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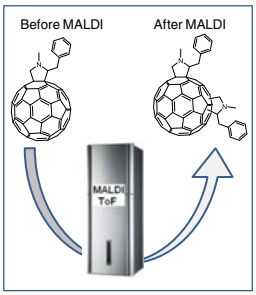
RESEARCH ARTICLE

Laser-Induced Azomethine Ylide Formation and Its Covalent Entrapment by Fulleropyrrolidine Derivatives During MALDI Analysis

Giovanni Bottari,[1,2] Claudia Dammann,[3] Tomás Torres,[1,2] Thomas Drewello[3]

[1]Departamento de Química Orgánica, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain
[2]IMDEA-Nanociencia, c/Faraday 9, Campus de Cantoblanco, 28049 Madrid, Spain
[3]Department of Chemistry and Pharmacy, Physical Chemistry I, University of Erlangen-Nuremberg, 91058 Erlangen, Germany



Abstract. Two novel monofunctionalized fulleropyrrolidine derivatives (Prato adducts) were prepared and characterized by matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). MALDI experiments conducted in the positive-ion mode on pure and mixed samples of both monofunctionalized fullerene derivatives revealed the efficient formation of bisadducts (in the case of the pure samples) and mixed bisadducts (in the case of a mixed sample). Bisadducts were not observed in the ESI experiments and thus not present in the sample. A mechanism for the MALDI formation of these bisadduct ions is proposed in which an azomethine ylide fragment is formed in situ from the monofunctionalized fulleropyrrolidine species upon laser irradiation. This fragment, which can survive as an intact moiety in the gas phase in the special environment provided by the MALDI experiment, is then able to attach to a fulleropyrrolidine monoadduct which acts as a dipolarophile, thus leading to the formation of a bisadduct fullerene derivative. The unprecedented re-attachment of the azomethine ylide implies that the establishment of the ligand attainment of Prato adducts based on MALDI analysis alone can lead to wrong assignments.

Key words: Azomethine ylide, ESI, Fulleropyrrolidine, MALDI, Retro-cycloaddition reaction

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Introduction

In the last two decades, soft ionization methods such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) have proved to be important tools for the characterization of fullerene derivatives. In ESI, derivatized fullerenes are sprayed from solution and the formation of ionic species is often achieved by electrochemical red/ox-processes occurring in the ion source [1, 2], as well as by proton transfer and/or a metal cation attachment to the functionality of the substituted fullerene [3]. In MALDI experiments, on the other hand, a solid mixture of the fullerene-based analyte embedded into a matrix material is activated by laser light. In this case, the matrix, which is normally more abundantly present than the analyte, plays a fundamental role in the transformation of the analyte from the solid to the gas phase [4]. In principle, MALDI may also take advantage of promoting the ion formation by attachment of a charge carrier to the functionality of the fullerene derivative [5, 6] or may involve electron transfer reactions [7, 8].

Among the large number of reactions that have been exploited in order to functionalize fullerenes [9, 10], the 1,3-dipolar cycloaddition reaction of azomethine ylides to fullerenes, also known as the Prato reaction, is one of the most frequently used methods, representing a straightforward approach for the preparation of fullerene derivatives [11, 12]. In this reaction, usually, the reactive azomethine ylide species is generated in situ by decarboxylation of an iminium salt derived from the condensation of an α-amino acid with an aldehyde or a ketone.

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Correspondence to: Giovanni Bottari; *e-mail:* giovanni.bottari@uam.es, Tomás Torres; *e-mail:* tomas.torres@uam.es, Thomas Drewello; *e-mail:* thomas.drewello@chemie.uni-erlangen.de

Fulleropyrrolidine derivatives are usually stable compounds that have found a wide range of applications in different technological fields, such as materials science and medicinal chemistry [13, 14]. However, the retro-cycloaddition reactions of this class of fullerene derivatives, quantitatively affording the parent unsubstituted fullerenes, can also occur under particular conditions, such as the use of thermal [15], or electrochemical [16] means as reported by Martín and Echegoyen and co-workers. Experimental studies and theoretical calculations on these derivatives have allowed to shed light on the mechanism of the thermal retro-cycloaddition reaction, confirming the generation of a reactive azomethine ylide which is covalently "trapped" in solution by using an excess of a dipolarophile such as maleic anhydride or pristine C_{60} fullerene in the presence, or absence, of a Lewis acid in the form of a metal salt [17]. More recently, retro-cycloaddition reactions of pyrrolidino and 2-pyrazolinofullerene derivatives have also been observed in collision-induced dissociations of negative ions following their generation by ESI [18, 19]. In MALDI, the retro-cycloaddition reaction has been observed for a pyrazolinopyrrolidino-bridged fullerene dimer [20].

We report here the unprecedented MALDI-promoted formation of bisadduct fulleropyrrolidine species from pure monoadduct fullerene derivatives (i.e., **1**, **2**, Figure 1). We propose a formation mechanism of such species, which involves the initial formation of an azomethine ylide via a retro-cycloaddition reaction of a monoadduct fulleropyrrolidine derivative. The ylide is then able to reattach to an intact monofunctionalized fulleropyrrolidine giving rise to the formation of a bisadduct.

Materials and Methods

The fulleropyrrolidine monoadducts **1** and **2** were prepared and characterized using standard spectroscopic techniques (see Supporting Information section). For the mass spectrometry experiments, two stock solutions of fulleropyrrolidines **1** and **2** in toluene (1 g/L) were prepared. ESI mass spectra were recorded with a quadrupole ion trap (Esquire 6000; Bruker) operating an orthogonal ESI source. The inlet flow rate (500 µL single-syringe infusion pump) was 240 µL/h. The pressure of the nebulizer gas (N_2) was 10 psi and the flow rate of the drying gas (N_2) was 5 L/min. The drying temperature was 300 °C. The voltage applied to the capillary was kept at 4000 V with 220 V at the capillary end. After the capillary the ions flew through a skimmer (40 V) into a dual octupole system. The first octupole voltage was 12 V and the second approximately 2,5 V. For the ESI measurements the stock toluene solutions of derivatives **1** and **2** were diluted with dichloromethane and methanol in a 1:1 ratio in order to obtain 5×10^{-5} mol/L solutions. To every 1 mL of solution, 10 µL of concentrated acetic acid was added in order to promote protonation.

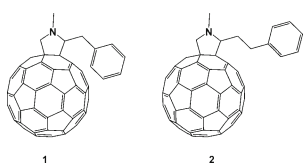


Figure 1. Molecular structures of fulleropyrrolidine monoadducts **1** and **2**

MALDI measurements were performed on two different time-of-flight mass spectrometers both confirming the presented observations. The positive-ion MALDI mass spectra shown in Figures 3 and 4 were obtained with an Applied Biosystems (Framingham, MA, USA) 4700 Proteonics Analyzer in reflectron mode, operating a solid state laser (repetition rate 200 Hz). The positive-ion MALDI mass spectrum shown in Figure 5 was obtained with a Shimadzu (Manchester, UK, England) Axima Confidence in linear detection mode, operating a nitrogen laser (repetition rate 50 Hz). The latter instrument was also used for the evaluation of the experimental parameters studying different analyte-to-matrix ratios (i.e., 1:10, 1:50, 1:100, 1:500, and 1:1000) in positive- and negative-ion mode, using linear and reflectron detection. For the MALDI experiments, a stock dichloromethane solution of the matrix (i.e., dithranol [8], Supplemental Figure S1) (1 g/L) was prepared and added to the stock toluene solution of compounds **1** and **2** (each at 1 g/L) in the appropriate ratio; 10 µL of the resulting solution were spotted on the sample plate and air-dried prior to analysis. The MALDI spectra of the pure samples were obtained at an analyte-to-matrix ratio of 1:500 and the mixed samples at an analyte(**1**)-to-analyte(**2**)-to-matrix ratio of 1:1:500.

Results and Discussion

The positive-ion ESI mass spectra of fulleropyrrolidines **1** and **2** are shown in Figure 2. Both spectra display the abundant formation of the protonated species $[\mathbf{1} + H]^+$ and $[\mathbf{2} + H]^+$, which appear at *m/z* 868 (Figure 2a) and 882 (Figure 2b), respectively.

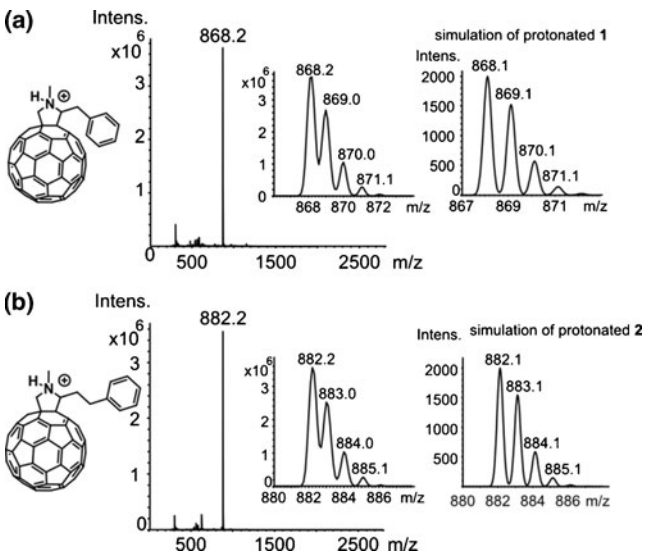


Figure 2. ESI mass spectra of fulleropyrrolidine monoadducts **(a) 1** and **(b) 2**. Insets: enlargement of the major peaks observed in the respective ESI mass spectra and comparison with the simulated isotope pattern

The absence, in both spectra, of the molecular ion peak $[M]^+$. suggests that the protonation, which presumably takes place at the nitrogen atom of the pyrrolidine moiety, is a very efficient process under the applied conditions. The ESI mass spectra of derivatives **1** and **2** show neither signs of higher adduct species, nor any major fragmentation peaks. This is a clear indication of (1) the purity and structural integrity of the fulleropyrrolidine monoadducts **1** and **2** and (2) the "softness" of the ESI process.

For further characterization of derivatives **1** and **2**, MALDI experiments were carried out. Dithranol, which was used earlier successfully with structurally similar fullerene derivatives [8], was employed as the matrix and spectra were recorded in the positive-ion mode. All MALDI mass spectra presented in this study have been carried out in positive-ion mode, since the negative-ion mode MALDI experiments provided only poor quality data. The performance of the MALDI matrix DCTB (i.e., *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile) (Supplemental Figure S1) was also tested [7, 21]. DCTB is widely used for the MALDI analysis of derivatized fullerenes which generally gives rise to abundant analyte peaks with only a small degree of unwanted fragmentation [7]. However, for the present samples, dithranol outperformed DCTB and was, therefore, used throughout. The analyte region of the MALDI mass spectra of derivatives **1** and **2** in positive-ion linear detection mode are shown in Figure 3a and b, respectively.

In both MALDI experiments is the protonated monoadduct $[M + H]^+$ at *m/z* 868 for **1** (Figure 3a) and *m/z* 882 for **2**

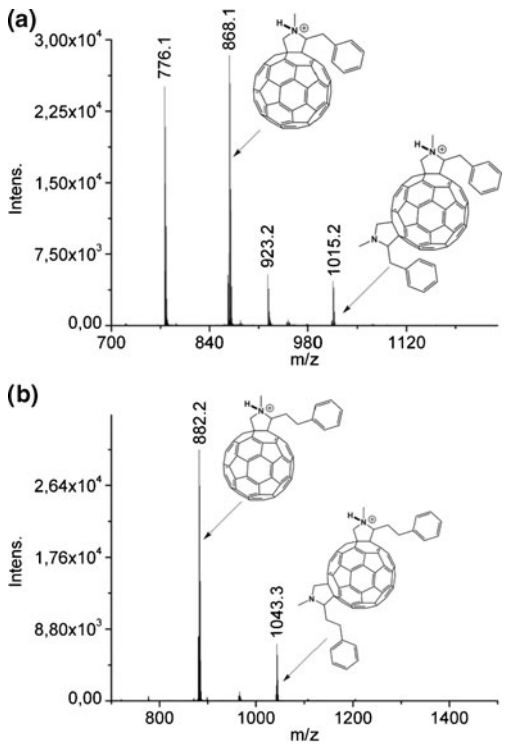


Figure 3. MALDI mass spectra (dithranol matrix, analyte-to-matrix ratio of approximately 1:500, positive reflectron mode) of fulleropyrrolidine monoadducts **(a) 1** and **(b) 2**

(Figure 3b) clearly prevailing and accompanied by a much less abundant molecular ion $[M]^{+\bullet}$. Protonation is obviously a more attractive alternative than electron transfer ionization. Unexpectedly, the MALDI mass spectra of both compounds **1** and **2** display signals, with sizeable intensities, which are representative of the protonated bisadducts (i.e., *m/z* 1015 for derivative **1** and *m/z* 1043 for derivative **2**). Supplementary Figure S2 provides confirmation of this assignment through the comparison of measured and simulated isotope patterns. The bisadducts were not detected in the accompanying ESI experiments of these compounds (vide supra) (Figure 2). It is therefore evident that the bisadducts were not present in the samples. Besides the fact that the monoadducts were chromatographically purified prior to analysis, there is no rational that would explain why bisadducts of such sizeable proportions would escape the detection by ESI.

In addition to the signals of the mono- and bisadduct species, additional signals at *m/z* 776 and *m/z* 923 were observed for derivative **1**, which were not present in the spectrum of derivative **2**. The ion of *m/z* 776, which is the most abundant ion when compound **1** is directly laser-activated (direct LDI, no matrix), is probably formed through the loss of a tolyl radical (i.e., $C_6H_5CH_2^{\bullet}$, $\Delta m=91$) from the monoadduct. The ion of *m/z* 923 corresponds to the ion of *m/z* 776 plus one additional ligand (Supplementary Figure S3). The exact formation mechanism of both these ions cannot be elucidated from the present data. However, these fragment ions do not originate from the protonated adducts, as evidenced by a daughter ion analysis by collision-induced dissociation (CID) of the protonated monoadduct of compound **1** (Supplemental Figure S4). The latter ion does indeed show the formation of *m/z* 776 upon CID, but this ion is always accompanied by other abundant daughter ions, which are not present in the MALDI mass spectrum. Since *m/z* 776-ion is most abundantly formed when no matrix is used, we assume that the ion may result from direct laser activation of the sample **1**. The ion formation by direct LDI may compete with the MALDI process, so that signals from both processes are seen in Figure 3a. Evidently, compound **2** is less fragile under the applied conditions.

In order to obtain further insight into the origin of the unexpected bisadduct formation, a MALDI experiment was carried out with a 1:1 mixture of compounds **1** and **2**. The resulting spectrum is displayed in Figure 4 and shows, in the region below *m/z* 900, a superposition of the signals observed in the MALDI spectra of the pure samples of compounds **1** and **2**. However, above *m/z* 900, two new signals appear. The first new signal at *m/z* 1029 is positioned between the signals of the protonated bisadducts at *m/z* 1015 (bisadduct of **1**) and *m/z* 1043 (bisadduct of **2**) and is assigned to the protonated bisadduct with mixed ligands (Figure 4b). The almost perfect 1:2:1 intensity pattern observed for the ions at *m/z* 1015, 1029, and 1043 (Figure 4b) seems to indicate that the bisadduct formation through covalent "entrapment" of the reactive azomethine ylide by the monoadducts is a statistical process. The statistical distribution of the two ligands on C_{60} strongly suggests that the ligands can freely attach to the C_{60}. This

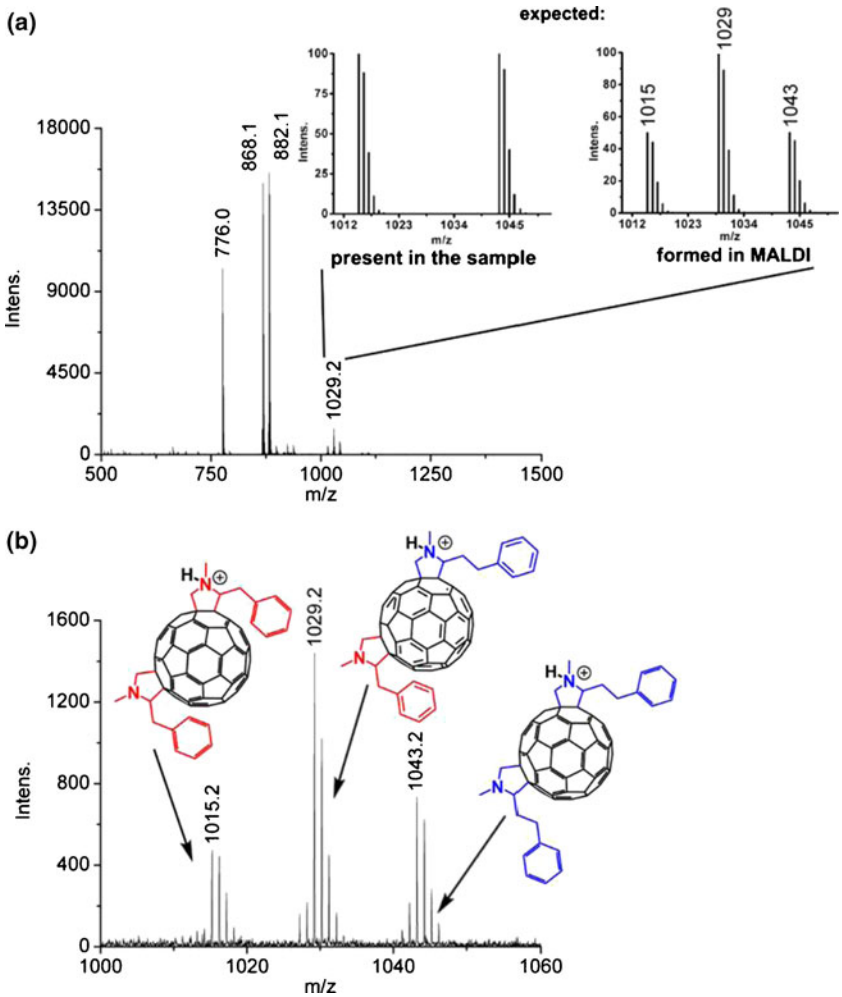


Figure 4. **(a)** MALDI mass spectrum (dithranol matrix, positive mode) of a mixed sample containing fulleropyrrolidine monoadducts **1** and **2** in a 1:1 ratio; **(b)** enlargement of the range *m/z* 1000–1060

finding does not support the presence of two constitutionally stable bisadduct impurities in the sample and rather indicates that the mixed bisadduct is formed during the MALDI experiment.

The second new signal is observed at *m/z* 937. This ion corresponds to the mixed ligand bisadduct, showing the above mentioned tolyl radical loss from the ligand of compound **1** (Supplemental Figure S5).

A straightforward mechanistic explanation of the bisadducts formation can be derived when considering the complete MALDI mass spectrum of compound **1** (Figure 5). The low mass region contains two major ion signals. First, at *m/z* 227 protonated dithranol is observed and at *m/z* 147 the ionised ligand is seen and constitutes the base peak of the spectrum. The ionized ligand was occasionally accompanied by its protonated form leading to a satellite signal at *m/z* 148 (Supplemental Figure S6). The signal of the liberated ligand is observed as the most abundant one, which may indicate that the retro-Prato reaction (ligand loss) has been fairly pronounced under the applied conditions. This statement assumes that the tendency to form ions under the applied conditions is similar for the free and the attached ligand. If this is the case, then the liberated ligand constitutes a major component in the material plume that follows the laser activation of the target materials in the MALDI experiment. It can be proposed that the liberated ligand survives as an isolated but reactive entity, until it may be trapped by appropriate partners, such as for instance by undissociated monoadduct **1**, which would result in the observed bisadduct. The reactions are assumed to occur in the laser ablation plume, representing a type of reaction that has been reviewed comprehensively [4].

While the actual structure of the neutral and ionic ligand cannot be elucidated by the present experiments, clear indication of the occurrence of the ligand attachment to other species is also seen in the spectra. The signal at *m/z* 372 is attributed to an ion composed of the ligand attached to the dithranol matrix (M) missing one hydrogen atom, $[L + M - H]^+$. The assignment of these low mass ions is corroborated by compound **2**, which shows for the ligand-

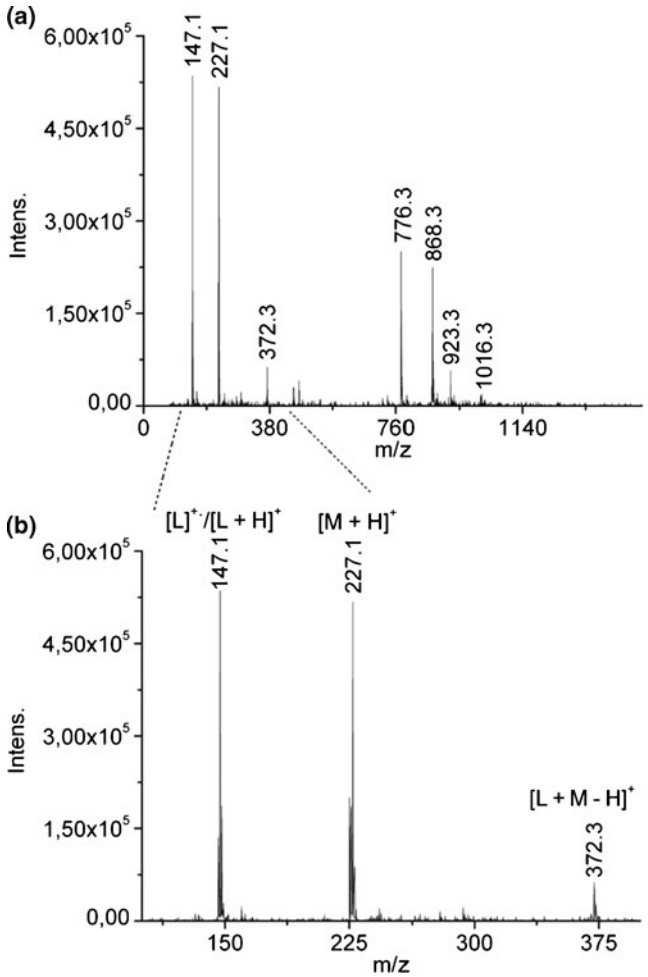
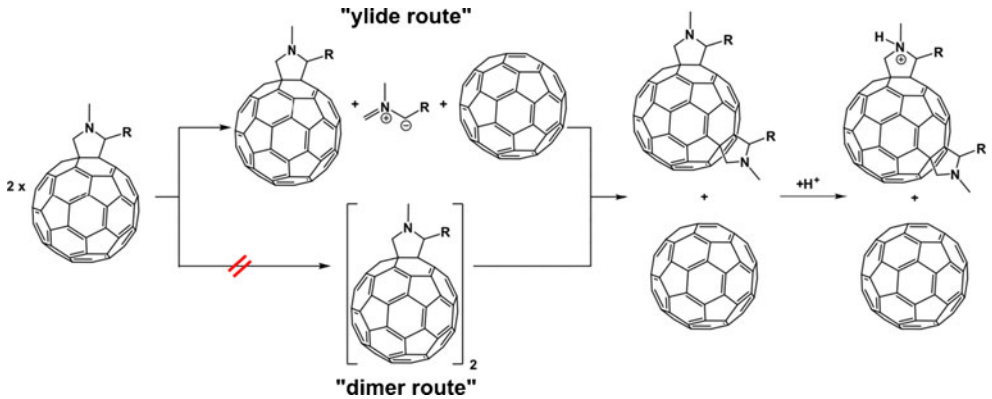


Figure 5. **(a)** Complete MALDI mass spectrum (dithranol matrix, analyte-to-matrix ratio of 1:500) of compound **1** and **(b)** zoom of the low mass *m/z* region of the spectrum in **(a)**

containing signals the expected mass shift of 14 Da (i.e., *m/z* 161 and 387) because of the additional CH_2 unit (Supplemental Figure S7).

Our interpretation is also supported by other recent investigations into the occurrence of the retro-cycloaddition of pyrrolidinofullerene ions in the gas phase. The collision-induced dissociation (CID, MS/MS) of related molecular radical anions led via a one-step loss of the ligand to the formation of the C_{60} radical anion fragment, providing evidence of the retro-addition [18, 19]. For protonated species similar to the ones studied here, the CID experiment provides a more complex dissociation pattern, whereby $C_{60}H^+$ results over a stepwise, dissociative loss of the ligand, discounting the retro-addition route [18, 19, 22]. Protonation of the nitrogen atom hinders the retro-cycloaddition of Prato adducts. These findings are confirmed by the CID mass spectrum of protonated compound **1** shown in Supplemental Figure S4. However, under MALDI conditions evidence has been provided of the retro-cycloaddition [20]. In MALDI, fullerene dimers linked by a pyrazolinopyrrolidino bridge showed, besides the protonated molecule, also the efficient formation of the protonated species which had lost a fullerene from the pyrollidino ligand by retro-cycloadditon.

Based on the observations discussed above, a mechanism for the bisadduct formation is proposed (Scheme 1). Laser activation induces a retro-cycloaddition reaction on a fulleropyrrolidine monoadduct leading to the formation of a reactive fragment, probably an azomethine ylide species, and unfunctionalized C_{60} fullerene ("ylide route," Scheme 1). This fragment is sufficiently stable to be eventually covalently "trapped" by a structurally intact monoadduct fullerene derivative, leading to a bisadduct species, which upon protonation is subsequently contributing to the MALDI mass spectrum. Contributions from a sequence in which the protonation precedes the ylide attachment cannot be ruled out. The present findings are unprecedented, as this is the first time that a molecular ligand has been transferred in MALDI from one fullerene to the other. The transfer events that were found in earlier investigations involved only mono-atomic species (mostly ionic), and included the Cl^- uptake by $C_{60}Cl_6$ [23], F^- uptake by $C_{60}F_{18}$ [24], and oxygen transfer from $C_{60}O_x$ to C_{70} [25].



Scheme **1**. Proposed mechanism for the formation of doubly-functionalized fulleropyrrolidine adducts from pure fulleropyrrolidine monoadducts

An alternative mechanism could involve the formation of a dimer constituted of two monoadducts ("dimer-route", Scheme 1). A likely motif of bonding within the dimer is the covalent bridging of both fullerene units by one or both ligands. Reminiscent of the "dimer route" is the proposed way by which monomalonate adducts of C_{60} convert into bisadducts under electrochemical conditions, in which a dimer is formed through the interaction of a neutral monoadduct and its anion radical [26]. In the dimer dissociation the two monoadducts may result from a straightforward back-reaction. Alternatively, since at least one of the ligands is bridging the two fullerenes, both ligands may remain on one C_{60} sphere, leading to a bisadduct species and unfunctionalized C_{60}. However, in the present case, we should discard this "dimer route" mechanism, as no traces of any dimer ion could be detected in the MALDI experiments.

Conclusions

MALDI experiments carried out with pure and mixed samples of monofunctionalized fullerene derivatives revealed the formation of bisadducts (in the case of the pure samples) and statistically mixed bisadducts (in the case of the mixed sample). The bisadducts were not present in the samples and, therefore, not observed in ESI experiments. A mechanism is proposed in order to explain the formation of such fullerene bisadducts, which involves the formation of an azomethine ylide species through a laser-induced retro-cycloaddition reaction of the pyrrolidine macrocycle. This reactive fragment is then able to attach via an intermolecular reaction to an undissociated fulleropyrrolidine monoadduct species, giving rise to the observed bisadduct products.

Evidently, the bisadduct formation is an artifact of the ionization method. Consequently, if the establishment of the number of ligands attained on a fullerene is based exclusively on the MALDI experiment, caution is of the essence. Fragmentation may suggest a too low attainment and, as shown by the present study, retro- and re-addition of appropriate ligands may indicate an attainment that is too high.

Acknowledgments

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References

1. Barrow, M.P., Feng, X., Wallace, J.I., Boltalina, O.V., Taylor, R., Derrick, P.J., Drewello, T.: Characterization of fullerenes and fullerene derivatives by nanospray. *Chem. Phys. Lett.* **330**, 267–274 (2000)
2. Kozlovski, V., Brusov, V., Sulimenkov, I., Pikhtelev, A., Dodonov, A.: Novel experimental arrangement developed for direct fullerene analysis by electrospray time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **18**, 780–786 (2004)
3. Greisch, J.F., De Pauw, E.: Mass spectrometric characterization of 3′-imino[60]fulleryl-3′-deoxythymidine by collision-induced dissociation. *J. Mass Spectrom.* **42**, 304–311 (2007)
4. Knochenmuss, R., Zenobi, R.: MALDI ionization: the role of in-plume processes. *Chem. Rev.* **103**, 441–452 (2003)
5. Fati, D., Leeman, V., Vasil'ev, Y.V., Drewello, T., Leyh, B., Hungerbühler, H.: Alkali cation attachment to derivatized fullerenes studied by matrix-assisted laser desorption/ionization. *J. Am. Soc. Mass Spectrom.* **13**, 1448–1458 (2002)
6. Fati, D., Vasil'ev, Y.V., Wachter, N.K., Taylor, R., Drewello, T.: MALDI mass spectrometric determination of molecular site-specific charge localization, and laser-induced coalescence reactivity of fullerenodendrimers. *Int. J. Mass Spectrom.* **229**, 3–10 (2003)
7. Vasil'ev, Y.V., Khvostenko, O.G., Streletskii, A.V., Boltalina, O.V., Kotsiris, S.G., Drewello, T.: Electron transfer reactivity in matrix-assisted laser desorption/ionization (MALDI): ionization energy, electron affinity and performance of the DCTB matrix within the thermochemical framework. *J. Phys. Chem. A* **110**, 5967–5972 (2006)
8. Zhou, L., Deng, H., Deng, Q., Zheng, L., Cao, Y.: Analysis of three different types of fullerene derivatives by laser desorption/ionization time-of-flight mass spectrometry with new matrices. *Rapid Commun. Mass Spectrom.* **19**, 3523–3530 (2005)
9. Hirsch, A., Brettreich, M.: The Chemistry of Fullerenes. Wiley-VCH, Weinheim, Germany (2005). pp. 141–151
10. Martín, N., Altable, M., Filippone, S., Martín-Domenech, A.: New reactions in fullerene chemistry. *Synlett* 3077–3095 (2007)
11. Maggini, M., Scorrano, G., Prato, M.: Addition of azomethine ylides to C_{60}: synthesis, characterization, and functionalization of fullerene pyrrolidines. *J. Am. Chem. Soc.* **115**, 9798–9799 (1993)
12. Prato, M., Maggini, M.: Fulleropyrrolidines: a family of full-fledged fullerene derivatives. *Acc. Chem. Res.* **31**, 519–526 (1998)
13. Guldi, D.M., Martín, N. (eds.): Fullerenes: From Synthesis to Optoelectronic Properties. Kluwer Academic, Dordrecht, The Netherlands (2002)
14. Langa, F., Nierengarten, J.-F. (eds.): Fullerenes: Principles and Applications. Nanoscience and Nanotechnology Series. The Royal Society of Chemistry, Cambridge, UK (2007)
15. Martin, N., Altable, M., Filippone, S., Martin-Domenech, A., Echegoyen, L., Cardona, C.M.: Retro-cycloaddition reaction of pyrrolidinofullerenes. *Angew. Chem. Int. Ed.* **45**, 110–104 (2006)
16. Lukoyanova, O., Cardona, C.M., Altable, M., Filippone, S., Domenech, A.M., Martin, N., Echegoyen, L.: Selective electrochemical retro-cycloaddition reaction of pyrrolidinofullerenes. *Angew. Chem. Int. Ed.* **45**, 7430–7433 (2006)
17. Filippone, S., Barroso, M.I., Martin-Domenech, A., Osuna, S., Sola, M., Martin, N.: On the mechanism of the thermal retrocycloaddition of pyrrolidinofullerenes (retro-Prato reaction). *Chem. Eur. J.* **14**, 5198–5206 (2008)
18. Delgado, J., Filippone, S., Martín-Domenech, A., Altable, M., Maroto, E., Langa, F., Martín, N., Martínez-Alvarez, R.: Mass spectrometry studies of the retro-cycloaddition reaction of pyrrolidino and 2-pyrazolinofullerene derivatives under negative ESI conditions. *J. Am. Soc. Mass Spectrom.* **22**, 557–567 (2011)
19. Maroto, E., Filipone, S., Martin-Domenech, A., Suarez, M., Martin, N., Martinez-Alvarez, R.: Effect of substituents and protonation on the mechanism of 1,3-dipolar retro-cycloaddition reaction of pyrrolidino[60]- and [70] fullerenes. *J. Mass Spectrom.* **46**, 1016–1029 (2011)
20. Delgado, J.L., Osuna, S., Bouit, P.-A., Martínez-Alvarez, R., Espíldora, E., Solà, M., Martín, N.: Competitive retro-cycloaddition reaction in fullerene dimers connected through pyrrolidinopyrazolino rings. *J. Org. Chem.* **74**, 8174–8180 (2009)
21. Ulmer, L., Mattay, J., Torres-Garcia, H.G., Luftmann, H.: The use of 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2- enylidene]malononitrile

as a matrix for matrix-assisted laser desorption/ionization mass spectrometry. *Eur. J. Mass Spectrom.* **6**, 49–52 (2000)
22. Ramos, C.I.V., Santana-Marques, M.G., Enes, R.F., Tomé, A.C., Cavaleiro, J.A.S., Nogueras, M.: Gas-phase fragmentation of protonated C_{60}-pyrimidine derivatives. *J. Mass Spectrom.* **44**, 911–919 (2009)
23. Kuvychko, I.V., Streletskii, A.V., Popov, A.A., Kotsiris, S.G., Drewello, T., Strauss, S.H., Boltalina, O.V.: Seven-minute synthesis of pure C_S-$C_{60}Cl_6$ from [60]fullerene and iodine monochloride: first IR, Raman, and mass spectra of 99 mol % $C_{60}Cl_6$. *Chem. Eur. J.* **11**, 5426–5436 (2005)
24. Streletskii, A.V., Ioffe, I.N., Kotsiris, S.G., Barrow, M.P., Drewello, T., Strauss, S.H., Boltalina, O.V.: In-plume thermodynamics of the MALDI generation of fluorofullerene anions. *J. Phys. Chem. A* **109**, 714–719 (2005)
25. Beck, R.D., Stroemer, C., Schulz, C., Michel, R., Weis, P., Bräuchle, G., Kappes, M.M.: Enhanced coalescence upon laser desorption of fullerene oxides. *J. Chem. Phys.* **101**, 3243–3249 (1994)
26. Beulen, M.W.J., Rivera, J.A., Herranz, M.A., Martin-Domenech, A., Martin, N., Echegoyen, L.: Reductive electrolysis of [60]fullerene mono-methanoadducts in THF leads to the formation of *bis*-adducts in high yields. *Chem. Commun* 407–408 (2001)