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# Enantioselective acylation of alcohols with fluorinated β-phenyl-β-lactams in the presence of *Burkholderia cepacia* lipase

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**Abstract**—This paper concentrates on studies of the acylation of alcohols with 3,3-difluoro-4-phenylazetidin-2-one *rac*-**1**, *trans*-3-fluoro-4-phenylazetidin-2-one *rac*-**2** and 4-phenylazetidin-2-one *rac*-**3** in the presence of immobilized lipase PS from *Burkholderia cepacia* in dry *tert*-butyl methyl ether (TBME). Fluorine activation in the compounds studied was essential in order to split the β-lactam ring with lipase PS. The highly enantioselective ring opening of *rac*-**1** and *rac*-**2** with methanol (1-butanol was also studied) allowed the preparation of the (*R*/(3*R*,4*R*))-β-lactams as the unreacted enantiomers and (*S*/(2*S*,3*S*))-β-amino esters as the product enantiomers with an ee >99%. Under the same conditions, *rac*-**3** was totally unreactive. The possibility for a competing hydrolysis caused by water in the enzyme preparations is also discussed.


## 1. Introduction

Natural organic compounds relatively rarely contain fluorine. On the other hand, fluorine has gained an important position as an isostere for hydrogen, especially in lead modifications to potent medicinal agents. This is based on various desirable effects caused by the presence of fluorine on the physiological activity and metabolism of compounds. Fluorine can also affect the binding of the substrate at the active site of the enzyme, for instance, by accepting hydrogen bonds from the protein or from the active site water. As a consequence, half of the top ten drugs sold in 2005 were fluorinated organic molecules, while 20% of the pharmaceuticals on the market were estimated to contain fluorine.[1] The α,α-difluoro-β-amino acid residue of (*S*)-**4** (Scheme 1) in the side chain of dodetaxel analogues is a good example of an important fluorine-containing β-amino acid.[2]

The enantiomers of various β-amino acids and their precursors and intermediates have widespread applications as building blocks and intermediates in the design of medicinally important compounds.[3] Results for the enzymatic kinetic resolution of nonfluorinated β-amino esters and their precursors have previously been reviewed.[4] The results clearly indicate that 2,2,2-trifluoroethyl butanoate is a typical achiral acyl donor and *Candida antarctica* lipase A (CAL-A), a catalyst for the asymmetric N-acylation of β-amino esters. On the other hand, 2,2,2-trifluoroethyl esters of β-amino acids were previously prepared by making use of *C. antarctica* lipase B (CAL-B)-catalyzed interesterification (two ester substrates give products with exchanged alkyl counterparts) between a β-amino ester and a 2,2,2-trifluoroethyl ester.[5] Accordingly, lipases will accept alkyl-fluorinated carboxylic esters as substrates. Enzymatic kinetic resolutions of β-amino acid derivatives and precursors containing fluorine in the acid moiety are rarely investigated. The studies seem to be restricted to the hydrolysis of *N*-phenylacetyl derivatives of β-fluoroalkyl- and β-fluoroaryl-β-amino acids by penicilline acylase[6–8] and to the hydrolysis of β-fluorophenyl-β-alanine ethyl ester by *Burkholderia cepacia* lipase (lipase PS).[9]

β-Lactams (2-azetidinones) are interesting precursors of β-amino acids, while the lipases are highly usable and economical catalysts in organic chemistry. The enantioselective hydrolyses of many β-lactams with CAL-B (Novozym 435 preparation) were previously reported with 1 equiv of water in diisopropyl ether (DIPE) at an elevated temperature.[10,11] Enantioselective alcoholysis of *rac*-4-phenyl-2-azetidinone with CAL-B and 2-octanol was also reported, although the ester product was not detected.[12]

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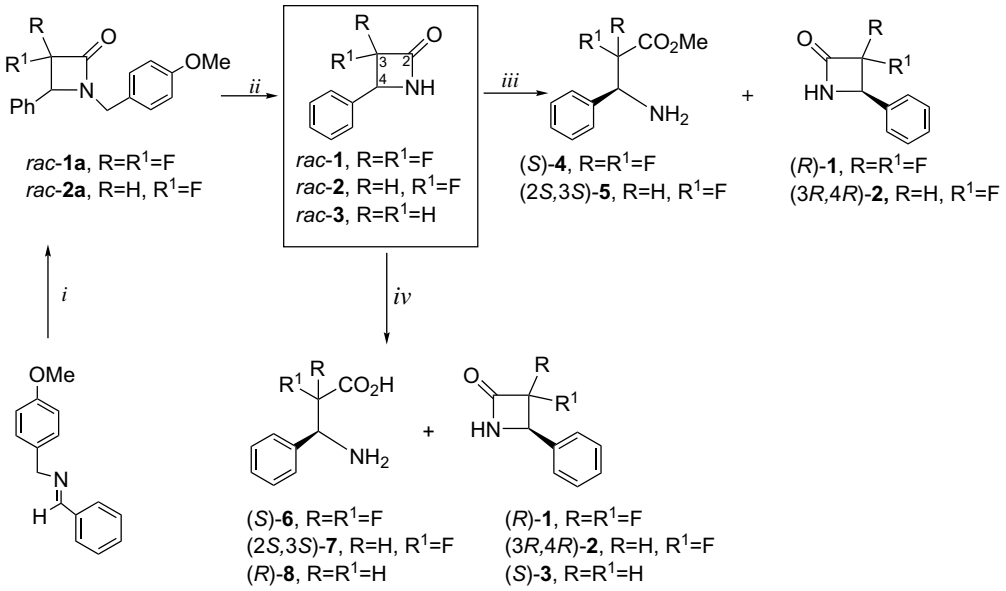
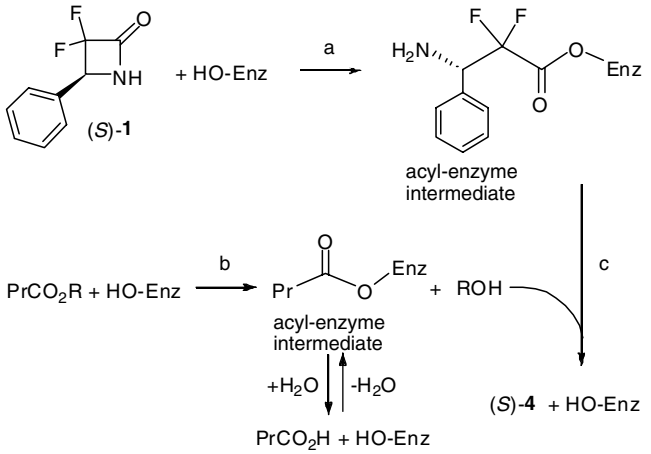



**Scheme 1.** Chemoenzymatic routes to the observed enantiomeric products of the work. Reagents: (i) $BrCF_2CO_2Et/BrCHFCO_2Et$, Zn, THF; (ii) $Ce(NH_4)_2(NO_3)_6$ (CAN), $CH_3CN$, $H_2O$; (iii) $CH_3OH$, lipase PS; (iv) $H_2O$, lipase PS-D or CAL-B.[10]

In our previous studies of the reaction of 4-benzyl-2-azetidinone in the presence of methanol and CAL-B in dry organic solvents, two important observations were evident: (1) the added methanol and the water in the enzyme preparation served as competitive nucleophiles for ring opening; (2) and the methyl ester produced by the action of methanol was further enzymatically hydrolyzed, the enantiopreference being opposite to that observed for the hydrolysis of the β-lactam ring.[13] Moreover, there was experimental evidence that the lipase PS preparation (lipase adsorbed on Celite in the presence of sucrose)[14] in dry organic solvents exposed the β-lactam to hydrolysis less than it did in the CAL-B preparation. This is in accordance with the observation that the lipophilic polymethacrylate carrier of Novozym 435 easily releases the attached water, giving hydrolysis of hydrolyzable compounds.[15] Sih et al. were the first able to open a β-lactam ring with methanol in *tert*-butyl methyl ether (TBME) in the presence of lipase PS.[16] In their study, the *N*-benzoyl activation of the β-lactam was supposed to make the ring susceptible to nucleophilic attack.

Herein, the ability of lipases to enantioselectively acylate alcohols to afford β-amino esters with fluorinated 4-phenyl-2-azetidinones *rac*-**1** and *rac*-**2** as well as nonfluorinated *rac*-**3** under mild conditions is considered (Scheme 1, route iii). The enzymatic preparation of β-amino esters directly from β-lactams offers an attractive alternative to the ring opening with alcohols by chemical means.[3b] More specifically, we have studied the importance of fluorine activation on the ring opening and kinetic resolution of our substrates by methanol and 1-butanol and the possibility to suppress the competing hydrolysis route iv by using mainly lipase PS adsorbed on Celite as a catalyst in TBME. The critical step for the ring opening by lipase catalysis is the formation of the so called acyl-enzyme intermediate when the active site serine hydroxyl reacts as a nucleophile to the carbonyl carbon of the lactam ring (Scheme 2).[4] During the following second addition–elimination process, the obtained ester intermediate reacts with a nucleophile, and leads to the formation of the corresponding final product (an acid with water and an ester with an alcohol).




**Scheme 2.** Ring opening by the alcohol generated in situ from an $PrCO_2R$ (R = Me or Bu, Enz = lipase). The water is from the enzyme preparation.

The synthesis of fluorinated β-amino acids has been reviewed.[3,17] Compound (*S*)-**6** was previously prepared, based on various Reformatsky-type reactions in the presence of chiral auxiliaries.[18,19] On the other hand, the key step to (2*S*,3*S*)-**7** was the tandem conjugate addition of lithium (*S*)-*N*-benzyl-*N*-α-methylbenzylamide to *tert*-butyl cinnamate.[20] Based on these articles, the absolute configurations of the present resolution products are known (see Section 4.5) and are shown in Scheme 1. The main benefit of the lipase-catalyzed kinetic resolution pathways over the published synthetic routes to enantiopure compounds[18–20]

is that chiral induction now is catalytic rather than stoichiometric, meaning there is no need to prepare enantiopure auxiliaries, and mild reaction conditions are possible.

## 2. Results and discussion

### 2.1. Synthesis of racemic β-lactams

N-Protected *rac*-**1a** and *rac*-**2a** were prepared with reasonable chemical yields (81% and 49%, respectively) via a Reformatsky addition, where the Schiff base between *p*-methoxylbenzylimine and benzaldehyde reacted with ethyl bromodifluoroacetate and bromofluoroacetate (2 equiv), respectively, in the presence of Zn dust as previously described for *rac*-**1a** (Scheme 1, i).[18] The following oxidative deprotection furnished *rac*-**1** with 80% and *rac*-**2** as the *trans*-diastereomer with 57% yield (route ii). In addition to the *trans*-product, the synthesis of *rac*-**2a** produced two unidentified products on the basis of TLC. The *trans*-stereochemistry in *rac*-**2** is based upon the lack of NOESY correlation between the protons on carbon atoms 3 and 4. As further evidence for the *trans*-assignment, the specific rotation and the coupling constants of (2*S*,3*S*)-**7** (obtained through hydrolysis of enzymatic acylation product (2*S*,3*S*)-**5**, see Section 4.5) were closely identical with the published data.[20] *rac*-**3** was obtained by the 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate to styrene as previously described.[10]

**Table 1.** Lipase screening for the alcoholysis of *rac*-**1** (0.05 M) by lipase preparations (30 mg/mL) with methanol (0.25 M) in TBME at room temperature

| Entry | Lipase | Time (h) | Conversion (%) | ee$^{(R)\text{-}1}$ (%) | ee$^{(S)\text{-}4}$ (%) | *E* |
|---|---|---|---|---|---|---|
| 1 | Lipase PS[a] | 7 | 33 | 49 | >99 | >200 |
| 2 | Lipase PS-D | 7 | 43 | 76 | >99 | >200 |
| 3 | Lipase PS[b] | 7 | 1 | 1 | >99 | >200 |
| 4 | Lipase PS-C II | 7 | 46 | 86 | >99 | >200 |
| 5 | CAL-B | 7 | 33 | 48 | >99 | >200 |
| 6 | CAL-A[a] | 24 | 0 | — | — | — |

[a] Lipase preparation on Celite containing 20% (w/w) of the commercial enzyme powder.
[b] Free lipase PS powder.

### 2.2. Enantioselective ring opening of *rac*-1–3 by alcoholysis

For optimization, *rac*-**1** was used as a model substrate. Free lipase PS and various immobilized lipase PS, CAL-B and CAL-A preparations were screened for the alcoholysis of *rac*-**1** with methanol (5 equiv) in dry TBME (Table 1; Scheme 1, route iii). CAL-A did not catalyze the reaction (entry 6) while all lipase PS preparations and CAL-B gave highly enantioselective reactions, which were not contaminated by hydrolysis (entries 1–5) on the basis of stoichiometry [the sum of the amounts of (*R*)-**1** and (*S*)-**4** equals to the initial amount of *rac*-**1**]. CAL-B and lipase PS preparations, other than unimmobilized lipase PS powder (entry 3), gave good reactivities as measured with the conversion reached after 7 h. Due to the well-known tendency of CAL-B to hydrolyze β-lactams,[10–13] lipase PS adsorbed on Celite in the presence of sucrose (called lipase PS preparation from this point on)[14] was the choice for further optimizations. Later, when commercial lipase PS-D (lipase PS also on Celite) from Amano was available, we decided to replace our own preparation as the results were very similar. In practise, lipase PS-D was used for the studies with *rac*-**2** and *rac*-**3** and in all preparative-scale reactions.

The effect of methanol concentration (0.1–0.5 M) was studied for the alcoholysis of *rac*-**1** (0.05 M) in TBME in the presence of lipase PS preparation (Table 2; Scheme 1, route iii). Enantioselectivity (measured by the enantiomer ratio, *E*) was excellent in each case, and accordingly the reaction was at (entries 1, 2 and 4) or was approaching (entry 7) 50% conversion after 24 h, producing enantiopure resolution products (*R*)-**1** and (*S*)-**4**. Enzymatic reactivity clearly decreased with increasing methanol concentration, as can be seen from the conversions between 41% and 28% after the reaction of 7 h (entries 1, 2, 4 and 7). Elevated temperatures (entry 3 compared to 2) and higher enzyme contents (entry 5 compared to 4) enhanced reactivity. The ring opening was somewhat faster in DIPE (entry 6) than in TBME (entry 4). For further studies TBME was preferred because it does not form peroxides. 1-Butanol (entry 8), when used as a nucleophile, was practically as effective as methanol (entry 4). Although hydrolysis was not a side reaction in the alcoholysis of *rac*-**1** with methanol or 1-butanol, the lipase PS preparation gave surprisingly fast and highly enantioselective hydrolysis when no alcohol was added

**Table 2.** Alcoholysis of *rac*-**1** (0.05 M) catalyzed by lipase PS preparation (30 mg/mL) in TBME at room temperature

| Entry | Alcohol (M) | Time (h) | Conversion (%) | ee$^{(R)\text{-}1}$ (%) | ee$^{(S)\text{-}4}$ (%) | *E* |
|---|---|---|---|---|---|---|
| 1 | $CH_3OH$ (0.1) | 7/24 | 41/50 | 70/>99 | >99 | >200 |
| 2 | $CH_3OH$ (0.15) | 7/24 | 40/50 | 66/>99 | >99 | >200 |
| 3 | $CH_3OH$ (0.15)[a] | 5 | 49 | 95 | >99 | >200 |
| 4 | $CH_3OH$ (0.25) | 7/24 | 33/50 | 49/>99 | >99 | >200 |
| 5 | $CH_3OH$ (0.25)[b] | 5/24 | 33/50 | 50/>99 | >99 | >200 |
| 6 | $CH_3OH$ (0.25)[c] | 7/24 | 46/50 | 86/>99 | >99 | >200 |
| 7 | $CH_3OH$ (0.5) | 7/24 | 28/47 | 38/90 | >99 | >200 |
| 8 | $CH_3(CH_2)_3OH$ (0.25) | 7/24 | 36/50 | 57/>99 | >99[d] | >200 |
| 9 | No alcohol | 24 | 47 | 90 | >99[e] | >200 |

[a] Temperature 47 °C.
[b] Lipase PS preparation (50 mg/mL).
[c] DIPE in the place of TBME as a solvent.
[d] ee for the (*S*)-butyl ester.
[e] ee$^{(S)-6}$.

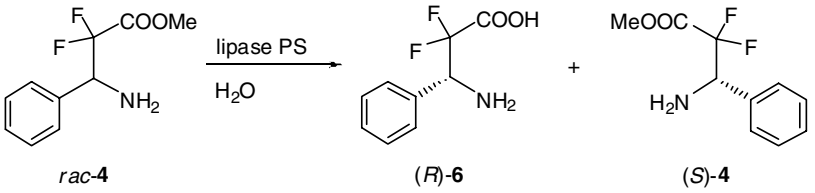
(entry 9; route vi). No reaction was observed in the absence of the enzyme.

The β-amino ester (*S*)-**4**, which was produced when *rac*-**1** reacts with methanol in the presence of the lipase PS preparation (Scheme 1, route iii), can be further enzymatically hydrolyzed. In order to study this, *rac*-**4** (prepared by opening the β-lactam ring of *rac*-**1** by sodium methoxide)[21] was incubated in TBME in the presence of the lipase PS preparation. As a result, the hydrolysis of (*S*)-**4** turned to be highly unlikely since *rac*-**4** was hydrolyzed with excellent (*R*)-enantiopreference (25% after 24 h, $E > 200$; Scheme 3). The observed opposite enantiopreference for the ring opening of the β-lactam and for the hydrolysis of the corresponding β-amino ester in the case of lipase PS-catalysis is in agreement with that which was observed earlier for 4-benzyl-2-azetidinone and CAL-B catalysis.[13] Interestingly, when *rac*-**4** was incubated in the presence of methanol (5 equiv) and the lipase PS preparation, its hydrolysis in TBME was totally suppressed.

In the next step, we chose 0.25 M methanol and subjected *rac*-**1**–**3** to alcoholysis in the presence of lipase PS-D in dry TBME at room temperature (Table 3). According to the results, the importance of fluorine activation for the opening of the lactam ring with methanol and/or water in the presence of lipase PS-D is evident. While the ring opening exclusively took place through alcoholysis in the case of *rac*-**1** (entry 1), 9% of the observed total conversion (46%) proceeded by hydrolysis in the case of *rac*-**2** under the same conditions (entry 3). In spite of the competitive hydrolysis, the formation of (2*S*,3*S*)-**5** was highly enantioselective ($ee^{ester}$ >99%). The proportion of the hydrolysis was reduced by using higher enzyme or methanol contents (entries 4–6). When competitive alcoholysis and hydrolysis were observed, enantioselectivity was not expressed using *E*-values. In the case of *rac*-**3**, there was no reaction at all in the presence or absence of methanol or added water (entries 8–11). When CAL-B replaced lipase PS-D, hydrolysis of *rac*-**3** with 1 equiv of water in TBME proceeded smoothly (entry 12). This is in accordance with the published data for the hydrolysis of *rac*-**3** in DIPE at 60 °C (entry 13).[10] The low enantioselectivity in our case at room temperature (entry 12) can be explained by the previous observation that elevated temperature is a common prerequisite for highly enantioselective hydrolysis of β-lactams in organic solvents.[10–12]

### 2.3. Enantioselective ring opening of *rac*-1 by an interesterification-type reaction

In a highly enantioselective alcoholysis of *rac*-**1** with methanol above, (*S*)-**1** as an acyl donor forms an acyl-enzyme intermediate with lipase PS which in the next mechanistic step reacts with methanol (ROH) giving the product (*S*)-**4** (Scheme 2). This gave us an idea to make use of our expe-




*rac*-**4** (*R*)-**6** (*S*)-**4**

**Scheme 3.** Hydrolysis of *rac*-**4** by the water in the enzyme preparation.

**Table 3.** Enantioselective ring opening of *rac*-(**1**–**3**) (0.05 M) with methanol in the presence of lipase PS-D (30 mg/mL) in TBME at room temperature

| Entry | Compound | Nucleophile (M) | Time (h) | Conversion/(a)[a] (%) | $ee^{1-3}$ (%) | $ee^{ester}$ (%) | *E* |
|---|---|---|---|---|---|---|---|
| 1 | *rac*-**1** | MeOH (0.25) | 24 | 50/(0) | >99 | >99 | >200 |
| 2 | *rac*-**1** | No alcohol | 24 | —/(47) | 90 | >99[b] | >200 |
| 3 | *rac*-**2** | MeOH (0.25) | 24 | 46/(9) | 59 | >99 | —[d] |
| 4 | *rac*-**2**[c] | MeOH (0.25) | 24 | 47/(3) | 83 | >99 | —[d] |
| 5 | *rac*-**2** | MeOH (0.5) | 24 | 24/(4) | 41 | >99 | —[d] |
| 6 | *rac*-**2**[c] | MeOH (0.5) | 24 | 40/(2) | 60 | >99 | —[d] |
| 7 | *rac*-**2** | No alcohol | 24 | —/(35) | 46 | — | 26 |
| 8 | *rac*-**3** | MeOH (0.25) | 24 | 0 | — | — | — |
| 9 | *rac*-**3** | No alcohol | 24 | 0 | — | — | — |
| 10 | *rac*-**3**[e] | No alcohol | 24 | 0 | — | — | — |
| 11 | *rac*-**3** | Water (1 equiv) | 48 | 0 | — | — | — |
| 12 | *rac*-**3**[f] | Water (1 equiv) | 24 | —/(46) | 66 | — | 15 |
| 13 | *rac*-**3**[g] | Water (1 equiv) | 24 | —/(50) | 99 | 99 | >200 |

[a] The proportion of hydrolysis (a).
[b] $ee_{(S)-6}$.
[c] Lipase PS preparation (50 mg/mL).
[d] *E* not given due to competitive alcoholysis and hydrolysis reactions.
[e] Temperature 47 °C.
[f] CAL-B in the place of lipase PS-D.
[g] Ref. 10; CAL-B in DIPE at 60 °C, $ee^{acid}$ is given.

rience from previous interesterification work with lipase catalysis[5,22] where the alcohol ROH was generated in situ from an achiral ester $PrCO_2R$ through route b and reacted further with a chiral ester. When *rac*-**1** was dissolved in TBME/$PrCO_2R$ (1:1; R = Me or Bu) and lipase PS-D was added, the formation of (*S*)-**4** (R = Me) or the corresponding butyl ester (R = Bu) took place with reasonable reactivity, especially in the case of butyl butanoate as the source of an alcohol (Scheme 2, Table 4). Surprisingly, the alcohol (methanol and 1-butanol) obtained from the butanoate ester in situ was able to suppress the competing hydrolysis of *rac*-**1** completely. This result supports our earlier explanation[5,22] that the alcohol ROH does not necessarily leave the active site as is expected according to the classic ping-pong bi-bi mechanism.[23] Instead the alcohol stays bound at the active site, replacing the active site water or is inserted into a possible water network in the vicinity of the active site. This process enables the equivalent amounts of ROH to react with the acyl-enzyme intermediate. This means that the water in the enzyme preparation first leads to the hydrolysis of the butanoate ester (route b), leaving an alcohol product in the active site. When (*S*)-**1** in *rac*-**1**forms an acyl-enzyme intermediate (route a) at the active site bearing the bound alcohol, the formation of (*S*)-**4** or its butyl ester analogue becomes possible through route c.

**Table 4.** Enantioselective ring opening of *rac*-**1** (0.05 M) in the mixture of TBME/$PrCO_2R$ (1:1) in the presence of lipase PS-D (20 mg/mL) at room temperature

| Entry | R | Time (h) | Conversion (%) | $ee^{(R)-1}$ (%) | $ee^{(S)-4}$ (%) | *E* |
|---|---|---|---|---|---|---|
| 1 | Me | 24 | 29 | 40 | >99 | >200 |
| 2 | Bu | 24 | 40 | 68 | >99[a] | >200 |

[a] ee for the (*S*)-butyl ester.

The results in Table 4 (reaction in TBME/$PrCO_2R$) are unoptimized and cannot be compared to those in Table 2 (reaction in TBME) except to the extent that reactions in both systems proceed with the same high enantioselectivity. As the most important conclusion, it is clear that an alcohol added can be replaced by the respective alcohol produced in situ at the active site of the lipase.

### 2.4. Preparative-scale kinetic resolutions

The preparative-scale kinetic resolution of *rac*-**1** and *rac*-**2** with methanol and lipase PS-D was performed as described in Section 4. Unreacted lactams (*R*)-**1**, (3*R*,4*R*)-**2** and amino esters (*S*)-**4** and (2*S*,3*S*)-**5** were obtained in enantiopure forms at 50% conversion (Scheme 1). The specific rotation $[\alpha]_D^{22} = -76.6$ (*c* 1.0, $CHCl_3$) for the prepared (*R*)-**1**disagreed with the literature value[18] $[\alpha]_D^{25} = +38.3$ (*c* 1.01, $CHCl_3$) for (*S*)-**1**. In order to show that our compound was correct, (*R*)-**1** was transformed into the hydrochloride of (*R*)-**6**, giving $[\alpha]_D^{20} = -3.4$ (*c* 0.84, MeOH) in agreement with the literature value[18] $[\alpha]_D^{20} = +3.3$ (*c* 0.84, MeOH) for the corresponding (*S*)-enantiomer. As *rac*-**3** and lipase PS-D were unable to acylate methanol, the gram-scale kinetic resolution was impossible.

## 3. Conclusions

Herein, we have reported a Reformatsky type reaction leading to fluorinated 4-phenyl-2-azetidinones, *rac*-**1** and *rac*-**2** (Scheme 1). The racemates and the corresponding nonfluorinated analogue *rac*-**3** were subjected to enantioselective alcoholysis in dry TBME in the presence of lipase PS-D (or lipase PS adsorbed on Celite in the presence of sucrose) from *B. cepacia*. The results indicate the importance of fluorine activation for the lipase PS-catalyzed ring opening in general and for the opening with alcohols in particular. As a result, fluorine activation was necessary for the acylation of methanol and 1-butanol with lipase PS-D, with *rac*-**3** being totally unreactive. Interestingly, hydrolysis by water in the seemingly dry enzyme preparation was totally suppressed in the case of *rac*-**1** and could be minimized in the case of *rac*-**2** allowing the kinetic resolution to produce the respective enantiopure resolution products (*R*)-**1** and (*S*)-**4** and (3*R*,4*R*)-**2** and (2*S*,3*S*)-**5** at 50% conversion. On the other hand, the relatively fast ring opening of *rac*-**1** and *rac*-**2** through hydrolysis was evident in the absence of an added alcohol while there was no hydrolysis with *rac*-**3** as the substrate.

The results with *rac*-**1** and achiral alkyl butanoates also showed highly enantioselective ring opening of the β-lactam, indicating that an added alcohol can be replaced by an alcohol produced in situ in the active site of the lipase.

## 4. Experimental

### 4.1. Materials and methods

Zinc, ethyl bromodifluoroacetate, ethyl bromofluoroacetate, cerium(IV) ammonium nitrate (CAN) and solvents were products of Aldrich or Fluka. *rac*-**1**[18] and *rac*-**3**[10] were prepared as previously described. All solvents were of the highest analytical grade and were dried by standard methods when necessary. Lipase PS from *B. cepacia* (previously *Pseudomonas cepacia*) as a free enzyme powder and when immobilized on Celite (lipase PS-D) and on ceramic particles (lipase PS-C II) was purchased from Amano Europe, England. *C. antarctica* lipase A (CAL-A) was the product of Roche. Before use, lipase PS and CAL-A powders were adsorbed on Celite by dissolving the enzyme (5 g) and sucrose (3 g) in Tris–HCl buffer (250 mL, 20 mM, pH 7.9) followed by the addition of Celite (17 g) as previously described.[14] The lipase preparation containing 20% (w/w) of the original enzyme powder was thus obtained. *C. antarctica* lipase B (CAL-B, Novozym 435) was a generous gift from Novo Nordisk. Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063–0.200 μm). TLC was carried out with Merck Kieselgel $60F_{254}$ sheets. Melting points were measured on a Sanyo instrument at a heating rate of 2 °C/min. Optical rotations were determined with a Perkin–Elmer polarimeter, and $[\alpha]_D$ values are given in units of $10^{-1}$ deg $cm^2$ $g^{-1}$. The determination of *E* was based on the equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$ with the use of linear regression, *E* being the slope of the line $\ln[(1-c)(1-ee_S)]$ versus $\ln[(1-c)(1+ee_S)]$.[24]

The $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker 500 spectrometer with tetramethylsilane (TMS) as an internal standard. $^{1}H$–$^{1}H$ COSY, $^{1}H$–$^{13}C$ HQSC and $^{1}H$–$^{13}C$ HMBC spectra were used for the assignment of the chemical shifts when necessary. 2,2,2-Trifluoroacetic acid was used as the external standard for measuring $^{19}F$ NMRs. Mass spectra were taken on a VG 7070E mass spectrometer.

In a typical small-scale experiment, one of the lipase preparations was added to one of the substrates *rac*-**1**–**3** (0.05 M) and a nucleophile (an alcohol, 0.05–0.5 M) or alkyl butanoate in TBME (1 mL). Unless stated otherwise, the enzymatic reactions were performed at room temperature (23 °C). The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by HPLC on a CHIRACEL-OD column (0.46 × 25 cm) and GC on a Chrompack CP-Chirasil-DEX CB column or a Chrompack CP-Chirasil-L-Valine column. 4-Fluorophenyl acetonitrile was used as an internal standard for quantitative analyses of the resolution mixture.

### 4.2. Preparation of *trans*-3-fluoro-4-phenylazetidin-2-one *rac*-2

*rac*-**2a** was prepared following the published method for *rac*-**1a**.[18] A solution of the imine (1.52 g, 6.77 mmol) and ethyl bromofluoroacetate (2.50 g, 13.54 mmol) in THF (2 mL) was added to a refluxing suspension of Zn dust (834 mg, 12.83 mmol) in THF (3 mL). After 2.5 h, the reaction mixture was cooled down and quenched by adding saturated $NH_4Cl$ (10 mL). The aqueous layer was extracted with $CH_2Cl_2$, and the organic layer dried over anhydrous $Na_2SO_4$ and filtered. After concentration, the residue was purified on a silica gel column eluting with petroleum ether/ethyl acetate (8:1) to afford *rac*-**2a** as an oil (944 mg, 3.31 mmol, 49%). $^{1}H$ NMR (500 MHz, $CDCl_3$) $\delta$ 3.74–3.77 (d, $J = 14.9$ Hz, 1H, C$H_2$), 3.82 (s, 3H, OC$H_3$), 4.46–4.48 (d, $J = 9.9$ Hz, 1H, C$H$), 4.82–4.85 (d, $J = 14.8$ Hz, 1H, C$H_2$), 5.19–5.30 (d, $J = 54.1$ Hz, 1H, C$H$F), 6.81–6.84 (m, 2arom. H), 7.05–7.07 (m, 2arom. H), 7.21–7.23 (m, 2.7 Hz, 2arom. H), 7.42–7.44 (m, 3arom. H); $^{13}C$ NMR (126 MHz, $CDCl_3$) $\delta$ 43.90 (*C*$H_2$), 55.30 (O*C*$H_3$), 62.22–62.41 (d, $J = 25.2$ Hz, *C*H), 96.91–98.70 (d, $J = 226.4$ Hz, *C*HF), 114.27, 126.30, 126.80, 129.24, 129.28, 130.01, 134.45, 159.42, 163.45–163.63 (d, $J = 22.9$ Hz, *C*O). $^{19}F$ NMR (471 MHz, $CDCl_3$) $\delta$ −203.08–(−203.22) (dd, $J = 56.5$ Hz, 14.1 Hz.). HRMS: $M^+$ found ($M^+$ calculated for $C_{17}H_{16}FNO_2$) 285.11600 (285.11651); MS: *m*/*z* (relative intensity) 285 (18), 226 (2), 163 (69), 131 (3), 121 (100), 107 (6), 91 (5), 77 (10).

CAN (2.55 g, 4.65 mmol) was added in small portions to a solution of *rac*-**2a** (441 mg, 1.55 mmol) in $CH_3CN/H_2O$ (9:1, v/v, 80 mL). After 6 h at room temperature, the mixture was poured into water (100 mL). The aqueous layer was extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$ (50 mL) and 10% $Na_2SO_3$ (50 mL) and dried over anhydrous $Na_2SO_4$. The mixture was filtered and concentrated under vacuum. The residue was purified on silica gel column with petroleum ether/ethyl acetate (6:1) being the eluent, affording *rac*-**2** as a solid product (145 mg, 0.88 mmol, 57%). Mp 107–108 °C. $^{1}H$ NMR (500 MHz, $CDCl_3$) $\delta$ 4.82–4.84 (dd, $J = 11.0$ Hz, 1.5 Hz, 1H, C$H$), 5.15–5.26 (dt, $J = 53.5$ Hz, 2.0 Hz, 1H, C$H$F), 6.61 (s, 1H, N$H$), 7.35–7.45 (m, 5arom. H); $^{13}C$ NMR (126 MHz, $CDCl_3$) $\delta$ 59.70–59.89 (d, $J = 23.9$ Hz, *C*H), 98.10–99.90 (d, $J = 226.8$ Hz, *C*HF), 125.85, 129.14, 129.16, 136.41 (arom. C), 163.81 (*C*O); HRMS: $M^+$ found ($M^+$ calculated for $C_9H_8FNO$) 165.05970 (165.05899); MS: *m*/*z* (relative intensity) 165 (2), 136 (4), 122 (100), 109 (3), 104 (10), 101 (5), 96 (11), 77 (7).

### 4.3. Kinetic resolution of *rac*-1 with methanol

*rac*-**1** (200 mg, 1.09 mmol) was dissolved in TBME (22 mL) after which methanol (174 mg, 5.45 mmol) and lipase PS-D (660 mg) were added. The reaction was stopped after 17.5 h by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residue purified on a silica gel column to afford (*R*)-**1** as a solid {98 mg, yield 98%, mp 62–63 °C, ee$^{(R)\text{-}\mathbf{1}}$ >99%, $[\alpha]_D^{22} = -76.6$ (*c* 1.0, $CHCl_3$); known data[18] for (*S*)-**1** $[\alpha]_D^{25} = +38.3$ (*c* 1.01, $CHCl_3$)}. (*S*)-**4** was obtained as an oily product {92 mg, yield 78%, ee$^{(S)\text{-}\mathbf{4}}$ >99%, $[\alpha]_D^{22} = -5.8$ (*c* 1.0, $CHCl_3$)} with $^{1}H$ NMR (500 MHz, $CDCl_3$) $\delta$ 3.80 (s, 3H, OC$H_3$), 4.44–4.49 (dd, $J = 15.5$ Hz, 10.5 Hz, 1H, C$H$), 7.34–7.37 (m, 5arom. H); $^{13}C$ NMR (126 MHz, $CDCl_3$) $\delta$ 53.25 (*C*$H_3$), 58.25–58.64 (t, $J = 23.9$ Hz, *C*H), 113.33–117.39 (t, $J = 255.8$ Hz, *C*$F_2$), 127.91, 128.61, 128.94, 136.22 (arom. C), 164.07–164.59 (t, $J = 32.8$ Hz, *C*O); $^{19}F$ NMR (471 MHz, $CDCl_3$) $\delta$ −99.25–(−98.67) (dd, $J = 253.8$ Hz, 14.1 Hz), −95.35–(−94.78) (dd, $J = 253.8$ Hz, 14.1 Hz); HRMS: $M^+$ found ($M^+$ calculated for $C_{10}H_{11}F_2NO_2$) 215.07470 (215.07579); MS: *m*/*z* (relative intensity) 215 (0.01), 200 (0.03), 195 (0.23), 180 (0.14), 164 (0.15), 140 (4), 106 (100), 79 (15).

### 4.4. Kinetic resolution of *rac*-2 with methanol

*rac*-**2** (200 mg, 1.21 mmol) was dissolved in TBME (24 mL) after which methanol (194 mg, 6.05 mmol) and lipase PS-D (1.20 g) were added. The reaction was stopped after 48 h by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residue purified on a silica gel column to afford (3*R*,4*R*)-**2** as a solid (96 mg, 0.58 mmol, yield 96%): mp 107–108 °C, ee$^{(3R,4R)\text{-}\mathbf{2}}$ >99%, $[\alpha]_D^{22} = -19.1$ (*c* 1.0, $CHCl_3$)}. Compound (2*S*,3*S*)-**5** was obtained as an oily product {108 mg, 0.55 mmol, yield 91%, ee$^{(2S,3S)\text{-}\mathbf{5}}$ >99%, $[\alpha]_D^{22} = +10.1$ (*c* 1.0, $CHCl_3$)} with $^{1}H$ NMR (500 MHz, $CDCl_3$) $\delta$ 3.68 (s, 3H, OC$H_3$), 4.39–4.44 (dd, $J = 20.6$ Hz, 4.7 Hz, 1H, C$H$), 5.03–5.13 (dd, $J = 48.6$ Hz, 4.8 Hz, 1H, C$H$F), 7.29–7.37 (m, 5arom. H); $^{13}C$ NMR (126 MHz, $CDCl_3$) $\delta$ 52.21 (*C*$H_3$), 57.22, 57.39 (d, $J = 20.9$ Hz, *C*H), 91.58, 93.09 (d, $J = 189.9$ Hz, *C*HF), 127.11, 128.15, 128.58, 139.50 (arom. C), 168.26–168.45 (d, $J = 23.2$ Hz, *C*O); MS: *m*/*z* (relative intensity) 191 (10), 123 (6), 106 (100), 91(6), 79 (15).

### 4.5. Determination of absolute configurations

The absolute configurations were determined by correlating the specific rotation data of (2*S*,3*S*)-**7** and the HCl salt of (*R*)-**6** to the reported data.[18,20] Accordingly, a solution of (*R*)-**1** (40 mg, 0.22 mmol) in aqueous HCl (6 M, 4 mL)

was refluxed for 2 h before the solvent was evaporated, affording the HCl salt of (*R*)-**6** (mp 160–161 °C) in a quantitative yield with $[\alpha]_D^{20} = -3.4$ (*c* 0.84, MeOH) {the literature value[18] for the HCl salt of (*S*)-**6** $[\alpha]_D^{20} = +3.3$ (*c* 0.84, MeOH)}. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 5.06–5.11 (dd, $J = 6.0$ Hz, 19.0 Hz, 1H, C*H*), 7.52–7.56 (m, 5arom. H); $^{13}$C NMR (126 MHz, $CD_3OD$) $\delta$ 56.72–57.10 (t, $J = 23.9$ Hz, *C*H), 112.15–116.27 (t, $J = 257.8$ Hz, $CF_2$), 128.24, 128.51, 129.31, 130.49 (arom. C), 166.6 (t, $J = 28.9$ Hz, *C*O).

A solution of (2*S*,3*S*)-**5** (30 mg, 0.15 mmol, ee >99%) in aqueous HCl (6 M, 2 mL) was refluxed for 4 h followed by evaporation of the solvent. The resulting solid was treated with $^i$PrOH (2 mL) and propylene oxide (35 mg, 0.60 mmol) for 3 h. The precipitate was collected and washed with $^i$PrOH (4 mL) to afford amino acid (2*S*,3*S*)-**7** as a solid (mp 178–180 °C) in a quantitative yield with $[\alpha]_D^{22} = -42.8$ (*c* 0.4, $CH_3OH$) {the literature value[20] for (2*S*,3*S*)-**7** $[\alpha]_D^{22} = -40$ (*c* 0.4, $CH_3OH$)}. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 4.68–4.73 (dd, $J = 24.2$ Hz, 3.8 Hz, 1H, C*H*), 4.98–5.09 (dd, $J = 50.9$, 3.9 Hz, 1H, C*H*F), 7.32–7.43 (m, 5 arom. H); $^{13}$C NMR (126 MHz, $CD_3OD$) $\delta$ 57.51, 57.68 (d, $J = 21.4$ Hz, *C*H), 89.99, 91.52 (d, $J = 192.3$ Hz, *C*HF), 129.73, 129.93, 130.52, 133.91 (arom. C), 172.02 (*C*O).

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## References

1. Thayer, A. M. *Chem. Eng. News* **2006**, *84*, 15–24.
2. Uoto, K.; Ohsuki, S.; Takenoshita, H.; Ishiyama, T.; Iimura, S.; Hirota, Y.; Mitsui, I.; Hirofumi, T.; Soga, T. *Chem. Pharm. Bull.* **1997**, *45*, 1793–1804.
3. (a) *Enantioselective Synthesis of β-Amino Acids*, 2nd ed.; Juaristi, E., Soloshonok, V., Eds.; Wiley-VHC: New York, 2005; (b) Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. In *Enantioselective Synthesis of β-Amino Acids*, 2nd ed.; Wiley-VHC: New York, 2005; pp 477–495.
4. Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831–5854.
5. Li, X.-G.; Kanerva, L. T. *Org. Lett.* **2006**, *8*, 5593–5596.
6. Soloshonok, V. A.; Kirilenko, A. G.; Fokina, N. A.; Shishkina, I. P.; Galushko, S. V.; Kukhar, V. P.; Svedas, V. K.; Kozlova, E. V. *Tetrahedron: Asymmetry* **1994**, *5*, 1119–1126.
7. Soloshonok, V. A.; Kirilenko, A. G.; Fokina, N. A.; Kukhar, V. P.; Galushko, S. V.; Svedas, V. K.; Resnati, G. *Tetrahedron: Asymmetry* **1994**, *5*, 1225–1228.
8. Soloshonok, V. A.; Fokina, A. F.; Rybakova, A. V.; Shishkina, I. P.; Galushko, S. V.; Sorochinsky, A. E.; Kukhar, V. P.; Savchenko, M. V.; Svedas, V. K. *Tetrahedron: Asymmetry* **1995**, *6*, 1601–1610.
9. Faulconbridge, S. J.; Holt, K. E.; Sevellano, L. G.; Lock, C. J.; Tiffin, P. D.; Tremayne, N.; Winter, S. *Tetrahedron Lett.* **2000**, *41*, 2679–2681.
10. Forró, E.; Paál, T.; Tasnádi, G.; Fülöp, F. *Adv. Synth. Catal.* **2006**, *348*, 917–923.
11. Forró, E.; Fülöp, F. *Mini-Rev. Org. Chem.* **2004**, *1*, 93–102.
12. Park, S.; Forró, E.; Grewal, H.; Fülöp, F.; Kazlauskas, R. J. *Adv. Synth. Catal.* **2003**, *345*, 986–995.
13. Li, X.-G.; Kanerva, L. T. *Adv. Synth. Catal.* **2006**, *348*, 197–205.
14. Sundholm, O.; Kanerva, L. T. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407–2410.
15. Veum, L.; Kanerva, L. T.; Halling, P. J.; Maschmeyer, T.; Hanefeld, U. *Adv. Synth. Catal.* **2005**, *347*, 1015–1021.
16. Brieva, R.; Crich, J. Z.; Sih, C. J. *J. Org. Chem.* **1993**, *58*, 1068–1075.
17. Qiu, X.-L.; Meng, W.-D.; Qing, F.-L. *Tetrahedron* **2004**, *60*, 6711–6745.
18. Marcotte, S.; Pannecoucke, X.; Feason, C.; Quirion, J.-C. *J. Org. Chem.* **1999**, *64*, 8461–8464.
19. Sorochinsky, A.; Voloshin, N.; Markovsky, A.; Belik, M.; Yasuda, N.; Uekusa, H.; Ono, T.; Berbasov, D. O.; Soloshonok, V. A. *J. Org. Chem.* **2003**, *68*, 7448–7454.
20. Andrews, P. C.; Bhaskar, V.; Bromfield, K. M.; Dodd, A. M.; Duggan, P. J.; Duggan, S. A. M.; McCarthy, T. D. *Synlett* **2004**, 791–794.
21. Gyónfalvi, S.; Szakonyi, Z.; Fülöp, F. *Tetrahedron: Asymmetry* **2003**, *14*, 3965–3972.
22. Liljeblad, A.; Kavenius, H.-M.; Tähtinen, P.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2007**, *18*, 181–191.
23. Straathof, A. J. J.; Rakels, J. L. L.; Heijnen, J. J. *Biocatalysis* **1992**, *7*, 13–27.
24. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.