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An Enzymatic Toolbox for the Kinetic Resolution of 2-(Pyridin-*x*-yl)but-3-yn-2-ols and Tertiary Cyanohydrins

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The kinetic resolution of a series of acetates of tertiary alcohols bearing a nitrogen substituent has been studied by using several recombinant carboxyl esterases and variants thereof expressed in *E. coli*. Most of the enzymes were active in the conversion of these tertiary alcohols and excellent enantioselectivities were achieved in the synthesis of three 2-(pyridin-x-yl)but-3-yn-2-ols with the nitrogen atom in the pyridine ring in the 2′-, 3′-, and 4′-positions. The resolution of tertiary cyanohydrins proved to be more difficult as the enantioselectivity of the enzymes was generally lower. Nevertheless, (*S*)-1-cyano-2,2,2-trifluoro-1-phenylethyl acetate was obtained with 99 % *ee*. The results show that the limited substrate range of the individual enzymes in the synthesis of a series of tertiary alcohols can be efficiently overcome by using a combination of different enzymes.

Introduction

Optically pure tertiary alcohols and tertiary cyanohydrins are versatile building blocks in organic synthesis. For instance, compound **1c** has been used in the synthesis of an A_{2A} receptor antagonist that was shown to be orally active in a mouse catalepsy model[1] or the acetylene moiety of **1a** can be used to synthesize useful heterocyclic structures (see Scheme 1).[2] The precursor for the synthesis of anticonvulsants can also be obtained from **1i**.[3] Moreover, optically pure tertiary alcohols have recently received attention as potential inhibitors of HIV protease[4] and reverse transcriptase.[5] The synthesis of these compounds by both chemical and biocatalytic routes is challenging and no standard method has been developed so far.[5–9]

The multitude of applications for aldehyde-derived cyanohydrins has been reviewed recently.[10,11] A straightforward biocatalytic approach for their preparation involves the addition of HCN to aldehydes catalyzed by hydroxynitrile lyases. Ketone-derived cyanohydrins have fewer applications owing to their difficult synthesis. Indeed, in the analogous synthesis of tertiary cyanohydrins by the addition of HCN to ketones, the equilibrium is on the side of the substrates. This makes the use of a large excess of HCN necessary. In addition, the stereodifferentiation becomes considerably more difficult when ketones are the substrates.[12,13] The alternative chemical asymmetric syntheses only give reasonable results with methyl ketones and are thus rather limited.[6,9] Ketone-derived cyanohydrins are tertiary alcohols. The enantioselective hydrolysis of their esters by carboxyl esterases is a promising alternative to the addition of HCN to ketones as the starting compounds are easy to prepare.[14,15] When a neutral pH is used in the reaction, the cyanohydrin formed decomposes into the corresponding ketone and HCN. The resulting ketones can be reused for the synthesis of the corresponding racemic esters. Therefore no racemization is needed in the kinetic resolution. Previous attempts with commercially available lipases, proteases,[15] and whole-cell catalysts[16–18] have met only moderate success so far or have been limited to aliphatic tertiary cyanohydrins.

In the last few years, the kinetic resolution of racemic tertiary alcohol acetates catalyzed by hydrolases has been established as an attractive method for the synthesis of tertiary alcohols.[8,15,19,20] In particular, the esterase-catalyzed hydrolysis of esters of tertiary alcohols is a highly enantioselective route to optically pure building blocks.[8,15] Only a very few commercially available enzymes, however, convert these sterically very demanding substrates. We have identified a series of esterases with confirmed activity towards tertiary alcohols, some of them showing excellent enantioselectivity towards a range of compounds.[20–22] All these esterases share a motif of three glycines or two glycines and an alanine in their active sites. This so-called GGG(A)X motif has been related to the ability of the esterases to convert tertiary alcohols.[22] The substrate specificity of the esterases, however, is very high and seems to be affected by

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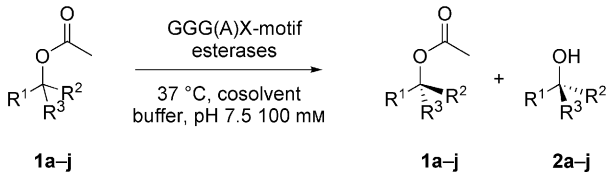


a: R^1 = 2'-pyridyl, R^2 = CH_3, R^3 = C≡CH
b: R^1 = 3'-pyridyl, R^2 = CH_3, R^3 = C≡CH
c: R^1 = 4'-pyridyl, R^2 = CH_3, R^3 = C≡CH
d: R^1 = 4'-pyridyl, R^2 = CH_3, R^3 = CH=CH_2
e: R^1 = Ph, R^2 = CF_3 , R^3 = CH=CH_2
f: R^1 = Ph, R^2 = CH_3, R^3 = CN
g: R^1 = 4-Cl-C_6H_4, R^2 = CH_3, R^3 = CN
h: R^1 = 4-MeO-C_6H_4, R^2 = CH_3, R^3 = CN
i: R^1 = Ph, R^2 = CF_3, R^3 = CN
j: R^1 = 2-thienyl, R^2 = CH_3, R^3 = CN

k: R^1 = Ph, R^2 = CH_3, R^3 = C≡CH
l: R^1 = Ph, R^2 = CF_3, R^3 = C≡CH

Scheme 1. Kinetic resolution of tertiary alcohol acetates **1a**–**j**. Compounds **1k** and **1l** are shown for comparison.

steric and electronic effects caused by minor changes in the substrate structure.[20,21] We attempted to overcome this limitation by providing a wide diversity of different GGG(A)X esterases consisting of novel biocatalysts from the metagenome[21] and highly enantioselective esterase variants created by protein design.[19,23,24] Esterase BS2 from *Bacillus subtilis* has previously been shown to be highly enantioselective towards tertiary alcohol acetates.[20] The enzyme variants BS2 G105A with increased enantioselectivity[19] and BS2 E188W/M193C with inverse enantiopreference[24] were created by protein design. The metagenome-derived esterase Est8[25] and esterase PEstE from the thermophilic archeon *Pyrobaculum calidifontis*[26] are both highly enantioselective in the kinetic resolution of tertiary alcohols bearing a *tert*-butylcarbamoyl substituent. A novel esterase from *Pseudomonas putida* has recently been cloned and overexpressed.[37] It was identified by the presence of the GGG(A)X motif as being active towards tertiary alcohol acetates. Recombinantly expressed isoenzymes of pig liver esterase (PLE) offer several advantages over the use of the commercially available crude extract, which consists of a mixture of different isoenzymes, such as a higher enantioselectivity, a higher reproducibility, and fewer regulatory issues.[27] The sequences of the two isoenzymes γPLE and PLE5 differ only in 21 amino acids, but this leads to a striking change in enantioselectivity and enantiopreference towards the acetates of secondary alcohols.

In the work reported herein, we prepared a series of tertiary alcohols containing a nitrogen atom in various positions, including pyridine-substituted aryl-substituted aliphatic tertiary alcohols and tertiary cyanohydrins (Scheme 1). The effect of variation of the position of a nitrogen heteroatom on the enantioselectivity of the different enzymes is discussed.

Results and Discussion

Substrate Synthesis, Analysis, and Stability

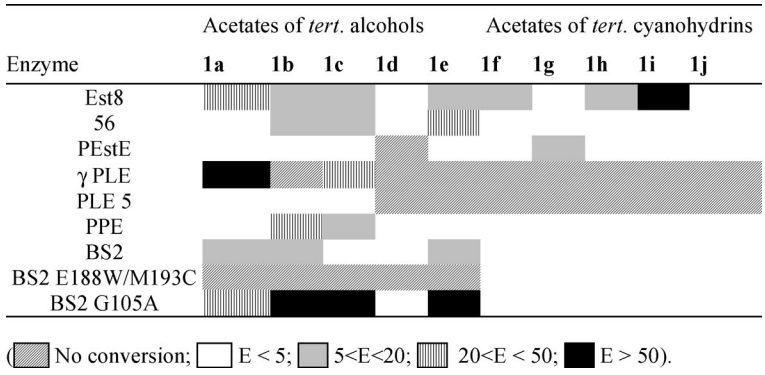
The racemic tertiary alcohols **2a**–**j** were prepared from the corresponding ketones by Grignard reaction or addition of TMSCN. Subsequent acetylation yielded the corresponding racemic acetates **1a**–**j**. For all compounds a method for the separation of their enantiomers by GC analysis was established (see the Supporting Information). The absolute configurations of the cyanohydrins **1f**–**h** were recently determined and the configurations of **1i** and **1j** were assigned on the basis of their elution order in chiral GC analysis.[15,18]

The acetates were subjected to analytical-scale kinetic resolution using nine esterases and variants thereof. The reactions were carried out at pH 7.5 and 37 °C in the presence of an appropriate cosolvent (Scheme 1). Aryl-substituted aliphatic tertiary alcohol acetate **1k** undergoes a nonenzymatic S_N1-type hydrolysis in aqueous systems.[28] Even though this can be suppressed to a certain degree by the addition of water-miscible cosolvents such as DMSO,[23] it still lowers the effective enantioselectivity of the kinetic resolution. The trifluorinated analogue **1l** is stable in buffer because of the electron-withdrawing effect of the trifluoromethyl group.[28] Pyridyl-substituted aliphatic tertiary alcohols can also be expected to have a higher stability in aqueous systems due to a lower electron density in the pyridine ring. Indeed, none of the acetates **1a**–**d** underwent measurable autohydrolysis during the reaction; neither did the cyanohydrins **1f**–**j**. As expected, the products of the kinetic resolutions of **1f**–**j** decomposed to the corresponding ketones under the screening conditions.

Activity of the Enzymes

The kinetic resolution of **1a**–**d** was investigated to study the effect of the pyridine substituent (R^1, Scheme 1) on the activity and enantioselectivity of the esterases. Acetate **1e** was included for a better understanding of the influence of R^2 and to facilitate a comparison with **1d** and **1l**.[20] The screening of **1f**–**j** allowed the performance of the enzymes to be determined in the conversion of tertiary alcohol acetates bearing a CN substituent in position R^3. Table 1 summarizes the enantioselectivity of the esterases towards **1a**–**1j**. For details, the reader is referred to the Supporting Information. All wild-type enzymes showed activity towards at least some of the substrates (Table 1). The enzyme activity towards tertiary alcohol acetates was determined relative to

Table 1. Enantioselectivity of GGG(A)X esterases towards tertiary alcohol acetates **1a**–**1j**.



the activity towards *p*-nitrophenyl acetate (pNPA), for example, the number of units necessary to reach 50% conversion in 1 h. The differences between the activities of the GGG(A)X esterases towards the structurally related compounds were striking. For instance, application of 0.5 U of PPE led to 58% conversion in the resolution of **1b** whereas 126 U of BS2 were necessary to achieve a similar conversion. Wild-type esterase BS2 and its mutant BS2 G105A converted the pyridyl aliphatic tertiary alcohol acetates **1a**–**d**, whereas double mutant E188W/M193C showed no activity. A ten-fold greater amount of mutant BS2 G105A was necessary to resolve the pyridyl aliphatic compounds **1a**–**c** (around 60 U) in comparison to the phenyl-substituted aliphatic **1l** (6 U).[20,24] The same holds true for the vinyl analogue of **1l**, **1e** (56 U). Both effects are combined in **1d**, with 160 U being necessary to achieve 50% conversion. In the case of the aryl-substituted aliphatic tertiary alcohol acetates **1a**–**1e**, the position of the nitrogen atom has a strong effect on the enantioselectivity of the esterases. Esterase variant BS2 G105A resolved **1a**–**1c** and **1e** with moderate-to-excellent enantioselectivity. Est8, PPE, and Est56 showed moderate enantioselectivity towards **1a**, **1b**, and **1e**, respectively (Table 1). With substrates **1a**–**1c**, the difference in enantioselectivities is assumed to reside not in the size effect of the substituents but in the electronic effects as the three compounds share a pyridine substituent of nearly identical size. This phenomenon has also been observed before with lipases in the kinetic resolution of secondary alcohols.[29,30] Furthermore, the effect could arise from differences in the interaction of the nitrogen atom of the pyridine ring with residues or water molecules in the active site of enzymes. Zhu et al.[31] observed this kind of effect in the reduction of aryl ketones with ketoreductases. Also, in the revised rule to predict the enantioselectivity in the kinetic resolution of secondary alcohols, Savile and Kazlauskas[32] described how substrate solvation could strongly effect the enantioselectivity.

Surprisingly, the enantioselectivity dropped dramatically in the conversion of **1d** in comparison to its analogue **1c** despite the small difference in the R^3 substituents. This can be explained by the difference in the nature of bond: The double bond CH_2=CH in **1d** is less rigid than the triple bond CH≡C in **1c** and this can lead to a different behavior of the compounds in the active site.

Previously, we modeled the substrate recognition of BS2 esterase in molecular dynamics studies.[23,24] The active site of this esterase is large and allows for the accommodation of the substrate in several binding modes, for which one productive configuration for each enantiomer could be identified. Given the large size of the active center, it is surprising that a subtle electronic change, such as the difference between a 2- and 3-pyridyl moiety in **1a** and **1b**, in the substrate structure might have such a strong impact on the activity and enantioselectivity of the enzyme. Several other esterases of the series also show remarkable differences in enantioselectivity between **1a** and **1b**. This led us to conclude that the substrate recognition of the esterases is strongly influenced by electronic interactions rather than mere steric effects.

The effect of electronic interactions on the enantioselectivity of carboxyl hydrolases was observed by Rotticci et al.[29] when they studied the kinetic resolution of secondary alcohols bearing methyl and bromo substituents catalyzed by lipase B from *Candida antarctica*. Similar catalytic behavior has previously been observed with GGG(A)X hydrolases[14,20,21] and commercially available lipases,[29] proteases,[15] and whole-cell biocatalysts[16–18] and seems to be the general rule in the conversion of these very difficult substrates.

Interactions with the solvent may also account for the differences in substrate recognition in the cases of both pyridine/phenyl and ethynyl/nitrile substituents. Savile and Kazlauskas[32] demonstrated that the solvation of the substrate contributes to the enantiorecognition of the protease subtilisin. They showed an inversion of the enantiopreference towards some aryl-substituted aliphatic secondary alcohols in the hydrolysis in buffer and in the esterification in organic solvent. This switch was not observed for substrates bearing a pyridine *N*-oxide substituent (Figure 1).

The active site of GGG(A)X esterases is generally open so that the tetrahedral intermediate is exposed to the solvent. Molecular modeling[23,24] suggested that both enantio-

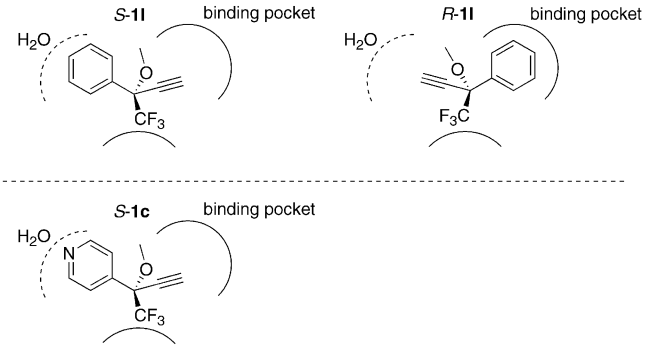


Figure 1. Accommodation of (*R*)-**1l** and (*S*)-**1l** in the active site of BS2 esterase.[24] A similar accommodation of pyridine-substituted substrates such as (*S*)-**1c** would result in an entirely different solvation of the substrate.

mers have an inverse orientation and that the phenyl ring of the *S* enantiomer is oriented towards the entrance of the active site. It is easy to imagine that the interactions of the pyridine ring of **1a**–**c** with the solvent would be quite different to that of a phenyl ring. Also, the position of the nitrogen atom in the ring might influence the solvation. Furthermore, molecular modeling indicated that the main difference in substrate recognition of esterase BS2 between tertiary alcohols with an ethynyl substituent and tertiary cyanohydrins lies in the ability of the nitrile group to form hydrogen bonds with water molecules in the active site of the enzyme.[14] These observations suggest that the solvation of solvent-exposed groups may be an important factor in the enantiorecognition of esterases.

The isoenzymes of pig liver esterase differ in their enantioselectivities towards **1a**–**1c**, whereas with **1d** and **1e** there was no conversion. Interestingly, an inverse enantiopreference of the two PLE isoenzymes was observed in the case of **1c**. This demonstrates, as has already been shown before for the resolution of secondary alcohol acetates,[27] how isoenzymes with high sequence similarity can vary in their properties thus broadening the substrate scope. Moreover, the isoenzyme γPLE showed excellent and good enantioselectivity towards compounds **1a** and **1c**, respectively.

The effect of the C≡CH or CN substituent on the enantioselectivity was strong. This has also been observed previously with commercially available lipases, proteases,[15] and microbial whole-cell catalysts.[16,17] Most of the enzymes showed at least some selectivity towards the tertiary alcohol acetates **1a**–**e** but only Est8 and PEstE were enantioselective towards the cyanohydrins **1f**–**j**. Pig liver esterase did not show any activity towards the tertiary cyanohydrins. Interestingly, Est8 and PEstE displayed an inverse enantiopreference. Small changes in substrate structure had a considerable effect on the enantioselectivity of the esterases. For instance, by changing the *p*-chloro substituent on the phenyl ring (**1g**) to *p*-methoxy (**1h**), the *eeS* value from PEstE decreased significantly from 91% *ee* (at 67% conversion) to 7% *ee* (at 71% conversion), respectively. Est8 also behaved differently in terms of enantioselectivity with **1f** and **1h**. Adding a *p*-methoxy group to the phenyl ring increased the enantioselectivity from 78% *ee* for **1f** to 91% *ee* for **1h** at similar conversions. This narrow substrate scope has also been observed with other enzymes used in the screening (Table 1). Est8 showed a promising *E* value (*E* = 10) in the kinetic resolution of **1i** at 37 °C, which could be increased to *E* = 18 by lowering the temperature to 4 °C (see the Supporting Information). The enantioselectivity of Est8 was strongly influenced by the choice of cosolvent [10% (v/v) of 2-propanol, toluene, dimethylformamide (DMF), or dioxane]. The addition of DMF led to an increase in the *E* value from 18 [10% (v/v) DMSO] to 116 [10% (v/v) DMF] at 4 °C. The influence of water-miscible cosolvents on the enantioselectivity of esterases has been known for a long time.[33] It is a complex phenomenon and has been attributed to conformational changes in the biocatalyst but also to an improved solubility of the substrate[33] and hence the effect is difficult to predict. In some cases, adding[23,30] or changing a cosolvent[34] could lead to dramatic changes (even inversion) in the enantioselectivity.

An increase in the enantioselectivity of Est8 towards tertiary alcohols after addition of DMF has been observed previously.[25] Surprisingly, the application of the optimized conditions for **1i** to other cyanohydrins did not show any improvement in enantioselectivity. This might be explained by the overall different reactivity of the CF_3-substituted cyanohydrin ester **1i**. Indeed, all the enzymes tested in this study had a remarkably lower activity towards **1i**, which confirms the results of a previous study.[15] The strong effect of the CN substituent on the enantioselectivity of esterases has already been observed for esterase BS2. A molecular dynamics study indicated that the main difference between tertiary alcohols with an ethynyl substituent and tertiary cyanohydrins lies in the ability of the nitrile group to form hydrogen bonds with water molecules in the active site of the enzyme.[14]

Table 2. Kinetic resolution of tertiary alcohol acetates on a preparative scale.

Entry	Compound	Enzyme	Conv. [%]	% *eeS*[a]	Yield [%]	% *eeP*[a]	Yield [%]	*E*[b]
1	**1a**[c]	γ PLE	46	98	31	97	30	>100
2	**1b**[c]	BS2 G105A	55	>99	35	86	34	51
3	**1c**[c]	BS2 G105A	52	>99	36	90	30	99
4	**1e**[c]	BS2 G105A	65	90	36	95	31	>100
5	**1i**[d]	Est8	52	>99	38	n.d	n.d	>100

[a] Determined by chiral GC. [b] Calculated from *eeS* and *eeP*. [c] Conditions: 10% DMSO, 37 °C, 1 h reaction time. [d] Conditions: 10% DMF, 4 °C, 4 h reaction time.

The kinetic resolution of **1a**, **1b**, **1c**, **1e**, and **1i** was also performed in experiments on the 100 mg scale. The reaction products were isolated in moderate-to-good yields and with excellent enantiopurity (Table 2). Owing to the low activity of the esterases towards the tertiary alcohol acetates, considerable amounts of biocatalyst were needed. This was particularly the case with Est8, for which the low reaction temperature made a greater amount of enzyme necessary. Note, however, that these esterases can be easily synthesized by recombinant expression and consequently sufficient amounts can be produced easily.

Conclusions

Several aryl-substituted aliphatic tertiary alcohols, including tertiary cyanohydrins, were resolved in good yields and with very high enantiopurity by GGG(A)X esterases although the resolution of the cyanohydrins proved to be more difficult. The substrate range of the individual enzymes in the synthesis of a given series of tertiary alcohols is very narrow. This study shows how this can be efficiently overcome by a combination of different enzymes. In addition, nonsteric interactions, especially electronic effects, can have a strong influence on the enantioselectivity of enzymes. A possible explanation for this observation is the interaction of enzyme-bound substrates with the solvent.

Experimental Section

Characterization data for all of the compounds, including chiral analysis, spectroscopic, and analytical data, and general details of the equipment used are provided in the Supporting Information

General Procedure for Expression of Recombinant Esterases:[35] *E. coli* strain BL21 was used as host for the transformation of the appropriate plasmid DNA.[19,23] The strains were grown in 400 mL LB liquid media supplemented with the appropriate antibiotics (100 $\mu g\,mL^{-1}$) ampicillin for BS2 variants[24] and PestE,[26] additionally 50 $\mu g\,mL^{-1}$ kanamycin for PPE and the PLE variants[27] at 37 °C and supplemented with the appropriate inductor to induce esterase production at an optical density of 0.5 at 600 nm. Cultivation continued for 4 h at the appropriate cultivation temperature. Cells were collected by centrifugation (15 min, 4 °C, 4000 g) and washed twice with sodium phosphate buffer (100 mM, pH 7.5, 4 °C). Cells were disrupted by using a French press (French Pressure cell press, Thermo spectronic, USA). Production of BS2 variants gave after induction with rhamnose (0.2% v/v) and 4 h growth at 37 °C 32000 units of wild-type BS2, 6000 units (pNPA assay) of BS2 G105A, and 5400 units of BS2 E188W/M193C. Production of PestE gave after induction with IPTG (1 mM) and 4 h growth at 37 °C and purification by heat-precipitation after cell disruption 8 000 units. Production of PPE gave after induction with IPTG (1 mM) and 4 h growth at 20 °C 3900 units. Production of PLE isoenzymes gave after induction with IPTG (1 mM) and 4 h growth at 30 °C 63 units of isoenzyme PLE5 and 77 units of the γ-isoenzyme. The metagenomic esterases were produced by BRAIN AG and used as glycerol-stabilized crude cell extracts or lyophylisate.

General Procedure for Small-Scale Esterase-Catalyzed Resolutions: Esterase solution [900 μL, with a crude esterase concentration of 1–3 $mg\,mL^{-1}$ dissolved in phosphate buffer (100 mM, pH 7.5)] was added to acetate **1a**–**i** (12 μmol) dissolved in cosolvent (100 μL) to form a total volume of 1 mL. The reaction mixture was shaken in a Thermoshaker (Eppendorf, Germany) at 37 °C for a certain time (0.5, 1, and 4 h) after which a sample (300 μL) was removed. The sample was extracted twice with dichloromethane (2 × 400 μL). The combined organic layers were dried with Na_2SO_4, filtered, and transferred to a GC vial. The enantioselectivities and conversions were calculated according to Chen et al.[36] The *E* values of compounds **1a**–**d** were calculated based on values of *eeS* and *eeP*. In the case of compounds **1e**–**i**, the products released by the enzymes decomposed immediately to the corresponding ketone and HCN; therefore their *E* values were calculated from *eeS* values and the conversions.

Preparative-Scale Esterase-Catalyzed Resolution: An appropriate amount of esterase dissolved in phosphate buffer (29.7 mL, 100 mM, pH 7.5) was added to a solution of the acetate (0.4 mmol) in DMSO or DMF (3.3 mL) to give a total volume of 33 mL. The solution was stirred for 4 h at 37 °C and the product was extracted three times with dichloromethane (3 × 300 mL). After drying with Na_2SO_4, the solvent was removed by distillation under reduced pressure and the products were purified by column chromatography.

2-(2-Pyridyl)but-3-yn-2-yl Acetate (1a): The reaction was carried out by following the general procedure (46% conversion) using 4700 U of esterase γPLE and 10% (v/v) DMSO. Acetate (+)-**1a** was obtained as a yellow liquid after column chromatography using aluminum oxide and *n*-hexane/ethyl acetate (15:1) as eluent (32 mg, 0.17 mmol, 31%, 98% *ee*). $[\alpha]_D^{20}$ = +92.9 (*c* = 1.25, $CHCl_3$). The GC–MS and NMR analyses matched the data given in the Supporting Information. Alcohol (+)-**2a** was obtained as a brown liquid (24 mg, 0.16 mmol, 30%, 97% *ee*). $[\alpha]_D^{20}$ = +119.9 (*c* = 1.35, $CHCl_3$). The GC–MS and NMR analyses matched the above given data.

2-(3-Pyridyl)but-3-yn-2-yl Acetate (1b): The reaction was carried out by following the general procedure (55% conversion) using 4700 U of esterase BS2 G105A and 10% (v/v) DMSO. Acetate (–)-**1b** was obtained as a brown liquid after column chromatography using aluminum oxide and *n*-hexane/ethyl acetate (3:1) as eluent (35 mg, 0.18 mmol, 35%, 99% *ee*). $[\alpha]_D^{20}$ = –53.8 (*c* = 1.00, $CHCl_3$). The GC–MS and NMR analyses matched the data given in the Supporting Information. Alcohol (+)-**2b** was obtained as a yellow liquid (27 mg, 0.18 mmol, 34%, 86% *ee*). $[\alpha]_D^{20}$ = +5.2 (*c* = 0.92, $CHCl_3$). The GC–MS and NMR analyses matched the above given data.

2-(4-Pyridyl)but-3-yn-2-yl Acetate (1c): The reaction was carried out by following the general procedure (52% conversion) using 3000 U of esterase BS2 G105A and 10% (v/v) DMSO. Acetate (–)-**1c** was obtained as a brown liquid after column chromatography using aluminum oxide and *n*-hexane/ethyl acetate (3:1) as eluent (36 mg, 0.19 mmol, 36%, 99% *ee*). $[\alpha]_D^{20}$ = –40.1 (*c* = 0.95, $CHCl_3$). The GC–MS and NMR analyses matched the data given in the Supporting Information. Alcohol (+)-**2c** was obtained was obtained as a white solid (24 mg, 0.16 mmol, 30%, 90% *ee*). $[\alpha]_D^{20}$ = +6.4 (*c* = 1.15, EtOH). The GC–MS and NMR analyses matched the above given data.

1,1,1-Trifluoro-2-phenylbut-3-en-2-yl Acetate (1e): The reaction was carried out by following the general procedure (65% conversion) using 2700 U of esterase BS2 G105A and 10% (v/v) DMSO. Acetate (–)-**1e** was obtained as a white solid after column chromatography using aluminum oxide and *n*-hexane/ethyl acetate (10:1) as eluent (36 mg, 0.15 mmol, 36%, 90% *ee*). $[\alpha]_D^{20}$ = –38.9 (*c* = 1.25, $CHCl_3$). The GC–MS and NMR analyses matched literature

data.[20] Alcohol (+)-**2c** was obtained as a transparent liquid (24 mg, 12 mmol, 31%, 95% *ee*). $[\alpha]_D^{20}$ = +21.8 (*c* = 1.75, $CHCl_3$). The GC–MS and NMR analyses matched the above given data.

(*S*)-1-Cyano-2,2,2-trifluoro-1-phenylethyl Acetate (1i): The reaction was carried out by following the general procedure (52% conversion) using 20550 U of esterase Est8 and 10% (v/v) DMF. Acetate (*S*)-**1i** was obtained as a colorless liquid at room temperature after column chromatography using silica gel and petroleum ether/ethyl acetate (10:1) as eluent (38 mg, 0.16 mmol, 38%, 99% *ee*). The GC–MS and NMR analyses matched literature data.[15]

Supporting Information (see also the footnote on the first page of this article): Chemical syntheses and spectroscopic data of alcohols and acetates are provided together with conditions for GC analysis.

Acknowledgments

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