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Chemoenzymatic resolution of epimeric *cis* 3-carboxycyclopentylglycine derivatives

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Abstract—Epimeric 3-carboxycyclopentylglycines (+)-**10**/(−)-**10** and (+)-**11**/(−)-**11** were efficiently prepared by the way of a sequence of Diels–Alder and retro-Claisen reactions. The synthesis incorporates a concise and inexpensive chemoenzymatic resolution of racemic compounds **4**,**5a**, the *N*,*O*-protected derivatives of amino acids **10**,**11**. Systematic screening with different enzymes and microorganisms was performed to select a very efficient catalyst for the separation of the racemic mixtures. The reaction conditions allowing deprotection of both ester and amino functions and to avoiding epimerization processes were studied. Enantiomers (i.e., (+)-**10**/(−)-**10** and (+)-**11**/(−)-**11**) were obtained in high enantiopurity. The absolute configuration of all stereocenters was unequivocally assigned.

1. Introduction

Unnatural amino acids have been recognized as major tools for the preparation of peptidomimetics with enhanced biological activity and proteolytic resistance. One of the modern approaches towards the synthesis of modified peptides is the incorporation of non-proteinogenic amino acids into the peptide backbone.[1] This is a powerful strategy to overcome the problem that peptides are generally flexible and do not take the defined conformation necessary for function.

3-Carboxycyclopentylglycines are very promising amino acids to be used in this field since they are non-proteinogenic amino acids containing conformational constraints. Furthermore, the skeleton of both 2-aminoadipic acid and 2-aminopimelic acid, two natural amino acids of biological importance, is included in their structure.[2–5] Heterosubstituted 3-carboxycyclopentylglycines were prepared and their biological activity toward neuraminidase was evaluated.[6] Recently, we reported on the chiral preparation of epimeric 1*S*,3*R*,1′*R*- and 1*S*,3*R*,1′*S*-(benzoylamino-ethoxycarbonylmethyl)cyclopentanecarboxylic acid derivatives **6** and **7** (Scheme 3) characterized by the cis relationship between the two carbon residues.[7]

Chiral synthesis is a powerful tool to produce a chiral compound, but there are some limitations such as the expensive chiral reagents, the necessity to repeat the synthetic protocol twice when both enantiomers are needed, and most of all and in most cases, the difficulty of separating the diasteromers. For these reasons, one alternative approach to overcome all these limitations is the enzymatic separation of racemic compounds and, in particular, of amino acids. In fact, isolated enzymes or microbial cells can catalyse the stereoselective hydrolysis of amino acids with *N*-protected amide group or *O*-protected ester group.[8] High selectivity and mild and safe conditions are typical. In particular, the use of whole microbial cells often shows good performance with regard to stability and selectivity and is advantageous to access intracellular esterase activity.[9]

The importance of amino acid chirality in biological interactions is well known. The impossibility to forecast a priori the biological activity of a single stereoisomer requests the availability of both pure enantiomers. For this reason, considering the potential biological interest of the above amino acids and the perspective of using the 3-carboxycyclopentylglycines in peptide syntheses, racemic epimers (±)-**4** and (±)-**5a** were prepared and the synthetic

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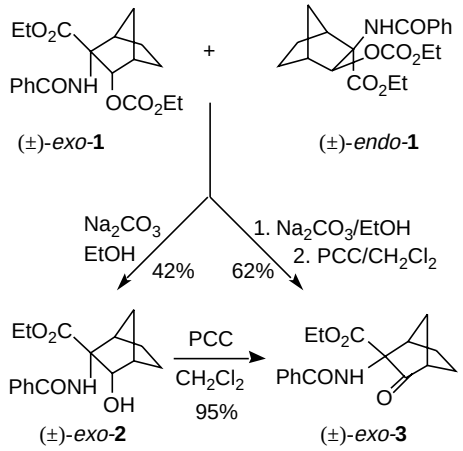
protocol for their preparation was considerably improved.[10] The chemoenzymatic resolution of each epimer (±)-**4** and (±)-**5a** was planned. Systematic screening with different enzymes and microorganisms was performed to select a very efficient catalyst for the separation of the racemic mixtures.

Furthermore, the absolute configuration of each stereocenter in the above amino acids was assigned taking advantage of the availability of chiral derivatives **6** and **7**.

2. Results

The key starting material for the preparation of the epimeric 3-carboxy-cyclopentylglycines **4**,**5a** is the racemic ethyl 2-benzoylamino-3-oxo-bicylo[2.2.1]heptane-2-carboxylate (±)-*exo*-**3** (Scheme 1).



Scheme 1.

The synthesis of the corresponding methyl ester, was already reported by us[11] but a more efficient protocol is described here which allowed to minimize us the reaction steps, to avoid chromatographic purifications and to improve the reaction yield (53% instead of 35%).

A mixture of the norbornane derivatives (±)-*exo*-**1** and (±)-*endo*-**1** (70:30) was the starting material for the preparation of **3**. (Scheme 1) The selective deprotection of the C-3 carbonate of the exo compound, using sodium carbonate in ethanol at room temperature, gave the 3-hydroxy derivative (±)-*exo*-**2** which was easily separated from the endo carbonate (±)-**1**. Compound (±)-*exo*-**2** was isolated in 42% yield and than oxidized to ketone (±)-*exo*-**3** (95%) using pyridinium chlorocromate (PCC). Alternatively, the oxidation reaction was performed directly on the mixture of alcohol (±)-*exo*-**2** and carbonate (±)-*endo*-**1**.

Ethyl 2-phenyl-5-oxo-oxazol-4-methylene-carbonate and cyclopentadiene were the starting reagents used for the synthesis of norbornane ring via a Diels–Alder reaction. Here, we prepared the ketone (±)-*exo*-**3** in four steps and 53% overall yield. This protocol allowed us to avoid one chromatographic step and, importantly, to perform an easier separation of carbonate *endo*-**1** from the ketone *exo*-**3**.

β-Ketoester (±)-*exo*-**3** was then transformed into a mixture of epimeric cyclopentylglycines (±)-**4** and (±)-**5a** (1:1; 82%) by a base catalysed retro-Claisen reaction (pyridine/water at reflux; 1:1) which ensured the cis relationship between the two carbon residues on the ring (Scheme 2).

2.1. Enzymatic resolution

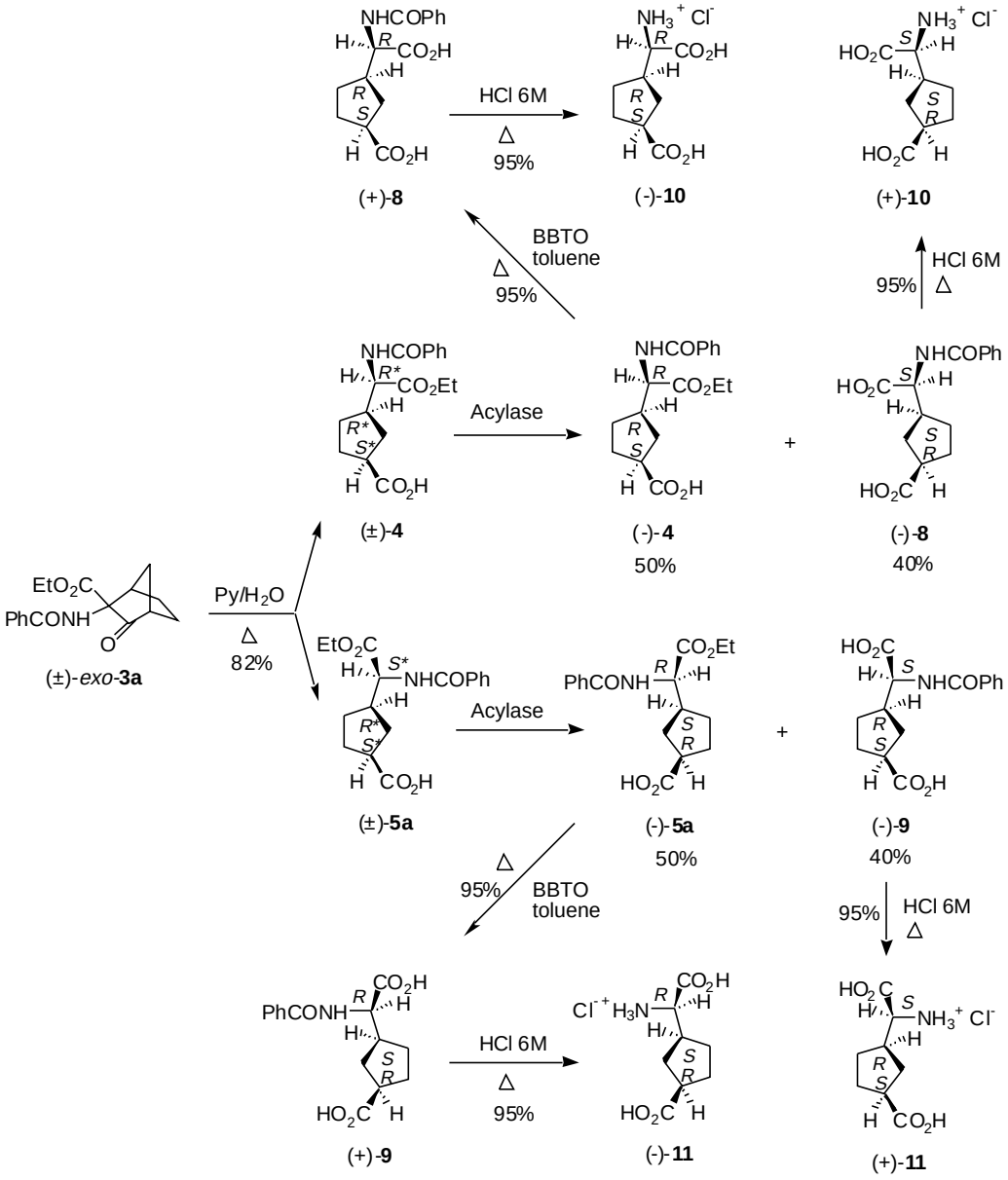
Aiming to obtain the enantiopure amino acids, an enzymatic study was performed starting from racemic compound (±)-**5a**. Twelve enzymes (lipases, acylases, proteases) and fifty microorganisms (yeasts, bacteria) belonging to different genera were tested. The screening was carried out using miniaturized systems and TLC as analytical technique allowing an evaluation of the activity of a large number of biocatalysts in a short time. Different enzymes and microbial cells were able to selectively hydrolyze the ester function of the cyclopentylglycine **5a** to obtain compound *S*-**9**. The enantiomeric excess was evaluated by chiral HPLC and the results are summarized in Table 1. The hydrolysis of the amide function was never observed.

As shown in Table 1, the best results were achieved by using PPL and acylase from *Aspergillus melleus*. In both cases it was possible to obtain the complete resolution of racemic (±)-**5a**. After the extraction of the reaction mixture, the bicarboxylic acid (−)-**9** was separated from the ester (−)-**5a** (50%, $[\alpha]_D^{25}$ −22) by simple crystallization from dichloromethane. (Scheme 2) Enantiopure acid (−)-**9** ($[\alpha]_D^{25}$ −14.5) was obtained in 40% yield. The same results were obtained starting from (±)-**4**. Enantiopure acid (−)-**8** ($[\alpha]_D^{25}$ −20) was separated from the ester (−)-**4** (50%, $[\alpha]_D^{25}$ −11) by crystallization. (Scheme 2) Interestingly, a different reaction rate was observed starting from two epimers, with the 1*S**,3*R**,1′*R** epimer being more reactive (24 h) than 1*S**,3*R**,1′*S** one (48 h).

The acylase from *Aspergillus melleus* was also most active also at low concentration (5 mg/mL) for both racemic compounds. For this reason this enzyme was selected for the semi-preparative resolution (250 mg) of both (±)-**4** and (±)-**5a** (Scheme 2).

One problem of biotransformations carried out with enzymes is the formation of emulsions during the work-up of the crude reaction mixture. For this reason we screened whole cells and the best results were found using *Pichia etchellsii* MIM and *Saccharomyces cerevisiae* ZEUS. (Table 1) This result appears very interesting in the perspective of scaling up the process to the gram scale.

Recently, we have synthesised the chiral epimeric cyclopentylglycines (+)-**6** and (+)-**7**. Since their separation had been achieved by semi-preparative HPLC, which required the use of considerable amounts of solvent and long times, we planned their separation using enzymes. This target was successfully achieved using a lipase from *Candida cylindracea*, which gave the best selectivity. In fact, this enzyme is both regioselective and stereoselective. It hydrolysed only the methyl ester function of stereoisomer (+)-**7** in 72 h with a diastereomeric excess of 97% and molar conversion of 49%. After column chromatography it was easily possible to obtain the pure compound (−)-**5b**



Scheme 2.

(30%, $[\alpha]_D^{25}$ −8) and the methyl ester derivative (+)-**6** (40%) (Scheme 3). Considering that both epimer **6** and **7** have the same 1-*S* stereocenter, we conclude that the remote amino acid function controls the selectivity of the enzyme.

2.2. Deprotection of the amino acid function

The deprotection of the ester and amide functions in compounds **4**,**5a** was critical because a partial epimerization of the amino acid C-α occurred when performing the above hydrolysis in standard conditions (see below). To avoid this problem, different reaction conditions were tested starting from the racemic (±)-**4** and (±)-**5a**. Results are summarized in Table 2 (Scheme 2).

The selective deprotection of the ester function of both (±)-**4** and (±)-**5a** with LiOH in MeOH at room temperature gave a mixture of epimeric amino acids (±)-**8** and (±)-**9**, respectively, in the ratio indicated in the Table (entries 1, 2).

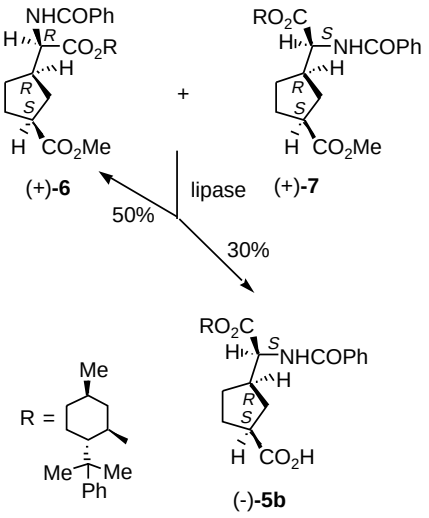
The epimerization was also observed by hydrolysis of the ester function of (±)-**5a** with BBr_3 (entry 3).[12] Attempts to first hydrolyze the amide function using Na_2O_2[13] gave only the hydrolysis of the ester function with epimerization (entry 4). Finally, the best result was achieved by using a catalytic amount of bis-tributyltin oxide (BBTO)[14] in refluxing toluene (entry 5). Compound (±)-**9** was obtained from **5a** with a small amount of **8** (7%). The direct hydrolysis of both ester and amide function of (±)-**5a** with 6 M HCl at reflux was possible and a mixture of amino acids **11** and **10** was obtained due to the epimerization process (entry 6). Instead, the hydrolysis (6 M HCl) of the amide function of the free bicarboxylic compounds did not undergo epimerization. In fact, starting from the mixture of acids **9**/**8** (93:7) the mixture of amino acids **11** and **10** was obtained in the same ratio (entry 7).

We can conclude that it is impossible to completely avoid the epimerization process in compounds in which both

Table 1. Hydrolysis of ester of racemic compound (±)-**5a** with different microbial cells or isolated enzymes

Microorganism	ee S (%)[a]	ee P (%)[a]	Molar conversion (%)[a]	E[a]	Time (h)
Streptomyces sp. 27	74	90	45	42	24
Streptomyces sp. 52	22	51	30	3,8	48
Streptomyces sp. 103	24	>99	29	>200	24
Streptomyces sp. 34	4	68	6	5,5	144
Pichia etchellsii MIM	36	>99	26	>200	4
Pichia etchellsii MIM	>99	80	56	65	24
Saccaromyces cerevisiae Zeus	66	>99	40	>200	4
Saccaromyces cerevisiae Zeus	>99	/	/	/	24
Geobacillus thermoleovorans ATCC 43513	41	42	49	3,6	24
Papaine	93	93	50	94	24
Papaine	>99	84	54	84	96
Acylase from *Aspergillus melleus*	>99	95	51	>200	24
Acylase from *Aspergillus melleus*	>99	84	54	84	96
Acylase from *Asp.* sp.	88	92	49	69	4
Acylase from *Asp.* sp.	>99	83	55	78	24
Porcine pancreatic lipase	77	>99	44	>200	4
Porcine pancreatic lipase	>99	99	50	>200	24

[a] Conversion and enantioselectivity factor (E) calculated from the ee of the substrate (ee S) and the product (ee P).



Scheme 3.

the amino and the carboxy groups are protected, probably because of the high acidity of the amino acid α-hydrogen. Instead, starting from the *N*-protected acids this problem is overcome.

With this information in mind, the chiral esters (−)-**4** and (−)-**5a** were hydrolyzed with BBTO. Bicarboxylic compounds (+)-**8** (de 86%) and (+)-**9** (de 86%) were obtained, respectively.

The hydrolysis of the amide function of the single stereomers (+)-**8**, (−)-**8**, (+)-**9** and (−)-**9** to the corresponding amino acids (−)-**10** (de 86%, $[\alpha]_D^{25}$ −4), (+)-**10** ($[\alpha]_D^{25}$ +5), (−)-**11** (de 86%, $[\alpha]_D^{25}$ −10) and (+)-**11** ($[\alpha]_D^{25}$ +11) was performed using 6 M HCl.

The absolute configuration of all stereocenters of bicarboxylic acids **8** and **9** was unequivocally assigned by correlation of the [α] values with authentic samples. We found that starting both from **7**,[7] by hydrolysis, and from (±)-**5a**, by enzymatic resolution, the same epimer (−)-*S*-**9** was obtained. The same *S* selectivity of the enzymes was observed in the case of compound (±)-**4**. Epimer (−)-*S*-**8** was obtained, having the opposite optical rotation with respect to that found for compound obtained through the chiral synthesis.

3. Conclusion

In conclusion, a highly efficient protocol for the preparation of four enantiopure stereoisomers of the 3-carboxycyclopentylglycine derivatives **10**,**11** was achieved starting from the racemic 3-oxo-norbornaneamino acid derivative **3**. The enzymatic separation of protected cyclopentylglycine

Table 2. Chemical deprotection of amino acid function

Entry	Reagent	Reaction conditions	Yield (%)	Products[a] (ratio)
1	(±)-**4**	LiOH/MeOH, 25 °C	80	**8/9** (86:14) [b]
2	(±)-**5a**	LiOH/MeOH, 25 °C	80	**9/8** (86:14)[b]
3	(±)-**5a**	BBr_3/CH_2Cl_2, 25 °C	75	**9/8** (85:15) [b]
4	(±)-**5a**	Na_2O_2/H_2O, 50 °C	80	**9/8** (80:20) [b]
5	(±)-**5a**	BBTO/toluene/reflux	95	**9/8** (93:7) [b]
6	(±)-**5a**	6 M HCl, reflux	95	**11/10** (87:13) [c]
7	(±)-**9/8** (93:7)	6 M HCl, reflux	95	**11/10** (93:7) [c]

[a] Isolated compounds.
[b] HPLC determination.
[c] ^{13}C NMR determination.

derivatives, obtained in high enantiomeric purity, was assured using the acylase from *Aspergillus melleus*, which also operates on semipreparative scale.

4. Experimental

4.1. General

Melting points were measured with a Büchi B-540 heating unit and are not corrected. NMR spectra were recorded with an AVANCE 500 Bruker at 500 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts, relative to TMS as internal standard, are given in δ values. IR spectra were taken with a Perkin–Elmer 1725X FT-IR spectrophotometer. $[\alpha]_D^{25}$ were measured with a Perkin–Elmer MODEL343 Plus Polarimeter. Ethanol-free CH_2Cl_2 was used in all experiments.

4.1.1. 'One Pot' synthesis of ethyl (1*R,2*R**,4*S**)-2-benzoylamino-3-oxo-bicyclo[2.2.1]heptane-2-carboxylate *exo*-3.** Compounds *exo*-**1a** and *endo*-**1a** were prepared according to known procedures.[11] Without the separation of diastereomers, the mixture of norbornane esters (5 g, 13.3 mmol, 7:3) was treated with lyophilized Na_2CO_3 (1.37 g, 13.3 mmol) in EtOH (50 mL) at room temperature under stirring for 24 h (TLC: CH_2Cl_2/Et_2O, 2:1). Na_2CO_3 was filtered over a Celite column and the alcohol was evaporated. Unreacted compound *endo*-**1** (1.2 g, 24%) was separated from hydroxy compound *exo*-**2** by column chromatography on silica gel (CH_2Cl_2/Et_2O, 10:1; R_f: *endo*-**1**=0.51, *exo*-**2**=0.37). Pure compound *exo*-**2** (1.7 g, 42%) was obtained after crystallization. Alternatively, the mixture of *endo*-**1** and *exo*-**2**, after filtration over Celite, was directly oxidized. Both, hydroxy compound *exo*-**3** and a mixture of *endo*-**2**/*exo*-**3** were treated under nitrogen atmosphere with PCC (6 mmol×1 mmol of reagent) in CH_2Cl_2 (80 mL). The solution, was stirred at room temperature for 2 h (TLC: cyclohexane/AcOEt, 1:1). The reaction mixture was filtered through a silica gel column (cyclohexane/AcOEt, 70:30). Starting from pure *exo*-**2** (1.7 g, 5.6 mmol), ketone *exo*-**3** (1.6 g, 95%) was obtained. Compound *exo*-**3** (2.58 g, 62%) and carbonate *endo*-**1** (1.25 g, 25%) (R_f: *exo*-**3**=0.6, *endo*-**1**=0.2) were isolated starting from the mixture of *endo*-**1**/*exo*-**2** (5 g, 13.3 mmol, 7:3). Mp 160 °C (cyclohexane/AcOEt). IR: ν_{max} 3320, 1720, 1700, 1640 cm^{-1}; ^{1}H NMR δ 7.83–7.42 (m, 5H, ArH), 6.69 (s, 1H, exch., NH), 4.30–4.10 (m, 2H, CH_2O), 3.73 (br s, 1H, H-4), 2.81 (dd, J=5.1, 1.2 Hz, 1H, H-1), 2.53, 1.83 (AB system, J=9.9 Hz, 2H, H-7), 2.06–1.94 (m, 1H, H-5), 1.75–1.28 (m, 3H, H-5, H-6), 1.22 (t, J=7.3 Hz, 3H, Me); ^{13}C NMR δ 14.3, 22.1, 27.6, 34.8, 44.4, 48.3, 62.5, 72.0, 127.4, 128.9, 132.3, 133.6, 166.3, 168.2, 210.2. Anal. Calcd: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.70; H, 6.38; N, 4.62.

4.2. General procedure for the retro-Claisen reaction of ketone *exo*-3

Pure ketone *exo*-**3** (301 mg, 1 mmol) was dissolved in pyridine (3 mL) and H_2O (1.5 mL). The reaction mixture was heated at reflux for 3 h (TLC: CH_2Cl_2/Et_2O, 2:1). The solvent was evaporated and the residue was taken up with a HCl solution (10%, 10 mL) which was then extracted with a mixture of THF/AcOEt (7.5 mL, 1:1). The organic layer was separated and dried over Na_2SO_4 giving a mixture of the two diastereomeric amino acid derivatives **4** and **5a** (1:1, 260 mg, 82%). Compounds (±)-**4** from (±)-**5a** were separate by flash chromatography (CH_2Cl_2/Et_2O/AcOH, 5:1:0.1, R_f: **4**=0.4, **5a**=0.32).

4.2.1. (1*S,3*R**,1′*R**) 3-(Benzoylamino-ethoxycarbonyl-methyl)cyclopentanecarboxylic acid (±)-4.** 95 °C. (CH_2Cl_2/Et_2O). IR: ν_{max} 3340, 1732, 1700, 1668 cm^{-1}; ^{1}H NMR δ 7.88–7.40 (m, 5H, ArH), 7.12 (d, J=8.1 Hz, 1H, exch., NH), 4.89 (dd, J=8.1, 5.1 Hz, 1H, CHN), 4.25 (q, J=7.0 Hz, 2H, CH_2O), 2.99–2.83 (m, 1H, H-1), 2.78–2.53 (m, 1H, H-3), 2.10–1.60 (m, 6H, H-2, H-4, H-5), 1.32 (t, J=7.0 Hz, 3H, Me); ^{13}C NMR δ 181.6, 172.5, 168.1, 134.3, 132.1, 129.0, 127.6, 61.9, 55.0, 43.2, 42.5, 30.9, 30.3, 28.7, 14.6. Anal. Calcd: C, 63.94; H, 6.63; N, 4.39. Found: C, 63.87; H, 6.59; N, 4.31.

4.2.2. (1*S,3*R**,1′*S**) 3-(Benzoylamino-ethoxycarbonyl-methyl)cyclopentanecarboxylic acid (±)-5a.** Mp 134 °C (CH_2Cl_2/iPr_2O). IR: ν_{max} 3340, 1730, 1700, 1665 cm^{-1}; ^{1}H NMR δ 7.85–7.42 (m, 5H, ArH), 6.87 (d, J=7.7 Hz, 1H, exch., NH), 4.88 (dd, J=8.0, 5.9 Hz, 1H, CHN), 4.26 (q, J=7.0 Hz, 2H, CH_2O), 2.93–2.80 (m, 1H, H-1), 2.80–2.45 (m, 1H, H-3), 2.30–1.60 (m, 6H, H-2, H-4, H-5), 1.32 (t, J=7.0 Hz, 3H, Me); ^{13}C NMR δ 181.7. 172.4, 168.1, 134.2, 132.2, 129.0, 127.5, 62.0, 55.1, 43.4, 43.1, 32.7, 29.4, 27.8, 14.6. Anal. Calcd: C, 63.94; H, 6.63; N, 4.39. Found: C, 63.89; H, 6.60; N, 4.33.

4.3. Media and culture conditions

Strains from an official collection (ATCC, American Type Culture Collection), from our collection (MIM, Microbiologia Industriale Milano) and *Streptomyces* kindly furnished by Prof. Flavia Marinelli were employed. The active biocatalysts are reported in Table 1. Yeasts were routinely maintained on malt extract (8 g/L, agar 15 g/L pH 5,5), non filamentous bacteria on Difco nutrient broth (8 g/L, agar 15 g/L, pH 7) and *Streptomyces* on oatmeal agar (60 g/L corn flaks, agar 20 g/L, pH 7). To obtain cells for biotransformations, microorganisms were cultured on different media: (a) yeast: malt extract 15 g/L, yeast extract 5 g/L, pH 5.8; (b) *Geobacillus* CYSP medium (casytone 15 g/L yeast extract 5 g/L, soytone3 g/L, peptone 2 g/L, $MgSO_4\cdot 7H_2O$ 15 mg/L, $FeCl_3\cdot 6H_2O$ 116 mg/L, $MnCl_2\cdot 4H_2O$ 20 mg/L, pH 7); (c) *Streptomyces*: AF/MS medium (glucose 20 g/L, yeast extract 2 g/L, soy meal 8 g/L, NaCl 4 g/L, $CaCO_3$ 1 g/L, pH 7). The cultures were inoculated into a 100 mL Erlenmeyer flask containing 20 mL of medium and incubated at 28 °C (45 °C for *Geobacillus*) for 24–48 h in the case of yeast and *Geobacillus* and 96 h for *Actinomyces*. The biomass production was carried out on rotary shaker at 200 rpm.

4.4. Biotransformations

The screening was carried out in 24 well microtitre plates (125×5×18 mm). Isolated enzyme or cells harvested by centrifugation were suspended (20 mg dry weight/mL) in a phosphate buffer (0.1 M, pH 7.0) and put into the wells. A mixture of (±)-**5a** or (±)-**4** (final concentration 4 mg/mL)

or of diastereomers **6** and **7** (final concentration 2 mg/mL) was directly added. The plates were incubated at 28 °C on a rotary shaker. The biotransformation was monitored by TLC at different times. To detect the enantiomeric excess, the biotransformations were carried out in a 10 mL screw capped test tube with the biocatalyst suspended in 5 mL of buffer and the samples were analysed by chiral HPLC. The diastereoisomeric excess was detected by reverse phase HPLC.

4.5. Analytical methods

In the Screening phase, 200 μL of the sample were brought to pH 2 with HCl and extracted with ethyl acetate and analysed by thin-layer chromatography ($CHCl_3$/Et_2O/AcOH, 50:25:1). The enantiomeric excess and the molar conversion were determined by chiral HPLC analysis (Chiralcel OD: hexane/*i*PrOH/TFA, 85:15:1; $T=25$ °C; flow=0,5 mL/min; $\lambda=230$ nm). The samples (0.5 mL) were brought to pH 2 with HCl and extracted twice with an equal volume of ethyl acetate. The organic phases were dried over Na_2SO_4, the solvent was removed and the sample was analysed. The diastereomeric excess for compounds **6** and **7** was determined by reverse phase HPLC analysis (Hypersil ODS: MeCN/H_2O, 70:30; $T=25$ °C; flow=1 mL/min; $\lambda=254$ nm).

4.6. Semipreparative biotransformations

Resolution of (±)-**4** *and* (±)-**5a**. Semi preparative resolution of mixture (±)-**4** or (±)-**5a** (250 mg, 0.8 mmol) was carried out resuspending acylase from *Aspergillus melleus* (5 mg/mL) in a phosphate buffer (0.1 M, pH 7.0, 60 mL). After 24 h (**5a**: HPLC monitoring) or 48 h (**4**, HPLC monitoring) the reaction was treated with HCl (pH 2) and extracted three times with ethyl acetate. The organic phase was separated and dried over Na_2SO_4 and the solvent removed under vacuum. The acid (−)-**8** (93 mg, 40%) or (−)-**9** (93 mg, 40%) was separated from unreacted compound (−)-**4** (125 mg, 50%) or (−)-**5a** (125 mg, 50%), respectively, by crystallization with CH_2Cl_2 (15 mL).

Resolution of **6**,**7**. A mixture of **6**,**7** (50 mg) was treated with lipase from *Candida cylindracea* (17 mg/mL) resuspended in phosphate buffer (0.1 M, pH 7.0) in the presence of MeCN (10%). The biotransformation systems were incubated at 30 °C under magnetic stirring. After 72 h the reaction mixture was extracted three times with ethyl acetate. The organic phase was separated and dried over Na_2SO_4 and the solvent removed under vacuum. Pure compound **5b** (15 mg, 30%) was separated from **6** (25 mg, 50%) by column chromatography ($CHCl_3$, Et_2O, 2:1).

4.6.1. 3-(Benzoylamino-carboxy-methyl)-cyclopentane-carboxylic acids.

(1*R*,3*S*,1′*S*) (−)-**8**. Mp 210 °C (CH_2Cl_2). $[\alpha]_D^{25}$ −20 (*c* 1, MeOH).

(1*S*,3*R*,1′*S*) (−)-**9**. Mp 193 °C (CH_2Cl_2). $[\alpha]_D^{25}$ −14.5 (*c* 1, MeOH) [Mp 193 °C (CH_2Cl_2); $[\alpha]_D^{25}$ −14.5 (*c* 1, MeOH)].[7]

4.6.2. 1*S*,3*R*,1′*S*-(Benzoylamino-(−)-8-phenylmenthoxy-carbonyl-methyl)cyclopentanecarboxylic acid (−)-5b.

Oil. $[\alpha]_D^{25}$ −8 (*c* 1, $CHCl_3$). IR (nujol) cm^{-1} 3300, 1725, 1660; ^1H NMR δ 7.82–7.78 (m, 2H, ArH), 7.52–7.16 (m, 8H, ArH), 6.58 (d, $J=8.0$ Hz, 1H, exch., NH), 4.87–4.79 (m, 1H, OCH), 4.17 (dd, $J=8.0$, 5.5 Hz, 1H, CHN), 2.79–2.71 (m, 1H, H-1), 2.17–0.79 (m, 15H, H-2, H-3, H-4, H-5, CH_{2menth}, CH_{menth}), 1.31 (s, 3H, Me), 1.21 (s, 3H, Me), 0.87 (d, $J=6.4$ Hz, 3H, Me); ^{13}C NMR δ ($CDCl_3$) 180.0, 171.4, 167.5, 151.7, 134.5, 131.8, 128.8, 128.3, 127.3, 125.5, 76.2, 54.2, 50.8, 43.0, 42.9, 41.6, 39.7, 34.7, 32.9, 31.5, 29.9, 27.1, 26,7, 24.4, 22.0. Anal. Calcd: C, 73.63; H, 7.77; N, 2.77. Found: C, 73.59; H, 7.80; N, 2.70.

4.7. General procedure for the deprotection of carboxylic group

Method (*a*). To a solution of compound (±)-**4**, or (±)-**5a** (319 mg, 1 mmol) in MeOH (4 mL), LiOH (48 mg, 2 mmol) was added. The reaction was stirred at room temperature for 24 h and extracted with AcOEt (3×4 mL). The aqueous layer was acidified with 2 M HCl (3 mL) and extracted with THF/AcOEt (1:1, 3×4 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuum to obtain the free carboxylic derivative ((±)-**4**: **8/9**, 86:14, 80%; (±)-**5a**: **9/8**, 86:14, 80%).

Method (*b*). In a sealed tube a solution of compound (±)-**5a**, or (−)-**5a** or (−)-**4** (319 mg, 1 mmol) and BBTO (1.19 g, 2 mmol) in toluene (10 mL) was stirred at 110 °C for 48 h. The solvent was removed under vacuum, the residue was taken up with AcOEt (10 mL) and extracted with a saturated solution of $NaHCO_3$ (3×5 mL). The aqueous layer was acidified with 2 N HCl (4 mL) and extracted with THF/AcOEt (1:1, 3×5 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuum to give the free carboxylic acid derivative ((±)-**5a**: **9/8**, 93:7, 90%; (−)-**5a**: (+)-**9**/(−)-**8**, 93:7, 90%; (−)-**4**: (+)-**8**/(−)-**9**, 93:7, 90%).

4.7.1. 3-(Benzoylamino-carboxymethyl)cyclopentane-carboxylic acid.

(1*S*,3*R*,1′*R*) (+)-**8**. Mp 200 °C. (CH_2Cl_2); $[\alpha]_D^{25}$ +17.5 (*c* 1, MeOH). [210 °C (CH_2Cl_2); $[\alpha]_D^{25}$ +20 (*c* 1, MeOH)].[7]

(1*R*,3*S*,1′*R*) (+)-**9**. Mp 189 °C. $[\alpha]_D^{25}$ +12 (*c* 1, MeOH).

4.7.2. 3-(Amino-carboxymethyl)cyclopentanecarboxylic acid hydrochloride.

In a sealed tube, pure compound (−)-**8**, or (−)-**9**, or a mixture of (+)-**8**/(−)-**9**, or of (+)-**9**/(−)-**8** (93:7) or their corresponding racemic mixture (291 mg, 1 mmol) was suspended in HCl (6 M, 3 mL) and heated at 105 °C for 12 h. The reaction mixture was cooled at 0 °C and the precipitated benzoic acid was filtered. The aqueous layer was washed with Et_2O (3×2 mL) and evaporated under vacuum giving the pure amino acid hydrochloride **10** or **11** (159 mg, 85%).

(1*R*,3*S*,1′*S*) (+)-**10**. Oil. $[\alpha]_D^{25}$ +5 (*c* 1, H_2O).

(1*S*,3*R*,1′*R*) (−)-**10**. Oil. $[\alpha]_D^{25}$ −4 (*c* 1, H_2O); [oil, $[\alpha]_D^{25}$ −5 (*c* 1, H_2O)].[7]

(1*S*,3*R*,1′*S*) (+)-**11**. Oil. $[\alpha]_D^{25}$ +11 (*c* 1, H_2O); [oil; $[\alpha]_D^{25}$ +9 (*c* 1, H_2O)].[7]

(1*R*,3*S*,1′*R*) (−)-**11**. Oil. $[\alpha]_D^{25}$ −10 (*c* 1, H_2O).

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