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Influence of ester chain length on lipase catalysed hydrolyses of *meso*-oxiranedimethanol esters

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Abstract

A range of *meso*-oxiranedimethanol diesters have been hydrolysed by a selection of microbial and porcine derived lipases. The results reveal the effects of ester substitution, particularly chain length, on the hydrolyses. General conclusions are made which demonstrate that ester chain length can be 'tuned' to achieve optimum enantiomeric enrichment for a given substrate. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

The desymmetrisation by enzymic hydrolysis of *meso*-diesters is an attractive strategy for the generation of enantio-enriched synthons from achiral precursors; the use of this 'meso trick'[1] can give a 100% chemical yield of the product in 100% enantiomeric excess (e.e.). Furthermore, both enantiomers are generally accessible, whatever the outcome of the hydrolysis, by subsequent manipulations of the differentiated functional groups.[2]

Although many workers have conducted limited investigations into the effects of ester substitutions on enzymic hydrolyses, there have been surprisingly few systematic studies.[3–5] Similar studies have been performed for PLE[6] and some other enzymes;[7] more frequently however, it is the core of the molecule that has been varied to demonstrate wide substrate tolerance for a specific enzyme and ester group, e.g. cyclic diesters;[2,8] glutaric esters;[8] 3-substituted cyclopentane diesters;[9] and bicyclic esters.[10] In addition, it seemed to us that much of the knowledge in this area was anecdotal in nature. Therefore, during our studies on the oxiranedimethanol system **2**, we set about a comprehensive investigation of the effects of ester side chain substitution on the results of enzymic hydrolyses (Scheme 1). Since both ester side chains are discarded in subsequent manipulations of alcohols **3**, there is considerable scope for optimising the hydrolyses by choosing the side chain substitution that best suits a given lipase. Other reports on this

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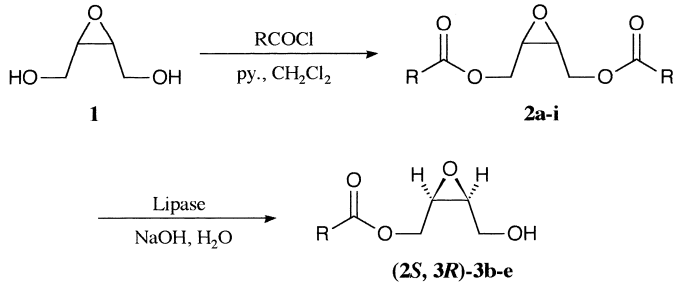
substrate would allow us to compare our results against external references[11–13] for what has proved to be a versatile synthetic intermediate.[13–16]



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

Scheme 1.

2. Results and discussion

A range of *meso*-diesters **2a–i** was readily synthesised by esterification of the known epoxide **1** (Scheme 1).[17] The propionate **2b**, *iso*-butyrate **2c**, *n*-butyrate **2d** and hexanoate **2e** diesters were then subjected to hydrolysis by a range of commercially available microbial and porcine-derived lipases and esterases.[18,19] The e.e.s were determined from the optical rotations of the purified alcohols **3b–e** and by Mosher's ester formation[20] of a representative sample of alcohols. The integration of the ^{19}F NMR signals confirmed all e.e.s derived by optical rotation measurements to within ±1%. Subsequent transformations to known compounds[14] further corroborated these figures, and also demonstrated that the sign of rotation and the absolute configuration was uniform across the series of alcohols **3b–e**. The results are collected in Table 1 and can be analysed both with respect to the ester side chain substituent and to the enzyme. We will discuss the side chain substitution first.

2.1. Discussion of ester chain length and substitution

The propionate diester **2b** proved to be a model substrate, with reactions proceeding in high yields and with reasonable reaction times for full conversion, although e.e.s were moderate (6–54%; see Table 1, entries 1–4). The *iso*-butyrate diester **2c**, which can be compared in length to the propionate diester **2b**, or by atom count to the butyrate diester **2d**, proved to be an unsuitable substrate for LRA and PPL, two enzymes which performed well for the butyrate diester (cf. entry 5 with 11, and 8 with 14). However, LPF and PLE digested **2c** in high yield at full conversion, albeit in low e.e.s (entries 6 and 7). The butyrate results were the best of the series with generally high yields and good e.e.s (entries 9–14). Lastly, the hexanoate diester **2e** was a moderate substrate with poor to fair reaction times and e.e.s (entries 15–18). High conversions with low isolated yields are indicative of over-hydrolysis of product **3e**, producing water-soluble diol **1** which is lost on work up.

The acetate diester **2a**, which was not studied in this work, gave yields and e.e.s for PLE and PPL comparable to the propionate diester **2b** as determined by Mori.[13] Grandjean reported slightly better figures with PPL, but noted that this enzyme/substrate pair seemed to be more sensitive to conditions

Table 1
Enzymic hydrolysis of diesters **2b–e**

Entry	Substrate	Enzyme[a]	Conv.[b] (%)	Time (hrs)	Yield[c] (%)	e.e.[d]	Product[e]
1	**2b**	LCL	100	27	61	6	-
2	**2b**	LPF	100	1.5	80	21	+
3	**2b**	PLE	100	3.5	65	27	+
4	**2b**	PPL	100	2.5	61	54	+
5	**2c**	LRA	25	8	[58][f]	20	+
6	**2c**	LPF	100	4.5	86	25	+
7	**2c**	PLE	100	2.5	67	14	+
8	**2c**	PPL	20	48	[47][f]	25	+
9	**2d**	LCL	100	1.5	65	6	-
10	**2d**	LMJ	100	10	62	71	+
11	**2d**	LRA	100	16	67	73	+
12	**2d**	LPF	100	1	65	10	+
13	**2d**	PLE	50	6	[60][f]	21	+
14	**2d**	PPL	100	6.5	73	84	+
15	**2e**	LMJ	80	24	36	55	+
16	**2e**	LPF	100	0.5	41	3	+
17	**2e**	PLE	40	24	[6][f]	10	+
18	**2e**	PPL	100	3.5	42	65	+

a. See reference 17 for abbreviations used. b. Conversion based on equivalents of NaOH needed to readjust pH to 7.0. c. Isolated yield of purified material after chromatography. d. Based on optical rotation and Mosher's ester determination. e. (+) = (2*S*, 3*R*); (-) = (2*R*, 3*S*) enantiomers. f. [ ] = calculated for 100% conversion.

than other combinations.[11] At the other end of the range, Marples[12] reported that the octanoate diester **2h** was "hydrolysed only sluggishly".

2.2. Discussion of enzymes

Unsurprisingly, all the enzymes studied displayed the same stereochemical preference across the series of diesters **2b–e**, although the results were of marginal significance in some cases (i.e. entries 1, 9 and 16). LPF proved to be least affected by the ester chain length; it was the fastest lipase for all but the branched case (entry 6) and gave good chemical yields but was not very discriminating for these substrates, the best result being a modest 25% e.e. The enantiotopic preference at the hydrolysed site (3*R*-position) is in agreement with the proposed model[21] although the selectivities are modest; however, high e.e.s can be achieved with LPF as noted.[22]

PLE also proved to be a fast but undiscriminating enzyme for these substrates (best result entry 3, 27% e.e.). The branched chain four carbon diester **2c** gave an almost identical result to the three carbon chain propionate diester **2b**, in contrast to the four carbon straight chain diester **2d** (entries 3, 7 and 13). This clearly demonstrates that PLE regards this substitution pattern as a three carbon chain. The wide substrate tolerance of PLE for short chain esters has been ascribed to the "varied diet of the pig".[23] Active site models have been proposed[2,24] and refuted [25] for this much studied enzyme.[6,26] However, the

simplified model proposed by Naemura[10] for bicyclic bis-alcohols appears to favour the preferred (3*R*) hydrolysis site for these monocyclic bis-alcohols **2**, although this is against the more general trend in favour of hydrolysis adjacent to the (2*S*) prochiral centre.

PPL comfortably gave the best results both in terms of e.e. and yield, with only the branched *iso*-butyrate diester **2c** giving a poor result (entry 8). Active site models have been proposed of which that by Jones is typical[27] in predicting hydrolysis at the (2*S*) centre. This does not fit with the observed results, which is unfortunate given that these hydrolyses were the most synthetically useful. Coupled with Grandjean's results for the diacetate **2a**, PPL has proved itself equal or superior to other lipases for unsubstituted ester side chains of two to six carbon atoms.

3. Conclusions

We report on the influence of ester substitution on the result of enzymic hydrolyses for a given central moiety. In principle, the ester chain can be selected to give the best result for any chosen core fragment. More specifically, we conclude that butyrate is an ideal chain length for hydrolyses by lipases, and propionate is also worth considering. With respect to the enzymes, PPL is the lipase of choice; PLE is known to work well for shorter chain esters (up to three carbons). Both PLE and LPF have potential for chemical hydrolyses where achiral products are required. In summary, this approach is ideal for process development work.

Acknowledgements

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18. All samples gave satisfactory elemental analyses after purification by flash silica gel chromatography and were fully characterised by standard physical and spectroscopic methods. For enzymic hydrolyses on a 5.0 mmol scale, 40 mg of

lipases were used except PPL (50 mg) and PLE (3 μl purified supension). A typical example for **2d** follows: a suspension of diester **2d** (1.22 g, 5.0 mmol) and PPL (Fluka, 50 mg) was vigourously stirred at room temperature 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 around 7.0 until nearly the full equivalent of NaOH had been consumed. The aqueous solution was promptly extracted with ethyl acetate (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%); m.p. 20–21°C (neat); $[\alpha]_D^{23}$ +15.1 (*c* 1.03, $CHCl_3$, 84% e.e.); analysis (found: C, 54.88; H, 8.01; $C_8H_{14}O_4$ requires C, 55.15; H, 8.12%).

19. All lipases commercialy available from Fluka; PLE from Sigma. Abbreviations: LCL, lipase from *Candida lipolytica*; LMJ, lipase from *Mucor javanicus*; LPF, lipase from *Pseudomonas fluorescens*; LRA, lipase from *Rhizopus arrhizus*; PLE, pig liver esterase; PPL, porcine pancreatic lipase.
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