COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Enantiomeric partitioning using fluorous biphase methodology for lipase-mediated (trans)esterifications†

Petr Beier and David O'Hagan*

School of Chemistry and Centre for Biomolecular Sciences, University of St Andrews, North Haugh, St Andrews, Fife, UK KY16 9ST. E-mail: do1@st-andrews.ac.uk

Received (in Cambridge, UK) 13th May 2002, Accepted 17th June 2002
First published as an Advance Article on the web 4th July 2002

Lipase-catalysed (trans)esterification reactions in homogenous perfluorocarbon–hydrocarbon solvents enabled direct enantiomeric partitioning (up to 95% ee) of the products by liquid–liquid separation.

The application of perfluorous solvents as a method for liquid–liquid separation technologies has attracted a huge level of interest since the seminal report of Horváth and Rábai in 1994.[1] It is well known that perfluorocarbon solvents such as perfluorohexane (PFH) are immiscible with hydrocarbon solvents such as hexane at low temperature. However these immiscible phases become homogenous at around 30 °C. Thus reactions can be carried out in the homogenous phase on warming, and partitioning is achieved by cooling after a particular reaction has been completed. In their original paper Horváth and Rábai successfully demonstrated that a catalyst, carrying long chain perfluoroalkyl groups, associated with the perfluorocarbon liquid phase, whereas the organic products of the reaction associated with the hydrocarbon solvent. As a consequence, their account has stimulated many studies in the interim exploring novel reagents and catalysts carrying appended perfluorocarbon tails, informally termed 'ponytails'.[2] Subsequent reactions in warmed homogenous perfluorocarbon–hydrocarbon solvent mixtures followed by cooling have proven a generally successful strategy for partitioning the perfluorocarbon tagged reagents into the fluorous phase.[3]

In this study we have explored lipase-mediated transesterification and esterification reactions in the fluorous phase. It is a remarkable feature of lipases that they retain their catalytic efficiency when suspended in dry hydrophobic solvents such as hexane.[4] The accumulated evidence indicates that the more hydrophobic the solvent the more efficient the lipase catalysed transesterifications are. It has been argued that this is due to the enzyme retaining 'essential water' hydrated to its surface, holding the protein in a catalytically competent structure,[5] and protecting the enzyme from being attacked by the solvent. The more polar the solvent then the greater it's ability to strip the 'essential water' from the surface of the protein[6] and therefore hexane has emerged as a widely used solvent for such reactions. Another attractive feature of using lipases in hydrocarbon solvents such as hexane is the ability to increase temperature. Enzymes operating in aqueous solution generally deactivate above 40 °C. This is attributed to water disrupting intramolecular hydrogen bonding and unravelling the protein as the temperature is raised. However, in hexane the hydrophobic solvent is less able to penetrate the protein and lipase mediated transesterifications, where a hydrophobic alcohol formally replaces water, are optimal between 45–60 °C.[7]

Perfluorocarbons such as perfluorohexane are more hydrophobic than hexane,[8] and it was anticipated that reactions using lipases in such media should not result in any increased deactivation relative to hexane, unlike the more polar solvents. Furthermore the perfluorocarbon–hydrocarbon solvents become homogenous at temperatures that are optimal for the lipase mediated (trans)esterification reactions in hexane. To achieve the maximum advantage from such a solvent system it was attractive to explore (trans)esterification reactions between esters–carboxylic acids and long chain polyfluorinated alcohols in the homogenous hydrocarbon–perfluorocarbon phase, to assess if the ester products will partition preferentially into the fluorous phase on cooling (Fig. 1). Clearly this opens up attractive prospects for enantiomeric resolution if product stereoisomers of the opposite enantiomeric series partition preferentially into the different liquid phases based on their partitioning coefficients.

A recent report[9] on a lipase mediated transesterification reaction in acetonitrile has used perfluorocarbon solvent extraction protocols during the reaction work up to partition highly fluorinated phenethanol ester products of one enantiomeric series from phenethanol of the other enantiomeric series. In this study we explore, for the first time, lipase-mediated transesterification reactions *directly* in perfluorocarbon–hydrocarbon solvent mixtures. Trial experiments (data not shown) indicated that a reaction between 2,2,2-trichloroethyl butyrate and hexan-1-ol catalyzed by the lipases from *Candida rugosa* (*CRL*)‡ and porcine pancreatic lipase (*PPL*) at 45 °C in the mixed solvent system [1 : 1 perfluorodecalin : hexane or 1 : 1 perfluorohexane : hexane] progress either with a similar (*CRL*) or slightly greater (*PPL*) efficiency than in hexane alone. The perfluoro solvents appear to have a neutral to enhancing property with respect to hexane. This is consistent with the high hydrophobicity associated with perfluorocarbon solvents and their inability to dehydrate the enzyme. It emerged from these trial experiments that the mixed solvent systems provide an excellent medium in which to carry out lipase-mediated transesterification reactions.

In order to investigate enantiomeric partitioning in these mixed solvent systems transesterification reactions with vinyl 2-methylpentanoate **1** and esterification reactions with 2-me-

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b2/b204607p/

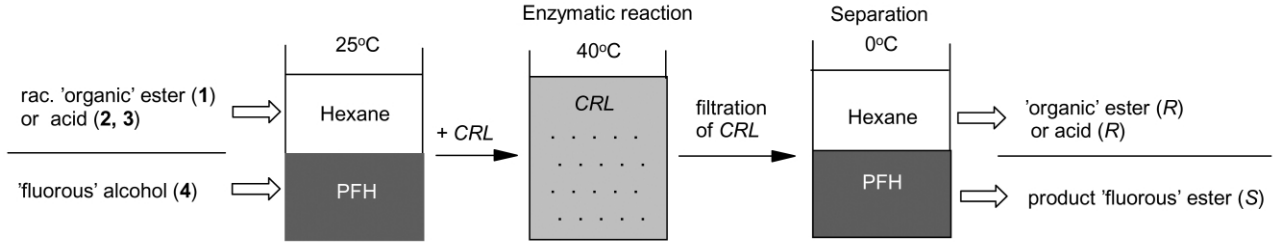
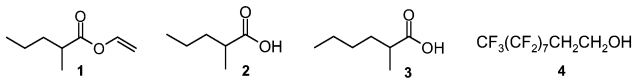


Fig. 1 General scheme for enantioselective partitioning in lipase-mediated (trans)esterifications using fluorous biphase methodology.

DOI: 10.1039/b204607p

thylpentanoic acid **2** and 2-methylhexanoic acid **3** were explored. The *CRL* lipase has previously been shown[10] to discriminate enantiomers of the corresponding acids in esterification reactions in organic solvents with moderate to good enantioselectivities 52–93% ee.§



For this study **1**, **2** and **3** were (trans)esterified with the highly fluorinated decanol **4** to explore (trans)esterification between 'organic' compatible esters–acids with a 'fluorous' alcohol. The two liquid phases (hexane–PFH) became homogenous at 30 °C and the reactions were carried out at 40 °C (Table 1). In all cases the reaction progress was monitored by GC-MS to approximately a 50% conversion. After completion, all reactions were filtered to remove the enzyme and the homogenous medium cooled (0 °C) and the phases left to partition (30 min). In general the enantiomeric excesses of the product esters were high. It is interesting that the enantiomeric enrichment values (Table 1) of the fluorous recovered products is generally higher to that from previous studies[10] for *CRL* esterification reactions in hexane, indicating an improved stereoselectivity under these novel conditions. It is also notable that the esterification reaction between **2** and **4** generates a product with a higher enantiomeric purity than that between the corresponding vinyl ester **1** and **4**, despite these being slower reactions. Efficient partitioning of the unreacted (*S*)-acids **2** and **3**, and the product (*R*)-esters of the opposite enantiomeric series, is compromised to some extent by some solubility of the product esters in hexane and trace amounts of the unreacted acids in the fluorous phase. This was overcome by washing the hexane layer with PFH for maximum recovery of the ester. The little acid in the fluorous phase was not problematical and could be removed by rotary evaporation on work up. Enantiomeric analysis of the ester carboxylate moiety was determined directly after ester hydrolysis of the products recovered from each phase without recourse to chromatographic separation.

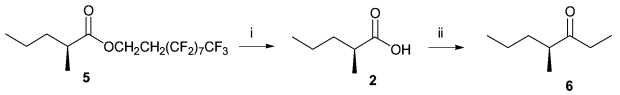
With these encouraging results a preparative scale experiment was conducted with *CRL* between acid **2** (6 g) and alcohol **4** (21.3 g) in a hexane–PFH solvent mix (1 : 1) at 40 °C. In this case dry Na_2SO_4 was added to absorb expelled water. The reaction was worked up after it had progressed to about 50% conversion. The product ester (*S*)-**5** (94% ee) was recovered after evaporation of the fluorous phase. The resultant acid **2** was recovered (1.97 g, 66%) after enzymatic hydrolysis (CRL) and extraction of the product from the buffer into hexane. The enantiomeric purity of this (*S*)-**2** was 96% ee. The unreacted acid was recovered from the PFH washed hexane layer with a 79% ee (*R*)-**2** (1.81 g, 57%,) contaminated with less than 2% of ester **5**. Decanol **4** was also recovered (13.3 g, 62%) from the fluorous phase and there was an 80% recovery of the fluorous solvent.

In order to demonstrate the synthetic utility of the resolution process the (*S*)-**5** ester which was hydrolysed in the second enzymatic reaction was used to prepare a sample of (*S*)-(+)-4-methylheptan-3-one **6** (94% ee) the principal alarm pheromone of the ant *Atta texana*.[11] These transformations are summarised in Scheme 1. The enantiomeric purity of the resultant ketone (*S*)-**6** was determined both by chiral GC analysis and optical rotation.[12]

Table 1 *CRL* (trans)esterification reactions with alcohol **4** and esters–acids **1**, **2** and **3**

Ester–acid	Reaction time/h[a]	Conversion (%)	(*S*)-Ester product[b] ee (%)	(*R*)-Ester–acid unreacted[c] ee (%)
1	44	48	72	44
2	95	53	95	79
3	149	49	95	94.5

[a] Reaction temperature 40 °C. [b] Combined fluorous phase. [c] Hexane phase after extractions.



Scheme 1 Synthesis of alarm pheromone of (*S*)-(+)-4-methylheptan-3-one **6**; i. *CRL*, Phosphate buffer, 0.2 M, pH 7; ii EtLi (2.2 equiv.), Et_2O, 54%.

In conclusion we have demonstrated that a perfluorocarbon–hydrocarbon solvent system offers an excellent medium for lipase catalysed (trans)esterification reactions. Further by judicious choice of 'organic' and 'fluorous' compatible substrates, products of different enantiomeric series are partitioned between the two phases. The product esters recovered from the fluorous phase had high enantiomeric purities and reactions can be conducted on a gramme scale. Clearly an optimal system will have reduced partitioning of the 'fluorous' esters into hexane with no post reaction washing, and this remains to be achieved, but there are good prospects now for the development of such reactor systems. The authors thank the European Commission for supporting a Studentship (PB) through Research Training Network, ERBFM-RXCT9.

Notes and references

‡ Lipase from *Candida rugosa* (*CRL*) was purchased from the Sigma Chemical Co. and had a specific activity of 724 U mg^{-1} solid. In all experiments the lipase was used 'straight from the bottle'.

§ *Experimental procedure*: Ester–acid (1 mmol), 'fluorous' alcohol (1 mmol), PFH (10 ml), hexane (10 ml) and *CRL*§ (0.2 g) were placed in a conical flask with a rubber septum. The mixture was shaken at 200 rpm at 40 °C and periodically aliquots (5 μl) of the reaction solution were analysed by GC-MS to determine the extent of conversion. For the calculation of conversion, the calibration curves of starting and product esters and alcohols were constructed. The enzyme was filtered, the solid on the filter was washed with 2 ml of PFH and 2 ml of hexane. Liquid phases were collected, cooled (0 °C, 30 min) and separated. The hexane phase was washed with PFH (5 × 15 ml). The washed hexane phase contained unreacted ester–acid and less than 2% of 'fluorous' ester. Unreacted vinyl ester **1** was hydrolysed in an aqueous acid (dil. H_2SO_4) solution of mercury(II) acetate. Combined fluorous phases were concentrated under reduced pressure (100 °C, 20 torr to remove traces of acid), hydrolysed with methanolic LiOH and then acidified. The % ee values (including **6**) of the resulting acids were determined by GC-MS using a chiral column (β-DEX 120, Supelco).

1 I. T. Horváth and J. Rábai, *Science*, 1994, **266**, 72.
2 I. T. Horváth, *Acc. Chem. Res.*, 1998, **31**, 641; I. T. Horváth, G. Kiss, R. A. Cook, J. E. Bond, P. A. Stevens, J. Rábai and E. J. Mozeleski, *J. Am. Chem. Soc.*, 1998, **120**, 3133; L. P. Barthel-Rosa and J. A. Gladysz, *Coord. Chem. Rev.*, 1999, **190–192**, 587; B. Cornils, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2057; D. P. Curran, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 1174; E. de Wolf, G. van Koten and B.-J. Deelman, *Chem. Soc. Rev.*, 1999, **28**, 37; C. Rocaboy, D. Rutherford, B. L. Bennett and J. A. Gladysz, *J. Phys. Org. Chem.*, 2000, **13**, 596.
3 R. H. Fish, *Chem. Eur. J.*, 1999, **5**, 1677; J. J. J. Juliette, D. Rutherford, I. T. Horváth and J. A. Gladysz, *J. Am. Chem. Soc.*, 1999, **121**, 2696; G. Pozzi, M. Cavazzini, F. Cinato, F. Montanari and S. Quici, *Eur. J. Org. Chem.*, 1999, 1947.
4 A. M. Klibanov, *Acc. Chem. Res.*, 1990, **23**, 114.
5 A. M. Klibanov, *Chem.-Tech. (Heidelberg)*, June 1986, 354.
6 P. A. Fitzpatrick and A. M. Klibanov, *J. Am. Chem. Soc.*, 1991, **113**, 3166.
7 V. F. Hogan, D. O'Hagan and J. Sanvoisin, *Ind. J. Chem., Sect. B*, 1992, **31**, 883.
8 L. E. Kiss, I. Kövesdi and J. Rábai, *J. Fluorine Chem.*, 2001, **108**, 95.
9 B. Hungerhoff, H. Sonnenschein and F. Theil, *Angew. Chem., Int. Ed.*, 2001, **40**, 2492; B. Hungerhoff, H. Sonnenschein and F. Theil, *J. Org. Chem.*, 2002, **67**, 1781.
10 B-V. Nguyen and E. Hedenström, *Tetrahedron: Asymmetry*, 1999, **10**, 1821; K.-H. Engel, *Tetrahedron: Asymmetry*, 1991, **2**, 165; P. Berglund, M. Holmquist, E. Hedenström, K. Hult and H.-E. Högberg, *Tetrahedron: Asymmetry*, 1993, **4**, 1869.
11 R. G. Riley and R. M. Silverstein, *Tetrahedron*, 1974, **30**, 1171.
12 Spectroscopic characterisation of (*S*)-**6** was entirely consistent with the literature. $[\alpha]_D^{24}$ = +19.1° (c, 2.9, hexane); (lit.[11] $[\alpha]_D^{27}$ = +21.0°).