Optimisation of lipase catalysed kinetic resolutions of 3-aryl alkanoic acids through variation of reaction conditions.

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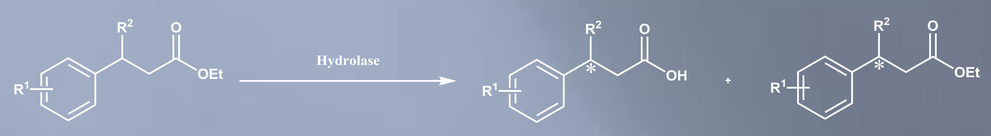
Background

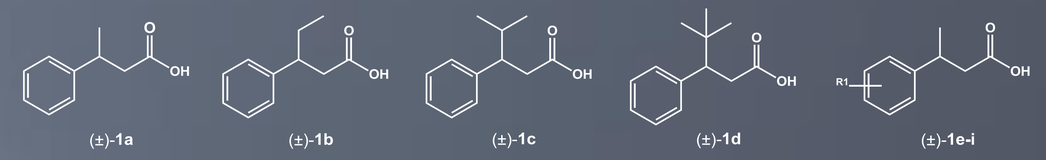
Hydrolases are excellent biocatalysts, combining wide substrate specificity with high regio- and enantioselectivity enabling the resolution of organic substrates with superb efficiency and selectivity.[1] Hydrolase catalysed kinetic resolution is widely used to provide highly enantioenriched chiral carboxylic acids, which are valuable synthetic intermediates for the preparation of a variety of compounds of biological interest (Scheme 1).



Scheme 1.

Enantiomerically pure 3-aryl alkanoic acids are used as chiral synthons in the asymmetric synthesis of antibacterial agents such as (-)-malyngolide,[2] curcumene and curcuphenol, biological important bisabolene sesquiterpenes[3] and in the synthesis of amino acids.[4-5] Within our own group, 3-aryl alkanoic acids are utilised in the synthesis of diazoketone derivatives, which in turn have been employed in Buchner cyclization reactions demonstrating excellent diastereoselectivity.[6-7]

Hydrolase catalysed non-aqueous enantioselective esterification of acids (±)-**1a-1d** (Figure 1) has previously been reported,[8] however substrate acids (±)-**1a** and (±)-**1b** were esterified with a modest to slow rate resulting in very low E values (E<2) and no ester was observed under any conditions for acids (±)-**1c** and (±)-**1d**.[8]



(±)-**1a** (±)-**1b** (±)-**1c** (±)-**1d** (±)-**1e-i**

Figure 1.

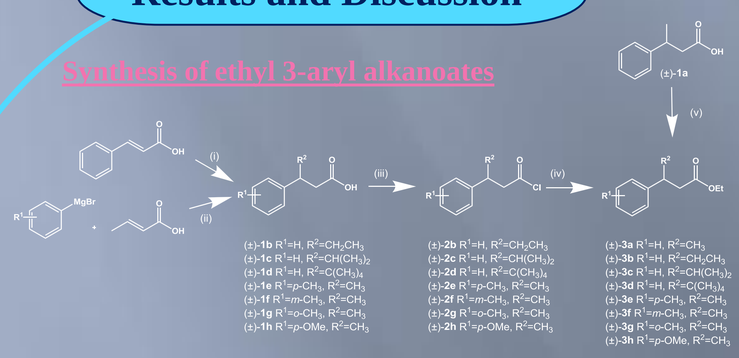
(±)-**1e** R^1=*p*-CH_3
(±)-**1f** R^1=*m*-CH_3
(±)-**1g** R^1=*o*-CH_3
(±)-**1h** R^1=*p*-OMe
(±)-**1i** R^1=*p*-F

In this study, a wide range of hydrolases were explored to establish if it is possible to generate the carboxylic acid (±)-**1a-i** in enantiopure form through kinetic resolution. Acids (±)-**1a-d**, were selected for investigation to determine the impact of steric effects at C3 on the efficiency of the kinetic resolution, while substrates (±)-**1e-i** were designed to explore both steric and electronic effects of substituents on the aromatic ring on the biotransformations.

In contrast to the limited reported success in enantioselective esterification, this study focussed on enantioslective hydrolysis and indeed it was found that through appropriate choice of biocatalyst and reaction conditions, each of the carboxylic acids could be obtained in highly enantioenriched form.

Results and Discussion

Synthesis of ethyl 3-aryl alkanoates

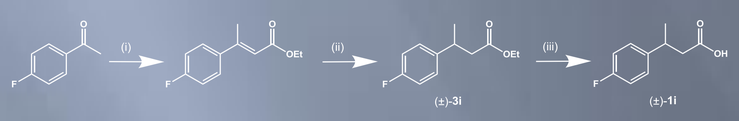


Scheme 2. Synthesis of ethyl 3-aryl alkanoate (±)-**3a-h**. Reagents: (i) R_2MgX, Et_2O, (±)-**1b-d**; (ii) Et_2O, (±)-**1e-h**; (iii) $SOCl_2$; (iv) Et_3N, EtOH, CH_2Cl_2; (v) EtOH, cat. H_2SO_4, (±)-**1a**.

Racemic ester (±)-**3a** was obtained *via* a simple Fischer esterification reaction from commercial 3-phenylbutanoic acid (±)-**1a**

The 3-aryl alkanoic esters (±)-**3b-h** were synthesized in a three step synthesis. *via* conjugate addition of a Grignard reagent to an α,β-unsaturated acid.[9] The crude carboxylic acid (±)-**1b-h** was transformed directly to the analogous acid chloride which was readily purified by vacuum distillation. Treatment of the pure acid chloride with ethanol in the presence of triethylamine led to analytically pure ester (Scheme 2).

An alternative route *via* a Wadsworth-Emmons reaction was employed in synthesising ethyl 3-(4-fluorophenyl)butanoate (±)-**3i** (Scheme 3).[10]



Scheme 3. Synthesis of ethyl 3-(4-fluorophenyl)butanoate (±)-**3i** and ethyl 3-(4-fluorophenyl)butanoic acid (±)-**1i**. Reagents: (i) $(C_2H_5O)_2P(O)CH_2CO_2Et$, NaH, THF; (ii) H_2, Pd/C, EtOH; (iii) NaOH.[10]

Chiral HPLC and crystallography studies

With racemic samples of both the esters and acids in hand, chiral HPLC conditions were developed for each ester hydrolysis in which both enantiomers of the ester and acid could be seen on a single trace. With a single injection, ready monitoring of both the efficiency and stereoselectivity of each of the hydrolase mediated transformations could be performed.

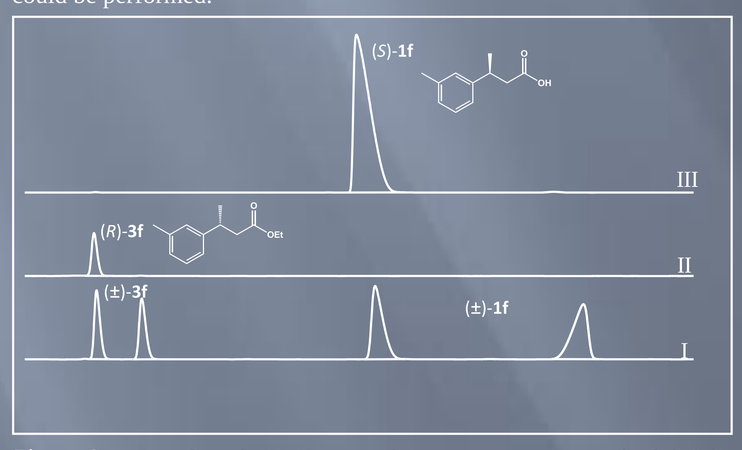


Figure 2. An overlay of HPLC traces of I: A racemic mixture of ethyl 3-(3-methylphenyl)butanoic acid (±)-**1f** and ethyl 3-(3-methylphenyl)butanoate (±)-**3f**. II: Enantiopure ethyl 3-(3-methylphenyl)butanoate (*R*)-**3f** and III: Enantiopure 3-(3-methylphenyl)butanoic acid (*S*)-**1f**.

Acids **1a-e** and **1h** have been previously reported in the literature in enantioenriched form and therefore the assignment of absolute stereochemistry for each of these compounds was made by comparison of specific rotation data. Acids **1f-g** and **1i** have not been previously reported in enantiopure form and the absolute stereochemistry was determined in each case through crystallography studies.

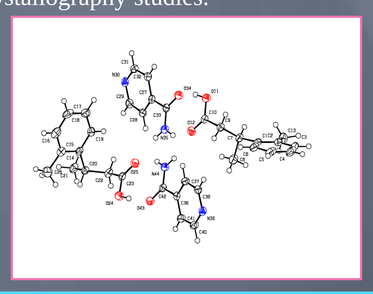
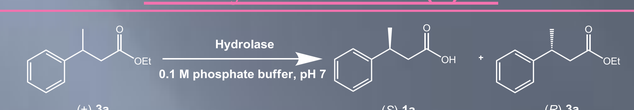


Figure 3. Co-crystal structure of (*S*)-3-(2-methylphenyl)butanoic acid (*S*)-**1g** and isonicotinamide.

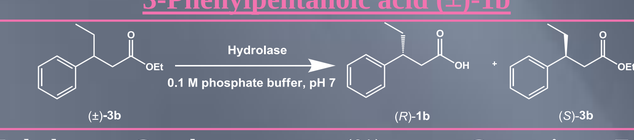
3-Phenylbutanoic acid (±)-1a



Hydrolase	ee (%)		Conversion (%)	E Value
	Ester 3a	Acid 1a		
Pseudomanas cepacia P1	99 (*R*)	94 (*S*)	51	170
Alcaligenes spp. 2	98 (*R*)	97 (*S*)	50	>200
Pseudomonas fluorescens	99 (*R*)	94 (*S*)	51	170

Burholderia cepacia hydrolysis of the methyl ester of (±)-**1a** had previously been reported (E>50) providing access to the acid (*S*)-**1a** with 89% ee.[11] In this study, *Alcaligenes spp*. yielded the acid (*S*)-**1a** with excellent improved enantioselectivity of 97% ee (E>200) by hydrolysis of the corresponding ethyl ester (±)-**3a**. Unreacted (*R*)-**3a** was recovered in 98% ee providing access to both enantiomeric series in a single resolution.

3-Phenylpentanoic acid (±)-1b



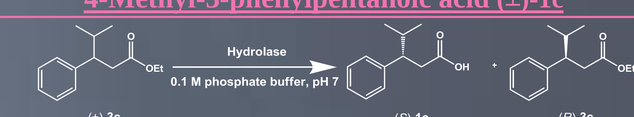
Hydrolase	Co-solvent (17% v/v)	ee (%)		Conversion (%)	E Value
		Ester 3b	Acid 1b		
Candida antarctica lipase B (immob)	-	85 (*S*)	81 (*R*)	51	25
Candida antarctica lipase B (immob)	Dioxane	72 (*S*)	92 (*R*)	44	51
Candida antarctica lipase B (immob)	Acetone	25 (*S*)	94 (*R*)	21	41
Candida antarctica lipase A	-	5 (*R*)	44 (*S*)	10	2.7

The enzymatic hydrolysis of (±)-**3b** proved to be less facile than with (±)-**3a**. Thus replacement of the methyl with the ethyl moiety at the stereogenic centre C3 resulted in a significant reduction of enzymatic activity.

The immobilised *Candida antarctica* lipase B provided the best results under aqueous conditions (E=25) and variation of reaction conditions for the hydrolysis was undertaken.

The utilisation of acetone as co-solvent, resulted in recovery of (*R*)-**1b** with 94% ee and E=41 while with dioxane E=51. Thus hydrolase catalysed resolution can be effective as a route to enantioenriched (*R*)-**1b**. The only prior report of hydrolase catalysed esterification of **1b** describes very low activity and enantioselectivity (E<2).[8] Furthermore, the acid (*S*)-**1b** has been resolved using amidase biocatalysis and again enantiopurity was lower (88% ee).[12]

4-Methyl-3-phenylpentanoic acid (±)-1c

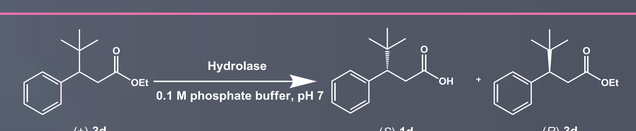


Hydrolase	ee (%)		Conversion (%)	E Value
	Ester 3c	Acid 1c		
Candida antarctica lipase B	12 (*R*)	99 (*S*)	11	>200
Candida antarctica lipase A	10 (*S*)	64 (*R*)	14	5
Candida antarctica lipase B (immob)	33 (*R*)	97 (*S*)	25	90

While the R, S labels in the acid (*S*)-**1c** are switched relative to acids (*R*)-**1a** and (*R*)-**1b** the sense of enantioselection is identical with the *R* enantiomer selectively isolated using *Candida antarctica* lipase A.

Significantly, the hydrolasses that were identified to hydrolyse (±)-**3b** were found to hydrolyse (±)-**3c** confirming that these biocatalysts can accommodate increased steric demand. In this instance (*S*)-**1c** was obtained in 99% ee using *Candida antartica* lipase B; hence no further optimisation was required.

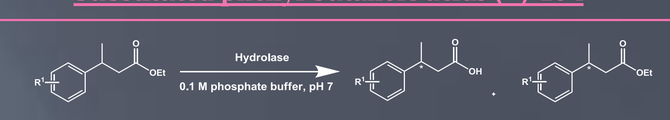
4,4-Dimethyl-3-phenylpentanoic acid (±)-1d



Hydrolase	Temperature (ºC)	ee (%)		Conversion (%)	E Value
		Ester 3d	Acid 1d		
Candida antarctica lipase B	Ambient	2 (R)	≥99 (S)	2	>200
	35 ºC-40 ºC	23 (R)	≥99 (S)	19	>200
Candida antarctica lipase A	Ambient	3 (S)	73 (R)	4	6.6
	35 ºC-40 ºC	7 (S)	81 (R)	8	10
Candida antarctica lipase B (immob)	Ambient	1 (R)	≥99 (S)	1	>200
	35 ºC-40 ºC	30 (R)	98 (S)	23	132

With both the free and immobilised *Candida antarctica* lipase B, while the extent of the hydrolyse is limited the enantioselectivity is excellent, with the acid (*S*)-**1d** isolated in enantiopure form. Increasing the temperature improved the conversion somewhat, thereby resulting in an increased enantiopurity of the unreacted ester (*R*)-**3d**.

Substituted phenyl butanoic acids (±)-1e-i



Ester substrate		Hydrolase	ee (%)		Conversion (%)	E Value
	R^1		Ester	Acid		
3e	*p*-CH_3	*Pseudomonas cepacia* P1	98 (*R*)	99 (*S*)	50	>200
3f	*m*-CH_3	*Pseudomonas fluorescens*	96 (*R*)	97 (*S*)	50	>200
3g	*o*-CH_3	*Pseudomonas fluorescens*	≥99 (*R*)	≥99 (*S*)	50	>200
3h	*p*-OCH_3	*Pseudomonas fluorescens*	≥200 (*R*)	97 (*S*)	51	>200
3i	*p*-F	*Pseudomonas fluorescens*	≥99 (*R*)	94 (*S*)	62	170

3-(4-Methylphenyl)butanoic acid (±)-**1e** had previously been resolved utilising *Pseudomonas cepacia* immobilized on ceramic particles to yield (*S*)-**1e** in 99% ee.[3] The results obtained in this study utilising the free hydrolase correlate strongly.

In all cases highly enantioenriched samples of the 3S acids and the 3R esters are readily obtained using the *Pseudomonas* biocatalysts resulting in successful hydrolysis of the *S* enantiomer with very similar outcomes to those seen with 3-phenylbutanoic acid (*S*)-**1a** indicating that the aryl substituent had little impact on the enzymatic hydrolysis.

Conclusion

In this study, a series of 3-aryl alkanoic acids (±)-**1a-i** were successfully resolved with enantiopurity ≥94% ee *via* hydrolase catalysed kinetic hydrolysis of the corresponding ethyl esters. It was apparent upon resolving acids (±)-**1a-d** that a large reduction in reaction rate and enantioselectivity was observed once the moiety at the C3 stereogenic centre increased in size greater than a methyl. Despite this, the highest obtained enantiopurities of hydrolase catalysed resolutions of 3-aryl alkanoic acids (±)-**1a-d** are reported through optimisation of reaction conditions and a viable route to both enantiomers has been identified.

Furthermore, substituents on the phenyl ring, acids (±)-**1e-i** were determined to have limited effect on the excellent enantioselectivities attainable concluding that the hydrolases can tolerate increased steric demand in the aryl group more readily than in the 3-alkyl group

Acknowledgements and References

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