: March 3, 2023