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Optimization of information content in a mass spectrometry based flow-chemistry system by investigating different ionization approaches

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ABSTRACT

Current development in catalyst discovery includes combinatorial synthesis methods for the rapid generation of compound libraries combined with high-throughput performance-screening methods to determine the associated activities. Of these novel methodologies, mass spectrometry (MS) based flow chemistry methods are especially attractive due to the ability to combine sensitive detection of the formed reaction product with identification of introduced catalyst complexes. Recently, such a mass spectrometry based continuous-flow reaction detection system was utilized to screen silver-adducted ferrocenyl bidentate catalyst complexes for activity in a multicomponent synthesis of a substituted 2-imidazoline. Here, we determine the merits of different ionization approaches by studying the combination of sensitive detection of product formation in the continuous-flow system with the ability to simultaneous characterize the introduced [ferrocenyl bidentate+Ag]$^+$ catalyst complexes. To this end, we study the ionization characteristics of electrospray ionization (ESI), atmospheric-pressure chemical ionization (APCI), no-discharge APCI, dual ESI/APCI, and dual APCI/no-discharge APCI. Finally, we investigated the application potential of the different ionization approaches by the investigation of ferrocenyl bidentate catalyst complex responses in different solvents.

1. Introduction

Modern developments in catalyst discovery involve combinatorial synthesis methods for the generation of large number of potential catalysts [1,2]. In order to synchronize the throughput of the activity assessment with the number of candidates, high-throughput performance-screening methods are increasingly being developed and utilized for the exploration and optimization of catalyst libraries and associated reaction characteristics. By monitoring the product formation, the influence of catalyst activities, substrate ratios, reaction temperatures and reaction pressures can be recorded and, in online flow chemistry systems, directly optimized. Of these novel methodologies, mass spectrometry (MS) based continuous-flow reaction detection systems offer exceptional throughput, ease of automation, selectivity and sensitivity [3]. The pertinence of this screening methodology is demonstrated in various publications concerning enzymatic (inhibitory) activities [4–6]. More recently, we introduced an MS-based continuous-flow reaction detection system that enables rapid evaluation of homogeneous catalyst performance in synthetic conversions [3,7]. In one of these studies [3], we used homogenous catalysis of a multicomponent reaction for the synthesis of a substituted 2-imidazoline using a Lewis acid catalyst (silver triflate) combined with Josiphos-type ferrocene diphosphine ligands. Such ferrocene-based catalysts provide an ideal template where the activity and selectivity can be adjusted by alteration of substituents [8,9]. The popularity of this exemplary metallocene in material science, organometallic and coordination chemistry is derived from the unique combination of properties associated with this electron-rich aromatic scaffold. These characteristics result in a catalyst ligand family that is associated with a wide variety of synthetic conversions comprising exceptional enantioselectivities, substrate-to-catalyst ratios and turnover frequencies [10]. Our MS-based continuous-flow system offers excellent specificity and selectivity for the sensitive detection of target (product) molecules in the presence of substrates and catalyst complexes. When a single time-point is used to assess catalyst activity, it is of eminent importance that the formed product is quantified after the conversion reaches the initial rate period. Then, it is unnecessary to determine complete reaction progress curves. Additionally, the quantification has to be performed at exactly the same reaction time. For this system with a fixed reactor volume and associated reaction time, it was demonstrated that a single time-point sufficed for an activity assessment. By correcting for solvent specific product responses, catalyst performance results obtained in various solvents could be directly compared [3]. Moreover, it was demonstrated that the same system could be utilized for a high-throughput optimization of rudimentary reaction parameters.

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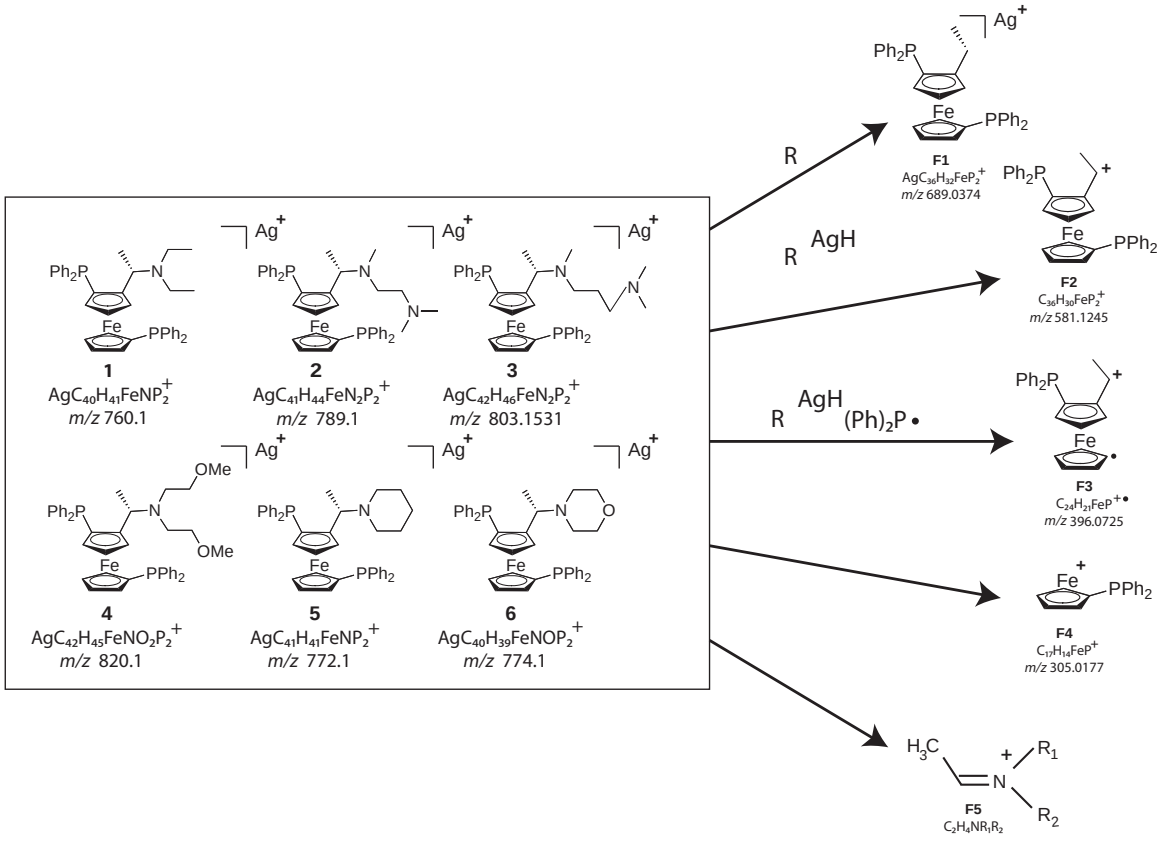

Scheme 1. The six ferrocenylbidentates (**1**–**6**) of the in-house produced catalyst library (left). In APCI (right), these complexes fragment into class specific fragments F1, F2, F3, F4 and F5.

It was found that the silver-adducted ferrocene diphosphine catalyst complexes show structure-informative fragmentation under atmospheric-pressure chemical ionization (APCI) MS conditions. This fragmentation of [ferrocenyl bidentate+Ag]$^+$ adduct ions was studied previously in detail using electrospray ionization (ESI) MS^n [11].

Although the promise of direct detection in flow chemistry systems was illustrated previously, the ability to use the incorporated MS detector for a provisional identification of introduced compounds is untapped. This feature is especially attractive in flow chemistry systems where focused libraries of analogues are produced on the fly. In that way, the synthesized structural analogues can be simultaneously (provisionally) identified.

Here, we describe the characteristics of the [ferrocenyl bidentate+Ag]$^+$ catalyst complexes in a variety of solvent systems in both ESI MS and APCI MS. The fragmentation of these complexes in APCI MS is compared to that in ESI MS^n. The potential of a flow chemistry reaction detection system equipped with single-stage quadrupole MS is evaluated with respect to its ability to screen for catalyst performance and to (provisionally) identify the (active) catalysts in a combinatorial synthesized library. In order to optimize the information content of the mass spectrometric detection, the potential of ESI in a dedicated ESI source, APCI, no-discharge APCI (ND-APCI), and rapid switching between APCI and ND-APCI in a dedicated APCI source, as well as rapid switching between ESI and APCI in a dual-ESI/APCI source was investigated.

2. Experimental

2.1. Solvents

The MS investigation of the catalyst complexes was conducted in six different solvents: GC-grade (>99%) tetrahydrofurane (THF) and dichloromethane (DCM) were purchased from Fluka (Buchs, Switzerland), methanol (MeOH, absolute ULC/MS grade) and acetonitrile (ACN, HPLC-S grade) from Biosolve (Valkenswaard, the Netherlands) and 2-propanol (IPA, >99.8%) and ethyl acetate (EtOAc, >99.5%) from Sigma–Aldrich (Steinheim, Germany).

2.2. Multicomponent reaction

The ferrocene catalyst complexes are injected into a stream of reactants. The applied multicomponent reaction involves three reactants: acetone (>99%) was purchased from J.T. Baker (Deventer, the Netherlands), benzylamine was purchased from Fluka, while *p*-nitrobenzylisocyanide and 2-imidazoline product standards were synthesized in-house [12]. In the experiments that demonstrate the MS performance in screening conditions, 4 mM reactant was used.

2.3. Ferrocene ligand library

The ferrocene ligand library studied here comprises six ferrocene ligands (see Scheme 1 for structures and elemental composition) that were synthesized in-house in 6 steps starting from ferrocene carboxaldehyde according to procedures described elsewhere [13]. The catalyst complex solutions were prepared by 1:1 mixing 200 μM silver trifluoromethanesulfonate (silver triflate, AgOTf, >98%, Fluka) and 200 μM of the ferrocene ligand in the appropriate solvent. The optimal concentration in an activity assessment was determined previously by determining product formation at different catalyst concentrations [3]. When a catalyst/reactant ratio exceeding 2.5% (=100 μM) of catalyst was used, no increased product formation could be detected due to saturation of the reaction mixture.

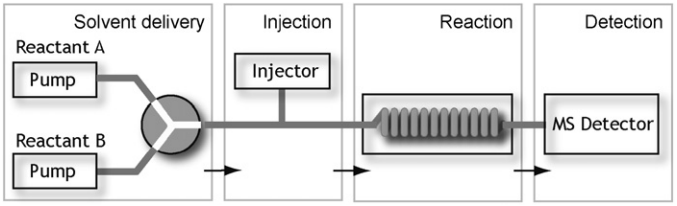

Fig. 1. Schematic overview of the continuous-flow reaction detection catalyst activity screening system. The system is composed of a substrate delivery unit comprising two HPLC pumps that pump the imine (Reactant A) formed after the condensation of acetone and benzylamine and the p-nitrobenzylisocyanide (Reactant B). When the reactants are mixed, an autoinjector is used to introduce the ferrocenyl bidentate-silver triflate catalyst complexes into the stream of substrates after which the catalyst-mediated conversion into the 2-imidazoline product occurs in the coiled open tubular reactor. Finally, product formation is monitored with a SIM trace of the product ion and the different catalyst complexes are determined using a full-spectrum TIC trace.

2.4. Reaction detection system

The flow chemistry reaction detection system consisted of an online coupled substrate delivery unit, a temperature-controlled reactor, and a mass spectrometric detector (Fig. 1). The substrate delivery unit was composed of two Shimadzu ('s-Hertogenbosch, the Netherlands) LC-20AD HPLC pumps that delivered the preformed imine and *p*-nitrobenzylisocyanide substrates at 200 μL min^{-1} into a mixer (Valco International, Schenkon, Switserland). With dichloromethane solutions, due to equipment incompatibilities, the substrate delivery was performed using two Gilson Model 302 (Villiers-le-Bel, France) HPLC pumps. Subsequently, 10 μL of the catalyst complex of interest was injected by employing a Spark Holland (Emmen, the Netherlands) Midas autosampler. The conversion into product occurred in an in-house produced coiled open tubular reactor immersed in a temperature controlled water bath at 60 °C (Grant Cambridge, UK).

2.5. Single-stage quadrupole MS detection in the reaction detection system

The system is equipped with a Shimadzu LCMS-2010EV single-stage quadrupole mass spectrometer, operated by alternating between two events. The first event (1.0 s) is used to assess catalyst complex activity by selected ion monitoring (SIM) of the 2-imidazoline product (*m/z* 310.2), while the second event (0.2 s) is used to study catalyst complexes with a full-spectrum analysis in the mass range *m/z* 140–1200. This set-up is optimized for the sensitive detection of the product that is formed in the catalysts activity screening experiments. However, the limited acquisition time for the full-spectrum analysis compromises the mass accuracy.

For the investigation of catalyst complexes, ESI, APCI and ND-APCI in positive ion mode were utilized. The MS was operated with a detector voltage of 1.7 kV. ESI settings included an interface and curved desolvation line (CDL) temperature of 250 °C, an interface voltage of 4.5 kV and a nebulizer gas-flow of 1.5 L min^{-1}. The optimal APCI and ND-APCI settings only differ from the ESI settings by the applied interface temperature of 450 °C and a nebulizer gas-flow of 2.5 L min^{-1}. In APCI, a corona discharge voltage of 4.5 kV was applied while in ND-APCI the corona discharge was kept at 0 kV.

2.6. Single-stage quadrupole MS detection with dual-ESI/APCI source

The potential of ESI/APCI switching in a continuous-flow reaction detection system was investigated by equipping the platform with a Shimadzu LCMS-2020 single-stage quadrupole MS fitted with a dual ion source (DUIS). The single quadrupole MS was operated with a single full-spectrum analysis event (0.2 s), a detector voltage of 1.2 kV, a mass range *m/z* 100–1000. The MS was operated with a desolvation line (DL) temperature of 300 °C, a heat block temperature of 450 °C, a nebulizer gas-flow of 1.5 L min^{-1}. For the ESI experiments, an interface voltage of 4.50 kV was applied, whereas for the APCI experiments, a corona discharge voltage of 4.5 kV was applied. In the additional in-source CID experiments, the optimal Q-array voltage was found to be 130 V.

3. Results and discussion

3.1. Continuous-flow reaction detection system

The current flow chemistry reaction detection system (Fig. 1) is a flow chemistry system designed for the assessment of catalyst (complex) performance towards a specific synthetic conversion in a system equipped with MS detection. In more traditional catalyst performance screening methods, catalyst performance is rated based on kinetics determined from the increased slope of the initial rate period at different reaction times. In our system, however, conversion into product occurs in a flow-through reactor with fixed internal volume and associated fixed reactor residence time. In the resulting one time-point activity assessment procedure, the reaction time only slightly exceeds the initial rate period, thereby accomplishing a significantly increased throughput [7]. The incorporated MS detector allows for the sensitive quantification of the synthesis product and assessing catalyst performance by an automated determination of the peak area from the SIM product trace (*m/z* 310.2). Moreover, because system parameters like substrate flow-rates and ratios, the amount of injected catalyst, reaction time, reaction pressure and reaction temperature are either fixed or very accurately controlled, the system provides the high system accuracy, repeatability and reproducibility required for such a one time-point catalyst assessment approach [3,7].

The atom-efficient three-component synthetic conversion that was studied as a model reaction involves the Lewis-acid catalyzed synthesis of a 2-imidazoline from an intermediate imine, preformed by condensation of a ketone with an amine, and a substituted isocyanide [14]. In this reaction, the involved condensation equilibrium requires the synthetic conversion to be conducted in a water-free environment. This exemplary multicomponent reaction is ideally suited for the generation of focused libraries of pharmaceutical interesting analogues. By varying substituents, molecular diversity is rapidly introduced and different 2-imidazolines generated.

3.2. Ionization of catalyst complexes in the reaction detection system

In the development of this MS-based screening platform, the merits of both APCI MS and ESI MS ionization were investigated. An important factor in the application perspective of any MS-based flow chemistry system for catalyst performance assessment is the possibility to be operated in the variety of (non-aqueous) solvents employed in synthetic organic chemistry (e.g. MeOH, ACN, IPA, EtOAc, THF and DCM). Dissimilar ionization efficiencies of the formed product in different solvents requires additional calibration efforts in order to be able to quantitatively compare screening results from different solvents. In previous studies, APCI MS was successfully employed for the sensitive detection of the 2-imidazoline product (*m/z* 310.2) in different solvents by calibrating solvent-specific 2-imidazoline responses of product standards [3]. In ESI MS with the non-aqueous solvents, the reaction product was detected with reduced sensitivity. However, in the current

discussion, next to reaction product detection, the characterization of the catalysts complexes and the optimization of the information content is considered important as well.

The spectra in Fig. 2 that were acquired using ESI MS and APCI MS illustrate the inherent characteristics of the two ionization methods when silver-adducted ferrocenyl bidentate ligands were introduced in THF as solvent. In the ESI MS spectrum, an abundant $[M+Ag]^+$ adduct ion is observed while the APCI MS spectrum shows only a minor $[M+Ag]^+$ adduct ion next to extensive fragmentation. Apparently, the limited amount of energy that is involved in the ESI process results in the efficient transfer of intact catalyst complexes from the solution to the gas phase. When APCI MS is utilized, the transfer of intact catalyst complexes is less efficient. The fragment ions observed in the APCI spectra can originate either from thermally induced solution-phase dissociation of the catalyst complex with subsequent ionization of the degradation products in the gas-phase or from transfer to the gas phase of the intact silver-adducted catalyst complex with the subsequent fragmentation of this complex in the APCI trajectory, induced by either the high temperatures in the ionization trajectory (nebulizer and source), the harsh corona discharge conditions, or both. As a result, ESI MS is generally considered to be better suited for the investigation of the solution-phase chemistry since many molecular processes and interactions are conserved in their transfer to the gas phase [15,16]. However, the goal of MS detection in our reaction detection system is not the conservation of the solution-phase chemistry but rather the ability to quantify the reaction product formation and to simultaneously characterize those injected catalysts that provide enhanced product formation. The extensive fragmentation that is generated with APCI may result in enhanced identification potential.

3.3. Comparison of fragment ions generated in APCI MS and in MS^n

The fragmentation of the studied silver-adducted ferrocene diphosphine catalysts (see Scheme 1 for their structures) in ESI MS^n was discussed in detail elsewhere [11]. The characteristic fragment ions are summarized in Scheme 1. The *m/z* value of $[M+Ag]^+$ and the fragments **F1** with *m/z* 689 and **F2** with *m/z* 581 are most relevant for catalyst characterization. The fragment ion **F1** with *m/z* 689 is due to the loss of the amine side chain as an imine. The generally more abundant fragment ion **F2** with *m/z* 581 is due to the loss of AgH and the amine side chain as an imine (see Scheme 1). The *m/z* difference between the $[M+Ag]^+$ and the fragment ions with *m/z* 689 and/or 581 may be used to calculate the mass of the amine substituent. In addition, in ESI MS^2, a low-*m/z* fragment due to the *N*-ethylidene immonium ion (**F5**) is observed as well [11].

Most of the fragment ions observed in ESI MS^2 spectra are actually also observed in the APCI spectra (*cf.* Fig. 2B). Next to a weak $[M+Ag]^+$ adduct ion, the structure-informative fragment ions with *m/z* 689 and 581 are readily observed. In addition, the backbone fragment ions with *m/z* 396 (**F3** in Scheme 1) and 305 (**F4**) are observed with low abundance. These data indicate that an APCI spectrum of a silver-adducted ferrocene diphosphine, acquired during activity assessment in the continuous-flow reaction detection system, can be applied in the initial characterization of (the more active) catalysts without the need for MS^n or high-resolution mass spectrometry.

Careful inspection of the APCI spectra (Fig. 2) reveals the presence of apparent fragment ions with *m/z* 597 and 613, that is *m/z* 581 + 16 and *m/z* 581 + 32, respectively. Accurate-mass determination (data not shown) indicates that these ions are due to oxidation. In some cases, especially with adulteration of the catalyst solutions, intact oxygenated impurities are observed as well, especially in ESI MS spectra (Fig. 2A), where the intact $[M+Ag]^+$ shows greater abundance. The occurrence of oxidative impurities with 1,1′-bis(diphenylphosphino)-ferrocenes was recently demonstrated by Wu et al. [17]. In a normal setting for activity screening, fresh solutions of the catalyst are used, and these additional ions are less abundant or even absent.

Finally, at the low-*m/z* end of the APCI MS spectrum, ions due to the reactants like the protonated imine reactant with *m/z* 148 and (intense) solvent background ions are observed. Unfortunately, the solvent background ions limit the detection of the low-*m/z* compound-specific *N*-ethylidene immonium ion (**F5**).

3.4. Solvent effects in APCI and ESI of catalyst complexes

The spectra of the catalyst complexes in Figs. 2 and 5 were acquired in THF and EtOAc, respectively. The solvent specific responses of the silver-adducted ferrocenyl bidentate catalyst complexes in APCI MS and ESI MS were determined by injecting the catalyst complexes in different solvents. Solvent and ionization method specific responses of intact catalyst complex and their fragment ions were determined from the peak areas in extracted ion chromatograms.

First, the response of the catalyst complexes in different solvents was compared between ESI MS and APCI MS. In order to remove the influence of the intense solvent-related background ions at low *m/z*, which is especially important in APCI, the response of the catalyst complexes was determined from the total ion current (TIC) in the range of *m/z* 500–850. In this way, the class-specific fragment ions with *m/z* 396 and 305, that are only present with low abundance, were ignored as well.

In ESI MS, the catalyst complexes showed best response in THF, IPA, and (perhaps somewhat surprisingly) DCM. The response in these solvents is up to four fold higher than in the other solvents, EtOAc, MeOH and ACN. An up to threefold difference in response may be observed with individual catalyst complexes in a particular solvent (data not shown).

In APCI MS, poor response for catalyst complexes is observed in IPA and DCM, whereas the responses in EtOAc, MeOH, THF, and ACN are about similar, spanning a range of a factor 2–3, but at least four times higher than in IPA or DCM. In THF, the best solvent for ESI MS, the response in APCI MS is about four times lower than in ESI MS. For EtOAc and MeOH, the response in APCI MS is slightly higher than in ESI MS; in ACN, similar responses are observed in ESI MS and APCI MS (data not shown). These differences appear not to be correlated to solvent properties such as proton-donor or proton-acceptor properties, dielectric constant, surface tension, boiling point and viscosity.

The poor response of the catalyst complexes in IPA with APCI MS may be disadvantageous in practical use of the continuous-flow reaction detection system, because previous data show that the best catalytic performance of these complexes is achieved in ACN, IPA and EtOAc [3].

The extent of fragmentation can be evaluated from the abundances for $[M+Ag]^+$ and the characteristic fragment ion **F2** with *m/z* 581. This is demonstrated for all six complexes in THF for ESI MS (Fig. 3A) and APCI MS (Fig. 3D) and for catalyst complex **5** in different solvents for ESI MS (Fig. 3B) and APCI MS (Fig. 3E). These data show that in ESI MS $[M+Ag]^+$ is generally ten times more abundant than the **F2** ion for all solvents, except MeOH. In APCI, the abundance of $[M+Ag]^+$ is more or less constant for the relevant solvents (EtOAc, MeOH, THF, and ACN) and significantly less (typically ten times) than that of the **F2** ion with *m/z* 581. Similar plots could have been shown for the other catalyst complexes and other solvents. This means that the trends indicated with Fig. 2 appears to be valid for all conditions, except ESI MS in MeOH. This is in fact also demonstrated for ESI MS in Fig. 3C and for APCI MS in Fig. 3F, where the averaged responses (for $[M+Ag]^+$ and **F2**) of the six catalyst complexes in the six solvents are compared. Interestingly, the average

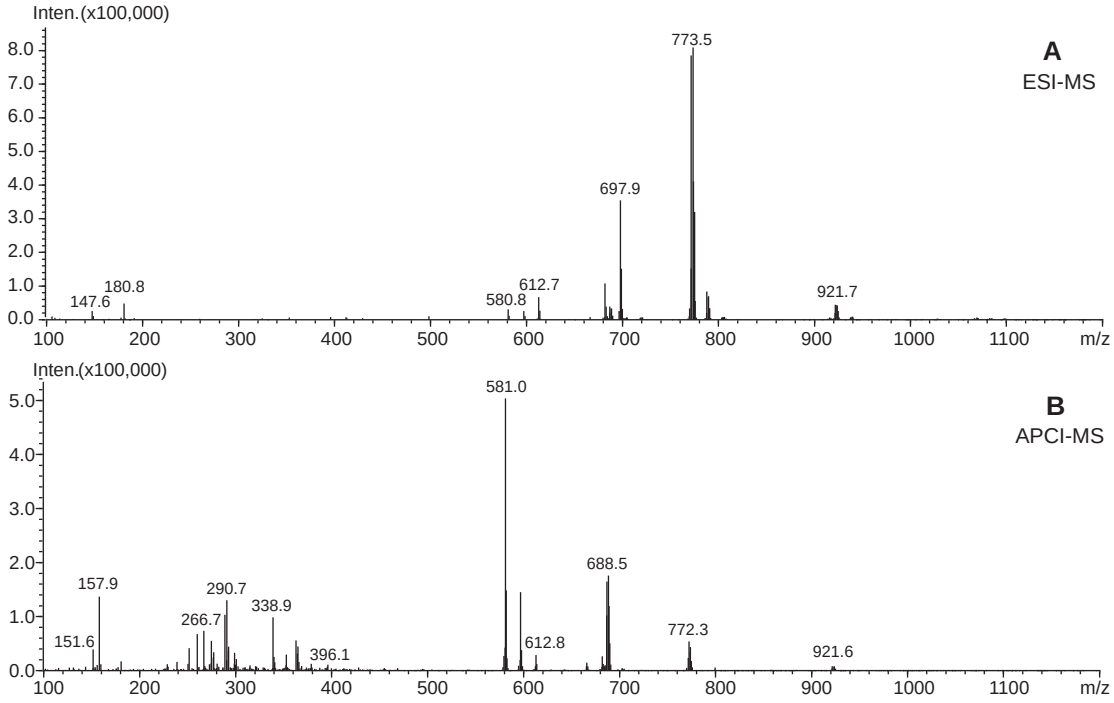

Fig. 2. Mass spectra of the silver adducted catalyst ferrocene complex 5 in THF recorded with a single-stage quadrupole MS equipped with an ESI (A) and APCI (B) interface.

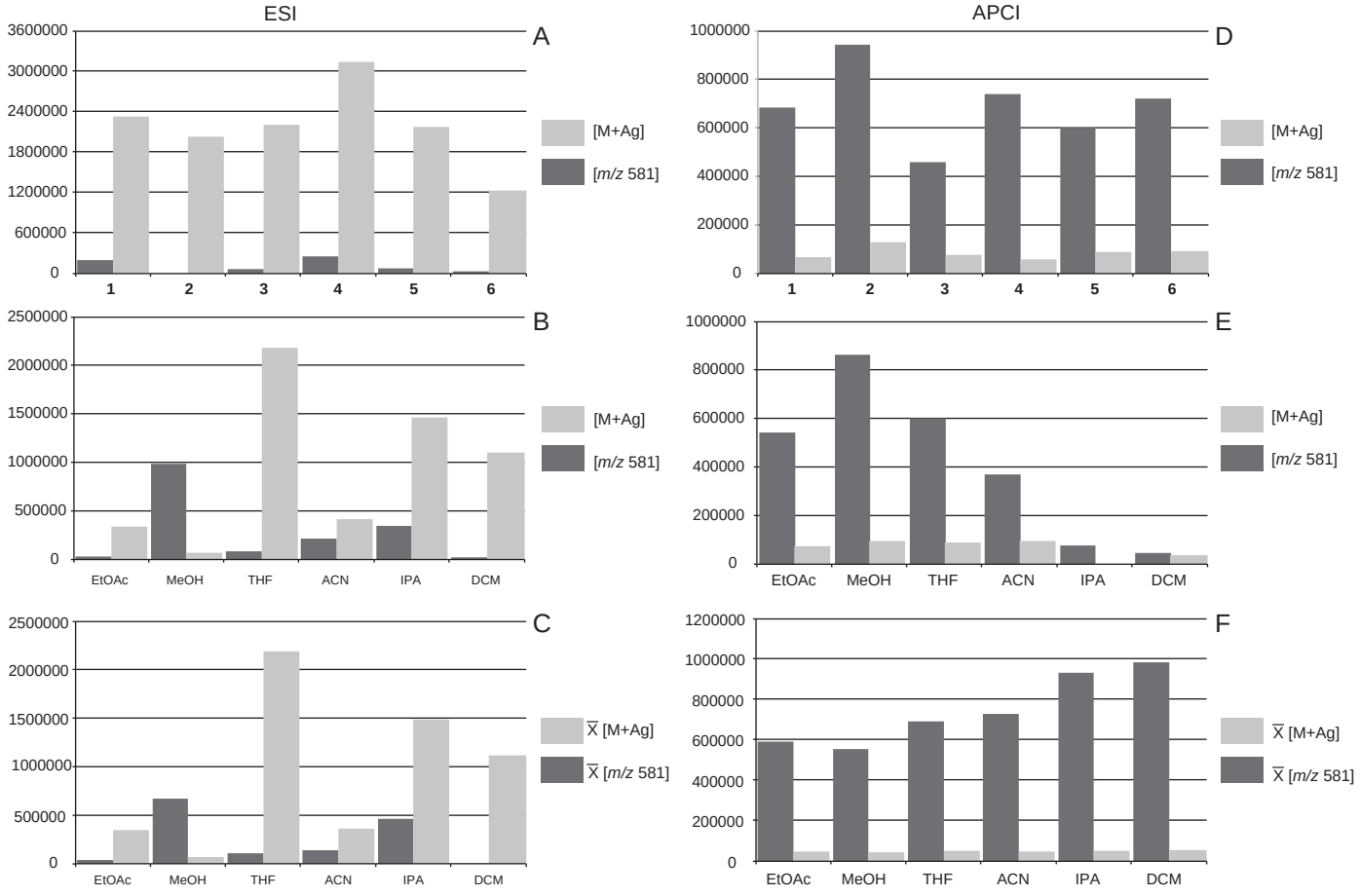

Fig. 3. ESI MS (A, B and C) and APCI MS (D, E and F) ionization efficiency of the ferrocenyl bidentate based catalyst complex library. In (B) and (E), the solvent specific efficiency of complex **5** is presented while in (C) and (F), the average response of the $[M+Ag]^+$ of catalyst and characteristic fragment ion with *m/z* 581 is presented. In (A) and (D), the catalyst complex specific efficiency in THF is shown.

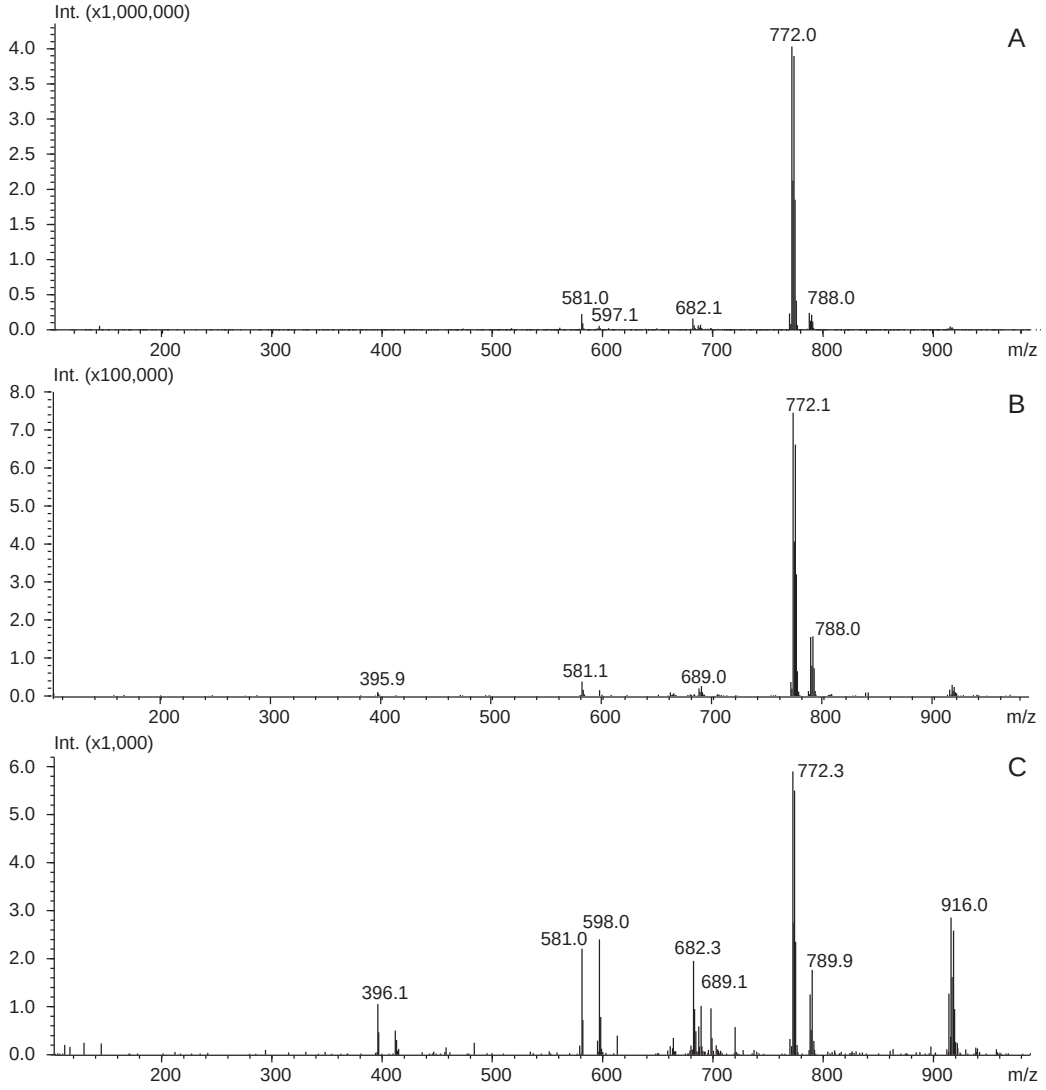

Fig. 4. Spectra of silver coordinated ferrocenylbidentate complex **5** in isopropylalcohol recorded with an ESI/APCI combined ionization source. (A) = ESI MS, (B) = APCI MS and, (C) = APCI MS with in-source CID.

ratio of $[M+Ag]^+$ and TIC (range of *m/z* 500–850) is almost constant for all solvents in both ESI MS and APCI MS (data not shown).

3.5. Alternative ionization strategies: dual ESI/APCI

In choosing the ionization conditions in the reaction detection system, one has to consider both the detection of the 2-imidazoline reaction product and the optimum characterization of the injected catalyst complex. In previous studies [3,7], it was found that best performance in detection of the reaction product is achieved with APCI MS rather than ESI MS. The response of the reaction product is also dependent on the solvent, with a 20-fold difference between the response in IPA (lowest) and DCM (highest) [3]. Most favorable solvents for product detection are DCM, ACN and EtOAc, although catalyst performance assessment is possible in all six solvents tested.

For optimum characterization of the catalyst complexes, we argued that one would like to detect both the intact [M+Ag]+ and the specific fragments **F1**, **F2** and preferably **F5**. This would require switching between ESI MS and APCI MS. In that case, ESI MS is used for the detection of the intact $[M+Ag]^+$ and APCI MS is used for the generation of the specific fragments **F1**, **F2**, **F5** and the sensitive detection of the formed product. In a simultaneous ESI/APCI source, the conditions of the continuous APCI ionization result in the undesired fragmentation of the intact $[M+Ag]^+$.

Dual ESI/APCI sources have become commercially available from most instrument manufacturers, especially to enhance the applicability range in screening of combinatorial libraries [18,19]. These dual sources either enable scan-wise switching between ESI MS and APCI MS or they perform ESI and APCI simultaneously in different regions within the same source housing. In the Dual ESI/APCI source available for our Shimadzu 2010 LC–MS system, ESI and APCI are performed simultaneously. Therefore, such a source is not useful to our needs. Interestingly, the Dual ESI/APCI source (DUIS) available for the more recent Shimadzu 2020 LC–MS system enables scan-wise switching between ESI and APCI modes. Therefore, some preliminary experiments were performed with the latter system in the characterization of the catalyst complexes.

Some results of the experiments with the DUIS are presented in Fig. 4. The catalyst complex **5** is injected into a 200 $\mu L\,min^{-1}$ flow of IPA and alternating ESI MS, APCI MS and APCI MS with in-source CID mass spectra were acquired. The ESI MS spectrum (Fig. 4A) closely resembles the spectrum acquired with a dedicated ESI MS source (Fig. 2A). The APCI MS spectrum (Fig. 4B), however, does not present the same extent of fragmentation as in the dedicated APCI

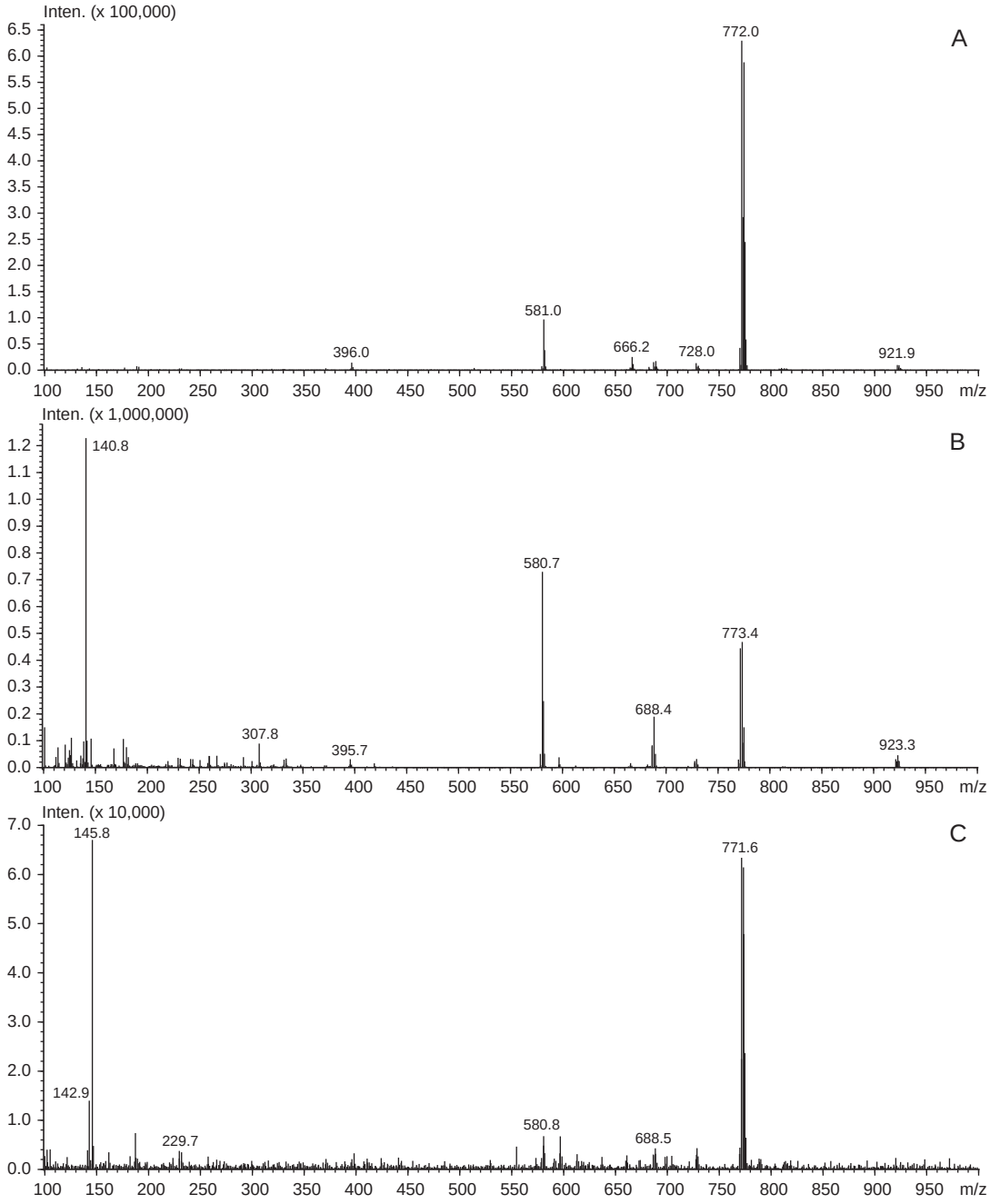

Fig. 5. ESI MS (A), APCI MS (B), and NDAPCI MS (C) spectra of silver coordinated ferrocenylbidentate complex **5** in IPA.

MS source. In the DUIS, the solvent is nebulized using pneumatically assisted electrospray, and not via the heated nebulizer of the dedicated APCI probe. These spectra thus indicate that (a part of the) fragmentation observed with the dedicated APCI source should be the result of thermally induced decomposition, either in the liquid phase during nebulization or in the gas phase after nebulization. Obviously, in-source CID with a higher voltage on the Q-array can be applied to induce fragmentation, as demonstrated for APCI MS in Fig. 4C. The relative abundance of the fragment ion with *m/z* 581 is still less than in the dedicated source, and the overall sensitivity is compromised. It must be added that, although in-source CID worked nicely in the LC–MS 2020 instrument, we were mostly not very successful with performing in-source CID in our LC–MS 2010 instrument. Finally, a careful inspection of the in-source CID spectra of all catalyst complexes reveals that next to the known fragments, a variety of other ions are observed, which may hamper straightforward identification of the injected catalyst.

3.6. Alternative ionization strategies: no-discharge APCI

An alternative solution-phase ionization approach is to utilize "no-discharge APCI" (ND-APCI). This method was developed by Cristoni et al. [20,21] and is essentially based on the nebulization of the solvent stream using the heated nebulizer applied in APCI. Solution-phase ions are transferred into the gas phase by thermal vaporization induced coulomb expansion [22]. As such, this technique closely resembles thermospray ionization [23,24], atmospheric pressure spray ionization (APSI) [25], and surface activated chemical ionization (SACI) [26]. Moreover, the same principle appears to be involved in atmospheric pressure photo ionization

(APPI) [27,28]. To our knowledge, ND-APCI has not yet been applied in the study of organometallic compounds.

As the silver-adducted ferrocene diphosphine complexes are present as preformed ion in solution, they should be readily amenable to ND-APCI. As ND-APCI uses the same hardware as dedicated APCI, scan-wise switching between the two modes would enable us to simultaneously, that is within one injection, detect the reaction product, the intact $[M+Ag]^+$ of the catalyst complex, and the structure-informative fragment ions.

In the spectra of the exemplary catalyst complex **5** presented in Fig. 5, two alternatingly acquired APCI/ND-APCI spectra are shown and compared to an ESI MS spectrum. As expected from the data in Fig. 3B, slightly more fragmentation is observed in ESI MS for **5** in IPA than in THF (Fig. 2A). In ESI MS and ND-APCI MS, similar spectra are obtained, but the response in ND-APCI MS is about tenfold lower than in ESI MS. These data indicate that the fragment ions observed in APCI MS spectra are primarily due to gas-phase fragmentation reactions, probably induced by the harsh condition of the corona discharge and the resulting reagent gas rather than liquid-phase thermally induced decomposition, as the same heated nebulizer is applied in the APCI and ND-APCI experiments. Similar observations were made in the comparison of APCI and ND-APCI in the analysis of peptides [20,21]. ND-APCI is applicable to ferrocene complexes in all solvents, except THF and DCM. By employing two acquisition events, it is possible to alternate between an ionization event that is optimized for the transfer of intact silver-adducted catalyst complexes (ND-APCI) and one for the optimum detection of the reaction product and providing enhanced identification potential for further characterization of active catalysts.

3.7. Sensitivities of the different ionization techniques

To investigate the ionization technique specific characteristics under real reaction detection conditions, the lower limit of detection (LLOD, S/N = 3), the linearity and the dynamic range for the reaction product was determined using triplicate injections of in-house synthesized product standards (100 pM to 10 μM) in a stream of reactants. The LLOD was 100 pM in APCI, 1 nM in ESI and 10 nM in ND-APCI. The linear dynamic range was between 10 and 100 nM for ND-APCI, between 1 nM and 100 nM for ESI, and between 0.1 nM and 1000 nM for APCI. These results indicate that APCI is the favored ionization technique to determine product formation. For simultaneous characterization of the injected catalyst complex, APCI is preferably combined with an alternative ionization approach, either APCI with in-source CID in a DUIS, or ND-APCI.

A quantitative comparison between the two approaches is presented in Fig. 6 (top bar chart) where the catalyst complex **5** is injected in IPA. As can be seen in Fig. 6A, APCI MS with the dedicated APCI probe provides the highest sensitivity for both $[M+Ag]^+$ and fragment **F2** with the highest intensity for fragment **F2**. The APCI spectrum obtained in the DUIS experiment predominantly resulted in the transfer of the intact $[M+Ag]^+$ accompanied by minor fragmentation. In ND-APCI, the intensity of both species is significantly lower. Finally, the in-source CID experiments in the DUIS source resulted in significantly lower intensity than in all other experiments.

As Fig. 6B (bottom bar chart) demonstrates, the lowest **F2**/$[M+Ag]^+$ ratio is achieved in the ND-APCI event and this ionization approach is thus best capable of transferring intact catalyst complexes with minor associated fragmentation. The highest **F2**/$[M+Ag]^+$ ratio is found in the dedicated APCI MS experiments, indicating the highest fragmentation yield. This indicates that APCI/ND-APCI is the best ionization strategy for the determination of these silver-adducted ferrocenyl catalyst complexes, although it cannot be operated in all solvents tested.

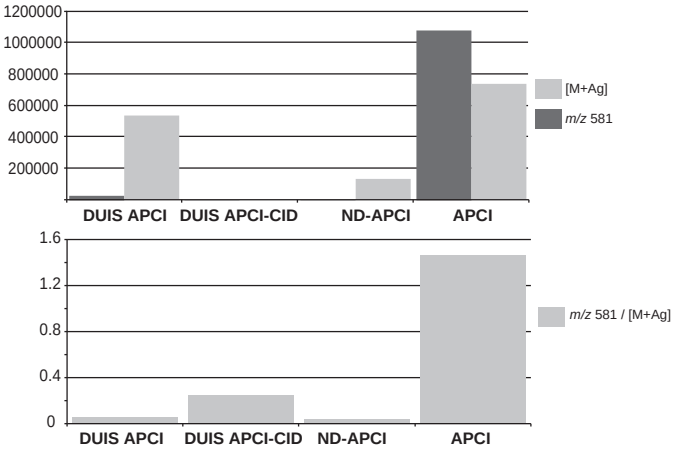

Fig. 6. Comparison of the different ionization strategies by the injection of catalyst complex **5** in IPA.

4. Conclusion

The ability to simultaneously assess catalyst performance and (provisionally) identify the introduced catalyst complexes was investigated for six different solvents with an MS-based continuous-flow reaction detection system equipped with different ionization sources. Although the obtained in-source fragmentation with APCI MS was significantly less profound than the fragmentation with dedicated (high-resolution) MS^n instruments, comparison of the fragmentation with the previously elucidated fragmentation patterns results in the ability to characterize the introduced catalyst complexes from APCI MS spectra acquired on a single-stage quadrupole MS system, especially if APCI is performed in combination with ND-APCI to determine the intact $[M+Ag]^+$. For final and more complete identification of highly active catalyst complexes, additional technology such as high-resolution MS^n will still be necessary.

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