Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 3197–3209

TETRAHEDRON: *ASYMMETRY*

Influence of ester chain length, enzyme, and physical parameters on lipase-catalysed hydrolyses of *meso*-oxiranedimethanol esters. Part 2

Jonathan D. Moseley*,† and James Staunton

University Chemical Laboratories, Lensfield Road, Cambridge, CB2 1EW, UK

Received 28 June 2000; accepted 11 July 2000

Abstract

A range of *meso*-oxiranedimethanol diesters were prepared and subjected to hydrolysis by a selection of microbial and porcine-derived lipases. The best chain length was then subjected to a wider range of lipases. The physical reaction parameters were further investigated for the best enzyme/substrate combination. The results revealed the effects of ester chain length, choice of enzyme, and physical reaction parameters on the enzymic hydrolysis. Using a combination of the these three variables, it is demonstrated that this is a versatile approach for the optimisation of an enzymic hydrolysis for a given substrate. Additionally, we also report on an efficient, multigram synthesis of oxiranedimethanol diesters from cheap achiral precursors, their successful enzymic hydrolyses to alcohols in good chemical yield and e.e., and their conversion to synthetically useful aldehydes in high yield. Both enantiomeric series can readily be accessed from a single enzymic hydrolysis result.

1. Introduction

In our initial communication,[1] we reported on the desymmetrisation of *meso*-diesters **3** by enzymic hydrolysis, and demonstrated that the ester side chain can be tailored to improve the enantiomeric excess (e.e.) of the resulting alcohols **4**. Since both ester side chains are discarded in subsequent manipulations, there is considerable scope for further optimisation of this hydrolysis by choice of the ester or other parameters. We now report on the extension of our investigations on the preferred butyrate diester **3d** which was subjected to hydrolysis by an extensive range of enzymes. The physical parameters were then varied for the preferred substrate/enzyme combination.

* Corresponding author. Tel: +44 117 938 5601; e-mail: jonathan.moseley@astrazeneca.com

† Present address: AstraZeneca, Process Research and Development, Avlon Works, Severn Road, Hallen, Bristol BS10 7ZE, UK.

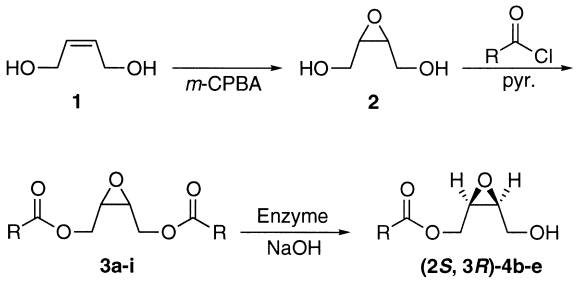
0957-4166/00/$ - see front matter
PII: S0957-4166(00)00276-7

Lastly, we detail the simple transformations required to generate further useful synthetic intermediates, and access to both enantiomeric series.

2. Results and discussion

2.1. Preparation of substrates for enzymic hydrolysis

A range of *meso*-diesters **3a–i** was readily prepared by esterification of the known epoxide **2** derived by *m*-CPBA epoxidation of *cis*-butene-1,4-diol **1** adapted from the method of Schloss[2] (Scheme 1). An alternative epoxidation method was also tried using *m*-CPBA in acetonitrile instead of chloroform.[3] Although this method gave a nominally higher yield of diol **2** (89% versus 60%), it performed poorly in the esterification step giving a lower overall yield for the two steps.[4] Schloss' epoxidation method was adequate on moderate scale-up and so this was used in preference. Surprisingly, the majority of the simple alkyl esters were unknown at the time of this work, although some more exotic derivatives had been prepared by Schloss.



Scheme 1. R = **a**, Me; **b**, Et; **c**, *i*Pr; **d**, *n*Pr; **e**, *n*Pen; **f**, *t*Bu; **g**, *t*Amyl; **h**, *n*Hep; **i** Ph

2.2. Variation of enzymes

In our earlier communication,[1] we reported the effects on the hydrolysis of varying the side chain substitution for the propionate **3b**, *iso*-butyrate **3c**, *n*-butyrate **3d** and hexanoate **3e** diesters. A limited range of enzymes was used, including the more common LPF, PLE and PPL.[5] In summary, it was concluded that the butyrate ester has an ideal chain length for many lipases, although the results for the propionate ester were also worth considering. The *iso*-butyrate and hexanoate esters gave only moderate results.[1] Other workers have achieved high e.e.s with the acetate diester **3a**.[6,7]

We therefore chose to assay the butyrate diester **3d** against a wider range of commercially available microbial and porcine derived lipases and esterases.[5] The e.e.s were determined from the specific rotations of the purified alcohol **4d** and correlated against Mosher's ester formation[8] of a number of representative samples, the integration of the ^{19}F NMR signals agreeing within ±1% of the specific rotation figures. The results are collected in Table 1, and where the same enzyme has been used, are generally similar to those of Marples.[9] The results varied from effectively racemic hydrolyses (entries 1–3 and 5) through to those displaying good enantiotopic discrimination in up to 85% e.e. (entries 8 and 11). The majority of the lipases, and all of the best

Table 1
Enzymic hydrolysis of butyrate diester **3d** with different enzymes

Entry	Enzyme[a]	Source[b]	Conv.[c] (%)	Time (hrs)	Yield[d] (%)	e.e.[e] (%)	Product[f]
1	CCL	S	100	8.0	70	12	-
2	LAN	F	100	100	27	9	-
3	LCL	F	100	1.5	65	6	-
4	LMJ	F	100	10	62	71	+
5	LPF	F	100	1.0	65	10	+
6	LPR	F	25	96	[50][g]	56	-
7	LRA	F	100	16	67	73	+
8	LRD	F	100	12	76	85	+
9	LRN	F	100	72	40	64	+
10	PLE	S	50	6.0	[60][g]	21	+
11	PPL	F	100	6.5	73	84	+
12	PPL	S	100	5.5	67	79	+

a. See reference 5 for abbreviations used. b. F = Fluka; S = Sigma. c. Based on equivalents of NaOH added to readjust pH to 7.0. d. Isolated yield after chromatography. e. Based on specific rotation and Mosher's ester determination. f. (+) = (2*S*, 3*R*); (-) = (2*R*, 3*S*) enantiomers. g. [ ] = calculated for 100% conversion.

results, yielded the (+)-(2*S*,3*R*) enantiomer **4d** in good chemical yield (62–76%). Only one lipase gave a fair result for the opposite enantiomer (LPR, 56%), and this was by far the slowest hydrolysis (entry 6). PLE was also slow and as expected did not cope well with a four carbon chain. The best results were achieved for LRD and PPL (Fluka).[10] Interestingly LRD, which is an extracellular fungal lipase that selectively hydrolyses triacylglycerols, has itself been the subject of mutagenesis studies to alter its side chain length selectivity over its regioselectivity.[11] Further work with this lipase was not continued due to its higher cost (£500/g against £0.30/g for PPL) and given the consistently good results achieved with PPL, we continued the investigation of physical parameters with this lipase alone.

2.3. Variation of physical parameters

We then investigated the effects of several physical parameters on the hydrolysis of butyrate diester **3d** specifically with PPL (Fluka) including pH, temperature, concentration, enzyme equivalents, and the use of phosphate buffers and co-solvent (Table 2). Grandjean[6] had conducted a limited study of the first of these parameters and found that generally for the butyrate (and acetate) diesters, a moderate lowering of both the pH (from 7.0 to 6.0) and temperature (from 20 to 4°C) resulted in a 5–10% improvement in e.e. This was not the case in our hands (entries 2, 3 and 8) with no significant improvement seen, although the results were effectively no worse, being within 5%.[12] The use of dilute phosphate buffer did however result in a detectably worse result at two pHs (entries 4 and 5). This may have been due to the higher ionic strength of the aqueous solvent forcing a less than ideal performance from the lipophilic lipase.

Table 2
Enzymic hydrolysis of butyrate diester **3d** with PPL under different reaction conditions

Entry	Variation[a]	pH	Temp. (°C)	Time (hrs)	Yield[b] (%)	e.e.[c] (%)
1	Standard	7.0	20	5.0	78	84
2	Lower pH	6.5	28	3.0	62	79
3	Lower pH	6.0	23	8.0	67	83
4	Phosphate buffer	7.0	20	1.5	75	60
5	Phosphate and lower pH	6.0	22	2.5	69	65
6	Double concentration	7.0	20	3.0	74	79
7	Higher temperature	7.0	40	1.5	80	68
8	Lower temperature	6.5	4	[7][d]	45	79
9	Portionwise addition	7.0	19	4.5	74	80
10	After storage	7.0	20	3.0	76	84

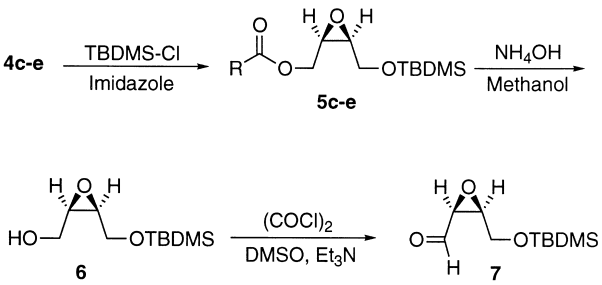
a. Entry describes variation from standard conditions (entry 1). b. Isolated yield after chromatography.
c. Based on optical rotation and Mosher's ester determination. d. 70% complete after 7 hours

Doubling the concentration (entry 6) resulted in a predictable halving of the reaction time, but with no benefit on the e.e. Increasing the temperature to 40°C (entry 7) also reduced the reaction time, but the resulting e.e. was lower.

A co-solvent of methanol (20% v/v with water) was tried with PPL and the hexanoate diester **3e**, a low melting solid (m.p. 28–29°C). However, an identical result in e.e. of 65% was achieved in similar reaction time (4 hours) to the control preparation without methanol,[1] but with the yield being lower (25% versus 42%), probably due to mechanical loss on work-up in the solubilising liquors. The use of a co-solvent therefore made no difference to the overall reaction profile. Portionwise addition was also used due to concern over enzyme inactivation through either poisoning or mechanical degradation (due to grinding by the magnetic stirrer),[13] but no improvement over the standard result was achieved (entry 9), indicating that this was not a factor. Finally, as a control, re-screening with the sample of Fluka PPL after 12 months storage at 4°C but without special precautions against moisture etc. gave a similar result (entry 10) also indicating no degradation over this period.

2.4. Derivatisation of hydrolysis products

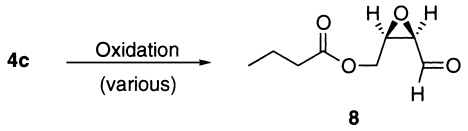
We converted the individual alcohols (+)-**4c**, (+)-**4d** and (+)-**4e** to the common alcohol **6** and aldehyde **7** via the protected esters **5c**–**e**, respectively. This was achieved by TBDMS-protection, mild alkaline hydrolysis and Swern oxidation[14] (Scheme 2). This provided a further check on e.e. values by comparison of the $[\alpha]_D$'s across the series for the common products **6** and **7**, and also with other literature sources with which they were found to be in good agreement.[15] This facile reaction sequence could be achieved in 89% yield over the three steps without the need for chromatography in a semi-telescoped fashion on the crude oils (conducted on the butyrate half ester (+)-**4d**). The largest scale hydrolysis undertaken on this work was 37 mmol, but there is no



Scheme 2.

obvious reason why this could not have been scaled up much further, as there appeared to be no agitation or physical form issues. Larger scale Swern oxidations would also not have been expected to present a problem. Finally, although the highest e.e.s achieved in this work were 80–84%, Grandjean has shown that e.e.s of these levels can be improved to effectively enantiomeric purity in one crystallisation for **4d**, irrespective of the potential improvement that might be achieved with other later intermediates i.e. **5**, **6** and **7**.

Alcohol **6** is effectively the enantiopode of the (+)-**4** alcohols but with a different protecting group, thus demonstrating that both enantiomeric series can be readily accessed from a single hydrolysis result, irrespective of the result of the enzymic hydrolysis. Direct oxidation of (+)-**4d** to the useful aldehyde **8** proved unsatisfactory under a wide range of mild oxidation conditions (i.e. TPAP, MnO_2, Moffat, Corey–Kim, PCC/PDC all gave $<25\%$ yields) (Scheme 3). The best yields for the isolated material were achieved using the Dess–Martin[16] reagent (50% impure) or Swern oxidation (45%, also impure). The product appears to be unstable to chromatographic purification since Pale[17] has generated the aldehyde **8** by modified Swern oxidation and reacted it in situ in higher overall yields.



Scheme 3.

3. Conclusions

In summary, we conclude our study of the influence of side chain substitution on the result of enzymic hydrolyses, showing that the side chain can be tailored to give potentially the best result for a given molecule. Investigation of both a range of enzymes and physical parameters provides a further option for optimisation. If the reverse *trans*-esterification process is considered as well,[18,19] then there are multiple options for optimisation, making this approach ideal for process development work, perhaps best conducted under a factorial experimental design (FED) approach.

We also report on an efficient, multigram synthesis of oxiranedimethanol diesters **3** from cheap achiral precursors; their successful enzymic hydrolyses in good chemical yield and e.e. to the

alcohols **4**; and demonstrate facile access to both enantiomeric series through conversion to synthetically useful derivatives **6** and **7**. These manipulations were carried out on a multigram scale, without purification of the intermediates, to give the valuable chiral aldehyde **7** in 90% over three steps.

4. Experimental

4.1. General methods

Optical rotations were measured on a Perkin–Elmer 241 polarimeter using the sodium D line at 589 nm. Melting points were determined on a Reichert hot-stage apparatus or a Büchi 510 melting point apparatus and are uncorrected. Infra red spectra were run on Perkin–Elmer 297 or 1310 spectrophotometers between 4000 and 600 cm^{-1} and only readily assigned absorbances above ~1500 cm^{-1} are listed. The following abbreviations are used: s = strong, m = medium, w = weak, b = broad. ^1H NMR spectra were recorded on Bruker WM-250 (250 MHz), AC-250 (250 MHz), or AM-400 (400 MHz) spectrometers. Chemical shifts are given in ppm relative to tetramethylsilane at $\delta = 0$, with tetramethysilane or the residual non-deuterated solvent signal as internal standard. In addition to the conventional abbreviations for multiplicity, the following have been used for first order spectra: qu = quintet, sx = sextet, sp = septet, m = multiplet, b = broad. ^{13}C NMR spectra were recorded on Bruker AM-400 (100.6 MHz) or AC-250 (62.9 MHz) spectrometers and were proton decoupled. Chemical shifts are given in ppm relative to tetramethylsilane at $\delta = 0$. An 'attached proton test' was routinely carried out on all samples to aid in the assignment of spectra. ^{19}F NMR spectra were recorded on a Bruker WM-250 spectrometer at 235 MHz with proton decoupling, and are given in ppm relative to $CFCl_3$ as external standard. Low resolution electron impact (EI^+) mass spectra were recorded on Kratos MS902 (ex A.E.I.) or MS 890 spectrometers. Fast atom bombardment (FAB^+) mass spectra (carrier gas Xe) were recorded on Kratos MS 50 or MS 890 spectrometers. High resolution EI^+ mass spectra were recorded on a Kratos MS 30 spectrometer. Elemental analyses (C and H) were performed by the 'in-house' analytical service.

Analytical TLC was carried out on commercially prepared plates coated with 0.25 mm of self-indicating Merck Kieselgel 60 F_{254} and were developed using a 5% solution of phosphomolybdic acid in ethanol, in addition to visualisation by UV and I_2. Preparative flash silica chromatography was performed using Merck Kieselgel 60 (230–400 mesh). Enzymes were purchased from Fluka or Sigma as noted.[5] All other reagents and solvents were purified and dried as necessary according to standard procedures.

4.2. cis-*2,3-Epoxybutane-1,4-diol* **2**[2]

cis-2-Butene-1,4-diol **1** (5.7 ml, 69 mmol) and *m*-CPBA (21.7 g of 50–55% technical grade stabilised with water, 69 mmol) were stirred in a solution of ether (80 ml) at 5°C in the dark for 3 hours. The resulting white precipitate was collected by filtration, washed with ice-cold ether (20 ml maximum) and dried in vacuo to give a hygroscopic white powder (4.45 g, 62%). Mp 50–52°C (lit.,[2] 60%, mp 50–52°C). ^1H NMR (250 MHz, D_2O) 3.86 (2H, dd, $J_{Gem} = 12.6$, $J_{Vic} = 2.9$ Hz, 2×OC*H*H), 3.60 (2H, dd, $J_{Gem} = 12.6$, $J_{Vic} = 2.9$ Hz, 2×OCH*H*), 3.32 (2H, m, epoxide); MS (EI^+) 104 (M^+, 5), 87 (18), 73 (66), 61 (100). Anal. calcd for $C_4H_8O_3$: C, 45.97; H, 7.63. Found: C, 46.14; H, 7.76.

4.3. General preparation of diesters **3a–i**

The following procedure, exemplified by the preparation of **3d** below, was applied to epoxy diol **2** with the appropriate acid chloride to generate in an analogous fashion the following diesters, for which physical and spectroscopic data is supplied.

4.3.1. cis-2,3-Epoxybutane-1,4-dibutyrate **3d**

Epoxy diol **2** (2.0 g, 19.2 mmol), pyridine (7.5 ml, 90 mmol) and DMAP (trace) were dissolved in dichloromethane (40 ml) and cooled to –5°C. Butyryl chloride (6.0 ml, 57.7 mmol) was added dropwise over 15 min keeping the temperature below 5°C. The reaction was allowed to warm to rt overnight and quenched with ice (5 g). After stirring for 2–3 hours at rt, the solution was diluted with dichlromethane (40 ml), washed sequentially with 1.0 M H_2SO_4 (3×40 ml), saturated $NaHCO_3$ (2×40 ml), dried (Na_2SO_4) and concentrated to an orange oil (7 g). The crude oil was purified by flash silica gel chromatography in 4:1 hexane:ether (R_f 0.28) to yield the title compound as a yellow oil or a low melting solid (4.1 g, 87%). Mp 13–14°C (neat); IR ($CHCl_3$) 2900m, 2840m, 1737s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.33 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=4.0$ Hz, 2×OC*H*H), 4.10 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.8$ Hz, 2×OCH*H*), 3.27 (2H, m, epoxide), 2.33 (4H, t, $J=7.4$ Hz, 2×CH_2CO), 1.65 (4H, sx, $J=7.4$ Hz, 2×CH_2CH_3), 0.94 (6H, t, $J=7.4$ Hz, 2×CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) 173.23 (C=O), 61.97 (CH_2O), 53.35 (epoxide), 35.84 (*C*H_2CO), 18.30 (*C*H_2CH_3), 13.59 (CH_3); MS (EI^+) 245 (M+1, 3%), 229 (15), 201 (45), 157 (100). Anal. calcd for $C_{12}H_{20}O_5$: C, 58.99; H, 8.27. Found: C, 59.10; H, 8.38.

4.3.2. cis-2,3-Epoxybutane-1,4-diacetate **3a**

A pale yellow solid, 87%. R_f 0.48 (3:2 hexane:EtOAc); mp 40–42°C (neat); IR ($CHCl_3$) 1743s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 4.31 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=3.8$ Hz, 2×OC*H*H), 4.08 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.7$ Hz, 2×OCH*H*), 3.27 (2H, m, epoxide), 2.09 (6H, s, 2×Me); ^{13}C NMR (100.6 MHz, $CDCl_3$) 170.59 (C=O), 62.16 (CH_2O), 53.26 (epoxide), 35.84 (*C*H_2CO), 20.67 (CH_3); MS (FAB^+) 189 (M+1, 100%), 154 (50), 137 (65), 129 (25), 107 (18), 87 (20), 69 (18). Anal. calcd for $C_8H_{12}O_5$: C, 51.05; H, 6.30. Found: C, 51.05; H, 6.44.

4.3.3. cis-2,3-Epoxybutane-1,4-dipropionate **3b**

A pale yellow solid, 75%. R_f 0.24 (2:1 hexane:ether); mp 24–26°C (neat); IR ($CHCl_3$) 1739s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.35 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=4.0$ Hz, 2×OC*H*H), 4.11 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.8$ Hz, 2×OCH*H*), 3.27–3.33 (2H, m, epoxide), 2.40 (4H, q, $J=7.5$ Hz, 2×CH_2CH_3), 1.17 (6H, t, $J=7.5$ Hz, 2×CH_2CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) 173.96 (C=O), 62.01 (CH_2O), 53.28 (epoxide), 27.21 (*C*H_2CO), 8.89 (CH_3); MS (FAB^+) 217 (M+1, 100%), 173 (8), 143 (34), 69 (18). Anal. calcd for $C_{10}H_{16}O_5$: C, 55.32; H, 7.62. Found: C, 55.53; H, 7.47.

4.3.4. cis-2,3-Epoxybutane-1,4-diisobutyrate **3c**

A pale yellow oil, 78%. R_f 0.28 (4:1 hexane:ether); IR ($CHCl_3$) 2900w, 1734s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 4.31 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=4.1$ Hz, 2×OC*H*H), 4.11 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.5$ Hz, 2×OCH*H*), 3.28 (2H, m, epoxide), 2.59 (2H, sp, $J=6.9$ Hz, 2×Me_2CH), 1.16 (12H, d, $J=6.9$ Hz, 2×$(CH_3)_2CH$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 176.74 (C=O), 62.08 (CH_2O), 53.32 (epoxide), 33.81 (Me_2CH), 18.90 (CH_3); MS (EI^+) 244 (M^+, 2%), 201 (20), 157 (100), 143 (100), 114 (25), 71 (100). Anal. calcd for $C_{12}H_{20}O_5$: C, 58.99; H, 8.27. Found: C, 59.22; H, 8.48.

4.3.5. cis-*2,3-Epoxybutane-1,4-dihexanoate* ***3e***

A yellow solid, 72%. R_f 0.21 (5:1 hexane:ether); mp 28.5–29.5°C; IR ($CHCl_3$) 2930m, 2865m, 1736s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.32 (2H, dd, $J_{Gem}=12.4$, $J_{Vic}=3.9$ Hz, 2×OC*H*H), 4.09 (2H, dd, $J_{Gem}=12.4$, $J_{Vic}=6.8$ Hz, 2×OCH*H*), 3.27 (2H, m, epoxide), 2.34 (4H, t, $J=7.5$ Hz, 2×CH_2CO), 1.63 (4H, qu, $J=7.4$ Hz, 2×CH_2CH_2CO), 1.29 (8H, m, 2×CH_2CH_2), 0.88 (6H, t, $J=6.7$ Hz, 2×CH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$) 173.42 (C=O), 61.948 (CH_2O), 53.36 (epoxide), 33.97, 31.22, 24.51 and 22.25 (4×CH_2), 13.84 (CH_3); MS (FAB^+) 301 (M+1, 100%), 185 (17), 154 (82), 137 (78), 136 (76). Anal. calcd for $C_{16}H_{28}O_5$: C, 64.07; H, 9.48. Found: C, 63.96; H, 9.41.

4.3.6. cis-*2,3-Epoxybutane-1,4-di*-tert-*butyrate* ***3f***

A pale yellow oil, 55%. R_f 0.34 (4:1 hexane:ether); IR ($CHCl_3$) 2988m, 2973m, 2874m, 1732s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.31 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=4.1$ Hz, 2×OC*H*H), 4.10 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.7$ Hz, 2×OCH*H*), 3.27 (2H, m, epoxide), 1.21 (18H, s, 2×*t*Bu); ^{13}C NMR (62.9 MHz, $CDCl_3$) 178.10 (C=O), 62.14 (CH_2O), 53.17 (epoxide), 38.66 (*C*Me_3), 27.02 (CH_3); MS (EI^+) 273 (M+1, 57%), 171 (77), 157 (53), 116 (25), 101 (20), 85 (51), 69 (37), 57 (100). Anal. calcd for $C_{14}H_{24}O_5$: C, 61.83; H, 9.06. Found: C, 61.73; H, 8.90.

4.3.7. cis-*2,3-Epoxybutane-1,4-di*-tert-*butylacetate* ***3g***

A yellow oil, 46%. R_f 0.32 (4:1 hexane:ether). Anal. calcd for $C_{16}H_{28}O_5$: C, 63.71; H, 9.46. Found: C, 63.96; H, 9.41.

4.3.8. cis-*2,3-Epoxybutane-1,4-dioctanoate* ***3h***

A white solid, 56%. R_f 0.23 (4:1 hexane:ether); mp 47–48°C; IR ($CHCl_3$) 2920s, 2860m, 1735s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.33 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=4.0$ Hz, 2×OC*H*H), 4.09 (2H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.8$ Hz, 2×OCH*H*), 3.27 (2H, m, epoxide), 2.34 (4H, t, $J=7.5$ Hz, 2×CH_2CO), 1.62 (4H, qu, $J=7.4$ Hz, 2×CH_2CH_2CO), 1.27 (16H, m, 2×$(CH_2)_4$), 0.86 (6H, t, $J=6.7$ Hz, 2×CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) 173.44 (C=O), 61.99 (CH_2O), 53.35 (epoxide), 34.00 (*C*H_2CO), 31.60, 29.03, 28.87, 24.82 and 22.56 (5×CH_2), 14.03 (CH_3); MS (EI^+) 357 (M+1, 2%), 285 (14), 213 (19), 199 (100), 188 (13), 141 (16), 127 (100), 99 (11), 83 (99). Anal. calcd for $C_{20}H_{36}O_5$: C, 67.57; H, 10.22. Found: C, 67.36; H, 10.20.

4.3.9. cis-*2,3-Epoxybutane-1,4-dibenzoate* ***3i***

A white powder, 63%. R_f 0.18 (4:1 hexane:ether); mp 118–119°C (neat). Anal. calcd for $C_{18}H_{16}O_5$: C, 69.18; H, 4.98. Found: C, 69.21; H, 5.17.

4.4. General method for Mosher's ester formation[8]

Samples (20 mg) of the enzyme hydrolysis products (alcohols **4b**–**e**) were converted to their Mosher's ester derivatives as follows: The alcohol (0.15 mmol) was stirred for between 3 and 24 hours with DMAP (18 mg, 0.15 mmol), Et_3N (100 μl) and (+)-(*S*)-MTPA-Cl (30 μl) in dichloromethane (0.5 ml) dried with a few 3 Å molecular sieves. The reaction was quenched with 3-dimethylaminopropylamine (50 μl) and applied directly to a small flash silica gel chromatography column, eluting with EtOAc–hexane mixtures (typical values, R_f 0.28 in 4:1 hexane:EtOAc), taking care to collect all high-running materials. The resulting combined fractions were assayed by ^{19}F NMR spectroscopy to determine the e.e. from the relative integration of the CF_3 signal. ^{19}F NMR (235 MHz, $CDCl_3$) –72.17 and –72.22 (varying ratios).

4.5. General procedure for enzymic hydrolyses

The reactions were carried out in an appropriately sized wide-necked beaker on the open bench. Rapid stirring was achieved by a flat magnetic stirrer bar. A pH meter electrode and a thermometer were immersed in the solution at all times. Careful positioning of these monitors was necessary to avoid the liquid's vortex, and also to avoid splashing, which over the long reaction times could result in substantial mechanical loss of solution volume and hence reagents and product. Glass-distilled water, adjusted to appropriate pH with either a few drops of 1.0 M NaOH or 0.1 M KH_2PO_4 solutions after the addition of the appropriate diester **3b–e**, was used. In a few cases a 0.1 M K_2HPO_4 buffered solution adjusted to pH 7.0 was used. On the 5.0 mmol scale, the following quantities of enzymes were used: microbial lipases (Fluka), 40 mg except LPF and LRD, 10 mg; PPL (Fluka), 50 mg; CCL and PPL (Sigma), 100 mg; PLE (Sigma), 3 μl.

The reactions started with the addition of the enzymes to the rapidly stirred suspension. A 1.0 M NaOH solution was added by burette to maintain the pH at close to or below the appropriate pH (usually pH 7.0). On addition of 1 equivalent of alkali, the reaction mixture was quickly extracted into an organic solvent to halt the reaction. The crude concentrates were then purified by flash silica gel chromatography. One standard reaction is given below for the dibutyrate ester **3d** as an example. The hydrolyses run in phosphate buffer (Table 2, entries 4 and 5) used 0.1 M KH_2PO_4 adjusted to the required pH with a few drops of 1.0 M NaOH. The portionwise addition reaction (Table 2, entry 9) was run at 37 mmol, and eight 40 mg portions of PPL were added at half hour intervals.

4.5.1. (+)-(2S,3R)-4-Butanoyloxy-2,3-epoxybutan-1-ol **4d**

A suspension of diester **3d** (1.22 g, 5.0 mmol) and PPL (Fluka, 50 mg) were vigourously stirred at rt in glass-distilled water (40 ml) adjusted to pH 7.0. A 1.0 M NaOH solution (5.0 ml, 5.0 mmol) was titrated into the reaction mixture to maintain the pH at or just below 7.0 until nearly the full equivalent of NaOH had been consumed. The aqueous solution was promptly extracted with EtOAc (6×30 ml). The combined organic extracts were washed once with a saturated brine solution (30 ml), dried (Na_2SO_4) and concentrated to dryness (763 mg). The crude oil was purified by flash silica gel chromatography in 2:1 ether:hexane (R_f 0.22) to yield the title compound as a pale yellow oil or a low melting solid (627 mg, 73%). $[\alpha]_D^{23}$ +15.1 (*c* 1.03, $CHCl_3$, 84% e.e.); mp 20–21°C (neat) (lit.[6] 20±3°C, pentane–CH_2Cl_2); IR ($CHCl_3$) 3200–3600bs, 2940m, 2870m, 1725s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 4.32 (1H, dd, $J_{Gem}=12.4$, $J_{Vic}=5.3$ Hz) and 4.14 (1H, dd, $J_{Gem}=12.4$, $J_{Vic}=5.6$ Hz, CO_2CH_2), 3.82 (1H, dd, $J_{Gem}=12.4$, $J_{Vic}=5.3$ Hz) and 3.78 (1H, dd, $J_{Gem}=12.7$, $J_{Vic}=5.4$ Hz, CH_2OH), 3.18–3.27 (2H, m, epoxide), 2.34 (2H, t, $J=7.4$ Hz, CH_2CO), 2.25 (~1H, bs, OH), 1.66 (2H, sx, $J=7.4$ Hz, CH_2CH_3), 0.95 (3H, t, $J=7.4$ Hz, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) 173.80 (C=O), 61.60 and 60.15 (2×CH_2O), 56.14 (C-2), 53.77 (C-3), 35.86 (CH_2CO), 18.27 (CH_2CH_3), 13.55 (CH_2CH_3); MS (FAB^+) 175 (M+1, 100). Anal. calcd for $C_8H_{14}O_4$: C, 54.88; H, 8.01. Found: C, 55.15; H, 8.12.

4.5.2. (+)-(2S,3R)-4-iso-Butanoyloxy-2,3-epoxybutan-1-ol **4c**

A pale yellow oil. $[\alpha]_D^{23}$ +14.4 (*c* 1.04, $CHCl_3$, 25% e.e.); R_f 0.14 (2:1 ether:hexane); oil; IR ($CHCl_3$) 3500bm, 2930w, 2870w, 1720s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.32 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=5.3$ Hz) and 4.14 (1H, dd, $J_{Gem}=12.2$, $J_{Vic}=5.5$ Hz, CO_2CH_2), 3.88 (2H, d, $J=4.5$ Hz, CH_2OH), 3.20 (2H, m, epoxide), 2.55 (1H, sp, $J=7.0$ Hz, $(CH_3)_2CH$), 2.30 (1H, bs, OH), 1.70 (6H, d, $J=7.0$ Hz, $(CH_3)_2CH$); ^{13}C NMR (100.6 MHz, $CDCl_3$) 177.33 (C=O), 61.54

and 60.19 (2×CH_2O), 56.09 (C-2), 53.32 (C-3), 33.67 (Me_2CH), 18.90 (CH_3); MS (EI^+) 175 (M+1, 4), 161 (62), 143 (85), 131 (100), 89 (100), 71 (100). Anal. calcd for $C_8H_{14}O_4$: C, 54.88; H, 8.01. Found: C, 54.61; H, 7.85.

4.5.3. (+)-(2S,3R)-4-Hexanoyloxy-2,3-epoxybutan-1-ol ***4e***

A low melting yellow solid. $[\alpha]_D^{23}$ +7.5 (*c* 0.59, CH_2Cl_2, 65% e.e.); R_f 0.31 (2:1 ether:hexane); mp 28–29°C (neat); IR ($CHCl_3$) 3400–3650bm, 2920m, 2860m, 1730s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.34 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=5.4$ Hz) and 4.15 (1H, dd, $J_{Gem}=12.2$, $J_{Vic}=5.6$ Hz, CO_2CH_2), 3.85 (1H, dd, $J_{Gem}=12.5$, $J_{Vic}=5.5$ Hz) and 3.79 (1H, dd, $J_{Gem}=12.5$, $J_{Vic}=5.2$ Hz, CH_2OH), 3.19–3.29 (2H, m, epoxide), 2.45–2.65 (1H, bs, OH), 2.37 (2H, t, $J=7.5$ Hz, CH_2CO), 1.65 (2H, qu, $J=7.4$ Hz, CH_2CH_2CO), 1.31 (4H, m, CH_2CH_2), 0.91 (3H, t, $J=6.4$ Hz, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) 174.02 (C=O), 61.56 and 60.15 (2×CH_2O), 56.14 (C-2), 53.77 (C-3), 34.02 (CH_2CO), 31.12, 24.51, 22.26 (CH_2), 13.87 (CH_3); MS (FAB^+) 203 (M+1, 39), 176 (10), 150 (19). Anal. calcd for $C_{10}H_{18}O_4$: C, 59.37; H, 8.99. Found: C, 59.64; H, 8.91.

4.6. General preparation of silyl esters ***5c–e***

The following procedure for the preparation of silyl esters **5c–e** is exemplified below for **5d**; only physical and spectroscopic data are provided for the other examples.

*4.6.1. (+)-(2R,3S)-4-(*tert*-Butyldimethylsilyloxy)-1-butanoloxy-2,3-butan-1-ol* ***5d***

Alcohol **4d** (4.84g, 27.8 mmol) was stirred with TBDMS-Cl (5.02g, 33.3 mmol) and imidazole (3.78g, 55.6 mmol) in DMF (70 ml) for 30 min. The mixture was diluted with hexane (150 ml), washed with a 5% $NaHCO_3$ solution (75 ml) and separated. The aqueous layer was further extracted with hexane (4×80 ml) and the combined organic portions dried and concentrated to a colourless oil (7.77 g, 97%). The product was often used without further purification. However, for analytical purposes it could be purified by flash silica chromatography in 9:1 hexane:ether (R_f 0.25). $[\alpha]_D^{18}$ +8.5 (*c* 1.07, CH_2Cl_2, 83% e.e.); oil; IR ($CHCl_3$) 2957s, 2932s, 2858m, 1733s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.34 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=3.6$ Hz) and 4.05 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=7.0$ Hz, CH_2CO), 3.81 (1H, dd, $J_{Gem}=11.9$, $J_{Vic}=4.8$ Hz) and 3.75 (1H, dd, $J_{Gem}=11.8$, $J_{Vic}=5.3$ Hz, CH_2OSi), 3.14–3.26 (2H, m, epoxide), 2.33 (2H, t, $J=7.4$ Hz, CH_2CO), 1.66 (2H, sx, $J=7.4$ Hz, CH_2CH_3), 0.95 (3H, t, $J=7.4$ Hz, CH_2CH_3), 0.89 (9H, s, *t*Bu), 0.08 (3H, s) and 0.07 (3H, s, Me_2Si); ^{13}C NMR (62.9 MHz, $CDCl_3$) 173.39 (C=O), 62.50 and 61.38 (2×CH_2O), 56.46 (C-2), 53.60 (C-3), 35.95 (CH_2CO), 25.82 (Me_3C), 18.36 (CH_2CH_3), 18.27 (Me_3C), 13.63 (CH_2CH_3), –5.30 and –5.42 (Me_2Si); MS (EI^+) 289 (M+1, 100%), 273 (64), 257 (16), 245 (22), 231 (100), 143 (100). HRMS (EI^+) $C_{14}H_{29}O_4Si$ requires M+H, 289.1835. Found: M+H, 289.1826.

*4.6.2. (+)-(2R,3S)-4-(*tert*-Butyldimethylsilyloxy)-1-*iso*-butanoyl-2,3-epoxybutan-1-ol* ***5c***

Data for the crude material: R_f 0.36 (4:1 hexane:ether); oil; $[\alpha]_D^{25}$ +4.6 (*c* 1.40, CH_2Cl_2, 25% e.e.); IR ($CHCl_3$) 2982w, 2955w, 2931m, 2857m, 1732s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.34 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=3.6$ Hz) and 4.05 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.9$ Hz, CH_2OSi), 3.82 (1H, dd, $J_{Gem}=11.8$, $J_{Vic}=4.7$ Hz) and 3.75 (1H, dd, $J_{Gem}=11.8$, $J_{Vic}=5.3$ Hz, CH_2OCO), 3.15–3.27 (2H, m, epoxide), 2.60 (1H, sp, $J=7.0$ Hz, $(CH_3)_2CH$), 1.18 (6H, $J=7.0$ Hz, $(CH_3)_2CH$), 0.89 (9H, s,*t*Bu), 0.08 (3H, s) and 0.07 (3H, s, Me_2Si); ^{13}C NMR (62.9 MHz, $CDCl_3$) 176.77 (C=O), 62.47 and 61.27 (2×CH_2O), 56.28 (C-2), 53.49 (C-3), 33.73 (Me_2CH), 25.69 (Me_3C),

18.82 and 18.79 (Me_2CH), 18.14 (Me_3C), –5.43 and –5.55 (Me_2Si); MS (EI^+) 289 (M+1, 72%), 231 (48), 185 (36), 143 (100).

*4.6.3. (+)-(2R,3S)-4-(*tert*-Butyldimethylsilyloxy)1-hexanoyl-2,3-epoxybutan-1-ol* ***5e***

Data for the crude material: R_f 0.39 (4:1 hexane:ether); oil; $[\alpha]_D$ n.d.; IR ($CHCl_3$) 2930m, 2860m, 1735 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 4.34 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=3.6$ Hz) and 4.05 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=6.9$ Hz, CH_2OSi), 3.82 (1H, dd, $J_{Gem}=11.8$, $J_{Vic}=4.7$ Hz) and 3.75 (1H, dd, $J_{Gem}=11.8$, $J_{Vic}=5.3$ Hz, CH_2OCO), 3.17–3.28 (2H, m, epoxide), 2.35 (2H, t, $J=7.4$ Hz, CH_2CO), 1.64 (2H, qu, $J=7.4$ Hz, CH_2CH_2CO), 1.32 (4H, m, CH_2CH_2), 0.89 (3H, t, $J=6.5$ Hz, CH_3), (9H, s, *t*Bu), 0.08 (3H, s) and 0.07 (3H, s, Me_2Si).

*4.7. (+)-(2R,3S)-4-(*tert*-Butyldimethylsilyloxy)-2,3-epoxybutan-1-ol* ***6***

The crude silyl ester **5d** (1.0 g, 3.47 mmol) was stirred in a sealed flask with concentrated '0.88' aqueous ammonia solution (520 μl, 10.3 mmol) and methanol (20 ml) at rt for 3 days or at reflux for 8 hours. The crude concentrate was purified by flash silica chromatography in 1:1 hexane:ether to yield the title compound as a light yellow oil (701 mg, 92%). R_f 0.28. $[\alpha]_D^{19}$ +9.9 (*c* 1.40, CH_2Cl_2, 84% e.e.); oil; IR ($CHCl_3$) 3300–3600bm, 2940s, 2865s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 3.90 (1H, dd, $J_{Gem}=11.8$, $J_{Vic}=5.5$ Hz, C*H*HOSi), 3.73–3.80 (2H, m, CH_2OH), 3.71 (1H, dd, $J_{Gem}=11.8$, $J_{Vic}=5.4$ Hz, CH*H*OSi), 3.15–3.25 (2H, m, epoxide), 2.29 (1H, bt, $J=6.5$ Hz, OH), 0.89 (9H, s, *t*Bu), 0.08 (3H, s) and 0.07 (3H, s, Me_2Si); ^{13}C NMR (100.6 MHz, $CDCl_3$) 61.58 and 60.83 ($2\times CH_2O$), 56.29 and 55.96 (epoxide), 25.79 (Me_3C), 18.23 (Me_3C), –5.23 and –5.47 (Me_2Si); MS (FAB^+) 219 (M+1, 46%). Anal. calcd for $C_{10}H_{22}O_3Si$: C, 54.97; H, 10.26. Found: C, 54.99; H, 10.17.

4.7.1. Compound ***6*** *from hydrolsis of silyl ester* ***5c***

Silyl ester **5c** was hydrolysed according to the preceding method described for **5d** above to yield the silyl-protected alcohol **6** in 90%. $[\alpha]_D^{23}$ +6.7 (*c* 2.18, CH_2Cl_2, 25% e.e.). Other data for **6** as described in Section 4.7.

4.7.2. Compound ***6*** *from hydrolsis of silyl ester* ***5e***

Silyl ester **5e** was hydrolysed according to the preceding method described for **5d** above to yield the silyl-protected alcohol **6** in 53%. $[\alpha]_D^{23}$ +7.9 (*c* 1.04, CH_2Cl_2, 65% e.e.). Other data for **6** as described in Section 4.7.

*4.8. (–)-(2R,3S)-4-(*tert*-Butyldimethylsilyloxy)-2,3-epoxybutan-1-al* ***7***[15]

All solutions were dried over 4 Å molecular sieves for 3–4 hours before addition to the main reaction vessel. Oxalyl chloride (2.2 ml, 25.4 mmol) in dichloromethane (150 ml) was cooled to –70°C and DMSO (3.9 ml, 55.4 mmol) in dichloromethane (15 ml) was added dropwise over 10 min. The solution was stirred for a further 10 min before the alcohol **6** (5.05 g, 23.1 mmol) in dichloromethane (25 ml) was likewise added over 10 min. The reaction was quenched after 1 hour by the careful addition of triethylamine (16.1 ml, 115.5 mmol), maintaining the temperature below –70°C and further stirring for 25 min. The mixture was warmed to rt and water (100 ml) added with stirring for 30 min. HCl (1.0 M, 100 ml) was added and the mixture separated. The organic layer was washed consecutively with 100 ml portions of water, 5% $NaHCO_3$, and water,

dried and concentrated to a pale yellow oil (4.51 g, 92%), $[\alpha]_D^{21}$ (crude) –59.8 (*c* 0.70, CH_2Cl_2). The material was often used without further purification. Analytically pure material could be obtained by flash silica chromatography in 3:1 hexane:ether (R_f 0.29). $[\alpha]_D^{18}$ (pure) –73.0 (*c* 0.93, CH_2Cl_2, 84% e.e.); oil; IR ($CHCl_3$) 2910m, 2845s, 2735w, 1715s cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) 9.49 (1H d, $J=4.4$ Hz, CHO), 4.01 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=2.6$ Hz) and 3.93 (1H, dd, $J_{Gem}=12.3$, $J_{Vic}=3.5$ Hz, CH_2OSi), 3.36–3.45 (2H, m, epoxide), 0.87 (9H, s, *t*Bu), 0.06 (6H, s Me_2Si); ^{13}C NMR (62.9 MHz, $CDCl_3$) 197.99 (C=O), 60.23 (C-2), 59.87 (C-4), 57.69 (C-3), 25.78 (Me_3C), 18.31 (Me_3C), –5.48 (Me_2Si); MS (FAB^+) 217 (M+1, 10), 215 (24), 201 (26), 187 (11), 159 (37), 75 (100). Anal. calcd for $C_{10}H_{20}O_3Si$: C, 55.50; H, 9.42. Found: C, 55.50; H, 9.33.

4.9. (2R,3R)-4-Butyryloxy-2,3-epoxybutan-1-al **8**

Alcohol **4d** (523 mg, 3.0 mmol) was oxidised by Swern[14] oxidation according to the method of Section 4.8 to generate the title compound as a crude oil, contaminated with ~25% unreacted alcohol as determined by ^1H NMR integration and TLC. Estimated yield of title compound by ^1H NMR, 45%. Data for crude product: R_f 0.28 (1:1 hexane:ether); $[\alpha]_D$ n.d.; oil; IR ($CHCl_3$) 1738bs, 1715bs cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 9.46 (1H, d, $J=4.8$ Hz, CHO), 4.34 (1H, dd, $J_{Gem}=12.6$, $J_{Vic}=4.3$ Hz) and 4.27 (1H, dd, $J_{Gem}=12.6$, $J_{Vic}=5.6$ Hz, CH_2O), 3.51 (1H, ddd, $J_{2-3}=4.8$, $J_{3-4}=4.3$ and 5.6 Hz, H-3), 3.45 (1H, t, $J=4.8$ Hz, H-2), 2.29 (2H, t, $J=7.4$ Hz, CH_2CO), 1.62 (2H, sx, $J=7.4$ Hz, CH_2CH_3), 0.91 (3H, t, $J=7.4$ Hz, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) 196.78 (CHO), 172.99 (C=O), 60.64 (CH_2O), 57.19 (C-2), 55.89 (C-3), 35.70 (CH_2CO), 18.20 (CH_2CH_3), 13.55 (CH_3).

Acknowledgements

We are grateful to the SERC for generous finance (to J.D.M.). J.D.M. thanks David R. Lewis for helpful discussions at the time of this work, former colleagues at Merck Sharp and Dohme (Terlings Park) for facilitating this research, and colleagues at AstraZeneca (Avlon) for helpful comments on the preparation of this manuscript.

References

1. Moseley, J. D.; Staunton, J. *Tetrahedron: Asymmetry* **1998**, *9*, 3619–3623.
2. Schloss, J. V.; Hartman, F. C. *Bioorg. Chem.* **1980**, *9*, 217–226.
3. Nelson, W. L.; Freeman, D. S.; Vincenzi, F. F. *J. Med. Chem.* **1976**, *19*, 153–158.
4. Aside from poor performance in the esterification step, the inferior quality was typified by its less crystalline appearance and lower melting point (47–49°C in our hands by Nelson's method against 50–52°C using Schloss' method; Nelson reported 44–47°C). This may be accounted for by the fact that the material was isolated from water by freeze drying, probably resulting in an amorphous or less crystalline form, and possibly retained water.
5. All enzymes are commercially available from Fluka (F) except CCL and PLE available from Sigma (S), and PPL from both. Abbreviations used: CCL, lipase from *Candida cylindracea*; LAN, lipase from *Aspergillus niger*; LCL, lipase from *Candida lipolytica*; LMJ, lipase from *Mucor javanicus*; LPF, lipase from *Pseudomonas fluorescens*; LPR, lipase from *Penicillium roqueforti*; LRA, lipase from *Rhizopus arrhizus*; LRD, lipase from *Rhizopus delemar*; LRN, lipase from *Rhizopus niveus*; PLE, pig liver esterase; PPL porcine pancreatic lipase.
6. Grandjean, D.; Pale, P.; Chuche, J. *Tetrahedron Lett.* **1991**, *32*, 3043–3046.
7. Brevet, J.-L.; Mori, K. *Synthesis* **1992**, 1007–1012.

8. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549.
9. Corser, D. A.; Dart, R. K.; Marples, B. A. *J. Chem. Res. (S)* **1992**, 66–67.
10. The Sigma derived PPL was moderately inferior, perhaps due to the source or method of isolation.
11. Joerger, R. D.; Haas, M. J. *Lipids* **1994**, *29*, 377.
12. In general, our results were 5–10% lower in e.e. compared to Grandjean[6] although they mirrored their results in all other respects. We offer three possibilities for the observed differences; (i) differences in enzyme source (Fluka versus Sigma); (ii) differences in the w/w quantity of enzyme used; and (iii) our preparations were run to 100% conversion, theirs to a maximum of 90%.
13. Kvittingen, L.; Sjursnes, B.; Halling, P.; Anthonsen, T. *Tetrahedron* **1992**, *48*, 5259–5264.
14. Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651–1660.
15. Roush, W. R.; Straub, J. A.; van Nieuwenhze, M. S. *J. Org. Chem.* **1991**, *56*, 1636–1648.
16. Dess, D. B.; Martin, J. C. *J. Org.Chem.* **1983**, *48*, 4155–4156.
17. Grandjean, D.; Pale, P.; Chuche, J. *Tetrahedron Lett.* **1992**, *33*, 5355–5358.
18. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1992**, *3*, 1529–1532.
19. Trincone, A.; Pagnotta, E. *Tetrahedron: Asymmetry* **1996**, *7*, 2773–2774.