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Lipase-catalysed kinetic resolutions of secondary alcohols in pressurised liquid hydrofluorocarbons

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ABSTRACT

Three model secondary alcohols have been subjected to enzymatic kinetic resolution using three common lipases and a typical acyl donor. The resolutions were performed in two pressurised low-boiling hydrofluorocarbons, which are novel media for enzymatic reactions, and five conventional organic solvents. In general, higher yields, ee's and rates of reaction were observed in the hydrofluorocarbons.

The kinetic resolution (KR) of secondary alcohols via enantioselective acylation, catalysed by hydrolase enzymes, is a well known procedure for production of chiral alcohols or esters for pharmaceutical synthesis and other applications.[1–3] The most common enzymatic method for performing kinetic resolutions of this type (Fig. 1) is to use a non-aqueous solvent and an irreversible acyl donor such as vinyl acetate, to drive the reaction in the synthetic direction.[4–6] The fact that common lipases tend to display opposite stereoselectivity to some proteases means that both enantiomers of the alcohol or ester product can in principle be obtained.[7–9] Enzymatic acylation of secondary alcohols has also been used to trigger enantioselective multistep (domino) reactions.[10] In addition kinetic resolution of primary alcohols with remote stereocentres has also been achieved.[11,12] Despite this product yield for kinetic resolution is limited to 50%. To overcome this problem, dynamic kinetic resolutions (DKRs)[13,14] have been introduced, where the unreacted secondary alcohol is racemised, for example, using a ruthenium catalyst[8,15,16] (e.g., **1**, Fig 1).

The selection of the non-aqueous solvent for enzymatic–kinetic resolutions is of critical importance. Indeed early work with organic solvents showed that enzyme activity, yields and enantioselectivities can all be affected by the solvent physical properties.[1,2,17] Similarly with DKRs, the choice of a solvent that can afford both high activity of the enzyme and the chosen racemisation catalyst is an important factor. As a result of the critical role of the media, on biocatalysis, the concept of solvent engineering[1,2,17] was introduced as a way to optimise and alter the course of enzymatic reactions in non-aqueous media including conventional organic solvents (COSs),[1,2] supercritical fluids[18] and ionic liquids.[19] To this end we introduced hydrofluorocarbon HFCs as alternative media for biocatalysis.[20] The use of pressurised liquid HFCs[21,22] as media

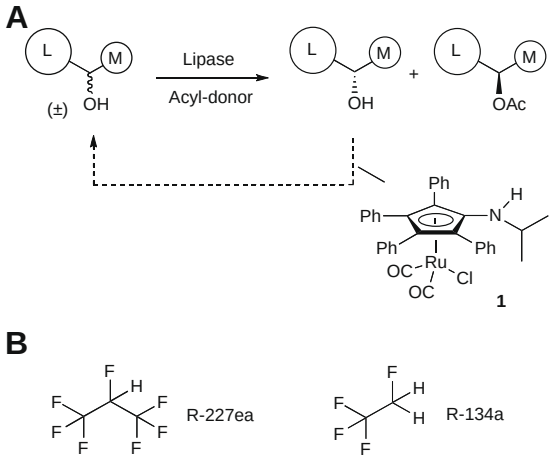

Figure 1. (A) Lipase-catalysed kinetic resolution (KR) following the 'Kazlauskas rule' and dynamic kinetic resolution (DKR) of secondary alcohols; (B) hydrofluorocarbons R-227ea and R-134a used in this study.

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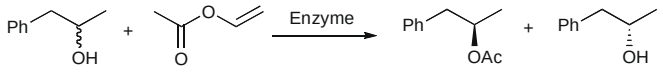
for biocatalysis was shown to give improved rates, yields and in some cases enantioselectivities in lipase and protease-catalysed model biotransformations.[20] In addition, pressurised liquid HFCs, which are used as refrigerants, propellants and as solvents for extraction, offer several major operational benefits for potential industrial processes, including ease of product recovery.[20,22]

Previously we showed that the kinetic resolution of racemic 1-phenylethanol[20] was particularly promising in HFCs. In light of this we chose to explore the wider utility of these media for kinetic resolution of secondary alcohols. Initially we focused on the kinetic resolution of 1-phenylpropan-2-ol (*rac*-**2**) with commercial *Candida antarctica* lipase B on acrylic resin (Novozyme 435) and *Pseudomonas cepacia* lipase supported on ceramic (Amano PS-CII), with vinyl acetate **3** as the acyl donor to give the acetylated product (*R*)-**4**. For comparison with the HFCs R134a and R227ea (Fig. 1), a series of conventional organic solvents (COSs) were selected which are most commonly used for similar kinetic resolutions using lipases and related enzymes. Through a series of preliminary experiments, conditions were established so that the progress of reactions could be monitored by GC allowing accurate initial rates to be determined during the early stages of the reaction when pseudolinear kinetics are observed. At a set time point (24 h), when the reaction has proceeded towards completion, the yield of the acetylated product (*R*)-**4** is recorded and it enantiomeric excess was determined by chiral HPLC. From this it can be seen (Table 1) that the reaction in R134a, catalysed by Novozyme 435, exhibited the highest initial rate and yield compared with all other solvents. Of the COSs hexane gave highest initial rates and yields, but slightly lower ee. In light of this full time course experiments were run, under optimised conditions, with higher quantities of enzyme in R134a and hexane. This confirmed higher rates and yields in the HFC and resulted in 42% yield of (*R*)-**4** (99% ee) in R134a, compared with 36% yield of (*R*)-**4** (99% ee) in hexane (see Supplementary data). In the kinetic resolution of 1-phenylpropan-2-ol (*rac*-**1**), catalysed by PS-CII, it is clear that the HFCs give rise to considerably higher initial rates compared with COSs. Again hexane gave significantly higher rates than other COS, and interestingly initial rates in toluene are considerably higher for PS-CII than for Novozyme 435. A full time course experiment was run in the best HFC (R227ea) and best COS (hexane) with PS-CII, resulting in 45% yield of (*R*)-**4** (99% ee) in R227ea, compared with 43% yield of (*R*)-**4** (99% ee) in hexane (see Supplementary data).

The kinetic resolution of 1-(2-furyl)ethanol *rac*-**5** was next explored, with the widely used *Pseudomonas fluorescens* lipase (PFL) supported on Eupergit C, in addition to PS-CII. Again the production of the acetyl product, (*R*)-**6**, was monitored by GC and chiral GC to give initial rates as well as yields and ee after 24 h (Table 2). In this case TBME gave the highest initial rate and yield, followed by R134a. With PS-CII hexane gave the highest initial rates, followed by R134a, although after 20 h the reaction with R227ea had progressed furthest. In this series a number of reactions had proceeded beyond the 50% optimum yield, due to additional acyl-

Table 1
Initial rates, yields, ee and enantiomeric ratio (*E*) for acetylation of *rac*-**2** to give (*R*)-**4** in various solvents, catalysed by Novozyme 435 and PS-CII

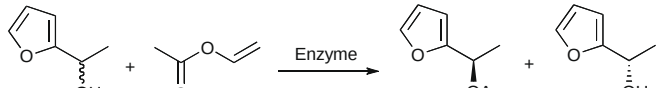


rac-**2** **3** (*R*)-**4** (*S*)-**2**

Solvent	Novozyme 435				PS-CI			
	Initial rate (nmol min^{-1} U^{-1})	Yield **4** (%)	ee **4** (%)	*E*	Initial rate (nmol min^{-1} U^{-1})	Yield **4** (%)	ee **4** (%)	*E*
R134a	7.40 ± 0.07	34	97	108	1419 ± 19.7	13	99	>200
R227ea	2.00 ± 0.08	27	97	93	2229 ± 30.4	25	99	>200
Hexane	3.50 ± 0.01	20	93	35	1088 ± 13.6	15	99	>200
Toluene	n.d.[a]	17	n.d.[b]	n.d.[b]	736 ± 1.39	8	n.d.[b]	n.d.[b]
Chloroform	0.90 ± 0.02	5	98	104	255 ± 1.65	2	99	>200
TBME	2.80 ± 0.15	20	96	62	354 ± 9.06	5	99	>200
Vinyl acetate	2.00 ± 0.02	18	95	48	440 ± 7.38	11	99	>200

Yields and ee for each reaction are recorded after 24 h. Conditions: 0.5 mmol *rac*-**2**, 10 mmol **3**, 95U Novozyme 435 or 0.6U PS-CII, 5 mL solvent.
[a] The rate was too low to be measured.
[b] The toluene peak overlapped with those of the product.

Table 2
Initial rates, yields, ee and enantiomeric ratio (*E*) for acetylation of *rac*-**5** to give (*R*)-**6** in various solvents, catalysed by PFL and PS-CII

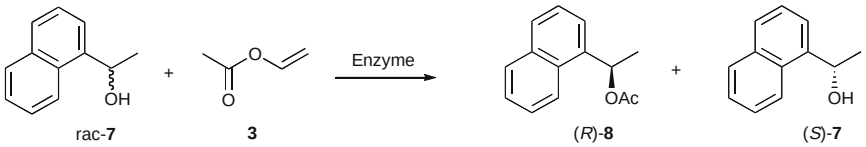


rac-**5** **3** (*R*)-**6** (*S*)-**5**

Solvent	PFL				PS-CII			
	Initial rate (nmol min^{-1} U^{-1})	Yield **6** (%)	ee **6** (%)	*E*	Initial rate (nmol min^{-1} U^{-1})	Yield **6** (%)	ee **6** (%)	*E*
R134a	9.10 ± 0.48	11	95	44	556 ± 14.2	50	99	>200
R227ea	n.d.	n.d.[a]	n.d.[a]	n.d.[a]	319 ± 10.8	61	80	34
Hexane	7.10 ± 0.25	10	97	73	695 ± 40.8	57	80	39
Toluene	1.80 ± 0.05	3	98	102	489 ± 12.4	54	92	118
Chloroform	0.60 ± 0.02	n.d.[a]	n.d.[a]	n.d.[a]	230 ± 5.50	8	99	>200
TBME	10.90 ± 0.13	17	96	59	287 ± 8.7	52	97	>200
Vinyl acetate	6.00 ± 0.17	7	93	30	133 ± 15.6	46	99	>200

Yields and ee for each reaction are recorded after 24 h. Conditions (5 mL total volume): 10U PFL, 2.0 mmol *rac*-**5**, 2.5 mmol **3**; 3U PS-CII, 0.5 mmol *rac*-**5**, 5 mmol **3**.
[a] Yields too low to be measured.

Table 3
Initial rates, yields, ee and enantiomeric ratio (*E*) for acetylation of *rac*-7 to give (*R*)-8 in various solvents, catalysed by PS-CII and Novozyme 435



rac-**7** **3** (*R*)-**8** (*S*)-**7**

Solvent	PS-CII				Novozyme 435			
	Initial rate (nmol min^{-1} U^{-1})	Yield **8** (%)	ee **8** (%)	*E*	Initial rate (nmol min^{-1} U^{-1})	Yield **8** (%)	ee **8** (%)	*E*
R134a	3.60 ± 0.12	53	89	123	9.6 ± 0.53	49	>99	>200
R227ea	6.20 ± 0.21	53	89	123	10.8 ± 0.21	51	96	>200
Hexane	7.30 ± 0.27	55	82	73	4.5 ± 0.20	55	82	73
Toluene	n.d.[a]	55	82	73	n.d.[a]	15	>99	>200
Chloroform	n.d.[b]	4	>99	>200	n.d.[b]	4	>99	>200
TBME	0.80 ± 0.01	31	99	>200	1.10 ± 0.04	13	>99	>200
Vinyl acetate	n.d.[b]	24	>99	>200	n.d.[b]	10	>99	>200

Yields and ee for each reaction are recorded after 24 h. Conditions: 0.5 mmol rac-**7**, 5 mmol **3**, 120U PS-CII or 118U Novozyme 435, 5 mL solvent.
[a] (*R*)-8 Peak obscured by solvent.
[b] Rates too low to be reliably measured.

ation of the (*S*)-**5** enantiomer resulting in significantly lower observed ee. After further optimisation, a full time course for the kinetic resolution of *rac*-**5** with PS-CII was performed, which gave a 45% of (*R*)-**6** (99% ee) in hexane and 43% of (*R*)-**6** (99% ee) R134a, under identical conditions (see Supplementary data).

Finally the kinetic resolution of 1-(1-naphthyl)ethanol *rac*-**7** was studied using PS-CII and Novozyme 435 (Table 3). With PS-CII, hexane exhibits highest initial rates and yields, closely followed by R227ea. Novozyme 435, on the other hand, exhibits highest initial rates in R227ea and R134a, but a higher yield was obtained in hexane. As with most of the other experiments chloroform proved to be the least effective solvent for these transformations. In order to rationalise the overall results from these kinetic resolution experiments it is useful to compare the physical properties of HFCs[22] with those of COSs.[23,24] Of the many physical parameters available viscosity, polarity as indicated by dielectric constants (ε) or dipole moments (DM), and hydrophobicity as reflected by log*P* are probably the most informative (Table 4). Considering these parameters and the data presented here some apparent trends can be observed. Firstly solvents that have lower viscosity tend to exhibit faster initial rates, which can be due to increased solute diffusivity and improved mass transfer, which is particularly important with immobilised enzymes. Secondly amongst COSs rates and yields tend to decrease with increasing solvent polarity, which can in part be attributed to the propensity of the more polar solvent to strip the enzyme of essential water.[1,2,17] In some cases the increased solubility of the substrate alcohol in the more polar solvents can have the opposite effect. Despite this, HFCs and R134a in particular are highly polar, yet generally afford higher rates and yields for the lipase-catalysed reactions described here and previously.[20] Most likely this is due to the fact that HFCs, as well as being polar, are also hydrophobic.[25] Generally COSs of higher polarity tend to be more hydrophilic.

Table 4
Physical properties of solvents used in this study[22–24]

Solvent	Viscosity[a]	ε [b]	DM[c]	Log*P*
R134a	0.21	9.5	2.05	1.42
R227ea	0.26	4.1	0.93	2.17
Hexane	0.29	1.9	0.08	4.00
Toluene	0.56	2.4	0.31	2.73
Chloroform	0.53	4.8	1.01	1.97
TBME	0.34	1.4	1.37	0.94
Vinyl acetate	0.41	3.0		0.73

[a] Viscosity (cP at the rate of 25 °C).
[b] Dielectric constant (ε/kHz).
[c] Dipole moment (DM).

In summary kinetic resolutions of three model, secondary alcohols using three well-known lipases, have been investigated in two HFC solvents and compared with five conventional organic solvents that would typically be used in such transformations. On the whole rates and yields for these reactions in HFCs were higher than COSs. It is likely that this can be attributed to the unusual physical properties of HFCs, which unlike COSs, possess both high polarity and hydrophobicity,[25] with low viscosity. The fact that HFCs also offer improved operational properties such as very low-boiling points[20] allowing easier product recovery, low toxicity and in many cases non-flammability, means that they offer significant advantages over COSs for enzymatic-production of chiral alcohols or esters of industrial importance. Finally we have shown, in a separate study, that HFCs are also good solvents for ruthenium-complexes (e.g., **1**) allowing fast racemisation of alcohols, which could be beneficial in DKRs of secondary alcohols.[8,15,16] Studies to explore the applications of HFCs as media for DKRs are currently underway and will be presented shortly.

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Supplementary data

Supplementary data (experimental procedures and additional data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.037.

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