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Chemoenzymatic Preparation of 1-Heteroarylethanamines of Low Solubility

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Both enantiomers of biologically and pharmaceutically interesting benzofuran-, benzothiophen-, and phenylfuran-based 1-heteroarylethanamines were prepared at close to theoretical yields by using *Candida antarctica* lipase B (Novozym 435) catalyzed (*R*)-selective *N*-acylation with isopropyl butanoate (enantiomeric ratio $E > 200$). The use of *N*-methyl-2-pyrrolidinone (NMP) as a cosolvent (1:30) in isopropyl butanoate solved the problem of low solubility of the compounds. Instability of the heterocyclic ring systems against traditional acid- and base-catalyzed hydrolysis was solved by using *Candida antarctica* lipase A as a commercial CAL-A-CLEA preparation for deprotection of the *N*-acylated (*R*) enantiomers in water. The slow, highly enantioselective ($E > 200$) hydrolyses of racemic butanamides was also observed in the presence of Novozym 435.

Introduction

Demand for enantiopure arylethanamines is continuously increasing, especially in the pharmaceutical and fine chemical industries. The activity of 1-arylethanamines **3a**–**g** (Scheme 1) have been tested for instance against varicella zoster virus[1] and as kinase inhibitors,[2] calcium-sensing receptor regulating agents,[3,4] pesticides,[5] and as compounds for the prevention and treatment of pain.[6] The nature of a benzofuran, benzothiophene, and phenylfuran ring in these and related compounds requires handling under mild reaction conditions to prevent ring degradation. Therefore, biocatalytic rather than traditional chemocatalytic preparative methods are favored. Lipases (E.C. 3.1.1.3) have been commonly used for the kinetic resolution of compounds with primary[7,8] and – although more rarely – secondary[9] amino groups by enantioselective *N*-acylation in organic solvents. *N*-Acylations of amines are typically irreversible, because most amide bonds are stable against lipases in organic media. Additional benefits of lipases include high stability, wide substrate scope, chemo-, enantio-, and regioselectivity, possibilities for modifications by genetic engineering, and easy recycling, affording applications in which amine enantiomers are prepared by lipases on a ton-scale.[10]

Various chemocatalytic methods are known to produce enantiomerically enriched 1-(benzofuran-2-yl)- and 1-(benzothien-2-yl)alkanamines. One of the methods describes the formation of (*S*)-1-(benzofuran-2-yl)ethanamine from *N*-protected α-L-amino acids by utilizing a microwave technique.[11] Another synthesis has been reported that involves rhodium-catalyzed hydrogenation of *N*-sulfonyl ketimine.[12] A similar compound with the amine frame, (*R*)-*N*-[1-(benzo[*b*]thiophen-2-yl)ethyl]-*O*-benzylhydroxylamine, has been prepared by using an alternative, expensive coupling and an intramolecular Wittig reaction.[13] (*S*)-*N*-Aryl-1-(benzofuran-2-yl)ethamine has been synthesized by Sigamide-catalyzed enantioselective reduction of ketimines with trichlorosilane.[14]

Our aim has been to prepare the enantiomers of seven benzofuran-, benzothiophen-, and phenylfuran-based 1-heteroarylethanamines by a chemoenzymatic pathway, the enantiopurity being introduced by the enantioselective *N*-acylation of *rac*-**3a**–**g** (Scheme 1). The emphasis on the enzymatic kinetic resolution step focused on solving two problems associated with **3a**–**g** and *N*-acylated **4a**–**g**. The first problem to be addressed was the extremely low solubility of the compounds in organic solvents commonly used in enzymatic *N*-acylation reactions. The second problem concerned the instability of heteroaryl rings and the deprotection of amides (*R*)-**4a**–**g** to release the corresponding (*R*)-**3a**–**g**, which is traditionally performed under acidic or basic conditions at high temperature. The use of polar cosolvents in enzymatic *N*-acylation and lipase-catalyzed hydrolysis of amides **4a**–**g** addressed these issues.

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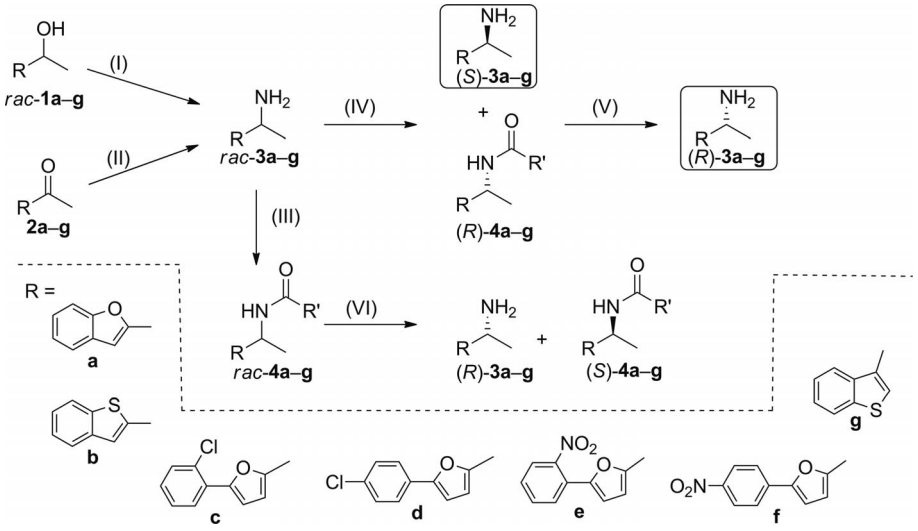


Scheme 1. Reagents and conditions: (I) (a) $(PhO)_2PON_3$, toluene; (b) Ph_3P, H_2O/THF. (II) (a) NH_3, $[Ti(OiPr)_4]$, MeOH; (b) $NaBH_4$, MeOH. (III) PrCOCl, DMAP, pyridine, CH_2Cl_2. (IV) CAL-B, $PrCO_2iPr$, NMP. (V) CAL-A, H_2O. (VI) CAL-B, H_2O.

Results and Discussion

Synthesis of Amines *rac*-3a–g and Amides *rac*-4a–g

The preparation of *rac*-**3a**–**g** was accomplished from the corresponding alcohols *rac*-**1a**–**g** and ketones **2a**–**g** by applying literature methods (routes I and II, Scheme 1).[15] With alcohols *rac*-**1a**–**g** as starting materials, low yields (10–55%) were obtained, because *rac*-**1a**–**g** appeared to be unstable during azide formation with diphenyl phosphorazidate under basic conditions (route I, step a) in which elimination of water was detected.[15a,15b] In step b, the transformation of azides into amines under basic conditions at high temperature (40–85 °C) led to amine dimerization. Hydrogenation of azides in the presence of Pd/C in EtOH/MeOH before the Staudinger reaction (route I, step b) gave similar low yields (45–55%), as did the Mitsunobu reaction of *rac*-**1a**–**g** in the presence of phthalimide (35–45% yields).[15c,15d] Finally, amines *rac*-**3a**–**g** were synthesized from the corresponding ketones **2a**–**g** by reductive amination with 52–84% isolated yields (route II, Scheme 1).[15e] The reactions were complete within 6–12 h at room temperature.

Amides *rac*-**4a**–**g** were obtained by chemical acylation with an acyl chloride or acyl anhydride in dichloromethane in the presence of pyridine and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) (route III, Scheme 1).

Enzymatic *N*-Acylation of *rac*-3a–f

Frequently used commercial lipase preparations [50 mg mL^{-1}; lipases from *Candida rugosa*, *Pseudomonas fluorescens*, *Burkholderia cepacia*, *Thermomyces lanuginosus*, and *Candida antarctica* (CAL-A and CAL-B)] and alcalase (protease from *Bacillus licheniformis*) were first screened for the *N*-acylation of *rac*-**3a** (0.01 M) in ethyl acetate at room temperature (route IV, Scheme 1). Surprisingly, only CAL-B as a Novozym 435 preparation gave both high enantioselectivity (measured as enantiomeric ratio *E*) and reactivity (measured as conversion after a certain time; data not shown).

Optimization was continued with Novozym 435 as a catalyst in anhydrous ethyl and isopropyl acetates, which are common solvents for the acylation of 1-phenylethanamine,[7f,8,16] in the presence of molecular sieves [4 Å; in a 1:1 (w/w) ratio to the catalyst]. Molecular sieves were necessary, because the so-called residual water in the seemingly dry enzyme catalyst or traces of water in the mixture may cause lipase-catalyzed hydrolysis of hydrolyzable compounds. Under the present *N*-acylation conditions, the hydrolysis of an acyl donor may lead to ammonium salt formation between an amine and the acid liberated, lowering the enantioselectivity of the kinetic resolution.[7d,17] The effect of water was clear when the *N*-acylation of *rac*-**3a** was performed in ethyl acetate saturated with water (E = 50) compared to the reaction in anhydrous ethyl or isopropyl acetate ($E > 100$; Table 1, Entries 1–3). The effect of water is often pointed out, especially in connection with Novozym 435 (CAL-B on hydrophobic macroporous divinylbenzene-crosslinked carrier), which readily releases adsorbed water into the anhydrous reaction medium.[18] Additionally, water tunnels, leading to the active site of CAL-B, were previously shown to allow access for water to the active site.[19]

One clear problem, especially with amines **3c**–**f**, concerns their low solubility. For instance, *rac*-**3c** was soluble in ethyl acetate only as a 1 mM solution at room temperature. Solubility also remained low in acetonitrile, dioxane, toluene, *tert*-amyl alcohol, 2-methyltetrahydrofuran, dimethoxyethane, diglyme, and tetraline. Common dipolar aprotic solvents such as tetraglyme, dimethyl sulfoxide (DMSO) and *N*-methylpyrrolidinone dissolved the substrate better, but enzymatic reactivity was then negligible. *N*-Methyl-2-pyrrolidinone (NMP), a suitable building block for ionic liquids[20] and pharmaceuticals such as Cefepime,[21] was se-

Table 1. *N*-Acylation of *rac*-**3a** (0.010 M) and *rac*-**3c** (0.020 M) with CAL-B (10 mg mL^{-1} for *rac*-**3a** and 20 mg mL^{-1} for *rac*-**3c**) at room temp. for *rac*-**3a** and 45 °C for *rac*-**3c**, with different solvents and acyl donors (t = 4 h).

Entry	Amine	Solvent/Acyl donor	Conv. [%]	(*S*)-**3** *ee* [%]	(*R*)-**4** *ee* [%]	E[a]
1	**3a**	EtOAc (wet)	43	70	92	50
2	**3a**	EtOAc	43	73	98	> 100
3	**3a**	*i*PrOAc	50	96	96	> 100
4	**3c**	NMP/*i*PrOAc (30:1)	6	5	82	10
5	**3c**	NMP/*i*PrOAc (1:1)	37	53	92	40
6	**3c**	NMP/*i*PrOAc (1:5)	51	97	91	90
7	**3c**	NMP/*i*PrOAc (1:30)	49	94	94	> 100
8	**3c**[b]	NMP/*i*PrOAc (1:30)	51	98	94	> 100
9	**3c**	NMP/EtOAc (1:30)	52	97	91	90
10	**3c**	NMP/$MeOCH_2CO_2Et$ (1:30)	51	94	91	75
11	**3c**	NMP/$PrCO_2Et$ (1:30)	48	89	98	> 200
12	**3c**	NMP/$PrCO_2$*i*Pr (1:30)	51	> 99	97	> 200

[a] Enantiomeric ratio. [b] The results with Novozym 435 reused in five cycles.

Table 2. Preparative-scale *N*-acylation of *rac*-**3a**–**f** (0.020 M) with CAL-B (20 mg mL^{-1}) at 45 °C in a mixture of NMP/isopropyl butanoate (1:30) and in the presence of molecular sieves (4 Å; 3 mg mL^{-1}). Reaction time 4 h.

Entry	Amine	Conv. [%]	(*S*)-**3** *ee* [%]	Yield [%][a]	(*R*)-**4** *ee* [%]	Yield [%][a]	E[b]
1	**3a**	50	> 99	94	98	97	> 200
2	**3b**	50	> 99	95	99	95	> 200
3	**3c**	50	> 99	93	97	93	> 200
4	**3d**	51	> 99	94	98	95	> 200
5	**3e**	51	> 99	95	97	95	> 200
6	**3f**	51	> 99	93	97	95	> 200

[a] Isolated yield. [b] Enantiomeric ratio.

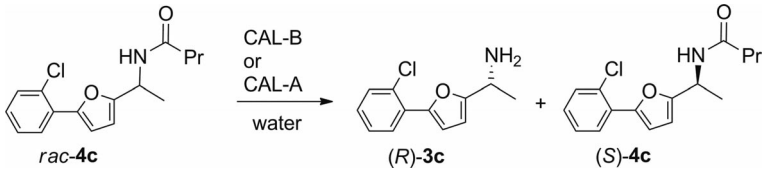
lected as a cosolvent to increase the solubility of the amine. Thus, the *N*-acylation of *rac*-**3c** (0.020 M) was carried out at 45 °C in various solvent mixtures of NMP and acetate, 2-methoxyacetate, and butanoate esters (Table 1, Entries 4–12). Enantioselectivity E (10 → > 100) and conversion after 4 h (6 → 49%) both increased considerably when the proportion of NMP was reduced from 30:1 to 1:30 with respect to the acyl donor (Table 1, Entries 4–7). When the *N*-acylation was repeated by reusing the same Novozym 435 catalyst, there was practically no change in the enzymatic efficiency even after five reuse cycles (compare Table 1, Entries 7 and 8), indicating that the amine and the small amount of NMP (1:30) were not harmful to the lipase. The enantioselectivity of the *N*-acylations with various esters as solvents and acyl donors varied slightly, with butanoate esters being clearly the best candidates (compare Table 1, Entries 7–10, 11, and 12). Disappointingly, the *N*-acylation of *rac*-**3c** with acyl-activated ethyl 2-methoxyacetate (Table 1, Entry 10), an excellent acyl donor under the solvent-free kinetic resolution conditions for arylamines,[8] proceeded even worse than with the other acyl donors (Table 1, Entries 7–9, 11, and 12). Thus, marked rate enhancements, caused by favorable hydrogen-bond formation between the β-oxygen atom of the methoxyacetate moiety and the amine hydrogen atom of the reactive amine counterpart during the progress of catalysis,[22] were not detected at this time. According to the results presented in Table 1, the *N*-acylation of *rac*-**3c** is the most enantioselective one in the NMP/isopropyl butanoate mixture (1:30; Table 1, Entry 12).

The kinetic-resolution method developed for *rac*-**3c** was then used for the preparative-scale kinetic resolution of *rac*-**3a**–**f** (0.020 M) with isopropyl butanoate and Novozym 435 (20 mg mL^{-1}; Table 2). The highly enantioselective *N*-acylations of the reactive (*R*)-**3a**–**f** (E > 200) were all complete within 4 h. The resolution products [the unreacted (*S*)-**3a**–**f** and the obtained (*R*)-**4a**–**f**] were all separated at close to 50% theoretical yields (93–97% from the theoretical amounts at 50% conversion) in highly enantiopure form (*ee* 97–99%). In conclusion, the results clearly show NMP to be a usable cosolvent for the lipase-catalyzed *N*-acylation of amines of limited solubility.

Deprotection and Kinetic Resolution of 4a–g by Lipase-Catalyzed Hydrolysis

The instability of especially (*R*)-**4e** and -**4f** during base-catalyzed hydrolysis, and that of (*R*)-**4a**–**g** in aqueous HCl, prevented their deprotection under traditional chemocatalyzed hydrolysis conditions. A small improvement was made when (*R*)-**4a**–**g** were heated to reflux overnight in dioxane containing HCl (8 equiv.), leading to acceptable yields (70–80%). Because environmentally more acceptable methods were desired, the studies were directed to the lipase-catalyzed amide hydrolysis of *rac*-**4c** (Table 3). The most important feature enabling amide hydrolysis by serine proteases was recently attributed to the hydrogen bond from the substrate amide nitrogen atom to the enzyme or substrate, facil-

Table 3. CAL-A and CAL-B-catalyzed (10 mg mL^{-1}) hydrolyses of *rac*-**4c** (R′ = Pr; 3.5 mM) in phosphate buffer (pH = 7.5, 0.100 M) or water at 45 °C (t = 3 d).



rac-**4c** (*R*)-**3c** (*S*)-**4c**

Entry	Enzyme	Solvent	Conv. [%]	(*S*)-**4c** *ee* [%]	(*R*)-**3c** *ee* [%]	E[a]
1	Novozym 435	buffer	26	35	98	> 100
2	Novozym 435	water	33	48	> 99	> 200
3	CAL-B (ICR-110)	water	27	38	> 99	> 200
4	CAL-B (LCAHN)	water	10	10	> 99	> 200
5	CAL-B-T1-1500	water	25	33	> 99	> 200
6	CAL-B-T2-150	water	3	3	> 99	> 200
7	CAL-B (IMB-111)	water	17	21	> 99	> 200
8	CAL-A-CLEA	buffer	60	30	12	4
9	CAL-A-CLEA	water	76	32	16	9

[a] Enantiomeric ratio.

itating the nitrogen inversion during the catalytic process.[23] Even though lipases have the same catalytic triad, they lack this particular hydrogen bond and, consequently, the ability to efficiently cleave amides. However, from a synthetic point of view, lipases are of particular interest because of their stability and other favorable properties and – as an encouraging result – CAL-A was previously found to hydrolyze amides in water.[24] The ability to hydrolyze amides has also been observed with CAL-B.[25]

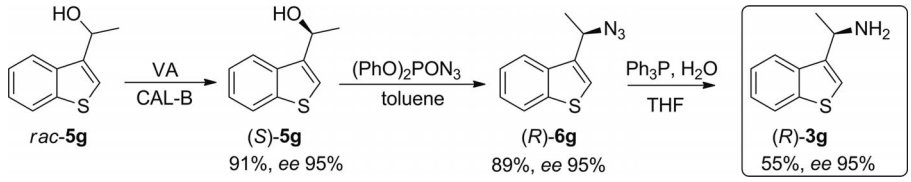
Before CAL-A and CAL-B could be used for the deprotection of (*R*)-butanamides by hydrolysis, it was important to know that the lipase catalyzes hydrolysis of the particular enantiomer. Thus, the hydrolysis of butanamide *rac*-**4c** (R′ = Pr in Scheme 1; 3.5 mmol/L) served as a model substrate in an aqueous medium (water or phosphate buffer, pH = 7.5, 0.100 M) in the presence of CAL-A-CLEA (crosslinked enzyme aggregate of CAL-A) and various CAL-B-preparations (Table 3). Because the amide hydrolyses tended to be slow, elevated temperature was used. At 60 °C, the thermal deactivation of CAL-A-CLEA was significant; therefore, the hydrolyses were performed at 45 °C. In general, hydrolyses in water were preferable to reactions in phosphate buffer (compare Table 3, Entries 1 to 2 and 8 to 9). CAL-A-CLEA gave good reactivity (conversions 60–76% in 3 d) but almost no enantioselectivity (Table 3, Entries 8 and 9), whereas various CAL-B catalysts gave excellent enantioselectivity (E > 200) with slow reactions (conversions 3–48% in 3 d; Table 3, Entries 1–7), with the highest reactivity being achieved with Novozym 435 (Table 3, Entry 2). Similar reactivities also appeared with CAL-B-T1-1500 (Table 3, Entry 5) and with free lyophilized CAL-B powder, ICR-110 (Table 3, Entry 3). CAL-B immobilized either through adsorption on a highly hydrophobic polymer (LCAHN; Table 3, Entry 4) or covalently (T2-150 and IMB-111; Table 3, Entries 6 and 7, respectively) gave the lowest reactivity. As the amounts of protein in the commercial lipase preparations are not known, comparison between reactivities are based simply on the amounts of catalysts weighed (10 mg mL^{-1}).

Finally, (*R*)-**4a**–**f** (3.5 mM; R′ = Pr) obtained in the *N*-acylation were subjected to preparative-scale hydrolysis in water in the presence of CAL-A-CLEA (Table 4). Accordingly, amines (*R*)-**3a**–**f** were obtained at approximately 100% conversions in 4–6 d. Additionally, preparative-scale hydrolysis of *rac*-**4a**–**g** with Novozym 435 proceeded with excellent enantioselectivities (E > 200; Table 4), albeit slowly (5–7 d to reach full 50% conversions). Thus, the Novozym 435 catalyzed hydrolysis allowed the preparation of the enantiomers of *rac*-**4g** as produced (*R*)-**3g** and unreacted (*S*)-**4g** (R′ = Me; Table 4, Entry 7) although the *N*-acylation method totally failed due to the extremely low solubility of the compounds in ester solvents even in the presence of NMP. The *N*-acylation was also unsuccessful when performed in ionic liquids [1-ethyl-3-methylimidazolium trifluoromethansulfonate, 1-hexyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-ethyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide, and tetrafluoroborate] in which the amine was soluble; in these cases low conversions or enantioselectivities were observed (data not shown).

Table 4. Use of CAL-A and CAL-B (10 mg mL^{-1}) on the preparative-scale hydrolyses of *rac*- or (*R*)-**4a**–**f** (R′ = Pr; 3.5 mM) and **4g** (R′ = Me; 3.5 mM) in water at 45 °C.

Entry	Substrate	Time	CAL-B		Substrate	Time	CAL-A
		[d]	c [%]	E[a]		[d]	c [%]
1	*rac*-**4a**	6	50	>> 200	(*R*)-**4a**	5	97
2	*rac*-**4b**	6	50	>> 200	(*R*)-**4b**	6	99
3	*rac*-**4c**	7	50	>> 200	(*R*)-**4c**	6	96
4	*rac*-**4d**	6	50	> 200	(*R*)-**4d**	4	98
5	*rac*-**4e**	5	50	> 200	(*R*)-**4e**	4	99
6	*rac*-**4f**	5	50	> 200	(*R*)-**4f**	5	98
7	*rac*-**4g**[b]	13	49	>> 200	(*R*)-**4g**	6	97

[a] Enantiomeric ratio. [b] Enzyme content 30 mg mL^{-1}.



Scheme 2. Chemoenzymatic synthesis of (*R*)- and (*S*)-**3g**.

The results clearly show that Novozym 435 can be used to produce (*R*)-**3a**–**g** from the corresponding butanamide as well as to resolve *rac*-**4a**–**g**, whereas the deprotection of (*S*)-**4a**–**g** was unsuccessful. On the other hand, the low enantioselectivity of CAL-A-CLEA allowed the deprotection of either (*R*)- or (*S*)-amides.

Confirmation of Absolute Configurations

The absolute configuration of (*R*)-**3g** was determined by first preparing the corresponding alcohol according to the previous method and resolving it with Novozym 435 in neat vinyl acetate to yield (*S*)-**5g** with 95% enantiomeric excess (Scheme 2).[26] Compound (*S*)-**5g** was converted into the azide (*R*)-**6g** with diphenyl phosphorazidate [$(PhO)_2PON_3$] in anhydrous toluene under argon (Scheme 1, route I, step a) followed by reduction to amine (*R*)-**3g** by the Staudinger reaction with triphenylphosphane (step b).[15a,15b] Details of the synthesis are provided in the Supporting Information. The enantiopurity was not affected by the transformation, giving $[a]_D^{25}$ = +16.8 (*c* = 1.0, CH_3OH) at 95% ee, which is in accordance with the value {$[a]_D^{25}$ = +17.3 (*c* = 1.0, CH_3OH) at 99% ee} obtained for the Novozym 435 catalyzed hydrolysis of *rac*-**4g** in water (Table 4). It is reasonable to assume that Novozym 435 also has the same enantiopreference with other substrates in the *N*-acylation and deacylation reactions. The (*R*) enantiopreference of Novozym 435 shown herein is in accordance with the enantiopreference observed previously for the enzyme in *N*-acylations of arylethanamines.[8]

Conclusions

Biologically and pharmaceutically interesting enantiomers of racemic benzofuran-, benzothiophen-, and phenylfuran-based 1-heteroarylethanamines (*rac*-**3a**–**f**) were separated by Novozym 435 catalyzed *N*-acylation with isopropyl butanoate (*t* = 3–4 h; *E* > 200) at close to theoretical 50% yields and *ee* > 97%. The use of NMP as a cosolvent in isopropyl butanoate (1:30) solved the problem of low solubility of the amines **3a**–**f** and the corresponding butanamides. However, the kinetic resolution of *rac*-**3g** was not possible by this method, because the compound was not soluble under the reaction conditions.

The instability of especially (*R*)-**4e** and (*R*)-**4f** during base-catalyzed hydrolysis and that of (*R*)-**4a**–**g** in aqueous HCl prevented the deprotection of butanamide products under traditional hydrolysis conditions. This problem, and the insolubility of *rac*-**3g** under *N*-acylation conditions, were addressed by utilizing CAL-A as CLEA and CAL-B as Novozym 435 preparations for the hydrolysis of butanamides (*R*)-**4a**–**g** and *rac*-**4a**–**g** in water. Hydrolysis by CAL-A-CLEA proceeded much faster than with Novozym 435, allowing the deprotection of (*R*)-**4a**–**g** to yield (*R*)-**3a**–**g** in 96–99% isolated yields. The enzyme is fascinating, because amides of the opposite enantiomers can also be deprotected. On the other hand, the excellent enantioselectivity (*E* >> 200) of Novozym 435 made the kinetic resolution of *rac*-**4a**–**g** in water possible, although not attractive, due to long reaction times compared to *N*-acylation. The method, although slow, made the preparation of (*R*)-**3g** (*ee* 99%) possible by hydrolyzing *rac*-**4g** in water, because the low solubility of *rac*-**3g** prevented its kinetic resolution through *N*-acylation; (*R*) enantioselectivity in Novozym 435 and CAL-A-CLEA catalyses was shown by preparing the corresponding (*S*)-alcohol and transforming it chemically into (*R*)-**3g**.

Experimental Section

Materials and Methods: Toluene was distilled from Na and stored under argon. Solvents and acyl donors for enzymatic reactions were stored over molecular sieves unless otherwise stated. All other reagents were purchased from Aldrich or Fluka and used as received. Lipases from *P. fluorescens* and *B. cepacia* were products of Amano Europe. Lipase A from *C. antarctica* as cross-linked enzyme aggregates (CAL-A-CLEA), alcalase (protease from *B. licheniformis*) and lipase from *C. rugosa* were obtained from Fluka, and lipase B from *C. antarctica* (CAL-B, Novozym 435) and *T. lanuginosus* lipase (Lipozyme TL IM) from Novozymes. CAL-B as IMMOZYME CAL-B-T2-150 (covalently immobilized) and IMMOZYME CAL-B-T1-1500 (immobilized by adsorbtion) were products of ChiralVision. CAL-B as IMB-111 (covalently immobilized) and ICR-110 (lyophilized) were from Biocatalytics and as LCAHN (on highly hydrophobic styrene and divinylbenzene copolymer) from Sprin technologies. The enzymatic reactions were performed at 23 °C (room temperature) and 45 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker spectrometer operating at 500 MHz with tetramethylsilane (TMS) as an internal standard. HRMS data were measured in ESI^+ mode with a Bruker Avance microOTOF-Q quadrupole-TOF spectrometer. Melting points were recorded with a Gallenkamp apparatus. Optical rotations were determined with a PerkinElmer 241 or 341 polarimeter, and $[a]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Determination of the enantiomeric ratio *E* was based on the equation $E = \ln[(1 - c)(1 - ee_S)]/\ln[(1 - c)(1 + ee_S)]$, with $c = ee_S/(ee_S + ee_P)$ by using linear regression {*E* as the slope of the line $\ln[(1 - c)(1 - ee_S)]$ vs. $\ln[(1 - c)(1 + ee_S)]$} (subscripts S and P refer to substrate and product, respectively).[27] Enantiomeric excesses for (*R*)- and (*S*)-**3a**–**g** were determined as the corresponding acet-, propan- or butanamides (amines derivatized with the corresponding anhydride in the presence of a catalytic amount of DMAP in pyridine) with

Agilent 1090 or 1050 HPLC instruments equipped with Daicel Chiracel OD-H or Chiralpak IA chiral columns. Enantiomeric excess values of (*S*)-**5g** and (*R*)-**6g** were determined with an Agilent 6850 gas chromatograph equipped with a Cyclosil-B chiral column. Thin layer chromatography (TLC) was carried out by using Merck Kieselgel $60F^{254}$ sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating.

Synthesis of *rac*-3a–g: Amines *rac*-**3a**–**g** were synthesized from ketones **2a**–**g** according to the literature.[15e,26a] In a Schlenk tube flushed with argon, a mixture of the corresponding ketone **2a**–**g** (20.0 mmol) was dissolved in a solution of ammonia in methanol (2 M, 50 mL, 100 mmol) and titanium(IV) isopropoxide (12.0 mL, 40 mmol). The mixture was stirred under argon at room temperature for 2–3 h and then cooled in an ice bath before sodium borohydride (1.2 g, 30 mmol) was added in small portions. The mixture was stirred at room temperature for 3 h and then quenched by pouring it into ammonium hydroxide (2 M, 100 mL). The inorganic precipitate was filtered off, the phases were separated, and the aqueous phase was extracted several times with ethyl acetate. The combined organic phases were washed with HCl solution (1 M, 150 mL) to separate the neutral materials. The acidic aqueous extracts were washed with ethyl acetate (250 mL), treated with aqueous sodium hydroxide (2 M) to obtain pH = 12 and then extracted with ethyl acetate (250 mL). The combined organic extracts were washed with brine (150 mL), dried with sodium sulfate, and concentrated in vacuo to give the corresponding crude amine. The amines were purified by vacuum chromatography on silica gel (dichloromethane/methanol, 7:3) to afford *rac*-**3a**–**g** as semisolids with 52–78% yields.

Synthesis of *rac*-4a–g: DMAP (1%) in pyridine (1.11 mmol, 87.8 mg, 89.4 μL) and the acylating agent (1.11 mmol) were added to a solution of *rac*-**3a**–**g** (1.00 mmol) in anhydrous CH_2Cl_2 (1.5 mL). The mixture was stirred at room temperature for 3 h and then quenched with water (15 mL). The isolated organic phase was dried with anhydrous sodium sulfate, the solvent was evaporated, and the crude product was purified by vacuum chromatography on silica gel (dichloromethane/methanol, 9.5:0.5) to give *rac*-**4a**–**g** as semisolids with 92–96% yields. NMR and MS data were indistinguishable from those of their (*R*) enantiomers.

Enzymatic Reactions

Analytical-Scale *N*-Acylation: Novozym 435 and 3–4 beads of molecular sieves (4 Å) were weighed in a reaction vial before *rac*-**3a** or *rac*-**3c** (10–100 mM) in anhydrous organic solvent (1 mL) and an acyl donor were added. Reactions proceeded under shaking (170 rpm) at 23 °C or (80 rpm) at 45 °C. Samples were taken to monitor the reaction by filtering off the enzyme and derivatizing the free amine with an appropriate acyl anhydride in the presence of DMAP in pyridine. The samples were diluted with acetonitrile and analyzed by HPLC.

Analytical-Scale Hydrolysis: Either CAL-B or CAL-A-CLEA (10 mg/mL) was weighed in a reaction vial before one of the amides *rac*-**4a**–**g** (3.5 mmol/L) and water or phosphate buffer (10 mL, pH = 7.5, 0.100 M) were added. The mixture was shaken at 45 °C. Samples were taken to monitor the reaction as above by freezing the sample (–20 °C) and removing the water by freeze drying. After derivatization, analysis by HPLC was performed as described above.

Preparative-Scale *N*-Acylation: Novozym 435 (860 mg, 20 mg/mL) and molecular sieves (4 Å, 120 mg) were weighed in a reaction vial before *rac*-**3a**–**f** (0.020 mM) in NMP/isopropyl butanoate (1:30) was added. The mixture was shaken at 45 °C for 4 h to reach 50% conversion. The enzyme was filtered off, washed with CH_2Cl_2, and the combined organic phases were concentrated in vacuo. The crude product was purified by silica gel chromatography [ethanol/CH_2Cl_2, 5–30% (v/v)].

Enzymatic Deprotection: CAL-A-CLEA (500 mg, 10 mg/mL) was weighed in a reaction vial before the butanamide (*R*)-**4a**–**g** (R′ = Pr; 50 mg) and water (50 mL) were added. The mixture was shaken at 45 °C. At close to 100% conversion (reaction monitored by TLC) the reaction was stopped by freezing the reaction mixture (–20 °C) and evaporating the water by lyophilization. The product was dissolved in CH_2Cl_2, and the enzyme was filtered off. The solution was concentrated in vacuo and the crude product was purified by silica gel chromatography [ethanol/CH_2Cl_2, 5–30% (v/v)] to yield (*R*)-**3a**–**f**. The NMR and MS data of the amines were identical to those of *rac*-**3a**–**f** above. The kinetic resolution of *rac*-**4a**–**g** (0.4 mmol) with Novozym 435 (1.25 g) was performed in water (125 mL) at 45 °C by otherwise applying the deprotection procedure with CAL-A-CLEA.

Enantiomerically Enriched Products

(*S*)-3a: Yield: 130 mg (0.80 mmol, 94%); yellow semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = –12.6 (*c* = 1.0, $CHCl_3$).

(*R*)-4a: Yield: 191 mg (0.82 mmol, 97%); yellow solid; m.p. (78 ± 1) °C; *ee* = 98%; $[\alpha]_D^{25}$ = +148 (*c* = 1.0, $CHCl_3$).

(*R*)-3a: CAL-A-CLEA-catalyzed hydrolysis of (*R*)-**4a** in water produced (*R*)-**3a**. Yield: 33 mg (0.20 mmol, 95%); yellow semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = +12.4 (*c* = 1.0, $CHCl_3$).

(*S*)-3b: Yield: 144 mg (0.81 mmol, 95%); yellow semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = –17.5 (*c* = 1.0, $CHCl_3$).

(*R*)-4b: Yield: 200 mg (0.81 mmol, 95%); white solid; m.p. (115 ± 1) °C; *ee* = 99%; $[\alpha]_D^{25}$ = +147 (*c* = 1.0, $CHCl_3$).

(*R*)-3b: CAL-A-CLEA-catalyzed hydrolysis of (*R*)-**4b** in water produced (*R*)-**3b**. Yield: 36 mg (0.20 mmol, 98%); yellow semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = +17.8 (*c* = 1.0, $CHCl_3$).

(*S*)-3c: Yield: 162 mg (0.79 mmol, 93%); white solid; m.p. (159 ± 1)°C; *ee* > 99%; $[\alpha]_D^{25}$ = –2.5 (*c* = 1.0, CH_3OH).

(*R*)-4c: Yield: 242 mg (0.83 mmol, 93%); white oil; *ee* = 97%; $[\alpha]_D^{25}$ = +28.5 (*c* = 0.5, CH_3OH)].

(*R*)-3c: CAL-A-CLEA-catalyzed hydrolysis of (*R*)-**4c** in water produced (*R*)-**3c**. Yield: 35 mg (0.16 mmol, 92%); white semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = +2.5 (*c* = 1.0, CH_3OH).

(*S*)-3d: Yield: 178 mg (0.80 mmol, 94%); white solid; m.p. (182 ± 1) °C; *ee* > 99%; $[\alpha]_D^{25}$ = +9.6 (*c* = 1.0, CH_3OH).

(*R*)-4d: Yield: 237 mg (0.81 mmol, 95%); white solid; m.p. (136 ± 1) °C; *ee* = 97.8%; $[\alpha]_D^{25}$ = 50.6 (*c* = 0.5, CH_3OH).

(*R*)-3d: CAL-A-CLEA-catalyzed hydrolysis of (*R*)-**4d** in water produced (*R*)-**3d**. Yield: 36 mg (0.16 mmol, 96%); white solid; m.p. (191 ± 1) °C; *ee* > 99%; $[\alpha]_D^{25}$ = –9.0 (*c* = 1.0, CH_3OH).

(*S*)-3e: Yield: 188 mg (0.81 mmol, 95%); yellow semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = –40.2 (*c* = 1.0, CH_3OH).

(*R*)-4e: Yield: 245 mg (0.81 mmol, 95%); yellow oil; *ee* = 97%; $[\alpha]_D^{25}$ = 53.0 (*c* = 0.5, CH_3OH).

(*R*)-3e: CAL-A-CLEA-catalyzed hydrolysis of (*R*)-**4e** in water produced (*R*)-**3e**. Yield: 37 mg (0.16 mmol, 96%); yellow semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = +41.3 (*c* = 1.0, CH_3OH).

(*S*)-3f: Yield: 184 mg (0.79 mmol, 93%); orange solid; m.p. (104 ± 1) °C; *ee* > 99%; $[\alpha]_D^{25}$ = +18.9 (*c* = 1.0, CH_3OH).

(*R*)-4f: Yield: 248 mg (0.82 mmol, 95%); yellow solid; m.p. (145 ± 1) °C; *ee* = 97%; $[\alpha]_D^{25}$ = 57.8 (*c* = 0.5, CH_3OH).

(*R*)-3f: CAL-A-CLEA-catalyzed hydrolysis of (*R*)-**4f** in water produced (*R*)-**3f**. Yield: 36 mg (0.16 mmol, 95%); yellow semisolid; *ee* > 99%; $[\alpha]_D^{25}$ = –19.0 (*c* = 1.0, CH_3OH).

(*R*)-3g: Yield: 34.3 mg (0.19 mmol, 85%); white solid; m.p. (218 ± 1) °C (dec.); *ee* = 99%; $[\alpha]_D^{25}$ = +17.3 (*c* = 1.0, CH_3OH).

(*S*)-4g: Yield: 40 mg (0.18 mmol, 81%); white solid; m.p. (152 ± 1) °C; *ee* = 89%; $[\alpha]_D^{25}$ = –13.6 (*c* = 1.0, CH_3OH).

Supporting Information (see footnote on the first page of this article): Retention times of the resolved compounds, ^1H and ^{13}C NMR spectra, and HRMS data.

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[1] M. J. Di Grandi, K. J. Curran, G. Feigelson, A. Prashad, A. Ross, A. R. Visalli, J. Fairhurst, B. Feld, J. D. Bloom, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4157–4160.
[2] F. E. Lovering, S. J. Kirincich, W. Wang, J.-B. Telliez, L. Resnick, J. E. Sabal-Ski, A. L. Banker, J. Butera, I. Mcfadyen, WO2009/012375, **2009**.
[3] X. Liang, T. Høyer, J. Fensholdt, S. E. Havez, B. Nørremark, WO2008/019690, **2008**.
[4] H. Shunichiro, I. Kazuhiro, I. Ryotaro, T. Taisuke, O. Makoto, S. Ryushi, T. Yoh, S. Masanao, US2010/0029687, **2010**.
[5] J. E. Drumm, R. M. Lett, T. M. Stevenson, WO1992/08704, **1992**.
[6] Y. Besidski, B.-M. Swahn, WO2000/76990, **2000**.
[7] For recent reviews, see: a) V. Gotor-Fernández, V. Gotor, *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 784–797; b) E. Busto, V. Gotor-Fernández, V. Gotor, *Chem. Rev.* **2011**, *111*, 3998–4035; c) A. Liljeblad, L. T. Kanerva, *Tetrahedron* **2006**, *62*, 5831–5854; d) M. Höhne, U. T. Bornscheuer, *ChemCatChem* **2009**, *1*, 42–51; e) N. J. Turner, *Curr. Opin. Chem. Biol.* **2010**, *14*, 115–121; f) J. H. Lee, K. Han, M.-J. Kim, J. Park, *Eur. J. Org. Chem.* **2010**, 999–1015.
[8] M. Päiviö, P. Perkiö, L. T. Kanerva, *Tetrahedron: Asymmetry* **2012**, *23*, 230–236.
[9] a) A. Liljeblad, A. Kiviniemi, L. T. Kanerva, *Tetrahedron* **2004**, *60*, 671–677; b) A. Hietanen, K. Lundell, L. T. Kanerva, A. Liljeblad, *ARKIVOC (Gainesville, FL, U.S.)* **2012**, *5*, 60–74; c) V. Gotor-Fernández, P. Fernández-Torres, V. Gotor, *Tetrahedron: Asymmetry* **2006**, *17*, 2558–2564.
[10] A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, *Nature* **2001**, *409*, 258–268.
[11] L. De Luca, G. Giacomelli, G. Nieddu, *J. Org. Chem.* **2007**, *72*, 3955–3957.
[12] S. H. Kwak, S. A. Lee, K.-I. Lee, *Tetrahedron: Asymmetry* **2010**, *21*, 800–804.
[13] C.-N. Hsiao, T. Kolasa, *Tetrahedron Lett.* **1992**, *33*, 2629–2632.
[14] A. V. Malkov, K. Vranková, S. Stončius, P. Kočovský, *J. Org. Chem.* **2009**, *74*, 5839–5849.
[15] a) A. S. Thompson, G. R. Humphrey, A. M. DeMarco, D. J. Mathre, E. J. J. Grabowski, *J. Org. Chem.* **1993**, *58*, 5886–5888; b) F. Felluga, W. Baratta, L. Fanfoni, G. Pitacco, P. Rigo, F. Benedetti, *J. Org. Chem.* **2009**, *74*, 3547–3550; c) S. Alatorre-Santamaría, V. Gotor-Fernández, V. Gotor, *Eur. J. Org. Chem.* **2009**, 2533–2538; d) O. Torre, E. Busto, V. Gotor-Fernández, V. Gotor, *Adv. Synth. Catal.* **2007**, *349*, 1481–1488; e) B. Miriyala, S. Bhattacharyya, J. S. Williamson, *Tetrahedron* **2004**, *60*, 1463–1471.
[16] M. T. Reetz, C. Dreisbach, *Chimia* **1994**, *48*, 570.
[17] A. Hietanen, T. Saloranta, S. Rosenberg, E. Laitinen, R. Leino, L. T. Kanerva, *Eur. J. Org. Chem.* **2010**, 909–010.
[18] L. Veum, L. T. Kanerva, P. J. Halling, T. Maschmeyer, U. Hanefeld, *Adv. Synth. Catal.* **2005**, *347*, 1015–1021.
[19] M. W. Larsen, D. F. Zielinska, M. Martinelle, A. Hidalgo, L. J. Jensen, U. T. Bornscheuer, K. Hult, *ChemBioChem* **2010**, *11*, 796–801.
[20] a) K. M. Johansson, E. I. Izgorodina, M. Forsyth, D. R. MacFarlane, K. R. Seddon, *Phys. Chem. Chem. Phys.* **2008**, *10*, 2972–2978; b) S. A. Forsyth, K. J. Fraser, P. C. Howlett, D. R. MacFarlane, M. Forsyth, *Green Chem.* **2006**, *8*, 256–261.
[21] M. A. Farajzadeh, L. Goushjuii, Y. Bashour, *J. Sep. Sci.* **2010**, *33*, 3767–3773.
[22] M. Cammenberg, K. Hult, P. Seongsoon, *ChemBioChem* **2006**, *7*, 1745–1749.
[23] P.-O. Syrén, K. Hult, *ChemCatChem* **2011**, *3*, 853–860.
[24] A. Liljeblad, P. Kallio, M. Vainio, J. Niemi, L. T. Kanerva, *Org. Biomol. Chem.* **2010**, *8*, 886–895.
[25] a) H. Smidt, A. Fischer, P. Fischer, R. D. Schmid, *Biotechnol. Tech.* **1996**, *10*, 335–338; b) T. Wagegg, M. M. Enzelberger, U. T. Bornscheuer, R. D. Schimd, *J. Biotechnol.* **1998**, *61*, 75–78; c) D. R. Duarte, E. L. Castillo, E. Bárzana, A. López-Munguía, *Biotechnol. Lett.* **2000**, *22*, 1811–1814; d) M. V. Sergeeva, V. V. Mozhaev, J. O. Rich, Y. L. Khmelnitsky, *Biotechnol. Lett.* **2000**, *22*, 1419–1422; e) A. Torres-Gavilán, J. Escalante, I. Regla, A. López-Munguía, E. Castillo, *Tetrahedron: Asymmetry* **2007**, *18*, 2621–2624; f) H. Ismail, R. M. Lau, F. van Rantwijk, R. A. Sheldon, *Adv. Synth. Catal.* **2008**, *350*, 1511–1516.
[26] a) M. I. Toşa, P. V. Podea, C. Paizs, F. D. Irimie, *Tetrahedron: Asymmetry* **2008**, *19*, 2068–2071; b) L. C. Bencze, C. Paizs, M. I. Tosa, F. D. Irimie, *Tetrahedron: Asymmetry* **2010**, *21*, 356–364.
[27] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.

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