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Reaction Mechanisms

Functionality, Effectiveness, and Mechanistic Evaluation of a Multicatalyst-Promoted Reaction Sequence by Electrospray Ionization Mass Spectrometry

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Abstract: A multicatalytic three-step reaction consisting of epoxidation, hydrolysis, and enantioselective monoacylation of cyclohexene was studied by using mass spectrometry (MS). The reaction sequence was carried out in a one-pot reaction using a multicatalyst. All reaction steps were thoroughly analyzed by electrospray ionization (ESI) MS (and MS/MS), as well as high-resolution MS for structure elucidation. These studies allow us to shed light on the individual mode of action of each catalytic moiety. Thus, we find that under the epoxidation conditions, the catalytically active *N*-methyl imidazole for the terminal acylation step is partially deactivated through oxidation. This observation helps to explain the lower efficiency of the catalyst in the last step compared to the monoacylation performed separately. All reactive intermediates and products of the reaction sequence, as well as of the side-reactions, were monitored, and we present a working mechanism of the reaction.

Introduction

Various synthetic methods have been developed utilizing small organic molecules as organocatalysts.[1] One especially intriguing development has been the use of cascade or tandem reactions,[2] in which several reactions are subsequently carried out in one pot. These types of reactions promise higher efficiency while minimizing resource and energy requirements. Typical cascade or tandem reactions combine the reactants from the beginning,[2] often giving rise to a broad variety of side-reactions. An alternative concept for cascade reactions is a catalyst bearing multiple catalytic moieties, separated by a spacer molecule, a so-called multicatalyst.[2l,3] This methodology is reminiscent of an assembly line, in which a complex molecule is consecutively assembled from simple starting materials in a manner that at the correct time each catalytic moiety is selectively activated.[3]

The elucidation of reaction mechanisms of such complex reactions is rather challenging, because many intermediates are short-lived and occur often only in minor quantities. Indeed, one advantage of such reactions is that isolation of short-lived intermediates can be neglected, because they are generated in situ and consumed rapidly. Methods allowing the characterization of structural changes typically are NMR[4] and, to a minor extend, IR spectroscopy.[4e,5] A powerful tool for the investigation of complex organocatalytic reactions is mass spectrometry (MS) due to its advantages to detect components at low concentrations within short lifetimes. Even structural data can be derived with MS/MS experiments.[6] The fragmentation of individual intermediates can be utilized for the characterization of catalytic reactions.[7]

Due to the novelty of the multicatalyst approach, no mechanistic studies have been carried out yet. Herein, we report a detailed mechanistic investigation concerning the catalytic epoxidation of olefins followed by hydrolysis and enantioselective kinetic resolution through acylation.[8] Each individual step can be defined by reaction intermediates as reaction markers that can be detected by ESI-MS. The multicatalyst **A0B0** used in this study consists of a chiral peptide backbone[9] including an adamantane spacer[3,10] separating two catalytic moieties. Dicarboxylic acid **A0** is responsible for the first reaction step, an epoxidation,[11] and *N*-methyl imidazole moiety **B0** catalyzes the terminal acylation.[3b] The individual catalytic moieties are activated by adding the corresponding reagent after a particular time interval, and this is the key difference to cascade reactions. Various catalysts have been developed, in which the catalytically active moieties were placed in different positions of the peptide backbone; herein, we employed the most effective catalyst **A0B0** (Scheme 1).[3b]

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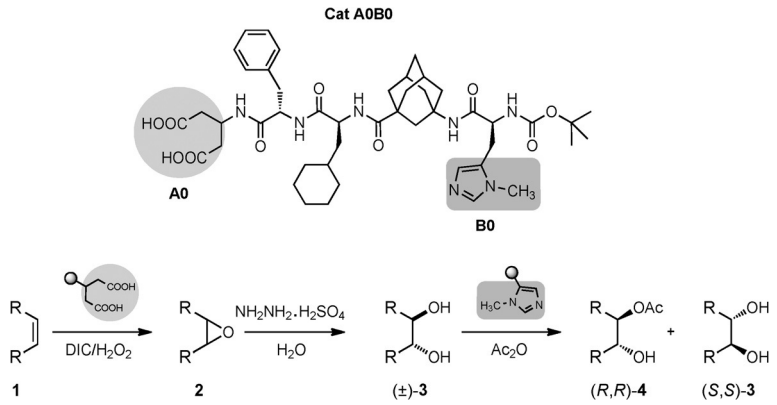


Scheme 1. Reaction sequence using multicatalyst **A0B0**.

Results and Discussion

The first step in the mechanistic evaluation of a catalytic reaction with ESI-MS is the determination and characterization of reaction markers that allow an unequivocal assignment of the reaction intermediates. The first step of the reaction under consideration is the epoxidation of cyclohexene (**1**) to cyclohexene oxide (**2**). Although the reaction itself is straightforward, the identification of catalytically active moieties is more challenging. For the activation of catalytic moiety **A0**, diisopropylcarbodiimid (DIC) was added forming the *O*-acylisourea (**A1**), which, after cleavage, removes a molecule of water providing the corresponding anhydride **A2**. This further reacts with hydrogen peroxide to give the catalytically active peracid moiety **A3**. After formation of peracid **A3**, the catalyst reacts with **1** to give **2** (Scheme 2). The ESI MS/MS spectra of **2** and the appearing catalytic species are shown in Figures 1 and 2, respectively. One potential side reaction of *O*-acylisourea **A1** attached to the peptide is the formation of *N*-acylurea **A1′**, which was reported by Montalbetti and co-workers.[12] We also observed this rearrangement, but the molecular ion is present in such low intensity that it is under standard conditions almost not detectable, implying that it is relatively unimportant for the catalytic step (Scheme 2; Figure 2). Due to the short lifetime of those and other intermediates, it was not possible to determine

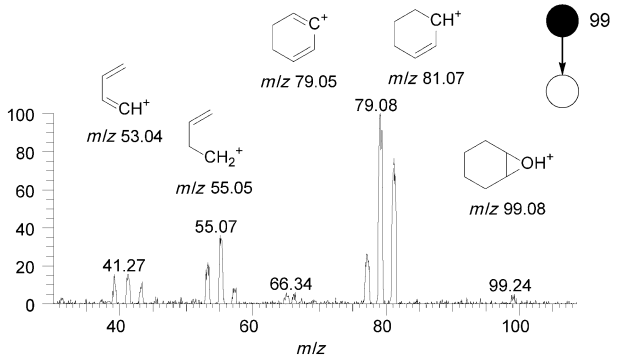
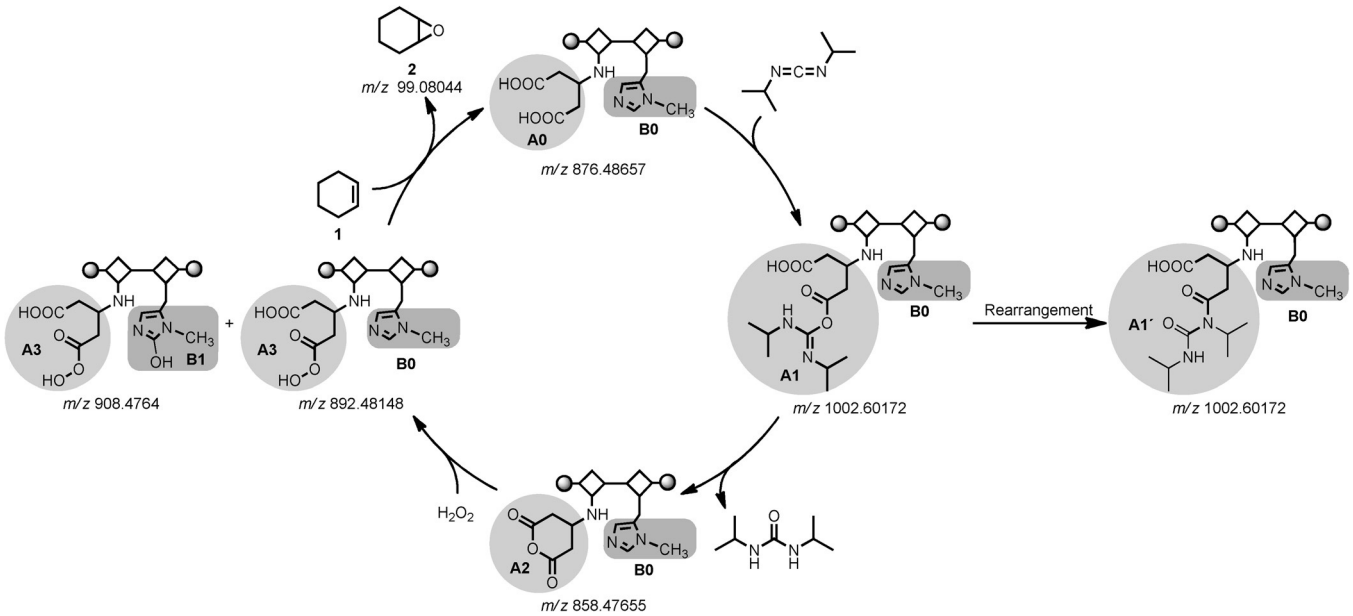


Figure 1. ESI MS/MS spectrum of epoxide intermediate **2** obtained with a triple quadrupole MS.

them from the reaction mixture alone. Therefore, some additional studies were carried out using an online microflow reactor as shown in Figure 6 in the Experimental Section. In these experiments, two different syringes were filled with different



Scheme 2. Reaction cycle for the epoxidation reaction.

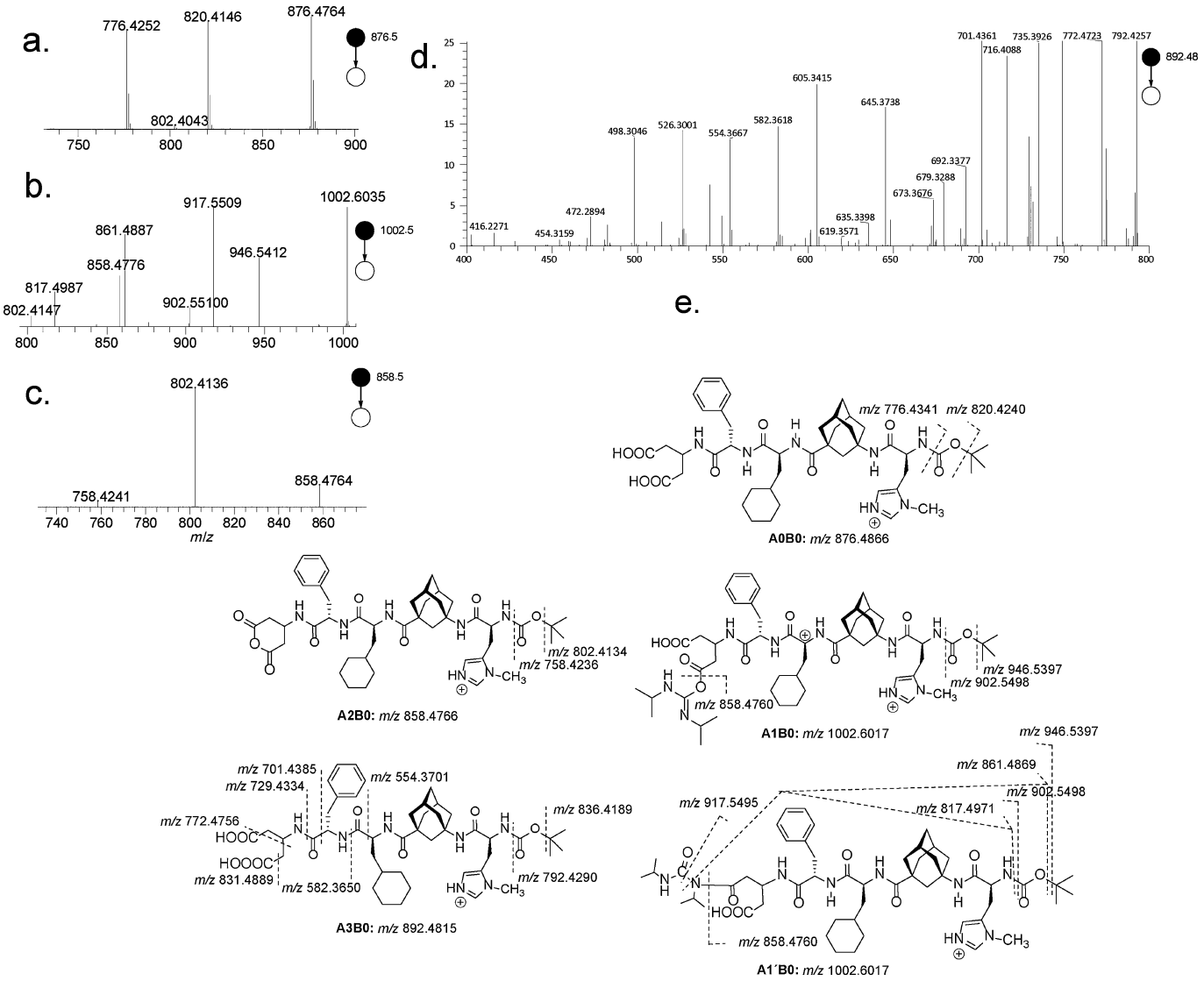


Figure 2. ESI MS/MS spectra of a) **A0B0**; b) **A1B0** and **A1′B0**; c) **A2B0**; and d) **A3B0**; and the associated fragmentation. The spectrum of intermediate **A1B0** was obtained by using a microflow reactor set-up by high-resolution MS.

reaction solutions. The reaction takes place after the two flows are combined in a mixing T-piece. The reaction time depends on the length of the capillary after the mixing T-piece and can be adjusted to the time frame of interest. This allows studying very reactive and short-lived intermediates. Due to the continuous formation of the same components, it is possible to study them in detail by MS and MS/MS methods.

Catalyst **A0B0** converts 57% (±**3**) into the final product **4** in the separately performed acylation reaction (2 mol% catalyst loading, 3 h). However, only 35% of the monoacetylated product **4** were obtained when the reaction was performed under multicatalysis conditions, even at 5 mol% catalyst loading and after longer reaction time (17 h), indicating a somewhat lower activity of the multicatalyst.[3b] When studying the formation of the moiety **A3**, we found that another reaction occurs that could have a direct impact on the effectiveness of the methyl imidazole group **B0** that is responsible for the subsequent catalytic acylation. During formation of peracid **A3**, an additional signal was found at *m/z* 908 (Figure 3a and b); the difference of 16 Da indicates oxidation of the catalyst. The position of the oxygenation was studied thoroughly with highly accurate MS/MS and MS^n experiments, in which the molecule was fragmented and the structural differences were analyzed (Figure 3c, d). We determined the oxidation to occur at the imidazole group: activation of moiety **A** also leads to oxidation of moiety **B**, resulting in the formation of a hydantoin derivative **B1** (Scheme 2 and Figure 3). The oxidation of the imidazole moiety leads to reduced effectiveness of the catalyst due to the resulting lower amount of active catalytic moiety utilizing the multicatalyst.

For the epoxide opening forming diol hydrazine bisulfate and water have to be added to the reaction mixture.[7b] The hydrolysis of **2** was not investigated mechanistically, because the peptide catalyst is not involved in this step.

The second catalytic step investigated in this reaction sequence is the kinetic resolution of racemic diol. At the begin-

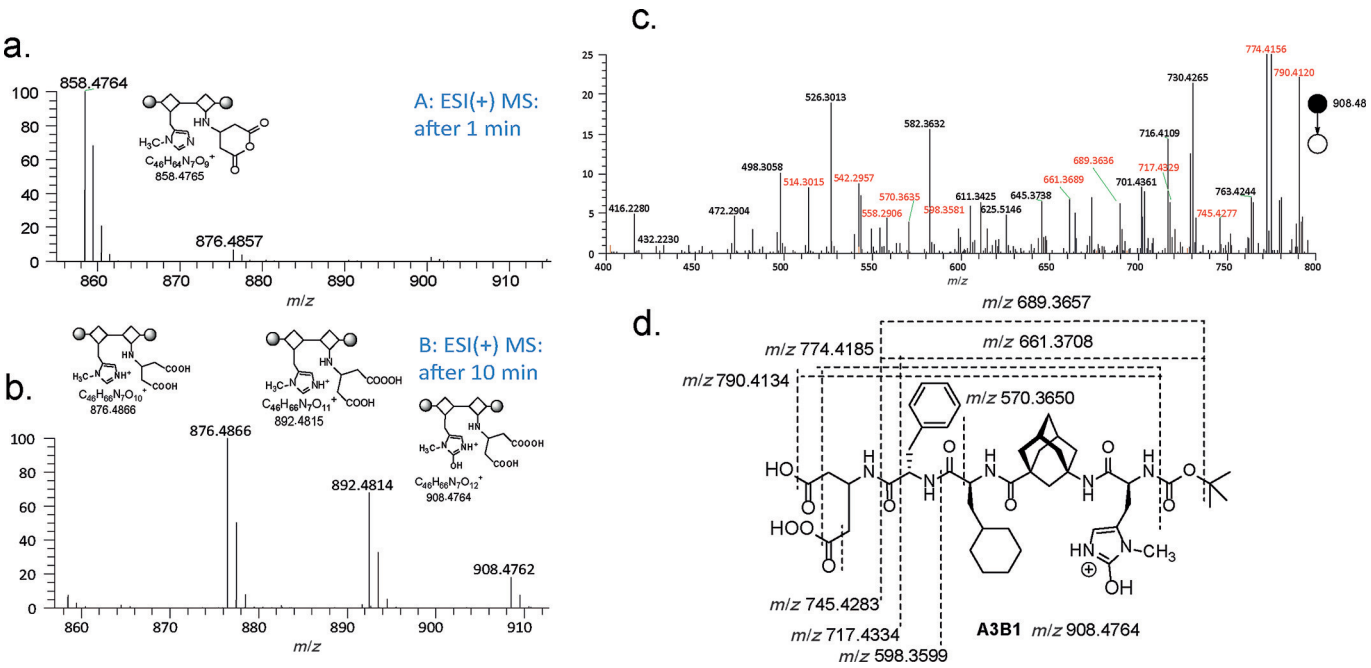


Figure 3. Characterization of completely oxidized catalyst **A3B1**: a) and b) MS spectra of the activation of catalytic moiety **A** [a) after adding DIC; b) after adding DIC and H_2O_2]; c) and d) ESI MS/MS spectra and the associated fragmentation[(1)] of **A3B1**.[(1)] Red numbers indicate the fragments of the oxidized imidazole functionality. Measuring was achieved by HRMS.

ning, both catalytic sites of multicatalyst **A0B0** are inactive. After completion of the preceding reactions, the activation of catalytic moiety **B0** is accomplished by adding acetic anhydride to the reaction mixture providing acylium ion **B2** (Figure 4).

After activation of the catalyst, monoacylation of diol takes place. The corresponding MS/MS spectrum is depicted in Figure 4.

Our results also indicate that during the last step of the reaction, not only moiety **B** was activated by acetic anhydride, but also moiety **A** was directly converted to the intramolecular anhydride **A2** (Figure 4). In a subsequent step, **A2B2** was oxidized to **A3B2** by hydrogen peroxide, which is still present due to the one-pot conditions. Figure 5 shows the MS, the MS/MS, as well as the MS^3 spectra of the detected catalytic species. The observed side-reactions may also have a direct influence on the enantioselectivity due to potential conformational changes in the peptide backbone. Indeed, the multicatalyst provides somewhat lower selectivities compared to the originally developed acylation catalyst.[3b,10a]

Conclusion

A triple-cascade sequence consisting of an epoxidation, an epoxide opening, and an enantioselective acylation reaction catalyzed by an oligopeptide multicatalyst was studied thoroughly by using ESI-MS. The key reaction intermediates were successfully characterized. In addition to the activation of the catalytic moieties **A0** to **A3** in the first reaction step by reagents DIC and H_2O_2, a side-reaction takes place on the catalytic moiety for the second reaction step **B0** into **B1** leading to a partially oxidized methyl imidazole moiety that causes reduction of the

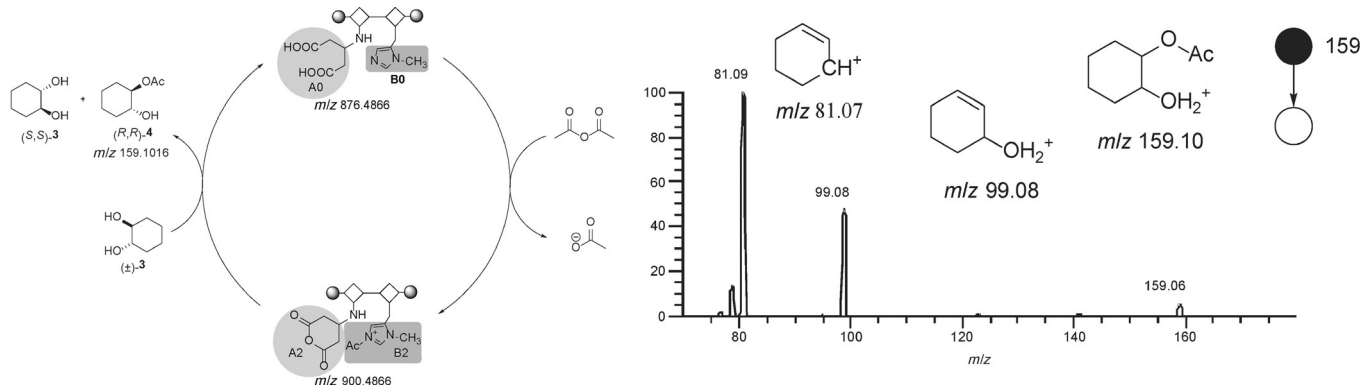


Figure 4. Activation of the catalytic moiety **B0** and MS/MS spectrum of the monoacetylated product by triple quadrupole MS/MS.

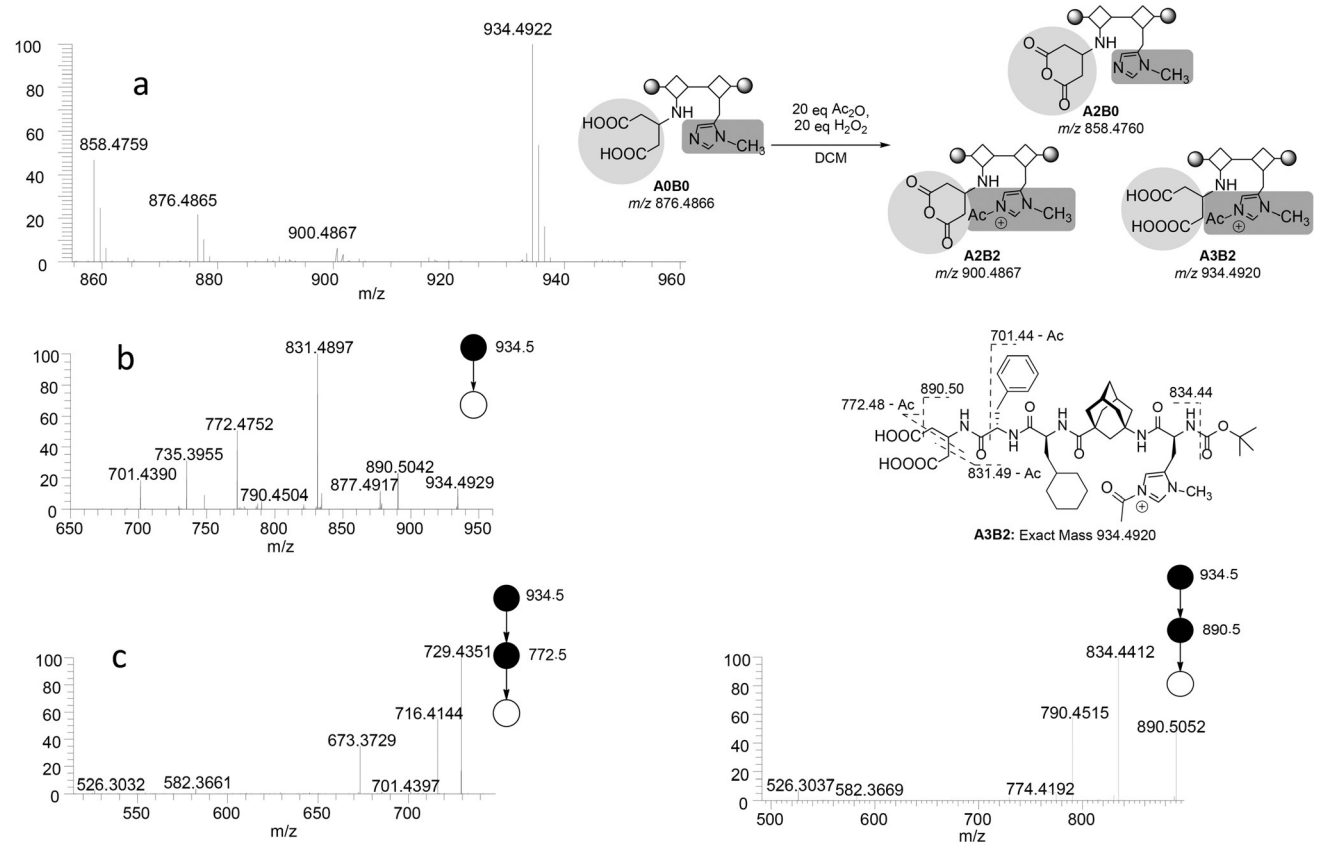


Figure 5. MS^n spectra of the catalytic species of the last reaction step by HRMS: a) MS spectrum of the activation sequence using acetic anhydride and hydrogen peroxide; b) MS/MS spectrum of catalyst **A3B2** after activation with acetic anhydride; c) MS^3 spectra of catalyst **A3B2** detailing the structural characterization of both catalytic moieties.

activity of catalytic moiety **B0** for the acetylation in the terminal step.

Our detailed mass spectrometric study of the present multicatalyst allows the individual characterization of both catalytic reactions. This is a prime example how detailed mechanistic studies using highly accurate MS and MS^n data can help in understanding complex organocatalytic reactions.

Experimental Section

Reaction procedure

Catalyst (0.05 mmol, 21.9 mg, 5 mol%), cyclohexene (1 mmol, 101 μL, 1 equiv), and DIC (1.2 mmol, 185 μL, 1.2 equiv) were dissolved in dichloromethane (DCM; 2 mL). To this mixture, hydrogen peroxide (30%; 130 μL, 1.2 equiv) was added, and the resulting reaction mixture was stirred at room temperature for 24 h. After this time, the addition of DIC (1.2 mmol, 185 μL, 1.2 equiv) and 30% hydrogen peroxide (130 μL, 1.2 equiv) was repeated, and the reaction mixture was stirred under the same conditions for additional 24 h. Then toluene (6 mL) was added, followed by the addition of H_2O (10 mmol, 180 μL, 10 equiv) and hydrazine bisulfate (0.1 mmol, 13 mg, 0.1 equiv), and the mixture was stirred at room temperature for 18 h. In the next step, toluene (180 mL) and iPr_2EtN (5.3 mmol, 901 μL, 5.3 equiv) were added, and the reaction mixture was cooled to 0 °C. Finally, Ac_2O (5.3 mmol, 501 μL, 5.3 equiv) was added, and the kinetic resolution was monitored by chiral GC. After 17 h, the reaction mixture was quenched by adding methanol (10 mL), the solvents were evaporated under reduced pressure, and the column chromatography on silica gel in hexane/EtOAc (1:1) gave 56 mg (35%) of 1-acetoxy-2-cyclohexane alcohol (60% yield).

Microreactor procedure

One example that could only be investigated by the microreactor experiment is the intermediate **A2B0**. For this experiment, syringe I was filled with catalyst (0.005 mmol, 4.5 mg) in DCM (1 mL), syringe II with reagent diisopropyl carbodiimide (DIC; 0.12 mmol, 15 mg, 18.7 μL) in DCM (1 mL; Figure 6). The reaction took place in polyether ether ketone (PEEK) capillary after combining reactants from syringes I and II in the mixing chamber. Syringe pumps for I and II were adjusted to flow rates of 5 $\mu L\,min^{-1}$ and connected directly to the ion source. It was not just possible to determine the intermediate itself, but also the rearrangement product of this intermediate as fragments at *m/z* 917.54, 861.48, and 817.49, respectively, using tandem MS.

Mass spectrometry

MS and MS/MS experiments were carried out using a Thermo TSQ Quantum Ultra AM triple quadrupole mass spectrometer (Thermo Scientific, Dreieich, Germany) equipped with an ESI source, which was controlled by Xcalibur software. The ESI spray voltages were

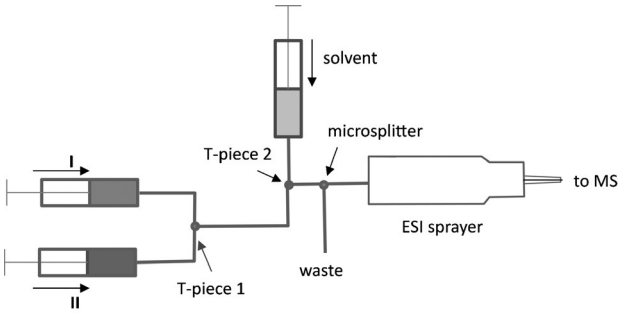


Figure 6. Experimental set-up of the microflow reactor.

set to 4000 and 3000 V for positive and negative ions, respectively. The heated capillary temperature was adjusted to 270 °C. For MS/MS analysis, the collision energy was increased from 10 to 50 eV. The mass spectrometer was operated in the Q1 scan and product ion scan modes, with the mass width for Q1 set at 0.5 Da and for Q3 set at 0.7 Da. The collision cell, Q2, contained argon and was adjusted to a pressure of 1.5 mTorr to induce collision-induced dissociation (CID). Spectra were collected by averaging ten scans with a scan time of 1 s. The mass range was adjusted between 50 and 1500 Da. HRMS data were acquired by using an LTQ-Orbitrap Elite mass spectrometer (Thermo Scientific, Bremen, Germany). All experimental parameters were the same as for the triple quadrupole experiments, except that MS/MS measurements were carried out with an isolation window of 1 Da and different collision energies.

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Keywords: electrospray ionization · mass spectrometry · organocatalysis · reaction mechanisms · structure elucidation

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