

Electrospray ionization mass spectrometry: a major tool to investigate reaction mechanisms in both solution and the gas phase

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Electrospray ionization mass spectrometry (ESI-MS), in conjunction with its tandem version ESI-MS/MS, is now established as a major tool to study reaction mechanisms in solution. This suitability results mainly from the ability of ESI to “fish” ions directly from solution to the gas phase environment of mass spectrometers. In this review, we summarize recent studies from our laboratory on the use of on-line monitoring by ESI-MS *ion fishing* of several types of reactions that permitted us to follow how these reactions progress as a function of both time and conditions using the ultra-high sensitivity and speed of ESI-MS to detect and even characterize transient reaction intermediates. We also show that the intrinsic reactivity of each key gaseous species *fished* by ESI can be further investigated via ESI-tandem mass spectrometry experiments, searching for the most active species via gas-phase ion/molecule reactions. In the gas-phase, solvent and counter-ion effects are absent. These studies often permit a detailed overview of major steps via the interception, characterization and reactivity investigation of key reaction players.

Keywords: reaction mechanisms, reaction intermediates, tandem mass spectrometry, direct infusion electrospray mass spectrometry, ion chemistry, gas-phase reactions

Reactions are the heart and soul of chemistry and chemists, to effectively control and improve reactions, need to know the mechanistic details by which these reactions evolve. The tremendous success and rapid overall acceptance of electrospray ionization mass spectrometry (ESI-MS)¹ result substantially from its ability to both transfer ions of many types, charge states and nearly unlimited masses from the “real-world” solution environment directly to the gas phase in a very gentle and efficient way and to characterize these gaseous ions with the outstanding speed, sensitivity and selectivity that only mass spectrometry is able to offer. ESI-MS has, therefore, rapidly become a major technique in many branches of science and the number of ESI-MS applications is still growing steadily. A novel application of ESI-MS is to study reaction mechanisms in solution. In this unique field, ESI-MS/MS has also been very successful and has been rapidly accepted as a major tool. This success results from the ability of ESI to rapidly and efficiently “fish” reactants, intermediates and products in ionic forms (regardless of their organic, inorganic or organometallic nature) directly from solution to the diluted gas-phase environment of mass

spectrometers in order to measure their masses and access their structures and intrinsic reactivities using tandem mass spectrometric (MS/MS) experiments. On-line monitoring by ESI-MS “ion fishing” (Figure 1) of many types of reaction permits the chemist to follow how these reactions progress as a function of time and reaction conditions and the ultra-high sensitivity and speed of ESI-MS allows even transient intermediates to be detected and well-characterized. The intrinsic reactivity of each key gaseous species fished by ESI can also be further investigated via ESI-MS/MS experiments in search of the most active species via gas-phase ion/molecule reactions in which solvent and counter-ion effects are absent. Therefore, the extraordinary ability to “fish” ionic species (or neutral, zwitterionic or radical species in their protonated or deprotonated forms) directly from the solution to the gas phase allows a detailed overview of the reaction steps via the interception, characterization and reactivity investigation of its key players.

The first review on the use of ESI-MS “ion fishing” for reaction mechanism studies was published recently² and discusses basic and general concepts, advantages and

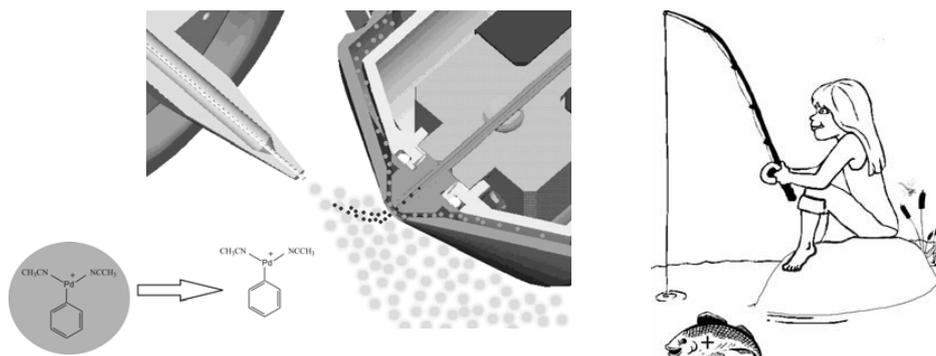


Figure 1. Left: a schematic of the ESI-MS process in which a reaction solution is electrospayed and ions from reactants, intermediates and products may evaporate from the charged droplets directly into the gas phase, being transferred to a mass spectrometer for characterization. Right: a cartoon of the ESI-MS "ion fishing" process.

applications of on-line and off-line screening. Therefore, in this review, although the area is currently blooming with many studies having been recently published by several groups, we have decided to focus on the more recent ESI-MS studies from our laboratory, summarizing briefly the important information on the mechanisms of various reactions that such studies have provided.

The Heck reaction

The first reaction we monitored by ESI-MS ion fishing was the Heck reaction with diazonium salts.³ This reaction occupies a prominent place among the synthetic tools available in organic synthesis for the construction of C–C bonds. Despite its great versatility and growing list of applications, the Heck reaction still displayed a number of unaccountable features and unverified mechanistic details. To "fish" for

the most important catalytic species, our investigation was performed first with an acetonitrile solution of the arene diazonium salt $4\text{-MeOPhN}_2^+\text{BF}_4^-$ using $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ (dba = dibenzylideneacetone) as the source of palladium. The reactant diazonium ion, together with four ionic species, were detected by ESI-MS after one to five minutes of reaction, all of them displaying the characteristic isotopic distributions of palladium-containing species. Interestingly, the composition of the cationic intermediates before olefin addition changes drastically with time but stabilizes after 90 min of mixing. All these potentially catalytic species were selected for ESI-MS/MS characterization and to investigate their intrinsic gas-phase reactivity towards model olefins.⁴ These olefins were then added to the reaction solution and, indeed, after 90 min of mixing, olefin insertion products were clearly detected, such as those of m/z 324, 517 and 558 formed by the addition of the active catalytic species of m/z 488 to 2,3-dihydrofuran (Figure 2).

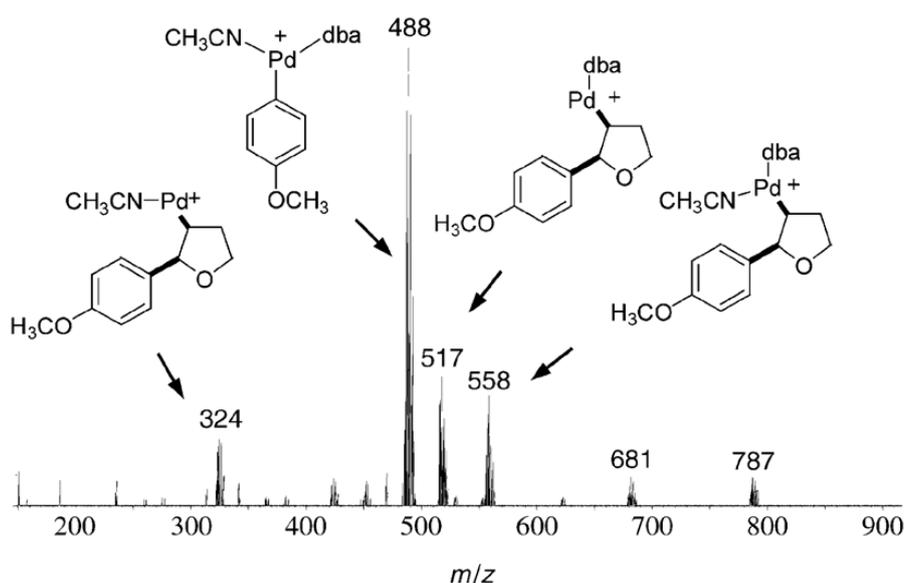


Figure 2. ESI(+) spectrum of a solution (2 nmol L^{-1}) of the arene diazonium ion $4\text{-MeOPhN}_2^+\text{BF}_4^-$ and $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ in acetonitrile after 90 min of mixing and the subsequent addition of a model olefin, 2,3-dihydrofuran.

For the first time, therefore, several cationic intermediates of the oxidative addition step of the Heck reaction involving arene diazonium salts have been detected and characterized by ESI-MS(/MS).⁴ A dynamic, time-dependent process with ligand equilibria between several ionic intermediates was observed for the oxidative addition step. The most reactive intermediate for olefin addition (the ion of m/z 488 in Figure 2) was also detected and characterized and this species dominates after mixing the arene diazonium salt and $[\text{Pd}_2(\text{dba})_3]\cdot\text{dba}$ after *ca* 90 min. Therefore, a novel protocol for the Heck reaction with a delay of 90 min before olefin addition was established for maximum yield. A detailed catalytic cycle for the Heck reaction with arene diazonium salts was therefore proposed (Scheme 1). This rich set of mechanistic information obtained for the Heck reaction nicely illustrates the power of ESI-MS(/MS) ion fishing for the study of reaction mechanisms both in solution and in the gas phase.

The Baylis–Hillman reaction

Another major reaction we investigated by ESI-MS ion fishing was the Baylis–Hillman reaction.⁵ This versatile multi-component reaction, which leads to the straightforward formation of new C–C bonds in a single step, has recently experienced an enormous growth in importance and use. It yields densely functionalized molecules that are conveniently manipulated as key synthons of a variety of natural and non-natural products. The BH reaction can be broadly defined as a coupling reaction between an alkene activated by an electron-withdrawing group and an electrophile that occurs under Lewis base catalysis, with 1,4-diazabicyclo[2.2.2]octane (DABCO) normally used as the base.

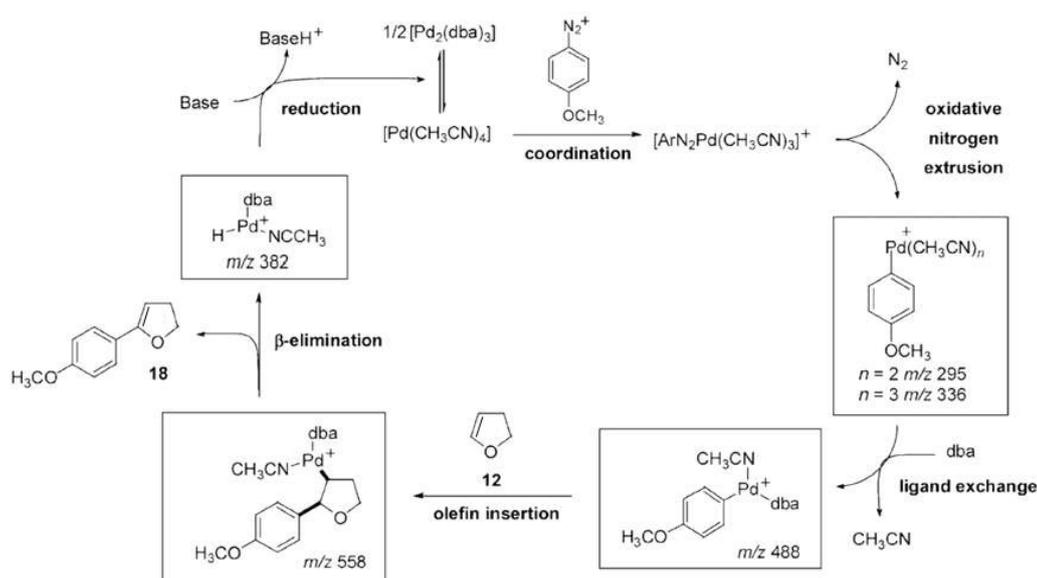
Via ESI-MS(/MS) ion fishing, we have been able to “fish” and to characterize all proposed intermediates for the catalytic cycle of the BH reaction (Scheme 2). Strong support for the currently accepted mechanism of this key reaction has, therefore, been provided by ESI-MS ion fishing.⁵

Co-catalysis by ionic liquids

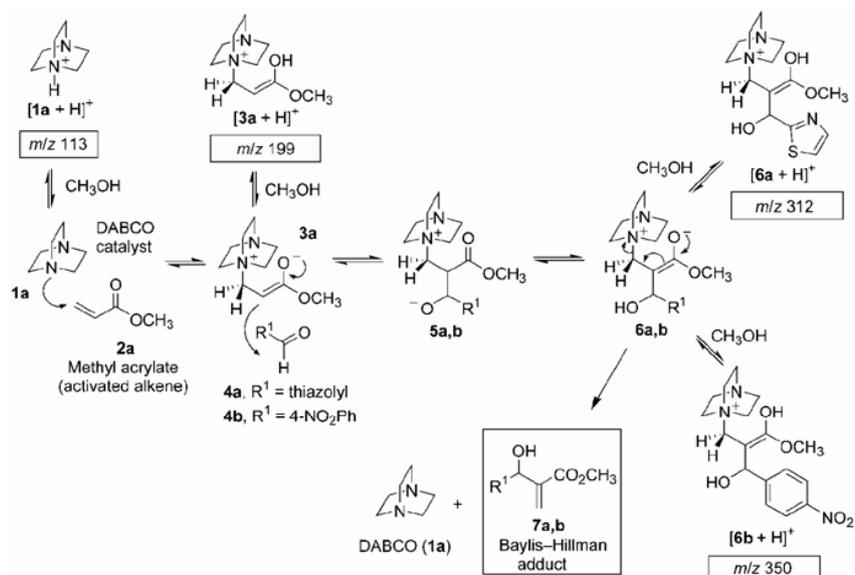
Using ESI-MS in both the positive and negative ion modes, we have also performed on-line monitoring of the BH reaction in the presence of ionic liquids as co-catalysts.⁶ Loosely bonded supramolecular species formed by coordination of neutral reagents, products and the protonated forms of zwitterionic BH intermediates and final product with cations and anions of ionic liquids have been gently and efficiently “fished” directly from the solution to the gas phase. Mass measurements and ESI-MS/MS characterization of these unprecedented species via collision-induced dissociation were performed. As Scheme 3 summarizes, the interception of several catalytically-active supramolecular species indicate that ionic liquids co-catalyze BH reactions by activating the aldehyde toward nucleophilic enolate attack and by stabilizing the zwitterionic species that acts as the main BH intermediates.

Troger’s bases

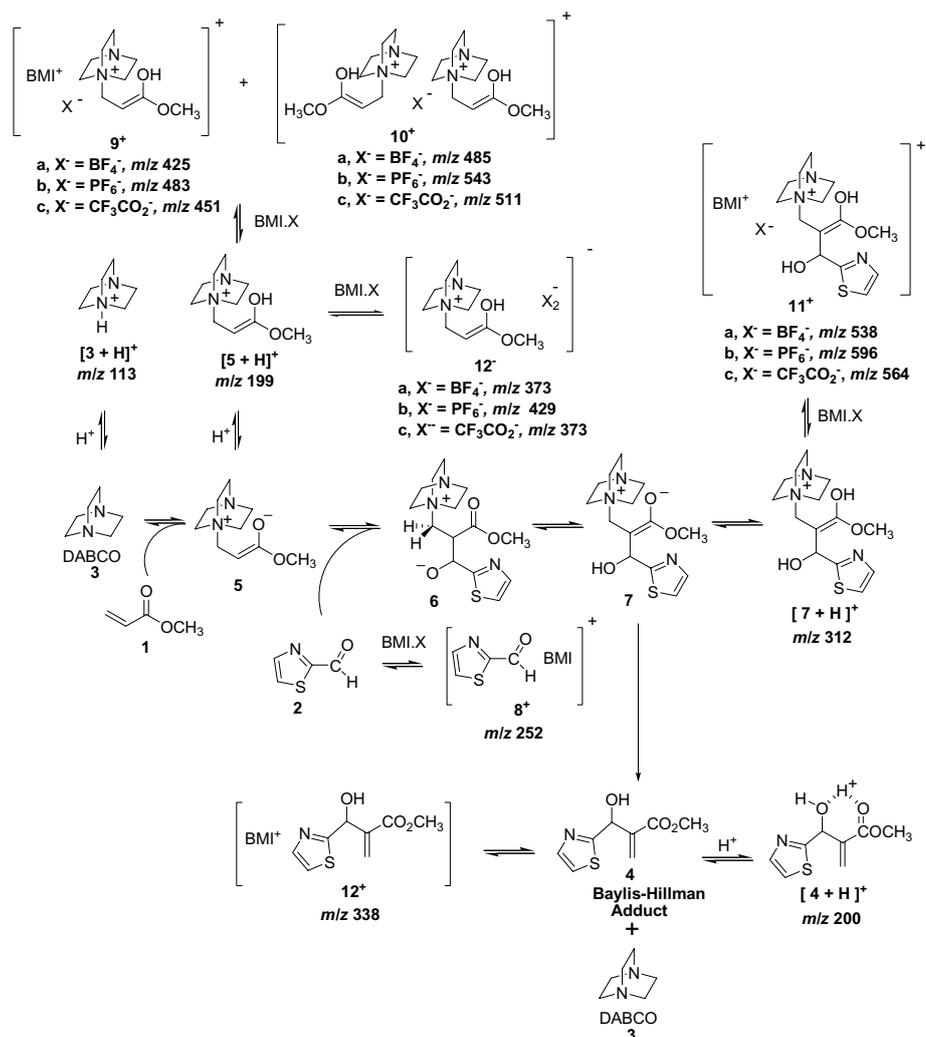
These bases constitute a relatively simple but geometrically-rich, V-shaped class of bicyclic molecules that are commonly formed under acid catalysis by the simple condensation of anilines and formaldehyde. They have found a variety of applications, such as their use as



Scheme 1. Proposed catalytic cycle for the Heck reaction with arene diazonium salts.



Scheme 2. Catalytic cycle for the Baylis-Hillman reaction of methyl acrylate and aldehydes in the presence of DABCO. The m/z values for reactants, intermediates and products detected and characterized in their protonated forms by ESI(+)-MS(/MS) are shown.



Scheme 3. Catalytic cycle for the Baylis-Hillman reaction co-catalyzed by ionic liquids.

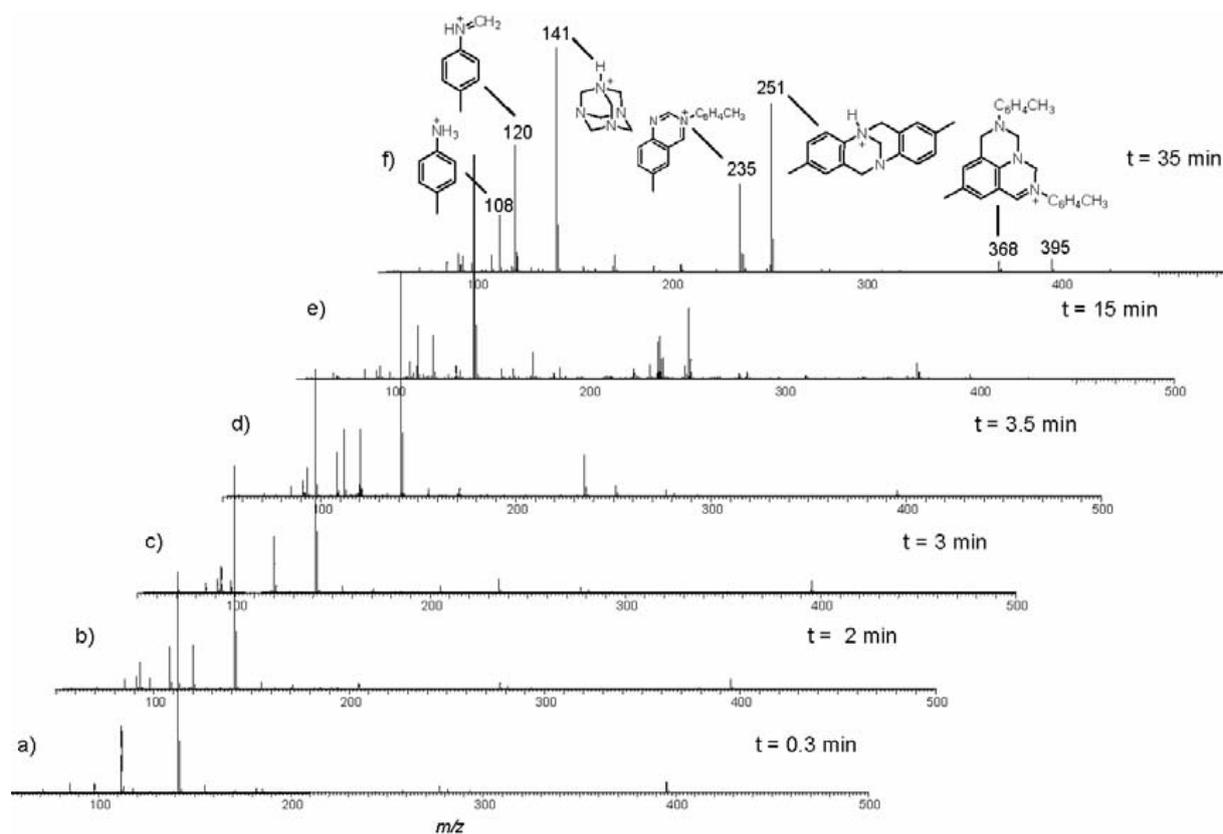


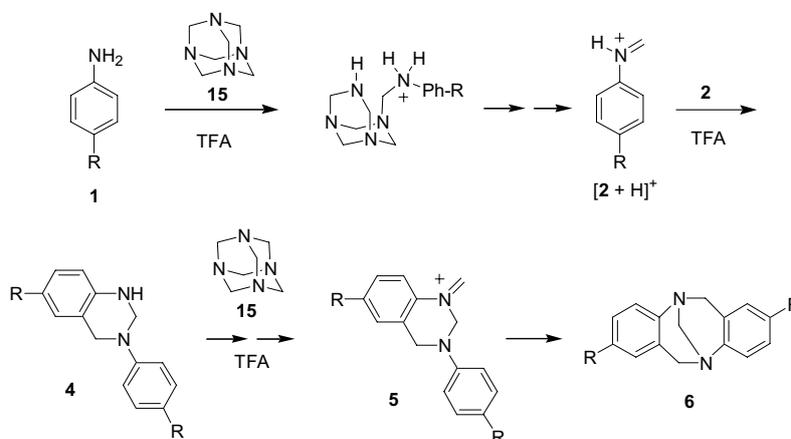
Figure 3. ESI(+)-MS of the reaction solution during the on-line monitoring of the Troger reaction of *p*-toluidine and urotropine in neat TFA after (a) 20 s, (b) 2 min, (c) 3 min, (d) 3.5 min, (e) 15 min and (f) 35 min of reaction.

relatively rigid chiral frameworks for the construction of chelating and biomimetic systems. We have used ESI-MS ion fishing to detect and characterize the key intermediates (as either cationic species or protonated forms of neutral species) of the reaction leading to the formation of Troger's bases (Figure 3).⁷ The unclear role of urotropine as the methylene source in these reactions has also been accessed and the pathways shown by ESI-MS have been probed by gas-phase ion/molecule reactions of selected key interme-

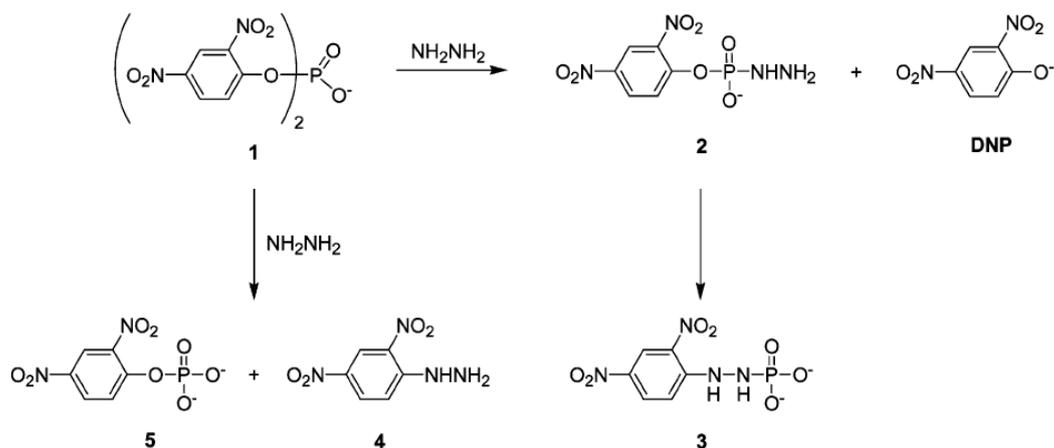
diates and reactants. An expanded mechanism for Troger's base formation, based on the ESI-MS ion fishing data, has also been proposed (Scheme 4).

Coupling of vinylic tellurides with alkynes

We have also monitored by ESI-MS ion fishing the coupling of vinylic tellurides with alkynes and have evaluated



Scheme 4. Mechanism for the formation of Troger's base based on ESI(+)-MS/MS monitoring.

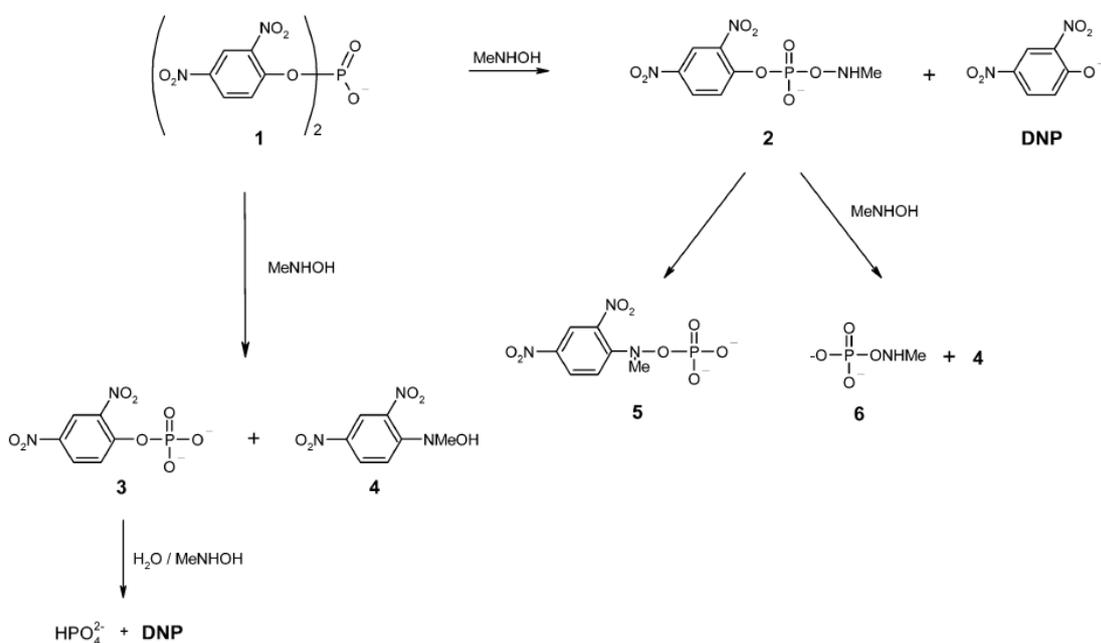


Scheme 5. Reaction of anionic (BDNPP) with hydrazine.

their synthetic and mechanistic details.⁸ Under palladium dichloride catalysis, vinylic tellurides couple efficiently with alkynes with retention of the double-bond geometry. Using ESI-MS(/MS) we were able to detect and characterize, again for the first time, the Pd–Te cationic intermediates of this reaction and the data permitted us to propose an expanded catalytic cycle for this important method for enyne synthesis. It is also known that tellurium tetrachloride adds to alkynes via two pathways: a concerted *syn*-addition that yields *Z*-tri- and tetra-substituted alkenes or by an *anti*-addition that yields *E*-alkenes. We have, therefore, investigated the mechanistic aspects of these divergent pathways for TeCl₄ addition to alkynes by “fishing”, directly from solution to the gas phase, the proposed intermediate species of Te(IV) in its cyclic form via ESI-MS followed by an ESI-MS/MS structural investigation.⁹

S_N2 reactions

We have also investigated by ESI-MS ion fishing the fundamental mechanistic aspects of S_N2 reactions.¹⁰ The reaction of bis(2,4-dinitrophenyl) phosphate with hydrazine (Scheme 5) and hydrogen peroxide were monitored, and *O*- versus *N*-phosphorylation were compared. Nonionic hydrazine was found to react with anionic bis(2,4-dinitrophenyl) phosphate (BDNPP) to give 2,4-dinitrophenylhydrazine and dianionic 2,4-dinitrophenyl phosphate by an S_N2(Ar) reaction and, at the phosphoryl center, to give 2,4-dinitrophenoxide ion and a transient phosphorylated hydrazine that rearranges intramolecularly to *N*-(2,4-dinitrophenyl)-*N*-phosphonohydrazine. ESI-MS(/MS) permitted us to fish the key reaction intermediates of these reactions, thus providing a detailed view of the reaction pathways and major players.



Scheme 6. Reaction of anionic (BDNPP) with MeNHOH.

We also studied by ESI-MS(/MS) the mechanisms of nucleophilic substitution reactions of methylated hydroxylamines with bis(2,4-dinitrophenyl)phosphate and were again able to detect and characterize the key intermediates (Scheme 6).

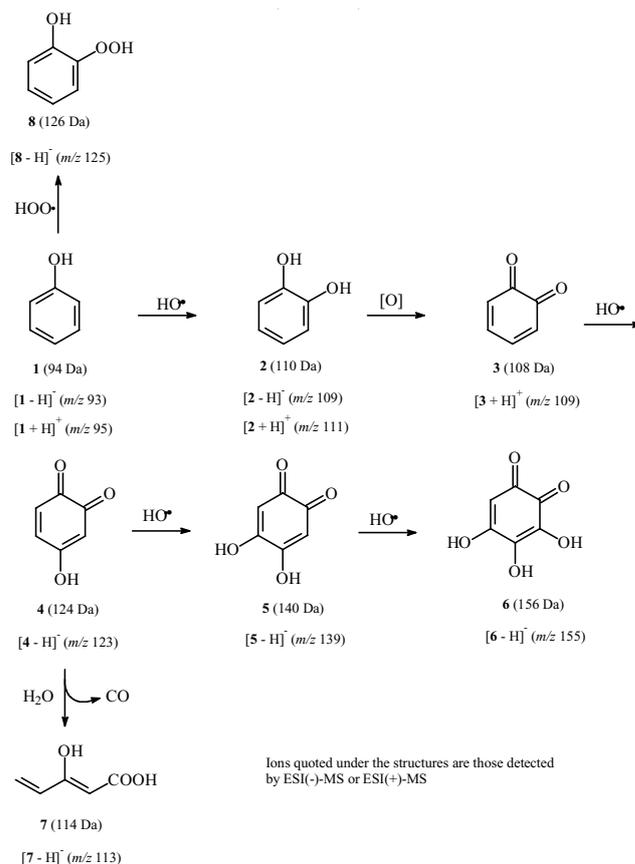
Advanced oxidation processes of environmental importance

Using caffeine as an example, we have also shown that ESI-MS ion fishing is suitable for on-line and real-time monitoring of advanced oxidation processes of drugs and other environmentally relevant compounds.¹¹ Whereas classical off-line approaches such as GC-MS can handle the more volatile and lighter species, the more polar, relatively unstable, or even transient intermediates or products that escape detection by these techniques may be detected and structurally characterized by on-line and real-time ESI-MS monitoring. Experimental and spectroscopic data show that caffeine is quickly and completely degraded under the oxidative conditions of the UV/H₂O₂, TiO₂/UV, and Fenton systems but the organic carbon content of the solution decreases much more slowly. ESI-MS(/MS) show that caffeine is in fact first oxidized to *N*-dimethylparabanic acid, and then to a second degradation intermediate, di(*N*-hydroxymethyl)parabanic acid. This second polar and, probably, relatively unstable intermediate, which is not detected by off-line GC-MS and which constitutes an unprecedented intermediate in the degradation of caffeine, is likely formed via oxidation of *N*-dimethylparabanic acid at both of its *N*-methyl groups.

We have also used on-line ESI-MS(/MS) to monitor the oxidation of phenol by a novel heterogeneous Fenton system based on a Fe⁰/Fe₃O₄ composite and H₂O₂.¹² It was shown that this heterogeneous system promotes prompt oxidation of phenol to hydroquinone, which is subsequently oxidized to quinone, other cyclic poly-hydroxylated intermediates and an acyclic carboxylic acid. A peroxide-type intermediate, probably formed via an electrophilic attack of HOO• on the phenol ring, was also intercepted and characterized. ESI-MS(/MS) monitoring of the oxidation of two other model aromatic compounds, benzene and chlorobenzene, indicates the participation of analogous intermediates. These results suggest that oxidation by the heterogeneous system is promoted by highly reactive HO• and HOO• radicals generated from H₂O₂ on the surface of the Fe⁰/Fe₃O₄ composite via a classical Fenton-like mechanism (Scheme 7).

OH-radical-mediated photo-oxidation of isoprene

Recently, we have also used ESI-MS ion fishing to monitor a process that tried to mimic the atmospheric OH-radical-mediated photo-oxidation of isoprene.¹³ This oxidation is believed to form polyols that act as cloud-condensation nuclei yielding two major secondary organic aerosol (SOA) components, i.e. 2-methylthreitol and 2-methylerythritol. These

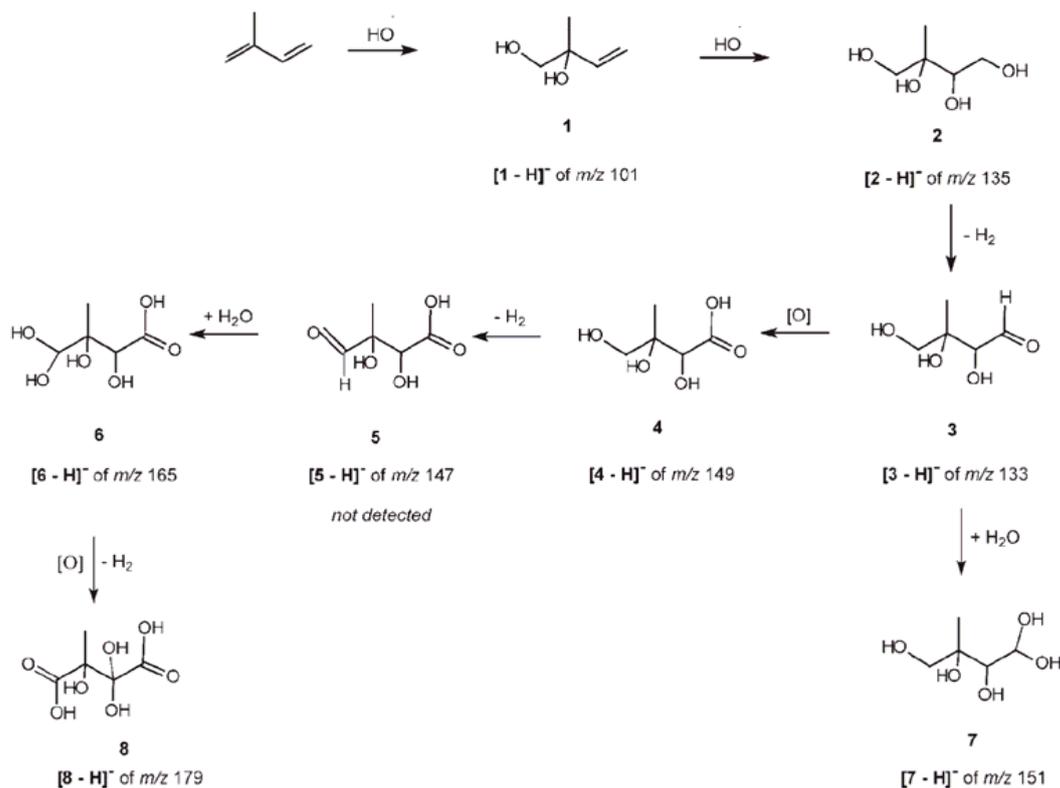


Scheme 7. Degradation of phenol by a novel heterogeneous Fenton system based on a Fe⁰/Fe₃O₄ composite and H₂O₂ as monitored by ESI-MS.

diastereoisomeric tetrols, which were characterized for the first time in the fine sized fraction (<2.5 mm aerodynamic diameter) of aerosols collected in the Amazon rain forest during the wet season, were proposed to enhance the capability of the aerosols to act as cloud-condensation nuclei. We performed the oxidation of isoprene in aqueous solution under conditions that attempted to mimic atmospheric OH-radical-induced photo-oxidation, and monitored and characterized on-line the reaction products via ESI(-)-MS(/MS). The results show that the reaction of isoprene with photo- or chemically-generated hydroxyl radicals indeed yields 2-methyltetrols, as proposed. Other polyols were also detected, and they may, therefore, be considered as plausible SOA components eventually formed in normal or more extreme OH-radical mediated photo-oxidation of biogenic isoprene (Scheme 8).

α-Methylenation of ketoesters

We also used ESI-MS ion fishing to investigate the mechanism of an efficient method for the α-methylenation of ketoesters.¹⁴ The method is useful to prepare α-methylene ketoesters in high yields using one-pot reactions with simple reagents *via*

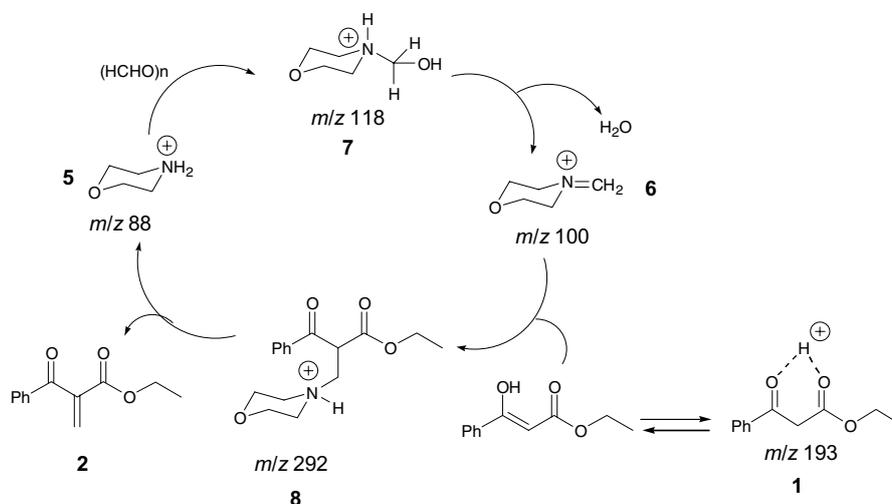


Scheme 8. Proposed route for the formation of polyols via reactions of isoprene with hydroxyl radicals.

direct Mannich-type α -methylenation of α , β and γ -ketoesters. The key intermediates of the catalytic cycle were detected and characterized and the ESI-MS information was used to guide optimization of the reaction conditions. Scheme 8 summarizes the catalytic cycle for direct Mannich-type α -methylenation of the ketoester that forms α -methylene ketoesters based on previous mechanistic interpretations but now showing three cationic intermediates detected by ESI-MS and characterized by ESI-MS/MS (Scheme 9).

N-oxide hydroxyquinolines from *o*-nitrophenyl Baylis–Hillman adducts

Very recently, we have also applied ESI-MS(/MS) ion fishing to investigate the mechanism by which substituted *N*-oxide hydroxyquinolines are formed from *o*-nitrophenyl Baylis–Hillman adducts and have been able to intercept a new key intermediate of this important reaction,¹⁵ that of m/z 334, as seen in Figure 4. This new intermediate corresponds



Scheme 9. Proposed mechanism for α -methylenation of ketoesters.

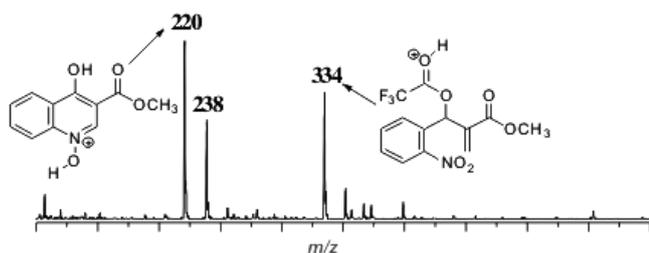


Figure 4. ESI(+)-MS for the reaction solution during formation of an *N*-oxide hydroxyquinoline (m/z 220) in which the new intermediate of m/z 334 formed upon TFA addition is clearly detected.

to an *ortho*-trifluoroacetylated BH adduct that seems to explain the requirement of TFA catalysis (Scheme 10).

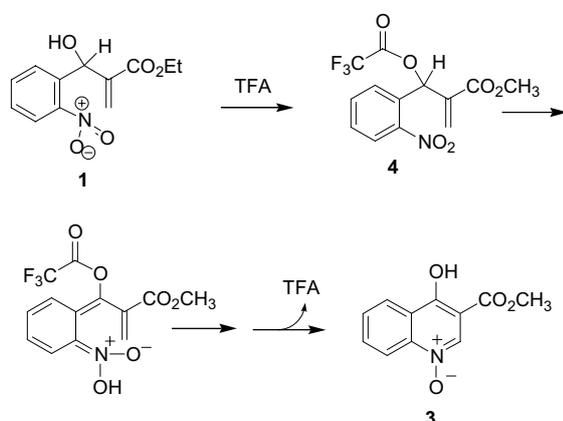
Conclusion

As illustrated in this article with recent results from our laboratory, ESI-MS(/MS) ion fishing has been used with great success to monitor a variety of key chemical reactions, today being one of the most powerful techniques available to study mechanisms of solution and gas-phase reactions.

Note added in proof: Very recently, we have also investigated the Stille reaction using ESI-MS(/MS) which has proved to be of great use in organic synthesis. Major intermediates were detected and characterized and a detailed catalytic cycle was proposed.¹⁶

Acknowledgments

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Scheme 10. New intermediate 4 identified by ESI-MS for the formation of the *N*-oxide hydroxyquinoline from the *o*-nitrophenyl Baylis–Hillman adduct 1.

ionization technique that has so extensively and positively revolutionized our field and that has allowed us to go ion fishing!

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