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Enzyme-mediated synthesis of EEHP and EMHP, useful pharmaceutical intermediates of PPAR agonists

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ABSTRACT

A new scaleable synthetic route to the title compounds has been developed. The reaction pathway is based on the α-chymotrypsin-catalysed hydrolysis of the racemic ethyl 2-ethoxy-3-(*p*-methoxyphenyl)propanoate or of the racemic ethyl 2-methoxy-3-(*p*-methoxyphenyl)propanoate to give the corresponding resolved (*S*)-esters with excellent ee. The acids were easily separated from the (*S*)-esters by a simple acid–base work-up. The overall yields of **1** and **2** were 16% and 17%, respectively.

1. Introduction

Both ethyl (*S*)-2-ethoxy-3-(4-hydroxyphenyl)propanoate (*S*)-**1** (EEHP) and ethyl (*S*)-2-methoxy-3-(4-hydroxyphenyl)propanoate (*S*)-**2** (EMHP) are useful intermediates for the synthesis of several Peroxisome Proliferator-Activated Receptors (PPARs) agonists.[1] Among these, the most popular are Tesaglitazar **3**[2] and Navaglitazar **4**[3] developed by AstraZeneca and Eli Lilly, respectively (Fig. 1).

These molecules have found their main therapeutic application in the treatment of type-2 diabetes (T2D), and, although most of them have recently been discontinued since the risk-benefit balance was not met,[4] (*S*)-**1** and (*S*)-**2** are still important pharmaceutical intermediates for the development of a safer and more efficient generation of PPAR agonists.[5]

So far, a large number of synthetic procedures for the preparation of (*S*)-**1**[6–9] and (*S*)-**2** or related precursors such as the α-hydroxyesters[10] or the glycidic esters[11] have been reported. However, although asymmetric catalysis has been greatly developed over the last few decades, the process based on the catalytic reduction of the α-ethoxycinnamic acid is not sufficiently efficient in terms of enantioselectivity (60–92% ee) and practicality (10% in weight of a Rh-based chiral catalyst).[8] Furthermore, the chiral pool approach, based on the diazotisation of the O-benzylated derivatives of the naturally occurring and commercially available L-tyrosine, seems to be an unappealing route from an industrial point of view because the overall yield is too low.[6a,b] Most industrial processes for the production of (*S*)-**1** or (*S*)-**2** are based on kinetic resolutions, using enzymes[7a,c] or chiral amines.[7b]

Thus, keeping this in mind, we have designed a new synthetic route based on an enzyme-mediated resolution. In addition, the entire process has been developed in such a way to avoid chromatographic purifications and expensive reagents,[7d] which are inconsistent with the typical large-scale pharmaceutical productions. Herein we report the details of our synthesis that has succeeded in meeting all the above goals.

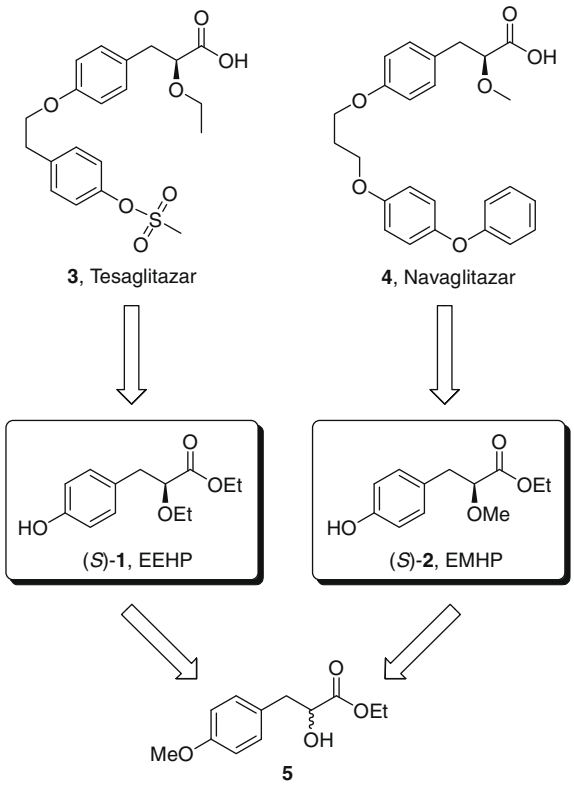

Figure 1. Structures of Tesaglitazar and Navaglitazar, PPAR agonists based on EEHP and EMHP intermediates, respectively.

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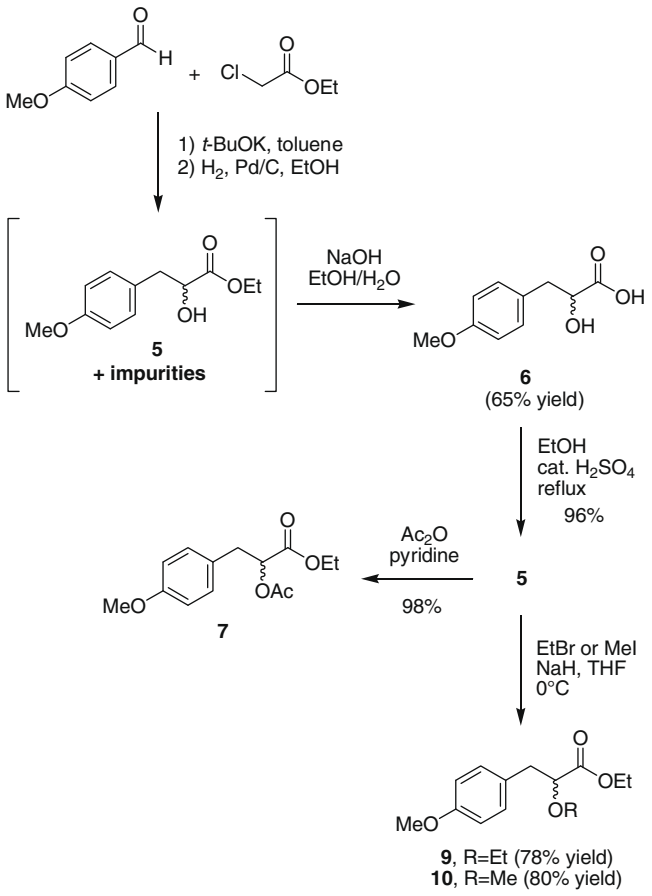
2. Results and discussion

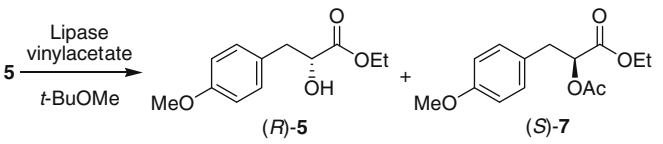
Initially, we envisaged ethyl 2-hydroxy-3-(4-methoxyphenyl)-propanoate **5** (Fig. 1) as an ideal intermediate on which to perform our preliminary enzyme-mediated resolutions[12] for three main reasons: (i) the lipase-mediated acylations, carried out on similar substrates such as the phenyl lactic ester,[13] often proceed with excellent enantiomeric excesses (ee); (ii) the alkylation of the hydroxyl group, which is the divergent step of the synthesis of the two target molecules, is strategically planned at the last stage giving clear benefits in terms of number of steps; (iii) and finally, the aryl methoxy-protecting group is more convenient, in terms of atom economy, than the *O*-benzyl ether, which has been used in almost all previously reported syntheses.

The preparation of racemic **5** is shown in Scheme 1. Darzens condensation of anisaldehyde with ethyl chloroacetate in toluene and in the presence of *t*-BuOK gave the crude glycidic ester which, without any purification, was submitted to the Pd/C catalytic hydrogenolysis in EtOH to afford a complex mixture composed mainly of **5** together with other unidentified impurities. The purification of **5** was achieved by treatment of the latter mixture with NaOH in EtOH/H_2O to give the corresponding acid **6** which could be easily separated, as a white powder, from the impurities by trituration in EtOAc. Finally, the acid, having a satisfactory purity (over 99% by ^{1}H NMR) was almost quantitatively reconverted to **5** by standard Fischer esterification and in an overall yield of 62%.



Scheme 1. Preparation of the racemic substrates **5**, **7**, **9** and **10**.

Next, **5** was submitted to enzymatic resolution screening of three different lipases (*Pseudomonas cepacia,* porcine pancreatic, *Candida rugosa*) in *t*-BuOMe, using vinyl acetate as the acyl donor (Scheme 2). The results are summarised in Table 1. The best ee was obtained using the *P. cepacia* lipase (*PS*-L). Indeed, after 5 days it was possible to isolate, via column chromatography separation, the hydroxyester (*R*)-**5** in 50.8% yield and 90.4% ee by HPLC ($[\alpha]_D^{25} = -8.2$ (*c* 1.50, EtOH)) from the acetoxyester (*S*)-**7** in 43.7% yield and 96.4% ee by HPLC {$[\alpha]_D^{25} = -7.8$ (*c* 1.23, EtOH) versus lit. $[\alpha]_D^{25} = -8.3$ (*c* 1.50, EtOH)[14]}.



Scheme 2. Lipase-mediated acetylation of **5**. For conversions and ee, see Table 1.

However, although this *PS*-L-catalysed resolution gave (*S*)-**7** with excellent ee, the use of chromatography makes such procedure not completely ideal for our requirements (efficiency, easy purification).

Instead, the use of the bovine digestive enzyme α-chymotrypsin (α-CT) could better match our aims. Indeed, it is known that this enzyme stereoselectively hydrolyses ethyl α-acetoxy-β-phenylpropionate,[15] allowing a column-chromatography-free separation of the resolved acid from the ester by means of an extremely simple and practical acid–base work-up. Unfortunately, treatment of **5** with α-CT at standard conditions (H_2O, pH 7.8) gave after a day acid **6** in an almost quantitative yield and without any appreciable stereoselectivity (Scheme 3, Table 1). In addition, the hydrolysis of the acetoxyester derivative **7**, under the same experimental conditions, furnished after 4 days and a conversion of 42.9% (by GC of the methyl ester derivative, obtained by treatment with CH_2N_2 in Et_2O) the acetoxyacid (*S*)-**8** (38.1% ee by HPLC of the methyl ester) and the acetoxyester (*R*)-**7** (28.0% ee by HPLC) with poor enantiomeric purities (Scheme 3, Table 1). However, it is interesting to note that α-CT hydrolyses **5** much quicker than **7**, and without stereoselectivity.

Thus, by combining these results, we set up a column-chromatography-free procedure for the separation of (*S*)-**5** from (*R*)-**7** coming from the *PS*-L-mediated resolution. Indeed, the α-CT-mediated hydrolysis of the latter produced a complex mixture consisting of esters (*S*)-**7** (96.3% ee) and (*R*)-**5** (89.2% ee) in the ratio 93:7, and acids (*R*)-**6** (95.5% ee) and (*S*)-**8** (97.5% ee) in the ratio of 91:9, which were separated by the usual acid–base work-up (Scheme 4).

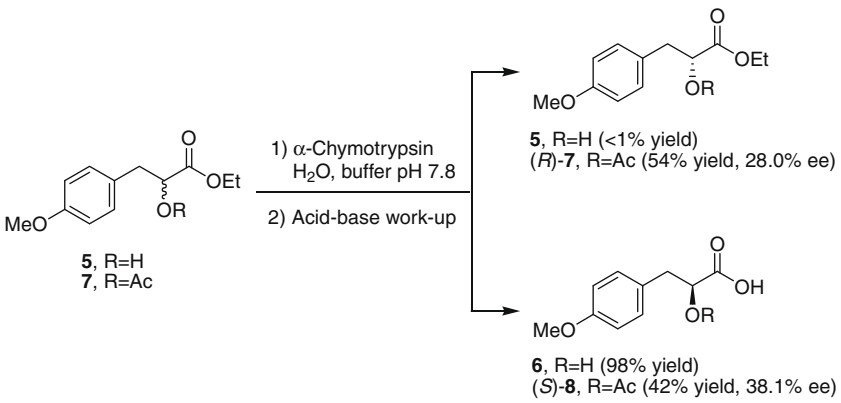
Next, the (*S*)-**7**-enriched mixture was hydrolysed (KOH, MeOH/H_2O, 96% yield) to give (*S*)-**6** (82.2% ee) in an overall yield of 37% over three steps (*PS*-L-mediated resolution, α-CT-mediated hydrolysis and chemical hydrolysis of the survived fraction). The latter was esterified in acetone with EtBr, K_2CO_3 and in the presence of a substoichiometric amount of tetrabutylammonium iodide (TBAI) to give (*S*)-**7** in a quantitative yield and without loss of enantiomeric purity. Finally, the hydroxyester (*S*)-**7** was converted to the ethoxyester (*S*)-**9** following a reported procedure (EtBr/EtI, NaH, THF/DMF).[6a,6b] However, we obtained (*S*)-**9** with a substantial loss of enantiomeric purity {73.1% ee by HPLC, $[\alpha]_D^{25} = -14.0$ (*c* 1.17, $CHCl_3$)}. This result was very disappointing, since such alkylations, carried out on similar/identical substrates, were described to proceed without any significant loss of enantiomeric purity and in high yields.

Recently, the alkylation of the hydroxyl group of a similar compound has been extensively studied using different solvents (DMF, THF/DMF), bases (NaH, NaHMDS, KHMDS, *t*-AmylONa) and electrophiles (EtBr, EtI, EtOTs, MeI) at different temperatures, however, the reagents that gave the lowest epimerisation extent are too

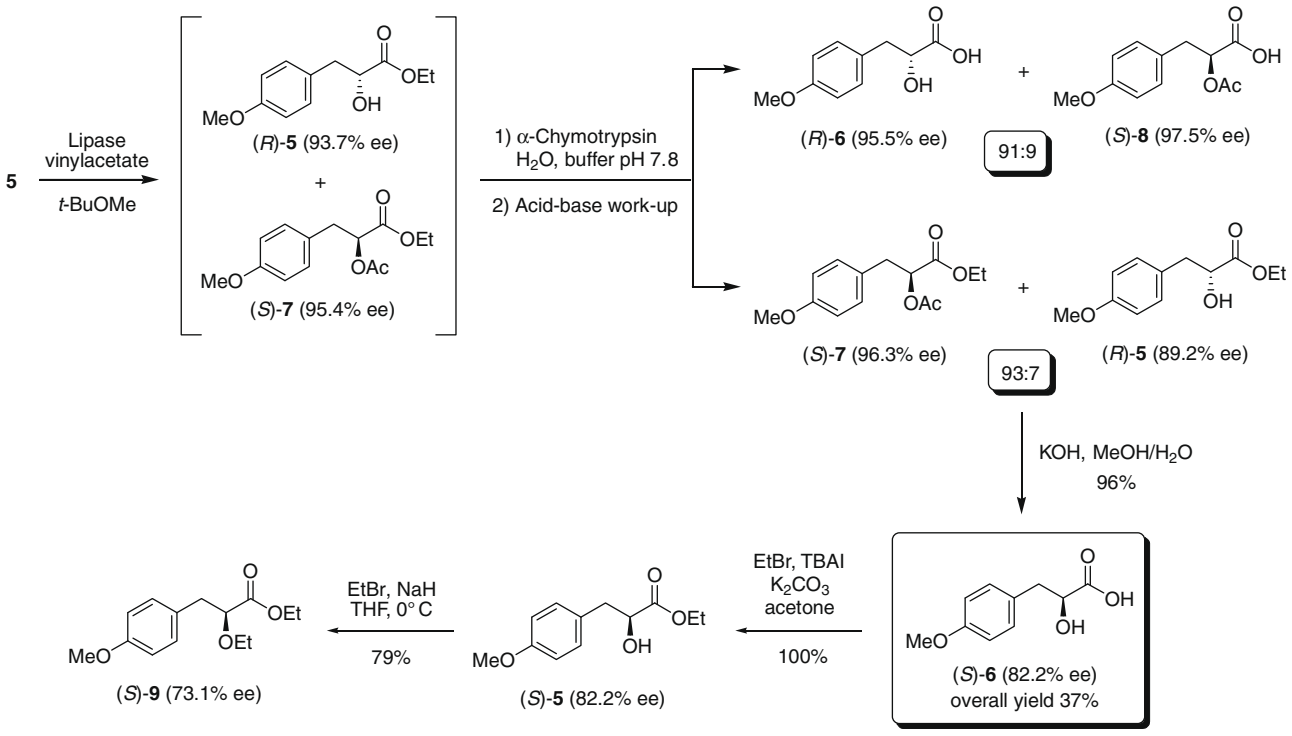
Table 1
Enzyme-mediated resolutions of substrates **5**, **7**, **9** and **10**

Entry	Enzyme	Substrate	Time (days)	Conv[a] (%)	Abs. Conf. *Transf.*	ee[b] (%) *Transf.*	ee[b] (%) *Surv.*	*E* (Enant. *S*)	Yield (%) *Transf.*	Yield (%) *Surv.*
1	*PS*-L	**5**	5	47.6	(*S*)	96.4	90.4	158	44	52
2	*CR*-L	**5**	20	27.5	(*S*)	12.4	3.4	1.3	–	–
3	*PP*-L	**5**	20	<1	–	–	–	–	–	–
4	α-CT	**5**	1	97.8	–	–	–	–	–	–
5	α-CT	**7**	4	42.9	(*S*)	38.1	28.0	2.9	42	54
6	α-CT	**9**	40	53.2	(*R*)	82.1	93.0	33.6	44	39
7	α-CT	**10**	40	57.1	(*R*)	74.2	99.9	50.5	51	40

[a] By GC (for α-CT resolution the analytical samples were treated with CH_2N_2/Et_2O).
[b] By HPLC.



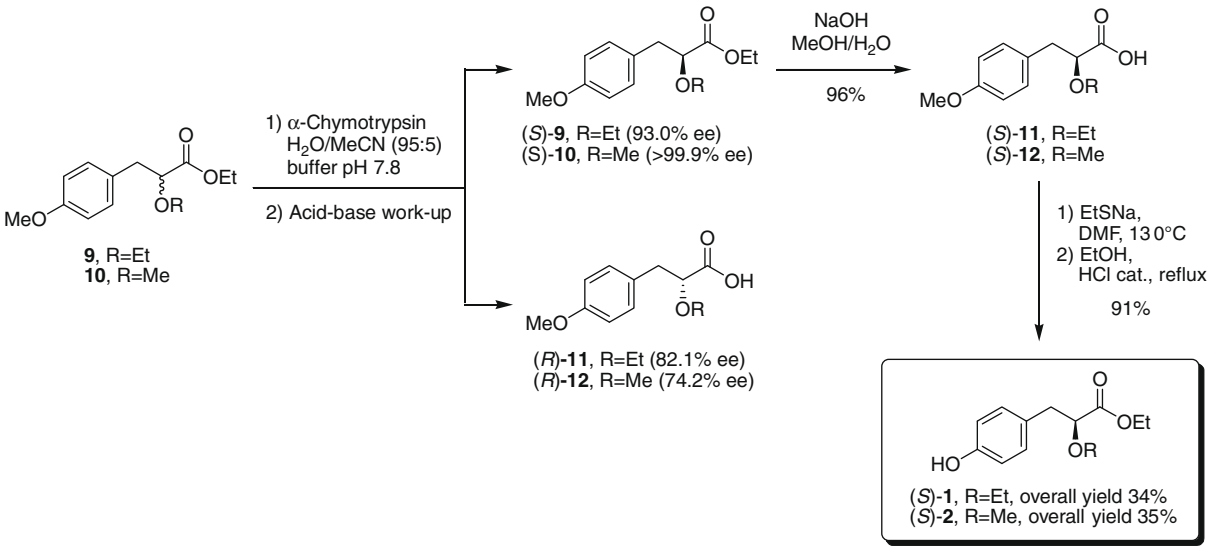
Scheme 3. α-CT-mediated hydrolysis of **5** and **7**.



Scheme 4. Column-chromatography-free synthesis of (*S*)-**6**.

expensive to be used on industrial scale.[16] Thus, we concluded that this step has to be carried out before the resolution process.

Accordingly, the racemic ethoxyester **9**, obtained by ethylation of **5** (Scheme 1), was submitted to the α-CT-catalysed hydrolysis using experimental conditions similar to those adopted for substrates **5** and **7** (H_2O/MeCN, 95:5, pH 7.8, for solubility reasons it was necessary to add some MeCN, Scheme 5). After 40 days and a conversion of 53.2% (by GC), ester (*S*)-**9** {93.0% ee, $[\alpha]_D^{25} = -18.1$ (*c* 1.34, $CHCl_3$)} was separated from acid (*R*)-**11** (82.1% ee by HPLC of methyl ester derivative) in a yield of 39% and 44%, respectively by the usual work-up.



Scheme 5. Preparation of **1** and **2** based on the α-CT-catalysed hydrolysis of **9** and **10**.

It is important to note that by substituting the acetoxy group with the less polar ethoxy group, the α-CT inverted its stereoselectivity [(*S*) vs (*R*)] and the reaction rate decreased dramatically (4 days vs 40 days); it should also be noted that a larger amount of enzyme was used (about four times more with respect to the previous hydrolyses).[17]

Finally, ester (*S*)-**9** was hydrolysed to give acid (*S*)-**11** ($[\alpha]_D^{25} = -16.6$ (*c* 1.21, $CHCl_3$) versus an authentical sample $[\alpha]_D^{25} = -16.8$ (*c* 1.08, $CHCl_3$)[18]) which in turn was submitted to a two-step sequence consisting of the cleavage of the aryl methoxy group followed by esterification of the carboxylic group to give (*S*)-**1** (93.0% ee by HPLC of aryl methoxy derivative obtained by treatment with Me_2SO_4, K_2CO_3) in an overall yield of 91% (Scheme 5).[7b]

Analogously the racemic methoxyester **10** was submitted to the same synthetic sequence to give (*S*)-**2** (>99.9% ee by HPLC) in an overall yield of 35% (Scheme 5).

3. Conclusion

In short, we have disclosed a new synthetic process for the preparation of EEHP (ee 93%) and EMHP (ee >99%) based on the α-CT-mediated resolution of racemic materials **9** and **10** in an overall yield of 16% and 17%, respectively. After the process's optimisation, this new synthetic route might be scaleable on an industrial scale because it avoids the use of expensive reagents and does not require the utilisation of chromatographic purifications. Furthermore, we have disclosed a new column-chromatography-free synthesis of the intermediates (*R*) and (*S*)-**6**.

4. Experimental

4.1. General remarks

GC–MS analyses were performed on an Agilent HP 6890 gas-chromatograph equipped with a 5973 mass-detector, using an Agilent HP-5MS column (30 m × 0.25 mm × 0.25 μm). The following temperature program was employed: 60 °C (1 min)/6 °C/min/150 °C (1 min)/12 °C/min/280 °C (5 min). ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX 400 spectrometer (400 MHz ^1H, 100.6 MHz ^{13}C) in $CDCl_3$ solution at rt, using TMS as internal standard for ^1H and $CDCl_3$ for ^{13}C; chemical shifts δ are expressed in ppm relative to TMS; *J* values are given in hertz. Optical rotations were determined on a Dr. Kernchen Propol digital automatic polarimeter. Chiral HPLC analyses were performed on a Merck-Hitachi L-4250 chromatograph equipped with a Chiralcel OD column and a UV detector (210 nm); mobile phase: *n*-hexane/*i*-PrOH 98:2 for compounds **5**, **7** and the corresponding methyl esters of acids **6**, **8**, **11**; 99:1 for compounds **9**, **10** and methyl ester of acid **12**; flow rate: 0.6 mL/min. TLC analyses were performed on Merck Kieselgel 60 F_{254} plates. All the chromatographic separations were carried out on silica gel columns. The α-CT-mediated hydrolyses were carried out on a Metrohm 718 STAT Titrino pH-stat. Lipase-PS from *P. cepacia* (*PS*-L, Amano Pharmaceuticals Co., Japan), *C. rugosa* lipase (*CR*-L; Sigma, type VII), porcine pancreatic lipase (*PP*-L, Sigma, type II) and α-chymotrypsin (α-CT, Sclavo S.p.A.) were employed as purchased. All reagents and solvents were purchased from Fluka or Aldrich and used without any further purification.

4.2. Preparation of racemic substrates

4.2.1. 2-Hydroxy-3-(4-methoxyphenyl)propanoic acid 6

To a mechanically stirred solution of anisaldehyde (150 g, 1.1 mol) and ethyl chloroacetate (117 mL, 1.1 mol) in toluene (500 mL) was added portionwise *t*-BuOK (63.9 g, 0.57 mol) at such a rate to keep the temperature below 30 °C by cooling in an ice-water bath. The complete conversion of the aldehyde was ensured by the addition of a 10% surplus of chloroacetate and *t*-BuOK in one portion. After 2 h at rt, the solution was quenched with ice (450 g) and NH_4Cl solution (satd, 500 mL) and extracted with EtOAc (3 × 400 mL). The organic phase was washed with water (3 × 100 mL) and brine (100 mL). The mixture was dried over Na_2SO_4 and concentrated under reduced pressure to give a brown oil, which was dissolved in EtOH (400 mL) and was added to a Parr reactor containing Pd/C (5% w/w, 70 g). The suspension was shaken under 70 psi of H_2 for 3 days. Afterwards, the mixture was filtered on a Celite pad and the filtrate was added to an aqueous solution of NaOH (100 mL, 80 g). After 4 h, most of the EtOH was removed under reduced pressure to give a suspension, which was dissolved in H_2O (500 mL) and washed with Et_2O (2 × 300 mL). The aqueous solution was acidified with HCl (6 M, 400 mL) and extracted with EtOAc (3 × 500 mL). The organic phase was washed with brine (500 mL) and dried over Na_2SO_4. Concentration under reduced pressure and trituration in ice-cold EtOAc (100 mL) gave **6** as a white powder (140 g, 65% yield, >99% purity by ^1H NMR). ^1H

NMR (400 MHz, $CDCl_3$: δ 7.16 (d, J = 8.5, 2H), 6.85 (d, J = 8.5 2H), 4.47 (m, 1H), 3.79 (s, 3H), 3.05 (m, *AB syst.*, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$: δ 177.7, 158.8, 130.5, 127.7, 114.1, 71.1, 55.3, 39.3.

4.2.2. Ethyl 2-hydroxy-3-(4-methoxyphenyl)propanoate 5

A solution of **6** (20.0 g, 0.102 mol) and H_2SO_4 (concd, 1 mL) in EtOH (abs., 100 ml) was refluxed for 10 h. Then it was concentrated under reduced pressure, diluted with H_2O (50 ml) and extracted with EtOAc (2 × 50 mL). The combined organic phase was washed with $NaHCO_3$ solution (satd, 50 mL), brine (2 × 50 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded **5** as a yellow oil (21.9 g, 96% yield, 62% overall yield from anisaldehyde, 99.4% purity by GC (t_R 21.0 min)). ^1H NMR (400 MHz, $CDCl_3$: δ 7.11 (d, J = 8.6, 2H), 6.80 (d, J = 8.6, 2H), 4.36 (dd, J = 6.6, 4.7, 1H), 4.18 (q, J = 7.1, 2H), 3.75 (s, 3H), 2.96 (m, *AB syst.*, 2H), 1.25 (t, J = 7.1, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$: δ 174.2, 158.6, 130.5, 128.5, 113.8, 71.4, 61.5, 55.2, 39.7, 14.1. MS: m/z (%) 224 $[M]^+$ (10), 206 (8), 151 (6), 121 (100), 91 (7), 77 (7).

4.2.3. Ethyl 2-acetoxy-3-(4-methoxyphenyl)propanoate 7

To an ice-cold stirred solution of **5** (10.0 g, 44.6 mmol) in pyridine/CH_2Cl_2 (1:1, 100 mL) was added Ac_2O (20 mL). After 10 h the mixture was concentrated under reduced pressure, diluted with water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was washed with HCl (1 M, 50 mL), H_2O (50 mL), $NaHCO_3$ solution (satd, 50 mL) and brine (2 × 50 mL), and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave **7** as a brownish oil (11.6 g, 98% yield, 98.9% purity by GC (t_R 22.4 min). ^1H NMR (400 MHz, $CDCl_3$: δ 7.13 (d, J = 8.6, 2H), 6.83 (d, J = 8.6, 2H), 5.15 (dd, J = 4.8, 8.4, 1H), 4.16 (q, J = 7.1, 2H), 3.78 (s, 3H), 3.06 (m, *AB syst.*, 2H), 2.07 (s, 3H), 1.22 (t, J = 7.1, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$: δ 170.2, 169.7, 158.6, 130.3, 127.9, 113.8, 73.2, 61.3, 55.2, 36.5, 20.5, 14.0. MS: m/z (%) 266 $[M]^+$ (2), 206 (95), 161 (47), 134 (38), 121 (100).

4.2.4. General procedure for the O-alkylation of 5

To a stirred and ice-cold suspension of NaH (60% disp. in min oil, 1.16 g, 28.9 mmol) in dry THF (50 mL) under an N_2 atmosphere, a solution of **5** (5.0 g, 22.3 mmol) in dry THF (20 mL) was added dropwise. After 15 min a solution of halide (28.9 mmol) in dry THF (10 mL) was added dropwise. A small surplus of NaH and halide was added to obtain complete conversion (by TLC). The mixture was quenched with ice (100 g) and NH_4Cl solution (satd, 50 mL) and extracted with Et_2O (3 × 150 mL). The combined organic phase was washed with brine (2 × 50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give a crude oil, which was used for the next steps without further purification. A sample was filtered on a silica gel pad in gradient elution (*n*-hexane→*n*-hexane/EtOAc 1:1) to give the corresponding O-alkylated ethyl ester.

Ethyl 2-ethoxy-3-(4-methoxyphenyl)propanoate **9**. Halide: EtBr. 4.38 g, 78% yield, 99.5% purity by GC (t_R 21.5 min). ^1H NMR (400 MHz, $CDCl_3$: δ 7.15 (d, J = 8.7, 2H), 6.81 (d, J = 8.7, 2H), 4.15 (q, J = 7.1, 2H), 3.97 (m, 1H), 3.77 (s, 3H), 3.59 (dq, J = 7.0, 9.2, 1H), 3.55 (dq, J = 7.0, 9.2, 1H), 2.95 (m, 2H), 1.22 (t, J = 7.1, 3H), 1.16 (t, J = 7.0, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$: δ 172.5, 158.4, 130.4, 129.3, 113.7, 80.4, 66.2, 60.7, 55.2, 38.5, 15.0, 14.2. MS: m/z (%) 252 $[M]^+$ (4), 206 (26), 179 (10), 151 (6), 121 (100), 91 (7).

Ethyl (*S*)-2-ethoxy-3-(4-methoxyphenyl)propanoate (*S*)-**9**. Halide: EtBr. Starting from 0.50 g of (*S*)-**5** (82.2% ee). 0.44 g, 79% yield, 73.1% ee by HPLC (t_R (*R*) 16.3 min, (*S*) 17.6 min), 99.5% purity by GC, $[\alpha]_D^{25} = -14.0$ (c 1.17, $CHCl_3$).

Ethyl 2-methoxy-3-(4-methoxyphenyl)propanoate **11**. Halide: MeI. 4.24 g, 80% yield, 98.3% purity by GC–MS (t_R 20.9 min). ^1H NMR (400 MHz, $CDCl_3$: δ 7.16 (d, J = 8.4, 2H), 6.84 (d, J = 8.6, 2H), 4.19 (q, J = 7.2, 2H), 3.97 (m, 1H), 3.79 (s, 3H), 3.37 (s, 3H), 2.98 (m, 2H), 1.16 (t, J = 7.2, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$: δ 171.6, 158.1, 129.9, 128.6, 113.4, 81.6, 60.3, 57.7, 54.8, 37.9, 13.8. MS: m/z (%) 238 $[M]^+$ (5), 206 (30), 165 (17), 121 (100).

4.3. Representative procedure for the lipase mediated acylation

A mixture of **5** (5.0 g, 22.2 mmol), vinyl acetate (20 mL), *t*-BuOMe (50 mL) and *PS*-L (2.5 g) was stirred at rt and the reaction was followed by HPLC. After 5 d the mixture was filtered and concentrated under reduced pressure. Column chromatography separation (*n*-hexane/EtOAc, 9:1) gave, in order of elution: (*S*)-**7**, 2.60 g, 44% yield, 99.7% purity by GC (t_R 22.4 min), 96.4% ee by HPLC (t_R 15.0 min (*S*), 17.1 min (*R*)), $[\alpha]_D^{25} = -7.8$ (c 1.23, EtOH) versus lit. $[\alpha]_D^{25} = -8.3$ (c 1.50, EtOH), and (*R*)-**5**, 2.62 g, 52% yield, 99.8% purity by GC (t_R 21.0 min), 90.4% ee by HPLC (t_R 26.5 min (*S*), 29.1 min (*R*)), $[\alpha]_D^{25} = -8.2$ (c 1.50, EtOH).

4.4. General procedure for the α-chymotrypsin mediated hydrolysis

α-CT (2.0 g) was suspended in aqueous NaCl solution (0.1 M, 200 mL) and $Na_2HPO_4{\cdot}2H_2O$ (0.47 g, 3.0 mmol) was added as a buffering agent. The mixture under magnetic stirring was brought to pH 7.8 by a pH-stat charged with 0.5 M aqueous NaOH. The substrate (20.0 mmol), if necessary dissolved in MeCN (10 mL), was added and the pH was maintained at 7.8 until about half of the theoretical amount of NaOH solution had been consumed. The mixture was then extracted with Et_2O (4 × 100 mL), centrifuging between the extractions to help break the emulsion. The combined ether extract was subsequently washed with brine (50 mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield the non-hydrolysed substrate (*survived fraction*). The aqueous phase was then brought to pH 2–3 with HCl (1 M). The resulting suspension was extracted with EtOAc (3 × 100 mL), centrifuging between the extractions. The reunited acetate extract was washed with brine (50 mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield the hydrolysed product (*transformed fraction*).

With substrate **7** (9.0 g): *transformed fraction* (*S*)-**8**, 3.42 g, 42% yield, 38.1% ee by HPLC of CH_2N_2 derivative (t_R (*S*) 18.4 min, (*R*) 20.0 min); *survived fraction* (*R*)-**7**, 4.85 g, 54% yield, 28.0% ee by HPLC (t_R (*S*) 15.3 min, (*R*) 17.1 min).

With substrate **9** (3.0 g): *transformed fraction* (*R*)-**11**, 1.18 g, 44% yield, 82.1% ee by HPLC of CH_2N_2 derivative (t_R (*R*) 16.1 min, (*S*) 17.0 min), $[\alpha]_D^{25} = +14.6$ (c 1.14, $CHCl_3$); *survived fraction* (*S*)-**9**, 1.34 g, 39% yield, 93.0% ee by HPLC (t_R (*R*) 16.3 min, (*S*) 17.6 min), $[\alpha]_D^{25} = -18.1$ (c 1.34, $CHCl_3$).

With substrate **10** (3.0 g): *transformed fraction* (*R*)-**12**, 1.16 g, 51% yield, 74.2% ee by HPLC of CH_2N_2 derivative (t_R (*R*) 22.5 min, (*S*) 27.4 min) $[\alpha]_D^{25} = +20.2$ (c 1.46, EtOH); *survived fraction* (*S*)-**10**, 1.20 g, 40% yield, 99.9% ee by HPLC (t_R (*R*) 18.9 min, (*S*) 19.4 min), $[\alpha]_D^{25} = -16.9$ (c 3.74, EtOH).

4.5. Column-chromatography-free preparation of (*S*)-6 from racemic 5

Hydroxyester **5** (5.0 g, 22.3 mmol) and vinyl acetate (20 mL) were dissolved in *t*-BuOMe (50 mL) and *PS*-L (2.5 g) was added. The suspension was stirred at rt until approaching 50% conversion by HPLC (about 10 days). The reaction mixture was then filtered to remove the enzyme and concentrated under reduced pressure, yielding a raw mixture of (*S*)-**7** and (*R*)-**5** in about equal proportions (5.22 g). Then, to a well-stirred suspension of α-CT (1.05 g) in NaCl solution (0.1 M, 180 mL) brought to pH 7.8 by a pH-stat charged with 0.5 M NaOH solution, was added the mixture of (*R*)-**5** and (*S*)-**7**. After about 50% consumption of the theoretical

amount of NaOH (less than 3 days), the mixture was treated according to the general procedure for the α-chymotrypsin-mediated hydrolysis, to give the (*S*)-**7**-enriched fraction (2.06 g, ratio 93:7, 96.3% ee of (*S*)-**7**, 89.2% ee of (*R*)-**5** by HPLC) and the (*R*)-**6**-enriched fraction (2.33 g, ratio 91:9, 95.5% ee of (*R*)-**6,** 97.5% ee of (*S*)-**8** by HPLC of CH_2N_2 derivative).

(*S*)-2-Hydroxy-3-(4-methoxyphenyl)propanoic acid (*S*)-**6**: To the (*S*)-**7** enriched fraction from the previous step (2.00 g, 7.5 mmol), dissolved in MeOH (35 mL), an aq solution of KOH (5% w/w, 35 mL) was added. After completion of the hydrolysis (by TLC), most of the MeOH has been removed under reduced pressure, the mixture was acidified with HCl (1 M) and extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The barely crystalline product obtained was then triturated under Et_2O and filtered under vacuum, yielding hydroxyacid (*S*)-**6** (1.44 g, 98% yield, >99% purity by ^{1}H NMR, 82.2% ee by HPLC of CH_2N_2 derivative) $[\alpha]_D^{25} = -9.6$ (*c* 1.03, EtOH).

4.6. Ethyl (*S*)-2-hydroxy-3-(4-methoxyphenyl)-propanoate (*S*)-5

To a solution of (*S*)-**6** (1.35 g, 6.9 mmol) in dry acetone (80 mL) were subsequently added anhydrous K_2CO_3 (1.10 g, 7.9 mmol), TBAI (2.00 g, 5.4 mmol) and EtBr (0.59 mL, 7.9 mmol). The white suspension was stirred at rt until complete consumption of the acid (by TLC) and was filtered over a Celite pad, washing the filter cake with Et_2O. The still milky filtrate was concentrated under reduced pressure, treated with *n*-hexane/Et_2O (9:1, 50 mL) to precipitate the remaining TBAI and filtered again (over the same Celite pad). Evaporation of the solvent afforded the pure hydroxyester (*S*)-**5** (1.41 g, 100% yield, 99.3 purity by GC, 82.2% ee by HPLC).

4.7. Hydrolysis of (*S*)-9 and (*S*)-10

Esters (*S*)-**9** and (*S*)-**10** were hydrolysed according to the procedure reported above for the synthesis of (*S*)-**6**.

(*S*)-2-Ethoxy-3-(4-methoxyphenyl)propanoic acid, (*S*)-**11**: white solid; 1.14 g, 96% yield, >99% purity by ^{1}H NMR; 93.0% ee by HPLC of CH_2N_2 derivative; $[\alpha]_D^{25} = -16.6$ (*c* 1.21, $CHCl_3$).

(*S*)-2-Methoxy-3-(4-methoxyphenyl)propanoic acid, (*S*)-**12**: white solid; 1.01 g, 95% yield, >99% purity by ^{1}H NMR; >99.9% ee by HPLC of CH_2N_2 derivative; $[\alpha]_D^{25} = -25.2$ (*c* 1.40, $CHCl_3$).

4.8. General procedure for the demethylation and esterification of (*S*)-11 and (*S*)-12

To a suspension of NaH (0.80 g, 60% disp. in min oil, 20.1 mmol) in DMF (5 mL) was added EtSH (1.49 g, 24.1 mmol) under an N_2 atmosphere. After 30 min, a solution of acid (4.0 mmol) in DMF (5 mL) was added. After 48 h at 130 °C, the reaction mixture was quenched with a solution of $NaHCO_3$ (satd, 40 mL) and was washed with CH_2Cl_2 (3 × 20 mL). The aq phase was acidified with HCl (1 M) and extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine (2 × 20 mL), dried over Na_2SO_4 and concentrated under reduced pressure to about a volume of 5 mL. Abs. EtOH (10 mL) and HCl (0.2 mL, 12 M) were added to the solution that was heated at 75 °C distilling off a mixture of EtOAc/EtOH/H_2O, occasionally restoring the volume of EtOH. After 3 h the reaction mixture was concentrated under reduced pressure, diluted with EtOAc (50 mL) and washed with $NaHCO_3$ solution (satd, 50 mL) and brine (2 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give an oil, which crystallises on standing.

Ethyl (*S*)-2-ethoxy-3-(4-hydroxyphenyl)propanoate, (*S*)-**1**: white solid; 0.87 g, 91% yield, 99.9% purity by GC (t_R 21.97 min); 93.0% ee by HPLC of Me_2SO_4 derivative; $[\alpha]_D^{25} = -21.3$ (*c* 1.45, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, *J* = 8.6, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 4.94 (s, 1H), 4.16 (q, J = 7.1, 2H), 3.97 (dd, *J* = 6.1, 7.1 Hz, 1H), 3.60 (dq, *J* = 7.0, 9.2 Hz, 1H), 3.36 (dq, *J* = 7.0, 9.2 Hz, 1H), 2.94 (m, 2H), 1.23 (t, *J* = 7.1, 3H), 1.16 (t, *J* = 7.0, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.6, 154.4, 130.6, 129.2, 115.1, 80.4, 66.2, 60.8, 38.5, 15.0, 14.2; MS: *m*/*z* (%) 238 $[M]^+$ (3), 192 (53), 165 (23), 132 (16), 107 (100).

Ethyl (*S*)-2-methoxy-3-(4-hydroxyphenyl)propanoate, (*S*)-**2**: white solid; 0.82 g, 92% yield, 99.8% purity by GC (t_R 21.51 min); >99.9% ee by HPLC of Me_2SO_4 derivative; $[\alpha]_D^{25} = -24.2$ (*c* 1.56, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.6, 2H), 6.72 (d, *J* = 8.6, 2H), 5.47 (s, 1H), 4.18 (q, *J* = 7.1, 2H), 3.93 (dd, *J* = 5.7, 7.1, 1H), 3.36 (s, 3H), 2.95 (m_{AB}, 2H), 1.23 (t, *J* = 7.1, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 173.1, 154.4, 130.8, 128.5, 115.7, 82.4, 61.5, 58.5, 38.6, 14.5; MS: *m*/*z* (%) 224 [M]+ (3), 192 (40), 151 (20), 119 (12), 107 (100).

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