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Combination of Olefin Metathesis and Enzymatic Ester Hydrolysis in Aqueous Media in a One-Pot Synthesis

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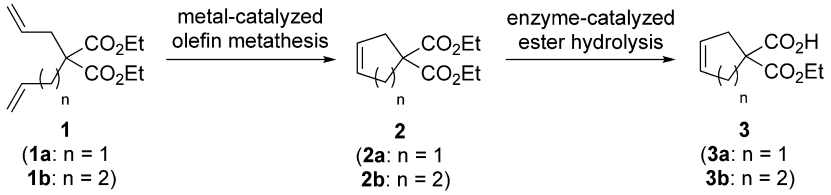
Abstract: A new synthetic method for the preparation of cyclic malonic acid monoesters in aqueous media was developed based on the combination of a metathesis reaction and subsequent biocatalytic hydrolysis with a pig liver esterase in a one-pot synthesis. Both reaction steps proceed smoothly under optimized conditions in aqueous media requiring only a low amount of the metal catalyst for the metathesis reaction. Notably, the applied biocatalyst turned out to be highly compatible with the metal catalyst showing no significant influence on the enzyme activity.

Keywords: aqueous media; biocatalysis; esters; olefin metathesis; one-pot synthesis

The metathesis reaction belongs to the most efficient and atom-economical metal-catalyzed syntheses and has already found important applications on the industrial scale.[1] Currently, intensive research efforts concentrate on metathesis reactions in water, which serves as a cheap, environmental friendly, easy to handle and industrially attractive solvent.[2] For this purpose tailor-made water-soluble Grubbs catalysts turned out to be suitable.[3] In addition, polymer- or membrane-bound,[4,5] PEGylated ligands[6] or micellar systems can be used.[7] Recently it was found that metathesis in water also proceeds with high conversion when using commercially available Grubbs or Hoveyda-Grubbs catalysts under heterogeneous conditions, albeit a high catalyst loading of 4 mol% is needed.[8–10]

A further research area of current interest is the development of multi-step one-pot processes as they are time and cost efficient since less work-up and purification steps and therefore less solvents are needed.[11] In this field the combination of man-made metal catalysts and enzymes is a particular challenge as such reactions typically require different reaction media. Metal-catalyzed reactions often take place in organic solvents whereas enzymes often need aqueous media (except, e.g., lipases). So far there are only a few examples for such one-pot syntheses.[12] Therefore the development of efficient chemocatalytic reactions in water, which are compatible with a biotransformation, is desirable.

In continuation with our studies on metathesis reactions in water[10] and chemoenzymatic one-pot synthesis in aqueous media,[12d,e,13] we became interested in combining metal-catalyzed olefin metathesis with a subsequent biotransformation in aqueous media. In the following we present such a one-pot synthesis as a new synthetic concept for the synthesis of cyclic malonic acid monoesters **3** (Scheme 1). This model reaction was chosen as the products **3** serve as possible in-



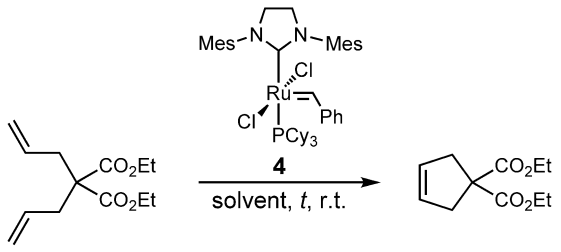
Scheme 1. General synthetic concept: metathesis and subsequent enzymatic ester hydrolysis.

termediates in the synthesis of non-natural amino acids bearing a quaternary carbon center.[14]

The combination of metal- and enzyme-catalyzed transformations in a one-pot synthesis as shown in Scheme 1 requires compatibility of the metathesis reaction as the first step or the resulting reaction mixture thereof with the subsequent biotransformation in aqueous medium. Accordingly we first focused on developing an olefin metathesis which is highly efficient in aqueous media (instead of using typical organic solvents), proceeding with very low amount of catalyst (<1 mol%).

Therefore diallyl malonate **1a** was chosen as a model substrate. Furthermore, we were interested in a comparison of the efficiency of the metathesis reaction in water with an organic solvent like dichloromethane (Table 1). First, we studied the efficiency of this reaction in D_2O on an analytical scale (1 mL of solvent, no work-up) according to a recently published protocol of Varma et al.,[15] which enabled a simple analysis of the reaction mixture by ^{1}H NMR spectroscopy (deuterated solvent: MeOD-d_4, Table 1, entries 1–3). We were pleased to find that even a very low catalyst loading of 0.2 mol% of Grubbs II catalyst, **4**, led to the formation of the desired product **2a** with quantitative conversion after 22 h (entry 3).

Table 1. Optimization of the metathesis reaction.[a]



1a **2a**

Entry	Solvent	**4** [mol%]	c(**1a**) [M]	t [h]	Conversion [%]
1	D_2O	5.0	0.150	4	100
2	D_2O	0.5	0.300	6	100
3	D_2O	0.2	0.300	22	100
4	H_2O	0.5	0.200	18	100
5	H_2O	0.5	0.033	6	98 (100)[b]
6	CH_2Cl_2	0.5	0.500	6	100
7	CH_2Cl_2	0.5	0.300	6	99
8	CH_2Cl_2	0.5	0.033	6	93

[a] Entries 1–3: these experiments were carried out on an analytical scale and the conversion was determined from the reaction mixture by ^{1}H NMR spectroscopy in MeOD-d_4. Entries 4 and 5: these experiments were carried out on a preparative scale and after extraction of the reaction mixture with CH_2Cl_2 the conversion was determined by ^{1}H NMR spectroscopy in $CDCl_3$. Entries 6–8: these experiments were carried out on a preparative scale and the conversion was determined by ^{1}H NMR spectroscopy in $CDCl_3$.

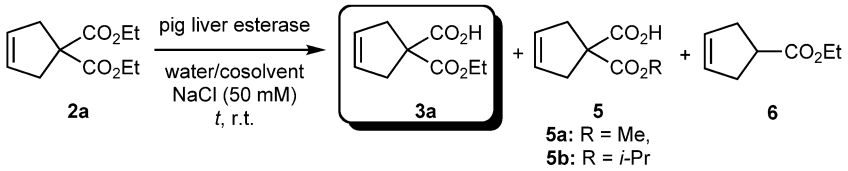
[b] In parenthesis the result of a repeat test is shown.

In general, excellent conversions were obtained also on preparative scale in water (5.0–7.5 mL H_2O, work-up with dichloromethane) independent of the substrate concentration, which was in the range of 33 mM to 200 mM (Table 1, entries 4 and 5). With respect to the mixture of the reactants in water we observed that the substrate, which is insoluble in water, forms droplets on water, in which the metal catalyst is dissolved. Thus, it appears to be more likely that such metathesis reactions proceed "on" water and not "in" water. Although the reaction also ran in dichloromethane as a solvent, at low substrate concentrations and low catalyst loading the conversion in water is quantitative after six hours (entry 5) whereas the same reaction in dichloromethane only reaches 93% conversion (entry 8). Thus, interestingly, water turned out to be the preferred solvent for the metathesis reaction, enabling a highly efficient synthesis of **2a** with excellent conversion at low catalyst loading of 0.2–0.5 mol% of **4**. In addition, the use of water as a solvent also fulfils a requirement for the desired one-pot synthesis.

After successfully optimizing the metathesis reaction in water we next focused on the development of an efficient enzymatic hydrolysis of **2a** under selective formation of monoester **3a**. As biocatalyst we chose pig liver esterase (PLE) due to its broad applicability and use for hydrolyses of a wide range of non-cyclic, disubstituted dialkyl malonates.[16,17] This type of biotransformation (shown in Table 2) gives access to the cyclic mono acid ester **3a** bearing a quaternary carbon center. To monitor and control the reaction and formation of product **3a**, the pH is kept at 7 by titration with an NaOH solution. When using pig liver esterase in pure water as a solvent the hydrolysis of **2a** proceeded with a conversion of 92%. However, a product mixture was isolated consisting of a 74:26 ratio of desired monoester **3a** and a significant amount of decarboxylated side product **6** (Table 2, entry 1). In order to avoid decarboxylation as a side reaction we systematically explored the influence of water-soluble organic solvents on the reaction course.[18] High yields in the range of 91–92% as well as suppression of decarboxylation were observed in all experiments (entries 2–5).

However, when using the water-soluble alcohols methanol and isopropanol transesterification was observed leading to significant formation of the undesired side products **5a** and **5b**, respectively (Table 2, entries 2 and 3). This effect might be due to the existence of lipases in the enzyme preparation of PLE. By engineering of the reaction medium and using an advantageous mixture of water and *tert*-butyl alcohol [3:1 (v/v)] we were pleased to find that both decarboxylation and transesterification were avoided and

Table 2. Development of an efficient biotransformation.[a]



Entry	Cosolvent [% (v/v)]	*t* [h]	Conversion [%][b]	**3a** [%][c]	**5** [%][c]	**6** [%][c]
1	–	25	92	74	0	26
2	MeOH (10)	12	91	13	84 (**5a**)	3
3	*i*-PrOH (10)	42	91	61	34 (**5b**)	5
4	*t*-BuOH (10)	23	92	97	0	3
5	*t*-BuOH (25)	50	92	100	0	0

[a] All reactions were carried out in a total volume of 10 mL with 0.25 mmol of substrate **2a** and 270 units of pig liver esterase.
[b] The conversion was determined *via* a Titrino apparatus by titration with 0.2 M NaOH solution and adjusting the pH to 7.
[c] The ratios of products **3a**, **5** and **6** (given in %) were determined by ^{1}H NMR spectroscopy from the crude product after work-up of the reaction mixture.

an excellent reaction yield of the product **3a** was obtained (92% conversion, 100% selectivity; entry 5).

In the next step we studied the (bio-)compatibility of the Grubbs II metathesis catalyst **4** with PLE used as a biocatalyst. Such types of investigations on the combination of chemocatalysts and biocatalysts towards one-pot, multi-step procedures in water are in general still rare up to now[12,13] but, at the same time, essential for the development of such a process. We investigated the reaction course of the enzymatic hydrolysis of **2a** under optimized conditions in the absence and presence of metal catalyst **4** (Figure 1). When adding the Grubbs catalyst **4**, the amount of **4** was in the same range (0.1 or 0.5 mol%) as in the metathesis reactions described in Table 1.

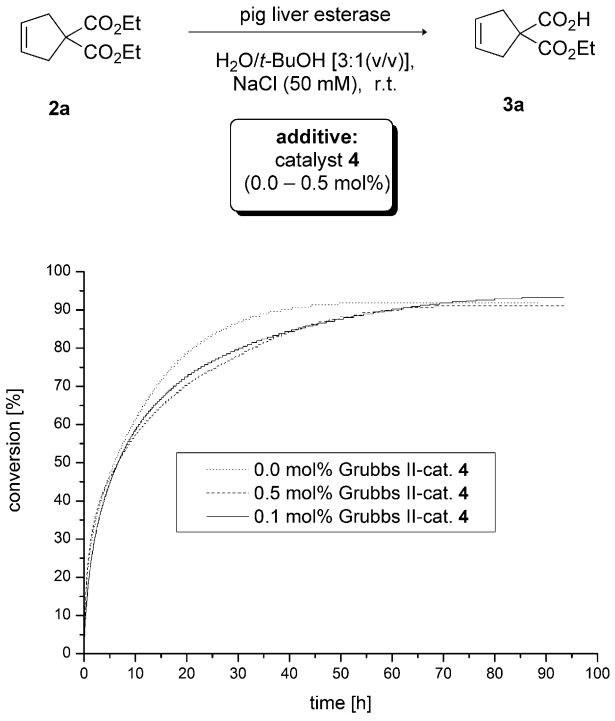
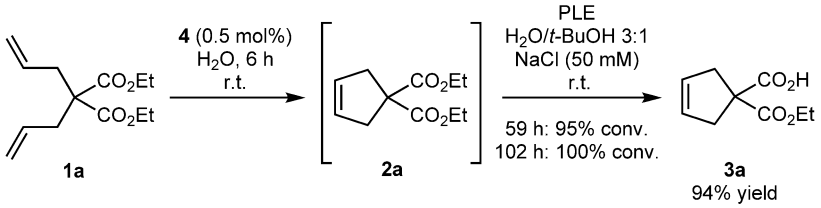


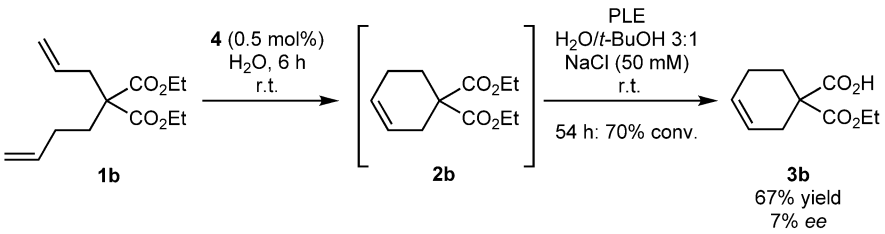
Figure 1. Compatibilty of catalyst **4** and enzyme PLE.

Interestingly, nearly the same reaction course was determined in the absence and presence of 0.1 mol% and 0.5 mol% of Grubbs II catalyst **4**, respectively, leading to high conversions and similar reaction rates in all cases. In the presence of 0.1 mol% of **4** the biotransformation proceeded with a conversion of 91%, and even at a higher loading of 0.5 mol% of metal catalyst **4** a high conversion of 91% was obtained although a slightly prolonged reaction time was required. Thus, the Grubbs II catalyst **4** seems to have a negligible influence on enzyme activity and turned out to be excellently compatible with the used esterase PLE.

Based on these promising results in optimization of both the metathesis and the biotransformation with proven excellent compatibility of the enzyme with the metal catalyst, a two-step, one-pot process in aqueous solution combining the metathesis and enzymatic hydrolysis was successfully developed (Scheme 2). The metathesis reaction was performed according to the optimized procedure (Table 1, entry 5) using 0.5 mol% of catalyst **4**. After a reaction time of 6 h (which turned out to be sufficient for 98% conversion) the enzyme, sodium chloride and *tert*-butyl alcohol were added. The desired monoester **3a**, which was formed with a high overall conversion of $\geq$95% in the one-pot process, was obtained in excellent yield of 94% after work-up (Scheme 2). The reaction time required for 95% conversion was 59 h (Scheme 2), thus



Scheme 2. One-pot synthesis of monoester **3a**.



Scheme 3. One-pot synthesis of monoester **3b.**

being in a similar range as the analogous biotransformation starting from the isolated product **2a** (50 h, 92% yield; Table 2, entry 5).

This synthetic one-pot process concept was also applied to diester **1b** as a further substrate (Scheme 3), using the same conditions for metathesis and biotransformation as has been done for substrate **1a**. A quantitative conversion was also observed for this metathesis after 6 h at room temperature in the presence of 0.5 mol% of Grubbs II catalyst **4**, and the enzymatic hydrolysis proceeds with a conversion of 70% in 54 h (Scheme 3). After work-up the desired product **3b** was isolated in 67% yield. The enantioselectivity of the hydrolytic process catalyzed by a commercially available wild-type preparation of pig liver esterase, however, was low with 7% *ee*.

In conclusion, we have reported the first example of a combination of a metal-catalyzed metathesis reaction with a biotransformation in a one-pot synthesis in aqueous media. With excellent conversion and without formation of any side products we obtained monoesters **3a** and **3b** in 94% and 67% yield, respectively. This one-pot synthesis also represents a new synthetic approach to unsaturated cyclic acids, which serve as interesting compounds for the synthesis of unusual amino acids. Current work focuses on the expansion of the substrate range for the chemoenzymatic one-pot syntheses of malonic acid monoesters, improvement of enantioselectivity of hydrolysis of **2b** by using recombinant isoenzymes of the pig liver esterase in isolated form[17b] as well as the synthesis of non-natural amino acids bearing a quaternary carbon center from the monoesters as intermediates. Further future work will be also related to the development of a one-pot process running in a "tandem mode", in which both catalyst components (namely the metathesis catalyst and the hydrolase) are present in the reaction mixture from the beginning.[19]

Experimental Section

Procedure for the One-Pot Synthesis of Malonic Acid Monoesters (According to Scheme 2 and Scheme 3)

In a 15-mL glass vial diethyl malonate **1a** or **1b** (0.25 mmol) was suspended in H_2O (7.5 mL) and Grubbs II catalyst (**4**, 0.5 mol%) was added. The reaction mixture was stirred for 6 h at room temperature before adding *t*-BuOH (2.5 mL) and NaCl (0.5 mmol). The subsequent enzymatic hydrolysis, which was performed in a Titrino apparatus (Metrohm), was started by addition of pig liver esterase (PLE, purchased from Sigma–Aldrich; 17 U/mg, 1080 U/mmol of **2a** or **2b**). The conversion was determined by the amount of consumed NaOH solution. The aqueous solution was extracted with CH_2Cl_2 (3×30 mL) and the combined organic phases were re-extracted with H_2O (2×30 mL). The combined aqueous phases were acidified with 2 M HCl to pH 2, and again extracted with CH_2Cl_2 (3×30 mL). After drying the combined organic phases over Na_2SO_4, the solvent was evaporated under vacuum. The products **3a** and **3b** were obtained as slightly yellowish oils; yield: 94% and 67%, respectively.

Acknowledgements

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[19] Notably, enzymatic hydrolysis of **1a** proceeds slower than for **2a**, thus fulfilling one prerequisite for such an envisioned tandem reaction in the future. For example, for **1a** we obtained a conversion of 4% after 1 h whereas 27% of **2a** were hydrolyzed in the same time in the presence of PLE. A further prerequisite for a one-pot process running in a tandem mode, however, is stability of the metathesis catalyst under conditions of the biotransformation, which is also currently under investigation.

