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Radical Cations in Electrospray Mass Spectrometry: Formation of Open-Shell Species, Examination of the Fragmentation Behaviour in ESI-MS^n and Reaction Mechanism Studies by Detection of Transient Radical Cations

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The ion formation mechanism in electrospray MS is reviewed, with special focus on the electrochemical red/ox reactions responsible for the formation of radical molecular ions. Prerequisites influencing the likelihood of formation and observation of a particular compound as an open-shell molecular species in ESI-MS (i.e., the structure and the oxidation potential of the analyte, the solvent and additives) are evaluated. For illustration of the ESI phenomena governing radical cation formation, an ESI-MS study of tetra(aryl)-benzidine compounds is presented. The facile formation of abundant radical molecular cations in ESI-MS demonstrates imposingly that the basicity of the analyte's nitrogen atoms is strongly overcompensated by the ability to stabilize unpaired electrons. ESI-MS^n spectra of the tetra(aryl)benzidine molecular ions exhibit a characteristic feature in the loss of radicals. This process is the major fragmentation pathway of open-shell molecular precursor ions in their MS^2 spectra, and also that of even-electron ions in sequential MS^n spectra. The collision-induced dissociation (CID) behaviour suggests a general assumption: easily oxidizable compounds (e.g., hydrocarbon polyenes, polycyclic aromatic hydrocarbons, porphyrins etc.) generating predominantly molecular radical cations in ESI-MS contradict the even electron rule in ESI-MS^n experiments. The strong ability to stabilize unpaired electrons is preserved in product ions and makes the formation of open-shell species energetically less demanding. A selection of solution-phase reaction mechanistic studies in which open-shell intermediates were detected and structurally characterized by ESI-MS and ESI-MS/MS, respectively, is presented. The merits of ESI-MS and ESI-MS^n for mechanistic studies of chemical reaction are critically discussed.

1. Introduction

Since its introduction,[1,2] electrospray ionization mass spectrometry (ESI-MS) has become a major technique for the analysis of a wide variety of analytes that are soluble in solvents of moderate conductivity.[3]

In ESI-MS,[4,5] a dilute solution of an analyte is pumped through a capillary (inner diameter typically 100 μm) at a low flow rate (1–10 μL min^{-1}). A high voltage (2–5 kV) is applied to the capillary. This voltage can be either positive (positive ion mode: generation of positively charged ions) or negative, if the formation of negatively charged ions is intended. The applied voltage provides the electric field gradient in the solution required to cause the migration of charged species to the capillary metal/solution interface, resulting in an electric double layer. Additionally, the applied high voltage repels charged particles of the same polarity, leading to their accumulation at the surface of the liquid. As a result the liquid protrudes from the capillary tip into the ES ion source at atmospheric pressure as the so called the "Taylor cone". At the point at which the coulombic repulsion of the surface charges equals the surface tension of the solution (i.e., the so called "Raleigh limit"), droplets that carry an excess of charge on their surfaces are detached from the tip. The charged droplets shrink by loss of solvent molecules as they move along with the electric field towards the counter electrode (i.e., the entrance to the mass spectrometer), undergo uneven fission processes as they repeatedly reach Raleigh limits, and finally generate charged gas-phase analyte ions.[3,6–8]

Compounds that are particularly suited for ESI-MS analysis are organic or inorganic salts, inherently charged and therefore completely involatile, which excludes them from MS techniques depending on thermal vaporization (e.g., Electron Ionization and Chemical Ionization MS). In those cases these compounds dissociate completely in solution and ESI efficiently ensures desolvation and phase

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transfer of the ions into the gas phase. Neutral compounds incorporating polar functionalities are ionized by solution-phase Brønsted or Lewis acid/base chemistry (e.g., proteins, peptides) and are therefore typically observed as pseudomolecular ions either protonated, sodiated or otherwise cationized in positive-ion mode ESI-MS or deprotonated in negative-ion mode ESI-MS.[6,7,9] Moreover, even the ion abundance distribution exhibited in an ESI spectrum can be at least qualitatively related to the abundances of the corresponding ionic species in solution, given that severe matrix-induced signal suppression or gas-phase charge-changing phenomena are excluded.[10–15] To avoid ambiguities it must be emphasized that the processes (i.e., protonation, deprotonation) leading to the solution-phase ions detected later in the ESI-MS spectra take place in the charged droplets formed at atmospheric pressure in the ES ion source. This is a direct consequence of the fact that the polarity applied to the ESI capillary determines the polarity of the charges residing on the surface of the droplets and the polarity of the corresponding ions in the gas phase irrespective of the pH of the analyte solution used for the ESI experiments.[16–20] Consequently, ESI of polar compounds is more a technique for efficient transfer of preformed solution-phase ions into the gas phase rather than a true ionization process in which neutral molecules are converted into ions.[4,9]

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Klaus Meerholz was born in Bielefeld in 1963 and studied chemistry at the Universities of Freiburg and Bielefeld. He obtained his Ph.D. from the University of Freiburg in 1991. From 1992 to 1995 he worked as a postdoctoral researcher at the State University of New York at Buffalo and the Optical Sciences Center at the University of Arizona. From 1995 to 1998 he performed his habilitation at the Physical Chemistry Department of the LMU Munich. Since 2002 Klaus Meerholz has been full professor at the University of Cologne. He is author of about 110 peer-reviewed, high-ranked publications and several patents.

An alternative ionization concept is demanded for ESI-MS of neutral, relatively apolar compounds,[21] such as hydrocarbon polyenes,[22–24] polycyclic aromatic hydrocarbons (PAHs),[25,26] porphyrins[27] and metalloporphyrins,[28] because these analytes lack basic or acidic sites amenable to solution-phase acid/base chemistry.[29] For the ionization of apolar but easily oxidizable compounds, electrochemical oxidation is relevant, since molecular radical cations of the corresponding analytes are frequently generated and observed in the positive-ion mode.[10,29–31] Oxidation of these analytes takes place in solution in contact with the internal side of the ESI capillary as a consequence of the high voltage applied. As mechanistic studies of reactions involving radical ionic species by ESI-MS and ESI-MS^n (reviewed in Section 4) are obviously strongly dependent on proper understanding of the processes governing formation of open-shell ions and phase transfer of preformed ions, we discuss this issue further in Section 2.

In Section 3, the collision-induced dissociation (CID)[32] behaviour of open-shell species is discussed, because multistage mass spectrometry (MS/MS and MS^n in quadrupole-ion traps: QIT)[33,34] is the method of choice for fast and reliable structure elucidation, especially in multi-component mixtures (e.g., reaction mixtures) without extensive sample cleanup or fractionation (e.g., by chromatography). Mixture analysis by ESI-MS^n requires the exclusive formation of intact molecular ions needed as precursors for subsequent CID experiments in order to achieve valuable analytical data sets and low detection limits. Consequently, ES ion source conditions are optimized for exclusive production of molecular ions (i.e., minimized loss of ion current due to collision-induced dissociation in the ion source).[35] Consequently, cold gas-phase molecular ions are generated and extensive fragmentation reactions are avoided.[1–5] Tetra(aryl)benzidine derivatives have been chosen as representative analytes for illustration of ESI-MS^n fragmentation reactions of open-shell molecular ions.

Understanding of the fundamentals of the ES ionization process, comprising the soft phase-transfer of preformed ions and the generation of open-shell ionic species, as well as their CID behaviour, has set the stage for many solution-phase mechanistic studies of chemical reactions of major importance.[36,37] This article highlights contributions in which transient odd-electron-number (OE) reaction intermediates were successfully detected and reliably identified.[38–45] In particular, for solution-phase mechanistic studies of chemical reactions it is very important to verify unambiguously that potentially indicative species detected in the gas phase by ESI-MS are definitely not a result of ESI red/ox ionization processes. Furthermore, it must be established that the ions of interest are indeed reactive intermediates of a predicted reaction path.[38] A straightforward MS analysis of reaction mechanism studies based on ESI therefore requires an exclusive, efficient and soft phase transfer of solution-phase species (i.e., radical reaction intermediates) into the gas phase, without any change in the ions or additional formation of ESI artefacts.

2. Formation of Open-Shell Cations in ESI-MS

All proposed and discussed ESI mechanisms explaining the formation of gas-phase ions have one fundamental and universally valid feature in common. That is the fact that the formation of relevant amounts of isolated gas-phase ions depends on the presence of the corresponding solvated analogues in the charged droplets formed upstream in the ESI source.[3,6,8,30] Consequently, all types of polar analytes that possess basic or acidic sites promoting easy solution-phase protonation, cationization or deprotonation through Brønsted or Lewis acid/base chemistry are ideally suited for ESI-MS, yielding low detection limits. Radical cations formed by oxidation of neutral organic molecules in solution are not typically observed in ESI-MS since these types of ions are not formed by simple acid/base chemistry, nor are these species generally stable in solution for long periods.[9,29] However, easily oxidizable compounds can be ionized by loss of an electron to form open-shell molecular radical cations by an electrochemical oxidation process inherent to positive-ion mode ESI, generating the ESI current and the continuous production of charged droplets.[46] Therefore, an ES ion source can be generally recognized as a special type of electrolytic cell in which electrolysis ensures the charge balance between the ESI capillary (i.e., the working electrode) and a counter electrode (e.g., the aperture of a heated capillary).[30,47,48] In a more detailed view, the ES ion source is a controlled-current electrolytic (CCE) flow cell,[48,49] in which the potential applied to the ESI capillary, which ultimately determines whether a particular analyte will undergo a red/ox reaction in the capillary, is a function of the magnitude of the ESI current, the concentration of the analyte in solution and its red/ox potential.[3]

These assumptions give rise to a set of criteria relevant for the oxidative formation of open-shell molecular cations (i.e., ions with uneven numbers of electrons: OE species) by ESI. Firstly, the potential applied to the ESI capillary has to be sufficiently high for the formation of red/ox-reaction products (i.e., radical cationic species in positive-ion mode ESI).[48,50] The oxidation potential of the analyte under examination has to be suitably low, indicating structural prerequisites that provide a distinct ability to stabilize unpaired electrons. Furthermore, the concentration of the analyte solution subjected to ESI-MS has to be sufficiently high and the properties of the solvent – mainly regarding the stabilization of radical ionic species – have to be considered as well. These criteria are discussed in detail below.

2.1. Important Criteria for the Formation of Open-Shell Molecular Ions by ESI-MS

2.1.1. Oxidation Potential ($E_{1/2(ox)}$) and Ionization Energy (IE) of the Analyte and Concentration of the Analyte Solution Used for ESI-MS

Analytes with oxidation potentials far below $E_{1/2(ox)}$ = 1 V (vs. saturated calomel electrode SCE) almost universally lose an electron at the ESI capillary and deliver abundant

open-shell species in the positive-ion mode.[9,29] However, the ESI response (i.e., the yield of radical molecular cations) decreases distinctly with increasing oxidation potentials as the analytes become oxidized much less effectively.[9,50] To enable such less favoured oxidation reactions, either the concentration of the analyte solution can be increased to relatively high levels (i.e., 0.1 mM or higher) or the voltage applied to the ESI capillary can be raised as far as possible.[3] However, ESI experiments with highly concentrated analyte solutions sometimes deliver only moderate abundances of the molecular radical cations, accompanied by protonated molecular ions (i.e., ions with even numbers of electrons: EE species). Oxidative ESI loses relevance for analytes with oxidation potentials exceeding $E_{1/2(ox)}$ = 1.25 V.[50]

It must be noted that the capability of an organic molecule to be oxidized basically depends on the energy level of the highest occupied molecular orbital (HOMO). This fundamental energy value can at least be estimated by theoretical calculations and approximated by determination of the oxidation potential $E_{1/2(ox)}$, mostly achieved by cyclic voltammetry (CV) experiments in solution or by gas-phase measurements of the ionization energy (IE). $E_{1/2(ox)}$ values determined by CV are strongly affected by solvation effects and other experimental constraints (nature of the working electrode and the counter electrode, nature and concentration of additional electrolytes, solvent, reference system), while IE values determined in the gas phase (e.g., by photoelectron spectroscopy,[51,52] examination of charge transfer equilibria[53] and electron ionization MS[54]) also suffer from experimental uncertainties. A comparison of $E_{1/2(ox)}$ values is therefore only allowed with appropriate caution (consideration of correct offset factors). In a rigorous sense it is only possible for $E_{1/2(ox)}$ values obtained by measurements under comparable conditions and with identical reference systems.[55]

2.1.2 Influence of Solvent

The solvent choice in ESI-MS is basically defined by the necessity for stable spray conditions and the promotion of effective solution-phase ionization through acid/base chemistry.[8,27,46] Solubility characteristics of the analytes, mostly polar hydrophilic compounds, sufficient volatility and a moderate surface tension of the solvents used round up the requirements of an ideal solvent.

For sensitive generation and detection of open-shell species in ESI-MS, the requirements regarding solvents have to be reconsidered. The needs for proper analyte solutions and stable spray conditions obviously remain valid. Additionally, the effective prevention of rapid scavenger reactions that might shorten the lifetime of the open-shell species in the charged droplets becomes vitally important and a significant requirement of the solvent.[25] Besides fast dimerization reactions of radical cationic species,[56,57] nucleophilic reactions with solvents and additives are found to be particularly relevant in this concern. Hence, solvents with both protic and nucleophilic properties (e.g., water, methanol) and nucleophilic additives (e.g., acetate anion) are typically not recommended. On the other hand, aprotic, nonnucleophilic solvents such as dry acetonitrile and dichloromethane are more appropriate for electrochemical formation of radical cations by ESI.

The influence of solvents on ESI-MS spectra can be exemplified by looking at the ESI-MS data for *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD).[25] Due to the distinct conjugation of the π-system of the phenyl ring with the free electron pairs of the two *para* nitrogen atoms, supported by the donor effect of four methyl substituents [$E_{1/2(ox)}$ –0.28 V vs. ferrocene; approx. 0.10–0.2 V vs. SCE], TMPD is extremely easily oxidized.[55,58,59] This captodative effect is responsible for the unique solution- and solid-phase stability of *N*,*N*,*N*′,*N*′-tetraalkyl-*p*-phenylenediamines, enabling the simple isolation of the radical cations as their corresponding salts (e.g., by treatment with bromine; so-called Wurster salts).[60,61]

Figure 1 shows the molecular ionic region of the ESI-MS spectrum of TMPD dissolved in CH_2Cl_2/methanol. In that spectrum the most abundant signal corresponds to the radical molecular cation $[M]^{+\cdot}$ at *m*/*z* 164. Obviously, the strong ability to stabilize the radical cation of TMPD overwhelmingly dominates the basicity of the free electron pairs of the two nitrogen atoms, allowing only a small but significant amount of $[M + H]^+$ to be formed and registered (see insert showing the theoretical isotopic pattern of $[M]^{+\cdot}$ with the additional contribution of the $[M + H]^+$ species). The origin of the protonated molecular ion $[M + H]^+$ can be explained in two different ways: either through protonation by solution-phase Brønsted acid/base chemistry or through an alternative route of formation (i.e., by a red/ox reaction with water molecules present in trace amounts, known from electrochemical experiments).[25,62]

$$2[TMPD]^{+\cdot} + H_2O \rightarrow 2[TMPD + H]^+ + \tfrac{1}{2}O_2$$

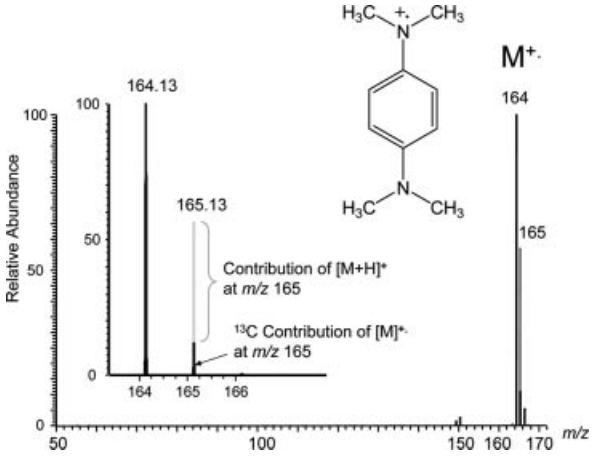


Figure 1. Positive-ion mode ESI-MS of TMPD dissolved in CH_2Cl_2/methanol.[25] The insert shows the molecular ion region of TMPD, highlighting the contribution of the calculated isotopic pattern to the open-shell molecular ion $[M]^{+\cdot}$ (black) and the additional contribution of the $[M + 1]^+$ species (grey; i.e., the protonated molecular ion $[M + H]^+$). ESI-MS spectrum recorded by M. Schäfer.

This reaction of open-shell radical cations with solvent species (probably water, methanol) is very probably of relevance for other analytes as well, wherever the corresponding radical cations in ESI-MS and noticeable amounts of the protonated analogue ions are detected concurrently.[25] Obviously, suitable solvents are of greater importance for analytes with higher oxidation potentials in which the formation of radical molecular species is less effective.

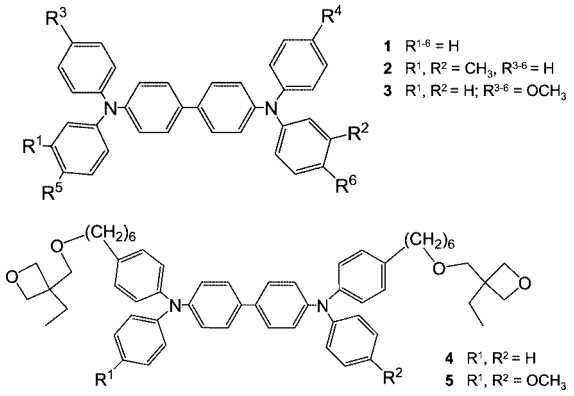
2.1.3. Solvent Additives and Electron Transfer Reagents

To expand the analytical use of the generation of molecular radical cations of compounds with highly conjugated π-systems by ESI, strong Brønsted acids such as trifluoroacetic acid (TFA),[23,25,63–65] Lewis acids (antimony pentafluoride, SbF_5)[9] and charge-transfer reagents (2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DDQ)[9,66] can be added to the analyte solutions to initiate and support solution-phase oxidation prior to and independently of ESI phase transfer. In contrast, negative radical anions of neutral analytes can be generated by one-electron reduction with NaK amalgam in negative ESI, as demonstrated successfully for fullerenes.[67,68]

Additionally, Siu et al. presented a widely recognized route for generating gas-phase molecular radical cations of peptides, a class of compounds not generally profusely suited for that kind of ionization by plain ESI, due to their distinctly negligible tendency towards oxidation.[69,70] It was shown that ESI of a methanolic mixture of a Cu^{II}–amine complex [e.g., $Cu^{II}(dien)(NO_3)_2$ (with dien = diethylenetriamine)], and an oligopeptide (M) yields the corresponding $[Cu^{II}(dien)M]^{2+\cdot}$ ion, which produces $[Cu^{I}(dien)]^{+}$ and $[M]^{\cdot+}$, the molecular radical cation of the oligopeptide upon CID. This interesting discovery triggered numerous studies on the formation and fragmentation behaviour of radical molecular ions of peptides[71–73] with different primary structures, and even nucleobases[74] and oligonucleotides,[74,75] together with examinations of different transition metal complexes as redox reagents for ESI-MS.[75,76]

2.2. Formation of Radical Molecular Cations of Tetra(aryl)-benzidine Derivatives by ESI-MS

A series of easily oxidizable tetra(aryl)benzidine derivatives (see Scheme 1 for structures) with $E_{1/2(ox)}$ values distinctively below 1 V (vs. ferrocene), synthesized for use as hole conductors in multilayer organic light-emitting devices (OLEDs),[77–79] were examined by ESI-MS.[58] The formation of molecular radical cations was tested in different solvents. Depending on the solvent used (i.e., toluene, methanol, CH_2Cl_2/methanol, methanol/toluene) the signal patterns of the molecular ion region changed, indicating varying ratios of molecular ionic species (i.e., radical cations $[M]^{+\cdot}$ and protonated molecular cations $[M + H]^{+}$).[58] Various solution mixtures of dichloromethane, toluene and methanol delivered nearly exclusive generation of the corresponding molecular radical cations of compounds **1**–**5** (i.e., $[M]^{+\cdot}$; Figure 2).



Scheme 1. Tetra(aryl)benzidine derivatives examined by ESI-MS and ESI-MS^n. Compounds **4** and **5** carry photopolymerizable oxetane moieties, allowing convenient formation of cross-linked polymers with hole-conducting properties.

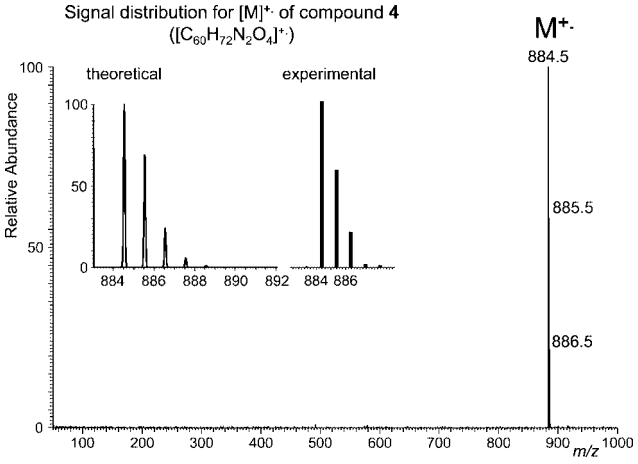


Figure 2. Positive-ion mode ESI-MS spectrum of tetra(aryl)-benzidine derivative **4** (CH_2Cl_2/CH_3OH solution, 1:10; $c = 10^{-5}$ mol L^{-1}).

Obviously, the radical cations of the selected series of compounds are sufficiently stable to prevent the presence of methanol in the analyte solutions having any noticeable influence on their detection.

3. ESI-MS^n Product Ion Experiments in a Quadrupole Ion Trap of Tetra(aryl)benzidine Derivative Radical Molecular Cations $[M]^{\cdot+}$

To elucidate their fragmentation behaviour upon CID, molecular radical cations of compounds **1**–**5** were examined by sequential ESI-MS^n product ion experiments in a quadrupole ion trap (QIT). Additional exact mass measurements of the product ions were conducted in a FTICR-MS instrument.[58]

Before the results of the QIT MS^n experiments are discussed, a short introduction to the workflow of these experiments is given. Prior to QIT MS^n experiments, precursor ion species are selected and all additional present ions are expelled from the trap. After that isolation step, the ki-

netic energy of the precursor ions is selectively enhanced by resonance interaction with a potential applied to the electrodes of the QIT. The ions experience multiple collisions at elevated velocity with He atoms, present as background buffer gas in the QIT.[80] These low-energy collisions convert a significant portion of the precursor ion's kinetic energy to internal excitation (i.e., the population of higher vibration levels), which in turn triggers extensive fragmentation reactions and therefore gives rise to instructive product ions (see Figures 3 and 4, Tables 1 and 2).[34,81–83]

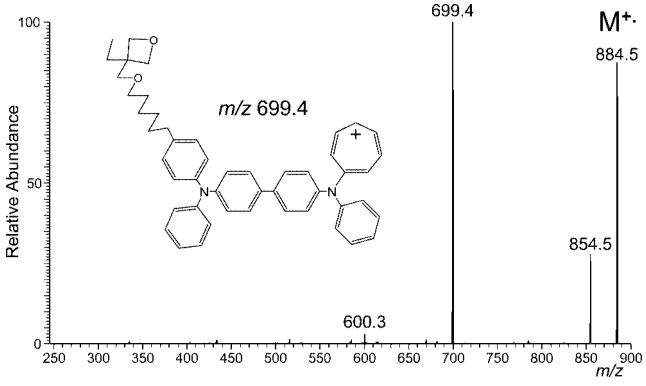


Figure 3. QIT-MS^2 product ion spectrum of the radical precursor ion $[M]^{+\cdot}$ of **4** at *m/z* 884.5.

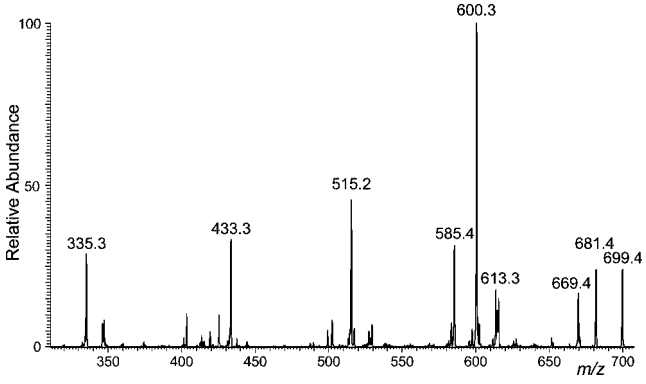


Figure 4. QIT-MS^3 product ion spectrum of *m/z* 699.5 generated by QIT-MS^2 of *m/z* 884.5 of compound **4**.

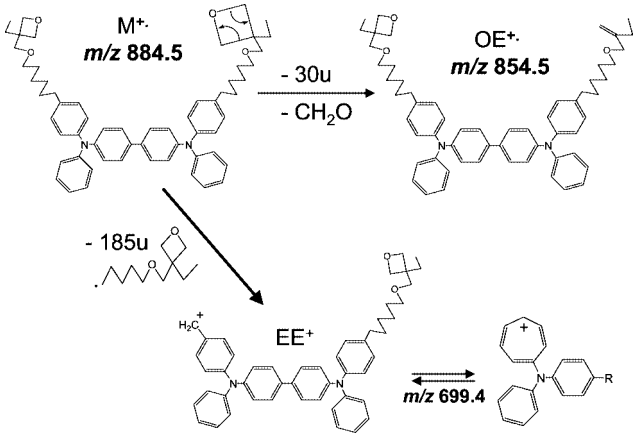
Table 1. MS^2 experiments in a QIT and a FTICR-MS of the precursor ion $[M]^{+\cdot}$ of **4** at *m/z* 884.54.

Precursor ion		Formula	Monoisotopic mass exp. [*m/z*]	Monoisotopic mass theo. [*m/z*]
$[M]^{+\cdot}$	$OE^{+\cdot}$	$[C_{60}H_{72}N_2O_4]^{+\cdot}$	884.5487	884.5485
Product ion				
$[M - CH_2O]^{+\cdot}$	$OE^{+\cdot}$	$[C_{59}H_{70}N_2O_3]^{+\cdot}$	854.5381	854.5380
$[M - C_{11}H_{21}O_2^{\cdot}]^+$	EE^+	$[C_{49}H_{51}N_2O_2]^+$	699.3945	699.3944

The ESI-MS^n spectra of radical molecular cations of compounds **1**–**5** exhibit an important similarity: loss of radicals. Open-shell precursor ions are known to show this CID behaviour, delivering closed-shell product ions of high abundance (Figure 3 and Scheme 2). Interestingly, these closed-shell product ions, when chosen as precursors for MS^3 CID experiments, decay again by loss of radical fragments to give abundant OE product ions (Figure 4 and Scheme 3).

Table 2. MS^3 experiments in a QIT and a FTICR-MS with exact mass measurement of the product ions of **4**: sequential CA of $[M]^{+\cdot}$ at *m/z* 884.54 and *m/z* 699.4.

Precursor ion		Formula	Monoisotopic mass exp. [*m/z*]	Monoisotopic mass theo. [*m/z*]
$[M]^+$	EE^+	$[C_{49}H_{51}N_2O_2]^+$	699.3945	699.3944
Product ion				
$[M - H_2O]^+$	EE^+	$[C_{49}H_{49}N_2O]^+$	681.3842	681.3841
$[M - CH_2O]^+$	EE^+	$[C_{48}H_{49}N_2O]^+$	669.3843	669.3845
$[M - C_5H_8O]^+$	EE^+	$[C_{44}H_{43}N_2O]^+$	615.3374	615.3376
$[M - C_5H_{10}O]^+$	EE^+	$[C_{44}H_{41}N_2O]^+$	613.3213	613.3219
$[M - C_6H_{11}O^{\cdot}]^{+\cdot}$	$OE^{+\cdot}$	$[C_{43}H_{40}N_2O]^{+\cdot}$	600.3140	600.3141
$[M - C_6H_{10}O_2]^+$	EE^+	$[C_{43}H_{41}N_2]^+$	585.3270	585.3270
$[M - C_{11}H_{20}O_2]^+$	EE^+	$[C_{38}H_{31}N_2]^+$	515.2487	515.2487

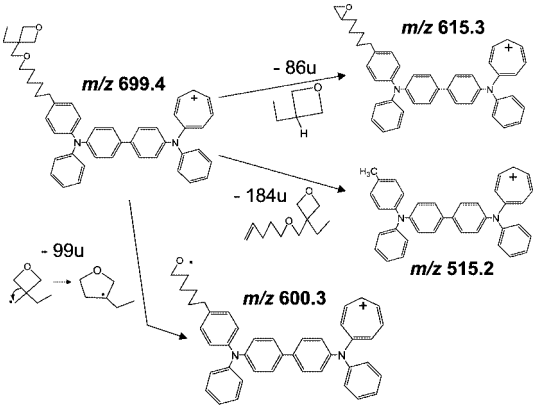


Scheme 2. Fragmentation reactions observed in a QIT-MS^2 product ion spectrum of the precursor ion $[M]^{+\cdot}$ of **4** at *m/z* 884.5.

The compositions of all relevant ions formed in these MS^n experiments were reliably confirmed by measurements of the exact ion masses with a Fourier Transform Ion Cyclotron Resonance-MS (FTICR-MS; see Tables 1 and 2).[84]

Figure 4 shows the MS^3 spectrum of *m/z* 699.4 of compound **4**. The loss of an oxetane radical (99 u) is the predominant fragmentation pathway delivering the prominent product ion at *m/z* 600.3 (Scheme 3).

For that ion we present a distonic structure with a terminal oxygen radical, the result of a homolytic dissociation of the bond next to the ether oxygen. This rather unusual charge-remote fragmentation process is not further examined and the actual structure of the ion remains a subject of speculation. However, the determination of the exact mass of the corresponding open-shell ion allows an unambiguous assignment of the loss of the oxetane radical, which probably rearranges as proposed in Scheme 3.



Scheme 3. Fragmentation reactions observed in a QIT-MS^3 product ion spectrum of the closed-shell precursor ion at *m*/*z* 699.4 generated by QIT-MS^2 of *m*/*z* 884.5 of compound **4**. The structure of the distonic product ion at *m*/*z* 600.3 is deduced from the loss of the oxetane radical (99 u).

The easy formation of abundant gas-phase OE molecular ions by ESI and the peculiar MS^n fragmentation behaviour of these radical molecular cations strongly indicates that the capability of stabilizing unpaired electron species, which is the basis for the hole-conducting properties of tetra(aryl)-benzidine compounds in the solid phase, is preserved in the gas phase as well.[79] In particular, the remarkable loss of radical fragments in low-energy CID experiments of EE precursor ions, observed here in the MS^3 product ion spectrum of **4**, is worth noting (see Table 2, Scheme 3 and Figure 3).[85–94] This CID behaviour obviously contradicts the "*even electron rule*",[95–97] which states that closed-shell EE cations preferentially decompose in CID experiments to give closed-shell product ions and neutral fragments. Although exceptions from this empirical prediction are documented,[85–94] it is far more often the case that the formation of two open-shell species by CID of a closed-shell precursor (i.e., $EE^+ \rightarrow OE^{+\cdot}$ + radical) is energetically less favoured than the loss of a closed-shell neutral and the formation of an EE product ion (i.e., $EE^+ \rightarrow EE^+$ + neutral).[85,94,96] Obviously, this assumption seems not to be valid for easily oxidizable compounds (e.g., hydrocarbon polyenes, polycyclic aromatic hydrocarbons, porphyrins etc.) because fragment ions are conserving the strong ability to stabilize unpaired electrons.

4. Investigation of Reaction Mechanisms by Detection and Characterization of Transient Radical Species by ESI-MS and ESI-MS/MS

Investigations of complete reaction mechanisms on the basis of the analysis of transient intermediates are complex analytical problems. Most importantly, the origin and identity of detected species as real transient intermediates of a predicted reaction path must be clarified unambiguously. For that purpose, control measurements are mandatory in order reliably to exclude any formation of ESI artefact ions, which can dramatically mislead the interpretations of the experiments.

A general experimental protocol includes an appropriate ESI-MS analysis of a given reaction mixture, followed by ESI-MS^n product ion experiments for structure elucidation and verification of the identity of those ions potentially relevant as transient species. Here, the determination of the exact ion masses of the species of interest is incontrovertibly recommended. Independently, neutral organic compounds related to the suspected intermediates have to be examined by ESI-MS to verify whether or not their corresponding radical cations detected in the reaction mixture analysis were generated by the ESI process itself. Such tests are indispensable if it is to be confirmed that ESI exclusively is transferring the ions formed in a solution-phase reaction mixture into the gas phase. Additional atmospheric pressure chemical ionization (APCI) experiments have been shown to be beneficial, because relevant radical cations of neutral organic compounds of interest could be effectively obtained from solutions to provide $[M]^{+\cdot}$ precursor ions for reference MS/MS experiments important for comparison and verification of the ESI-MS/MS results.

An early example of reaction mechanism examinations by ESI-MS is to be found in the work of Viscontini et al., who examined the oxidative formation of tetrahydropterine radical cations.[98]

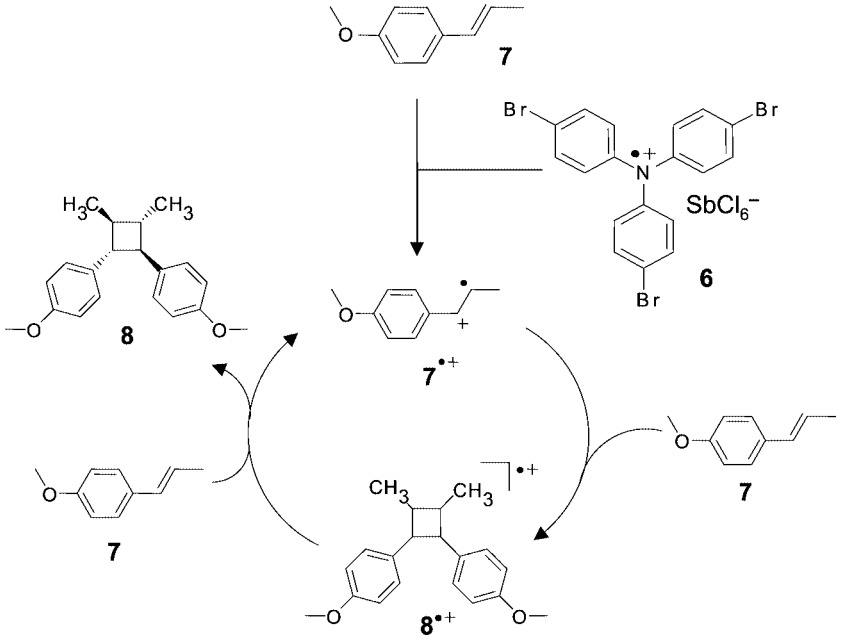
Expanding the classical focus of charged species analysis by MS, even reactions that progress through uncharged radical intermediates were examined successfully by ESI-MS. For that purpose, nitroxide spin traps such as 5,5-dimethyl-1-pyrrolidine *N*-oxide (DMPO) are utilized to transform transient radicals into stable spin-trapped adducts amenable to ESI-MS analysis and structure elucidation by ESI-MS/MS.[99–104] Additionally, neutral radical intermediates can be detected by complexation with charged species: Lewis-acid controlled radical chain reactions, for example.[41,42]

In another interesting example of reaction mechanism examinations, not only are the unique properties of ESI for soft ion transfer from the solution into the gas phase included in the analytical strategy, but the special experimental setup of ESI is consciously incorporated as well. Here, peroxide is added to solutions of selected reagents and these mixtures experience the effects of the high voltage applied to the needle tip of the ESI capillary and are transferred further on into charged droplets in the ES ion source. Either circumstance initiates the solution-phase reaction under examination by providing hydroxyl radicals originating from decomposed peroxide. In doing so, OH-radical-induced reactions mimicking photooxidation processes could successfully be examined by ESI-MS.[105]

Three examples of reaction mechanistic studies illustrating the highlighted analytical strategy are selected and discussed more closely below.

4.1. Stereoselective [2 + 2] Cycloaddition of *trans*-Anethole to 1,2-Bis(4-methoxyphenyl)-3,4-dimethylcyclobutane

Metzger et al. investigated a number of solution-phase reactions of great importance for synthetic organic chemis-



Scheme 4. Radical chain-reaction of *trans*-anethole (**7**) mediated by tris(*p*-bromophenyl)aminium hexachloroantimonate (**6**) to give the dimer product 1,2-bis(4-methoxyphenyl)-3,4-dimethylcyclobutane (**8**) via the reaction intermediates $[7]^{\bullet+}$ and $[8]^{\bullet+}$ (reproduction of the original scheme with permission of the publisher and the author).

try: the Diels–Alder reaction and electron-transfer-initiated reactions exhibiting radical cations as reactive intermediates.[37–42] For these studies a microreactor was coupled directly to a mass spectrometer fitted either with an ES or with an atmospheric pressure chemical ion source.[37–42]

To investigate the electron-transfer-initiated reaction in solution, which proceeds via radical cations as reactive intermediates, catalytic amounts of tris(*p*-bromophenyl)aminium hexachloroantimonate (**6**) were used as radical mediator to initiate the stereoselective [2 + 2] cycloaddition of *trans*-anethole (**7**) to give 1,2-bis(4-methoxyphenyl)-3,4-dimethylcyclobutane (**8**; see Scheme 4).[38,40]

Kinetic studies regarding the stereo- and regioselective dimerization process for this conversion suggested a radical chain reaction mechanism. Scheme 4 shows the catalytic circle of the reaction: the aminium salt **6** oxidizes the substrate **7** to give the radical cation $[7]^{\bullet+}$, which directly combines with a second equivalent of **7** to form the dimeric radical cation $[8]^{\bullet+}$. Finally product **8** is formed in a reaction with a molecule of **7**, which itself regenerates a corresponding radical cation $[7]^{\bullet+}$.

To investigate the reaction mechanism ESI-MS spectra of the reaction directly, solutions that would exhibit only an intensive signal of $[6]^{\bullet+}$ were acquired (see Figure 5). The concentrations of the quasi-stationary amounts of the intermediates $[7]^{\bullet+}$ and $[8]^{\bullet+}$ are obviously too low for single ESI-MS. However, weak ion currents appear at the corresponding *m*/*z* values for these intermediates, and product ion spectra of both suspected radical cations $[7]^{\bullet+}$ and $[8]^{\bullet+}$ could be acquired by ESI-MS/MS.

Comparison of the ESI-MS/MS spectra with reference APCI-MS/MS spectra of the particular radical cationic precursor ions generated independently by APCI of compounds **7** and **8** confirmed the identity of the intermediates $[7]^{\bullet+}$ and $[8]^{\bullet+}$ on the basis of the significant fragmentation patterns. Finally, the formation of the radical cations $[7]^{\bullet+}$ and $[8]^{\bullet+}$ by ESI had to be excluded. Several control experiments with plain solutions of compounds **7** and **8** without the presence of the radical mediator **6** inhibiting the chemical formation of radical cation $[7]^{\bullet+}$ were run by ESI-MS. As the relevant radical cations $[7]^{\bullet+}$ and $[8]^{\bullet+}$ could not be observed, it is reasonable to assume that they are present in the solution phase as true reaction intermediates, supporting the proposed reaction.

4.2. Tributyltin-Mediated Addition of *tert*-Butyl Iodide to Dimethyl 2-Cyclohexyl-4-methyleneglutarate

In this study the complex tributyltin-mediated addition of *tert*-butyl radicals to dimethyl 2-cyclohexyl-4-methyleneglutarate, initiated by triethylborane in the presence of $Sc(OTf)_3$ to give dimethyl 2-cyclohexyl-4-neopentylglutarate (**11**) was investigated by ESI-MS (Scheme 5).[41,42,106]

Examination of this reaction was carried out with an experimental setup comprising a microreactor coupled to an ESI-MS instrument. A solution of *tert*-butyl iodide (**9**), dimethyl 2-cyclohexyl-4-methyleneglutarate (**10**) and $Sc(OTf)_3$ in diethyl ether saturated with air and a solution of tributyltin hydride containing triethylborane under argon were mixed and immediately introduced into the ESI source. The ESI-MS spectrum of the reacting solution exhibits several complex ions containing $Sc(OTf)_2$ with one or more substrate and product molecules. Additionally, signals of heterodimeric species of substrate **10** and product **11** were registered (Figure 6). With progressing reaction the

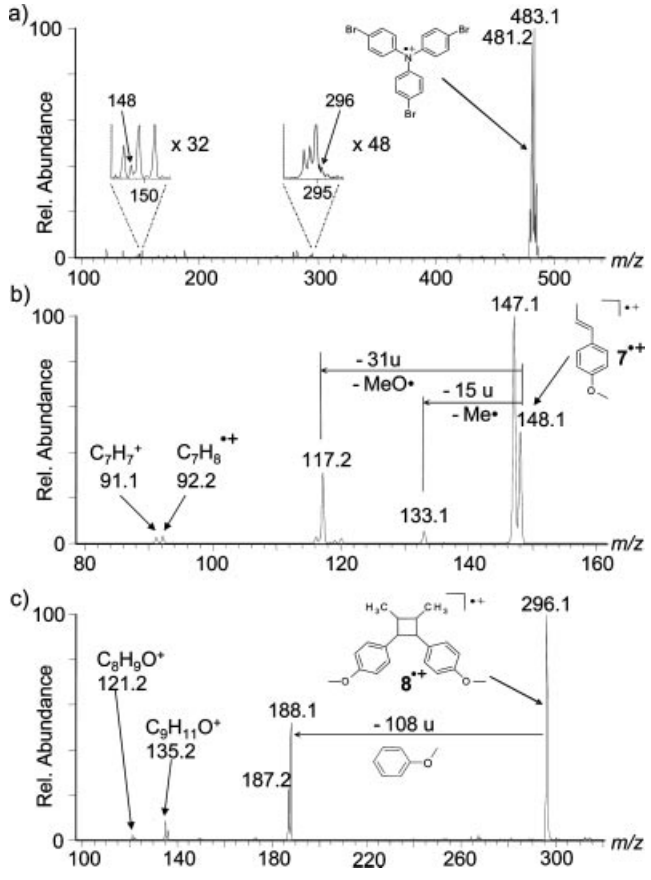
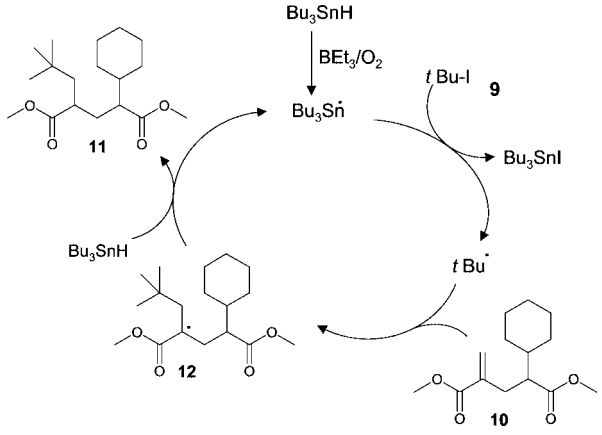


Figure 5. **a**) Positive ESI mass spectrum of the reacting solution of *trans*-anethole (**7**) and tris(*p*-bromophenyl)aminium hexachloroantimonate (**6**) in CH_2Cl_2. **b**) ESI-MS/MS spectrum of signal *m*/*z* 148 of the reacting solution showing fragmentations identical to those of an APCI-MS/MS spectrum of the authentic radical cation $[\mathbf{7}]^{\cdot+}$. **c**) ESI-MS/MS spectrum of the signal *m*/*z* 296 of the same reacting solution, showing fragmentations identical to those of a reference APCI-MS/MS spectrum of the authentic radical cation $[\mathbf{8}]^{\cdot+}$. Original spectra are reproduced with permission of the publisher and of the author.



Scheme 5. Tributyltin hydride-mediated addition of *tert*-butyl iodide (**9**) to dimethyl 2-cyclohexyl-4-methyleneglutarate (**10**). The stereoselective reaction yields dimethyl *syn*-2-cyclohexyl-4-neopentylglutarate (**11**) via a transient adduct radical **12** in the presence of $Sc(OTf)_3$. Original scheme is reproduced with permission of the publisher and of the author.

ions representing the substrate compound decrease in abundance whereas the abundance of the ions relating to the reaction product increases.

The low stationary concentration of the transient radical in the reaction mixture, estimated to be approximately 10^{-7} M, prevented unambiguous detection of the open-shell complex $[\mathbf{12}{\cdot}Sc(OTf)_2]^{+\cdot}$ at *m*/*z* 654 by single stage ESI-MS. However, with cautious accumulation and selection of the ions at *m*/*z* 654 it was possible to acquire a valuable product ion spectrum of that complex ion, containing the transient radical **12** and $Sc(OTf)_2$. The assignment was confirmed by a characteristic loss of CF_2SO_3 (–130 u), giving rise to the respective product ion at *m*/*z* 524 (Figure 7, a). The unusual adduct ions of *m*/*z* 524 with water and diethyl ether are

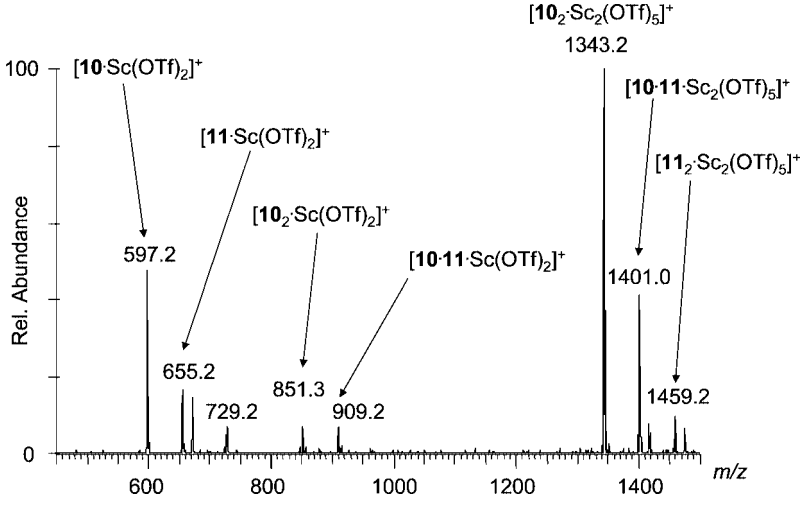


Figure 6. Positive ESI mass spectrum of the reacting solution of the tributyltin hydride-mediated addition of *tert*-butyl iodide (**9**) to dimethyl 2-cyclohexyl-4-methyleneglutarate (**10**) in the presence of scandium triflate in diethyl ether resulting in addition product **11** after a reaction time of approximately 30 s. Original spectrum is reproduced with permission of the publisher and of the author.

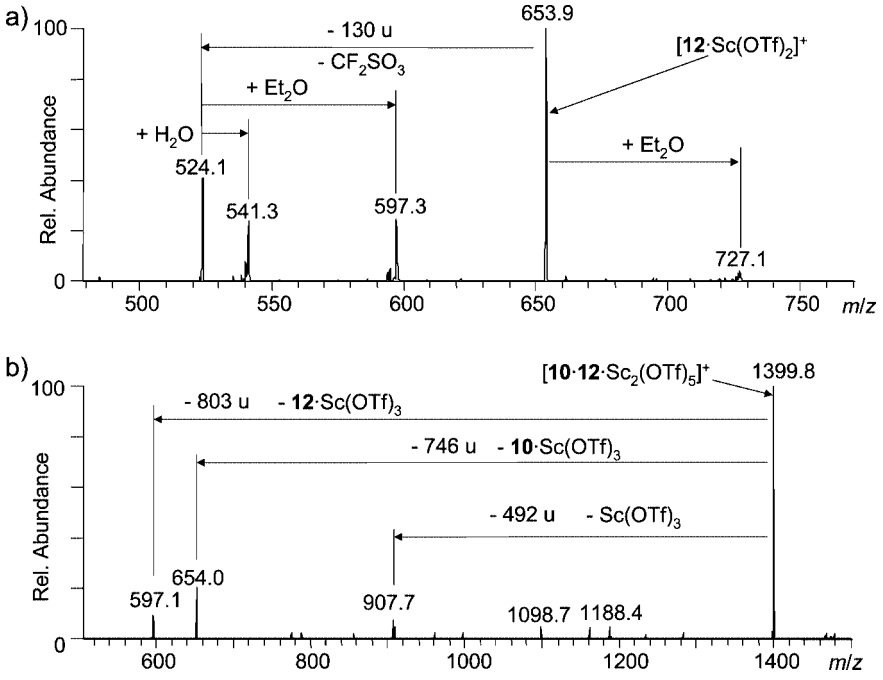


Figure 7. **a**) ESI-MS/MS spectrum of the radical complex ion $[\mathbf{12}\cdot Sc(OTf)_2]^+$ (*m*/*z* 654) formed by the tributyltin hydride-mediated addition of *tert*-butyl iodide (**9**) to dimethyl 2-cyclohexyl-4-methyleneglutarate (**10**) in the presence of scandium triflate in diethyl ether. **b**) ESI-MS/MS spectrum of the substrate-radical complex ion $[\mathbf{10}\cdot\mathbf{12}\cdot Sc(OTf)_5]^+$ (*m*/*z* 1400) from the same reacting solution. Original spectra are reproduced with permission of the publisher and of the author.

formed by ion–molecule reactions in the ion trap due to long residence times and are detected at the respective *m*/*z* values (see Figure 7, a).

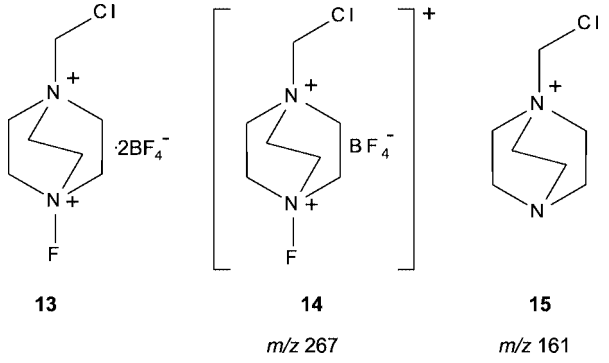
Additionally, a signal at *m*/*z* 1400 was found and identified as the heterodimeric complex of radical **12** with substrate **10** $[\mathbf{10}\cdot\mathbf{12}\cdot Sc_2(OTf)_5]^+$ (Figure 7, b). This assignment was confirmed by determination of the exact ion mass with an orthogonal ToF system and further MS/MS experiments that yielded characteristic fragmentations, such as the complementary losses either of the neutral radical **12** accompanied with $Sc(OTf)_3$ (–803 u), forming the complex ion $[\mathbf{10}\cdot Sc(OTf)_2]^+$, or the loss of substrate **10** with $Sc(OTf)_3$ (–746 u), leading to the radical complex ion $[\mathbf{12}\cdot Sc(OTf)_2]^+$ (see Figure 7, b).

Finally, the formation of the heterodimeric complex ions containing radical **12** in the reaction cycle was examined by several ESI-MS control measurements to exclude the formation of the corresponding intermediate by ESI. Different substrate mixtures with strict exclusion of oxygen were examined. Radical **12** could not be detected in any ionic species in these control experiments, confirming the assumption that **12** is a key intermediate of the depicted reaction mechanism.

4.3. Fluorination of Triphenylethylene and Tetraphenylethylene

Guo et al. examined the electrophilic fluorination of multiply phenyl-substituted olefins by ESI-MS and ESI-MS/MS and found ample evidence for a single-electron transfer (SET) mechanism of that reaction.[44,102,103]

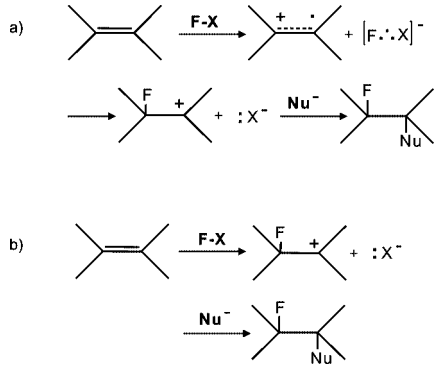
For the fluorination 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis-tetrafluoroborate (Selectfluor, F-TEDA-BF_4) was used (Scheme 6).[107]



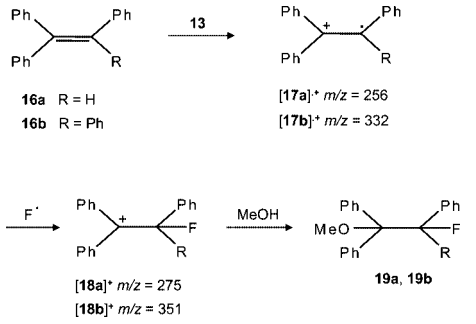
Scheme 6. Fluorination reagent Selectfluor (**13**). Ions derived from **13** observed in ESI-MS. Original scheme is reproduced with permission of the publisher and of the author.

Two alternative mechanisms for the reaction [i.e., a radical conversion (Scheme 7, a) and a S_N2 reaction (Scheme 7, b)] were postulated and examined.

In the latter reaction pathway an initial charge-transfer complex undergoes sequential electron and fluorine radical-transfer steps, involving a radical cation as key intermediate species.[108] However, both proposed mechanisms proceed via fluorinated carbocations [starting materials for **16a** and **16b** would be $(\mathbf{18a})^+$ and $(\mathbf{18b})^+$ respectively; see Scheme 8].



Scheme 7. Two alternative mechanisms considered for the reaction of **13** with olefins. Original scheme is reproduced with permission of the publisher and of the author.



Scheme 8. Examined fluorination reaction of aryl-substituted olefins by Selectfluor (**13**). Original scheme is reproduced with permission of the publisher and of the author.

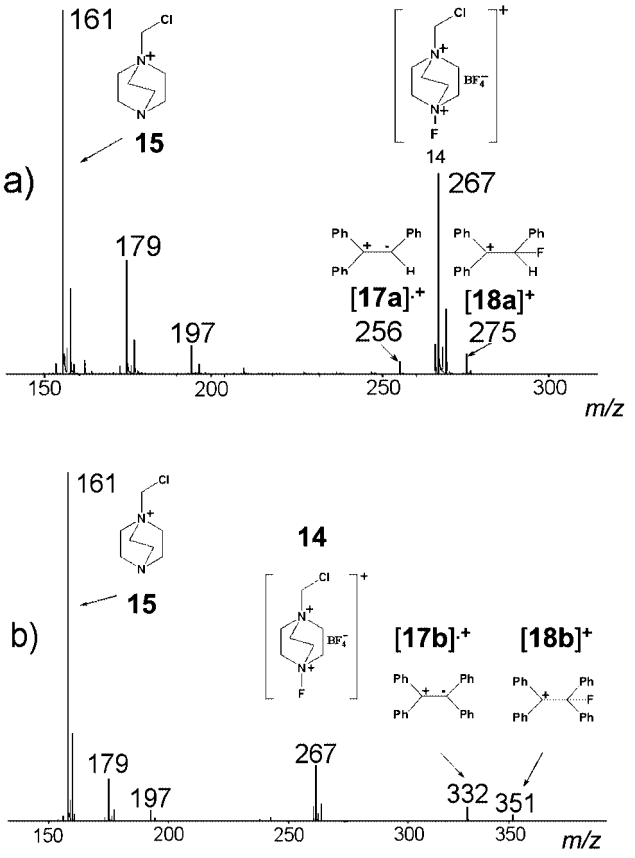


Figure 8. Positive ESI mass spectra of methanolic reacting solutions: **a) 16a** + **13** and **b) 16b** + **13**. Original spectra are reproduced with permission of the publisher and of the author.

Prior to the actual reaction, analysis control measurements of solutions of the aryl-substituted olefins **16a** and **16b** were performed by ESI-MS. The corresponding signals of the radical cations $[\mathbf{17a}]^{\cdot+}$ and $[\mathbf{17b}]^{\cdot+}$ were not detected in the ESI mass spectra, so oxidative formation of the corresponding radical molecular cations of **16a** and **16b** under ES ion source conditions can be ruled out. For the examination of the fluorination reaction by ESI-MS, methanolic solutions of Selectfluor was added to acetonitrile solutions of substrates **16a** and **16b**. After 1–3 min the reaction mixtures were analysed by ESI-MS (see part a in Figure 8 and b in Figure 8). The ESI mass spectrum of olefin **16a** reacting with Selectfluor shows abundant signals of ions derived from Selectfluor at *m*/*z* 161, *m*/*z* 267, $[\mathbf{15} + F - H]^+$ at *m*/*z* 179 and $[\mathbf{15} + 2F - 2H]^+$ at *m*/*z* 197. Additionally, signals of the radical cation $[\mathbf{17a}]^{\cdot+}$ at *m*/*z* 256 and of the fluorinated carbocation $[\mathbf{18a}]^+$ at *m*/*z* 275 are observed. The reaction of olefin **16b** was investigated analogously and yielded similar results (see Figure 8, b). Here, the transient radical cation $[\mathbf{17b}]^{\cdot+}$ appears at *m*/*z* 332 and the fluorinated carbocation $[\mathbf{18b}]^+$ shows a signal at *m*/*z* 351 in the corresponding ESI mass spectrum.

The identities of the radical intermediates $[\mathbf{17a}]^{\cdot+}$ and $[\mathbf{17b}]^{\cdot+}$ were further confirmed by the determination of their corresponding exact ion masses in a FTICR-MS and on the basis of characteristic fragment ions of the former generated by CID.

Altogether, reliable characterization of the reaction intermediates was accomplished by multidimensional MS experiments, confirming the assumption that the fluorination of multiply phenyl-substituted olefins follows a radical conversion mechanism.

Conclusions

The formation of radical molecular cations in ESI-MS relies on the solution-phase electrochemical oxidation of the analyte in the ESI capillary. Sensitive detection of open-shell molecular ions in ESI-MS is therefore limited to the analysis of compounds with low oxidation potentials, indicating a strong ability to stabilize the corresponding open-shell cations. That characteristic feature is also documented in CID experiments with the corresponding radical molecular cations and of closed-shell ions in sequential MS^n experiments dominated by the loss of radicals. It is therefore reasonable to assume that whenever easily oxidizable compounds (e.g., hydrocarbon polyenes, polycyclic aromatic hy-

drocarbons, porphyrins etc.) form molecular radical ions in ESI-MS, the fragmentation behaviour upon CID may show exceptions from the *even electron rule*.

The fundamental understanding of the ES ionization mechanism and the CID fragmentation behaviour of ions with open and closed shells sets the stage for the analysis of solution-phase reaction mixtures. The presented examples clearly demonstrate that the successful elucidation of complex reaction mechanisms demands the sensitive transfer of transient intermediates from the solution to the gas phase, which can be accomplished by ESI-MS. Subsequent selection of relevant precursor ions for MS^n experiments offers extensive sets of data for structure elucidation. It must be noted that structure assignments based solely on fragmentation patterns need further confirmation by exact mass measurements of the product ions to assure reliable assumptions.

However, reaction mechanism studies based on ESI-MS and MS^n require appropriate control experiments to clarify the origins and identities of detected species unambiguously as real transient intermediates of a reaction path under consideration. It is of special importance that these control experiments be carefully chosen and properly conducted to reliably exclude the formation of artefact ions by ESI, which can dramatically mislead the interpretations.

Acknowledgments

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