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Chemoenzymatic synthesis of orthogonally protected (3*R*,4*R*)- and (3*S*,4*S*)-*trans*-3-amino-4-hydroxypyrrolidines

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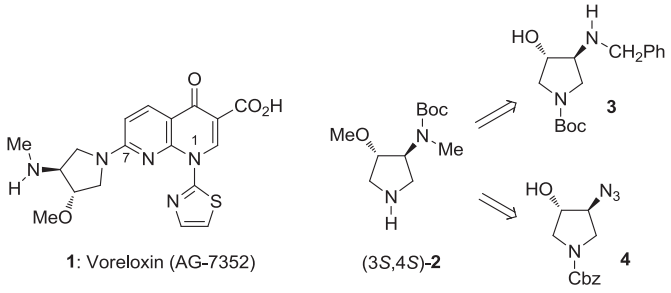
ABSTRACT

Several orthogonally protected racemic *trans*-3-amino-4-hydroxypyrrolidines have been easily prepared from *N*-Cbz-3,4-epoxypyrrolidine. Resolution of each racemic compound was accomplished by means of lipase-catalyzed aminolysis, transesterification or hydrolysis reactions. In most cases, the corresponding remaining substrates and the products were obtained with very high enantiomeric excesses.

1. Introduction

The synthesis of optically active vicinal amino alcohols is a very important task for organic chemists in view of the fact that these compounds have been successfully used as chiral auxiliaries, ligands, and building blocks for complex molecules.[1] Furthermore, non-racemic pyrrolidines constitute key structural units found in a variety of bioactive natural products and drugs.[2] An excellent representative example combining both a vicinal amino alcohol moiety and a pyrrolidine ring is Voreloxin (**1**, Fig. 1), a first-in-class anticancer quinolone derivative, that is, currently completing phase two clinical trials in acute myeloid leukemia and platinum-resistant ovarian cancer.[3] Compound **1** is easily obtained by the coupling of the selectively protected pyrrolidine (3*S*,4*S*)-**2** (Fig. 1) with the appropriate 7-chloro-1,8-naphthyridine derivative.[3a]

Some synthetic approaches aimed at preparing optically active (3*S*,4*S*)-**2** include the resolution of (±)-**3** with mandelic acid,[4] or the enzymatic resolution of the azido derivative (±)-**4**[5] as key steps (Fig. 1). In addition, the chiral pool synthesis of (3*S*,4*S*)-**2** from D-isoascorbic acid has also been described through a 10-step strategy.[6]



1: Voreloxin (AG-7352) (3*S*,4*S*)-**2**

Fig. 1. Optically active pyrrolidine derivatives as precursors of Voreloxin.

As a part of our research on the chemoenzymatic synthesis of optically active functionalized heterocycles,[7] we planned the synthesis and enzymatic resolution of different orthogonally protected *trans*-3-amino-4-hydroxypyrrolidines[8] that can be used as precursors of the optically active pyrrolidine *trans*-**2**. The synthesis strategy reported here avoids the use of azide as a reagent and allows us to study the influence of the protecting group on the exocyclic nitrogen in the enzymatic resolution. Moreover, some of these optically active derivatives are also precursors of diastereomeric *cis*-4-amino-3-hydroxypyrrolidine,[9] which, in turn, has been used in the synthesis of biologically active pyrrolidine nucleotides.[10]

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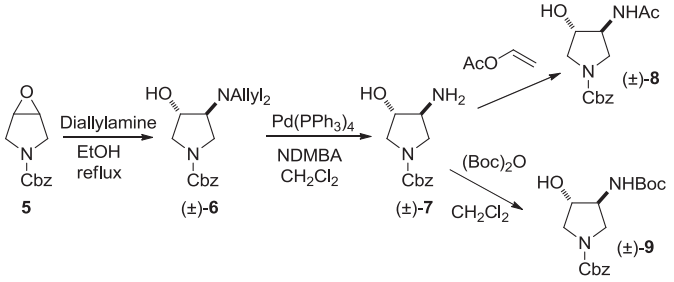
2. Results and discussion

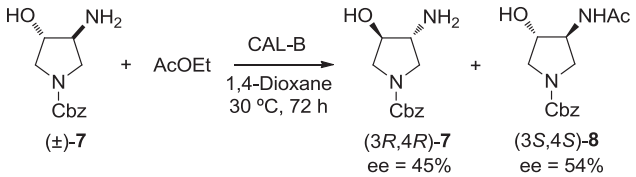
2.1. Synthesis of orthogonally protected racemic *trans*-4-amino-3-hydroxypyrrolidine derivatives, and enzymatic aminolysis of (±)-7

We initially carried out the synthesis of racemic *trans*-3-(diallylamino)-4-hydroxypyrrolidine (±)-**6** by opening the epoxide **5**[7a] with diallylamine. The selection of diallylamine to incorporate the amine function at the 3-position of the pyrrolidine was based on the following reasons: (1) allyl groups are easily removed in the presence of other protecting groups, (2) the opening reaction affords a good yield, and (3) no side-products are formed.[11] Moreover, the presence of a bulky substituent adjacent to the secondary carbon bearing the hydroxyl group could favor the enzymatic resolution of this compound. As our aim in this study was also to investigate the influence of the protecting group on the exocyclic nitrogen, (±)-**6** was converted into the unsubstituted amino alcohol (±)-**7** by removal of the allyl groups with *N*,*N*′-dimethylbarbituric acid (NDMBA) in the presence of Pd(0)[11,12] (Scheme 1). In addition, (±)-**7** was selectively *N*-acylated by treatment with vinyl acetate, and also converted into the *N*-Boc derivative (±)-**9** by reaction with di-*tert*-butyl pyrocarbonate.



Scheme 1. Synthesis of some (±)-*trans*-3-amino-4-hydroxypyrrolidine derivatives.

Compound (±)-**7** contains two groups—amino and hydroxyl—capable of being transformed by a lipase in aminolysis and transesterification reactions, respectively. Given that aminolysis requires less activated esters than transesterification, we started off by studying the aminolysis process. For this purpose, ethyl acetate was used as the acyl donor, and several enzymes [lipases A and B from *Candida antarctica* (CAL-A and CAL-B), lipase from *Burkholderia cepacia* (PSL-IM)], and solvents [*tert*-butyl methyl ether (TBME), toluene, and 1,4-dioxane] were tested. All the processes were carried out on a 25 mg scale monitoring the progress of each reaction by chiral-HPLC. Unfortunately, all the tested enzymes were poor catalysts. The best result is the one shown in Scheme 2. After 3 days of reaction at 30 °C, CAL-B catalyzed the selective acylation of the amino group of **7** (the degree of conversion was 45%), though with very low enantioselectivity (E=5).[13] In this process, the enzyme showed preference for the (3*S*,4*S*) enantiomer of the amino alcohol **7**, contrary to what might be expected on the basis of Kazlauskas' rule.[14]

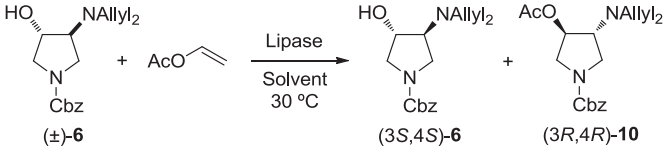


Scheme 2. CAL-B-catalyzed aminolysis of AcOEt with (±)-**7**.

2.2. Enzymatic transesterification of pyrrolidines (±)-6, (±)-8, and (±)-9

Resolution of the *N*,*N*-diallyl derivative (±)-**6** via the transesterification reaction was attempted using vinyl acetate as the acyl donor and employing different lipase–solvent combinations. Initially, these reactions were carried out on a 25 mg scale; a selection of the results obtained is shown in Table 1. As can be seen, the solvent has a strong influence on both the enantioselectivity and the catalytic activity exhibited by CAL-B. This enzyme catalyzed the acetylation of **6** with very high enantioselectivity in TBME and 1,4-dioxane (Table 1, entries 2 and 8, respectively), though only the reaction in TBME was found to be of practical utility. Furthermore, PSL-IM showed very high enantioselectivity in all the tested solvents, and was found to be a more efficient catalyst than CAL-B in toluene and TBME. It is also of note the low catalytic activity exhibited by the PSL toward **6** in comparison with that shown in the transesterification, under analogues conditions, of (±)-*trans*-2-(diallylamino)cyclopentanol (after 3 h of reaction at 28 °C, the conversion degree was 50%; E>200).[11] This means that the benzyloxycarbonyl group on the pyrrolidinic nitrogen has a dramatical effect on the catalytic activity, but not on the enantioselectivity.

Table 1
Lipase-catalyzed transesterification of (±)-**6**[a]

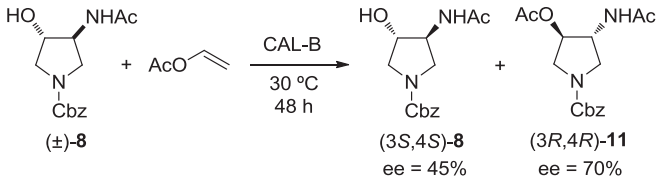


Entry	Enzyme	Solvent	t[b]	ee$_S$ (%)[c]	ee$_P$ (%)[c]	c[d]	E[e]
1	CAL-A	TBME	1	4	29	12	2
2	CAL-B	TBME	3	57	99	37	>200
3	PSL-IM	TBME	3	92	99	48	>200
4	CAL-B	Toluene	5	18	91	17	26
5	PSL-IM	Toluene	5	42	>99	29	>200
6	CAL-B	THF	5	5	90	5	19
7	PSL-IM	THF	5	5	>99	5	>200
8	CAL-B	1,4-Dioxane	5	4	>99	4	>200
9	PSL-IM	1,4-Dioxane	5	4	>99	4	>200

[a] Reactions were carried out using 25 mg (entries 1, 2, 4–9) or 1.0 mmol (entry 3) of substrate. 5 equiv of vinyl acetate was employed in all cases.
[b] Reaction time (days).
[c] Determined by chiral-HPLC (see Section 4.12).
[d] The degree of conversion (%) was calculated from the enantiomeric excesses of the remaining substrate **6** (ee$_S$) and the product **10** (ee$_P$): c=ee$_S$/(ee$_S$+ee$_P$).
[e] See Ref. 13.

In view of the results included in Table 1, the best option to accomplish the resolution of (±)-**6** is to use PSL-IM in TBME (Table 1, entry 3). In this case, both the remaining substrate (3*S*,4*S*)-**6** and the product (3*R*,4*R*)-**10** were isolated with very high enantiomeric excesses (ee≥92%) and yields (49 and 47%, respectively) after 3 days of reaction.

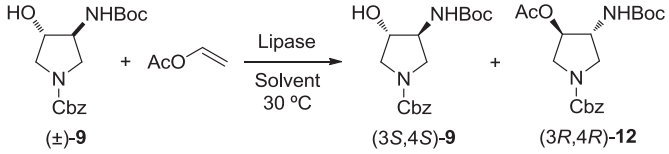
Some attempts to achieve the enantioselective transesterification of the acetamide derivative (±)-**8** were unsuccessful. A drawback of these processes was the low solubility of the substrate in the usual organic solvents. After a screening of solvents, we decided to employ vinyl acetate both as acyl donor and solvent. Under these conditions, CAL-A and CAL-B catalyzed the reaction (a conversion degree of 20 and 39%, respectively, was obtained after 48 h of reaction at 30 °C), though with very low enantioselectivity (E=9). The reaction with PSL-IM was even less enantioselective. In these processes, CAL-A and CAL-B showed opposing enantiopreference,[15] although CAL-B preferentially catalyzed the acetylation of the (3*R*,4*R*) enantiomer (Scheme 3), as predicted by Kazlauskas' rule.



Scheme 3. CAL-B catalyzed transesterification of (±)-**8**.

Similarly to the aforementioned pyrrolidine derivatives, resolution of dicarbamate (±)-**9** was evaluated using several lipases and solvents. A selection of the obtained results is shown in Table 2. For this substrate, CAL-B was found to be the most efficient enzyme in all the tested solvents. Thus, the reaction in 1,4-dioxane, though slow, occurred with high enantioselectivity, and both the remaining substrate and the product were obtained with high ee after 5 days of reaction at 30 °C (Table 2, entry 5). In spite of employing anhydrous conditions in all the transesterification reactions, the prolonged reaction time of this process favors the formation of trace amount of acetic acid as a consequence of the lipase-catalyzed hydrolysis of vinyl acetate. As the resulting acetic acid could negatively affect enzymatic activity,[16] we attempted the transesterification in the presence of triethylamine. Effectively, under these conditions, the enantioselectivity of the reaction continued to be high, the *E* value being similar to that obtained in the absence of Et_3N (Table 2, entry 7). However, a moderate increase in the reaction rate was observed, as the time required to achieve a 50% conversion degree was reduced to 3 days.

Table 2
Lipase-catalyzed transesterification of (±)-**9**[a]



	Enzyme	Solvent	t[b]	ee_S (%)[c]	ee_P (%)[c]	c	E
1	CAL-B	TBME	7	96	58	62	13
2	PSL-IM	TBME	7	97	36	73	8
3	CAL-B	Toluene	5	73	50	59	6
4	PSL-IM	Toluene	5	47	78	38	12
5	CAL-B	1,4-Dioxane	5	93	94	50	110
6	PSL-IM	1,4-Dioxane	5	23	92	20	30
7	CAL-B	1,4-Dioxane[d]	3	94	94	50	120
8	CAL-B	1,4-Dioxane[d]	3.5	>99	91	52	123

[a] Reactions were carried out with 5 equiv of vinyl acetate and 25 mg (entries 1–6) or 200 mg (entries 7 and 8) of substrate. The degree of conversion (*c*, %) and the *E* values were calculated as in Table 1.
[b] Reaction time (days).
[c] Determined by chiral-HPLC (see Section 4.12).
[d] Triethylamine (10% v/v) was added.

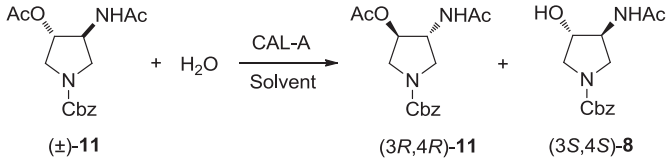
When comparing the results obtained in the transesterification of the analogous racemic substrates (±)-**6**, (±)-**8**, and (±)-**9**, some trends regarding the behavior of the different lipases can be inferred. For instance, PSL-IM is very efficient for **6**, the bearer of a basic diallylamino group, whereas CAL-B was the most active catalyst in the reactions with **9** and **8**, both of which bear a non-basic NHBoc or NHAc group. In addition, CAL-B showed higher enantioselectivity with **9** than with **8**, i.e., with the substrate bearer of the bulkiest substituent.

2.3. Enzymatic hydrolysis reactions of pyrrolidines (±)-10–12

Treatment of the racemic pyrrolidinols (±)-**6**, (±)-**7**, and (±)-**9** with acetyl chloride yielded the acetyl derivatives (±)-**10**–**12**, respectively, which were subsequently submitted to enzymatic hydrolysis. Reactions were carried out in different organic solvents using 5 equiv of H_2O. No satisfactory results were obtained in the hydrolysis of the ester derivatives (±)-**10** and (±)-**12** (the reactions were either very slow or only slightly enantioselective), in contrast to the good results obtained in the transesterification of the corresponding hydroxyl derivative (see Tables 1 and 2).

However, CAL-B-catalyzed hydrolysis of (±)-**11** in 1,4-dioxane was more efficient than the transesterification of its precursor (±)-**8**. After 2 days of reaction at 30 °C, the degree of conversion was 49% and both the product (3*R*,4*R*)-**8** and the remaining substrate (3*S*,4*S*)-**11** were obtained with 84% and 80% ee, respectively, (*E*=28). When the hydrolysis of the diacetyl derivative was attempted with CAL-A in 1,4-dioxane, the reaction was very slow, though the enantioselectivity was very high (Table 3, entry 1). Raising the temperature to 45 °C accelerated the process, but caused a drastic fall in enantioselectivity (entry 2). Other solvents were tested and the best conditions were obtained when the hydrolysis was carried out in toluene at 30 °C (Table 3, entry 3). In this process, both the reaction rate and the enantioselectivity were moderate, which allowed us to isolate the product (3*S*,4*S*)-**8** with high ee (94%). Analogously to the transesterification of (±)-**8**, CAL-A preferentially hydrolyzed the (3*S*,4*S*) enantiomer of **11**, and CAL-B, its counterpart (3*R*,4*R*).

Table 3
CAL-A-catalyzed hydrolysis of (±)-**11**[a]



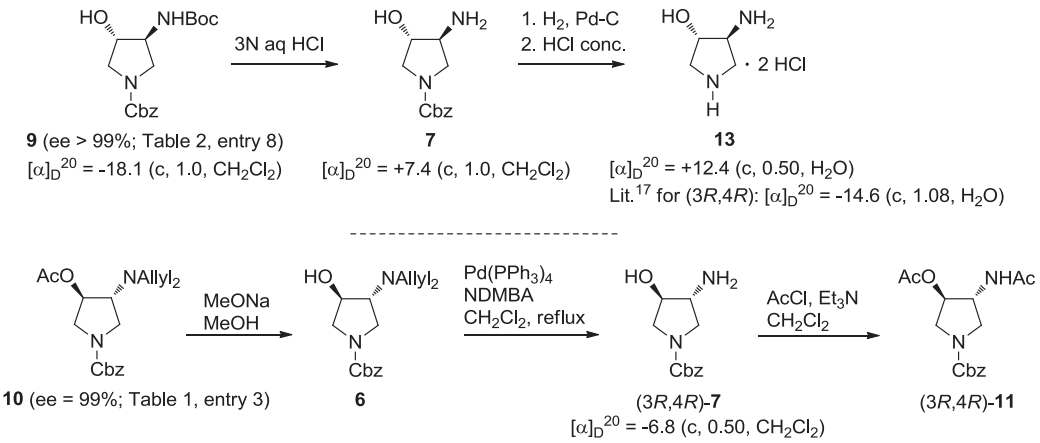
	Solvent	T (°C)	Time (days)	ee_S (%)[b]	ee_P (%)[b]	c	E
1	1,4-Dioxane[c]	30	7	8	>99	8	>200
2	1,4-Dioxane[c]	45	6	38	92	29	35
3	Toluene[d]	30	2	62	94	40	63
4	Toluene[d]	45	2	>99	34	75	16
5	TBME[d]	30	1	21	90	19	23
6	TBME[d]	45	1	>99	19	84	11

[a] 25 mg (entries 1, 2, 4–6) or 100 mg (entry 3) of substrate were employed. The degree of conversion (*c*, %) and the *E* values were calculated as in Table 1.
[b] Determined by chiral-HPLC (see Section 4.12).
[c] 5.0 equiv of water was used.
[d] 2.5 equiv of water was used.

2.4. Assignment of the absolute configuration to the enzymatically prepared compounds

The remaining substrate (−)-**9** (isolated in the enzymatic transesterification reaction with ee>99%: see Table 2, entry 8) was treated with aq 3 N HCl at room temperature to selectively remove the Boc group and isolate (+)-**7**. The subsequent hydrogenolysis of the Cbz group of **7** afforded the unprotected 3-amino-4-hydroxypyrrolidine, which was isolated as its dihydrochloride salt (+)-**13** (Scheme 4, first row). Comparison of the sign of the specific rotation of the obtained **13** with the reported value for (3*R*,4*R*)-(−)-**13**[17] establishes the absolute configuration (3*S*,4*S*) for its precursor (−)-**9**, and hence the (3*R*,4*R*) configuration for the enzymatically produced ester **12**. Once the configuration of (−)-**9** had been assigned, the (3*S*,4*S*) configuration for the intermediate (+)-**7** was also established.

The optically active *N*,*N*-diallyl derivative **10** [produced in the enzymatic transesterification reaction of (±)-**6**] was selectively hydrolyzed using a methanolic solution of sodium methoxide



Scheme 4. Assignment of the absolute configuration of the enzymatically prepared enantioenriched compounds.

(Scheme 4, second row). Removal of the allyl groups of the resulting pyrrolidinol **6** afforded (−)-**7**, which has the (3*R*,4*R*) configuration, as was established after comparison of the sign of its optical rotation with that of the preceding compound **7** (Scheme 4, first row). Thus, the absolute configuration (3*R*,4*R*) was assigned to the enzymatically produced **10**. In addition, (3*R*,4*R*)-**7** was transformed into (3*R*,4*R*)-**11** by conventional acetylation (Scheme 4, second row). No racemization or epimerization was observed for compounds showed in Scheme 4, such it was proven by the chiral-HPLC and ^{1}H NMR analyses.

Configuration of compound **11** arising from the enzymatic processes shown in Scheme 3 and Table 3 was assigned by comparison of the corresponding chiral-HPLC chromatogram with that obtained for (3*R*,4*R*)-**11**. Once the configuration for the diacetyl derivative **11** had been established, the configuration of the acetamide **8** was also assigned. Lastly, configuration of both the remaining substrate **7** and the product **8**, isolated in the enzymatic aminolysis of (±)-**7** (Scheme 2), was also determined by chiral-HPLC analysis.

3. Conclusions

Different orthogonally protected racemic *trans*-3-amino-4-hydroxypyrrolidine derivatives have been easily prepared starting out from *N*-Cbz-3,4-epoxipyrrolidine. After testing different lipases, solvents and reaction conditions, a successful enzymatic resolution was obtained for each derivative. The transesterification catalyzed by PSL-IM and CAL-B was found to be the most adequate via to resolve the 3-diallylamino (±)-**6** and 3-*tert*-butoxycarbonylamino (±)-**9** derivatives, respectively. The presence of a bulky substituent in the neighboring position to the reactive OH group could be responsible for the high enantioselectivities obtained in these processes. In contrast, these lipases were ineffective in the resolution of the acetylamino derivative (±)-**8**, which bears a smaller substituent. Finally, CAL-A showed moderate enantioselectivity in the hydrolysis of the diacetyl derivative (±)-**11**. Furthermore, the selective deprotection of the different groups present in these compounds has also been shown.

4. Experimental section

4.1. General

Lipase B from *C. antarctica* (CAL-B, Novozyme 435, available immobilized on polyacrylamide, 7300 PLU/g) was gifted by Novo Nordisk Co. Immobilized lipase A from *C. antarctica* (CAL-A, NZL-101, 6.2 U/g) was purchased from Codexis. Immobilized lipase from *B. cepacia* (PSL-IM, 783 U/g), which previously was classified as *Pseudomonas cepacia*, was purchased from Amano Pharmaceutical Co. For the enzymatic reactions, anhydrous solvents were used. Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded using KBr pellets (for solids) or neat (for liquids). ^{1}H NMR and proton-decoupled ^{13}C NMR spectra ($CDCl_3$ solutions) were obtained using AC-300 or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometer using the δ scale (parts per million) for chemical shifts; calibration was made on the $CDCl_3$ (^{13}C; 76.95 ppm) or the residual $CHCl_3$ (^{1}H; 7.26 ppm) signals. In almost all cases duplicity of some carbon signals (especially for those of the ring) was observed due to both the presence of different conformers for the pyrrolidine ring and/or *cis*–*trans* isomers around the N(1)–(C=O) bond.[18] In Fig. 2 pyrrolidinic compounds with the numbering used in the assignation of the NMR signals are shown.

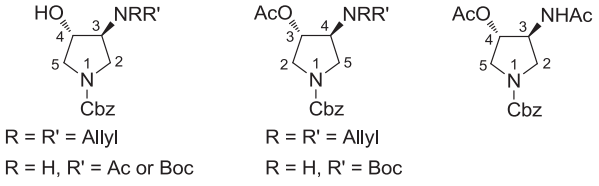


Fig. 2. Pyrrolidinic compounds.

4.2. Synthesis of (±)-*trans*-1-benzyloxycarbonyl-3-(diallylamino)-4-hydroxypyrrolidine [(±)-6]

Diallylamine (3.00 g, 30.6 mmol) was added to a solution of epoxide **5**[7a] (3.20 g, 14.6 mmol) in ethanol (35 mL). After heating at 90 °C for 48 h in a sealing tube, the solvent and the excess of amine were evaporated in vacuo to give the crude product, which was purified by flash chromatography (hexane/AcOEt 1:1). Compound (±)-**6** was obtained as a pale yellow oil (80% yield); ν_{max}(neat) 3422, 1703 cm^{-1}; δ_H (300.13 MHz, $CDCl_3$) 7.41–7.27 (m, 5H, Ph), 5.92–5.73 (m, 2H, 2× C*H*=CH$_2$), 5.29–5.09 (m, 6H, O–CH$_2$, 2× CH=C*H*$_2$), 4.26 (q, 1H, ^3J 6.9 Hz, H-4), 3.88–3.75 (m, 1H, H-5), 3.70–3.55 (m, 1H, H-2), 3.48–3.05 (m, 7H, H-2′, H-3, H-5′, and 2× C*H*$_2$–CH=CH$_2$), 2.71 (br s, 1H, OH); δ_C (75.5 MHz, $CDCl_3$) 154.8 (C=O), 136.6 (C), [135.4 and 135.3 (*C*H=CH$_2$)], 128.4 (CH), 127.9 (CH), 127.7 (CH), [117.8 and 117.7 (CH=*C*H$_2$)], 71.2 and 70.6 (C-4), 66.8 (O–CH$_2$), [66.6 and 66.0 (C-3)], 53.5 (N–CH$_2$), [51.1, 50.7, 44.9, and 44.7 (C-2 and C-5)]; HRMS (ESI$^+$): MH$^+$, found 317.1868. $C_{18}H_{25}N_2O_3$ requires 317.1860.

4.3. Synthesis of (±)-*trans*-3-amino-1-benzyloxycarbonyl-4-hydroxypyrrolidine [(±)-7]

It was prepared from (±)-**6** following the methodology described in Ref. 19. The crude material was purified by flash chromatography (ethyl acetate/methanol 4:1) to yield (±)-**7** (85%) as a colorless oil; ν_{max}(neat) 3351, 3292, 1696 cm^{-1}; δ_H (300.13 MHz, $CDCl_3$) 7.40–7.27 (m, 5H, Ph), 5.11 (s, 2H, O–CH_2), 3.98–3.88 (m, 1H, H-4), 3.79–3.67 (m, 2H, H-2, H-5), 3.38–3.25 (m, 2H, H-2′, H-5′), 3.21–3.08 (m, 1H, H-3), 2.40–2.08 (br s, 3H, OH, NH_2); δ_C (75.5 MHz, $CDCl_3$) 155.1 (C=O), 136.6 (C), 128.4 (CH), 127.9 (CH), 127.7 (CH), [76.1 and 75.5 (C-4)], 66.8 (O–CH_2), [57.4 and 56.8 (C-3)], [53.1, 51.9, 51.7, and 51.4 (C-2, C-5)]; HRMS (ESI$^+$): MNa^+, found 259.1043. $C_{12}H_{16}N_2NaO_3$ requires 259.1053.

4.4. Synthesis of (±)-*trans*-3-(acetamido)-1-benzyloxycarbonyl-4-hydroxypyrrolidine [(±)-8]

A solution of (±)-**7** (0.227 g, 0.961 mmol) in vinyl acetate (5.0 mL) was stirred at 30 °C for 24 h. After this time, solvent was evaporated under reduced pressure and the resulting crude was purified by flash chromatography (ethyl acetate as eluent) to yield (±)-**8** (69%) as a white solid; mp 159–160 °C; ν_{max}(KBr) 3244, 1702, 1647 cm^{-1}; δ_H (300.13 MHz, CD_3OD) 7.42–7.26 (m, 5H, Ph), 5.13 (s, 2H, O–CH_2), 4.18–4.07 (m, 2H, H-3, H-4), 3.80–3.70 (m, 1H, H-2 or H-5), 3.65–3.53 (m, 1H, H-5 or H-2), 3.42–3.30 (m, H-2′, H-5′ overlapped with the signal of the solvent), 1.93 (s, 3H, CH_3); δ_C (75.5 MHz, $CDCl_3$) 173.3 (C=O), 156.8 (C=O), 138.1 (C), 129.5 (CH), 129.1 (CH), 129.0 (CH), [74.7 and 73.9 (C-4)], 68.2 (O–CH_2), [57.3 and 56.5 (C-3)], [53.0, 52.7, 50.2, and 50.1 (C-2, C-5)], 22.4 (CH_3); HRMS (ESI$^+$): MH^+, found 279.1333. $C_{14}H_{19}N_2O_4$ requires 279.1339.

4.5. Synthesis of (±)-*trans*-1-benzyloxycarbonyl-3-(*tert*-butoxycarbonyl)amino-4-hydroxypyrrolidine [(±)-9]

To a solution of (±)-**7** (0.300 g, 1.27 mmol) in anhydrous dichloromethane (12 mL), di-*tert*-butyl pyrocarbonate (0.360 g, 1.64 mmol) was added. After 24 h of reaction at room temperature, solvents were eliminated and the residue was subjected to flash chromatography (hexane/ethyl acetate 1:1). Compound (±)-**9** was isolated as a white solid; yield 87%; mp 140–142 °C; ν_{max}(KBr) 3323, 1682 cm^{-1}; δ_H (300.13 MHz, $CDCl_3$) 7.40–7.30 (m, 5H, Ph), 5.13 (s, 2H, O–CH_2), [4.86 and 4.77 (br s, 1H, NH)], 4.30–4.18 (m, 1H, H-4 or H-3), 4.03–3.89 (m, 1H, H-3 or H-4), 3.84 (dd, 1H, ^3J 6.8, $|^2J|$ 11.0 Hz, H-2 or H-5), 3.72 (dd, 1H, ^3J 5.7, $|^2J|$ 11.8 Hz, H-5 or H-2), 3.48–3.23 (m, 2H, H-2′, H-5′), 2.90 (br s, 1H, OH), 1.44 (s, 9H, ^{t}Bu); δ_C (75.5 MHz, $CDCl_3$) [156.2 and 155.7 (C=O)], 154.8 (C=O), 136.4 (C), 128.4 (CH), 128.0 (CH), 127.8 (CH), 80.4 (C), [74.8 and 73.7 (C-4)], 67.0 (O–CH_2), [57.1 and 56.6 (C-3)], [51.5, 51.2, and 49.2 (C-2, C-5)], 28.2 (CH_3); HRMS (ESI$^+$): MNa^+, found 359.1589. $C_{17}H_{24}N_2NaO_5$ requires 359.1577.

4.6. Synthesis of (±)-*trans*-3-(acetamido)-4-acetoxy-1-(benzyloxycarbonyl)pyrrolidine [(±)-11]

Anhydrous pyridine (1.6 mL, 20 mmol) and acetyl chloride (1.4 mL, 20 mmol) were added to a solution of (±)-**7** (1.0 g, 4.2 mmol) in anhydrous dichloromethane (50 mL). After stirring at room temperature for 12 h, the reaction mixture was successively washed with aq 1 N HCl (3×50 mL) and brine (40 mL). The organic phase was dried with Na_2SO_4 and evaporated to reduced pressure to give a residue, which was subjected to flash chromatography (ethyl acetate as eluent). White solid; yield 79%; mp 119–121 °C; ν_{max}(neat) 1743, 1707, 1683 cm^{-1}; δ_H (300.13 MHz, $CDCl_3$) 7.41–7.28 (m, 5H, Ph), 6.45 (br s, 1H, NH), 5.19–5.02 (m, 3H, O–CH_2, H-4), 4.44–4.33 (m, 1H, H-3), 3.90–3.69 (m, 2H, H-2, H-5), 3.53–3.32 (m, 2H, H-2′, H-5′), 2.06 (s, 3H, CH_3), 1.97 (s, 3H, CH_3); δ_C (75.5 MHz, $CDCl_3$) 170.2 (C=O), 154.7 (C=O), 136.1 (C), 128.5 (CH), 128.1 (CH), 127.9 (CH), [75.4 and 74.4 (C-4)], 67.1 (O–CH_2), [53.5 and 52.7 (C-3)], 49.6 (C-2, C-5)], 22.9 (CH_3), 20.9 (CH_3); HRMS (ESI$^+$): MNa^+, found 343.1271. $C_{16}H_{20}N_2NaO_5$ requires 343.1264.

4.7. General procedure for the enzymatic aminolysis and transesterification reactions on a 25 mg scale

To a mixture of racemic substrate (25 mg) and lipase (25 mg), under a nitrogen atmosphere, the corresponding anhydrous organic solvent (0.8 mL) and the acyl donor (5 equiv) were added. The mixture was stirred at 30 °C and 250 rpm, and the progress of the reaction was analyzed by chiral-HPLC. After the time indicated in Scheme 2, and Tables 1 and 2, the enzyme was filtered and washed with methanol. Solvents were eliminated under reduced pressure and the crude material analyzed by chiral-HPLC.

4.8. Enzymatic transesterification of (±)-6 on a 1 mmol scale

The mixture containing substrate (±)-**6** (0.316 g, 1.00 mmol), PSL-IM (0.316 g), TBME (10 mL), and vinyl acetate (0.461 mL, 5.00 mmol) was allowed to react until achieve a degree of conversion nearly 50% (3 days at 30 °C; Table 1, entry 3). After this time, the enzyme was filtered and washed with methanol. Solvents were eliminated under reduced pressure and the resulting crude material, which was formed by the optically active (3*S*,4*S*)-**6** and the ester (3*R*,4*R*)-**10**, was submitted to flash chromatography (hexane/ethyl acetate 3:1).

*4.8.1. (3S,4S)-1-Benzyloxycarbonyl-3-(diallylamino)-4-hydroxypyrrolidine [(3S,4S)-**6**].* Yield 49% (0.155 g, 0.49 mmol); $[\alpha]_D^{20}$ +47.8 (*c* 1.0, $CHCl_3$), ee 92%.

*4.8.2. (3R,4R)-3-Acetoxy-1-(benzyloxycarbonyl)-4-(diallylamino) pyrrolidine [(3R,4R)-**10**].* Colorless oil; yield 47% (0.168 g, 0.47 mmol); $[\alpha]_D^{20}$ −17.2 (*c* 1.0, CH_2Cl_2), ee 99%; ν_{max}(neat) 1742, 1711 cm^{-1}; δ_H (300.13 MHz, $CDCl_3$) 7.42–7.27 (m, 5H, Ph), 5.87–5.69 (m, 2H, 2× C*H*=CH_2), 5.29–5.07 (m, 7H, O–CH_2, H-3, 2× CH=CH_2), 3.87 (dd, 1H, ^3J 6.4, $|^2J|$ 12.0 Hz, H-2), 3.72–3.58 (m, 1H, H-5), 3.52–3.26 (m, 3H, H-2′, H-4, H-5′), 3.24–3.08 (m, 4H, 2× CH_2–CH=CH_2), 2.06 (s, 3H, CH_3); δ_C (75.5 MHz, $CDCl_3$) 170.2 (C=O), 154.5 (C=O), 136.5 (C), [135.3 and 135.1 (*C*H=CH_2)], 128.4 (CH), 127.9 (CH), 127.8 (CH), 117.5 (CH=CH_2), [74.1 and 73.1 (C-3)], 66.9 (O–CH_2), [64.2 and 63.3 (C-4)], 53.2 (N–CH_2), [50.4 and 50.0 (C-2)], 46.5 (C-5), 20.9 (CH_3); HRMS (ESI$^+$): MNa^+, found 381.1787. $C_{20}H_{26}N_2NaO_4$ requires 381.1785.

4.9. Enzymatic transesterification of (±)-9 on a 200 mg scale

The mixture containing substrate (±)-**9** (0.200 g, 0.595 mmol), CAL-B (0.200 g), 1,4-dioxane (6.0 mL), triethylamine (0.60 mL), and vinyl acetate (0.274 mL, 2.98 mmol) was allowed to react at 30 °C. The reaction was monitorized by chiral-HPLC and stopped when the enantiomeric excess for the substrate was >99% (3.5 days; Table 2, entry 8). After this time, the enzyme was filtered and washed with methanol. Solvents were eliminated under reduced pressure and the resulting crude material, which was formed by the optically active (3*S*,4*S*)-**9** and the ester (3*R*,4*R*)-**12**, was submitted to flash chromatography (hexane/ethyl acetate 1:1).

*4.9.1. (3S,4S)-1-Benzyloxycarbonyl-3-(tert-butoxycarbonylamino)-4-hydroxypyrrolidine [(3S,4S)-**9**].* Yield 45% (90 mg, 0.268 mmol); $[\alpha]_D^{20}$ −18.1 (*c* 1.0, CH_2Cl_2), ee>99%.

*4.9.2. (3R,4R)-3-Acetoxy-1-(benzyloxycarbonyl)-4-(tert-butoxycarbonylamino)pyrrolidine [(3R,4R)-**12**].* Yield 46% (0.103 g, 0.274 mmol); $[\alpha]_D^{20}$ −0.7 (*c* 1.0, CH_2Cl_2), ee 91%.; ν_{max}(KBr) 1744,

1719, 1706 cm^{-1}; δ_H (300.13 MHz, $CDCl_3$) 7.41–7.28 (m, 5H, Ph), 6.45 (br s, 1H, NH), 5.19–5.02 (m, 3H, H-4, O–CH_2), 4.44–4.33 (m, 1H, H-3), 3.90–3.69 (m, 2H, H-2, H-5), 3.53–3.32 (m, 2H, H-2′, H-5′), 2.06 (s, 3H, CH_3), 1.97 (s, 3H, CH_3); δ_C (75.5 MHz, $CDCl_3$) 170.2 (C=O), 154.7 (C=O), 136.1 (C), 128.5 (CH), 128.1 (CH), 127.9 (CH), [75.4 and 74.4 (C-4)], 67.1 (O–CH_2), [53.5 and 52.7 (C-3)], 49.6 (C-2, C-5)], 22.9 (CH_3), 20.9 (CH_3); HRMS (ESI^+): MNa^+, found 401.1684. $C_{19}H_{26}N_2NaO_6$ requires 401.1683.

4.10. Enzymatic hydrolysis of (±)-11

To a solution of (±)-**11** (100 mg, 0.313 mmol) in toluene (3.2 mL), CAL-A (200 mg), and H_2O (15 μL) were added. The mixture was allowed to react during 2 days at 30 °C (Table 3, entry 3) as indicated in the general procedure (Section 4.7). The resulting crude material, which was formed by the optically active (3*S*,4*S*)-**8** and (3*R*,4*R*)-**11**, was submitted to flash chromatography (ethyl acetate as the eluent).

*4.10.1. (3S,4S)-3-(Acetamido)-1-benzyloxycarbonyl-4-hydroxypyrrolidine [(3S,4S)-**8**].* Yield 36% (31 mg, 0.113 mmol); $[\alpha]_D^{20}$ −5.9 (*c* 1.0, MeOH), ee 94%.

*4.10.2. (3R,4R)-3-(Acetamido)-4-acetoxy-1-(benzyloxycarbonyl)pyrrolidine [(3R,4R)-**11**].* Yield 55% (55 mg, 0.172 mmol); ee 62%.

4.11. Optically active compounds involved in the determination of the absolute configuration

*4.11.1. (3S,4S)-**7**.* A mixture of (3*S*,4*S*)-**9** (100 mg, 0.297 mmol; ee>99%) and aq 3 N HCl (4.0 mL) was stirred at room temperature during 24 h. Aqueous solution was washed with CH_2Cl_2 (2×5 mL), and then, aq 4 N NaOH was added until pH was strongly basic. The aqueous phase was extracted with CH_2Cl_2 (3×10 mL). Organic phase was dried with Na_2SO_4, and the organic solvents were eliminated to yield enantiopure (3*S*,4*S*)-**7** (yield 70%). $[\alpha]_D^{20}$ +7.4 (*c* 1.0, CH_2Cl_2).

*4.11.2. (3S,4S)-3-Amino-4-hydroxypyrrolidine dihydrochloride [(3S,4S)-**13**].* To a solution of (3*S*,4*S*)-**7** (50 mg, 0.21 mmol) in deoxygenated methanol (2.0 mL), 10% Pd–C (10 mg) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for 7 h. After this time, the catalyst was filtered through a pad of Celite® and washed with methanol. Conc. HCl (several drops) was added to the solution and solvents were evaporated giving (3*S*,4*S*)-**13**·2HCl. Yield 58%; $[\alpha]_D^{20}$ +12.4 (*c* 0.5, H_2O); ee>99%. Ref. 17 for (3*R*,4*R*)-**13**: $[\alpha]_D^{20}$ −14.6 (*c* 1.08, H_2O).

*4.11.3. (3R,4R)-**6**.* Compound (3*R*,4*R*)-**10** (145 mg, 0.405 mmol) was dissolved in a 0.50 M methanolic solution of sodium methoxide (4.0 mL, 2.0 mmol) and the mixture was stirred at room temperature for 4 h. Elimination of the solvent yielded a residue, which was purified by flash chromatography (hexane/ethyl acetate 1:1) as eluent. Yield 93%; $[\alpha]_D^{20}$ −52.6 (*c* 1.0, CH_2Cl_2), ee 99%.

*4.11.4. (3R,4R)-**7**.* It was prepared from (3*R*,4*R*)-**6** as indicated for the racemic compound (±)-**7**. $[\alpha]_D^{20}$ −6.8 (*c* 1.0, CH_2Cl_2), ee 99%.

*4.11.5. (3R,4R)-**11**.* It was obtained from (3*R*,4*R*)-**7** following the procedure described for the racemic compound (±)-**11**. $[\alpha]_D^{20}$ +2.7 (*c* 1.0, CH_2Cl_2), ee 99%.

4.12. HPLC determination of the enantiomeric excesses

The enantiomeric excess for each compound obtained in the enzymatic reactions was determined by chiral-HPLC. Results obtained in the analysis of racemic samples are as follows:

(±)-**6**: Chiralcel OJ-H; hexane/propan-2-ol 94:6, 0.8 mL/min, 30 °C; t_R=14.9 (3*R*,4*R*) and 16.5 (3*S*,4*S*) min; *RS*=1.7.
(±)-**10**: Chiralcel OJ-H; hexane/propan-2-ol 94:6, 0.8 mL/min, 30 °C; t_R=12.1 (3*R*,4*R*) and 13.5 (3*S*,4*S*) min; R_S=1.7.
(±)-**7**: Chiralcel OJ-H; hexane/ethanol 93:7, 0.8 mL/min, 40 °C; t_R=25.4 (3*S*,4*S*) and 28.0 (3*R*,4*R*) min; R_S=2.1.
(±)-**8**: Chiralcel OJ-H; hexane/ethanol 93:7, 0.8 mL/min, 40 °C; t_R=19.7 (3*S*,4*S*) and 22.5 (3*R*,4*R*) min; R_S=3.0. This compound was also analyzed using: Chiralcel OJ-H; hexane/propan-2-ol 92:8, 0.8 mL/min, 40 °C; t_R=17.2 (3*S*,4*S*) and 30.9 (3*R*,4*R*) min; R_S=10.4.
(±)-**11**: Chiralcel OJ-H; hexane/propan-2-ol 92:8, 0.8 mL/min, 40 °C; t_R=35.9 (3*S*,4*S*) and 40.5 (3*R*,4*R*) min; R_S=2.1.
(±)-**9**: Chiralpack IA; hexane/ethanol 91:9, 0.8 mL/min, 30 °C; t_R=19.1 (3*R*,4*R*) and 22.1 (3*S*,4*S*) min; R_S=3.1.
(±)-**12**: Chiralpack IA; hexane:ethanol 91:9, 0.8 mL/min, 30 °C; t_R=15.0 (3*R*,4*R*) and 20.3 (3*S*,4*S*) min; R_S=6.4.

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