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Chemoenzymatic Synthesis of Vitamin B5-Intermediate (*R*)-Pantolactone via Combined Asymmetric Organo- and Biocatalysis

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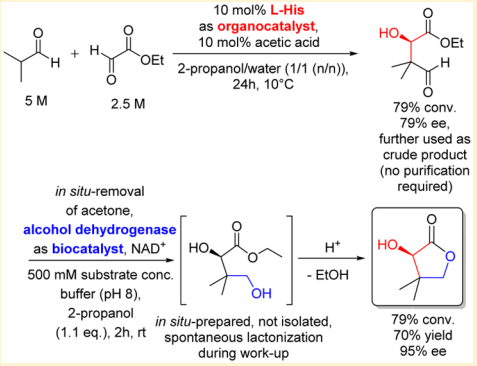
Supporting Information

ABSTRACT: The combination of an asymmetric organocatalytic aldol reaction with a subsequent biotransformation toward a "one-pot-like" process for the synthesis of (*R*)-pantolactone, which to date is industrially produced by a resolution process, is demonstrated. This process consists of an initial aldol reaction catalyzed by readily available L-histidine followed by biotransformation of the aldol adduct by an alcohol dehydrogenase without the need for intermediate isolation. Employing the industrially attractive starting material isobutanal, a chemoenzymatic three-step process without intermediate purification is established allowing the synthesis of (*R*)-pantolactone in an overall yield of 55% (three steps) and high enantiomeric excess of 95%.



INTRODUCTION

The asymmetric synthesis of (*R*)-pantolactone ((*R*)-**1**, Figure 1) continues to be of interest to organic chemists due to its wide use as a chiral auxiliary[1−4] and key industrial intermediate for the production of pantothenate.[5,6] Besides the inhibitory effects of optically pure (*R*)-**1** toward lactic acid bacteria and malaria,[7,8] it serves as a starting material for the preparation of (*R*)-panthenol,[9] (*R*)-pantetheine,[10] and pantoyl taurine, which has been shown to inhibit the growth of certain strains of diphtheria bacilli,[11] among others. Furthermore, (*R*)-**1** is a key building block for natural products like 4′-phosphopantetheine, the prosthetic group of fatty acid synthase,[12] and pantothenic acid (vitamin B5, Figure 1), which is needed for the biosynthesis of coenzyme A (CoA).[12,13]

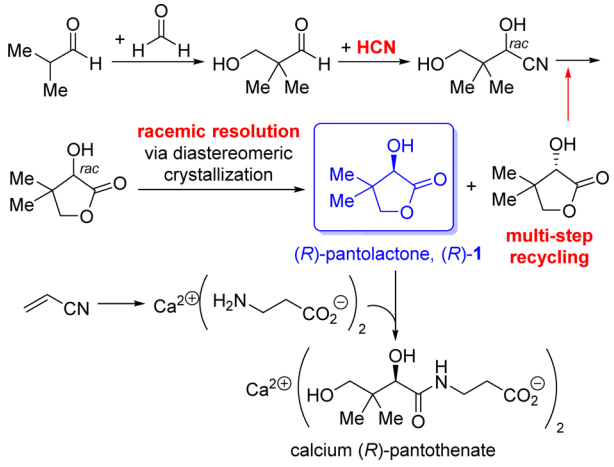


Figure 1. Industrial production of (*R*)-pantolactone ((*R*)-**1**) and (*R*)-pantothenic acid.

For industrial production of (*R*)-pantothenic acid and its commercial form calcium (*R*)-pantothenate, first racemic pantolactone (*rac*-**1**) is synthesized via aldol condensation using isobutanal and formaldehyde, followed by addition of hydrogen cyanide and saponification under acidic conditions.[13,14] Resolution of *rac*-**1** is conducted through diastereomeric crystallization using chiral auxiliaries such as (+)-3-aminoethylpinane,[15] cinchona alkaloids[16] or quinine,[17] with the latter method being applied on an industrial scale.[6,18] Enzymatic approaches for the kinetic resolution of *rac*-**1** make use of lipase-catalyzed esterification of the unwanted *S*-enantiomer[19] or hydrolysis of acylated pantoyl lactone,[20] whereas microbial methods focus on either *S*-[21] or *R*-specific lactonase-catalyzed hydrolysis of the racemate.[22,23] Notably, the latter biocatalytic resolution has been in commercial operation since 1995.[24,25] Apart from chiral resolution, different concepts for the direct synthesis of (*R*)-**1** are known, relying on either

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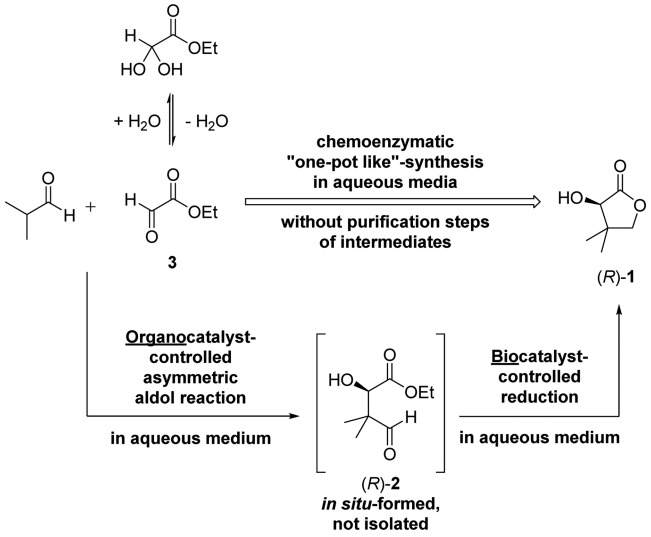
"pure" chemical procedures, such as Sharpless asymmetric epoxidation[26] and Sharpless dihydroxylation,[27] or an enzymatic approach that uses oxynitrilase-catalyzed synthesis of cyanohydrin, starting from pantolactone precursors.[28,29]

To the best of our knowledge, there is to date only one method that describes combining both chemo- and biocatalysis using the industrially well-established starting material isobutanal.[30] However, in this process, chemical preparation of the precursor ketopantoyl lactone suffers from side product formation, and it therefore has to be purified via vacuum distillation (65% yield). The subsequent microbial reduction effectively provides (*R*)-**1** in high optical purity (99% conversion, 94% enantiomeric excess (ee)) but is limited to low substrate concentrations of <80 mM, which makes this route less attractive industrially.

In this study, we present a chemoenzymatic "one-pot-like" process for the two-step production of (*R*)-**1** with high enantioselectivity starting from readily available isobutanal and glyoxylate (which is in an equilibrium with its hydrate as the predominant form in aqueous medium) as starting materials (Scheme 1; in the following schemes, only glyoxylate

Scheme 1. Chemoenzymatic Synthesis of (*R*)-Pantolactone ((*R*)-1)

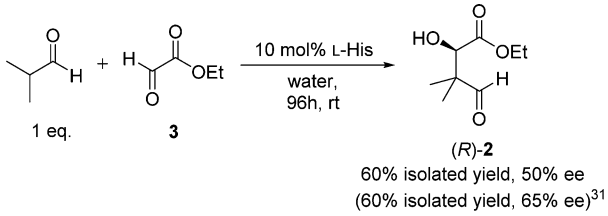


form **3** is shown, and the structure of the corresponding hydrate is omitted for simplicity). This process is based on a combination of asymmetric organo- and biocatalytic transformations, employing high substrate concentrations (e.g., up to 500 mM) for the biotransformation step. Notably, in contrast to today's existing industrialized syntheses, this chemoenzymatic route does not require the use of highly toxic cyanide.

RESULTS AND DISCUSSION

Organocatalytic Aldol Reaction. As a starting point for the synthesis of pantolactone precursor (*R*)-**2**, we chose a procedure recently published by some authors of this study,[31,32] which is based on an asymmetric histidine-catalyzed cross-aldol reaction yielding aldehyde (*R*)-**2** in 60% with 65% ee. Our first goal was to improve this asymmetric aldol reaction in terms of conversion, yield, and ee of (*R*)-**2**. Analogous to the previous procedure,[31,32] we initially used equimolar amounts of isobutanal and ethyl glyoxylate **3** (technical grade, containing 50% w/w toluene) and 10 mol % of L-histidine as an organocatalyst in water (Scheme 2).

Scheme 2. Organocatalytic Synthesis of (*R*)-2 Using Histidine



Although proline and its derivatives are widely applied catalysts for asymmetric aldol reactions,[33,34] α-branched aldehydes only react as electrophiles in proline-catalyzed cross-aldol additions, ruling out their use for optimizing this synthetic route.[35] Accordingly, similar to an attempt made by Lu,[36] we investigated natural amino acids other than histidine and proline with respect to their ability to act as organocatalysts in the aldol reaction using isobutanal as an aldol donor. However, none of the tested amino acids gave better results than histidine (data not shown). As histidine remained the organocatalyst of choice, we next conducted kinetic studies to gain a better insight into the reaction course of this aldol reaction (Figure 2). Within 24 h, product formation already

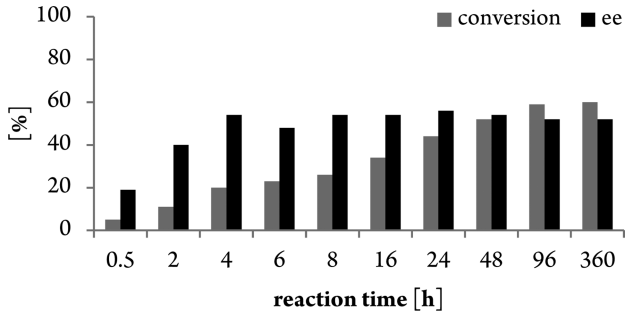


Figure 2. Reaction course of the histidine-catalyzed aldol reaction of isobutanal and ethyl glyoxylate.

reached 75% of its final value (60%), which did not increase further even over a prolonged reaction time. The enantiomeric excess of (*R*)-**2** interestingly increases initially, reaching its maximum after 24 h before slightly dropping afterward. For further optimization, a reaction time of 24 h was chosen.

Encouraged by analogous results to that of Singh et al.,[37] we then tried to enhance the reaction performance by substituting water for brine, which is known to have a positive effect on conversion rate as well as enantiomeric excess of aldol addition products in proline-catalyzed reactions. However, due to the low solubility of L-histidine in water, adding brine to the reaction mixture led to partial precipitation of the catalyst, resulting in lower conversions compared to the benchmark system (see also Scheme 2).

In general, the conversion data (in %) given in this manuscript are defined as the percentage of substrate consumption leading to the formation of the corresponding product, and determination of the conversion was done by measuring the absolute amount of product in the crude product by means of a calibration line.

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To clarify the role of water in the organocatalytic synthesis of (*R*)-**2**, we varied the water-to-substrate molar ratio (Figure 3).

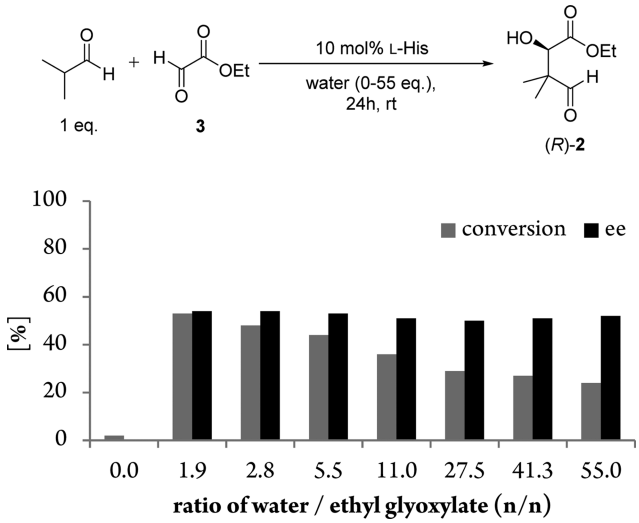


Figure 3. Influence of water on the reaction rate.

Notably, the absence of water nearly completely stopped the reaction, most likely due to insufficient solubility of the catalyst. Whereas maximum conversion was found at a water/substrate ratio of 1.9, the amount of water had no effect on the enantiomeric excess of the aldol reaction product (*R*)-**2**.

We also evaluated the impact of the reaction temperature on the reaction course. In agreement with data in the literature,[37−39] lowering the temperature resulted in lower conversions. Interestingly, enantioselectivity was not a linear correlation and reached a maximum at 10 °C (Figure 4).

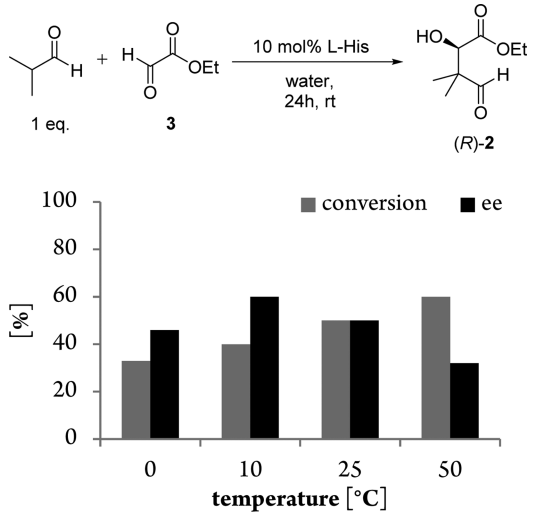
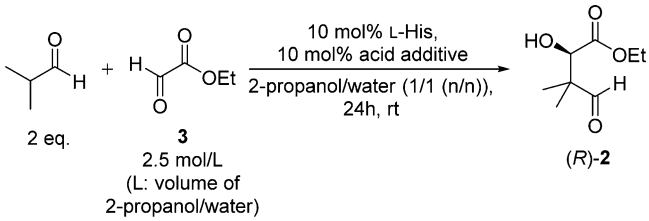


Figure 4. Effect of reaction temperature on conversion.

Varying the stoichiometry of the starting materials showed no significant increase when switching the ratio of isobutanal/**3** from 3:1 to 4:1 or to 5:1 (for details, see the Supporting Information). With respect to atom economy, a molar ratio of 2:1 donor-to-acceptor aldehyde **3** was chosen for final optimizations.

From mechanistic studies on proline-catalyzed asymmetric aldol addition[40,41] and histidine-catalyzed analogue reactions,[32,42] the importance of the formation of an enamine adduct (as an intermediate) for the stereoselective reaction course is well known. In addition, reaction kinetics are dominated by the formation and disaggregation of the enamine intermediate adduct consisting of the organocatalyst and both carbonyl compounds. As disaggregation is controlled by proton transfer, it can be assumed that (i) media that support proton transfer and (ii) the addition of Brønsted acids can boost the reaction. Accordingly, adding acid cocatalysts to a mixture comprised of a polar protic solvent (e.g., 2-propanol) and water resulted in both higher conversion and enantiomeric excess of the aldol addition product (*R*)-**2** (Table 1).

Table 1. Acid Cocatalysts Improve Conversion and Selectivity



entry	acid	pK_a	conv. [%]	ee of (*R*)-**2** [%]
1	TFA	0.26	65	65
2	salicylic	2.75	75	72
3	formic	3.77	84	67
4	L-lactic	3.87	86	71
5	benzoic	4.17	90	75
6	*m*-toluic	4.28	85	75
7	cinnamic	4.44	84	75
8	acetic	4.76	83	75
9	pivalic	5.02	85	75
10	lauric	5.78	82	72
11	uric	5.82	74	74

When applying equimolar amounts of organocatalyst and acid additive, the best results in terms of accelerating the reaction as well as improving optical purity of (*R*)-**2** are found with cocatalysts that have pK_a-values between 4 and 5 (Table 1, entries 5−9). These findings are in good agreement with results published by Jia et al. regarding improved activities and enantioselectivities of amino acid-derived primary amine organocatalysts in asymmetric aldol reactions upon the addition of acid additives.[43]

In parallel to amino acids as organocatalysts leading to up to 75% ee when using L-histidine, a second screening of various types of other organocatalysts was carried out (e.g., sulfonamides, amino acids, alkaloids, and squareamides), revealing bifunctional primary-amine thiourea organocatalysts as further suitable catalysts for the desired transformation (Table 2).

Catalysts **4**, **6**, and **7**, which gave the best results, are based on (*R*,*R*)-diaminocyclohexane (*trans*-DACH). The presence of an aromatic substituent on the thiourea moiety proved crucial for achieving good activity and enantioselectivity. Catalysts **4**, **6**, and **7** can be prepared in one step from (*R*,*R*)-DACH-monohydrochloride by reaction with the appropriate isocyanate, yielding the desired organocatalyst after basic workup.[44]

In general, urea-type organocatalysts gave enantioselectivities that were higher than their thiourea analogues as exemplified by, for example, reactions with compounds **4**[45] and **7** (Table 2,

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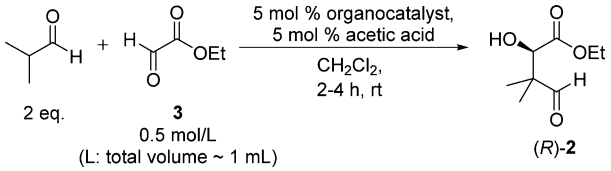
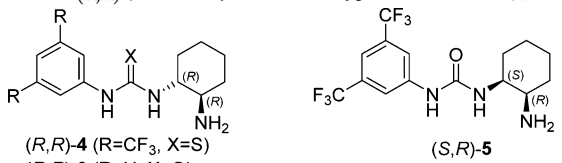
J. Org. Chem. 2015, 80, 3387−3396

Table 2. Screening of Amino-Urea and Amino-Thiourea Organocatalysts



entry	catalyst	time [h]	conv.[a] [%]	ee of (*R*)-**2**[a] [%]
1[b]	(*R*,*R*)-**4**	1	85	55
2[b]	(*S*,*R*)-**5**	1	74	75
3	(*R*,*R*)-**6**	3	86	75
4	(*R*,*R*)-**7**	2.5	94	79
5[c]	(*R*,*R*)-**7**	3	77	81
6[d]	(*R*,*R*)-**7**	4	95	81



[a]Experiments were run on a 0.4 mmol scale. Yield and ee were determined via chiral gas chromatography with dodecane as the internal standard. [b]Used 10 mol % catalyst. [c]Used 25 mol % acetic acid. [d]Used 50 mol % acetic acid.

entries 1 and 4). Urea-type catalyst **5**,[46] derived from *cis*-(*S*,*R*)-DACH, gave an enantioselectivity of 75% ee (entry 2), and when using the simple phenyl-substituted (*R*,*R*)-DACH-based urea **6**[47] as an organocatalyst, a similar enantioselectivity and improved yield was obtained (entry 3).

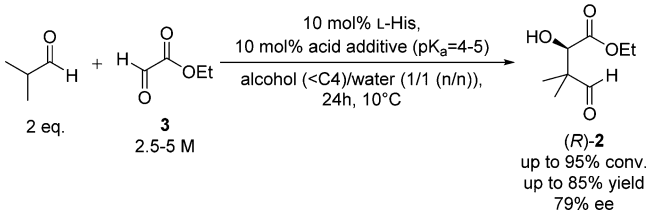
Amino-urea **7**, which was introduced by Jacobsen in 2007,[44] showed the best result with an enantioselectivity of 79% ee. By increasing the amount of cocatalyst acid to 50 mol %, a product yield of 95% and an enantiomeric excess of 81% were finally achieved (Table 2, entries 4–6). It is noteworthy that these results were achieved at catalyst loading of only 5 mol % and within a 4 h reaction time.

Although preparation of organocatalyst **7** is just one step from commercially available starting materials, after detailed evaluation of the potential of all the applied organocatalysts, we decided to use the natural amino acid L-histidine as the organocatalyst of choice for further process optimization due to its direct commercial availability, attractive price, and similar enantioselectivities (75% ee, see Table 1, entries 5–9), which were obtained in the asymmetric aldol reaction for synthesis of (*R*)-**2** when using this natural L-amino acid.

Next, with L-histidine as the prioritized organocatalyst component, we investigated the influence of solvent properties on the aldol reaction in a solvent engineering study. Although polar solvents like DMF or DMSO gave better results than unpolar cosolvents (data not shown), the best results were gained when applying polar protic solvents, such as alcohols, in the aldol reaction (for details, see the Supporting Information). Low molecular-weight alcohols, such as methanol, ethanol, and 2-propanol, were favorable because of their positive effects on catalyst activity and selectivity and for their attractiveness from economic and sustainability perspectives.[48] Furthermore, when studying the impact of the molar ratio of water and a selected alcohol (e.g., 2-propanol; for details, see Supporting Information) on the L-histidine-catalyzed aldol addition, maximum conversion is observed at an alcohol/water ratio in the range of 0.7–1.3, whereas enantioselectivity was almost unaffected.

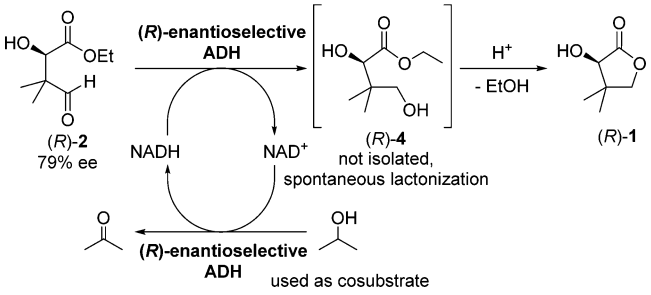
In summary, for the organocatalytic asymmetric aldol addition step starting from isobutanal and ethyl glyoxylate **3**, optimized reaction conditions were found, which consist of the use of 2 equiv of the donor aldehyde isobutanal, 1 equiv of **3** (technical grade, containing 50% w/w toluene), 10 mol % of L-histidine, 1 equiv of Brønsted acid additive relative to the amount of organocatalyst, 0.2–0.4 mL/mmol **3** mixture of a monovalent alcohol (<C4) with water in a molar ratio of 1:1, reacted for 24 h at 10 °C (Scheme 3). Applying such optimized conditions enabled the synthesis of (*R*)-**2** with up to 95% conversion, 85% yield, and 79% ee.

Scheme 3. Optimized Conditions for the L-Histidine-Catalyzed Asymmetric Aldol Reaction Step



Biocatalytic Reduction. With respect to the second step of this chemoenzymatic synthesis, we used an alcohol dehydrogenase (ADH) for reduction of the (*R*)-pantolactone precursor (*R*)-**2** with formation of α-hydroxy ester (*R*)-**4**, which should then undergo spontaneous cyclization to desired product (*R*)-**1** (Scheme 4).

Scheme 4. Enzymatic Reduction of (*R*)-2 to (*R*)-Pantolactone ((*R*)-1)



It has to be emphasized that the natural cofactor nicotinamide adenine dinucleotide (NADH in its reduced form) does not have to be added in stoichiometric amounts when following this strategy. Instead, the reducing agent NADH can be applied in catalytic amounts and is regenerated via in situ oxidation of the cosubstrate 2-propanol to acetone (as a process catalyzed by the same ADH), which makes cheap 2-propanol the reducing equivalent used in a stoichiometric amount (Scheme 4). Additionally, stereorecognition of the precursor aldehyde (*R*)-**2** by the ADH would lead to enantiomeric enrichment of (*R*)-**1**. Accordingly, the first challenge was to find an appropriate ADH that would (i) have a high activity for the reduction of (*R*)-**2**, thus efficiently convert this precursor to pantolactone, (ii) possess a stereopreference for (*R*)-**2** over (*S*)-**2**, and (iii) ensure effective substrate-coupled cofactor regeneration.

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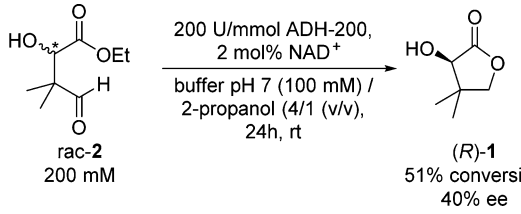
When we screened a limited set of commercially available, robust recombinant alcohol dehydrogenases, such an enzyme was found in ADH-200[49] from evocatal (evo-1.1.200), which in an initial preliminary experiment converted 51% of aldehyde *rac*-**2** and gave (*R*)-**1** in 40% ee (Scheme 5). In contrast, under

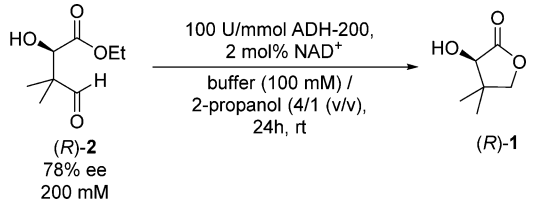
Scheme 5. Enzymatic Reduction of *rac*-2 to (*R*)-Pantolactone ((*R*)-1)



the same reaction conditions, the ADH from *Rhodococcus* sp.[49] gave somewhat lower conversion (36%) and 36% ee, whereas the ADH from *L. kefir*,[50] as a widely applied ADH, led to <5% conversion (reactions with these two enzymes not shown in detail).

The following pH-optimization study is shown in Table 3. Utilizing the enantiomerically enriched precursor aldehyde (*R*)-**2** (78% ee) then led to desired pantolactone (*R*)-**1** in high enantiomeric excess when slighty basic buffer solutions of pH (8.0−8.5) were used (entries 6 and 7).

Table 3. pH-Optimization Tests

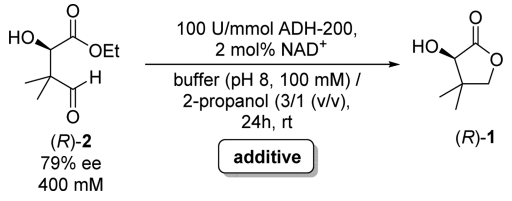


entry	pH	conv. [%]	ee of (*R*)-**2** [%]
1	3.8	11	n.d.
2	4.6	30	96
3	6.3	54	93
4	7.0	49	93
5	7.6	65	93
6	8.0	67	95
7	8.5	67	95
8	9.0	63	96
9	9.6	58	94

Having achieved high enantiomeric excess of (*R*)-**1** via enzymatic reduction, we next focused on overcoming the thermodynamic limitations of the system due to the reverse reaction, in which acetone is reduced by oxidizing the product, to improve the overall conversion and thus the process economy. First, we changed the reaction temperature or used 2-propanol in high excess to shift the equilibrium toward the direction of the product. Raising the temperature led to higher conversion (e.g., 77% at 45 °C vs 67% at room temperature (rt); see Table 3, entry 6) but at the expense of selectivity (89% ee at 45 °C vs 95% ee at rt). Applying a higher amount of 2-propanol can shift the equilibrium toward the formation of alcohol **4**, however, only to a certain extent because high concentrations of organic solvents tend to have a negative impact on enzyme stability. A volumetric ratio of 3:1 (v/v) buffer to 2-propanol can be applied without causing significant stability or activity problems, even up to a substrate concentration of 800 mM (*R*)-**2**.

Chemoenzymatic One-Pot Synthesis of (*R*)-1. With these optimized reactions in hand, we next evaluated a possible "one-pot-like" reaction concept, which is based on direct use of the reaction mixture resulting from the organocatalytic step for the subsequent enzymatic reduction, thus avoiding the need to isolate intermediate (*R*)-**2**. Compared to the benchmark enzymatic reduction system using purified aldol product (*R*)-**2** (Table 4, entry 1), this process with the two coupled reactions unfortunately drops the conversion from 69 to 33%, while enantioselectivity remains unchanged. Obviously, components remaining from the initial organocatalytic step suppress efficient enzymatic reduction of (*R*)-**2**, thus making a "standard" tandem-type one-pot synthesis a less-favorable option in further process development.

Table 4. Preliminary Tests for "One-Pot-Like" Synthesis



entry	conditions[a]	additive	conv. [%]	ee of (*R*)-**2** [%]
1	A		69	95
2	B		33	94
3	C		64	96
4[b]	A	toluene	63	95
5[b]	A	acetic acid	67	95
6[b]	A	L-His	71	94
7	A	isobutyraldehyde (1 equiv)	42	96
8	A	acetone (1 equiv)	48	97

[a]Reaction conditions: A, purified aldehyde (*R*)-**2**; B, reaction mixture derived according to Scheme 3 using 2-propanol as cosolvent and acetic acid as additive; C, similar to B but with all volatile components removed in vacuo prior to introducing the residual mixture (crude product) to the enzymatic reduction. [b]Additive amounts as described in Scheme 3.

However, we were pleased to find that when removing all volatile materials from the reaction mixture of the aldol reaction prior to its use for biotransformation, conversion is in the same range as that of the benchmark reaction (entry 3). This illustrates that a workup and purification of intermediate (*R*)-**2** is not required and that simple evaporation of the volatile materials after conducting the organocatalytic transformation is sufficient for a subsequent efficient biocatalytic reduction process.

Next, we evaluated which component was responsible for the negative impact on the biotransformation with the observed strong drop of conversion. Besides residual acid additive, L-histidine, toluene, and the aldol product, at least one equiv of isobutanal remains in the reaction mixture, even at a maximum conversion of 95% to (*R*)-**2** when using benzoic acid as the cocatalyst. Interestingly, when adding toluene, acid additive, or histidine to purified (*R*)-**2**, no effect on biotransformation was detected (entries 4−6). However, when isobutanal (entry 7) or acetone (entry 8) is present in the mixture, a significant drop of conversion after 24 h to <50% occurs, possibly due to the

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J. Org. Chem. 2015, 80, 3387−3396

ability of these additives to act as competitive substrates for aldehyde (*R*)-**2**.

Although isobutanal (as well as acetone from the aldol reaction) can easily be removed in vacuo before starting the enzymatic reduction, acetone is also formed in situ as a stoichiometric side-product during cofactor regeneration with isopropanol as the reducing agent (Scheme 3). On the basis of the low boiling point of acetone, we thus became interested in applying an "in situ product removal" (ISPR) of acetone to effectively withdraw this ketone byproduct during the reaction and thus shift the equilibrium in the direction of the desired product.[51,52]

In the chosen setting, air saturated with water/2-propanol (5 vol %) was continuously passed through the biotransformation reaction mixture during the reaction, thus effectively removing the acetone formed during the course of the reaction. As the 2-propanol concentration is kept stable, applying 1.1 equiv of the alcohol is sufficient for effective substrate ((*R*)-**2**)-coupled in situ cofactor regeneration. We were pleased to find that by means of this methodology, the constant removal of acetone resulted in the reaction equilibrium shifting significantly to the product side. Notably, this enabled us to further decrease catalyst loading to an excellent value of only 10 U of alcohol dehydrogenase per mmol of substrate (*R*)-**2**, while conversion was further improved to 85% (Figure 5).

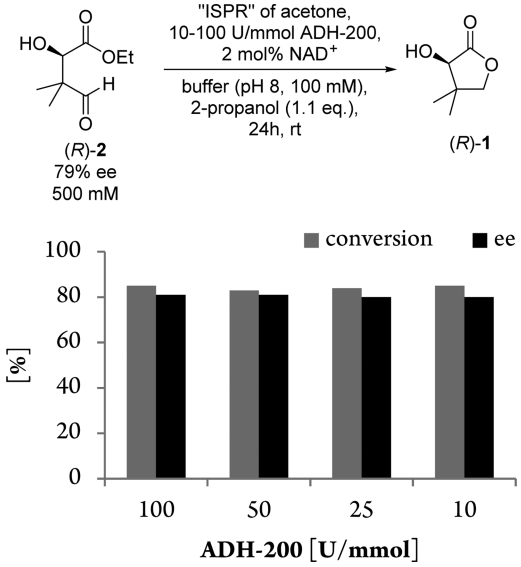
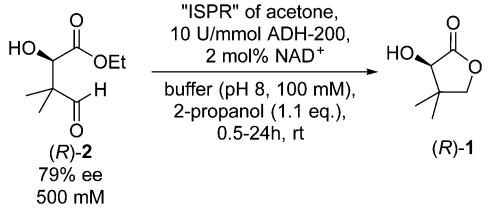


Figure 5. ISPR-settings of catalyst loading (24 h reaction time) and in situ removal of acetone.

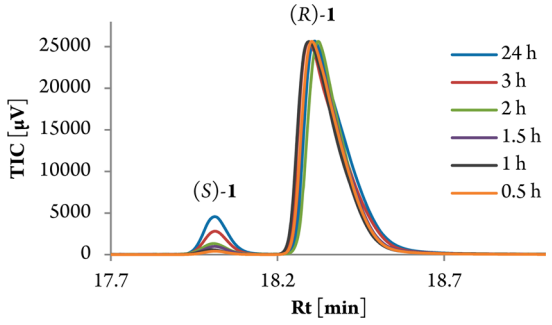
It has to be mentioned that the increase in conversion, however, was initially at the expense of enantiomeric excess of reduction product (*R*)-**1**, which dropped from 95% (Table 4, entry 1; reaction without ISPR of acetone) to 80% with acetone being constantly removed. This value resembles the ee of starting material (*R*)-**2**; thus, in this case, there is no further enrichment of the enantiomeric excess via enantioselective enzymatic reduction.

Interestingly, however, reducing the reaction time upon applying 10 U/mmol ADH led to the formation of (*R*)-pantolactone with high conversion and in excellent enantiomeric excess (Table 5, entry 4). Furthermore, the highest ee values were seen at the beginning of the process (entries 1 and 2), thus suggesting a typical kinetically controlled reaction course of biotransformation.

Table 5. Biotransformation Using ISPR at Low Catalyst Loadings[a]



entry	time [h]	conv. [%]	ee of (*R*)-**2** [%]
1	0.5	58	98
2	1.0	75	98
3	1.5	81	96
4	2.0	86	95
5	3.0	87	88
6	24.0	85	80



[a]Graph shows analytical total ion current (TIC) gas chromatograms for ee control at different reaction times normalized to the (*R*)-**1** peak (for details, see "General Information" in the Experimental Section).

Finally, with respect to the overall process economy, we also addressed the issue of enzyme recycling. Notably, when applying a molecular weight cutoff (MWCO) membrane of 10 kDa in our ISPR-type enzymatic reduction setup (Table 5) at 2 h of reaction time, no biocatalyst leaching, deactivation, or destabilization was observed (Figure 6). Furthermore, conversion (77 ± 2%) to and optical purity (95% ee) of (*R*)-**1** remained unchanged over 5 cycles, thus further decreasing the required overall catalyst loading to a very low value of only 2 U/mmol of substrate (*R*)-**2**. As no negative effects on conversion or selectivity were observed during 5 cycles of the enzymatic reduction of (*R*)-**2**, it can be assumed that catalyst loading can be further decreased by extension of the recycling cycles.

Combining both reaction steps led to a chemoenzymatic "one-pot-like" process (Scheme 6). As it concerns the organocatalytic aldol reaction, we chose a setup with acetic acid as cocatalyst because it can be removed in vacuo together with all of the other volatile compounds that are present in the crude product. Conducting the organocatalytic aldol reaction under these conditions, followed by subsequent biotransformation under ISPR conditions and spontaneous ring-cyclization then afforded (*R*)-**1** with 79% conversion, 70% yield, and high enantiomeric excess of 95%. The overall yield of this chemoenzymatic synthesis of (*R*)-pantolactone, (*R*)-**1**, based on the economically attractive starting material ethyl glyoxylate **3** was 55% over three steps (organocatalytic aldol reaction, enzymatic reduction, and spontaneous cyclization; Scheme 6). Although to date the chemoenzymatic one-pot synthesis

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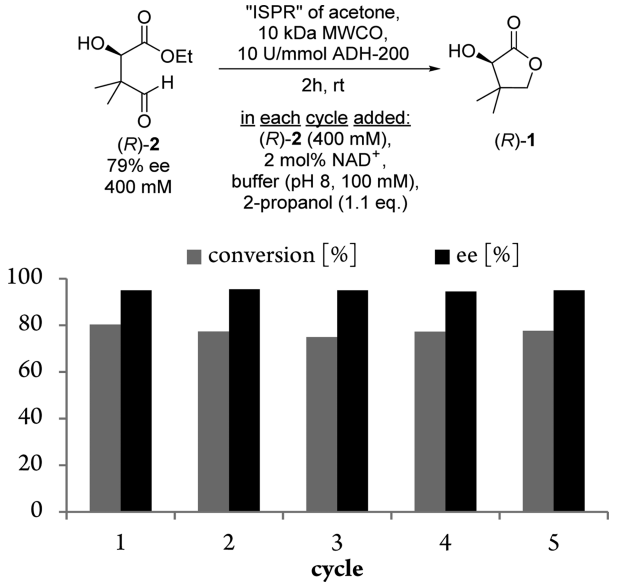
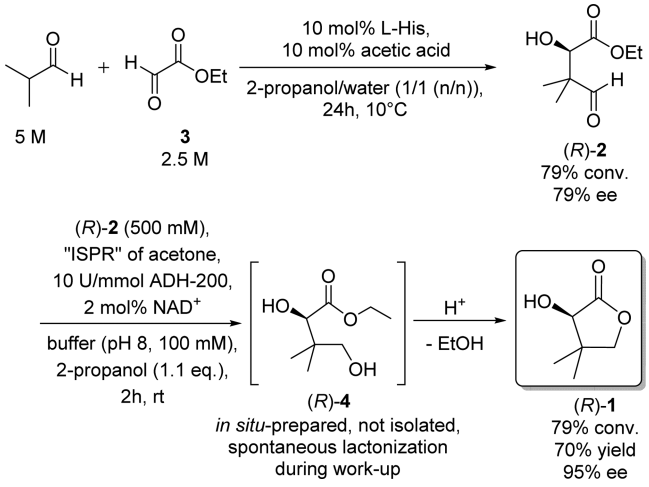


Figure 6. ISPR-type enzymatic reduction with recycling and reuse of the alcohol dehydrogenase.

Scheme 6. Chemoenzymatic "One-Pot-Like" Process for the Synthesis of (*R*)-Pantolactone (*R*)-1



(according to Scheme 6) has been performed on a smaller lab scale (1.28 mmol), the feasibility of scaling up this process is expected because this process does not contain unit operation steps that are known to be critical when applied on a larger scale.

CONCLUSION

In summary, we have developed an alternative route for the synthesis of (*R*)-pantolactone, (*R*)-**1**, using the combination of an organocatalytic asymmetric aldol reaction with subsequent stereoselective biocatalytic reduction of the intermediate **2** and spontaneous ring-cyclization to the desired (*R*)-pantolactone, (*R*)-**1**. This chemoenzymatic three-step sequence, which is based on industrially attractive isobutanal as the starting material, can be carried out without intermediate purification, leading to a "one-pot-like" process in which the biocatalyst ADH can be fully reused during 5 consecutive reaction cycles without leaching or deactivation effects. Notably, in contrast to the existing multistep industrial process based on resolution, the use of highly toxic cyanide is not required in the process we developed. With considerably high substrate concentrations (2.5 M ethyl glyoxylate **3** for step 1, 500 mM intermediate **2** for the biotransformation step; Scheme 5), the chemoenzymatic "one-pot-like" process for the synthesis of (*R*)-pantolactone, (*R*)-**1**, gave an overall yield of 55% of (*R*)-**1** with a high enantioselectivity of 95%.

EXPERIMENTAL SECTION

General Information. Reagents were purchased from commercial sources and used without further purification. Isobutanal was freshly distilled before use (75 °C at atmospheric pressure). Ethyl glyoxylate (**3**) was used in technical grade containing 50% (w/w) toluene. All buffers used were 100 mM (acetate buffer for pH 3.8 and 4.6; phosphate buffer for pH 6.3, 7.0, 7.6, and 8.0; tris(hydroxymethyl)-aminomethane buffer for pH 8.5 and 9.0; ethanolamine buffer for pH 9.6). All products were characterized by their NMR spectra. ^{1}H and ^{13}C NMR spectra were recorded in deuterochloroform ($CDCl_3$) on a 500 MHz instrument. Chemical shifts were reported in parts per million (ppm, δ) downfield from residual $CHCl_3$ signal ($\delta(^{1}H)$, 7.26 ppm; $\delta(^{13}C)$, 77.16 ppm). Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Enzyme recycling experiments were performed in a stirred cell with 10 mL of overall volume using Ultracel PL-10 ultrafiltration discs. Analytical HPLC was carried out with a supercritical fluid chromatography setup consisting of a sampler/injector (AS-2059-SF Plus), pressure regulator (BP-2080 Plus), detector (MD-2010 Plus), cryostat (F250), pumps (PU-2080 Plus), line degasser (DG-2080-53), net box (LC-Net II/ADC), and column oven (CO-2060 Plus). A mixture of carbon dioxide, hexane, and 2-propanol was used as the solvent. The enantiomeric excess (ee) of (*R*)-**2** was determined using a Daicel Chiralpak column OD-H with the above-mentioned HPLC settings. Quantitative gas chromatography (GC) for conversion determination was carried out on an Rxi-5 ms column (25 m, 0.2 mm inner diameter) at 140 °C (isotherm, 3 min) with N_2 as a carrier gas using a straight calibration line. Conversion was determined before crude products were purified. In general, the conversion data (in %) given in this study are defined as the percentage of substrate consumption leading to the formation of the corresponding product, and the conversion was determined measuring the absolute amount of product in the crude product by means of a calibration line. The enantiomeric excess of (*R*)-**2** could also be determined using chiral GC analysis (HP 6890 Series GC System; Lipodex E (25 m, 0.25 mm inner diameter) column; N_2 as carrier gas; isothermic at 85 °C). Both yield and ee given in Table 2 were determined from crude reaction mixtures with the former calibrated against *n*-dodecane as an internal standard. Enantiomeric excess of (*R*)-**1** was determined by chiral GC using an Rt-βDEXm column (30 m, 0.25 mm inner diameter). The temperature was regulated at 80 °C for 10 min, heated to 120 °C at 10 °C/min, and held at 120 °C for 10 min.

Procedure for the Analytical Method to Determine Product Formation of (*R*)-1 and (*R*)-2 by Means of a Calibration Curve. In 100 mL volumetric flasks, stock solutions in ethyl acetate were prepared containing the aldol addition product (*R*)-**2** and the biotransformation product (*R*)-**1**, each in mass concentrations of 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/mL. Each analyte solution was measured in a 6-fold determination via GC using an Rxi-5 ms column (25 m, 0.2 mm inner diameter) at 140 °C (isotherm, 3 min). For each mass concentration, a leveled value based on the area peaks (total integration) was calculated, and a linear regression line (correlation factor $R^2 > 0.999$) was applied to all monitored data points. From this straight calibration line, the unknown mass concentrations of (*R*)-**1** and (*R*)-**2** were identified by their area integrals. In all of the experiments, stock solutions of crude reaction mixtures were examined.

General Procedure for the L-Histidine-Catalyzed Synthesis of (*R*)-2 Using Equimolar Amounts of the Starting Materials Ethyl Glyoxylate 3 and Isobutanal. To a solution of 155 mg (1 mmol) of L-histidine in 3 mL of water were added 2.05 g (10 mmol) of

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J. Org. Chem. 2015, 80, 3387−3396

ethyl glyoxylate **3** and 0.9 mL (10 mmol) of isobutanal. The reaction mixture was stirred at room temperature (rt) for 0.5−360h (see Scheme 2 and Figure 2), extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. From the crude product mixture, a stock solution in ethyl acetate was prepared and measured via GC to determine conversion (see Scheme 2 and Figure 2).

Influence of Water on the Reaction Rate of the L-Histidine-Catalyzed Synthesis of (*R*)-2 Using Equimolar Amounts of the Starting Materials Ethyl Glyoxylate 3 and Isobutanal. To 155 mg (1 mmol) of L-histidine and 0−10 mL (0−55 equiv relative to ethyl glyoxylate **3**) of water were added 2.05 g (10 mmol) of ethyl glyoxylate **3** and 0.9 mL (10 mmol) of isobutanal. The reaction mixture was stirred at rt for 24 h (see Figure 3), extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. From the crude product mixture, a stock solution in ethyl acetate was prepared and measured via GC to determine conversion (see Figure 3).

Effect of Reaction Temperature on Conversion in the L-Histidine-Catalyzed Synthesis of (*R*)-2 Using Equimolar Amounts of the Starting Materials Ethyl Glyoxylate (3) and Isobutanal. To a solution of 155 mg (1 mmol) of L-histidine in 0.333 mL of water were added 2.05 g (10 mmol) of ethyl glyoxylate **3** and 0.9 mL (10 mmol) of isobutanal. The reaction mixture was stirred at 0−50 °C for 24 h (see Figure 4), extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. From the crude product mixture, a stock solution in ethyl acetate was prepared and measured via GC to determine conversion (see Figure 4).

Influence of Donor Aldehyde Equivalents on the Conversion to (*R*)-2. To a solution of 155 mg (1 mmol) of L-histidine in 0.333 mL of water were added 2.05 g (10 mmol) of ethyl glyoxylate **3** and 721−3605 mg (10−50 mmol, 1−5 equiv with reference to ethyl glyoxylate **3**) of isobutanal. The reaction mixture was stirred at rt for 24 h (see Figure S1 in the Supporting Information), extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. From the crude product mixture, a stock solution in ethyl acetate was prepared and measured via GC to determine conversion (see Figure S1 in the Supporting Information).

General Procedure for the Screening of Acid Cocatalysts for the Organocatalytic Synthesis of (*R*)-2. To a solution of 1.02 g (5 mmol) of ethyl glyoxylate **3** and 0.9 mL (10 mmol) of isobutanal in 1.6 mL of 2-propanol were added 0.4 mL of a 1.25 M L-histidine solution and 10 mol % (with reference to ethyl glyoxylate **3**) of an acid additive (see Table 1). The reaction mixture was stirred at rt for 24 h, extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. From the crude product mixture, a stock solution in ethyl acetate was prepared and measured via GC to determine conversion (see Table 1).

Synthesis of the Amino-Thiourea Organocatalysts 4−7. Organocatalyst **4** was prepared according to the protocol in ref 45. Amino-urea **5** was synthesized according to methods in ref 46, and catalysts **6** and **7** were assembled following methods in ref 44. The analytical data were in accordance with the literature.[47] Compound **5**, which was not described in the literature, was prepared in the same manner as its thiourea analogue.[46]

Characterization of *cis*-(*S*,*R*)-Amino-Urea 5. Colorless oil. ^{1}H NMR (300 MHz, MeOD-d4): δ 7.99 (s, 2H), 7.47 (s, 1H), 3.94−3.90 (m, 1H), 2.99−2.96 (m, 1H), 1.77−1.60 (m, 4H), 1.55−1.37 (m, 4H). ^{13}C NMR (300 MHz, MeOD-d4): δ 157.1, 143.4, 133.1 (q, J = 32.9 Hz, CCF_3), 124.8 (q, J = 271.9 Hz, CCF_3), 118.9, 115.4, 52.0, 51.9, 31.5, 29.8, 23.4, 22.9. FT-IR (ATR) ν [cm^{-1}]: 3312 (w), 2934 (w), 2860 (w), 1655 (w), 1560 (w), 1474 (w), 1443 (w), 1387 (m), 1275 (s), 1245 (w), 1171 (m), 1125 (s), 1043 (w), 999 (w). ESI-MS M$^+$ [MeOH] = 370.1 g/mol (M+H$^+$).

General Procedure for Thiourea Catalyzed Synthesis of (*R*)-2. Stock solutions in dry dichloromethane were prepared from isobutanal, ethyl glyoxylate, *n*-dodecane, acetic acid, and catalysts **4**−**7** (see Table 2). In the order given, to a GC-vial were added 0.2 mmol *n*-dodecane, 0.8 mmol isobutyraldehyde, the respective amount of acetic acid, 0.4 mmol ethyl glyoxylate, and finally the respective amount of catalyst. The stock solutions were prepared in such a manner that a total volume of 1 mL resulted. The GC vial was shaken at 500 rpm under an inert atmosphere. The reaction progress was followed by chiral GC (see Table 2) in which t_r (*n*-dodecane) = 8.41 min, t_r ((*S*)-**2**) = 45.35 min, and t_r ((*R*)-**2**) = 45.52 min.

Organocatalytic Synthesis of (*R*)-2: General Procedure for Solvent Engineering Experiments. To a solution of 1.02 g (5 mmol) of ethyl glyoxylate **3** and 0.9 mL (10 mmol) of isobutanal in 20 mmol organic solvent (with reference to ethyl glyoxylate **3**, see Figure S2 in the Supporting Information) was added 0.4 mL (corresponding to 20 mmol of water) of a 1.25 M catalyst solution containing L-histidine and acetic acid (10 mol % each with reference to ethyl glyoxylate **3**). The reaction mixture was stirred at rt for 24 h, extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. From the crude product mixture, a stock solution in ethyl acetate was prepared and measured via GC to determine conversion (see Figure S2 in the Supporting Information).

Effect of Differing Water to Alcohol Ratios on the Synthesis of (*R*)-2. To 1.02 g (5 mmol) of ethyl glyoxylate **3** and 0.9 mL (10 mmol) of isobutanal was added 2.0 mL of a mixture of 2-propanol and water in volumetric ratios of 90:10 to 70:30 (see Figure S3 in the Supporting Information). After the addition of 77.6 mg (0.5 mmol, 10 mol % with reference to ethyl glyoxylate **3**) of L-histidine and 30 mg (0.5 mmol, 10 mol % with reference to ethyl glyoxylate **3**) of acetic acid (10 mol % each with reference to ethyl glyoxylate **3**), the reaction mixture was stirred at rt for 18 h, extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. From the crude product mixture, a stock solution in ethyl acetate was prepared and measured via GC to determine conversion (see Figure S3 in the Supporting Information).

Optimized Procedure for the Organocatalytic Synthesis of (*R*)-2. To a solution of 2.05 g (10 mmol) of ethyl glyoxylate **3** and 1.44 g (20 mmol) of isobutanal in 40 mmol alcohol (see Figure 4) was added 0.8 mL of a 1.25 M catalyst solution containing L-histidine and acid additive (see Table 1). The reaction mixture was stirred at 10 °C for 24 h, extracted with ethyl acetate, and dried over anhydrous magnesium sulfate. The solvent was removed, and the crude product was purified by distillation (0.2 mbar, 73 °C). Yield: 95% (MeOH, benzoic acid), 79% (2-propanol, acetic acid); ee: 79%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.57 (s, 1H), 4.33 (s, 1H), 4.31−4.18 (m, 2H), 3.05 (s, 1H), 1.27 (t, J = 7.2 Hz, 3H), 1.14 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 202.7, 172.8, 73.6, 62.2, 50.4, 18.2, 16.9, 14.1. Spectral data are in accordance with literature values.[31,32] GC (quantitative, GC 2010 Plus): t_r = 2.4 min. HPLC (Chiralpak column OD-H at 20 °C, CO_2/hexane/2-propanol 90:9:1, flow 1.0 mL/min, 212 nm): t_r = 8.9 min for (*S*)-**2**, 9.7 min for (*R*)-**2**.

General Procedure for the Enzymatic Synthesis of (*R*)-1. To an emulsion of 175 mg (1 mmol) of precursor (*R*)-**2** (79% ee; used in purified form (condition A), used as reaction mixture prepared according to the optimized procedure for the organocatalytic synthesis utilizing 2-propanol as alcohol and acetic acid as additive components (condition B) or used as crude product obtained from such a reaction mixture after subsequent removal of volatile components in vacuo (condition C)) in 4 mL of buffer (100 mM, pH 3.8−9.6; see Table 3) were added 1 mL of 2-propanol, 14 mg (2 mol %) of NAD$^+$, and 100 U/mmol ADH-200. The reaction mixture was stirred for 24 h at rt prior to the addition of 1 mL of diluted hydrochloric acid. The mixture was extracted with ethyl acetate and separated via centrifugation (10000 rpm, 5 min, 15 °C) three times. A stock solution in ethyl acetate was prepared in a 100 mL volumetric flask, and conversion was determined by quantitative GC (see Table 3). The solvent was removed, and the crude product was purified by column chromatography (chloroform/acetone 9:1 (v/v), 3.5 cm column diameter, 100 mL silica gel). Determination of ee values was done by a direct measurement of pure product-containing fractions via chiral GC (see Table 3).

Preliminary Tests for "One-Pot-Like" Synthesis: Influence of Additives on the Biocatalytic Reduction of (*R*)-2. To an emulsion of 175 mg (1 mmol) of precursor (*R*)-**2** (78% ee) in 1.875 mL of buffer (100 mM, pH 8) were added 0.625 mL of 2-propanol, 14 mg (2 mol %) of NAD$^+$, and 100 U/mmol ADH-200. To this mixture no additive or (under condition A only) one of the following additives was added: 10−20 vol% toluene (additive 1) or 0.1 mmol (10 mol %

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J. Org. Chem. 2015, 80, 3387−3396

with reference to (*R*)-**2**) of acetic acid (additive 2) or 15.5 mg (10 mol % with reference to (*R*)-**2**) of L-histidine (additive 3) or 72 mg (1 mmol) of isobutanal (additive 4) or 58 mg (1 mmol) of acetone (additive 5) (see Table 4). The reaction mixture was stirred for 24 h at rt prior to the addition of 1 mL of diluted hydrochloric acid. The mixture was extracted with ethyl acetate and separated via centrifugation (10000 rpm, 5 min, 15 °C) three times. A stock solution in ethyl acetate was prepared in a 100 mL volumetric flask, and the conversion was determined by quantitative GC (see Table 4). The solvent was removed, and the crude product was purified by column chromatography (chloroform/acetone 9:1 (v/v), 3.5 cm column diameter, 100 mL silica gel). Determination of ee values was done by direct measurement of pure product-containing fractions via chiral GC (see Table 4).

Evaluation of Catalyst Loading for the Enzymatic Synthesis of (*R*)-1 using ISPR of Acetone. To an emulsion of 175 mg (1 mmol) of precursor (*R*)-**2** (79% ee) in 1.875 mL of buffer (100 mM, pH 8) were added 85 μL of 2-propanol, 14 mg (2 mol %) of NAD^+, and 10−100 U/mmol ADH-200 (see Figure 5). A water/2-propanol (5 vol %) saturated air sparge was attached, and the reaction mixture is stirred for 24 h at rt prior to adding 1 mL diluted hydrochloric acid. The mixture is extracted with ethyl acetate and separated via centrifugation (10000 rpm, 5 min, 15 °C) three times. A stock solution in ethyl acetate is prepared in a 100 mL volumetric flask and the conversion is determined via quantitative GC (see Figure 5). The solvent is removed and the crude product is purified by column chromatography (chloroform/acetone 9/1 (v/v), 3.5 cm column diameter, 100 mL silica gel). Determination of ee-values is done by direct measurement of pure product containing fractions via chiral GC (see Figure 5).

General Procedure for the Enzymatic Synthesis of (*R*)-1 Using ISPR of Acetone. To an emulsion of 175 mg (1 mmol) of precursor (*R*)-**2** (79% ee) in 1.875 mL of buffer (100 mM, pH 8) were added 85 μL of 2-propanol, 14 mg (2 mol %) of NAD^+, and 10 U/mmol ADH-200. A water/2-propanol (5 vol %) saturated air sparge was attached, and the reaction mixture was stirred for 0.5−24 h at rt prior to the addition of 1 mL of diluted hydrochloric acid. The mixture was extracted with ethyl acetate and separated via centrifugation (10000 rpm, 5 min, 15 °C) three times. A stock solution in ethyl acetate was prepared in a 100 mL volumetric flask, and conversion was determined by quantitative GC. The solvent was removed, and the crude product was purified by column chromatography (chloroform/acetone 9:1 (v/v), 3.5 cm column diameter, 100 mL silica gel) to give (*R*)-**1** as a colorless solid. Conversion: 58−85% (see Table 5). Yield: 76% (2 h); ee: 80−98% (see Table 5).

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.11 (d, *J* = 3.4 Hz, 1H), 4.03 (d, *J* = 8.9 Hz, 1H), 3.95 (d, *J* = 9.0 Hz, 1H), 2.50 (d, *J* = 3.4 Hz, 1H), 1.24 (s, 3H), 1.08 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 177.8, 76.6, 75.9, 41.0, 23.0, 18.9. Spectral data are in accordance with literature values.[27,31,32] GC (quantitative, GC 2010 Plus): t_r = 1.7 min. GC (analytical, GC 2010): t_r = 17.9 min for (*S*)-**1**, 18.2 min for (*R*)-**1**.

General Procedure for the Synthesis of (*R*)-1 using ISPR of Acetone under Enzyme Recycling. A 10 mL stirred cell, equipped with a 10 kDa MWCO membrane, was equilibrated by flushing with buffer (100 mM, pH 8). The cell was loaded with 350 mg (2 mmol) of precursor (*R*)-**2** (79% ee), 4.7 mL of buffer (100 mM, pH 8), and 190 μL of 2-propanol. To it were added 28 mg (2 mol %) of NAD^+ and 10 U/mmol ADH-200. A water/2-propanol (5 vol %) saturated air sparge was attached, and the reaction mixture was stirred for 2 h at rt. The solution was flushed from the cell by applying 35 psi of argon pressure and mixed with 1 mL of diluted hydrochloric acid. The mixture was extracted with ethyl acetate and separated via centrifugation (10000 rpm, 5 min, 15 °C) three times. The solvent was removed in vacuo. For each following cycle, the cell was again loaded with 2 mmol aldehyde **1**, 4.7 mL of buffer, 190 μL of 2-propanol, and 28 mg of NAD^+. No additional ADH was added. The reaction times, conditions, and workup remained unchanged as described above. The crude products were purified by column chromatography (chloroform/acetone 9:1 (v/v), 3.5 cm column diameter, 100 mL silica gel) to give (*R*)-**1** as a colorless solid. Conversion per cycle: 77 ± 2% (see Figure 6). Yield: 70%; ee: 95%.

For the analytical data, please refer to the section "General Procedure for the Enzymatic Synthesis of (R)-1 Using ISPR of Acetone".

Chemoenzymatic Synthesis of (*R*)-1. To a solution of 263 mg (1.28 mmol) of ethyl glyoxylate **3** and 184 mg (2.56 mmol) of isobutanal in 410 μL (5.1 mmol) 2-propanol were added 103 μL of 1.25 M catalyst solution containing L-histidine and acetic acid. The reaction mixture was stirred at 10 °C for 24 h prior to removing all volatile materials in vacuo. The highly viscous, yellow residue was emulsified in 1.875 mL of buffer (100 mM, pH 8) and 85 μL of 2-propanol. Then, 14 mg (2 mol %) of NAD^+ and 10 U/mmol ADH-200 were added, and a water/2-propanol (5 vol %) saturated air sparge was attached. The reaction mixture was stirred for 2 h prior to adding 1 mL of diluted hydrochloric acid. The mixture was extracted with ethyl acetate and separated via centrifugation (10000 rpm, 5 min, 15 °C) three times. The solvent was removed, and the crude product was purified by column chromatography (chloroform/acetone 9:1 (v/v), 3.5 cm column diameter, 100 mL silica gel) to give (*R*)-**1** as colorless solid with an overall yield of 55% (95% ee) with reference to ethyl glyoxylate **3** (see Scheme 6).

For the analytical data, please refer to the section "General Procedure for the Enzymatic Synthesis of (R)-1 Using ISPR of Acetone".

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C spectra, gas and HPL chromatograms of synthesized compounds, GC straight calibration lines used for quantitative analysis, scheme for ISPR settings, and figures illustrating the influence of several reaction parameters on conversion and enantioselectivity of the organocatalytic aldol reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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