# Resolution of Racemic Acids by Irreversible Lipase-Catalyzed Esterification in Organic Solvents

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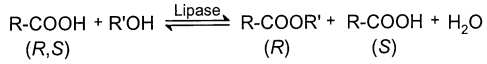
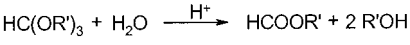
The reversible nature of the direct esterification of acids with alcohols limits the use of this process in the biocatalytic kinetic resolution of chiral acids. A new irreversible procedure, using orthoesters, has been developed.

The pioneering work of Klibanov[1] has paved the way for enzymatic catalysis in predominantly organic media, and lipases (triacylglycerol acyl hydrolases, E. C. 3.1.1.3) have been used to efficiently catalyze a multitude of esterification, transesterification and interesterification reactions. In particular, highly enantioselective direct esterification has been widely applied to the kinetic resolution of racemic alcohols and acids.[2] However, the reversibility of the reaction makes it impossible to obtain the slower reacting enantiomer in high optical purity simply by extending the conversion at the expense of chemical yield. In fact, since the same enantiomer is the fast reacting one in both the forward (esterification) and the reverse (hydrolysis) reaction, the optical purity of the unchanged substrate drops rapidly when the conversion is extended much beyond 50%.[3] In order to overcome this detrimental effect in the resolution of alcohols, irreversible esterification procedures have been resorted to, involving the use of activated or enol esters as acyl donors.[4] In the case of the resolution of racemic acids (Scheme 1) it is essential to decrease the influence of the reverse process by keeping the water content to a low level.




Scheme 1. Esterification of a racemic acid catalyzed by a lipase with *R*-stereopreference

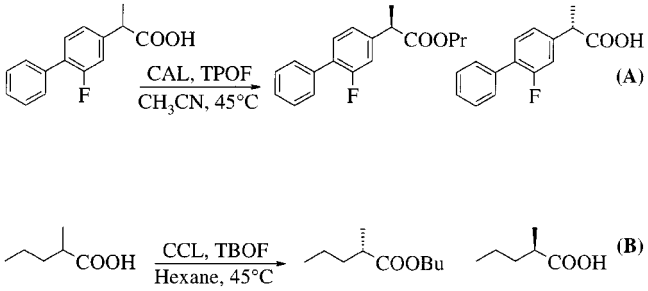
Many approaches have been proposed for reducing water levels during esterification. They include the use of molecular sieves[5] or the coupling of a hydrated salt and its lower hydrated or anhydrous form,[6] evaporation from solvent-free reaction mixtures,[7] or sorption of water into a polymeric membrane followed by selective diffusion and evaporation into a vapor phase.[8] As these physical methods appeared to us to have their drawbacks or to be of limited applicability, we considered the possibility of resorting to a chemical method, and here we report the successful biocatalyzed resolution of racemic acids in the presence of orthoformates. The decision to use orthoformates in the resolution of racemic acids was based on the consideration that these orthoesters are known to be easily hydrolyzed in acidic conditions according to Equation (1):[9]




(1)

As such, orthoformates were expected to preclude accumulation of water in the reaction mixture and thus limit the reverse reaction, and at the same time furnish the nucleophile for the forward reaction.

In this study two substrates were used as models (Scheme 2), flurbiprofen [(*R,S*)-2-(2-fluoro-4-biphenyl)propanoic acid], a member of the nonsteroidal anti-inflammatory drugs (NSAIDs),[10] and (*R,S*)-2-methylvaleric acid, an intermediate in the synthesis of branched-chain biologically active compounds.[11]




Scheme 2. Enzymatic esterification of (±)-flurbiprofen (**A**), and (±)-2-methylvaleric acid (**B**)

Trimethyl (TMOF), triethyl (TEOF), tripropyl (TPOF) and tributyl orthoformate (TBOF) were the orthoesters examined to investigate the influence of the alkoxy groups on the course of the reaction. Flurbiprofen was esterified in acetonitrile in the presence of lipase from *Candida antartica* (Novozym 435®), while for 2-methylvaleric acid a different solvent (hexane), and catalyst (lipase from *Candida rugosa*, CRL), were used. Each experiment was run in parallel with a control, in which the orthoformate was replaced by the corresponding alcohol. At the end of the incubation period the values for conversion and enantiomeric excess (*ee*) were determined by chiral HPLC or GC.

The results of the lipase-catalyzed esterification with orthoformates, relative to those obtained with the "normal" procedure with the corresponding alcohols as reagents, are

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shown graphically in Figure 1 and 2. Inspection of Figure 1a shows that in the normal esterification of flurbiprofen the *ee* of the unchanged substrate reaches a maximum value of 80−85% after ca. 40 h and then begins to drop. In contrast, when orthoformates are used the course of the reaction is similar to an irreversible reaction (Figure 1b) and the *ee* values continue to increase upon extension of the incubation period, and, consequently, the conversion value. With all the orthoformates tested, after 140 h of reaction time the conversion was between 60 and 70%, with an *ee* value for the unchanged acid of 95−98%.

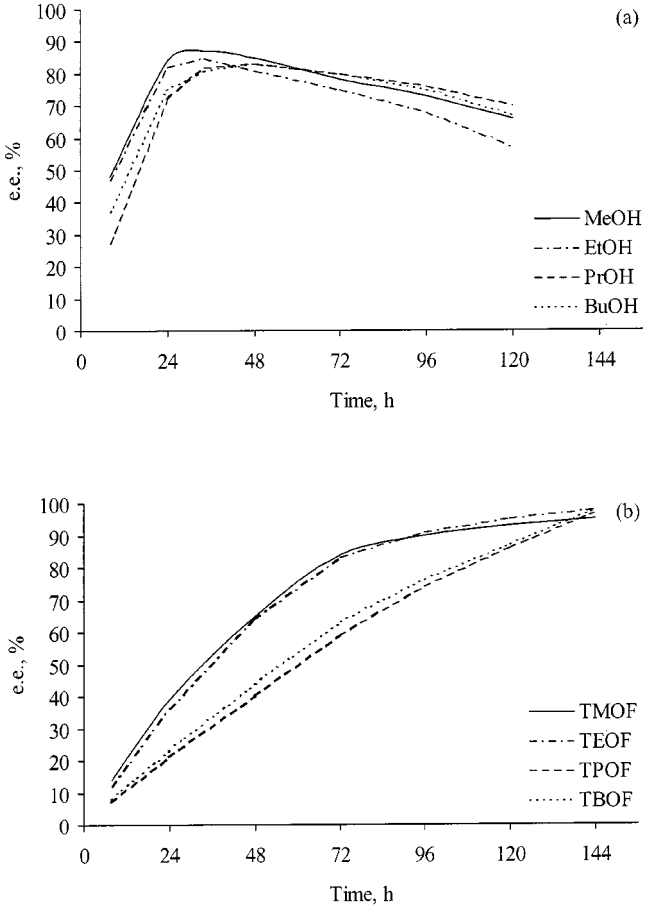


Figure 1. Enantiomeric excess (*ee*) value of unchanged flurbiprofen versus reaction time with (a) different alcohols, and (b) orthoformates

For 2-methylvaleric acid the trend was similar, in that the normal esterification (Figure 2a) followed the expected course for a reversible reaction, where the *ee* of the residual acid decreased when conversion was extended much beyond 50%. The esterification of 2-methylvaleric acid with orthoformates also proceeded as an irreversible reaction (Figure 2b). Of the four orthoformates tested, tributyl orthoformate was found to be superior, giving *ee* values for the remaining substrate of 97 and >98% at conversions of 65 and 70%, respectively.

Finally, ancillary experiments on the hydrolysis of tripropyl orthoformate under different conditions demonstrated the purely chemical nature of the reaction, without the participation of the enzyme.

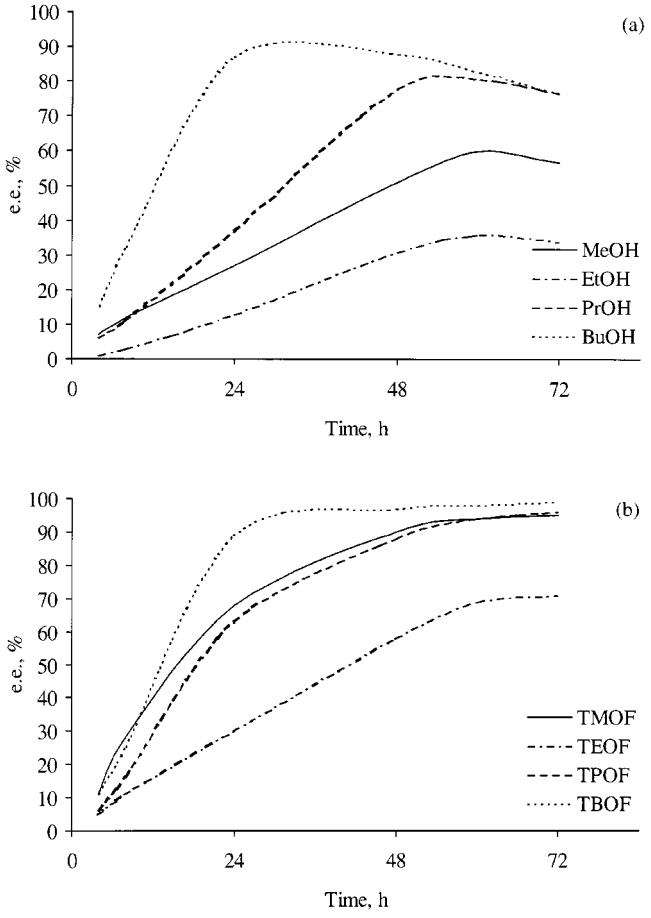


Figure 2. Enantiomeric excess (*ee*) value of unchanged 2-methylvaleric acid versus reaction time with (a) different alcohols, and (b) orthoformates

In summary, this study shows that orthoformates can be used in the lipase-catalyzed esterification aimed at the kinetic resolution of racemic acids, circumventing the adverse effects of the water formed in the course of the reaction. Water not only affects negatively the position of the equilibrium, but also acts on the optical purity of the remaining substrate, which cannot be raised to high levels simply by extending the conversion. Orthoformates trap the water through hydrolysis and therefore prevent the reverse reaction, and at the same time provide the nucleophile for the esterification. Obviously, the method proposed here can be used not only in the resolution of chiral acids, but also in the esterification of achiral acids to increase the yield of ester by pushing the equilibrium toward completion. Increasing the yield can be of great value when the achiral acids are expensive.

## Experimental Section

**General:** $^1$H NMR spectra were recorded in $CDCl_3$ solution at 250 MHz on a Bruker AC 250 instrument. − Mass spectra were recorded on a Hewlett−Packard GC-MS instrument, Mod. 5971A. − Optical rotation data were measured at 25 °C on a Jasco DIP 370 digital polarimeter. − All reactions were monitored (conversion and *ee* values) by HPLC using a Chirex R-NGLY &

DNB (250 × 4.0 mm) column (0.02 M ammonium acetate in methanol as the eluent) or GC using a β-cyclodextrin (dimethylpenthyl-β-cyclodextrin/OV1701 3:7) column. – Lipase from *Candida rugosa* (CRL) was purchased from Sigma. Novozym 435® (lipase from *Candida antarctica*, CAL) was a gift from Novo Nordisk. – Elemental analyses were performed on a Carlo Erba Instrument CHNS-O EA1108 Elemental Analyzer.

**Preparation of Enantiopure (*S*)-(+)-Flurbiprofen:** Racemic flurbiprofen (41 mmol, 10 g) was added to a solution of $CH_3CN$ (1 l) containing tripropyl orthoformate (123 mmol, 26.5 mL), 0.1 mL of *n*-propanol and Novozym 435® (100 g). The mixture was incubated by shaking at 45 °C (300 rpm); the conversion and *ee* of unchanged flurbiprofen were followed by chiral HPLC analysis. After 6 days conversion had reached 60% and the reaction was stopped by filtering off the enzyme. Removal of the solvent in vacuo left a residue that was partitioned between hexane and aq. $NaHCO_3$ (3 g in 200 mL of water). The organic phase was washed with water, dried over $Na_2SO_4$ and the solvent removed to afford (*R*)-flurbiprofen propyl ester (6.8 g, yield 58%, *ee* 64%). – $^1$H NMR ($CDCl_3$): δ = 0.89 (t, *J* = 7 Hz, 3 H), 1.54 (d, *J* = 7 Hz, 3 H), 1.65 (m, 2 H), 3.78 (q, *J* = 7 Hz, 1 H), 4.06 (t, *J* = 6 Hz, 2 H), 7.1–7.6 (m, 8 H). – $C_{18}H_{19}FO_2$: calcd. C 75.50, H 6.69; found C 75.62, H 6.89.

Acidification of the aqueous phase with $H_2SO_4$ gave a precipitate of (*S*)-(+)-flurbiprofen (3.9 g, yield 39%, *ee* >98%). – $C_{15}H_{13}FO_2$ (244.26): calcd. C 73.76, H 5.36; found C 73.90, H 5.52.

**Preparation of Enantiopure (*R*)-(–)-2-Methylvaleric Acid:** Racemic 2-methylvaleric acid (86.2 mmol, 10 g) was dissolved in hexane (500 mL) containing tributyl orthoformate (86.2 mmol, 23 mL), 0.1 mL of *n*-butyl alcohol and *Candida rugosa* lipase (CRL) (50 g). The mixture was incubated by shaking at 45 °C (300 rpm); the conversion and *ee* of the butyl ester were followed by chiral GC analysis. After 48 h conversion had reached 65% and reaction was stopped by filtering off the enzyme. After partition with aq. $NaHCO_3$ (3 g in 200 mL of water) the hexane phase was dried over $Na_2SO_4$ and evaporated under vacuum to furnish (*S*)-2-methylvaleric butyl ester (9.6 g, yield 65%, *ee* 53%). MS data agreed with those reported in the literature.[12] – $C_{10}H_{20}O_2$ (172.27): calcd. C 69.72, H 11.70; found C 69.98, H 11.84.

The aqueous phase was acidified with $H_2SO_4$, extracted three times with hexane and the organic phases were pooled. Removal of hexane under vacuum gave (*R*)-(–)-2-methylvaleric acid (3.5 g, yield 35%, *ee* > 97%). $[\alpha]_D^{20} = -18.2$ (neat); [ref. $[\alpha]_D^{20} = -18.4$ (neat)].[13] – $C_6H_{12}O_2$ (116.19): calcd. C 62.04, H 10.41; found C 62.31, H 10.52.

## Acknowledgments

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[1] [1a] A. Zaks, A. M. Klibanov, *Science* **1984**, *224*, 1249–1251. – [1b] A. Zaks, A. M. Klibanov, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 3192–3196. – [1c] A. Zaks, A. M. Klibanov, *J. Am. Chem. Soc.* **1986**, *108*, 2767–2768. – [1d]A. M. Klibanov, CHEMTECH **1986**, *16*, 354–359.

[2] [2a] C. H. Wong, G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Tetrahedron Organic Series, Vol. 12, Pergamon, Oxford, **1994**. – [2b] K. Faber, *Biotransformations in Organic Chemistry*, 4th ed., Springer Verlag, Berlin, **2000**. – [2c] G. Carrea, S. Riva, *Angew, Chem.* **2000**, *112*, 2312–2341; *Angew. Chem. Int. Ed.* **2000**, *39*, 2226–2254.

[3] [3a] C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299. – [3b] C. S. Chen, S. H. Wu, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1987**, *109*, 2812–2817. – [3c] C. S. Chen, C. J. Sih, *Angew. Chem.* **1989**, *101*, 711–724; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695–707.

[4] [4a] M. Deguil-Castaing, B. De Jeso, S. Drouilard, B. Maillard, *Tetrahedron Lett.* **1987**, *28*, 953–954. – [4b] Y. F. Wang, C. H. Wong, *J. Org. Chem.* **1988**, *53*, 3127–3129. – [4c] J. Hiratake, M. Inagaki, T. Nishioka, J. Oda, *J. Org. Chem.* **1988**, *53*, 6130–6133. – [4d] Y. F. Wang, J. J. Lalonde, M. Momogan, D. E. Bergbreiter, C. H. Wong, *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.

[5] F. Fonteyn, C. Blecker, G. Lognay, M. Marlier, M. Severin, *Biotechnol. Lett.* **1994**, *16*, 693–696.

[6] [6a] P. Kuhl, P. J. Halling, *Biochim. Biophys. Acta* **1991**, *1078*, 326–328. – [6b] L. Kvittingen, B. Sjursnes, T. Anthonsen, P. Halling, *Tetrahedron* **1992**, *48*, 2793–2802. – [6c] M. Zarevúcka, M. Rejzek, M. Hoskovec, A. Svatoš, Z. Wimmer, B. Koutek, M. D. Legoy, *Biotechnol. Lett.* **1997**, *19*, 745–750.

[7] S. M. Kim, J. S. Rhee, *J. Am. Oil Chem. Soc.* **1991**, *68*, 499–503.

[8] S. J. Kwon, K. M. Song, W. H. Hong, J. S. Rhee, *Biotechnol. Bioeng.* **1995**, *46*, 393–395.

[9] D. Barton, W. D. Ollis, *Comprehensive Organic Chemistry, Vol. 2* (Ed.: I. O. Sutherland), Pergamon Press, Oxford, **1979**.

[10] J. G. Lombardino, *Nonsteroidal anti-inflammatory drugs*, Wiley, New York, **1985**.

[11] K. Mori, *Tetrahedron* **1989**, *45*, 3233–3298.

[12] J. Kim Ha, R.C. Lindsay, *J. Food Compos. Anal.* **1989**, *2*, 118–131.

[13] P. A. Levene, R. E. Marker, *J. Biol. Chem.* **1932**, *98*, 1–7.

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