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New chemo-enzymatic approaches for the synthesis of (*R*)- and (*S*)-bufuralol

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ABSTRACT

Both enantiomers of bufuralol are pharmaceutically important molecules. While the (*S*)-isomer with a higher β-blocking activity is recommended for hypertension treatment, the (*R*)-enantiomer can be used as marker of hepatic activity. In this paper two new alternative approaches are described for their chemo-enzymatic synthesis, providing both highly enantiomerically enriched stereoisomers of the target molecule (ee 96–98%). One route is based on the baker's yeast mediated stereoselective biotransformation of α-substituted ketones, and the other one on the lipase mediated kinetic resolution of the racemic bromoethanol.

1. Introduction

The preparation of enantiopure compounds has gained great importance since the enantiomers of pharmaceuticals often display different physiological properties or metabolic behaviors and may have their own effects on drug–ligand interactions.

Amino alcohol moieties with a certain stereochemistry can lead to important biological activities showing antiarrhythmic, enzymatic inhibitory, antihypertensive, and β-adrenoacceptor blocking activity.[1]

Bufuralol, developed by Hoffman-La Roche, is a widely studied potent, nonselective, β-adrenergic receptor antagonist.[2] It is efficient in hypertension treatment,[3] acts as inhibitor of testosterone 6β-hydroxylase,[4] and it was also used in studies of cytochrome P450.

Bufuralol is a chiral molecule with an asymmetric carbon in the ethanol amine side chain and its oxidative degradation takes place enantioselectively and regioselectively in the liver.[5] While the β-blocking potency resides mainly in (*S*)-bufuralol (100 times greater than that of the (*R*)-enantiomer), (*R*)-bufuralol is a commonly used marker of hepatic CYP 2D6 activity.[6]

Various stereoselective procedures for the synthesis of both enantiomers of bufuralol have been already described in the literature.

(*S*)-Bufuralol has been obtained with acceptable enantiomeric excess (ee 87%) from 3-ethyl-2-hydroxy-benzaldehyde, via the stereoselective reduction of 2-bromo-1-(7-ethylbenzofuran-2-yl) ethanone with (−)-*B*-chlorodiisopino-campheylborane as the key step.[1a] Lee et al. have described a similar method involving the asymmetric transfer hydrogenation of 1-(7-ethylbenzofuran-2-yl)-2-mesyloxyethanol mediated by Cp*RhCl[*S*,*S*-TsDPEN] using an azeotropic mixture of formic acid/triethylamine.[7] The (*R*)-enantiomer was successfully obtained from the corresponding chloro-ketone[8] or keto-imine[9] using a sequential transfer hydrogenation–substitution procedure or through a combined ruthenium-enzyme-catalyzed dynamic kinetic resolution of chlorohydrin.[10]

Recently, the stereoselective synthesis of (*S*)-bufuralol (95% yield, ee 98%) via the corresponding (*S*)-cyanohydrin, was reported.[11] The latest compound was prepared by a homochiral metal–organic catalyst mediated enantiotope selective cyanation of the corresponding aldehyde.

Due to their generally large substrate tolerance, high stereoselectivity, effective catalytic activity without cofactors, and good commercial availability, lipases (EC 3.1.1.3) are especially attractive biocatalysts for synthetic purposes. A highly enantioselective lipase-catalyzed kinetic resolution affords both enantiomers simultaneously from a racemic mixture (one as the unreacted enantiomer and the other as a new reaction product).

Consequently Turner et al. employed an enzymatic enantiomer selective acylation of *rac*-7-ethylbenzofuran-2-yl-chloro-alcohol.[12] It was shown that lipases from *Pseudomonas* and *Candida* species can lead to successful resolution of the racemic chloro-alcohol providing (*R*)-bufuralol.

Herein we present two new chemo-enzymatic approaches for the stereoselective synthesis of both enantiomers of bufuralol. The first route is based on a baker's yeast catalyzed biotransformation of 2-(7-ethylbenzofuran-2-yl)-2-oxoethyl acetate **5** and 1-(7-ethylbenzofuran-2-yl)-2-hydroxyethanone **6**, affording both

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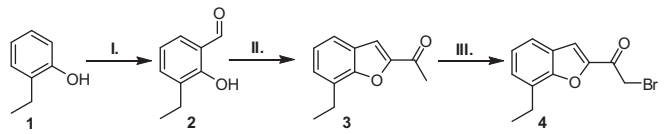
enantiomers of the corresponding 1,2-diols. The other procedure is based on the enantiomer selective lipase-mediated acylation of *rac*-2-bromo-1-(7-ethylbenzofuran-2-yl)ethanol *rac*-**8**, affording at 50% conversion one enantiomer as an ester and the other (slowly reacting) enantiomer as untransformed alcohol. All of the enantiomerically enriched intermediates obtained were further chemically transformed into the both desired enantiomers of bufuralol with good yields and without significant alteration of the enantiopurity.

2. Results and discussion

2.1. Chemical synthesis

The chemical synthesis of the starting materials uses the commercially available 2-ethylphenol **1** which was *ortho*-formylated with paraformaldehyde affording 3-ethyl-salicylaldehyde **2** in good yield (Scheme 1). The aldehyde **2** was transformed with chloroacetone into 7-ethyl-benzofuran-2yl-ethanone **3** which was further brominated with pyridinium tribromide providing the bromo-ketone **4**.



Scheme 1. Chemical synthesis of the α-bromo-ketone. (I) Paraformaldehyde, NEt_3, $MgCl_2$, CH_3CN; (II) chloroacetone, K_2CO_3, CH_3CN: (III) $C_5H_6NBr_3$, CH_3COOH.

2.2. Synthesis of (*R*)- and (*S*)-bufuralol via the corresponding ethane diols obtained by Baker's yeast-mediated biotransformations

Prochiral ketones **5** and **6**, used as substrates for the baker's yeast mediated biotransformation, were obtained by starting from 2-bromo-1-(7-ethylbenzofuran-2-yl)-ethanone **4**, using a procedure previously developed in our laboratories (Scheme 2).[13] Under anhydrous conditions bromo-ethanone **4** was transformed with sodium acetate (in the presence of crown ether 18-C6 as a phase-transfer catalyst) into the corresponding α-acetoxymethyl-ketone **5**. The *Candida antarctica* lipase B (Novozym 435) mediated ethanolysis of the ketoester **5** allowed us to obtain α-hydroxymethyl-ketone **6** in excellent yield. Finally the latest compound was reduced with sodium borohydride to yield the *rac*-1,2-ethanediol *rac*-**7** (84% overall yield starting from **4**) used to set up the chiral analytical chromatographic separation of the racemic diol, allowing to investigate the stereochemical outcome of the baker's yeast-catalyzed biotransformations.

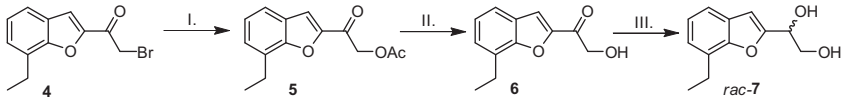
The baker's yeast mediated procedure has gained popularity for several reasons such as mild reaction conditions, low cost, and easy availability of the whole-cell system. Besides these facts the main advantage of this cellular biotransformation consists in the large substrate acceptability and stereoselectivity of the hydrolases and oxido-reductases from *Saccharomyces cerevisiae* cells.

One of the most reliable biocatalytic procedures for forming enantiopure aryl-ethanediols is the cellular biotransformation of various α-substituted ketones, since their enantiotope selective reduction produces one enantiomer with 100% theoretical yield.

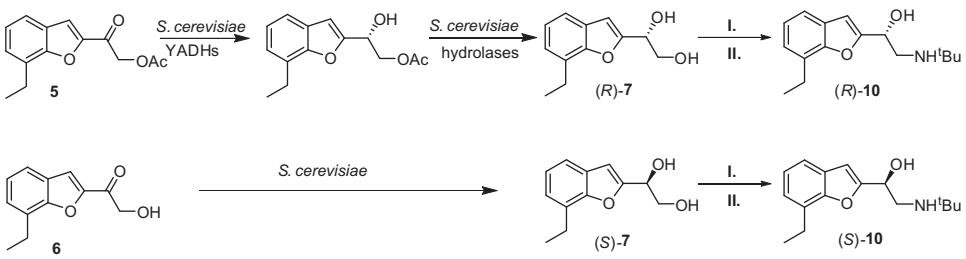
It is known that α-hydroxy and α-acetoxymethyl ketones can be transformed by *Saccharomyces cerevisiae* with opposite stereopreference into both enantiomers of the corresponding diols.[14] Moreover, the baker's yeast mediated bioreduction of the α-acetoxymethyl ketones usually is followed by a subsequent enzymatic hydrolysis of the reduction product. Thus the hydroxy-monoacetate obtained by bioreduction is further hydrolyzed into 1,2-ethanediol by the hydrolases also present in baker's yeast. Our previous results[15] have demonstrated that the enantioselective bioreduction of 1-(benzofuran-2-yl)-2-hydroxyethanones and 2-acetoxy-1-(benzofuran-2-yl)ethanones provided the opposite enantiomeric forms of the diols with high enantiomeric purity (ee 84–93%). Ketones with the relatively small and hydrophilic hydroxymethyl group were all reduced from the same face, whereas baker's yeast has an opposite enantiotopic face preference for the acetoxymethyl ketones (Scheme 3).

These observations proved to be valid also for the biotransformations of ketones **5** and **6** (Scheme 3), which were performed under fermenting and non-fermenting conditions. In order to increase the enantiopurity of the products, the influence of various additives upon the stereoselectivity of the reactions was also tested (Table 1).

As was expected, the enantiomeric excess of the products was significantly influenced by the nature of the additives used. Generally it was observed that, while the use of additives increases the selectivity in the biotransformation of acetoxy-ketone **5**, in the case of hydroxy-ketone **6** most of the additives decreased the selectivity of the bioreduction. Higher selectivity was obtained in the fermenting system only in the presence of L-cysteine (Table 1,



Scheme 2. Chemical synthesis of the α-substituted ketones **5** and **6** and of the racemic diol **7**. (I) $CH_3COO^-Na^+$, 18-C6, 1,4-dioxane; (II) Novozym 435, EtOH; (III) $NaBH_4$, MeOH, rt.



Scheme 3. Chemo-enzymatic transformation of α-substituted ketones **5** and **6** into (*R*)- and (*S*)-bufuralol. (I) TsCl, Et_3N, Bu_2SnO, CH_2Cl_2, rt; (II) *tert*-butylamine, EtOH, reflux.

Table 1
Fermenting and non-fermenting cellular biotransformation of **5** and **6**

Entry	Additives (0.5% w/w)	$ee_{(R)\text{-}7}$[a]		$ee_{(S)\text{-}7}$[b]	
		Fermenting	Non-fermenting	Fermenting	Non-fermenting
1	Without additive	92	90	86	87
2	Allyl alcohol	96	96	80	80
3	*n*-Hexane	98	95	50	78
4	L-Cysteine	96	95	96	76
5	Ethyl bromoacetate	–	–	–	–
6	$MgCl_2$	96	92	62	50
7	DMSO	95	94	76	77

[a] From prochiral acetoxy-ketone **5** as the substrate.
[b] From prochiral hydroxy-ketone **6** as the substrate.

entry 4). While the use of ethyl bromoacetate inhibits the biotransformation of both ketones **5** and **6**, the best result for the transformation of **5** was obtained in the fermenting system using *n*-hexane as an additive (Table 1, entry 3).

The isolated and purified (*R*)- and (*S*)-heteroaryl-1,2-diols (*R*)- and (*S*)-**7** served as valuable precursors for the chemical synthesis of both enantiomers of bufuralol. In the first step the regioselective tosylation of the primary alcohol group of (*R*)- and (*S*)-**7** with *para*-toluenesulfonyl chloride in the presence of a catalytic amount of dibutyltin(IV) oxide[16] was performed. In the second step the tosyl group was substituted with *tert*-butylamine (Scheme 3) yielding the end-product with a small decrease of the enantiopurity (ee 96% for (*R*)-**10** and 93% for (*S*)-**10**) compared with those of (*R*)- and (*S*)-heteroaryl-1,2-diols (ee 98% for (*R*)-**7** and 96% for (*S*)-**7**) (40% overall yield starting from the diols).

2.3. Synthesis of (*R*)- and (*S*)-bufuralol from the lipase-catalyzed enantiopure resolution products of 2-bromo-1-(7-ethylbenzofuran-2-yl)-ethanol

The other approach for the synthesis of (*R*)- and (*S*)-bufuralol involved the use of the lipase-catalyzed stereoselective O-acylation of racemic 2-bromo-1-(7-ethylbenzofuran-2-yl)ethanol *rac*-**8** (Scheme 4), which was previously prepared by reduction of **4** with $NaBH_4$.

Commercially available immobilized or lyophilized lipases, such as lipases A and B from *Candida antarctica* (CaL-A on Celite and CaL-B immobilized on Eupergite commercialized as Novozyme 435), lipase from *Pseudomonas fluorescens* (L-AK), *Candida rugosa* (CRL), and lipase from *Pseudomonas sp.* (LPS) were tested for the analytical scale enantioselective acylation of *rac*-**8** with vinyl acetate and vinyl laurate as acyl donors in various solvents. Lipase A from *Candida antarctica* displayed excellent reactivity but poor selectivity, whereas L-AK, CRL, and LPS showed good selectivity but reduced activity in all solvents tested in the presence of both acyl donors.

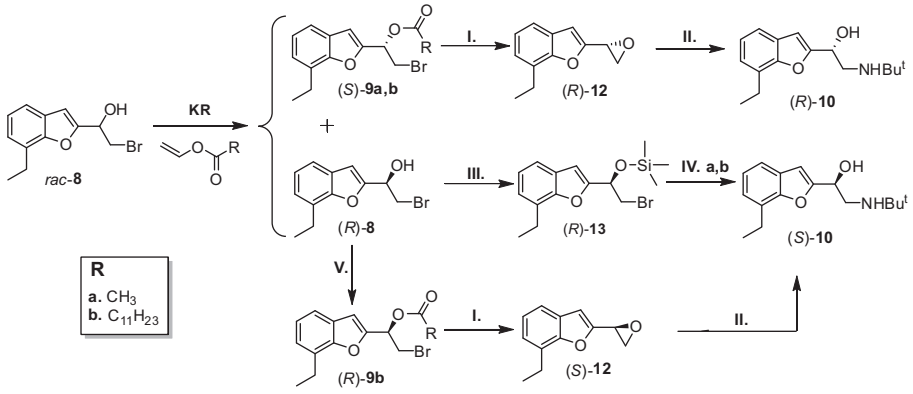
CaL-B proved to be the best biocatalyst. The reaction selectivity was high in all solvents tested with both vinyl esters (Table 2). Excellent enantiomeric excesses of the products and also high reaction rates were obtained in diisopropyl ether and *n*-octane using vinyl acetate as an acyl donor (Table 2, entries 2 and 9) and in toluene using vinyl laurate, respectively, (Table 2, entry 8).

Furthermore a scale-up of the previously mentioned optimal analytical scale reactions was set up, using the same substrate–biocatalyst *ratio*. The reactions were stopped at an approximately 50% conversion (checked by TLC and HPLC). While a small decrease of the ee (~97%) of the resolution products was detected when vinyl acetate was used as the acyl-donor, the ee values were the same as those found for small scale reactions with vinyl dodecan-

Table 2
CaL-B catalyzed enantioselective acylation of *rac*-**8** with vinyl acetate and vinyl dodecanoate after 16 h

Entry	Solvent	ee_p (%)	ee_s (%)	*c* (%)	*E*
1	MTBE[a]	99	49	33	»200
2	MTBE[b]	99	41	29	>200
3	DIPE[a]	>99	>99	50	»200
4	*n*-Hexane[a]	>99	69	41	>200
5	*n*-Hexane[b]	>99	93	48	»200
6	Acetonitrile[a]	>99	42	30	>200
7	Toluene[a]	>99	71	41	»200
8	Toluene[b]	>99	99	50	»200
9	*n*-Octane[a]	>99	99	50	»200

[a] Vinyl acetate.
[b] Vinyl dodecanoate.



Scheme 4. KR. Enzyme, acyl donor, rt, 800 rpm; (I) LiOH, EtOH, rt; (II) *tert*-butylamine, reflux; (III) DMCTMS, Et_2O, rt; (IV) (a) *tert*-butylamine , MeOH, rt; (b) HF, MeOH; (V) vinyl dodecanoate, CaL-A, DIPE.

oate. Both enantiopure compounds were isolated in excellent yields and used for further chemical transformations.

In order to prevent significant racemization and undesired secondary reactions, two particular chemical paths were developed for the efficient transformation of the resolution products into (*R*)- and (*S*)-bufuralol.

In our first attempt the reaction of *tert*-butylamine[12] with enantiomerically pure bromohydrin (*R*)-**8** was investigated in various conditions (data not given). Due to the instability of bromo-ethanol (*R*)-**8** under basic conditions, in each case almost racemic bufuralol was isolated with poor yields. The mild protection of the hydroxy group from (*R*)-**8** with trimethylsilyl-*N*,*N*-dimethylcarbamate (DMCTMS, Scheme 4) allowed the substitution of bromine with *tert*-butyl amine on O-silylated bromohydrin (*R*)-**13**, avoiding racemization. Since the deprotection of (*R*)-**13** with mild acids failed, the HF catalyzed deprotection of the hydroxy group occurred with partial racemization, yielding the desired (*S*)-bufuralol (*S*)-**10** with reduced enantiopurity (ee 90%) and 35% overall yield.

For the synthesis of (*R*)-bufuralol (*R*)-**10** the enantiomerically pure acylated bromohydrins (*S*)-**9a**,**b** were transformed into the enantiopure epoxide (*R*)-**12** in the presence of LiOH. The latest compound was transformed in neat *tert*-butylamine into (*R*)-**10** with 53% overall yield and with excellent enantiomeric excess (ee 98%).

Based on this result, with the aim to improve the enantiopurity of (*S*)-bufuralol, we decided to reinvestigate the chemical transformation of (*R*)-**8** applying the strategy described earlier. Since CaL-A proved to be a highly active but unselective biocatalyst for the acylation of *rac*-**8**, the acylation of (*R*)-**8** with vinyl laurate in DIPE in the presence of this lipase was performed and the isolated ester (*R*)-**9b** (ee 99%) was further transformed into the target (*S*)-**10** (ee 98%) as shown in Scheme 4.

3. Conclusions

Using two alternative biocatalytic approaches based on baker's yeast mediated reactions (through the biotransformation of prochiral α-substituted 7-ethyl-benzofuran-2-yl-ethanones) and on a lipase catalyzed kinetic resolution of (7-ethyl-benzofuran-2-yl)-bromohydrin (by enantiomer selective O-acylation), highly enantiomerically enriched compounds were obtained. These compounds serve as building blocks for the synthesis of both enantiomers of bufuralol. Due to partial racemization occurring during the chemical transformation of the chiral intermediates, the enantiomeric excesses of the target (*R*)- and (*S*)-bufuralol were decreased to 96–98%.

4. Experimental

4.1. Analytical methods

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker spectrometers operating at 400 MHz, 101 MHz and 600 MHz, 151 MHz, respectively. Spectra were recorded at 21 °C and were referenced internally to the solvent signal. Mass spectra were recorded on a GC–MS Shimadzu QP 2010 Plus spectrometer using direct injection and EI^{+} ionization at 70 eV. IR spectra were recorded on a Bruker Vector 22 spectrometer. High performance liquid chromatography (HPLC) analyses were conducted with an Agilent 1200 instrument using a Chiralpak IB column and a mixture of *n*-hexane–2-propanol 95:5 (v/v) as eluent for the enantiomeric separation of *rac*-**7** ($t_{r(S)\text{-}7}$ 19.2 min; $t_{r(R)\text{-}7}$ 21.5 min), and a (*R*,*R*)-Welk-O1 column and a mixture of *n*-hexane–2-propanol 99:1 (v/v) as eluent for the separation of *rac*-**8** and *rac*-**9a**,**b** respectively, ($t_{r(S)\text{-}8}$ 10.6 min; $t_{r(R)\text{-}8}$ 12.2 min; $t_{r(R)\text{-}9a}$ 3.8 min; $t_{r(S)\text{-}9a}$ 5.5 min; $t_{r(R)\text{-}9b}$ 2.9 min; $t_{r(S)\text{-}9b}$ 4.1 min). The enantiomeric purities of the bufuralol enantiomers were determined using a Chirobiotic V2 column in polar ionic mode with methanol–acetic acid–triethylamine 100:0.02:0.015 (v/v/v) as eluent ($t_{r(S)\text{-}12}$ 19.6 min; $t_{r(R)\text{-}12}$ 22.4 min). Optical rotations were measured with a Perkin–Elmer 201 polarimeter and $[\alpha]_D^{25}$ values are given in units 10^{-1} deg cm^2 g^{-1}.

4.2. Reagents, solvents, and biocatalysts

2-Ethylphenol, all reagents used, and solvents were products of Sigma–Aldrich and Alfa Aesar. Lipase from *Pseudomonas fluorescens* (L-AK) and lipase from *Pseudomonas* sp. (LPS) were obtained from Amano, England, while lipase from *Candida rugosa* (CRL) was purchased from Fluka. Immobilized lipase B from *Candida antarctica* (CaL-B) was purchased from Novozyme, Denmark, while lipase A from *Candida antarctica* immobilized by adsorption on Celite (CaL-A) was a gift from Professor Liisa T. Kanerva, University of Turku.

4.3. Chemical transformations

4.3.1. Synthesis of 3-ethyl-2-hydroxybenzaldehyde 2

Into the mixture of 2-ethylphenol **1** (9.77 g, 80 mmol), magnesium chloride (11.42 g, 120 mmol) and triethylamine (30.36 g, 300 mmol) in acetonitrile (100 mL) paraformaldehyde (16.21 g, 540 mmol) was added in small portions at rt. The mixture was refluxed for 5 h, stirred for another 12 h at rt and finally acidified with a dilute HCl solution (5%, 200 mL). The mixture was extracted with dichloromethane (2 × 100 mL) and then the organic layer was dried over Na_2SO_4. The solvent was removed in vacuo and the product was isolated by vacuum distillation with 98% yield. Spectral data were in accordance with those reported in the literature.[1a]

4.3.2. Synthesis of 1-(7-ethylbenzofuran-2-yl)ethanone 3

Chloroacetone (7.32 g, 79.2 mmol) was added dropwise to a mixture of **2** (10.8 g, 72 mmol) and anhydrous potassium carbonate (9.95 g, 72 mmol) in acetonitrile (60 mL) at 35 °C. The mixture was refluxed for 5 h and cooled to rt. The precipitated solid was filtered off and washed with acetonitrile (2 × 10 mL). After removing the solvent the crude product was purified by distillation followed by recrystallization from *n*-hexane affording the pure desired product **3** (9.9 g, 73% yield). Spectral data were in accordance with those reported in the literature.[1a]

4.3.3. Synthesis of 2-bromo-1-(7-ethylbenzofuran-2-yl)ethanone 4

Into a solution of **3** (16 g, 0.1 mol) in acetic acid (100 mL) pyridinium tribromide (28.7 g, 0.9 equiv.) was added in small portions at 35 °C. The reaction was completed at 50 °C (controlled by TLC with dichloromethane as eluent, approx. 2 h). The reaction mixture was poured on ice-cooled water; the precipitate formed was filtered and redissolved in dichloromethane. The organic solution was dried over Na_2SO_4, the solvent was further removed in vacuo, and the crude product was purified by column chromatography using *n*-hexane–dichloromethane 1:4 (v/v) as eluent.

4.3.3.1. 2-Bromo-1-(7-ethylbenzofuran-2-yl)ethanone 4. Yield 85%, 19.4 g; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 7.2 Hz, 1H), 7.27 (dd, J = 7.8, 7.2 Hz, 1H), 4.46 (s, 2H), 2.99 (q, J = 7.6 Hz, 2H), 1.38 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.33, 154.65, 149.95, 129.08, 127.89, 126.62, 124.47, 120.92, 115.08, 30.30, 22.80, 13.99; MS *m*/*z* 268 (M+2, 25.74), 267 (M^{+}, 8.72), 266 (M−1, 26.75), 174 (12.86), 173 (100), 159 (17.26), 144 (12.36), 117 (12.25), 115 (29.8), 91 (9.74); IR (KBr, cm^{-1}): 1693, 1558, 1376, 1313, 1138, 1001.

4.3.4. Synthesis of 2-(7-ethylbenzofuran-2-yl)-2-oxoethyl acetate 5

Into a solution of **4** (900 mg, 3.4 mmol) in 1,4-dioxane (10 mL) anhydrous sodium acetate (830 mg, 10.1 mmol) and phase transfer catalyst 18-C6 (0.1 equiv, 89 mg) were added. The resulting mixture was heated at reflux until the reaction was completed (controlled by TLC with dichloromethane as eluent, approx. 5 h). After cooling, the deposited solid was filtered off and washed with dioxane (3 × 10 mL). The solvent was removed in vacuo and the crude product was recrystallized from ethanol.

4.3.4.1. 2-(7-Ethylbenzofuran-2-yl)-2-oxoethyl acetate 5. Yield 90%, 735 mg; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.30 (d, J = 6.9 Hz, 1H), 7.24 (dd, J = 7.7, 6.9 Hz, 1H), 5.32 (s, 2H), 2.96 (q, J = 7.6 Hz, 2H), 2.23 (s, 3H), 1.35 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.72, 170.34, 154.35, 150.16, 128.92, 127.63, 126.37, 124.39, 120.85, 113.67, 65.82, 22.71, 20.52, 13.96; MS m/z 246 (M^+, 5.96), 173 (16.37), 115 (9.21), 43 (100), 29 (8.06); IR (KBr, cm^{-1}): 1745, 1563, 1378, 1230, 1160, 1008.

4.3.5. Synthesis of 1-(7-ethylbenzofuran-2-yl)-2-hydroxyethanone 6

The mixture of **5** (300 mg, 1.2 mmol) and CaL-B (300 mg) in ethanol (23 mL) was shaken at 300 rpm and rt until the reaction was completed (controlled by TLC with dichloromethane–methanol 95:5 (v/v) as eluent, approx. 4 h). The enzyme was filtered off and washed with ethanol (3 × 10 mL). The solvent was removed in vacuo from the filtrate and the crude product was recrystallized from ethanol.

4.3.5.1. 1-(7-Ethylbenzofuran-2-yl)-2-hydroxyethanone 6. Yield 98%, 244 mg; ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.76 (s, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 7.3 Hz, 1H), 7.28 (dd, J = 7.8, 7.3 Hz, 1H), 4.86 (s, 2H), 2.95 (q, J = 7.6 Hz, 2H), 1.33 (t, J = 7.6 Hz, 3H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 190.55, 154.88, 151.19, 129.41, 128.15, 127.58, 125.11, 121.87, 114.31, 66.39, 23.28, 14.34. MS m/z 204 (M^+, 16.58), 173 (100), 115 (21.9), 91 (10.24); IR (KBr, cm^{-1}): 3444, 1679, 1562, 1388, 1223, 1154, 1097, 996.

4.3.6. Synthesis of *rac*-1-(7-ethylbenzofuran-2-yl)ethane-1,2-diol *rac*-7

Sodium borohydride (2 equiv, 1.11 g) was added in small portions into a stirred mixture of **6** (2 g, 9.8 mmol) in methanol (40 mL), until all of the ketone was transformed (controlled by TLC with (dichloromethane–methanol 9:1 (v/v) as eluent, approx. 1 h). The methanol was removed in vacuo and the mixture was acidified with 5% HCl, followed by extraction with dichloromethane (3 × 50 mL) of the crude product. The organic layer was dried over anhydrous Na_2SO_4, concentrated in vacuo, and the crude product was recrystallized from *n*-hexane.

4.3.6.1. *rac*-1-(7-Ethylbenzofuran-2-yl)ethane-1,2-diol *rac*-7. Yield 95%, 1.9 g; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 7.5 Hz, 1H), 7.16 (dd, J = 7.5, 7.1 Hz, 1H), 7.11 (d, J = 7.1 Hz, 1H), 6.71 (s, 1H), 4.98 (t, J = 4.7 Hz, 1H), 4.00 (d, J = 4.9 Hz, 2H), 2.92 (q, J = 7.6 Hz, 2H), 1.34 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.79, 153.42, 127.74, 127.54, 123.61, 123.12, 118.62, 104.07, 68.96, 65.17, 22.80, 14.10. MS m/z 206 (M^+ 38.53), 189 (23.39), 175 (100), 131 (11.67), 119 (17.11), 91 (43.98), 77 (12.29), 31 (16.25); IR (KBr, cm^{-1}): 3283, 3262; 1463, 1427, 1272, 1180, 1142, 1032; $[\alpha]_D^{25}$ = +38.3 (c 1.0, $CHCl_3$) for (*R*)-**7**, $[\alpha]_D^{25}$ = −37.4 (c 1.0, $CHCl_3$) for (*S*)-**7**.

4.3.7. Synthesis of *rac*-2-bromo-1-(7-ethylbenzofuran-2-yl)-ethanol *rac*-8

Sodium borohydride (1 equiv, 0.8 g) was added in small portions to a stirred solution of **4** (5.61 g, 21.1 mmol) in methanol (40 mL) at 0 °C under an argon atmosphere. The reduction was completed under stirring at room temperature (controlled by TLC with dichloromethane as eluent, approx. 30 min). After distillation of methanol in vacuo at room temperature, the crude product was resuspended in water (150 mL), acidified with 10% HCl, and extracted with dichloromethane (3 × 150 mL). The organic layer was dried over anhydrous Na_2SO_4 and *c*oncentrated in vacuo, affording the product as a yellow semisolid.

4.3.7.1. *rac*-2-Bromo-1-(7-ethylbenzofuran-2-yl)ethanol *rac*-8. Yield 97%, 5.48 g; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 7.6 Hz, 1H), 7.21 (dd, J = 7.6, 6.9 Hz, 1H), 7.16 (d, J = 6.9 Hz, 1H), 6.76 (s, 1H), 5.11 (dd, J = 6.7, 4.4 Hz, 1H), 3.87 (dd, J = 10.5, 4.3 Hz, 1H), 3.80 (dd, J = 10.5, 7.0 Hz, 1H), 2.96 (q, J = 7.6 Hz, 3H), 1.38 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.17, 153.49, 127.84, 127.50, 123.84, 123.26, 118.84, 104.46, 68.25, 36.57, 22.89, 14.20. MS m/z 271 (M+2, 11.97), 270 (M+1, 68.24), 269 (M^+, 15.05), 268 (64.69), 253 (50), 251 (50.53), 175 (100), 119 (22.59), 91 (40); IR (KBr, cm^{-1}): 3400, 1602, 1426, 1300, 1274, 1184, 1073, 988; $[\alpha]_D^{25}$ = −43.7 (c 1.0, $CHCl_3$) for (*R*)-**8**.

4.4. Cellular biotransformation of 2-(7-ethylbenzo-furan-2-yl)-2-oxoethyl acetate 5 and 1-(7-ethylbenzofuran-2-yl)-2-hydroxyethanone 6 with baker's yeast cells

4.4.1. Non-fermenting biotransformation

Baker's yeast (2 g) was suspended in 15 mL water. After stirring for 15 min, the corresponding additive (see Table 1) and the ketone **5** or **6** (10 mg) dissolved in ethanol (0.5 mL) were added into the resulting cell suspension under stirring. Samples (1 mL) were taken after 18 h and 40 h, respectively, extracted with ethyl acetate and dried over anhydrous Na_2SO_4. The solvent was evaporated, the crude solid was redissolved in *n*-hexane–isopropanol 9:1 (v/v) and analyzed by HPLC.

4.4.2. Fermenting biotransformation

A fresh wet cake of baker's yeast (2 g) and sucrose (1 g) were added in water (15 mL). The resulting suspension was stirred for 30 min, the corresponding additive (see Table 1) and the ketone **5** or **6** (10 mg) dissolved in ethanol (0.5 mL) were added. Further experiments were performed as described in the previous section.

4.4.3. Synthesis of (*R*)- and (*S*)-2-*tert*-butylamino-1-(7-ethylbenzofuran-2-yl)ethanol (*R*)- and (*S*)-10

To a solution of the enantiomerically pure alcohol (*R*)- or (*S*)-**7** (200 mg, 1 mmol) in dichloromethane (20 mL) Bu_2SnO (50 mg, 0.2 mmol), *p*-TsCl (190 mg, 1 mmol), and Et_3N (100 mg, 1 mmol) were added. The reaction mixture was stirred until TLC indicated the complete consumption of the starting material (dichloromethane–methanol 9:1 (v/v) as eluent, approx. 30 min). The mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography using dichloromethane as eluent.

4.4.3.1. 2-Tosyloxy-1-(7-ethylbenzofuran-2-yl)ethanol. Yield 74%, 260 mg; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 7.6 Hz, 1H), 7.20 (d, J = 8.1 Hz, 2H), 7.16 (t, J = 7.5 Hz, 1H), 7.10 (d, J = 7.1 Hz, 1H), 6.67 (s, 1H), 2.84 (q, J = 7.6 Hz, 2H), 2.37 (s, 3H), 1.30 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.79, 153.39, 145.13, 132.24, 129.86, 127.87, 127.68, 127.39, 123.70, 123.14, 118.75, 104.87, 71.40, 66.52, 22.69, 21.62, 14.03. MS m/z 360 (M^+ 5.38), 343 (10.40), 188 (79.32), 175 (100), 159 (24.84), 91 (60.21), 65 (34.00); IR (KBr, cm^{-1}): 3536, 1599, 1360, 1180, 1097, 983.

Further, into the solution of the tosylated diol (145 mg, 0.5 mmol) in absolute ethanol (10 mL) *tert*-butylamine (110 mg,

1.5 mmol) was added and the mixture was heated to reflux overnight. The volatile materials were removed in vacuo and the crude product was purified by column chromatography using dichloromethane–methanol 8:2 (v/v) as eluent. The product was obtained as a light-yellow solid.

4.4.3.2. 2-(*tert*-Butylamino)-1-(7-ethylbenzofuran-2-yl)ethanol *rac*-10. Yield 54%, 56 mg; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, J = 7.6, 1.1 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 7.09 (d, J = 7.3 Hz, 1H), 6.69 (s, 1H), 5.00 (dd, J = 6.9, 4.9 Hz, 1H), 4.02 (br s, 2H), 3.11–3.02 (m, 2H), 2.94 (q, J = 7.6 Hz, 2H), 1.35 (t, J = 7.6 Hz, 3H), 1.17 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.21, 153.40, 127.83, 127.63, 123.18, 122.90, 118.51, 103.23, 66.24, 51.31, 46.53, 28.60, 22.83, 14.18; MS m/z 262 (M+1, 23.9), 261 (M^+, 2.0), 228 (14.7), 175 (13.6), 131 (11.5), 115 (11.2), 91 (16.5), 86 (100.0), 57 (18.2); IR: (KBr, cm^{-1}): 3285, 3130, 1601, 1477, 1426, 1364, 1320, 1270, 1262, 1094, 1019.

4.5. Kinetic resolution of 2-bromo-1-(7-ethylbenzofuran-2-yl) ethanol *rac*-8

4.5.1. Analytical scale enzymatic acylation of *rac*-8

One of the lipases (30 mg/mL) and an acyl donor (vinyl acetate or vinyl dodecanoate, 2 equiv) were added into the solution of *rac*-**8** (30 mg) in an organic solvent (1 mL). The reaction mixture was shaken (1350 rpm) at room temperature. For HPLC analysis, the samples (25 μL) were concentrated, diluted to 700 μL with 2-propanol and *n*-hexane, and filtered before injection.

4.5.2. Preparative scale enzymatic acylation of *rac*-8

CaL-B (1 g) was added into the solution of **8** (1 g), vinyl acetate, or dodecanoate (2 equiv) in the appropriate solvent (33 mL). The reaction mixture was shaken for 24 h at room temperature. The solvent was evaporated in vacuo and the crude product was purified by column chromatography, using dichloromethane as eluent.

4.5.2.1. (*S*)-2-Bromo-1-(7-ethylbenzofuran-2-yl)ethyl acetate (*S*)-9a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 7.4 Hz, 1H), 7.24–7.16 (m, 2H), 6.83 (s, 1H), 6.25 (t, J = 6.5 Hz, 1H), 3.87 (d, J = 6.5 Hz, 2H), 2.99 (q, J = 7.6 Hz, 2H), 2.19 (s, 3H), 1.40 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.66, 153.54, 151.61, 127.94, 127.20, 124.21, 123.35, 118.99, 106.57, 68.64, 30.57, 22.89, 20.87, 14.11; MS (70 eV) m/z 312 (M+2, 1.67), 310 (M^+, 1.73), 230 (6.68), 188 (46.26), 175 (13.44), 157 (11.37), 115 (13.11), 87 (23.16), 74 (30.95), 43 (100); IR (KBr, cm^{-1}): 1749, 1462, 1428, 1372, 1272, 1176; $[\alpha]_D^{25}$ = +92.8 (c 1.0, $CHCl_3$).

4.5.2.2. (*S*)-2-Bromo-1-(7-ethylbenzofuran-2-yl)ethyl dodecanoate (*S*)-9b. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 7.3 Hz, 1H), 7.20–7.11 (m, 2H), 6.77 (s, 1H), 6.21 (t, J = 6.5 Hz, 1H), 3.83 (d, J = 6.5 Hz, 2H), 2.93 (q, J = 7.5 Hz, 2H), 2.47–2.31 (m, 2H), 1.71–1.62 (m, 2H), 1.34 (t, J = 7.6 Hz, 3H) overloaded with 1.2–1.34 (m, 16H) 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.68, 153.62, 151.88, 128.06, 127.33, 124.24, 123.40, 119.05, 106.47, 68.50, 34.34, 32.05, 30.82, 29.73, 29.57, 29.47, 29.37, 29.20, 25.05, 22.99, 22.83, 14.27, 14.20; MS (70 eV) m/z 452 (M^+, 0.46), 370 (4.73), 251 (4.47), 188 (100), 172 (29.22), 157 (15.99), 43 (29.03); IR (KBr, cm^{-1}): 1748, 1709, 1463, 1426, 1377, 1148, 1112, 1069; $[\alpha]_D^{25}$ = +81.0 (c 1.0, $CHCl_3$).

4.5.3. Synthesis of (*S*)-bufuralol from (*R*)-8

Trimethylsilyl *N*,*N*-dimethylcarbamate (640 μL, 3.7 mmol) was added to a solution of (*R*)-**8** (500 mg, 1.86 mmol) in dry diethyl ether (15 mL) under an argon atmosphere. The solution was stirred at rt until no starting material was observed (controlled by TLC with dichloromethane, approx. 20 min). The product was obtained after removing the volatile materials in vacuo (589 mg, yield 93%).

tert-Butylamine (300 μL, 3 mmol) was added to a solution of (*R*)-**13** (500 mg, 1.46 mmol) in methanol (15 mL) and then stirred at rt until the reaction was finished (approx. 30 min). In order to remove the protecting trimethyl silyl group hydrofluoric acid (1 M, 1.5 mL) was added dropwise into the reaction mixture. The methanol was evaporated in vacuo, the residue was dissolved in ethyl acetate (40 mL) and washed with water (2 × 40 mL). The organic layer was dried over anhydrous Na_2SO_4, the solvent was removed in vacuo affording pure (*S*)-bufuralol in 35% yield (based on (*R*)-**8**).

4.5.4. Synthesis of (*R*)-bufuralol from the corresponding acylated bromohydrins (*S*)-9a,b

Into the solution of the acylated bromohydrin (350 mg) in EtOH (10 mL), $LiOH \times H_2O$ (1.2 equiv) was added and the mixture was stirred at room temperature. After 20 min no starting material was observed (controlled by TLC with dichloromethane as eluent). The reaction was quenched with sat. $NaHCO_3$ solution (2 mL) and the EtOH was removed in vacuo. Brine (2 mL) was added to the residue and the mixture was extracted with diethyl ether (3 × 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and concentrated.

The mixture of crude epoxide (*R*)-**12** (approx. 200 mg) and *tert*-butylamine (300 μL, 3 mmol) was stirred overnight at rt. The excess of *tert*-butylamine was removed under reduced pressure. Chromatographic purification (dichloromethane–methanol, 8:2 (v/v) as eluent) afforded 158 mg (*R*)-bufuralol (54% overall yield).

4.5.5. Synthesis of (*S*)-bufuralol from (*R*)-8 via the epoxide intermediate (*S*)-12

Into a solution of (*R*)-**8** (200 mg) in DIPE (5 mL), CaL-A on Celite (50 mg) and vinyl dodecanoate (330 μL, 2 equiv) were added. The reaction was completed at room temperature under shaking (1350 rpm), for approx. 16 h. The enzyme was filtered off and washed with DIPE (2 × 0.5 mL). The solvent was removed in vacuo and the crude product obtained (*R*)-**9b** (325 mg, 97%) was used further without purification in the synthesis of epoxide (*S*)-**12** and of (*S*)-bufuralol, as described for the (*R*) enantiomer in Section 4.5.4.

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References

1. (a) Zaidlewicz, M.; Tafelska-Kaczmarek, A.; Prewysz-Kwinto, A.; Chechłowska, A. *Tetrahedron: Asymmetry* **2003**, *14*, 1659–1664; (b) Hou, X.-L.; Yang, Z.; Wong, H. N. C. In *Progress in Heterocyclic Chemistry, Furans and Benzofurans*; Gribble, G. W., Gilchrist, T. L., Eds.; ; Springer: Berlin, 2002; Vol. 14, pp 139–179.
2. (a) Fothergill, G. A.; Osbond, J. M.; Wickens, J. C. *Arzneim.-Forsch. Drug. Res.* **1977**, *27*, 978–981; (b) Blaber, L. C.; Burden, D. T.; Eigeumann, R.; Gerold, M. *Cardiovasc. Pharmacol.* **1984**, *6*, 165–175.
3. (a) Magometschnigg, D.; Bonelli, J.; Hitzenberger, G.; Kaik, G.; Korn, A. *Int. J. Clin. Pharmacol. Biopharm.* **1978**, *16*, 54–58; (b) Magometschnigg, D.; Bonelli, J.; Kaik, G.; Rameis, H. *Int. J. Clin. Pharmacol. Biopharm.* **1979**, *17*, 334–340.
4. Li, D. N.; Pritchard, P. M.; Hanlon, S. P.; Burchell, B.; Wolf, C. R.; Friedberg, T. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 661–667.
5. Narimatsu, S.; Takemi, C.; Kuramoto, S.; Tsuzuki, D.; Hichiya, H.; Tamagake, K.; Yamamoto, S. *Chirality* **2003**, *15*, 333–339.
6. Pruksaritanont, T.; Dwyer, L. M.; Cribb, A. E. *Biochem. Pharmacol.* **1995**, *50*, 1521–1525.
7. Kwak, S. H.; Lee, D. M.; Lee, K. I. *Tetrahedron: Asymmetry* **2009**, *20*, 2639–2645.
8. Zaidlewicz, M.; Tafelska-Kaczmarek, A.; Prewysz-Kwinto, A. *Tetrahedron: Asymmetry* **2005**, *16*, 3205–3210.
9. Tafelska-Kaczmarek, A.; Prewysz-Kwinto, A.; Skowerski, K.; Petrasiak, K.; Kozakiewicz, A.; Zaidlewicz, M. *Tetrahedron: Asymmetry* **2010**, *21*, 2244–2248.

10. Johnston, V. E.; Bogár, K.; Bäckvall, J. E. *J. Org. Chem.* **2010**, *75*, 4596–4599.
11. Mo, K.; Yang, Y.; Cui, Y. *J. Am. Chem. Soc.* **2014**, *136*, 1746–1749.
12. Hull, J. D.; Scheinmann, F.; Turner, N. J. *Tetrahedron: Asymmetry* **2003**, *14*, 567–576.
13. Paizs, C.; Toşa, M. I.; Majdik, C.; Bodai, V.; Novák, L.; Irimie, F. D.; Poppe, L. *J. Chem. Soc., Perkin Trans. 1* **2002**, *21*, 2000–2002.
14. (a) Podea, P. V.; Paizs, C.; Toşa, M. I.; Irimie, F. D. *Tetrahedron: Asymmetry* **2008**, *19*, 1959–1964; (b) Bencze, L. C.; Paizs, C.; Toşa, M. I.; Irimie, F. D. *Tetrahedron: Asymmetry* **2010**, *21*, 356–364.
15. Paizs, Cs; Tosa, M.; Majdik, C.; Moldovan, P.; Novák, L.; Kolonits, P.; Marcovici, A.; Irimie, F. D.; Poppe, L. *Tetrahedron: Asymmetry* **2003**, *14*, 1495–1501.
16. Martonelli, J. M.; Nayyar, K. N.; Moher, D. E.; Dhokte, P. U.; Pawlak, M. J.; Vaidyanathan, R. *Org. Lett.* **1999**, *1*, 447–450.