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

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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.

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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 targets18 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
demonstrated that the hydrolysis of acid substrates encompassing more sterically demanding substituents at the C2 stereogenic centre is much less successful. 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$_2$2 approach employing potassium carbonate and ethyl iodide was adopted for the esterification of the sterically hindered 1c (Scheme 3). The 3-arylalkanoic acid 1b was not commercially accessible and thus 2b was synthesised via α-alkylation of ethyl butyrate employing lithium disopropylamide 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 of 2-methyl-3-phenylpropanoic acid (S)-enantiomer of ethyl 2-methyl-3-phenylpropanoate 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.1

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 cylindracea 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. The acyl binding site of Candida antarctica lipase B is a shallow crevice. It is likely that the lower enantioselectivity towards stereo-centres 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...
(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-phenylpropanoate 2a. The conversion and enantiopurity of ester 2a and acid 1a were analysed by utilising chiral HPLC (>98% ee) was recovered in 27% yield (Table 2, entry 1).

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 reports21,22 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.7

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 1

Hydrolase-mediated hydrolysis of ethyl 2-methyl-3-phenylpropanoate 2a

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<th>Entry</th>
<th>Enzyme source</th>
<th>Time (h)</th>
<th>ee (%)</th>
<th>Conversion (%)</th>
<th>E value</th>
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* Determined by chiral HPLC analysis [Daicel Chromatography, 0.5 mm, Step gradient: 45°C, injection volume 2 μL, λ = 209.8 nm, hexane/i-ProOH (3% trifluoroacetic acid), 0–49 min; 99.5:0.5, flow rate 0.2 mL/min, 50 min; 99.5:5, flow rate 1.0 mL/min].

* Conversion and the enantiomeric excess (E value) was calculated from the enantiomeric excess of substrate ester (S)-1a (ee,1) and product acid (S)-1a (ee,1)22.

* Limited enantipurity observed, thus the direction of enantioselection should be interpreted with caution.

* Reaction went to 100% completion (conversion determined by 1H NMR), no enantioselectivity observed.

### Scheme 4

Hydrolase-mediated hydrolysis of ethyl 2-methyl-3-phenylpropanoate 2d.7
It should be noted that 4 of the 5 hydrolases screened which resulted in conversion (Table 3), also displayed enantioselective activity. 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. 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 (30% vs 51%). Increasing the reaction time from 17 h to 43 h (Table 3, entry 4) did result in a higher conversion rate (30% vs 51%).

Errors occurred due to reaction sampling.

Although the β-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.

It should be noted that 4 of the 5 hydrolases screened which resulted in conversion (Table 3), also displayed enantioselective activity. 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. 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),
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 $\alpha$-ethyl acid (R)-1b was somewhat less efficient than that in the corresponding $\beta$-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 $\alpha$-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.2 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 $\alpha$-tert-butyl substituted ester 2c. Given the decrease in efficiency and enantioselectivity for the $\alpha$-ethyl substituted (R)-2-benzylbutanoic acid (R)-1b relative to the $\alpha$-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.5 We have previously demonstrated the ability of Candida antarctica lipase B to resolve sterically demanding $\alpha$- and $\beta$-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 $\alpha$-position dramatically reduced the efficiency of the enzymatic hydrolysis. Under identical reaction conditions, the extent of the resolution of the $\beta$-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 $\alpha$-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 $\alpha$-substituent. Thus, when the $\alpha$-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 literature20,21,26 and with the results observed with the $\beta$-substituted 3-aryalkanoic 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 $\alpha$-ethyl substituted 3-aryalkanoic acid (R)-1b (82% ee) with improved enantioselection relative to that achieved by Sih et al. (53% ee)7 (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 $\alpha$- and $\beta$-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. $^1$H$-^1$H and $^1$H$-^{13}$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

5 The following hydrolases gave no conversion Pseudomonas cepacia P1, Pseudomonas cepacia P2, Candida antarctica B, Candida antarctica B (immob) and Pseudomonas fluorescens.
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. \(\varphi_0\) is the specific rotation of a compound and is expressed in units of 10\(^{-1}\) deg cm\(^2\) g\(^{-1}\). All haldroses 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 (conc 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 hexanee/ethyl acetate 60/40 as eluent gave the pure ester 2a (1.62 g, 63%) as a clear oil; \(c_{\text{max}}\)/cm\(^{-1}\) (film) 2979 (CH), 1733 (CO), 1605, 1496, 1455 (Ar), 1176 (C–O); \(\delta_{\text{H}}\) (400 MHz) 1.15 [3H, d, J 6.8, C(2)CH\(_3\)], 1.18 [3H, t, J 7.2, OCH\(_2\)CH\(_3\)], 2.63–2.76 (2H, m, AB of ABX, CH\(_2\)Ph), 3.01 [1H, dd, X of ABX, CH\(_2\)Ph], 4.08 (2H, q, J 7.2, OCH\(_2\)CH\(_3\)), 7.15–7.21 [3H, m, C(3)\(^{3}\)H, C(4)\(^{3}\)H and C(5)\(^{3}\)H, ArH], 7.25–7.28 [2H, m, C(2)\(^{3}\)H and C(1)\(^{3}\)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, 0.6065 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\(_{22}\)O\(_{2}\) requires C, 75.69; H, 8.80%); \(c_{\text{max}}\)/cm\(^{-1}\) (film) 2966 (CH), 1732 (CO), 1605, 1496, 1456 (Ar), 1163 (C–O); \(\delta_{\text{H}}\) (300 MHz) 0.92 [3H, t, J 7.4, C(4)\(^{3}\)H\(_3\)], 1.15 [3H, t, J 7.2, OCH\(_2\)CH\(_3\)], 1.44–1.77 [2H, m, C(3)H\(_3\)], 2.53–2.62 [1H, m, X of ABX, C(2)\(^{3}\)H], 2.74 [1H, dd, D of ABX, J\(_{AB}\) 13.5, J\(_{AX}\) 6.6, one of CH\(_2\)Ph], 2.93 [1H, dd, B of ABX, J\(_{AB}\) 13.5, J\(_{AX}\) 8.4, one of CH\(_2\)Ph], 4.06 (2H, q, J 7.1, OCH\(_2\)CH\(_3\)), 7.15–7.29 (5H, m, ArH); \(\delta_{\text{C}}\) (75.5 MHz) 11.7 [CH\(_3\), C(4)\(^{3}\)H\(_3\)], 14.2 [CH\(_2\), OCH\(_2\)CH\(_3\)], 25.2 [CH\(_2\), C(3)\(^{3}\)H\(_3\)] 38.2 [CH\(_2\), OCH\(_2\)CH\(_3\)], 49.2 [CH, C(2)\(^{3}\)H], 60.1 [CH\(_2\), OCH\(_2\)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\(_{20}\)O\(_{2}\) [M+H]\(^{+}\) 207.1385. Found: 207.1388.
4.2.3. Ethyl 2-benzyl-3,3-dimethylbutanoate 2c
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; \( \nu \text{max/cm}^{-1} \) (film) 2963 (CH), 1729 (CO), 1605, 1456 (Ar), 1152 (C–O); \( \delta \text{H} \) (300 MHz) 1.04 (3H, t, J = 7.1 Hz, C(CH₃)₂), 2.79–2.93 (2H, m, AB of ABX, CH₂Ph), 3.88–4.03 (2H, sym. m, OC(CH₃)₂), 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
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 more (3 × 5 mL) ethyl acetate. The combined organic extracts were washed with brine (10 mL), dried, filtered and concentrated under reduced pressure to give the pure acid 1b (0.53 g, 66%) as a light orange oil; \( \nu \text{max/cm}^{-1} \) (film) 2966 (OH), 1705 (CO), 1605, 1501, 1486 (CH₃), 1252, 1152 (C–O); \( \delta \text{H} \) (300 MHz) 0.94 [3H, t, J = 7.1 Hz, CH₃], 2.57–2.66 [1H, m, X of ABX, CH₂], 2.75 [1H, dd, X of ABX, JAX = 13.8, JBX = 6.9, one of CH₂Ph], 2.98 [1H, dd, B of ABX, JAB = 13.5, JBX = 7.8, one of CH₂Ph], 7.09–7.34 (5H, m, ArH).

4.4. Enzyme screening

4.4.1. General procedure for the hydrolyse-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 \(^1\)H NMR spectroscopy, reconstituted and dissolved in a mixture of isopropanol/hexane [10:90 (HPLC grade)]. The enantiomeric excess was determined by chiral HPLC. The results of the screens are summarised in Tables 1 and 3.

4.4.2. Synthetic scale hydrolyse-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%. The reaction mixture was filtered at 20 h through a pad of Celite® and the hydrolyse 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 eluent gave the pure ester (R)-2a (63.7 mg, 27%) as a clear oil; \( \nu \text{D/cm}^{-1} = -36.4 \) (c 1.0, CHCl₃), >98% ee, lit. \(^{24}\) \( [\alpha]_D = +28.4 \) (c 1.0, CHCl₃), (S)-isomer, 82% ee and the pure acid (S)-1a (76.6 mg, 37%) as a clear oil; \( [\alpha]_D = +28.0 \) (c 0.82, CHCl₃), 96% ee, lit. \(^{24}\) \( [\alpha]_D = +30.2 \) (c 0.82, CHCl₃), 99% ee. \(^1\)H NMR spectra were identical to those for the racemic materials previously prepared.

4.4.3. Synthetic scale hydrolyse-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 hydrolyse 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; \( [\alpha]_D = +6.8 \) (c 1.0, CH₂Cl₂), 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; \( [\alpha]_D = -43.8 \) (c 1.0, CH₂Cl₂), 82% ee, lit. \(^{25}\) \( [\alpha]_D = -40.0 \) (c 1.0, CH₂Cl₂), >99% ee. The conversion was estimated by an \( E \)-value calculator at 24%. \(^1\)H NMR spectra were identical to those for the racemic materials previously prepared.

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References