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Resolution of racemic amines via lipase-catalyzed benzoylation: Chemoenzymatic synthesis of the pharmacologically active isomers of labetalol

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ABSTRACT

Lipase-catalyzed benzoylation of amines was shown to be feasible, in some cases with high enantioselectivity, and the best results were obtained using immobilized lipase from *Candida antarctica* (Novozym 435) and methyl benzoate as acyl donor in the presence of molecular sieves. The procedure was optimized for the resolution of (±)-1-methyl-3-phenylpropylamine, a key intermediate in the synthesis of antihypertensive drug labetalol, and the enantiopure (*R*)-benzamide was then converted into the pharmacologically active isomers of the drug. In comparison with the reported synthesis of chiral isomers of labetalol, this chemoenzymatic route offers the advantage in the lack of any chiral stoichiometric auxiliary.

1. Introduction

Biocatalysis is nowadays a well-accepted methodology for the preparation of pure isomers of chiral drugs providing a clean, economical and sustainable alternative to conventional chemical processes. In this context, lipases have received growing interest for their remarkable characteristics of regio-, chemo- and enantioselectivity in the resolution process of racemates [1,2] as well as in the desymmetrization of prochiral substrates [3], without the use of cofactors, and for their excellent stability in organic solvents, that facilitate the solubilization of organic substrates and makes transesterification reactions feasible.

Many chiral drugs or intermediates for the synthesis of pharmaceuticals contains secondary alcohol groups and most of the reported lipase-catalyzed processes deal with the kinetic resolution of such alcohols through enantioselective transesterification in organic solvent and a large structural diversity is well tolerated [4,5].

Although amine and/or amide groups are present in many pharmacologically active compounds, the kinetic resolution of amine drugs or intermediates by lipase-catalyzed acylation is still limited [6,7] and enantiopure amines are usually obtained by differential crystallization of their diastereoisomeric salts with chiral acids [8,9] or by asymmetric synthesis [10]. However, the development of effective lipase-catalyzed acylation of amines could overcome the need of stoichiometric chiral reagents or expensive and synthetically demanding catalysts.

Lipase from *Candida antarctica* has proved the most effective catalyst for the enantioselective acylation of amines [11], with a special focus on (±)-1-phenylethylamine and related α-branched primary amines. Esters of alkylcarboxylic acids [12], *O*-methoxyesters [13] or dialkylcarbonates [14] are usually employed as acyl donors, since vinyl esters, the best choice in the enzymatic resolution of alcohols, are not suitable for amines due to their high reactivity that promotes spontaneous side reactions.

Since in the enzymatic resolution process one enantiomer of the amine substrate is converted into the corresponding amide, wherefrom it can be recovered by acid hydrolysis in some cases using harsh conditions, the search of alternative acyl donors could be valuable.

In spite of the interest in benzamides [15–17], that can be easily converted into *N*-benzyl protected amines for further synthetic manipulations and/or into the corresponding free amines by simple hydrogenolysis, enzymatic aminolysis reactions of benzoate esters have not previously reported.

Labetalol **1** (Fig. 1) is an antihypertensive drug with dual α_1- and β_1-adrenoceptor antagonist activities [18] commercially available as an equimolecular mixture of the four possible isomers. It has been shown that the *SS*- and *RS*- isomers are inactive, whereas the most α_1-blocking activity is due to the *S,R*-enantiomer and the *R,R*-enantiomer displays the highest potency in the β_1- adrenoceptor blocking activity [19]. Dilevalol (*R,R*)-**1** never reached the market owing to its hepatoxicity not seen to the same extent with labetalol, while the combination of *RR*- and *SR*- isomers, with a shared *R*-configuration of the aminic carbon, may be a valuable substitute for the current drug in the treatment of systemic hypertension [20].

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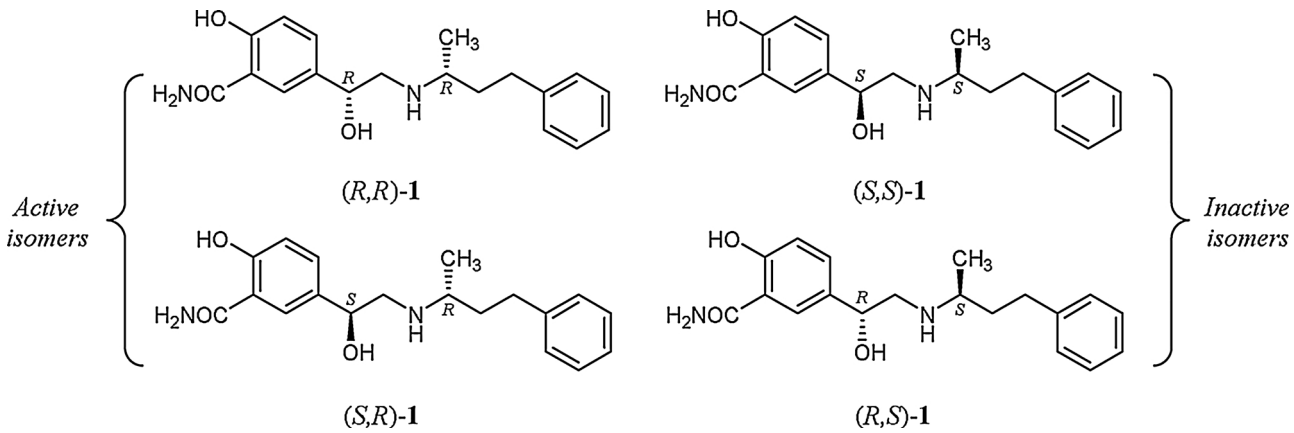


Fig. 1. Chemical structures of the four stereoisomers of labetalol drug.

Since the reported synthetic routes to single enantiomers of labetalol start from *N*-benzyl protected amine **2a** [18] or **2b** [19,21] (Fig. 2), obtained in optically pure forms by diastereoisomeric differential crystallization with suitable acids or by derivatization with chiral α-methylbenzylamine, we envisaged that kinetic resolution of 1-methyl-3-phenylpropylamine, (±)-**3** via enzymatic benzoylation could give an alternative access to the key intermediate **2a** without the need of any chiral

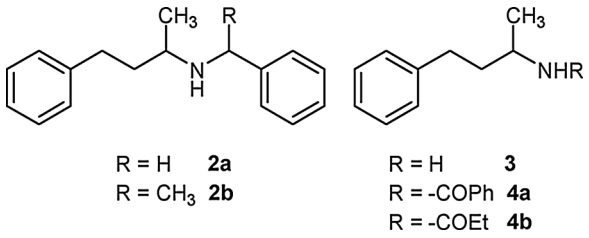


Fig. 2. Synthetic precursors of labetalol.

In this context, the feasibility of a lipase-catalyzed benzoylation was checked on some selected amines and herein we report the obtained results. The optimization of the process for kinetic resolution of amine (±)-**3** allowed us to obtain benzamide (*R*)-**4a** as a precursor of (*R*)-**2a**, that was then chemically converted into the 1:1 mixture of pharmacologically active *R,R*- and *S,R*-isomers of labetalol.

2. Experimental

2.1. Materials

5-(2-Bromoacetyl)-2-hydroxybenzamide was purchased from Alfa Aesar, (±)-α-methylbenzylamine from Acros Organics and the other chemicals from Aldrich. Lipases from *Candida antarctica* B immobilized on acrylic resin (Novozym 435) and from *Mucor miehei* immobilized on macroporous ion-exchange resin (Lipozyme) were obtained from Sigma. Amano Lipase PS-C II (immobilized on ceramic) and lipase A from *Candida antarctica* cross-linked aggregate (CAL-A) were purchased from Fluka. Solvents for enzymatic reactions were dried over activated molecular sieves (3 Å, Aldrich) prior the use. Thin layer chromatography (TLC) was carried out on Merck silica gel 60-F254 precoated glass plates.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance™ 400 instrument at 400.13 and 100.03 MHz respectively. Chemical shifts (δ) are reported in ppm relative to TMS and coupling constants (*J*) are in Hz.

The enantiomeric excesses of chiral compounds were determined by HPLC using Lux Cellulose columns (250 × 4.60 mm, Phenomenex) isocratically eluted at 23 °C with *n*-hexane/2-propanol mixtures at 0.5 mL/min flow rate and simultaneous detection at 220, 250, 266 and 275 nm. Optical rotations were recorded on a DIP 135 JASCO instrument using a f 3.5′ 100 mm cell.

High resolution mass spectra (HR-MS) were acquired by a Thermo Scientific Exactive Plu Orbitrap MS (Thermo Fisher Scientific, Inc., Milan, Italy) instrument with ESI ionization mode using a Thermofisher Orbitrap QExactive instrument, set with 3.0 kV source voltage and 300 °C capillary temperature.

2.2. General procedure for aminolysis of methyl benzoate catalyzed by Novozym 435

To an equimolecular (0.62 mmol) solution of amine substrate (see Table 1) and methyl benzoate in 1 mL of anhydrous toluene, Novozym 435 (50 mg) and molecular sieves (40 mg) were added. The suspension was then stirred in a shaker at 300 rpm at 45 °C. The reaction was monitored by TLC analysis (*n*-hexane/EtOAc 70:30) and quenched at suitable time by filtering off the solids. The solution was taken to dryness and an aliquot of the residue analysed by ^{1}H NMR for the determination of substrate conversion, by means of integration of the resonances for C*H*-NH_2 and C*H*-NHCOPh protons. The residue was then partitioned between EtOAc and aqueous HCl to give unreacted amine in the aqueous phase. The organic phase was washed with $NaHCO_3$, dried over Na_2SO_4 and taken to dryness to give pure benzamides **4a** and **8**-**10**. The NMR data of all the obtained benzamides were in agreement with reported data [16,22,23].

The enantiomeric excesses of **4a**, **9** and **10** were measured by chiral HPLC chiral analyses on Lux Cellulose-1 column: **4a** (*n*-hexane/2-PrOH 85:15) t_R/min: 24.21 [(*R*)-**4a**] and 35.59 [(*S*)-**4a**]; **9** (*n*-hexane/2-PrOH 90:10) t_R/min: 32.09 [(*R*)-**9**] and 42.92 [(*S*)-**9**], **10** (*n*-hexane/2-PrOH 85:15) t_R/min: 17.45 [(*S*)-**10**] and 22.49 [(*R*)-**10**].

The absolute configuration of (*R*)-**9** was assigned by comparison of the HPLC retention time of the product of enzymatic reaction with an authentic sample obtained by chemical benzoylation of commercially available (*R*)-**6** and confirmed by its optical rotatory power $[\alpha]_D^{25} = +19.7$ (*c* 1.0, $CHCl_3$), lit. $[\alpha]_D^{19} = -20.1$ (*c* 1.0, $CHCl_3$) [24]. The *R*-configuration was also assigned to the enzymatically obtained **4a** on the basis of its optical rotation, $[\alpha]_D^{25} = -6.3$ (c 0.9, CH_2Cl_2), lit. $[\alpha]_D^{25} = +6.0$ (c 0.25, CH_2Cl_2) for the (*S*)-**4a**, [22].

2.3. General procedure for lipase-catalyzed kinetic resolution of (±)-3

To a solution of (±)-**3** (100 µL, 0.62 mmol) and methyl benzoate (78 µL, 0.62 mmol) in 1 mL of dry toluene 50 mg of the lipase of choice and 40 mg of molecular sieves were added. The resulting heterogeneous mixture was shaken at 300 rpm and 45 °C. The reaction progress was monitored by TLC chromatography (*n*-hexane/EtOAc 70:30) and at

suitable time the solids were filtered off. To an aliquot (0.5 mL) of the solution propionic anhydride (40 μL) was added and the mixture left to react for 24 h at room temperature. After standard workup, this mixture was analysed by chiral HPLC to determine the enantiomeric excesses of unreacted amine (*S*)-**3** (as the corresponding propionic amide **4b**) and the formed (*R*)-**4a** (ee_s and ee_p, respectively) from which the substrate conversion was calculated according the equation $c = [ee_s/(ee_s + ee_p)] \times 100$ [25]. HPLC conditions: Lux Cellulose-1 column, *n*-hexane/2-PrOH 90:10 eluent, t_R/min: 24.97 [(*R*)-**4b**] and 30.10 [(*S*)-**4b**]; 36.41 [(*R*)-**4a**] and 54.00 [(*S*)-**4a**].

2.4. Screening of solvents for the kinetic resolution of (±)-3 with Novozym 435

To a solution of (±)-**3** (100 μL, 0.62 mmol) and methyl benzoate (78 μL, 0.62 mmol) in 1 mL of the solvent of choice Novozym 435 (50 mg) and molecular sieves (40 mg) were added. The suspension was then shaken at 300 rpm at 45 °C until a suitable conversion of substrate was reached, as stated by TLC chromatography (*n*-hexane/EtOAc 70:30). The reaction mixture was then treated as above in order to determine the substrate conversion and the enantiomeric excesses of (*R*)-**4a** and (*S*)-**3** by chiral HPLC analysis.

2.5. Enzymatic synthesis of (R)-N-(1-methyl-3-phenylpropyl)-benzamide, (R)-4a

Amine (±)-**3** (0.5 mL, 3.09 mmol) was dissolved in 5 mL of dry toluene containing 500 mg of molecular sieves and 0.3 mL methyl benzoate (0.3 mL, 2.77 mmol). The reaction was started by addition of Novozym 435 (600 mg) and was stirred at 45 °C and 300 rpm. After 5 days (45% conversion) the enzyme was filtered off and the solution taken to dryness under reduced pressure to partially remove the unreacted amine and methyl benzoate. The residue was dissolved in diethyl ether and the solution extracted three times with 1N HCl. The organic phase was washed with satd. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent evaporated to give (*R*)-**4a** as a white solid (328 mg, 1.3 mmol, 42% yield, ee > 99%), ^{1}H NMR ($CDCl_3$) δ: 1.26 (d, J = 6.8, 3H), 1.87 (m, 2H), 2.71 (t, J = 8.0, 2H), 4.26 (m, 1H), 5.91 (d, J = 8.0, 1H), 7.18 (m, 3H), 7.27 (m, 2H), 7.40 (m, 2H), 7.48 (m, 1H), 7.66 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 20.99, 32.48, 38.51, 45.68, 125.87, 126.77, 128.29, 128.43, 131.21, 134.88, 141.71, 166.79.

2.6. Synthesis of (R)-N-benzyl-N-(1-methyl-3-phenylpropyl)-amine, (R)-2a

To a solution of (*R*)-**4a** (300 mg, 1.2 mmol, > 99% ee) in diethyl ether (8 mL) $LiAlH_4$ (45 mg, 1.2 mmol) was added portionwise and the suspension was stirred at room temperature. The reaction was monitored by TLC analysis (*n*-hexane/EtOAc 60:40) and quenched at substrate disappearance by careful dropwise addition of 15% aqueous sodium hydroxide. The organic phase was washed with water, then brine and dried on Na_2SO_4. After the evaporation of solvent under reduced pressure (*R*)-**2a** (243 mg, 1.03 mmol, 86% yield, > 99% ee) was isolated of a pale yellow oil. $[\alpha]_D^{25}$ for (*R*)-**2a**·HCl = + 3.8 (c 0.5, EtOH), lit. $[\alpha]_D^{25}$ = +5.0 (solvent and concentration not given) [19]. NMR data were in agreement with literature values [26]: ^{1}H NMR ($CDCl_3$) δ: 1.09 (d, J = 6.4, 3H), 1.63 (m, 1H), 1.76 (m, 1H), 2.60 (m, 2H), 2.67 (m, 1H), 3.66 (d, J = 13.2, 1H), 3.75 (d, J = 13.2, 1H), 7.19 (m, 10H); ^{13}C NMR ($CDCl_3$) δ: 20.05, 32.07, 38.39, 51.00, 51.74, 125.51, 126.64, 126.67, 126.95, 127.97, 128.13, 128.19, 140.41, 142.16. The enantiomeric purity of (*R*)-**2a** was checked by chiral HPLC analysis on Lux Cellulose-3 column (*n*-hexane/2-PrOH 90:10) of the corresponding acetamide, t_R/min: 23.02 [(*S*)-enantiomer] and 25.24 [(*R*)-enantiomer].

2.7. Synthesis of (R)-2-hydroxy-5-[[N-(1-methyl-3-phenylpropyl)-N-(phenylmethy)amino]acetyl] benzamide, (R)-12

To a solution of (*R*)-**2a** (500 mg, 2.11 mmol, > 99% ee) in anhydrous methyl ethyl ketone (10 mL) 5-(2-bromoacetyl)-2-hydroxybenzamide **11** (272 mg, 1.06 mmol) was added and the mixture stirred at reflux until disappearance of the ketone reagent (1 h), as stated from TLC analysis (*n*-hexane/EtOAc 60:40). The solution was left to cool at room temperature and taken to dryness in vacuo. The residue was suspended in diethyl ether, from which the hydrobromide of unreacted amine was separated as insoluble salt and removed by filtration. Excess of HCl in ethanol was added to the filtrate solution to give (*R*)-**12**·HCl (203 mg, 0.45 mmol, 43% yield) as a white powder, $[\alpha]_D^{25}$ = +16.8 (c 0.5, EtOH), lit. $[\alpha]_D^{25}$ = +18 (solvent and concentration not given) [19]. Structural characterization was carried out on the free base (*R*)-**12**: ^{1}H NMR ($CDCl_3$) δ: 1.11 (d, J = 6.4, 3H), 1.64 (m, 1H), 1.93 (m, 1H), 2.48 (m, 1H) 2.66 (m, 1H), 2.79 (q, J = 6.4, 1H), 3.43 and 3.77 (d, AB system, J = 13.2, each 1H), 3.63 and 3.85 (d, AB system, J = 14.4, each 1H), 6.91 (d, J = 8.4, 1H), 7.06 (d, J = 7.2, 1H), 7.18-7.33 (m, 9H), 7.92 (d, J = 7.2, 1H), 8.03 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 13.72, 33.03, 35.76, 53.90, 55.31, 57.87, 112.72, 118.08, 125.72, 127.29, 128.17, 128.32, 128.40, 129.48, 135.57, 139.33, 142.21, 166.03, 172.06, 197.32. HRMS ESI–MS calcd for $C_{26}H_{29}N_2O_3$ $[M + H]^+$ 417.2172, found 417.1749.

2.8. Synthesis of optically active labetalol [diastereoisomeric mixture of (R,R)-1 and (R,S)-1]

In a 50-mL round-bottomed flask equipped with a gas inlet tube with valve stopcock 10% Pd-C (20 mg) was added to a solution of (*R*)-**12**·HCl (203 mg, 0.45 mmol) in absolute EtOH (6 mL). The flask was then filled with hydrogen gas (1 atm) and the suspension magnetically stirred at room temperature. When the complete conversion of substrate was reached (4 h, TLC analysis, *n*-hexane/EtOAc 60:40) the catalyst was filtered off over a short pad of Celite and the solution taken to dryness at reduced pressure to afford 1:1 diastereoisomeric mixture of (*R,R*)-**1** and (*S,R*)-**1** hydrochloride salts (156 mg, 0.42 mmol) in quantitative yield. ^{1}H NMR (DMSO-d_6) d: 1.31 (t, sum of two partially overlapped doublets for diastereoisomeric $-CH_3$, 2 × 3H), 1.78 (m, 2 × 1H), 2.11 (m, 2 × 1H), 2.56 (m, 2 × 1H), 2.71 (m, 2 × 1H), 3.05 (m, 2 × 2H), 3.19 (m, 2 × 1H), 4.86 (br t, 2 × 1H), 6.09 (s, 2 × 1H, −OH), 6.89 (d, J = 8.4, 2 × 1H), 7.19-7.31 (m, 2 × 6H), 7.44 (d, J = 8.4, 2 × 1H), 7.90 (s, 2 × 2H), 8.47 (br, 2 × 1H), 9.05 (br, 2 × 1H); ^{13}C NMR (DMSO-d_6) d: 15.55 and 16.05 (doubled signals due to diastereoisomerism, *d**), 31.32 and 31.42 (*d**), 34.38, 36.25, 50.39 and 50.48 (*d**), 53.69 and 53.79 (*d**), 68.28 and 68.49 (*d**), 114.71, 117.66, 126.39 and 126.57 (*d**), 128.63 and 128.77 (*d**), 132.05 and 132.39 (*d**), 141.22, 160.82, 172.01. HRMS ESI–MS calcd for $C_{19}H_{23}N_2O_3$ $[M + H]^+$ 329.1860, found 329.1842.

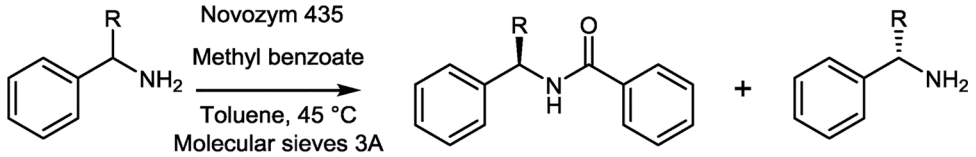
3. Results and discussion

At the onset of our work, the enzymatic benzoylation of some selected amines was carried out in dry toluene in the presence of *Candida antarctica* lipase (CAL-B) immobilized on acrylic resin (Novozym 435), reported as the most effective catalyst in the acyl transfer reactions with amines in non-aqueous media [11]. Methyl benzoate was used as acyl donor and molecular sieves were also added to the reaction mixture in order to prevent any competitive enzymatic hydrolysis of the benzoate ester.

Achiral benzylamine **5**, (±)-1-phenylethylamine **6**, (±)-1-methyl-3-phenylpropylamine **3** and (±)-2-amino-2-phenylethanol **7** were chosen as simple models for arylalkyl amines and aminoalcohols and the progress of reactions was monitored by ^{1}H NMR while the optical purity of the formed amides was determined by chiral HPLC (Table 1).

In all the cases the benzoylation reaction gave the expected amide

Table 1
Enzymatic benzoylation of amines[a].



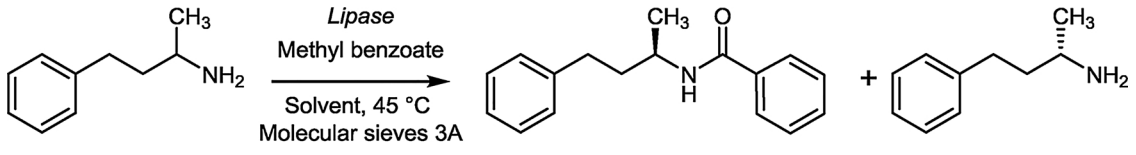
R = H **5**
R = CH_3 (±)-**6**
R = CH_2OH (±)-**7**

R = H **8**
R = CH_3 **9**
R = CH_2OH **10**

Entry	Substrate	Time (h)	Conversion (%)[b]	Product	*ee* of product (%)[c]
1	**5**	20	30	**8**	–
2	**6**	144	19	(*R*)-**9**	99
3	**3**	96	24	(*R*)-**4a**	99
4	**7**	24	20	**10**	0

[a] Conditions: substrate (0.62 mmol), methyl benzoate (0.62 mmol), toluene (1 mL), Novozym 435 (50 mg), molecular sieves (40 mg), 45 °C, 300 rpm.
[b] Determined by 1H-NMR of the reaction mixture.
[c] Determined by chiral HPLC.

Table 2
Optimization of lipase-catalyzed kinetic resolution of (±)-**3**[a].



(±)-**3** (*R*)-**4a** (*S*)-**3**

Entry	Lipase	Solvent	Time (d)	*ee* of (*S*) − 3 (%)[b]	*ee* of (*R*)-4a (%)[b]	Conversion (%)[c]
1	Novozym 435	Toluene	4	31	99	24
2	Lipase AK	Toluene	7	–	–	–
3	PS-CII	Toluene	7	4	91	4
4	CAL-A	Toluene	7	–	–	–
5	Lipozyme	Toluene	7	–	–	–
6	Novozym 435	CH_2Cl_2	7	4	99	4
7	Novozym 435	TBME	4	18	99	15
8	Novozym 435	THF	4	11	99	9
9	Novozym 435	Dioxane	4	16	99	14
10	Novozym 435	CH_3CN	7	6	99	6
11	Novozym 435[d]	Toluene	7	9	99	8
12	Novozym 435[e]	Toluene	7	12	99	11
13	Novozym 435[f]	Toluene	2	26	99	21

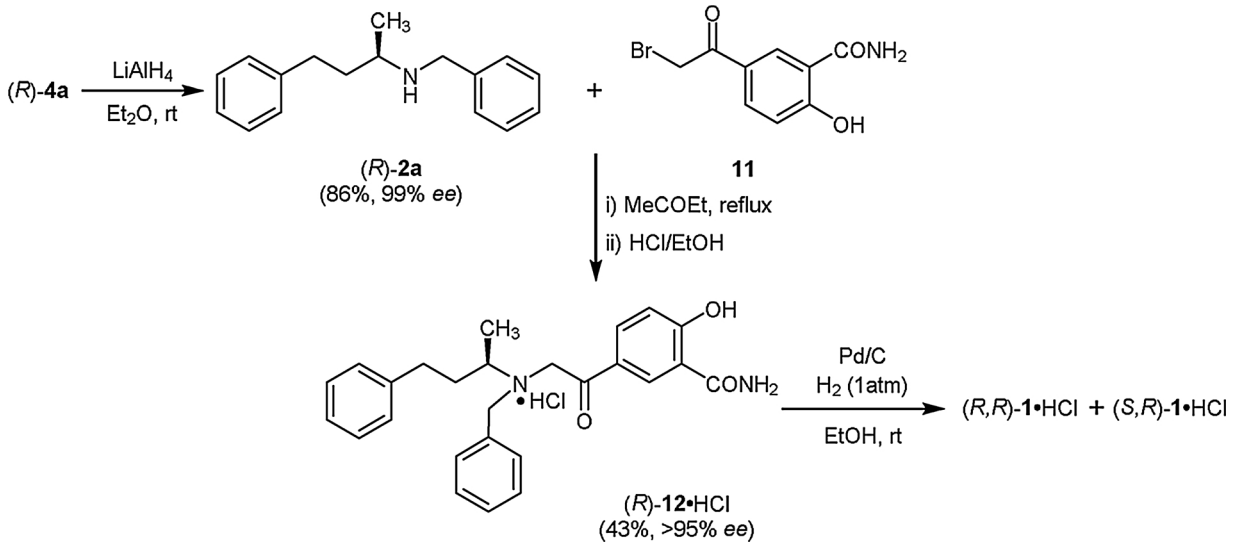
[a] Conditions: substrate (0.62 mmol), methyl benzoate (0.62 mmol), toluene (1 mL), lipase (50 mg), molecular sieves (40 mg), 45 °C, 300 rpm.
[b] Determined by chiral HPLC.
[c] Calculated from the equation $c = \frac{ee_S}{ee_P + ee_S} \times 100$.
[d] Ethyl benzoate as acyl donor.
[e] The reaction was carried out at 55 °C.
[f] Doubled amounts of enzyme and molecular sieves.

products and moderate reaction rates were observed for amines **5** and (±)-**7**, that were fully converted in 72–96 h. Amines (±)-**3** and (±)-**6** reacted in quite lower rate (Table 1, entries 2 and 3), but the enzyme displayed excellent enantioselectivity E [24] (E > 200) with *R*-stereopreference so allowing the kinetic resolution of the racemates and the preparation of optically pure benzamides (*R*)-**4a** and (*R*)-**9**.

The benzoylation of aminoalcohol (±)-**7** with Novozym 435 proceeded without any stereoselectivity, but the reaction was highly chemoselective affording only the amide product **10** even in presence of an excess of methyl benzoate. Comparable reaction course had been previously reported in the presence of porcine pancreatic lipase and ethyl acetate as solvent and acylating agent [27].

Encouraged by these preliminary results we focused our attention on benzamide (*R*)-**4a** as a key intermediate for a chemo-enzymatic synthesis of the pharmacologically active diastereoisomers of labetalol, (*R*,*R*)-**1** and (*S*,*R*)-**1**. An optimization study of the lipase-catalyzed resolution of (±)-**3** via benzoylation was then carried out by screening different lipases in the same experimental conditions described above and the obtained results are summarized in Table 2.

Among the different tested enzymes only lipase from *Candida antarctica B* (CAL-B, Novozym 435) successfully catalyzed the aminolysis of methyl benzoate, whereas lipase from *Pseudomonas cepacia* (PS-CII) displayed negligible activity and low enantioselectivity (E = 22, Table 2, entry 3). The other assayed lipases, including CAL-A which is an isoenzyme of CAL-B lipase produced by the same yeast, were fully inactive. In a further investigation the kinetic resolution of (±)-**3** in



Scheme 1. Synthesis of pharmacologically active isomers of labetalol.

the presence of Novozym 435 was performed in different solvents. The high *R*-stereoselectivity of the enzyme (E > 200) was maintained in all the conditions but, in comparison with the reaction in toluene, acceptable reaction rate was observed only in the presence of oxygenated solvents (Table 2, compare entry 1 and entries 7–9) and sensibly decreased in CH_2Cl_2 and CH_3CN (Table 2, entries 6 and 10) without any apparent correlation with the polarity of the solvent (expressed as logP).

Changing the working temperature and methyl benzoate with ethyl benzoate were not effective in improving the productivity of the reaction. The increase in the amount of enzyme resulted in a proportional variation of the reaction rate (Table 2, compare entries 1 and 13), providing that also the amount of molecular sieves, which play an essential role in the reaction outcome, was adequately modified.

In a preparative run, the reaction of (±)-**3** with methyl benzoate in the presence of Novozym 435 and molecular sieves for 5 days gave enantiopure (*R*)-**4a** together with unreacted (*S*)-**3** (80% *ee*), that were separated by simple extraction to afford the target benzamide in 42% isolated yield.

With the enantiopure (*R*)-**4a** in the hand, the synthesis of labetalol diastereoisomers (*R,R*)-**1** and (*S,R*)-**1**, was then carried out in three step following a reported procedure (Scheme 1) [19] in which it is assumed that the optical purity of the starting material is preserved in the products.

Conventional reduction of (*R*)-**4a** with $LiAlH_4$ afforded amine (*R*)-**2a** which was in turn reacted with **11** to give the *N*-benzyl protected ketone (*R*)-**12**, easily separated from the reaction mixture by precipitation of the corresponding hydrochloride salt. The maintenance of the optical purity of the starting material was confirmed by chiral HPLC analysis for amine (*R*)-**2a** and by comparison of the optical rotatory power with literature data for (*R*)-**12**·HCl. Indeed, in spite of different chiral columns and solvent combinations were tested, all attempts to determine the enantiomeric excess of compound (*R*)-**12**·HCl (as well as the corresponding free base) by HPLC were unsuccessful.

Hydrogenation over Pd/C of (*R*)-**12**·HCl allowed the simultaneous carbonyl reduction and debenzylation of the substrate and the target products (*R,R*)-**1** and (*S,R*)-**1**were obtained as an equimolar mixture of their hydrochloride salts, which are suitable for drug formulations, without any chromatographic purification. Apart from the partially overlapped doublets for the methyl group, the two diastereoisomers gave indistinguishable proton resonances, but their ratio was easily deduced by the presence of many 1:1 splitted signals in the ^{13}C NMR spectrum of the mixture.

4. Conclusions

Lipase from Candida Antarctica (Novozym 435) was found effective in promoting the benzoylation of amines with methyl benzoate. The procedure was optimized for the resolution of 1-methyl-3-phenylpropylamine, (±)-**3** to give corresponding benzamide (*R*)-**4a** in enantiopure form and applied to the preparation of pharmacologically active isomers of labetalol. Since benzamides can be easily converted into *N*-benzyl protected amines, this previously unreported enzymatic process shows potential practical application in amine resolution, in particular with hydrolysis-sensitive substrates or when synthetic manipulations of amine intermediates are required.

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