Tetrahedron: *Asymmetry* 24 (2013) 1480–1487

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

# Influence of the position of the substituent on the efficiency of lipase-mediated resolutions of 3-aryl alkanoic acids

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ARTICLE INFO

*Article history:*
Received 17 August 2013
Accepted 19 September 2013
Available online 29 October 2013

ABSTRACT

Hydrolase-catalysed kinetic resolutions to provide enantioenriched α-substituted 3-aryl alkanoic acids are described. (*S*)-2-Methyl-3-phenylpropanoic acid (*S*)-**1a** was prepared in 96% ee by *Pseudomonas fluorescens* catalysed ester hydrolysis, while, *Candida antarctica* lipase B (immob) resolved the α-ethyl substituted 3-arylalkanoic acid (*R*)-**1b** in 82% ee. The influence of the position of the substituent relative to the ester site on the efficiency and enantioselectivity of the biotransformation is also explored; the same lipases were found to resolve both the α- and β-substituted alkanoic acids. Furthermore, the steric effect of substituents at the C2 stereogenic centre relative to that for their C3 substituted counterparts on the efficiency and stereoselectivity is discussed.



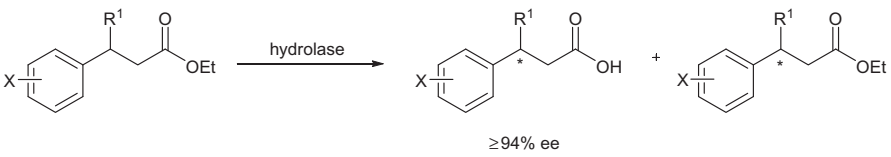
## 1. Introduction

The use of hydrolases in kinetic resolution has grown enormously in popularity, from an academic curiosity a century ago to a transformation that is routinely used in industry.[1–3] These environmentally benign, biodegradable catalysts are effective under mild reaction conditions, and combine wide substrate specificity with high regio- and enantioselectivity enabling the resolution of organic substrates with superb efficiency.[4–6]

We have recently reported on successful hydrolase-catalysed kinetic resolutions leading to a series of highly enantioenriched 3-aryl alkanoic acids through optimisation of the reaction conditions (Scheme 1).[7] Hydrolysis of the ethyl esters with a series of hydrolases was undertaken and it was found that through an appropriate choice of biocatalyst and careful control of the reaction conditions, the corresponding β-substituted acids were formed with excellent enantiopurity in each case (⩾94% ee). The steric and electronic effects on the efficiency and enantioselectivity of the biocatalytic transformation were also explored.

Leading on from the success of this lipase-mediated hydrolysis study, we extended this work to a series of 3-aryl alkanoic acids that were alkylated α to the ester moiety. Unlike our earlier study on the β-substituted series,[7] the chiral resolution of carboxylic acids with a stereogenic centre at the α-position has been examined extensively.[8–10] In particular, the literature has been dominated by the successful resolution of commercially important 2-aryl or 2-aryloxypropionic acids, the former are non-steroidal anti-inflammatory drugs while the latter are an important class of herbicides.[11–17]

Enantiopure α-substituted 3-aryl alkanoic acids are attractive synthetic targets[18] and traditional aqueous hydrolase-catalysed ester hydrolysis has been previously described for the resolution of 2-methyl-3-phenylpropanoic acid (*S*)-**1a** with excellent enantioselectivity being achieved (95% ee).[19] However, it has been




**Scheme 1.** Lipase-catalysed kinetic resolutions of β-substituted 3-aryl alkanoic acids.[7]

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http://dx.doi.org/10.1016/j.tetasy.2013.09.019

demonstrated that the hydrolysis of acid substrates encompassing more sterically demanding substituents at the C2 stereogenic centre is much less successful.[20] In fact, a dramatic reduction of activity and enantioselectivity was reported in the lipase-mediated resolution of 2-benzylbutanoic acid (*S*)-**1b** (53% ee), in comparison to **1a**, upon replacement of the methyl with the bulkier ethyl substituent.[21]

Herein we report our studies exploring a range of alkyl substituents at the C2 stereogenic centre and examining the effect of the position of the substituent relative to the active site on the efficiency of the bioresolution. The steric effect of the substituents at C2 relative to that previously reported for their C3 substituted counterparts upon enantioselection was also examined. The C2 substituted methyl, ethyl and *tert*-butyl 3-arylalkanoic acids **1a–c** were selected for investigation (Scheme 2).

## 2. Results and discussion

### 2.1. Synthesis of ethyl 3-aryl alkanoates

The 3-arylalkanoic acids **1a** and **1c** were commercially available and esterification yielded the ethyl 3-arylalkanoates **2a** and **2c** which were obtained as substrates in moderate yield (63% and 53%, respectively). Racemic ester **2a** was obtained via a Fischer esterification reaction while an alternative $S_N2$ approach employing potassium carbonate and ethyl iodide was adopted for the esterification of the sterically hindered **1c** (Scheme 3). The 3-aryl alkanoic acid **1b** was not commercially accessible and thus **2b** was synthesised via α-alkylation of ethyl butyrate employing lithium diisopropylamide and benzyl bromide (50% yield) (Scheme 3). With racemic samples of both the esters **2a–c** and acids **1a–c** in hand, chiral HPLC conditions were developed to enable determination of the enantiopurity of the substrate and product through a single injection of the reaction mixture.

### 2.2. Hydrolase-catalysed kinetic resolution to provide enantioenriched 2-methyl-3-phenylpropanoic acid 1a

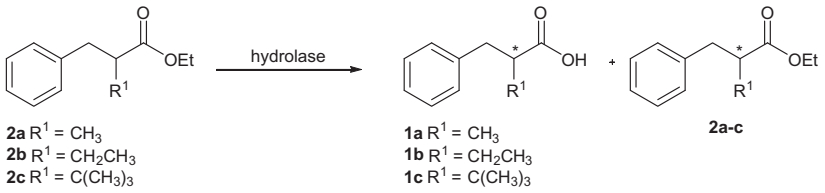
In total, 18 lipases were screened in the lipase-catalysed kinetic resolution to provide enantioenriched 2-methyl-3-phenylpropanoic acid **1a** as summarised in Table 1. All of the enzymatic resolutions investigated resulted in at least partial hydrolysis of the (*S*)-enantiomer of ethyl 2-methyl-3-phenylpropanaote **2a**. Margolin et al. previously reported the *Pseudomonas* sp. (Amano) mediated hydrolysis of the corresponding methyl ester of **1a** providing the acid (*S*)-**1a** with high enantiomeric excess (95% ee).[19] It should be noted Margolin's study was limited to the screening of the lipase *Pseudomonas* sp. (Amano), while a broad series of lipases were examined herein which identified *Alcaligenes* spp. 2 in addition to *Pseudomonas cepacia* P2 and *Pseudomonas fluorescens* for the enzymatic-catalysed hydrolysis of **2a** providing highly enantioenriched (*S*)-**1a**.[†]

The *Pseudomonas* lipases afforded the (*S*)-acid **1a** (⩾92% ee) and (*R*)-ester **2a** (>98% ee) in excellent enantiopurity (Table 1, entries 10a and 13a). *Alcaligenes* spp. 2 generated the (*S*)-acid **1a** with an improved enantioselectivity of 97% ee (Table 1, entry 9). However the rate of the resolution was decreased (conversion 41%) and therefore the enantiopurity of the recovered (*R*)-ester **2a** (67% ee) was compromised.

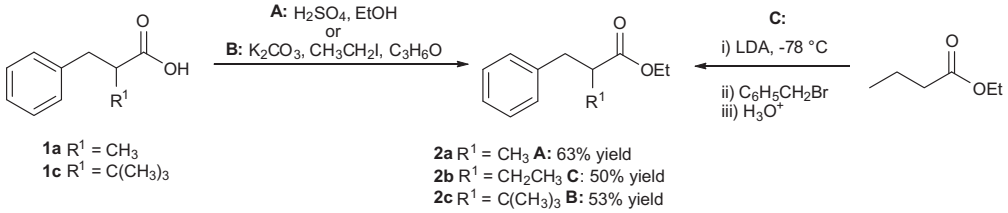
The *Candida cyclindracea* C2 and *Candida antarctica* lipase B (free and immobilised) (Table 1, entries 2, 14 and 16, respectively) mediated hydrolysis proceeded with 100% conversion to racemic acid **1a** exhibiting a lack of discrimination between the enantiomers. Bornscheuer and Kazlauskas reported that *Candida antarctica* lipase B usually displays low to moderate enantioselectivity towards carboxylic acids with a stereocentre at the α-position.[5] The acyl binding site of *Candida antarctica* lipase B is a shallow crevice. It is likely that the lower enantioselectivity towards stereocentres in the acyl part of an ester stems from fewer and/or weaker contacts between the acyl part and its binding site.

Significantly, *Alcaligenes* spp. 2 and the *Pseudomonas* lipases were also identified to yield the highest enantiopurity upon resolution of the structurally related β-substituted 3-phenylbutanoic acid (*S*)-**1d** (⩾94% ee) and the analogous ethyl ester (*R*)-**2d** (>98% ee) albeit at extended reaction times (65 h) (Scheme 4).[7] Thus, the position of the chiral methyl substituent relative to the reactive ester moiety has a limited effect on the choice of biocatalyst or high enantiopurity obtainable; however, the reaction rate is altered with efficient resolution achieved within 20 h for (*S*)-**1a** versus 65 h for (*S*)-**1d**.

Based on the screening results in Table 1, the use of *Pseudomonas cepacia* P2 and *Pseudomonas fluorescens* (entries 10a and 13a, respectively) was evidently the most attractive from the perspective of preparing enantioenriched samples of the acid



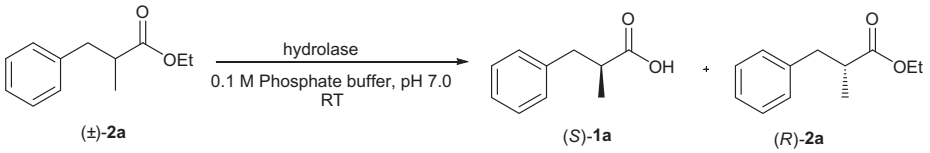

**Scheme 2.** Lipase-catalysed kinetic resolutions of α-substituted 3-aryl alkanoic acids.




**Scheme 3.** Synthesis of ethyl 3-aryl alkanoates **2a–2c**.

[†] The bacterium *Pseudomonas cepacia* is now known as *Burkholderia cepacia*.

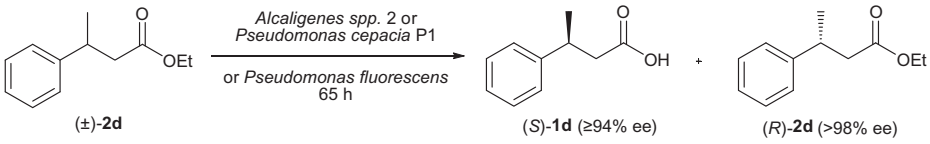
**Table 1**
Hydrolase-mediated hydrolysis of ethyl 2-methyl-3-phenylpropanoate **2a**




(±)-**2a** (*S*)-**1a** (*R*)-**2a**

| Entry | Enzyme source | Time (h) | ee[a] (%) | | Conversion[b] (%) | *E* value[b] |
|---|---|---|---|---|---|---|
| | | | Ester (R)-**2a** | Acid (S)-**1a** | | |
| 1 | *Candida cyclindracea* C1 | 20 | 3[c] | 3[c] | 50 | 1.1 |
| 2 | *Candida cyclindracea* C2 | 20 | 0[d] | 0[d] | *Complete conversion* | |
| 3 | *Alcaligenes* spp. 1 | 72 | 2 | 11 | 15 | 1.3 |
| 4 | *Pseudomonas cepacia* P1 | 72 | 53 | 68 | 44 | 6.4 |
| 5 | *Pseudomonas stutzeri* | 72 | 83 | 50 | 62 | 17 |
| 6 | *Rhizopus* spp. | 72 | 5 | 30 | 14 | 1.9 |
| 7 | *Rhizopus niveus* | 72 | 20 | 21 | 49 | 1.8 |
| 8 | *Aspergillus niger* | 72 | 5 | 33 | 13 | 2.1 |
| 9 | *Alcaligenes* spp. 2 | 20 | 67 | 97 | 41 | 132 |
| 10a | *Pseudomonas cepacia* P2 | 20 | >98 | 93 | 52 | >200 |
| *10b* | | *10* | *95* | *96* | *50* | *183* |
| 11 | *Mucor javanicus* | 20 | 14 | 21 | 40 | 1.7 |
| 12 | *Penicillium camembertii* | 72 | 3 | 17 | 15 | 1.5 |
| 13a | *Pseudomonas fluorescens* | 20 | >98 | 92 | 52 | 179 |
| *13b* | | *10* | *81* | *97* | *46* | *164* |
| 14 | *Candida antarctica* B | 20 | 0[d] | 0[d] | *Complete conversion* | |
| 15 | *Mucor meihei* | 20 | 23 | 0 | 52 | 1.9 |
| 16 | *Candida antarctica* B (immob) | 20 | 0[d] | 0[d] | *Complete conversion* | |
| 17 | *Porcine pancrease* Type II | 72 | 58 | 90 | 39 | 34 |
| 18 | *Porcine pancrease* Grade II | 72 | 11 | 47 | 19 | 3.1 |

[a] Determined by chiral HPLC analysis [Daicel Chiralcel OJ-H, Step gradient: 45 °C, injection volume 2 μL, $\lambda$ = 209.8 nm, hexane/*i*-PrOH (3% trifluoroacetic acid), 0–49 min; 99.5:0.5, flow rate 0.2 mL/min, 50 min; 95:5, flow rate 1.0 mL/min].
[b] Conversion and the enantiomeric excess (*E* value) was calculated from the enantiomeric excess of substrate ester **2a** ($ee_s$) and product acid **1a** ($ee_p$).[22]
[c] Limited enantiopurity observed, thus the direction of enantioselection should be interpreted with caution.
[d] Reaction went to 100% completion (conversion determined by $^1$H NMR), no enantioselectivity observed.




(±)-**2d** (*S*)-**1d** (≥94% ee) (*R*)-**2d** (>98% ee)

**Scheme 4.** Hydrolase-mediated hydrolysis of ethyl 2-methyl-3-phenylpropanoate **2d**.[7]

(*S*)-**1a**. It is clear from the extent of conversion (>50%) and the slightly low enantiopurity of the acid (*S*)-**1a** (92 and 93% ee) that a small amount of the ester (*R*)-**2a** undergoes hydrolysis during the kinetic resolution. Accordingly, kinetic resolutions were undertaken with a shorter reaction time of 10 h, under otherwise identical reaction conditions, and significantly, as we anticipated, the enantiopurity of the recovered acids (*S*)-**1a** was enhanced to 96% and 97% ee (entries 10b and 13b, respectively). This observation highlights that with optimisation, highly enantioenriched samples of (*S*)-**1a** could be obtained.

In order to demonstrate the practical viability of this process, *Pseudomonas fluorescens* was selected as the most suitable hydrolase for the preparative scale (1.21 mmol) hydrolysis of ethyl 2-methyl-3-phenylpropanaote **2a**. The conversion and enantiopurity of ester **2a** and acid **1a** were analysed by utilising chiral HPLC and an optimum 50% conversion was achieved with both enantiomers being produced in ⩾96% ee. The highly enantioenriched acid (*S*)-**1a** (96% ee) was isolated in 37% yield after column chromatography and the ester (*R*)-**2a** (>98% ee) was recovered in 27% yield (Table 2, entry 1).[‡]

[‡] Yields may be reduced due to reaction sampling.

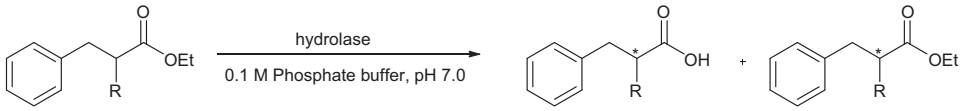
## 2.3. Hydrolase-catalysed kinetic resolution to provide enantioenriched 2-benzylbutanoic acid 1b

On increasing the size of the methyl moiety at the C2 site to the bulkier ethyl group, a sharp decrease in both efficiency and enantioselection was observed, with a number of hydrolases displaying no hydrolysis. This correlated strongly with the literature reports[20,21,26] and with the trends observed with the C3 substituted 3-arylalkanoic acids,[7] demonstrating the dramatic dependence of the synthetic and stereochemical outcome of the reaction upon the size of the alkyl group at the stereogenic centre.

Herein *Candida antarctica* lipase B (Table 3, entry 3) provided the highest enantiopurity of 2-benzylbutanoic acid (*R*)-**1b** (83% ee) via lipase-mediated hydrolysis. Previous to this result, the highest reported lipase-mediated resolution of (*S*)-**1b** was 53% ee.[21] Herein *Candida antarctica* lipase B (free and immobilised) provided the (*R*)-enantiomer selectively, while all of the other reported hydrolases preferentially hydrolysed the (*S*)-enantiomer, albeit with low to modest enantioselectivity.

The decreased efficiency in the kinetic resolution in the *Candida antarctica* lipase B mediated hydrolysis of **2b** relative to **2a** is notable. This may be due to the fact that in both the ethyl and benzyl substituents of **2b**, a methylene group is adjacent to the stereogenic centre, with discrimination of the groups only at the next carbon.

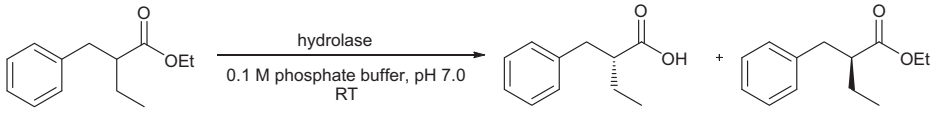
**Table 2**
Synthetic scale hydrolase-mediated kinetic resolution of C2 substituted alkanoic esters **2a–b**




| Entry | R | Hydrolase | Conversion[a] (%) | Acid | Ester | Yield (%) | ee (%) | Specific rotation[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | Me | *P. fluorescens* | 51 | (*S*)-**1a** | | 37[c] | 96 | $[\alpha]_D^{20} = +28.0$ (*c* 0.82, $CHCl_3$)[23] |
| | | | | | (*R*)-**2a** | 27[c] | >98 | $[\alpha]_D^{20} = -36.4$ (*c* 1.0, $CHCl_3$)[24] |
| 2 | Et | *Candida antarctica* B (immob) | 24 | (*R*)-**1b** | | 19 | 82 | $[\alpha]_D^{20} = -43.8$ (*c* 1.0, $CH_2Cl_2$)[25] |
| | | | | | (*S*)-**2b** | 43 | 26 | $[\alpha]_D^{20} = +6.8$ (*c* 1.0, $CH_2Cl_2$)[d] |

[a] Conversion was calculated from the enantiomeric excess of substrate ester **2a** or **2b** ($ee_s$) and product acid **1a** or **1b** ($ee_p$).[22]
[b] The direction of enantioselection of each of the acids (*S*)-**1a**[23] and (*R*)-**1b**[25] and recovered ester (*R*)-**2a**[24] was determined by comparing the specific rotation data obtained in this study with those reported in the literature.
[c] Yield may be reduced due to reaction sampling.
[d] Although the α-ethyl substituted ester (*S*)-**2b** has not previously been reported in its enantioenriched form, its absolute stereochemistry was assigned as (*S*) as it must be opposite to that of the recovered acid (*R*)-**1b**.

**Table 3**
Hydrolase-mediated hydrolysis of ethyl 2-benzylbutanoate **2b**



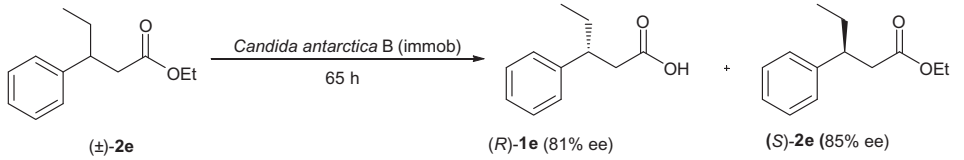

(±)-**2b** (*R*)-**1b** (*S*)-**2b**

| Entry | Enzyme source[a] | Time (h) | ee[b] (%) | | Conversion[c] (%) | *E* value[c] |
|---|---|---|---|---|---|---|
| | | | Ester **2b** | Acid **1b** | | |
| 1 | *Candida cyclindracea* C1 | 43 | 3 (*R*) | 13 (*S*) | 19 | 1.3 |
| 2 | *Candida cyclindracea* C2 | 17 | 20 (*R*) | 4 (*S*) | 83 | 1.3 |
| 3 | *Candida antarctica* B | 17 | 35 (*S*) | 83 (*R*) | 30 | 15 |
| 4 | *Candida antarctica* B | 43 | 74 (*S*) | 71 (*R*) | 49 | 14 |
| 5 | *Candida antarctica* B (immob) | 43 | 17 (*S*) | 73 (*R*) | 19 | 7.6 |
| 6 | Pig Liver esterase | 17 | 6 (*R*)[d] | 3 (*S*)[d] | 67 | 1.1 |

[a] The following hydrolases gave no conversion *Pseudomonas cepacia* P2, *Pseudomonas cepacia* P1, *Mucor javanicus*, *Pseudomonas fluorescens*, *Porcine Pancrease* Type II, *Pseudomonas stutzeri*, *Rhizopus niveus* and *Penicillium camembertii*.
[b] Determined by chiral HPLC analysis [Daicel Chiralcel OJ-H, hexane/*i*-PrOH (3% trifluoroacetic acid) = 95:5, flow rate 0.5 mL/min, ambient temperature, injection volume 2 μL, $\lambda$ = 209.8 nm].
[c] Conversion and the enantiomeric excess (*E* value) was calculated from the enantiomeric excess of substrate ester **2b** ($ee_s$) and product acid **1b** ($ee_p$).[22]
[d] Limited enantiopurity was observed, thus the direction of enantioselection should be interpreted with caution.

It should be noted that 4 of the 5 hydrolases screened which resulted in conversion (Table 3), also displayed enantioselection for the resolution of the β-ethyl substituted 3-phenylpentanoic acid **1e**, the exception being *Candida cylindracea* C1 which recorded no catalytic activity for the hydrolysis of **2e**.[7] In addition, under similar reaction conditions, the same source of enzyme *Candida antarctica* lipase B, although the immobilised version rather than the free enzyme, afforded the highest enantiopurity of (*R*)-**1e** (81% ee) (Scheme 5) comparable to the enantioselectivity obtained herein for (*R*)-**1b** (83% ee) (Table 3, entry 3). Thus, the position of the ethyl moiety had a limited impact on the choice of the biocatalyst.

Herein the enantiomeric excess of the unreacted ester (*S*)-**2b** was poor (35% ee) (Table 3, entry 3) relative to that of the β-substituted analogue (*S*)-**2e** (85% ee) (Scheme 5), due to the decreased conversion rate (30% vs 51%). Increasing the reaction time from 17 h to 43 h (Table 3, entry 4) did result in a higher conversion rate (49%) and increased the enantiomeric purity of (*S*)-**2b** (74% ee),




(±)-**2e** (*R*)-**1e** (81% ee) (*S*)-**2e** (85% ee)

**Scheme 5.** Hydrolase-mediated hydrolysis of ethyl 3-phenylpentanoate **2e**.[7]

however the enantiopurity of the acid (*R*)-**1b** was compromised (71% ee) due to the partial hydrolysis of ester (*S*)-**2b** at the extended reaction time.

Thus, the enantiodiscrimination in the hydrolysis to form the α-ethyl acid (*R*)-**1b** was somewhat less efficient than that in the corresponding β-ethyl acid (*R*)-**1e** despite the increased proximity of the stereocentre. This may again be due to the fact that in both the ethyl and benzyl substituents, a methylene group is adjacent to the stereogenic centre, with discrimination of the groups only at the next carbon.

The synthetic scale (1.95 mmol) hydrolysis of ethyl 2-benzylbutanoate **2b** was performed next. In the analytical screens, free *Candida antarctica* lipase B resulted in the highest enantioselectivity (83% ee) of the sterically hindered α-ethyl acid (*R*)-**1b** (Table 3, entry 3). However, *Candida antarctica* lipase B (immob) was utilised in the preparative-scale since immobilised lipases offer significant advantages over their free counterparts from the perspective of large-scale process efficiency.[5] Herein the *Candida antarctica* lipase B (immob) mediated resolution of **2b** on a preparative-scale resulted in a slight increase in conversion rate (24% vs 19%) and thus improved the enantiopurity of (*R*)-**1b** (82% ee) and (*S*)-**2b** (26% ee) relative to that from the small scale screen (73% ee and 17% ee, respectively) (Table 2, entry 2 vs Table 3, entry 5).

### 2.4. Hydrolase-catalysed kinetic resolution to provide enantioenriched 2-benzyl-3,3-dimethylbutanoic acid 1c

The next substrate investigated was the α-*tert*-butyl substituted ester **2c**. Given the decrease in efficiency and enantioselectivity for the α-ethyl substituted (*R*)-2-benzylbutanoic acid (*R*)-**1b** relative to the α-methyl substituted (*S*)-2-methyl-3-phenylpropanoic acid (*S*)-**1a**, challenges in efficiency were anticipated. In the screening assays, none of the lipases, including *Candida antarctica* lipase B, successfully catalysed the hydrolysis of **2c** to any extent.[§] We have previously demonstrated the ability of *Candida antarctica* lipase B to resolve sterically demanding α- and β-substituted substrates, thus proving to be the lipase of choice for the mediated resolution of (*R*)-**1b**, (*R*)-**1e** and (*S*)-**1f**.

The presence of the *tert*-butyl substituent at the α-position dramatically reduced the efficiency of the enzymatic hydrolysis. Under identical reaction conditions, the extent of the resolution of the β-substituted derivative, 4,4-dimethyl-3-phenylpentanoic acid **1f** was also extremely limited and only by increasing the reaction temperature and extending the incubation period, was the isolation of enantiopure samples of acid (*S*)-**1f** achieved, albeit with a low extent of biotransformation (Scheme 6).[7]

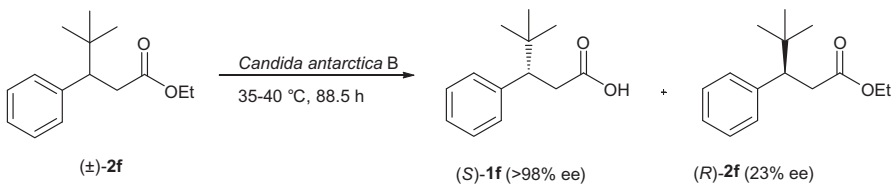
## 3. Conclusion

While α-substituted phenyl propanoic acids can be resolved using a lipase-mediated kinetic resolution, the outcome in terms of both efficiency and selectivity is strongly dependent upon the steric features of the α-substituent. Thus, when the α-substituent increased in size from a methyl to an ethyl or *tert*-butyl group, a dramatic decrease in the rate of conversion and enantioselectivity of the hydrolysis was recorded. This correlated with earlier reports in the literature[20,21,26] and with the results observed with the β-substituted 3-arylalkanoic acids.[7] Despite the steric hindrance within the active site, (*S*)-2-methyl-3-phenylpropanoic acid (*S*)-**1a** was obtained in 97% ee, via *Alcaligenes* spp. 2 catalysed hydrolysis of **2a** while *Candida antarctica* lipase B was identified as resolving the α-ethyl substituted 3-arylalkanoic acid (*R*)-**1b** (82% ee) with improved enantioselection relative to that achieved by Sih et al. (53% ee)[21] (Fig. 1). Significantly we have demonstrated that through variation of reaction time, the enantiopurity of the recovered acids (*S*)-**1a** and (*R*)-**1b** can be optimised. Thus these biotransformations have the potential to be synthetically useful processes since the hydrolysis of the slower reacting enantiomer of the esters (*R*)-**2a** and (*S*)-**2b** can be minimised by careful reaction control. Furthermore, the choice of biocatalyst was determined to be independent of the position of the substituent relative to the reactive site, with the same lipases identified for the resolution of both the α- and β-substituted 3-aryl alkanoic acids.

## 4. Experimental

### 4.1. General

Solvents were distilled prior to use as follows: dichloromethane was distilled from phosphorus pentoxide. Ethyl acetate was distilled from potassium carbonate and hexane was distilled prior to use. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Diisopropylamine was distilled from calcium hydride. The organic phases were dried over anhydrous magnesium sulfate. Infrared spectra were recorded as thin films on sodium chloride plates on a Perkin Elmer Paragon 1000 FT-IR spectrometer. NMR spectra were recorded on a 300 MHz or 400 MHz NMR spectrometer. All spectra were recorded at room temperature (~20 °C) in deuterated chloroform ($CDCl_3$), unless otherwise stated, using tetramethylsilane (TMS) as an internal standard. $^1H$–$^1H$ and $^1H$–$^{13}C$ correlations were used to confirm the NMR peak assignments. All spectroscopic details for compounds previously made were in agreement with those previously reported. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer in electrospray ionisation (ESI) mode using




(±)-**2f** (*S*)-**1f** (>98% ee) (*R*)-**2f** (23% ee)

**Scheme 6.** Hydrolase-mediated hydrolysis of ethyl 4,4-dimethyl-3-phenylpentanoate **2f**.[7]

[§] The following hydrolases gave no conversion *Pseudomonas cepacia* P1, *Pseudomonas cepacia* P2, *Candida antarctica* B, *Candida antarctica* B (immob) and *Pseudomonas fluorescens*.

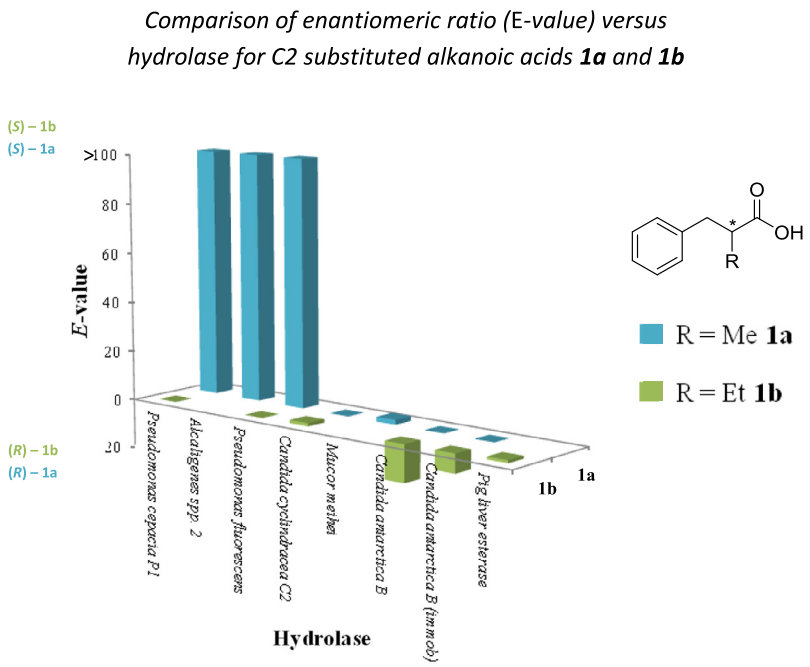



**Figure 1.** Comparison of enantiomeric ratio (*E*-value) versus hydrolase for C2 substituted alkanoic acids **1a** and **1b**.

50% water/acetonitrile containing 0.1% formic acid as eluant; samples were made up in acetonitrile. Elemental analysis was performed by the Microanalysis Laboratory, National University of Ireland, Cork, using Perkin–Elmer 240 and Exeter Analytical CE440 elemental analysers. Enantiomeric excesses were measured by high performance liquid chromatography (HPLC), using a Chiralcel® OJ-H column (5 × 250 mm) from Daicel Chemical Industries Limited. Mobile phase, flow rate, detection wavelength and temperature are included in the appropriate Tables 1 and 3. HPLC analysis was performed on a Waters alliance 2690 separations module with a PDA detector. When only one single enantiomer could be detected, the enantiomeric excess is quoted as >98%. Optical rotations were measured on a Perkin–Elmer 141 polarimeter at 589 nm in a 10 cm cell; concentrations (*c*) are expressed in g/100 mL. $[\alpha]_D^{20}$ is the specific rotation of a compound and is expressed in units of $10^{-1}$ deg cm$^2$ g$^{-1}$. All hydrolases were kindly donated by Almac Sciences and all enzymatic reactions were performed on a VWR Incubating Mini Shaker 4450.

### 4.2. Synthesis of ethyl esters

#### 4.2.1. Ethyl 2-methyl-3-phenylpropanoate 2a[27]

Sulfuric acid (concd 95–97%, 2.1 mL, 39.4 mmol) was added to a solution of 2-methyl-3-phenylpropanoic acid **1a** (2.21 g, 13.46 mmol) in absolute ethanol (40 mL) and refluxed overnight. Excess ethanol was evaporated off under reduced pressure. The crude product was dissolved in dichloromethane (45 mL) and washed with water (2 × 45 mL), a saturated aqueous solution of sodium bicarbonate (2 × 45 mL) and brine (50 mL), dried, filtered and concentrated under reduced pressure to give the crude ester **2a** (1.67 g) as a clear oil. Purification by column chromatography on silica gel using hexane/ethyl acetate 60/40 as eluent gave the pure ester **2a** (1.62 g, 63%) as a clear oil; $\upsilon_{max}$/cm$^{-1}$ (film) 2979 (CH), 1733 (CO), 1605, 1496, 1455 (Ar), 1176 (C–O); $\delta_H$ (400 MHz) 1.15 [3H, d, *J* 6.8, C(2)CH$_3$], 1.18 (3H, t, *J* 7.2, OCH$_2$C***H*****$_3$**), 2.63–2.76 (2H, m, AB of ABX, C***H*****$_2$**Ph), 3.01 [1H, dd, X of ABX, $J_{AX}$ 6.4, $J_{BX}$ 12.8, C(2)H], 4.08 (2H, q, *J* 7.2, OC***H*****$_2$**CH$_3$), 7.15–7.21 [3H, m, C(3′)H, C(4′)H and C(5′)H, ArH], 7.25–7.28 [2H, m, C(2′)H and C(6′)H, ArH].

#### 4.2.2. Ethyl 2-benzylbutanoate 2b[28]

At first, *n*-butyllithium (1.9 M in hexanes, 27 mL, 51.45 mmol) was added dropwise to freshly distilled diisopropylamine (8.5 mL, 60.65 mmol) in freshly distilled tetrahydrofuran (40 mL) at −78 °C under an atmosphere of nitrogen. Once the addition was complete, the reaction mixture was warmed to −35 °C. Ethyl butyrate (6.5 mL, 49.13 mmol) in tetrahydrofuran (35 mL) was then added dropwise to the solution and once the addition was complete, the reaction mixture was stirred for 1.5 h at −35 °C. Benzyl bromide (6.4 mL, 53.81 mmol) was then added in one portion. The reaction mixture was stirred overnight at −35 °C. The reaction was quenched by pouring the mixture onto aqueous hydrochloric acid (10%, 400 mL) and diethyl ether (200 mL). The layers were separated and the aqueous layer extracted with diethyl ether (2 × 100 mL). The combined organic layer was washed with water (100 mL), brine (100 mL), dried, filtered and concentrated under reduced pressure to give the crude ester **2b** (10.14 g) as a yellow oil. Purification by column chromatography on silica gel using dichloromethane as eluent gave the pure ester **2b** (5.08 g, 50%) as a clear oil; (found: C, 74.80; H, 8.73. $C_{13}H_{18}O_2$ requires C, 75.69; H, 8.80%); $\upsilon_{max}$/cm$^{-1}$ (film) 2966 (CH), 1732 (CO), 1605, 1496, 1456 (Ar), 1163 (C–O); $\delta_H$ (300 MHz) 0.92 [3H, t, *J* 7.4, C(4)H$_3$], 1.15 (3H, t, *J* 7.2, OCH$_2$C***H*****$_3$**), 1.44–1.77 [2H, m, C(3)H$_2$], 2.53–2.62 [1H, m, X of ABX, C(2)H], 2.74 [1H, dd, A of ABX, $J_{AB}$ 13.5, $J_{AX}$ 6.6, one of C***H*****$_2$**Ph], 2.93 [1H, dd, B of ABX, $J_{AB}$ 13.5, $J_{BX}$ 8.4, one of C***H*****$_2$**Ph], 4.06 (2H, q, *J* 7.1, OC***H*****$_2$**CH$_3$), 7.15–7.29 (5H, m, ArH); $\delta_C$ (75.5 MHz) 11.7 [CH$_3$, C(4)H$_3$], 14.2 (CH$_3$, OCH$_2$***C***H$_3$), 25.2 [CH$_2$, C(3)H$_2$] 38.2 (CH$_2$, ***C***H$_2$Ph), 49.2 [CH, C(2)H], 60.1 (CH$_2$, O***C***H$_2$CH$_3$), 126.2 [CH, C(4′)H, ArCH], 128.3, 128.9 [4 × CH, C(2′)H, C(6′)H, C(3′)H and C(5′)H, ArCH], 139.6 [C, C(1′), ArC], 175.5 [C, C(1)]; HRMS (ES$^+$): Exact mass calculated for $C_{13}H_{18}O_2$ [M+H]$^+$ 207.1385. Found: 207.1388.

#### 4.2.3. Ethyl 2-benzyl-3,3-dimethylbutanoate 2c[29]

Potassium carbonate (0.63 g, 4.58 mmol) was added to a solution of 2-benzyl-3,3-dimethylbutanoic acid **1c** (0.94 g, 4.58 mmol) in HPLC grade acetone (40 mL). Once the addition was complete, the reaction mixture was stirred for 10 min before iodoethane (1.53 g, 9.81 mmol) was added in one portion. The reaction mixture was stirred at room temperature overnight, and then filtered to remove the potassium carbonate. Acetone was evaporated under reduced pressure and at this point further filtration was performed to remove excess potassium carbonate. The crude product was dissolved in dichloromethane (50 mL) and washed with water (2 × 20 mL), a saturated aqueous solution of sodium bicarbonate (2 × 20 mL), aqueous hydrochloric acid (5%, 2 × 25 mL) and brine (30 mL). The organic extract was dried, filtered and concentrated under reduced pressure to give a mixture of 2-benzyl-3,3-dimethylbutanoic acid **1c** and ethyl 2-benzyl-3,3-dimethylbutanoate **2c** (0.67 g) as a clear oil in the ratio 13:87. Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as eluent gave the pure ester **2c** (0.57 g, 53%) as a clear oil; $\upsilon_{max}/cm^{-1}$ (film) 2963 (CH), 1729 (CO), 1605, 1496, 1456 (Ar), 1152 (C–O); $\delta_H$ (300 MHz) 1.04 (3H, t, *J* 7.1, $OCH_2C\boldsymbol{H_3}$), 1.05 [9H, s, $C(CH_3)_3$], 2.45 [1H, dd, X of ABX, $J_{AX}$ 11.4, $J_{BX}$ 3.9, C(2)H], 2.79–2.93 (2H, m, AB of ABX, $C\boldsymbol{H_2}Ph$), 3.88–4.03 (2H, sym. m, $OC\boldsymbol{H_2}CH_3$), 7.13–7.27 (5H, m, ArH).

### 4.3. Preparation of the analytically pure acid 1b by basic hydrolysis of the corresponding ethyl ester 2b

#### 4.3.1. 2-Benzylbutanoic acid 1b[30]

Aqueous sodium hydroxide (1 M, 6 mL) was added to ethyl 2-benzylbutanoate **2b** (88.5 mg, 0.43 mmol). The reaction mixture was heated at reflux while stirring overnight, then allowed cool to room temperature and extracted with diethyl ether (2 × 5 mL). The ether solution was discarded. The aqueous phase was acidified to pH 1 with aqueous hydrochloric acid (10%) and then extracted with diethyl ether (3 × 5 mL) and the combined organic extracts were washed with brine (10 mL), dried, filtered and concentrated under reduced pressure to give the pure acid **1b** (50.3 mg, 66%) as a light orange oil; $\upsilon_{max}/cm^{-1}$ (film) 2966 (OH), 1705 (CO), 1605, 1496, 1456 (Ar); $\delta_H$ (300 MHz) 0.96 [3H, t, *J* 7.5, $C(4)H_3$], 1.50–1.77 [2H, m, $C(3)H_2$], 2.57–2.66 [1H, m, X of ABX, C(2)H], 2.75 [1H, dd, A of ABX, $J_{AB}$ 13.8, $J_{AX}$ 6.9, one of $C\boldsymbol{H_2}Ph$], 2.98 [1H, dd, B of ABX, $J_{AB}$ 13.5, $J_{BX}$ 7.8, one of $C\boldsymbol{H_2}Ph$], 7.09–7.34 (5H, m, ArH).

### 4.4. Enzyme screening

#### 4.4.1. General procedure for the hydrolase-catalysed kinetic resolution of the 3-aryl alkanoic ethyl esters 2a–c (analytical scale)

A spatula tip of enzyme (~5–10 mg, amount not critical) was added to the ester substrate **2a–c** (~50 mg) in a 0.1 M phosphate buffer, pH 7 (4.5 mL). The small test tubes were sealed and agitated at 700–750 rpm and incubated for the appropriate length of time and temperature. The aqueous layer was extracted with diethyl ether (3 × 5 mL) and the combined organic extracts were filtered through Celite® and concentrated under reduced pressure. The sample was analysed by $^1H$ NMR spectroscopy, reconcentrated and dissolved in a mixture of isopropanol/hexane [10:90 (HPLC grade)]. The enantioselectivity was determined by chiral HPLC. The results of the screens are summarised in Tables 1 and 3.

#### 4.4.2. Synthetic scale hydrolase-mediated hydrolysis of ethyl 2-methyl-3-phenylpropanoate 2a

At first, *Pseudomonas fluorescens* (48.0 mg) was added to ethyl 2-methyl-3-phenylpropanoate **2a** (232.0 mg, 1.21 mmol) in a 0.1 M phosphate buffer, pH 7 (20 mL) and the reaction mixture was shaken at 750 rpm at 24 °C. An aliquot of the reaction mixture (1 mL) was withdrawn at 20 h. Following a mini work-up, chiral HPLC analysis was conducted. Conversion was estimated by an *E*-value calculator at 51%.[22] The reaction mixture was filtered at 20 h through a pad of Celite® and the hydrolase was washed with water (2 × 20 mL) and ethyl acetate (10 × 10 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 30 mL) and then acidified with aqueous hydrochloric solution (10%) and extracted with more (3 × 30 mL) ethyl acetate. The combined organic layers were washed with brine (1 × 100 mL) dried, filtered and concentrated under reduced pressure to produce a light yellow oil (186.8 mg). Purification by column chromatography on silica gel using hexane/ethyl acetate 90/10 as the eluent gave the pure ester (*R*)-**2a** (63.7 mg, 27%) as a clear oil $[\alpha]_D^{20} = -36.4$ (*c* 1.0, $CHCl_3$), >98% ee, lit.[24] $[\alpha]_D^{20} = +28.4$ (*c* 1.0, $CHCl_3$), (*S*)-isomer, 82% ee and the pure acid (*S*)-**1a** (76.6 mg, 37%) as a clear oil $[\alpha]_D^{20} = +28.0$ (*c* 0.82, $CHCl_3$), 96% ee, lit.[23] $[\alpha]_D^{20} = +30.2$ (*c* 0.82, $CHCl_3$), 99% ee. $^1H$ NMR spectra were identical to those for the racemic materials previously prepared.

#### 4.4.3. Synthetic scale hydrolase-mediated hydrolysis of ethyl 2-benzylbutanoate 2b

At first, *Candida antarctica* lipase B (immob) (407.8 mg) was added to ethyl 2-benzylbutanoate **2b** (401.4 mg, 1.95 mmol) in a 0.1 M phosphate buffer, pH 7 (20 mL). The reaction mixture was shaken at 750 rpm for 45 h at 24 °C, then the solution was filtered through a pad of Celite® and the hydrolase washed with water (2 × 20 mL) and heptane (10 × 10 mL). The layers were separated and the aqueous layer was extracted with heptane (3 × 30 mL). The combined organic layers were washed with brine (1 × 100 mL), dried, filtered and concentrated under reduced pressure to produce the pure ester (*S*)-**2b** (172.1 mg, 43%) as a clear oil $[\alpha]_D^{20} = +6.8$ (*c* 1.0, $CH_2Cl_2$), 26% ee. The aqueous layer was acidified with aqueous hydrochloric solution (10%) and extracted with (3 × 30 mL) ethyl acetate. The combined organic layers were washed with brine (1 × 100 mL), dried, filtered and concentrated under reduced pressure to produce the pure acid (*R*)-**1b** (66.2 mg, 19%) as a clear oil $[\alpha]_D^{20} = -43.8$ (*c* 1.0, $CH_2Cl_2$), 82% ee, lit.[25] $[\alpha]_D^{20} = -40.0$ (*c* 1.0, $CH_2Cl_2$), >99% ee. The conversion was estimated by an *E*-value calculator at 24%.[22] $^1H$ NMR spectra were identical to those for the racemic materials previously prepared.

## Acknowledgements

This work was carried out with the financial support of IRCSET and Eli Lilly. Nuala Maguire and Tom O'Mahony are acknowledged for their technical assistance.

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