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A chemoenzymatic scalable route to optically active (*R*)-1-(pyridin-3-yl)-2-aminoethanol, valuable moiety of β_3-adrenergic receptor agonists

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Abstract—Enantiomerically pure (*R*)-2-chloro-1-(pyridin-3-yl)ethanol has been prepared by kinetic resolution performed in the presence of *Candida antarctica* SP435-L lipase immobilized on a macroporous polyacrylate resin (Novozym 435®). It was converted into (*R*)-1-(pyridin-3-yl)-2-aminoethanol, left-hand side of β_3-adrenergic receptor ligands.

1. Introduction

β_3-Adrenergic receptor agonists are potential drugs for the treatment of obesity.[1] These agents activating such specific receptors, located on the surface of adipocytes, stimulate lipolysis and increase in metabolic rate.[2] A number of different structural classes of β_3-adrenoceptor agonists have been developed.[1] Among these, pyridylethanolamines (**A**, Fig. 1) were found to be potent and selective human β_3-adrenergic receptor agonists.[3–5] For instance, L-770,644 was selected for pre-clinical evaluation due to its favourable oral bioavailability.[6]

The activity of such compounds is strictly dependent on the absolute configuration at C_1 which must be (*R*).[7,8] Pure enantiomers of β-adrenenoceptor ligands have been prepared by 'chemical' asymmetric synthesis,[9–17] crystallization of diastereoisomeric derivatives,[18–20] preparative chiral chromatography[21–25] and enzymatic procedures that in the last 20 years have become more and more attractive.[26–28] In this field, hydrolases are widely used as chiral catalysts for asymmetric acyl transfer reactions between a number of acyl-acceptors and -donors.[29] The broad range of accepted substrates, the numerous reaction types (hydrolysis, esterification, transesterification, amidation, etc.) mediated by these enzymes, along with their high stability in aqueous solutions and in organic solvents, has made hydrolases important catalysts for the preparation of enantiomerically pure building blocks, as valuable precursors of fine chemicals and pharmaceuticals.[28–35]

Enantioselectivity of hydrolase-induced reactions, so far reported, for a direct preparation of optically active β-aminoalcohols depends dramatically on the nature of the substrate, and the enantiomeric excess of the reaction products is often not very high. An exception is the kinetic resolution of propranolol, a β_1-blocker,[36,37] for which suitable reaction conditions (hydrolysis in phosphate buffer/DMF/Tween 80 medium in the presence of porcine pancreas esterase) and computer-aided substrate design (**B**, Fig. 2), not widely usable,[38,39] were identified [in the series of aryloxy propanolamines the higher activity resides in the (*S*)-enantiomer].

Hence, enzyme-catalysed kinetic resolution towards the preparation of β-aminoalcohols is usually performed on their precursors or derivatives, such as β-chlorohydrins,[40–44] β-bromohydrins,[43–45] β-azidohydrins,[46] oxazolidines[47] and oxazolidinones.[48–54]

Herein, we report lipase-mediated kinetic resolution of *rac*-2-chloro-1-(pyridin-3-yl)ethanol [*rac*-**3**], to afford (*R*)-**3**, precursor of (*R*)-1-(pyridin-3-yl)-2-aminoethanol

Keywords: Pyridylethanolamines; Kinetic resolution; *Candida antarctica* SP435-L lipase; β_3-Adrenergic receptor agonist.
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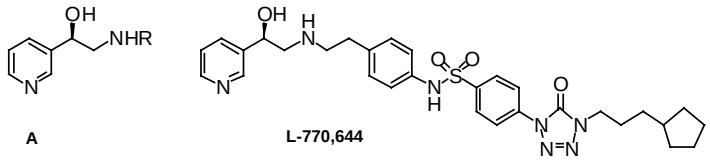


Figure 1.

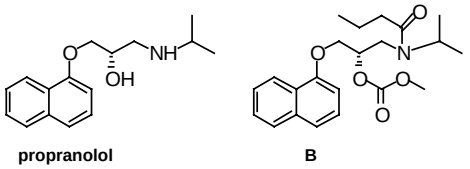


Figure 2.

(**5**), valuable moiety of β_3-adrenergic receptor agonists.[6,55–59]

2. Results and discussion

3-Chloroacetylpyridine hydrochloride (**2**), prepared in good yield (75%) by reacting commercially available 3-acetylpyridine (**1**) with *N*-chlorosuccinimide in acetic acid saturated with gaseous hydrochloric acid,[60] was quantitatively reduced to the corresponding *rac*-2-chloro-1-(pyridin-3-yl)ethanol [*rac*-**3**] with $NaBH_4$ (Scheme 1).[61]

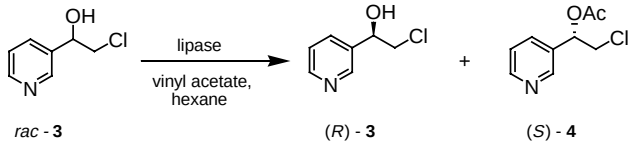
(*R*)-**3** was prepared, as a reference sample for HPLC analysis and absolute configuration assignment, in 93% enantiomeric excess and 4% yield in 4 days of reaction time, by treating **2** with (−)-B-chlorodiisopinocampheylborane [(−)-DIP-Cl] (Scheme 1),[55] a widely used reagent in organic synthesis for asymmetric reduction of prostereogenic ketones.[62]

Since our research group is interested in the development of biocatalytic procedures and biotransformations to prepare enantiomerically pure active ingredients for pharmaceuticals,[31,63–65] a chemoenzymatic synthetic strategy to obtain the highly enantiomerically enriched (*R*)-**3** was chosen.

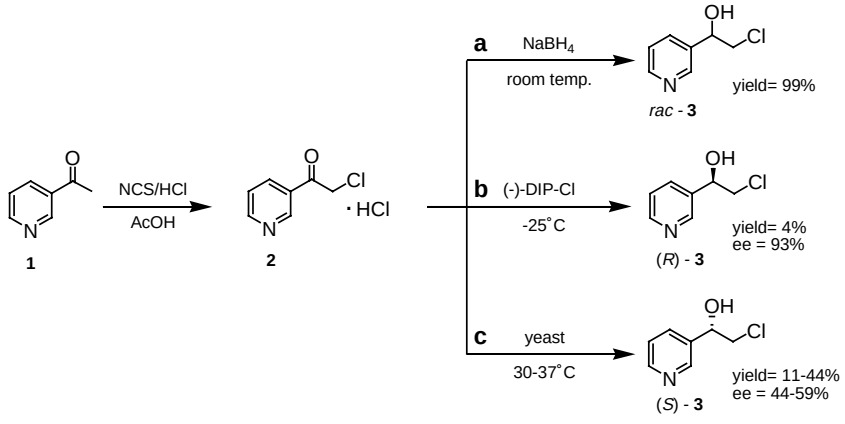
Reduction of **2** in the presence of yeasts with reductive properties such as Baker's yeast and *Kluyveromyces marxianus* CBS 6556 was performed as a first attempt to prepare (*R*)-**3**. As expected, Baker's yeast induced the formation of (*S*)-**3** (44% yield and ee = 59%).[66,67] It is known that *K. marxianus* KCTC 7155 mediated the conversion of prostereogenic ketones into the corresponding (*R*)-alcohols.[68–70] Since we have investigated the reducing capabilities of *K. marxianus* CBS 6556,[64,65] we used this yeast in the bioreduction of **2**. Unfortunately, this reaction afforded again (*S*)-**3** (11% yield and ee = 44%) (Scheme 1).

(*S*)-**3** was previously obtained in quantitative yield and 99.8% ee by using enzymes from other yeasts or recombinant *Escherichia coli* HB101 (pNTSSIG).[70,71]

As a further attempt to prepare (*R*)-2-chloro-1-(pyridin-3-yl)ethanol [(*R*)-**3**] in higher yield and with a methodology suitable for a scale-up, *rac*-**3** was incubated in hexane, in the presence of different lipases and vinyl acetate as acyl donor (Scheme 2). Lipases isolated from the following sources were screened: *Penicillium cyclopium* (G), *Rhizopus delemar* (Rd), *Humicola* sp. (CE-10),



Scheme 2. Lipase-mediated resolution of *rac*-**3**.



Scheme 1. Preparation of 3-chloroacetylpyridine hydrochloride (**2**) and its reduction by $NaBH_4$, (−)-DIP-Cl or yeast.

Candida cylindracea (Type VII CCL), *Candida rugosa* (CRL), *Mucor javanicus* (MAP), *Pseudomonas fluorescens* (PF), *Aspergillus niger* (AP), *Pseudomonas* sp. (K-10), *Pseudomonas* sp. (K-10 immobilized on XAD-8), *Candida antarctica* (SP435-L), *Humicola lanuginosa* (R10-Amano), *Pseudomonas* sp. (AK, immobilized on XAD-8), *Candida cylindracea* (AY-Amano 30), *Mucor meihei* (M-Amano), *Candida cylindracea* (OF-360) and *Rhizopus niveus* (N conc).

Lipases from *Aspergillus niger* (AP), *Penicillium cyclopium* (G), *Pseudomonas fluorescens* (PF), and *Candida antarctica* (SP435-L) were chosen for the detailed investigation, which led to the results reported in Table 1.

Reaction times did not exceed 2 days for all the selected lipases, except for that from *Aspergillus niger* (4 days) for which a very low enantioselectivity ($E = 4$), the lowest enantiomeric excesses for both unreacted substrate (8%) and product (30%), and a 21% extent of conversion were observed. $C = 22\%$ was obtained in the presence of lipase from *Penicillium cyclopium* which mediated the transesterification reaction with a similar enantioselectivity factor value ($E = 5$), and 17% ee for the unreacted substrate and 60% ee for the ester **4**. Enantioselectivity was greatly improved when lipase from *Pseudomonas fluorescens* ($E = 56$) and lipase from *Candida antarctica* SP435-L ($E = 47$) were employed: enantiomeric excess ranged between 64% and 70% for (*R*)-**3** and it was 93% for (*S*)-**4**, while conversion was about twofold higher.

Lipase from *Candida antarctica* is well recognized for carrying out highly enantioselective acylations of racemic secondary alcohols.[73–79] Recovery of enzymes from reaction solutions and often separation of the enzymes from substrates and products are generally difficult. These problems can be successfully tackled by immobilization of the enzyme or by performing the reaction in organic solvents in which the enzyme results not soluble. For these reasons, further investigation of the reaction conditions was carried out for lipase SP435-L from *Candida antarctica* (Table 2), a lipase immobilized on a macroporous polyacrylate resin (Novozym 435®). An immobilized enzyme is definitely more suitable for an automated multi-gram scale reaction.

Performing the kinetic resolution using the reaction conditions **A** and **B** (Table 2), in which lipase/substrate ratio and acyl-donor/substrate ratio are, respectively, 1:10 and 8.5:1, *rac*-**3** concentration was determinant for enantioselectivity: by doubling substrate concentration, the enantioselectivity factor E goes from 62 (**A**) to >100 (**B**). Notwithstanding the high enantioselectivity factor value observed, in both conditions the unreacted recovered substrate showed only a modest enantiomeric enrichment (ee = 24% and 16%, respectively), of course due to the low extent of conversion (C up to 20%).

Since the enzyme reaction with acetaldehyde formed in the transesterification reaction could be responsible for activity and enantioselectivity lowering,[80] we reduced the vinyl acetate/substrate ratio from 8.5:1 to 5:1 (**C**, Table 2). Enantiomeric excess of the unreacted

Table 1. Summary of the results of the lipase-mediated kinetic resolution of *rac*-**3**[a]

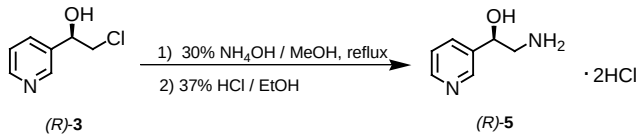
Lipase source	Reaction time (days)	Ee-**3**[b] (%)	Ee-**4**[c] (%)	C[d] (%)	E[e]
		(Absolute configuration)			
Aspergillus niger (AP)	4	8 (*R*)	30 (*S*)	21	4
Penicillum cyclopium (G)	2	17 (*R*)	60 (*S*)	22	5
Pseudomonas fluorescens (PF)	2	70 (*R*)	93 (*S*)	43	56
Candida antarctica (SP435-L)	2	64 (*R*)	93 (*S*)	41	47

[a] All the reactions were carried out as described in Section 4: screening conditions.
[b] Enantiomeric excesses of the remaining unreacted substrate **3** were determined by HPLC.
[c] Enantiomeric excesses of the product **4** were determined by HPLC.
[d] Conversion was determined according to the following equation: $C = ee_s/(ee_s + ee_p)$, where ee_s is the enantiomeric excess of remaining unreacted alcohol **3** and ee_p is the enantiomeric excess of the produced ester **4**.[72]
[e] E, enantioselectivity factor value calculated according to the following equation: $E = \ln[(1 - C)(1 - ee_s)]/\ln[(1 - C)(1 + ee_s)]$.[72]

Table 2. Scale-up of kinetic resolution of *rac*-**3** mediated by lipase from *Candida antarctica* (SP435-L)[a]

Reaction conditions	Lipase/*rac*-**3** (w/w)	Vinyl acetate/*rac*-**3** (mol ratio)	*rac*-**3** Concn. (g/100 mL)	Ee-**3**[b] (%)	Ee-**4**[c] (%)	C[d] (%)	E[e]
A	1:10	8.5:1	1	24	96	20	62
B	1:10	8.5:1	2	16	95	14	>100
C	1:10	5:1	2	13	95	12	48
D	1:1	8.5:1	2	57	97	37	>100
E	1.5:1	8.5:1	2	93	97	49	>100
F	1.5:1	8.5:1	2	>98	87	53	>100

[a] All the reactions here described were incubated at 37 °C for 6 days, with the exception of reaction **F** that took 7 days.
[b] Enantiomeric excesses of the unreacted substrate **3** were determined by HPLC.
[c] Enantiomeric excesses of the product **4** were determined by HPLC.
[d] Conversion determined according to the following equation: $C = ee_s/(ee_s + ee_p)$, where ee_s is the enantiomeric excess of remaining unreacted alcohol **3** and ee_p is the enantiomeric excess of the produced ester **4**.[72]
[e] E, enantioselectivity factor calculated according to the following equation: $E = \ln[(1 - C)(1 - ee_s)]/\ln[(1 - C)(1 + ee_s)]$.[72]



(*R*)-**3** (*R*)-**5**

Scheme 3. Preparation of (*R*)-1-(pyridin-3-yl)-2-aminoethanol [(*R*)-**5**].

substrate was still low (13%, $C = 12\%$) and the reaction had the lowest enantioselectivity factor value ($E = 48$).

To increase the extent of conversion, a higher enzyme/substrate ratio was used (**D**, Table 2) and ee = 57% for the alcohol after 6 reaction days ($E > 100$). This result prompted us to still increase the amount of the enzyme (enzyme/substrate = 1.5:1, **E**, Table 2). In these conditions, after the same reaction time, an almost enantiopure alcohol was obtained with the desired absolute configuration (ee = 93%). The enantioselectivity factor was again very high ($E > 100$). Hence, this procedure allowed the preparation of (*R*)-2-chloro-1-(pyridin-3-yl)ethanol [(*R*)-**3**] with the same enantiomeric purity (ee = 93%) as in the reduction of **2** with (−)-DIP-Cl (Scheme 1), but with a yield 10-fold higher (40% yield), even by costless and environmentally friendly procedure.

Enantiomerically pure (*R*)-**3** (ee > 98%) was obtained just by increasing to seven days the reaction time (row **F** in Table 2).

It has been recently reported that polymeric matrix can affect the enantioselectivity of the reaction.[81] Its swelling properties might, in fact, influence substrate and product diffusion ability. For this reason, enantiomeric excess of compounds **3** and **4** obtained from the suspension and those obtained by washing with methanol the immobilized lipase recovered by filtration were determined. Ee_s of **3** and **4** recovered from both the polymeric matrix and the reaction medium were, respectively, identical. Thus, it seems that in our conditions the presence of the polymer in the reaction suspension has no impact, at least apparently, on the enantioselectivity showed by *Candida antarctica* lipase in catalyzing the kinetic resolution of racemic 2-chloro-1-(pyridin-3-yl)ethanol (**3**), probably due to a rapid dynamic equilibrium that occurs between the polymeric matrix and the reaction medium.[82]

(*R*)-2-Chloro-1-(pyridin-3-yl)ethanol [(*R*)-**3**] was, then, converted into the desired (*R*)-1-(pyridin-3-yl)-2-aminoethanol [(*R*)-**5**],[83] a useful intermediate for the preparation of β_3-adrenergic receptor agonist (Scheme 3).

3. Conclusions

Kinetic resolution of *rac*-2-chloro-1-(pyridin-3-yl)ethanol [*rac*-**3**] was achieved by a transesterification reaction performed in the presence of a lipase from *Candida antarctica* (SP435-L) immobilized on a macroporous polyacrylate resin (Novozym 435®). (*R*)-**3**, obtained with ee > 98%, was then converted into (*R*)-2-amino-1-(pyridin-3-yl)ethanol [(*R*)-**5**]. The lipase from *Candida antarctica* (SP435-L) is one of the most often used enzymes in preparative organic chemistry[84] since it represents a valid alternative to the use of 'classical chemicals' to promote enantioselective reactions. The use of immobilized enzymes has also some advantages because they can be recovered by simply filtering the reaction medium and reused several times. Further studies are currently in progress to optimize the reaction conditions, mainly reaction time, enzyme–substrate ratio and temperature.

The obtained product (*R*)-**5** constitutes the left-hand side of the class of pyridylethanolamines endowed with β_3-adrenoceptor agonist activity. Such compounds are potential thermogenetic agents that might be used for the treatment of obesity, which still represents an open field of pharmaceutical research.

4. Experimental

4.1. General methods

Melting points were taken on an electrothermal apparatus and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ on a VARIAN Mercury 300 MHz or BRUKER AM 500 MHz spectrometer and chemical shifts are reported in parts per million (δ). Absolute values of the coupling constant (J) are reported. IR spectra were recorded on a Perkin-Elmer 681 spectrometer. Reaction progress was monitored by TLC and GC analysis. Thin-layer chromatography (TLC) was performed on silica gel sheets with a fluorescent indicator (Statocrom SIF, 60 F_{254} MERK). GC analyses were performed by using a HP-5MS column (5% phenyl methyl siloxane; 30 m × 0.321 mm × 0.25 μm) on an Agilent 6850 SERIES GC SYSTEM. GC–MS analyses were performed on a HEWLETT PACKARD 6890–5793MSD, and microanalyses on a Elemental Analyzer 1106-Carlo Erba instrument. ESI-MS analyses were performed on an Agilent 1100 LC/MSD trap system VL. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter. The enantiomeric excesses and absolute configurations of the reaction products were determined by the HPLC analysis performed on a Perkin-Elmer 200 series with a UV–vis detector 785A on a commercially available Chiralcel OD (Daicel) in isocratic conditions employing *n*-hexane/2-propanol = 95:5, flow rate 1 mL/min, $\lambda = 254$ nm. The extent of conversion was determined according to the following equation: $C = ee_s/(ee_s + ee_p)$, where ee_s is the enantiomeric excess of the remaining unreacted alcohol **3** and ee_p is the enantiomeric excess of the produced ester **4** determined by HPLC. The ee_s and the extent of conversion (C) were used to calculate the enantioselectivity factor values (E).

4.2. Materials

Lipases used were supplied from either Amano Enzyme Co. Meito Sangyo, or Sigma Chemical Co. In particular, lipase from *Candida antarctica* (SP435-L) was a gift of

Novozymes (Denmark). The baker's yeast used to reduce **2** was supplied by Lievitalia. *Kluyveromyces marxianus* CBS 6556 was cultivated under aerobic conditions in a medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1% glucose. Agar-agar (2%) was added to the same medium for cell preservation on agar slants.

All other chemicals and solvents were of the highest quality grade commercially available and purchased from Aldrich Chemical Co. or Sigma Chemical Co.

4.3. Synthesis of 3-chloroacetylpyridine hydrochloride (2)[60]

Gaseous HCl (28 g, 0.77 mol) was slowly introduced into glacial acetic acid (51 mL) kept at 0 °C. The temperature was raised to 20 °C and 3-acetylpyridine (4.5 mL, 0.04 mol) was added dropwise. *N*-chlorosuccinimide (6.85 g, 0.05 mol) was added to the solution. The mixture was stirred overnight at room temperature, then cooled to 15 °C and filtered. The solid was washed with acetic acid and with ethyl acetate, and dried in high vacuum affording a white powder in 76% yield. Mp 200–201 °C (dec). FT-IR (KBr): 3116, 3084, 3055, 3017, 2975, 2930, 2869, 2860-2200, 2109, 1988, 1873, 1717, 1628, 1607, 1548, 1465, 1377, 1345, 1258, 1234, 1194, 1176, 1107, 1065, 1011, 938, 902, 807, 680, 658, 624 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, δ): 14.50–13.20 (br s, 1H, NH^+: exchanges with D_2O); 9.33–9.32 (d, $J = 1.9$ Hz, 1H, aromatic proton); 9.02–9.00 (dd, $J = 5.4$ and 1.5 Hz, 1H, aromatic proton); 8.77–8.73 (m, 1H, aromatic proton); 8.02–7.98 (dd, 1H, $J = 8.1$ and 5.4 Hz, aromatic proton); 5.32 (s, 2H, CH_2Cl). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 190.26, 148.88, 145.32, 142.15, 132.49, 127.00, 48.74. Anal. Calcd for $C_7H_7Cl_2NO$: C, 43.78; H, 3.67; N, 7.29. Found: C, 43.75; H, 3.70; N, 7.29.

4.4. Synthesis of *rac*-2-chloro-1-(pyridin-3-yl)ethanol [*rac*-3][61]

To an ice-bath cooled solution of 3-chloroacetylpyridine hydrochloride (**2**) (3 g, 15.7 mmol) in water (10 mL) and methanol (20 mL) was slowly added $NaBH_4$ (775 mg, 20 mmol). The resulting reaction mixture was stirred at 0 °C for 30 min, warmed at room temperature and stirred for further 2 h. Solvent was removed under reduced pressure. The residue was dissolved in Na_2CO_3 and extracted with ethyl acetate. The organic layer was dried over anhyd Na_2SO_4 and the solvent was evaporated under reduced pressure to afford a viscous yellow oil in quantitative yield. FT-IR (neat): 3600–3000, 2956, 2924, 2853, 1596, 1581, 1479, 1426, 1341, 1319, 1257, 1217, 1192, 1168, 1081, 1046, 1029, 975, 956, 874, 811, 745, 710, 644 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.56 (s, 1H, aromatic proton); 8.51–8.50 (d, $J = 4.7$ Hz, 1H, aromatic proton); 7.78–7.77 (d, $J = 7.9$ Hz, 1H, aromatic proton); 7.33–7.30 (dd, $J = 4.7$ and 7.9 Hz, 1H, aromatic proton); 4.96–4.94 (dd, $J = 8.1$ and 4.2 Hz, 1H, C*H*OH); 4.00–3.50 (s, 1H, O*H*: exchanges with D_2O); 3.76–3.72 (dd, $J = 4.2$ and 11.2 Hz, 1H, CH_2Cl); 3.69–3.65 (dd, $J = 8.1$ and 11.2 Hz, 1H, CH_2Cl). ^{13}C NMR (75 MHz, CD_3OD, δ): 148.04, 147.24, 137.98, 135.07, 123.71, 71.04, 48.80. GC–MS (70 eV) (*m*/*z*) (rel int.) 159 [$M(^{37}Cl)^+$, 2], 157 [$M(^{35}Cl)^+$, 5], 108 (100), 80 (28), 78 (10), 53 (11), 51 (9).

Chromatographic data:[a]

Compound	t_R	k'	α
(−)-(*R*)-**3**	31.72	9.47 (k'_1)	1.13
(+)-(*S*)-**3**	35.41	10.69 (k'_2)	

[a]Stationary phase: Chiralcel OD (Daicel). Mobile phase: *n*-hexane/*iso*-propanol = 95:5; flow rate, 1 mL/min; UV-λ, 254 nm; t_R, retention time (min); k'_1 and k'_2, capacity factors of (−)-(*R*)-**3** and (+)-(*S*)-**3**, respectively; α, separation factor. Capacity factors (k') were calculated by using as t_R° the retention time of the mobile phase prepared replacing *i*-PrOH with *i*-PrOH-d_8 revealed by a refractive index detector.

4.5. (*R*)-3 prepared with (−)-B-chloro-diisopinocampheylborane [(−)-DIP-Cl][55]

Oil (4% yield). $[\alpha]_D^{20} = -34.47$ (*c* 0.55, CH_3OH). Ee = 93%.

4.6. Bioreduction procedures of 3-chloroacetylpyridine hydrochloride (2)

4.6.1. Baker's yeast-mediated reduction of 2. To baker's yeast (250 g) dispersed in tap water (180 mL) 3-chloroacetylpyridine hydrochloride (**2**, 200 mg) in absolute ethanol (5 mL) was added. The suspension was stirred at 250 rpm and kept at 37 °C. Reaction progress was monitored by the GC analysis: 0.5 mL samples were extracted by adding ethyl acetate (1 mL) and then centrifuged at 10,000*g* for 5 min; the supernatant (organic phase) was separated, dried over anhyd Na_2SO_4 and then analysed by GC and/or TLC. The reaction was stopped after 3 h. NaOH (1 N) was added to the mixture to alkaline pH. The mixture so obtained was extracted several times with EtOAc. The extracts were combined and dried over anhyd Na_2SO_4. A yellow oil (44% yield, ee = 59%) was obtained after removal of the solvent under reduced pressure.

4.6.2. *Kluyveromyces marxianus* CBS 6556-mediated reduction of 2. Cells preserved on agar slants at 4 °C were used to inoculate 250-mL flasks containing the cultivation medium (100 mL). The flasks were incubated aerobically at 30 °C on an orbital shaker and stirred at 250 rpm. 250-mL Flasks containing the cultivation medium (100 mL) were then inoculated with 5 mL of the 24 h-old suspension and incubated under the same conditions for 24 h. 1 L-Flask containing the cultivation medium (400 mL) was then inoculated with 5 mL of the latter suspension and incubated for 24 h, and **2** (152 mg) dissolved in ethanol (1 mL) was added when the culture had the optical density of 7.2. Reaction progress was monitored by the GC analysis: 0.5 mL samples were extracted by adding ethyl acetate (1 mL) and then centrifuged at 10,000*g* for 5 min. The supernatant (organic phase) was separated, dried over anhyd Na_2SO_4 and then analysed by GC and TLC. The reaction was stopped after 113 h (~5 days). The content of the flask

was centrifuged. 1 N NaOH was added to the mixture to alkaline pH. The reaction mixture was extracted several times with EtOAc. The extracts were combined and dried over anhyd Na_2SO_4. A yellow oil (11% yield, ee = 44%) was obtained after removal of the solvent under reduced pressure.

4.7. Lipase-catalyzed acylation of *rac*-2-chloro-1-(pyridin-3-yl)ethanol 3: screening conditions

Crude lipase (20 mg) and vinyl acetate (0.1 mL) were added to a suspension of racemic alcohol (20 mg) in hexane (1 mL). The heterogeneous mixture was incubated at 37 °C and stirred at 250 rpm. The reaction progress was monitored by TLC. The enantiomeric excesses of the product formation **4** and remaining unreacted substrate **3** were determined analysing directly the suspension by chiral chromatography.

Chromatographic data:[a]

Compound	t_R	k'	α
(−)-(*R*)-**4**	25.39	7.46 (k'_1)	1.09
(+)-(*S*)-**4**	27.39	8.13 (k'_2)	
(−)-(*R*)-**3**	35.34	10.78 (k'_1)	1.16
(+)-(*S*)-**3**	40.69	12.53 (k'_2)	

[a]Stationary phase: Chiralcel OD (Daicel). Mobile phase: *n*-hexane/*iso*-propanol = 95:5; flow rate, 1 mL/min; UV-λ, 254 nm; t_R, retention time (min); k'_1 and k'_2, capacity factors of (−)-(*R*)-**4**, (+)-(*S*)-**4**, (−)-(*R*)-**3**, (+)-(*S*)-**3**, respectively; α, separation factor. Capacity factors (k') were calculated by using as t_R° the retention time of the mobile phase prepared replacing *i*-PrOH with *i*-PrOH-d_8 revealed by refractive index detector.

4.7.1. Gram-scale Novozym 435®-catalyzed acylation of *rac*-2-chloro-1-(pyridin-3-yl)ethanol (3). Lipase from *Candida antarctica* (SP435-L, Novozym 435®) (2.4 g) and vinyl acetate (8 mL) were added to a suspension of racemic alcohol (1.6 g) in hexane (100 mL). The heterogeneous mixture was incubated at 37 °C and stirred at 250 rpm. The reaction was monitored by TLC and stopped at the time indicated in the Table 1. The mixture was filtered through a sintered glass funnel to recover the lipase. The hexane was removed under reduced pressure. Product (*S*)-**4** and remaining unreacted substrate (*R*)-**3** were separated by chromatography (silica gel, eluent: petroleum ether/ethyl acetate = 8:2).

4.7.1.1. (*S*)-3-[(1-Acetoxy-2-chloro)ethyl]pyridine [(*S*)-4]. Oil (41% yield, ee = 97%). $[\alpha]_D$ +63.28 (*c* 1, $CHCl_3$). IR (neat): 3037, 2961, 1747, 1594, 1579, 1481, 1429, 1373, 1325, 1231, 1045, 1024, 986, 941, 812, 748, 712 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, δ): 8.60–8.59 (d, J = 2.2 Hz, 1H, aromatic proton); 8.57–8.55 (dd, J = 4.9 and 1.8 Hz, 1H, aromatic proton); 7.69–7.65 (dt, J = 8.0 and 1.8 Hz, 1H, aromatic proton); 7.31–7.26 (dd, J = 8.0 and 4.9 Hz, 1H, aromatic proton); 5.97–5.92 (dd, J = 7.0 and 5.2 Hz, 1H, C*H*); 3.82–3.75$_0$ (dd, J = 11.6 and 7.0 Hz, 1H, CH_2); 3.74$_7$–3.69 (dd, J = 11.6 and 5.2 Hz, 1H, CH_2); 2.11 (s, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$, δ): 169.94, 150.32, 148.54, 134.78, 133.06, 123.74, 73.06, 46.18, 21.10. GC–MS (70 eV) *m*/*z* (rel. int.) 201 [M(^{37}Cl)$^+$, 0.2], 199 [M(^{35}Cl)$^+$, 0.4], 163 (21), 150 (6), 140 (6), 121 (73), 108 (100), 104 (30), 78 (16), 51 (15), 43 (36).

4.7.1.2. (*R*)-2-Chloro-1-(pyridin-3-yl)ethanol [(*R*)-3]. Oil (40% yield, ee > 98%). $[\alpha]_D^{20}$ −40.4 (*c* 0.90, CH_3OH).

4.8. Preparation of (*R*)-1-(pyridin-3-yl)-2-aminoethanol dihydrochloride [(*R*)-5][60]

NH_4OH (9.5 mL) was added to a solution of (*R*)-**3** (426 mg, 2.71 mmol) in methanol (12 mL) kept at room temperature. The reaction mixture was stirred for 3 h under reflux and then concentrated under reduced pressure. *n*-Butanol was added to the residue and removed as azeotropic mixture (*n*-butanol, remaining water and excess ammonia) by distillation under reduced pressure. 37% HCl (0.5 mL) was added to the solution and it was concentrated under reduced pressure. The product was recrystallized from absolute ethanol obtaining white crystals in 47% yield. Mp = 198.3–199.2 °C (ethanol). $[\alpha]_D^{20}$ −45.31 (*c* 0.6, H_2O). IR (KBr): 3600–3300, 3209, 3158, 3119, 3098, 3044, 3017, 2960, 2932, 2882, 1633, 1609, 1547, 1490, 1470, 1440, 1259, 1135, 1044, 997, 808, 685 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, δ): 14.35–13.20 (br s, 1H, NH^+: exchanges with D_2O); 8.89–8.88 (d, J = 1.6 Hz, 1H, aromatic proton); 8.86–8.84 (d, J = 5.6 Hz, 1H, aromatic proton); 8.58–8.56 (d, J = 8.0 Hz, 1H, aromatic proton); 8.45–8.10 (br s, 3H, $CH_2NH_3^+$: exchange with D_2O); 8.06–8.01 (dd, J = 8.0 and 5.6 Hz, 1H, aromatic proton); 7.20–5.80 (br s, 1H, OH: exchanges with D_2O); 5.18–5.14 (dd, J = 7.7 and 3.8 Hz, 1H, C*H*OH); 3.23–3.16 (m, 1H, C$H_2NH_3^+$); 3.08–2.99 (m, 1H, C$H_2NH_3^+$). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 143.93, 141.88, 141.81, 140.88, 127.49, 66.86, 45.27. MS-ESI *m*/*z* (%): 139 $[M+H]^+$ (100%).

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