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Asymmetric Chemoenzymatic One-Pot Synthesis of α-Hydroxy Half-Esters

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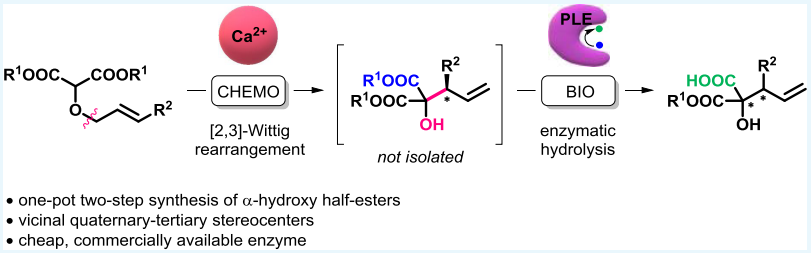

ABSTRACT: A new chemoenzymatic one-pot strategy has been developed for the synthesis of α-hydroxy half-esters containing consecutive quaternary and tertiary stereocenters using asymmetric cascade catalysis. In this study, an asymmetric Ca^{2+}-catalyzed [2,3]-Wittig rearrangement reaction was proven to be suitable for a combination with porcine liver esterase-mediated hydrolysis resulting in the enhanced enantiomeric purity of the obtained products in a one-pot synthesis compared to the stepwise method.

INTRODUCTION

An α-hydroxy-β-dicarbonyl moiety is a ubiquitous fragment in naturally occurring biomolecules and their precursors.[1] These densely functionalized motifs, including α-hydroxy half-esters are synthetically pliable building blocks and, therefore, are widely used as intermediates in numerous synthetic routes for the preparation of different pharmaceuticals.[2]

Despite the fact that stereoselective chemical transformations of α-hydroxy-β-dicarbonyl compounds have been intensively studied, most of the methods have been developed for preparation of α-hydroxy-β-ketoesters.[3] There have been only limited studies on the asymmetric synthesis of α-hydroxy half-esters, especially of α-hydroxy malonate derivatives. Both metal-catalyzed[4,5] and organocatalytic[6−8] methods have been exploited. The described methods show a synthetic path to obtain α-hydroxy diesters, which can be readily transformed to α-hydroxy half-esters by basic hydrolysis. On the other hand, biotransformations provide a wide range of opportunities to catalyze the desymmetrization of prochiral mono- and disubstituted diesters utilizing both transesterification reactions and hydrolysis. The most suitable and commonly used class of enzymes for these transformations are hydrolases. Broad substrate specificity and high stereoselectivity, in addition to the fact that hydrolases are mostly commercially available and do not require additional cofactors, have made hydrolases widely used catalysts in asymmetric synthesis.[9] Despite all of these tremendous advantages of hydrolases, the literature currently lacks proper coverage of synthetic pathways for obtaining α-hydroxy half-esters enantioselectively utilizing enzymes. Among the few reported methods, α-hydroxy half-esters were prepared enantioselectively with methodologies proposed by the Tamm[10] and Kikelj[11] groups. All of the described examples provide attractive methods to synthesize α-hydroxy half-esters, although containing only one stereogenic center.

One-pot reactions have been demonstrated to be environmentally sustainable and economically viable techniques as no isolation of the intermediate is required, which leads to increased efficiency due to the reduction of energy and time consumption and the production of waste.[12] Combinations of asymmetric organocatalytic or metal-catalytic reactions with biotransformation in a chemoenzymatic one-pot process offer a great potential to achieve this goal. However, the possible incompatibility of the different reagents or solvents used to catalyze one of the steps and the corresponding reaction conditions with an enzyme make chemoenzymatic one-pot reactions challenging.[13,14]

To the best of our knowledge, a systematic study of the creation of multiple stereogenic centers containing α-hydroxy half-esters has not been reported in the literature. In our ongoing endeavor to achieve this goal, herein, we report the asymmetric chemoenzymatic one-pot strategy for the formation of α-hydroxy half-esters bearing vicinal quaternary and tertiary stereocenters. We combine our previous study of the

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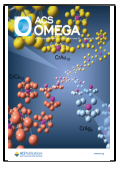

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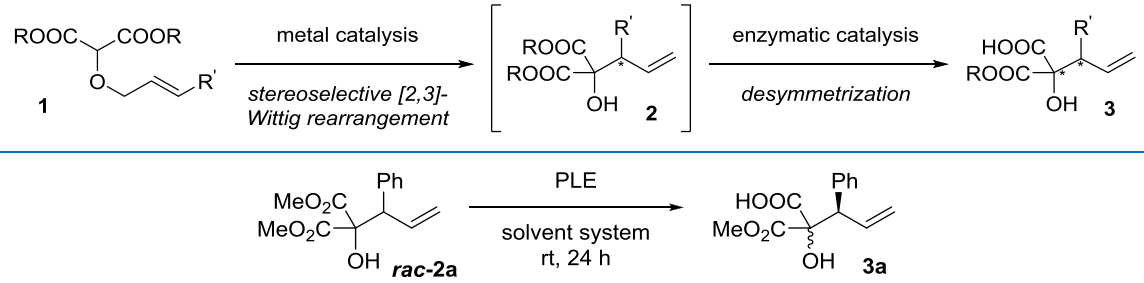
Scheme 1. Chemoenzymatic Approach to Half-Esters 3



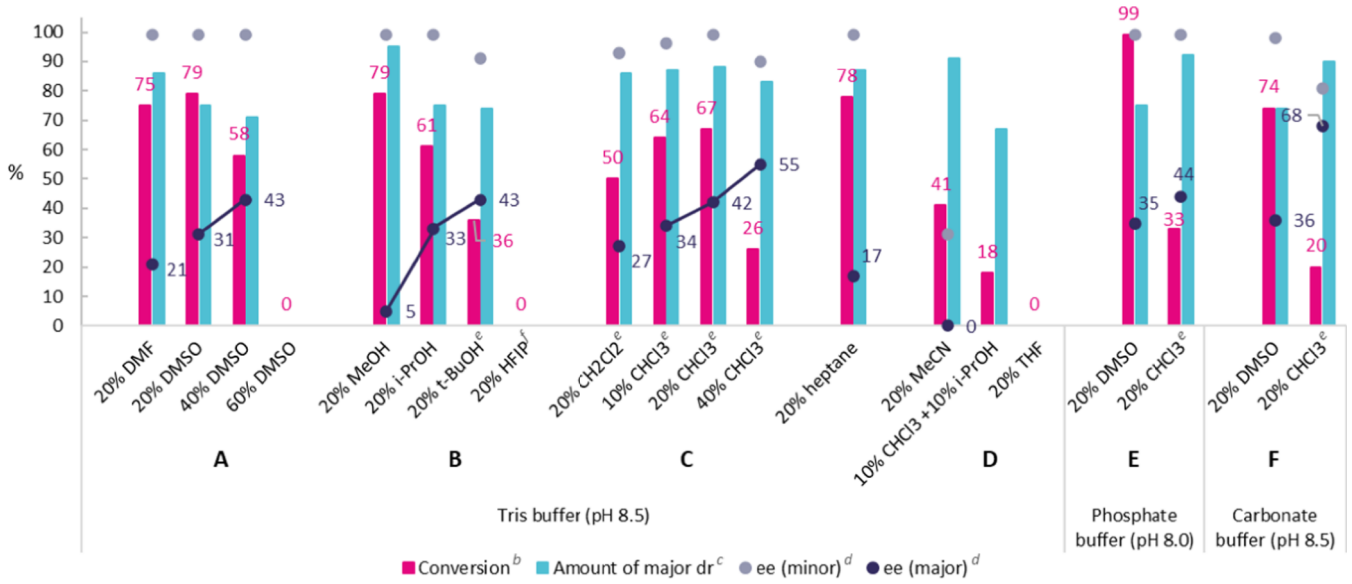


Figure 1. (A–F) Effect of co-solvents on the PLE-mediated hydrolysis of ***rac*-2a**.[a] ([a]Reaction conditions: 0.1 mmol scale, 85 EU of crude PLE, solvent system (0.2 M). [b]Conversion was determined by ^{1}H NMR analysis of the crude mixture and referred to the ratio of starting material and product. [c]Diastereoisomeric ratio was determined by ^{1}H NMR analysis of the crude mixture. [d]Enantiomeric excess was determined by chiral high-performance liquid chromatography (HPLC) analysis of the sample obtained by preparative thin-layer chromatography (TLC). [e]Reaction was completed after 72 h. [f]HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol).

metal-catalyzed asymmetric [2,3]-Wittig rearrangement of allyloxy malonates **1**[15] with the following enzymatic desymmetrization of sterically demanding α-substituted diesters **2** (Scheme 1). The Wittig rearrangement affords α-hydroxy malonic derivative **2** with a single tertiary stereogenic center. In the second step of the sequence, a quaternary stereogenic center is formed affording product **3** with two contiguous highly crowded stereocenters.

RESULTS AND DISCUSSION

In our initial study, we set the benchmarks for the asymmetric [2,3]-Wittig rearrangement reaction and enzymatic hydrolysis separately. As the asymmetric [2,3]-Wittig rearrangement reaction was based on our previous experience, we first investigated the enzymatic hydrolysis of the racemic [2,3]-Wittig rearrangement reaction product ***rac*-2a** (Figure 1). Of a number of widely used hydrolase family enzymes,[16] we tested immobilized lipase B from *Candida antarctica* (CALB), esterase from porcine liver (PLE), lipase from porcine liver (PLL), lipase from *Candida rugosa* (CRL), and lipase from *Rhizomucor miehei* (RML). For the enantioselective desymmetrization of the racemic rearrangement product **2a**, only PLE showed catalytic activity (the conversion of the starting material under unoptimized conditions in phosphate buffer was 34%) and it was used in all further experiments (see Table S1, Supporting Information (SI)). From the screening of the most commonly used buffers at different pH values, phosphate buffer (pH 8.0, 0.2 M), Tris buffer (pH 8.5, 0.2 M), and carbonate buffer (pH 8.5, 0.2 M) were selected as starting points for the optimization of the reaction conditions. Of them, Tris buffer showed a greater diastereoisomeric ratio (dr, 1:8) with comparably high conversion (91%), and therefore it was chosen as the primary buffer (see Table S2, SI). Additionally, the influence of organic co-solvents on the rate and the selectivity of the hydrolysis step was evaluated (Figure 1). In PLE-catalyzed hydrolysis, in general, the presence of the co-solvent inhibited the enzyme activity but the stereoselectivity was very dependent on the co-solvent used. The enantioselectivity of the minor diastereoisomer was high (>90%) in almost all cases, but the enantiomeric excess (ee) of the major diastereoisomer was influenced more. The addition of 20% of dimethylformamide (DMF) or dimethyl sulfoxide (DMSO) gave a similar conversion, but the latter showed slightly higher enantioselectivity for the major diastereoisomer (ee 31%) (Figure 1A). The enantiomeric purity of the major diastereoisomer was increased using 40% of DMSO (ee 41%), although it decreased the reaction rate significantly. Upon further increasing the DMSO percentage to 60%, no

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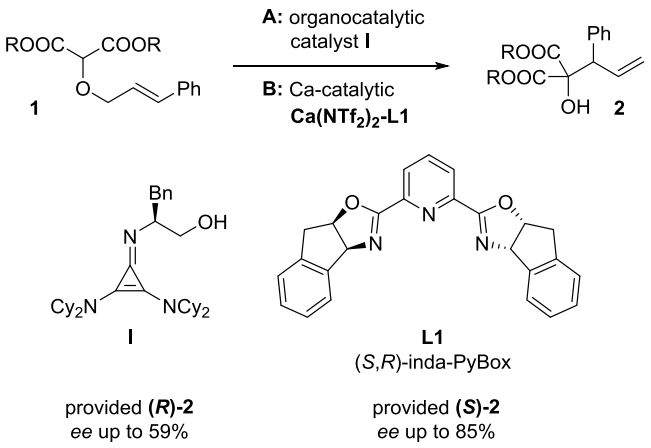
reaction was observed. When alcohols were employed, there was a strong correlation between the reaction rate, selectivity, and the bulkiness of the alcohol used (Figure 1B). The reaction in MeOH proceeded with higher conversion, diastereoselectivity, and enantioselectivity of the minor diastereoisomer than the reaction in *t*-BuOH, although the enantioselectivity of the major diastereoisomer decreased significantly (ee 5%). Surprisingly, chlorinated solvents were found to be compatible with the biotransformation, although they led to longer reaction times (Figure 1C). Also, as in the case of DMSO, a larger amount of $CHCl_3$ caused a decrease in the rate of reaction, while it increased the ee of the major diastereoisomer (ee 55%). In combination with Tris buffer at pH 8.4, 20% of heptane was able to enhance the enzyme activity resulting in 78% of conversion in 2 h, but, unfortunately, the ee of the major diastereoisomer was only 17%. Other co-solvents or combinations of them (shown in Figure 1D) either showed a detrimental effect on the enantioselectivity of the hydrolysis or did not have a reaction at all.

Our preliminary results demonstrated that enzymatic reactions using an 8:2 ratio of Tris buffer/co-solvent (DMSO or $CHCl_3$) showed an optimal conversion-to-ee ratio. Thus, similar conditions were applied to phosphate (pH 8.0) and carbonate (pH 8.5) buffers (Figure 1E,F, respectively). The reaction in phosphate buffer/DMSO reached full conversion within 24 h without a decrease in the enantioselectivity of the major diastereoisomer compared to Tris buffer.

Since the desymmetrization of the racemic Wittig product ***rac*-2a** via enzymatic hydrolysis gave poor results, we studied a one-pot process where the first rearrangement reaction afforded an enantiomerically enriched rearranged product **2**. This was achievable by exploiting our earlier study where we used either an organocatalytic approach or a Ca^{2+}-catalyzed system in the [2,3]-Wittig rearrangement reaction (Scheme 2).[15] Having observed the inhibition of enzyme with

Scheme 2. Cyclopropenimine and Ca^{2+}-Catalyzed [2,3]-Wittig Rearrangement Reaction

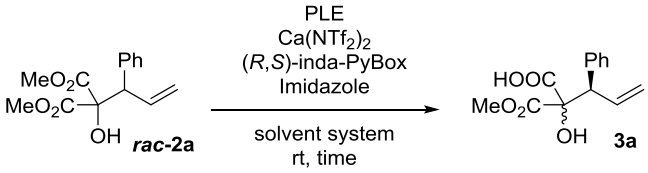


unacceptably low enantioselectivity (see Tables S3 and S4, SI) in a one-pot synthesis conducted using cyclopropenimine catalyst **I** in the first step, we moved on to the combination of the Ca^{2+}-catalyzed [2,3]-Wittig rearrangement reaction with hydrolysis.

Implementation of Ca^{2+}-Catalyzed [2,3]-Wittig Rearrangement/PLE-Mediated Desymmetrization in a One-Pot Reaction. First, an investigation of the Ca^{2+}-catalytic system compatibility with PLE-mediated hydrolysis was performed. In our earlier study,[15] Ca^{2+}-catalyzed [2,3]-Wittig rearrangement reactions were conducted in *i*PrOH. However, *i*PrOH caused transesterification of the starting material **1** and the intermediate **2**, which also participated in the hydrolysis step, thereby complicating the purification process of product **3**. To exclude the formation of these side products, we rescreened the solvents in the [2,3]-Wittig rearrangement reaction of **1a** to **2a**. As a result, MeOH showed equal activity to *i*PrOH, without the formation of any side products, although low enantioselectivity was observed (*i*PrOH showed 75% ee, and MeOH showed 24% ee; see Table S5, SI). Thus, to reach quantitative conversion and to avoid the formation of the transesterification products $Ca(NTf_2)_2$, (*R*,*S*)-inda-PyBox, and imidazole in MeOH were further used in the first step.

To this end, we carried out experiments to verify whether the Ca-inda-PyBox catalytic system used to perform the [2,3]-Wittig rearrangement reaction was compatible with PLE. For this, PLE-mediated hydrolysis was conducted in the selected solvent systems using the isolated racemic [2,3]-Wittig rearrangement reaction product ***rac*-2a** as a starting material in the presence of a catalytic amount of $Ca(NTf_2)_2$, (*R*,*S*)-inda-PyBox, and imidazole. The Tris buffer (pH 8.5)/$CHCl_3$ biphasic system was found to be ineffective by showing total inhibition of the enzyme activity (Table 1, entry 1; compare with Figure 1). We were delighted to find that PLE is sufficiently active in the phosphate buffer (Table 1, entries 2 and 3).

Table 1. Ca^{2+}-Catalytic System Compatibility with PLE[a]



entry	solvent system	time (h)	conversion (%)[b]	dr[c]	ee (%)[d] maj/min
1	Tris buffer (pH 8.5)/20% $CHCl_3$	72	7	n.d.[e]	n.d.[e]
2	phosphate buffer (pH 8.0)	24	75	7:1	13/99
3	phosphate buffer (pH 8.0)/20% DMSO	24	100	4:1	30/99

[a]Reaction conditions: 0.1 mmol scale, 5 mol % $Ca(NTf_2)_2$, 5 mol % (*R*,*S*)-inda-PyBox, 5 mol % imidazole, 85 EU of crude PLE, and the solvent system (0.2 M). [b]Conversion was determined by ^{1}H NMR analysis of the crude mixture and referred to the ratio of the starting material and the product. [c]Diastereoisomeric ratio was determined by ^{1}H NMR analysis of the crude mixture. [d]Enantiomeric excess was determined by the chiral HPLC analysis of the sample obtained by preparative TLC; maj, major diastereoisomer and min, minor diastereoisomer. [e]n.d., not determined.

We then combined a Ca^{2+}-catalyzed [2,3]-Wittig rearrangement reaction and hydrolysis in a consecutive one-pot process. The results presented in Table 2 show the influence of the solvent system used in the second step on the rate and the selectivity of the one-pot reaction. During the one-pot synthesis, intermediate **2a** was not isolated, although solvent

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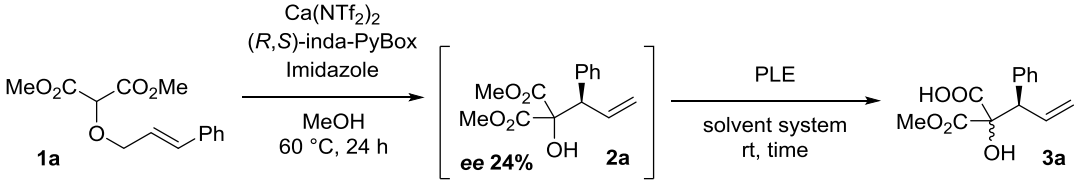
Table 2. Combination of a Ca^{2+}-Catalyzed [2,3]-Wittig Rearrangement Reaction with PLE-Mediated Hydrolysis in a One-Pot Reaction[a]



		conversion (%)[b]			ee (%)[d]
entry	solvent system	24 h	72 h	dr[c]	maj/min
1	carbonate buffer (pH 8.5)/20% $CHCl_3$	7	8	4.5:1	n.d.[e]
2	Tris buffer (pH 8.5)	69	81	6.3:1	51/95
3	Tris buffer (pH 8.5)/20% DMSO	78	83	4:1	64/96
4	carbonate buffer (pH 8.5)	47	86	3:1	64/99
5	carbonate buffer (pH 8.5)/20% DMSO	79[f]		4.3:1	59/99
6	carbonate buffer (pH 8.5)/20% DMSO (5 °C)	72[f]		4.5:1	57/99
7	phosphate buffer (pH 8.0)	36	46	7.6:1	63/99
8	phosphate buffer (pH 8.0)/20% DMSO	9	43	3.6:1	85/99
9	phosphate buffer (pH 8.0)/20% DMSO (35 °C)	100		2.9:1	73/99

[a]Reaction conditions: 0.1 mmol scale, 5 mol % $Ca(NTf_2)_2$, 5 mol % (*R*,*S*)-inda-PyBox, 5 mol % imidazole, and MeOH (0.1 M) were stirred at 60 °C for 24 h, followed by solvent evaporation and addition of 85 EU of crude PLE; solvent system (0.2 M). [b]Conversion was determined by ^{1}H NMR analysis of the crude mixture and referred to the ratio of starting material and the product. [c]Diastereoisomeric ratio was determined by ^{1}H NMR analysis of the crude mixture. [d]Enantiomeric excess was determined by chiral HPLC analysis of the sample obtained by preparative TLC; maj, major diastereoisomer and min, minor diastereoisomer. [e]n.d., not determined. [f]Reaction conversion did not change after 2 h.

exchange was performed after the first step was complete (such a multistep process is still considered a one-pot reaction).[12,17] Hence, relying on our preliminary screening of buffers and co-solvents (see Table S2, SI; and Figure 1), we conducted the reactions in Tris (pH 8.5), carbonate (pH 8.5), and phosphate (pH 8.0) buffers. It was confirmed that the hydrolysis step could not be carried out in the biphasic system using a carbonate buffer (pH 8.5)/$CHCl_3$ solvent system (Table 2, entry 1). High conversion was achieved using Tris buffer (pH 8.5) without co-solvent as the reaction medium, as well as with DMSO, but in both cases product **3a** was formed in moderate enantioselectivity of the major diastereoisomer (ee 51 and 64%, respectively) (Table 2, entries 2 and 3). The reaction carried out in a carbonate buffer (pH 8.5) showed a moderate reactivity reaching 86% conversion in 72 h with the enantioselectivity of the major diastereoisomer of 64% (Table 2, entry 4). In addition to the enhancement of enzyme activity, higher diastereoselectivity was observed in the reaction utilizing DMSO as a co-solvent. However, it led to a lower ee of the major diastereoisomer (Table 2, entry 5). Surprisingly, a decrease in temperature did not influence the results obtained (Table 2, entry 6). The use of a phosphate buffer (pH 8.0) and a phosphate buffer (pH 8.0)/DMSO system decreased the rate of the reaction. However, promising enantioselectivity of the major diastereoisomer (85%) was detected (Table 2, entries 7 and 8). Therefore, by increasing the temperature to 35 °C, we were now able to run the one-pot reaction with full conversion (Table 2, entry 9).

To obtain a better understanding of the reaction, we decided to run the one-pot synthesis in optimal conditions and to follow the hydrolysis step using ^{1}H NMR. By monitoring the progress of the hydrolysis step (after the completion of the first step), we found that the reaction exhibited a sigmoidal curve leading to full conversion in 24 h (Figure 2a). For comparison, the kinetic study of the enzymatic reaction of isolated [2,3]-Wittig rearrangement product **2a** (ee 25%) was carried out under the same reaction conditions (Figure 2b). In this case, the reaction reached full conversion in 3 h. The presence of a lag phase and extended reaction time (Figure 2a) suggests that the inhibition of the enzyme occurred during the hydrolysis step in the one-pot reaction. However, it promotes the stereoselectivity of the reaction affording the major diastereoisomer in noticeably higher enantiomeric purity than in the case of the hydrolysis of the isolated [2,3]-Wittig rearrangement product **2a** (ee 73 and 52%, respectively).

Having successfully developed a one-pot procedure, the substrate scope was evaluated using $Ca(NTf_2)_2$, (*R*,*S*)-inda-PyBox, and imidazole in MeOH at 60 °C for the first step and PLE in a phosphate buffer (pH 8.0) with 20% of DMSO at 35 °C for the second step (Scheme 3). First, we used (*R*,*S*)- and (*S*,*R*)-inda-PyBox ligands for the consecutive one-pot reaction to gain access to both enantiomers of the rearrangement intermediate **2a**. In the case of intermediate (*S*)-**2a**, the hydrolysis step was slightly slower than expected compared to the hydrolysis with its enantiomer (*R*)-**2a**. Additionally, the enantiomeric excess of the major diastereoisomer of product **3a** from the reaction with intermediate (*S*)-**2a** dropped to 27% compared to 73% of *R*-enantiomer. From the obtained results it was clear that the PLE preferred *R*-enantiomer of compound **2a** providing notably higher ee of the major diastereoisomer in a shorter time; thus, the following one-pot reactions were carried out using (*R*,*S*)-inda-PyBox in the first step.

The aromatic substitution pattern in substrates **1a**−**i** did not affect the results of the [2,3]-Wittig rearrangement step substantially (full conversion was achieved in 24 h, and the ee of the intermediates **2a**−**i** were in the range 14−24%; detailed information in Table S7, SI), although the results of the hydrolysis step were dependent on it.

The hydrolysis step of *o*-chlorophenyl derivative **2b** was less efficient compared to the reaction with unsubstituted **2a** and reached only 56% of conversion in 48 h. Surprisingly, product **3b** was isolated with excellent diastereoselectivity but with low enantiomeric excesses of both diastereoisomers. Reactions with *m*- and *p*-chlorophenyl substituted intermediates **2c** and **2d**

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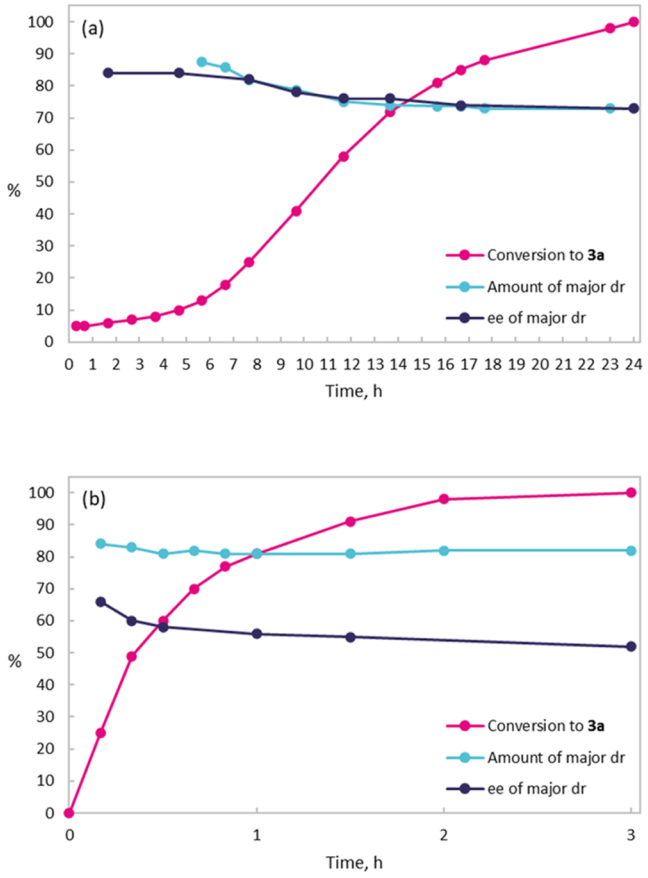

Figure 2. (a) Reaction profile for the hydrolysis step of the one-pot reaction without isolation of the [2,3]-Wittig rearrangement product **2a**. Enantiomeric excess of the minor diastereoisomer remained 99% during the reaction. (b) Reaction profile for the hydrolysis reaction using isolated [2,3]-Wittig rearrangement product **2a** as the starting material. Enantiomeric excess of the minor diastereoisomer remained 99% during the reaction.

stopped before reaching full conversion, affording products **3c** and **3d** with 68 and 64% yields, respectively. However, similar diastereo- and enantioselectivities were detected. Compounds **1e** and **1f** with electron-donating and electron-withdrawing substituents were tolerated under the reaction conditions, but in both cases, the hydrolysis step was slower. While the electron-donating substituent had no notable influence on the selectivities of the obtained product **3e**, the electron-withdrawing nitro group containing intermediate **2f** provided product **3f** with a diminished enantiomeric purity of both diastereoisomers. A significant decrease in the rate of the hydrolysis step was detected using bulkier naphthyl- and indole-substituted intermediates **2g** and **2h** instead of the phenyl substituent. Surprisingly, the naphthyl-derived product **3g** was obtained with even better selectivity, although in the case of the indole-derived product **3h**, the enantioselectivities of both diastereoisomers dropped drastically. It was assumed that due to variation in the spatial arrangement of the substituents (2-naphtyl and 1-indolyl), substrates fit the active site of the enzyme differently, which led to differences in enantioselectivity. When a heteroaromatic derivative **1i** was used as a starting material, a full conversion in the hydrolysis step was achieved within 24 h, resulting in product **3i** with higher diastereoselectivity (6.7:1), although, unfortunately, the enantiomeric purity of the major diastereoisomer decreased. In the case of crotyl-substituted substrate **1j**, an unreasonably long reaction time (7 days) was required for the [2,3]-Wittig rearrangement reaction. The conversion of the hydrolysis step reached a plateau at 93% in 24 h, but the one-pot product **3j** was isolated with a moderate 62% yield due to its instability during workup. The absence of the aromatic ring strongly affected the selectivities of the obtained product, leading to diminished diastereo- and enantioselectivities.

The rearrangement-hydrolysis protocol of other malonic esters was studied briefly. Due to the formation of transesterification products in the [2,3]-Wittig rearrangement step, the solvents were rescreened with substrates **1k** and **1l** (see Table S6, SI), indicating EtOH and *i*PrOH as the optimal solvents, respectively. The reaction with diethyl malonic derivative **1k** proceeded smoothly, providing half-ester **3k** with high diastereo- and enantioselectivities. In this case, higher enantioselectivity (ee 55%) of the intermediate **2k** was observed in the first step, which influenced the selectivities of the obtained one-pot product **3k**. Bulky dibenzyl derivative **1l** showed the highest enantioselectivity in the [2,3]-Wittig rearrangement, but unfortunately, only 17% of conversion was observed in the hydrolysis step within 72 h. The mixed ester **1m** did not reach full conversion in the first step even in 5 days; therefore, a mixture of the starting material **1m** and intermediate **2m** was used in the hydrolysis step leading to no reaction. The additional substituent in the double bond suppressed the reactivity of the starting material **1n**; thus, only 24% of conversion in the [2,3]-Wittig rearrangement reaction was detected.

Unfortunately, we were not able to determine the absolute configuration of product **3**. All our attempts to crystallize product **3** or amides derived from them using various solvent systems at different temperatures, slow evaporation technique, vapor diffusion method, seeding procedure, as well as co-crystallization and the crystalline sponge method with metal–organic frameworks[18] were unsuccessful. Also, we performed a conformational analysis and found that differences in energies of enantiomers were too small to determine the preferred configuration.

To show the synthetic utility of the proposed method, compound **3a** was converted into amide with benzylamine in the presence of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) in nearly quantitative yield (Scheme 4). The diastereoisomers of the obtained amide were chromatographically separable affording single enantiomers in high-to-very-high enantiomeric purities.

CONCLUSIONS

We developed an effective one-pot method by combining an asymmetric Ca^{2+}-catalyzed [2,3]-Wittig rearrangement reaction with an enzymatic desymmetrization of malonic esters. Such an implementation of the cascade catalysis in a one-pot manner provided access for the synthesis of vicinal quaternary and tertiary stereogenic centers containing α-hydroxy half-esters in high yields and good enantioselectivities. Different α-branched sterically demanding esters could be easily desymmetrized to the corresponding half-esters using the same one-pot procedure. Due to the compatibility of the Ca^{2+}-catalytic system with PLE, no isolation of the intermediate was required. In addition, the hydrolysis step is efficiently catalyzed by commercially available, crude PLE; thus, no expensive

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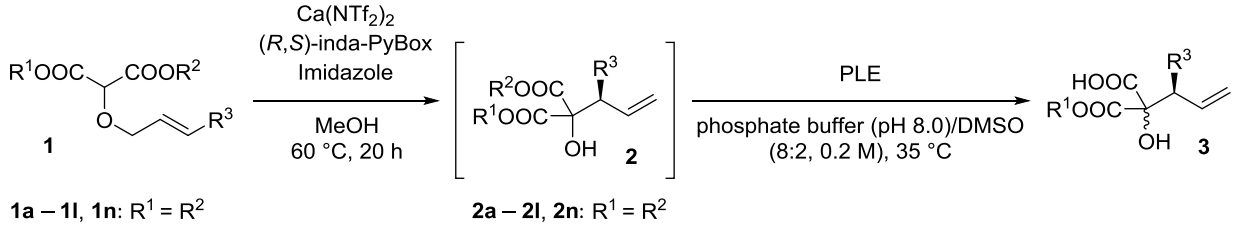
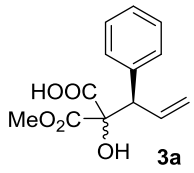
Scheme 3. Scope of the Reaction[a–l]

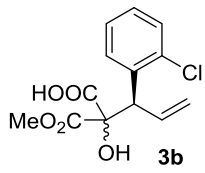




3a

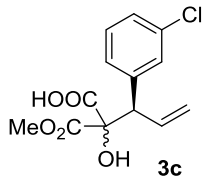
*via (R)-**2a***
yield 85%[b]
dr 2.9:1
ee 73%/99%

*via (S)-**2a***
yield 81%[c,d]
dr 1.6:1
ee 27%/99%



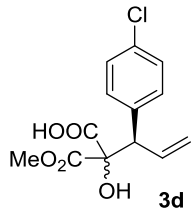
3b

yield 46%[d]
dr 20:1[e]
ee 31%/46%



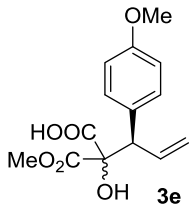
3c

yield 68%[b]
dr 2.6:1
ee 73%/99%



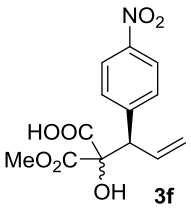
3d

yield 64%[b]
dr 2.9:1
ee 75%/96%



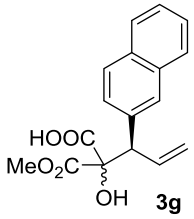
3e

yield 82%[d]
dr 2.5:1
ee 72%/97%



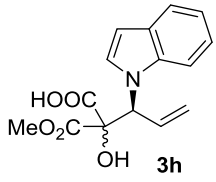
3f

yield 71%[d]
dr 2.7:1
ee 65%/91%



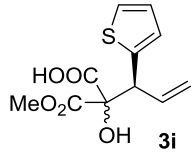
3g

yield 43%[d]
dr 2.6:1
ee 81%/99%



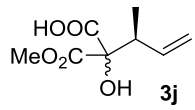
3h

yield 45%[d,f]
dr 3:1
ee 28%/39%



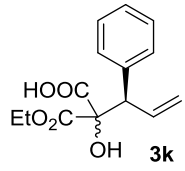
3i

yield 86%[b]
dr 6.7:1
ee 40%/99%



3j

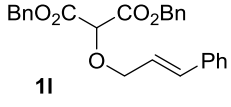
yield 62%[b,g]
dr 1.4:1
ee 64%/61%



3k

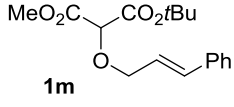
yield 46%[d,h]
dr 8:1
ee 73%/99%

limitations



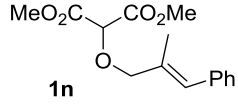
1l

step 1: conv 100%[i]
ee 78%
step 2: conv 17%[j]



1m

step 1: conv 85%[k]
dr 4.7:1
step 2: nr



1n

step 1: conv 24%[l]

[a]Reaction conditions: 0.3 mmol scale, 5 mol % $Ca(NTf_2)_2$, 5 mol % (*R*,*S*)-inda-PyBox, 5 mol % imidazole, and MeOH (0.1 M) were stirred at 60 °C for 20 h (if not stated otherwise), followed by solvent evaporation and addition of 255 EU of crude PLE, phosphate buffer (pH 8.0)/DMSO (8:2, 0.2 M). Diastereoisomeric ratio was determined by ^{1}H NMR analysis of the crude mixture. Enantiomeric excess was determined by chiral HPLC of the isolated product. Major diastereoisomers are depicted in the scheme. [b]Hydrolysis was completed after 24 h when the plateau phase was reached. [c](*S*,*R*)-Inda-PyBox was used. [d]Hydrolysis was completed after 48 h when the plateau phase was reached. [e]Diastereoisomeric ratio of the isolated product is presented. [f]Reaction was conducted in 0.1 mmol scale. [g]The [2,3]-Wittig rearrangement reaction was completed after 7 days. [h]The [2,3]-Wittig rearrangement reaction was conducted in absolute EtOH. [i]The [2,3]-Wittig rearrangement reaction was conducted in *i*PrOH. [j]Conversion of the hydrolysis was determined after 72 h. [k]The [2,3]-Wittig rearrangement reaction was stopped after 5 days. [l]Conversion of the [2,3]-Wittig rearrangement reaction was determined after 48 h.

purified isoenzyme forms nor modification of the enzyme were needed.

EXPERIMENTAL SECTION

Full assignment of ^{1}H and ^{13}C chemical shifts is based on the one-dimensional (1D) and two-dimensional (2D) Fourier transform (FT) NMR spectra measured on a Bruker Avance III 400 MHz instrument. Residual solvent signals were used [$CDCl_3$ δ = 7.26 (^{1}H NMR), 77.16 (^{13}C NMR), and DMSO-d_6 δ = 2.50 (^{1}H NMR), 39.52 (^{13}C NMR)] as internal standards. All peak assignments are confirmed by 2D experiments (^{1}H–^{1}H correlated spectroscopy (COSY), ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC), ^{1}H–^{13}C heteronuclear multiple bond correlation (HMBC)). In ^{13}C NMR, 2C in brackets refers to either two chemically equivalent or two overlapping unique carbon signals. The determination of the diastereomeric ratio in the reaction mixture was based on CH_2 integrals of the double bonds (^{1}H NMR in DMSO-d_6). If possible, the obtained ratio of diastereomers was also determined using the CH integrals of

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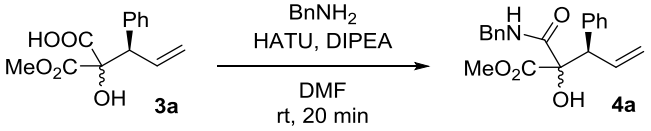
ACS Omega 2021, 6, 20686–20698

Scheme 4. Amidation of Compound 3a[a]



[a]Reaction conditions: 0.2 mmol scale, 1.1 equiv of $BnNH_2$, 1.1 equiv of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxid hexafluorophosphate (HATU), and 1.1 equiv of *N*,*N*-diisopropylethylamine (DIPEA) in DMF (0.1 M) were stirred at room temperature (rt) for 20 min. Diastereoisomeric ratio was determined by ^{1}H NMR analysis of the crude mixture. Enantiomeric excess was determined by chiral HPLC of the isolated diastereoisomers of product **4a**.

the double bonds or CHAr integrals. The determination of the diastereomeric ratio of the isolated products was based on $COOCH_3$ integrals (^{1}H NMR in $CDCl_3$). High-resolution mass spectra (HRMS) were recorded using an Agilent Technologies 6540 UHD Accurate-Mass Q-TOF LC/MS spectrometer and electrospray ionization (ESI). Chiral HPLC was performed using Chiralpak AD-H (250 × 4.6 mm) and Chiralcel OJ-H (250 × 4.6 mm) columns. In the case of carboxylic acids **3**, trifluoroacetic acid (TFA) was used as a mobile phase additive in chiral HPLC. Absolute ethanol (ABSE) was used as received. Precoated silica gel 60 F254 plates were used for TLC. Column chromatography was performed on a Biotage Isolera Prime preparative purification system with silica gel Kieselgel 40−63 μm. Purchased chemicals and solvents were used as received. Petroleum ether (PE) had a boiling point of 40−60 °C. Esterase from porcine liver (PLE, lyophilized powder; 18 units/mg, 20 units/mg, 24 units/mg) was purchased from Sigma-Aldrich. The reactions were performed under an air atmosphere without additional moisture elimination unless stated otherwise.

Ligands (*S*,*R*)- and (*R*,*S*)-inda-PyBox were prepared according to the literature procedures.[19]

Synthesis of Starting Materials 1a−n. Compounds **1a−g**, **1i**, and **1l** were synthesized according to the procedure previously described in the literature. The analytical data of compounds **1a−g**, **1i**, and **1l** are in agreement with the literature data.[15]

The same general procedure was used for the synthesis of compounds **1h**, **1j**, **1k**, **1m**, and **1n**, which is described below.

General Procedure. Rhodium(II) acetate dimer (0.005 equiv) and dichloromethane (DCM) (0.4 M) were added to an alcohol (1.2 equiv) under an argon atmosphere. The corresponding 2-diazomalonate (1 equiv) solution in DCM (0.4 M) was added for over 5 min at 0 °C. The reaction was stirred overnight at rt. The solvent was removed under reduced pressure, and the crude mixture was purified by column chromatography on silica gel.

Dimethyl (E)-2-((3-(1H-Indol-1-yl)allyl)oxy)malonate ***1h.*** Indole (1171 mg, 10 mmol) and sodium *tert*-butoxide (481 mg, 5 mmol) were added to DMF (63 mL, 0.16 M) under an argon atmosphere for 30 min. Then, ethyl propionate (1.21 mL, 12 mmol) was added for over 2 min, and the reaction was stirred for 4 h at rt. The solvent was removed under reduced pressure and the crude mixture was purified twice by column chromatography on silica gel (3−5% EtOAc in DCM and 30−65% DCM in PE), providing ethyl (*E*)-3-(1*H*-indol-1-yl)-acrylate (570 mg, 26%) as a dark yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 14.0 Hz, 1H), 7.64−7.56 (m, 2H), 7.38 (d, J = 3.5 Hz, 1H), 7.33 (ddd, J = 8.4, 7.2, 1.2 Hz, 1H), 7.23 (ddd, J = 7.9, 7.2, 0.9 Hz, 1H), 6.73 (dd, J = 3.7, 0.8 Hz, 1H), 5.96 (d, J = 14.0 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 1.35 (t, J = 7.1 Hz, 3H). Analytical data are in agreement with the literature data.[20]

Diisobutylaluminum hydride (DIBAL-H) (1 M solution in toluene, 5.7 mL, 5.73 mmol) was added to an ethyl (*E*)-3-(1*H*-indol-1-yl)acrylate (561 mg, 2.61 mmol) solution in DCM (13 mL, 0.2 M) under an argon atmosphere at −78 °C. The reaction was stirred for 2 h at −78 °C. Then, the reaction mixture was quenched by 1 M aq HCl solution and extracted with DCM (6 × 15 mL). The combined organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (3−8% EtOAc in DCM), providing (*E*)-3-(1*H*-indol-1-yl)prop-2-en-1-ol (247 mg, 55%) as a dark orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64−7.60 (m, 1H, Ar−C4), 7.50−7.45 (m, 1H, Ar−C7), 7.39 (d, J = 3.4 Hz, 1H, Ar−C2), 7.30−7.22 (m, 2H, CHAr and Ar−C6), 7.17 (ddd, J = 8.0, 7.1, 1.0 Hz, 1H, Ar−C5), 6.63 (d, J = 3.4 Hz, 1H, Ar−C3), 5.92 (dt, J = 14.1, 6.6 Hz, 1H, $CHCH_2$), 4.36 (ddd, J = 6.7, 5.6, 1.3 Hz, 2H, CH_2OH), 1.47 (t, J = 5.7 Hz, 1H, OH). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 135.7, 129.2, 126.6, 124.0, 122.9, 121.4, 121.0, 112.1, 109.6, 105.3, 62.1. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{11}H_{12}NO$ 174.0913; found 174.0905.

Following the general procedure, starting from (*E*)-3-(1*H*-indol-1-yl)prop-2-en-1-ol (289 mg, 1.67 mmol) and dimethyl 2-diazomalonate (220 mg, 1.39 mmol), the crude product was purified by column chromatography (2−8% EtOAc in PE/DCM 3/1 mixture), providing dimethyl (*E*)-2-((3-(1*H*-indol-1-yl)allyl)oxy)malonate **1h** (60 mg, 14%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dt, J = 7.9, 1.1 Hz, 1H, Ar−C4), 7.49−7.44 (m, 1H, Ar−C7), 7.38 (d, J = 3.5 Hz, 1H, Ar−C2), 7.31−7.23 (m, 2H, CHAr and Ar−C6), 7.17 (ddd, J = 8.0, 7.1, 1.0 Hz, 1H, Ar−C5), 6.64 (d, J = 3.4 Hz, 1H, Ar−C3), 5.82 (dt, J = 14.2, 7.2 Hz, 1H, CH_2CH), 4.68 (s, 1H, OCH), 4.37 (dd, J = 7.1, 1.1 Hz, 2H, CH_2CH), 3.82 (s, 6H, $COOCH_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 167.1 (2C), 135.6, 129.3, 129.1, 123.8, 123.1, 121.4, 121.2, 109.6, 106.8, 105.9, 77.4, 70.2, 53.1 (2C). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{16}H_{17}NaNO_5$ 326.0999; found 326.0987.

Dimethyl (E)-2-(But-2-en-1-yloxy)malonate ***1j.*** Following the general procedure, starting from (*E*)-but-2-en-1-ol (185 mg, 2.57 mmol) and dimethyl 2-diazomalonate (338 mg, 2.14 mmol), the crude product was purified by column chromatography (2−8% EtOAc in PE/DCM 3/1 mixture), providing dimethyl (*E*)-2-(but-2-en-1-yloxy)malonate **1j** (176 mg, 41%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82−5.72 (m, 1H, $CHCH_3$), 5.62−5.53 (m, 1H, $CHCH_2$), 4.57 (s, 1H, OCH), 4.09 (dt, J = 6.6, 1.0 Hz, 2H, $CHCH_2$), 3.81 (s, 6H, $COOCH_3$), 1.75−1.69 (m, 3H, $CHCH_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 167.3 (2C), 132.7, 125.8, 77.3, 71.9, 53.1 (2C), 17.9. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_9H_{14}NaO_5$ 225.0733; found 225.0735.

Diethyl 2-(Cinnamyloxy)malonate ***1k.*** Following the general procedure, starting from (*E*)-3-phenylprop-2-en-1-ol (259 mg, 1.93 mmol) and diethyl 2-diazomalonate (300 mg, 1.61 mmol), the crude product was purified by column chromatography (0−2% diethyl ether in toluene), providing diethyl 2-(cinnamyloxy)malonate **1k** (136 mg, 29%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.36 (m, 2H), 7.35−7.29 (m, 2H), 7.28−7.23 (m, 1H), 6.63 (d, J = 16.1

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ACS Omega 2021, 6, 20686−20698

Hz, 1H), 6.29 (dt, J = 15.8, 6.5 Hz, 1H), 4.59 (s, 1H), 4.34 (dd, J = 6.5, 1.2 Hz, 2H), 4.32−4.22 (m, 4H), 1.29 (t, J = 7.2 Hz, 6H). Analytical data are in agreement with the literature data.[21]

1-(tert-Butyl) 3-Methyl 2-(cinnamyloxy)malonate ***1m.*** To a solution of tosyl azide (374 mg, 1.89 mmol) and triethylamine (0.3 mL, 2.15 mmol) in acetonitrile (ACN) (1.72 mL, 1 M) was added *tert*-butyl methyl malonate (300 mg, 1.72 mmol) in ACN (1.72 mL, 1 M) at 0 °C. The reaction mixture was warmed to rt and stirred for 24 h. After evaporating the solvent, the crude mixture was purified by column chromatography on silica gel (3−7% EtOAc in PE) providing 1-(*tert*-butyl) 3-methyl 2-diazomalonate (279 mg, 81%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 3H), 1.51 (s, 9H). Analytical data are in agreement with the literature data.[22]

Following the general procedure, starting from (*E*)-3-phenylprop-2-en-1-ol (224 mg, 1.67 mmol) and 1-(*tert*-butyl) 3-methyl 2-diazomalonate (279 mg, 1.39 mmol), after evaporating the solvent, the crude mixture was purified by column chromatography on silica gel (3% EtOAc in PE/DCM 3/1 mixture). The impure fractions were repurified by column chromatography on silica gel (2−4% EtOAc in PE/DCM 3/1 mixture), providing 1-(*tert*-butyl) 3-methyl 2-(cinnamyloxy)-malonate **1m** (total yield: 203 mg, 48%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.36 (m, 2H, ArH), 7.35−7.29 (m, 2H, ArH), 7.28−7.22 (m, 1H, ArH), 6.63 (d, J = 15.9 Hz, 1H, CHAr), 6.29 (dt, J = 15.9, 6.4 Hz, 1H, CH_2CH), 4.50 (s, 1H, OCH), 4.37−4.28 (m, 2H, CH_2CH), 3.79 (s, 3H, $COOCH_3$), 1.48 (s, 9H, *t*Bu). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 167.6, 165.7, 136.4, 134.7, 128.7 (2C), 128.2, 126.8 (2C), 124.3, 83.4, 78.3, 71.7, 52.8, 28.0 (3C). HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{17}H_{22}NaO_5$ 329.1359; found 329.1350.

Dimethyl (E)-2-((2-Methyl-3-phenylallyl)oxy)malonate ***1n.*** Following the general procedure, starting from (*E*)-2-methyl-3-phenylprop-2-en-1-ol (274 mg, 1.85 mmol) and dimethyl 2-diazomalonate (244 mg, 1.54 mmol), the crude mixture was purified by column chromatography (3−7% EtOAc in PE/DCM 3/1 mixture), providing dimethyl (*E*)-2-((2-methyl-3-phenylallyl)oxy)malonate **1n** (200 mg, 47%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.31 (m, 2H), 7.30−7.21 (m, 3H), 6.52 (s, 1H), 4.61 (s, 1H), 4.22 (d, J = 1.1 Hz, 2H), 3.82 (s, 6H), 1.93 (d, J = 1.4 Hz, 3H). Analytical data are in agreement with the literature data.[21]

General Procedure for the Chemoenzymatic One-Pot Synthesis of α-Hydroxy Half-Esters 3. To a solution of allyloxy 1,3-dicarbonyl compound **1** (0.3 mmol) in methanol (unless stated otherwise, 3 mL), $Ca(NTf_2)_2$ (0.015 mmol, 9 mg), (*R*,*S*)-inda-PyBox (0.015 mmol, 5.9 mg), and imidazole (0.015 mmol, 1 mg) were added. The reaction mixture was stirred at 60 °C until full conversion to the [2,3]-Wittig rearrangement product **2** was observed (^{1}H NMR). After evaporation of the solvent, the crude intermediate was suspended in DMSO (0.3 mL) and sodium phosphate buffer (pH 8.0, 1.2 mL). PLE (255 EU) was added, and the mixture was stirred at 35 °C until the plateau phase was reached. It was then acidified to pH 2 with 1 M aq HCl solution and extracted with diethyl ether (a drop of ethanol can be used to prevent emulsion formation). The combined organic layers were dried over $MgSO_4$. After filtration, the solvent was removed in vacuo, and the crude carboxylic acid was purified by column chromatography on silica gel (2% EtOAc in DCM (to elute the possible residual starting material), followed by 2% EtOAc in the DCM/formic acid 99/1 mixture), providing the desired product **3**. Since the diastereoisomers were chromatographically inseparable, a diastereomeric ratio after purification is shown. The enantioselectivities of intermediates **2** (see Table S7, SI) were determined by HPLC analysis according to the literature procedure[15] unless stated otherwise. The enantioselectivities were determined by HPLC analysis of the purified products **3**.

2-Hydroxy-2-(methoxycarbonyl)-3-phenylpent-4-enoic Acid ***3a.*** The title compound was synthesized following the general procedure from dimethyl 2-(cinnamyloxy)malonate **1a** (79 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 24 h. Compound **3a** was obtained as an off-white solid in 85% yield (64 mg); dr 2.9:1 (^{1}H NMR).

(3R)-Major Diastereoisomer. ee 73% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 39.0 min and t_R (minor) = 34.9 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.21 (m, 5H, ArH), 6.15 (ddd, J = 17.1, 10.2, 9.0 Hz, 1H, $CHCH_2$), 5.30−5.19 (m, 2H, CH_2), 4.32 (d, J = 9.0 Hz, 1H, CHAr), 3.69 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.8, 169.5, 137.3, 134.0, 129.0 (2C), 128.7 (2C), 128.0, 119.8, 83.1, 55.3, 54.3.

(3S)-Minor Diastereoisomer. ee 99% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 68.7 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.20 (m, 5H, ArH), 6.21−6.07 (m, 1H, $CHCH_2$), 5.22−5.17 (m, 2H, CH_2), 4.31 (d, J = 9.3 Hz, 1H, CHAr), 3.92 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.4, 169.3, 137.0, 134.7, 129.4 (2C), 128.6 (2C), 127.9, 119.1, 82.9, 55.2, 54.7.

HRMS (ESI) m/z: $[M - H]^-$ calcd for $C_{13}H_{13}O_5$ 249.0768; found 249.0772.

3-(2-Chlorophenyl)-2-hydroxy-2-(methoxycarbonyl)pent-4-enoic Acid ***3b.*** The title compound was synthesized following the general procedure from dimethyl (*E*)-2-((3-(2-chlorophenyl)allyl)oxy)malonate **1b** (90 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 48 h. Compound **3b** was obtained as an off-white solid in 46% yield (39 mg); dr 20:1 (^{1}H NMR).

(3R)-Major Diastereoisomer. ee 31% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 9:1, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 16.3 min and t_R (minor) = 20.7 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (dd, J = 7.7, 1.9 Hz, 1H, ArH), 7.37 (dd, J = 7.7, 1.6 Hz, 1H, ArH), 7.26−7.15 (m, 2H, ArH), 6.01 (ddd, J = 17.1, 10.1, 8.5 Hz, 1H, $CHCH_2$), 5.31−5.20 (m, 2H, CH_2), 5.09 (d, J = 8.5 Hz, 1H, CHAr), 3.66 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.2, 169.8, 135.3, 134.1, 133.7, 130.1, 129.9, 128.9, 127.2, 120.1, 82.5, 54.3, 49.5.

(3S)-Minor Diastereoisomer. ee 46% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 9:1, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 26.0 min and t_R (minor) = 24.1 min].

HRMS (ESI) m/z: $[M - H]^-$ calcd for $C_{13}H_{12}ClO_5$ 283.0379; found 283.0384.

3-(3-Chlorophenyl)-2-hydroxy-2-(methoxycarbonyl)pent-4-enoic Acid ***3c.*** The title compound was synthesized following the general procedure from dimethyl (*E*)-2-((3-(3-

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ACS Omega 2021, 6, 20686−20698

chlorophenyl)allyl)oxy)malonate **1c** (90 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 24 h. Compound **3c** was obtained as a yellow solid in 68% yield (58 mg); dr 2.5:1 (^{1}H NMR).

(3R)-Major Diastereoisomer. ee 73% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 97:3, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 89.6 min and t_R (minor) = 118.9 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.31 (m, 1H, CCHCCl), 7.29−7.19 (m, 3H, ArH), 6.10 (ddd, J = 16.9, 10.2, 9.0 Hz, 1H, C*H*CH$_2$), 5.31−5.20 (m, 2H, CH$_2$), 4.30 (d, J = 9.0 Hz, 1H, CHAr), 3.72 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.7, 169.6, 139.4, 134.4, 133.5, 129.9, 129.2, 128.1, 127.2, 120.3, 82.8, 54.7, 54.4.

(3S)-Minor Diastereoisomer. ee 99% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 97:3, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 77.4 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.41 (m, 1H, CCHCCl), 7.29−7.19 (m, 3H, ArH), 6.12−6.00 (m, 1H, C*H*CH$_2$), 5.26−5.15 (m, 2H, CH$_2$), 4.29 (d, J = 9.4 Hz, 1H, CHAr), 3.91 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.1, 169.4, 139.2, 134.3, 134.2, 129.85, 129.6, 128.0, 127.8, 119.6, 82.6, 54.8, 54.6.

HRMS (ESI) m/z: [M − H]$^-$ calcd for $C_{13}H_{12}ClO_5$ 283.0379; found 283.0383.

3-(4-Chlorophenyl)-2-hydroxy-2-(methoxycarbonyl)pent-4-enoic Acid ***3d***. The title compound was synthesized following the general procedure from dimethyl (*E*)-2-((3-(4-chlorophenyl)allyl)oxy)malonate **1d** (90 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 24 h. Compound **3d** was obtained as a white solid in 64% yield (55 mg); dr 2.4:1 (^{1}H NMR).

(3R)-Major Diastereoisomer. ee 75% [Chiralpak OJ-H, hexane (TFA 0.01%)/*i*PrOH = 97:3, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 35.1 min and t_R (minor) = 32.4 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.21 (m, 4H, ArH), 6.08 (ddd, J = 16.9, 10.2, 8.9 Hz, 1H, C*H*CH$_2$), 5.27−5.18 (m, 2H, CH$_2$), 4.29 (d, J = 8.9 Hz, 1H, CHAr), 3.68 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.2, 169.6, 135.9, 133.9, 133.82, 130.4 (2C), 128.9 (2C), 120.0, 82.8, 54.4 (2C).

(3S)-Minor Diastereoisomer. ee 96% [Chiralpak OJ-H, hexane (TFA 0.01%)/*i*PrOH = 97:3, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 30.0 min and t_R (minor) = 22.2 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.21 (m, 4H, ArH), 6.10−5.99 (m, 1H, C*H*CH$_2$), 5.21−5.14 (m, 2H, CH$_2$), 4.28 (d, J = 9.3 Hz, 1H, CHAr), 3.89 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.1, 169.9, 135.7, 134.4, 133.77, 130.9 (2C), 128.8 (2C), 119.4, 82.6, 54.7, 54.3.

HRMS (ESI) m/z: [M − H]$^-$ calcd for $C_{13}H_{12}ClO_5$ 283.0379; found 283.0383.

2-Hydroxy-2-(methoxycarbonyl)-3-(4-methoxyphenyl)-pent-4-enoic Acid ***3e***. The title compound was synthesized following the general procedure from dimethyl (*E*)-2-((3-(4-methoxyphenyl)allyl)oxy)malonate **1e** (88 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 48 h. Compound **3e** was obtained as an off-white solid in 82% yield (69 mg); dr 2.5:1 (^{1}H NMR).

(3R)-Major Diastereoisomer. ee 72% [Chiralpak OJ-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 50.2 min and t_R (minor) = 57.2 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 8.9 Hz, 2H, ArH), 6.83 (d, J = 8.9 Hz, 2H, ArH), 6.11 (ddd, J = 16.9, 10.2, 8.8 Hz, 1H, C*H*CH$_2$), 5.27−5.16 (m, 2H, CH$_2$), 4.28 (d, J = 8.8 Hz, 1H, CHAr), 3.78 (s, 3H, CH$_3$), 3.71 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.9, 169.0, 159.24, 134.2, 130.0 (2C), 129.2, 119.5, 114.1 (2C), 83.2, 55.3, 54.6, 54.4.

(3S)-Minor Diastereoisomer. ee 97% [Chiralpak OJ-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 61.9 min and t_R (minor) = 36.6 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, J = 8.9 Hz, 2H, ArH), 6.86−6.80 (m, 2H, ArH), 6.14−6.05 (m, 1H, C*H*CH$_2$), 5.20−5.15 (m, 2H, CH$_2$), 4.26 (d, J = 9.5 Hz, 1H, CHAr), 3.92 (s, 3H, CH$_3$), 3.77 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.5, 170.2, 159.22, 134.8, 130.5 (2C), 128.9, 118.8, 114.05 (2C), 83.0, 55.3, 54.7, 54.5.

HRMS (ESI) m/z: [M − H]$^-$ calcd for $C_{14}H_{15}O_6$ 279.0874; found 279.0875.

2-Hydroxy-2-(methoxycarbonyl)-3-(4-nitrophenyl)pent-4-enoic Acid ***3f***. The title compound was synthesized following the general procedure from dimethyl (*E*)-2-((3-(4-nitrophenyl)allyl)oxy)malonate **1f** (93 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 48 h. Compound **3f** was obtained as an off-white solid in 71% yield (63 mg); dr 2.7:1 (^{1}H NMR).

(3R)-Major Diastereoisomer. ee 65% [Chiralpak AD-H, hexane (TFA 0.01%)/ABSE/*i*PrOH = 95:4:1, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 84.7 min and t_R (minor) = 97.4 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.8 Hz, 2H, ArH), 7.55 (d, J = 8.8 Hz, 2H, ArH), 6.17−6.02 (m, 1H, C*H*CH$_2$), 5.28−5.32 (m, 1H, CH$_2$), 5.28−5.25 (m, 1H, CH$_2$), 4.45 (d, J = 8.9 Hz, 1H, CHAr), 3.71 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.8, 169.3, 147.58, 145.0, 133.0, 130.1 (2C), 123.8 (2C), 120.9, 82.4, 54.5, 54.5.

(3S)-Minor Diastereoisomer. ee 91% [Chiralpak AD-H, hexane (TFA 0.01%)/ABSE/*i*PrOH = 95:4:1, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 76.4 min and t_R (minor) = 153.0 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21−8.11 (m, 2H, ArH), 7.58 (d, J = 8.8 Hz, 2H, ArH), 6.17−6.02 (m, 1H, C*H*CH$_2$), 5.28−5.25 (m, 1H, CH$_2$), 5.25−5.22 (m, 1H, CH$_2$), 4.45 (d, J = 9.2 Hz, 1H, CHAr), 3.94 (s, 3H, CH$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.7, 169.6, 147.55, 144.7, 133.5, 130.5 (2C), 123.7 (2C), 120.4, 82.3, 54.9, 54.4.

HRMS (ESI) m/z: [M − H]$^-$ calcd for $C_{13}H_{12}NO_7$ 294.0619; found 294.0626.

2-Hydroxy-2-(methoxycarbonyl)-3-(naphthalen-2-yl)-pent-4-enoic Acid ***3g***. The title compound was synthesized following the general procedure from dimethyl (*E*)-2-((3-(naphthalen-2-yl)allyl)oxy)malonate **1g** (94 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 48 h. Compound **3g** was obtained as an off-white solid in 43% yield (39 mg); dr 2.3:1 (^{1}H NMR).

(3R)-Major Diastereoisomer. ee 81% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 66.9 min and t_R (minor) = 60.1 min]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80−7.72 (m, 4H, ArH), 7.50−7.39 (m, 3H, ArH), 6.25 (ddd, J = 17.2, 10.1, 8.7

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Hz, 1H, C*H*CH_2), 5.34−5.19 (m, 2H, CH_2), 4.51 (d, *J* = 8.8 Hz, 1H, CHAr), 3.64 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 169.9, 169.8, 134.9, 134.1, 133.5, 133.0, 128.3, 128.1, 128.0, 127.8, 126.9, 126.3, 126.23, 119.9, 83.2, 55.3, 54.3.

(3S)-Minor Diastereoisomer. ee 99% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 130.4 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.89−7.72 (m, 4H, ArH), 7.59−7.39 (m, 3H, ArH), 6.33−6.15 (m, 1H, C*H*CH_2), 5.34−5.19 (m, 2H, CH_2), 4.50 (d, *J* = 9.2 Hz, 1H, CHAr), 3.93 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.4, 169.6, 134.8, 134.6, 133.4, 132.9, 128.6, 128.2, 128.18, 127.7, 127.3, 126.16 (2C), 119.2, 83.0, 55.1, 54.7.

HRMS (ESI) *m*/*z*: $[M - H]^-$ calcd for $C_{17}H_{15}O_5$ 299.0925; found 299.0928.

2-Hydroxy-3-(1H-indol-1-yl)-2-(methoxycarbonyl)pent-4-enoic Acid ***3h.*** The title compound was synthesized following the general procedure in a 0.1 mmol scale from dimethyl (*E*)-2-((3-(1*H*-indol-1-yl)allyl)oxy)malonate **1h** (35 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 48 h. Compound **3h** was obtained as an off-white solid in 45% yield (15 mg); dr 2.6:1 (^1H NMR).

(3S)-Major Diastereoisomer. ee 28% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 47.9 min and t_R (minor) = 40.1 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (dt, *J* = 7.9, 1.0 Hz, 1H, Ar−C4), 7.47 (d, *J* = 8.4 Hz, 1H, Ar−C7), 7.38 (d, *J* = 3.4 Hz, 1H, Ar−C2), 7.25−7.19 (m, 1H, Ar−C6), 7.13−7.08 (m, 1H, Ar−C5), 6.54 (d, *J* = 3.3 Hz, 1H, Ar−C3), 6.16 (ddd, *J* = 17.2, 10.4, 7.0 Hz, C*H*CH_2), 5.95 (d, *J* = 6.7 Hz, 1H, CHAr), 5.38−5.26 (m, 2H, CH_2), 3.40 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 168.5, 168.4, 136.0, 130.4, 128.2, 126.1, 121.79, 121.2, 121.0, 120.0, 109.4, 103.4, 82.5, 61.1, 54.3.

(3R)-Minor Diastereoisomer. ee 39% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 61.1 min and t_R (minor) = 101.3 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.8 Hz, 1H, Ar−C4), 7.51 (d, *J* = 8.2 Hz, 1H, Ar−C7), 7.48 (d, *J* = 3.3 Hz, 1H, Ar−C2), 7.23−7.16 (m, 1H, Ar−C6), 7.12−7.06 (m, 1H, Ar−C5), 6.52 (d, *J* = 3.3 Hz, 1H, Ar−C3), 6.17−6.06 (m, 1H, C*H*CH_2), 5.94 (d, *J* = 6.1 Hz, 1H, CHAr), 5.34−5.28 (m, 1H, CH_2), 5.20 (dt, *J* = 16.9, 1.1 Hz, 1H, CH_2), 3.94 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 168.8, 168.1, 136.6, 131.2, 128.2, 126.9, 121.77, 120.8, 120.6, 119.9, 109.9, 103.3, 82.3, 60.9, 54.5.

HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{15}H_{15}NaNO_5$ 312.0842; found 312.0833.

2-Hydroxy-2-(methoxycarbonyl)-3-(thiophen-2-yl)pent-4-enoic Acid ***3i.*** The title compound was synthesized following the general procedure from dimethyl (*E*)-2-((3-(thiophen-2-yl)allyl)oxy)malonate **1i** (81 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 48 h. Compound **3i** was obtained as an off-white solid in 86% yield (66 mg); dr 6:1 (^1H NMR).

(3S)-Major Diastereoisomer. ee 40% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 93:7, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 33.4 min and t_R (minor) = 28.5 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.22 (dd, *J* = 4.9, 1.5 Hz, 1H, ArH), 6.99−6.91 (m, 2H, ArH), 6.06 (ddd, *J* = 17.0, 10.1, 8.9 Hz, 1H, C*H*CH_2), 5.33−5.25 (m, 1H, CH_2), 5.22 (dd, *J* = 10.1 Hz, 1H, CH_2), 4.69 (d, *J* = 8.9 Hz, 1H, CHAr), 3.77 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 169.9, 169.4, 138.8, 134.0, 126.8, 126.5, 125.5, 119.8, 82.7, 54.5, 50.85.

(3R)-Minor Diastereoisomer. ee 99% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 93:7, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 59.9 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.25−7.16 (m, 1H, ArH), 7.04−7.00 (m, 1H, ArH), 6.99−6.91 (m, 1H, ArH), 6.12−5.97 (m, 1H, C*H*CH_2), 5.33−5.16 (m, 2H, CH_2), 4.68 (d, *J* = 9.2 Hz, 1H, CHAr), 3.90 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.2, 169.7, 138.7, 134.7, 127.0, 126.7, 125.6, 119.2, 82.5, 54.6, 50.88.

HRMS (ESI) *m*/*z*: $[M - H]^-$ calcd for $C_{11}H_{11}O_5S$ 255.0333; found 255.0334.

2-Hydroxy-2-(methoxycarbonyl)-3-methylpent-4-enoic Acid ***3j.*** The title compound was synthesized following the general procedure from dimethyl (*E*)-2-(but-2-en-1-yloxy)-malonate **1j** (61 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 7 days, the enzymatic hydrolysis reaction mixture was stirred for an additional 24 h. Compound **3j** was obtained as a yellow oil in 62% yield (35 mg); dr 1.4:1 (^1H NMR).

(3S)-Major Diastereoisomer. ee 64% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 93:7, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 30.5 min and t_R (minor) = 18.9 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 5.77−5.67 (m, 1H, C*H*CH_2), 5.24−5.11 (m, 2H, CH_2), 3.91 (s, 3H, $COOCH_3$), 3.25−3.12 (m, 1H, C*H*CH_3), 1.02 (d, *J* = 6.8 Hz, 3H, CHC*H*$_3$). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 171.0, 170.6, 136.2, 118.4, 82.33, 54.4, 43.9, 14.37.

(3R)-Minor Diastereoisomer. ee 61% [Chiralpak AD-H, hexane (TFA 0.01%)/*i*PrOH = 93:7, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 25.0 min and t_R (minor) = 22.0 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 5.67−5.59 (m, 1H, C*H*CH_2), 5.19−5.07 (m, 2H, CH_2), 3.86 (s, 3H, $COOCH_3$), 3.25−3.12 (m, 1H, C*H*CH_3), 1.09 (d, *J* = 6.8 Hz, 3H, CHC*H*$_3$). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.7, 170.5, 136.6, 118.0, 82.26, 54.4, 44.3, 14.43.

HRMS (ESI) *m*/*z*: $[M - H]^-$ calcd for $C_8H_{11}O_5$ 187.0612; found 187.0615.

2-(Ethoxycarbonyl)-2-hydroxy-3-phenylpent-4-enoic Acid ***3k.*** HPLC analysis conditions for the [2,3]-Wittig rearrangement intermediate **2k**: ee 55% [Chiralpak AD-H, hexane/ABSE/*i*PrOH = 95:3:2, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 12.0 min and t_R (minor) = 10.8 min].

The title compound was synthesized following the general procedure from diethyl 2-(cinnamyloxy)malonate **1k** (88 mg). After 20 h, full conversion was achieved in the [2,3]-Wittig rearrangement reaction conducted in ethanol, then the enzymatic hydrolysis reaction mixture was stirred for 48 h. Compound **3k** was obtained as an off-white solid in 85% yield (68 mg); dr 8.3:1 (^1H NMR).

(3R)-Major Diastereoisomer. ee 73% [Chiralpak OJ-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 19.2 min and t_R (minor) = 23.9 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41−7.33 (m, 2H, ArH), 7.32−7.22 (m, 3H, ArH), 6.15 (ddd, *J* = 17.1, 10.0, 9.0 Hz, 1H, C*H*CH_2), 5.29−5.23 (m, 1H, CHC*H*$_2$), 5.21 (dd, *J* = 10.1 Hz, 2.1 Hz, 1H, CHC*H*$_2$), 4.33 (d, *J* = 8.9 Hz, 1H, CHAr), 4.11 (q, *J* = 7.0 Hz, 2H, C*H*$_2$CH$_3$), 1.16 (t, *J* = 7.2 Hz,

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3H, CH_2CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.1, 169.3, 137.4, 134.4, 129.2 (2C), 128.6 (2C), 127.9, 119.5, 82.8, 64.1, 55.1, 13.9.

(3S)-Minor Diastereoisomer. ee 99% [Chiralpak OJ-H, hexane (TFA 0.01%)/*i*PrOH = 95:5, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 29.7 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41−7.33 (m, 2H, ArH), 7.32−7.22 (m, 3H, ArH), 6.18−6.08 (m, 1H, $CHCH_2$), 5.25−5.17 (m, 2H, $CHCH_2$), 4.37 (q, J = 7.2 Hz, 2H, CH_2CH_3), 4.32 (d, J = 9.4 Hz, 1H, CHAr), 1.38 (t, J = 7.2 Hz, 3H, CH_2CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.0, 169.9, 137.2, 134.6, 129.4 (2C), 128.6 (2C), 127.8, 119.1, 82.5, 64.4, 55.0, 14.2.

HRMS (ESI) m/z: $[M - H]^-$ calcd for $C_{14}H_{15}O_5$ 263.0925; found 263.0926.

1 mmol Scale Chemoenzymatic One-Pot Synthesis of 2-Hydroxy-2-(methoxycarbonyl)-3-phenylpent-4-enoic Acid ***3a.*** The experiment was conducted following the general procedure in a 1 mmol scale from dimethyl 2-(cinnamyloxy)-malonate **1a** (264 mg, 1 mmol). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 24 h. Compound **3a** was obtained as an off-white solid in 83% yield (208 mg); dr 2.2:1 (^1H NMR), ee 76/99% (HPLC).

General Procedure for the Synthesis of Racemic α-Hydroxy Half-Esters 3a−k. To a solution of allyloxy 1,3-dicarbonyl compound **1** (1 equiv) in $CHCl_3$ (0.2 M), 1,5,7-triazabicyclo[4.4.0]dec-5-ene (0.2 equiv) was added. After stirring at rt for 5 min, the solvent was evaporated. The crude mixture was purified by column chromatography on silica gel (4−10% EtOAc in PE/DCM 3/1) providing racemic homoallyl alcohol **2**.

To a solution of homoallyl alcohol **2** (1 equiv) in MeOH (0.1 M), KOH (1 equiv) was added. The reaction mixture was stirred at rt until the completion of the reaction, monitored by TLC. Then, the pH of the mixture was adjusted to 9−10 using 1 M aq NaOH solution and extracted twice with diethyl ether. The water layer was then acidified to pH 2 with 1 M aq HCl solution and extracted with diethyl ether. The combined organic layers (extracted at pH 2) were dried over $MgSO_4$. Evaporation of the solvent provided the racemic product **3**. If necessary, additional purification by column chromatography was performed using 2% EtOAc in DCM/formic acid 99/1 mixture.

Methyl 2-(Benzylcarbamoyl)-2-hydroxy-3-phenylpent-4-enoate ***4a.*** To a solution of 2-hydroxy-2-(methoxycarbonyl)-3-phenylpent-4-enoic acid **3a** (50 mg, 0.2 mmol, dr 2.9:1) and HATU (84 mg, 0.22 mmol) in DMF (2 mL, 0.1 M), DIPEA (38 μL, 0.22 mmol) and benzylamine (24 mg, 0.22 mmol) were added. The reaction was stirred for 20 min at rt. Then, the reaction mixture was extracted once with distilled water and EtOAc. The organic phase was collected, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude mixture was purified twice by column chromatography on silica gel (5−20% EtOAc in PE), providing the major diastereoisomer (46 mg, 68%) and the minor diastereoisomer (18 mg, 27%) of compound **4a** as white solids.

(3R)-Major Diastereoisomer. ee 72% [Chiralpak AD-H, hexane/*i*PrOH = 9:1, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 19.1 min and t_R (minor) = 22.2 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 (t, J = 5.9 Hz, 1H, NH), 7.37−7.20 (m, 10H, Ar), 6.11 (ddd, J = 17.1, 10.2, 8.8 Hz, 1H, $CHCH_2$), 5.19 (dt, J = 17.1, 1.3 Hz, 1H, $CHCH_2$), 5.14 (dd, J = 10.3, 1.6 Hz, 1H, $CHCH_2$), 4.56 (dd, J = 14.8, 6.3 Hz, 1H, $NHCH_2Ar$), 4.37 (d, J = 8.8 Hz, 1H, CHAr), 4.36 (dd, J = 14.8, 5.5 Hz, 1H, $NHCH_2Ar$), 4.29 (s, 1H, OH), 3.64 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 171.9, 167.5, 138.3, 137.7, 134.8, 129.1 (2C), 128.8 (2C), 128.5 (2C), 128.0 (2C), 127.8, 127.6, 118.9, 82.9, 55.5, 54.0, 44.0.

(3S)-Minor Diastereoisomer. ee 99% [Chiralpak AD-H, hexane/*i*PrOH = 9:1, flow rate = 1.0 mL/min, 25 °C, λ = 210 nm; t_R (major) = 15.5 min]. ^1H NMR (400 MHz, $CDCl_3$) δ 7.41−7.36 (m, 2H, CH-*m*-Ar), 7.31−7.25 (m, 4H, CH-*o*-Ar, CH-*p*-Ar, $NHCH_2$-*p*-Ar), 7.21−7.14 (m, 2H, $NHCH_2$-*o*-Ar), 7.12−7.01 (m, 1H, NH), 6.76−6.64 (m, 2H, $NHCH_2$-*m*-Ar), 6.14 (dt, J = 17.1, 9.9 Hz, 1H, $CHCH_2$), 5.21 (ddd, J = 17.1, 1.6, 0.8 Hz, 1H, $CHCH_2$), 5.15 (dd, J = 10.2, 1.8 Hz, 1H, $CHCH_2$), 4.40 (dd, J = 15.0, 7.3 Hz, 1H, $NHCH_2Ar$), 4.38 (s, 1H, OH), 4.39−4.33 (m, 1H, CHAr), 4.00 (dd, J = 15.0, 4.6 Hz, 1H, $NHCH_2Ar$), 3.92 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 172.5, 167.1, 137.9, 137.4, 135.6, 129.7 (2C), 128.6 (2C), 128.4 (2C), 127.5 (2C), 127.4 (2C), 118.5, 83.0, 55.4, 54.5, 43.4.

HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{20}H_{21}NO_4$ 340.1543; found 340.1536.

General Procedure for the Kinetic Study. *Reaction Profile for the Hydrolysis Step of One-Pot Reaction without Isolation of the [2,3]-Wittig Rearrangement Product* ***2a.*** The experiment was conducted following the general procedure for the chemoenzymatic one-pot synthesis of α-hydroxy half-esters **3**, starting from dimethyl 2-(cinnamyloxy)malonate **1a** (79 mg). After full conversion was achieved in the [2,3]-Wittig rearrangement reaction within 20 h, the enzymatic hydrolysis reaction mixture was stirred for an additional 24 h. During this period, samples were taken from the reaction mixture for ^1H NMR (20 μL in 600 μL DMSO-d_6) and HPLC (30 μL). HPLC samples were acidified to pH 2 with 1 M aq HCl solution and extracted with diethyl ether. Enantiomeric excess was determined by chiral HPLC analysis of the sample obtained by preparative TLC.

Reaction Profile for the Hydrolysis Reaction Using Isolated [2,3]-Wittig Rearrangement Product ***2a*** *as a Starting Material.* To a solution of dimethyl 2-(cinnamyloxy)-malonate **1a** (53 mg, 0.2 mmol) in methanol (2 mL), $Ca(NTf_2)_2$ (0.01 mmol, 6 mg), (*R*,*S*)-inda-PyBox (0.01 mmol, 3.9 mg), and imidazole (0.01 mmol, 0.7 mg) were added. The reaction mixture was stirred at 60 °C for 20 h. After evaporating the solvent, the crude mixture was purified by column chromatography on silica gel (3−7% EtOAc in PE/DCM 3/1 mixture), providing dimethyl (*R*)-2-hydroxy-2-(1-phenylallyl)malonate **2a** (48 mg, 91%) as a white solid. To a suspension of dimethyl (*R*)-2-hydroxy-2-(1-phenylallyl)-malonate **2a** (47 mg, 0.17 mmol, ee 26%) in DMSO (0.18 mL) and sodium phosphate buffer (pH 8.0, 0.71 mL), PLE (150 EU) was added, and the mixture was stirred at 35 °C for 3 h. During this period, samples were taken from the reaction mixture for ^1H NMR (20 μL in 600 μL DMSO-d_6) and HPLC (30 μL). HPLC samples were acidified to pH 2 with 1 M aq HCl solution and extracted with diethyl ether. Enantiomeric excess was determined by chiral HPLC analysis of the sample obtained by preparative TLC.

■ ASSOCIATED CONTENT

Supporting Information

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Synthesis of starting compounds; optimization procedures; copies of ^{1}H and ^{13}C spectra; and HPLC chromatograms (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Christoffers, J.; Baro, A.; Werner, T. α-Hydroxylation of β-Dicarbonyl Compounds. *Adv. Synth. Catal.* **2004**, *346*, 143−151.

(2) (a) Yang, C.; Shen, H. C.; Wu, Z.; Chu, H. D.; Cox, J. M.; Balsells, J.; Crespo, A.; Brown, P.; Zamlynny, B.; Wiltsie, J.; et al. Discovery of Novel Oxazolidinedione Derivatives as Potent and Selective Mineralocorticoid Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4388−4392. (b) Yang, C.; Balsells, J.; Chu, H. D.; Cox, J. M.; Crespo, A.; Ma, X.; Contino, L.; Brown, P.; Gao, S.; Zamlynny, B.; et al. Discovery of Benzimidazole Oxazolidinediones as Novel and Selective Nonsteroidal Mineralocorticoid Receptor Antagonists. *ACS Med. Chem. Lett.* **2015**, *6*, 461−465. (c) Rubio, O. H.; Taouil, R.; Muñiz, F. M.; Monleón, L. M.; Simón, L.; Sanzd, F.; Morána, J. R. A molecular receptor selective for zwitterionic alanine. *Org. Biomol. Chem.* **2017**, *15*, 477−485.

(3) Selected references for enantioselective synthesis of α-hydroxy ketoesters: (a) Acocella, M. R.; Mancheno, O. G.; Bella, M.; Jørgensen, K. A. Organocatalytic Asymmetric Hydroxylation of β-Keto Esters: Metal-Free Synthesis of Optically Active anti-Diols. *J. Org. Chem.* **2004**, *69*, 8165−8167. (b) Lu, M.; Zhu, D.; Lu, Y.; Zeng, X.; Tan, B.; Xu, Z.; Zhong, G. Chiral Brønsted Acid-Catalyzed Enantioselective r-Hydroxylation of β-Dicarbonyl Compounds. *J. Am. Chem. Soc.* **2009**, *131*, 4562−4563. (c) Baidya, M.; Griffin, K. A.; Yamamoto, H. Catalytic Enantioselective O-Nitrosocarbonyl Aldol Reaction of β-Dicarbonyl Compounds. *J. Am. Chem. Soc.* **2012**, *134*, 18566−18569. (d) Rose, C. A.; Gundala, S.; Fagan, C.-L.; Franz, J. F.; Connon, S. J.; Zeitler, K. NHC-catalysed, chemoselective crossed-acyloin reactions. *Chem. Sci.* **2012**, *3*, 735−740. (e) Yao, H.; Lian, M.; Li, Z.; Wang, Y.; Meng, Q. Asymmetric Direct α-Hydroxylation of β-Oxo Esters Catalyzed by Chiral Quaternary Ammonium Salts Derived from Cinchona Alkaloids. *J. Org. Chem.* **2012**, *77*, 9601−9608. (f) Zou, L.; Wang, B.; Mu, H.; Zhang, H.; Song, Y.; Qu, J. Development of Tartaric Acid Derived Chiral Guanidines and Their Application to Catalytic Enantioselective α-Hydroxylation of β-Dicarbonyl Compounds. *Org. Lett.* **2013**, *15*, 3106−3109.

(4) Reddy, D. S.; Shibata, N.; Nagai, J.; Nakamura, S.; Toru, T. A Dynamic Kinetic Asymmetric Transformation in the α-Hydroxylation of Racemic Malonates and Its Application to Biologically Active Molecules. *Angew. Chem., Int. Ed.* **2009**, *48*, 803−806.

(5) Wu, H.; Wang, Q.; Zhu, J. Catalytic Enantioselective Benzilic Ester Rearrangement. *Angew. Chem., Int. Ed.* **2020**, *59*, 7261−7265.

(6) Kano, T.; Song, S.; Maruoka, K. Molecular Recognition of Ketomalonates by Asymmetric Aldol Reaction of Aldehydes with Secondary-Amine Organocatalysts. *Chem. Commun.* **2012**, *48*, 7037−7039.

(7) Wang, C.; Zong, L.; Tan, C.-H. Enantioselective Oxidation of Alkenes with Potassium Permanganate Catalyzed by Chiral Dicationic Bisguanidinium. *J. Am. Chem. Soc.* **2015**, *137*, 10677−10682.

(8) Ha, M. W.; Choi, S.; Kim, S.; Lee, J. Y.; Lee, J. K.; Lee, J.; Hong, S.; Park, H. Phase-transfer catalyzed enantioselective α-alkylation of α-acyloxymalonates: construction of chiral α-hydroxy quaternary stereogenic centers. *RSC Adv.* **2016**, *6*, 77427−77430.

(9) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations*, 1st ed.; Wiley, 2005.

(10) Mohr, P.; Waespe-Šarcevic, N.; Tamm, C.; Gawronska, K.; Gawronski, J. K. A Study of Stereoselective Hydrolysis of Symmetrical Diesters with Pig Liver Esterase. *Helv. Chim. Acta* **1983**, *66*, 2501−2511.

(11) (a) Breznik, M.; Kikelj, D. Pig liver esterase catalyzed hydrolysis of dimethyl and diethyl 2-methyl-2-(o-nitrophenoxy)malonates. *Tetrahedron: Asymmetry* **1997**, *8*, 425−434. (b) Breznik, M.; Mrcina, A.; Kikelj, D. Enantioselective synthesis of (R)- and (S)-2-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-2-carboxamides. *Tetrahedron: Asymmetry* **1998**, *9*, 1115−1116. (c) Breznik, M.; Hrast, V.; Mrcina, A.; Kikelj, D. Stereoselective synthesis of (R)- and (S)-2-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-2-carboxylic acids, -carboxylates and -carboxamides. *Tetrahedron: Asymmetry* **1999**, *10*, 153−167. (d) Breznik, M.; Grdadolnik, S. G.; Giester, G.; Leban, I.; Kikelj, D. Influence of Chirality of the Preceding Acyl Moiety on the cis/trans Ratio of the Proline Peptide Bond. *J. Org. Chem.* **2001**, *66*, 7044−7050.

(12) Hayashi, Y. Pot economy and one-pot synthesis. *Chem. Sci.* **2016**, *7*, 866−880.

(13) (a) Denard, C. A.; Hartwig, J. F.; Zhao, H. Multistep One-Pot Reactions Combining Biocatalysts and Chemical Catalysts for Asymmetric Synthesis. *ACS Catal.* **2013**, *3*, 2856−2864. (b) Rudroff, F.; Mihovilovic, M. D.; Gröger, H.; Snajdrova, R.; Iding, H.; Bornscheuer, U. T. Opportunities and challenges for combining chemo- and biocatalysis. *Nat. Catal.* **2018**, *1*, 12−22.

(14) Selected references for combination of organocatalytic or metal-catalytic reactions with hydrolases in one-pot reaction: (a) Tenbrink, K.; Seßler, M.; Schatz, J.; Gröger, H. Combination of Olefin Metathesis and Enzymatic Ester Hydrolysis in Aqueous Media in a One-Pot Synthesis. *Adv. Synth. Catal.* **2011**, *353*, 2363−2367. (b) Pauly, J.; Gröger, H.; Patel, A. V. Catalysts Encapsulated in Biopolymer Hydrogels for Chemoenzymatic One-Pot Processes in Aqueous Media. *ChemCatChem* **2019**, *11*, 1503−1509.

(15) Ošeka, M.; Kimm, M.; Järving, I.; Lippur, K.; Kanger, T. Two Catalytic Methods of an Asymmetric Wittig [2,3]-Rearrangement. *J. Org. Chem.* **2017**, *82*, 2889−2897.

(16) Palomo, J. M.; Cabrera, Z. Enzymatic Desymmetrization of Prochiral Molecules. *Curr. Org. Synth.* **2012**, *9*, 791−805.

(17) Albrecht, Ł.; Jiang, H.; Jørgensen, K. A. A Simple Recipe for Sophisticated Cocktails: Organocatalytic One-Pot Reactions—Concept, Nomenclature, and Future Perspectives. *Angew. Chem., Int. Ed.* **2011**, *50*, 8492−8509.

(18) Inokuma, Y.; Yoshioka, S.; Ariyoshi, J.; Arai, T.; Hitora, Y.; Takada, K.; Matsunaga, S.; Rissanen, K.; Fujita, M. X-ray analysis on the nanogram to microgram scale using porous complexes. *Nature* **2013**, *495*, 461−466.

(19) (a) Meng, J.-C.; Fokin, V. V.; Finn, M. G. Kinetic resolution by copper-catalyzed azide−alkyne cycloaddition. *Tetrahedron Lett.* **2005**, *46*, 4543−4546. (b) Cornejo, A.; Fraile, J. M.; García, J. I.; Gil, M. J.; Martínez-Merino, V.; Mayoral, J. A.; Pires, E.; Villalba, I. An Efficient and General One-Pot Method for the Synthesis of Chiral Bis(oxazoline) and Pyridine Bis(oxazoline) Ligands. *Synlett* **2005**, *15*, 2321−2324.

(20) Kabir, M. S.; Namjoshi, O. A.; Verma, R.; Lorenz, M.; Phani Babu Tiruveedhula, V. V. N.; Monte, A.; Bertz, S. H.; Schwabacher, A. W.; Cook, J. M. Base-Mediated Stereospecific Synthesis of Aryloxy and Amino Substituted Ethyl Acrylates. *J. Org. Chem.* **2012**, *77*, 300−310.

(21) Kennedy, C. R.; Guidera, J. A.; Jacobsen, E. N. Synergistic Ion-Binding Catalysis Demonstrated via an Enantioselective, Catalytic [2,3]-Wittig Rearrangement. *ACS Cent. Sci.* **2016**, *2*, 416−423.

(22) Stokes, S.; Mustain, R.; Pickle, L.; Mead, K. T. Rhodium-catalyzed cyclopropanations of 2-aryl-2H-chromenes with dialkyl malonate esters. A comparison of α-diazo derivatives and phenyl-iodonium ylides. *Tetrahedron Lett.* **2012**, *53*, 3890−3893.

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