Supporting Information for:

ESI-MS Study on the Aldol Reaction Catalyzed by L-proline

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Experimental

The mass spectrometric measurements were performed on the quadrupole-time of flight (Q-Tof) instrument Micromass Premier (Waters, Manchester) equipped with a standard ESI ion source containing a stainless steel metal spray capillary (127μm inner diameter, 229μm outer diameter, 181mm of length). A capillary voltage of 2.5kV, and source and desolvation temperatures of 100°C were used as standard ESI operation conditions. The collision-induced dissociation (CID, collision gas argon) was performed in the collision cell region.

MS continuous-flow experiments were performed using two syringes containing solutions of the different reactants, mixing them before entering into the ionization source. For this, a mixing tee (Techlab, PEEK mixing tee) was directly connected to the ESI spray capillary. The reaction occurred in the metal spray capillary of the ion source, and different reaction times were achieved by varying the flow rate in the syringe pumps.

When the MS experiments required reactants at high concentrations, such as those used in the synthetic procedures described in the literature, a second mixing tee was attached between the above commented and the ionization source to reduce 50 times the final sample concentration entering the mass spectrometer. The reaction volume was then that of the capillary connecting both mixing tees; the volume of the metal spray capillary is rendered negligible, as the dilution slows the bimolecular reaction approximately 2500 times.

The *on-going* MS experiment designed to follow the reactions in time were performed using two syringes, one containing the on-going reaction with the reactants at the standard concentrations described in the literature, the other containing a diluting solvent, *e.g.*, methanol. For reactions reaching the equilibrium in a time range of minutes, the

process was monitored continuously, meanwhile for reactions requiring hours or even days, samples were taken at different times.

Acetone **1**, 4-acetamidobenzaldehyde **2a**, 4-nitrobenzaldehyde **2b**, 2-chlorobenzaldehyde **2c**, isobutyraldehyde **2d**, L-proline **4**, methanol, and DMSO were obtained from Aldrich (Steinheim, Germany). All chemicals were used without further purification.

General Procedure for the MS Study of the Aldol Reaction. 4 (3.45mg, 20mM) was added to a mixture of DMSO and acetone (4:1 v/v, 1.5mL), followed by the addition of the corresponding aldehyde **2** (100mM). The resulting solution was then injected into the API source after being diluted 50 times using a mixing tee attached before the source and methanol as diluting solvent. For experiments requiring longer than 4 hours, samples of 37μL of the reacting solution were collected at different intervals (the first hour every ten minutes, the following six hours every hour, and then a sample every 24h up till 168h) and diluted 50 times with methanol. Once complete, the samples were injected directly into the ESI source.

5•H$^+$ and **6•H$^+$.** *Continuous-flow.* A solution of **4** (3.45mg, 20mM)in methanol was prepared and, by using two syringe pumps feeding a mixing tee coupled directly to the ESI-MS ion source, mixed with acetone. An injection speed of 0.25μLmin^{-1} was settled for the methanol solution, and 20μLmin^{-1} for the acetone. These conditions allowed the study of the medium approximately 6s after initiating the reaction. For the interception of transient **5•H$^+$** the source and cone temperatures of the spectrometer were set up to 40°C, and a capillary voltage of 2.5kV was used.

6•H$^+$. *On-going reaction.* A solution 0.1mM of **4** in a mixture of methanol and acetone (4:1 v/v, 1.5mL) was prepared and injected directly into the ESI source.

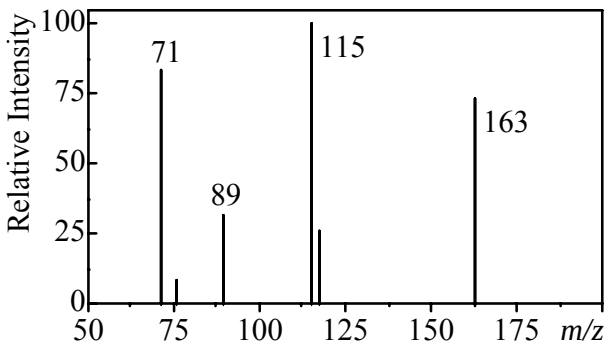
7•H$^+$. *Continuous-flow*. Using two syringe pumps feeding a mixing tee, a solution of **4** (40mM) in a mixture of anhydrous DMSO and acetone (4:1 v/v, 1.5mL) was allowed to react with a solution of the corresponding aldehyde **2** (200mM) in the same mixture. An injection speed of 0.12μLmin^{-1} was settled for both solutions. In order to reduce the concentration, a second mixing tee was attached between that above and the ESI-MS source, diluting the reacting mixture using methanol with a flow rate of 10μLmin^{-1} These conditions allowed the study of the medium 2s after the reaction initiation.

On-going reaction. **4** (3.45mg, 20mM) was added to a mixture of anhydrous DMSO and acetone (4:1 v/v, 1.5mL), followed by the addition of the corresponding aldehyde **2**(100mM). The resulting solution was then injected into the ESI-MS source after diluting it 50 times using a mixing tee attached before the source, and methanol was used as diluting solvent. The derivatives were intercepted at different times during the progress of the reaction.

8•H$^+$. *On-going reaction.* **4** (3.45mg, 20mM) was added to a mixture of anhydrous DMSO and acetone (4:1 v/v, 1.5mL), followed by the addition of the corresponding aldehyde **2** (100mM). The reaction was left to proceed, and the reaction mixture was injected into the ESI-MS source after diluting it 50 times by using a mixing tee attached before the source. Methanol was used as diluting solvent. The derivatives were intercepted at different times during the progress of the reaction.

Enamine Recognition.

It is possible to distinguish the enamine **6**$^{\cdot}$**H**$^+$ from the corresponding isomers iminium ion and protonated oxazolidinone by the different ESI-MS/MS fragmentation pattern expected. When a solution 0.1mM of **4** in a mixture of methanol-d_4 and acetone-d_6 (4:1 v/v, 1.5mL) was prepared and injected directly into the ESI source, it was possible to intercept the per-deuterated enamine D_6-**6**$^{\cdot}$**D**$^+$ and analyze its ESI-MS/MS spectrum –*see below*–.



ESI-MS/MS spectrum for per-deuterated enamine D_6-**6**$^{\cdot}$**D**$^+$.

The main fragmentation for the enamine is the release of mainly formic acid, as shown in Figure 1. In this fragmentation, DCOOD – by elimination of the N-bound deuterium- and DCOOH – by elimination of a β-C-bound hydrogen – should be released for the per-deuterated enamine, meanwhile DCOOH should be exclusively released for the iminium. As it can be observed, the ion peak with m/z 115 clearly discards the iminium as main responsible for the signal with m/z 163 –m/z 156 in case of not deuterated solvents–.

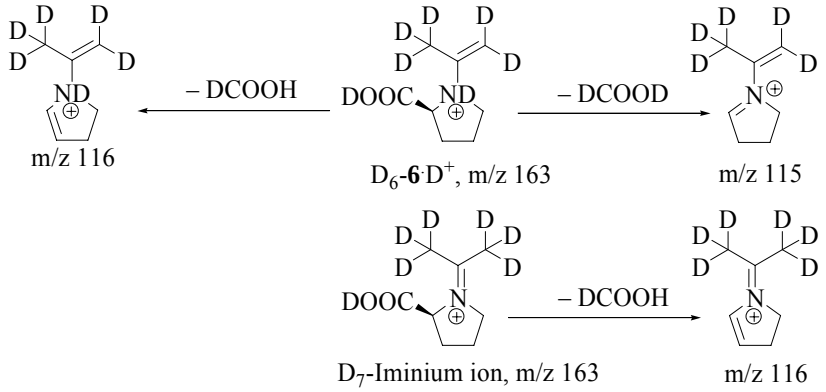
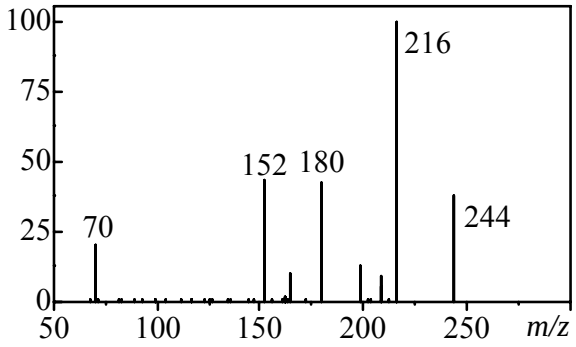


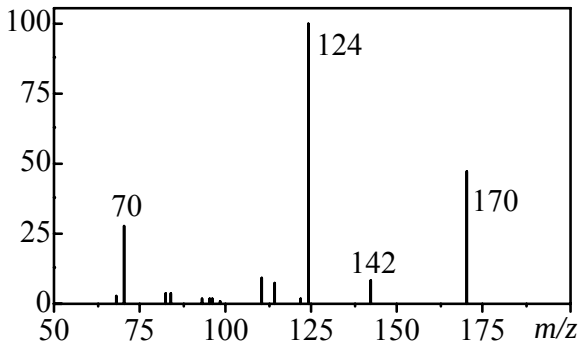
Figure 1. Expected fragmentation patterns for the per-deuterated enamine D_6-**6**·**D**$^+$ and isomeric iminium ion.

It has been described that oxazolidinones tend to fragment releasing CO_2 when ammonia introduced ionization is used – Iwamura, H.; Mathew, S. P.; Blackmond, D. G. *J. Am. Chem. Soc.* **2004**, *126*, 11770–. However, when we recorded the ESI-MS/MS spectrum in acetonitrile of (2*R*,5*S*)-2-trichloromethyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one **9** synthesized as described in the literature – Orsini, F; Pelizzoni, F.; Forte, M.; Sisti, M.; Bombieri, G.; Benetollo, F *J. Heterocyclic Chem.* **1989**, *26*, 837– we observed the release of CO as main fragmentation, to produce the ion with m/z 216 –see below–.



ESI-MS/MS spectrum of protonated oxazolidinone **9**•$\mathbf{H}^+$.

In the reaction of acetone **1** and *iso*-butyraldehyde **2d** in the presence of L-proline the fragmentation to release CO is also observed in the ESI-MS/MS spectrum of the ion with m/z 170, corresponding to the adduct formed by **2d** and L-proline –see below-.



ESI-MS/MS spectrum of protonated adduct formed by **2d** and L-proline.

In this case we observed, apart of the expected release of formic acid to give the ion with m/z 124 –corresponding to the enamine isomer **10**-, another ion at m/z 142 produced by the release of CO. This suggests a mixture of the isomer enamine **10** and the oxazolidinone **11** in solution, as it is shown in Figure 2.

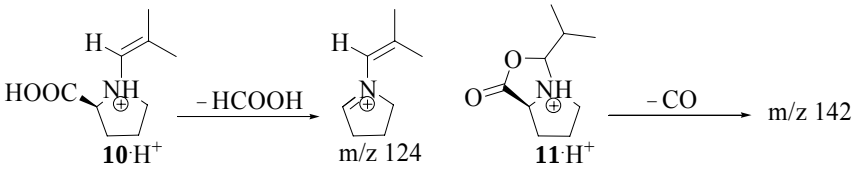


Figure 2. Expected fragmentation patterns of enamine **10**·H^+ and isomer oxazolidinone **11**·H^+.

This fragmentation to release CO should produce a fragment ion with m/z 128 in case of the oxazolidinone formed by L-proline and acetone, as shown in Figure 3. The lack of this ion signal suggest the oxazolidinone is not present in the reaction mixture.

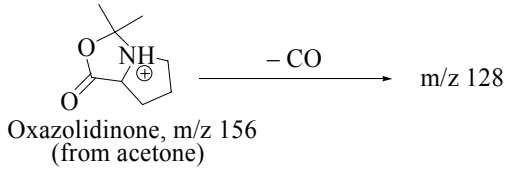
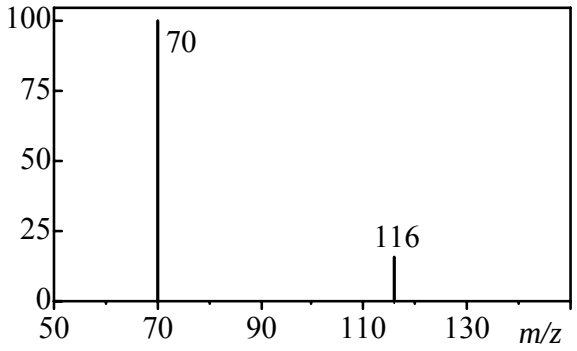
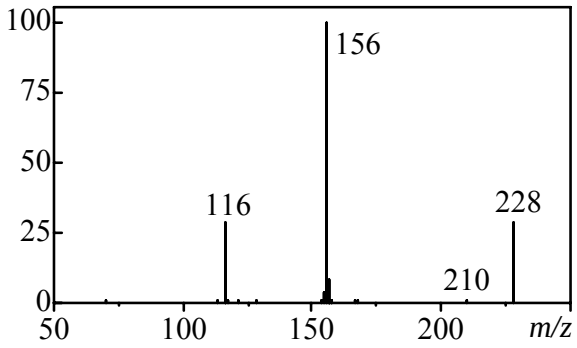


Figure 3. Expected fragmentation pattern of oxazolidinone formed with **1** and **4**.

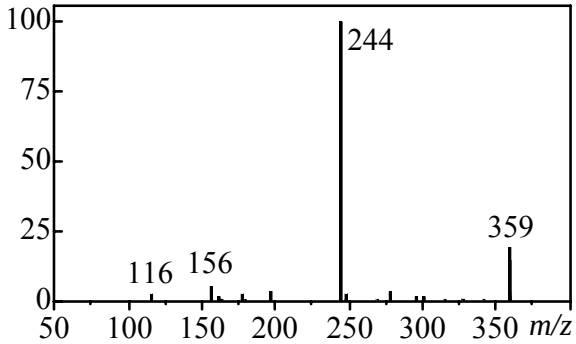
ESI-MS/MS Spectra of L-proline 4 and of some intermediates.



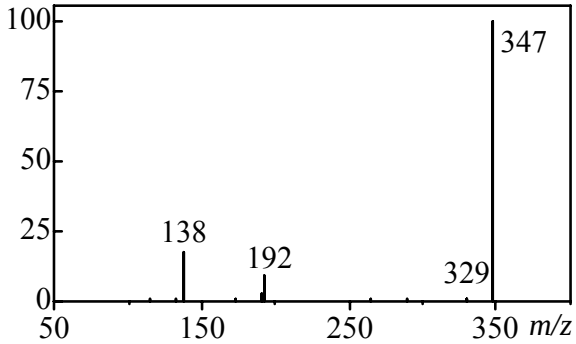
ESI-MS/MS spectrum of protonated L-proline **4•H**$^+$



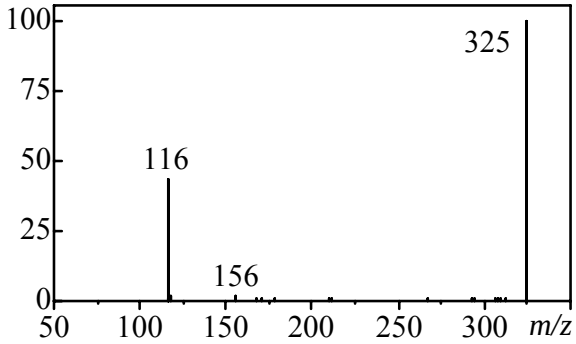
ESI-MS/MS spectrum of **7d•H**$^+$.



ESI-MS/MS spectrum of **8a•Na**$^+$.



ESI-MS/MS spectrum of **8b•Na**$^+$.



ESI-MS/MS spectrum of **8c•H**$^+$.