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Hydrolase-catalyzed stereoselective preparation of protected α,α-dialkyl-α-hydroxycarboxylic acids

Robert Kourist [a], Giang-Son Nguyen [a], Dirk Strübing [a], Dominique Böttcher [a], Klaus Liebeton [b], Christian Naumer [b], Jürgen Eck [b], Uwe T. Bornscheuer [a,*]

[a] *Department of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, Greifswald University, Felix-Hausdorff-Strasse 4, D-17487 Greifswald, Germany*
[b] *B.R.A.I.N. AG, Darmstädter Strasse 34–36, D-64673 Zwingenberg, Germany*

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ABSTRACT

The title compounds were prepared by a Passerini multi-component reaction and subjected to enzymatic kinetic resolution using 40 enzymes known to be active towards esters of tertiary alcohols. This identified a protease (P1), a thermophilic esterase (PestE) and an esterase of metagenome origin (esterase 8) as the most active and enantioselective biocatalysts. After further optimization of reaction conditions, two compounds were prepared in preparative scale using esterase 8 yielding (+)-1-(*tert*-butylamino)-2-methyl-1-oxobutyl-2-yl acetate with 93%ee and (−)-1-(*tert*-butylamino)-2-trifluoro-methyl-1-oxobutyl-2-yl acetate with 47%ee.

1. Introduction

The synthesis of enantiomerically pure tertiary alcohols is an important, but also challenging task in organic synthesis.[1,2] Nevertheless, in recent years a number of enzymatic processes have emerged, including the hydrolase-catalyzed kinetic resolution of tertiary structures such as cyanohydrins,[3] epoxides[4] and carboxyl esters.[5] The identification of esterases and lipases acting on tertiary alcohols was greatly facilitated by the discovery of an amino acid motif, the so-called GGG(A)X-motif, consisting of three glycines (or two glycines and an alanine) in the active site,[6,7] which was identified in a computer-based study of structure–function relationships of a large number of hydrolases[8] and later related to the ability to convert esters of tertiary alcohols.[9] The application of the GGG(A)X-motif in a guided screening led to the identification of several highly enantioselective esterases in databases[5,6] and metagenome-derived enzyme libraries.[10]

Enantiomerically pure α,α-dialkyl-α-hydroxycarboxylic acids are important building blocks in organic synthesis. For instance, (*S*)-2-hydroxy-2-methylbutyric acid **1** is present in the natural product clerodendrin-A[11] and has been applied to the synthesis of a cyclooxygenase inhibitor.[12] Only very recently has a biocatalytic route using oxynitrilases that affords **1** with high enantiomeric purity (ee >99%) been reported.[13] It is, however, hampered by the required use of HCN as a substrate. Enzyme-catalyzed resolution would represent an environmentally more beneficial process. The enzymatic conversion is difficult because the crowded chiral centre significantly lowers the reaction rates. Hence the enzymatic synthesis of enantiopure α,α-disubstituted carboxylic acids has so far been mainly attempted by enantioselective hydrolysis of carboxylic acid esters,[14] while the resolution of the tertiary hydroxyesters has received much less attention. To overcome steric hindrance, Recuero et al. used a linker between the stereogenic centre and the reaction centre, that allowed them to prepare key precursors of (*S*)-oxybutynin with high enantioselectivity using lipase from *Pseudomonas cepacia* or from *Candida antarctica* B.[15] Ohta et al. studied the kinetic resolution of benzyloxy-protected esters of **1** and several other derivatives. Whilst the enantioselectivities for several substrates varied up to $E = 52$, (*S*)-**1** was prepared with an enantioselectivity of only $E = 10$.[16]

We attempted to resolve this class of compounds by resolving the tertiary alcohol and therefore protecting the acid with an amide group. Even though the reaction mechanisms of enzymatic hydrolysis of amides and esters are highly similar, promiscuous amidase activity of esterases, if present, is usually very low,[17,18] which has already been successfully applied to the selective enzymatic deprotection of *tert*-butyl esters in the presence of other protected functional groups.[19]

Preparation of the protected esters can be easily accomplished in one step by the Passerini-reaction, a multi-component reaction (MCR) widely applied in combinatorial chemistry.[20] Enzymatic kinetic resolution and subsequent deprotection would directly give the desired α,α-dialkyl-α-hydroxycarboxylic acid. MCRs have already been efficiently combined with biocatalytic methods, both for the synthesis of enantiopure precursors for the Ugi MCR[21] and for the synthesis of racemic secondary alcohols by Passerini-MCR and subsequent enantioselective enzymatic hydrolysis.[22]

Herein we report the synthesis of protected α,α-dialkyl-α-hydroxycarboxylic acid esters by the Passerini-reaction and the

* Corresponding author. Tel.: +49 3834 86 4367; fax: +49 3834 86 80066.
E-mail address: uwe.bornscheuer@uni-greifswald.de (U.T. Bornscheuer).

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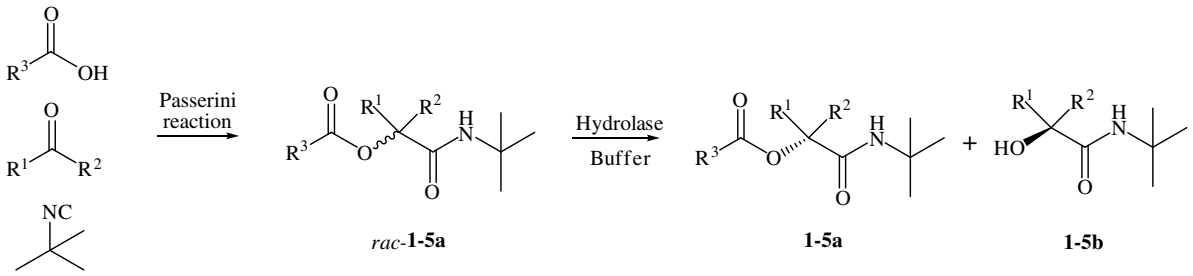


1a R^1=Et, R^2=CF_3, R^3=Me
2a R^1=Et, R^2=Me, R^3=Me
3a R^1=Ph, R^2=CF_3, R^3=Me
4a R^1=Et, R^2=CF_3, R^3=*n*-Pr
5a R^1=Et, R^2=CF_3, R^3=Phenyl

Scheme 1. Synthesis of protected α,α-dialkyl-α-hydroxycarboxylic acid esters by Passerini-MCR and subsequent enzymatic kinetic resolution.

subsequent enantioselective hydrolysis and deprotection in a simple two-step route (Scheme 1) to afford these building blocks.

2. Results and discussion

2.1. Synthesis of protected α,α-dialkyl-α-hydroxy-carboxylic acids

As reported in the literature,[20] the Passerini-reaction proceeded considerably faster in aqueous media than in an organic solvent. In aqueous solution, however, a certain amount of hydrolysis of the substrate to the alcohol was observed, which made purification by column chromatography necessary. Alternatively, the reactions were performed in organic solvent and here the products could be directly isolated with excellent purity although longer reaction times were required.

2.2. Identification of metagenome-derived hydrolases acting on tertiary alcohols

Activity towards tertiary alcohols has been found in several α,β-fold hydrolases bearing the GGG(A)X-motif in the active site[9] or with high structural similarity to peptidases.[23,24] We have already reported on the screening of 35 esterases and lipases from metagenome origin, previously identified by a high-throughput screening for activity in the hydrolysis of several esters of tertiary alcohols including **6a**.[10]

In a similar approach, we investigated 58 enzymes from another metagenomic library initially for principle hydrolytic activity against the acetates of tertiary alcohols **6a** and **7a**, as these compounds were readily available in our laboratory. In this screening 14 enzymes were identified as active and five enzymes having the highest activity were used further for biocatalysis experiments. Three enzymes, esterases 8, 56 and M48cD5, had low to moderate enantioselectivity in the hydrolysis of the arylaliphatic tertiary esters **6a** and **7a** (Table 1, Scheme 2).

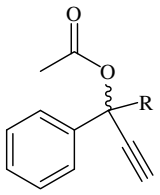
2.3. Identification of enzymes with activity towards amide-protected α,α-dialkyl-α-hydroxycarboxylic acid acetates

For the kinetic resolution of the Passerini-MCR reaction products, 40 carboxyl hydrolases were screened for activity towards **1a** by small-scale biocatalysis experiments, including the metagenome-derived enzymes, commercially available hydrolases such as *Candida rugosa* lipase, pig liver esterase, *Candida antarctica* lipase A and Chirazyme P1. Furthermore, recombinantly expressed esterase BS2 from *Bacillus subtilis*[5,6] and esterase PestE from the thermophilic organism *Pyrobaculum calidifontis* were used.[26,27] Only three

Table 1
Enantioselectivity of metagenome-derived GGG(A)X-hydrolases in the hydrolysis of acetates of arylaliphatic tertiary alcohols **6a** and **7a**

Entry	Enzyme	ee_S[a] (%)	ee_P[a] (%)	c[b] (%)	E[c] (–)
6a	Esterase 56	27	43	40	3
6a	Esterase 63	4	4	52	1
6a	Esterase 8	60	44	58	4
6a	M48cD5	40	37	52	3
6a	Esterase 5	0	0	37	1
7a	Esterase 56	39	91	56	35
7a	Esterase 8	62	63	51	8
7a	M48cD5	2	8	25	1

[a] As determined by GC analysis.
[b] Conversion calculated as $c = ee_S/(ee_S + ee_P)$.
[c] Calculated according to Chen et al.[25]



6a R=CH_3
7a R=CF_3

Scheme 2. Esters of tertiary alcohols used in the initial screening of metagenome-derived hydrolases.

enzymes, esterase 8, esterase PestE and Chirazyme P1 showed activity and thus were investigated further.

Chirazyme P1 displayed low enantioselectivity towards substrates **1a** and **2a**. The thermophilic esterase PestE showed activity towards **1a** at temperatures above 50 °C but at this temperature low chemical hydrolysis was observed, which led to a decrease in the enantiomeric purity of the product. Interestingly, the enantiopreference of PestE was inverse to that of esterase 8 but the enantioselectivity (E = 10) was too low for preparative purposes and the overall activity was unsatisfactory. Surprisingly, PestE had almost no activity towards analogue **2a** (E = 3) and was inactive towards the bulky compound **3a**.

Esterase 8 showed a promising E-value (E = 19) in the kinetic resolution of **1a** at 30 °C. Optimization of the reaction conditions including the addition of water-miscible co-solvents [*tert*-butanol, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF)] and a decrease of the temperature slightly increased the enantioselectivity to E = 22 for **1a** and E = 42 for **2a** at 4 °C and in the presence of 5% DMF (v/v). The kinetic resolution of **2a** was performed under opti-

Table 2
Results of kinetic resolution of **1a** and **2a** after 4 h reaction time

Entry	Enzyme	U[a]	T (°C)	Co-solvent	ee_S[d] (%)	ee_P[e] (%)	c (%)	E[g] (–)
1a	PestE	40[b]	50	10% DMSO	n.d.	80 (–)	11[f]	10
2a	PestE	100[b]	50	10% DMSO	n.d.	54 (+)	2[f]	3
1a	P1	856[b]	37	10% DMSO	n.d.	49 (+)	40[f]	4
2a	P1	856[b]	37	10% DMSO	n.d.	38 (–)	24[f]	2
1a	Esterase 8	230[b]	30	5% DMF	n.d.	73 (+)	54[f]	19
1a	Esterase 8	680[c]	4	5% DMF	47 (–)	87 (+)	36[g]	22
2a	Esterase 8	400[c]	4	5% DMF	93 (+)	85 (–)	53[g]	42

[a] Determined using *p*-nitrophenyl acetate.
[b] 0.055 mmol substrate.
[c] 0.55 mmol substrate.
[d] Determined after hydrolysis to **2b**.
[e] Determined by GC analysis.
[f] Determined by NMR spectroscopy.
[g] Calculated according to Chen et al.[25]

mized conditions on a 120-mg scale and yielded the remaining substrate (+)-**2a** (38%, 93%ee) and the product (–)-**2b** (46%, 85%ee) with good yields and enantiomeric purities (Table 2). The attempt to vary the chain length and structure of the acyl moiety was not successful, as **4a** and **5a** were not converted by esterase 8. The bulky **3a** was not converted by any of the enzymes.

3. Conclusion

It has been shown that a structure-guided search for hydrolases acting on tertiary alcohols can provide enzymes active towards difficult compounds such as the bulky amide-protected α,α-dialkyl-α-hydroxycarboxylic acids. Two esterases bearing the GGG(A)X-motif in their active sites, and a protease were active and enantioselective in the hydrolysis of the tertiary alcohol acetates and one enzyme, esterase 8, displayed good enantioselectivity. This underlines the importance of the GGG(A)X-motif for the identification of enzymes for the conversion of tertiary alcohols. In connection with the easy preparation of the substrates by the Passerini-reaction, hydrolase-catalyzed kinetic resolution provides a fast, straight-forward route for the synthesis of enantiomerically enriched protected α,α-dialkyl-α-hydroxycarboxylic acids.

4. Experimental

4.1. General

All chemicals were purchased from Fluka (Buchs, Switzerland), Sigma (Steinheim, Germany) and Merck (Darmstadt, Germany), unless stated otherwise. NMR spectroscopy experiments were performed on an ARX300 (300.13 MHz for ^{1}H and 75.5 MHz for ^{13}C, Bruker, Karlsruhe, Germany), using the δ scale (ppm) for chemical shifts; ^{13}C-spectra were edited using DEPT techniques. Mass spectra were recorded on a QP2010 GC–MS device (electron impact, 70 eV, Shimadzu, Japan). High-resolution MS was determined by direct injection on a micrOTOF device (Bruker, Karlsruhe, Germany) with nitrogen as nebulizer gas and drygas. Chiral GC-analyses were performed by using a Heptakis-(2,3-di-*O*-acetyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin-column (Machey-Nagel, Düren, Germany) on a GC-14A gas chromatograph (Shimadzu, Tokyo, Japan). Chiral HPLC-analyses were performed by using the column OD-H (Daicel Chemical Industries Ltd., Osaka, Japan) with *n*-hexane and 2-propanol as solvent on a Hitachi Elite LaChrom device (Hitachi High Technologies America Inc., San Jose, USA). The sign of the optical rotation was detemined by using a chiral HPLC-detector (IBZ-Messtechnik, Hannover, Germany). Chirazyme P1 was obtained from Roche Diagnostics GmbH (Mannheim, Germany). Production of esterase Pest E from *Pyrobaculus calidifontis*[26] and synthesis, analytical-scale biocatalysis and chiral analytics of **6a** and **7a** were performed as described.[7] All metagenomic esterases were produced by B.R.A.I.N. AG and used as glycerol-stabilized crude cell extracts or lyophylizate.[28]

4.2. General procedure for the synthesis of protected racemic α,α-dialkyl-α-hydroxycarboxylic acids

These were prepared by adapting a previously reported procedure:[20] (a) in organic solvent: to a stirred solution of 3.0 mmol isocyanide in dichloromethane (20 mL), ketone (3.3 mmol, 1.1 equiv) and acid (3.3 mmol, 1.1 equiv) were added. After 7 d, the organic phase was washed three times with distilled water. (b) In aqueous solution: to a stirred solution of 3.0 mmol isocyanide in an aqueous LiCl-solution (1 M, 20 mL), ketone (3.3 mmol, 1.1 equiv) and acid (3.3 mmol, 1.1 equiv) was added. The product was extracted three times with dichloromethane. In both cases, after drying over Na_2SO_4, the solvent and the remaining reactants were removed by distillation under reduced pressure.

4.2.1. (*R,S*)-2-(*tert*-Butylcarbamoyl)-1,1,1-trifluorobut-2-yl acetate, 1a

Compound **1a** was obtained after 7 d in dichloromethane as a white powder (1.5 g, 5.6 mmol, 84%, mp 52 °C). ^{1}H NMR: δ = 0.92 (t, J = 7.48, 3H, CH_3), 1.4 (s, 9H $(CH_3)_3$), 2.2 (s, 3H, CH_3), 2.5 (m, 2H, CH_2), 5.8 (s, 1H, N). ^{13}C NMR: δ = 7.5, 22.4, 21.2, 28.3, 51.9, 83.9 (q, J = 27.9), 123.3, (q, J = 285.9), 163.1, 167.3. MS (EI): 269 (M^+), 254, 226, 212, 194, 171, 154, 97, 43. Anal. Calcd for $C_{11}H_{18}F_3NO_3$ (269.26): C, 49.07; H, 6.74; N, 5.20. Found: C, 49.18; H, 6.68; N, 5.04. HRMS: 292.111[M + Na^+].

4.2.2. 1-(*tert*-Butylamino)-2-methyl-1-oxobut-2-yl acetate, 2a

Compound **2a** was obtained after 4 h in aqueous LiCl-solution and by column chromatography (*n*-pentane/ethyl acetate) as white powder (775 mg, 3.6 mmol, 31%, mp 46 °C). ^{1}H NMR: δ = 0.8 (t, J = 7.45, 3H, CH_3), 1.7 (s, 3H, CH_3), 1.4 (s, 9H, $C(CH_3)_3$), 2.0 (m, 2H, CH_2), 2.1 (s, 3H, CH_3), 6.0 (s, 1H, NH). ^{13}C NMR: δ = 8.0, 22.0, 22.1, 28.7, 29.7, 51.0, 85.5, 168.7, 171.3. MS (EI): 215 (M^+), 187, 172, 155, 116, 100. Anal. Calcd for $C_{11}H_{21}NO_3$ (215.15): C, 61.37; H, 9.83; N, 22.29. Found: C, 60.55; H, 9.98; N, 6.33. HRMS: 238.139 [M+Na^+].

4.2.3. (*R,S*)-3-(*tert*-Butylamino)-1,1,1-trifluoro-3-oxo-2-phenylpropan-2-yl acetate, 3a

Compound **3a** was obtained after 4 h in aqueous LiCl-solution and by column chromatography (*n*-pentane/ethyl acetate) as white powder (464.7 mg, 1.5 mmol, 48%, mp 88 °C). In dichloromethane, **3a** was obtained after 7 d with higher yield (666 mg, 2.1 mmol,

70%). ^{1}H NMR: δ = 1.4 (s, 9H, C(CH_3)$_3$), 2.3 (s, 3H, CH_3), 5.9 (s, 1H, NH), 7.4 (m, 3H, H-Ar), 7.5 (m, 2H, H-Ar).^{13}C NMR: δ = 21.4, 28.3, 52.3, 83.6 (q, J = 28.3), 122.7 (q, J = 285.5), 127.3, 128.4, 129.6, 131.3, 162.8, 167.9. MS (EI): 318 (M^+), 274, 260, 242, 219, 156, 77, 57, 43. Anal. Calcd for $C_{15}H_{18}F_3NO_3$: C, 56.78; H, 5.72; N, 4.41. Found: C, 57.07; H, 55.74; N, 4.33. HRMS: 298.101 [M−CH_3COOH+Na^+].

4.2.4. (*R,S*)-2-(*tert*-Butylcarbamoyl)-1,1,1-trifluorobut-2-yl butyrate, 4a

Compound **4a** was obtained after 7 d in dichloromethane as a white powder (1064 mg, 3.6 mmol, 92%, mp 72 °C). ^{1}H NMR: δ = 0.9 (t, J = 7.52, 3H, CH_3), 1.0 (t, 3H, CH_3), 1.4 (s, 9H, (CH_3)$_3$), 1.7 (m, 2H, CH_2), 2.5 (t, 2H, CH_2), 2.6 (m, 2H, CH_2), 5.8 (s. 1H, NH). ^{13}C NMR: δ = 7.5, 22.4, 13.5, 18.3, 28.3, 36.6, 51.9, 83.8, (q, J = 27.8), 123.4 (q, J = 285.6), 163.2, 170.0. MS (EI): 297 (M^+), 279, 254, 242, 172, 100, 71, 57. Anal. Calcd for $C_{13}H_{22}F_3NO_3$: C, 52.52; H, 7.46; N, 4.71; O, 16.14. Found: C, 52.52; H, 7.46; N, 4.71. HRMS: 320.144 [M+Na^+].

4.2.5. (*R,S*)-2-(*tert*-Butylcarbamoyl)-1,1,1-trifluorobut-2-yl benzoate, 5a

Compound **5a** was obtained after 2 d in dichloromethane and column chromatography (*n*-pentane/ethyl acetate) as colourless oil (845 mg, 2.8 mmol, 93%, mp 102 °C). ^{1}H NMR: δ = 0.9 (t, J = 7.45, 3H, CH_3), 2.5 (m, 2H, CH_2), 5.9 (s, 1H, NH), 7.3 (m, 2H, H-Ar), 7.6 (m, 1H, H-Ar), 8.1 (m, 1H, H-Ar). ^{13}C-NMR: δ = 7.6, 22.6, 28.3, 52.0, 84.1 (q, J = 27.9), 123.6 (q, J = 283.2), 129.3, 129.8, 130.1, 134.0, 163.2, 163.3. MS (EI): 332 (M^+), 316, 259, 232, 105, 77, 57. Anal. Calcd for $C_{15}H_{17}F_3NO_3$: C, 58.00; H, 6.08; N, 4.23. Found: C, 58.45; H, 6.03; N, 4.215. HRMS: 354.129 [M + Na^+].

4.3. General procedure for the synthesis of racemic *N-tert*-butyl-2-hydroxyamides (serving as standards)

General procedure for the preparation of hydroxyamides: 2 mmol of tertiary ester of protected α,α-dialkyl-α-hydroxycarboxylic acid were dissolved in an aqueous solution of NaOH (0.1 N, 50 mL) with DMSO (10%, v/v) as co-solvent and stirred for 1 h. The product was extracted three times with dichloromethane. After drying over Na_2SO_4, the solvent was removed by distillation under reduced pressure.

4.3.1. (*R,S*)-*N-tert*-Butyl-2-hydroxy-2-(trifluoromethyl)-butanamide, 1b

Compound **1b** was obtained after as yellowish powder (69 mg, 0.3 mmol, 83%). ^{1}H NMR: δ = 0.92 (t, J = 7.41, 3H, CH_3), 1.4 (s, 9H, (CH_3)$_3$), 1.9 (m, 2H, CH_2), 4.5 (s, 1H, OH), 6.1 (s,1H, NH). ^{13}C NMR: δ = 6.33, 25.61, 28.35, 52.18, 77.15 (t, J = 28.0), 126.2 (t, J = 285.4), 166.0. MS (EI): 212 (M^+−15). HRMS: 228.119 [M+H^+]. GC analysis: 5.3 min (+), 8.0 min (−) at a column temperature of 75 °C.

4.3.2. (*R,S*)-*N-tert*-Butyl-2-hydroxy-2-methyl butanamide, 2b

Compound **2b** was obtained after as white powder 72 mg, 0.4 mmol, 90%, mp 71 °C. ^{1}H NMR: δ = 0.9 (t, J = 7.44, 3H, CH_3), 1.4 (s, 3H, CH_3), 1.3 (s, 9H, (CH_3)$_3$), 1.7 (m, 2H, CH_2), 2.4 (2H, CH_2), 2.9 (s, 1H, OH), 6.5 (s, 1H, NH). ^{13}C NMR: δ = 7.8, 26.6, 33.4, 27.7, 50.7, 75.9, 174.8. MS (EI): 144 (M^+−29), 130, 101, 73, 58, 41. HRMS: 174.150 [M^+H^+]. GC analysis: 41.2 min (−), 44.0 min (+) at a column temperature of 90 °C.

4.3.3. (*R,S*)-*N-tert*-Butyl-3,3,3-trifluoro-2-hydroxy-2-phenylpropanamide, 3b

Compound **3b** was obtained after as white powder (61 mg, 0.26 mmol, 67%, mp 115 °C). ^{1}H NMR: δ = 1.31 (s, 9H, (CH_3)$_3$), 5.3 (s, 1H, OH), 6.2 (s, 1H, NH), 7.4 (m, 3H, H-Ar), 7.7 (m, 2H, H-Ar). ^{13}C NMR: δ = 28.4, 52.9, 77.8 (q, J = 28.5), 123.9 (q, J = 286.1), 128.8, 128.9, 129.6, 134.8, 166.0. MS (EI): 232 (M−43), 176, 156, 105, 84, 77, 57. HRMS: 298.121 [M+Na^+].

4.4. High-throughput screening

The test kit for the determination of released acetic acid was from R-Biopharm GmbH (Darmstadt, Germany) and applied according to the manufacturer's protocol as described previously.[29] Enzyme solution (20 μL) and substrate solution (20 μL; 7.5 mg/mL) of racemic **6a** were added to a mixture (150 μL) of the test kit components. The increase of NADH was monitored at 340 nm by using the Fluostar Galaxy or Fluostar Optima (BMG, Offenburg, Germany). Mixtures of test kit components with buffer or cell lysate from induced *Escherichia coli*, that contained pUC18 without an esterase gene, served as controls.

4.5. General procedure for esterase-catalyzed small-scale resolutions

To a stirred solution of the acetate (25 mM) in a phosphate buffer (100 mM, pH 7.5) and the appropriate amount of co-solvent, esterase solution was added to a total volume of 2 mL. The reaction mixture was stirred in a thermoshaker (Eppendorf, Hamburg, Germany) at the appropriate temperature. Samples were taken after 1 h, 4 h and 24 h. The reaction mixture was extracted twice with 400 μL dichloromethane, the combined organic layers were dried over anhydrous sodium sulfate and the organic solvent was removed under nitrogen. For the determination of the enantiomeric purity of the remaining substrate **a** it was hydrolyzed to the corresponding alcohol **b**. Enantioselectivity and conversion were calculated according to Chen et al.[25]

4.6. Esterase-catalyzed preparative-scale resolution of 2a

To a solution of **2a** (120 mg, 0.55 mmol) in phosphate buffer (100 mM, pH 7.5) and 5% (v/v) dimethyl formamide, esterase solution was added to a total volume of 40 mL. The solution was stirred for 4 h at 4 °C, and the product was extracted three times with methyl *tert*-butyl ether. After drying over Na_2SO_4 the solvent was removed by distillation under reduced pressure, and the product was purified by column chromatography (*n*-pentane/ethyl acetate).

4.6.1. (−)-2-(*tert*-Butylcarbamoyl)-1,1,1-trifluorobut-2-yl acetate, 1a

Compound (−)-**1a** was obtained after column chromatography (*n*-pentane/ethyl acetate) as a white powder (30 mg, 0.11 mmol, 30%). Enantiomeric excess was determined after hydrolysis to **1b** as 47%ee.

4.6.2. (+)-*N-tert*-Butyl-2-hydroxy-2-(trifluoromethyl)-butanamide, 1b

Compound (+)-**1b** was obtained after column chromatography (*n*-pentane/ethyl acetate) as a white powder (25 mg, 0.12 mmol, 32%, 87%ee).

4.6.3. (+)-1-(*tert*-Butylamino)-2-methyl-1-oxobutyl-2-yl acetate, 2a

Compound (+)-**2a** was obtained after column chromatography (*n*-pentane/ethyl acetate) as a white powder (46 mg, 0.22 mmol, 38%). Enantiomeric excess was determined after hydrolysis to **2b** as 93%ee.

4.6.4. (−)-*N-tert*-Butyl-2-hydroxy-2-methylbutanamide, 2b

Compound (−)-**2b** was obtained after column chromatography (*n*-pentane/ethyl acetate) as a white powder (44 mg, 0.25 mmol, 46%, 85%ee).

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