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Biocatalytic preparation of chiral 3,4-dihydroxypyrrolidines

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Dedicated to Professor Carmen Nájera in the occasion of her 60th birthday

ABSTRACT

Enzymatic acylations and alcoxycarbonylations of *cis*- and *trans*-3,4-dihydroxypyrrolidines and hydrolysis of their diacylated or dialcoxycarbonylated derivatives have been studied. High enantioselectivity is obtained using *Candida antarctica* lipase B as catalyst in the hydrolysis of the *trans*-diacetyl derivative, while for the desymmetrization of the *cis*-3,4-dihydroxypyrrolidines the best results are obtained in the acylation process catalyzed by *C. antarctica* lipase A.

1. Introduction

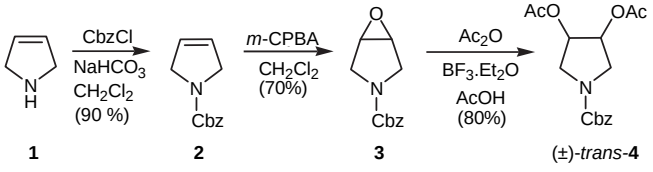
Optically pure 3,4-dihydroxypyrrolidines form an integral feature of many natural compounds and of synthetic derivatives that exhibit interesting biological activities.[1] They are immediate precursors to a promising new class of 3,4-disubstituted pyrrolidine HIV-1 protease inhibitors,[2] as well as to diverse analogues of natural nucleosides[3] and aza-sugars;[4] the often potent inhibitory activity of these compounds toward glycosidases, suggests an enormous therapeutic potential in the treatment of many diseases. Moreover, *trans*-vicinal diols, diamines, and their derivatives occupy a central role in catalytic asymmetry synthesis: 3,4-dihydroxypyrrolidines have been used in the preparation of chiral ligands for asymmetric catalysis[5] or chiral ionic liquids;[6] a novel single-isomer of β-cyclodextrin has been employed as chiral selector in capillary electrophoresis.[7]

Typically, enantiomerically enriched 3,4-dihydroxypyrrolidine derivatives have been synthesized by cyclization of tartaric acid, followed by a low yield hydride reduction step.[1d,5c,8] Biocatalytic methods, such as the use of microbial epoxide hydrolases, have also been successfully employed for the asymmetric ring opening of the *meso*-epoxide, to obtain optically pure *trans*-3,4-dihydroxypyrrolidines.[9]

As a part of our research on the enzymatic preparation of optically active polyhydroxyheterocycles, we are very interested in the enzymatic resolution of *trans*-3,4-dihydroxypyrrolidines, and in the desymmetrization of the *cis*-isomer. Lipase-catalyzed processes are a very advantageous alternative to chemical or microbial methods because they allow the selective functionalization of one of the two hydroxyl groups and constitute an easy access to optically pure functionalized pyrrolidines.

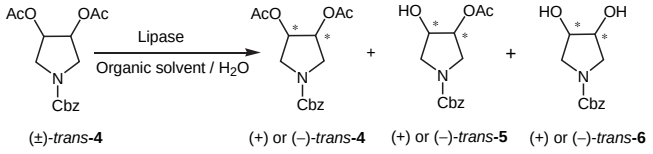
2. Results and discussion

Our initial experiments were designed to find the most suitable lipase for catalyzing the hydrolysis of the diacetylated derivative (±)-*trans*-**4**. This substrate has been prepared in high yield by treatment of 1-benzyloxy-carbonyl-3-pyrroline (**2**) with *m*-chloroperbenzoic acid in CH_2Cl_2[10] and subsequent opening of the resulting epoxide **3** with acetic anhydride and $BF_3 \cdot Et_2O$ (Scheme 1).



Scheme 1. Synthesis of (±)-*trans*-**4**.

Taking into account our previous results in the biocatalytic resolution of *trans*-3,4-dihydroxytetrahydrofuran[11] and *trans*-1-benzyloxycarbonyl-3,4-dihydroxypiperidines,[12] we studied the hydrolysis of (±)-*trans*-**4** in 1,4-dioxane using a small amount of water (5 equiv) as nucleophile (Scheme 2). We tested several commercially available lipases [*Pseudomonas cepacia* (PSL-C and PSL-D) and *Candida antarctica* lipase A and B (CAL-A and CAL-B)]. All the processes were carried out at 30 °C.



Scheme 2. Enzymatic hydrolysis of (±)-*trans*-**4**.

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Under these conditions only lipases CAL-A and CAL-B catalyzed the sequential hydrolysis of substrate (±)-*trans*-**4** (Table 1, entries 1 and 2), whereas, in processes catalyzed by lipases PSL-C and PSL-D no product was observed after 7 days of reaction. The enantioselectivity of the processes catalyzed by lipases CAL-A and CAL-B was very high, even though very low conversion was achieved. Interestingly, a different behavior and an opposite stereochemical preference of the two lipases was observed: in the process catalyzed by CAL-A only the monoacetate (3*S*,4*S*)-**5** was detected (entry 1), whereas a mixture of the monoacetate (3*R*,4*R*)-**5** and the diol (3*R*,4*R*)-**6** was achieved in the hydrolysis catalyzed by CAL-B (entry 2).

Table 1
Lipase catalyzed hydrolysis of (±)-*trans*-**4** at 30 °C in 1,4-dioxane

Entry	Lipase[a]	H_2O (equiv)	*t* (h)	Yield[b] (%) *trans*-**4**	ee_s[c] (%) *trans*-**4**	Yield[b] (%) *trans*-**5**	ee_p[c] (%) *trans*-**5**	Yield (%)[b] *trans*-**6**	ee_p[c] (%) *trans*-**6**	*c*[d] (%)	*E*[d]
1	CAL-A	5	65	—	7	—	>99	—	—	7	>200
2	CAL-B	5	26	90	12	7	>99	4	>99	—	—
3	CAL-A	10	12	—	23	—	>99	—	—	19	>200
4	CAL-A	10	20	69	38	28	79	3	>99	—	—
5	CAL-B	10	4.6	—	20	—	>99	—	—	17	>200
6	CAL-B	10	6.6	83	29	8	>99	8	>99	—	—
7	CAL-B	10	20	63	73	4	>99	33	>99	—	—

[a] Weight ratio enzyme/substrate 1:1.
[b] Determined by GC.
[c] Determined by chiral HPLC.
[d] Conversion, $c=ee_s/(ee_s+ee_p)$, enantiomeric ratio, $E=\ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.[13] These equations are used when only one product is achieved in the kinetic resolution.

The absolute configurations of the products and the remaining substrate were assigned as follows. The hydrolysis of the remaining substrate-*trans*-**4** or the monoacetate *trans*-**5** using NaOMe in MeOH, afforded the diol *trans*-**6**, whose specific rotation sign was compared with that reported for (3*R*,4*R*)-(+)-**6**.[9b]

First, we increased the weight ratio enzyme/substrate to 2:1 (200 mg of enzyme/100 mg substrate in a typical experiment). Table 2 summarizes the results of these experiments. For both enzymatic processes, the reaction rate increased considerably in these conditions.

Table 2
Lipase catalyzed hydrolysis of (±)-*trans*-**4** in 1,4-dioxane, using 5 equiv of H_2O and a weight ratio enzyme/substrate 2:1

Entry	Lipase	*T* (°C)	*t* (days)	Yield[a] (%) *trans*-**4**	ee_s[b] (%) *trans*-**4**	Yield[a] (%) *trans*-**5**	ee_p[b](%) *trans*-**5**	Yield[a] (%) *trans*-**6**	ee_p[b] (%) *trans*-**6**	*c*[c](%)	*E*[c]
1	CAL-A	30	5	—	28	—	81	—	—	25	12
2	CAL-A	30	6	71	33	27	76	2	>99	—	—
3	CAL-B	30	5	56	79	5	>99	40	>99	—	—
4	CAL-B	30	11	45	99	2	>99	55	92	—	—
5	CAL-B	50	1	55	87	5	>99	40	92	—	—
6	CAL-B	50	2	49	98	6	39	45	87	—	—
7	CAL-B	50	5	39	>99	8	58	53	62	—	—

[a] Determined by GC in the processes where two products are achieved.
[b] Determined by chiral HPLC.
[c] Conversion, $c=ee_s/(ee_s+ee_p)$, enantiomeric ratio, $E=\ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.[13] These equations are used when only one product is achieved in the kinetic resolution.

In order to improve the conversion of this enzymatic hydrolysis we increased the amount of water to 10 equiv. Entries 3–7 of Table 1 show the progress of the reactions catalyzed by CAL-A and CAL-B in these conditions. Even though the rates of these processes were slightly lower using a double amount of water, one can establish several conclusions from the obtained results. In the hydrolysis catalyzed by CAL-A high enantioselectivity was attained. After 12 days of reaction the optically pure monoacetate (3*S*,4*S*)-**5** was achieved with 19% conversion, (Table 1, entry 3). When this reaction was allowed to continue, the second hydrolytic step took place affording the corresponding diol (3*S*,4*S*)-**6**, (Table 1, entry 4).

The process catalyzed by CAL-B was also slow, after 4.6 days 17% of optically pure monoacetate (3*R*,4*R*)-**5** was achieved (Table 1, entry 5). As in the case of CAL-A, when this reaction was allowed to continue, the second hydrolytic step takes place affording the corresponding diol (3*R*,4*R*)-**6** (Table 1, entries 6 and 7). The two sequential hydrolytic steps showed very high enantioselectivity. Therefore, it seems that CAL-B shows identical stereochemical preference in the two sequential hydrolytic steps.

Taking into account that an increment of the water concentration does not improve the reaction rate (enzymatic processes carried out in a mixture 1,4-dioxane/H_2O 2:1 were slower that using 10 equiv of water), we decided to study the influence of different reaction parameters in the reaction.

In the reaction catalyzed by CAL-A a conversion of 25% had been achieved after 5 days of reaction (Table 2, entry 1); the reaction product showed a moderate enantioselectivity [(3*S*,4*S*)-**5**, ee=81%]. The selectivity of the process was also moderate and after 6 days of reaction the diol (3*S*,4*S*)-**6** was detected, and the enantiomeric excess of monoacetate (3*S*,4*S*)-**5** decreased considerably, (Table 2, entry 2).

Better results were obtained in the process catalyzed by CAL-B (Table 2, entries 3 and 4). A 40% yield of the optically pure diol (3*R*,4*R*)-**6** was achieved after 5 days of reaction (entry 3). When the reaction was incubated for 11 days, the remaining substrate (3*S*,4*S*)-**4** was obtained in an isolated yield higher than 40% and ee of 99% (entry 4).

For a better appreciation of the reaction progress with time, Figures 1 and 2 represent percent of substrate and products and the enantiomeric excess versus time for the reaction catalyzed by CAL-B at 30 °C.

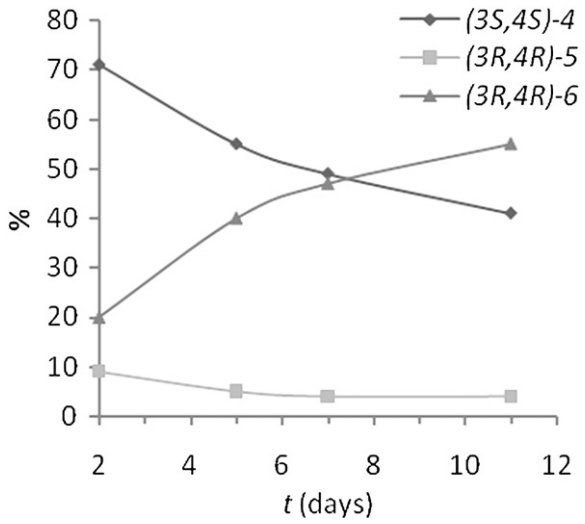


Figure 1. Percent of substrate and products versus time for the CAL-B catalyzed hydrolysis 30 °C in 1,4-dioxane, using 5 equiv of H_2O and a weight ratio enzyme/substrate 2:1.

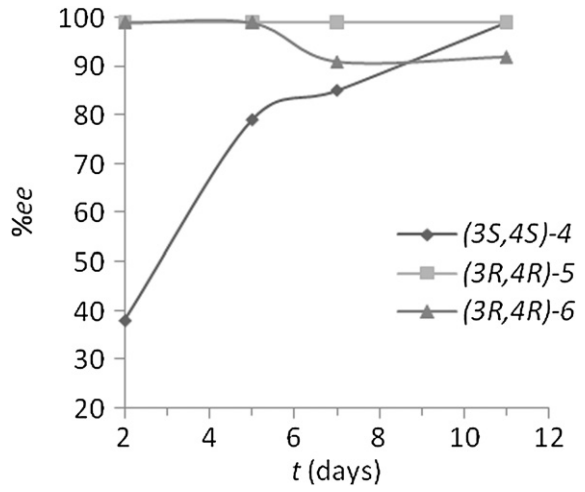


Figure 2. Enantiomeric excess of substrate and products versus time for the CAL-B catalyzed hydrolysis 30 °C in 1,4-dioxane, using 5 equiv of H_2O and a weight ratio enzyme/substrate 2:1.

The effect of the temperature was also analyzed in the process catalyzed by CAL-B (Table 2, entries 5–7). An increase of the temperature to 50 °C enhanced the reaction rate, even though the enantiomeric excess of the diol (3*R*,4*R*)-**6** was slightly lower (entry 5) than at 30 °C. Nevertheless, these are the best conditions to obtain the remaining substrate (3*S*,4*S*)-**4** in an enantiopure form and an excellent yield; after 5 days of reaction 39% of the optically pure substrate is achieved (entry 7). A considerable improvement of the yield is possible (49%) with only a slight loss of enantiomeric excess if the reaction is stopped after 2 days of reaction (entry 6).

Taking into account the good results obtained using 5 equiv of H_2O and a weight ratio enzyme/substrate of 2:1 at 30 °C, a further study of the influence of the organic solvent was carried out for both lipases, in the same conditions. Table 3 shows the results obtained for both enzymes after 5.5 and 13 days in different solvents. In general, processes catalyzed by CAL-A (entries 1–8) were slower and less selective than those catalyzed by CAL-B (entries 9–15). Nevertheless, the result obtained with CAL-A in acetonitrile was noteworthy, because these conditions allowed us to obtain the monoacetate (3*S*,4*S*)-**5** in an enantiopure form with a moderate yield of 21% (entry 3).

For the processes catalyzed by CAL-B, the best results were obtained in THF: 30% of the optically pure diol (3*R*,4*R*)-**6** was achieved after 5.5 days (entry 14) and a 53% of the remaining substrate (3*S*,4*S*)-**4** can be obtained in a 92% of enantiomeric excess after 13 days (entry 15). In general, the results obtained in the processes catalyzed by CAL-B in different solvents did not improve those obtained using 1,4-dioxane (Table 2).

In order to better understand the enantiomeric preference and the enantioselectivity of CAL-A and B in the two sequential hydrolytic steps, we studied the second step by carrying out the enzymatic hydrolysis of the racemic monoacetate (±)-*trans*-**5** in the optimum conditions for each enzyme. As one can see from Scheme 3, the reaction rate was moderate in the process catalyzed by CAL-A and fast in the case of CAL-B. In both processes the enantioselectivity was low.

These data are in accordance with the results obtained in the sequential hydrolysis of substrate (±)-*trans*-**4**. For both enzymes, the first hydrolysis to give the monoacetate **5** must be the slow and enantioselective step of the process. In the process catalyzed by CAL-B, the second step was so fast that only a small amount (less than 10%) of the monoacetate intermediate is detected during the reaction. In the case of CAL-A, the second step was slower and, depending on the reaction conditions, a moderate yield of the optically pure monoacetate intermediate can be obtained, as we indicated previously (Table 3, entry 3).

After the study of the enzymatic hydrolysis of the diacetylated derivative (±)-*trans*-**4** we examined the diol (±)-*trans*-**6** as substrate in enzymatic acylations. We carried out the processes at 30 °C, using different acylating agents (5 equiv) and 1,4-dioxane as solvent, except when ethyl acetate was used itself as solvent (Scheme 4).

The results obtained in the processes catalyzed by CAL-A, CAL-B and PSL-C are summarized in Table 4. In all cases, the stereochemical preferences of the tested enzymes were the same as for the hydrolytic processes, but lower conversions and enantioselectivities were achieved. When ethyl acetate was used as acyl donor (Table 4, entries 1–3), only the monoacetylated product was obtained but the three tested lipases showed low enantioselectivity. The conversion was low in processes catalyzed by CAL-A and PSL-C but a 47% of conversion can be obtained after 15 days of reaction using CAL-B (entry 2). When the ethyl acetate was used itself as solvent (Table 4, entries 4–6), the enzymatic processes were faster but, in the case of CAL-A and CAL-B, a mixture of the mono and diacetylated products were obtained, with moderate enantioselectivity.

Finally, when the vinyl acetate was used as acyl donor (Table 4, entries 7–9), faster processes were achieved and the optically pure remaining substrate was obtained, even thought only in a moderate yield of 19% in the reaction catalyzed by CAL-A (Table 4, entry 7).

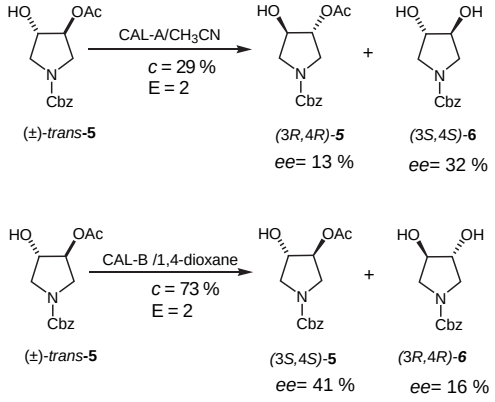
It is remarkable that when the acylation was catalyzed by CAL-B, a 92% yield of monoacetylated (±)-*trans*-**5** was obtained after 12 h of reaction (Table 4, entry 8). This lipase-catalyzed process is a very advantageous alternative to the chemical selective functionalization of one of the two hydroxyl groups.

In order to establish the scope of this enantiomer separation methodology, the best conditions used in the biocatalytic resolution of *trans*-3,4-dihydroxypyrrolidines, were applied in the

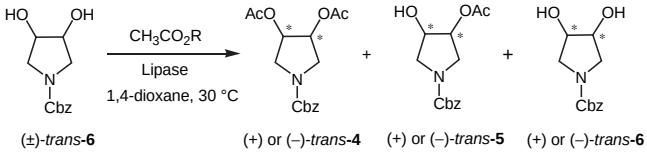
Table 3
Lipase catalyzed hydrolysis of (±)-*trans*-**4** at 30 °C in organic solvents, using 5 equiv of H_2O and a weight ratio enzyme/substrate 2:1

Entry	Lipase	Solvent	*t* (days)	Yield[a] (%) *trans*-**4**	ee_s[b] (%) *trans*-**4**	Yield[a] (%) *trans*-**5**	ee_p[b] (%) *trans*-**5**	Yield[a] (%) *trans*-**6**	ee_p[b] (%) *trans*-**6**	*c*[c] (%)	*E*[c]
1	CAL-A	Toluene	5.5	64	87	29	18	7	>99	—	—
2	CAL-A	Toluene	13	55	90	37	33	8	61	—	—
3	CAL-A	*CH_3CN*	5.5	—	*26*	—	*>99*	—	—	*21*	*>200*
4	CAL-A	CH_3CN	13	66	42	23	62	11	>99	—	—
5	CAL-A	^{t}BuOMe	5.5	7	72	37	36	55	41	—	—
6	CAL-A	^{t}BuOMe	13	—	—	17	23	83	5	—	—
7	CAL-A	THF	5.5	—	10	—	>99	—	—	9	>200
8	CAL-A	THF	13	71	30	25	71	4	>99	—	—
9	CAL-B	Toluene	13	>99	—	—	—	—	—	—	—
10	CAL-B	CH_3CN	5.5	72	53	7	>99	19	>99	—	—
11	CAL-B	CH_3CN	13	54	84	6	>99	40	85	—	—
12	CAL-B	^{t}BuOMe	5.5	7	72	37	36	55	41	—	—
13	CAL-B	^{t}BuOMe	13	12	85	10	6	78	7	—	—
14	CAL-B	THF	5.5	69	57	2	>99	30	>99	—	—
15	CAL-B	THF	13	53	92	2	>99	46	91	—	—

[a] Determined by GC in the processes where two products are achieved.
[b] Determined by chiral HPLC.
[c] Conversion, $c=ee_s/(ee_s+ee_p)$, enantiomeric ratio, $E=\ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.[13] These equations are used when only one product is achieved in the kinetic resolution.



Scheme 3. Enzymatic hydrolysis of (±)-*trans*-**5** at 30 °C in organic solvent, using 5 equiv of H_2O and a weight ratio enzyme/substrate 2:1; reaction time 18 h.



Scheme 4. Enzymatic acetylation of (±)-*trans*-**6**.

hydrolysis or acylation of their *cis*-analogues. Substrates *cis*-**4a**–**c** and *cis*-**6** were synthesized according to Scheme 5. Treatment of 1-benzyloxycarbonyl-3-pyrroline **2** with OsO_4 and *N*-methylmorpholine *N*-oxide (NMMO) yielded the *cis*-**6**, which was acylated or alcoxycarbonylated to the corresponding derivative *cis*-**4a**–**c**.

The enzymatic hydrolysis was carried out at 30 °C in 1,4-dioxane using 5 equiv of H_2O (Scheme 6). The results obtained are summarized in Table 5. For the substrate *cis*-**4a** both enzymes, CAL-A and CAL-B showed low selectivity (Table 5, entries 1 and 2); the process catalyzed by CAL-B was faster but a high percent of the dihydroxylated product *cis*-**6** was achieved.

Next we tested substrates *cis*-**4b** and *cis*-**4c** in the same conditions (Table 5, entries 3–6). No reaction was observed in the enzymatic hydrolysis of *cis*-**4b**. In the hydrolysis of the dicarbonate *cis*-**4c** the best result was obtained in the reaction catalyzed by CAL-A, even though only a 35% yield of the monohydrolyzed product was achieved, with a low enantiomeric excess of 44% (Table 5, entry 5).

Enzymatic transesterification or alcoxycarbonylation of substrate *cis*-**6** were also tested. As for the trans-analogues, the processes were carried out in 1,4-dioxane at 30 °C using 5 equiv of the corresponding ester or carbonate (Scheme 7, Table 6).

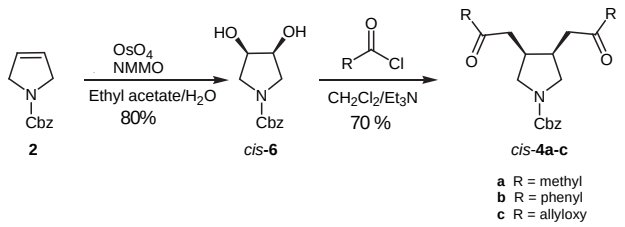
No reaction or very low yields were achieved when ethyl acetate or ethyl benzoate were used as acylating agents (results not show in table).

The results obtained with activated acylating agents are summarized in Table 6. When CAL-A was used as catalyst and vinyl

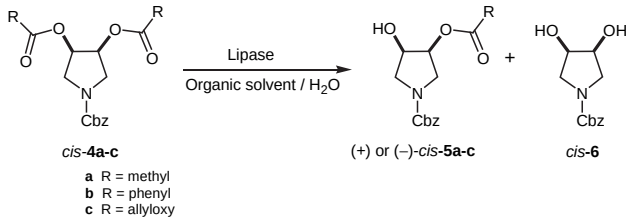
Table 4
Lipase catalyzed transesterification of (±)-*trans*-**6** at 30 °C in organic solvents

Entry	Lipase[a]	R	Solvent	*t* (days)	Yield[b] (%) *trans*-**4**	ee_s[c] (%) *trans*-**4**	Yield[b] (%) *trans*-**5**	ee_p[c] (%) *trans*-**5**	Yield[b] (%) *trans*-**6**	ee_p[c] (%) *trans*-**6**	*c*[d] (%)	*E*[d]
1	CAL-A	Ethyl	1,4-Dioxane	15	—	—	—	78	—	3	4	8
2	CAL-B	Ethyl	1,4-Dioxane	15	—	—	—	4	—	3	47	1
3	PSL-C	Ethyl	1,4-Dioxane	15	—	—	—	85	—	4	5	13
4	CAL-A	Ethyl acetate		10	72	14	2	69	38	17	—	—
5	CAL-B	Ethyl acetate		10	80	13	9	71	7	16	—	—
6	PSL-C	Ethyl acetate		10	—	—	56	—	22	—	29	4
7	CAL-A	Vinyl	1,4-Dioxane	4.5	29	91	52	1	19	>99	—	—
8	CAL-B	Vinyl	1,4-Dioxane	0.5	6	79	92	3	2	>99	—	—
9	PSL-C	Vinyl	1,4-Dioxane	4.5	8	47	80	13	12	>99	—	—

[a] Weight ratio enzyme/substrate 2:1.
[b] Determined by GC in the processes where two products are achieved.
[c] Determined by chiral HPLC.
[d] Conversion, $c=ee_s/(ee_s+ee_p)$, enantiomeric ratio, $E=\ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.[13] These equations are used when only one product is achieved in the kinetic resolution.



Scheme 5. Synthesis of *cis*-**4** and *cis*-**6**.

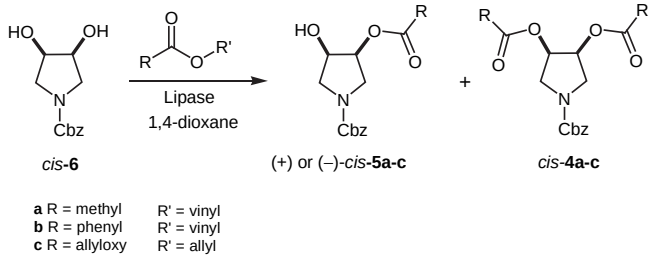


Scheme 6. Enzymatic hydrolysis of *cis*-**4a**–**c**.

Table 5
Lipase catalyzed hydrolysis of *cis*-**4a**–**c** at 30 °C in 1,4-dioxane. using 5 equiv of H_2O

Entry	Lipase[a]	R	*t* (days)	(%)[b] *cis*-**5**	ee_p[c] (%) *cis*-**5**	(%)[b] *cis*-**6**
1	CAL-A	Me	3	13	8	73
2	CAL-B	Me	3	5	6	15
3	CAL-A	Ph	5	—	—	—
4	CAL-B	Ph	5	—	—	—
5	CAL-A	Allyloxy	8	35	44	13
6	CAL-B	Allyloxy	8	6	4	7

[a] Weight ratio enzyme/substrate 2:1.
[b] Determined by HPLC.
[c] Determined by chiral HPLC.



Scheme 7. Enzymatic acylation or alcoxycarbonylation of *cis*-**6**.

Table 6
Lipase catalyzed acylation or alcoxycarbonylation of *cis*-**6**, using 5 equiv of acylating agent and a weight ratio enzyme/substrate 2:1

Entry	Lipase	R	R′	*t* (h)	(%)[a] *cis*-**5**	ee_s[b] (%) *cis*-**5**	(%)[a] *trans*-**4**
1	CAL-A	Me	Vinyl	24	7	10	93
2	CAL-A	Me	Vinyl	2	71	33	2
3	CAL-B	Me	Vinyl	24	89	41	9
4	CAL-A	Ph	Vinyl	168	31	75	—
5	CAL-B	Ph	Vinyl	216	9	0	—
6	CAL-A	Allyloxy	Allyl	96	8	73	—
7	CAL-B	Allyloxy	Allyl	95	12	13	—

[a] Determined by HPLC.
[b] Determined by chiral HPLC.

acetate as acyl donor, a fast reaction was observed; in 24 h a 93% yield of diacetylated product *cis*-**4a** was achieved (Table 6, entry 1). When the process was allowed to react for only 2 h, a 71% yield of (−)-*cis*-**5a** was obtained, but the enantioselectivity of the process was low (ee=33%, entry 2).

When CAL-B was used as catalyst, in the same conditions, the formation of the diacetylated product *cis*-**4a** was slower and the monoacetylated derivative (−)-*cis*-**5a** was obtained in 89% yield; however, the enantioselectivity of the process was only moderate (ee=41%, Table 6, entry 3).

For both catalysts, slower processes were achieved when vinyl benzoate was used as acyl donor (Table 6, entries 4 and 5). However, it is noteworthy the enantomeric excess of 75% of the monobezoylated derivative (−)-*cis*-**5b** obtained in the process catalyzed by CAL-A. Unfortunately, attempts to improve the yield of (−)-*cis*-**5b** by increasing the reaction time failed because of the formation of diol *cis*-**6**.

Finally, an alcoxycarbonylation was carried out using diallylcarbonate as acylating agent. Product (−)-*cis*-**5c** was obtained in low yield in both enzymatic processes (Table 6, entries 6 and 7). The process catalyzed by CAL-A showed a moderate enantioselectivity. However, as in the case of substrate (−)-*cis*-**5b**, attempts to improve the yield of the monoalcoxycarbonylated product by increasing the reaction time, were not possible because of the formation of the diol *cis*-**6**.

3. Conclusion

This paper describes an easy methodology for the enantioselective preparation of both enantiomers of *trans*-1-benzyloxycarbonyl-3,4-dihydroxypyrrolidines via a lipase-catalyzed process. The optically pure diol (3*R*,4*R*)-**6** can be prepared in an excellent yield by the CAL-B catalyzed hydrolysis of the diacetyl derivative (±)-*trans*-**4**, in 1,4-dioxane at 30 °C. The optically pure (3*S*,4*S*)-derivative is obtained as the remaining substrate in the same process; in order to obtain this isomer in good yield, the reaction must be carried out at 50 °C.

Monoacetate (3*S*,4*S*)-**5** can be obtained in an enantiopure form using CAL-A in acetonitrile, although only with a moderate yield of 21%.

On the other hand, the CAL-B catalyzed transesterification of the dihydroxyderivative (±)-*trans*-**6** allows the preparation the monoacetylated derivative (±)-*trans*-**5** in an almost quantitative yield.

Finally, the methodology was applied to the preparation of cis derivatives. Moderate yields and enantiomeric excesses were obtained in the conditions tested. A more exhaustive study of the lipase catalyzed desymmetrization of these substrates needs to be carried out in the future.

4. Experimental

4.1. General remarks

Enzymatic reactions were carried out in a Gallenkamp incubatory orbital shaker. Immobilized *C. antarctica* lipase B, CAL-B (Novozym 435, 7300 PLU/g), was a gift from Novo Nordisk co., immobilized CAL-A (lipase NZL-101, 6,2 U/g) is commercialized by Codexis, immobilized *P. cepacia* lipase (PSL-C, 783 U/g) is commercialized by Amano Pharmaceuticals. Chemical reagents were commercialized by Aldrich, Fluka, Lancaster or Prolabo. Solvents were distilled over an appropriate desiccant under nitrogen. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh). Optical rotations were measured using a Perkin/Elmer 343 polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}.^{1}H NMR, ^{13}C NMR, and DEPT spectra were recorded in a Bruker AC-300, Bruker AC-300 DPX or Bruker NAV-400 spectrometer using $CDCl_3$ as solvent. The chemical shift

values (δ) are given in parts per million. $APCI^+$ and ESI^+ using a Hewlett/Packard 1100 chromatograph mass detector or EI^+ with a Hewlett/Packard 5973 mass spectrometer were used to record mass spectra (MS). IR spectra were recorded in a UNICAM Mattson 3000 FT. The enantiomeric excesses were determined by chiral HPLC analysis on a Hewlett/Packard 1100, LC liquid chromatograph, using a CHIRALPCK IA column (4.5×250 mm) and concentrations by GC analyses on a Hewlett Packard 6890 Series II chromatograph equipped with a column HP-1 (30 m×0.32 mm×0.25 μm, 1.0 bar N_2). For all the analyses, the injector temperature was 70 °C and 5 °C min^{-1} until the FID temperature (230 °C).

4.2. Synthesis of 1-benzyloxycarbonyl-3-pyrroline, (2)

Benzyl chloroformate (3.0 mL, 36.3 mmol) was added drop wise to a stirred mixture of 3-pyrroline **1** (1.0 g, 14.5 mmol) and pyridine (5 mL, 36.3 mmol) in dry CH_2Cl_2 (50 ml) under N_2 atmosphere, the resulting mixture was stirred 4 h at room temperature. After this time, 50 mL of 1 N HCl was added, the organic phase was separated and dried over Na_2SO_4; the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 4:1) to afford the Cbz-protected product **2** as a colorless oil (2.8 g, 95%).

4.3. Synthesis of 1-benzyloxycarbonyl-3,4-epoxy pyrrolidine, (3)

A solution of *m*-CPBA (1.7 g, 14.8 mmol) in dry CH_2Cl_2 (9 mL) was added drop wise to a stirred solution of **2** (1.5 g, 7.4 mmol) in dry CH_2Cl_2 (8 mL) and the mixture was stirred at room temperature for 12 h. The formed solid was removed by filtration and the solution was washed with a 10% aqueous NaOH solution (2×20 mL). The organic phase was dried over Na_2SO_4, the solvent was removed under reduced pressure to afford (**3**) as a crude oil residue in a 70% yield. The residue was used for the preparation of (±)-*trans*-**4** without a further purification. Analytical and spectroscopical data agree with those reported in the literature.[10]

4.4. Preparation of (±)-*trans*-3,4-diacetoxy-1-benzyloxycarbonylpyrrolidine, [(±)-*trans*-4]

To a solution of **3** (1.4 g, 6.5 mmol) in acetic acid (55 mL), acetic anhydride was added (2.5 mL, 26.1 mmol). Boron trifluoride etherate (600 μL, 6.5 mmol) was slowly added, and the mixture was stirred at room temperature for 12 h. An aqueous saturated solution of $NaHCO_3$ (10 mL) was added, and the mixture extracted with EtOAc (20 mL). The organic phase was washed with saturated aqueous Na_2CO_3 solution (20 mL) followed by saturated aqueous NaCl solution (20 mL), and then was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to afford the product (±)-*trans*-**4** as a white solid (1.7 g, 80%). Mp (hexane/EtOAc) 61–62 °C. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 7.39–7.35 (m, 5H), 5.16 (br s, 4H), 3.79–3.74 (m, 2H), 3.64–3.54 (m, 2H), 2.08 (s, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.6 (CO), 154.6 (CO), 136.4 (C), 128.4 (CH), 128.0 (CH), 127.9 (CH), 74.9 (CH), 74.0 (CH), 67.0 (CH_2), 50.2 (CH_2), 50.0 (CH_2), 20.8 (CH_3); IR (KBr): ν 1744, 1708 cm^{-1}; HRMS-ESI^+ calcd for $[C_{16}H_{19}NNaO_6]^+$ $(M+Na)^+$ m/z 344.1105, found 344.1110.

4.5. Preparation of (±)-*trans*-3-acetoxy-1-benzyloxycarbonyl-4-hydroxypyrrolidine, [(±)-*trans*-5]

To a solution of (±)-*trans*-**6** (30 mg, 0.13 mmol) in CH_2Cl_2 (1 mL) and pyridine (11 μL, 0.14 mmol), acetyl chloride (10 μL, 0.14 mmol) was added under nitrogen atmosphere. The mixture was stirred at room temperature for 4 h. Then, the mixture was washed with 1 N HCl (3×1 mL), the organic phase was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 2:3) to afford the product (±)-*trans*-**5** as a colorless oil (13 mg, 36%). This compound can be obtained almost quantitatively by the enzymatic procedure as stated above. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 7.34–7.31 (m, 5H), 5.14 (s, 2H), 5.04–5.02 (m, 1H), 4.27–4.26 (m, 1H), 3.85–3.80 (m, 1H), 3.60–3.48 (m, 2H), 2.72 (br s, 1H, OH), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.4 (CO), 154.9 (CO), 136.5 (C), 128.4 (CH), 127.9 (CH), 127.8 (CH), 77.7 (CH), 77.1 (CH), 67.0 (CH_2), 52.1 (CH_2), 49.3 (CH_2), 20.9 (CH_3); IR (neat, NaCl): ν 3421, 1743, 1687 cm^{-1}; HRMS-ESI^+ calcd for $[C_{14}H_{17}NNaO_5]^+$ $(M+Na)^+$ m/z 302.0999, found 302.1002.

4.6. Preparation of (±)-*trans*-1-benzyloxycarbonyl-3,4-dihydroxypyrrolidine, [(±)-*trans*-6]

To a solution of (±)-*trans*-**4** (1.6 g, 5.07 mmol) in methanol (50 mL), a 0.5 M solution of sodium methoxide in methanol was added. The mixture was stirred at room temperature for 5 h. Then, the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (EtOAc) to afford the product (±)-*trans*-**6** as a colorless oil (0.84 g, 70%). Analytical and spectroscopical data agree with those reported in the literature.[9a]

4.7. General procedure for the enzymatic hydrolysis of (±)-*trans*-4

The reaction mixture, containing (±)-*trans*-**4** (50 mg, 0.15 mmol), the appropriate amount of H_2O and the lipase (100 mg) in the corresponding organic solvent (2 mL), was shaken (generally at 30 °C and 250 rpm) in an orbital shaker. The progress of the reaction was monitored by HPLC analysis of samples that were prepared by taking aliquots (0.1–0.2 mL) until achievement of the required conversion. The enzyme was then removed by filtration and washed with the corresponding organic solvent. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 2:3). The proportion of products and the remaining substrate was determined by GC: t_R 31.98 min product-*trans*-**6**; t_R 32.86 min product-*trans*-**5**; t_R 33.96 min substrate-*trans*-**4**.

4.7.1. Determination of the ee by HPLC analysis. Chiralpack IA, 20 °C, hexane/2-propanol (95:5), UV 210 nm, 0.8 mL min^{-1}, t_R 23.85 min [(3*R*,4*R*)-4]; t_R 25.93 min [(3*S*,4*S*)-4] t_R 40.18 min [(3*S*,4*S*)-5]; t_R 43.29 min [(3*R*,4*R*)-5]; t_R 51.75 min [(3*R*,4*R*)-6]; t_R 58.63 min [(3*S*,4*S*)-4].

(+)-(3*S*,4*S*)-3,4-*Diacetoxy*-1-*benzyloxycarbonyl-pyrrolidine*, [(+)-(3*S*,4*S*)-4]. $[\alpha]_D^{25}$ +17.0 (*c* 1, EtOH), ee >99%.

(+)-(3*R*,4*R*)-1-*Benzyloxycarbonyl*-3,4-*dihydroxy-pyrrolidine* [(+)-(3*R*,4*R*)-6]. $[\alpha]_D^{25}$ +6.7 (*c* 1, $CHCl_3$), ee >99%.

4.8. General procedure for the enzymatic acylation of (±)-*trans*-6

The reaction mixture containing (±)-*trans*-**6** (25 mg, 0.10 mmol), the lipase (50 mg) and the acylating agent in the corresponding organic solvent (1 mL), was shaken (generally at 30 °C and 250 rpm) in an orbital shaker. The progress of the reaction was monitored by HPLC analysis of samples that were prepared by taking aliquots (0.1–0.2 mL) until achievement of

the required conversion. The enzyme was then removed by filtration and washed with the corresponding organic solvent. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 2:3).

4.9. Synthesis of *cis*-3,4-diacetoxy-1-benzyloxycarbonylpyrrolidine, (*cis*-4a)

To a solution of *cis*-**6** (0.2 g, 0.8 mmol) in CH_2Cl_2 (10 mL) and pyridine (0.34 mL, 4.2 mmol), acetyl chloride (0.3 mL, 4.2 mmol) was added. The resulting mixture was stirred at room temperature for 12 h. The solvent was then removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 3:2) to afford the product *cis*-**4** as a colorless oil (1.5 g, 70%); ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.4–7.3 (m, 5H), 5.4–5.3 (m, 2H), 5.15 (br s, 2H), 3.81–3.74 (m, 2H), 3.56–3.45 (m, 2H), 2.08 (s, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 168.9 (CO), 153.6 (CO), 135.4 (C), 127.5 (CH), 127.1 (CH), 127.0 (CH), 69.7 (CH), 69.1 (CH), 66.1 (CH_2), 47.2 (CH_2), 47.0 (CH_2), 19.6 (CH_3); IR (neat, NaCl): ν 1744, 1708 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{16}H_{19}NNaO_6]^+$ $(M+Na)^+$ m/z 344.1105, found 344.1112.

4.10. Synthesis of *cis*-3,4-dibenzoyloxy-1-benzyloxycarbonylpyrrolidine, (*cis*-4b)

To a solution of *cis*-**6** (0.1 g, 0.42 mmol) in CH_2Cl_2 (5 mL) and pyridine (169 μL, 2.1 mmol), benzoylchloride (245 μL, 2.1 mmol) was added. The resulting mixture was stirred at room temperature for 12 h. Then, 5 mL of CH_2Cl_2 was added and the resulting mixture was washed with 1 N HCl (3×10 mL). The organic phase was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 7:3) to afford the product *cis*-**4b** as a white solid (131 mg, 70%). Mp (hexane/EtOAc) 124–127 °C. ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ 8.14–7.33 (m, 15H), 5.76–5.68 (m, 2H), 5.24–5.15 (m, 2H), 4.06–3.99 (m, 2H), 3.83–3.70 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 165.5 (CO), 154.7 (CO), 136.3 (C), 133.6 (CH), 133.4 (CH), 130.1 (CH), 129.7 (CH), 129.5 (CH), 128.5 (C), 128.4 (CH), 128.1 (CH), 128.0 (CH), 71.6 (CH), 70.9 (CH), 67.2 (CH_2), 48.5 (CH_2), 48.3 (CH_2); IR (KBr): ν 1733, 1700 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{26}H_{24}NO_6]^+$ $(M+H)^+$ m/z 446.1598, found 446.1600.

4.11. Synthesis of *cis*-3,4-diallyloxy-1-benzyloxycarbonylpyrrolidine, (*cis*-4c)

To a solution of *cis*-**6** (0.1 g, 0.42 mmol) in CH_2Cl_2 (5 mL), pyridine (169 μL, 2.1 mmol), allyl chloroformate (224 μL, 2.1 mmol) was added under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 24 h. Then, 5 mL of CH_2Cl_2 was added and the resulting mixture was washed with 1 N HCl (3×10 mL). The organic phase was separated and dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the product *cis*-**4c** as colorless oil (119 mg, 70%); ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.39–7.32 (m, 5H), 6.00–5.87 (m, 2H), 5.41–5.10 (m, 6H), 5.20–5.10 (m, 2H), 4.66–4.64 (m, 4H), 3.85–3.81 (m, 2H), 3.68–3.57 (m, 2H); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 154.4 (CO), 154.0 (CO), 136.3 (C), 131.1 (CH), 128.5 (CH), 128.1 (CH), 128.0 (CH), 127.1 (CH), 119.3 (CH_2), 73.9 (CH), 73.4 (CH), 69.0 (CH_2), 66.2 (CH_2), 48.0 (CH_2), 47.6 (CH_2); IR (neat, NaCl): ν 1756, 1712 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{20}H_{23}NNaO_8]^+$ $(M+Na)^+$ m/z 428.1321, found 428.1336.

4.12. Synthesis of *cis*-3-acetoxy-1-benzyloxycarbonyl-4-hydroxypyrrolidine, (±)-*cis*-5a

Analogous procedure to the synthesis of (±)-*trans*-**5**. Colorless oil, yield 35%. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.34–7.33 (m, 5H), 5.19–5.07 (m, 3H), 4.44–4.39 (m, 1H), 3.79–3.69 (m, 2H), 3.57–3.38 (m, 2H), 2.14 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.5 MHz:) δ 169.4 (CO), 153.9 (CO), 135.3 (C), 127.5 (CH), 127.0 (CH), 126.8 (CH), 76.9 (CH), 76.1 (CH), 66.1 (CH_2), 51.1 (CH_2), 48.4 (CH_2), 19.8 (CH_3); IR (neat, NaCl): ν 3421, 1743, 1687 cm^{-1}; MS (APCI$^+$, m/z): 280 [$(M+H)^+$, 84%], 236 (100%). HRMS-ESI$^+$ calcd for $[C_{14}H_{17}NNaO_5]^+$ $(M+Na)^+$ m/z 302.0999, found 302.0992.

4.13. Synthesis of *cis*-3-benzoyloxy-1-benzyloxycarbonyl-4-hydroxypyrrolidine, (±)-*cis*-5b

To a solution of *cis*-**6** (0.1 g, 0.42 mmol) in CH_2Cl_2 (4 mL), pyridine (67 μL, 0.84 mmol), benzoylchloride (98 μL, 0.84 mmol) was added. The resulting mixture was stirred under nitrogen atmosphere at room temperature for 12 h. Then, 5 mL of CH_2Cl_2 was added and the resulting mixture was washed with 1 N HCl (3×10 mL). The organic phase was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 2:3) to afford the product (±)-*cis*-**5b** as a colorless oil (53 mg, 37%); ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 8.12–7.96 (m, 2H), 7.58–7.57 (m, 1H), 7.48–7.34 (m, 2H), 7.37–7.35 (m, 5H), 5.45–5.39 (m, 1H), 5.22–5.08 (m, 2H), 4.57–4.52 (m, 1H), 3.83–3.46 (m, 4H), 2.51 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 171.13 (CO), 166.1 (CO), 154.8 (C), 136.5 (C), 136.4 (CH), 130.0 (CH), 129.7 (CH), 129.1 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.0 (CH), 73.7 (CH), 70.5 (CH), 67.0 (CH_2), 50.4 (CH_2), 48.6 (CH_2); IR (KBr): ν 3423, 1706 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{19}H_{19}NNaO_5]^+$ $(M+Na)^+$ m/z 364.1155, found 364.1153.

4.14. Synthesis of *cis*-3-allyloxy-1-benzyloxycarbonyl-4-hydroxypyrrolidine, (±)-*cis*-5c

To a solution of *cis*-**6** (0.1 g, 0.42 mmol) in CH_2Cl_2 (4 mL), pyridine (101 μL, 1.26 mmol), allyl chloroformate (135 μL, 1.26 mmol) was added under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 2 h. Then, 6 mL of CH_2Cl_2 was added and the resulting mixture was washed with 1 N HCl (3×10 mL). The organic phase was separated and dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 2:3) to afford the product (±)-*cis*-**5c** as a colorless oil (55 mg, 41%); ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 7.43–7.30 (m, 5H), 6.02–5.88 (m, 1H), 5.42–5.41 (m, 1H), 5.37–5.27 (m, 1H), 5.19–5.14 (m, 2H), 5.09–5.02 (m, 1H), 4.70–4.61 (m, 1H), 4.47–4.11 (m, 1H), 4.17–4.09 (m, 1H), 3.82–3.36 (m, 4H), 1.84 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 154.2 (CO), 135.8 (CO), 130.9 (C), 128.6 (CH), 128.4 (CH), 128.3 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 119.6 (CH_2), 69.1 (CH), 68.9 (CH), 67.0 (CH_2), 48.6 (CH_2), 48.2 (CH_2); IR (neat, NaCl): ν 1807, 1743, 1703 cm^{-1}; HRMS-ESI$^+$ calcd for $[C_{16}H_{19}NNaO_6]^+$ $(M+Na)^+$ m/z 344.1105, found 344.1109.

4.15. Synthesis of *cis*-1-benzyloxycarbonyl-3,4-dihydroxypyrrolidine, (*cis*-6)

To a solution of **2** (1 g, 5 mmol) in acetone/H_2O (1:1, 10 mL), *N*-methylmorpholine *N*-oxide (0.87 g, 7.4 mmol) was added, followed by a solution of OsO_4 in *tert*-butanol (0.87 mL, 2.5% w/v).

The mixture was stirred for 2 days at room temperature. A saturated solution of $Na_2S_2O_5$ (50 mL) was added and the mixture was extracted with EtOAc (3×50 mL). The organic phase was dried over Na_2SO_4, the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography on silica gel (EtOAc) to afford the product *cis*-**4** as a white solid (0.95 g, 80%). Mp (hexane/EtOAc) 78–80 °C. Analytical and spectroscopical data agree with those reported in the literature.[14]

4.16. General procedure for the enzymatic hydrolysis of *cis*-**4a**–**c**

The reaction mixture containing *cis*-**4a**–**c** (50 mg), the appropriate amount of H_2O, and the lipase (100 mg) in 1,4-dioxane (2 mL), was shaken (generally at 30 °C and 250 rpm) in an orbital shaker. The progress of the reaction was monitored by HPLC analysis of samples that were prepared by taking aliquots (0.1–0.2 mL) until achievement of the required conversion. The enzyme was then removed by filtration and washed with 1,4-dioxane. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 2:3). The proportion of products and the remaining substrate was determined by GC: t_R 31.98 min product-*cis*-**6**; t_R 32.86 min product-*cis*-**5a**; t_R 33.96 min substrates-*cis*-**4a**.

4.17. General procedure for the enzymatic acylation or alcoxycarbonylation of *cis*-**6**

The reaction mixture containing *cis*-**6** (25 mg, 0.10 mmol), the lipase (50 mg), 1,4-dioxane (1 mL), and the corresponding acylation or alcoxycarbonylation agent (5 equiv), was shaken (generally at 30 °C and 250 rpm) in an orbital shaker. The progress of the reaction was monitored by HPLC analysis of samples that were prepared by taking aliquots (0.1–0.2 mL) until the achievement of the required conversion. The enzyme was then removed by filtration and washed with 1,4-dioxane. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/EtOAc 2:3).

4.17.1. Determination of the ee by HPLC analysis. Chiralpack AS, 30 °C, hexane/2-propanol (90:10), UV 210 nm, 0.8 mL min^{-1}, t_R 21.18 min *cis*-**4b**; t_R 26.21 min [(−)-**5b**]; t_R 31.84 min [(+)-**5b**]; t_R 23.69 min *cis*-**6b**.

(−)-3-*Benzoyloxy*-1-*benzyloxycarbonyl*-4-*hydroxypyrrolidine*, [(−)-**5b**]. $[\alpha]_D^{25}$ −12.7 (*c* 1, EtOH), ee=75%.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.06.060.

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