Enzymatic Hydrolysis and Selective Racemisation Reactions of α-Chloro Esters

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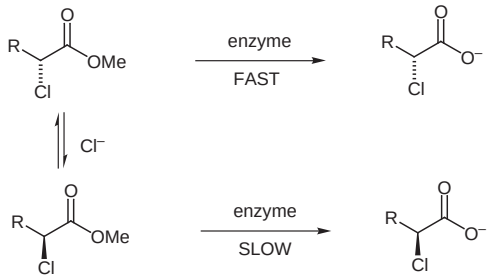
Abstract: The kinetic resolution of α-chloro esters was effected with good selectivity using CLEC (Cross-Linked Enzyme Crystals) enzymes. The selective racemisation of α-chloro esters in the presence of α-chloro acids enabled a successful dynamic kinetic resolution reaction to be performed.

Key words: enzymes, kinetic resolution, asymmetric synthesis, racemisation

The use of enzymes to hydrolyse esters under kinetic resolution conditions is a widely used method for the preparation of enantiomerically enriched carboxylic acids.[1] Herein, we report on the use of enzymatic hydrolysis for α-chloro esters. Although not widely used as substrates, there is reasonable precedent for reactions of this type,[2] as well as for the corresponding resolution of α-bromo esters.[3] We were interested in the possibility of establishing a dynamic kinetic resolution,[4] according to Scheme 1.

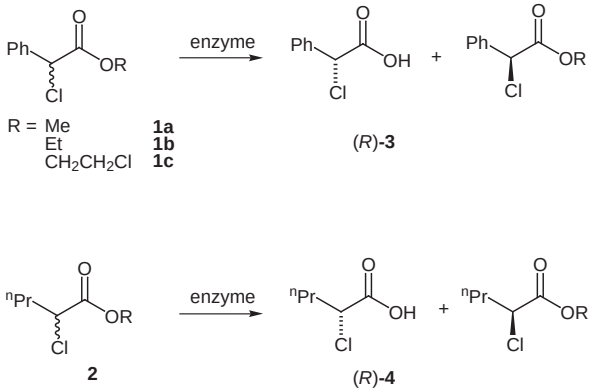


Scheme 1

In order for this approach to be successful, it is necessary to find suitable conditions for a simple kinetic resolution of the α-chloro ester substrate, and to find conditions that facilitate racemisation of the α-chloro ester selectively in the presence of the α-chloro acid. Additionally, the dynamic resolution will only be effective if the enzyme and racemising agent do not interfere with each other. These points are addressed in turn.

Firstly, the α-chloro esters **1a–c** and **2** were prepared by conventional methodology. Starting materials **1a–c** were prepared by the reaction of an appropriate alcohol with commercially available α-chlorophenylacetyl chloride (which could also be prepared by treatment of racemic mandelic acid with thionyl chloride). Starting material **2** was prepared by diazotisation of racemic norvaline with sodium nitrite in ethereal hydrochloric acid followed by acid-catalysed esterification in methanol.[5]

Various enzymes and reaction conditions were investigated for the hydrolysis of substrates **1a–c** and **2** (Scheme 2). We found that commercially available CLEC's (Cross-Linked Enzyme Crystals) were particularly reliable, and that conditions using phosphate buffer were the most satisfactory. Ethyl acetate was employed as a co-solvent, and the reactions stirred vigorously to ensure thorough mixing during the course of the reactions. The results of these kinetic resolution reactions afforded reasonable to good levels of enantioselectivity in the acid and recovered ester, as shown in Table 1. It is noteworthy that the Altus 17 and Altus 20 CLEC's (derived from *Candida cylindracea* lipase and *Pseudomonas cepacia* lipase, respectively) afford opposite selectivity to each other, although Altus 17 is generally more selective. Altus 20 did not catalyse the hydrolysis of ester **2**. Similar results could be obtained using water in place of phosphate buffer, regulating the pH at 7–8 using an autotitrator.



Scheme 2

With good procedures in hand for the kinetic resolution of α-chloro esters, we turned our attention to the selective racemisation of α-chloro esters in the presence of α-chloro acids (as their carboxylate salts).

The displacement of halide by halide can lead to racemisation or epimerisation in suitable substrates. Literature examples involving chloride, bromide and iodide have all been reported.[6–8]

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Table 1 Enzyme Hydrolysis of Esters **1** and **2**

Substrate[a]	Enzyme	Acid ee (%)	Ester ee (%)	Conversion (%)
1a	Altus 17[b]	91 (*R*)	89 (*S*)	47
1a	Altus 20[c]	78 (*S*)	32 (*R*)	26
1b	Altus 17	86 (*R*)	62 (*S*)	35
1b	Altus 20	67 (*S*)	65 (*R*)	50
1c	Altus 17	87 (*R*)	72 (*S*)	42
1c	Altus 20	39 (*S*)	22 (*R*)	30
2	Altus 17	80 (*R*)	73 (*S*)	48
2	Altus 20			0

[a] All reactions were run in phosphate buffer/EtOAc (4:1), r.t., 48 h.
[b] Altus 17 is *Candida cylindracea* lipase (in CLEC form).
[c] Altus 20 is *Pseudomonas cepacia* lipase (in CLEC form).

Table 2 Competitive Racemization Study of Ester **1a** and Acid **3**

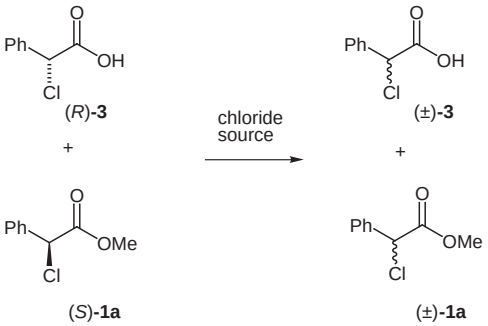
Racemising Agent[a]	Initial ee (%)		Final ee (%)	
	Ester **1a**	Acid **3**	Ester **1a**	Acid **3**
Aliquat 336[b]	31	64	0	42
Adogen 464[b]	31	64	0	41
Bu_4NCl	31	64	20	61
Bu_4NCl	31	64	11	55
$MePh_3PCi$	31	64	22	36
ResinCl	31	64	0	53

[a] All reactions were run in water, adjusted to pH 7 with added KOH, 40 °C, 16 h.
[b] Aliquat 336 and Adogen 464 are commercially available methyltrialkylammonium chloride salts.

We anticipated that racemisation would be faster for α-chloro esters than for α-chlorocarboxylates, since for the ester, π*(C=O) is able to stabilise the S_N2 transition state by accepting electron density. However, the carboxylate is more electron-rich and therefore less able to facilitate an S_N2 reaction. The partitioning of chloride, carboxylate and ester between the aqueous and organic phases is also expected to influence the relative rates of hydrolysis.

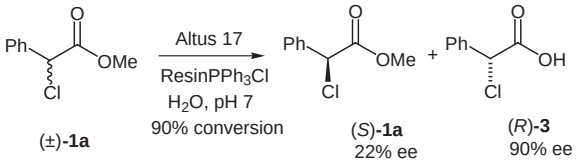
A competitive racemisation study of ester **1a** and acid **3** confirmed that the ester underwent racemisation more easily than the acid (as its carboxylate salt) (Scheme 3). Several sources of chloride were employed, and the results are provided in Table 2. The resin bound phosphonium chloride was prepared by treating Merrifield resin (containing a benzyl chloride group) with triphenylphosphine. The resin bound racemising agent was especially effective, although as this material aged, its ability to also racemise the carboxylate increased. Racemisation experiments in phosphate buffer were found to be less satisfactory, as racemisation of the acid occurred at a similar rate to racemisation of the ester.

The aliphatic chloro ester and chloro acid/carboxylate were inert to racemisation under these conditions, and it is clear that a different racemisation method will be required for substrates of this type.[6]



Scheme 3

Combining the kinetic resolution reaction with the selective racemisation procedure proved to be dependent upon the choice of chloride source. Only the resin bound phosphonium chloride provided good results, with other chloride sources leading to low hydrolysis rates or low selectivities. However, use of 0.6 equivalent of the freshly prepared resin-bound phosphonium chloride, allowed a useful dynamic resolution of ester **1a** to be achieved using an autotitrator (pH 7 controlled by the addition of KOH). Further evidence that the ester was being selectively racemised under the reaction conditions was provided by the fact that the small amount of recovered ester had a low enantiomeric excess (Scheme 4).



Scheme 4

In summary, CLEC enzymes derived from *Candida cylindracea* lipase and *Pseudomonas cepacia* lipase are able to catalyse the kinetic resolution of α-choro esters, affording good levels of selectivity. The racemisation of α-chloro esters was found to proceed more quickly than for the corresponding carboxylates with a variety of chloride sources. A successful dynamic kinetic resolution was achieved by combining the enzyme and racemisation chemistry.

Methyl 2-Chloro-2-phenylethanoate (1a);[9] Typical Procedure

α-Chlorophenylacetyl chloride (5.0 g, 0.027 mol) was added slowly to a solution of Et_3N (4.45 mL, 0.032 mol) in MeOH (30 mL) at 0 °C. After 3 h at r.t., the reaction was poured into H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo. The resultant residue

was distilled under reduced pressure to give **63** (4.7 g, 95%) as a colourless oil; bp 70 °C/3 Torr.

IR (film): ν = 1756 (C=O), 1455 (C–O), 1163 cm^{-1} (C–O).

^1H NMR (400 MHz, $CDCl_3$): δ = 7.5 (2 H, m, C_6H_5), 7.3 (3 H, m, C_6H_5) 5.4 (1 H, s, CHCl), 3.8 (1 H, s, CH_3).

^{13}C NMR ($CDCl_3$): δ = 168.9 (C=O), 135.9 (CH), 129.5 (CH), 129.1 (CH), 128.1 (CH), 59.3 (CHCl), 53.7 CH_3).

MS (EI): *m/z* (%) = 184 (23, M^+), 125 (97).

A similar procedure was employed for the synthesis of esters **1b** and **1c**, using EtOH or 2-chloroethanol (1.2 equiv) in THF, respectively.

Ester **1b**
Yield: 4.9g (95%); colourless oil; bp 98 °C/1.5 Torr.

IR (film): ν = 1752 (C=O), 1455 (C–O), 1158 (C–O), 727 cm^{-1} (C–Cl).

^1H NMR (400 MHz, $CDCl_3$): δ = 7.5 (2 H, m, C_6H_5), 7.3 (3 H, m, C_6H_5) 5.4 (1 H, s, CHCl), 4.2 (2 H, q, *J* = 7.0 Hz, CH_2), 1.2 (3 H, t, *J* = 7.0 Hz, CH_3).

^{13}C NMR ($CDCl_3$): δ = 168.4 (C=O), 136.1 (CH), 129.4 (CH), 129.0 (CH), 128.1 (CH), 62.8 (CHCl), 59.5 (CH_2), 14.4 (CH_3).

MS (CI): *m/z* (%) = 199 (89, MH^+), 178 (66), 163 (100), 102 (73).

HRMS: *m/z* found for MH^+ 199.0526. $C_{10}H_{11}ClO_2$ requires MH 199.0527.

Ester **1c**
Yield: 5.3g (91%); pale yellow oil; bp 120 °C/1.5 Torr.

IR (film): ν = 1756 (C=O), 1455 (C–O), 1161 (C–O), 728 cm^{-1} (C–Cl).

^1H NMR (400 MHz, $CDCl_3$): δ = 7.5 (2 H, m, C_6H_5), 7.4 (3 H, m, C_6H_5) 5.4 (1 H, s, CHCl), 4.4 (2 H, t, *J* = 6.5 Hz, CH_2O), 3.7 (2 H, t, *J* = 6.5 Hz, CHCl).

^{13}C NMR ($CDCl_3$): δ = 168.1 (C=O), 135.5 (CH), 129.6 (CH), 129.1 (CH), 128.1 (CH), 65.9 (CHCl), 59.1 (CH_2) 41.4 (CH_2Cl).

MS (EI): *m/z* = 233 (10%, M^+).

Anal. Calcd for $C_{10}H_{10}Cl_2O_2$: C, 51.5; H, 4.3. Found: C, 51.2; H, 4.3.

2-Chloropentanoic Acid (4)[5]
$NaNO_2$ (2.97 g, 0.043 mol) was added in portions (slowly) to a solution of norvaline (4.46 g, 0.038 mol) in ethereal HCl at 0 °C. After 16 h at r.t., the reaction was poured into H_2O and extracted with CH_2Cl_2 (3 × 10 mL). The extract was washed with $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled by Kugelrohr (132 °C/2 Torr) giving acid **4** (3.67g, 71%) as a colourless oil.

IR (film): ν = 3170 (COO–H), 1713 (C=O), 772 (C–Cl), 751 cm^{-1} (C–Cl).

^1H NMR (400 MHz, $CDCl_3$): δ = 9.1 (br s, 1 H, CO_2H), 4.3 (dd, 1 H, *J* = 8.2, 5.9 Hz, CHCl), 2.0 (m, 1 H, CH_2), 1.5 (m, 2 H, CH_2), 1.0 (t, 3 H, *J* = 7.4 Hz, CH_3).

^{13}C NMR ($CDCl_3$): δ = 175.2 (C=O), 57.0 (CHCl), 36.7 (CH_2), 19.3 (CH_2), 13.3 (CH_3).

MS (CI): *m/z* = 136.9 (72%, M^+).

Methyl 2-Chloropentanoate (2)
Concd HCl (3 drops) were added to a stirred solution of **4** (1.0g, 8.33 mmol) in MeOH (10 mL) at r.t. After 16 h, the reaction was poured into aq sat. $NaHCO_3$ solution (10 mL) and extracted with EtOAc (3 × 10 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled by Kugelrohr to afford ester **2** (1.1 g, 82%) as a colourless oil.

IR (film): ν = 2963 (C–H), 1652 (C=O), 1464 (C–O), 1171 (C–O), 769 cm^{-1} (C–Cl).

^1H NMR (400 MHz, $CDCl_3$): δ = 0.9 (t, *J* = 7 Hz, 3 H, CH_3), 1.1–1.6 (m, 2 H, CH_2), 1.6–2.2 (m, 2 H, CH_2CHCl), 3.7 (s, 3 H, OCH_3), 4.1 (t, *J* = 7 Hz, 1 H, CHCl).

^{13}C NMR ($CDCl_3$): δ = 159.1 (C=O), 56.6 (CHCl), 44.4 (CH_3), 38.4 (CH_3), 38.4 (CH_2), 35.0 (CH_2), 27.0 (CH_2), 19.8 (CH_3).

MS (CI): *m/z* = 166.1 (95%, M^+).

Enzymatic Hydrolysis of α-Chloro Esters; General Procedure
Candida cylindracea lipase (Altus 17, CLEC) (3 mg, 10% w/w) was added to a solution of ester (25 mg, 0.14 mmol) in phosphate buffer/solvent (5 mL, 80:20) at r.t. At the suitable time (48 h, see Table 1) the resulting solution was filtered through Celite, acidified with 0.1 M HCl, and extracted with EtOAc (3 × 5 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo, giving ester and acid. Alternatively, reactions were run in H_2O–solvent controlled at pH 7–8 using an autotitrator. The enantiomeric excess was determined by HPLC or GC analysis (Table 1).

Resin Bound Phosphonium Salt
Ph_3P (1.0 g, 3.8 mmol, 10 equiv), was added to a solution of Merrifield resin (0.5 g, 1.26 nM/g, 1 equiv) in toluene (15 mL). The mixture was heated at reflux for 16 h, filtered, then washed successively with toluene (10 mL), H_2O (10 mL), EtOH (10 mL) and finally with CH_2Cl_2 (10 mL). The resin was used immediately.

Competitive Racemisation Studies; General Procedure
Racemising agent (1 equiv) was added to a solution of ester (*S*)-**1a** (25 mg, 0.14 mmol) and acid (*R*)-**3** (25 mg, 0.15 mmol) in phosphate buffer–solvent (5 mL, 80:20) or alternatively pH controlled H_2O–solvent (80:20). At the suitable time (16 h, see Table 2) the resulting solution was filtered through silica gel, the phases separated and the aqueous layer acidified with 0.1 M HCl and extracted with EtOAc (3 × 5 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo, giving ester (*R/S*)-**1a** and (*R*)-**3** that was analysed using chiral HPLC (Table 2).

The Dynamic Kinetic Resolution of Ester 1a
Candida cylindracea lipase (Altus 17, CLEC) (3 mg, 10% w/w) was added to a solution of **1a** (25 mg, 0.14 mmol) and racemising agent (0.1–0.6 equiv) in phosphate buffer–solvent (5 mL, 80:20) or pH controlled H_2O–solvent (5 mL, 80:20). At a suitable time (24 h) the resulting solution was filtered through Celite, the phases separated and the aqueous phase acidified with 0.1 M HCl and extracted with EtOAc (3 × 5 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo, to give ester **1a** and acid **3** which were analysed by chiral HPLC or GC.

Selected HPLC and GC Data

Ester **1a**
Chiralcel OD, 25 cm, 99:1 hexane–isopropanol, 1 mL/min, *R*: 6.5 min, *S*: 7.1 min.

Acid **3**

Chiralcel OD, 25 cm, 99:1 hexane–isopropanol–formic acid, 240:10:1, 1 mL/min, *R*: 13.97 min, *S*: 15.08 min.

Ester **2** and acid **4** were more easily analysed by chiral GC.

Ester **2**
Gammadex-120, 30 m, 110 °C, 15.90 min and 16.21 min.

Acid **4**

Gammadex-120, 30 m, 150 °C, 44.01 min and 44.45 min.

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