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Enantioselective acylation of *rac*-2-phenylcycloalkanamines catalyzed by lipases

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Abstract—The kinetic resolution of some 2-phenylcycloalkanamines was performed by means of aminolysis reactions catalyzed by lipases, with Kazlauskas' rule being obeyed in all cases. The size of the ring and the stereochemistry of the stereogenic centers of the amines had a strong influence on both the enantiomeric ratio and the reaction rate of these aminolysis processes. Lipase B from *Candida antarctica* (CAL-B) showed excellent enantioselectivities toward *trans*-2-phenylcyclohexanamine in a variety of reaction conditions ($E > 150$), whereas lipase A from *C. antarctica* (CAL-A) was the best catalyst for the acylation of *cis*-2-phenylcyclohexanamine ($E = 34$) and *trans*-2-phenylcyclopropanamine ($E = 9$).
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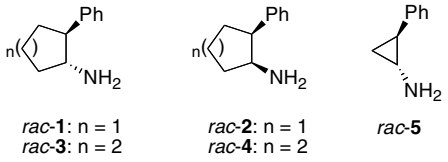
1. Introduction

2-Phenylcycloalkanamines (Fig. 1) are interesting compounds because of their pharmacological properties. For example, *trans*-2-phenylcyclopentanamine **1**, 'cypenamine', and *trans*-2-phenylcyclopropanamine **5**, 'tranylcypromine' are well-known antidepressants.[1] In addition, the *cis*- and *trans*-isomers of 2-phenylcyclopentanamine and 2-phenylcyclohexanamine **1**–**4** are important building blocks of semicyclic amidines with potent hypoglycemic activity[2] and have also been used as starting materials in the synthesis of sulfonamide potentiators of AMPA receptors, the activity being dependent on the stereochemistry of the stereogenic centers.[3] Besides the pharmacological importance of these compounds, optically active amines are also of great synthetic applicability and have been used as chiral auxiliaries, bases and ligands, and for asymmetric synthesis.[4] For these reasons, the development of efficient methods for the preparation of optically active amines is an area of continuous interest in organic chemistry.



rac-**1**: n = 1 *rac*-**3**: n = 2 *rac*-**2**: n = 1 *rac*-**4**: n = 2 *rac*-**5**

Figure 1. 2-Phenylcycloalkanamines.

In recent years, we have investigated the utility of some lipases, especially lipase B from *Candida antarctica* (CAL-B) to catalyze the aminolysis of esters.[5] Thus, in a very recent report, we reported the CAL-B-catalyzed kinetic resolution of racemic *trans*- and *cis*-2-phenylcyclopentanamine **1** and **2**.[6] The adequate selection of the reaction conditions allowed us to obtain the corresponding amines in very high enantiomeric excess and chemical yield, thus resulting in an interesting alternative to other reported methods.[7] These findings prompted us to extend our study to other important 2-phenylcycloalkanamines (Fig. 1). Herein, we report our results on the enzymatic resolution of *trans*- and *cis*-2-phenylcyclohexanamine *rac*-**3** and *rac*-**4**, and the commercially available *trans*-2-phenylcyclopropanamine *rac*-**5**.

2. Results and discussion

2.1. Synthesis of 2-phenylcyclohexanamines 3 and 4

The first attempt to prepare the *cis*-isomer *rac*-**4** consisted of the reductive amination of 2-phenylcyclohexanone **6** in methanol using ammonium formate and 10% Pd–C as catalyst (Scheme 1).[8] However, the reaction

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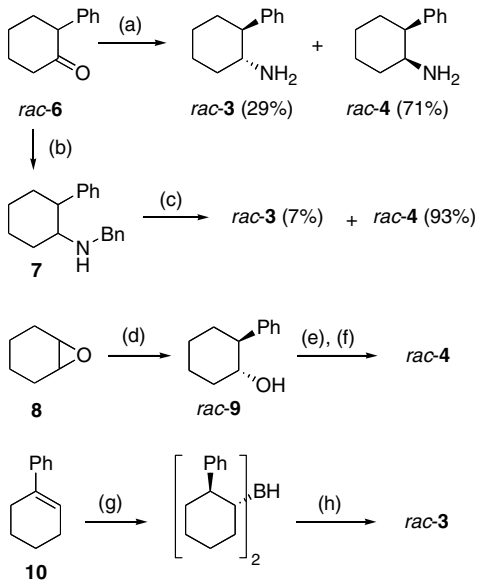


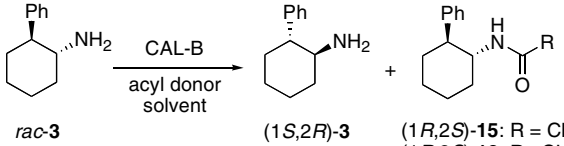
Scheme 1. Synthesis of *rac*-**3** and *rac*-**4**. Reagents: (a) $HCO_2^-NH_4^+$, 10% Pd–C, MeOH–H_2O; (b) $BnNH_2$, $Na(AcO)_3BH$, glacial AcOH, 1,2-dichloroethane; (c) 4.4% HCO_2H in MeOH, Pd-black; (d) PhMgBr, Et_2O; (e) PPh_3, DIAD, phthalimide, THF; (f) H_2N–NH_2, toluene; (g) $NaBH_4$, BF_3–Et_2O, diglyme; (h) H_2N–OSO_3H, diglyme.

happened with low diastereoselectivity, and a 71:29 mixture of *cis*- and *trans*-isomers was obtained. Further changes of the solvent and the reaction temperature did not modify this diastereomeric ratio. The second attempt involved several steps: the formation of an imine from **6** and benzylamine, in situ reduction of this imine with sodium triacetoxyborohydride, and hydrogenolysis of the benzyl group of the resulting amine **7** (see Scheme 1). With this methodology, 2-phenylcyclohexanamine was obtained in good yield and with a promising isomeric ratio (93:7, *cis*/*trans*). However, it was not possible to increase this ratio. Finally, the stereoselective synthesis of *rac*-**4** was efficiently achieved by a three-step sequence of a known procedure: reaction of cyclohexene oxide **8** with phenyl magnesium bromide, Mitsunobu reaction of the resulting *trans*-2-phenylcyclohexanol *rac*-**9** with phthalimide and subsequent aminolysis with hydrazine.[2,8] In addition, the preparation of the trans-isomer *rac*-**3** (see Scheme 1) was accomplished by hydroboration–oxidation of 1-phenylcyclohexene **10** following the method developed by Brown et al.[9]

2.2. Enzymatic resolution of racemic 2-phenylcycloalkanamines *rac*-3-5

So far, CAL-B (Novozyme SP-435) has proven to be the most effective catalyst for the aminolysis reaction in organic solvents.[5] Thus, as a first attempt, the resolution of racemic *trans*-2-phenylcyclohexanamine *rac*-**3** was carried out by CAL-B-catalyzed acylation under the simplest reaction conditions, that is, using ethyl acetate as the acyl donor and the solvent (Table 1, entry 1). Under these conditions, the enzyme preferentially catalyzed the acylation of the (1*R*,2*S*)-enantiomer of the amine, the produced acetamide (1*R*,2*S*)-**15** and the unreacted amine (1*S*,2*R*)-**3** being easily separated by selective extraction and isolated with very high yields. The high enantiomeric ratio allowed us to obtain the acetamide with very high ee (98%). The remaining amine was obtained with only moderate ee (67%), but a longer reaction time allowed us to isolate the enantiopure (1*S*,2*R*)-**3** at conversions (*c*) near to 50% (Table 1, entry 2). In spite of the good results obtained, we decided to optimize the enantioselectivity checking *tert*-butyl methyl ether (TBME) as solvent and other acyl donors (Fig. 2), according to the results previously described with the 2-phenylcyclopentanamines.[6] Thus, in all cases the enantiomeric ratios (*E*)[10] were very high (Table 1, entries 3–5), allowing the isolation of amides (1*R*,2*S*)-**15** and (1*R*,2*S*)-**16** with ee $\geqslant$96%. As shown in Table 1, the reactions with α-methylbenzyl acetate *rac*-**12** and

Table 1. Enzymatic resolution of racemic *trans*-2-phenylcyclohexanamine *rac*-**3**[a]



Entry	Acyl donor	Solvent	Time [h]	c[b] [h]	(1*S*,2*R*)-**3** ee_s[c] [%]	(1*R*,2*S*)-**15**, **16** ee_p[c] [%]	E[d]
1	**11**	AcOEt	20	41	67	98[e]	166
2	**11**	AcOEt	47	52	>99	91[e]	159[f]
3[g]	*rac*-**12**	TBME	43	36	56	99[e]	>200
4[g]	**11**	TBME	44	17	20	99[e]	160
5[g]	**13**	TBME	24	51	>99	96[h]	>200[f]

[a] All the reactions were carried out at 28 °C and 200 rpm.
[b] Conversion: $c = ee_s/(ee_s + ee_p)$.
[c] Enantiomeric excess determined by chiral HPLC.
[d] Determined from ee_s and ee_p according to Ref. 10.
[e] Amide (1*R*,2*S*)-**15**.
[f] Value obtained from $ee_s = 99.9\%$.
[g] 1:3 Molar ratio amine/acyl donor.
[h] Amide (1*R*,2*S*)-**16**.

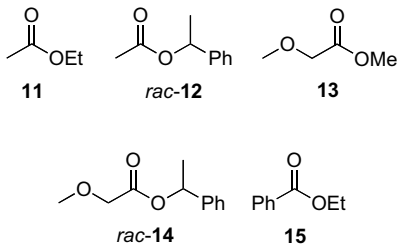


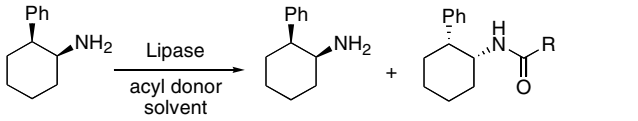
Figure 2. Selected acyl donors **11**–**15** used in the enzymatic reactions.

methyl methoxyacetate **13** showed the highest enantioselectivities, and were the most suitable acyl donors to produce the optically active (1*R*,2*S*)-amide in good yields. In addition, if the amine of the opposite configuration was required, that is (1*S*,2*R*)-**3**, the fastest reaction happened with **13** in TBME. However, the use of ethyl acetate as an acyl donor and solvent is an interesting alternative since the enantiopure amine was also obtained from this reaction. The (1*S*,2*R*)-configuration for the remaining amine **3** was assigned after comparison of its specific rotation with that reported.[11] This means that CAL-B follows the Kazlauskas' rule with the (1*R*)-enantiomer of the substrate preferentially being transformed.[12]

In the same way, we tried the resolution of racemic *cis*-2-phenylcyclohexanamine *rac*-**4** by CAL-B-catalyzed acylation employing ethyl acetate as the acyl donor and solvent (Table 2, entry 1). However, great differences in the reaction rate and enantioselectivity were observed with respect to the *trans*-isomer. In contrast to the excellent results above, the reaction with the *cis*-isomer *rac*-**4** was markedly slower and happened with very poor enantioselectivity. This result reveals a very strong influence of the relative configuration of the two stereogenic centers of the amine on the enzyme activity. In our previous work, we also observed differences in behavior of the enzyme toward the *cis*- and *trans*-isomers of 2-phenylcyclopentanamine, but to a much lower degree.[6] Trying to improve this preliminary result, two different lipases [PSL-C (immobilized lipase PS from Amano, recently classified as *Burkholderia cepacia* lipase) and CAL-A (*C. antarctica* lipase A)] were screened in combination with ethyl acetate (Table 2, entries 2 and 3). Unfortunately, in both cases unsuccessful results were obtained. Although with PSL-C the reaction rate was significantly increased, no selectivity was observed. The next attempt was to test different combinations of acyl donors and enzymes with *tert*-butyl methyl ether as solvent (entries 4–7). Reactions again took place very slowly, and the enantioselectivities were poor, except when the reaction was carried out with CAL-A and α-methylbenzyl acetate *rac*-**12** (entry 6) for which a significant enhancement of the enantioselectivity was achieved ($E = 34$). This moderate value of E allowed us to obtain the amide (1*R*,2*R*)-**17** in high ee (94%). Nevertheless, the reaction was very slow, with five days of reaction being required to attain a conversion degree of 6%.

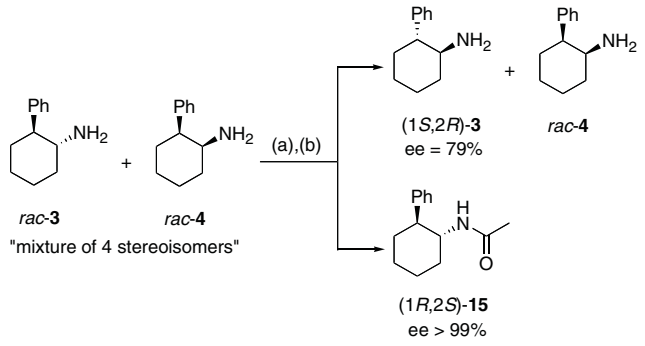
The strong differences in reactivity between the *cis*- and *trans*-isomers of 2-phenylcyclohexanamine in the lipase-catalyzed aminolysis of esters indicate that CAL-B is also highly diastereoselective. Thus, when the easily prepared 29:71 mixture of racemic *trans*-**3** and *cis*-**4** (see Scheme 1) was submitted to acylation with CAL-B, α-methylbenzyl acetate *rac*-**12** as acyl donor and TBME as solvent, only amine *trans*-(1*R*,2*S*)-**3** was acylated (Scheme 2). Moreover, acetamide (1*R*,2*S*)-**15** was produced to similar reaction rate and enantioselectivity as when the starting material was the single diastereomer *rac*-**3**. So, after 49 h of reaction, 44% of the *trans*-amine was converted with the enantiomeric excesses of the remaining *trans*-amine (1*S*,2*R*)-**3** and the produced *trans*-amide (1*R*,2*S*)-**15** being 79% and >99%,

Table 2. Enzymatic resolution of racemic *cis*-2-phenylcyclohexanamine *rac*-**4**[a]



Entry	Acyl donor	Lipase	Solvent	Time [h]	c[b] [%]	(1*S*,2*S*)-**4** ee_S[c] [%]	(1*R*,2*R*)-**17**, **18** ee_P[c] [%]	E[d]
1	**11**	CAL-B	AcOEt	47	5	<3	<3[e]	1
2	**11**	PSL-C	AcOEt	96	40	<3	<3[e]	1
3	**11**	CAL-A	AcOEt	185	20	<3	<3[e]	1
4[f]	**13**	CAL-B	TBME	33	1	<3	<3[g]	1
5[f]	*rac*-**12**	CAL-B	TBME	168	2	<3	24[e]	2
6[f]	*rac*-**12**	CAL-A	TBME	120	6	6.0	94[e]	34
7[f]	*rac*-**14**	CAL-A	TBME	134	13	8.0	55[g]	4

[a] All the reactions were carried out at 28 °C and 200 rpm.
[b] Determined from the isolated yields of amides (entries 1–5) or from ee_s and ee_p (entries 6 and 7).
[c] Enantiomeric excess determined by chiral HPLC.
[d] Determined from ee_s and ee_p according to Ref. 10.
[e] Amide (1*R*,2*R*)-**17**.
[f] 1:3 Molar ratio amine/acyl donor.
[g] Amide (1*R*,2*R*)-**18**.



Scheme 2. Reagents and conditions: (a) CAL-B, *rac*-**12**, TBME; (b) aq 1 M HCl followed by extraction.

respectively ($E > 200$). These results indicate that the *cis*-diastereomer is not an inhibitor of the enzyme. Finally, by a simple extraction, enantiopure (1*R*,2*S*)-**15** was separated from the mixture of the unreacted amines. This method, which takes advantage of the diastereoselective and enantioselective properties of the CAL-B, could have interesting applications with analogous substrates.

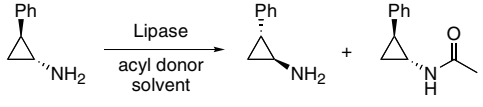
On the other hand, we tried to find out the origin of the drastic differences in reactivity between the *cis*- and *trans*-isomers of 2-phenylcyclohexanamine in the enzymatic reaction. These differences could be motivated for the different accommodation of both isomers in the active site of the enzyme or they could proceed from a different chemical reactivity of the *trans*- and *cis*-amine.[13] To prove the last point, the 'pK_a' values for both isomers were measured since basicity and nucleophilicity are parallel properties of the amines in most cases. The values obtained were practically identical, that is, 9.95 for the *trans*-isomer and 9.90 for the *cis*-isomer. On the other hand, we prepared an equimolar mixture of both isomers and checked the acetylation with a variety of reagents and conditions. However, the reactivity of both isomers was identical in all cases. These results clearly indicate that the reason for the different reactivities of the isomers in the enzymatic reaction is the capacity of the enzyme to accommodate them into the active site.

Finally, racemic *trans*-2-phenylcyclopropanamine *rac*-**5** was submitted to CAL-B-catalyzed acetylation using ethyl acetate as the acyl donor and solvent (Table 3, entry 1). However, the enantioselectivity of the process was very low ($E = 4$) and a high conversion (90%) was necessary to isolate the unreacted amine (1*S*,2*R*)-**5** with high ee. The (1*S*,2*R*)-absolute configuration for this amine was established by the comparison of its specific rotation with that reported.[14] It is noteworthy the great reactivity of this amine in comparison to the previous 2-phenylcycloalkanamines studied, a very high conversion degree ($c = 90\%$) being obtained after only 2 h of reaction. Further attempts to improve the enantioselectivity were carried out. When the reaction was checked at 15 °C, no difference was observed in the selectivity, despite a slight lower conversion (Table 3, entry 2). Employing other lipases (CAL-A and PSL-C) and ethyl acetate, caused the reaction rates to strikingly decrease and the enantiomeric ratio only slightly improved with PSL-C, although it still remained very low (Table 3, entries 3 and 4). Finally, we tried the resolution of *rac*-**5** with α-methylbenzyl acetate as acyl donor in TBME (Table 3, entries 5–7), but the lipases tested showed very low enantioselectivities.

3. Conclusions

Results obtained in the enzymatic resolution of some 2-phenylcycloalkanamines show that the size of the ring and the relative configuration of the stereogenic centers play an important role in determining both the reaction rate and enantioselectivity. CAL-B was the best catalyst for the resolution of racemic *trans*-2-phenylcyclohexanamine, and for its *cis*-isomer and for *trans*-2-phenylcyclopropanamine the best results were achieved with CAL-A. Further studies of molecular modeling of these

Table 3. Enzymatic resolution of racemic *trans*-2-phenylcyclopropanamine *rac*-**5**



rac-**5** (1*S*,2*R*)-**5** (1*R*,2*S*)-**19**

Entry	Acyl donor	Lipase	Solvent	Time [h]	c[a] [%]	(1*S*,2*R*)-**5** ee_S[b] [%]	(1*R*,2*S*)-**19** ee_p[b] [%]	E[c]
1	**11**	CAL-B	EtOAc	2	90	96	11	4
2[d]	**11**	CAL-B	EtOAc	3	86	91	15	3
3	**11**	CAL-A	EtOAc	72	40	4.5	6.2	1
4	**11**	PSL-C	EtOAc	8	21	18	69	7
5[e]	*rac*-**12**	CAL-B	TBME	1	48	21	23	2
6[e]	*rac*-**12**	CAL-A	TBME	94	49	62	65	9
7[e]	*rac*-**12**	PSL-C	TBME	94	40	31	47	4

[a] Conversion determined from the isolated yields of amides and from ee_s and ee_p.
[b] Enantiomeric excess determined by chiral HPLC.
[c] Determined from ee_s and ee_p according to Ref. 10.
[d] Reaction carried out at 15 °C.
[e] 1:3 Molar ratio amine/acyl donor.

processes are currently being carried out in order to explain the observed stereoselectivities.

4. Experimental

4.1. General

C. antarctica lipase B (Novozyme 435, available immobilized on polyacrylamide, 7300 PLU/g) was gifted by Novo Nordisk Co. CAL-A (Chirazyme® L-5, 2600 U/g lyo) supplied by Roche. Immobilized lipase from *Pseudomonas cepacia* (PSL-C, 783 U/g), recently classified as *B. cepacia* lipase, was purchased from Amano Pharmaceutical Co. For the enzymatic reactions, ethyl acetate of spectrophotometric grade (stored with 4 Å molecular sieves) and commercial anhydrous *tert*-butyl methyl ether (99.8%) were used. Thin-layer chromatography was performed on precoated TLC plates of Merck silica gel $60F_{254}$, using potassium permanganate as the developing reagent. For column chromatography, Merck silica gel 60 (particle size, 40–63 μm) was used. Melting points were taken on samples in open capillary tubes and are uncorrected. Optical rotations were measured using a Perkin–Elmer 343 polarimeter. IR spectra were recorded on an Infrared FT spectrophotometer in dichloromethane. Mass spectra were recorded on a Hewlett–Packard 1100 HPLC/MS (electrospray) instrument. The C, H, and N analyses were performed on a Perkin–Elmer 2400 analyzer. ^{1}H NMR and proton-decoupled ^{13}C NMR spectra ($CDCl_3$ solutions) were obtained using Bruker AC-200 (200.13 MHz for the ^{1}H and 50.3 MHz for the ^{13}C nuclei), and AC-300 (300.13 MHz for the ^{1}H and 75.5 MHz for the ^{13}C nuclei) spectrometers using the δ scale (ppm) for chemical shifts; calibration was made on the $CDCl_3$ (^{13}C; 76.95 ppm) or the residual $CHCl_3$ (^{1}H; 7.26 ppm) signals; ^{13}C NMR spectra were edited using DEPT techniques. Most of enantiomeric excesses were determined with a LC-10AD Shimadzu high performance liquid chromatograph, using a Chiralcel OD column (Daicel). For the pH-metric titrations, a Metrohm TITROPROCESSOR-636 titrimeter was used, the reference electrode was an Ag/AgCl electrode in satd aq KCl, the cell was thermostated at 298 ± 0.1 K, and the measurements were performed under nitrogen atmosphere. The protonation constants were determined by titration with 0.1 M NaOH of a solution containing 10^{-3} M of the HCl salt of the amine in the presence of Me_4NCl (0.1 M). The measurements were carried out twice and the data analysis performed with the computer program SUPERQUAD.[15]

4.2. Reductive amination of 2-phenylcyclohexanone

Method A: To a solution of 2-phenylcyclohexanone (20 mmol) in deoxygenated methanol (50 mL), water (6 mL), and ammonium formate (0.21 mol) were added. After complete dissolution, 10% Pd–C (0.80 g, 0.75 mmol) was added and the mixture stirred at room temperature until the disappearance of ketone (TLC control, ethyl acetate–methanol, 4:1). Then, the catalyst was filtered on Celite®, washed with methanol and the solvents evaporated. The residue was treated with concd aq HCl (4 mL) and water (30 mL) and extracted with diethyl ether (2×20 mL). The aqueous phase was treated with solid NaOH until pH basic and extracted with diethyl ether (3×25 mL). Evaporation of the organic phase yielded the crude amine which was purified by distillation under reduced pressure (bp 80 °C, 0.5 Torr). In this way, we obtained a 71:29 mixture of the *cis*- and *trans*-isomers of 2-phenylcyclohexanamine in 75% yield (^{1}H NMR spectra analysis).

Method B: To a mixture of 2-phenylcyclohexanone (5 mmol) and benzylamine (5.5 mmol) dissolved in dichloroethane (20 mL) under nitrogen was added sodium triacetoxyborohydride (7.5 mmol) and glacial acetic acid (5 mmol). After being stirred for two days, the mixture was treated with 3 M H_2SO_4 solution in order to remove the excess of sodium triacetoxyborohydride and benzylamine. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting residue was dissolved in a 4.4% solution of formic acid in methanol (50 mL) and Pd-black added (250 mg). The mixture was stirred at room temperature for 6 h. Then, palladium was filtered on Celite®, washed with methanol, and the solvents evaporated. The crude was treated with aq 3 M HCl and the mixture was stirred at room temperature overnight. The aqueous phase was washed with dichloromethane (20 mL), basified with solid NaOH and extracted with dichloromethane (3×25 mL). Further evaporation of solvent yielded the desired 2-phenylcyclohexanamine (70%) with a diastereomeric ratio of 93:7, *cis*/*trans*-isomers.

4.3. Stereospecific synthesis of racemic *cis*-2-phenylcyclohexanamine *rac*-3

To a mixture of copper iodide (2.0 mmol) and a 3 M diethyl ether solution of phenylmagnesium bromide (18 mL, 54 mmol,) under a nitrogen atmosphere was added dropwise over a period of 15 min a solution of cyclohexene oxide (30 mmol) in THF (15 mL) (exothermic reaction, reaching solvent reflux by the end of the addition). The reaction mixture was allowed to stir 7 h at room temperature and quenched with saturated ammonium chloride (40 mL). Diethyl ether was added (40 mL), and the organic layer separated and washed with saturated ammonium chloride (20 mL), dried, filtered, and concentrated to provide the corresponding *trans*-2-phenylcyclohexanol as a brown solid (95%).

To a solution of triphenylphosphine (30 mmol) in THF (70 mL) was added dropwise, at 0 °C and under a nitrogen atmosphere, a solution of diisopropyl azodicarboxylate (30 mmol) in THF (10 mL). A massive precipitate formed immediately, making it difficult to stir the reaction mixture. To the slurry was added phthalimide (30 mmol) and a solution of *trans*-2-phenylcyclohexanol (29 mmol) in THF (10 mL). The reaction mixture was then stirred at 0 °C for 4 h and brought to room temperature overnight. Solvent was evaporated under reduced pressure and dichloromethane (100 mL) and water (50 mL) were added. The organic layer was separated, washed with water (2×30 mL) and dried over

anhydrous magnesium sulfate. Subsequent concentration under reduced pressure afforded a precipitate. Then, hexane was added with intense stirring. The triphenylphosphine oxide precipitate was filtered off and the filtrate was concentrated to a yellow oil. Further purification by flash chromatography of the residue (hexane–AcOEt, 9:1) yielded *cis*-2-(2-phenylcyclohexyl)isoindole-1,3-dione (50%).

To a solution of *cis*-2-(2-phenylcyclohexyl)isoindole-1,3-dione (14.5 mmol) in toluene (50 mL) was added monohydrated hydrazine (145 mmol) and the resulting mixture refluxed for 8 h. Then, the reaction was cooled and the precipitates filtered. The cake was washed with toluene (10 mL), and the filtrate was concentrated to provide *cis*-2-phenylcyclohexanamine (>99%).

4.4. Enzymatic acetylation of racemic amines *rac*-3–5 with ethyl acetate. General procedure

To a mixture of a racemic amine *rac*-**3**–**5** (1.0 mmol) and CAL-B (Novozyme 435, 100 mg) under nitrogen atmosphere, ethyl acetate (5 mL) was added. The resulting mixture was circularly shaken at 28 °C and 200 rpm for the time shown in Tables 1–3. The enzyme was filtered off through a 1 cm pad of Celite, successively washed with ethyl acetate and methanol, and the organic solvent evaporated under reduced pressure. The residue was treated with 3 M aq H_2SO_4 (10 mL) and extracted with dichloromethane (3 × 15 mL). The aqueous phase was treated with solid NaOH until pH basic, and extracted with dichloromethane (4 × 15 mL). Evaporation of both organic phases allowed us to isolate the pure amide and amine respectively, in excellent yields. To avoid the oxidation of the amines, they were stored under a nitrogen atmosphere.

4.5. Enzymatic acylation of racemic amines *rac*-3-5 with acyl donors 11–14. General procedure

The corresponding acyl donors **11**–**14** (3.0 mmol) and solvents (6.5 mL) indicated in Tables 1–3 were added to a mixture of amine (1.0 mmol) and lipase (100 mg) under a nitrogen atmosphere. The suspension was circularly shaken at 28 °C and 200 rpm for the time shown in Tables 1–3. Afterwards, the procedure was similar to that for the enzymatic acetylation shown above, but once the remaining amine was extracted with 3 M aq H_2SO_4, combined organic layers were concentrated in vacuo to give a residue containing a mixture of amide, alcohol, and ester. Flash chromatography (hexane–AcOEt mixture) of the residue yielded pure amide.

4.5.1. (1*S*,2*R*)-2-Phenylcyclohexanamine (1*S*,2*R*)-3. $[\alpha]_D^{20} = +37.3$ (*c* 0.40, CH_3OH); ee = 67%. Lit.[11] for (1*S*,2*R*)-(+)-**4**: $[\alpha]_D^{20} = +45.0$ (*c* 0.074, CH_3OH); ee = >99%.

4.5.2. (1*R*,2*S*)-*N*-(2-Phenylcyclohexyl)acetamide (1*R*,2*S*)-15. White solid, mp = 144–146 °C; $[\alpha]_D^{20} = +27.9$ (*c* 1.0, $CHCl_3$); ee = 98%. Lit.[16] for (1*R*,2*S*)-**15**: $[\alpha]_D^{20} = +24$ (*c* 0.39, $CHCl_3$), ee = 98%. IR (CH_2Cl_2) 3424, 3321, 1646 cm^{-1}; ^1H NMR (300 MHz): $\delta = 1.15–1.55$ (m, 4H), 1.66 (s, 3H, CH_3), 1.70–1.90 (m, 3H), 2.18 (d, 1H, $J = 12.2$ Hz), 2.39 (dt, 1H, $J = 11.7$ and 3.2 Hz), 4.03 (dq, 1H, $J = 11.7$ and 3.8 Hz), 5.43 (br s, 1H, NH), 7.10–7.25 (m, 5H, Ph); ^{13}C NMR (75.5 MHz): $\delta = 20.70$ (CH_3), 23.28 (CH_2), 25.35 (CH_2), 25.71 (CH_2), 30.79 (CH_2), 44.51 (CH), 49.48 (CH), 126.32 (CH), 127.19 (CH), 128.20 (CH), 142.77 (C), 169.28 (C=O); MS (ESI^+), *m*/*z* (%) = 218 ($[M+H]^+$, 7), 240 ($[M+Na]^+$, 65). Anal. Calcd for $C_{14}H_{19}NO$ (217.3): C, 77.38; H, 8.81; N, 6.45. Found: C, 77.41; H, 8.71; N, 6.38.

4.5.3. (1*R*,2*S*)-*N*-(2-Phenylcyclohexyl)methoxyacetamide (1*R*,2*S*)-16. White solid, mp = 121–123 °C; $[\alpha]_D^{20} = +27.4$ (*c* 0.87, $CHCl_3$); ee = 96%; IR (CH_2Cl_2) 3309, 1648 cm^{-1}; ^1H NMR (300 MHz): $\delta = 1.20–1.60$ (m, 4H), 1.70–1.95 (m, 3H), 2.10–2.20 (m, 1H), 2.44 (dt, 1H, $J = 11.7$ and 3.1 Hz), 3.00 (s, 3H, CH_3), 3.43 (d, 1H, $J = 15.4$ Hz), 3.75 (d, 1H, $J = 15.1$ Hz), 4.06 (dq, 1H, $J = 11.4$ and 3.9 Hz), 6.19 (br d, 1H, NH, $J = 8.0$ Hz), 7.10–7.30 (m, 5H, Ph); ^{13}C NMR (75.5 MHz): $\delta = 25.12$ (CH_2), 25.97 (CH_2), 33.76 (CH_2), 35.04 (CH_2), 50.51 (CH), 51.54 (CH), 58.69 (CH_3), 71.62 (CH_2), 126.32 (CH), 127.26 (CH), 128.28 (CH), 143.33 (C), 168.49 (C=O); MS (ESI^+), *m*/*z* (%) = 248 ($[M+H]^+$, 20), 270 ($[M+Na]^+$, 45). Anal. Calcd for $C_{15}H_{21}NO_2$ (247.3): C, 72.84; H, 8.56; N, 5.66. Found: C, 72.90; H, 8.50; N, 5.61.

4.5.4. (1*S*,2*S*)-2-Phenylcyclohexanamine (1*S*,2*S*)-4. $[\alpha]_D^{20} = +10.4$ (*c* 0.45, CH_3OH); ee = 8%. Lit.[11] for (1*S*,2*S*)-(+)-**2**: $[\alpha]_D^{20} = +59.0$ (*c* 0.26, CH_3OH); ee = >99%.

4.5.5. (1*R*,2*R*)-*N*-(2-Phenylcyclohexyl)acetamide (1*R*,2*R*)-17. White solid, mp = 114–116 °C; $[\alpha]_D^{20} = -26.9$ (*c* 0.40, $CHCl_3$); ee = 94%. Lit.[16] for (1*S*,2*S*)-**17**: $[\alpha]_D^{20} = +30$ (c 0.66, $CHCl_3$), ee = 98%. IR (CH_2Cl_2) 3434, 3317, 1644 cm^{-1}; ^1H NMR (300 MHz): $\delta = 1.30–1.55$ (m, 2H), 1.55–2.05 (m, 9H), 2.94 (dt, 1H, $J = 11.7$ and 3.4 Hz), 4.39 (m, 1H), 5.52 (br s, 1H, NH), 7.10–7.25 (m, 5H, Ph); ^{13}C NMR (75.5 MHz): $\delta = 23.07$ (CH_2), 25.14 (CH_3), 26.05 (CH_2), 33.86 (CH_2), 35.42 (CH_2), 50.36 (CH), 51.92 (CH), 126.30 (CH), 127.36 (CH), 128.30 (CH), 143.50 (C), 169.08 (C=O); MS (ESI^+), *m*/*z* (%) = 218 ($[M+H]^+$, 5), 240 ($[M+Na]^+$, 100). Anal. Calcd for $C_{14}H_{19}NO$ (217.3): C, 7.38; H, 8.81; N, 6.45. Found: C, 77.43; H, 8.75; N, 6.43.

4.5.6. (1*R*,2*R*)-*N*-(2-Phenylcyclohexyl)methoxyacetamide (1*R*,2*R*)-18. White solid, mp = 70–72 °C; $[\alpha]_D^{20} = -22.9$ (*c* 0.60, $CHCl_3$); ee = 55%. IR (CH_2Cl_2) 3419, 1652 cm^{-1}; ^1H NMR (300 MHz): $\delta = 1.35–2.05$ (m, 8H), 3.00–2.90 (m, 1H), 3.23 (s, 3H, CH_3), 3.55 (d, 1H, $J = 15.1$ Hz), 3.80 (d, 1H, $J = 15.1$ Hz), 4.35–4.45 (m, 1H), 6.64 (br d, 1H, NH, $J = 6.8$ Hz), 7.10–7.30 (m, 5H, Ph); ^{13}C NMR (75.5 MHz): $\delta = 20.54$ (CH_2), 25.35 (CH_2), 25.55 (CH_2), 31.02 (CH_2), 44.81 (CH), 49.01 (CH), 59.12 (CH_3), 71.96 (CH_2), 126.35 (CH), 127.24 (CH), 128.20 (CH), 142.77 (C), 168.71 (C=O); MS (ESI^+), *m*/*z* (%) = 248 ($[M+H]^+$, 30), 270 ($[M+Na]^+$, 75). Anal. Calcd for $C_{15}H_{21}NO_2$ (247.3):

C, 72.84; H, 8.56; N, 5.66. Found: C, 72.81; H, 8.60; N, 5.65.

4.5.7. (1*S*,2*R*)-2-Phenylcyclopropanamine (1*S*,2*R*)-5. $[\alpha]_D^{20} = -105.1$ (*c* 1.0, $CHCl_3$); ee = 96%. Lit.[14] for (1*S*,2*R*)-(−)-**5**: $[\alpha]_D^{25} = -115.8$ (*c* 1.13, $CHCl_3$); ee = >99%.

4.5.8. (1*R*,2*S*)-*N*-(2-Phenylcyclopropyl)acetamide (1*R*,2*S*)-19. White solid, mp = 100–101.5 °C; $[\alpha]_D^{20} = -76.2$ (*c* 0.66, $CHCl_3$); ee = 69%. IR (CH_2Cl_2) 3424, 1644 cm^{-1}; ^1H NMR (200 MHz): major conformer δ = 1.05–1.20 (m, 2H), 1.95 (s, 3H, CH_3), 1.95–2.10 (m, 1H), 2.89 (dt, 1H, *J* = 7.8 and 3.7 Hz), 6.75 (br s, 1H, NH), 7.05–7.26 (m, 5H, Ph); minor conformer δ = 1.20–1.30 (m, 2H), 1.95–2.10 (m, 1H), 2.12 (s, 3H, CH_3), 2.73 (m, 1H), 6.21 (br s, 1H, NH), 7.05–7.26 (m, 5H, Ph); ^{13}C NMR (75.5 MHz): major conformer δ = 15.95 (CH_2), 22.84 (CH_3), 24.21 (CH), 32.07 (CH), 126.06 (CH), 126.23 (CH), 128.16 (CH), 140.44 (C), 171.46 (C=O); minor conformer δ = 17.14 (CH_2), 21.09 (CH_3), 26.36 (CH), 34.24 (CH), 125.41 (CH), 126.06 (CH), 128.41 (CH), 140.44 (C), 171.46 (C=O); MS (ESI^+), *m*/*z* (%) = 176 ($[M+H]^+$, 5), 198 ($[M+Na]^+$, 100). Anal. Calcd for $C_{11}H_{13}NO$ (175.2): C, 75.40; H, 7.48; N, 7.99. Found: C, 75.49; H, 7.40; N, 8.02.

4.6. Assignment of the absolute configuration

The absolute configuration of the remaining amines **3**–**5** obtained in the enzymatic processes were determined by comparison of the sign of their specific rotations with those reported. Consequently, the absolute configuration for the amides was also established. In all cases, lipases tested following Kazlauskas' rule showed the (1*R*)-enantiomer of the substrate being preferentially transformed.

4.7. Determination of the enantiomeric excesses

The enantiomeric excess for each optically active compound isolated from the enzymatic reactions was determined by HPLC. Acetamides **15**, **17**, and **19** were directly analyzed using a Chiralcel OD column. Amines **3**–**5** were transformed into the corresponding acetamides **15**, **17**, and **19** by conventional treatment with acetyl chloride (1.2 equiv), DMAP (1.0 equiv) in dichloromethane (>95% yield). To determine the ee of the methoxyacetamides, these were previously hydrolyzed (3 M aq NaOH, reflux, 12 h, 95% yield) and the resulting amines transformed into the corresponding acetamides **15**, **17**, and **19**.

4.8. HPLC method

A Chiralcel OD column was used in all cases. Hexane–isopropyl alcohol (H–IPA) mixtures were employed as eluents, with a flow value of 0.8 mL/min. For **15**: H–IPA, 92:8; *T* = 20 °C; t_R = 10.01 (1*R*,2*S*) and 11.29 (1*S*,2*R*) min; R_S = 2.4. For **17**: H–IPA, 92:8; *T* = 20 °C; t_R = 12.96 (1*R*,2*R*) and 20.44 (1*S*,2*S*) min; R_S = 7.0. For **19**: H–IPA, 90:10; *T* = 30 °C; t_R = 19.34 (1*R*,2*S*) and 23.29 (1*S*,2*R*) min; R_S = 2.5.

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References

1. (a) McGrath, W. R.; Kuhn, W. L. *Arch. Int. Pharmacodyn. Ther.* **1968**, *172*, 405–413; (b) Riley, T. N.; Brier, C. G. *J. Med. Chem.* **1972**, *15*, 1187–1188.
2. (a) Hartmann, S.; Ullrich, S.; Hupfer, C.; Frahm, A. W. *Eur. J. Med. Chem.* **2000**, *35*, 377–392; (b) Grisar, J. M.; Claxton, G. P.; Carr, A. A.; Wiech, N. L. *J. Med. Chem.* **1973**, *16*, 679–683.
3. Shepherd, T. A.; Aikins, J. A.; Bleakman, D.; Cantrell, B. E.; Rearick, J. P.; Simon, R. L.; Smith, E. C. R.; Stephenson, G. A.; Zimmerman, D. M. *J. Med. Chem.* **2002**, *45*, 2101–2111.
4. (a) Juaristi, E.; León-Romo, J. L.; Reyes, A.; Escalante, J. *Tetrahedron: Asymmetry* **1999**, *10*, 2441–2495; (b) Juaristi, E.; Escalante, J.; León-Romo, J. L.; Reyes, A. *Tetrahedron: Asymmetry* **1998**, *9*, 715–740; (c) Angelo, J.; Desmaële, D.; Dumas, F.; Guingant, A. *Tetrahedron: Asymmetry* **1992**, *3*, 459–505.
5. (a) Skupinska, K. A.; McEachern, E. J.; Baird, I. R.; Skerlj, R. T.; Bridger, G. J. *J. Org. Chem.* **2003**, *68*, 3546–3551; (b) González-Sabín, J.; Gotor, V.; Rebolledo, F. *Tetrahedron: Asymmetry* **2002**, *13*, 1315–1320; (c) Choi, Y. K.; Kim, M. J.; Ahn, Y.; Kim, M.-J. *Org. Lett.* **2001**, *3*, 4099–4101; (d) López-Serrano, P.; Jongejan, J. A.; Rantwijk, F.; Sheldon, R. A. *Tetrahedron: Asymmetry* **2001**, *12*, 219–228; (e) Sánchez, V. M.; Rebolledo, F.; Gotor, V. *J. Org. Chem.* **1999**, *64*, 1464–1470; (f) Gotor, V. *Bioorg. Med. Chem.* **1999**, *7*, 2189–2197.
6. González-Sabín, J.; Gotor, V.; Rebolledo, F. *Tetrahedron: Asymmetry* **2004**, *15*, 481–488.
7. For the synthesis of *cis*-isomers, see: (a) Knupp, G.; Frahm, A. W. *Tetrahedron Lett.* **1981**, *22*, 2633–2636; (b) Wiehl, W.; Frahm, A. W. *Chem. Ber.* **1986**, *119*, 2668–2677; For the synthesis of *trans*-isomers, see: (c) Brown, H. C.; Kim, K.-W.; Cole, T. E.; Singaram, B. *J. Am. Chem. Soc.* **1986**, *108*, 6761–6764.
8. Allegretti, M.; Berdini, V.; Cesta, M. C.; Curti, R.; Nicolini, L.; Topai, A. *Tetrahedron Lett.* **2001**, *42*, 4257–4259.
9. Brown, H. C.; Kim, K.-W.; Srebnik, M.; Singaram, B. *Tetrahedron* **1987**, *43*, 4071–4078.
10. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
11. Verbit, L.; Price, H. C. *J. Am. Chem. Soc.* **1972**, *94*, 5143–5152.
12. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.
13. Great differences in reactivity between the *cis*- and *trans*-isomers of 2-alkylcyclohexanols have been observed in some non-biocatalyzed processes: (a) Pasto, D. J.; Rao, D. R. *J. Am. Chem. Soc.* **1970**, *92*, 5151–5160; (b) Eliel, E. L.; Biros, F. J. *J. Am. Chem. Soc.* **1966**, *88*, 3334–3343.
14. Csuk, R.; Schabel, M. J.; von Scholz, Y. *Tetrahedron: Asymmetry* **1996**, *7*, 3505–3512.
15. Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195–1200.
16. Hayashi, T.; Senda, T.; Ogasawara, M. *J. Am. Chem. Soc.* **2000**, *122*, 10716–10717.