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# Enantioselective bacterial hydrolysis of amido esters and diamides derived from (±)-*trans*-cyclopropane-1,2-dicarboxylic acid†

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Different optically active amido esters, mixed acid esters, amido acids, and diamides derived from *trans*-cyclopropane-1,2-dicarboxylic acid were prepared from the commercially available diethyl (±)-*trans*-cyclopropane-1,2-dicarboxylate. The key step was the *Rhodococcus rhodochrous* IFO 15564 catalyzed hydrolysis of the corresponding racemic amide. The amidase present in this microorganism showed moderate to high enantioselectivity towards these substrates. In addition a simple and efficient Curtius rearrangement of some of the enzymatically prepared cyclopropanecarboxylic acids allowed us to obtain optically active β-aminocyclopropanecarboxylic acid derivatives with high yields and enantiomeric excesses.

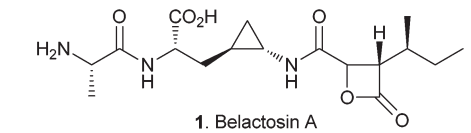
## Introduction

The cyclopropane ring is present in a variety of naturally occurring and synthetic products with a broad array of biological properties.[1] Two representative examples are shown in Fig. 1: the antitumor agent belactosin A (**1**)[2] and the cyclopropyl peptidomimetic **2**, which is an HIV protease inhibitor.[3] The synthesis of compound **2** was easily achieved from an optically active *trans*-cyclopropane-1,2-dicarboxylic acid derivative,[3] and an intermediate in the synthesis of **1**, the β-amino acid (1*S*,2*S*)-*N*,*N*-di(Boc)-*trans*-2-aminocyclopropanecarboxylic acid,[4] could be prepared from (1*S*,2*S*)-*trans*-2-(ethoxycarbonyl)cyclopropanecarboxylic acid.[5]

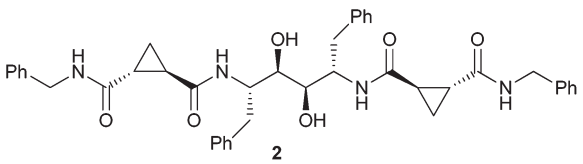
Given that the biological activity of a compound is closely related to the configuration of its chiral centres, the development of simple and efficient methods for the stereoselective preparation of these synthetically useful cyclopropane derivatives is of great interest. In this sense, enzymes are recognized as excellent tools for preparing optically active compounds, either by kinetic resolution (KR) of racemic mixtures or by desymmetrization of *meso* compounds.

Concerning cyclopropane derivatives, the common pig liver esterase (PLE) has been successfully used for the desymmetrization of dimethyl *cis*-cyclopropane-1,2-dicarboxylate;[6] however, this enzyme did not show enantioselectivity in the hydrolysis of its *trans* diastereomer. The KR of the last compound was carried out with an esterase from *Streptomyces diastatochromogenes* (ESD, recombinant from *E. coli*).[7] In addition, Wang *et al.*[8] have explored the potential of the amidase from *Rhodococcus eritropolis* AJ270 to catalyse the kinetic resolution of several *trans*-2-arylcyclopropanecarboxamides. Good enantioselectivities were only obtained in these biotransformations when two additional geminal methyl substituents were present in the cyclopropane ring.[8b] Subsequently, the authors applied this methodology to the resolution of (±)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)-cyclopropanecarboxamide.[8c]

In addition, it has also been demonstrated in our laboratory that the amidase contained in the whole-cells from *Rhodococcus rhodochrous* IFO 15564 enantioselectively catalyzes the hydrolysis of (±)-*trans*-1(or 3)-arylaziridine-2-carboxamides.[9] Based on these results, we planned to study an analogue




**1**. Belactosin A




**2**

**Fig. 1** Biologically active cyclopropane derivatives.

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microbial resolution using different carboxamides derived from (±)-*trans*-cyclopropane-1,2-dicarboxylic acid as substrates. All investigated compounds, lacking substituents at the 3-position, are easily obtained from the commercially available diethyl (±)-*trans*-cyclopropane-1,2-dicarboxylate ((±)-**3a**). Some of the optically active compounds here prepared have previously been used as precursors in the synthesis of **1** and **2**. Moreover, the synthetic utility of these compounds has been proven due to the development of a Curtius rearrangement free of epimerization.

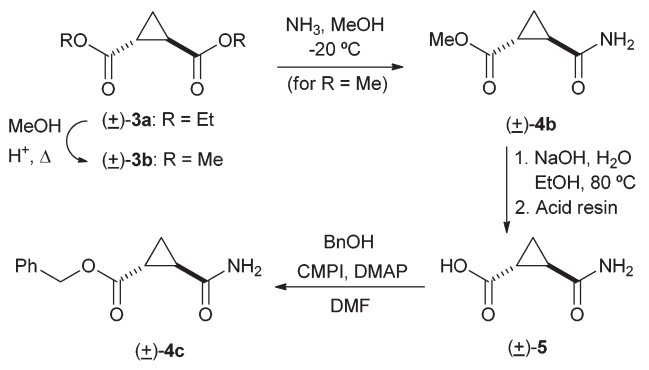
## Results and discussion

Two kinds of racemic substrates, two amido esters (**4b** and **4c**, Scheme 1) and three diamides (**10a–c**) bearing easily removable groups (benzyl and allyl), have been prepared from the commercially available (±)-**3a**. Apart from the above-mentioned importance of these compounds, with this selection we also intended to study the influence of the substrate structure on the enantioselectivity of the microbial amidase. The synthesis and resolution of each type of compound will be described in two different sections.

### Synthesis and resolution of alkyl (±)-*trans*-2-carbamoylcyclopropanecarboxylates

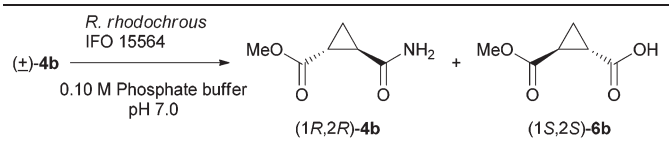
Ammonolysis of (±)-**3a** with a saturated solution of ammonia in methanol slowly yielded a mixture of ethyl and methyl (±)-*trans*-2-carbamoylcyclopropanecarboxylates ((±)-**4a** and (±)-**4b**). However, the dimethyl ester (±)-**3b** (prepared by acid-catalyzed transesterification of (±)-**3a** with methanol) underwent the ammonolysis quicker, and the amido ester (±)-**4b** was isolated with high yield (81%) after 48 h of reaction at −20 °C (Scheme 1).

To study the influence of the alkyl moiety of amido ester **4** in the microbial biotransformation, we also planned the synthesis of benzyl ester (±)-**4c**. After several failed attempts to prepare it by ammonolysis of the dibenzyl diester (±)-**3c** (R = Bn) in THF, (±)-**4c** was obtained with high yield from (±)-**4b** (Scheme 1). First, the ester function was selectively hydrolyzed at 80 °C with 1 equiv. of NaOH in a 1 : 1 water–ethanol mixture. Thus, the monoacid (±)-**5** was isolated with 85% yield




**Scheme 1** Synthesis of amido esters (±)-**4b** and (±)-**4c**.

**Table 1** Biotransformation of (±)-**4b**[a]




| Entry | $A_{650}$ | $t$ (h) | $T$ (°C) | (1*R*,2*R*)-**4b** $ee_S$[d] (%) | (1*S*,2*S*)-**6b** $ee_P$[e] (%) | $c$[f] (%) | $E$[g] |
|---|---|---|---|---|---|---|---|
| 1[b] | 1.5 | 2 | 28 | >99 | 82 | 55 | 59 |
| 2[b] | 1.5 | 2 | 10 | 80 | 95 | 46 | 96 |
| 3[b] | 0.50 | 5.5 | 10 | 68 | 96 | 42 | 100 |
| 4[b] | 0.25 | 23 | 10 | >99 | 91 | 52 | 158 |
| 5 | 0.25 | 21 | 10 | >99 | 71 | 58 | 41 |
| 6[c] | 0.25 | 23 | 10 | 66 | 87 | 43 | 29 |

[a] Reactions were carried out using 100 mg of the substrate, in phosphate buffer (100 mL). [b] Ethanol (1.0 mL) was used as a co-solvent. [c] Acetone (1.0 mL) was used as a co-solvent. [d] Enantiomeric excess of remaining substrate **4b** ($ee_S$) was determined by chiral HPLC (see the ESI). [e] Enantiomeric excess of product **6b** ($ee_P$) was determined by previous treatment with diazomethane and subsequent chiral GC of the resulting dimethyl ester **3b** (see the ESI). [f] The degree of conversion ($c$) was calculated from $ee_S$ and $ee_P$: $c = ee_S/(ee_S + ee_P)$. [g] See ref. 13.

after treatment with an acid ion-exchange resin.[10] The subsequent esterification of (±)-**5** with benzyl alcohol was tried under several conditions. Employing *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling reagent or the application of the Mitsunobu conditions (DEAD and $PPh_3$)[11] gave the required (±)-**4c** with very low yield (20 and 32%, respectively). However, with the Mukaiyama's reagent (CMPI: 2-chloro-1-methylpyridinium iodide)[12] as an activating agent of (±)-**5** and DMAP (4-dimethylaminopyridine) as a base, the benzyl ester (±)-**4c** was isolated with very high yield (98%).

Firstly, the hydrolysis of (±)-**4b** catalysed by the amidase-containing *Rhodococcus rhodochrous* IFO 15564 was carried out using a bacterial suspension in phosphate buffer of an absorbance of $A_{650}$ = 1.5. Ethanol was used as a co-solvent and the reaction mixture was incubated at 28 °C (Table 1, entry 1). Under these conditions the enzyme catalysed the hydrolysis of the amide function of **4b** displaying a high catalytic activity (55% of substrate was transformed after only 2 h of reaction) and a moderate enantioselectivity ($E$ = 59).[13] This $E$-value allowed us to obtain the remaining substrate (1*R*,2*R*)-**4b** with very high enantiomeric excess ($ee_S$ > 99%) at a degree of conversion near to the optimal value of 50%. It is of note that both enantioenriched compounds, (1*R*,2*R*)-**4b** and the hydrolysis product (1*S*,2*S*)-**6b**, could be easily isolated and purified from the aqueous reaction medium by successive basic–acid extractions.

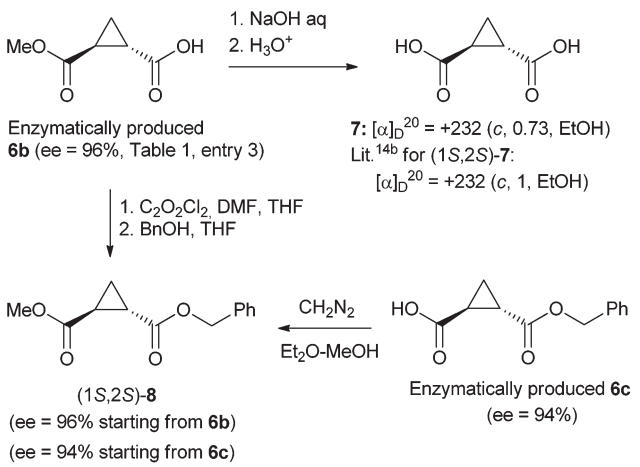
Taking the dependence of $E$ with the temperature into account, and considering that in most reactions the $E$ value increases as the temperature decreases, we tried the hydrolysis of (±)-**4b** lowering the temperature of reaction to 10 °C. Under these conditions, the enzyme showed a higher enantioselectivity and the rate of reaction was only slightly diminished (entry 2). This last fact encouraged us to try the reaction with a smaller amount of enzyme (entries 3 and 4). As indicated in

Table 1, an improved enantioselectivity was achieved using $A_{650}$ = 0.25, though almost 1 day of reaction was necessary to obtain a degree of conversion near to 50%. Nevertheless, from this process both the substrate (1*R*,2*R*)-**4b** and product (1*S*,2*S*)-**6b** were obtained with high ee (entry 4) and yields (41% and 51%, respectively). Finally, the influence of the co-solvent was also investigated in the biotransformation of (±)-**4b**. For this, reactions were performed in the absence of the co-solvent (entry 5) and with acetone (entry 6) as a co-solvent. In both cases, the enantioselectivity was lower than with ethanol, although the reaction rate was slightly enhanced when no co-solvent was employed.

When the biotransformation of the mixed amido benzyl ester (±)-**4c** was carried out with a bacterial suspension of $A_{650}$ = 0.25 at 10 °C, the starting material was recovered unaltered due to the low solubility of this substrate in the reaction medium. After several attempts, the biotransformation of (±)-**4c** was finally carried out at 28 °C and with a bacterial suspension of $A_{650}$ = 1.5. Under these conditions the remaining substrate (1*R*,2*R*)-**4c** and the product (1*S*,2*S*)-2-(benzyloxycarbonyl)cyclopropanecarboxylic acid ((1*S*,2*S*)-**6c**) were obtained with ee = 65% and 94%, respectively ($c$ = 41%, $E$ = 63) after 6 h of reaction. Comparing this result with that obtained for the mixed amido methyl ester **4b** under similar reaction conditions (see Table 1, entry 1), it can be concluded that the microbial amidase displays a lower catalytic activity with the bulkier benzyl derivative **4c** than with **4b**. However, the enantioselectivity exhibited towards both substrates is similar.

The (1*S*,2*S*) absolute configuration for the enzymatically produced **6b**, and thus the enantiopreference of the amidase of *R. rhodochrous* IFO 15564 toward **4b**, was determined after its conversion into *trans*-cyclopropane-1,2-dicarboxylic acid **7** (Scheme 2). Optical rotation of this **7** matched with that published for (1*S*,2*S*)-**7**.[14] Once the (1*S*,2*S*) configuration was established for **6b**, it was transformed into the mixed benzyl methyl diester (1*S*,2*S*)-**8** (Scheme 2). Comparison of the chiral HPLC chromatogram of this compound **8** with that obtained by derivatization of the enzymatically prepared **6c** with diazomethane also established the (1*S*,2*S*) configuration for **6c** and, consequently, the same enantiopreference of the amidase towards both substrates **4c** and **4b**.




**Scheme 2** Assignment of the absolute configuration to the enzymatically obtained products **6b** and **6c**.

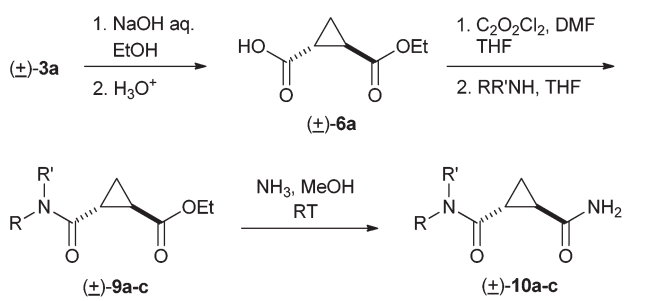
## Synthesis and resolution of *N*-substituted (±)-*trans*-cyclopropane-1,2-dicarboxamides

Three racemic *trans*-*N*-alkyl- or *trans*-*N*,*N*-dialkylcyclopropane-1,2-dicarboxamides (±)-**10a–c** were prepared starting from the diethyl ester (±)-**3a**. Diamide **10a** is structurally analogous to the amido ester **4c**, while **10b** and **10c** are adequate to investigate the influence of the substituents on the nitrogen on the enantioselectivity of the amidase. In addition, they bear easily removable groups, and an optically active monoacid derived from the *N*-benzyl diamide **10a** is a precursor used in the synthesis of **2** (Fig. 1).

Selective hydrolysis of (±)-**3a** (Scheme 3) was carried out in ethanol using 1 equiv. of NaOH (14 M aqueous solution).[15] The resulting monoacid (±)-**6a** was transformed into the acid chloride, which was then treated with an excess of the corresponding amine to yield amido esters (±)-**9a–c**. Finally, the ammonolysis of the ester function of (±)-**9a–c** employing a saturated solution of ammonia in methanol at room temperature yielded the diamides (±)-**10a–c**.

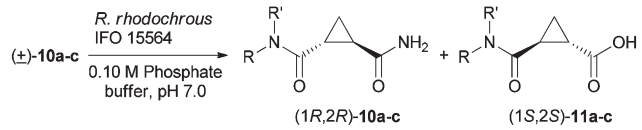
Some initial attempts to carry out the biotransformation of the *N*-benzyldiamide (±)-**10a** at 10 °C with a bacterial suspension of $A_{650}$ = 0.25 were unsuccessful. Under these conditions a high amount of racemic starting material (near 60%) remained undissolved after 21 h of reaction, such as it was proven after filtration of the residual solid and analysis by chiral HPLC. Moreover, *ca*. 30% of unreacted substrate **10a** was also isolated from the solution, which is indicative of a very low rate of reaction. Because this diamide is structurally analogous to substrate **4c**, we decided to try the biotransformation of **10a** under the reaction conditions found for **4c**, that is, using a bacterial suspension with a higher absorbance ($A_{650}$ = 1.5) at 28 °C, and with ethanol as a co-solvent. Solubilization of the substrate in the reaction medium was finally achieved if, prior to the addition of the concentrated bacterial suspension, the substrate is submitted to ultrasound in the phosphate buffer (see the Experimental section).

The most significant result obtained with **10a** is shown in Table 2, entry 1. After 12 h of reaction and a conversion of




**Scheme 3** Synthesis of diamides (±)-**10a–c** (**a**: R = Bn, R′ = H; **b**: R = allyl, R′ = H; **c**: R = allyl, R′ = Me).

Table 2 Biotransformations of diamides (±)-**10a–c**[a]




**a**: R = Bn, R' = H; **b**: R = Allyl, R' = H; **c**: R = Allyl, R' = Me.

| Diamide | *t* (h) | Remaining substrate[b] | $ee_S$[c] (%) | Product[d] | $ee_P$[c] (%) | *c*[e] (%) | *E*[e] |
|---|---|---|---|---|---|---|---|
| (±)-**10a** | 12 | (1*R*,2*R*)-**10a** | 75 | (1*S*,2*S*)-**11a** | 95 | 44 | 88 |
| (±)-**10b** | 22 | (1*R*,2*R*)-**10b** | 93 | (1*S*,2*S*)-**11b** | 92 | 50 | 81 |
| (±)-**10c** | 22 | (1*R*,2*R*)-**10c** | 97 | (1*S*,2*S*)-**11c** | 99 | 50 | >200 |

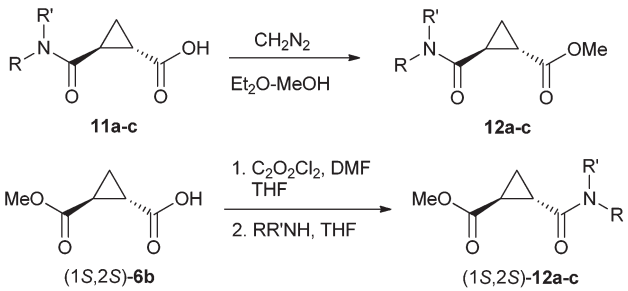
[a] Reactions were carried out using 100 mg of the substrate in phosphate buffer (100 mL), with a bacterial suspension of $A_{650}$ = 1.5, at 28 °C and ethanol (1.0 mL) as a co-solvent. [b] Isolated yields: **10a** (51%), **10b** (44%), and **10c** (47%). [c] Enantiomeric excess (%) of remaining substrate **10** ($ee_S$) and product **11** ($ee_P$) was determined by chiral HPLC (see the ESI). [d] Isolated yields: **11a** (40%), **11b** (43%), and **11c** (48%). [e] The degree of conversion (*c*) and the enantiomeric ratio (*E*) were calculated as in Table 1.

44%, the hydrolysis product (1*S*,2*S*)-**11a** was obtained with a high ee (95%), which means a moderate–high enantioselectivity of the amidase (*E* = 88). If the reaction is allowed to progress to a higher degree of conversion (56% after 17 h of reaction), the remaining substrate is isolated with ee >99% (yield = 40%). The change of ethanol by DMSO as a co-solvent facilitated the solubilization of the substrate, but the resulting enantioenriched compounds were contaminated with DMSO and the enantioselectivity value of the reaction (*E* = 44) was lower than with ethanol.

Enzymatic hydrolysis of *N*-allyldiamide (±)-**10b** and *N*-allyl-*N*-methyldiamide (±)-**10c** was conducted under the best reaction conditions found for **10a**. The most significant results obtained in these processes are collected in Table 2, entries 2 and 3, respectively. In both cases, 22 h of incubation were necessary to obtain a 50% degree of conversion. The *E*-value obtained in the reaction of **10b** was similar to that attained with the other *N*-monosubstituted diamide **10a**. However, the *N*,*N*-disubstituted diamide **10c** was an excellent substrate for the enzyme, which catalysed its hydrolysis with a very high enantioselectivity (*E* > 200). As shown in Table 2 both remaining substrates (1*R*,2*R*)-**10b,c** and the products (1*S*,2*S*)-**11b,c** were obtained with ee >90%. In addition, enantiopure (1*S*,2*S*)-**11c** can be isolated if the time of incubation of (±)-**10c** is shortened to 17 h ($ee_S$ = 80%; *c* = 44%; *E* > 200).

The enantiomeric excesses of products **11a–c** (Table 2) were determined by chiral HPLC of their methyl ester derivatives **12a–c** (Scheme 4). To establish the absolute configuration of products **11a–c**, the monoacid **6b** of known (1*S*,2*S*) configuration was converted into the amido esters (1*S*,2*S*)-**12a–c** *via* the acyl chloride intermediate (Scheme 4). From the comparison of the chromatograms of **12a–c** obtained in both routes, we concluded that the enzymatically produced **11a–c** have the (1*S*,2*S*) configuration. Thus, the enantiopreference of the amidase of *R. rhodochrous* towards diamides **10a–c** is the same as that shown with diesters **4b** and **4c**.

From the results obtained with amido esters and diamides under the same reaction conditions, it can be concluded that the amidase from *R. rhodochrous* is more efficient with amido




Scheme 4 Assignment of the absolute configuration to the enzymatically prepared **11a–c** (**a**: R = Bn, R′ = H; **b**: R = allyl, R′ = H; **c**: R = allyl, R′ = Me).

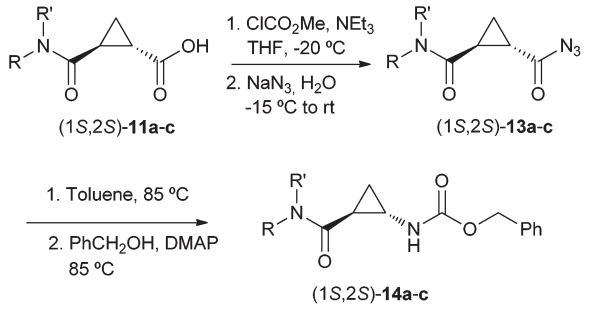
esters than with diamides. For instance, 6 h of reaction were required to attain 41% of conversion of the benzyl amido ester **4c**, whereas a similar degree of conversion (44%) of the analogue benzyl diamide was obtained after 12 h of reaction. However, the enzyme showed a higher enantioselectivity towards diamides, especially towards the bulkier *N*-allyl-*N*-methyl derivative **10c**. The major rigidity of the amide function in comparison with the ester function could be the cause of the higher enantioselective activity of the amidase towards diamides **10**.

## Curtius rearrangement of optically active carboxylic acids 11a–c

For the purpose to widen the synthetic utility of the optically active compounds here prepared, we decided to investigate the Curtius rearrangement of carboxylic acids **11a–c**. This strategy could lead to optically active β-amino acid derivatives incorporating the conformationally constrained cyclopropane ring. Apart from the already mentioned use as a precursor in the synthesis of belactosin A,[4] β-aminocyclopropanecarboxylic acids are attractive building blocks to induce defined conformations in peptides.[16]

The Curtius rearrangement of the optically active mixed ethyl ester acid **6a** has been published previously.[5] A Lewis acid such as CuCl was required in the carbamate formation




Scheme 5 Curtius rearrangement of the optically active (1*S*,2*S*)-**11a–c**.

step in order to avoid the epimerization of its chiral centres. When we applied this method to the carboxylic acid **11b**, the acyl azide intermediate was obtained in low yield and no rearrangement product was observed. For this reason, we tried some changes in this methodology. Thus, the formation of the acyl azides (1*S*,2*S*)-**13a–c** from (1*S*,2*S*)-**11a–c** was carried out using an excess of both methyl chloroformate (2.5 equiv.) and anhydrous triethylamine (2.5 equiv.), in THF as a solvent, at −20 °C (Scheme 5). After the addition of the aqueous solution of sodium azide, also at low temperature, acyl azides (1*S*,2*S*)-**13a–c** were obtained with high yields (82–96%), but in all cases a small amount of the corresponding mixed amide methyl ester **12a–c** (4–7%) was also formed. As the reaction progresses, the leaving methanol could compete with the azide anion as a nucleophile in the attack to the mixed anhydride intermediate giving the methyl ester **12**. Nevertheless, the corresponding crude acyl azide can be used without purification in the following steps. Next, a solution of the corresponding acyl azide in toluene was heated at 85 °C and, once the intermediate isocyanate was formed, benzyl alcohol (2.5 equiv.) and a catalytic amount of DMAP (10% molar) were added. DMAP was used to accelerate the formation of the benzyl carbamate.[17] Immediately, a white solid began to form in the reactions corresponding to the carbamates **14a** and **14b**. The very low solubility of these carbamates **14a,b** in toluene facilitates its isolation in pure form by simple filtration. No carbamate remained in the mother liquor. Carbamate **14c** is an oil and its purification was achieved by flash column chromatography of the crude reaction mixture. It is noteworthy that epimerization of the chiral centres was never observed as could be shown by spectroscopic and chiral HPLC analyses. In all cases the carbamate was obtained with the same enantiomeric excess as that of the starting optically active carboxylic acids **11a–c** (ee = 95, 92, and 99%, respectively). This fact together with the high overall yield obtained (86%, 77%, and 73% for **14a–c**, respectively) make the method an excellent approach for the synthesis of these carbamate derivatives.

# Experimental

### General information

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 ($CDCl_3$, $CD_3OD$ or DMSO-$d_6$ solutions) were obtained using AC-300 or DPX300 ($^1$H, 300.13 MHz and $^{13}$C, 75.5 MHz) spectrometers using the $\delta$ scale (ppm) for chemical shifts. Calibration was made on the signal of the solvent ($^{13}$C: $CDCl_3$, 76.95; $CD_3OD$, 49.00; DMSO-$d_6$, 39.52 ppm) or the residual solvent partially or non-deuterated ($^1$H: $CHCl_3$, 7.26; $CHD_2OD$, 3.31; DMSO-$d_5$, 2.50 ppm).

### Synthesis of compounds implied in the preparation of racemic substrates used in the biotransformations

**Dimethyl (±)-*trans*-cyclopropane-1,2-dicarboxylate ((±)-3b).** Diethyl (±)-*trans*-cyclopropane-1,2-dicarboxylate ((±)-**3a**) (2.00 mL, 11.4 mmol) was dissolved in methanol (12 mL). Two drops of conc. $H_2SO_4$ were added and the solution was refluxed for 24 h. Then water (30 mL) was added and the mixture was extracted with $CH_2Cl_2$ (2 × 30 mL). After washing the organic phase successively with aq. saturated $NaHCO_3$ and brine, solvents were eliminated to give a residue consisting of a mixture of compound (±)-**3b** and the ethyl methyl diester (89 : 11 ratio). This mixture was newly allowed to react with methanol (12 mL) and conc. $H_2SO_4$ (two drops) giving the pure dimethyl ester (±)-**3b** (1.68 g, 93%) after the work-up of the reaction mixture. Spectroscopic data for this compound are in good agreement with that published for (±)-**3b**.[18]

**Methyl (±)-*trans*-2-carbamoylcyclopropanecarboxylate ((±)-4b).** Ammonia was bubbled through methanol (55 mL) at 0 °C for 15 min. Then compound (±)-**3b** (1.58 g, 10.0 mmol) was added and the resulting solution was maintained at −20 °C for 48 h. The solvent was evaporated *in vacuo* to give the crude product, which was purified by recrystallisation in a mixture of $CH_2Cl_2$–hexane. White solid (1.16 g, 81%); mp 105–106 °C (lit.[19] mp 102–103 °C); IR (KBr) $\nu$ (cm$^{-1}$) 3401, 3336, 3304, 3214, 3062, 2999, 2956, 1717, 1663, 1617; $\delta_H$ (300.13 MHz, $CDCl_3$) 5.90 and 5.72 (2 H, two overlapped broad singlets, $NH_2$), 3.71 (3 H, s, $OCH_3$), 2.19 (1 H, ddd, $J_1$ = 9.2 Hz, $J_2$ = 5.6 Hz, $J_3$ = 3.6 Hz, CH), 2.00 (1 H, ddd, $J_1$ = 9.1 Hz, $J_2$ = 5.5 Hz, $J_3$ = 3.6 Hz, CH), 1.47 (1 H, ddd, $J_1$ = 9.1 Hz, $J_2$ = 5.5 Hz, $J_3$ = 3.6 Hz, C*H*H), 1.38 (1 H, ddd, $J_1$ = 9.1 Hz, $J_2$ = 5.5 Hz, $J_3$ = 3.6 Hz, CH*H*); $\delta_C$ (75.5 MHz, $CDCl_3$) 173.0 (C═O), 172.6 (C═O), 52.1 ($OCH_3$), 23.5 (CH), 21.6 (CH), 15.0 ($CH_2$). HRMS (ESI+) Calcd for $C_6H_{10}NO_3$ ($(M + H)^+$): 144.0655. Found: 144.0657.

**(±)-*trans*-2-Carbamoylcyclopropanecarboxylic acid ((±)-5).** It was obtained from (±)-**4b** (200 mg, 1.40 mmol) following the procedure described by Kennewell *et al.*[10] White solid (154 mg, 85%). Characterization data matched that reported.

**Benzyl (±)-*trans*-2-carbamoylcyclopropanecarboxylate ((±)-4c).** A solution of (±)-**5** (142 mg, 1.10 mmol) and benzyl alcohol (0.206 mL, 2.00 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF, 2.0 mL) was cooled at 0 °C, and then 2-chloro-1-methylpyridinium iodide (370 mg, 1.45 mmol) and 4-dimethylaminopyridine (DMAP, 354 mg, 2.90 mmol) were added. The reaction mixture was allowed to reach room temperature. After 5 hours of reaction, water (15 mL) was added and the mixture was extracted with AcOEt (2 × 15 mL). The organic phase was successively washed with aq. 1.5 M HCl and brine. Evaporation of

the solvent gave compound (±)-**4c** (235 mg, 98%), which was recrystallized from a mixture of $CH_2Cl_2$–hexane. Mp 117–118 °C. IR (KBr) $\nu$ ($cm^{-1}$) 3401, 3347, 3304, 3202, 1734, 1658, 1617; $\delta_H$ (300.13 MHz, $CDCl_3$) 7.44–7.30 (5 H, m, Ph), 5.72 and 5.51 (2 H, two overlapped broad singlets, $NH_2$), AB system centered to 5.13 ppm (2 H, $|J_{A,B}|$ = 15.0 Hz, C$H_2$–Ph), 2.24 (1 H, ddd, $J_1$ = 8.6 Hz, $J_2$ = 5.7 Hz, $J_3$ = 3.8 Hz, CH), 2.02 (1 H, ddd, $J_1$ = 8.6 Hz, $J_2$ = 5.7 Hz, $J_3$ = 3.7 Hz, CH), 1.49 (1 H, ddd, $J_1$ = 8.7 Hz, $J_2$ = 5.8 Hz, $J_3$ = 3.8 Hz, C*H*H), 1.40 (1 H, ddd, $J_1$ = 8.6 Hz, $J_2$ = 5.8 Hz, $J_3$ = 3.8 Hz, CH*H*); $\delta_C$ (75.5 MHz, $CDCl_3$) 172.4 (C=O), 172.3 (C=O), 135.5 (C), 128.5 (CH), 128.3 (CH), 128.1 (CH), 66.8 ($CH_2$), 23.5 (CH), 21.8 (CH), 15.1 ($CH_2$); MS (ESI+) *m*/*z*: 242 $(M + Na)^+$. HRMS (ESI+) Calcd for $C_{12}H_{13}NNaO_3$ $((M + Na)^+)$: 242.0788. Found: 242.0773.

**(±)-*trans*-2-(Ethoxycarbonyl)cyclopropanecarboxylic acid ((±)-6a).** A slight modification of the method described by Wiberg *et al.*[15] was followed: a solution of (±)-**3a** (1.25 g, 6.72 mmol) in ethanol (2.7 mL) was heated at reflux. Aqueous 14 M NaOH (0.480 mL, 6.72 mmol) was added over a period of 2 min. The reflux was maintained for 5 min. After this time, the reaction mixture was cooled and water (30 mL) was added. The aqueous solution was manually extracted with $CH_2Cl_2$ (2 × 20 mL). From the combined organic phases, the unreacted starting material was recovered (19%). The aqueous layer was acidified with aq. 3 M HCl until pH 0.7, and continuously extracted with $CH_2Cl_2$ for 8 h. The new organic phase was dried ($Na_2SO_4$) and the solvent was removed to yield (±)-**6a** (0.786 g, 74%) as a white solid; mp 54–56 °C (lit.[15] mp 58–60 °C; lit.[20] mp 52–54 °C). Spectroscopy data matched with that reported by Csuk and von Scholz.[20]

**Ethyl (±)-*trans*-2-(*N*-alkyl- or *N*,*N*-dialkylcarbamoyl)cyclopropanecarboxylate ((±)-9a–c). Typical procedure.** To a solution of (±)-**6a** (0.316 g, 2.00 mmol) in anhydrous THF (3.5 mL), DMF (one drop) was added. The solution was cooled at 0 °C and oxalyl chloride (0.34 mL, 4.0 mmol) was slowly added. After 12 h at room temperature, both the solvent and excess oxalyl chloride were removed under reduced pressure to give the corresponding acid chloride which was used in the next step without purification. This crude acid chloride was dissolved in anhydrous THF (5.0 mL) and the corresponding amine (4.0 mmol) was added at 0 °C, under a nitrogen atmosphere. The mixture was stirred at room temperature for 12 h. $CH_2Cl_2$ and water were added to the mixture, the phases were separated, and the aqueous layer was newly extracted with $CH_2Cl_2$. The organic phases were combined, and washed successively with aq. 1 M HCl, aq. saturated $NaHCO_3$, and brine. Evaporation of solvents yielded a residue, which was submitted to flash chromatography using *n*-hexane–ethyl acetate 2 : 1 (for (±)-**9a**) or 3 : 1 (for (±)-**9b,c**) as an eluent.

*Ethyl (±)*-trans-*2-(*N-*benzylcarbamoyl)cyclopropanecarboxylate ((±)-**9a**).* White solid (0.425g, 86%); mp 88–89 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3305, 2979, 2930, 1723, 1636, 1550; $\delta_H$ (300.13 MHz, $CDCl_3$) 7.38–7.20 (5 H, m, Ph), 6.61 (1 H, br s, NH), 4.39 (2 H, d, $J$ = 5.5 Hz, C$H_2$Ph), 4.03 (2 H, q, $J$ = 7.1 Hz, $OCH_2$), 2.20–2.11 (1 H, m, CH), 2.03–1.92 (1 H, m, CH), 1.49–1.38 (1 H, m, C*H*H), 1.33–1.17 (4 H, m + t, CH*H* and $CH_3$ (t centered to 1.22 ppm, $J$ = 7.1 Hz); $\delta_C$ (75.5 MHz, $CDCl_3$) 172.8 (C=O), 170.0 (C=O), 137.9 (C), 128.5 (CH), 127.8 (CH), 127.4 (CH), 60.9 ($CH_2$), 43.8 ($CH_2$), 24.0 (CH), 21.3 (CH), 14.7 ($CH_2$), 14.0 ($CH_3$). HRMS (ESI+) Calcd for $C_{14}H_{18}NO_3$: $((M + H)^+)$: 248.1281. Found: 248.1283.

*Ethyl (±)*-trans-*2-(*N-*allylcarbamoyl)cyclopropanecarboxylate ((±)-**9b**).* White solid (0.288 g, 73%); mp 75–76 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3298, 3087, 2985, 1724, 1642, 1550; $\delta_H$ (300.13 MHz, $CDCl_3$) 5.90 (1 H, broad s, NH), 5.84 (1 H, ddt, $J_{trans}$ = 17.2 Hz (d), $J_{cis}$ = 10.2 Hz (d), $J$ = 5.7 Hz (t), C*H*=$CH_2$), 5.24–5.12 (2 H, m, CH=C$H_2$), 4.14 (2 H, q, $J$ = 7.1 Hz, $OCH_2$), 3.89 (2 H, tt, $J_1$ = 5.8 Hz, $J_2$ = 1.5 Hz, $NCH_2$), 2.17 (1 H, ddd, $J_1$ = 8.8 Hz, $J_2$ = 5.7 Hz, $J_3$ = 3.8 Hz, CH), 1.93 (1 H, ddd, $J_1$ = 8.8 Hz, $J_2$ = 5.9 Hz, $J_3$ = 3.8 Hz, CH), 1.46 (1 H, ddd, $J_1$ = 8.8 Hz, $J_2$ = 5.9 Hz, $J_3$ = 3.8 Hz, C*H*H), 1.37–1.18 (4 H, ddd and t partially overlapped, CH*H* and $CH_3$ (t centered at 1.26 ppm, $J$ = 7.1 Hz)); $\delta_C$ (75.5 MHz, $CDCl_3$) 172.7 (C=O), 170.0 (C=O), 133.9 (CH), 116.6 ($CH_2$), 60.9 ($CH_2$), 42.2 ($CH_2$), 24.1 (CH), 21.4 (CH), 14.7 ($CH_2$), 14.1 ($CH_3$). HRMS (ESI+) Calcd for $C_{10}H_{16}NO_3$ $((M + H)^+)$: 198.1125. Found: 198.1130.

*Ethyl (±)*-trans-*2-(*N-*allyl*-N-*methylcarbamoyl)cyclopropanecarboxylate ((±)-**9c**).* Colorless oil (0.338 g, 80%); IR (neat) $\nu$ ($cm^{-1}$) 3079, 2986, 2933, 1725, 1644; $\delta_H$ (300.13 MHz, $CDCl_3$) corresponding to a 47 : 53 mixture of rotamers: 5.88–5.64 (1 H, m, C*H*=$CH_2$), 5.27–5.10 (2 H, m, CH=C$H_2$), 4.20–4.10 (2 H, two overlapped q corresponding to both rotamers, $J$ = 7.1 Hz, $OCH_2$), 4.07–3.92 (2 H, m, $NCH_2$), 3.10 (3 H for minor rotamer, s, $NCH_3$), 2.95 (3 H for major rotamer, s, $NCH_3$), 2.40–2.10 (2 H, m, 2 × CH), 1.55–1.10 (5 H, m, $CH_2$ and $CH_3$); $\delta_C$ (75.5 MHz, $CDCl_3$) corresponding to a mixture of rotamers: 172.9, 172.8, 170.4, 170.0, 132.7, 132.4, 117.4, 116.7, 60.9, 60.8, 52.2, 50.4, 34.7, 34.2, 21.9, 21.8, 21.1, 21.0, 15.4, 15.2, 14.1. HRMS (ESI+) Calcd for $C_{11}H_{17}NNaO_3$ $((M + Na)^+)$: 234.1101. Found: 234.1091.

**(±)-*trans*-*N*-Alkyl(or *N*,*N*-dialkyl)cyclopropane-1,2-dicarboxamides ((±)-10a–c). General procedure.** The corresponding amidoester (±)-**9a–c** (1.0 mmol) was dissolved in 5.0 mL of a methanolic solution of ammonia (obtained by bubbling of ammonia through methanol at 0 °C) in a sealed flask, and the mixture was stirred at room temperature until the disappearance of the starting material (TLC control, hexane–ethyl acetate 1 : 1). Evaporation of the solvents yielded the corresponding diamide (±)-**10a–c**, which was purified as indicated below.

*(±)*-trans-N-*Benzylcyclopropane-1,2-dicarboxamide ((±)-**10a**).* The crude material was washed with dichloromethane to give pure (±)-**10a** as a white solid (0.183 g, 84%). Mp 232–233 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3392, 3248, 3195, 3081, 1659, 1631, 1564; $\delta_H$ (300.13 MHz, $CD_3OD$) 7.40–7.18 (5 H, m, Ph), AB system centered to 4.37 ppm (2 H, $|J_{A,B}|$ = 15.3 Hz, C$H_2$-Ph), 2.18–1.97 (2 H, m, 2 × CH), 1.37–1.16 (2 H, m, $CH_2$); $\delta_C$ (75.5 MHz, DMSO-$d_6$) 172.7 (C=O), 170.7 (C=O), 139.5 (C), 128.4 (CH), 127.4 (CH), 126.9 (CH), 42.4 ($CH_2$), 22.0 (CH), 21.8 (CH), 12.6 ($CH_2$); HRMS (ESI+) Calcd for $C_{12}H_{14}N_2NaO_2$ $((M + Na)^+)$: 241.0947. Found: 241.0962.

*(±)*-trans-N-*Allylcyclopropane-1,2-dicarboxamide ((±)-**10b**).* The crude material was recrystallized in a mixture of $CH_2Cl_2$–MeOH to give (±)-**10b** as a white solid (0.153 g, 91%). Mp 222–223 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3393, 3248, 3199, 3081, 1662, 1632, 1617, 1569; $\delta_H$ (300.13 MHz, $CD_3OD$) 5.84 (1 H, ddt, $J_{trans}$ = 17.2 Hz (d), $J_{cis}$ = 10.3 Hz (d), $J$ = 5.5 Hz (t), C*H*═$CH_2$), 5.18 (1 H, dq, $J_{trans}$ = 17.2 Hz (d), $J$ = 1.6 Hz (q), CH═C*H*H), 5.10 (1 H, dq, $J_{cis}$ = 10.3 Hz (d), $J$ = 1.6 Hz (q), CH═CH*H*), 3.81 (2 H, dt, $J$ = 5.5 Hz (d), $J$ = 1.6 Hz (t), $NCH_2$), 2.09–2.00 (2 H, m, 2 × CH), 1.35–1.20 (2 H, m, $CH_2$); $\delta_C$ (75.5 MHz, $CD_3OD$) 176.3 (C═O), 173.3 (C═O), 135.4 (CH), 116.2 ($CH_2$), 43.0 ($CH_2$), 23.7 (CH), 23.0 (CH), 13.8 ($CH_2$); HRMS (ESI+) Calcd for $C_8H_{12}N_2NaO_3$ ($(M + Na)^+$): 191.0791. Found: 191.0794.

*(±)*-trans-N-*Allyl*-N-*methylcyclopropane-1,2-dicarboxamide ((±)-**10c**).* The crude material was purified by flash chromatography (*n*-hexane–AcOEt 1 : 1 and then, AcOEt–MeOH 4 : 1) to give (±)-**10c** as a white solid (0.158 g, 87%). Mp 125–127 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3344, 3161, 2943, 1685, 1642, 1612; $\delta_H$ (300.13 MHz, $CD_3OD$) corresponding to a 43 : 57 mixture of rotamers: 6.00–5.66 (1 H, m, C*H*═$CH_2$), 5.28–5.10 (2 H, m, CH═C$H_2$), 4.25–4.04 (2 H for the major rotamer, m, $NCH_2$), 4.02–3.96 (2 H for the minor rotamer, m, $NCH_2$), 3.15 (3 H for minor rotamer, s, $CH_3$), 2.95 (3 H for major rotamer, s, $CH_3$), 2.40–2.22 (1 H, m, CH), 2.12–2.02 (1 H, m, CH), 1.35–1.20 (m, 2H, $CH_2$); $\delta_C$ (75.5 MHz, $CD_3OD$) corresponding to a mixture of rotamers: 176.2, 173.4, 172.8, 134.0, 133.8, 117.7, 116.9, 53.3, 51.5, 35.4, 34.8, 23.9, 23.6, 21.1, 21.0, 14.7; HRMS (ESI+) Calcd for $C_9H_{14}N_2NaO_2$ ($(M + Na)^+$): 205.0947. Found: 205.0961.

### Microbial hydrolysis of (±)-4b

A solution of (±)-**4b** (100 mg) in ethanol (1.0 mL) was added to a bacterial suspension ($A_{650}$ = 0.25) in fresh 0.10 M potassium phosphate buffer pH 7.0 (100 mL), and the reaction mixture was incubated in an orbital shaker at 10 °C. After 23 h, the reaction was stopped by centrifugation (5000 rpm, 3 min). The cells were discarded. The supernatant aqueous solution was basified (pH = 8.2) with aq. 3 M NaOH and subjected to continuous extraction with $CH_2Cl_2$ for 6 h. The organic phase was dried with $Na_2SO_4$ and evaporated to yield the remaining substrate (1*R*,2*R*)-**4b** as a white solid (41%); mp 97–98 °C; $[\alpha]_D^{20}$ −238.2 (*c* 0.51, $CH_2Cl_2$), ee > 99%.

The remaining aqueous phase was acidified (pH = 1.7) with conc. HCl and again continuously extracted (11 h) with $CH_2Cl_2$. From this organic phase (1*S*,2*S*)-**6b** was isolated as a colorless oil with 51% yield; $[\alpha]_D^{20}$ +147.0 (*c* 1.48, $CHCl_3$), ee 91%. Spectroscopic data are in good agreement with that published for (±)-*trans*-**6b**.[21]

### Microbial hydrolysis of (±)-4c

Substrate (±)-**4c** (48 mg) and ethanol (0.48 mL) were added to 48 mL of bacterial suspension of $A_{650}$ = 1.5. The mixture was incubated in an orbital shaker at 28 °C for 6 h. Isolation of optically active compounds (**4c** and **6c**) was carried out as described in the microbial hydrolysis of (±)-**4b**.

The remaining substrate (1*R*,2*R*)-**4c** (27 mg, 56%) was isolated as a white solid ($[\alpha]_D^{20}$ −83.3 (*c* 0.56, $CHCl_3$), ee = 65%).

The product (1*S*,2*S*)-**6c** (16 mg, 33%) was isolated as a colorless oil ($[\alpha]_D^{20}$ +133.9 (*c* 0.75, MeOH), ee = 94%). IR (neat) $\nu$ ($cm^{-1}$) 3500–2500 (broad band), 3067, 3034, 2956, 1738, 1703; $\delta_H$ (300.13 MHz, $CD_3OD$) 7.43–7.28 (5 H, m, Ph), AB system centered to 5.14 ppm (2 H, $|J_{A,B}|$ = 12.5 Hz, C$H_2$–Ph), 2.18–2.04 (2 H, m, 2 × CH), 1.43–1.37 (2 H, m, $CH_2$); $\delta_C$ (75.5 MHz, $CD_3OD$) 174.9 (C═O), 173.1 (C═O), 137.3 (C), 129.6 (CH), 129.33 (CH), 129.29 (CH), 67.9 ($CH_2$), 23.04 (CH), 22.99 (CH), 15.7 ($CH_2$); HRMS (ESI+) Calcd for $C_{12}H_{12}NaO_4$ ($(M + Na)^+$): 243.0628. Found 243.0625.

### Microbial hydrolysis of (±)-10a

A mixture consisting of substrate (±)-**10a** (100 mg), ethanol (1.0 mL) and fresh potassium phosphate buffer pH 7.0 (50 mL) was sonicated for 25 min at room temperature. Then, the mixture was incubated in an orbital shaker for 30 min for its re-oxygenation. After this time, a concentrated bacterial suspension and fresh buffer were added to the mixture in order to attain an $A_{650}$ = 1.5. The reaction was incubated at 28 °C for 12 h. Isolation of optically active compounds (**10a** and **11a**) was carried out as described for the microbial hydrolysis of (±)-**4b**.

The remaining substrate (1*R*,2*R*)-**10a** (51 mg, 51%) was isolated as a white solid ($[\alpha]_D^{20}$ −110.3 (*c* 0.49, MeOH), ee = 75%).

The product (1*S*,2*S*)-**11a** (40 mg, 40%) was isolated as a white solid ($[\alpha]_D^{20}$ +166.3 (*c* 0.51, MeOH), ee = 95%). Mp 153–154 °C; IR (KBr) $\nu$ ($cm^{-1}$) 3500–2500 (broad band), 3303, 1693, 1638, 1550; $\delta_H$ (300.13 MHz, $CD_3OD$) 7.40–20 (5 H, m, Ph), 4.38 (2 H, m, C$H_2$–Ph), 2.24–2.10 (1 H, m, CH), 2.10–1.90 (1 H, m, CH), 1.43–1.22 (2 H, m, $CH_2$); $\delta_C$ (75.5 MHz, $CD_3OD$) 175.8 (C═O), 172.8 (C═O), 139.8 (C), 129.6 (CH), 128.6 (CH), 128.3 (CH), 44.4 ($CH_2$), 24.5 (CH), 22.0 (CH), 14.7 ($CH_2$); HRMS (ESI+) Calcd for $C_{12}H_{13}NNaO_3$ ($(M + Na)^+$): 242.0788. Found: 242.0793.

### Microbial hydrolysis of (±)-10b

The method, starting from (±)-**10b** (100 mg), was identical to that followed in the microbial hydrolysis of (±)-**10a**, except that the reaction time was 22 h. From this process, the optically active compounds **10b** and **11b** were isolated.

The remaining substrate (1*R*,2*R*)-**10b** (44 mg, 44%) was isolated as a white solid ($[\alpha]_D^{20}$ −172.6 (*c* 0.80, MeOH), ee = 93%).

The product (1*S*,2*S*)-**11b** (43 mg, 43%) was isolated as a viscous liquid ($[\alpha]_D^{20}$ +152.9 (*c* 0.75, MeOH), ee = 92%). IR (neat) $\nu$ ($cm^{-1}$) 3500–2500 (broad band), 3297, 3104, 3085, 1696, 1642, 1560; $\delta_H$ (300.13 MHz, $CD_3OD$) 5.95–5.75 (1 H, m, C*H*═$CH_2$), 5.28–5.04 (2H, m, CH═C$H_2$), 3.90–3.75 (2 H, m, $NCH_2$), 2.20–1.90 (2 H, m, 2 × CH), 1.40–1.20 (2 H, m, $CH_2$); $\delta_C$ (75.5 MHz, $CD_3OD$) 175.8 (C═O), 172.8 (C═O), 135.3 (CH), 116.3 ($CH_2$), 43.0 ($CH_2$), 24.5 (CH), 21.9 (CH), 14.7 ($CH_2$); HRMS (ESI+) Calcd for $C_8H_{10}NNa_2O_3$ ($(M + 2Na - H)^+$): 214.0451. Found: 214.0453.

### Microbial hydrolysis of (±)-10c

A solution of substrate (±)-**10c** (100 mg) in ethanol (1 mL) was added to a bacterial suspension ($A_{650}$ = 1.5) in fresh 0.10 M potassium phosphate buffer pH 7.0 (100 mL), and the reaction mixture was incubated in an orbital shaker at 28 °C. After 22 h of reaction, the optically active compounds **10c** and **11c** were isolated as described for the microbial hydrolysis of (±)-**4b**.

The remaining substrate (1*R*,2*R*)-**10c** (47 mg, 47%) was isolated as a white solid ($[\alpha]_D^{20}$ −108.4 (*c* 0.73, MeOH), ee = 97%); mp 112–114 °C.

The product (1*S*,2*S*)-**11c** (48 mg, 48%) was isolated as a colourless oil ($[\alpha]_D^{20}$ +140.7 (*c* 0.81, MeOH), ee = 99%). IR (neat) $\nu$ ($cm^{-1}$) 3500–2500 (broad band), 3091, 1723, 1601; $\delta_H$ (300.13 MHz, $CD_3OD$) corresponding to a 43 : 57 mixture of rotamers: 6.00–5.67 (1 H, m, C*H*=$CH_2$), 5.27–5.13 (2 H, m, CH=C$H_2$), 4.25–4.04 (2 H for the major rotamer, m, $NCH_2$), 4.03–3.97 (2 H for the minor rotamer, m, $NCH_2$), 3.16 (3 H for the minor rotamer, s, $CH_3$), 2.95 (3 H for the major rotamer, s, $CH_3$), 2.45–2.27 (1 H, m, CH), 2.07–1.93 (1 H, m, CH), 1.40–1.23 (2 H, m, $CH_2$); $\delta_C$ (75.5 MHz, $CD_3OD$) corresponding to a mixture of rotamers: 175.7, 172.8, 172.3, 134.0, 133.7, 117.7, 116.9, 53.3, 51.5, 35.4, 34.9, 22.8, 22.6, 21.93, 21.88, 15.5; HRMS (ESI+) Calcd for $C_9H_{13}NNaO_3$ ($(M + Na)^+$): 206.0788. Found: 206.0791.

### Curtius rearrangement of (1*S*,2*S*)-11a–c

**Benzyl (1*S*,2*S*)-*N*-[2-(*N*′-benzylcarbamoyl)cyclopropyl]carbamate ((1*S*,2*S*)-14a). General procedure.** Under a nitrogen atmosphere, a mixture of carboxylic acid (1*S*,2*S*)-**11a** (ee = 95%; 29 mg, 0.13 mmol) and anhydrous triethylamine (54 μL, 0.39 mmol) was dissolved in anhydrous THF (0.5 mL) and the solution was cooled at −20 °C. Then, methyl chloroformate (25 μL, 0.32 mmol) was added. After 90 min of stirring at −20 °C, an aqueous solution of sodium azide (25 mg, 0.39 mmol) in water (150 μL) was added and the reaction was allowed to reach room temperature. After 5 h, water and ethyl acetate were added. The organic phase was separated and the aqueous layer was extracted with ethyl acetate. The organic phases were combined and successively washed with saturated aq. solution of $NaHCO_3$ and brine. Evaporation of solvents gave the acyl azide (1*S*,2*S*)-**13a** (96%) contaminated with a small amount of methyl ester (1*S*,2*S*)-**12a**. The yields and ratio (96 : 4) of both compounds were calculated from the $^1$H NMR spectrum of the crude of reaction (see the ESI†).

The crude acyl azide (1*S*,2*S*)-**13a** (30.5 mg, 0.125 mmol) was dissolved in anhydrous toluene (1.0 mL) and the solution was heated at 85 °C for 90 min. Then benzyl alcohol (32 μL, 0.31 mmol) and DMAP (2.0 mg) were added and the heating was continued for 5 h. After this time, the reaction mixture was cooled and the white solid was filtered and washed successively with toluene and *n*-hexane. The solid was dried under vacuum to yield (1*S*,2*S*)-**14a** (36.5 mg, 90%); $[\alpha]_D^{20}$ +22.9 (*c* 0.32, MeOH), ee = 95%; IR (KBr) $\nu$ ($cm^{-1}$) 3318, 3033, 1688, 1635, 1565, 1536; $\delta_H$ (300.13 MHz, DMSO-$d_6$) 8.60 (1 H, t, *J* = 5.9 Hz, N*H*-$CH_2$), 7.63 (1 H, d, N*H*-CH), 7.45–7.20 (5 H, m, Ph), 5.02 (2 H, s, O-$CH_2$), AB signal of an ABX system (2 H, $|J_{A,B}|$ = 15.2 Hz, $J_{A,X} = J_{B,X}$ = 5.9 Hz, NH–C$H_2$), 2.83 (1 H, m, H-1), 1.68 (1 H, br s, H-2), 1.07 (1 H, m, H-3), 0.97 (1 H, m, H-3′); $\delta_C$ (75.5 MHz, DMSO-$d_6$) 170.7 (C=O), 156.4 (C=O), 139.5 (C), 137.0 (C), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.3 (CH), 126.8 (CH), 65.4 ($OCH_2$), 42.2 ($NCH_2$), 30.8 (C-1), 22.5 (C-2), 12.7 (C-3); HRMS (ESI+) Calcd for $C_{19}H_{21}N_2O_3$ ($(M + H)^+$): 325.1547. Found: 325.1535.

**Benzyl (1*S*,2*S*)-*N*-[2-(*N*′-allylcarbamoyl)cyclopropyl]carbamate ((1*S*,2*S*)-14b).** The general procedure described for **14a** was followed. From (1*S*,2*S*)-**11b** (ee = 92%; 30 mg, 0.18 mmol), a mixture of acyl azide (1*S*,2*S*)-**13b** (85% yield) and the methyl ester (1*S*,2*S*)-**12b** was obtained (**13b : 12b** ratio of 96 : 4; see the ESI†). After starting from the crude (1*S*,2*S*)-**13b** (25 mg, 0.13 mmol), (1*S*,2*S*)-**14b** (32 mg, 90%) was obtained as a white solid; mp 170–172 °C; $[\alpha]_D^{20}$ +20.9 (*c* 0.54, MeOH), ee = 92%; IR (KBr) $\nu$ ($cm^{-1}$) 3327, 3299, 1689, 1633, 1536; $\delta_H$ (300.13 MHz, $CDCl_3$) 7.34 (5 H, br s, Ph), 6.03 (1 H, br s, NH), 5.82 (1 H, m, C*H*=$CH_2$), 5.30–4.90 (5 H, m, CH=C$H_2$, NH and C$H_2$–Ph), 4.00–3.65 (2 H, m, $NCH_2$), 2.93 (1 H, m, H-1), 1.70 (1 H, br s, H-2), 1.44 (1 H, m, H-3), 1.00 (1 H, m, H-3′); $\delta_C$ (75.5 MHz, $CDCl_3$) 170.4 (C=O), 156.7 (C=O), 136.1 (C), 134.1 (CH), 128.5 (CH), 128.2 (CH), 128.0 (CH), 116.3 ($CH_2$), 66.9 ($OCH_2$), 42.1 ($NCH_2$), 30.8 (C-1), 24.5 (C-2), 13.9 (C-3); HRMS (ESI+) Calcd for $C_{15}H_{19}N_2O_3$ ($(M + H)^+$): 275.1390. Found: 275.1373.

**Benzyl (1*S*,2*S*)-*N*-[2-(*N*′-allyl-*N*′-methylcarbamoyl)cyclopropyl]carbamate ((1*S*,2*S*)-14c).** The general procedure described for **14a** was followed. From (1*S*,2*S*)-**11c** (ee = 99%; 44 mg, 0.24 mmol), a mixture of acyl azide (1*S*,2*S*)-**13c** (82% yield) and the methyl ester (1*S*,2*S*)-**12c** was obtained (**13c : 12c** ratio of 93 : 7; see the ESI†). After the general procedure was applied to the crude (1*S*,2*S*)-**13c** (37 mg, 0.18 mmol) except that once the reaction mixture was cooled, the organic solvent was eliminated and the resulting residue was submitted to flash column chromatography (*n*-hexane–AcOEt 2 : 3). Thus, (1*S*,2*S*)-**14c** (44 mg, 89%) was obtained as a colourless oil; $[\alpha]_D^{20}$ −39.2 (*c* 1.5, $CHCl_3$), ee = 99%; IR (neat) $\nu$ ($cm^{-1}$) 3290, 3063, 3039, 2933, 1721, 1626, 1529; $\delta_H$ (300.13 MHz, $CDCl_3$) corresponding to a mixture of rotamers 7.45–7.27 (5 H, m, Ph), 5.95–5.40 (1 H, m, C*H*=$CH_2$), 5.30–4.80 (5 H, m, CH=C$H_2$, NH and C$H_2$–Ph), 4.50–4.30 and 4.10–3.80 (2 H, br m, $NCH_2$), 3.25–2.80 (4 H, m, H-1, $NCH_3$), 2.00–1.80 (1 H, m, H-2), 1.50 (1 H, m, H-3), 0.99 (1 H, m, H-3′); $\delta_C$ (75.5 MHz, $CDCl_3$) corresponding to a mixture of rotamers: 170.8, 170.4, 156.6, 136.1, 132.9, 128.4, 128.1, 128.0, 117.0, 116.2, 66.7, 52.0, 50.3, 34.7, 34.0, 31.5, 21.4, 21.2, 14.4; HRMS (ESI+) Calcd for $C_{16}H_{20}N_2NaO_3$ ($(M + Na)^+$): 311.1366. Found: 311.1382.

## Conclusions

Starting from the commercially available diethyl (±)-*trans*-cyclopropane-1,2-dicarboxylate a series of racemic amido esters and diamides have been prepared. We have demonstrated that the amidase from *Rodococcus rhodochrous* IFO 15564 is an adequate catalyst to promote the enantioselective hydrolysis of

these substrates. The moderate to high enantioselectivity exhibited by the enzyme allowed us to obtain a great variety of optically active *trans*-cyclopropane-1,2-dicarboxylic acid derivatives with high enantiomeric excesses and yields. Whereas catalytic activity of amidase towards amido esters is higher than with diamides, the enzyme showed a higher enantioselectivity with diamides, especially with the *N*,*N*-disubstituted diamide. Nevertheless, with the highly soluble amido methyl ester **4b**, it was possible to conduct the biotransformation at a lower temperature. It was shown that the enantioselectivity of the enzyme in this biotransformation notably increased when the temperature decreased from 28 to 10 °C. Some of the optically active compounds here prepared are precursors of biologically active cyclopropanic compounds such as belactosin A. Moreover, the Curtius rearrangement of some carboxylic acids has been carried out. The efficacy of these processes was based on the high ee and yields obtained for the resulting β-amino cyclopropanecarboxamides. The ee of the starting carboxylic acids remains unaltered during the process.

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