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Chemoenzymatic enantioselective route to get (+) and (-) 4-acetoxy-azetidin-2-one by Lipase-catalysed kinetic resolution and their applications.

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Highlights

- Kinetic resolution of azetidinones by Lipases
- *Pseudomonas fluorescens* lipase promoted the resolution by transesterification
- Synthesis of enantiopure 4-acetoxy-azetidinones.
- Synthesis of enantiopure *N*-methylthio-4-acetoxy-azetidinones

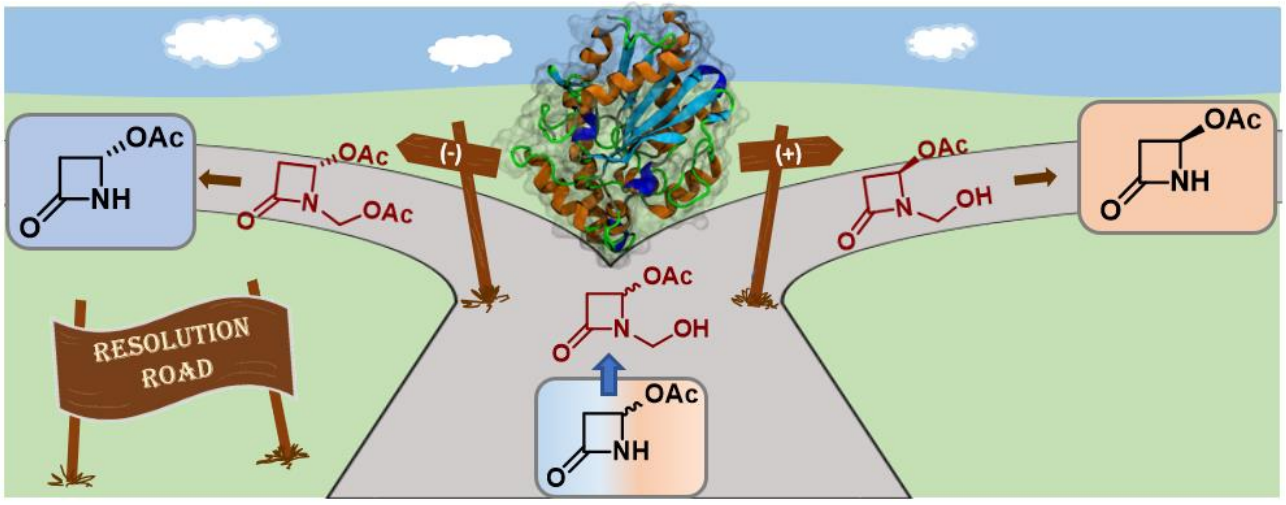
Abstract

4-Acetoxy-azetidin-2-one is an extremely useful intermediate widely applied for the synthesis of several biologically active β-lactam compounds. However, it is available as a racemic mixture that could limit its application in the synthesis of enantiopure products. Herein we evaluated the use of lipases in a kinetic resolution (KR) process to finally obtain 4-acetoxy-zetidin-2-one as separated pure enantiomers. From a preliminary screening on a set of commercial enzymes, *Pseudomonas fluorescens* emerged as the most suitable lipase that allowed to obtain good conversions and excellent enantiomeric excesses. On the enantiomerically pure 4-acetoxy-azetidin-2-ones some nucleophilic substitutions and *N*-thio-alkylation reactions were tested in order to evaluate the stereochemical integrity at the C-4 position.

Keywords:

Biocatalysis, Kinetic resolution, Lactams, Azetidinones, Lipases, 4-acetoxy-azetidinone, Single-enantiomer drugs.

Graphical Abstract



Introduction

β-Lactam compounds have been generating an increasing interest in medicinal chemistry thanks to their varied biological activities and versatility as intermediates for the synthesis of other biologically active compounds [1-3]. β-Lactams are widely known and exploited as antibiotics and inhibitors of β-lactamases [4]. As a structure-based classification, they fall into two main groups: bicyclic compounds such as penicillins, cephalosporins, carbapenems or clavams, and monocyclic compounds, such as Aztreonam, the only clinically available monobactam antibiotic [5].

Monocyclic β-lactams are considered unique structures because of different substituents on the ring that could address different biological activities [6, 7]. Accordingly, over the last 20 years great interest has been focused on the synthesis of properly substituted monocyclic derivatives that showed various pharmacological properties as anticancer, antidiabetic, anti-tubercular, anti-inflammatory, antiparkinsonian and anti-HIV activities [8-10]. Moreover, azetidinones were found promising as cholesterol absorption inhibitors, as thrombin, chymase Cathepsin K inhibitors, as human tryptase vasopressin V1a antagonists [9, 10], and as ligands of integrin receptors able to modulate cell adhesion and signalling [11, 12]. As a contribution in the development of new antimicrobial agents against resistant bacteria, we synthesized 4-alkylidene-azetidinones, a new group of monocyclic β-lactams that were functionalized with phytochemical polyphenolics and explored as dual-target antibacterial- and antioxidant compounds [13-15]. Moreover, the introduction of the so-called β-lactam synthon method by Ojima [16], has further contributed to a greatly increased interest in the synthesis of monocyclic β-lactams as useful intermediates in organic synthesis [17, 18].

As useful β-lactam intermediates, (3*R*,4*R*)-4-acetoxy-3-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)-ethyl]-azetidin-2-one (**A**) and 4-acetoxy azetidin-2-one (**rac-1**) are commercially available and have been widely used as starting materials for the synthesis of important bioactive compounds [19] (Figure 1). Intermediate **A** is available as enantiopure compound, **rac-1** instead is purchased only as a racemic mixture. Although the great usefulness of **rac-1** as starting material in the synthesis of new bioactive β-lactam compounds [20-27], this reactant could only provide racemic derivatives (selected examples in Figure 1). Since it has been well attested that two enantiomeric drugs could give different pharmacologic responses and diverse pharmacokinetic, pharmacodynamic and therapeutic profiles, or could even reveal adverse effects [28, 29], the possibility to gain access to the enantiopure form of 4-acetoxy-azetidinone **rac-1** would be of utmost importance in the synthesis of stereo-chemically defined bioactive β-lactams [30]. To the best of our knowledge, only (+)4-acetoxy-azetidinone has been reported and obtained by chiral recognition process upon separation of chiral host-guest inclusion complexes [31].

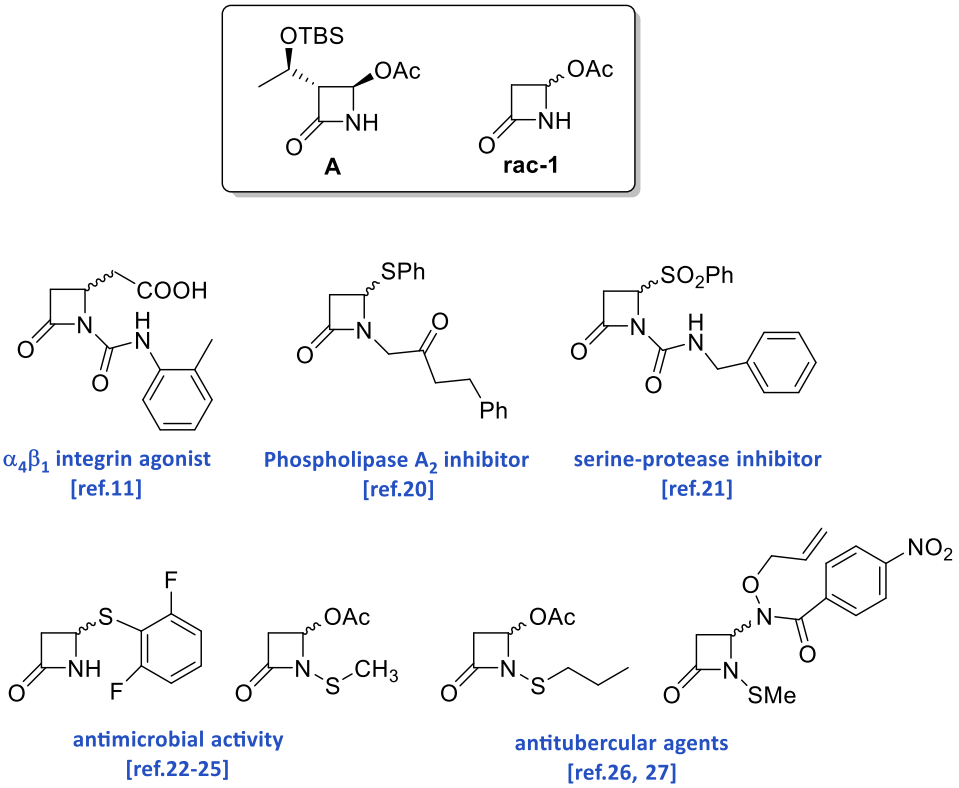


Figure 1. 4-acetoxy-azetidinones **A**, **rac-1,** and some selected racemic bioactive β-lactam compounds obtained from **rac-1**.

Enzymatic kinetic resolution (KR) is a leading approach among the biocatalytic methods for the conversion of racemates into single enantiomers, and lipases proved to be a powerful tool for the obtainment of enantiopure fine chemicals and pharmaceutical compounds [32, 33].

The aim of the present work is to establish a chemoenzymatic route starting from the commercially available **rac-1** to obtain the two enantiomers of 4-acetoxyazetidin-2-one from the racemic **rac-2** by means of a kinetic resolution by lipases, and to preliminary test the single enantiomers in some typical reactions as C4 substitutions and *N*-thioalkylation in order to evaluate the stereochemical integrity of the corresponding products (Figure 2).

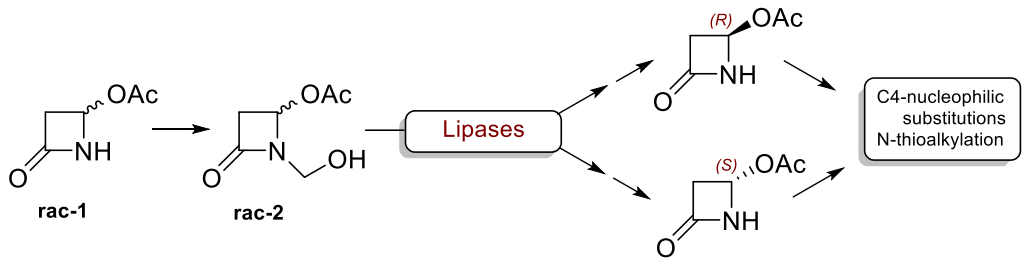


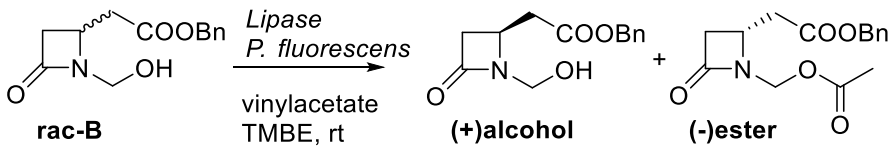
Figure 2. Enzymatic kinetic resolution on functionalized 4-acetoxy-azetidinone and evaluation of the stereochemical outcome in C4 and N-functionalization.

We thus chose to take advantage of the *N*-hydroxymethyl functionalization of the beta-lactam which has been already explored in the asymmetric acylation of the primary alcohol. Nagai *et al.* first reported the application of this functionalization in the kinetic resolution of some *N*-hydroxymethyl-4-aryl-azetidinones by *Burkholderia cepacia* and *Candida antarctica* B lipases in transesterification with vinylacetate or vinylbutyrate obtaining good results in term of yields of single enantiomers and enantiomeric excesses [34, 35]; the same process was then further exploited by Fülöp, Kanerva and coworkers. [36-40]. This strategy was also applied on other *N*-hydroxymethyl-azetidinones bridged in bicyclic systems on the C3 and C4 of the β-lactam ring by means of Lipase, among which also Lipase AK (*Pseudomonas sp.*), with trifluoroethyl butyrate or vinyl butyrate in various solvents with good enantioselectivities [41-44].

From these studies, it appeared a certain narrowness in the C4 substituents of the *N*-hydroxymethyl azetidinones that are mainly 4-aryl or bicyclic groups. However, the challenge of our project is to face the resolution of **rac-1** which presents the 4-acetoxy moiety, well known for its reactivity as excellent leaving group and widely used in substitution reactions on the C-4 position of azetidinones [19] as above mentioned. Exploiting also our previous experience in the kinetic resolution by lipases of racemic *N*-hydroxymethyl azetidinones with a carboxymethyl group on the C4 [45], we were able to find the best conditions and to successfully obtain the two separated enantiomers of 4-acetoxy-azetidinone.

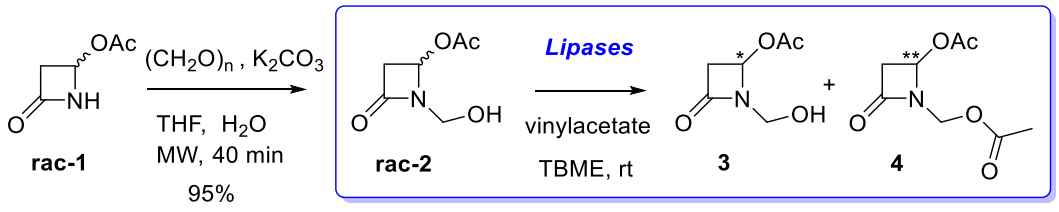
Results and discussion

To approach the resolution of **rac-1**, the possibility to directly perform a lipase-mediated hydrolytic kinetic resolution on the 4-acetoxy ester group was excluded because of an alleged low stability of the corresponding 4-hydroxy-azetidinone which could undergo a ring-opening reaction *via* breakdown of the N-C4 bond [46]. In a previous work, we took advantage of an N-hydroxymethyl-functionalization to successfully develop a KR on a racemic β-lactam intermediate for the synthesis of enantiopure integrin ligands (Scheme 1) [45].



Scheme 1. Kinetic enzymatic resolution on the β-lactam intermediate **rac-B** previously reported.

Thus, we applied the same strategy on the racemic 4-acetoxy-azetidinone **rac-1**, which was accordingly treated with paraformaldehyde and catalytic potassium carbonate under microwave irradiation to obtain the *N*-methylene-hydroxy derivative **rac-2** in excellent yields (Scheme 2).



Scheme 2. Synthesis of *N*-methylene-hydroxy-azetidinone **rac-2** and its enzymatic KR by transesterification reaction.

In order to perform a KR on **rac-2**, some lipases were tested in the transesterification reaction with vinyl acetate in *t*-butylmethylether (TBME) at room temperature. After filtration of the enzymes, the crude reaction mixtures were monitored by chiral HPLC analysis for evaluating conversions and enantiomeric excesses (Table 1).

Table 1. Screening of lipases in KR on **rac-2**.[a]

Entry	***Lipases*** **(activity)[b]**	Solvent	Enzyme (U)	Time (h)	Conv. (%)[c]	3 ee%[c]	4 ee%[c]
1	*Burkholderia cepacia* (30 U/mg)	TBME	300	20	3.5	6	>99
2	*Burkholderia cepacia* (30 U/mg)	TBME	300	72	13	24	>99
3	CAL B (10 U/mg)	TBME	75	16	>99	-	0
4	CAL B (10 U/mg)	TBME	75	6	84	>99	22
5	CAL B (10 U/mg)	TBME	75	2	65	>99	65
6	CAL B (10 U/mg)	TBME	75	1	54	78	60
7[d]	*Pseudomonas fluorescens* (20 U/mg)	dry THF	223	72	39	>99	70
8	*Pseudomonas fluorescens* (20 U/mg)	THF	223	72	44	>99	82
9	*Pseudomonas fluorescens* (20 U/mg)	CH_3CN	223	72	44	>99	81
10	*Pseudomonas fluorescens* (20 U/mg)	Toluene	223	72	46	>99	79

11	*Pseudomonas fluorescens* (20 U/mg)	TBME	223	72	51	>99	91
12[e]	*Pseudomonas fluorescens* (20 U/mg)	TBME	223	24	53	84	>99
13	*Pseudomonas fluorescens* (36 U/mg)	TBME	48	48	55	89	96
14	*Pseudomonas fluorescens* (309 U/mg)	TBME	150	23	55	>99	90
15	*Pseudomonas fluorescens* (309 U/mg)	TBME	117	8	52	>99	90

[a] Reaction conditions: **rac-2** (0.063 mmol), vinyl acetate (0.38 mmol), TBME (1.5 mL), enzyme (U in table), rt.

[b] Activity of the commercial enzymes.

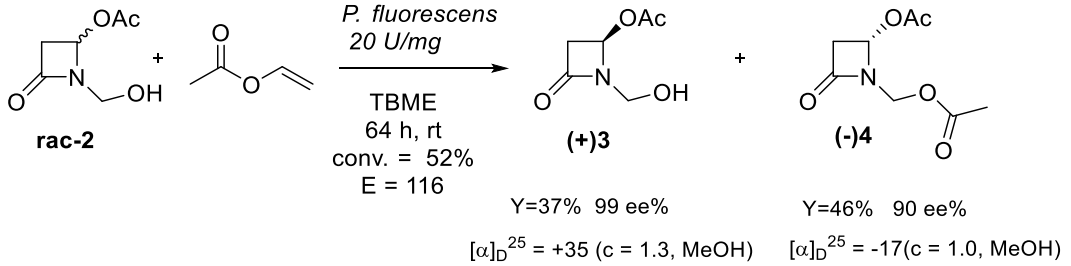
[c] Determined by chiral HPLC analysis on the crude.

[d] Reaction conducted under N_2 atmosphere.

[e] T=40°C

Lipase from *Burkholderia cepacia* or *Candida antarctica B* (*CAL B*) which achieved very good results with other *N*-hydroxymethyl-azetidinones [38-39], behaved differently on **rac-2**: *Burkholderia cepacia* gave low conversions with poor ee % of the unreacted alcohol **3**, even after 72 h (Table 1, entries 1 and 2); on the contrary, CAL B showed high conversions, but null or poor enantioselectivities in the ester **4** even at lower conversions (Table 1, entries 3-6). The best results were obtained with *Pseudomonas fluorescens* lipase and some conditions were then studied (Table 1, entries 7-15). Three *Pseudomonas fluorescens* lipase preparations with different activities (20, 36, and 309 U/mg) were tested, and on changing activity, units and reaction time, satisfactory conversions (45-55%) and good to excellent ee% for both **3** and **4** were obtained (Table 1, entries 11-13-15). On changing the reaction solvent, THF, acetonitrile and toluene gave good results for the obtainment of **3** with high enantiomeric excess, but low ee% were detected for **4** (Table 1 entries 7-10). TBME was confirmed as the best solvent to obtain good ee% for both **3** and **4** (Table 1, entry 11). When we tested the resolution in anhydrous THF under N_2 atmosphere to check an eventual influence of moisture or water, the reaction was slower and with lower ee% for compound **4** (Table 1, entry 7 *vs* 8). Also in a tentative exploration at 40 °C the process was faster but with lower ee% of the unreacted enantiomer **3** (Table 1, entry 12), thus confirming the room temperature as the best condition.

The conditions of Table 1 entry 11 were then applied in a preparative KR in order to isolate discrete amounts of **3** and **4**.

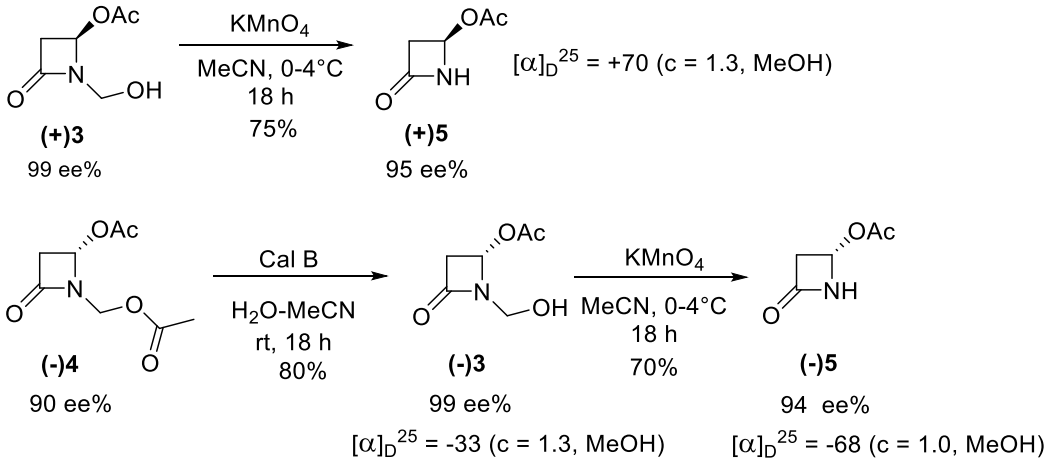


Scheme 3. Kinetic resolution of β-lactam **rac-2**; isolated yields % after column chromatography, ee% and specific optical rotations for compounds **3** and **4** are reported. The enzymatic selectivity E was calculated by the ENANTIO tool of the Elk group Graz University.

The reaction was stopped at a 52% conversion: the two products were isolated and separated by flash-chromatography, characterized and analysed by chiral HPLC analysis and polarimetry; their specific optical rotations and enantiomeric excesses resulted as **(+)3** (99 % ee) and **(-)4** (90 % ee) with an excellent enzymatic selectivity (E) of 116 (Scheme 3).

Concerning the absolute configuration of the two enantiomers, it can be only tentatively attributed according to the enantio-preference of lipases on some C-3 unsubstituted β-lactam compounds bearing primary alcohols with a β-stereocenter [45, 38]. As above mentioned for the kinetic resolution on the racemic β-lactam **rac-B** [45], *P. fluorescens* gave (*S*)-ester as the preferred enantiomer (Scheme 1). In that case the absolute configuration was assigned by comparison with an already known chiral azetidinone obtained from (*S*)-aspartic acid [47]. On assuming the same (*S*)-enantio-preference by *P. fluorescens* for the C4 substituent, it could be tentatively assigned a (*S*) configuration to the **(-)4** ester as the preferred enantiomer also in this KR.

Alcohol **(+)3** and ester **(-)4** were then further elaborated in order to eliminate the substituents on the β-lactam nitrogen atom and to obtain the final separated enantiomers **(+)5** and **(-)5** (Scheme 4).

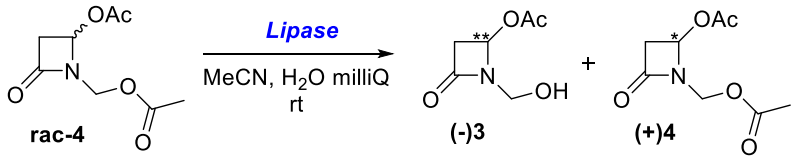


Scheme 4. Synthesis of β-lactam enantiomers **(+)5** and **(-)5**. Isolated yields, ee%, and specific optical rotation powers are reported.

For this purpose, it was necessary to eliminate the *N*-hydroxymethyl group on **(+)3** and different conditions were tested: NH_4OH (25%)/MeOH, $KMnO_4$/acetone-H_2O, and $KMnO_4$/MeCN [48]. Only oxidative conditions were effective, and the use of $KMnO_4$ in acetonitrile allowed to isolate compound **(+)5** in satisfactory yields. As mentioned in the introduction, the (+)4-acetoxy-azetidinone **(+)5** has been already reported in the literature, as an enantiopure compound obtained by means of host-guest inclusion complexes [29]. However, we observed a higher specific rotation for **(+)5** $[\alpha]_D^{25}$ = +70 (c = 1.3, MeOH) than that reported in the literature [31]: $[\alpha]_D^{25}$ = +6.5 (c = 0.68, MeOH), but the latter has a lower concentration and its enantiomeric purity as ee% by chiral chromatography was not reported.

The **(-)5** enantiomer was obtained from **(-)4** with a two steps procedure: ester hydrolysis to get intermediate **(-)3** followed by oxidative cleavage of the oxymethylene group. Concerning the hydrolysis, biocatalysis fulfilled the requirement of a high regioselectivity among the two acetates present on **(-)5**. The effectiveness of lipases to satisfy this requirement was preliminary evaluated on the racemic ester **rac-4** (Table 2), easily obtained from **rac-2** with acetic anhydride and triethylamine, and used as racemic standard for chiral HPLC analyses (see Supplementary Material).

Table 2. Screening of lipases for enzymatic hydrolysis on **rac-4**.[a]



Entry	*Lipases* (U/mg)[b]	Enzyme amount (U)	Time (h)	T (°C)	Conv. (%)[c]	(-)3 ee%[c]	(+)4 ee%[c]
1	*Burkholderia cepacia* (30 U/mg)	180	144	rt	30	>99	40
2	*Pseudomonas fluorescens* (309 U/mg)	326	21	rt	25	>99	24
3[d]	*CAL B* (10 U/mg)	100	3	rt	55	>99	66
4[d]	*CAL B* (10 U/mg)	100	24	rt	99	>99	-
5[e]	*CAL B* (10 U/mg)	35	24	rt	95	96	99[f]
6[d, e]	*CAL B* (10 U/mg)	100	3	60	95	>99	99[f]

7[d, e]	*CAL B* (10 U/mg)	100	1	60	58	>99	30

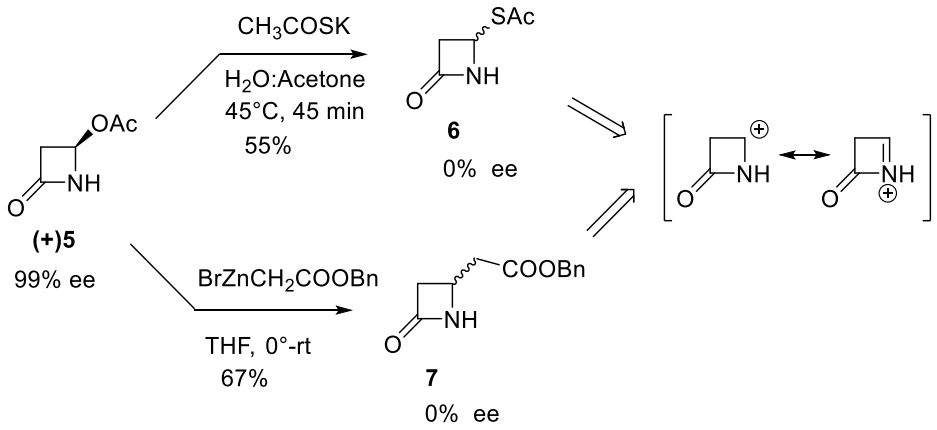
[a] Reaction conditions: **rac-4** (0.06 mmol), H_2O/MeCN 11:1 (2.5 mL), enzyme (U in table), rt.
[b] Activity of the commercial enzymes.
[c] Determined by chiral HPLC analysis on the crude.
[d] Reaction conditions: **rac-4** (0.174 mmol), H_2O/MeCN 11:1 (3 mL), enzyme (U in table), rt.
[e] The recovery of the organic fraction is around 60%.
[f] The ester (+)4 was detected only in traces in the chiral HPLC analysis

In this ester hydrolysis, *Burkholderia cepacia* and *Pseudomonas fluorescens* lipases gave unsatisfactory results with low conversions even for longer reaction time (Table 2, entries 1 and 2). *CAL B* was instead successful and furnished a 55% conversion and excellent enantiomeric excess (>99 %) for the alcohol **3** in only 3 hours (Table 2, entry 3). This result is quite interesting: the excellent ee for the product **(-)3** >99% at a conversion exceeding the 50% (i.e. 55 %) and a lower ee (66%) for the ester **(+)4** catches a glimpse of a possible racemization of the reactant at the C-4 position that could be temporarily ascribed to the tentative formation of a C-4 cation (see for instance Scheme 5), thus revealing the possibility of a prospective dynamic kinetic resolution under controlled conditions. On extending the hydrolysis for 24 h with *CAL B*, the conversion was quantitative, only the alcohol **3** was isolated as single enantiomer with ee >99 %, but no traces of the ester **4** were isolated in the organic fraction (Table 2, entry 4). This could probably due to a hydrolysis of the β-lactam ring in the ester **(+)4** and its further degradation in the aqueous phase due to a longer reaction time. The hydrolysis with *CAL B* was further examined at 60 °C at short reaction time, 1 and 3 h (Table 2, entries 6 and 7) obtaining 95% and 58% conversion, respectively. The product **3** was isolated with high ee%, but with a poor recovery in the organic extract (60%) with traces (entry 6) or low amounts (entry 7) of the ester. This result confirms that harsher reaction conditions could give significant amounts of hydrolysis of the β-lactam ring [49-52] leading to a yield drop.

The hydrolysis was repeated with *CAL B* on the enantiomerically enriched ester **(-)4** (Scheme 4). The process was carried out in MilliQ water with a small amount of acetonitrile necessary to dissolve **(-)4** (H_2O/CH_3CN = 11:1). Alcohol **(-3)** was obtained in an 80% yield with a 99% optical purity; remarkably, its enantiomeric excess was greatly enriched if compared to that of the starting ester **(-)4** (90% ee). The final oxidation of **(-)3** with $KMnO_4$ in acetonitrile afforded chiral (-)4-acetoxy-azetidinone **(-)5** in good yields (Scheme 4). Careful attention should be paid for a cold aqueous work-up of the crude because lowering of the enantiomeric excess was otherwise observed, and an ee = 88% was reached instead of 94% (see supplementary information for HPLC analyses).

Introduction or transformation of functional groups on C4 position of azetidinones is a common step in the synthesis of β-lactam-based compounds [19]. Once obtained the enantiomerically pure 4-acetoxy-azetidinones **(+)5** and **(-)5**, some nucleophilic substitution reactions were tested on these substrates. We then examined the stereochemical outcome in two C4 substitution reactions (Scheme 5) on enantiomer **(+)5**, with potassium thioacetate and with Reformatsky reagent

246 $BrZnCH_2COOBn$, previously applied for the synthesis of integrin ligands with a β-lactam scaffold [10,
247 11].



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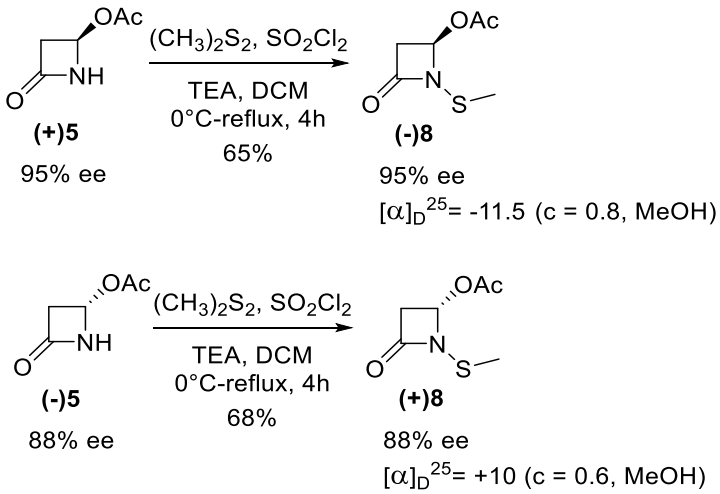
249 **Scheme 5.** Nucleophilic substitution reactions at C4 position of the enantiomerically
250 pure 4-acetoxy-azetidinone **(+)5**.

251

252 However, starting from enantiopure compound **(+)5**, the C4 substitution products **6** and **7** were
253 obtained as racemic mixtures (chiral HPLC analyses in Supplementary information), confirming the
254 mechanism of this substitution as an addition-elimination pathway *via* the short-living intermediate
255 1-azetin-2-one (Scheme 5), as determined by Gavina *et al.* [53].

256 As a further stereochemical investigation, the **(+)5** and **(-)5** enantiomers were considered for a
257 functionalization on the nitrogen atom of the β-lactam ring (Scheme 6). For this purpose, we
258 selected a *N*-thioalkylation reaction, since *N*-alkyl-thio-4-acetoxy-azetidinones demonstrated to
259 have interesting antibacterial activities [24, 26] and were also successfully applied in the
260 development of new antibacterial functional materials [25]. Enantiomers **(+)5** and **(-)5** were
261 separately subjected to a *N*-thiolation reaction using dimethyl disulfide in the presence of sulfuryl
262 chloride and triethylamine (TEA) in DCM (Scheme 6), according to a previously reported procedure
263 [25].



Scheme 6. *N*-thiomethylation on enantiopure 4-acetoxy-azetidinone **(-)5**.

Compounds **(-)8** and **(+)8** were isolated by flash chromatography and then analysed by chiral HPLC (see Supplementary Material). The chromatographic analysis showed the preservation of the ee at 95% and 88%, as that of the precursors **(+)5** and **(-)5**, respectively, thus confirming that the *N*-thioalkylation reaction did not affect the configuration at C4 of the β-lactam. The optical rotations of the *N*-methylthio derivatives **(-)8** and **(+)8** have opposite signs compared to the starting compounds **(+)5** and **(-)5**, however, optical power is an inherent property of a molecule, and a change in sign between different molecules does not entail an inversion of the configuration [54, 55].

Conclusion

The syntheses of several biologically active β-lactam-based compounds share as starting material the 4-acetoxy-azetidin-2-one, a cheap and readily available compound, but marketed as a racemic mixture. Given the effect of chirality on biological activity and the need to synthesize enantiopure β-lactams for pharmacological use it would be advisable to obtain 4-acetoxy-azetidin-2-one in its separated single enantiomers. Biocatalysis is becoming a valid and an increasingly employed technique for achieving enantiomerically pure products. Among a series of lipases, *Pseudomonas fluorescens* was selected as the most suitable enzyme for performing a kinetic resolution by transesterification on the *N*-hydroxymethyl β-lactam **rac-2**, giving the corresponding ester **(–)4** and the residual starting alcohol **(+)3** with excellent enantiomeric excesses. The successful ester hydrolysis of **(–)4** was obtained with *Candida antarctica lipase B* (*CAL B*) with a significant enrichment of the enantiomeric excess of the *N*-hydroxymethyl-azetidinone **(-)3**. The potential dynamic kinetic resolution of the β-lactam **rac-4** by *CAL B* is currently under investigation. Following the optimized deprotection steps, 4-acetoxy-azetidin-2-ones **(+)5** and **(–)5** were thus obtained as single enantiomers with excellent optical purities. Nucleophilic substitution reactions were then studied on the pure enantiomers in order to evaluate their stereochemical outcome. The C4

substitution on the enantiomer **(+)5** with potassium thioacetate or $BrZnCH_2COOBn$ gave the corresponding products **6** and **7** as racemic mixtures, confirming the propensity of 4-acetoxy-azetidinones to undergo a S_N1 mechanism. On the contrary, the C4 configuration is not affected by functionalization of the β-lactam nitrogen atom upon sulfenylation reaction, and optically active *N*-methylthio-4-acetoxy-β-lactams were successfully obtained. With this result we report for the first time the synthesis of an enantiopure *N*-alkylthio-4-acetoxy-azetidinone, hence paving the way to the development of novel chiral β-lactams that could be employed in medicinal chemistry as antimicrobial agents as pure enantiomers instead of racemic mixtures.

Experimental

Solvents and reagents were obtained commercially and used as received. Deionized water was obtained from a Millipore analytical deionization system (MilliQ). For TLC monitoring Merck 60 F254 plates were used and for liquid chromatography Merck silica gel 200–300 mesh was used. ^1H and ^{13}C NMR spectra were recorded with an INOVA 400 instrument with a 5 mm probe. All chemical shifts are quoted relative to deuterated solvent signals (δ in ppm and J in Hz). FTIR spectra were recorded with Alpha FT IR Bruker spectrometer. Polarimetric analyses were conducted on Unipol L 1000 Polarimeter at 598 nm. The purities of the target compounds were assessed as being > 95% using HPLC-MS. HPLC-MS: Agilent Technologies HP1100 instrument, equipped with a ZORBAX-Eclipse XDB-C8 Agilent Technologies column; mobile phase: H_2O/CH_3CN, 0.4 mL/min, gradient from 30 to 80% of CH_3CN in 8 min, 80% of CH_3CN until 25 min, coupled with an Agilent Technologies MSD1100 single-quadrupole mass spectrometer, full scan mode from m/z = 50 to 2600, in positive ion mode, ESI spray voltage 4500 V, nitrogen gas 35psi, drying gas flow 11.5 mL/min, fragmentor voltage 20 V. Enantiomeric excesses were determined by chiral-HPLC: Agilent Technologies 1200 instrument equipped with a diode array UV detector on Daicel Chiralcel column IA (25 cm, I.D. 0.46 cm, 5 μm) with HPLC grade isopropanol and *n*-hexane as eluting solvents. Racemic compounds were used for comparison (see supplementary material). The commercially available enzymes used in this work are: *Burkholderia cepacia lipase* (BCL) powder, ≥30 U/mg by Sigma Aldrich; Lipase from *Pseudomonas fluorescens*, powder, 309 U/mg by Fluka; Lipase from *Pseudomonas fluorescens*, powder, 20-36 U/mg by Sigma Aldrich; Lipase from *Candida antarctica B*, immobilized on acrylic resin, 10 U/mg, by Sigma Aldrich; Lipase B from *Candida antarctica,* immobilized on Immobead 150, 4.4 U/mg, by Sigma Aldrich.

Synthesis of racemic β-lactams

Preparation of racemic esters **rac-4** for the optimization of an analytic method by chiral HPLC is reported in the Supplementary Material.

1-(hydroxymethyl)-4-oxoazetidin-2-yl acetate (**rac-2**).

To a solution of compound **rac-1** (200 mg, 1.55 mmol, 1 equiv) in THF (3.4 mL), paraformaldehyde (72 mg, 2.48 mmol, 1.6 equiv), K_2CO_3 (2 mg, 0.05 mmol, 0.03 equiv) and water (140 µL) were added. The system was subjected to microwave irradiation at 180 W for 40 minutes**.** At completion, the reaction mixture was diluted with EtOAc (2.5 mL), dried on anhydrous Na_2SO_4, filtered and concentrated to yield compound **rac-2** as a colorless oil (240 mg, 95%) without further purifications. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.01 (dd, *J* = 4.2, 1.2 Hz, 1H), 4.78 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.5 Hz, 1H), 3.23 (dd, *J* = 15.3, 4.2 Hz, 1H), 2.96 (dd, *J* = 15.3, 1.2 Hz, 1H), 2.10 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.6, 165.3, 74.5, 64.1, 44.3, 20.7. IR (film, cm^{-1}) = 3425, 2950, 1752, 1649, 1378, 1241, 1120, 1043. HPLC-MS (ESI^+) t_r=2.9 min, m/z=100 $[M\text{-}OAc]^+$, 160 $[M+H]^+$, 177 $[M+H_2O]^+$, 182 $[M+Na]^+$.

*Procedure for lipases screening in enzymatic kinetic resolution on **rac-2** (see Table 1).*

In a glass vial with a screw cap, to a solution of alcohol **rac-2** (10 mg, 0.063 mmol, 1 equiv) and vinyl acetate (45 µL, 0.38 mmol, 6 equiv) in TBME (1.5 mL), the selected enzyme (see Units in Table 1) was added. The mixture was stirred at room temperature. At set time intervals, the substrate conversion and the enantiomeric excesses of unreacted alcohol **(+)3** and ester product **(-)4** were monitored by chiral HPLC. Chiral HPLC samples were prepared as follows: 0.5 mL of mixture was filtered through regenerated cellulose syringe filters (diameter = 25 mm, pore diameter = 0.45 µm); the filtrate was then concentrated, re-suspended in a solution of *n*-hexane/isopropanol 1:1 and directly analysed.

*Preparative enzymatic KR of compound **rac-2**.*

In a glass vial with a screw cap, to a solution of alcohol **rac-2** (164 mg, 1.03 mmol, 1 equiv) and vinyl acetate (0.57 mL, 6.18 mmol, 6 equiv) in TBME (24.5 mL), Amano Lipase from *Pseudomonas luorescens (20 U/mg,* 1640 U, 82 mg*)* was added. The mixture was then kept under magnetic stirring at room temperature and monitored by chiral HPLC. At 52% conversion, after 64 h, the mixture was filtered through regenerated cellulose syringe filters and the organic solvent was removed under reduced pressure. Compounds **(+)3** and **(-)4** were separated by flash chromatography (Cyclohexane/EtOAc 1:1 then 35:65); target ester **(-)4** was obtained as a colorless oil (46%, ee = 90%, $[\alpha]_D^{25}$ = −17 (c = 1.0, MeOH)) and residual alcohol **(+)3** was isolated as a colorless oil (37%, ee = 99%, $[\alpha]_D^{25}$ = +35 (c = 1.3, MeOH)).

*Procedure for lipases screening in the enzymatic hydrolysis on **rac-4** (see Table 2).*

In a glass vial with a screw cap, to a solution of **rac-4** (12 mg, 0.06 mmol) in CH_3CN and Milli Q H_2O (1:11 ratio, total volume 2.5 mL), the selected enzyme (see Units in Table 2) was added. The mixture was stirred at room temperature under orbital shaking (450 rpm). The conversion and the enantiomeric excesses of **(-)3** and **(+)4** were monitored by chiral HPLC at set time intervals. Chiral HPLC samples were prepared as follows: 200 µL of the mixture were extracted with 0.5 mL EtOAc, organic solvent was dried, re-suspended in a solution of *n*-hexane/isopropanol 1:1 and directly analysed.

*Preparative enzymatic hydrolysis of compound **(−)4**.*

In a glass vial with a screw cap, to a solution of ester **(-)4** (102 mg, 0.51 mmol, 1 equiv) in CH_3CN and Milli Q H_2O (1:11 ratio, total volume 17 mL), *Lipase B from Candida Antarctica immobilized on Immobead 150* (4.4 U/mg, 224 U, 51 mg) was added. The mixture was stirred at room temperature under orbital shaking (450 rpm) overnight and monitored by chiral HPLC. At reaction completion (18 h), the enzyme was filtered and the aqueous mixture was saturated with brine and extracted with EtOAc (3×10 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The target alcohol **(-)3** was obtained without further purification as a colorless oil (65 mg, Y =80%, ee = 99%, $[\alpha]_D^{25} = -33$ (c = 1.3, MeOH)). Spectroscopic data of **(−)3** were in fully accordance with those reported for its corresponding racemic analogue **rac-2**.

*Oxidation with $KMnO_4$ to give **(+)5** and **(−)5**.*

To a solution of the alcohol **(+)3** or **(−)3** (1 equiv) in CH_3CN (30 mL/mmol), $KMnO_4$ (6 equiv) was added portionwise at 0°C. The reaction flask was maintained at 4°C overnight (refrigerator). The reaction mixture was quenched at 0°C with a saturated solution of $Na_2S_2O_5$ until complete decoloring. The mixture was then filtered and acetonitrile evaporated under reduced pressure. The residual aqueous solution was then extracted with DCM (3×10 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated. The desired products **(+)5** and **(-)5** were obtained without further purification as sticky solid. **(+)5**: Y = 75%, ee = 99%, $[\alpha]_D^{25} = +70$ (c = 1.3, MeOH); **(−)5**: 70%, ee = 99%, $[\alpha]_D^{25} = -68$ (c = 1.0, MeOH). Spectroscopic data of **(+)5** and **(−)5** were in fully accordance with those reported for their corresponding racemic analogue **rac-1**.

*Synthesis of S-(4-oxoazetidin-2-yl) ethanethioate (**6**).*

To a solution of CH_3COSK (27 mg, 0.24 mmol, 1.2 equiv) in H_2O (1.5 mL) warmed at 45°C, compound **(+)5** (25 mg, 0.2 mmol, 1 equiv) dissolved in acetone (0.5 mL) was added dropwise. At completion (50 min, TLC monitoring), acetone was evaporated under reduced pressure and the residual aqueous solution was then extracted with EtOAc (5×10 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated. Compound **6** was yielded as a yellow oil (16 mg,

55%) without further purification. Spectroscopic data were in fully accordance with those reported in literature [24].

*Synthesis of benzyl 2-(4-oxo-azetidin-2-yl) acetate (**7**).*

In a 25 mL 3-neck flask under inert atmosphere (N_2), Zn powder (203 mg, 3.12 mmol, 8 equiv) and THF (1 mL) were introduced followed by TMSCl (20 µL, 0.155 mmol, 0.39 equiv). After 30 min of stirring the temperature was raised to 30-32 °C and a solution of benzylbromoacetate (247 µL, 1.56 mmol, 4 equiv) in THF (2 mL) was slowly added dropwise. After 30 min of stirring the mixture was cooled to rt and decanted. The limpid grey supernatant was slowly added dropwise into a 25 mL flask under nitrogen containing a solution of **(+)5** (50 mg, 0.39 mmol, 1 equiv) in anhydrous THF (2.2 mL) at 0°C. The mixture was stirred at rt for 3 h, quenched with a saturated Seignette salt (potassium sodium tartrate) solution and extracted with EtOAc (3x10 mL). The organic layers were dried on Na_2SO_4, filtered and concentrated in vacuum. The crude was purified by flash chromatography (Cyclohexane/AcOEt = 1:1) yielding **7** as a white solid (57 mg, 67%). Spectroscopic data were in fully accordance with those reported in literature. [11]

*Synthesis of compounds **(+) 8** and **(-8).***

In a round bottom flask under inert atmosphere (N_2), to a solution of dimethyl disulfide (10 µL, 0.11 mmol, 1 equiv) in dry DCM (1 mL), SO_2Cl_2 (13 µL, 0.16 mmol, 1.5 equiv) was slowly added at 0°C. After 10 minutes, **(+) 5** or **(-) 5** (14 mg, 0.11 mmol, 1 equiv) dissolved in DCM (1 mL) was added, followed by dropwise addiction of TEA (28 µL, 0.22 mmol, 2 equiv). After 10 minutes at 0°C, the reaction mixture was warmed to rt and then refluxed for 4 h. At completion (TLC monitoring), the reaction was quenched with saturated aqueous solution of NH_4Cl and the mixture extracted with DCM (3x5 mL). The collected organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The desired product was obtained as a yellow oil after purification by flash chromatography on silica gel (Cyclohexane/EtOAc = 7:3). **(+)8**: 13 mg, Y=68%, ee = 88%, $[\alpha]_D^{20}$ = +10 (c = 0.6, MeOH); **(–)8**: 12.5 mg, Y=65%, ee = 95%, $[\alpha]_D^{25}$ = −11.5 (c = 0.8, MeOH). Spectroscopic data were in fully accordance with those reported in literature [24, 25].

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