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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

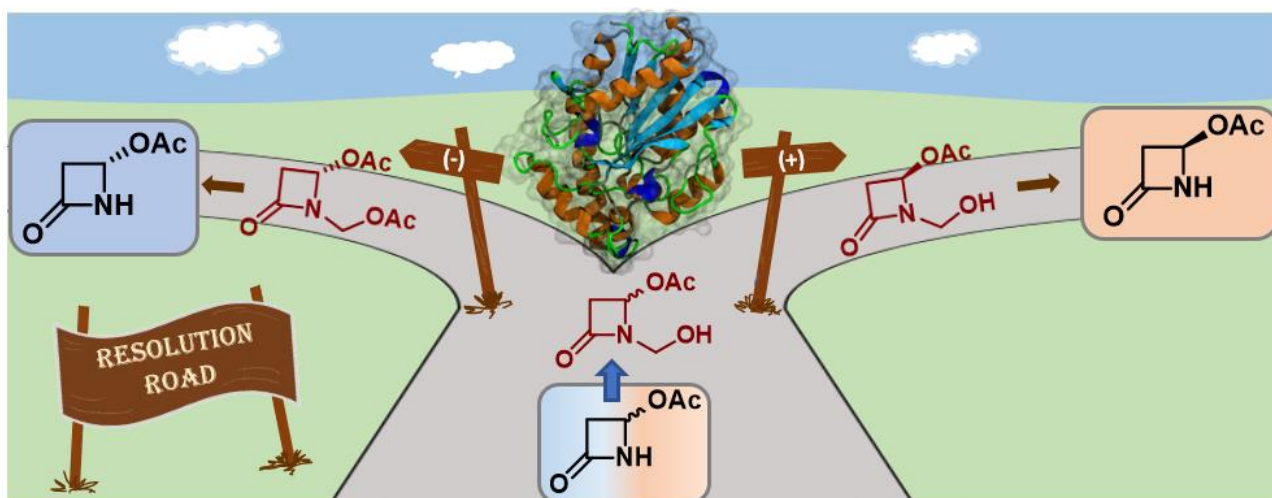
Abstract

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-azetidin-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



45 Introduction

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

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

66 As useful β -lactam intermediates, (3*R*,4*R*)-4-acetoxy-3-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)-ethyl]-
67 azetidin-2-one (**A**) and 4-acetoxy azetidin-2-one (**rac-1**) are commercially available and have been
68 widely used as starting materials for the synthesis of important bioactive compounds [19] (Figure
69 1). Intermediate **A** is available as enantiopure compound, **rac-1** instead is purchased only as a
70 racemic mixture. Although the great usefulness of **rac-1** as starting material in the synthesis of new
71 bioactive β -lactam compounds [20-27], this reactant could only provide racemic derivatives
72 (selected examples in Figure 1). Since it has been well attested that two enantiomeric drugs could
73 give different pharmacologic responses and diverse pharmacokinetic, pharmacodynamic and
74 therapeutic profiles, or could even reveal adverse effects [28, 29], the possibility to gain access to
75 the enantiopure form of 4-acetoxy-azetidinone **rac-1** would be of utmost importance in the
76 synthesis of stereo-chemically defined bioactive β -lactams [30]. To the best of our knowledge, only
77 (+)4-acetoxy-azetidinone has been reported and obtained by chiral recognition process upon
78 separation of chiral host-guest inclusion complexes [31].

79

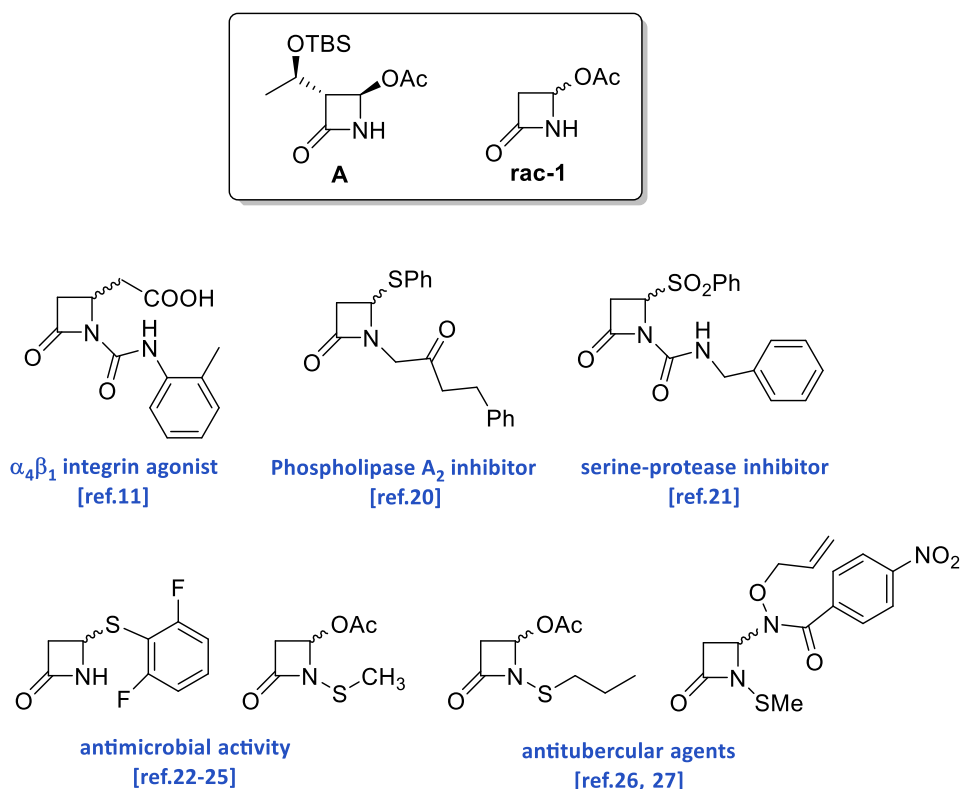


Figure 1. 4-acetoxy-azetidinones **A**, **rac-1**, and some selected racemic bioactive β -lactam compounds obtained from **rac-1**.

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).

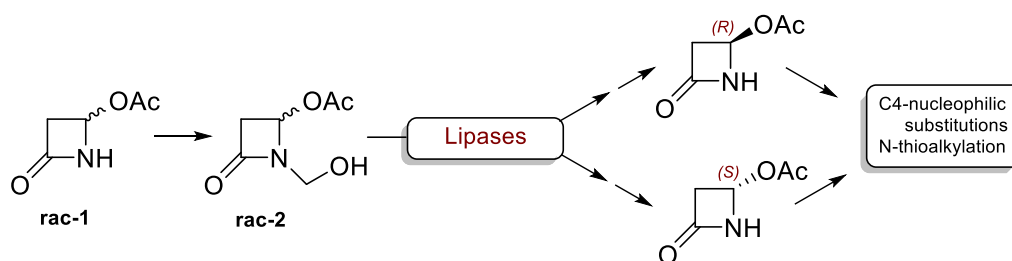


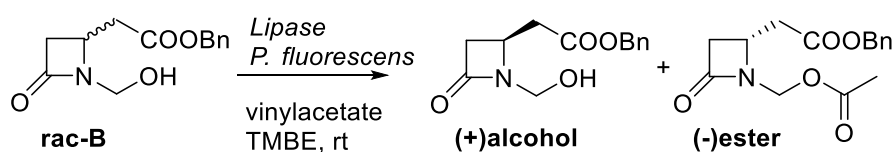
Figure 2. Enzymatic kinetic resolution on functionalized 4-acetoxy-azetidinone and evaluation of the stereochemical outcome in C4 and N-functionalization.

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.

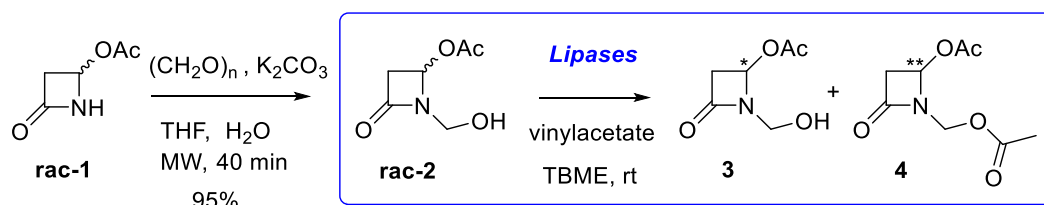
Results and discussion

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	<i>Burkholderia cepacia</i> (30 U/mg)	TBME	300	20	3.5	6	>99
2	<i>Burkholderia cepacia</i> (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	<i>Pseudomonas fluorescens</i> (20 U/mg)	dry THF	223	72	39	>99	70
8	<i>Pseudomonas fluorescens</i> (20 U/mg)	THF	223	72	44	>99	82
9	<i>Pseudomonas fluorescens</i> (20 U/mg)	CH ₃ CN	223	72	44	>99	81
10	<i>Pseudomonas fluorescens</i> (20 U/mg)	Toluene	223	72	46	>99	79

11	<i>Pseudomonas fluorescens</i> (20 U/mg)	TBME	223	72	51	>99	91
12^e	<i>Pseudomonas fluorescens</i> (20 U/mg)	TBME	223	24	53	84	>99
13	<i>Pseudomonas fluorescens</i> (36 U/mg)	TBME	48	48	55	89	96
14	<i>Pseudomonas fluorescens</i> (309 U/mg)	TBME	150	23	55	>99	90
15	<i>Pseudomonas fluorescens</i> (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.

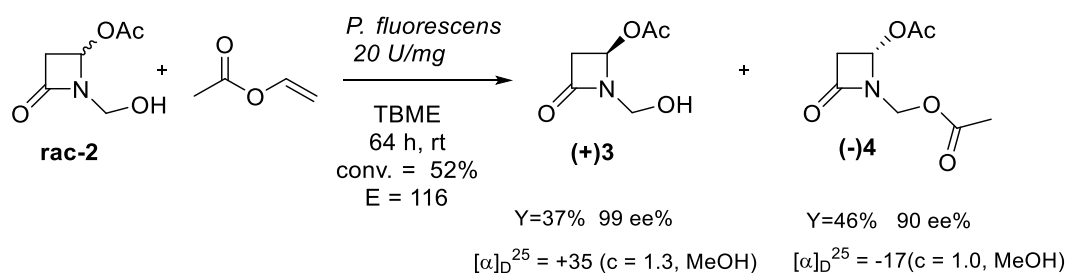
^c Determined by chiral HPLC analysis on the crude.

^d Reaction conducted under N₂ 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 N₂ 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**.

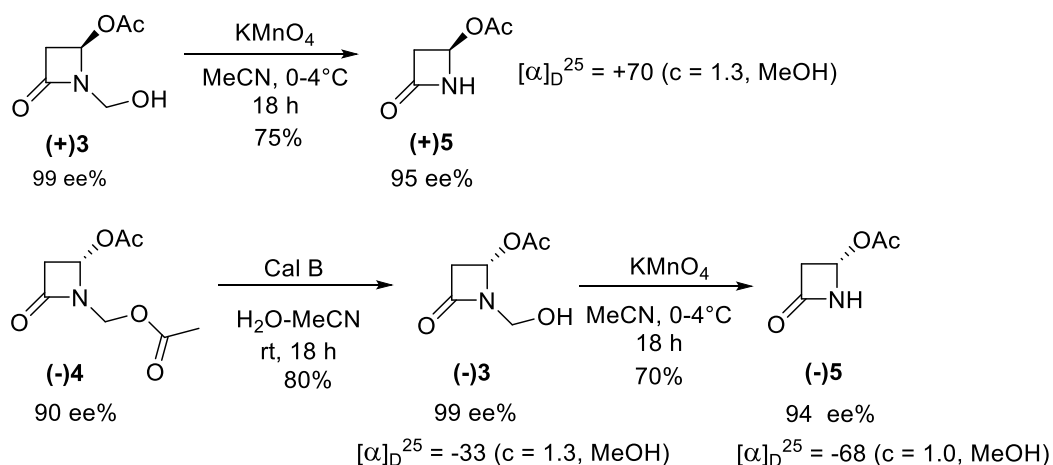


Scheme 3. Kinetic resolution of β -lactam **rac-2**; isolated yields % after column chromatography, ee% and specific optical rotations for compounds **3** and **4** are reported. The enzymatic selectivity E was calculated by the ENANTIO tool of the Elk group Graz University.

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).

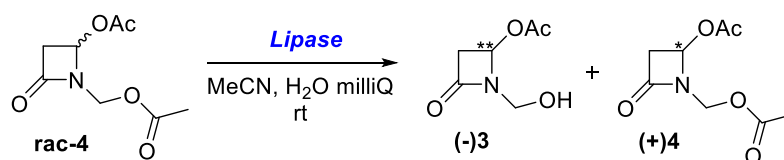


Scheme 4. Synthesis of β -lactam enantiomers **(+)**5 and **(-)**5. Isolated yields, ee%, and specific optical rotation powers are reported.

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-acetoxyazetidinone **(+)**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 $[\alpha]_{\text{D}}^{25} = +70$ ($c = 1.3$, MeOH) than that reported in the literature [31]: $[\alpha]_{\text{D}}^{25} = +6.5$ ($c = 0.68$, 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).

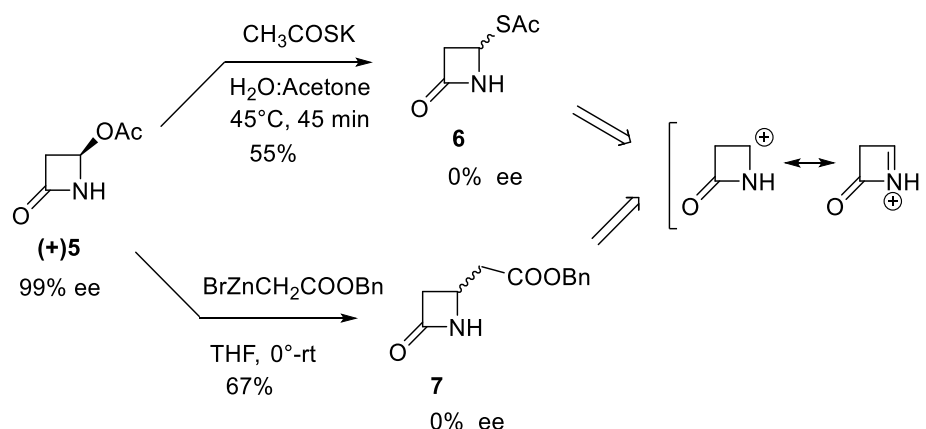
Table 2. Screening of lipases for enzymatic hydrolysis on **rac-4**.^a



Entry	Lipases (U/mg) ^b	Enzyme amount (U)	Time (h)	T (°C)	Conv. (%) ^c	(-) 3 ee% ^c	(+) 4 ee% ^c
1	<i>Burkholderia cepacia</i> (30 U/mg)	180	144	rt	30	>99	40
2	<i>Pseudomonas fluorescens</i> (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
209	^a Reaction conditions: rac-4 (0.06 mmol), H ₂ O/MeCN 11:1 (2.5 mL), enzyme (U in table), rt.							
210	^b Activity of the commercial enzymes.							
211	^c Determined by chiral HPLC analysis on the crude.							
212	^d Reaction conditions: rac-4 (0.174 mmol), H ₂ O/MeCN 11:1 (3 mL), enzyme (U in table), rt.							
213	^e The recovery of the organic fraction is around 60%.							
214	^f The ester (+) 4 was detected only in traces in the chiral HPLC analysis							
215	In this ester hydrolysis, <i>Burkholderia cepacia</i> and <i>Pseudomonas fluorescens</i> lipases gave							
216	unsatisfactory results with low conversions even for longer reaction time (Table 2, entries 1 and 2).							
217	CAL B was instead successful and furnished a 55% conversion and excellent enantiomeric excess							
218	(>99 %) for the alcohol 3 in only 3 hours (Table 2, entry 3). This result is quite interesting: the							
219	excellent ee for the product (-) 3 >99% at a conversion exceeding the 50% (i.e. 55 %) and a lower ee							
220	(66%) for the ester (+) 4 catches a glimpse of a possible racemization of the reactant at the C-4							
221	position that could be temporarily ascribed to the tentative formation of a C-4 cation (see for							
222	instance Scheme 5), thus revealing the possibility of a prospective dynamic kinetic resolution under							
223	controlled conditions. On extending the hydrolysis for 24 h with CAL B, the conversion was							
224	quantitative, only the alcohol 3 was isolated as single enantiomer with ee >99 %, but no traces of							
225	the ester 4 were isolated in the organic fraction (Table 2, entry 4). This could probably due to a							
226	hydrolysis of the β-lactam ring in the ester (+) 4 and its further degradation in the aqueous phase							
227	due to a longer reaction time. The hydrolysis with CAL B was further examined at 60 °C at short							
228	reaction time, 1 and 3 h (Table 2, entries 6 and 7) obtaining 95% and 58% conversion, respectively.							
229	The product 3 was isolated with high ee%, but with a poor recovery in the organic extract (60%) with							
230	traces (entry 6) or low amounts (entry 7) of the ester. This result confirms that harsher reaction							
231	conditions could give significant amounts of hydrolysis of the β-lactam ring [49-52] leading to a yield							
232	drop.							
233	The hydrolysis was repeated with CAL B on the enantiomerically enriched ester (-) 4 (Scheme 4). The							
234	process was carried out in MilliQ water with a small amount of acetonitrile necessary to dissolve (-							
235	4 (H ₂ O/CH ₃ CN = 11:1). Alcohol (-) 3 was obtained in an 80% yield with a 99% optical purity;							
236	remarkably, its enantiomeric excess was greatly enriched if compared to that of the starting ester (-							
237	4 (90% ee). The final oxidation of (-) 3 with KMnO ₄ in acetonitrile afforded chiral (-)4-acetoxy-							
238	azetidinone (-) 5 in good yields (Scheme 4). Careful attention should be paid for a cold aqueous work-							
239	up of the crude because lowering of the enantiomeric excess was otherwise observed, and an ee =							
240	88% was reached instead of 94% (see supplementary information for HPLC analyses).							
241	Introduction or transformation of functional groups on C4 position of azetidinones is a common step							
242	in the synthesis of β-lactam-based compounds [19]. Once obtained the enantiomerically pure 4-							
243	acetoxy-azetidinones (+) 5 and (-) 5 , some nucleophilic substitution reactions were tested on these							
244	substrates. We then examined the stereochemical outcome in two C4 substitution reactions							
245	(Scheme 5) on enantiomer (+) 5 , with potassium thioacetate and with Reformatsky reagent							

246 BrZnCH₂COOBn, previously applied for the synthesis of integrin ligands with a β-lactam scaffold [10,
247 11].



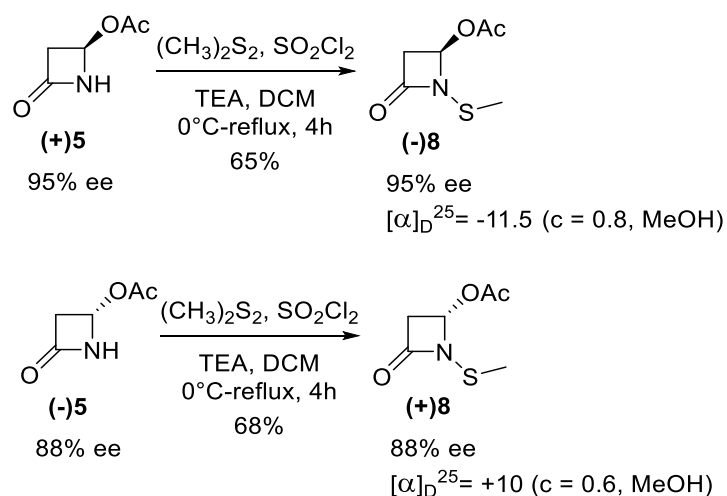
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249 **Scheme 5.** Nucleophilic substitution reactions at C4 position of the enantiomerically
250 pure 4-acetoxy-azetidinone (**(+)5**).

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 sulfur
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 BrZnCH₂COOBn 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 ¹³C 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: H₂O/CH₃CN, 0.4 mL/min, gradient from 30 to 80% of CH₃CN in 8 min, 80% of CH₃CN 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.

327 1-(hydroxymethyl)-4-oxoazetidin-2-yl acetate (**rac-2**).

328 To a solution of compound **rac-1** (200 mg, 1.55 mmol, 1 equiv) in THF (3.4 mL), paraformaldehyde
329 (72 mg, 2.48 mmol, 1.6 equiv), K₂CO₃ (2 mg, 0.05 mmol, 0.03 equiv) and water (140 µL) were added.
330 The system was subjected to microwave irradiation at 180 W for 40 minutes. At completion, the
331 reaction mixture was diluted with EtOAc (2.5 mL), dried on anhydrous Na₂SO₄, filtered and
332 concentrated to yield compound **rac-2** as a colorless oil (240 mg, 95%) without further purifications.
333 ¹H NMR (400 MHz, CDCl₃) δ (ppm) 6.01 (dd, *J* = 4.2, 1.2 Hz, 1H), 4.78 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* =
334 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). ¹³C NMR (100
335 MHz, CDCl₃) δ (ppm) 171.6, 165.3, 74.5, 64.1, 44.3, 20.7. IR (film, cm⁻¹) = 3425, 2950, 1752, 1649,
336 1378, 1241, 1120, 1043. HPLC-MS (ESI⁺) *t*_r=2.9 min, *m/z*=100 [M-OAc]⁺, 160 [M+H]⁺, 177 [M+H₂O]⁺,
337 182 [M+Na]⁺.

338

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

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

348

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

350 In a glass vial with a screw cap, to a solution of alcohol **rac-2** (164 mg, 1.03 mmol, 1 equiv) and vinyl
351 acetate (0.57 mL, 6.18 mmol, 6 equiv) in TBME (24.5 mL), Amano Lipase from *Pseudomonas*
352 *luorescens* (20 U/mg, 1640 U, 82 mg) was added. The mixture was then kept under magnetic stirring
353 at room temperature and monitored by chiral HPLC. At 52% conversion, after 64 h, the mixture was
354 filtered through regenerated cellulose syringe filters and the organic solvent was removed under
355 reduced pressure. Compounds **(+)**3 and **(-)**4 were separated by flash chromatography
356 (Cyclohexane/EtOAc 1:1 then 35:65); target ester **(-)**4 was obtained as a colorless oil (46%, ee = 90%,
357 [α]_D²⁵ = -17 (*c* = 1.0, MeOH)) and residual alcohol **(+)**3 was isolated as a colorless oil (37%, ee = 99%,
358 [α]_D²⁵ = +35 (*c* = 1.3, MeOH)).

359

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

361 In a glass vial with a screw cap, to a solution of **rac-4** (12 mg, 0.06 mmol) in CH₃CN and Milli Q H₂O
362 (1:11 ratio, total volume 2.5 mL), the selected enzyme (see Units in Table 2) was added. The mixture
363 was stirred at room temperature under orbital shaking (450 rpm). The conversion and the
364 enantiomeric excesses of **(-)-3** and **(+)-4** were monitored by chiral HPLC at set time intervals. Chiral
365 HPLC samples were prepared as follows: 200 μ L of the mixture were extracted with 0.5 mL EtOAc,
366 organic solvent was dried, re-suspended in a solution of *n*-hexane/isopropanol 1:1 and directly
367 analysed.

368

369 *Preparative enzymatic hydrolysis of compound (-)-4.*

370 In a glass vial with a screw cap, to a solution of ester **(-)-4** (102 mg, 0.51 mmol, 1 equiv) in CH₃CN and
371 Milli Q H₂O (1:11 ratio, total volume 17 mL), *Lipase B from Candida Antarctica immobilized on*
372 *Immobead 150* (4.4 U/mg, 224 U, 51 mg) was added. The mixture was stirred at room temperature
373 under orbital shaking (450 rpm) overnight and monitored by chiral HPLC. At reaction completion (18
374 h), the enzyme was filtered and the aqueous mixture was saturated with brine and extracted with
375 EtOAc (3 \times 10 mL). The collected organic phases were dried over anhydrous Na₂SO₄, filtered and
376 concentrated under vacuum. The target alcohol **(-)-3** was obtained without further purification as a
377 colorless oil (65 mg, Y = 80%, ee = 99%, [α]_D²⁵ = -33 (c = 1.3, MeOH)). Spectroscopic data of **(-)-3** were
378 in fully accordance with those reported for its corresponding racemic analogue **rac-2**.

379

380 *Oxidation with KMnO₄ to give (+)-5 and (-)-5.*

381 To a solution of the alcohol **(+)-3** or **(-)-3** (1 equiv) in CH₃CN (30 mL/mmol), KMnO₄ (6 equiv) was
382 added portionwise at 0°C. The reaction flask was maintained at 4°C overnight (refrigerator). The
383 reaction mixture was quenched at 0°C with a saturated solution of Na₂S₂O₅ until complete
384 decoloring. The mixture was then filtered and acetonitrile evaporated under reduced pressure. The
385 residual aqueous solution was then extracted with DCM (3 \times 10 mL). The collected organic phases
386 were dried over anhydrous Na₂SO₄, filtered and concentrated. The desired products **(+)-5** and **(-)-5**
387 were obtained without further purification as sticky solid. **(+)-5**: Y = 75%, ee = 99%, [α]_D²⁵ = +70 (c =
388 1.3, MeOH); **(-)-5**: 70%, ee = 99%, [α]_D²⁵ = -68 (c = 1.0, MeOH). Spectroscopic data of **(+)-5** and **(-)-5**
389 were in fully accordance with those reported for their corresponding racemic analogue **rac-1**.

390

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

392 To a solution of CH₃COSK (27 mg, 0.24 mmol, 1.2 equiv) in H₂O (1.5 mL) warmed at 45°C, compound
393 **(+)-5** (25 mg, 0.2 mmol, 1 equiv) dissolved in acetone (0.5 mL) was added dropwise. At completion
394 (50 min, TLC monitoring), acetone was evaporated under reduced pressure and the residual
395 aqueous solution was then extracted with EtOAc (5 \times 10 mL). The collected organic phases were dried
396 over anhydrous Na₂SO₄, filtered and concentrated. Compound **6** was yielded as a yellow oil (16 mg,

397 55%) without further purification. Spectroscopic data were in fully accordance with those reported
398 in literature [24].

399

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

401 In a 25 mL 3-neck flask under inert atmosphere (N₂), Zn powder (203 mg, 3.12 mmol, 8 equiv) and
402 THF (1 mL) were introduced followed by TMSCl (20 μ L, 0.155 mmol, 0.39 equiv). After 30 min of
403 stirring the temperature was raised to 30-32 °C and a solution of benzylbromoacetate (247 μ L, 1.56
404 mmol, 4 equiv) in THF (2 mL) was slowly added dropwise. After 30 min of stirring the mixture was
405 cooled to rt and decanted. The limpid grey supernatant was slowly added dropwise into a 25 mL
406 flask under nitrogen containing a solution of **(+)**5 (50 mg, 0.39 mmol, 1 equiv) in anhydrous THF (2.2
407 mL) at 0°C. The mixture was stirred at rt for 3 h, quenched with a saturated Seignette salt (potassium
408 sodium tartrate) solution and extracted with EtOAc (3x10 mL). The organic layers were dried on
409 Na₂SO₄, filtered and concentrated in vacuum. The crude was purified by flash chromatography
410 (Cyclohexane/AcOEt = 1:1) yielding **7** as a white solid (57 mg, 67%). Spectroscopic data were in fully
411 accordance with those reported in literature. [11]

412

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

414 In a round bottom flask under inert atmosphere (N₂), to a solution of dimethyl disulfide (10 μ L, 0.11
415 mmol, 1 equiv) in dry DCM (1 mL), SO₂Cl₂ (13 μ L, 0.16 mmol, 1.5 equiv) was slowly added at 0°C.
416 After 10 minutes, **(+)** 5 or **(-)** 5 (14 mg, 0.11 mmol, 1 equiv) dissolved in DCM (1 mL) was added,
417 followed by dropwise addition of TEA (28 μ L, 0.22 mmol, 2 equiv). After 10 minutes at 0°C, the
418 reaction mixture was warmed to rt and then refluxed for 4 h. At completion (TLC monitoring), the
419 reaction was quenched with saturated aqueous solution of NH₄Cl and the mixture extracted with
420 DCM (3x5 mL). The collected organic phases were dried over anhydrous Na₂SO₄, filtered and
421 concentrated under vacuum. The desired product was obtained as a yellow oil after purification by
422 flash chromatography on silica gel (Cyclohexane/EtOAc = 7:3). **(+)**8: 13 mg, Y=68%, ee = 88%, [α]_D²⁰
423 = +10 (c = 0.6, MeOH); **(-)**8: 12.5 mg, Y=65%, ee = 95%, [α]_D²⁵ = -11.5 (c = 0.8, MeOH). Spectroscopic
424 data were in fully accordance with those reported in literature [24, 25].

425

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431

432

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