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The synthesis of (*R*)- and (*S*)-α-trifluoromethyl-α-hydroxycarboxylic acids via enzymatic resolutions

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Abstract

Kinetic resolution of 1,1,1-trifluoro-2-alkanone cyanohydrin acyl derivatives with *Candida rugosa* lipase afforded the remaining (*R*)-enantiomer in high selectivity (E from 30 to >200). *Candida rugosa* lipases from several suppliers were compared and found to differ remarkably in their selectivity. The (*R*)-enantiomer was hydrolyzed in one step to yield optically pure (*R*)-α-trifluoromethyl-α-hydroxycarboxylic acids in excellent yield. The (*S*)-acids were obtained in good *e.e.* by subtilisin-catalyzed resolution of the corresponding racemic esters followed by chemical hydrolysis of the remaining (*S*)-esters.

1. Introduction

The technique of kinetic resolution of racemic substrates with enzymes is now a well-established tool in preparative organic chemistry. Books and reviews abound in this area,[1] and the commercial impact of this methodology is rising.[2] While the traditional focus in utilizing enzymes has been centered on food processing, detergent additives, and diagnostic applications, new companies are gearing up their attention to supply new enzymes and to make single enantiomers for the pharmaceutical industry on a large scale. However, certain areas, such as resolution of tertiary alcohols and α,α-disubstituted hydroxyacids, are less explored. During our work to find a practical method for the preparation of pure enantiomers of α-trifluoromethyl-α-hydroxycarboxylic acids (*R*)-**1a** and (*S*)-**1a**, we investigated the kinetic resolution of different substrates by enzymatic hydrolysis. Our results, which led to a very practical preparation of (*R*)-**1a**, are reported in this paper.

Chemical literature offers several methods to obtain enantiopure α-trifluoromethyl-α-hydroxypropionic acid (**1a**): resolution of *rac*-**1a** by crystallization of its salt with brucine in very low yield (5%) has been described as early as 1949.[3] In a recent report, multiple crystallizations of the

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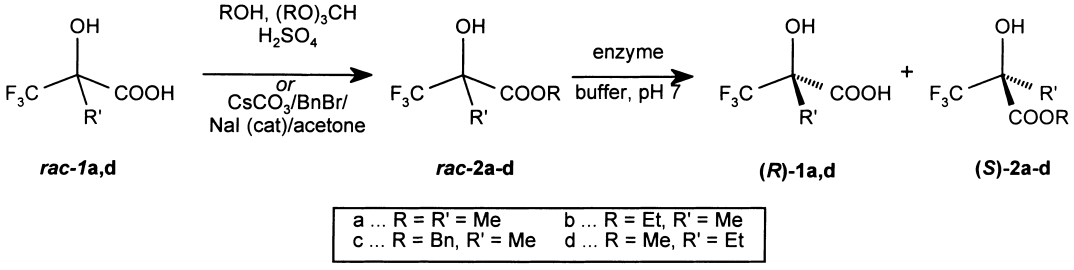
(*S*)-α-methylbenzylamine salt from 1-butanol afforded (*S*)-**1a** (*e.e.* >99%) in a 15–20% yield (theoretical yield 50%).[4] In addition, two recently released patents complement our results on the synthesis of (*S*)-**1a** utilizing enzymes.[5] These methods suffer from low yields, utilize expensive enzymes, or show lower enantiomeric excess, especially for the (*R*)-acid.

2. Results and discussion

We considered enzymatic hydrolysis of esters of *rac*-**1a** as the first option in preparing multigram quantities of enantiopure acids **1a** (Scheme 1).[6] Esterification of *rac*-**1a**, according to standard procedures, afforded esters *rac*-**2** in moderate to good yields. A screening of a series of approximately 30 hydrolases revealed subtilisin as the most promising lead. The selectivities obtained during these studies were rather disappointing (E=7),[7] but consistent with similar findings in one of the patents mentioned.[5b] However, at high conversion, the remaining (*S*)-**2a** could be obtained in >95% *e.e.* with subtilisin (Table 1). Hydrolysis of (*S*)-**2a** provided (*S*)-**1a** whose configuration was determined by comparison of the sign of the optical rotation with the literature value.[4] The selectivity of enzymatic hydrolysis of related *rac*-**2d** was assumed to be the same because the elution order of unreacted ester **2d** on chiral GC was the same as for (*S*)-**2a**.



Scheme 1.

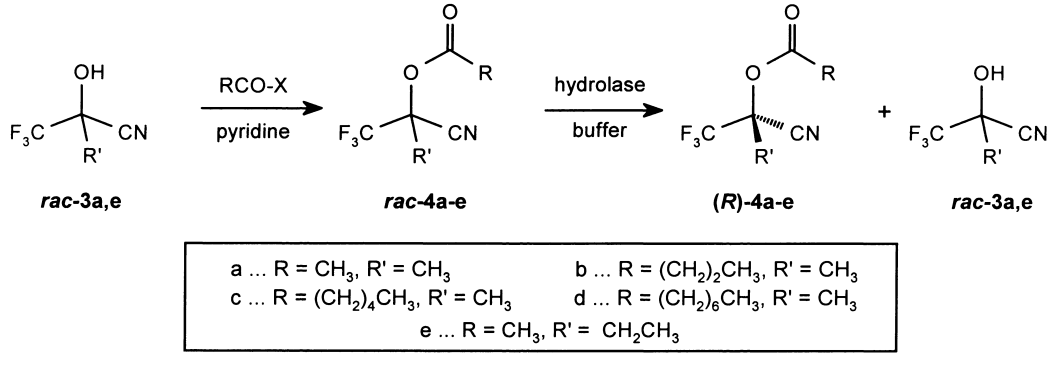
As the selectivity in the hydrolysis of esters *rac*-**2a–c** is low, and enantiomerically pure (*R*)-**1a** cannot be accessed in a practical manner by this approach, we changed our strategy and attempted the resolution of α,α,α-trifluoroalkanone cyanohydrins *rac*-**3**, the synthetic precursors of acids **1**. Some precedent for the enzymatic resolution of tertiary cyanohydrin esters exists: hydrolysis of 1-cyano-1-phenyl-2,2,2-trifluoroethyl acetate with lyophilized *Bacillus coagulans* afforded unreacted optically pure (*R*)-ester (30%),[8] and a series of 2-alkanone cyanohydrin acetates was resolved with resting cells of *Pichia miso* yielding the remaining ester in (*S*)-configuration.[9] We prepared acylated cyanohydrins *rac*-**4** from cyanohydrins *rac*-**3** using either anhydrides (1.05 equiv.)/pyridine (1.1 equiv.) or acid chlorides (1.05 equiv.)/pyridine/THF (Scheme 2, Table 2). For the lower acylated products, the solvent-free procedure was found to be the method of choice as the products could be isolated without the need for a tedious distillative separation from the reaction solvent. An initial screening of the hydrolysis of *rac*-**4a** by some 30 hydrolases revealed the lipase from *Candida rugosa* as the most interesting one for resolving the tertiary acylated intermediate. Determination of the *e.e.* of **4a** was accomplished by chiral GC allowing estimation of selectivity during the microscale screening. Further optimization reactions were run on a preparative scale with this enzyme (Table 3). The rate of hydrolysis of the initially tested acetate *rac*-**4a** was quite low, and the optimum pH for the hydrolysis of the acetate was determined to be 6. At this pH, we found a maximum rate of hydrolysis with the butyrate, which was used for further studies, while the rates for the corresponding hexanoate and octanoate were lower. The selectivity of hydrolysis (E) was comparable for all acyl residues tested, ranging from 18 to 55 in the methyl series (from **3a**) and >100 for the ethyl series (from **3e**). We were thus able to obtain the remaining esters in up to 37% yield and

Table 1
Hydrolysis of esters *rac*-**2a–d** by proteases

Substrate	Protease	Conditions	Ester 2 [a] *e.e.*	Acid 1 Yield	Acid 1 *e.e.*	Conversion [b] (calculated)	E [b]
(*rac*)-**2a**	Subtilisin Carlsberg 5 wt%	pH 7.2, 110 min	>95% (*S*) [c]	49%	33% (*R*)	74%	**6**
(*rac*)-**2a**	Subtilisin Carlsberg 0.7 wt%	pH 7.1, 90 min	25% (*S*)	24%	71% (*R*)	26%	**7**
(*rac*)-**2b**	Subtilisin Carlsberg 3 wt%	pH 7.2, 200 min	>90% (*S*)	51%	44% (*R*)	67%	**7**
(*rac*)-**2c**	Subtilisin Carlsberg 1 wt%	pH 7.1, 100 min	35% (*S*)	28%	64% (*R*)	30%	**6**
(*rac*)-**2a**	ChiroCLEC-BL (from S. Carlsberg) 6 wt%	pH 7.2, 63 min	83% (*S*)	59%	44% (*R*)	65%	**6**
(*rac*)-**2a**	Subtilisin BPN' 3 wt%	pH 7.1, 55 min	50% (*S*)	nd	60% (*R*)	45%	**6**
(*rac*)-**2a**	Streptomyces caespitosus, 50 wt%	pH 7.1, 17 h	72% (*S*)	nd	48% (*R*)	60%	**6**
(*rac*)-**2d**	Subtilisin Carlsberg 4 wt%	pH 7.0, 16 h	63% (*S*) [d]	47% [e]	60% (*R*)	51%	**7**

[a] Isolated yields of small scale experiments are not representative due to volatility of the ester.
[b] Calculated from *e.e.* of acid and ester.[7]
[c] Yield 19% (10 g scale); $[\alpha]_D^{22}$ –14.4 (*c* 3.65, CH_3OH).
[d] Yield 37% (6 g scale); $[\alpha]_D^{22}$ –8.1 (*c* 4.45, CH_3OH).
[e] $[\alpha]_D^{22}$ +7.3 (*c* 5.33, CH_3OH).

e.e. >98%. The isolated yield increased considerably (up to 40% in the case of **4b**) with the scale of the reaction, because losses of the volatile cyanohydrin esters were minimized on larger scales. The products of the enzymatic reaction, cyanohydrins **3**, are known to racemize under the reaction conditions, and isolation of cyanohydrins or extremely volatile α,α,α-trifluoroketones, present in equilibrium with the cyanohydrins at pH 6 as shown by a ^{19}F-NMR study, was not attempted. The octanoate exhibited quite an interesting reaction pattern: after fast initial hydrolysis, the reaction slowed down dramatically at 5 to 10% conversion, probably due to inhibition of further hydrolysis by the fatty acid precipitated at pH 6. The reaction rate can be enhanced by using heptane (100–200 wt%) as a cosolvent to dissolve the octanoic acid formed. However, the conversion, and thus the selectivity coefficient E for hydrolysis of the octanoate, could not be measured because octanoic acid formed is not readily neutralized with NaOH at pH 6.



Scheme 2.

Following the well known fact that crude lipases may contain other active enzymes,[10] we studied the hydrolysis of butyrate *rac*-**4b** with *Candida rugosa* lipases obtained from different commercial sources.

Table 2
Synthesis of acylated cyanohydrins

Entry	RCO-X	Conditions	Product	Yield	Phys. Data
3a	$(Ac)_2O$ (1.15 eq)	pyridine (1.3 eq) 18 h, 20-60 °C	***rac*-4a**	78%	bp 74 °C, 60 mm Hg
3a	$[CH_3(CH_2)_2CO]_2O$ (1.05 eq)	pyridine (1.1.eq) 14 h, 10-60 °C	***rac*-4b**	86 - 93%[a]	bp 76-78 °C, 20 mm Hg
3a	$CH_3(CH_2)_6COCl$ (1.02 eq)	pyridine (2.1 eq) THF, 0 °C-rt, 1 h	***rac*-4c**	59%	bp 109-11 °C, 22 mm Hg
3a	$CH_3(CH_2)_4COCl$ (1.02 eq)	pyridine (2.1 eq), CH_2Cl_2, 0 °C-rt, 1 h	***rac*-4d**	64%	bp 85-93 °C, 0.5 mm Hg
3e	$(Ac)_2O$ (1.1 eq)	pyridine (1.2 eq) 16 h, 10-60 °C	***rac*-4e**	72%	bp 78-80 °C, 12 mm Hg

[a] optimized yield, crude material (> 97% pure).

Table 3
Enzymatic hydrolyses of *rac*-**4** with *Candida rugosa* lipase

Entry	Lipase	Conversion[a]	Product[b]	Yield	*e.e.*	E	$[\alpha]_D^{22}$
(*rac*)-4a	Sigma (crude), 44 wt%	61% (72 h)	**(*R*)-4a**	23%	98%	**18**	−22.4 (*c* 5.12, CH_3OH)
(*rac*)-4b	Sigma (crude), 12 wt%	59% (9 h)	**(*R*)-4b**	37%	99%	**27**	−15.1 (*c* 3.7, CH_3OH)
(*rac*)-4c	Sigma (crude), 57 wt%	53% (20 h)	**(*R*)-4c**	36%	97%	**55**	−12.5 (*c* 4.3, CH_3OH)
(*rac*)-4d[d]	Sigma (crude), 70 wt%	nd[c] (15 h)	**(*R*)-4d**	30%	99%	**nd**[c]	−10.8 (*c* 3.68, CH_3OH)
(*rac*)-4e	Amano AY30, 12 wt%	49.5% (22 h)	**(*R*)-4e**	40%	99%	**>100**	+ 6.3 (*c* 3.75, CH_3OH)

[a] rt, pH 6.0, 0.1 M Na-phosphate buffer, substrate concentration 0.2 mol/L.
[b] Absolute configuration determined by comparison of the optical rotation of acid **1a,** derived by acidic hydrolysis from **(*R*)-4a** or **(*R*)-4b** with the literature value.[4] The order of elution of the individual enantiomers on GC (Cyclodex B column), compared with **(*R*)-4a** and **(*R*)-4b**, was used to determine the absolute configuration of **(*R*)-4c-e**.
[c] Insolubility of octanoic acids at pH 6 prevented accurate determination of conversion by NaOH consumption and calculation of E.
[d] In the presence of 150 wt% heptane.

The results of these experiments are shown in Fig. 1 in the form of a conversion/*e.e.* plot. Clearly, there is a group of lipase preparations, i.e., Lipase AY30, cholesterol esterase, and crude lipase (Sigma) exhibiting a relatively similar selectivity (E) of 50–70 while the pure, crystallized and cross-linked lipase CR (Altus Chiro-CLEC CR) offered very low selectivity (E=5). Unexpectedly, the crude lipase from the same source (Altus) was not selective either (E=8). An immobilized preparation of purified lipase (Chirazyme L-3, Boehringer) exhibited similar selectivity (E=30) as the crude lipase from the same vendor. These studies clearly illustrate the importance of the source of an enzyme for the selectivity of hydrolysis. Fig. 1 also shows the calculated relationship of conversion and enantiomeric excess according to the kinetic model proposed by Chen et al.[7] for irreversible Michaelis–Menten kinetics. The discrepancy in slope between these theoretical curves and our experimental results may be caused by errors in the determination of conversion, or the inadequacy of the model for crude enzymes with more than one enzyme active on the substrate.

The isolation of the remaining enantiopure ester from the reaction mixture was performed as follows. After extraction of the hydrolysis mixture with hexanes (emulsions can be broken by addition of acetone),

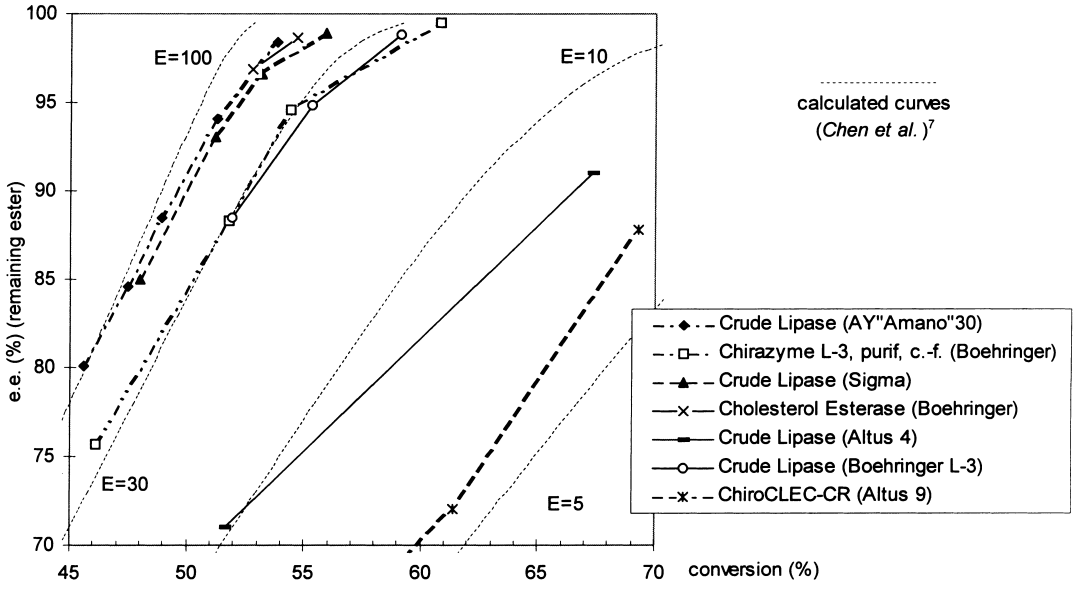


Figure 1. Enantiomeric excess:conversion in the hydrolysis of *rac*-**4b** for various lipase preparations from *Candida rugosa*

the organic phase was washed with water to remove cyanohydrin, and the solvent was distilled off to leave the butyrate (*R*)-**4b** (>98% *e.e.*) in 37–40% yield (from the racemate). Acid catalyzed hydrolysis of the (*R*)-butyrate (6 N HCl, 110°C, 8 h) afforded the acid (*R*)-**1a** in an 84% isolated yield. Separation from the butyric acid was accomplished by extraction under pH-controlled conditions, utilizing a pK_a difference of approximately 2.5 between acid **1a** and butyric acid.

To summarize, starting from 515 g of commercial racemic cyanohydrin **3a**, 170.5 g (58% of theoretical yield) of pure (*R*)-**1a** can be obtained in three steps in >98% *e.e.* using inexpensive *Candida rugosa* lipase, thus attesting the usefulness of enzymatic resolution methods in making enantiopure building blocks.

3. Experimental

3.1. General

Non-hydrolytic reactions were run under a nitrogen atmosphere. All chemicals used in the present study were of reagent grade and were used without further purification or drying. Due to the volatility of many of the substrates and products, concentration operations were performed by slow distillation over a Vigreux column, not using a rotary evaporator. The enzymatic reactions were followed with an autotitrator; interference in the pH-measurement from cyanide can be minimized by using a pH-electrode with a double junction reference cell. The enantiomeric excess of the remaining ester was in every case measured by GC using a 30 m×0.32 mm Cyclodex B column (permethyl-β-cyclodextrin in OV1701 stationary phase). NMR spectra of the following nuclei were determined in $CDCl_3$ using a Bruker Avance spectrometer: ^1H (300 MHz), referenced to tetramethylsilane (δ 0.0 ppm); ^{19}F (272 MHz), referenced to α,α,α-trifluorotoluene (δ −64.0 ppm); ^{13}C (75 MHz), referenced to the middle line of the $CDCl_3$-triplet (δ 77.0 ppm).

3.2. 2-Hydroxy-2-methyl-3,3,3-trifluoropropionic acid methyl ester rac-**2a**

A solution of the acid *rac*-**1a** (5.0 g, 31.6 mmol) and trimethyl orthoformate (7.7 g, 63 mmol) in anhydrous methanol (40 mL) containing sulfuric acid (200 μL) was heated at reflux for 4 h. Finely powdered $CaCO_3$ (2 g) was added, and the mixture was stirred at rt for 1 h. Filtration and distillation of the filtrate afforded the ester *rac*-**2a** (4.17 g, 76% yield) as a colorless liquid: bp 65–75°C (55 mmHg); ^{1}H-NMR δ 3.91 (s, 3H), 3.8 (br s, 1H), 1.60 (s, 3H); ^{13}C-NMR δ 170.78, 123.52 (q, J=285 Hz), 74.84 (q, J=30 Hz), 54.12, 18.87; ^{19}F-NMR δ −81.3.

3.3. 2-Hydroxy-2-methyl-3,3,3-trifluoropropionic acid ethyl ester rac-**2b**

A solution of the acid *rac*-**1a** (3.0 g, 19.0 mmol) and triethyl orthoformate (5.2 g, 35 mmol) in anhydrous ethanol (10 mL) containing sulfuric acid (200 μL) was heated at reflux for 4 h. The reaction was allowed to cool to rt, saturated $NaHCO_3$ solution (30 mL) was added, and the mixture was extracted with *t*-butyl methyl ether (tBME) (2×30 mL). The combined extracts were dried over $MgSO_4$ and distilled in vacuo over a Vigreux column to afford *rac*-**2b** (1.3 g, 37%) as a colorless liquid: bp 54–55°C (19 mmHg); ^{1}H-NMR δ 4.36 (m, 2H), 3.97 (br s, 1H), 1.59 (s, 3H), 1.34 (t, J=7.2 Hz, 3H); ^{13}C-NMR δ 170.19, 123.48 (q, J=285 Hz), 74.64 (q, J=30 Hz), 63.59, 18.75, 13.66; ^{19}F-NMR δ −81.2.

3.4. 2-Hydroxy-2-methyl-3,3,3-trifluoropropionic acid benzyl ester rac-**2c**

Finely powdered Cs_2CO_3 (4.6 g, 14 mmol) was added to a stirred solution of the acid *rac*-**1a** (3.0 g, 19.0 mmol) in acetone (30 mL). After the evolution of CO_2 ceased, benzyl bromide (4.9 g, 29 mmol) was added followed by sodium iodide (100 mg). The mixture was refluxed for 3 h. A voluminous solid was filtered off, the residue rinsed with tBME, and the filtrate concentrated in vacuo. The remaining oil was distilled to afford ester *rac*-**2c** (3.98 g, 77%) as a clear liquid: ^{1}H-NMR δ 7.35 (m, 5H), 5.28 (s, 2H), 3.9 (br s, 1H), 1.58 (s, 3H); ^{13}C-NMR δ 170.13, 134.19, 128.78, 128.68 (2C), 128.06 (2C), 123.54 (q, J=286 Hz), 74.90 (q, J=30 Hz), 69.05, 18.87; ^{19}F-NMR δ −81.0.

3.5. 2-Hydroxy-2-(trifluoromethyl)butyric acid methyl ester rac-**2d**

Prepared analogous to *rac*-**2a** from 10.1 g (59 mmol) of acid *rac*-**1d**. Yield 8.9 g (48 mmol, 81%), colorless liquid: bp 79–80°C (50 mmHg); ^{1}H-NMR δ 3.91 (s, 3H), 3.89 (s, 1H), 2.0 (m, 2H), 0.91 (t, J=7.3 Hz, 3H); ^{13}C-NMR δ 170.45, 123.52 (q, J=286 Hz), 78.23 (q, J=29 Hz), 54.08, 24.84, 6.68; ^{19}F-NMR δ −79.8.

3.6. General procedure for the enzymatic hydrolysis of rac-**2**

A 3-necked flask equipped with mechanical stirrer and a gel-filled double-junction pH electrode was charged with the enzyme (for amounts see Table 1) and sodium phosphate buffer (0.1 M, pH 7.0; adjusting the volume to achieve a substrate concentration of 0.2–0.3 mol/L), and the pH was set to the desired value with H_3PO_4 or NaOH. After addition of the substrate, the pH was held constant by addition of 1 N NaOH using an autotitration system. Samples (100 μL) were taken periodically, extracted with tBME (1 mL), and analyzed by GC using a chiral stationary phase. At a conversion of around 50%, determined by consumption of NaOH, the mixture was brought to pH 8, extracted with tBME (4×), the organic layers concentrated by distillation, and the residue distilled to obtain the remaining ester (*S*)-**2**.

The aqueous layer was brought to pH 1 with 6 N HCl, extracted twice with tBME, the combined organic layers dried over $MgSO_4$, concentrated, and the solid residue dried in vacuo at rt to afford the acid (*R*)-**1**. The *e.e.* of the acid (*R*)-**1** was determined by GC after derivatization with TMS–CHN_2 in CH_3OH/THF. For results see Table 1.

3.7. General procedure for the synthesis of acylated cyanohydrin

3.7.1. With acid chlorides (Method A)

Acid chloride (1.05 equiv.) was added to the cyanohydrin (**3a**) (20% solution in THF) at 0°C. While cooling with an ice bath, pyridine (2 equiv.) was added slowly to keep the internal temperature below 20°C. The reaction was allowed to warm to rt and stirred for 1 h. Water (1 mL/mmol), hexane (0.5 mL/mmol) and excess hydrochloric acid was added, and the organic layer was washed with aqueous Na_2CO_3 (10%) containing NaOH (1 equiv.). Drying of the organic phase with magnesium sulfate and distillation in vacuo afforded the ester as a colorless liquid.

3.7.2. With acid anhydrides (Method B)

Cyanohydrin (**3a** or **3e**) was added to acid anhydride (1.05 equiv.) at 0°C. While cooling with an ice bath, pyridine (1.2 equiv.) was added slowly to keep the internal temperature below 20°C. The reaction was slowly heated to 60°C and stirred until ^{19}F-NMR showed complete consumption of the cyanohydrin (4–8 h). Water (1 mL/mmol) was added, and extractive workup with hydrochloric acid and aqueous (10%) Na_2CO_3 containing NaOH (0.8 equiv.) afforded the crude ester as a colorless liquid. The crude ester was used in large scale hydrolysis experiments. Purification by filtration over a pad of silica followed by distillation in vacuo afforded the pure ester as a colorless liquid.

3.8. rac-*2-Cyano-1,1,1-trifluoroprop-2-yl acetate* rac-***4a***

Method B: yield 74% after distillation; bp 74°C (60 mmHg); ^{1}H-NMR δ 2.21 (s, 3H), 1.95 (s, 3H); ^{13}C-NMR δ 167.23, 121.37 (q, J=284 Hz), 112.56, 70.52 (q, J=34 Hz), 20.47, 18.59; ^{19}F-NMR δ −81.2.

3.9. rac-*2-Cyano-1,1,1-trifluoroprop-2-yl butyrate* rac-***4b***

Method B: yield 86–93% crude, 80.5% after distillation; bp 76–78°C (20 mmHg); ^{1}H-NMR δ 2.45 (t, J=7.3 Hz, 2H), 1.96 (s, 3H), 1.71 (sextet, J=7.3 Hz, 2H), 1.00 (t, J=7.3 Hz, 3H); ^{13}C-NMR δ 170.00, 121.44 (q, J=284 Hz), 112.66, 70.42 (q, J=34 Hz), 35.59, 18.74, 17.97, 13.25; ^{19}F-NMR δ −81.2.

3.10. rac-*2-Cyano-1,1,1-trifluoroprop-2-yl hexanoate* rac-***4c***

Method A: yield 59% after distillation; bp 109–111°C (22 mmHg); ^{1}H-NMR δ 2.45 (t, J=7.3 Hz, 2H), 1.96 (s, 3H), 1.69 (m, 2H), 1.33 (m, 4H), 0.91 (m, 3H); ^{13}C-NMR δ 170.17, 121.44 (q, J=284 Hz), 112.65, 70.43 (q, J=35 Hz), 33.73, 30.87, 24.04, 22.11, 18.73, 13.70; ^{19}F-NMR δ −81.2.

3.11. rac-*2-Cyano-1,1,1-trifluoroprop-2-yl octanoate* rac-***4d***

Method A: yield 64% after distillation; bp 85–93°C (0.5 mmHg); ^{1}H-NMR δ 2.45 (t, J=7.3 Hz, 2H), 1.94 (s, 3H), 1.67 (m, 2H), 1.30 (m, 8H), 0.91 (m, 3H); ^{13}C-NMR δ 170.18, 121.45 (q, J=284 Hz), 112.65, 70.42 (q, J=34 Hz), 33.77, 31.49, 28.71 (2C), 24.37, 22.48, 18.73, 13.93; ^{19}F-NMR δ −81.2.

3.12. rac-*2-Cyano-1,1,1-trifluorobut-2-yl butyrate* rac-***4e***

Method B: yield 72% after distillation; bp 78–80°C (12 mmHg); ^{1}H-NMR δ 2.43 (t, J=7.4 Hz, 2H), 2.30 (q, J=7.5, 2H), 1.72 (m, J=7.3 Hz, 2H), 1.16 (t, J=7.5 Hz, 2H), 1.00 (t, J=7.4 Hz, 3H); ^{13}C-NMR δ 169.81, 121.59 (q, J=286 Hz), 112.10, 73.57 (q, J=33 Hz), 35.49, 26.38, 17.95, 13.18, 7.61; ^{19}F-NMR δ −77.5.

3.13. *General procedure for small-scale hydrolysis of cyanohydrin esters*

Using a 100 mL reactor immersed in a constant temperature bath (25°C), with mechanical stirrer and a gel-filled double-junction pH electrode, the enzyme (for amount see Table 3) was suspended in 50 mL sodium phosphate buffer (0.1 M, pH 7.0) and brought to the desired pH value by addition of 85% phosphoric acid or 1 N NaOH. The substrate was added, and the pH was held constant by addition of 1 N NaOH using an autotitration system. At conversions of 45% and above, samples (100 μL) were taken periodically, extracted with tBME (1 mL), and analyzed by GC using a chiral stationary phase. Conversion, as measured by the consumption of base, and *e.e.* at this conversion were used to calculate E according to Chen et al.[7] Product isolation was accomplished by basification (5 N NaOH) to pH 9, extraction with hexanes (2×50 mL), and washing of the combined organic layers with water (2×50 mL) followed by distillation.

3.14. *Preparative-scale hydrolysis of* rac-*2-cyano-1,1,1-trifluoroprop-2-yl butyrate* rac-***4b***

A solution of $NaH_2PO_4 \cdot H_2O$ (121.0 g) in deionized water (9.0 L) was brought to pH 7.0 by addition of KOH pellets (approx. 36 g). Lipase Amano AY30 (91.3 g) was homogenously suspended. The pH was adjusted to 6 by addition of phosphoric acid (85%) (approx. 48 g). After stirring for 5 min, crude *rac*-2-cyano-2-methyl-1,1,1-trifluoroprop-2-yl butyrate (*rac*-**4b**) (697 g) was added in one portion. The autotitrator was switched on immediately to keep the pH constant by addition of 5 N NaOH and the reaction was monitored by tracking the consumption of sodium hydroxide solution. When the conversion approached 55%, the end point of the reaction was determined by GC analysis of the enantiomeric composition of the product. The kinetic resolution was considered complete when the *e.e.* of the remaining starting material was >98% (8–9 h). At this point the mixture was immediately brought to pH 9 by addition of 50% aqueous NaOH (approx. 100 mL), hexanes (6 L) were added, and the mixture was stirred vigorously, then allowed to settle. The lower, slightly turbid phase was separated from the upper, emulsified layer, and discarded. While stirring slowly, acetone (2000 mL) was added, and the phases were allowed to settle. (a) The lower, almost clear aqueous layer was discarded, the emulsified middle layer and the top layer were stirred vigorously for 1 min, and the phases were allowed to settle. The procedure was repeated from (a), until the amount of emulsion layer did not decrease any more (4 times typically). Then the emulsion layer was removed leaving the organic layer (7 L), which was washed with deionized water (2×4 L). At this point, ^{19}F-NMR was used to check for complete removal of the cyanohydrin. Hexanes were distilled off over a vacuum-jacketed bubble-plate column (10 plates), first at normal pressure, then under vacuum, to leave (*R*)-2-cyano-1,1,1-trifluoroprop-2-yl butyrate [(*R*)-**4b**], 280 g [1.34 mol, 40% yield (theoretical yield 50%), *e.e.* >98%], which was used in the hydrolysis step without further purification.

3.15. Synthesis of (R)-2-hydroxy-2-methyl-3,3,3-trifluoropropionic acid [(R)-**1a**]

(*R*)-2-Cyano-1,1,1-trifluoroprop-2-yl butyrate (*R*)-**4b** (211.8 g, 1.01 mol), water (400 mL) and HCl conc. (400 mL) were stirred vigorously and heated under reflux for 8 h. At this point, ^{19}F-NMR indicated quantitative conversion to the product. Aqueous NaOH, 50% (w/w) (480 g) was added over 30 min at or below 25°C to bring the pH to 4.8. tBME (1000 mL) was added and the mixture stirred vigorously while adjusting the pH to 4.8 with 6 N HCl. The organic phase was discarded, and the aqueous phase was washed once more with tBME (500 mL) at pH 4.8 as described above. After adjusting the pH of the aqueous phase to 1 with 6 N HCl and extraction with tBME (2×500 mL), the combined organic layers were concentrated to dryness (bath 30°C, vacuum ≤20 mmHg), at which point the product crystallized. Heptane (500 mL) was added and removed in vacuo. The heptane treatment was repeated once, then just enough heptane (approx. 180 mL) was added to allow slurrying of the crystalline material with a mechanical stirrer. The slurry was cooled in an ice bath and stirred for 2 h. Filtration afforded (*R*)-2-hydroxy-2-methyl-3,3,3-trifluoropropionic acid [(*R*)-**1a**] (134 g, 84%) as a white crystalline solid. Mp 106–110°C; $[\alpha]_D^{22}$ +18.4 (c 2.55, CH_3OH); *e.e.* assayed by GC after derivatization as the methyl ester with Cs_2CO_3/MeI/acetone: >99%.

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