Chemoenzymatic Synthesis of Optically Active Alcohols Possessing 1,2,3,4-Tetrahydroquinoline Moiety Employing Lipases or Variants of the Acyltransferase from Mycobacterium smegmatis †

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† Tribute to Prof. Zoltan George Hajos (1926–2022)—A groundbreaking pioneer of organocatalysis.

Abstract: The enzymatic kinetic resolution (EKR) of racemic alcohols or esters is a broadly recognized methodology for the preparation of these compounds in optically active form. Although EKR approaches have been developed for the enantioselective transesterification of a vast number of secondary alcohols or hydrolysis of their respective esters, to date, there is no report of bio- or chemo-catalytic asymmetric synthesis of non-racemic alcohols possessing 1,2,3,4-tetrahydroquinoline moiety, which are valuable building blocks for the pharmaceutical industry. In this work, the kinetic resolution of a set of racemic 1,2,3,4-tetrahydroquinoline-propan-2-ols was successfully carried out in neat organic solvents (in the case of CAL-B and BCL) or in water (in the case of MsAcT single variants) using immobilized lipases from Candida antarctica type B (CAL-B) and Burkholderia cepacia (BCL) or engineered acyltransferase variants from Mycobacterium smegmatis (MsAcT) as the biocatalysts and vinyl acetate as irreversible acyl donor, yielding enantiomerically enriched (S)-alcohols and the corresponding (R)-acetates with E-values up to 328 and excellent optical purities (>99% ee). In general, higher ee-values were observed in the reactions catalyzed by lipases; however, the rates of the reactions were significantly better in the case of MsAcT-catalyzed enantioselective transesterifications. Interestingly, we have experimentally proved that enantiomerically enriched 1-(7-nitro-3,4-dihydroquinolin-1(2H)-yl)propan-2-ol undergoes spontaneous amplification of optical purity under achiral chromatographic conditions.

Keywords: biocatalysis; lipases; acyltransferases; kinetic resolution; chiral alcohols; 1,2,3,4-tetrahydroquinoline-based alcohols; self-disproportionation of enantiomer (SDE)

1. Introduction

The implementation of simple, cost-efficient, and environmentally benign synthetic methods that allow the preparation of chiral compounds in high chemical and optical purities is of paramount interest to academia and industry. In this regard, biocatalytic processes which rely on biodegradable and relatively inexpensive enzymes, which can be easily produced in significant amounts by microbial fermentation, are of interest to possibly satisfy the economic and sustainable demand [1–5]. The great attractiveness of enzymes in synthetic organic chemistry mainly stems from their ability to catalyze the transformation of a broad scope of xenobiotic substrates with excellent regio-, chemo- and enantio-selectivity [6]. These particular features enable the synthesis of chiral products
with excellent optical purities by following shortened synthetic routes and generating less waste to be recycled, which all together constitute a strong driving force in a vast number of industrial applications [7,8], including the manufacturing of pharmaceuticals [9–15], agrochemicals [16,17] and cosmetic ingredients [18].

The most popular enzymes used in biotechnological applications worldwide are lipases (EC 3.1.1.3, triacylglycerol ester hydrolases), which global market was estimated at 425 mln USD in 2018 with a Compound Annual Growth Rate (CAGR) of 6–8% [19,20]. Lipases outperform enzymes from other classes mainly because they exhibit remarkable catalytic activity and high operational stability under non-physiological conditions, including nearly anhydrous organic solvents [21–26]. Moreover, an increased preference for the use of lipases in industry, and especially in the production of pharmaceuticals and their building blocks [27–30], is due to the fact that the reactions catalyzed by these enzymes do not require elevated temperatures, high pressures, costly and/or difficult-to-regenerate cofactors, anhydrous and oxygen-free conditions, special handling techniques or sophisticated laboratory equipment. Furthermore, the relatively low price and wide commercial availability of lipase preparations, either in a native or immobilized form, is also a key advantage for the development of enzyme-based biotechnologies.

The most prestigious status of lipases lies in their ability to resolve enantiomers of secondary alcohols/esters under kinetically-controlled conditions using (trans)esterification or hydrolytic methodologies. To date, a plethora of synthetically useful hydroxylated/acylated derivatives have been investigated as the racemic substrates for the lipase-catalyzed kinetic resolution (KR) and/or dynamic kinetic resolution (DKR). Among the most important are the secondary alcohols and the corresponding esters possessing (hetero)aromatic substituents, such as imidazole [31], 1,2,4-triazole [32–34], pyrazole [35], isoxazole [36], thiazole [37,38], tetrazole [39,40], thiophene [41,42], tetrahydrothiophene [43], furan [44–47], benzimidazole [48], benzotriazole [49,50], benzothiazole [51], benzothiophene [52,53], benzofuran [54–56], tetrahydrofuran [57], pyridine [58,59], pipерidine [44,60], piperazine [61], pyrrolidine [62,63], pyran [64], quinoline [65], phenoxythiazine [66–70], 1,10-phenanthroline [71], 1,3-dimethylxanthine [72,73], azetidine [74], benzazepine [75], cyclopyrrolone [76,77], phthalimide [78,79], morpholine [80,81], indole [82,83], (2,3-dihydroindole) [84–86], isoxindole [87], carbazole [88], etc.

On the other hand, the acyltransferase from Mycobacterium smegmatis (MsAcT) is a recently identified alternative catalyst with extraordinary catalytic activity for a wide array of reactions, including (trans)esterification, (trans)amidation, and perhydrolysis [89–91]. Amazingly, a promiscuous MsAcT offers efficient acyl transfer between various esters (i.e., ethyl acetate, benzyl acetate, vinyl acetate, etc.) and a broad range of alcohols in water as an environmentally benign solvent [92–96]. Such outstanding ability to perform transesterification in an aqueous solution is relatively uncommon since water usually promotes hydrolysis over synthesis, thus leading to the unfavorable equilibrium of the synthesis of the ester. Strikingly, the unique advantage of catalyzing (trans)esterification of alcohols in aqueous media can reduce the usage of toxic organic solvents and thus lower waste generation. Because of this reason, MsAcT has also attracted attention for its ability to catalyze selective transacylation of racemic alcohols [97,98] performed under kinetically-controlled conditions.

Interestingly, although lipases and MsAcT have proven beneficial in enantioselective C–O functional group chemistry of racemic substrates bearing hydroxyl or acyl moiety, their application in asymmetric biocatalytic transformations of alcohols/esters substituted with 1,2,3,4-tetrahydroquinoline is still unexplored. Moreover, since chiral tetrahydroquinoline-based derivatives serve as essential building blocks for various natural products [99,100], pharmaceuticals [101], and promising drug candidates [102–104] (Figure 1), the elaboration of novel synthetic routes toward obtaining these compounds in enantiomerically pure form is highly required.
Therefore, as a part of our ongoing research program, which is focused on biocatalytic preparation of optically active heteroaromatic alcohols, and with the aim to widen the biosynthetic repertoire of both titled enzymes towards racemic substrates, an efficient chemoenzymatic synthesis of enantiomerically enriched 1-(hydroxypropyl)-1,2,3,4-tetrahydroquinolines is reported for the first time.

2. Results and Discussion

Herein, we report a straightforward chemoenzymatic method for the preparation of optically active alcohols possessing 1,2,3,4-tetrahydroquinoline moiety \((S)-(+)-\) and \((R)-(-)-2a-c\) (Scheme 1). In order to obtain the desired products, two alternative biocatalytic routes, relying on enantioselective transesterification of racemic \(-\text{(3,4-dihydroquinolin-1(2H)-yl)propan-2-ols}\) \(\text{rac-2a-c}\) were performed under kinetically-controlled conditions. In this context, one of the approaches focused on lipase-catalyzed KR of \(\text{rac-2a-c}\) carried out in neat organic solvents. In contrast, the second attempt was realized by employing engineered variants of the acyltransferase from *Mycobacterium smegmatis* (MsAcT) in a water-abundant medium.

![Diagram](https://example.com/diagram.png)

**Figure 1.** Examples of pharmaceutical (I) and biologically active compounds (II–IV) incorporating 1,2,3,4-tetrahydroquinoline scaffold. The center of asymmetry was marked by the asterisk symbol (*).

**Scheme 1.** Enzyme-catalyzed kinetic resolution of racemic alcohols possessing 1,2,3,4-tetrahydroquinoline moiety \(\text{rac-2a-c}\) using lipases or acyltransferases as the biocatalysts.

In the first two steps of the synthesis, the required racemic alcohols \(\text{rac-2a-c}\) and their respective esters \(\text{rac-3a-c}\) (used as analytical standards for monitoring the reactions’ progress and establishment of enantiomeric excess of KR products) were prepared by using standard procedures. In this regard, a regioselective oxirane-ring opening of racemic propylene oxide with the respective 1,2,3,4-tetrahydroquinoline \((\text{1a–b})\) was performed in...
the presence of sodium bicarbonate (NaHCO₃) as a base in anhydrous ethanol (EtOH) at 100 °C for 72 h, leading to rac-2a-b in the 49–82% yield range. In the case of the nitro-derivative 1c, extended reaction time (168 h), as well as increased amounts of propylene oxide (8 equiv) and NaHCO₃ (4.5 equiv), were necessary to obtain rac-2c in 83% yield. Due to the low boiling temperature of propylene oxide, all the reactions were performed in a high-pressure glass cell. In turn, N,N-dimethylpyridin-4-amine (DMAP)-catalyzed esterification of rac-2a-c with an excess of acetyl chloride in the presence of triethylamine (Et₃N) as a base in dry dichloromethane (CH₂Cl₂) furnished racemic acetates rac-3a-c in an acceptable 36–52% yield range, respectively.

2.1. Lipase-Catalyzed KR of Racemic 1,2,3,4-Tetrahydroquinoline-Based Alcohols rac-2a-c Using Vinyl Acetate in Organic Solvents

The conditions for the lipase-catalyzed transesterification of racemic 1-(3,4-dihydroquinolin-1(2H)-yl)propan-2-ols rac-2a-c were optimized according to the conventional stepwise method reported previously [105]. Shortly, the effects of lipase, co-solvent, temperature, and time-course of the enzymatic acetylation of alcohols were evaluated on the KR outcome.

Preliminary enzyme-catalyzed KR studies were performed toward a model racemic substrate rac-2a by using a set of commercially available lipase preparations originating from fungi, bacteria, or plants. The respective biocatalytic system for the resolution of rac-2a consisted of the appropriate biocatalyst suspended in a mixture of vinyl acetate as an irreversible acyl donor and methyl tert-butyl ether (MTBE) as a standard solvent at 40 °C for 24 h (Table 1).

Table 1. Lipase screening for lipase-catalyzed KR of rac-2a with vinyl acetate in MTBE.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase Preparation a</th>
<th>Conv. (%) b</th>
<th>ee s (%) c</th>
<th>ee p (%) c</th>
<th>E d</th>
</tr>
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<tbody>
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<td>1</td>
<td>Novozym 435</td>
<td>66</td>
<td>&gt;99</td>
<td>51</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Novozym 435-STREM</td>
<td>75</td>
<td>&gt;99</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Lipolysyme 435</td>
<td>71</td>
<td>&gt;99</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>Chirazyme L-2, C-2</td>
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<td>&gt;99</td>
<td>65</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>Chirazyme L-2, C-3</td>
<td>54</td>
<td>&gt;99</td>
<td>87</td>
<td>107</td>
</tr>
<tr>
<td>6</td>
<td>Lipolysyme TL IM</td>
<td>35</td>
<td>48</td>
<td>89</td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>Lipolysyme RM IM</td>
<td>20</td>
<td>25</td>
<td>98</td>
<td>126</td>
</tr>
<tr>
<td>8</td>
<td>Amano PS-IM</td>
<td>45</td>
<td>79</td>
<td>98</td>
<td>240</td>
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<td>9</td>
<td>Amano PS</td>
<td>&lt;10</td>
<td>N.D. e</td>
<td>N.D. e</td>
<td>N.D. e</td>
</tr>
<tr>
<td>10</td>
<td>Amano AK</td>
<td>0</td>
<td>N.D. e</td>
<td>N.D. e</td>
<td>N.D. e</td>
</tr>
<tr>
<td>11</td>
<td>Lipase from Candida rugosa Type VII</td>
<td>0</td>
<td>N.D. e</td>
<td>N.D. e</td>
<td>N.D. e</td>
</tr>
<tr>
<td>12</td>
<td>Lipase from wheat germ Type I</td>
<td>0</td>
<td>N.D. e</td>
<td>N.D. e</td>
<td>N.D. e</td>
</tr>
<tr>
<td>13</td>
<td>Lipase from Rhizopus nivus</td>
<td>0</td>
<td>N.D. e</td>
<td>N.D. e</td>
<td>N.D. e</td>
</tr>
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</table>

a Conditions: rac-2a 25 mg, lipase 25 mg, MTBE 2 mL, vinyl acetate 1 mL (83 equiv), 24 h at 40 °C, 800 rpm (magnetic stirrer); b Based on GC, for confirmation, the % conversion was calculated from the enantiomeric excess of the slower-reacting (S)-alcohol (ee s) and the formed (R)-acetate (ee p) according to the formula conv. = ee s/(ee s + ee p); c Determined by chiral HPLC analysis by using a Chiralcel OD-H column; d Calculated according to Chen et al. [106], using the equation: E = |ln[(1 − conv.)/(1 − ee s)]|/|ln[(1 − conv.)]; e Not determined.

The enzymes exhibiting enantioselectivity toward rac-2a good enough for practical purpose (E = 20–33) were Candida antarctica lipase type B (CAL-B) immobilized on various carriers, including macro-porous acrylic resin (Novozym 435 and Novozym 435-STREM), Lewatit VP OC 1600® (Lipolysyme 435), and Carrier 2® (Chirazyme L-2, c-f., C2, Lyo.; here denoted as Chirazyme L-2, C-2), as well as Thermomyces lanuginosus lipase (TLL)
immobilized on a non-compressible silica gel carrier (Lipozyme TL IM). The other examined biocatalysts, which exhibited very promising enantioselectivity ($E = 107-240$), included CAL-B immobilized on Carrier 5® (Chirazyme L-2, c.-f., C3, Lyo.; here indicated as Chirazyme L-2, C-3), Rhizomucor miehei lipase (RML) immobilized on a macro-porous anion-exchange resin (Lipozyme RM IM), and Burkholderia cepacia lipase (BCL) immobilized on diatomaceous earth (Amano PS-IM). Among the investigated enzymes, CAL-B showed the highest catalytic activity, allowing the obtaining of optically active alcohol (S)-(+)-2a (>99% ee) and acetate (R)-(−)-3a (34–87% ee) with 54–75% conv.; BCL provided reasonable results in terms of activity, furnishing (S)-(−)-2a (79% ee) and (R)-(−)-3a (98% ee) with 45% conv.; whereas RML and TML preparations showed moderate activity, yielding (S)-(−)-2a (25–48% ee) and (R)-(−)-3a (89–98% ee) with only 20–35% conv., respectively. On the contrary, native lipases isolated from *Pseudomonas fluorescens* (Amano AK), *Candida rugosa* Type VII (CRL), *Rhizopus niveus* (RNL), and wheat germ Type I were inactive toward rac-2a. Notably, although Amano PS-IM catalyzed KR of rac-2a with the highest $E$-values compared with other lipases, a native BCL (Amano PS) showed no catalytic performance in KR of rac-2a. In control experiments, it was ensured that the reaction did not proceed without lipase preparation. The enantiomeric excesses of the slower-reacting alcohol (S)-(−)-2a and the formed acetate (R)-(−)-3a were determined by HPLC using a column packed with a chiral stationary phase.

According to rational rules of optimizing lipase-catalyzed kinetic resolutions, we then investigated the proper choice of organic solvent. The selection of the appropriate reaction medium is a critical factor for biocatalysis [107], as solvent variation in many cases of lipase-catalyzed KR is known to influence the reaction rate and the equilibrium position [108,109], as well as enantioselectivity [110–114], regioselectivity [115], enantiotopic (prochiral) selectivity [116], and thermal stability [117] of the enzymes. Noteworthy, in rare cases, the organic solvent can also reverse stereo-preference of the lipases [118].

With the aim to obtain both enantiomeric forms of benchmark alcohol rac-2a with the highest possible enantiomeric enrichment, acetylations of rac-2a with vinyl acetate were performed in various organic solvents of varying polarity ($\log P$ from 0.20 to 2.52) in the presence of the most enantioselective enzymes, either Chirazyme L-2, C-3 or Amano PS-IM, at 40 °C for 24 h, respectively (Table 2). Moreover, since in the course of transesterification reactions with vinyl acetate, a highly volatile and toxic acetaldehyde is generated as a by-product, we decided to reduce the amounts of acyl donor from >80 equiv to 5 equiv with respect to rac-2a. Decreasing vinyl acetate is also reasonable since in situ forming acetaldehyde deactivates enzymes due to the formation of stable Schiff base-type adducts with amino groups of the side chains belonging to peripheral lysine residues [119]. Furthermore, to minimize the costs of the overall enzymatic process, the reduction of biocatalyst loading (from 100% to 20% $w/w$ with respect to rac-2a) was investigated under optimal EKR conditions.

The results presented in Table 2 show that enzyme selectivity ($E > 100$) was sufficient for practical use in most of the tested organic solvents except acetone for the reaction catalyzed by Amano PS-IM, or acetone, ethyl acetate (EtOAc), 2-methyltetrahydrofuran (2-MeTHF), and toluene (PhCH$_3$) in the case of Chirazyme L-2, C-3. Among the solvents tested, MTBE gave the best results regarding enantioselectivity and reaction rate and, thus, was considered a suitable solvent for further optimization studies. In the case of Chirazyme L-2, C-3-catalyzed KR of rac-2a, it was observed that the effective resolution also occurred in tert-amyl alcohol (2-methyl-2-butanol) ($E = 175$), which resulted in the isolation of (S)-(−)-2a (94% ee) and (R)-(−)-3a (96% ee) with 50% conv. Interestingly, when comparing KR reactions conducted with both lipases suspended in PhCH$_3$, one can see that $E$-values were radically different (Table 2, entry 7 vs. entry 14). Changing the reaction’s stoichiometry by using less vinyl acetate significantly altered the reaction rates, leading to lower conversions than in the previous step. On the other hand, thanks to this change, the enantioselectivity of the reactions carried out in MTBE and other studied solvents improved to achieve $E$-value
up to 307. This time, since the conversions did not exceed 50%, alcohol (S)-(+)-2a was isolated with 5–94% ee. Conversely, acetate (R)-(+)-3a was obtained with up to >99% ee.

Table 2. Co-solvent screening for lipase-catalyzed KR of rac-2a with vinyl acetate.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase Preparation a</th>
<th>Solvent (log P) b</th>
<th>Conv. (%) c</th>
<th>ee ( s ) (%) d</th>
<th>ee ( p ) (%) d</th>
<th>( E ) c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chirazyme L-2, C-3</td>
<td>Acetone (0.20)</td>
<td>23</td>
<td>29</td>
<td>97</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>EtOAc (0.29)</td>
<td>46</td>
<td>81</td>
<td>94</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Vinyl acetate (0.54)</td>
<td>35</td>
<td>52</td>
<td>97</td>
<td>111</td>
</tr>
<tr>
<td>4</td>
<td>tert-Amyl alcohol (1.09)</td>
<td>2-MeTHF (0.72)</td>
<td>50</td>
<td>93</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>MTBE (0.96)</td>
<td>48</td>
<td>88</td>
<td>96</td>
<td>143</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>PhCH(2) (2.52)</td>
<td>22</td>
<td>27</td>
<td>96</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Amano PS-IM</td>
<td>Acetone (0.20)</td>
<td>5</td>
<td>5</td>
<td>&gt;99</td>
<td>226</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>EtOAc (0.29)</td>
<td>12</td>
<td>13</td>
<td>&gt;99</td>
<td>226</td>
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<td>8</td>
<td></td>
<td>Vinyl acetate (0.54)</td>
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<td>&gt;99</td>
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<tr>
<td>9</td>
<td>tert-Amyl alcohol (1.09)</td>
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<td>&gt;99</td>
<td>222</td>
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<tr>
<td>10</td>
<td></td>
<td>MTBE (0.96)</td>
<td>31</td>
<td>44</td>
<td>&gt;99</td>
<td>307</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>PhCH(2) (2.52)</td>
<td>26</td>
<td>26</td>
<td>&gt;99</td>
<td>257</td>
</tr>
</tbody>
</table>

- Conditions: rac-2a 25 mg, lipase 5 mg, organic solvent 2 mL, vinyl acetate 56 mg (60 µL, 5 equiv), 24 h at 40 °C, 800 rpm (magnetic stirrer); b Logarithm of the partition coefficient of a given solvent between n-octanol and water according to ChemBioDraw Ultra 13.0 software indications; c Based on GC, the % conversion was calculated from the enantiomeric excess of the slower-reacting (S)-alcohol (ee\( s \)) and the formed (R)-acetate (ee\( p \)) according to the formula conv. \( = \frac{\text{ee} \cdot (1 - \text{conv.})}{\text{ee} + \text{conv.}} \); d Determined by chiral HPLC analysis by using a Chiralcel OJ-H column; e Calculated according to Chen et al. [106], using the equation: \( E = \left[ \ln\left(1 - \text{conv.}\right)(1 - \text{ee} \cdot s)\right] / \left[ \ln\left(1 - \text{conv.}\right)(1 + \text{ee} \cdot s)\right] \).

To achieve a good conversion in a reasonable time span, we further tested the influence of temperature on Amano PS-IM-catalyzed KR of rac-2a with 5 equiv of vinyl acetate at temperatures ranging from 40 °C to 60 °C (Table 3). Since decreasing amounts of vinyl acetate lowered the reaction rates, all the acetylations of rac-2a were carried out for 72 h. These experiments revealed that elevated temperatures did not induce a notable acceleration of the reaction rate, but the enantioselectivity decreased. Since we could not observe any advantages of heating KR of rac-2a at higher temperatures in terms of reaction rates and enantioselectivity, the subsequent optimization attempts were performed at 40 °C. In turn, intensifying the CAL-B-catalyzed reactions by using elevated temperatures was not investigated since this lipase allowed the obtaining of very high conversions after relatively short reaction times (Table 2).

Kinetic control of enzymatic reactions is crucial for the outcome of the enantiomers’ resolution processes in terms of optical purity and yield of the products [6,120]. In the case of lipase-catalyzed KR, the product of the faster-reacting enantiomer is obtained with higher % ee-values when the reaction is arrested at <50% conv. On the other hand, a slower-reacting enantiomer is received with higher ee-values when KR of the racemic mixture is terminated at >50% conv. Depending on the time course of lipase-catalyzed KR, the yield of the products can vary significantly according to substrate conversion. Therefore, it is fundamental to select for KR process the most enantioselective enzyme (\( E >> 500 \)), which would allow for the obtaining of both non-racemic products in a yield close to the maximum theoretical value (50%) and with excellent enantiomeric purity (>99% ee).
Table 3. Effect of the temperature on Amano PS-IM-catalyzed KR of rac-2a with vinyl acetate after 72 h.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temperature (°C) a</th>
<th>Conv. (%) b</th>
<th>ee_s (%) c</th>
<th>ee_p (%) c</th>
<th>E d</th>
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<tr>
<td>1</td>
<td>40</td>
<td>47</td>
<td>87</td>
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<tr>
<td>2</td>
<td>50</td>
<td>49</td>
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<td>97</td>
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<tr>
<td>3</td>
<td>60</td>
<td>48</td>
<td>89</td>
<td>97</td>
<td>198</td>
</tr>
</tbody>
</table>

a Conditions: rac-2a 25 mg, Amano PS-IM 5 mg, MTBE 2 mL, vinyl acetate 86 mg (60 μL, 5 equiv), 72 h at 40–60 °C, 800 rpm (magnetic stirrer); b Based on GC, the % conversion was calculated from the enantiomeric excess of the slower-reacting (S)-alcohol (ee_s) and the formed (R)-acetate (ee_p) according to the formula conv. = ee_s/(ee_s + ee_p); c Determined by chiral HPLC analysis by using a Chiralcel OJ-H column; d Calculated according to Chen et al. [106], using the equation: $E = \ln[(1 - \text{conv.})(1 - ee_p)]/\ln[(1 - \text{conv.})(1 + ee_s)]$.

Considering the above-mentioned facts, our next objective was to establish the appropriate time/conversion after which enzymatic reaction could afford KR products (S)-(+)2a and (R)-(+)3a with the highest possible enantiomeric excesses (Table 4).

Table 4. Time-course of lipase-catalyzed KR of rac-2a with vinyl acetate as acyl donor.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase Preparation a</th>
<th>Time (h)</th>
<th>Conv. (%) b</th>
<th>ee_s (%) c</th>
<th>ee_p (%) c</th>
<th>E d</th>
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<td>1</td>
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<td>8</td>
<td>43</td>
<td>70</td>
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<td>2</td>
<td>Novozym 435</td>
<td>12</td>
<td>52</td>
<td>95</td>
<td>87</td>
<td>53</td>
</tr>
<tr>
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<td></td>
<td>18</td>
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<td>&gt;99</td>
<td>74</td>
<td>34</td>
</tr>
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<td>24</td>
<td>31</td>
<td>&gt;99</td>
<td>&gt;99</td>
<td>307</td>
</tr>
<tr>
<td>5</td>
<td>Amano PS-IM</td>
<td>72</td>
<td>47</td>
<td>87</td>
<td>98</td>
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<td>120</td>
<td>50</td>
<td>97</td>
<td>97</td>
<td>278</td>
</tr>
</tbody>
</table>

a Conditions: rac-2a 25 mg, lipase 5 mg, MTBE 2 mL, vinyl acetate 56 mg (60 μL, 5 equiv), 40 °C for 8–120 h, 800 rpm (magnetic stirrer); b Based on GC, the % conversion was calculated from the enantiomeric excess of the slower-reacting (S)-alcohol (ee_s) and the formed (R)-acetate (ee_p) according to the formula conv. = ee_s/(ee_s + ee_p); c Determined by chiral HPLC analysis by using a Chiralcel OJ-H column; d Calculated according to Chen et al. [106], using the equation: $E = \ln[(1 - \text{conv.})(1 - ee_p)]/\ln[(1 - \text{conv.})(1 + ee_s)]$.

Consequently, Novozym 435 and Amano PS-IM were arbitrarily selected for kinetic studies on KR of rac-2a. Replacing Chirazyme L-2, C-3 with Novozym 435 was found crucial for further experiments since Chirazyme L-2, C-3 is no longer available at chemical suppliers. Moreover, the performed change among the CAL-B biocatalysts was dictated by the fact that Novozym 435 was significantly more active toward the model racemic substrate rac-2a than Chirazyme L-2, C-3. All the acylations of rac-2a were carried out by using 20% w/w of lipase preparation with respect to rac-2a in the presence of 5 equiv of vinyl acetate in MTBE at 40 °C and terminated after specific time intervals optimal/tailored for each biocatalyst. From the results collected in Table 4, it was clear that in order to obtain enantiomerically pure alcohol (5)-(+)2a (>99% ee), the reaction catalyzed by Novozym 435 had to be stopped after 18 h when 57% conv. was achieved. In the case of Amano PS-IM-catalyzed KR of rac-2a, termination of the process after 24 h led to enantiomerically pure acetate (R)-(+)3a (>99% ee) with 31% conv. It is evident that further elongation of
the reaction time deteriorated the enantiomeric purity of (R)-(+)\-3a. Moreover, Amano PS-IM-catalyzed acetylation of rac-2a was impossible to run with higher conversions than 50%, even after extending the time to 120 h.

For the purpose of examining the synthetic potential of the present approach, a preparative-scale lipase-catalyzed KR of rac-2a was conducted under optimal reaction conditions by using either Novozym 435 or Amano PS-IM as a biocatalyst (Table 5). The performed experiments revealed that Novozym 435-catalyzed KR of rac-2a afforded (S)-(+)-2a in 39% yield and with 98% ee. On the other hand, the reaction catalyzed by Amano PS-IM allowed the obtaining of optically active acetate (R)-(+)\-3a in 25% yield and 99% ee.

**Table 5.** Preparative-scale lipase-catalyzed KR of rac-2a\-c with vinyl acetate as acyl donor.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate (^d)</th>
<th>Lipase</th>
<th>Time (h)</th>
<th>Conv. (%) (^b)</th>
<th>ee(_R) (%) (^b) / Yield (%) (^d)</th>
<th>ee(_P) (%) (^b) / Yield (%) (^d)</th>
<th>(E) (^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>54</td>
<td>98/39</td>
<td>82/48</td>
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<td>Amano PS-IM</td>
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<td>36/59</td>
<td>99/25</td>
<td>283</td>
</tr>
<tr>
<td>3</td>
<td>rac-2b</td>
<td>Novozym 435</td>
<td>18</td>
<td>62</td>
<td>&gt;99/29</td>
<td>62/49</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td></td>
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<td>24</td>
<td>26</td>
<td>35/51</td>
<td>98/23</td>
<td>140</td>
</tr>
<tr>
<td>5</td>
<td>rac-2c</td>
<td>Novozym 435</td>
<td>18</td>
<td>60</td>
<td>&gt;99/35</td>
<td>67/52</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Amano PS-IM</td>
<td>24</td>
<td>46</td>
<td>79/49</td>
<td>94/40</td>
<td>78</td>
</tr>
</tbody>
</table>

\(^d\) Conditions: rac-2a\-c 2.61 mmol, lipase 100 mg, MTBE 40 mL, vinyl acetate 1.13 g (1.20 mL, 5 equiv), 40 °C for 18–24 h, 800 rpm (magnetic stirrer); \(^b\) Based on GC, the % conversion was calculated from the enantiomeric excess of the slower-reacting (S)-alcohol (ee\(_R\)) and the formed (R)-acetate (ee\(_P\)) according to the formula conv. = ee\(_R\)/(ee\(_R\) + ee\(_P\)); \(^c\) Determined by chiral HPLC analysis by using a Chiralcel OJ-H or Chiralcel OD-H column, respectively; \(^d\) Isolated yield after column chromatography; \(^e\) Calculated according to Chen et al. [106], using the equation: \(E = [\ln(1 - conv.)/(1 - ee\(_R\))]/[\ln(1 - conv.)/(1 + ee\(_R\))].

Moreover, to study the effect of diverse substituents on the benzene ring of the 1,2,3,4-tetrahydroquinoline unit, two other derivatives rac-2b\-c were subjected to preparative-scale enzymatic KR. The obtained results show that both examined lipases were less enantioselective toward substituted derivatives rac-2b\-c \((E = 21–140)\) than when tested along with rac-2a. Moreover, the racemic substrate bearing strong electron-withdrawing nitro group rac-2c turned out to be more challenging for lipases in terms of selectivity in enantiomeric discrimination than the one possessing electron-donating methyl group rac-2b. In the best reaction scenario, optically pure alcohol (S)-(+)-2c (>99% ee) was isolated from the reaction catalyzed by Novozym 435 in 35% isolated yield. Noteworthy, lipase-catalyzed KR employing both the racemic substrates rac-2b and rac-2c proceeded with higher reaction rates when compared to rac-2a, thus leading to 60–62% conv. when Novozym 435 was applied. The nitro-derivative rac-2c was also faster transformed by Amano PS-IM, leading to 46% conv. after 24 h. Since the nitro group present in rac-2c boosted the reaction rates, a drop in enantiomeric excess of the formed acetate (R)-(+)\-3c was observed.

Finally, to obtain the counterpart alcohols (R)-(−)-2a\-c, the respective acetates (R)-(+)\-3a\-c were subjected to \(K_2CO_3\)-catalyzed methanolysis. The cleavage of the acetyl group carried out for 24 h at 25 °C furnished (R)-(−)-2a in a quantitative yield and 99% ee, (R)-(−)-2b in 27% yield and 98% ee, and (R)-(−)-2c in 69% yield and with 99% ee, respectively.

The most interesting result was obtained in the case of (R)-(−)-2c, which has been isolated with a higher enantiomeric excess (99% ee) than the initial substrate (R)-(+)\-3c (94% ee). As we were puzzled by such an outcome, the hydrolytic conditions have also been applied toward less enantioenriched acetate (R)-(+)\-3c (67% ee) obtained via biocatalytic resolution. According to chiral HPLC, this attempt resulted in the isolation
of the corresponding alcohol (R)-(−)-2c with 72% ee, which unambiguously proved that enantiomeric enrichment occurs in the case of nitro-derivative. Such phenomenon might be attributed to the spontaneous amplification of optical purity under achiral chromatographic conditions, which have already been reported for various ‘not-completely-racemic’ chiral compounds [121–124]. More in detail, the reason for the observed chiral recognition may lay in the ability of (R)-(−)-2c to act as a donor and an acceptor of hydrogen bonds and hence the intermolecular interactions between the enantiomers, leading to the formation of homochiral (R,R or S,S) and heterochiral (R,S)-associations. In turn, such dimeric species are able to interact selectively with active centers of the silica gel, as is often observed in the case of diastereoisomers. However, the experimental observations should be correlated with the thermodynamic analysis of the potential equilibrium between possible stereoisomeric aggregates to prove that the effect of ‘self-disproportionation of enantiomer’ (SDE) occurred in the case of non-racemic alcohol (R)-(−)-2c. Interestingly, when we additionally performed KR of rac-2c and analyzed the crude reaction mixture using HPLC, it turned out that the % ee-values of (S)-(+)−2c and (R)-(+)−3c were the same as for the reaction, which KR products were purified using SiO2-based column chromatography. These results revealed that the SDE phenomenon is absent in more complex mixtures containing corresponding alcohol-ester pairs, providing clear proof that optical purities of the KR products, and thus E-values calculated for enzymatic reactions, are not affected.

2.2. Acyltransferase-Catalyzed KR of Racemic 1,2,3,4-Tetrahydroquinoline-Based Alcohols rac-2a-c Using Vinyl Acetate in Water

It is well-known fact that wild-type acyltransferase from Mycobacterium smegmatis (wt-MsAcT) displays poor enantioselectivity toward secondary alcohols (E < 20), and low catalytic activity, especially for aromatic substrates, thus catalyzing acyl transfer with unsatisfactory rates and substrate conversions [97]. Recently, to overcome these drawbacks, novel variants of MsAcT have been developed by means of site-directed mutagenesis [98]. The best variants possessed a less bulky amino acid instead of phenylalanine in the active site in positions (F150, F154, F174) [98,125]. Especially single and double variants, having in these positions an alanine or valine, offered exquisite control in enantioselective acyl transfer (up to E > 200) between ethyl acetate or vinyl acetate and various racemic alcohols (i.e., phenyl or aliphatic secondary alkanols and alkynols at varying sizes).

Taking this advantage into account, in the next step of the biocatalytic studies, we have investigated MsAcT-catalyzed KR of rac-2a-c using the library of made-in-home MsAcT variants in an aqueous 200 mM potassium phosphate buffer (pH 7.5) supplemented with vinyl acetate (10%, v/v, 1 M final conc.) as acyl donor (Table 6).

The KR of the respective racemic substrate rac-2a-c (50 mM final conc.) was performed on an analytical scale utilizing the appropriate crude MsAcT lyophilized cell lysate (cell-free extract, CFE, 0.5 mg/mL) as biocatalysts at 30 °C for 5 h with vigorous shaking (500 rpm). Under these conditions, we found that the F150A/F154A variant outperformed other engineered MsAcTs in terms of enantioselectivity (E = 149) when rac-2a was used as the substrate. In this regard, F150A/F154A-catalyzed KR of rac-2a afforded (S)-(+)−2a with 98% ee and (R)-(+)−3a with 94% ee at 51% conv.

In turn, the most enantioselective MsAcT-catalyzed transformations of rac-2b were observed for F154A (E = 328) and F150A/F154A (E = 198) variants, respectively. For example, when F154A was applied in KR of rac-2b, the enantiomers were resolved very well to afford (S)-(+)−2b with 50% ee and (R)-(+)−3b with 99% ee at 34% conv. Additionally, F150A/F154A-catalyzed biotransformation of rac-2b resulted in the isolation of (S)-(+)−2b with 89% ee and (R)-(+)−3b with 97% ee at 48% conv.

In the case of rac-2c, all the examined MsAcT variants displayed low-to-moderate enantioselectivity (E = 1–27), showing that enantiomers of racemic substrate possessing the nitro group in the benzene ring were difficult to distinguish. For the most enantioselective F154A variant, a conversion of 22% was observed, while the (R)-enantiomer of the corresponding acetate (R)-(+)−3c was formed with 91% ee and an E-value of 27. In
In turn, F150A/F154A variant turned out to be significantly more active toward rac-2c than F154A. In this case, 58% of alcohol rac-2c was converted to the (R)-configured acetate (R)-(+)−3c with 55% ee resulting in an E-value of 8. Further experiments with novel MsAcT variants are needed to achieve more enantioselective biotransformations of racemic 1-(3,4-dihydroquinolin-1(2H)-yl)propan-2-ols rac-2a–c.

Table 6. Screening conditions for MsAcT-catalyzed KR of rac-2a–c using vinyl acetate in an aqueous buffer.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate a</th>
<th>MsAcT</th>
<th>Conv. (%) b</th>
<th>ee&lt;sub&gt;s&lt;/sub&gt; (%) c</th>
<th>ee&lt;sub&gt;p&lt;/sub&gt; (%) c</th>
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<tr>
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<td>95</td>
<td>57</td>
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</tr>
<tr>
<td>3</td>
<td>rac-2a</td>
<td>F150A/F154A</td>
<td>51</td>
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<td>F150V/F154V</td>
<td>37</td>
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<td>52</td>
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<tr>
<td>5</td>
<td>F154V/F174V</td>
<td>44</td>
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<td>75</td>
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<td>39</td>
<td>7</td>
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<td>1</td>
</tr>
<tr>
<td>15</td>
<td>F154V/F174V</td>
<td>32</td>
<td>32</td>
<td>17</td>
<td>36</td>
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</table>

a Conditions: rac-2a–c (50 mM final conc.), MsAcT single variant used as crude lyophilized cell lysate (cell-free extract, CFE 0.5 mg/mL), vinyl acetate (10%, v/v), 200 mM KPi (pH 7.5, 0.5 mL), 5 h, 30 °C, 500 rpm (laboratory shaker). b Based on GC, the % conversion was calculated from the enantiomeric excess of the slower-reacting (S)-alcohol (ee<sub>s</sub>) and the formed (R)-acetate (ee<sub>p</sub>) according to the formula conv. = ee<sub>s</sub>/[(1 − conv.) (1 + ee<sub>s</sub>)]. c Determined by chiral HPLC analysis by using a Chiralcel OJ-H or Chiralcel OD-H column, respectively; d Calculated according to Chen et al. [106], using the equation: E = ln[(1 conv.)/(1 − conv.)]/ln[(1 + conv.)/(1 + conv.)].

2.3. The Assignment of the Absolute Configuration of Enantiomeric Products 2a–c and 3a–c

To the best of our knowledge, no data are available for the absolute configurations of chiral alcohols 2a–c or their acetates 3a–c. Therefore, the absolute configurations of the KR products were determined by comparison of the peak elution order registered with chiral HPLC using optically active chemical standards, (R)-(−)−2a (>99% ee), (R)-(−)−2b (59% ee), and (R)-(−)−2c (>99% ee), synthesized from commercial (R)-(−)-propylene oxide (>99% ee). According to our investigation, the slower-reacting alcohols 2a–c and their acetates 3a–c have the (S)- (+)- and (R)-(+) -configurations, respectively. This assignment agrees well with the Kazlauskas-rule postulated for lipases [126]. In this context, all tested MsAcT variants also showed a clear stereo-preference for the (R)-enantiomer of the employed racemic substrates rac-2a–c. Moreover, as a proof-of-principle, the absolute configuration of the synthesized nitro-alcohol (S)-(−)-2c (>99% ee) was unambiguously confirmed by determining the crystal structure from single crystals using X-ray diffraction (XRD) analysis (Figure 2). For details concerning the crystal growth conditions and routine crystal structure determination using single-crystal XRD analysis, please see Experimental Section 3.11 and Supporting Information.
without further purification. (Darmstadt, Germany).

The following crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC-2217269. Ellipsoids are set to 50% probability. Nitrogen atoms are presented in blue, oxygen atoms in maroon, carbon atoms in black, whereas hydrogen atoms in gray.

3. Materials and Methods

All commercially available reagents [purchased from Merck KGaA, (Darmstadt, Germany), Tokyo Chemical Industry Co., Ltd. (TCI) (Fukaya, Japan), Thermo Fisher (Kandel), GmbH (Kandel, Germany) and Fluorochem Ltd. (Hadfield Derbyshire, UK)] were used without further purification. (R)-(+)-Propylene oxide (>99% ee) employed for the synthesis of analytical standards was purchased from Merck KGaA (Darmstadt, Germany) (Catalog No. 540048). Chromatography grade n-hexane and isopropanol/pronan-2-ol (2-PrOH) used in high-performance liquid chromatography (HPLC) were purchased from Avantor Performance Materials Poland S.A. (formerly POCH Polish Chemicals Reagents).

The enzyme preparations were purchased from Novozymes A/S ( Bagsvaerd, Denmark), STREM Chemicals, Inc. (Newburyport, MA, USA), Amano Pharmaceutical Co., Ltd. (Nagoya, Japan), Sigma-Aldrich (currently Merck) (Darmstadt, Germany), Roche (Basel, Switzerland), Boehringer Mannheim (currently Roche Diagnostics) (Basel, Switzerland), and were used without pre-treatment (for details, see Table S1 appended in Supplementary Materials).

Analytical scale lipase-catalyzed reactions were performed in thermo-stated glass vials (V = 4 mL) placed in Chemglass CG-1991-04 GOD Anodized Aluminum Reaction Block, 48 Position, 19 mm Hole Depth, For Circular Top Hot Plate Stirrer.

Melting point (mp) ranges, uncorrected, were determined with a commercial apparatus (Thomas-Hoover “UNI-MELT” capillary melting point apparatus (Philadelphia, PA, USA)) on samples contained in rotating capillary glass tubes open on one side (1.35 mm inner diam. and 80 mm length).

Analytical thin-layer chromatography was carried on TLC aluminum plates with silica gel Kieselgel 60 F254 from Merck (Darmstadt, Germany) (0.2 mm thickness film containing a fluorescence indicator green 254 nm (F254) using UV light as a visualizing agent.

Preparative separations were carried out by column chromatography using Merck silica gel 60 (230–400 mesh), with grain size 40–63 µm or by PLC PSC-Fertigplatten Kieselgel 60 F254 (20 × 20 cm with 2 mm thickness layer) glass plates purchased from Merck (Darmstadt, Germany).
Optical rotations ([α]) were measured with a PolAAr 32 polarimeter from Optical Activity Ltd. (Ramsey, Huntingdon, England) in a 2 dm long cuvette using the sodium D line (589 nm) at 26 °C or 28 °C, respectively; [α]D are given in units of: deg dm⁻¹ cm⁻³ g⁻¹; the concentration c is in g/100 mL (for details, see Table S2 appended in Supplementary Materials).

The gas chromatography (GC) analyses were performed with an Agilent Technologies 6890N instrument from Agilent Technologies (Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and fitted with HP-50+ (30 m) semi-polar column (50% phenyl-50% methyl-poly-siloxane); the GC injector was maintained at 250 °C; helium (2 mL/min) was used as carrier gas; retention times (tR) were given in minutes under these conditions; column temperature programs were given in Table S3 appended in Supplementary Materials.

The enantiomeric excesses (% ee) of optically active compounds were determined by high-performance liquid chromatography (HPLC) analyses performed on Shimadzu Nexera-i (LC-2040C 3D) chromatograph from Shimadzu Corporation (Tokyo, Japan) equipped with a photodiode array detector (PAD) and Chiralcel OD-H or Chiralcel OJ-H (4.6 mm × 250 mm, coated on 5 µm silica gel grain size) chiral columns (Daicel Chemical Industries Ltd., Osaka, Japan) equipped with dedicated pre-columns (4 mm × 10 mm, 5 µm) using mixtures of n-hexane/2-PrOH as the respective mobile phases in the appropriate ratios; the HPLC analyses were executed in an isocratic and isothermal (30 °C) manner; flow (f) is given in mL/min; racemic compounds were used as standards; HPLC conditions and retention times (tR) are given in Table S4 appended in Supplementary Materials.

¹H NMR and ¹³C NMR spectra were recorded on a Spektrometr Varian NMR System 500 MHz from Varian, Inc. (Palo Alto, CA, USA); chemical shifts (δ) are given in parts per million (ppm) on the delta scale related to the solvent peak used as reference value (CDCl₃, δH (residual CHCl₃) 7.26 ppm, δC 77.16 ppm); chemical shifts concerning signal multiplicity assignment are quoted as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; coupling constant (J) are given in hertz (Hz); all samples were recorded as solutions in fully deuterated chloroform (CDCl₃). All NMR reports for the Supplementary Materials document were created by MestReNova (Version: 6.0.2-5475) which was purchased from Mestrelab Research, S.L. (Santiago de Compostela, Spain).

High-resolution mass spectrometry (HRMS) was recorded on a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer both from Waters Corporation (Milford, MA, USA), ESI source: electrospray with a spray voltage 4.00 kV for FTMS analysis; all samples were prepared by dilution of MeOH (0.5 mL) and additives of mixtures of CH₃CN/MeOH/H₂O (50:25:25, v/v/v) + 0.5% formic acid (HCO₂H) each.

Infrared (IR) spectra were recorded on Specord M80 from Carl Zeiss (Jena, Germany) in transmittance mode in the 300–4000 cm⁻¹ range, in ambient air at room temperature, with 2 cm⁻¹ resolution and accumulation of 32 scans; wavenumber (frequency, ν) is given in cm⁻¹; all samples were prepared as Nujol suspensions.

X-ray diffraction (XRD) analysis of the selected single crystal was measured with mirror mono-chromated CuKα radiation on an Oxford Diffraction k-CCD Gemini A Ultra diffractometer (Oxford, UK). The XRD data collection, data reduction, scaling, and absorption correction were performed using CrysAlisPro software (Rigaku Oxford Diffraction, V1.17.1.272a, 2022; Oxford, UK).

3.1. General Procedure for the Synthesis of Racemic Alcohols rac-2a–c

To a solution of the respective 1,2,3,4-tetrahydroquinoline (1a–c, 20.0 mmol) in anhydrous EtOH (10 mL), racemic propylene oxide (4.64 g, 80.0 mmol, 5.59 mL for 1a–b or 9.31 g, 0.16 mol, 11.22 mL for 1c) and NaHCO₃ (5.03 g, 60.0 mmol for 1a–b or 7.57 g, 90.2 mmol for 1c) were added. The reaction mixture was placed in an Ace Glass Front Seal Pressure Bottle (V = 150 mL; Catalog No. 8648–245) and vigorously stirred (1000 rpm) at 100 °C for 72 h for 1a–b or 168 h for 1c. After this time, the content of the flask was filtered under a vacuum, and the remaining permeate was concentrated using a rotary evaporator.
The remaining oil was subjected to SiO2 column chromatography (120 g of silica gel) using a gradient of n-hexane/EtOAc (90:10, 70:30 v/v) for purification of rac-2a–b or 95:5, 90:10, 80:20, 70:30 v/v for purification of rac-2c) mixture as eluent, thus yielding desired racemic alcohol rac-2a–c.

1-(3,4-Dihydroquinolin-1(2H)-yl)propan-2-ol (rac-2a). Yield 82% (3.13 g); yellowish oil; Rf [n-hexane/EtOAc (70:30 v/v)] 0.45; 1H NMR (500 MHz, CDCl3): δ 7.09–7.03 (m, 1H), 7.00–6.95 (m, 1H), 6.71–6.62 (m, 2H), 4.18–4.08 (m, 1H), 3.40–3.26 (m, 2H), 3.25–3.14 (m, 2H), 2.79 (t, J = 6.4 Hz, 2H), 2.20–1.95 (m, 2H), 1.25 (d, J = 6.2 Hz, 3H); 13C NMR (126 MHz, CDCl3): δ 146.3, 129.5, 127.2, 123.2, 117.0, 112.1, 65.5, 60.8, 51.1, 28.2, 22.3, 20.3; IR (nujol): νmax = 3452, 2932, 1788, 1504, 1096, 743, 774; HRMS (ESI-TOF) m/z: [M + H]+Calcd for C12H10NO+ m/z: 192.13829, Found 192.13821; GC [150–260 (10 °C/min)]: tR = 6.04 min; HPLC [n-hexane-i-PrOH (90:10, v/v); f = 0.8 mL/min; λ = 260 nm, Chiralcel OJ-H]; tR = 8.495 (S-isomer) and 9.120 min (R-isomer).

1-(6-Methyl-3,4-dihydroquinolin-1(2H)-yl)propan-2-ol (rac-2b). Yield 49% (2.03 g); violet oil; Rf [n-hexane/EtOAc (70:30 v/v)] 0.58; 1H NMR (500 MHz, CDCl3): δ 6.89–6.87 (m, 1H), 6.84–6.79 (s, 1H), 6.67–6.61 (m, 1H), 4.16–4.06 (m, 1H), 3.32 (dd, J = 11.2, 7.0, 4.1 Hz, 1H), 3.24 (ddd, J = 11.3, 7.0, 4.1 Hz, 1H), 3.20–3.10 (m, 2H), 2.77 (t, J = 6.2 Hz, 2H), 2.33 (d, J = 4.1 Hz, 1H), 2.23 (s, 3H), 2.03–1.90 (m, 2H), 1.24 (d, J = 6.2 Hz, 3H); 13C NMR (126 MHz, CDCl3): δ 144.2, 130.2, 127.6, 126.4, 123.4, 112.6, 65.4, 61.2, 51.1, 28.1, 22.4, 20.3, 20.2; IR (nujol): νmax = 3404, 2932, 2368, 1620, 1512, 1096, 1012, 940, 800; HRMS (ESI-TOF) m/z: [M + H]+Calcd for C13H12NO+ m/z: 206.13594, Found 206.13581; GC [150–260 (10 °C/min)]: tR = 6.71 min; HPLC [n-hexane-i-PrOH (97.3, v/v); f = 0.8 mL/min; λ = 260 nm, Chiralcel OJ-H]; tR = 15.023 (S-isomer) and 16.400 min (R-isomer).

1-(7-Nitro-3,4-dihydroquinolin-1(2H)-yl)propan-2-ol (rac-2c). Yield 83% (3.91 g); orange solid; mp 59–61 °C [for rac-2c (n-hexane/EtOAc) or 86–88 °C [for (S)–(S)-2c (n-hexane/EtOAc)]; Rf [n-hexane/EtOAc (70:30 v/v)] 0.25; 1H NMR (500 MHz, CDCl3): δ 7.43–7.36 (m, 2H), 7.02–7.01 (m, 1H), 4.21–4.17 (m, 1H), 3.47–3.37 (m, 2H), 3.31–3.27 (m, 2H), 2.82 (t, J = 6.4 Hz, 2H), 2.03–1.93 (m, 2H), 1.92 (br s, 1H), 1.65 (br s, 1H), 1.28 (d, J = 6.2 Hz, 3H); 13C NMR (126 MHz, CDCl3): δ 147.8, 146.4, 129.9, 129.5, 111.0, 105.2, 65.6, 59.7, 50.8, 28.4, 21.5, 21.0; IR (nujol): νmax = 3428, 2904, 1612, 1512, 1461, 1064, 1020, 864, 796, 740; HRMS (ESI-TOF) m/z: [M + H]+Calcd for C12H12N2O3+ m/z: 237.12337, Found 237.12336; GC [200–260 (10 °C/min)]: tR = 7.28 min; HPLC [n-hexane-i-PrOH (98.2, v/v); f = 0.8 mL/min; λ = 260 nm, Chiralcel OD-H]; tR = 38.392 (S-isomer) and 41.245 min (R-isomer).

3.2. General Procedure for the Synthesis of Racemic Esters rac-3a–c

To a solution of the respective racemic alcohol rac-2a–c (1.00 mmol) in dry CH2Cl2 (10 mL), Et3N (152 mg, 1.50 mmol, 209 μL) and DMAP (10 mg) were added. The mixture was cooled to 0–5 °C in an ice bath. Next, acetyl chloride (118 mg, 1.50 mmol, 106 μL for rac-2a–b or 157 mg, 2.00 mmol, 142 μL for rac-2c) was dissolved in dry CH2Cl2 (5 mL) and added dropwise to the reaction mixture by using a syringe. Afterward, the cooling bath was removed, and the resulting mixture was stirred at 25 °C for 24 h for rac-2a–b or 72 h for rac-2c. The crude mixture was diluted with CH2Cl2 (20 mL), subsequently quenched with H2O (40 mL), and the water phase was exchanged with CH2Cl2 (3 × 20 mL). The combined organic layer was washed with a saturated aqueous solution of NaHCO3 (80 mL) and brine (80 mL) and dried over anhydrous MgSO4. After evaporation of the residuals of solvent under reduced pressure, the crude product was purified by column chromatography on silica gel, using a gradient of n-hexane/EtOAc (70:30 v/v) mixture as eluent, thus yielding desired racemic ester rac-3a–c.

1-(3,4-Dihydroquinolin-1(2H)-yl)propan-2-yl acetate (rac-3a). Yield 36% (83 mg); yellowish oil; Rf [n-hexane/EtOAc (70:30 v/v)] 0.84; 1H NMR (500 MHz, CDCl3): δ 7.10–7.01 (m, 1H), 6.97–6.91 (m, 1H), 6.70–6.68 (m, 1H), 6.60–6.57 (m, 1H), 5.28–5.19 (m, 1H), 3.49 (dd, J = 15.0, 7.6 Hz, 1H), 3.41–3.27 (m, 2H), 3.19 (dd, J = 15.0, 5.3 Hz, 1H), 2.75 (t, J = 6.3 Hz, 2H), 1.98 (s, 3H), 1.96–1.89 (m, 2H), 1.28 (d, J = 6.4 Hz, 3H); 13C NMR (126 MHz, CDCl3): δ
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170.7, 145.2, 129.4, 127.2, 122.5, 116.3, 111.4, 68.8, 56.6, 50.8, 28.2, 22.1, 21.4, 18.1; IR (nujol): \( v_{\text{max}} = 2932, 2340, 1736, 1600, 1504, 1456, 1240, 1060, 744 \); HRMS (ESI-TOF) \( m/z: [M + H]^+ \) Calcd for \( \text{C}_{14}\text{H}_{20}\text{NO}_4^+ \) \( m/z: 234.14886 \), Found 234.14897; GC [150–260 (10 °C/min)]: \( t_R = 6.86 \text{ min}; \) HPLC \( [n\text{-hexane-i-PrOH} (90:10, \nu/v); f = 0.8 \text{ mL/min}; \lambda = 260 \text{ nm, Chiralcel OJ-H}]; t_R = 7.369 \text{ (S-isomer) and 7.955 min (R-isomer).} \)

1-(6-Methyl-3,4-dihydroquinolin-1(2H)-yl)propan-2-yl acetate (rac-3b). Yield 40% (99 mg); yellowish oil; \( R_l \) [\( n\text{-hexane/EtOAc} (70:30 \nu/v) \) 0.87; \( ^1\text{H NMR} (500 \text{ MHz, CDCCl}_3): \delta 6.87–6.85 \text{ (m, 1H), 6.77 (s, 1H), 6.62–6.21 (m, 1H), 5.26–5.17 (m, 1H), 3.45 (dd, } J = 15.0, 7.4 \text{ Hz, 1H), 3.38–3.24 (m, 2H), 3.16 (dd, } J = 15.0, 5.4 \text{ Hz, 1H), 2.72 (t, } J = 6.3 \text{ Hz, 2H), 2.20 (s, 3H), 1.98 (s, 3H), 1.95–1.88 \text{ (m, 2H), 1.27 (d, } J = 6.4 \text{ Hz, 3H); } ^{13}\text{C NMR (126 MHz, CDCCl}_3): \delta 170.7, 143.3, 130.0, 127.6, 125.1, 122.3, 111.3, 69.0, 56.7, 50.9, 28.2, 22.4, 21.4, 20.3, 18.1; \) IR (nujol): \( v_{\text{max}} = 2932, 2368, 1740, 1620, 1512, 1456, 1244, 1060, 800; \) HRMS (ESI-TOF) \( m/z: [M + H]^+ \) Calcd for \( \text{C}_{15}\text{H}_{22}\text{NO}_4^+ \) \( m/z: 248.16451 \), Found 248.16418; GC 150–260 (10 °C/min): \( t_R = 7.61 \text{ min; HPLC} \[n\text{-hexane-i-PrOH} (97.3, \nu/v); f = 0.8 \text{ mL/min}; \lambda = 260 \text{ nm, Chiralcel OJ-H}]; t_R = 8.831 \text{ (S-isomer) and 9.866 min (R-isomer).} \)

1-(7-Nitro-3,4-dihydroquinolin-1(2H)-yl)propan-2-yl acetate (rac-3c). Yield 52% (144 mg); orange solid; mp 69–71 °C \[ for \text{rac-3c} \text{ (n-hexane/EtOAc) \text{ or } 51–53 °C \text{ (for } \text{(R)-(+)-3c} \text{ (n-hexane/EtOAc)\text{)}}; R_l \) [\( n\text{-hexane/EtOAc} (70:30 \nu/v) \) 0.73; \( ^1\text{H NMR} (500 \text{ MHz, CDCCl}_3): \delta 7.54–7.53 \text{ (m, 1H), 7.39–7.38 (m, 1H), 7.01–6.99 (m, 1H), 5.33–5.22 (m, 1H), 3.58 (dd, } J = 15.4, 8.3 \text{ Hz, 1H), 3.43 (dt, } J = 11.2, 4.9 \text{ Hz, 1H), 3.37–3.31 (m, 1H), 3.28 (dd, } J = 15.4, 4.1 \text{ Hz, 1H), 2.79 (t, } J = 6.3 \text{ Hz, 2H), 1.98–1.89 \text{ (m, 5H), 1.61 (br s, 1H), 1.31 (d, } J = 6.4 \text{ Hz, 3H); } ^{13}\text{C NMR (126 MHz, CDCCl}_3): \delta 170.7, 147.8, 145.9, 129.5, 129.4, 110.7, 105.1, 68.5, 56.3, 50.1, 28.4, 21.3, 21.3, 18.1; \) IR (nujol): \( v_{\text{max}} = 2920, 1736, 1616, 1512, 852, 732; \) HRMS (ESI-TOF) \( m/z: [M + H]^+ \) Calcd for \( \text{C}_{14}\text{H}_{19}\text{NO}_4^+ \) \( m/z: 279.13393, \) Found 279.13371; GC [200–260 (10 °C/min)]: \( t_R = 7.50 \text{ min; HPLC} \[n\text{-hexane-i-PrOH} (99.1, \nu/v); f = 0.7 \text{ mL/min}; \lambda = 260 \text{ nm, Chiralcel OD-H}]; t_R = 23.062 \text{ (S-isomer) and 24.774 min (R-isomer).} \)

3.3. General Procedure for Analytical Scale Lipase-Catalyzed KR of rac-2a—Enzyme Screening

To a solution of racemic 1-(3,4-dihydroquinolin-1(2H)-yl)propan-2-ol (rac-2a, 25 mg, 0.13 mmol) in MTBE (2 mL), vinyl acetate (934 mg, 10.85 mmol, 1 mL) and the respective lipase preparation [25 mg, 100%, \( w/w \) (catalyst/substrate rac-2a)] were added. The reaction mixture was stirred for 24 h at 40 °C and 800 rpm. The enzymatic reaction was stopped by filtering off the enzyme under suction and rinsing the filtrate cake of exploited lipase with MTBE (2 mL). After evaporation of the volatiles from the permeate, the crude oil was subjected to SiO\(_2\) column chromatography using a gradient of \( n\)-hexane/EtOAc (90:10, 80:20, 70:30 \( \nu/v \)) mixture, yielding desired optically active (\( S \))-(+)-2a and (\( R \))-(+)-3a. For details, see Table 1.

3.4. General Procedure for Analytical Scale Lipase-Catalyzed KR of rac-2a—Co-Solvent Screening

To a solution of racemic 1-(3,4-dihydroquinolin-1(2H)-yl)propan-2-ol (rac-2a, 25 mg, 0.13 mmol) in organic solvent (2 mL), vinyl acetate (56 mg, 0.65 mmol, 60 \( \mu \)L) and the respective lipase preparation [5 mg, 20%, \( w/w \) (catalyst/substrate rac-2a)] were added. The reaction mixture was stirred for 24 h at 40 °C and 800 rpm. The enzymatic reaction was stopped by filtering off the enzyme under suction and rinsing the filtrate cake of exploited lipase with MTBE (2 mL). After evaporation of the volatiles from the permeate, the crude oil was subjected to SiO\(_2\) column chromatography using a gradient of \( n\)-hexane/EtOAc (90:10, 80:20, 70:30 \( \nu/v \)) mixture, yielding desired optically active (\( S \))-(+)-2a and (\( R \))-(+)-3a. For details, see Table 2.

3.5. General Procedure for Analytical Scale Lipase-Catalyzed KR of rac-2a—Effect of Temperature

To a solution of racemic 1-(3,4-dihydroquinolin-1(2H)-yl)propan-2-ol (rac-2a, 25 mg, 0.13 mmol) in organic solvent (2 mL), vinyl acetate (56 mg, 0.65 mmol, 60 \( \mu \)L) and Amano PS-IM [5 mg, 20%, \( w/w \) (catalyst/substrate rac-2a)] were added. The reaction mixture was...
stirred for 72 h (800 rpm) at 40 °C, 50 °C, and 60 °C, respectively. The rest of the procedure was essentially the same as in the previous sections. For details, see Table 3.


To a solution of racemic 1-(3,4-dihydroquinolin-1(2H)-yl)propan-2-ol (rac-2a, 25 mg, 0.13 mmol) in MTBE (2 mL), vinyl acetate (56 mg, 0.65 mmol, 60 µL) and Novozym 435 or Amano PS-IM [5 mg, 20%, w/w (catalyst/substrate rac-2a)] were added, respectively. The reaction mixture was stirred at 40 °C (800 rpm) and terminated after 8 h, 12 h, and 18 h (in the case of Novozym 435) or after 24 h, 72 h, and 120 h (in the case of Amano PS-IM). The rest of the procedure was essentially the same as in the previous sections. For details, see Table 4.

3.7. General Procedure for Preparative Scale Lipase-Catalyzed KR of rac-2a–c

To a solution of racemic alcohol rac-2a–c (2.61 mmol); 500 mg in the case of rac-2a; 536 mg in the case of rac-2b; 618 mg in the case of rac-2c) in MTBE (40 mL), vinyl acetate (1.13 g, 13.05 mmol, 1.20 mL) and Novozym 435 or Amano PS-IM (100 mg) were added. The reaction mixture was stirred at 40 °C and 800 rpm for 18 h (in the case of Novozym 435) or 24 h (in the case of Amano PS-IM). Next, the enzymatic reaction was stopped by filtering off the enzyme under suction and rinsing the filtrate cake of exploited lipase with MTBE (60 mL). After evaporation of the volatiles from the permeate, the crude oil was subjected to SiO2 column chromatography using a gradient of n-hexane/EtOAc (90:10, 80:20, 70:30 v/v) mixture, yielding desired optically active alcohol (S)-(+)–2a [195 mg, 39% yield, 98% ee (in the case of Novozym 435) and 297 mg, 59% yield, 36% ee (in the case of Amano PS-IM)], (S)-(+)–2b [153 mg, 29% yield, >99% ee (in the case of Novozym 435) and 275 mg, 51% yield, 35% ee (in the case of Amano PS-IM)], (S)-(+)–2c [219 mg, 35% yield, >99% ee (in the case of Novozym 435) and 300 mg, 49% yield, 79% ee (in the case of Amano PS-IM)] and acetate (R)-(+)–3a [292 mg, 48% yield, 82% ee (in the case of Novozym 435) and 154 mg, 25% yield, 99% ee (in the case of Amano PS-IM)], (R)-(+)–3b [319 mg, 49% yield, 62% ee (in the case of Novozym 435) and 146 mg, 23% yield, 98% ee (in the case of Amano PS-IM)], (R)-(+)–3c [379 mg, 52% yield, 67% ee (in the case of Novozym 435) and 294 mg, 40% yield, 94% ee (in the case of Amano PS-IM)], respectively. For details, see Table 5.

3.8. General Procedure for K2CO3-Catalyzed Methanolysis of (R)-(+)–3a–c

To a solution of optically active ester (R)-(+)–3a–c (0.11 mmol; 25 mg in the case of (R)-(+)–3a (99% ee); 27 mg in the case of (R)-(+)–3b (98% ee); 31 mg in the case of (R)-(+)–3c (94% ee)) in MeOH (2 mL), anhydrous K2CO3 (30 mg, 0.22 mmol) was added in one portion. The resulting mixture was stirred for 24 h at 25 °C. Next, the volatile compounds were evaporated under vacuum, and the crude oil was subjected directly to column chromatography and purified using a gradient of a mixture of n-hexane/EtOAc (70:30 v/v) as an eluent yielding optically active (R)-(–)–2a (21 mg, >99% yield, 99% ee), (R)-(–)–2b (6 mg, 27% yield, 98% ee), or (R)-(–)–2c (18 mg, 69% yield, 99% ee), respectively.

3.9. General Procedure for the Synthesis of (R)-(–)–2a–c Using Commercially Available (R)-(+)–Propylene Oxide (99% ee)

To a solution of the respective 1,2,3,4-tetrahydroquinoline (1a–c, 2.0 mmol) in anhydrous EtOH (1 mL), optically pure (R)-(+)–propylene oxide (464 mg, 8.0 mmol, 559 µL for 1a–b or 931 mg, 16 mmol, 1.12 mL for 1c) and NaHCO3 (503 mg, 6.0 mmol for 1a–b or 757 mg, 9.0 mmol for 1c) were added. The reaction mixture was placed in an Ace pressure tube (V = 15 mL; Catalog No. Z181099) and vigorously stirred (1000 rpm) at 100 °C for 72 h. After this time, the content of the flask was filtered under a vacuum, and the remaining permeate was concentrated using a rotary evaporator. The remaining oil was subjected to SiO2 column chromatography using a gradient of n-hexane/EtOAc (90:10, 70:30 v/v for purification of (R)-(–)–2a or 95:5, 90:10, 80:20, 70:30 v/v for purification of (R)-(–)–2c) mixture as eluent. In turn, optically active alcohol (R)-(–)–2b was sequentially purified
on plates dedicated for preparative layer chromatography (PLC) using \textit{n}-hexane/EtOAc (80:20, \textit{v/v}) as eluent for the first development, \textit{n}-hexane/EtOAc (90:10 \textit{v/v}) for the second development, and \textit{n}-hexane/EtOAc (80:20 \textit{v/v}) for the last development. The following alcohols as HPLC-analytical standards were afforded: (\textit{R})-(–)-2a (208 mg, 54\% yield, >99\% ee), (\textit{R})-(–)-2b (10 mg, 2\% yield, 59\% ee), (\textit{R})-(–)-2c (338 mg, 71\% yield, >99\% ee).

3.10. Screening Conditions for MsAcT-Catalyzed KR of rac-2a–c Using Vinyl Acetate in Water

The EKR reaction was initiated by the addition of substrate rac-2a–c (50 \textmu L) from a 0.5 M stock solution prepared by dissolving rac-2a (95.6 mg) or rac-2b (102.6 mg) or rac-2c (118.1 mg) in vinyl acetate (1 mL), and the lyophilized cell-free extract (CFE) of the respective MsAcT (5 \textmu L) from a stock solution prepared by suspending CFE (25 mg) in 200 mM KPi buffer (pH 7.5, 0.5 mL). In general, biotransformations were conducted in 0.5 mL final volume composed of 200 mM KPi buffer (pH 7.5)/vinyl acetate (90:10, \textit{v/v}) in glass vials (\textit{V} = 1.5 mL) at 30 \textdegree C using a laboratory shaker (500 rpm) for 5 h. After this time, each reaction was stopped by extracting the content of the vial with MTBE (3 \x 0.5 mL). The combined organic phase was dried over anhydrous MgSO\textsubscript{4}, the filtrate was additionally centrifuged (5 min, 6000 rpm), and only then was the supernatant was transferred into separate HPLC vials and concentrated under vacuum. The oil residue in one of the vials was used to determine \% conv. by using GC analysis, and the other oil residue was redissolved in an HPLC-grade mixture of \textit{n}-hexane/2-PrOH (1.5 mL; 3:1, \textit{v/v}), passed through a short pad of silica gel loaded into a Pasteur pipette with cotton wool (in order to remove residuals of the cell components), and analyzed directly by HPLC on a chiral stationary phase to establish enantiomeric excesses of optically active alcohol (\textit{S})-(+)–2a–c and acetate (\textit{R})-(+)–3a–c. Blank reactions were prepared with buffer (5 \textmu L, KPi 200 mM, pH 7.5) instead of MsAcT enzyme solution. For details, see Table 6.

3.11. XRD Analyses

3.11.1. Conditions for Crystal Growth of (2S)-1-(7-Nitro-3,4-dihydroquinolin-1(2H)-yl)propan-2-ol ((\textit{S})-(+)-2c)

A single crystal of sufficient quality for X-ray diffraction (XRD) analysis was prepared using solvent evaporation methodology. In this regard, (\textit{S})-(+)–2c (15 mg, >99\% ee) was dissolved in EtOAc (1 mL) and transferred into an open-neck glass vial (\textit{V} = 4 mL). The vessel was sealed with Parafilm M (Sigma Aldrich) with a small hole punctured to enable very slow evaporation of the solvent and stored at room temperature. After two weeks, single crystals of morphology suitable to perform reliable XRD analysis were grown.

3.11.2. Crystal Structure Determination of (\textit{S})-(+)-2c

A suitable crystal of (\textit{S})-(+)-2c with dimensions 0.83 \times 0.50 \times 0.17 mm\textsuperscript{3} was selected and mounted on an Oxford Diffraction \kappa-CCD Gemini A Ultra diffractometer. The crystal was measured with mirror mono-chromated CuK\textalpha radiation at room temperature. Data collection, data reduction, scaling, and absorption correction were performed using CrysalisPro 1.171.42.72a [127]. The final completeness is 100.00\% out to 67.002 in \textdegree. Empirical absorption correction using spherical harmonics, implemented in the SCALE3 ABSPACK scaling algorithm, was applied. The structure was solved with the ShelXT 2018/2 [128] solution program using dual methods and Olex2 1.5 [129] as the graphical interface. The model was refined with ShelXL 2019/2 [130] using full matrix least squares minimization on \textit{F}\textsuperscript{2}. All non-hydrogen atoms were refined aniso-tropically. Hydrogen atom positions were calculated geometrically and refined using the riding model, except hydrogen atom from the hydroxy group, which was picked from the electronic differential map and refined freely. The Flack parameter was refined to 0.03(9). A slightly increased error on this parameter is caused by a disorder observed in the crystal structure of (\textit{S})-(+)-2c with two conformations of a six-membered aza ring. For more detail, see Supporting Information. An absolute configuration (\textit{S}) for the molecule was successfully determined using anomalous dispersion effects. The Flack parameter calculated from selected 849 quotients...
Further analysis of the absolute structure was performed using likelihood methods with PLATON using Bijvoet pairs to obtain the Hooft parameter. A total of 915 Bijvoet pairs (coverage of 0.99) were included in the calculations. The resulting value of the Hooft parameter was 0.05(8), with a P3 probability for an inverted structure smaller than $10^{-27}$. CCDC 2217269 contains the supplementary crystallographic data for compound (S)-(+)−2c. This can be obtained free of charge on application to CDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: (+44)1223-336-033; email: deposit@ccdc.cam.ac.uk).

3.11.3. Crystal Data for (S)-(+)−2c

$$C_{12}H_{16}N_2O_3, M_r = 236.27, \text{monoclinic, } P2_1/a, a = 9.1028(2) \text{ Å, } b = 5.18900(10) \text{ Å, } c = 12.4899(3) \text{ Å, } \beta = 97.670(2)^\circ, V = 584.68(2) \text{ Å}^3, T = 293 K, Z = 2, Z' = 1, m(Cu K\alpha) = 0.803, 18271 reflections measured, 2086 unique (R_{int} = 0.0673) which were used in all calculations. The final wR_2 was 0.0773 (all data) and R_1 was 0.0290 (I \geq 2 s(I)), Flack parameter = 0.03(9), Hooft parameter = 0.05(8) from 915 pairs (99% coverage).

4. Conclusions

Herein, we present our efforts to design novel alternative chemoenzymatic routes to access optically active alcohols possessing 1,2,3,4-tetrahydroquinoline moiety. Interestingly, the asymmetric synthesis of such optically active alcohols has not been reported before, either via chemo-catalytic or biocatalytic methods. At the same time, these synthons are potentially useful in the development of novel therapeutics. To fill this gap, enzyme-catalyzed kinetic resolution of titled racemic compounds was accomplished using either commercially available lipases from *Candida antarctica* type B or *Burkholderia cepacia* or made-in-home engineered variants of acyltransferase originating from *Mycobacterium smegmatis* (MsAcT).

In general, in terms of the optical purities of KR products, higher ee-values were observed in the reactions catalyzed by lipases. However, it seems interesting that MsAcT variants exhibited improved enantioselectivity toward methyl-substituted derivative rac-2b ($E = 328$) than the lipases ($E = 140$). Moreover, it has to be mentioned that MsAcT variants were significantly influenced by the electronic properties caused by the substituents present in the benzene ring of the 1,2,3,4-tetrahydroquinoline moiety. In this context, the nitro group in rac-2c displayed an even higher adverse effect on $E$-values than for the reactions catalyzed by lipases. Considering the above conclusions, it is clear that both methods can be used complementarily depending on the employed racemic substrate; however, the attempted MsAcT-catalyzed KR should be considered more environmentally benign since it generates less volatile and toxic organic solvents as wastes and accelerates the enantiomers resolutions significantly. Thus, we believe that examined variants of MsAcT are valuable additions to the biocatalysts library useful in the kinetic resolution of secondary alcohols and that this study will lead to more practical biocatalytic applications.

**Supplementary Materials:** The following supporting information can be downloaded at: [https://www.mdpi.com/article/10.3390/catal12121610/s1](https://www.mdpi.com/article/10.3390/catal12121610/s1), Figure S1. The geometry of two conformations observed for (S)-(+)−2c molecule; Figure S2. Crystal packing of (S)-(+)−2c showing H-bond topology; Figure S3: Crystal structure of (S)-(+)−2c with the atom numbering scheme; Table S1: List of commercial enzyme preparations employed in these studies; Table S2: The results of specific rotation values for the EKR products; Table S3: Analytical separation conditions of studied compounds by GC column; Table S4: HPLC analytical separation conditions of racemic alcohols and acetates; Table S5: Crystal data and structure refinement parameters for (S)-(+)−2c; Table S6: Atomic Occupancies for carbon atoms that are not fully occupied in (S)-(+)−2c; Table S7: Hydrogen Bond information for (S)-(+)−2c; Table S8: Bond Lengths in Å for (S)-(+)−2c; Table S9: Bond Angles in ° for (S)-(+)−2c; Table S10: Torsion Angles in ° for (S)-(+)−2c; Copies of HPLC chromatograms; Copies of NMR, FTMS, and IR spectra.
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