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Enzymatic resolution of 5-hydroxy- and 8-hydroxy-2-tetralols using immobilized lipases

Paolo Bonomi [a], Paola Cairoli [a], Daniela Ubiali [b], Carlo F. Morelli [a], Marco Filice [b], Ines Nieto [b,c], Massimo Pregnolato [b], Paolo Manitto [a], Marco Terreni [b], Giovanna Speranza [a,*]

[a] *Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano and Italian Biocatalysis Center, via Venezian 21, 20133 Milano, Italy*
[b] *Dipartimento di Chimica Farmaceutica, Pharmaceutical Biocatalysis Laboratories, Università degli Studi di Pavia and Italian Biocatalysis Center, via Taramelli 12, 27100 Pavia, Italy*
[c] *Innovate Biotechnology srl and Italian Biocatalysis Center, Parco Scientifico Tecnologico, Strada Savonesa 9, 15050 Rivalta Scrivia (AL), Italy*

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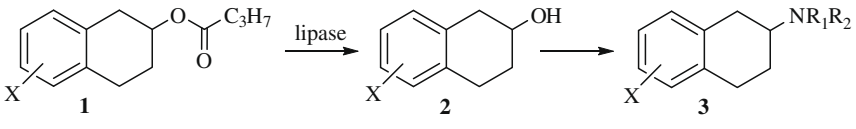
ABSTRACT

(*R*)-2-Tetralol (*R*)-**2a**, (*R*)-5-hydroxy-2-tetralol (*R*)-**2b** and (*R*)-8-hydroxy-2-tetralol (*R*)-**2c**, which are key intermediates in the synthesis of pharmacologically active 2-aminotetralins **3**, were prepared in moderate to very high enantiomeric excess (up to 99% ee) by enzymatic resolution of the corresponding racemic butyrates *rac*-**1a**, *rac*-**1b** and *rac*-**1c**, respectively, using lipases immobilized on octyl agarose. This methodology is an alternative to the microbial reduction of 2-tetralones.

1. Introduction

2-Tetralols (1,2,3,4-tetrahydro-2-naphthols, **2**, Scheme 1) are useful intermediates in the synthesis of pharmacologically active 2-aminotetralins **3**.[1,2] The 2-aminotetralin system represents the structural base of many dopaminergic, serotoninergic and adrenergic agents[3,4] as well as compounds that interact with melatonin receptors.[5] It has been found that the 2-aminotetralin activity is strongly dependent on both the substitution pattern of the aromatic ring and the configuration of the stereogenic centre at the 2-position.[3,6,7] For example, 5-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin **3b** (5-OH–DPAT) (R_1, R_2 = *n*-Pr, Scheme 1) is a highly potent dopamine receptor agonist which has affinity for both the dopamine D_2 and the dopamine D_3 receptors,[3,6,8] whereas the 8-hydroxy analogue **3c** (8-OH–DPAT) (R_1, R_2 = *n*-Pr) is a very potent and selective serotonin 5-HT_{1A} receptor agonist, but it is devoid of dopaminergic activity.[3,4,6,9] In the case of 5-OH–DPAT, the (*S*)-(−)-enantiomer is more active than the (*R*)-(+)-one.[7,8] The opposite holds true for 8-OH–DPAT, with the (*R*)-(+)-enantiomer being the most active one.[7,9] It should be noted that an affinity for the melatonin receptor requires a methoxy group at the 8-position of the 2-aminotetralin system and an amide instead of an amine moiety.[5]

2-Aminotetralin derivatives have found applications for the treatment of many central nervous system related disorders,[5–7,10] and have been reported to possess a variety of actions including cardiovascular effects[11] and potent antifungal activities against pathogenic fungi.[12]



Scheme 1.

* Corresponding author. Tel.: +39 02 5031 4097; fax: +39 02 5031 4072.
E-mail address: giovanna.speranza@unimi.it (G. Speranza).

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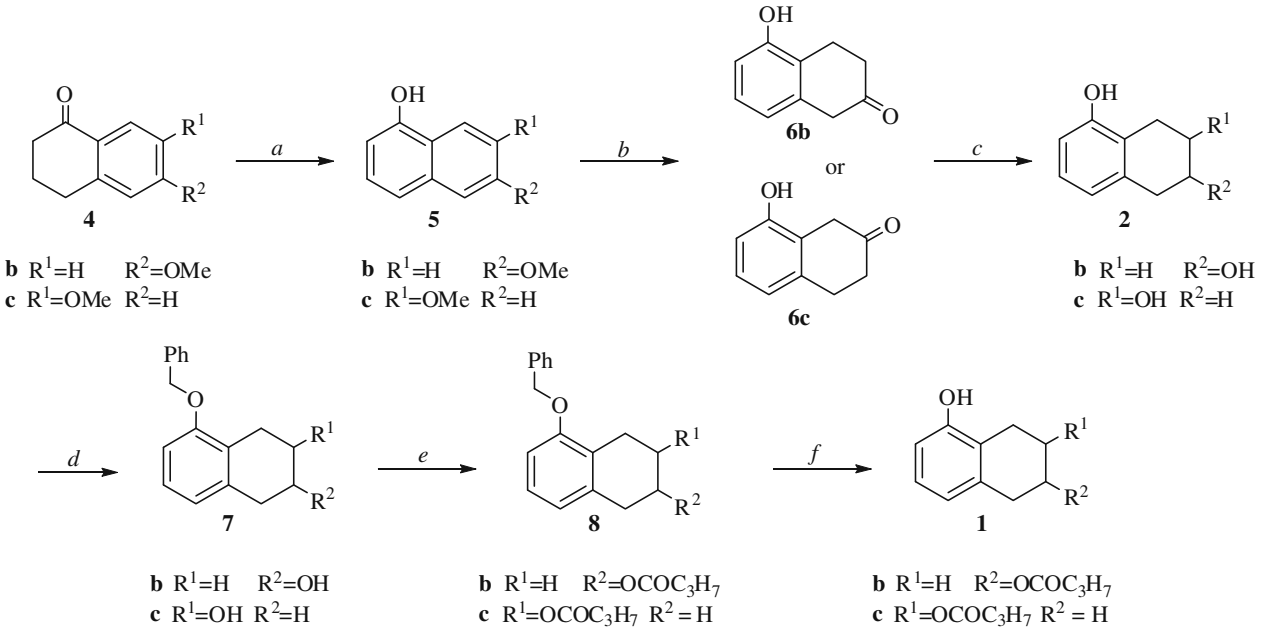


Scheme 2. Reagents and conditions: (a) Pd/C (10%), dry *p*-cymene; (b) Na, EtOH reflux; then *p*-TsOH, EtOH–H_2O, 1:1; (c) $NaBH_4$, MeOH; (d) $PhCH_2Br$, K_2CO_3, CH_3CN; (e) butyryl chloride, TEA, DMAP, CH_2Cl_2; (f) H_2, Pd/C (10%), MeOH.

Despite the synthetic relevance of optically active 2-tetralols **2**, their synthesis has been poorly investigated so far and mostly limited to the bioreduction of the corresponding 2-tetralones.[13–21]

Recently, we found that the hydrolysis of racemic 2-tetralyl butyrate *rac*-**1a** catalyzed by *Rhizomucor miehei* lipase (*RML*) immobilized on octyl agarose affords (*R*)-alcohol (*R*)-**2a** with high enantiomeric excess as determined by MTPA ester method.[22]

This prompted us to explore the possibility of extending such a methodology to prepare the synthetically useful 5-hydroxy- **2b** and 8-hydroxy-2-tetralols **2c** in enantiomerically pure form. Herein, we report that (*R*)-**2b** and (*R*)-**2c** can be obtained in moderate to very high enantiomeric excess (up to 99% ee) by lipase-catalyzed hydrolysis of the corresponding racemic butyrates *rac*-**1b** and *rac*-**1c**, respectively.

2. Results and discussion

2.1. Synthesis of substrates

5-Hydroxy-2-tetralyl butyrate *rac*-**1b** was prepared from 6-methoxy-1-tetralone **4b** as reported in Scheme 2, that is, by dehydrogenation over Pd/C in refluxing *p*-cymene, followed by Birch reduction of the intermediate 6-methoxy-1-naphthol **5b**.[15] Further reduction of the resulting 5-hydroxy-2-tetralone **6b** with sodium borohydride gave racemic 5-hydroxy-2-tetralol *rac*-**2b** which was selectively protected at the phenolic hydroxyl group as a benzyl ether **7b** and then treated with butyryl chloride. Finally, the hydrogenolytic removal of the benzyl group from *rac*-**8b** afforded *rac*-**1b** in 80% yield from **6b**.

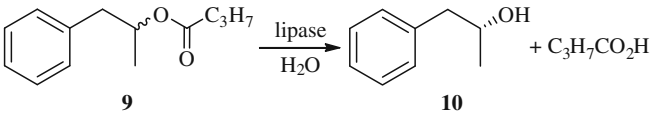
In the same way, 8-hydroxy-2-tetralyl butyrate *rac*-**1c** was synthesized starting from 7-methoxy-1-tetralone **4c** in 75% yield from **6c**. The ester *rac*-**1a** was prepared as previously reported.[22]

2.2. Enzymatic resolution of 2-tetralols 2a–c

Six lipases from different biological sources (*Termomyces lanuginosus, Candida rugosa, Porcine pancreas, Pseudomonas fluorescens, Pseudomonas cepacia* and *R. miehei*), known for their hydrolytic activity, were immobilized on octyl agarose and tested for their enantioselectivity using 1-methylphenethyl butyrate *rac*-**9** as a 'model substrate'. The results of this preliminary screening are reported in Table 1.[23] The enantioselectivity (*E*) was calculated according to Sih's method[24] from the reaction conversion and the enantiomeric excess of the product (ee_p). The extent of conversion was estimated by reversed phase HPLC, ee's by chiral HPLC, since the 1-phenyl-2-propanol **10** enantiomers could be easily separated on a OD-Chiralcel stationary phase. In all cases, the (*R*)-ester was preferentially hydrolyzed.

From these screening tests, lipases from *P. cepacia* (*PCL*), *P. fluorescens* (*PFL*) and *R. miehei* (*RML*) were selected and further evaluated as biocatalysts in the enantioselective hydrolysis of 2-tetralol butyryl esters *rac*-**1a–c**. Before submitting the racemic substrates to

Table 1
Screening of lipases immobilized on octyl agarose



9 10

Biological source of lipases	Immobilization yield[b] (%)	Activity[c] (U/g)	*c*[d] (%)	ee^e_p (%)	*E*[f]
Termomyces lanuginosus	76	8	12	78 (*R*)	9
Candida rugosa	82	128	44	30 (*R*)	2
Porcine pancreas	84	38	50	2 (*R*)	1
Pseudomonas cepacia	98	40	46	99 (*R*)	>200
Pseudomonas fluorescens	96	47	50	98 (*R*)	>200
Rhizomucor miehei	40	917	49	99 (*R*)	>200

Hydrolysis of 1-methylphenethyl butyrate (*rac*-**9**) in MeCN—25 mM phosphate buffer, pH 7 (2:8) at 25 °C[a].

[a] Substrate concentration, 2 mM.

[b] Immobilization conditions: 42 mg of protein/g support, 25 °C.

[c] The activity was measured at 25 °C towards a solution of ethyl butyrate (4 mL) in 100 mM phosphate buffer pH 7 (16 mL). The activity is expressed as U/g of support, U = μmol of substrate hydrolyzed per minute.

[d] *c* (conversion), product % after 24 h.

[e] Percentage of enantiomeric excess and absolute configuration of the alcohol **10** were determined by chiral HPLC, using (*R*)- and (*S*)-1-phenyl-2-propanol as reference samples.

[f] E (enantioselectivity) = $\ln[1 - c(1 + ee_p)]/\ln[(1 - c(1 - ee_p)]$; *c*, conversion.[24]

enzymatic resolution under the experimental conditions used for *rac*-**9**, we investigated the influence of the organic solvent on the catalytic properties of the immobilized lipases by incubating the enzyme preparations in the presence of different concentrations of acetonitrile in 25 mM phosphate buffer at pH 7 and 25 °C for 24 h. As it can be seen from Figure 1, increasing amount of acetonitrile results in a decrease of the lipase activities towards ethyl butyrate. In the case of *PFL* and *RML*, at 30% MeCN the enzymes lose their activities, whereas the immobilized *PCL* appears to be the most stable one considering that it retains a half of its maximum activity at 40% MeCN. On the basis of these data, the reaction conditions reported in Table 1 were chosen to test the enzymatic hydrolysis of *rac*-**1a–c**.

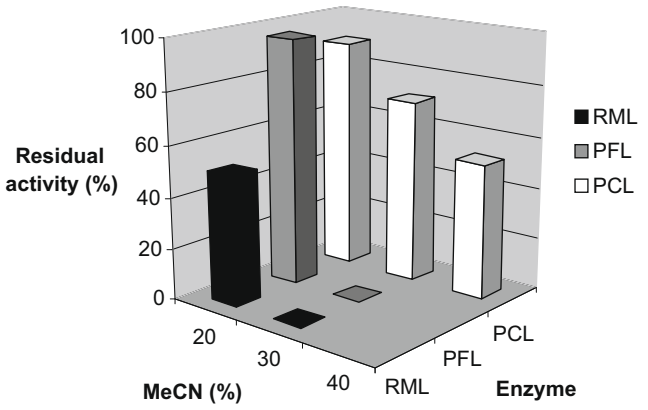


Figure 1. Residual hydrolytic activity towards ethyl butyrate of immobilized lipases in 25 mM phosphate buffer (pH 7) containing increasing amounts of MeCN after 24 h (see Section 4 for details).

Since neither 2-tetralols *rac*-**2a–c** nor their butyrates *rac*-**1a–c** were separated into enantiomers using the chiral HPLC column reported above for **10**, a number of chiral stationary phases were screened.[25] We succeeded in separating 2-tetralol *rac*-**2a** and 5-hydroxy-2-tetralol *rac*-**2b** as well as its butyrate *rac*-**1b**, but not 8-hydroxy-2-tetralol *rac*-**2c** on an S,S-WhelkO1 column. However, this column could be used to resolve 8-hydroxy-2-tetralyl butyrate *rac*-**1c** into enantiomers. This fact brought about the conversion of 8-hydroxy-2-tetralol, obtained by enzymatic hydrolysis, into its butyrate.

Thus, the absolute configuration of the alcohols obtained in the lipase-catalyzed hydrolysis of esters *rac*-**1a–c** was assigned by chiral HPLC comparison with authentic samples of (*S*)-2-tetralol (*S*)-**2a**, (*S*)-5-hydroxy-2-tetralol (*S*)-**2b** and (*S*)-8-hydroxy-2-tetralyl butyrate (*S*)-**1c**. Compounds (*S*)-**2a** and (*S*)-**2b** were obtained in 30% ee and 60% ee, respectively, by baker's yeast mediated reduction of the corresponding 2-tetralones.[15] Analogous bioreduction of **6c** afforded (*S*)-**2c** (21% ee)[15] which was converted into (*S*)-**1c** using the sequence of reactions outlined in Scheme 2.

The results of lipase-catalyzed hydrolysis of *rac*-**1a–c** (2 mM concentration) are summarized in Table 2.

Data of Table 2 indicate that the stereochemical course of resolutions performed by immobilized lipases is strongly dependent on both the biological source of the enzyme and the substitution pattern of the aromatic ring of the substrate. The most relevant result is the *PCL*-catalyzed hydrolysis of 8-hydroxy-2-tetralyl butyrate *rac*-**1c** to give the (*R*)-alcohol and the residual (*S*)-ester in almost enantiopure form (99% ee). Also the unsubstituted tetralol **2a** is resolved with good selectivity, particularly when *PFL* is used as the biocatalyst (92% ee). In contrast, a hydroxyl group at the 5-position seems to be scarcely tolerated and *rac*-**1b** was found to be a poor substrate. The best resolution (71% ee of the formed (*R*)-alcohol) was obtained using *PCL*. In all cases, the (*R*)-enantiomer is preferably hydrolyzed.

Table 2
Enzymatic hydrolysis of esters **1a–c** by lipases immobilized on octyl agarose[a]

Substrate	Enzyme[b]	c[c] (%)	ee_p[d] (%)	E[e]
1a	*PCL*	46	75 (*R*)	13
	PFL	40	92 (*R*)	45
	RML	44	91 (*R*)	45
1b	*PCL*	46	71 (*R*)	11
	PFL	41	59 (*R*)	6
	RML	48	12 (*R*)	1
1c	*PCL*	50	99 (*R*)	>200
	PFL	50	87 (*R*)	41
	RML	50	52 (*R*)	5

[a] In MeCN–25 mM phosphate buffer pH 7 (2:8) at 25 °C, concentration of substrate 2 mM.
[b] *Pseudomonas cepacia* lipase (*PCL*), *Pseudomonas fluorescens* lipase (*PFL*), *Rhizomucor miehei* lipase (*RML*); activity as in Table 1.
[c] c (conversion), product % after 24 h.
[d] Ee's and absolute configuration of (*R*)-**2a** and (*R*)-**2b** were determined by chiral HPLC of the reaction mixture on a S,S-WhelkO1 column; in the case of **2c**, its ee and absolute configuration were estimated via conversion into its ester (*R*)-**1c**.
[e] E (enantioselectivity), see note of Table 1.

The enzymatic hydrolysis of 8-hydroxy-2-tetralyl butyrate *rac*-**1c** was scaled up to 20 mM using immobilized *PCL* as the biocatalyst in the presence of variable amounts of MeCN (Table 3). Under all the conditions employed, the alcohol (*R*)-**2c** and the remaining ester (*S*)-**1c** were obtained in almost enantiopure form (99% ee) and with excellent yield (45% and 43% isolated yield for (*R*)-**2c** and (*S*)-**1c**, respectively, at 20 mM concentration).

Table 3
Scale-up of *PCL*-catalyzed enzymatic hydrolysis of **1c** in MeCN–25 mM phosphate buffer pH 7

Concentration of **1c** (mM)	% MeCN	c[a] (%)	Time (h)	ee_p[b] (%)	ee_s[d] (%)	E[e]
2	20	50	6	99	99	>200
5	20	50	7	99	99	>200
5	30	50	8	99	99	>200
20	40	50	23	99	99	>200

[a] % Conversion.
[b] Enantiomeric excess of the (*R*)-**2c** was evaluated by chiral HPLC after conversion into the corresponding butyrate (*R*)-**1c** (an S,S-WhelkO1 column).
[d] Enantiomeric excess of the remaining ester (*S*)-**1c** was evaluated by chiral HPLC.
[e] E (enantioselectivity), see note of Table 1.

Also in the case of the 5-hydroxy isomer *rac*-**1b**, the enantioselectivity of the *PCL*-catalyzed hydrolysis was not affected by increasing amounts of acetonitrile. For example, at 20 mM concentration and in the presence of 40% MeCN, the isolated (*R*)-alcohol (*R*)-**2b** and (*S*)-ester (*S*)-**1b** were obtained with 71% ee and 65% ee, respectively (% conversion, 47; *E*, 11). A study of immobilization of *PCL* is under evaluation with the goal to highlight a possible influence of the matrix on the enantioselectivity. The results are forthcoming and will be disclosed in due course.

3. Conclusion

Optically active 2-tetralols (*R*)-**2b,c** that are useful intermediates in the synthesis of pharmacologically active 2-aminotetralins **3**, as well as their corresponding (*S*)-butyrates, have been prepared in high (or moderate) ee's by enantioselective hydrolysis of racemic esters *rac*-**1b,c** using lipases immobilized onto octyl agarose. Among the commercial lipases we tested, *PCL* appears to be the best biocatalyst for preparative purposes. It should be noted that the enzymatic resolution of *rac*-**2a–c** by properly selected lipases appears to be an alternative to microbial reductions of the corresponding 2-tetralones.[13,15,19–21] In addition, the use of immobilized

enzymes allows the easy recovery and the reuse of the biocatalyst with advantages in terms of product purification and process costs.

4. Experimental

4.1. General experimental

Analytical TLC was performed on silica gel F_{254} precoated aluminium sheets (0.2 mm layer, Merck, Darmstadt, Germany) using the following eluents: A, hexane/EtOAc (1:1); B, hexane/EtOAc (2:1); components were detected under an UV lamp and by spraying with a ceric sulfate/ammonium molybdate solution, followed by heating to ca. 150°. Silica gel 60, 40–63 μm (Merck) was used for flash chromatography. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 400.1 and 100.6, respectively, on a Bruker AVANCE 400 Spectrometer equipped with a XWIN-NMR software package (Bruker, Karlsruhe, Germany). Chemical shifts (δ) are given in ppm, and were referenced to the solvent signals (δ_H 7.25, δ_C 77.00 from TMS). EIMS spectra were run on a VG 7070 EQ mass spectrometer operating at 70 eV. A Merck-Hitachi L-7100 liquid chromatograph equipped with L-7300 column oven and a L-7400 UV detector was used for analytical HPLC. The OD-Chiralcel column was from Daicel® Chemical Industries Ltd; the S,S-WhelkO1 column was from Regis Technologies Inc. Optical rotations (α_D) were measured in MeOH at 25 °C on a Jasco P-1030 polarimeter.

All reagents were of commercial quality or purified prior to use by standard methods. Lipases from *T. lanuginosus* (TLL), *C. rugosa* (CRL), *P. pancreas* (*PPL*) were from Sigma–Aldrich (Milano, Italy). Lipases from *P. cepacia* (*PCL*) and *P. fluorescens* (*PFL*) were kindly donated by Amano Enzyme Inc. (Japan). *R. miehei* lipase (*RML*) (Chirazyme® L-9, sol.) was from Roche Diagnostics GmbH (Germany). Octyl agarose (Octyl Sepharose CL-4B) was from Sigma–Aldrich (Milano, Italy).

4.2. Synthesis of substrates

5-Hydroxy-2-tetralone **6b** and 8-hydroxy-2-tetralone **6c** were prepared from 6-methoxy- **4b** and 7-methoxy-1-tetralone **4c**, respectively, as described in Ref. 15. 1-Methylphenethyl butyrate **9** and 2-tetralyl butyrate **1a** were prepared as reported in Ref. 22.

4.2.1. Racemic 5-hydroxy-2-tetralol *rac*-2b

To a solution of 5-hydroxy-2-tetralone **6b** (1.35 g, 8.3 mmol) in anhydrous MeOH (100 mL) at 0 °C under N_2, $NaBH_4$ (600 mg, 15.9 mmol) was added portion wise. The reaction mixture was allowed to warm to rt, stirred for 3 h (TLC control) and then quenched by the addition of acetone (3 mL), water (100 mL) and a few drops of concd HCl. After removal of MeOH under reduced pressure, the remaining aqueous phase was extracted with EtOAc (3 × 100 ml). The organic extracts were dried (Na_2SO_4), evaporated under reduced pressure, and the residue was purified by flash chromatography (eluent A) to give racemic 5-hydroxy-2-tetralol *rac*-**2b** as a white solid (1.28 g, 7.8 mmol, 94% yield). TLC (eluent A), R_f = 0.32; ^{1}H NMR, ^{13}C NMR and EI MS as in Ref. 15.

4.2.2. Racemic 8-hydroxy-2-tetralol *rac*-2c

The title compound was prepared by $NaBH_4$ reduction of 8-hydroxy-2-tetralone **6c** in 91% yield as described above for 5-hydroxy-2-tetralol *rac*-**2b**. TLC (eluent A), R_f = 0.29; ^{1}H NMR, ^{13}C NMR and EI MS as in Ref. 15.

4.2.3. 5-Benzyloxy-2-tetralol *rac*-7b

To a stirred solution of 5-hydroxy-2-tetralol *rac*-**2b** (1.0 g, 6.1 mmol) in dry CH_3CN (100 mL), K_2CO_3 (840 mg, 6.1 mmol) and benzyl bromide (0.73 mL, 6.1 mmol) were added, and the reaction mixture was heated at 70 °C for 24 h under an N_2 atmosphere. The solution was then cooled to rt and concentrated under reduced pressure. The resulting residue was triturated with Et_2O (3 × 100 mL), filtered and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography (eluent B) to obtain pure 5-benzyloxy-2-tetralol *rac*-**7b** (1.43 g, 5.6 mmol, 92% yield). TLC (eluent A), R_f = 0.49; ^{1}H NMR ($CDCl_3$) δ: 1.65 (s, 1H, OH), 1.78–1.88 (m, 1H, H-3$_a$), 2.09–2.12 (m, 1H, H-3$_b$), 2.74–2.83 (m, 2H, H-1$_a$, H-4$_a$), 3.00–3.13 (m, 2H, H-1$_b$, H-4$_b$), 4.14–4.20 (m, 1H, H-2), 5.09 (s, 2H, OCH_2), 6.75 (d, 1H, J = 7.6 Hz) and 6.77 (d, 1H, J = 8.0 Hz) (H-6 and H-8), 7.12 (app.t, 1H, J = 7.9 Hz, H-7), 7.33–7.47 (m, 5H, aromatic Hs); ^{13}C NMR ($CDCl_3$) δ: 21.4 (CH_2), 31.1 (CH_2), 38.5 (CH_2), 67.1 (CH), 69.7 (CH_2), 108.6 (CH), 121.9 (CH), 125.0 (C), 126.4 (CH), 127.1 (CH), 127.8 (CH), 128.5 (CH), 135.8 (C), 137.4 (C), 156.3 (C); EI MS *m/z* (rel. int.) 254 (M^+, 10), 236 (7), 162 (5), 144 (5), 91 (100).

4.2.4. 8-Benzyloxy-2-tetralol *rac*-7c

The title compound was prepared from 8-hydroxy-2-tetralol *rac*-**2c** in 88% yield as described for 5-benzyloxy-2-tetralol *rac*-**7b**. TLC (eluent A), R_f = 0.55; ^{1}H NMR ($CDCl_3$) δ: 1.61 (s, 1H, OH), 1.80–1.89 (m, 1H, H-3a), 2.02–2.09 (m, 1H, H-3$_b$), 2.67 (dd, 1H, J = 17.2, 7.8 Hz, H-1$_a$), 2.82–2.90 (m, 1H, H-4$_a$), 2.95–3.02 (m, 1H, H-4$_b$), 3.22 (dd, 1H, J = 17.2, 5.2 Hz, H-1$_b$), 4.16–4.22 (m, 1H, H-2), 5.09 (s, 2H, OCH_2), 6.76 (d, 1H, J = 8.1 Hz) and 6.77 (d, 1H, J = 7.9 Hz) (H-5 and H-7), 7.12 (app.t, 1H, J = 8.0 Hz, H-6), 7.33–7.48 (m, 5H, aromatic Hs); ^{13}C NMR ($CDCl_3$) δ: 27.1 (CH_2), 31.0 (CH_2), 32.6 (CH_2), 67.3 (CH), 69.7 (CH_2), 108.4 (CH), 121.1 (CH), 123.6 (C), 126.3 (CH), 127.1 (CH), 127.8 (CH), 128.5 (CH), 137.2 (C), 137.4 (C), 156.7 (C); EI MS *m/z* (rel. int.) 254 (M^+, 10), 162 (40), 91 (100).

4.2.5. 5-Benzyloxy-2-tetralyl butyrate *rac*-8b

A solution of 5-benzyloxy-2-tetralol *rac*-**7b** (510 mg, 2.0 mmol), triethylamine (0.30 mL, 2.1 mmol) and a catalytic amount of DMAP in dry CH_2Cl_2 (10 mL) was treated dropwise with butyryl chloride (0.3 mL, 2.6 mmol). The reaction mixture was stirred at rt under an N_2 atmosphere until the complete disappearance of the starting material (TLC analysis, eluent B). Usual work-up afforded 5-benzyloxy-2-tetralyl butyrate *rac*-**8b**, pure by TLC, which was used for the next step without further purification (600 mg, 1.85 mmol, 93% yield). R_f = 0.76 (eluent B); ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, 3H, J = 7.3 Hz), 1.63–1.73 (m, 2H) and 2.31 (t, 2H, J = 7.5 Hz) (Hs of $CH_3CH_2CH_2$ moiety), 1.93–2.09 (m, 2H, H_2-3), 2.79–2.00 (m, 3H, H_2-4 and H-1$_a$), 3.14 (dd, 1H, J = 16.6, 4.80 Hz, H-1$_b$), 5.10 (s, 2H, OCH_2), 5.21–5.26 (m, 1H, H-2), 6.74 (d, 1H, J = 8.2 Hz) and 6.77 (d, 1H, J = 7.7 Hz) (H-6 and H-8), 7.1 (app.t, 1H, J = 8.0 Hz, H-7), 7.35–7.48 (m, 5H, aromatic Hs); ^{13}C NMR ($CDCl_3$) δ: 13.6 (CH_3), 18.5 (CH_2), 20.8 (CH_2), 27.4 (CH_2), 34.7 (CH_2), 36.5 (CH_2), 69.3 (CH), 69.8 (CH_2), 108.6 (CH), 121.7 (CH), 124.9 (C), 126.4 (CH), 127.1 (CH), 127.8 (CH), 128.5 (CH), 135.3 (C), 137.4 (C), 156.2 (C), 173.4 (C); EI MS *m/z* (rel. int.) 324 (M^+, 1), 236 (20), 91 (100).

4.2.6. 8-Benzyloxy-2-tetralyl butyrate *rac*-8c

The title compound was prepared from 8-benzyloxy-2-tetralol *rac*-**7c** in 94% yield as described above for 5-benzyloxy-2-tetralyl butyrate *rac*-**8b**. R_f = 0.78 (eluent B); ^{1}H NMR ($CDCl_3$) δ: 0.94 (t, 3H, J = 7.1 Hz), 1.59–1.71 (m, 2H) and 2.25 (t, 2H, J = 7.3 Hz) (Hs of $CH_3CH_2CH_2$ moiety), 1.90–2.03 (m, 2H, H_2-3), 2.77 (dd, 1H, J = 17.5 and 6.6 Hz, H-1$_a$), 2.82–2.97 (m, 2H, H_2-4), 3.11 (dd, 1H, J = 17.5 and 5.4 Hz, H-1$_b$), 5.06 (s, 2H, OCH_2), 5.24–5.29 (m, 1H, H-2), 6.73 (d, 1H, J = 8.1 Hz) and 6.74 (d, 1H, J = 7.7 Hz) (H-5 and H-7), 7.12 (app.t, 1H, J = 7.9 Hz, H-6), 7.30–7.46 (m, 5H, aromatic Hs); ^{13}C NMR ($CDCl_3$) δ: 13.4 (CH_3), 18.3 (CH_2), 26.3 (CH_2), 27.3 (CH_2), 28.8 (CH_2), 36.4 (CH_2), 69.1 (CH), 69.5 (CH_2), 108.2

(CH), 120.8 (CH), 122.9 (C), 126.1 (CH), 126.9 (CH), 127.6 (CH), 128.3 (CH), 136.9 (C), 137.1 (C), 156.4 (C), 173.1 (C); EI MS *m/z* (rel. int.) 236 (40), 145 (10), 91 (100).

4.2.7. 5-Hydroxy-2-tetralyl butyrate *rac*-1b

5-Benzyloxy-2-tetralyl butyrate *rac*-**8b** (500 mg, 1.5 mmol was dissolved in MeOH (40 mL) and hydrogenated in the presence of 10% Pd/C (320 mg) under 1 atm of H_2 at rt for 4 h (TLC control, eluent B). Filtration of the catalyst and removal of the solvent under reduced pressure furnished a residue, which was purified by passing through a short column of silica gel (eluent B) to give the desired product in quantitative yield (350 mg). TLC $R_f = 0.59$ (eluent B); ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, 3H, $J = 7.4$ Hz), 1.63–1.72 (m, 2H) and 2.31 (t, 2H, $J = 7.3$ Hz) (Hs of $CH_3CH_2CH_2$ moiety), 1.96–2.12 (m, 2H, H_2-3), 2.72–2.91 (m, 3H, H_2-4 and H-1_a), 3.10 (dd, 1H, $J = 16.4$, 4.8 Hz, H-1_b), 5.20–5.26 (m, 1H, H-2), 6.64 (d, 1H, $J = 8.0$ Hz) and 6.70 (d, 1H, $J = 8.0$ Hz) (H-6 and H-8), 7.03 (app.t, 1H, $J = 8.0$ Hz, H-7); ^{13}C NMR ($CDCl_3$) δ: 14.3 (CH_3), 18.9 (CH_2), 20.7 (CH_2), 27.7 (CH_2), 35.0 (CH_2), 36.9 (CH_2), 69.6 (CH), 112.7 (CH), 122.0 (CH), 122.7 (C), 127.0 (CH), 135.9 (C), 153.6 (C), 173.9 (C); EI MS *m/z* (rel. int.) 234 (M^+, 2), 163 (3), 147 (60), 146 (100).

4.2.8. 8-Hydroxy-2-tetralyl butyrate *rac*-1c

The title compound was prepared from 8-benzyloxy-2-tetralyl butyrate *rac*-**8c** in quantitative yield as described above for 8-hydroxy-2-tetralyl butyrate. TLC $R_f = 0.57$ (eluent B); ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, 3H, $J = 7.4$ Hz), 1.64–1.73 (m, 2H) and 2.31 (t, 2H, $J = 7.5$ Hz) (Hs of $CH_3CH_2CH_2$ moiety), 1.93–2.05 (m, 2H, H_2-3), 2.72 (dd, 1H, $J = 16.9$, 6.9 Hz, H-1_a), 2.82–2.96 (m, 2H, H_2-4), 3.10 (dd, 1H, $J = 16.9$, 5.0 Hz, H-1_b), 5.25–5.31 (m, 1H, H-2), 6.63 (d, 1H, $J = 8.0$ Hz, H-7), 6.72 (d,1H, $J = 8.0$ Hz, H-5), 7.1 (app t, 1H, $J = 8.0$ Hz, H-6); ^{13}C NMR ($CDCl_3$) δ: 13.7 (CH_3), 18.5 (CH_2), 26.5 (CH_2), 27.5 (CH_2), 28.7 (CH_2), 36.6 (CH_2), 69.5 (CH), 112.1 (CH), 120.7 (C), 120.8 (CH), 126.5 (CH), 137.5 (C), 153.7 (C), 173.7 (C); EI MS *m/z* (rel. int.) 234 (M^+, 2), 163 (3), 147 (80), 146 (100).

4.2.9. (*S*)-2-Tetralol (*S*)-2a, (*S*)-5-hydroxy-2-tetralol (*S*)-2b, (*S*)-8-hydroxy-2-tetralol (*S*)-2c and (*S*)-8-hydroxy-2-tetralyl butyrate (*S*)-1c

(*S*)-2-Tetralol (*S*)-**2a** (30% ee, $[\alpha]_D^{25} = -21.6$ (*c* 0.5, MeOH)), (*S*)-5-hydroxy-2-tetralol (*S*)-**2b** {60% ee, $[\alpha]_D^{25} = -42.8$ (*c* 0.5, MeOH)} and (*S*)-8-hydroxy-2-tetralol (*S*)-**2c** {21% ee, $[\alpha]_D^{25} = -19.3$ (*c* 0.5, MeOH)} were prepared by baker's yeast reduction of 2-tetralone, 5-hydroxy-2-tetralone **6b** and 8-hydroxy-2-tetralone **6c**, respectively, according to Ref. 15.

(*S*)-8-Hydroxy-2-tetralyl butyrate (*S*)-**1c** {$[\alpha]_D^{25} = -8.4$ (*c* 0.5, MeOH)} was prepared from (*S*)-8-hydroxy-2-tetralol (*S*)-**2c** as reported above for the racemic ester.

4.3. Immobilization of lipases on octyl agarose

Immobilization of lipases on octyl agarose was performed as previously reported.[22] The amount of immobilized enzyme was determined by Bradford assay.[26] The enzymatic activity of each enzyme preparation was determined by ethyl butyrate hydrolysis as previously described.[22]

4.4. Enzyme stability assays

PCL, *PFL* and *RML* on octyl agarose were incubated in 25 mM phosphate buffer (pH 7.0) containing different concentrations of MeCN at 25 °C for 24 h. The residual activities of the enzymes were determined by ethyl butyrate hydrolysis[22] and were compared with those of the enzyme preparations prior to incubation.

4.5. Enzymatic hydrolysis

4.5.1. General procedure for lipase-mediated resolution of 1-methylphenethyl butyrate *rac*-9 and esters *rac*-1a–c

The enzyme preparation was added to a solution of the ester *rac*-**9** and *rac*-**1a–c** in MeCN and 25 mM phosphate buffer pH 7 (20:80 v/v) and the reaction mixture was mechanically stirred at 25 °C. During the reaction, the pH was maintained constant by automatic titration (Metrohm 718 STAT Tritino) and the hydrolytic activity was evaluated by measuring the NaOH (100 mM) consumption (μmol NaOH/min × mL of enzyme preparation). The progress of the reaction was monitored both by automatic titrator and reversed HPLC analysis of the organic phase obtained by extracting 500 μL aliquots of the reaction mixture with EtOAc (800 μL). Chromatographic conditions: column, LiChroCART 250-4 select-B (Merck, Darmstadt, Germany); eluent, MeCN/H_2O (8:2); flow rate, 0.8 mL/min for the hydrolysis of ester *rac*-**9**, and 1.0 mL/min for the hydrolysis of esters *rac*-**1a–c**; UV detector, λ 220 nm; temperature, 25 °C; retention times: 1-methylphenethyl butyrate **9** R_t 10.3 min; 1-phenyl-2-propanol **10**, R_t 3.4 min; 2-tetralyl butyrate **1a** R_t 11.2 min; 2-tetralol **2a**, R_t 3.7 min; 5-hydroxy-2-tetralyl butyrate **1b**, R_t 8.6 min; 5-hydroxy-2-tetralol **2b**, R_t 3.0 min; 8-hydroxy-2-tetralyl butyrate **1c**, R_t 9.1 min; 8-hydroxy-2-tetralol (**2c**), R_t 3.1 min.

4.5.2. Determination of enantiomeric excess and absolute configuration assignment of the alcohols 10, 2a and 2b

The enantiomeric excesses of the products arising from the enzymatic hydrolysis of 1-methylphenethyl butyrate (*rac*-**9**) and racemic esters **1a, b** were determined by chiral HPLC analysis of the organic phase obtained by extracting 500 μL aliquots of the reaction mixture with EtOAc (800 μL).

1-Phenyl-2-propanol **10** enantiomers were resolved on a OD-Chiralcel column. Chromatographic conditions: eluent, hexane/isopropanol (99:1); flow rate, 1 mL/min; *T*, 25 °C; detector, $\lambda = 220$ nm; (*S*)-**10**, R_t 16.3 min and (*R*)-**10**, R_t 19.0 min.

Enantioseparation of 2-tetralols **2a**,**b** and of 5-hydroxy-2-tetralyl butyrate **1b** was carried out using a S,S-WhelkO1 column at 25 °C and the detector set at $\lambda = 280$ nm.

In the case of **2a**, the eluent was *iso*-PrOH/*n*-hexane (2:98) at a flow rate of 1 mL/min; retention times (R_t): (*R*)-**2a** 11.6 min, (*S*)-**2a** 13.1 min.

In the case of **1b** and **2b**, a mixture of EtOH/*n*-hexane (2:98) was used as eluent at a flow rate of 2 mL/min; retention times (R_t): 5-hydroxy-2-tetralol, (*R*)-**2b** 26.6 min, (*S*)-**2b** 30.1 min; 5-hydroxy-2-tetralyl butyrate, (*R*)-**1b** 18.1 min, (*S*)-**1b** 16.8 min.

The absolute configurations of the alcohols **10**, **2a** and **2b** obtained by hydrolysis of *rac*-**9**, *rac*-**1a** and *rac*-**1b**, respectively, were assigned using (*R*)- and (*S*)-1-phenyl-2-propanol (**10**), (*S*)-2-tetralol ((*S*)-**2a**) and (*S*)-5-hydroxy-2-tetralol ((*S*)-**2b**) as reference compounds.

4.5.3. Isolation of (*R*)-8-hydroxy-2-tetralol (*R*)-2c and determination of its enantiomeric excess and absolute configuration

Hydrolysis of the 8-hydroxy-2-tetralyl butyrate *rac*-**1c** was stopped by filtering off the enzyme at about 50% conversion. MeCN was removed under vacuum, and the resulting aqueous solution was extracted with EtOAc. The organic phase was dried over Na_2SO_4, evaporated under reduced pressure and the residue was separated by flash chromatography using *n*-hexane/EtOAc (4:1) as eluent to give (*R*)-8-hydroxy-2-tetralol (*R*)-**2c** and (*S*)-8-hydroxy-2-tetralyl butyrate (*S*)-**1c**. (*R*)-8-Hydroxy-2-tetralol was converted into its butyrate as reported above for the racemic ester. Its ee and absolute configuration was determined by chiral HPLC comparison with an authentic sample of (*S*)-**1c** (S,S-WhelkO1

column, chromatographic conditions: eluent, *n*-hexane/EtOH (98:2); flow rate, 1 mL/min; *T*, 25 °C; detector, λ = 280 nm, (*R*)-**1c**, R_t 13.7 min, (*S*)-**1c**, R_t 17.6 min).

When enzymatic hydrolysis of *rac*-**1c** was carried out on 20 mM scale using *PCL* as a biocatalyst, at 50% conversion (*R*)-8-hydroxy-2-tetralol (*R*)-**2c** {99% ee, $[\alpha]_D^{25} = +92.5$ (*c* 0.5, MeOH)} and (*S*)-8-hydroxy-2-tetralyl butyrate (*S*)-**1c** {99% ee, $[\alpha]_D^{25} = -39.2$ (*c* 0.5, MeOH)} were isolated in 45% and 43% yield, respectively.

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