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A novel lipase enzyme panel exhibiting superior activity and selectivity over lipase B from *Candida antarctica* for the kinetic resolution of secondary alcohols

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ABSTRACT

A novel, commercially available lipase enzyme panel performing kinetic bioresolutions of a number of secondary alcohols is reported. The secondary alcohols that have been chosen are known from the literature to be particularly challenging substrates to resolve. Following initial screening, four co-solvents were investigated for each lead enzyme in an effort to assess their tolerance to common organic solvents. The superiority of these novel enzymes over lipase B from *Candida antarctica* (CALB) has been demonstrated.

1. Introduction

Although the growth of carbonyl reductase technology[1–3] offers increasing access to chiral alcohols, often in quantitative yield and in enantiopure form, there are cases in which the formation of the racemic alcohol can be achieved in a far more facile manner than the synthesis of the analogous prochiral ketone. In this instance, racemate resolution can prove to be more economically viable than asymmetric reduction. This fact, coupled with the value of hydrolase enzymes as a tool to perform bio-polish ee enhancement when required, should ensure their longevity for many years to come.

The use of hydrolase enzyme mediated esterification in the resolution of chiral secondary alcohols has been well documented in the literature over the past few decades and has found widespread use both in industry and academia.[4–6] The vast majority of literature examples report lipase B from *Candida antarctica* (CALB) as being the most suitable lipase enzyme for this purpose.[7–13] Nevertheless, there are a number of substrates against which CALB displays either very poor activity, or selectivity, or both.[14,15] It was our intention to test a novel lipase panel containing 23 enzymes against substrates **1a**–**5a** (Fig. 1) that are known to be particularly difficult to resolve with the hope of achieving their successful kinetic resolution. Reported data on the analogous CALB mediated reactions are shown in Table 1.

Figure 1 depicts the substrates chosen to test the ability of the lipase panel to successfully resolve compounds that are not currently

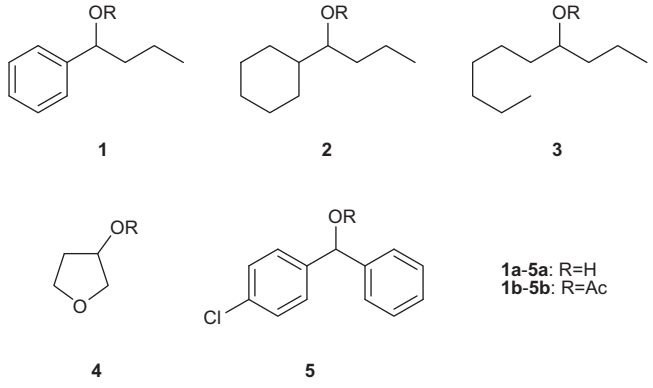

Figure 1. Secondary alcohols **1a**–**5a** that are known to be difficult to kinetically resolve, and their corresponding acetyl esters **1b**–**5b**.

catered for by the industry benchmark, CALB. For example, the CALB mediated kinetic resolution of compounds **1a**–**3a** generally requires high enzyme loading, long reaction times, and achieves only moderate E values (with compound **2a** being the exception exhibiting a reasonably good E value albeit with an extremely slow reaction rate). On the other hand, the CALB mediated acylation of compound **4a** proceeds rapidly but with little or no selectivity. This is perhaps unsurprising considering the lack of steric discrimination available to the enzyme on the tetrahydrofuran ring system. No literature data on the resolution of compound **5a** could be found. This compound has been synthesized in 81% yield and 76 % ee by coupling 4-chlorophenylboronic acid to benzaldehyde using diethylzinc in

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Table 1
Literature data on the CALB resolution of the assembled selection of secondary alcohols

Compound	Conditions	Selectivity		Conversion (%)
		(*R*)/(*S*) (ester)	*E*	
1a[a]	30 °C, 163 h	(*R*)	19.4	17.3 (1 mmol sub; 200 mg CALB)
2a[a]	30 °C, 168 h	(*R*)	65.1	5.9 (1 mmol sub; 200 mg CALB)
3a[a]	30 °C, 96 h	(*R*)	9.0	33.7 (1 mmol sub; 200 mg CALB)
4a[b]	37 °C, 5 min	(*S*)	3.0	(1.5 mmol sub; 25 mg CALB)
5a[c]	N/A	(*R*)/(*S*) depending on the ligand, with ee up to 75.8%		N/A

[a] Reported.[14]
[b] Reported.[15]
[c] No literature precedent with CALB. Synthesised using chiral organozinc reagents.[21]

the presence of 20 mol % of chiral amino alcohols derived from *cis*-(1*R*,2*S*)-2-benzamidocyclohexanecarboxylic acid. However, this is not an atom efficient process and lacks economical applicability on a larger scale.

2. Results and discussion

In order to assess the enantioselectivity obtained in the enzymatic screening reactions, racemic standards of the acetylated alcohols **1b**–**5b** were prepared using established chemistry. Analytical methods (chiral HPLC/GC) were developed based on the characteristics of each compound and are discussed in detail in the Experimental. Selected results from the 23 lipase enzyme screen are outlined in Table 2.

The initial screening results were extremely encouraging. The stereopreference of the novel lipase enzymes for each substrate tested was found to be the same as that exhibited by CALB. However, a number of distinct lead enzymes emerged as being more active and/or more selective catalysts for a number of these substrates.

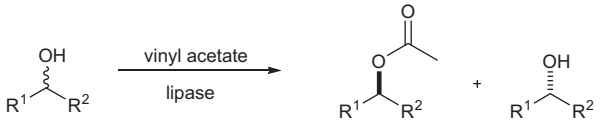
For compound **1a**, EL-01, EL-03, and EL-68 exhibited excellent activity versus the CALB control but while the use of EL-01 and EL-68 resulted in only a modest selectivity factor, EL-03 appeared to also facilitate excellent selectivity (E ~110) for the desired bioresolution.

In the same manner, EL-01 and EL-70 served to acetylate compound **2a** with excellent selectivity and activity when compared to the CALB control. The results from the lipase screen on compound **3a** were somewhat less conclusive with no lipase found that possessed the desired level of activity and selectivity. It was thought that following reaction optimization, EL-12 might prove to be a successful candidate for the desired transformation.

Compounds **4a** and **5a** were expected to be the most challenging of the substrates screened. Compound **4a**, possessing a completely unhindered alcohol moiety was overacylated in our initial screening experiments, which were allowed to react for 4 h. A reduction in the reaction time to one hour allowed the extraction of genuine selectivity factor data (<50% conversion) on the reactions in question. Although EL-14 displayed selectivity that was below a synthetically useful level, this result was still marginally superior to the analogous reaction mediated by CALB.

Interesting results were obtained from the lipase screening of compound **5a**. Extreme diversity in this lipase panel was evident from the fact that EL-13 and EL-14 displayed high, non-selective activity for the acetylation of compound **5a**, while EL-12 exhibited mild selectivity but produced only 5% conversion over the course of this reaction. The CALB control experiment produced only trace levels of racemic ester.

Table 2
Selected screening results from the lipase screen of compounds **1a**–**5a**



Compound	Enzyme number	Time (h)	Results			
			Alcohol (% ee)	Ester (% ee)	Conversion (%)	*E*
1a	EL-01	101	11	97	11	30
	EL-03	101	11	98	10	110
	EL-13	101	3	91	3	21
	EL-14	101	2	90	2	19
	EL-68	101	10	92	10	26
	CALB	101	3	>99	<1	>200
2a	EL-01	16	23	>99	22	>200
	EL-70	16	30	99	27	>200
	CALB	16	<1	94	1	32
3a	EL-01	16	26	33	43	2.5
	EL-12	16	2	84	2	11
	EL-70	16	34	33	51	2.7
	CALB	16	27	79	25	11
4a	EL-13	0.08	2	40	5	2.5
	EL-14	1	7	48	15	3.3
	EL-15	1	12	47	21	3.1
	CALB	1	8	52	14	3.4
5a	EL-01	101	3	27	1	1.8
	EL-12	101	3	53	5	3.4
	EL-13	101	3	2	60	1.1
	EL-14	101	3	1	75	1.0
	EL-68	87	3	30	9	1.9
	CALB	101	N/A	N/A	<1	N/A

It has been well documented that the presence of organic co-solvents can have a large effect on the activity and selectivity of enzymatic processes.[16–21] As a result, we thought that a brief investigation into the tolerance of this lipase enzyme panel to the most commonly used organic co-solvents was warranted. The lead enzyme from each of the acetylation reactions on compounds **1a**–**5a** was screened in the presence of hexane, MTBE, 2-methyl-THF, and toluene. CALB control reactions were carried out in parallel. The results are outlined in Table 3.

As was expected, the organic co-solvents screened against each lead enzyme produced a broad range of results. MtBE appears to be the most generally applicable organic co-solvent for reactions of this type. This reaffirmed our choice of MtBE as the co-solvent for all initial enzyme screens but the value of subsequent co-solvent screening is also highlighted in these results. For the conversion of **3a** to **3b**, hexane produces a notable increase in conversion with EL-12 compared to the analogous MTBE reaction while hexane produces a significant increase in selectivity (~50%) with CALB when compared to the analogous reaction with MtBE. Similarly, for the conversion of **5a** to **5b**, MeTHF produces a significant increase in selectivity (albeit with a marked loss in activity) with both EL-12 and CALB when compared to the analogous reactions with MtBE.

3. Conclusion

A number of secondary alcohols that are known to be difficult to obtain in enantioenriched form from their racemates via enzymatic kinetic resolution have been screened against 23 novel lipase

Table 3
Co-solvent screening results for the enzymatic acetylation of **1a–5a** to **1b–5b**, respectively, using lead enzymes identified from the initial screening

	Enzyme number	Time (h)	Solvent	Results			
				Alcohol (% ee)	Ester (% ee)	Conversion (%)	*E*
1a	EL-03	16	Hexane	14	92	3	24
			MTBE	14	98	4	103
			Me-THF	No reaction			
			Toluene	10	88	1	15
	CALB	16	Hexane	9	87	4	14
			MTBE	18	98	2	100
			Me-THF	No reaction			
			Toluene	10	97	3	67
2a	EL-70	16	Hexane	8	97	11	73
			MTBE	36	98	31	152
			Me-THF	<1	43	<1	2.5
			Toluene	6	97	8	71
	CALB	16	Hexane	<1	84	1	11
			MTBE	<1	82	1	10
			Me-THF	No Reaction			
			Toluene	<1	68	<1	5.2
3a	EL-12	16	Hexane	6	20	24	1.6
			MTBE	2	77	3	7.9
			Me-THF	No Reaction			
			Toluene	5	24	17	1.7
	CALB	16	Hexane	13	86	13	15
			MTBE	11	81	12	10
			Me-THF	No Reaction			
			Toluene	8	81	7	10
4a[a]	EL-14	16	Hexane	41	46	46	4.0
			MTBE	8	52	14	3.4
			Me-THF	1	16	4	1.4
			Toluene	6	45	12	2.8
	CALB	1	Hexane	9	23	29	1.7
		1	MTBE	7	31	19	2.0
		16	Me-THF	6	30	17	2.0
		1	Toluene	8	40	16	2.5
5a	EL-12	40	Hexane	4	48	8	3.0
			MTBE	2.5	45	6	2.7
			Me-THF	1	72	1	6.2
			Toluene	1	12	8	1.3
	CALB	40	Hexane	3	20	13	1.5
			MTBE	2	10	17	1.2
			Me-THF	2	52	4	3.2
			Toluene	No Reaction			

[a] Reactions with **4a** were carried out at 4 °C. All other reactions were carried out at 30 °C.

enzymes. Following initial enzyme screening using MtBE as the organic co-solvent, a lead enzyme was selected for each desired transformation and a small co-solvent screen was performed with 2-MeTHF, toluene, and hexane, along with a control reaction in MtBE. CALB was used throughout this entire study as a benchmark for activity and selectivity. For all five compounds **1a–5a** screened at least one novel lipase from the panel was found to outperform CALB in terms of activity and/or selectivity.

4. Experimental

4.1. Chemicals and enzymes

The novel lipase enzymes discussed herein are commercially available from both Almac and Eucodis Bioscience GmbH. Chemicals were purchased from Sigma Aldrich, Alfa Aesar and TCI. Lipase enzyme CALB (Novozym 435) was obtained from Enzagen Ltd.

4.2. Analytical methods

^{1}H NMR spectra were recorded at 400 MHz on a Bruker AV-400 spectrometer; shifts are relative to internal TMS. Conversion and enantiomeric excess measurements of compounds **1a–4a** and **1b–4b** were determined by GC analysis using a PerkinElmer Auto-System XLGC.

For 1-phenylbutan-1-ol **1a**, analysis was performed on a Varian Chirasil DEX CB column (25 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas. Method: 100 °C hold for 40 min, 5 °C min^{-1} until 180 °C, hold for 3 min; inlet temperature 250 °C; detector temperature 280 °C; flow rate: 3.4 mL min^{-1}. Retention times for (*R*)-**1a** and (*S*)-**1a** were 38.9 and 40.2 min, respectively. Retention times for (*S*)-**1b** and (*R*)-**1b** were 19.4 and 19.7 min, respectively. The assignment of the absolute stereochemistry for each enantiomer was achieved by comparison with the literature data for the analogous CALB mediated reaction.[14]

For 1-cyclohexyl-1-butanol **2a**, analysis was performed on a Varian Chirasil DEX CB column (25 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas. Method: 80 °C hold for 3 min, 1 °C min^{-1} until 130 °C, 10 °C min^{-1} until 180 °C, hold for 3 min; inlet temperature 250 °C; detector temperature 280 °C; flow rate: 3.4 mL min^{-1}. Retention times for (*S*)-**2a** and (*R*)-**2a** were 33.2 and 34.3 min, respectively. Retention times for (*S*)-**2b** and (*R*)-**2b** were 29.3 and 30.0 min, respectively. The assignment of the absolute stereochemistry for each enantiomer was achieved by comparison with the literature data for the analogous CALB mediated reaction.[14]

For 4-decanol **3a**, analysis was performed on a Varian Chirasil DEX CB column (25 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas. Method: 80 °C hold for 3 min, 1 °C min^{-1} until 130 °C, 10 °C min^{-1} until 180 °C hold for 3 min; inlet temperature 250 °C; detector temperature 280 °C; flow rate: 3.4 mL min^{-1}. Retention time for **3a** was 23.9 min. Retention times for (*S*)-**3b** and (*R*)-**3b** were 22.6 and 23.6 min, respectively. The enantiomeric excess of **3a** was determined theoretically from reaction conversion and the ee measured for **3b**. The assignment of the absolute stereochemistry for each enantiomer was achieved by comparison with the literature data for the analogous CALB mediated reaction.[14]

For 3-hydroxytetrahydrofuran **4a**, analysis was performed on a Supelco β-DEX 225 column (30 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas. Method: 80 °C hold for 3 min, 5 °C min^{-1} until 180 °C, hold for 10 min; inlet temperature 250 °C; detector temperature 280 °C; flow rate: 3.4 mL min^{-1}. Retention times for (*S*)-**4a** and (*R*)-**4a** were 13.2 and 13.5 min, respectively. For tetrahydrofuran-3-yl acetate **4b**, analysis was performed on a Varian Chirasil DEX CB column (25 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas. Method: 80 °C hold for 3 min, 5 °C min^{-1} until 140 °C, 10 °C min^{-1} until 180 °C, hold for 3 min; inlet temperature 250 °C; detector temperature 280 °C; flow rate: 3.4 mL min^{-1}. Retention times for (*R*)-**4b** and (*S*)-**4b** were 7.0 and 7.3 min, respectively. The assignment of the absolute stereochemistry for each enantiomer was achieved by comparison with the literature data for the analogous CALB mediated reaction.[15]

Enantiomeric excess measurements of (4-chlorophenyl)(phenyl)methanol **5a** were determined by normal phase HPLC on a Chiralcel IA column (250 mm × 4.6 mm × 10 μm, Daicel Chemical Industries) with UV detection (λ, 254 nm), eluent hexane/ethanol (95:5); flow rate 0.7 mL/min. Retention times for (*R*)-**5a** and (*S*)-**5a** were 13.5 and 14.3 min, respectively. Enantiomeric excess measurements of **5b** were determined by normal phase HPLC on a Chiralcel OJ column (250 mm × 4.6 mm × 10 μm, Daicel Chemical Industries) with UV detection (λ, 254 nm), eluent hexane/ethanol (95:5); flow rate 1.0 mL/min. Retention times for (*S*)-**5b** and (*R*)-**5b** esters were 9.27 and 10.42 min, respectively. It should be noted that although routine screening analysis was carried out on the IA and OJ columns as these provided excellent peak resolution, the assignment of the absolute stereochemistry for each enantiomer was achieved by comparison with the literature data

for the analogous CALB mediated reaction, using a Chiralpak AD-H column.[16]

4.2.1. (±)-1-Acetoxy-1-phenylbutane 1b

To a stirring solution of 4-dimethylaminopyridine (81 mg, 0.66 mmol) in dichloromethane (5 mL) was added triethylamine (0.51 mL, 3.66 mmol) followed by addition of 1-phenylbutan-1-ol (**1a**, 0.50 g, 3.33 mmol). Acetic anhydride (0.38 mL, 3.99 mmol) was added dropwise and the resultant solution was stirred at room temperature for two hours. The reaction mixture was washed with 10% HCl (2 × 5 mL) and 1 M $NaHCO_3$ (5 mL) and the organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to afford a clear oil (0.40 g, 63%). δ_H (400 MHz): 0.91 (3H, t, J = 7.6), 1.19–1.42 (2H, m), 1.69–1.78 (1H, m), 1.85–1.94 (1H, m), 2.07 (3H, s), 5.73 (1H, dd, J = 7.6 and 6.3), 7.26–7.36 (5H, m).

4.2.2. (±)-1-Cyclohexylbutyl acetate 2b

The title compound was prepared following the procedure for the preparation of (±)-1-acetoxy-1-phenylbutane **1b**, from 4-dimethylaminopyridine (31 mg, 0.26 mmol), triethylamine (0.24 mL, 0.18 mmol), 1-cyclohexyl-1-butanol **2a** (0.20 g, 0.13 mmol), and acetic anhydride (0.15 mL, 0.15 mmol) to afford a clear oil (156 mg, 61 %). δ_H (400 MHz): 0.90 (3H, K, J = 7.0), 0.94–1.05 (2H, m), 1.10–1.37 (5H, m), 1.42–1.52 (3H, m), 1.63–1.76 (5H, m), 2.05 (3H, s), 4.75 (1H, dd, J = 12.4 and 7.0).

4.2.3. (±)-Decan-4-yl acetate 3b

The title compound was prepared following the procedure for the preparation of (±)-1-acetoxy-1-phenylbutane (**1b**), from 4-dimethylaminopyridine (31 mg, 0.25 mmol), triethylamine (0.19 mL, 0.15 mmol), 4-decanol (**3a**, 0.20 g, 0.13 mmol), and acetic anhydride (0.15 mL, 0.15 mmol) to afford a clear oil (110 mg, 43%). δ_H (400 MHz): 0.86–0.92 (6H, m), 1.22–1.38 (10H, m), 1.44–1.56 (4H, m), 2.04 (3H, s), 4.85–4.91 (1H, m).

4.2.4. (±)-Tetrahydrofuran-3-yl acetate 4b

The title compound was prepared following the procedure for the preparation of (±)-1-acetoxy-1-phenylbutane **1b**, from 4-dimethylaminopyridine (55 mg, 0.15 mmol), triethylamine (0.35 mL, 0.25 mmol), 3-hydroxyterahydrofuran **4a** (0.20 g, 0.23 mmol), and acetic anhydride (0.26 mL, 0.27 mmol) to afford a clear oil (180 mg, 61 %). δ_H (400 MHz): 1.98-2.05 (1H, m), 2.07 (3H, s), 2.13-2.23 (1H, m), 3.81–3.96 (4H, m), 5.27-5.31 (1H, m).

4.2.5. (±)-(4-Chlorophenyl)(phenyl)methyl acetate 5b

The title compound was prepared following the procedure for the preparation of (±)-1-acetoxy-1-phenylbutane **1b**, from 4-dimethylaminopyridine (381 mg, 0.67 mmol), triethylamine (0.51 mL, 3.67 mmol), 4-chlorobenzohydrol **5a** (0.73 g, 3.33 mmol), and acetic anhydride (0.38 mL, 3.99 mmol) to afford a clear oil (516 mg, 60 %). δ_H (400 MHz): 2.16 (3H, s), 6.84 (1H, s), 7.26–7.37 (9H, m).

4.3. General enzyme screening conditions

Lipase enzyme lyophilized powder (2 mg), was added to a solution of the substrate (13.3 μmol) in the reaction solvent (0.5 mL), followed by addition of a solution of vinyl acetate (66.5 μmol) in the reaction solvent (0.5 mL). The reactions were shaken at 150 rpm at 30 °C for an appropriate amount of time. The reactions were filtered through anhydrous magnesium sulfate and analyzed directly by chiral GC or evaporated, redissolved in *iso*-propyl alcohol (HPLC grade) and analyzed by chiral HPLC. The screening results are summarized in Tables 2 and 3.

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