Mass Spectrometry

DOI: 10.1002/anie.200804353

Characterization of Key Intermediates in a Complex Organocatalytic Cascade Reaction Using Mass Spectrometry**

Wolfgang Schrader, Peni Purwa Handayani, Jian Zhou, and Benjamin List**

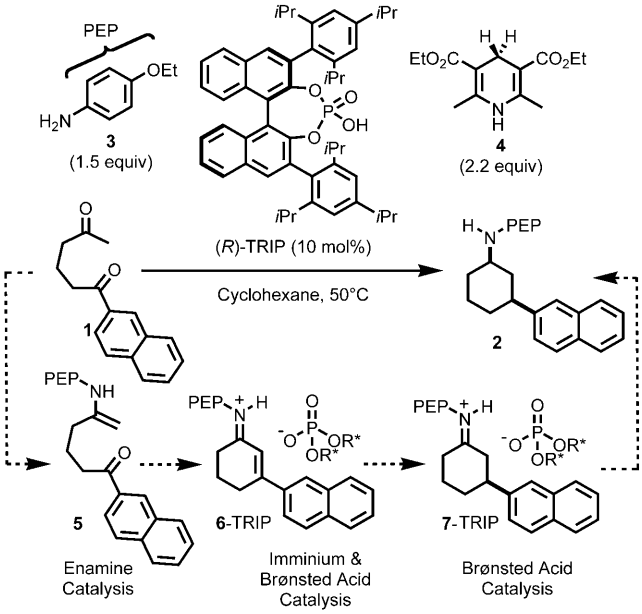
Dedicated to Professor Jan T. Andersson on the occasion of his 60th birthday

Modular combinations of organocatalytic reactions into cascades has attracted increasing attention in organic synthesis, as it enables the efficient and stereoselective construction of complex molecules from simple precursors, and greatly circumvents time, energy, and yield losses associated with traditional multistep syntheses.[1] Although the underlying mechanistic principles of individual steps seem to be well understood and even used in the design of new cascades, the mechanistic details of these complex reactions are largely unexplored. Isolation and characterization of reaction intermediates can be tedious, and widely used analytical techniques, such as NMR and IR spectroscopy, often fail in case of complex cascade reactions. Electrospray ionization mass spectrometry (ESI-MS) is an analytical technique that is rapidly and widely growing as an important tool in molecular analysis.[2] However, despite its success in elucidating reaction mechanisms, the implementation of ESI-MS for mechanistic studies of a complex organocascade reaction involving several compounds and intermediates has to the best of our knowledge not been reported. Herein we use ESI-MS analysis as a powerful method for the characterization of key intermediates in a quadruple organocatalytic cascade reaction, facilitating its detailed mechanistic understanding.

ESI-MS is a soft ionization method that allows the characterization of species that are actually present in solution. Additionally, ESI-MS/MS techniques enable selection and fragmentation of one specific ion using collision induced dissociation (CID) to gain structural information about important components. ESI-MS has been used in bioanalysis,[3] chemical reactions in solution[4] or in the gas phase,[5] and also for analyzing exotic problems, such as the formation of silicate oligomers.[6] ESI-MS and its tandem version ESI-MS/MS are particularly useful for the investigation of catalytic processes, that is, homogenously catalyzed reactions[7] and in the high-throughput screening of chiral catalysts.[8] We have previously used mass spectrometry and ESI-MS/MS techniques for the investigation of organocatalytic reaction mechanisms, such as the intramolecular Michael reaction of aldehydes[9] and a vinylogous Umpolung reaction.[10] We felt that ESI-MS may provide an ideal solution to the challenges posed by organocatalytic multistep cascade reactions as it allows for the individual characterization of all possible intermediates.

Concepts for designing new organocatalytic cascade reactions that involve enamine catalysis, iminium catalysis, and Brønsted acid catalysis have been proposed recently.[11] For example, a highly enantioselective synthesis of 3-substituted cyclohexylamines **2** from 2,6-diketones, such as **1** using a combination of catalytic quantities of the chiral Brønsted acid (*R*)-TRIP[12] with an achiral amine **3** and Hantzsch ester **4** has been developed.[13] This cascade reaction is believed to proceed via intermediates **5**, **6**, and **7,** and to involve an aldol condensation using enamine catalysis, a conjugate reduction using iminium catalysis and Brønsted acid catalysis, and a final reductive amination by Brønsted acid catalysis (Scheme 1).

We have now carefully investigated the reaction of diketone **1** to amine **2** using ESI-MS (for additional reactions, see Supporting Information), which enabled us to identify all critical intermediates.



Scheme 1. Quadruple organocatalytic cascade reaction for the synthesis of 3-substituted cyclohexylamines from 2,6-diketones via proposed intermediates. PEP = *p*-ethoxyphenyl.

[*] Priv.-Doz. Dr. W. Schrader, P. P. Handayani, Dr. J. Zhou, Prof. Dr. B. List
Max-Planck-Institut für Kohlenforschung
Kaiser Wilhelm-Platz 1, 45470 Mülheim an der Ruhr (Germany)
E-mail: wschrader@mpi-muelheim.mpg.de
list@mpi-muelheim.mpg.de

[**] We acknowledge generous funding from the Max-Planck-Society and the DFG SPP 1179 (Organocatalysis). We also thank H. W. Klein and W. Joppek for technical assistance.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200804353.

One of our questions concerned the mechanism of the initial aldolization: does this step proceed via an enamine aldol or via a Mannich-condensation? The aldol mechanism would involve intermediates **5** and **10**, both at *m*/*z* 360, whereas a Mannich mechanism would require two equivalents of the amine and would proceed via intermediates **11** and **12** (*m*/*z* 479). Under our standard conditions, the aldolization is rapid and full conversion of diketone **1** into imine **6** is observed within minutes.

Under these conditions the intermediates were hard to intercept, but by slightly modifying the reaction conditions, that is, not using molecular sieves (see Supporting Information), we were able to slow down this rapid conversion step such that intermediates could actually be observed. A typical spectrum obtained after five minutes is shown in Figures 1 and 2. The spectrum shows the signals for the starting materials, the reagents, the catalyst, and the cyclodehydration product **9** (*m*/*z* 223), and its imine **6** (*m*/*z* 342). Moreover, a characteristic signal at *m*/*z* 360 is observed. This signal is consistent with enamine intermediate **5**, its imine tautomer (not shown), or aldolization product **10**. Judging from the fragmentation pattern of the ion *m*/*z* 360 obtained from an ESI-MS/MS (positive mode) experiment, this intermediate corresponds mostly to enamine **5**, although additional minor signals point to the presence of **10**. Peaks at *m*/*z* 479, which would support a Mannich pathway via intermediates **11** and **12**, are entirely absent. This fact, along with our observation of aldolization intermediates[14] let us conclude that the initial aldol condensation does in fact proceed via an aldolization to β-hydroxy ketone intermediate **8** rather than a Mannich condensation (Scheme 2). For a better understanding we have collected the detailed fragmentation experiments from the reactions in the Supporting Information together with GC/MS data that support the findings.

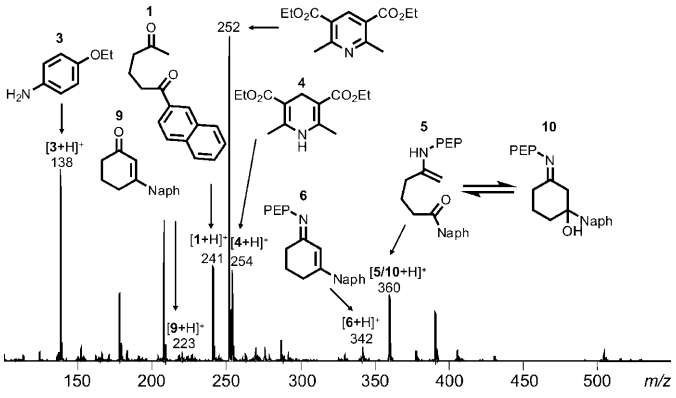


Figure 1. ESI-MS spectrum (positive mode) of the cascade reaction for the synthesis of 3-substituted cyclohexylamines, 5 min after the start of the reaction.

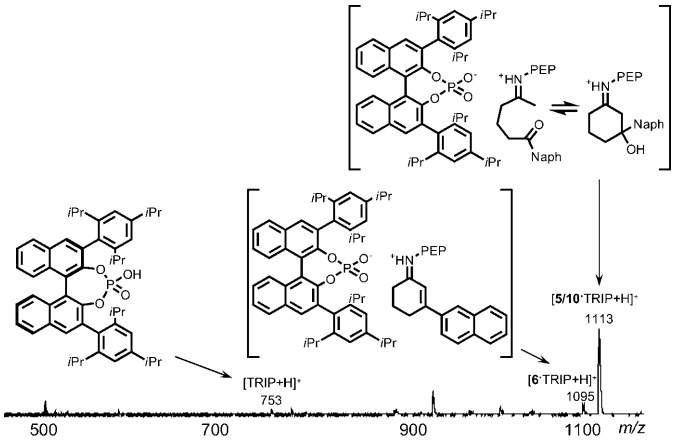
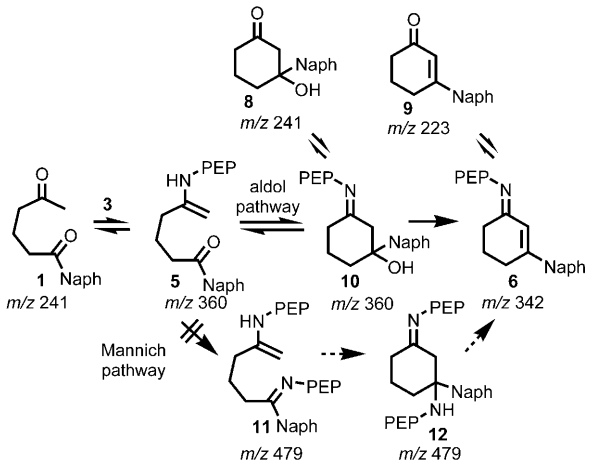
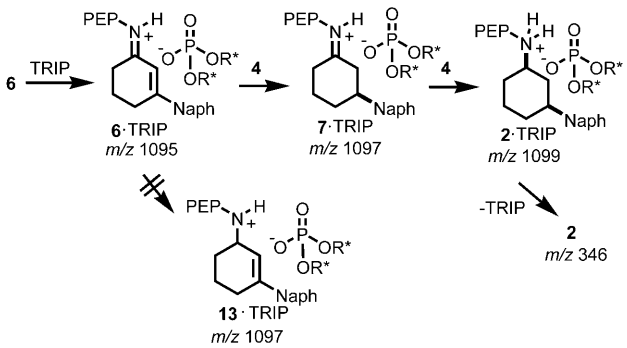


Figure 2. ESI-MS (positive mode) spectrum of the cascade reaction for the synthesis of 3-substituted cyclohexylamines, 5 min after the start of the reaction. Detection of protonated ion-pair intermediates are presented in the spectrum.



Scheme 2. Plausible and detected intermediates of the aldolization step; *m*/*z* ratios refer to protonated species. PEP = *p*-ethoxyphenyl, Naph = naphthyl.

The following reduction steps[15] in the cascade are proposed to involve ion-pair intermediates, such as **6**·TRIP and **7**·TRIP (Scheme 3). Rather to our delight and surprise, we not only detected all critical cationic iminium ions and ammonium ions (protonated **6** [*m*/*z* 342], **7** [*m*/*z* 344], **2** [*m*/*z* 346], **10** [*m*/*z* 360]), but also the correlating ion pairs. For example, in addition to free $[TRIP+H]^+$ (*m*/*z* 753), peaks at *m*/*z* 1113 and *m*/*z* 1095, which are consistent with iminium salts $[\mathbf{10}\cdot TRIP+H]^+$ and $[\mathbf{6}\cdot TRIP+H]^+$ are already seen



Scheme 3. Formation of ion pair intermediates; *m*/*z* ratios refer to protonated species. PEP = *p*-ethoxyphenyl, Naph = naphthyl.

after five minutes (Figure 2). Moreover, after 4 h the first hydrogenation product at m/z 344 $[\mathbf{7}+H]^+$ appeared and replaced its precursor $[\mathbf{6}+H]^+$ at m/z 342. The corresponding TRIP salt of this intermediate $[\mathbf{6}\cdot TRIP+H]^+$ was also detected at m/z 1097. After further conversion, the final product was also detected as a protonated ion pair $[\mathbf{2}\cdot TRIP+H]^+$ at m/z 1099. For a detailed evaluation of these presumed ion pairs,[16] a number of different experiments were conducted, with one example of $[\mathbf{7}\cdot TRIP+H]^+$ displayed in Figure 3. CID studies in both positive and negative mode were performed using different collision energies from 0 up to 30 eV (see Supporting Information).

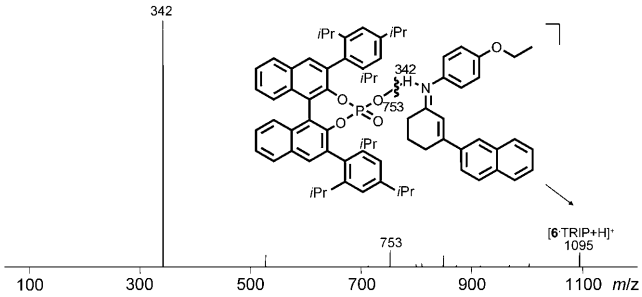


Figure 3. ESI-MS/MS (positive mode) spectrum of ion pair $[\mathbf{7}\cdot TRIP+H]^+$.

In the negative ESI mode, only the dissociated acid was found, as expected, whereas the ion pair complex could not be detected. In the positive ESI mode, even the relatively low collision energy of 10 eV leads to fragmentation into both $[TRIP+H]^+$ (m/z 753) and iminium ion $[\mathbf{7}+H]^+$ (m/z 344). The rather weak stability of these ions and similar complexes, and the detection of the ion pair of product **2**, led us to conclude that the higher mass peaks observed correspond to ion pairs rather than possible covalent adducts. These results are further confirmed using accurate mass data that have been obtained for all cationic intermediates, product, and ion pairs using Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS, Table 1).

The peak at m/z 344 is consistent with either 1,4-reduction product **7** or with the corresponding 1,2-reduction product **13** (Scheme 3). Interpretation of the corresponding positive-mode ESI-MS/MS (see Supporting Information) let us conclude that the reaction proceeds along a 1,4-reduction pathway. Allyl amine **13** should be unreactive towards further reduction; however, the peak at m/z 344 (as well as that at m/z 342) was clearly shown to correspond to an intermediate, which is converted into product **2**, by following the course of the reaction over time. The data are shown in Figure 4 with data points taken every five minutes during the first hour of the reaction and every two hours for the rest of the reaction time. Similar to the first intermediate **6**, which is formed and then disappears relatively fast, the second intermediate **7** ultimately disappears, during the formation of product **2**. Remarkably, similar results are obtained with the corresponding ion pairs $[\mathbf{6}\cdot TRIP+H]^+$ (m/z 1095), $[\mathbf{7}\cdot TRIP+H]^+$ (m/z 1097), and $[\mathbf{2}\cdot TRIP+H]^+$ (m/z 1099) (inset in Figure 4).

Table 1: High resolution mass data of detected intermediates.

Species	Mass (measured)	Mass (theoretical)	Formula	Error [ppm]
$[\mathbf{5}/\mathbf{10}+H]^+$	360.19637	360.19581	$C_{24}H_{26}NO_2$	1.6
$[\mathbf{6}+H]^+$	342.18478	342.18524	$C_{24}H_{24}NO$	1.3
$[\mathbf{7}+H]^+$	344.20083	344.20089	$C_{24}H_{26}NO$	0.2
$[\mathbf{2}+H]^+$	346.21654	346.21654	$C_{24}H_{28}NO$	0.0
$[\mathbf{6}\cdot TRIP+H]^+$	1094.58363	1094.58469	$C_{74}H_{81}NO_5P$	1.0
$[\mathbf{7}\cdot TRIP+H]^+$	1096.60141	1096.60034	$C_{74}H_{83}NO_5P$	1.0
$[\mathbf{2}\cdot TRIP+H]^+$	1098.61543	1098.61599	$C_{74}H_{85}NO_5P$	0.5

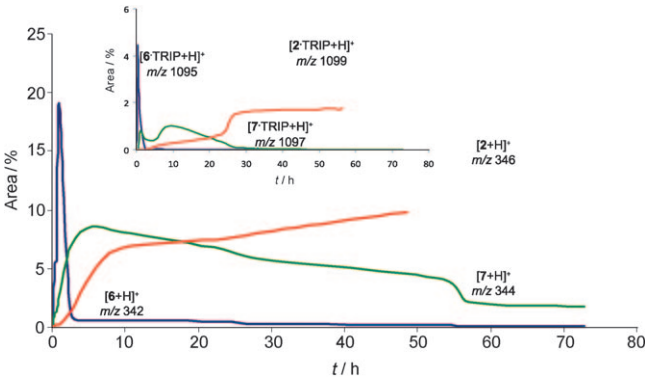


Figure 4. Formation of ions and ion pairs during 80 h of reaction time.

In conclusion, we have successfully intercepted and structurally characterized important intermediates of an organocatalytic cascade reaction. Fragmentation experiments as a function of time, and accurate mass data from FT-ICR MS experiments, allow insights into the mechanistic details of this complex reaction and confirm the previously proposed catalytic cycle. With our methods, chiral ion pair structures of similar reactions can be easily detected.[17] Further studies along these lines are under investigation in our laboratories.

Received: September 3, 2008
Published online: January 15, 2009

Keywords: cascade reactions · complexe reactions · electrospray mass spectrometry · iminium catalysis · organocatalysis

[1] a) K. C. Nicolaou, T. Montagnon, S. A. Snyder, *Chem. Commun.* **2003**, 551; b) J. C. Wasilke, S. J. Obrey, R. T. Baker, G. C. Bazan, *Chem. Rev.* **2005**, *105*, 1001.
[2] 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.
[3] a) W. Schrader, S. Döhring, W. Joppek, *Angew. Chem.* **2004**, *116*, 6826; *Angew. Chem. Int. Ed.* **2004**, *43*, 6657; b) W. Schrader, H. W. Klein, *Anal. Bioanal. Chem.* **2004**, *379*, 1013.
[4] a) J. Griep-Raming, S. Meyer, T. Bruhn, J. O. Metzger, *Angew. Chem.* **2002**, *114*, 2863; *Angew. Chem. Int. Ed.* **2002**, *41*, 2738; b) S. Meyer, R. Koch, J. O. Metzger, *Angew. Chem.* **2003**, *115*, 4848; *Angew. Chem. Int. Ed.* **2003**, *42*, 4700; c) S. Meyer, J. O. Metzger, *Anal. Bioanal. Chem.* **2003**, *377*, 1108; d) L. S. Santos,

L. Knaack, J. O. Metzger, *Int. J. Mass Spectrom.* **2005**, *246*, 155; e) L. S. Santos, *Eur. J. Org. Chem.* **2008**, 235.
[5] a) C. Hinderling, C. Adlhart, P. Chen, *Angew. Chem.* **1998**, *110*, 2831; *Angew. Chem. Int. Ed.* **1998**, *37*, 2685; b) D. M. Tomazela, L. A. B. Moraes, R. A. Pilli, M. N. Eberlin, *J. Org. Chem.* **2002**, *67*, 4652; c) D. Schröder, J. Roithova, H. Schwartz, *Int. J. Mass Spectrom.* **2006**, *254*, 197.
[6] a) S. A. Pelster, W. Schrader, F. Schüth, *J. Am. Chem. Soc.* **2006**, *128*, 4310; b) S. A. Pelster, F. Schüth, W. Schrader, *Anal. Chem.* **2007**, *79*, 6005.
[7] a) E. C. Meurer, L. S. Santos, R. A. Pilli, M. N. Eberlin, *Org. Lett.* **2003**, *5*, 1391; b) A. Sabino, A. H. L. Machaldo, C. R. D. Correia, M. N. Eberlin, *Angew. Chem.* **2004**, *116*, 2568; *Chem. Int. Ed.* **2004**, *43*, 2514; c) L. S. Santos, C. H. Pavam, W. P. Almeida, F. Coelho, M. N. Eberlin, *Angew. Chem.* **2004**, *116*, 4430; *Angew. Chem. Int. Ed.* **2004**, *43*, 4330; d) L. S. Santos, J. O. Metzger, *Angew. Chem.* **2006**, *118*, 991; *Angew. Chem. Int. Ed.* **2006**, *45*, 977; e) C. A. Marquez, F. Fabbretti, J. O. Metzger, *Angew. Chem.* **2007**, *119*, 7040; *Angew. Chem. Int. Ed.* **2007**, *46*, 6915; f) D. A. Yalalov, S. B. Tsogoeva, T. E. Shubina, I. M. Martynova, T. Clark, *Angew. Chem.* **2008**, *120*, 6726; *Angew. Chem. Int. Ed.* **2008**, *47*, 6624.
[8] a) P. Chen, *Angew. Chem.* **2003**, *115*, 2938; *Angew. Chem. Int. Ed.* **2003**, *42*, 2832; b) C. Markert, A. Pfalz, *Angew. Chem.* **2004**, *116*, 2552; *Angew. Chem. Int. Ed.* **2004**, *43*, 2498.
[9] M. T. Hechavarria Fonseca, B. List, *Angew. Chem.* **2004**, *116*, 4048; *Angew. Chem. Int. Ed.* **2004**, *43*, 3958.
[10] a) C. Burstein, F. Glorius, *Angew. Chem.* **2004**, *116*, 6331; *Angew. Chem. Int. Ed.* **2004**, *43*, 6205; b) W. Schrader, P. P. Handayani, C. Burstein, F. Glorius, *Chem. Commun.* **2007**, 716.
[11] a) J. W. Yang, M. T. H. Fonseca, B. List, *J. Am. Chem. Soc.* **2005**, *127*, 15036; b) Y. Huang, A. M. Walji, C. H. Larsen, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2005**, *127*, 15051; c) M. Marigo, T. Schulte, J. Franzen, K. A. Jørgensen, *J. Am. Chem. Soc.* **2005**, *127*, 15710; d) S. Brandau, E. Maerten, K. A. Jørgensen, *J. Am. Chem. Soc.* **2006**, *128*, 14986; e) B. List, *Chem. Commun.* **2006**, 819; f) C. Palomo, A. Mielgo, *Angew. Chem.* **2006**, *118*, 8042; *Angew. Chem. Int. Ed.* **2006**, *45*, 7876; g) A. Carlone, S. Cabrera, M. Marigo, K. A. Jørgensen, *Angew. Chem.* **2007**, *119*, 1119; *Angew. Chem. Int. Ed.* **2007**, *46*, 1101; h) D. Enders, C. Grondal, M. R. M. Huettl, *Angew. Chem.* **2007**, *119*, 1590; *Angew. Chem. Int. Ed.* **2007**, *46*, 1570.
[12] G. Adair, S. Mukherjee, B. List, *Aldrichimica Acta* **2008**, *41*, 31.
[13] J. Zhou, B. List, *J. Am. Chem. Soc.* **2007**, *129*, 7498.
[14] The results from GC supported this fact (for details, see the Supporting Information).
[15] For a review on asymmetric Brønsted acid catalysis, see: T. Akiyama, *Chem. Rev.* **2007**, *107*, 5744, and references therein.
[16] Also see: M. Sickert, C. Schneider, *Angew. Chem.* **2008**, *120*, 3687; *Angew. Chem. Int. Ed.* **2008**, *47*, 3631.
[17] P. P. Handayani, S. Hoffmann, W. Schrader, B. List, unpublished results.

