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# Online Mechanistic Investigations of Catalyzed Reactions by Electrospray Ionization Mass Spectrometry: A Tool to Intercept Transient Species in Solution

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**Keywords:** Mass spectrometry / ESI-MS / Reaction mechanisms / Intermediates / Organometallic catalysis

During the last two decades there has been considerable growth in the development of electrospray ionization mass spectrometry (ESI-MS) as a practical method for studying reaction mechanisms. This tool allows interception and characterization of several key intermediates, either as transient species or as protonated/deprotonated forms of neutral species. Reaction pathways shown by ESI-MS(/MS) have been probed by gas-phase ion/molecule reactions, and expanded mechanisms have been elaborated based on mass spectrometric data. The successful application of ESI-MS in revealing, elucidating, and helping to consolidate proposed mechanisms of organic reactions is emphasized. This is meant to serve as a foundation for future investigations in this rapidly developing area of research. Outstanding features and advantages of ESI-MS make it one of the most suitable tools for the fast screening of intermediates directly from solution; it has significantly increased the chemical information available to organic chemists.
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## 1. Introduction

In the last decade mass spectrometry has developed at a tremendous rate. This expansion has been driven by improved atmospheric pressure ionization (API) methods, especially electrospray ionization (ESI),[1] which enable the investigation of liquid solutions by mass spectrometry. ESI is used for ionic species in solution, and this "ionization" method has opened up access to the direct investigation of chemical reactions in solution by mass spectrometry. In principle, ESI makes possible the detection and study not only of reaction substrates and products, but even short-lived reaction intermediates as they are present in solution. It thus provides new insights into the mechanisms of many reactions.

ESI-MS and its tandem version ESI-MS/MS are rapidly becoming the techniques of choice for solution-phase mechanistic studies in chemistry. Depending on the settings of the equipment, the ESI process can be used to transfer analyte species generally ionized in the condensed phase into the gas phase as isolated entities. An interesting method to study intermediates using ESI is the "ion-fishing" technique applied by Chen.[2] The suspected catalytic ionic species is "fished" from solution and transferred to the collision cell of the mass spectrometer, where gas-phase catalytic reactions can be further studied by ion/molecule reactions.[3]

This microreview deals with the use of ESI-MS in studies of organic reaction mechanisms in the condensed phase. It is focused on the interception of organic reaction intermedi-

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ates previously proposed based on experimental evidence, and isolation of the different products, thus validating or undermining the empirical proposals. More detailed information about the technique can be found in some excellent reviews[4] that summarize the current thinking on the various stages of the ESI process.[5,6] However, a brief comment on the ESI mechanism must be emphasized.

## 2. Preservation of Charge During Transit of Ions from Solution to the Gas Phase Using the ESI Technique

The electrospray process can be described with relative simplicity. A solution of the analyte is passed through a capillary held at high potential. The high voltage generates a mist of highly charged droplets which passes through potential and pressure gradients toward the analyzer portion of the mass spectrometer. As they travel, the size of the droplets is reduced as the solvent evaporates, and further reduced as coulombic repulsions (from increasing charge density in the shrinking particles) cause the droplets to subdivide. As a final result, the ions become completely desolvated.[7]

The charge state of the isolated ions is expected to closely reflect the charge state in solution (multiply charged species are sometimes observed due to ion/molecule reactions at the interface), since the transfer of ions to the gas phase is not an energetic process − the desolvation is indeed a process that effectively cools the ion.[8,10] Therefore, it can be assumed that ESI involves only the stepwise disruption of non-covalent interactions, principally the removal of molecules of solvation, and interception of this process may allow the preservation of relatively strong non-covalent interactions of analytical significance.[9] The experimental information available so far on preservation of charge is not clear.[10] Single-electron transfer can occur during the electrospray process, because the ESI capillary can act as an electrolytic half-cell when extreme conditions are employed.[11] Redox reactions have been reported in ferrocene derivatives,[12] metalloporphyrins,[13] metal complexes of amino acids,[14] and some organic molecules. Some neutral organic molecules that possess no sites of protonation/deprotonation have also been analyzed by using unconventional ESI conditions.[15] In all these examples high ion source voltages or modifications in the equipment configuration were carried out to achieve the electrolytic-cell-like ESI-MS.

ESI-MS is not just a qualitative technique: the intensity of the detected gas-phase ions and the corresponding concentrations of these ions in the electrosprayed solution are related. However, in cases where the solution contains compounds able to react with these ions, the intensities may change drastically and much more complex relationships may prevail. The coordination properties of supramolecular compounds may also differ between the solution and the gas phase. It is well-known that polydentate ligands capable of forming stable solution complexes with transition metal ions favor the transfer of the metal into the gas phase by stabilizing it.[16,17] All these cases suggest the necessity of rigorous analysis to prove unambiguously that the species detected by ESI are the ones prevailing in solution, and more importantly to confirm that they are indeed intermediates on the reaction path.

## 3. Developing Methods to Study Reaction Mechanisms

### 3.1. Monitoring Methods

There are two different ways to study a reaction using API methods: offline and online screenings. Early investigations using API techniques were usually performed offline.[18,19] An offline MS study of a reaction in solution involves multiple steps, for example: (i) investigate the specific reaction conditions by mixing the reagents to produce the different intermediates; then (ii) determine the solution composition over time as the reactants are progressively transformed into products.[20,21] The latter operation can be accomplished by direct MS screening of the reaction intermediates during predefined intervals, and characterization by MS/MS. This is achievable if there is a reasonable concentration of intermediates in solution, and if these species are not degraded within a few seconds/minutes. The overall time resolution has to match the rate of the process to yield the desired information. One of the inherent limitations of this approach, therefore, is that transient species cannot be analyzed due to their short lifetime in solution.

In a second scenario, the kinetic and mechanistic information about the reactions in solution can be studied by using reactors coupled to the ion source. The first online mass spectrometric investigation of electrochemical reactions using thermospray ionization was reported in 1986.[22] It proved the potential-dependent formation of dimers and trimers in the electrooxidation of *N*,*N*-dimethylaniline in aqueous solution. Another example of online investigations is the reaction of ferric bleomycin and iodosylbenzene that was given by Sam and coworkers[23] in 1995, in which a low dead volume mixing tee directly attached to the spray source was used. This feature provides mass-specific characterization of stable products and reactive intermediates with lifetimes down to the millisecond time regime, and subtle changes in the reaction medium can also be observed. The simplest online reactor coupled to the mass spectrometer is the syringe itself. This allows screening of the reaction in real time, and trapping of transient species. Several groups have developed different devices to study the solution-phase mechanism of radical-initiated, photochemical, electrochemical and organometallic reactions, reducing the transit time of the species during the experiments. An interesting commercially available microreactor (Alltech, PEEK mixing tee, Figure 1)[24,25] can be directly connected to the ESI spray capillary, allowing analysis at reaction times from 0.7 to 28 s in continuous-flow mode. Longer reaction times can easily be covered by introducing a fused silica transfer capil-

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lary of variable length between the microreactor and the spray capillary. The chemical reaction in this system takes place by mixing two liquid flows containing the substrate and the reagent in close proximity of the ionization source. At the moment of mixing the two solutions, the reaction is initiated and the mass spectra of the reacting solution under steady state conditions can be acquired (Figure 1).

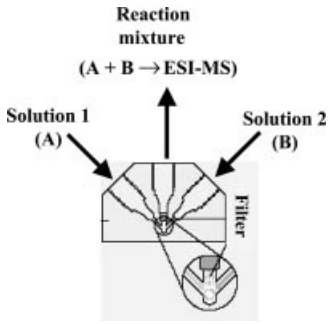


Figure 1. A microreactor that allows the effective mixing of the reactants in solution and is coupled directly to the electrospray ion source.

### 3.2. Probing Reactivity of Intermediates

We and others introduced an important methodology based on the isolation in the gas phase of species assumed to participate in the reaction mechanism and perform ion/molecule reactions with neutral substrates (in the collision cell) of the reaction solution. This methodology seems to be very useful to discard side products and to assure the reliability of the analyses.

The traditional method used for gas-phase ion/molecule reactions was limited to neutral compounds that were sufficiently volatile and thermostable to be transferred to the gas phase by heating. Some years ago it was shown that by using API conditions (ESI or APCI – atmospheric pressure chemical ionization), the range of neutral molecules participating in ion/molecule reactions could be expanded toward those of lower volatility and thermal stability.

Today this new approach is widely used by mass spectrometrists to exploit the outstanding speed, sensitivity and selectivity of the diverse environments that only mass spectrometry offers. The rational relationship between structure and chemical reactivity are used to investigate the structure of gaseous neutral molecules and ions through an arsenal of selective ion/molecule reactions. These investigations, which have been reviewed mainly from the EI (electron ionization) perspective,[26] cover the intrinsic mechanistic details of these reactions that occur under the unique solvent- and counterion-free environments of mass spectrometers. Now, the effectiveness of ion/molecule reactions is used to probe complex problems in organometallic chemistry. However, there are few examples of relevance using this approach, with a focus on those reactions performed under the well-defined conditions of sequential mass spectrometry using mass-selected ions. Key mechanistic details and potential applications of ion/molecule reactions have been shown to occur efficiently under API conditions. These innovative strategies greatly widened the scope of applications of such ion/molecule reactions to charged molecules in solution, which can now be studied easily by using API techniques (ESI, APCI, APPI – atmospheric pressure photoionization).

From a synthetic point of view, mass spectrometrists use triple quadrupoles to further refine mechanistic studies (Figure 2). Triple quadrupoles, Q-traps, and other devices certainly afford one of the most complete laboratories for the studies of gas-phase ion/molecule reactions. The remarkable success of this concept inspired many scientists to devote more interest to this potentially advantageous strategy. Thus, using triple quadrupoles, ions are obtained from the source (ESI, APCI), purified by mass selection in Q1, and reacted in q2 (collision cell) under controlled conditions, then further mass analysis of the reaction products is performed in Q3. Figure 2 illustrates a triple quadrupole instrument that can be used in this kind of study. The main advantage is that all these steps are carried out online in very short time intervals, and under conditions that can be maintained for long periods and easily described and reproduced in different equipment. Another important method is the study of well-known reactions and comparison of the data obtained by ESI-MS with that obtained by other spectroscopic techniques.

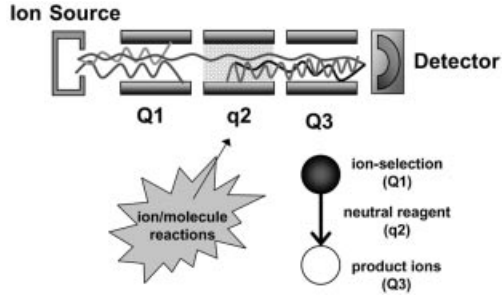


Figure 2. Representative triple quadrupole equipment that is used to study reaction mechanism and further ion/molecule reactions to probe reactivities of trapped species from solutions.

## 4. Reaction Mechanism Studies

### 4.1. The Baylis–Hillman Reaction

In a representative example of online monitoring, the Baylis–Hillman reaction was studied by ESI-MS in both the positive and negative ion modes. The reactions were performed in a sealed syringe directly coupled to the ion source.[27] The proposed intermediates for the catalytic cycle of the reaction (**1**–**7**, Scheme 1) were successfully intercepted and structurally characterized for the first time using ESI-MS and MS/MS. Strong evidence was collected corroborating the currently accepted mechanism.[28]

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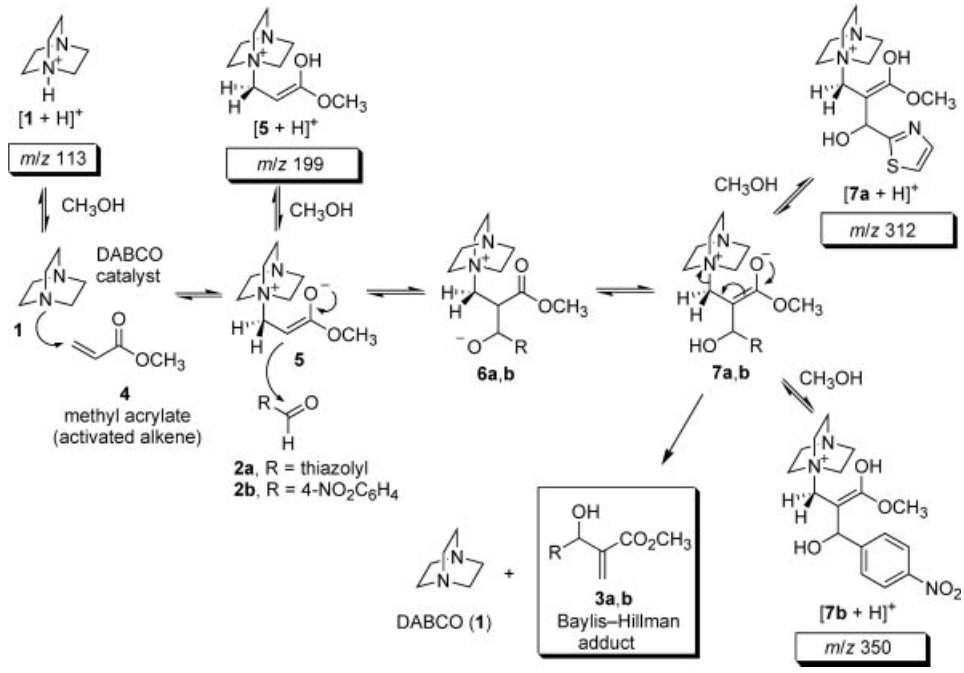


Scheme 1. Mechanism of the Baylis–Hillman reaction of methyl acrylate and aldehydes catalyzed by DABCO. The protonated species intercepted and structurally characterized by ESI(+)-MS/MS are shown, with their respective *m*/*z* ratios.

### 4.2. The Baylis–Hillman Reaction Cocatalyzed by Ionic Liquids

In a similar manner, online monitoring of Baylis–Hillman reactions cocatalyzed by ionic liquids was applied to identify supramolecular species responsible for the cocatalytic role of ionic liquids in the reaction, by gently fishing them from solution to the gas phase (Scheme 2).[29] Several supramolecular species formed by coordination of reagents and products were trapped, identified and characterized by MS analysis and MS/MS dissociations. By using competitive experiments, the relative efficiency of different cocatalysts was determined to be BMI·$CF_3CO_2$ > BMI·$BF_4$ > BMI·$PF_6$, which was the opposite of that observed by Afonso et al.[30] in the liquid phase. Based on the interception of these unprecedented supramolecular species, it was proposed that 1,3-dialkylimidazolium ionic liquids function as efficient cocatalysts for the reaction by: (i) activating the aldehyde toward nucleophilic attack by BMI coordination (species $\mathbf{15}^+$), and (ii) stabilizing the zwitterionic species that act as the main Baylis–Hillman intermediates through supramolecular coordination (species $\mathbf{16}^+$, $\mathbf{17}^+$, $\mathbf{18}^+$, and $\mathbf{20}^+$).

### 4.3. Ring-Contraction Reaction

Looking for experimental support to validate the mechanism of ring contraction for the reaction depicted in Scheme 3, online monitoring of the reaction was performed by ESI-MS and ESI-MS/MS using the syringe as the reaction vessel.[31] A bicyclic iminium ion intermediate $\mathbf{23}^+$ was proposed to participate in the reaction (Scheme 4), but both reactant **21** and product **22** were neutral molecules. These neutral species were, however, expected to be in equilibrium with their protonated forms when dissolved in protic solvents such as methanol. Therefore, ESI could transfer both reactants and products to the gas phase as $[M + H]^+$ species for MS analysis. Even a disfavored equilibrium was helpful because of the exceptionally high sensitivity of the ESI-MS technique. ESI-MS monitoring was first used to study the reaction of iodo-β-enamino esters **21a**–**c** with $Et_3N$ as the base. Iodo-β-enamino esters **21** (1.0 equiv.) and $Et_3N$ (1.0 equiv.) were mixed in 1:1 toluene/methanol (2 mL) at 25 °C, and the reaction was monitored by ESI-MS. Major ions were clearly detected by ESI-MS, corresponding to the protonated reactant [**21a** + H]$^+$ of *m*/*z* 386, intermediate $\mathbf{23a}^+$ of *m*/*z* 258, and the protonated product [**22a** + H]$^+$ of *m*/*z* 276. Likewise for **21b** and **21c**, the protonated reactants [**21b**–**c** + H]$^+$, the intermediates $\mathbf{23b}$–$\mathbf{c}^+$, and the final protonated products [**22b**–**c** + H]$^+$ were clearly detected. The detected cations, as shown by continuous ESI-MS monitoring, were the same from 1 to 60 min of reaction. Scheme 4 summarizes a general reaction pathway showing neutral and protonated reactants and protonated products, as well as the key bicyclic iminium ion intermediates $\mathbf{23a}$–$\mathbf{c}^+$ (with their respective *m*/*z* ratios) that have been intercepted and structurally characterized by ESI-MS(/MS).

### 4.4. Nucleophilic Substitution Reactions

An interesting approach using ESI(–)-MS (negative-mode ESI-MS) was used to probe the mechanism of nucleophilic substitution reactions of methylated hydroxylamines,

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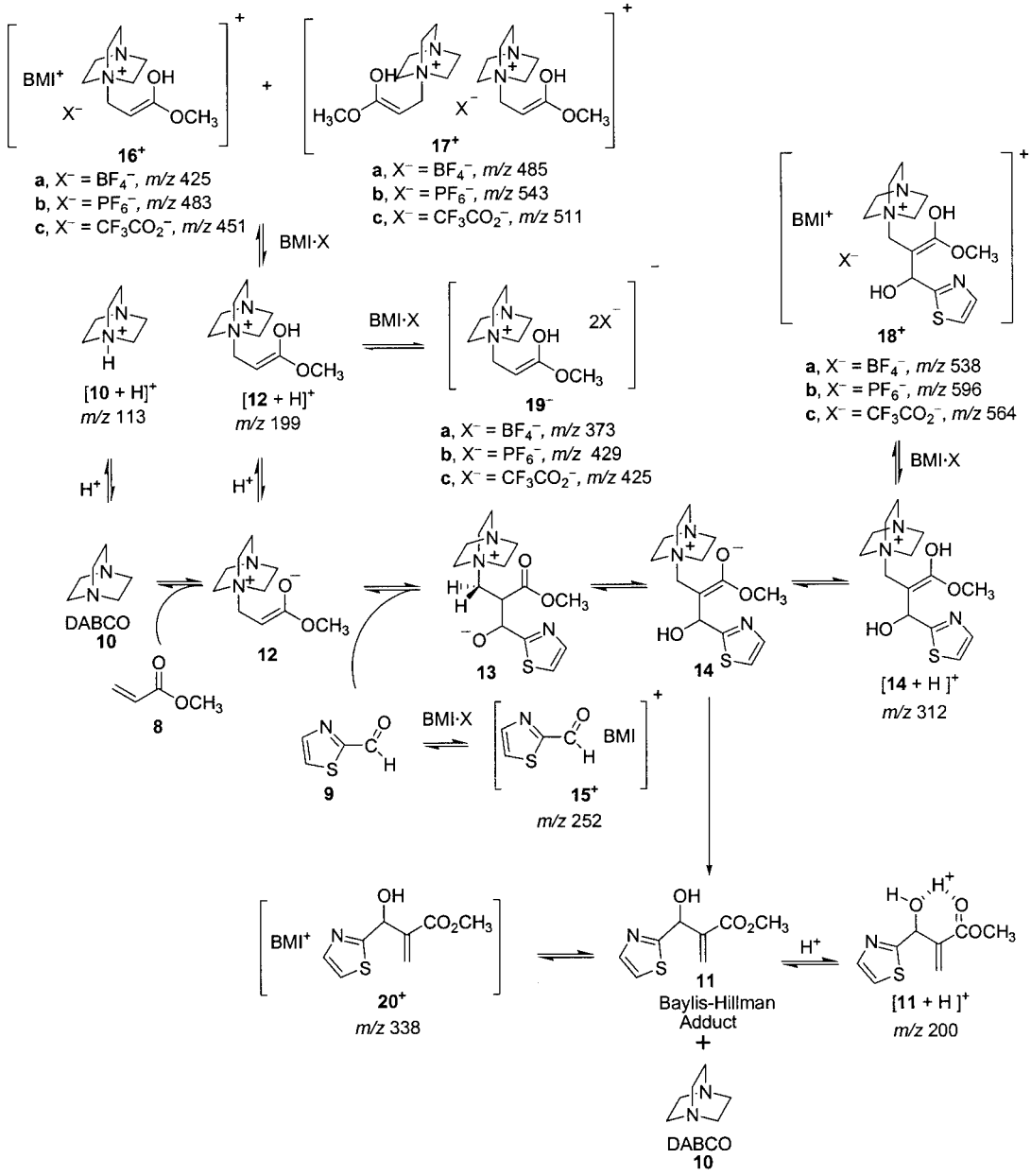
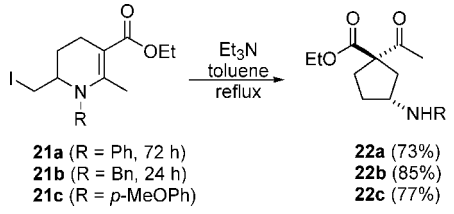



Scheme 2. Baylis–Hillman reaction of methyl acrylate (**8**) and 2-thiazolecarboxaldehyde (**9**) cocatalyzed by both DABCO (**10**) and an ionic liquid BMI·X (X = $BF_4^-$, $PF_6^-$, $CF_3CO_2^-$).




Scheme 3. Ring contraction reaction.

hydrazine and hydrogen peroxide with bis(2,4-dinitrophenyl)phosphate (BDNPP).[32] ESI-MS screening of the reaction was performed to fish ionic intermediates and products directly from solution into the gas phase, and the key intermediates were fully characterized by ESI-MS/MS experiments. Along with the ESI(–)-MS(/MS) results, kinetic and NMR spectroscopic data were collected, and the reaction of $NH_2OH$ with BDNPP was also studied. It was concluded that there was both aromatic substitution, generating DNPP **26** and compound **27**, and initial phosphorylation of the OH group of NHMeOH by BDNPP (Scheme 5, Figure 3). The latter generates dinitrophenyl oxide (DNP), forming intermediate **25**, which breaks down slowly by two distinct pathways: (a) aromatic nucleophilic substitution described above, giving **27** and **29**, or (b) spontaneous rearrangement where the terminal NHMe group attacks the dinitrophenyl moiety to form a transient cyclic Meisenheimer complex **30**, as for the reaction with $NH_2OH$.

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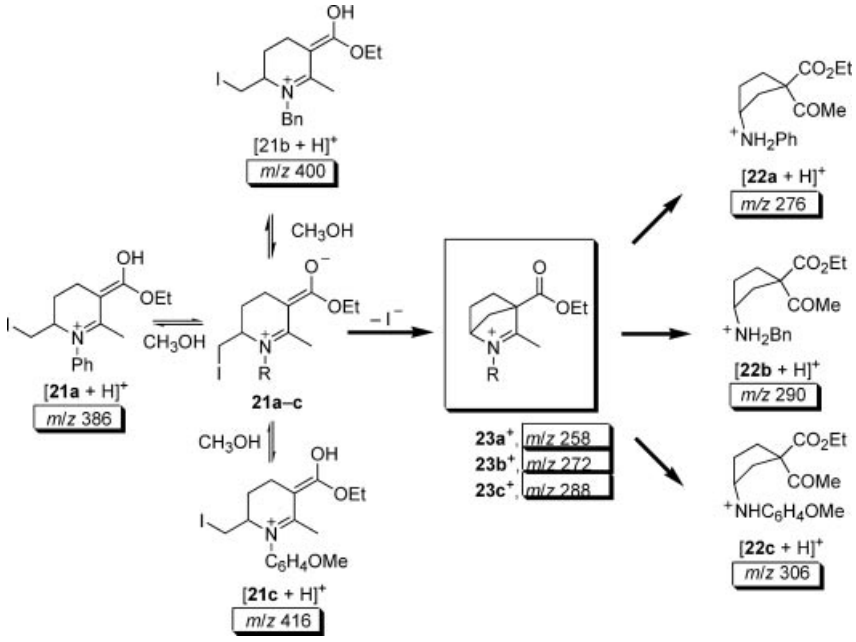
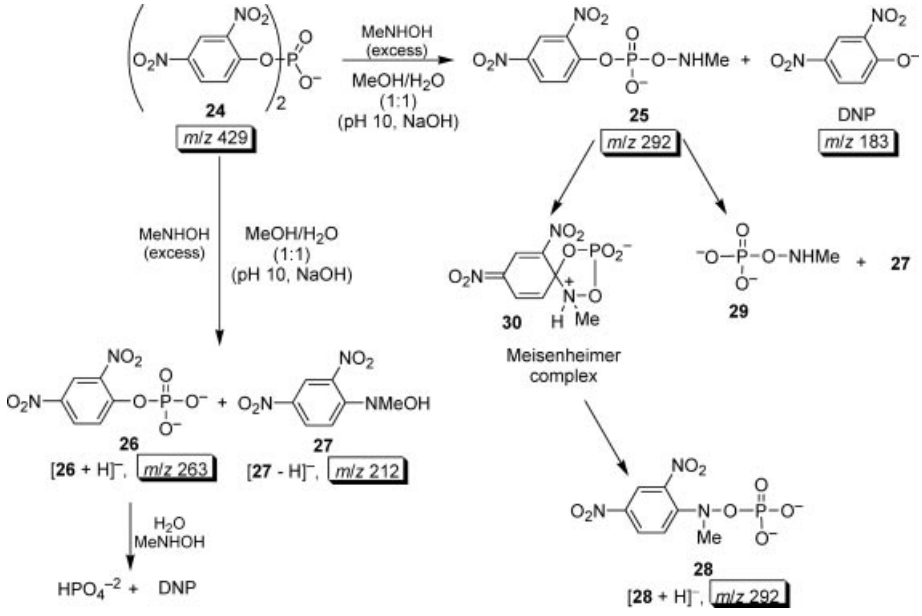



Scheme 4. Mechanism probed by ESI-MS for ring contraction reaction of iodo-β-enamino esters. The screening was performed using **21a**–**c** (1 mmol), $Et_3N$ (1 mmol) in toluene/methanol (1:1, 20 mL) at a flow rate of 0.01 mL min$^{-1}$.




Scheme 5. Mechanism for the reaction of MeNHOH and **24** probed by ESI(–)-MS experiments, which corroborated kinetics studies.

This complex **30** rapidly ring opens, giving **27** as a long-lived product, perhaps through the mechanism proposed in Scheme 5. Ionic intermediates **25** and **28** (Scheme 5 and Scheme 6) are isomers, and their relative amounts and therefore the ESI-MS/MS of the ions of *m*/*z* 292 changed drastically with time. Samples taken after 10 min of reaction in solution showed that these ions dissociated in the mass spectrometer by CID (collision-induced ionization) mainly into four fragment ions of *m*/*z* 263, 183, 97, and 79. The fragment ions, of *m*/*z* 263 and 183, were formed in the gas phase from **25** (Scheme 6, a), whereas dissociation to ions of *m*/*z* 97 and 79 was evidence for the presence of **28** (Scheme 6, b). Therefore, both **25** and **28** were present in the reaction mixture at an early stage of the reaction. After 30 or 60 min of reaction, however, the ion of *m*/*z* 292 generated only the two fragment ions of *m*/*z* 97 (**31**) and 79. This temporal change in product ion distribution from the CID of the ion of *m*/*z* 292 confirmed that at a later stage of the reaction, intermediate **28**, rather than **25**, became a dominant species through rearrangement from **25** in solution.

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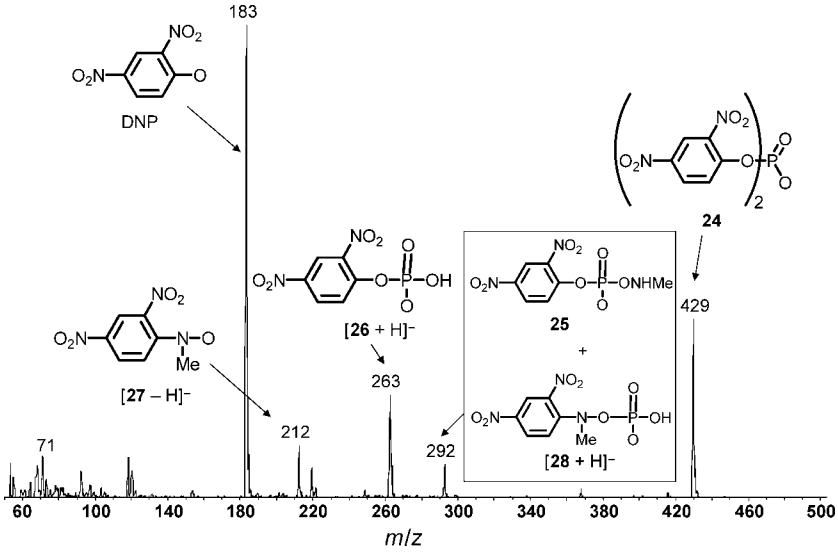
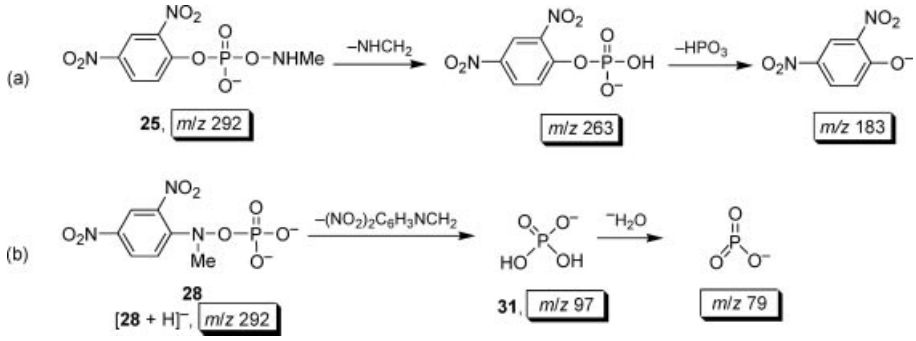



Figure 3. ESI-MS spectrum of the reaction mixture of 0.01 mol L$^{-1}$ BDNPP with 0.1 mol L$^{-1}$ NHMeOH, in aqueous methanol (50% v/v) at pH 10.




Scheme 6. Differentiation of the isomers **25** (a) and **28** (b) by fragmentation pathways (MS/MS experiments). The isomer **25** (*m*/*z* 292) was proposed to achieve **28** (*m*/*z* 292) through a Meisenheimer complex intermediate.

### 4.5. Oxidative Degradation of Caffeine

Caffeine **32** is quickly and completely degraded under the oxidative conditions of the $H_2O_2$/UV, $TiO_2$/UV, and Fenton systems to $CO_2$, $NH_3$ and $NH_2Me$. Augusti and coworkers[33] reported in an interesting article the mechanism of degradation of caffeine by $TiO_2$/UV, $H_2O_2$/UV and Fenton systems (Scheme 7). Continuous online and real-time monitoring by ESI-MS and ESI-MS/MS experiments, as well as high accuracy MS measurements, show that caffeine is first oxidized to *N*,*N*′-dimethylparabanic acid **33**, likely after initial OH insertion to the C4=C8 double bond. A second degradation intermediate, bis(*N*-hydroxymethyl)parabanic acid **34**, was identified by ESI-MS and characterized by ESI-MS/MS and high accuracy mass measurements (with errors of around 5–7 ppm). This polar and likely relatively unstable compound, which is not detected by other techniques, is likely formed by further oxidation of *N*,*N*′-dimethylparabanic acid at both of its *N*-methyl groups and constitutes an unprecedented intermediate in the degradation of caffeine.

After UV irradiation for 90 min as well as 150 min, ESI was able to gently and efficiently fish directly from the solution to the gas phase for MS analysis two increasingly abundant ions of *m*/*z* 143 (*N*,*N*′-dimethylparabanic acid, **33**) and *m*/*z* 175 [bis(*N*-hydroxymethyl)parabanic acid, **34**]. The conversion of **32** to **33** and then to **34** (an unprecedented intermediate in the oxidation of caffeine) is further supported by the ESI tandem mass spectra of their protonated molecules, which display a series of structurally diagnostic fragment ions. The authors suggested that the degradation of organic compounds by the $TiO_2$/UV, $H_2O_2$/UV, and Fenton systems goes through the mechanism for the conversion of caffeine (**32**) to *N*,*N*′-dimethylparabanic acid (**33**) involving an initial (fast) attack of the hydroxyl radicals to the C4=C8 double bond of caffeine (Scheme 7). After successive hydroxylations and oxidations, **33** and **34** are formed, and **34** is slowly mineralized to $CO_2$, $NH_3$, and $NH_2Me$.

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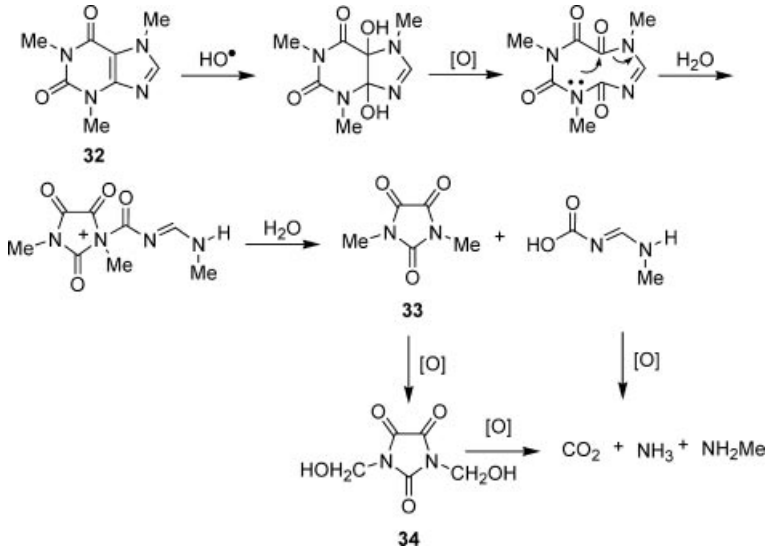
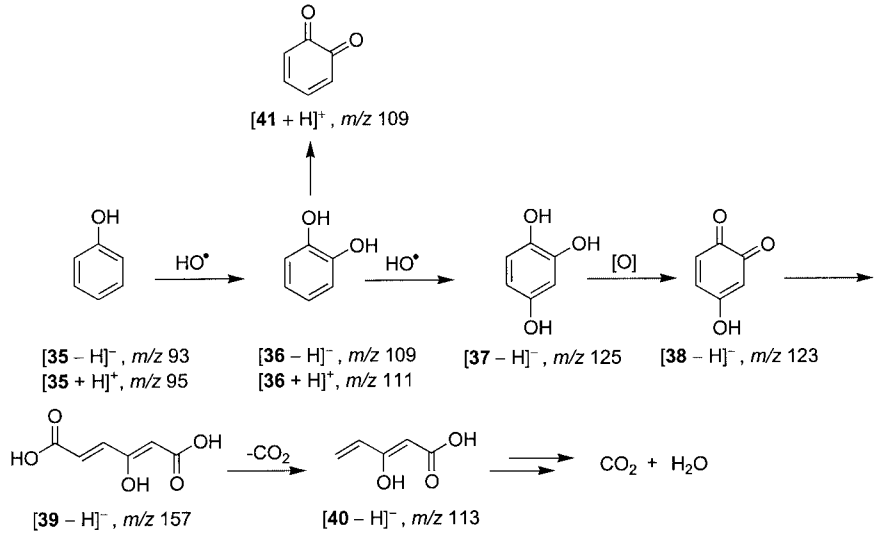



Scheme 7. Mechanism of caffeine degradation by $TiO_2$/UV, $H_2O_2$/UV or Fenton systems, as determined by ESI-MS online monitoring.

### 4.6. Heterogeneous Fenton System

Relatively few examples of radical-monitoring reactions have appeared in the literature.[25,34] Structural investigation of a novel heterogeneous Fenton system based on a FeO/$Fe_3O_4$ composite and $H_2O_2$ was reported.[35] Online ESI-MS and ESI-MS/MS show that model aromatic compounds (phenol, benzene and chlorobenzene) are successively oxidized to phenolic, benzoquinonic and ring-open carboxylic acid type intermediates. These results suggest that highly reactive hydroxyl radicals are generated from $H_2O_2$ on the surface of the FeO/$Fe_3O_4$ catalyst by a classical Fenton-like mechanism.

Oxidation reactions were performed with FeO/$Fe_3O_4$/$H_2O_2$ using phenol, benzene and chlorobenzene as model organic contaminants with on line sequential monitoring by using both ESI(–)-MS and ESI(+)-MS. In the case of phenol **35**, ESI-MS "fished" a single anion from the aqueous solution at zero reaction time. As expected, this corresponded to deprotonated phenol [**35** - H]$^-$ (*m*/*z* 93). Relatively intense anions of *m*/*z* 109, 113, 123, 125, and 157 were detected as the degradation proceeded for 20–40 min. These anionic intermediates most likely arise from deprotonation of their respective neutral forms in aqueous solutions. Despite the presumably low aqueous concentrations of these anions (because their neutral forms should predominate at pH ~6), clear ESI(–)-MS detection was achieved. The ESI(–)-MS interception of the anionic species suggested successive hydroxyl radical attacks, likely at the activated *ortho* or *para* position of the phenol ring, or both. A simple reaction scheme was rationalized for the oxidation of phenol by the FeO/$Fe_3O_4$/$H_2O_2$ system, showing intermediates **35**–**40** (Scheme 8). This additional example of online ESI-




Scheme 8. ESI-MS monitoring of phenol degradation by a heterogeneous Fenton system.

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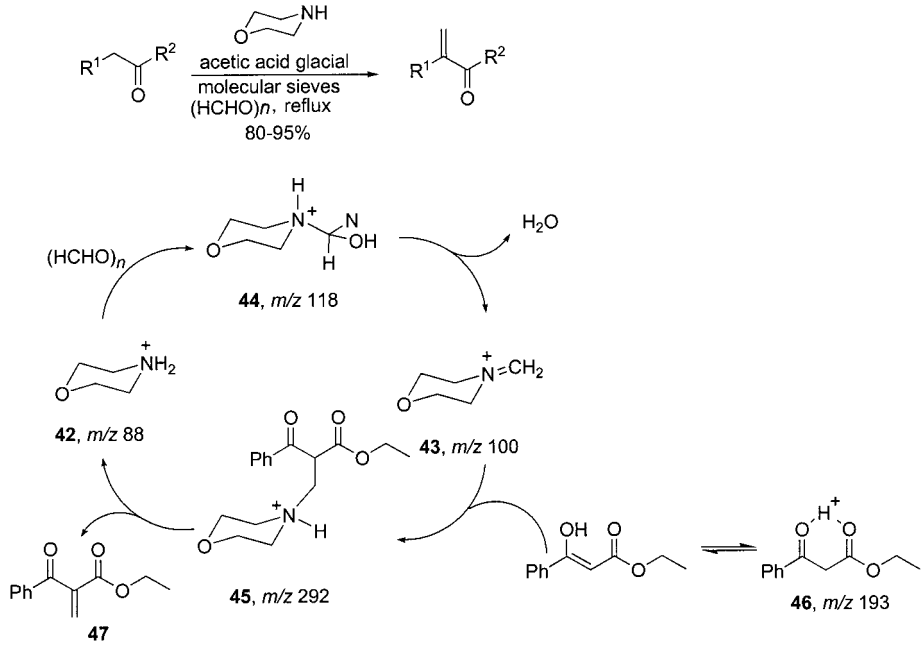
MS(/MS) monitoring of degradation of model organic molecules in aqueous solutions shows the consecutive formation of phenolic, benzoquinonic and carboxylic intermediates. This series of polyhydroxylated intermediates indicates degradation by successive attack of HO radicals, the latter formed likely from reactions of $H_2O_2$ with $Fe^{2+}$ surface species. The $FeO/Fe_3O_4/H_2O_2$ system is suggested to act by a classical Fenton-like mechanism.

### 4.7. α-Methylenation of Keto Esters

Extensive studies have been devoted to optimize the synthesis of α-methylene carbonyl compounds. Not only are they useful as synthetic intermediates, but they may also mimic biologically active natural products and be used as potential antitumor drugs. Eberlin and coworkers reported a convenient one-pot method to prepare α-methylene keto esters by direct α-methylenation of keto esters as well a mechanistic study of this interesting reaction by ESI-MS (Scheme 9).[36] They investigated by ESI(+)-MS monitoring the morpholine-catalyzed reaction of **46** with *p*-formaldehyde in acetic acid/acetonitrile solution (Scheme 9). The key players of the catalytic cycle could be either ionic or neutral species, but the authors hoped to intercept the neutral species in their protonated form from the acetic acid solutions, as achieved before.[27] Few examples of disfavored proton-




Scheme 9. Direct Mannich-type α-methylenation of carbonyl compounds.

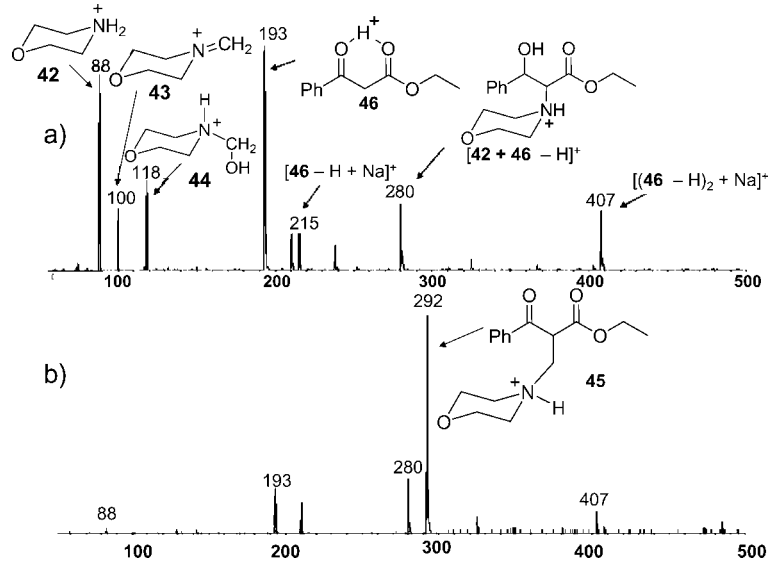



Figure 4. ESI(+)-MS for the α-methylenation reaction after (a) 6 s and (b) 12 min.

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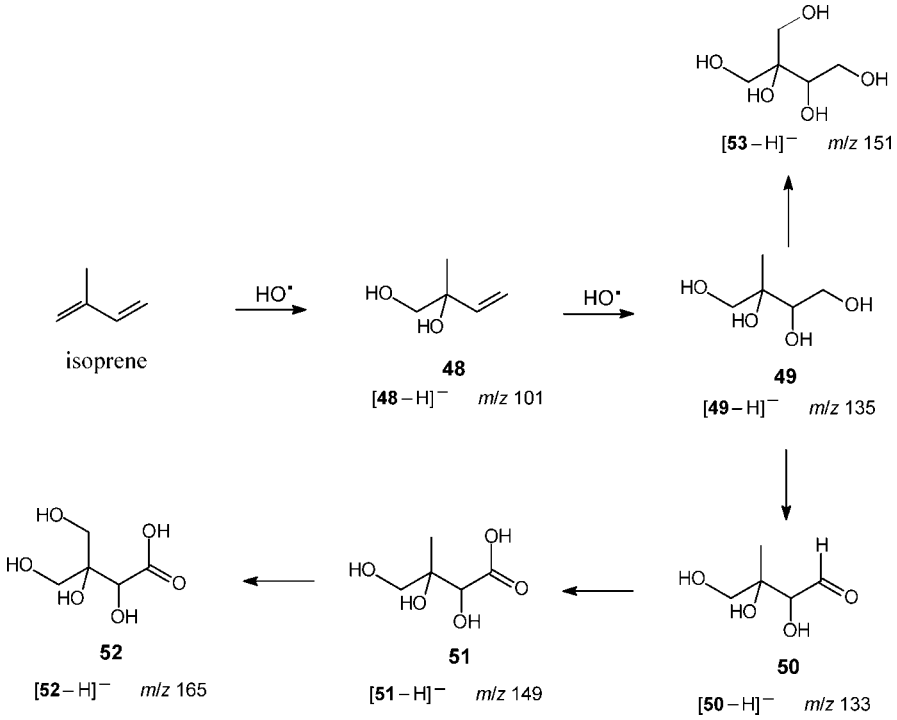
transfer equilibrium are described, however it is useful for intercepting neutral species in this case because of the high sensitivity of ESI-MS in transferring ionic intermediates from solution to the gas phase.

The ESI-MS data collected for the reaction was quite clean and mechanistically enlightening (Figure 4). As soon as the reaction mixture was formed and electrosprayed, ESI-MS detected the reactants, that is, protonated morpholine **42** (*m*/*z* 88) and protonated ethyl benzoylacetate **46** (*m*/*z* 193), and additionally two key cationic intermediates: **44** (*m*/*z* 118) and the iminium ion **43** (*m*/*z* 100) (Scheme 9). Previously, only kinetic evidence was available to support the intermediacy of iminium ions such as **43** in Mannich reactions. Another key piece of information was provided by continuous monitoring; after approximately 12 min of reaction, the ESI-MS spectrum changed considerably as another key intermediate was detected, the aldol **45** in its protonated form (*m*/*z* 292, part b of Figure 4). The abundance of the starting reagents [**46** + H]$^+$ (*m*/*z* 193) and **42** (*m*/*z* 88) as well as intermediates **43** (*m*/*z* 100) and **44** (*m*/*z* 118) had decreased accordingly. The online screening depicted in Figure 4 demonstrates for [**46** + H]$^+$ (*m*/*z* =193) and [**45** + H]$^+$ (*m*/*z* 292) that α-methylenations can be continuously monitored by ESI-MS; key changes are the consumption of the starting carbonyl compound and the formation of the final product via its immediate aldol precursor. Scheme 9 summarizes the catalytic cycle proposed for direct Mannich-type α-methylenation of keto ester **46** in the presence of **42** to form the α-methylene keto ester **47**.

The scheme is based on previous mechanistic interpretations but shows the three cationic intermediates **43**–**45** now intercepted and structurally characterized by ESI-MS(/MS). Metzger recently demonstrated a similar approach for L-proline-catalyzed reactions.[37]

### 4.8. Mimicking Atmospheric Oxidation of Isoprene

Photooxidation of isoprene in the atmosphere was thought to yield only lighter and more volatile products such as formaldehyde, methacrolein, and methyl vinyl ketone. Evidence for the formation of hygroscopic polar products such as **49** that can give rise to aerosols by gas-to-particle formation processes opened up a new panoroma for the role of isoprene in the atmosphere. Under simulated atmospheric conditions, photooxidation of isoprene by ozone was shown to be relatively slow and to occur mainly by reaction with OH radicals. Furthermore, when the OH radical initiated photooxidation of isoprene was performed in the absence of $NO_x$, 1,2-diol derivatives **48** were formed (Scheme 10). Santos and coworkers tried to mimic the OH radical mediated oxidation of isoprene in solution, and to monitor the process online and in real-time by using negative-mode ESI-MS(/MS).[39] The OH radical-mediated oxidation of isoprene by $H_2O_2$ in solution (initiated either photochemically or by AIBN) seems to mimic adequately the atmospheric oxidation of isoprene, as revealed by ESI(–)-MS(/MS) monitoring. The in situ fishing, detection and structural characterization of **49** in its deprotonated form supports the "$C_5$ isoprene hypothesis" formulated by Claeys for the formation of the secondary organic aerosol (SOA) marker compounds, the diastereomeric 2-methylte-




Scheme 10. Proposed route for the formation of the oxygenated products **48**–**53** in the AIBN-initiated reaction of isoprene with hydroxyl radicals from hydrogen peroxide.

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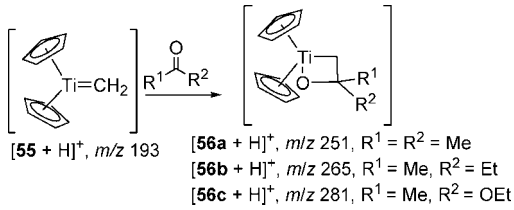
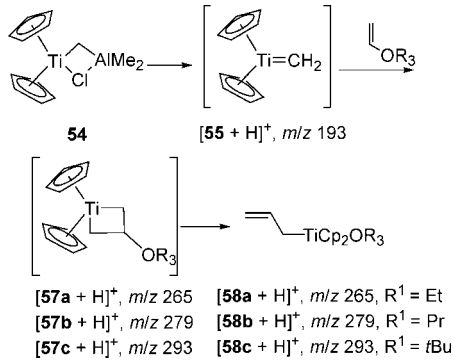
trols **49**.[38] These diasteromeric polyols have been recently identified in atmospheric aerosols of the Amazon forest, and their highly hygroscopic nature likely enhances the capability of aerosols to act as cloud condensation nuclei. Other polyoxygenated compounds assigned as **48** (the diol precursors of **49**) and **50**–**53** have also been intercepted and structurally characterized. It is suggested that products **50**–**53** are formed by further oxidation of **48** and **49**, and they may therefore also be considered as plausible SOA components eventually formed in normal or more extreme OH radical mediated photooxidation of biogenic isoprene (Scheme 10). This study also revealed that ESI(–)-MS(/MS) monitoring also has potential to mimic and therefore to study the OH radical mediated oxidation of first-generation gas-phase oxidation products of isoprene and α-pinene, such as methacrolein and pinic acid.

### 4.9. Transient Intermediates of Petasis and Tebbe Reactions

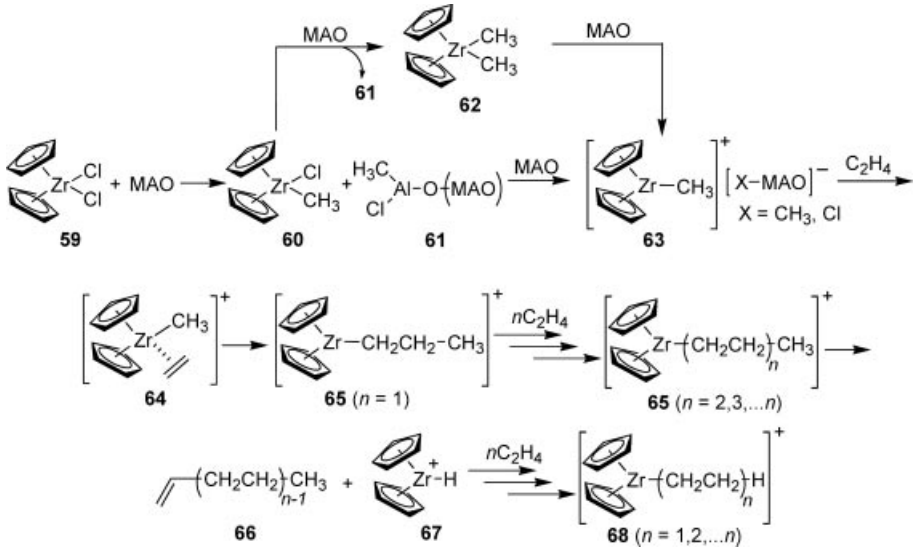
After confirming that methylenetitanocene **55** participates in Petasis reactions (Scheme 11),[40] Eberlin, Santos and coworkers decided to extend the same methodology to other related reactions for which there is no conclusive mechanistic data. This was the case for Tebbe olefination reactions.[41] In APCI, neutral molecules are transferred from evaporating droplets to the gas phase, where these molecules are ionized at atmospheric pressure by either electron abstraction, protonation or deprotonation (or a combination of these processes) due to ion/molecule reactions initiated by corona discharge. Therefore, the set of ions detected by APCI-MS is expected to closely reflect the solution composition of neutral species, and the gentle evaporation process is also likely to preserve the metal coordination spheres of organometallic species. Reagents, intermediates, and products are likely therefore to be transferred intact from solution to the gas phase, and then ionized gently and mass analyzed during APCI-MS of a reaction solution. APCI-MS in the positive ion mode efficiently protonated the postulated reaction intermediates **56** and **57** (Schemes 11 and 12). The same authors also investigated the proposal that allylic titanocene complexes are generated from non-allylic starting materials (vinyl ether derivatives) in a related Tebbe-like reaction. Key titanacycle intermediates **56** and **57** support both the classical mechanism of Ti-mediated Tebbe olefination reactions as properly postulated by Tebbe as well as the mechanism for the Tebbe-like [2 + 2] reaction postulated by Hanzawa.[42] As postulated by Tebbe, **54** generates **55**, which reacts further with the ketone to yield the oxatitanacyclic intermediates **56a**–**c**. To test the




Scheme 11. Transient intermediates of Petasis and Tebbe reactions intercepted by APCI-MS.




Scheme 12. Transient intermediates from the reaction of Tebbe reagent and vinyl ethers intercepted by APCI-MS.




Scheme 13. Proposed mechanism of Ziegler–Natta polymerization of $C_2H_4$ using the homogeneous catalyst $Cp_2ZrCl_2$/MAO.

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feasibility of this reaction toward ketones, the group applied APCI-MS to the screening of Tebbe olefination reactions, and unequivocally detected [**56** + H]$^+$ as a characteristic cluster of isotopologue ions, the most abundant signals (for $^{48}$Ti) being those at *m*/*z* 251 for [**56a** + H]$^+$, *m*/*z* 265 for [**56b** + H]$^+$, and *m*/*z* 281 for [**56c** + H]$^+$. The group then investigated the Tebbe-like [2 + 2] reaction of **54** with vinyl ethers known to afford allyl titanocenes **58** (Scheme 12). Using different vinyl ethers, the expected Ti-intermediates [**57a**–**c** + H]$^+$ were unambiguously detected by characteristic clusters of Ti-containing ions. This study further illustrates the broad potential of API mass spectrometry to study mechanisms of organometallic reactions by fishing and structural characterization of their key intermediates, a vast but still underexplored field.

### 4.10. Online Screening of Ziegler–Natta Polymerization

Significant remarkable mechanistic investigation has been performed on the Ziegler–Natta polymerization reaction. Several mechanistic and kinetic studies led to the gen-

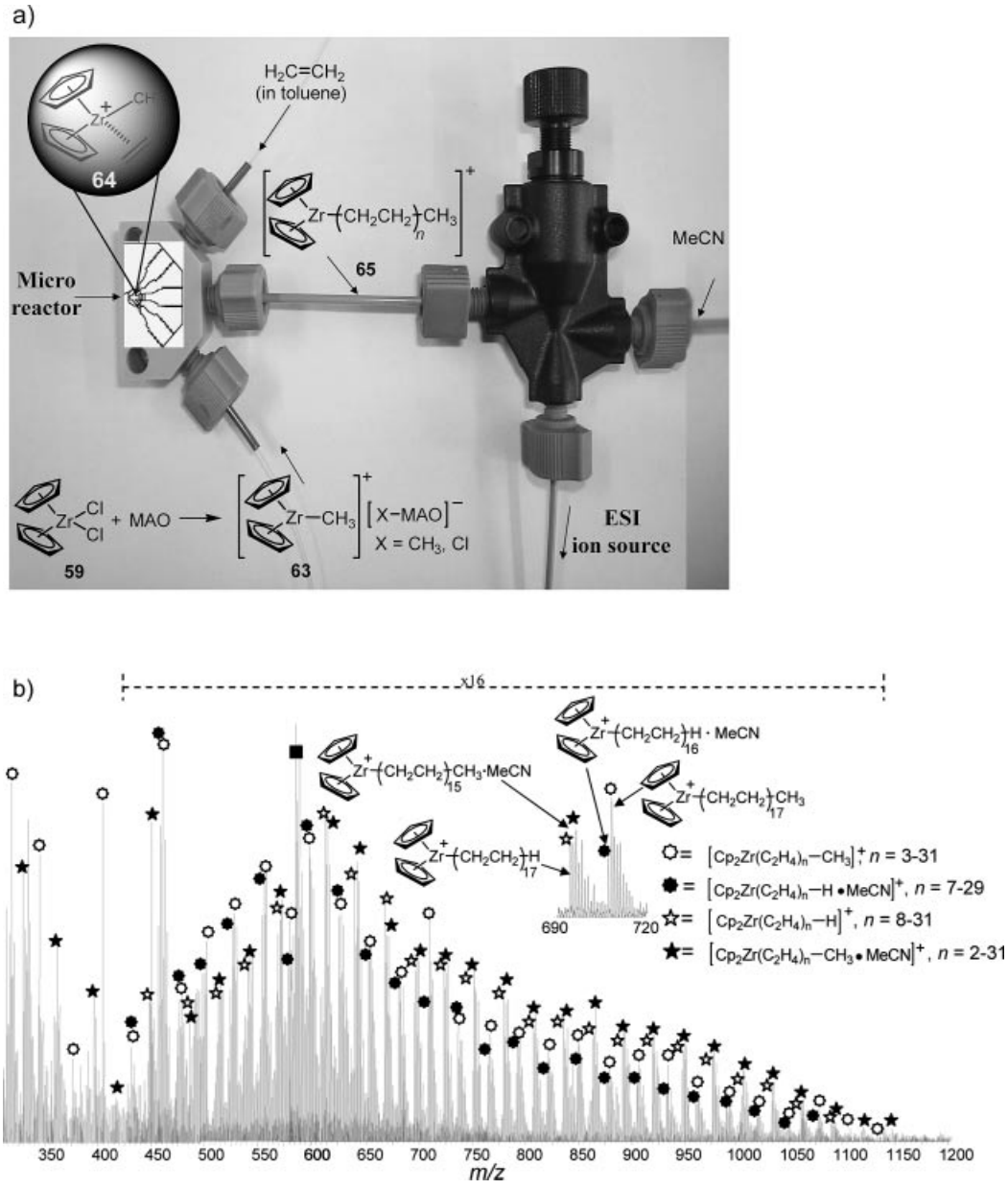


Figure 5. (a): A microreactor coupled online to the ESI source. In the first micromixer (left), toluene solutions of the preformed catalyst [$Cp_2ZrCl_2$]/MAO (1:1.2 equiv.) and of $C_2H_4$ were mixed continuously to initiate the polymerization. The reaction occurred in the capillary, transferring the reacting solution to the second micromixer (right), where it is quenched by MeCN and from which it is fed directly to the ESI source. (b): Ziegler–Natta polymerization of ethene: Positive-mode ESI mass spectrum of the MeCN-quenched reaction solution of [$Cp_2ZrCl_2$]/MAO (1:1.2 equiv.) and $C_2H_4$, using the methodology of part (a). The spectrum shows the odd- and even-numbered chain {$Cp_2Zr$}-alkyl species, as well as the MeCN adducts. The overall reaction time was approximately 1.7 s.

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erally accepted mechanism of Ziegler–Natta polymerization, which is depicted in Scheme 13. It is seen that treating a toluene solution of $Cp_2ZrCl_2$ (**59**) with methylaluminoxan (MAO) causes a rapid initial ligand exchange reaction that generates the monomethyl complex $Cp_2ZrCH_3Cl$ (**60**).[43] An excess of MAO leads to $Cp_2ZrMe_2$ (**62**).[43a] Abstraction of chloride from **60** or methyl from **62** by MAO gives the catalytically active ion-paired species $[Cp_2ZrCH_3]^+$ (**63**) with the counterion $[X–Al(Me)O–]_n^-$ (X = Cl, Me)[43b] based on solid-state XPS[44a] and $^{13}C$ NMR[44b] studies, as well as $^{91}Zr$ and $^{13}C$ NMR spectroscopic investigations of $Cp_2Zr(CH_3)_2$/MAO solutions.[45] The cation **63** in the presence of ethene gives, via π complex **64**, the insertion product **65** ($n$ = 1) as the first intermediate of the polymerization process, which is followed by step-by-step insertion of ethene to form the cationic alkyl zirconocenes **65** ($n$ = 2, 3, ... $n$). β-Elimination gives the uneven chain polymer **66** containing a terminal C=C bond and cationic zirconocene hydride **67**, which is able to start polymerization to give the even chain polymer via zirconocene cation **68**.[46] However, there is some experimental evidence that the general classification of zirconocene/MAO catalyst systems as single-site catalysts may be an oversimplification.[47]

Santos and Metzger[48] demonstrated that $Cp_2ZrCH_3^+$ (**63**) was easily detected in the reaction solution of $Cp_2ZrCl_2$ and MAO by ESI-MS. Cation **63** was characterized by MS/MS and the catalytic activity was directly demonstrated by an ion/molecule reaction of **63** and ethene in the gas phase. Furthermore, by using a microreactor coupled to the ESI source (Figure 5), they were able to intercept for the first time the intermediate alkyl zirconium cations **65** of the growing polymer chain of the homogeneous Ziegler–Natta polymerization of ethane (Figure 5). These were fished directly from solution and characterized mass spectrometrically, and their catalytic activity was proven directly by gas-phase reactions with ethene.

### 4.11. $TeCl_4$ Addition to Propargyl Alcohols

$TeCl_4$ is found to display reactivity towards alkynes similar to that of *p*-methoxyphenyltellurium trichloride, but it reacts with aromatic and 3-hydroxy alkynes by different mechanisms as shown by characteristic stereochemistries of the products. The complete *anti* stereospecificity of the additions of $TeCl_4$ to all propargyl alcohols studied is consistent with a cyclic chelated telluronium ion intermediate **71** (Figure 6). Using ESI-MS(/MS), Santos and coworkers[49] were able to intercept and characterize the active electrophile $TeCl_3^+$ in a THF solution of $TeCl_4$, as well as its THF complex and several $TeCl_x(OH)_y^+$ derivatives. For the first time, online ESI-MS(/MS) monitoring permitted key $Te^{IV}$ cationic intermediates in the electrophilic addition of $TeCl_4$ to alkynes to be captured from the solution and to be gently and directly transferred to the gas phase for mass measurement, determination of isotopic patterns, and structural investigation by collision-induced dissociation (Scheme 14). Two of the reaction products reported herein (the cyclic telluranes) have been recently found to act as cysteine protease inhibitors,[50] hence the mechanistic aspects of such reactions revealed in this study may assist in the design of new members of this class of potential antimetastasis agents. To date, despite their chemical and biological interest, only a few tellurium compounds have been investigated by mass spectrometry.[51]

### 4.12. Stille Reaction

For a Stille reaction, online ESI(+)-MS(/MS) monitoring has allowed Santos and coworkers[52] to intercept and char-

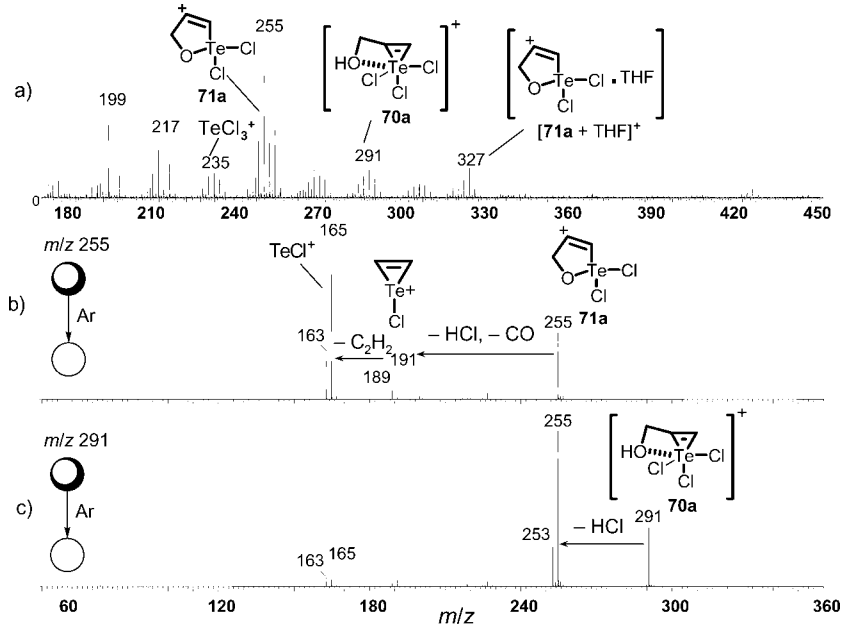


Figure 6. (a) ESI(+)-MS for online screening of a solution of **69a**/$TeCl_4$ (1.0:0.9 equiv.) in THF. ESI-MS/MS for CID of the intermediate species (b) **71a** (*m*/*z* 255) and (c) **70a** (*m*/*z* 291) intercepted from the reaction solution.

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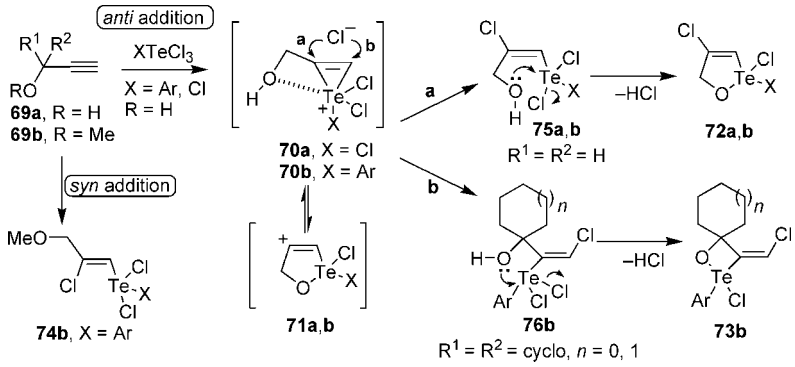


Scheme 14. Proposed mechanism of $TeCl_4$ addition to propargyl alcohols.

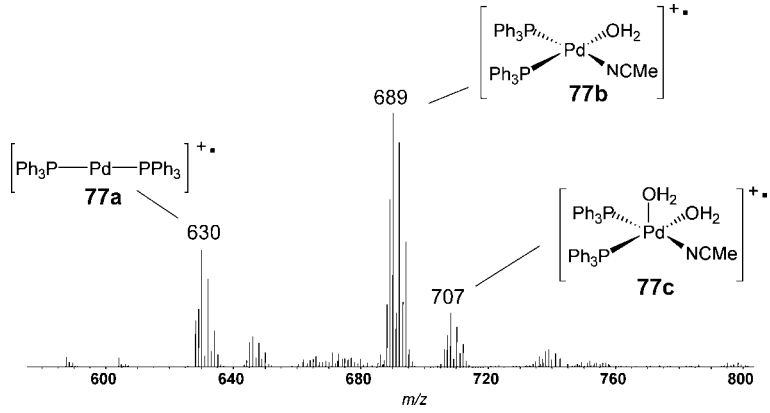


Figure 7. ESI(+)-MS of an acetonitrile solution of $Pd(OAc)_2/PPh_3$.

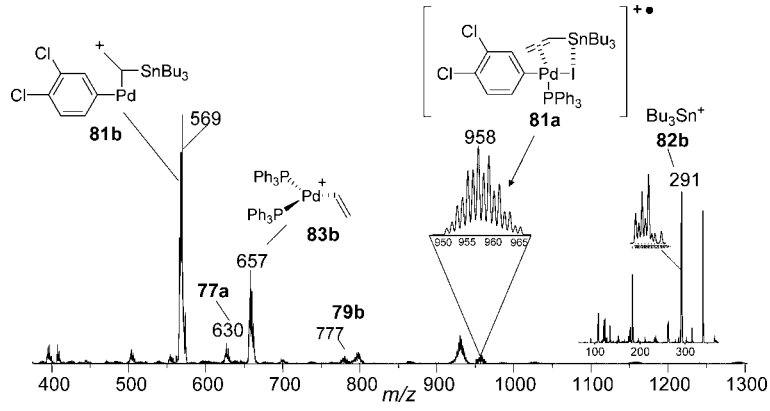


Figure 8. ESI(+)-MS of the Stille reaction of 3,4-dichloroiodobenzene (**78a**) and vinyltributyltin (**80a**) in acetonitrile mediated by $Pd(PPh_3)_4$.

acterize: a) the actual catalytically active species $Pd^0(PPh_3)_2$ (Figure 7), b) the oxidative addition product **79a** and c) the transmetalation intermediate **81a** and two products of this process **82a** and **83a** (see Figure 8 and Scheme 15). Gas-phase reductive elimination (for $\mathbf{83b^+}$) has also been observed. Therefore for the first time, most (if not all) major intermediates of a Stille reaction have been intercepted, isolated and characterized. Using ESI(–)-MS, the counterion $I^-$ was the only species detected. Such straightforward experiments further illustrate the applicability of direct injection ESI-MS(/MS) in revealing, elucidating, and helping to consolidate mechanisms of organic reactions as depicted in Scheme 15.

### 4.13. Alkynylatin of Tellurides Mediated by $Pd^{II}$

Under palladium dichloride catalysis, vinylic tellurides couple efficiently with alkynes with retention of the double-bond geometry. Looking for experimental support to validate the catalytic cycle proposed for this reaction, Raminelli and coworkers[53] decided to investigate the $PdCl_2$-pro-

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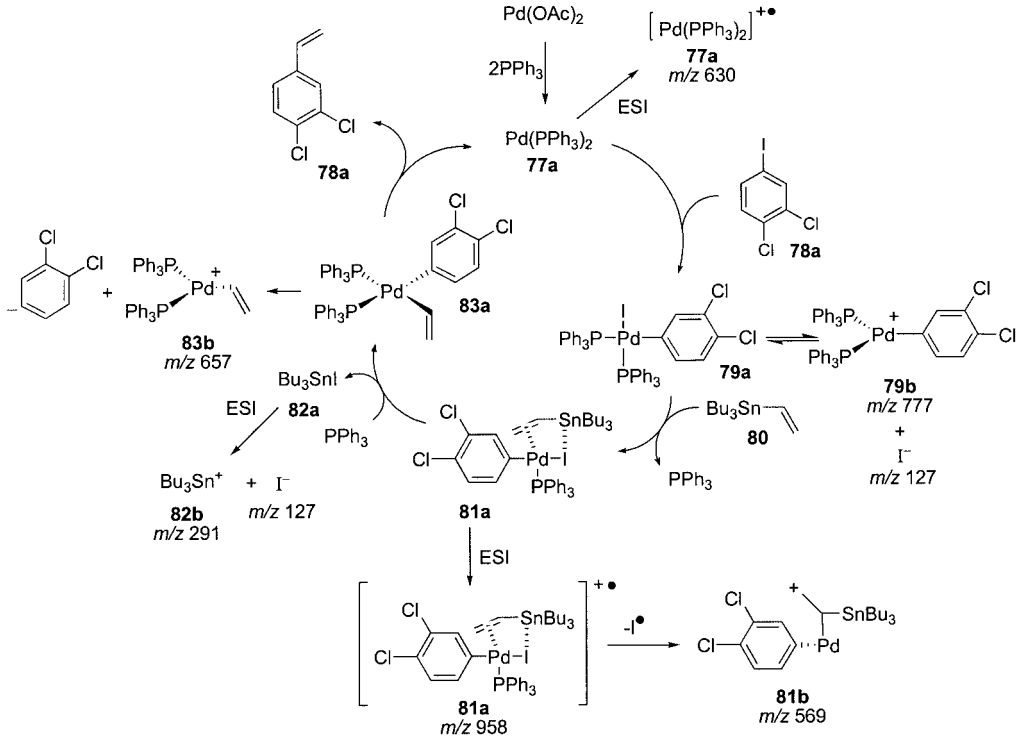
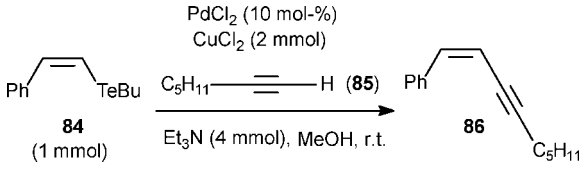



Scheme 15. Expanded mechanism (using ESI(+)-MS data) of the $Pd(OAc)_2$-mediated Stille reaction of 3,4-dichloroiodobenzene (**78a**) and vinyltributyltin (**80**) in acetonitrile.

moted coupling reaction of vinylic tellurides with alkynes by MS techniques. Pd- and Te-containing cationic intermediates were fished directly from the reaction medium to the gas phase for ESI-MS/(MS) analysis of the reaction described in Scheme 16. Reaction of telluride **84** with alkyne **85** was performed according to Scheme 16 to give **86**. The reaction mixture was electrosprayed by using the ESI source in the positive ion mode. The reaction mixture was stirred for 1 h and gave a number of ions that were attributed to the species shown in Scheme 17.




Scheme 16. Coupling of vinylic tellurides with alkynes catalyzed by $PdCl_2$ and $CuCl_2$.

The most relevant data for the validation of the proposed catalytic cycle was the detection of three Te and Pd-containing cationic complexes, **87** (*m*/*z* 717), **88** (*m*/*z* 499), and **89** (*m*/*z* 627). Ions **87** and **88** were likely formed by solution ionization of the neutral species $L_nPdCl_2$, that is, $L_nPdCl_2 \rightarrow L_nPdCl^+ + Cl^-$. Cations analogous to **87** and **88** have been suggested[54] using NMR and cyclic voltammetry in reactions where $ArPd^+$ complexes were postulated. ESI fishing of **87** and **88** in their cationic forms suggested that an equilibrium exists between these two species in solution in the palladium insertion process. It was also suggested that BuTeCl acts as a ligand to stabilize **87**, which may be formed by coordination of two styryl butyl tellurides to $PdCl_2$ followed by transmetalation. Since the relative intensity of cations **87** and **88** remained nearly constant during up to 36 h of reaction, as shown by continuous ESI-MS monitoring, the ligand exchange equilibrium between **87** and **88** is likely dynamic. The mechanism by which cation **89** is formed was not so straightforwardly rationalized, but a proposed route to **89** involves **88** in a ligand exchange process: that is, exchange of **84** by [**84** + Bu]$^+$ and further association with neutral HCl present in the reaction medium. The species **87** and **88** are Pd-styryl complexes that can undergo β-hydrogen elimination to yield PhC≡CH. However, this acetylene was not observed as a byproduct in the coupling reaction. It is known that cations such as $Li^+$, $Ag^+$, and $Tl^+$ can inhibit such β-hydrogen eliminations.[55] It is also known that cation association (**88**→**89**) enhances the solubility of metal complexes.[56] Organocopper cluster intermediates were also identified by ESI-MS and characterized as alkynylcopper species by characteristic Cu isotopic patterns and ESI-MS/MS structural analysis. An expanded catalytic cycle for the coupling of vinylic tellurides with alkynes catalyzed by palladium dichloride was proposed as depicted in Scheme 17.[57]

### 4.14. Mechanism of Tröger's Base Formation

In general, it is now accepted that the mechanism for Tröger's base formation involves a series of in situ Friedel–Crafts reactions of **92**, but the detailed course of the reac-

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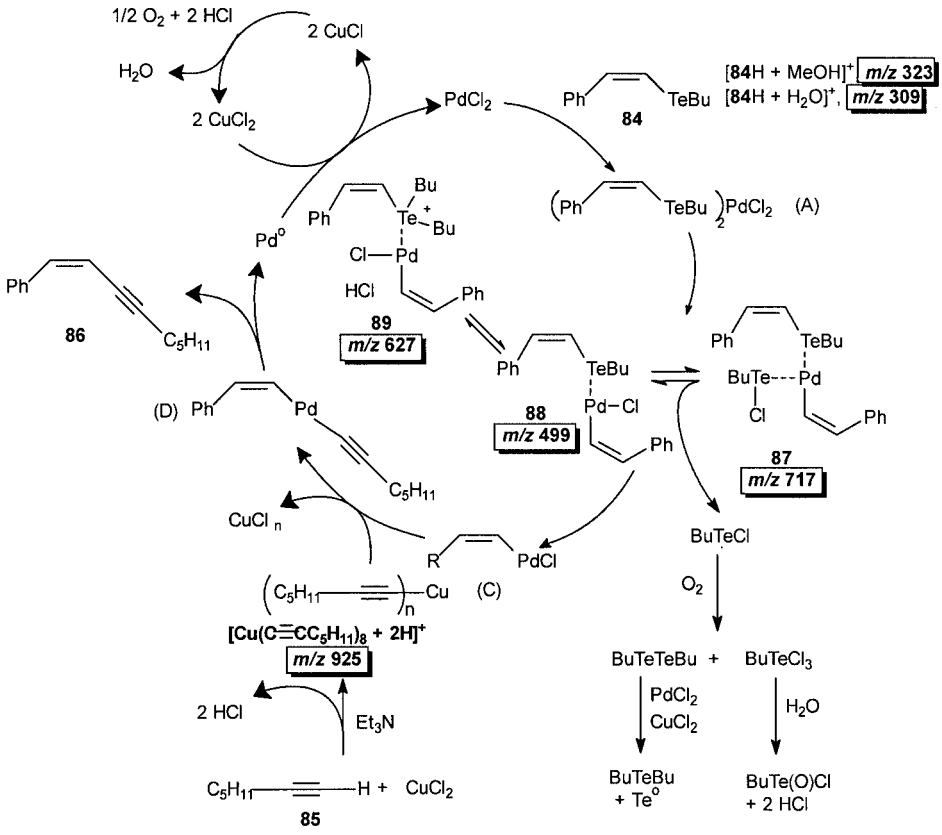


Scheme 17. Expanded mechanism based on ESI(+)-MS tandem-MS/MS experiments for the reaction of **84** with alkyne **85** to give **86** using $PdCl_2$ and $CuCl_2$ in the absence of an inert atmosphere.

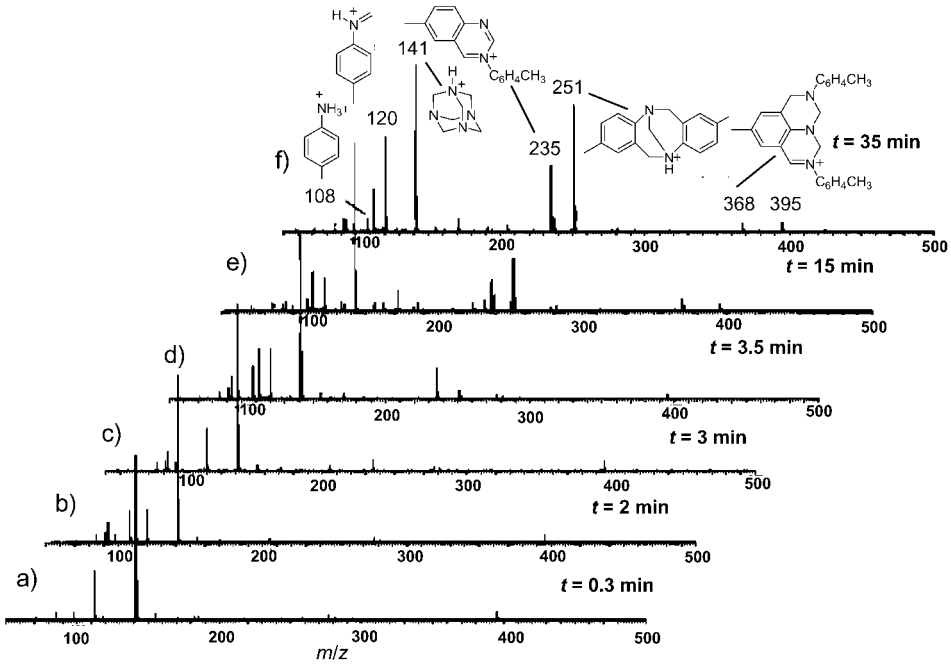


Figure 9. ESI(+)-MS acquired for the reaction solution during the online monitoring of the reaction of *p*-toluidine and urotropine in neat TFA after (a) 20 s; (b) 2 min; (c) 3 min; (d) 3.5 min; (e) 15 min; and (f) 35 min of reaction.

tion has not yet been fully established.[58] In trying to elucidate major mechanistic aspects, different methylene sources as well as different anilines have been used to form Tröger's bases by Abella and coworkers.[59] In this study, direct injection ESI-MS/MS was used to monitor Tröger's base formation from different anilines (*p*-toluidine and 4-aminovera-

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trol), using both formaldehyde and urotropine (**90**) as the methylene source (Figure 9). To gain further evidence for the methylene transfer step, gas-phase ion/molecule reactions of protonated urotropine with two volatile amines were also performed. Hybrid linear ion-trap equipment was used in which $N_2$ gas was replaced with reactive neutrals by needle valve adaptors that allowed the introduction of reactive gases into the collision cell. Interestingly, urotropine (*m*/*z* 141) was indeed found to react with ethylamine and aniline to form directly the corresponding iminium ions of *m*/*z* 56 and 106 (Figure 10). Figure 10 (part a) shows that the imine $C_2H_5$–NH=$CH_2^+$ (as for **92**, Scheme 18) of *m*/*z* 56 is formed in high yield in the ion/molecule reactions. Herein, the adduct of *m*/*z* 186, the first transient intermediate leading to methylene transfer, is obtained from nucleophilic addition of the amine to protonated urotropine. Furthermore, in reactions with aniline (Figure 10, b), the corresponding imine of *m*/*z* 106 (as for **92**, Scheme 18), that is, Ph–NH=$CH_2^+$, is formed as an abundant ion, and the transient adduct (*m*/*z* 234) is detected as a low abundance ion. On the basis of the mechanistic data collected from online ESI-MS(/MS) monitoring (Figures 9 and 10) and the gas-phase ion/molecule reactions, an experimentally tested mechanism for the formation of Tröger's bases using either formaldehyde or, more particularly, urotropine as the source of methylene was presented (Scheme 18). This mechanism proposes the participation of all of the intermediates intercepted by ESI-MS and fully characterized by ESI-MS/MS.

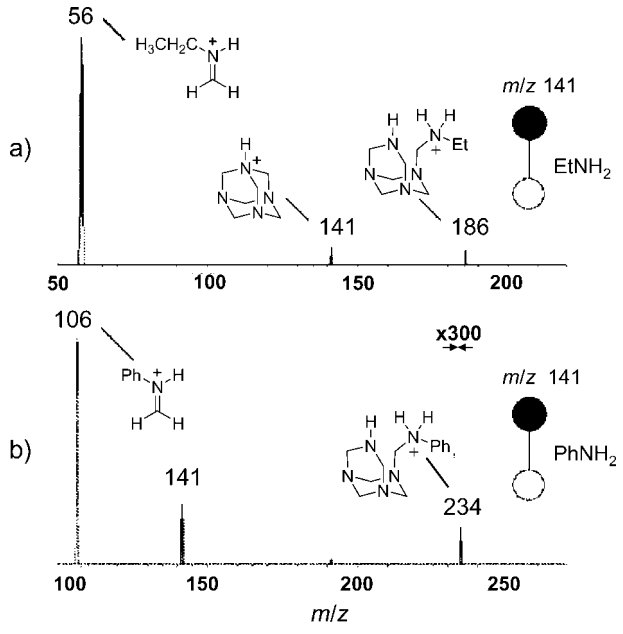


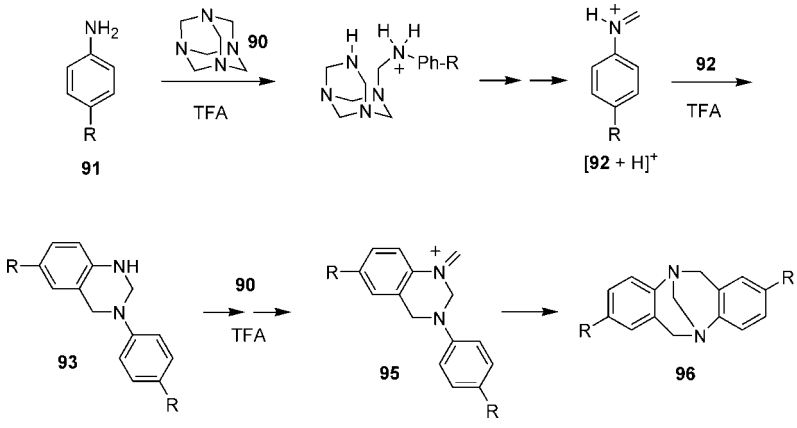

Figure 10. ESI-MS/MS for gas-phase reactions of protonated urotropine (*m*/*z* 141) with (a) ethylamine and (b) aniline. The insert x300 in (b) shows that the intensity of the ion of *m*/*z* 234 was increased by a factor of 300.

## Conclusions

Over the past few years, there has been a rapid increase in the application of API-MS techniques to the study of organic reaction mechanisms, mainly to intercept for the first time reactive intermediates from these reactions. The ability to isolate ions directly from crude reaction mixtures is an outstanding advantage and allows new applications in the mechanistic chemistry of transient species. Online purification will undoubtedly contribute to the importance of API-MS for future studies of labile and sensitive intermediates in solution, with no need for previous purification or isolation for further characterization of active species, reactive intermediates, and products. Such new MS techniques are also beginning to be used to probe mechanisms of reactions of fundamental importance and practical use, once again by transferring the reaction intermediates directly from solution to the gas phase. Furthermore, using API-MS many intermediates were intercepted, isolated, detected, and structurally characterized.

## Acknowledgments

Financial support from the International Foundation for Science (F/4195-1) and the Organisation for Prohibition of Chemical Weapons is gratefully acknowledged. The Laboratory of Asymmetric




Scheme 18. Mechanism for the formation of Tröger's base as probed by ESI(+)-MS(/MS) and ion/molecule reactions.

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Synthesis is sponsored by Programa de Investigación en Productos Bioactivos–UTalca.


[1] a) C. M. Whitehouse, R. N. Dreyer, M. Yamashita, J. B. Fenn, *Anal. Chem.* **1985**, *57*, 675; b) J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Science* **1989**, *246*, 64; c) R. B. Cole, (Ed.), *Electrospray Ionization Mass Spectrometry – Fundamentals, Instrumentation, and Applications*, Wiley, New York, **1997**; d) J. B. Fenn, *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 524.

[2] C. Adlhart, P. Chen, *Helv. Chim. Acta* **2000**, *83*, 2192.

[3] a) D. A. Plattner, *Int. J. Mass Spectrom.* **2001**, *207*, 125; b) D. A. Plattner, *Top. Curr. Chem.* **2003**, *225*, 153.

[4] a) L. S. Santos, L. Knaack, J. O. Metzger, *Int. J. Mass Spectrom.* **2005**, *246*, 84; b) P. Chen, *Angew. Chem. Int. Ed.* **2003**, *42*, 2832; c) M. N. Eberlin, *Eur. J. Mass Spectrom.* **2007**, *13*, 19.

[5] a) J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Mass Spectrom. Rev.* **1990**, *9*, 37; b) J. B. Fenn, *Angew. Chem. Int. Ed.* **2003**, *42*, 3871.

[6] P. Kebarle, L. Tang, *Anal. Chem.* **1993**, *65*, 972.

[7] S. J. Gaskell, *J. Mass Spectrom.* **1997**, *32*, 677.

[8] J. L. Jones, A. R. Dongre, A. Somogyi, V. H. Wysocki, *J. Am. Chem. Soc.* **1994**, *116*, 8368.

[9] P. Kebarle, Y. Ho in *Electrospray Ionization Mass Spectrometry*, (Ed.: R. B. Cole), Wiley: New York, **1997**, pp. 3–63.

[10] J. F. de la Mora, G. J. Van Berckel, C. G. Enke, R. B. Cole, M. Martinez-Sanchez, J. B. Fenn, *J. Mass Spectrom.* **2000**, *35*, 939.

[11] G. J. Van Berkel in *Electrospray Ionization Mass Spectrometry*, (Ed.: R. B. Cole, ), Wiley: New York, **1997**, pp. 65–105.

[12] L. A. P. Kane-Maguire, R. Kanitz, M. M. Sheil, *Inorg. Chim. Acta* **1996**, *245*, 209.

[13] V. E. Vandell, P. A. Limbach, *J. Mass Spectrom.* **1998**, *33*, 212.

[14] H. Lavanant, E. Hecquet, Y. Hoppilliard, *Int. J. Mass Spectrom.* **1999**, *187*, 11.

[15] a) G. L. Van Berkel, K. G. Asano, V. Kertesz, *Anal. Chem.* **2002**, *74*, 5047; b) G. L. Van Berkel, K. G. Asano, M. C. Gragner, *Anal. Chem.* **2004**, *76*, 1493; c) G. L. Van Berkel, K. G. Asano, P. D. Schnier, *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 853; d) G. L. Van Berkel, G. E. Giles, J. S. Bullock, L. J. Gray, *Anal. Chem.* **1999**, *71*, 5288.

[16] L. Gianelli, V. Amendola, L. Fabbrizzi, P. Pallavicini, G. G. Mellerio, *Rapid. Commun. Mass Spectrom.* **2001**, *15*, 2347.

[17] a) R. W. Vachet, J. A. R. Hartman, J. H. Callahan, *J. Mass Spectrom.* **1998**, *33*, 1209; b) R. W. Vachet, J. R. Hartman, J. W. Gertner, J. H. Callahan, *Int. J. Mass Spectrom.* **2001**, *204*, 101; c) J. R. Hartman, R. W. Vachet, J. H. Callahan, *Inorg. Chim. Acta* **2000**, *297*, 79; d) J. R. Hartman, R. W. Vachet, W. Pearson, R. J. Wheat, J. H. Callahan, *Inorg. Chim. Acta* **2003**, *343*, 119.

[18] a) A. Schäfer, B. Fischer, H. Paul, R. Bosshard, M. Hesse, M. Viscontini, *Helv. Chim. Acta* **1992**, *75*, 1955; b) A. Schäfer, H. Paul, B. Fischer, M. Hesse, M. Viscontini, *Helv. Chim. Acta* **1995**, *78*, 1763.

[19] S. R. Wilson, J. Perez, A. Pasternak, *J. Am. Chem. Soc.* **1993**, *115*, 1994.

[20] A. O. Aliprantis, J. W. Canary, *J. Am. Chem. Soc.* **1994**, *116*, 6985.

[21] M. Moreno-Manãs, M. Pérez, R. Pleixats, *J. Org. Chem.* **1996**, *61*, 2346.

[22] G. Hambitzer, J. Heitbaum, *Anal. Chem.* **1986**, *58*, 1067.

[23] J. W. Sam, X. J. Tang, R. S. Magliozzo, J. Peisach, *J. Am. Chem. Soc.* **1995**, *117*, 1012.

[24] J. Griep-Raming, S. Meyer, T. Bruhn, J. O. Metzger, *Angew. Chem. Int. Ed.* **2002**, *41*, 2738.

[25] S. Meyer, J. O. Metzger, *Anal. Bioanal. Chem.* **2003**, *377*, 1108.

[26] a) K. M. Stirk, M. L. K. Kiminkinen, H. I. Kenttamaa, *Chem. Rev.* **1992**, *92*, 1649; b) J. S. Brodbelt, *Mass Spectrom. Rev.* **1997**, *16*, 91; c) M. N. Eberlin, *Mass Spectrom. Rev.* **1997**, *16*, 113; d) S. Gronert, *Chem. Rev.* **2001**, *101*, 329; e) M. Speranza, *Int. J. Mass Spectrom.* **2004**, *232*, 277; f) R. G. Cooks, M. N. Eberlin, X. Zheng, H. Chen, A. Tao, *Chem. Rev.* **2006**, *106*, 188; g) M. K. Green, C. B. Lebrilla, *Mass Spectrom. Rev.* **1997**, *16*, 53.

[27] L. S. Santos, C. H. Pavam, W. P. Almeida, F. Coelho, M. N. Eberlin, *Angew. Chem. Int. Ed.* **2004**, *43*, 4330.

[28] a) J. S. Hill, N. S. Isaacs, *J. Phys. Org. Chem.* **1990**, *3*, 285; b) H. M. R. Hoffmann, J. Rabe, *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 796; c) M. L. Bode, P. T. Kaye, *Tetrahedron Lett.* **1991**, *32*, 5611; d) Y. Fort, M. C. Berthe, P. Caubere, *Tetrahedron* **1992**, *48*, 6371.

[29] L. S. Santos, B. A. DaSilveira Neto, C. S. Consorti, C. H. Pavam, W. P. Almeida, F. Coelho, J. Dupont, M. N. Eberlin, *J. Phys. Org. Chem.* **2006**, *19*, 731.

[30] J. N. Rosa, C. A. M. Afonso, A. G. Santos, *Tetrahedron* **2001**, *57*, 4189.

[31] H. M. C. Ferraz, F. L. C. Pereira, E. R. S. Goncalo, L. S. Santos, M. N. Eberlin, *J. Org. Chem.* **2005**, *70*, 110.

[32] a) J. B. Domingos, E. Longhinotti, T. A. S. Brandao, C. A. Bunton, L. S. Santos, M. N. Eberlin, F. Nome, *J. Org. Chem.* **2004**, *69*, 6024; b) J. B. Domingos, E. Longhinotti, T. A. S. Brandao, L. S. Santos, M. N. Eberlin, C. A. Bunton, F. Nome, *J. Org. Chem.* **2004**, *69*, 7898.

[33] I. Dalmazio, L. S. Santos, R. P. Lopes, M. N. Eberlin, R. Augusti, *Environ. Sci. Technol.* **2005**, *39*, 5982.

[34] a) S. Meyer, R. Koch, J. O. Metzger, *Angew. Chem. Int. Ed.* **2003**, *42*, 4700; b) S. Furmeier, J. O. Metzger, *J. Am. Chem. Soc.* **2004**, *126*, 14485; c) R. Arakawa, S. Tachiyashiki, T. Matsuo, *Anal. Chem.* **1995**, *67*, 4133; d) A. D. Modestov, J. Gun, I. Savotine, O. Lev, *J. Electroanal. Chem.* **2004**, *565*, 7; e) X. Zhang, H. Y. Wang, Y. L. Guo, *Rapid. Commun. Mass Spectrom.* **2006**, *20*, 1877; f) X. Zhang, Y. X. Liao, R. Qian, H. Y. Wang, Y. L. Guo, *Org. Lett.* **2005**, *7*, 3877.

[35] F. C. C. Moura, M. H. Araujo, I. Dalmazio, T. M. A. Alves, L. S. Santos, M. N. Eberlin, R. Augusti, R. M. Lago, *Rapid. Commun. Mass Spectrom.* **2006**, *20*, 1859.

[36] C. D. F. Milagre, H. M. S. Milagre, L. S. Santos, M. L. A. Lopes, P. J. S. Moran, M. N. Eberlin, J. A. R. Rodrigues, *J. Mass Spectrom.* **2007**, *42*, 1287.

[37] a) C. Marquez, J. O. Metzger, *Chem. Commun.* **2006**, 1539. For similar studies, see:; b) H. Y. Wang, X. Zhang, Y. L. Guo, L. Lu, *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1561; c) X. Zhang, Y. L. Guo, *Rapid. Commun. Mass Spectrom.* **2006**, *20*, 3477.

[38] M. Claeys, B. Graham, G. Vas, W. Wang, R. Vermeylen, V. Pashynska, J. Cafmeyer, P. Guyon, M. O. Andreae, P. Artaxo, W. Maenhaut, *Science* **2004**, *303*, 1173.

[39] L. S. Santos, I. Dalmazio, M. N. Eberlin, M. Claeys, R. Augusti, *Rapid. Commun. Mass Spectrom.* **2006**, *20*, 2104.

[40] E. C. Meurer, L. S. Santos, R. A. Pilli, M. N. Eberlin, *Org. Lett.* **2003**, *5*, 1391.

[41] E. C. Meurer, L. L. Rocha, R. A. Pilli, M. N. Eberlin, L. S. Santos, *Rapid. Commun. Mass Spectrom.* **2006**, *20*, 2626.

[42] Y. Hanzawa, N. Kowase, S. Momose, T. Taguchi, *Tetrahedron* **1998**, *54*, 11387.

[43] a) W. Kaminsky, R. Steiger, *Polyhedron* **1988**, *7*, 2375; b) D. Cam, U. Giannini, *Makromol. Chem.* **1992**, *193*, 1049.

[44] a) XPS: P. G. Gassman, M. R. Callstrom, *J. Am. Chem. Soc.* **1987**, *109*, 7875; b) $^{13}C$ NMR: C. Sishta, R. M. Hathorn, T. J. Marks, *J. Am. Chem. Soc.* **1992**, *114*, 1112.

[45] a) A. R. Siedle, W. M. Lamanna, R. A. Newmark, J. N. Schroepfer, *J. Mol. Catal. A* **1998**, *128*, 257; b) I. Tritto, S. X. Li, M. C. Sacchi, P. Locatelli, G. Zannoni, *Macromolecules* **1995**, *28*, 5358; c) I. Tritto, S. Li, M. C. Sacchi, G. Zannoni, *Macromolecules* **1993**, *26*, 7111.

[46] a) W. Kaminsky, *Macromol. Chem. Phys.* **1996**, *197*, 3907; b) W. Kaminsky, C. Strubel, *J. Mol. Catal. A* **1998**, *128*, 191.

[47] a) D. Coevoet, H. Cramail, A. Deffieux, *Macromol. Chem. Phys.* **1999**, *200*, 1208; b) J.-N. Pedeutour, D. Coevoet, H. Cramail, A. Deffieux, *Macromol. Chem. Phys.* **1999**, *200*, 1215; c)


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D. E. Babushkin, N. V. Semikolenova, V. A. Zakharov, E. P. Talsi, *Macromol. Chem. Phys.* **2000**, *201*, 558.

[48] L. S. Santos, J. O. Metzger, *Angew. Chem. Int. Ed.* **2006**, *45*, 977.

[49] L. S. Santos, R. L. O. R. Cunha, J. V. Comasseto, M. N. Eberlin, *Rapid. Commun. Mass Spectrom.* **2007**, *21*, 1479.

[50] R. L. O. R. Cunha, M. E. Urano, J. R. Chagas, P. C. Almeida, C. Bincoletto, I. L. S. Tersariol, J. V. Comasseto, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 755.

[51] a) M. Cojocaru, I. Elyashiv, M. Albeck, *J. Mass Spectrom.* **1997**, *32*, 705; b) F. D. Williams, F. X. Dunbar, *Chem. Commun. (London)* **1968**, 459; c) A. M. Duffied, H. Budzikiewicz, C. Djerassi, *J. Am. Chem. Soc.* **1965**, *87*, 2920; d) M. Wieber, E. Kauzinger, *J. Organomet. Chem.* **1977**, *129*, 339.

[52] L. S. Santos, G. B. Rosso, R. A. Pilli, M. N. Eberlin, *J. Org. Chem.* **2007**, *72*, 5809.

[53] C. Raminelli, M. H. G. Prechtl, L. S. Santos, M. N. Eberlin, J. V. Comasseto, *Organometallics* **2004**, *23*, 3990.

[54] a) L. Ripa, A. Hallberg, *J. Org. Chem.* **1996**, *61*, 7147; b) J. M. Brown, K. K. Hii, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 657; c) K. K. Hii, T. D. W. Claridge, J. M. Brown, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 984; d) A. O. Aliprantis, J. W. Canary, *J. Am. Chem. Soc.* **1994**, *116*, 6985; e) M. A. Aramendıa, A. Lafont, *J. Org. Chem.* **1999**, *64*, 3592.

[55] a) Z. Wang, Z. G. Zhang, X. Y. Lu, *Organometallics* **2000**, *19*, 775; b) Z. G. Zhang, X. Y. Lu, Q. H. Zang, X. L. Han, *Organometallics* **2001**, *20*, 3724; c) G. S. Liu, X. Y. Lu, *Tetrahedron Lett.* **2002**, *43*, 6791; d) A. de Meijere, F. E. Meyer, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2379, and references therein.

[56] A. Alexakis, J. Berlan, Y. Besace, *Tetrahedron Lett.* **1986**, *27*, 1047.

[57] For other examples of Pd studies, see: a) H. Guo, R. Qian, Y. X. Liao, S. M. Ma, Y. L. Guo, *J. Am. Chem. Soc.* **2005**, *127*, 13060; b) R. Qian, H. Guo, Y. X. Liao, Y. L. Guo, S. M. Ma, *Angew. Chem. Int. Ed.* **2005**, *44*, 4771; c) J. Masllorens, M. Moreno-Mañas, A. Pla-Quintana, A. Roglans, *Org. Lett.* **2003**, *5*, 1559; d) M. Moreno-Mañas, R. Pleixats, R. M. Sebastian, A. Vallribera, A. Roglans, *J. Organomet. Chem.* **2004**, *689*, 3669; e) A. O. Aliprantis, J. W. Canary, *J. Am. Chem. Soc.* **1994**, *116*, 6985; f) C. Markert, A. Pfaltz, *Angew. Chem. Int. Ed.* **2004**, *43*, 2498; g) C. Chevrin, J. Le Bras, F. Henin, J. Muzart, A. Pla-Quintana, A. Roglans, R. Pleixats, *Organometallics* **2004**, *23*, 4796.

[58] a) E. C. Wagner, *J. Am. Chem. Soc.* **1935**, *57*, 1296; b) T. R. Miller, E. C. Wagner, *J. Am. Chem. Soc.* **1941**, *63*, 832.

[59] C. A. M. Abella, M. Benassi, L. S. Santos, M. N. Eberlin, F. Coelho, *J. Org. Chem.* **2007**, *72*, 4048.

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