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# Lipase-mediated kinetic resolution of rigid clofibrate analogues with lipid-modifying activity

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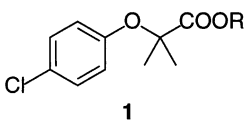
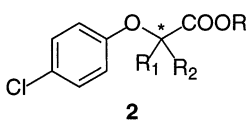
**Abstract**—The lipase-catalysed kinetic resolution of methyl esters of (±)-5-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid, (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-2-carboxylic acid, and (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid, rigid analogues of clofibrate, was effected with fair to moderate enantioselectivities ($E = 1.0$–$4.8$), enantiomeric excesses of up to 86% and workable reaction rates. Enantiomerically pure (*R*)- and (*S*)-5-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acids were obtained by fractional crystallisation of the diastereomeric salts of the corresponding racemic acid with (+)- and (−)-amphetamine from ethanol; the absolute configuration of the products were established by chemical correlation. 

## 1. Introduction

Fibrates (Fig. 1, structures **1** and **2**) constitute a widely used class of lipid-modifying agents.[1] Treatment with fibrates results in a substantial decrease in plasma triglycerides and is usually associated with a moderate decrease in LDL cholesterol and an increase in HDL cholesterol concentration. Recent studies indicate that the effects of fibrates are mediated, at least in part, through alterations in the transcription of genes encoding for proteins that control lipoprotein metabolism. Fibrates activate specific transcription factors belonging to the nuclear hormone receptor superfamily, termed peroxisome proliferator-activated receptors (PPARs).[2]




**1**




**2**

**1a** R = $CH_2CH_3$
**1b** R = H

**Figure 1.**

2,2-Dialkyl-2-(4-chlorophenoxy)ethanoic acids **2** (R = H) are analogues of the clofibric acid **1b**, the active metabolite of clofibrate **1a** which is still on the market in spite of side adverse effects on skeletal muscle (myalgias and myotonias)[3–7] and on the liver (carcinogenicity).[8,9] The two enantiomers of **2** in which $R_1 = H$ and $R_2$ = alkyl [e.g. (*R*)- and (*S*)-2-(4-chlorophenoxy)propanoic acids, $R_2 = CH_3$] exhibit different pharmacological profiles. In particular, the (*R*)-enantiomers have higher anti-aggregatory activity,[10] lower myotonic[11] and hepatocarcinogenicity[12–14] than the (*S*)-enantiomers, whilst both have the same antilipidemic effect as clofibric acid.

Rigid analogues of clofibric acid (Fig. 2) show an antilipolytic activity comparable to that of 2-alkyl-2-(4-chlorophenoxy)ethanoic acids.[15,16] These investigations were performed using the racemic compounds **3**–**5**, but nothing is known, so far, about the pharmacological behaviour of their pure enantiomers. This prompted us to investigate the preparation of homochiral **3**–**5**.

Several methods have already been reported for the resolution of racemic phenoxyalkanoic acid derivatives including chemical kinetic resolution,[17] crystallisation of diastereomeric salts,[18–28] and biological methods involving enantioselective microbial reduction,[29,30] enantioselective esterification of acids[31,32] and enan-

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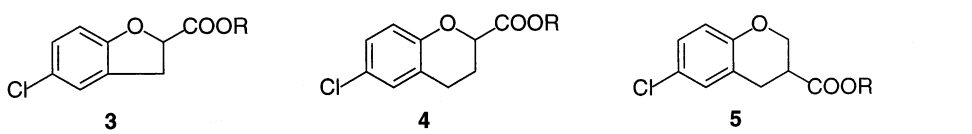



**3a** R = $CH_3$
**3b** R = H
**3c** R = $CH_2CH{=}CH_2$

**4a** R = $CH_3$
**4b** R = H
**4c** R = $CH_2CH{=}CH_2$

**5a** R = $CH_3$
**5b** R = H
**5c** R = $CH_2CH{=}CH_2$

**Figure 2.**

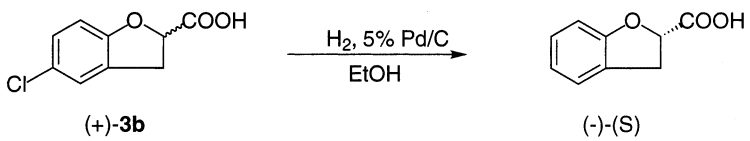
tioselective hydrolysis and/or transesterification of their esters.[33–36] In contrast, rigid analogues of clofibric acid have received less attention.

In the course of our previous investigations we accomplished the resolution of **4b** and **5b** by crystallisation of their diastereomeric salts with homochiral amines,[37] and the lipase-mediated kinetic resolution of 2-monoalkyl-2-aryloxyethanoic acids and 2-methyl-2-(*n*-propyl)-2-(4-chlorophenoxy)ethanoic acid.[38] Lipases, beyond their biological function, are widely used as chiral catalysts for asymmetric acyl transfer reactions between a number of acyl acceptors and donors. The broad range of accepted substrates (and consequently the broad range of possible reaction types including hydrolysis, esterification, transesterification, etc.) together with the high stability of most lipases in aqueous solutions and in organic solvents, has made lipases important catalysts for the preparation of highly enantiopure building blocks and intermediates.

We have investigated the pharmacological activity of clofibric acid derivatives [such as 2-alkyl-2-(4-chlorophenoxy)ethanoic acids] in order to find and, possibly, dissociate the structural determinants of the different effects. Thus, we decided to synthesise optically active rigid analogues of aryloxyalkanoic acids by chemical and chemoenzymatic routes and compare the two methods. In this paper the results of this investigation are reported, focusing on the e.e.s of the products and the enantioselectivity factor ($E$) values of the lipase-catalysed hydrolysis, esterification, and transesterification reactions.

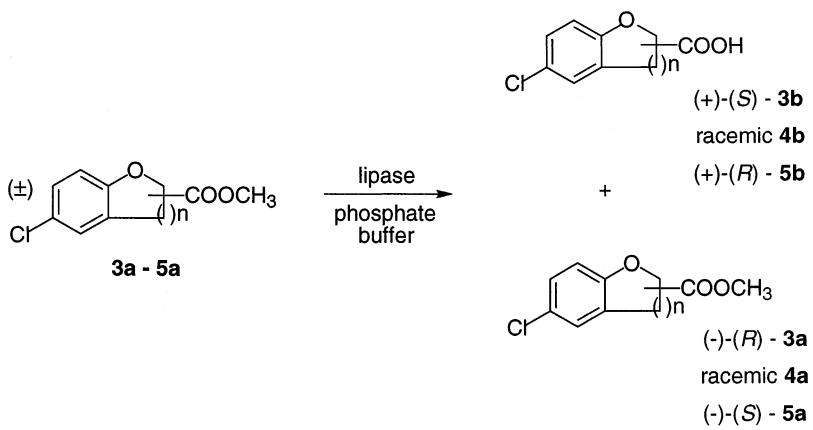
## 2. Results and discussion

Herein, we report the resolution of (±)-**3b** by the classical method of crystallisation of the diastereomeric salts, the establishment of the absolute configuration of (+)-**3b** and (−)-**3b**, and the lipase-catalysed kinetic resolution results of (±)-**3**, (±)-**4** and (±)-**5**. (±)-5-Chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid **3b**, prepared by the literature method,[15] was resolved by fractional crystallisation of the diastereomeric salts with (−)- and (+)-amphetamine from ethanol. Interestingly, the crystallisation of a partially resolved mixture of the antipodal forms led to the separation of the racemate similarly to our previous report on other 2-aryloxyalkanoic acids.[37,39] The absolute configuration was established by chemical correlation as depicted in Scheme 1. For this purpose, the *dextro*-acid was converted, by catalytic hydrogenation, into the corresponding dehalogenated compound (−)-2,3-dihydro-1-benzofuran-2-carboxylic acid of known ($S$)-configuration.[40]




(+)-**3b** (-)-(S)

**Scheme 1.**




**Scheme 2.**

**Table 1.** Hydrolysis of (±)-5-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid methyl ester **3a**, (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-2-carboxylic acid methyl ester **4a**, and (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid methyl ester **5a** in the presence of lipase[a]

| Comp. | Lipase (source) | Reaction time (h) | $C$[b] (%) | E.e.$_s$[c] (%) (absolute configuration) | E.e.$_p$[c] (%) (absolute configuration) | $E$[d] |
|---|---|---|---|---|---|---|
| **3a** | *Candida cylindracea* (AY-Amano) | 2.5 | 37 | 27 (*R*) | 46 (*S*) | 3.5 |
| **3a** | *Candida cylindracea* (AY-Amano) | 7 | 77 | 60 (*R*) | 19 (*S*) | 2.4 |
| **3a** | *Humicola* sp. (CE-10) | 3 | 79 | 45 (*R*) | 12 (*S*) | 1.8 |
| **3a** | *Mucor javanicus* (MAP) | 9 | 85 | 78 (*R*) | 14 (*S*) | 2.6 |
| **3a** | *Humicola lanuginosa* (R-10) | 30 | 91 | 27 (*R*) | 3 (*S*) | 1.3 |
| **3a** | *Humicola lanuginosa* (R-10)[e] | 51 | 44 | 17 (*R*) | 22 (*S*) | 1.8 |
| **3a** | *Rhizopus delemar* | 56 | 43 | 35 (*R*) | 46 (*S*) | 3.8 |
| **4a** | *Candida cylindracea* (AY-Amano) | 24 | 93 | 3 | –[f] | 1.0[g] |
| **5a** | *Candida cylindracea* (OF-MeitoSanjo) | 72 | 94 | 39 (*S*) | 3[h] (*R*) | 1.3 |
| **5a** | *Humicola* sp. (CE-10) | 72 | 51 | 35 (*S*) | 34[h] (*R*) | 2.8 |
| **5a** | *Mucor javanicus* (MAP) | 72 | 73 | 75 (*S*) | 28[h] (*R*) | 3.6 |
| **5a** | *Humicola lanuginosa* (R-10) | 72 | 6 | 27 (*S*) | –[f] | 1.0 |
| **5a** | *Rhizopus niveus* (*N*-conc) | 72 | 91 | 46 (*S*) | 5[h] (*R*) | 1.5 |
| **5a** | *Rhizopus delemar* | 72 | 30 | 40 (*S*) | 86[h] (*R*) | 1.8 |

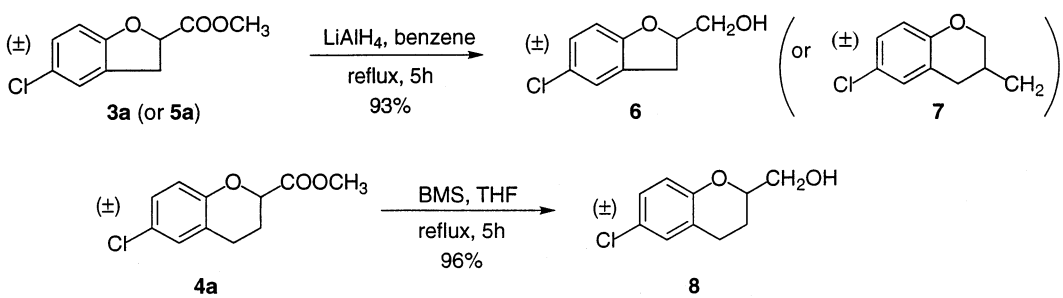
[a] Unless otherwise indicated the substrate:lipase ratio = 1:5 (w/w).
[b] C = conversion determined by HPLC.
[c] Unless otherwise indicated enantiomeric excess of unreacted substrate (e.e.$_s$) and product (e.e.$_p$) was determined by HPLC (see Section 3).
[d] $E$ = enantioselectivity factor.
[e] Substrate:lipase ratio = 1:1 (w/w).
[f] The product was recovered as a racemate.
[g] The same result was obtained performing the reaction in the presence of other lipases such as those ones from *Humicola* sp. (CE-10), *Mucor javanicus* (MAP), *Humicola lanuginosa* (R-10), *Rhizopus delemar*.
[h] Calculated according to the following equation: $C$ = e.e.$_s$/(e.e.$_s$+e.e.$_p$), where e.e.$_s$ is the enantiomeric excess of remaining unreacted ester substrate and e.e.$_p$ is the enantiomeric excess of the acid product.

Compounds **4b**–**5b** were synthesised as previously reported.[15,16] Their resolution was accomplished by crystallisation of their diastereomeric salts obtained by reaction with optically active amines,[37] and the enantiomers used as standards for the present investigation.

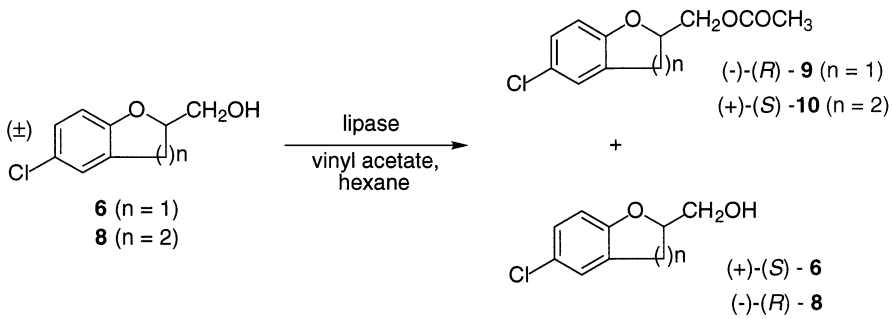
As far as the lipase-mediated kinetic resolution of compounds **3**–**5** is concerned, a variety of experimental conditions were investigated. Initially, hydrolysis of the methyl esters **3a**–**5a** was performed in the presence of several lipases (Scheme 2). A summary of the results obtained is listed in Table 1. It seems that reactions of the three compounds **3a**, **4a** and **5a** are strongly dependent upon the lipase used.

The enantioselectivity factor $E$ value for **3a** ranges from 1.3 (lipase from *Humicola lanuginosa*) to 3.8 (lipase from *Rhizopus delemar*), whereas the e.e. of the unreacted substrate (e.e.$_s$) ranges between 17% (lipase from *Humicola lanuginosa*) and 78% (using the lipase from *Mucor javanicus*). The e.e. of the product (e.e.$_p$) varied between 3% (lipase from *Humicola lanuginosa*) and 46% (lipase from *Rhizopus delemar* and *Candida cylindracea*). No enantioselectivity was found in the hydrolysis of substrate **4a**, indicating that both (*R*)- and (*S*)-enantiomers of **4a** react at very similar rates irrespective of the lipase chosen to catalyse the hydrolysis, so racemic unreacted substrate **4a** and racemic product **4b** are always isolated. The $E$ value is approximately 1.0 with all the lipases examined (see also footnote g of Table 1).

It is noteworthy that the hydrolysis of **3a** occurred almost always faster than for **4a**, for the reaction executed in the presence of the same lipase. The hydrolysis of (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid methyl ester **5a** gave an $E$ of 1.0–3.6, with e.e.$_s$ from 27% (using the lipase from *Humicola*




**Scheme 3.**




**Scheme 4.**

*lanuginosa*) to 75% (using lipase from *Mucor javanicus*) and e.e.$_p$ from 3% (lipase from *Candida cylindracea*) to 86% (lipase from *Rhizopus delemar*).

It is known that the enantioselectivity of the lipase-catalysed kinetic resolution of racemic acids (and their derivatives) can be enhanced by substrate modification.[41] Hence, the allyl esters **3c** and **5c** were prepared and incubated with the lipase from *Pseudomonas* sp. (AK-Amano) in phosphate buffer. The lipase from *Pseudomonas* sp. (AK-Amano) was chosen for further investigation because in the preliminary screening its reaction proceeded at an appreciable rate. No marked improvement was observed. Similar results were obtained when **3c** (or **5c**) was incubated in hexane, in the presence of lipase from *Pseudomonas* sp. (AK-Amano) and methanol; a transesterification reaction occurred and methyl ester **3a** (or **5a**, respectively) formed.

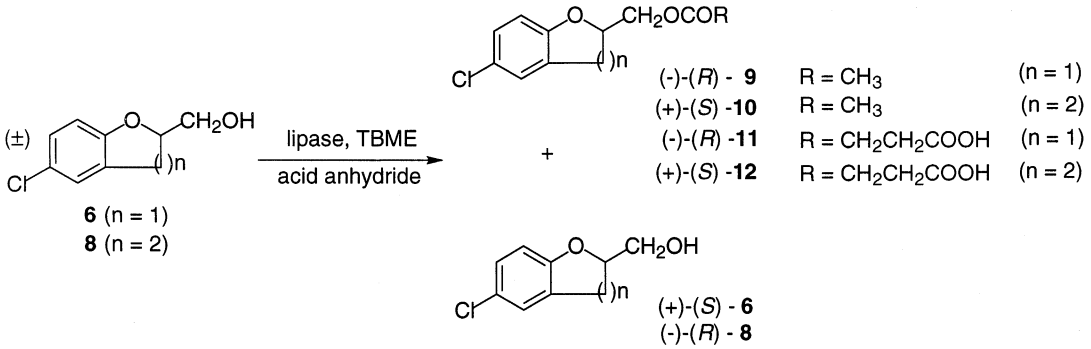
In a further attempt to develop a more enantioselective method for the preparation of this class of compounds, it was decided to investigate the kinetic resolution of the alcohols **6**–**8**, which can be considered as precursors of **3**–**5**. (±)-**6**–**8** were prepared in 93–96% yield, by reducing the corresponding esters **3a**–**5a** (Scheme 3). These were incubated in hexane, in the presence of a lipase and vinyl acetate acyl donor (Scheme 4 and Table 2).

An enzymatic screening was performed and the best results obtained in the case of compound **6** were found to be as follows: e.e.s of the unreacted substrate (e.e.$_s$)

**Table 2.** Transesterification reaction of (±)-2-hydroxymethyl-5-chloro-2,3-dihydro-1-benzofuran **6** and (±)-2-hydroxymethyl-6-chloro-2,3-dihydro-4*H*-1-benzopyran **8** with vinyl acetate in hexane in the presence of lipase[a]

| Comp. | Lipase (source) | Reaction time (h) | $C$[b] (%) | E.e.$_s$[c] (%) (absolute configuration) | E.e.$_p$[d] (%) (absolute configuration) | $E$[e] |
|---|---|---|---|---|---|---|
| **6** | *Candida cylindracea* (AY) | 1 | 47 | 6 (*S*) | 3 (*R*) | 1.2 |
| **6** | *Rhizopus niveus* (*N-conc*) | 30 | 8 | –[f] | –[f] | – |
| **6** | *Mucor javanicus* (MAP) | 23 | 50 | 20 (*S*) | 20 (*R*) | 1.8 |
| **6** | *Pseudomonas* sp. (K-10) | 7 | 30 | 14 (*S*) | 10 (*R*) | 2.2 |
| **6** | *Aspergillus niger* (AP) | 8 | 43 | 17 (*S*) | 22 (*R*) | 1.8 |
| **6** | *Geotrichum candidum* (GC) | 8.5 | 10 | 5 (*S*) | 45 (*R*) | 2.8 |
| **6** | *Penicillium cyclopium* (G) | 8.5 | 33 | 16 (*S*) | 32 (*R*) | 2.3 |
| **6** | *Candida antarctica* (S 435 L) | 0.25 | 71 | 23 (*S*) | 9 (*R*) | 1.5 |
| **6** | *Candida lipolytica* (L) | 32 | 19 | 14 (*S*) | 60 (*R*) | 4.5 |
| **6** | *Pseudomonas* sp. (AK) | 1 | 51 | 11 (*S*) | 11 (*R*) | 1.4 |
| **6** | Porcine pancreas (PPL) | 1.5 | 22 | 7 (*S*) | 25 (*R*) | 1.8 |
| **8** | *Rhizopus niveus* (N-conc) | 7.5 | 14 | –[g] | –[g] | – |
| **8** | *Humicola* sp. (CE-10) | 2 | 43 | 20 (*R*) | 26 (*S*) | 2.1 |
| **8** | *Mucor meihei* (M) | 6 | 25 | 19 (*R*) | 57 (*S*) | 4.4 |
| **8** | *Humicola* sp. (R-10) | 24 | 52 | 7 (*R*) | 6 (*S*) | 1.1 |
| **8** | *Mucor javanicus* (MAP) | 5 | 26 | 10 (*R*) | 28 (*S*) | 2.0 |
| **8** | *Penicillium cyclopium* (G) | 5 | 40 | 6 (*R*) | 9 (*S*) | 1.3 |
| **8** | *Pseudomonas* sp. (K-10) | 3 | 43 | 14 (*R*) | 18 (*S*) | 1.7 |
| **8** | *Pseudomonas* sp. (AK) | 1.5 | 35 | 29 (*R*) | 54 (*S*) | 4.4 |

[a] Substrate:lipase = 1:5 (w/w).
[b] $C$ = conversion determined by HPLC.
[c] E.e. of the unreacted substrate determined by HPLC.
[d] E.e. of the product calculated according to the following equation: $C = \text{e.e.}_s/(\text{e.e.}_s+\text{e.e.}_p)$, where e.e.$_s$ is the enantiomeric excess of remaining unreacted alcohol and e.e.$_p$ is the enantiomeric excess of the produced ester.
[e] $E$ = enantioselectivity factor.
[f] Both unreacted substrate and product were recovered as a racemate. *Humicola* sp. (CE-10), *Pseudomonas fluorescens* (Sigma), and *Candida cylindracea* (Meito-Sanjo) afforded the same results.
[g] Both unreacted substrate and product were recovered as a racemate. *Aspergillus niger* (AP), *Geotrichum candidum* (GC), *Rhizopus delemar*, and *Pseudomonas fluorescens* (Sigma) afforded the same results.




**Scheme 5.**

ranged between 5% (lipase from *Geotrichum candidum*) and 23% (lipase from *Candida antartica*), while the e.e.$_p$ varied between 3% (using lipase from *Candida cylindracea*) and 60% (with lipase from *Candida lipolytica*), and $E=1.2$–4.5; whereas for **8**: e.e.$_s$ ranges between 6% (lipase from *Penicillium cyclopium*) and 29% (lipase from *Pseudomonas* sp.-AK), e.e.$_p$ = between 6% (lipase from *Humicola* sp.) and 57% (lipase from *Mucor meihei*), and $E=1.1$–4.4. The reaction with **7** was unselective and always afforded the racemic unreacted substrate and a racemic product. By comparing the results in Tables 1 and 2, it can be seen that the hydrolysis of substrates **3a**–**5a** gave higher e.e. values than the transesterification reactions of **6**–**8** with vinyl acetate.

It has been reported that in some cases better results (higher e.e.) can be obtained by using an anhydride acyl donor such as acetic anhydride or, even better, succinic anhydride, in the presence of $KHCO_3$.[42] The lipase from *Pseudomonas* sp. (AK- and K-10-Amano) was chosen (Scheme 5). The $E$ values obtained from these reactions (Table 3) were similar to those obtained previously (Tables 1 and 2) and no marked difference was seen in the reaction performed with both anhydrides in the presence or absence of $KHCO_3$. E.e.$_s$ values of 3–76% for **6** and 23–78% for **8** were obtained.

In summary, using the methods presented, **5a** can be prepared with an e.e. of up to 86%, for **4a** and **7** the e.e. was close to zero, while for **3a**, **6** and **8** the e.e.s ranged from 10 to 80%. Such results could be improved by recycling the reaction product, as has been done already for other substrates and also for analogues of compound **4**, which is an intermediate for the synthesis of natural tocols.[43]

The enzymatic hydrolyses (Scheme 2 and Table 1) are complementary to the enzymatic transesterifications (Schemes 4 and 5, Tables 2 and 3), in terms of stereochemical preference. In fact, all of the lipases examined preferentially promoted the hydrolysis of (*S*)-5-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid methyl ester **3a**. The same hydrolysis reactions performed on the benzopyran derivative (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid methyl ester **5a** proceeded with an (*R*)-preference.

**Table 3.** Enzymatic acylation of (±)-2-hydroxymethyl-5-chloro-2,3-dihydro-1-benzofuran **6** and (±)-2-hydroxymethyl-6-chloro-2,3-dihydro-4*H*-1-benzopyran **8** using succinic or acetic anhydride in TBME in the presence of lipase[a]

| Comp. | Lipase (source) | Reaction time (h) | $C$[b] (%) | E.e.$_s$[c] (%) | E.e.$_p$[c] (%) | $E$[d] |
|---|---|---|---|---|---|---|
| | | | | (absolute configuration) | | |
| **6** | *Candida cylindracea* (AY) | 24 | 37 | 11 (*S*) | 19 (*R*) | 1.6 |
| **6** | *Pseudomonas* sp. (K-10) | 24 | 67 | 66 (*S*) | 32 (*R*) | 3.6 |
| **6** | *Pseudomonas* sp. (K-10)[e] | 24 | 34 | 38 (*S*) | 74 (*R*) | 4.8 |
| **6** | *Pseudomonas* sp. (K-10)[e,f] | 24 | 54 | 76 (*S*) | 65 (*R*) | 2.4 |
| **6** | *Pseudomonas* sp. (AK) | 25 | 55 | 31 (*S*) | 25 (*R*) | 2.2 |
| **6** | *Rhizopus delemar* | 48 | 26 | 3 | 8 (*R*) | 1.2 |
| **8** | *Candida cylindracea* (AY) | 24 | 37 | 23 (*R*) | 39 (*S*) | 1.8 |
| **8** | *Pseudomonas* sp. (AK) | 48 | 70 | 78 (*R*) | 34 (*S*) | 4.3 |
| **8** | *Pseudomonas sp.* (K = 10) | 48 | 70 | 74 (*R*) | 60 (*S*) | 3.9 |

[a] Substrate:lipase = 1:1 (w:w). Unless otherwise indicated the acyl donor used was the succinic anhydride.
[b] $C$ = conversion determined by HPLC.
[c] E.e.$_s$ is the enantiomeric excess of remaining unreacted alcohol and e.e.$_p$ is the enantiomeric excess of the produced ester, both determined by HPLC.
[d] $E$ = enantioselectivity factor.
[e] $KHCO_3$ was added to the medium in ratio 1:1 (w/w) with the substrate.
[f] Acetic anhydride was used.

**Table 4.** Chromatographic data[a]

| Comp. | Abs. conf. | $t_R$ | $k'$ | $k''$ | $\alpha$ | Mobile phase, flow rate, UV-$\lambda$ |
|---|---|---|---|---|---|---|
| **3a** | (*S*)-(+) | 20.05 | 2.13 | 2.62 | 1.23 | Hexane-*iso*-propanol (98:2), flow 0.8 mL/min, $\lambda=230$ nm |
| | (*R*)-(−) | 23.22 | | | | |
| **3b** | (*S*)-(+) | 18.51 | 0.92 | 1.50 | 1.63 | Hexane-*iso*-propanol-trifluoroacetic acid (90:10:0.75), flow 0.7 mL/min, $\lambda=280$ nm[b] |
| | (*R*)-(−) | 14.18 | | | | |
| **3c** | (*S*)-(+) | 21.28 | 3.46 | 3.99 | 1.15 | Hexane-*iso*-propanol (99:1), flow 0.8 mL/min, $\lambda=280$ nm |
| | (*R*)-(−) | 23.83 | | | | |
| **4a** | (*S*)-(+) | 9.33 | 0.44 | 1.02 | 2.31 | Hexane-*iso*-propanol (90:10), flow 0.8 mL/min; $\lambda=230$ nm |
| | (*R*)-(−) | 13.07 | | | | |
| **4b** | (*S*)-(+) | 12.07 | 1.38 | 1.74 | 1.26 | Hexane-*iso*-propanol-trifluoroacetic acid (90:10:0.5), flow 1 mL/min, $\lambda=230$ nm |
| | (*R*)-(−) | 10.51 | | | | |
| **5a** | (S)-(−) | 14.54 | 0.95 | 1.06 | 1.18 | Hexane-*iso*-propanol (98:2), flow 0.8 mL/min, $\lambda=230$ nm[c] |
| | (*R*)-(+) | 15.33 | | | | |
| **5b** | (*S*)-(−) | 9.61 | 1.40 | 1.50 | 1.07 | Hexane-*iso*-propanol-trifluoroacetic acid (99:1:0.1), flow 0.5 mL/min, $\lambda=254$ nm |
| | (*R*)-(+) | 10.00 | | | | |
| **5c** | (*S*)-(−) | 14.00 | 2.50 | 2.74 | 1.09 | Hexane-*iso*-propanol (99:1), flow 1 mL/min, $\lambda=280$ nm |
| | (*R*)-(+) | 14.97 | | | | |
| **6** | (*S*)-(+) | 23.02 | 4.05 | 5.40 | 1.33 | Hexane-*iso*-propanol (95:5), flow 1 mL/min, $\lambda=230$ nm[d] |
| | (*R*)-(−) | 18.19 | | | | |
| **8** | (*S*)-(+) | 34.55 | 3.04 | 3.42 | 1.12 | Hexane-*iso*-propanol (95:5), flow 0.5 mL/min, $\lambda=230$ nm[d] |
| | (*R*)-(−) | 31.61 | | | | |
| **10** | (*S*)-(+) | 13.30 | 1.42 | 1.57 | 1.10 | Hexane-*iso*-propanol (98:2), flow 1 mL/min, $\lambda=254$ nm |
| | (*R*)-(−) | 14.12 | | | | |
| **11**[e] | (*S*)-(+) | 40.81 | 1.35 | 1.93 | 1.43 | Hexane-*iso*-propanol (95:5), flow 0.7 mL/min, $\lambda=230$ nm[f] |
| | (*R*)-(−) | 32.71 | | | | |
| **12**[e] | (*S*)-(+) | 39.01 | 4.01 | 4.61 | 1.15 | Hexane-*iso*-propanol (90:10), flow 0.5 mL/min, $\lambda=230$ nm[g] |
| | (*R*)-(−) | 34.84 | | | | |

[a] Stationary phase = Chiralcel OD (Daicel), $t_R$ = retention time (min), $k'$ and $k''$ = capacity factors of the first and second eluted enantiomer, respectively, $\alpha$ = separation factor.

[b] By using hexane–isopropanol–trifluoroacetic acid (95:5:0.75), flow 1.0 mL/min, $\lambda=280$ nm, **3a** and **3b** were eluted in the same analysis: (*S*)-**3a** $t_R=9.35$ min, (*R*)-**3a** $t_R=10.19$ min, (*S*)-**3b** $t_R=18.75$ min, (*R*)-**3b** $t_R=13.61$ min.

[c] By using hexane–isopropanol–trifluoroacetic acid (90:10:5.0), flow 0.4 mL/min, $\lambda=230$ nm, **5a** and **5b** were eluted in the same analysis: (*S*)-**5a** $t_R=21.45$ min, (*R*)-**5a** $t_R=23.95$ min, (*S*)-**5b** $t_R=17.91$ min, (*R*)-**5b** $t_R=22.95$ min.

[d] Chromatogram of the transesterification reaction crude with vinyl acetate (Scheme 4) or acetic anhydride (Scheme 5) contained also **9** or **10** not resolved with $t_R=10.29$ min and $t_R=11.50$ min, respectively.

[e] Analysed after its conversion into methyl ester by $CH_2N_2$.

[f] The analysis could be performed directly on the reaction crude (Scheme 5) after its treatment with $CH_2N_2$ [hexane–isopropanol (90:10), flow 1.0 mL/min, $\lambda=230$ nm]: (*S*)-**6** $t_R=42.67$ min, (*R*)-**6** $t_R=34.29$ min, (*S*)-**11** methyl ester $t_R=44.04$ min, (*R*)-**11** methyl ester $t_R=52.23$ min.

[g] The analysis could be performed directly on the reaction crude (Scheme 5) after its treatment with $CH_2N_2$ [hexane–isopropanol (90:10), flow 1.0 mL/min, $\lambda=230$ nm]: (*S*)-**8** $t_R=39.01$ min, (*R*)-**8** $t_R=34.84$ min, (*S*)-**12** methyl ester $t_R=20.57$ min, (*R*)-**12** methyl ester $t_R=18.98$ min.

In contrast, in the case of the transesterification reactions, (*R*)-2-hydroxymethyl-5-chloro-2,3-dihydro-1-benzofuran **6** and (*S*)-2-hydroxymethyl-6-chloro-2,3-dihydro-4*H*-1-benzopyran **8** were preferred, respectively.

The pharmacological activity of compounds **3–5** and **6–8** is currently under evaluation and will be reported separately.

## 3. Experimental

### 3.1. General methods

Melting points were taken on electrothermal apparatus and are uncorrected. $^1$H NMR spectra were recorded in $CDCl_3$ on a Varian EM 390 or Mercury 300 MHz spectrometer and chemical shifts are reported in parts per million ($\delta$ ppm). Absolute values of the coupling constant ($J$) are reported. IR spectra were recorded on a Perkin–Elmer 681 spectrometer. GC analyses were performed by using a HP1 column (methyl silicone gum; 5 m×0.53 mm×2.65 μm film thickness) on a HP 5890 model, Series II. HPLC analyses (Table 4) for the determination of e.e.s were carried out using a DAICEL Chiralcel OD column (tris-3,5-dimethylphenylcarbamate, derivatised cellulose film) on a HP series 1050 instrument. Optical rotation measurements were obtained using a Perkin–Elmer digital polarimeter, model 241 MC. Thin-layer chromatography (TLC) was performed on silica gel sheets with fluorescent indicator (Statocrom SIF, Carlo Erba), the spots on the TLC were observed under ultraviolet light or were visualised with $I_2$ vapour. Flash chromatography was conducted by using silica gel with an average particle size of 60 μm, a particle size distribution of 40–63 μm and 230–400 ASTM. GC–MS analyses were performed on a HP 5995C model and microanalyses on an elemental analyser 1106-Carlo Erba-instrument.

### 3.2. Materials

The enzymes used in this paper were obtained from either Amano Enzyme Co., Meito Sangyo, or Sigma Chemical Co. All other chemicals and solvents were of the highest quality grade available and purchased from Aldrich Chemical Co. or Sigma Chemical Co.

### 3.3. Resolution of (±)-5-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid 3b

The racemic acid[9] (11.2 g, 0.056 mol) and (−)-amphetamine (7.6 g, 0.056 mol) were mixed and crystallised from EtOH. After three crystallisations the salt had $[\alpha]_D$ −17.0 (*c* 1.0, MeOH), which remained constant after two more crystallisations. This salt was treated with 5% $H_2SO_4$ and extracted with $CHCl_3$ affording the *dextro*-acid, which was recrystallised from $CHCl_3$:petroleum ether; mp 123–125°C; $[\alpha]_D$ = +14.7 (*c* 1.0, MeOH). The mother liquors from the first crystallisation of the (−)-amphetamine salt were evaporated to dryness; the residue was treated with 5% $H_2SO_4$ and extracted with $CHCl_3$ obtaining a partially enriched mixture of the *levo*-acid, which was mixed with an equivalent amount of (+)-amphetamine and crystallised from EtOH. After two crystallisations, the salt had $[\alpha]_D$ = +17.0 (*c* 1.0, MeOH). This salt was treated with 5% $H_2SO_4$ and extracted with $CHCl_3$ affording the *levo*-acid which was crystallised from $CHCl_3$:petroleum ether; mp 123–125°C; $[\alpha]_D$ = −14.5 (*c* 1.0, MeOH). $^1$H NMR ($CDCl_3$, $\delta$): 3.33–3.66 (m, 2H, $CH_2$); 5.25 (dd, 1H, CH); 5.84 (bs, 1H, COOH: exchanged with $D_2O$); 6.77–7.20 (m, 3H, aromatic protons).

### 3.4. Assignment of the absolute configuration to 3b (Scheme 1)

The *dextro*-acid (430 mg, 2.17 mmol) with $[\alpha]_D$ +12.3 (*c* 1.5, MeOH) was dissolved in EtOH (15 mL) and hydrogenated at atmospheric pressure overnight in the presence of catalytic 5% Pd/C at room temperature. The solution was filtered through Celite and the solvent removed under reduced pressure. The residue was dissolved in $Et_2O$ and extracted with 10% $NaHCO_3$ aqueous solution. The aqueous phase was acidified with 2N HCl and extracted with $Et_2O$. The organic layer was washed with water, dried over anhydrous $Na_2SO_4$ and evaporated to dryness affording a solid (100 mg) with $[\alpha]_D$ = −16.0 (*c* 1.1, EtOH).[40] $^1$H NMR ($CDCl_3$, $\delta$): 3.35–3.69 (m, 2H, $CH_2$); 5.24 (dd, 1H, CH); 5.65 (bs, 1H, COOH); 6.85–7.22 (m, 4H, aromatic protons).

### 3.5. General procedure for the esterification of (±)-5-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid 3b, (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-2-carboxylic acid 4b and (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid 5b with $CH_2N_2$

A slight excess of diazomethane in ethereal solution, was added to a solution of the acid in ethyl ether. The resulting solution was stirred at room temperature for 30 min and evaporated under reduced pressure. The resulting oil was dissolved in chloroform and washed with 5% aqueous $NaHCO_3$ to remove trace of the unreacted acid. The chloroform solution was dried over anhydrous $Na_2SO_4$ and then evaporated, affording the ester as an oil (yield 93–98%).

**3.5.1. (±)-5-Chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid methyl ester 3a**. $^1$H NMR ($CDCl_3$, $\delta$): 3.35–3.60 (m, 2H, $CH_2$); 3.80 (s, 3H, $CH_3$); 5.15–5.38 (m, 1H, CH); 6.81–7.30 (m, 3H, aromatic protons). GC–MS (70 eV) *m*/*z* (rel. int.): 212 ($M^+$, 79), 125 (100).

**3.5.2. (±)-6-Chloro-2,3-dihydro-4*H*-1-benzopyran-2-carboxylic acid methyl ester 4a**. $^1$H NMR ($CDCl_3$, $\delta$): 2.05–2.35 (m, 2H, $CH_2CHCOOCH_3$), 2.60–2.91 (m, 2H, $ArCH_2CH_2$), 3.81 (s, 3H, $COOCH_3$), 4.65–4.85 (m, 1H, $CHCOOCH_3$), 6.80–7.21 (m, 3H, aromatic protons). GC–MS (70 eV) *m*/*z* (rel. int.): 226 ($M^+$, 64), 167 (100).

**3.5.3. (±)-6-Chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid methyl ester 5a**. $^1$H NMR ($CDCl_3$, $\delta$): 3.05 (d, 2H, $ArCH_2CH$), 3.71 (s, 3H, $COOCH_3$), 4.10–4.61 (m, 3H, $OCH_2CH$ and, $CH_2CHCOOCH_3$), 6.75–7.40 (m, 3H, aromatic protons). GC–MS (70 eV) *m*/*z* (rel. int.): 226 ($M^+$, 83), 166 (100).

### 3.6. General procedure for the esterification of (±)-5-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid 3b, (±)-6-chloro-2,3-dihydro-(4*H*)-1-benzopyran-3-carboxylic acid 5b with allyl alcohol

A solution of the acid (1.4 mmol) and $SOCl_2$ (1.6 mmol) was stirred under reflux for 5 h. After cooling at room temperature, the reaction mixture was concentrated and the crude acyl chloride was dissolved in allyl alcohol (4.2 mmol). The solution was stirred for 1 h at room temperature. Then, the solution was concentrated under reduced pressure. The resulting oil was dissolved in chloroform, washed with 10% aqueous $NaHCO_3$ and then with $H_2O$. The organic phase was dried over anhydrous $Na_2SO_4$ and concentrated affording the ester as a colourless oil (yield 80–85%).

**3.6.1. (±)-5-Chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid, allyl ester 3c**. $^1$H NMR ($CDCl_3$, $\delta$): 3.32 (dd, 1H, *J* = 10.5 Hz and 16.0 Hz, benzylic $CH_2$); 3.50 (dd, 1H, *J* = 9.6 Hz and 16.0 Hz, benzylic $CH_2$); 4.65 (d, 2H, $CH_2CH{=}CH2$); 5.10–5.38 (m, 3H, $CH{=}CH_2$ and OCH-COO); 5.78–5.98 (m, 1H, $CH{=}CH_2$), 6.70–7.15 (m, 3H, aromatic protons). GC–MS (70 eV) *m*/*z* (rel. int.): 238 ($M^+$, 50), 125 (100).

**3.6.2. (±)-6-Chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid, allyl ester 5c**. $^1$H NMR ($CDCl_3$, $\delta$): 2.90–3.25 (m, 2H, benzylic $CH_2$); 3.95–4.92 (m, 3H, $COOCH_2$ and CHCOO); 4.50–4.72 (m, 2H, $OCH_2$); 5.18–5.50 (m, 2H, $CH{=}CH_2$); 5.68–6.25 (m, 1H, $CH{=}CH_2$), 6.65–7.25 (m, 3H, aromatic protons). GC–MS (70 eV) *m*/*z* (rel. int.): 252 ($M^+$, 2), 167 (100).

### 3.7. Enzymatic enantioselective hydrolysis of 3a–5a

The reaction mixture containing the powdered enzyme (1 g) in phosphate buffer, (0.2N, 20 mL, pH 7.0), the racemic substrate (200 mg), $CaCl_2$ (10 mg) and NaCl (30 mg) was placed in a screw-cap bottle and shaken on an orbit shaker at 250 rpm and 37°C. After the time specified in Table 1, the reaction mixture was extracted with $CHCl_3$. The organic phase was washed with 10% aqueous $NaHCO_3$, dried over anhydrous $Na_2SO_4$ and the solvent evaporated under reduced pressure. The residue consisted of the remaining unreacted ester. The aqueous phase was acidified with 6N HCl and then extracted three times with $CHCl_3$. The chloroform solution was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to afford a white solid residue. The recovered substrates and products were then analysed by HPLC (Table 4) for the determination of the e.e.$_s$ and e.e.$_p$ (the product was methylated by $CH_2N_2$ treatment at 0°C and then used for the determination of e.e.$_p$ values), and by $^1$H NMR to compare their spectra with those of authentic samples. Reaction times, e.e. and $E$ values for the three compounds are reported in Table 1.

Similar conditions were used to hydrolyse allyl esters **3c** and **5c**. In these reactions 15% of methanol was added as co-solvent. Enantiomeric excesses were determined as reported in Table 4.

### 3.8. General procedure for the reduction of (±)-6-chloro-2,3-dihydro-1-benzofuran-2-carboxylic acid methyl ester 3a and (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-3-carboxylic acid methyl ester 5a with $LiAlH_4$

A suspension of the methyl ester (2.35 mmol) and $LiAlH_4$ (3.06 mmol) in benzene (14 mL) was stirred under reflux and $N_2$ atmosphere for 5 h. After cooling at room temperature, $H_2O$ was added to the reaction mixture which was then extracted with ethyl acetate. The organic phase was dried over anhydrous $Na_2SO_4$ and the solvent was evaporated under reduced pressure affording a colourless oil (yield 93%).

**3.8.1. (±)-2-Hydroxymethyl-5-chloro-2,3-dihydro-1-benzofuran 6**. $^1$H NMR ($CDCl_3$, $\delta$): 1.79 (bs, 1H, OH, $D_2O$ exchanged); 3.01 (dd, 1H, $J=16.0$ Hz and 7.6 Hz, benzylic $CH_2$); 3.22 (dd, 1H, $J=16.0$ Hz and 9.5 Hz, benzylic $CH_2$); 3.70 (dd, 1H, $J=12.0$ Hz and 6.0 Hz, 1 of C$H_2$OH); 3.84 (dd, 1H, $J=12.0$ Hz and 3.3 Hz, 1 of C$H_2$OH); 4.86–4.97 (m, 1H, $CH_2OCH$); 6.65–7.13 (m, 3H, aromatic protons). GC–MS (70 eV) $m/z$ (rel. int.): 184 ($M^+$, 100), 165 (85), 125 (99).

**3.8.2. (±)-3-Hydroxymethyl-6-chloro-2,3-dihydro-4*H*-1-benzopyran 7**. $^1$H NMR ($CDCl_3$, $\delta$): 2.18–2.40 (bs, 1H, OH, $D_2O$ exchanged); 2.45–2.76 (m, 1H, benzylic $CH_2$); 2.78–3.10 (m, 1H, benzylic $CH_2$), 3.50–3.88 (m, 3H, $OCH_2CH$); 3.90–4.15 (m, 1H, 1 of C$H_2$OH); 4.18–4.45 (m, 1H, 1 of C$H_2$OH); 6.60–7.20 (m, 3H, aromatic protons). GC–MS (70 eV) $m/z$ (rel. int.): 198 ($M^+$, 65), 167 (100).

### 3.9. Reduction of (±)-6-chloro-2,3-dihydro-4*H*-1-benzopyran-2-carboxylic acid methyl ester 4a with BMS

A solution of methyl ester (2.74 mmol) and BMS (5.48 mmol) in anhydrous THF (10 mL) was stirred under reflux for 5 h. After cooling to room temperature $H_2O$ (0.57 mL) was added followed by 3N aqueous NaOH (0.4 mL) and 30% aqueous $H_2O_2$ (0.4 mL). After stirring at room temperature for 0.5 h, the reaction mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $Na_2SO_4$ and the solvent evaporated under reduced pressure to afford a colourless oil (yield 96%).

**3.9.1. (±)-2-Hydroxymethyl-6-chloro-2,3-dihydro-4*H*-1-benzopyran 8**. $^1$H NMR ($CDCl_3$, $\delta$): 1.73–1.99 (m, 2H, C$H_2$CHC$H_2$OH); 2.00–2.25 (bs, 1H, OH, $D_2O$ exchanged); 2.66–2.91 (m, 2H, benzylic $CH_2$); 3.74 (dd, 1H, $J=12.0$ Hz and 6.0 Hz, C$H_2$OH); 3.83 (dd, 1H, $J=12.0$ Hz and 3.5 Hz, C$H_2$OH), 4.03–4.13 (m, 1H, C$H$C$H_2$OH), 6.70–7.05 (m, 3H, aromatic protons). GC–MS (70 eV) $m/z$ (rel. int.): 198 ($M^+$, 78), 167 (100).

### 3.10. Lipase-catalysed acylation of 6–8 using vinyl acetate in hexane

Crude lipase (500 mg) and vinyl acetate (1 mL) were added to a solution of racemic alcohol (100 mg) in hexane (10 mL). The heterogeneous mixture was incubated at 37°C with stirring at 250 rpm. The reaction was followed by TLC and stopped at the time indicated in the Table 2. The mixture was filtered through a sintered glass funnel to recover the enzyme extract. The hexane was removed under reduced pressure. Product and remaining unreacted substrate were separated by chromatography (silica gel, eluent: petroleum ether:ethyl acetate = 8:2). The enantiomeric excesses were determined by chiral HPLC (Table 4) on a column supplied from DAICEL (Chiralcel OD) on the mixture containing the remaining substrate and product, just after the filtration. The extent of the conversion was also determined from the same HPLC chromatogram. The e.e. of the remaining alcohol (e.e.$_s$) and the extent of the conversion (C) were used to calculate the enantioselectivity factor $E$ (Table 2).

### 3.11. Enzymatic acylation using succinic (or acetic) anhydride in TBME

To a TBME (10 mL) solution of the substrate (±)-**6** (100 mg) or (±)-**8** (100 mg) and acid anhydride (100 mg) was added lipase (100 mg). When $KHCO_3$ was added (100 mg), a finely powdered anhydrous compound was used. The mixture was shaken at 250 rpm on a rotatory shaker at 35°C for the time indicated in Table 3. After filtration of the solid (lipase powder and $KHCO_3$), $H_2O$ and ethyl acetate were added to the organic solution. The organic layer was extracted with 10% $NaHCO_3$ aqueous solution, dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure affording the unreacted alcohol. The alkaline solution was acidified with

6N HCl and extracted three times with ethyl acetate. The organic layers were dried and the solvent was evaporated, affording the acid as a white solid. For the reaction performed with acetic anhydride, TBME was removed under reduced pressure after filtration of the lipase powder. Product and unreacted substrate were separated by chromatography (silica gel, eluent: petroleum ether:ethyl acetate = 8:2). Reaction times and e.e.s are reported in Table 3 (see Table 4 in which are listed the HPLC conditions for e.e. determination).

### 3.12. General procedure for the esterification of racemic alcohols (±)-6–8 with acetic anhydride

A slight excess of acetic anhydride (10 mL) was added to a solution of racemic alcohol (100 mg) in pyridine (10 mL). The resulting solution was stirred at room temperature for 1 h. Then, $CHCl_3$ (10 mL) was added. The organic solution was washed three times with $H_2O$, dried over anhydrous $Na_2SO_4$ and concentrated affording an oil. Chromatography of the residue using silica gel and ethyl acetate:petroleum ether (1:4) afforded the ester as a colourless oil (yield 95–98%).

**3.12.1. (±)-2-Methylacetoxy-5-chloro-2,3-dihydro-1-benzofuran 9**. $^1$H NMR ($CDCl_3$, $\delta$): 2.11 (s, 3H, $CH_3$); 2.96 (dd, 1H, $J$ = 16.0 Hz and 7.4 Hz, benzylic $CH_2$); 3.32 (dd, 1H, $J$ = 16.0 Hz and 9.0 Hz, benzylic $CH_2$); 4.23 (dd, 1H, $J$ = 12.0 Hz and 7.0 Hz, $CH_2OCO$); 4.37 (dd, 1H, $J$ = 12.0 Hz and 3.5 Hz, $CH_2OCO$); 4.92–5.12 (m, 1H, OCH); 6.68–7.22 (m, 3H, aromatic protons). GC–MS (70 eV) $m/z$ (rel. int.): 226 ($M^+$, 27), 165 (100).

**3.12.2. (±)-2-Methylacetoxy-6-chloro-2,3-dihydro-4*H*-1-benzopyran 10**. $^1$H NMR ($CDCl_3$, $\delta$): 1.15–1.40 (m, 2H, endo $CH_2$CH); 1.80–2.40 (m, 5H, $CH_3$ and benzylic $CH_2$); 3.98–4.20 (m, 3H, $OCHCH_2$); 6.68–7.10 (m, 3H, aromatic protons). GC–MS (70 eV) $m/z$ (rel. int.): 240 ($M^+$, 34), 180 (100).

**3.12.3. (±)-3-Methylacetoxy-6-chloro-2,3-dihydro-4*H*-1-benzopyran 13**. $^1$H NMR ($CDCl_3$, $\delta$): 2.12 (s, 3H, $CH_3$), 2.26–2.46 (m, 1H, CH); 2.48–2.70 (m, 1H, benzylic $CH_2$); 2.78–2.98 (m, 1H, benzylic $CH_2$); 3.85–4.37 (m, 4H, $ArOCH_2$ and $CH_2OCO$); 6.70–7.18 (m, 3H, aromatic protons). GC–MS (70 eV) $m/z$ (rel. int.): 240 ($M^+$, 54), 145 (100).

### 3.13. General procedure for the esterification of racemic alcohols 6 and 8 with succinic anhydride

A slight excess of succinic anhydride (10 mL) was added to a solution of racemic alcohol (100 mg) in pyridine (10 mL). The resulting solution was stirred at room temperature for 1 h. Ethyl acetate (10 mL) was added. The organic solution was washed three times with $H_2O$, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. Chromatography of the residue using silica gel and ethyl acetate:petroleum ether:acetic acid (1:1:0.1) afforded the ester as a colourless oil (yield 90–95%).

**3.13.1. (±)-Mono (5-chloro-2,3-dihydro-1-benzofuran-2-methyl)butanedioate 11**. $^1$H NMR ($CDCl_3$, $\delta$): 2.55–2.75 (m, 4H, $CH_2CH_2$COOH); 2.97 (dd, 1H, $J$ = 16.0 Hz and 7.4 Hz, benzylic $CH_2$); 3.20–3.35 (dd, 1H, $J$ = 16.0 Hz and 9.6 Hz, benzylic $CH_2$); 4.18–4.26 (dd, 1H, $J$ = 12.0 Hz and 6.4 Hz, $CH_2OCO$); 4.28–4.38 (dd, 1H, $J$ = 12.0 Hz and 3.6 Hz, $CH_2OCO$); 4.92–5.05 (m, 1H, CH); 6.65–7.25 (m, 3H, aromatic protons), 7.5–9.4 (bs, COOH, $D_2O$ exchanged). GC–MS (70 eV) $m/z$ (rel. int.): 284 ($M^+$, 2), 166 (100), 153 (15), 131 (25), 101 (27); (methyl ester) 298 ($M^+$, 34), 166 (100).

**3.13.2. (±)-Mono (6-chloro-2,3-dihydro-4*H*-1-benzopyran-2-methyl)butanedioate 12**. $^1$H NMR ($CDCl_3$, $\delta$): 1.62–1.90 (m, 1H, 1 of $CH_2CH_2$Ar); 1.93–2.06 (m, 1H, $CH_2CH_2$Ar); 2.58–2.94 (m, 6H, $CH_2CH_2$COOH and benzylic $CH_2$); 4.15–4.27 (m, 1H, CH); 4.29–4.35 (m, 2H, $CH_2OCO$); 6.69–7.08 (m, 3H, aromatic protons); 8.50–9.95 (bs, COOH, $D_2O$ exchanged). GC–MS (70 eV) $m/z$ (rel. int.): (methyl ester) 312 ($M^+$, 34), 180 (100).

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## References

1. Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J. C. *Circulation* **1998**, *98*, 2088.
2. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
3. Mastaglia, F. L. *Drugs* **1982**, *24*, 304.
4. Conte-Camerino, D.; Tortorella, V.; Ferrannini, E.; Bryant, S. H. *Arch. Toxicol.* **1984**, *7*, 482.
5. Margarian, G. J.; Lucas, L. M.; Colley, C. *Arch. Inter. Med.* **1991**, *151*, 1873.
6. Lavarenne, J.; Poinascaillaud, H. *Therapie* **1991**, *46*, 347.
7. London, S. F.; Gross, K. F.; Ringel, S. P. *Neurology* **1991**, *41*, 1159.
8. Reddy, J. K.; Azarnoff, D. C.; Hignite, C. E. *Nature* **1980**, *283*, 397.
9. Vouillamoz, D.; Schaller, M. D.; Bianchi, L.; Chaubert, P.; Reinhart, W.; Armstrong, D.; Thorens, J.; Blum, A. L. *Lancet* **1991**, *338*, 581.
10. Feller, D. R.; Kamanna, V. S.; Newman, H. A. I.; Romstedt, K. J.; Witiak, D. T.; Bettoni, G.; Bryant, S. H.; Conte-Camerino, D.; Loiodice, F.; Tortorella, V. *J. Med. Chem.* **1987**, *30*, 1265.
11. Bettoni, G.; Loiodice, F.; Tortorella, V.; Conte-Camerino, D.; Mambrini, M.; Ferrannini, E.; Bryant, S. H. *J. Med. Chem.* **1987**, *30*, 1267.
12. Esbenshade, T. A.; Kamanna, V. S.; Newman, H. A. I.; Tortorella, V.; Witiak, D. T.; Feller, D. R. *Biochem. Pharmacol.* **1990**, *40*, 1263.

13. Inomata, N.; Yoshida, H.; Aoki, Y.; Tsunoda, M.; Yamamoto, M. *Tohoku J. Exp. Med.* **1991**, *165*, 171.
14. Ciolek, E.; Dauca, M. *Biol. Cell.* **1991**, *71*, 313.
15. Witiak, D. T.; Feller, D. R.; Stratford, E. S.; Hackney, R. E.; Nazareth, R.; Wagner, G. *J. Med. Chem.* **1971**, *14*, 754.
16. Witiak, D. T.; Stratford, E. S.; Nazareth, R.; Wagner, G.; Feller, D. R. *J. Med. Chem.* **1971**, *14*, 758.
17. Salz, U.; Ruchardt, C. *Chem. Ber.* **1984**, *117*, 3457.
18. Jacques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates and Resolution*; Wiley: New York, 1981; p. 167.
19. Fourneau, E.; Sandulescu, G. *Bull. Soc. Chim. Fr.* **1922**, *31*, 991.
20. Fourneau, E.; Sandulescu, G. *Bull. Soc. Chim. Fr.* **1923**, *33*, 459.
21. Fredga, A.; Matell, M. *Arkiv Kemi* **1952**, *4*, 325.
22. Smith, S. M.; Wain, R. L.; Wightman, F. *Ann. Appl. Biol.* **1952**, *39*, 295.
23. Matell, M. *Arkiv Kemi* **1952**, *4*, 473.
24. Matell, M. *Arkiv Kemi* **1954**, *7*, 437.
25. Fredga, A.; Weider, A. M.; Gronwall, C. *Arkiv Kemi* **1961**, *17*, 265.
26. Sjoberg, B.; Sjoberg, S. *Arkiv Kemi* **1964**, *22*, 447.
27. Gottstein, W. J.; Cheney, L. C. *J. Org. Chem.* **1965**, *30*, 2072.
28. Azzolina, O.; Collina, S.; Ghislandi, V. *Farmaco* **1993**, *48*, 1401.
29. Tsuda, Y.; Kawai, S.; Nakajima, S. *Chem. Pharm. Bull.* **1985**, *33*, 1985.
30. Tsuda, Y.; Kawai, S.; Nakajima, S. *Agric. Biol. Chem.* **1984**, *48*, 1373.
31. Cambou, B.; Klibanov, A. M. *Biotechnol. Bioeng.* **1984**, *26*, 1449.
32. Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072.
33. Chen, C. S.; Wu, S. H.; Girdaucas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812.
34. Dernoucour, R.; Azerard, R. *Tetrahedron Lett.* **1987**, *28*, 4661.
35. Azzolina, O.; Vercesi, D.; Collina, S.; Ghislandi, V. *Farmaco* **1995**, *50*, 221.
36. Azzolina, O.; Ghislandi, V. *Farmaco* **1995**, *50*, 713.
37. Loiodice, F.; Longo, A.; Bianco, P.; Tortorella, V. *Tetrahedron: Asymmetry* **1995**, *6*, 1001.
38. Chiaia Noya, F.; Ferorelli, S.; Franchini, C.; Scilimati, A.; Sinicropi, M. S.; Tortorella, P. *Farmaco* **1996**, *51*, 293.
39. Bettoni, G.; Ferorelli, S.; Loiodice, F.; Tangari, N.; Tortorella, V.; Gasparrini, F.; Misiti, D.; Villani, C. *Chirality* **1992**, *4*, 193.
40. Bonner, W. A.; Burke, N. I.; Fleck, W. E.; Hill, R. K.; Joule, R. K.; Sjöberg, B.; Zalkow, J. H. *Tetrahedron* **1964**, *20*, 1419.
41. Scilimati, A.; Ngooi, T. K.; Sih, J. C. *Tetrahedron Lett.* **1988**, *29*, 4927.
42. Berger, B.; Rabiller, C. G.; Konigsberger, K.; Faber, K.; Griengel, H. *Tetrahedron: Asymmetry* **1990**, *1*, 541.
43. Hyatt, J. A.; Skelton, C. *Tetrahedron: Asymmetry* **1997**, *8*, 523.