Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 7, pp. 1773-1778, 1995
Elsevier Science Ltd
Printed in Great Britain
0957-4166/95 $9.50+0.00

0957-4166(95)00223-5

Resolution of Racemic Flurbiprofen by Lipase-Mediated Esterification in Organic Solvent

Raffaele Morrone, Giovanni Nicolosi,* Angela Patti and Mario Piattelli

Istituto CNR per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico
Via del Santuario 110, 95028 Valverde CT, Italy

Abstract: Resolution of *rac*-flurbiprofen, 2-fluoro-α-methyl-[1,1'-biphenyl]-4-acetic acid (**1**), by biocatalytic methodologies has been studied. Enzymatic hydrolysis of *rac*-flurbiprofen methyl ester (**2**) in aqueous-organic medium gave poor results. Transesterification of the same ester mediated by immobilized lipase from *Candida antarctica* (Novozym® 435) in organic solvent proceeded with good enantiomeric excess but the isolation of the product required chromatographic separation and therefore was unsuitable for large scale preparation. Direct esterification of **1** with methanol in acetonitrile promoted by Novozym® 435 proved to be the best method since it gave, *via* a twofold kinetic resolution, *S*-flurbiprofen with excellent enantiomeric excess. The *R*-flurbiprofen methyl ester formed in the reaction can be converted into the starting *rac*-flurbiprofen by alkaline hydrolysis or, alternatively, into *R*-flurbiprofen by hydrolysis with acid.

INTRODUCTION

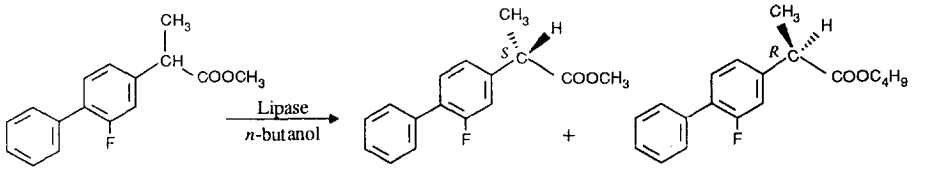
The difference in the pharmacological properties of the enantiomers of a chiral drug is by no means a new notion. However, racemic compounds have been and are still frequently marketed for medical applications, assuming that the unwanted isomer (the distomer) is only a "stereochemical ballast", devoid of any biological activity, accompanying the isomer in which the biological activity resides (the eutomer). Not so long ago the thalidomide incident dramatically drew attention to the consequences of chirality on pharmacological activity and the need to use enantiomerically pure chiral compounds for pharmaceutical purposes. Therefore, the demand for single-isomer chiral drugs is now growing rapidly and methods for their production are being actively investigated. Members of the 2-aryl propanoic acid (APA) class of pharmaceuticals (the "profen" family) are non steroidal anti-inflammatory drugs (NSAIDs) that, by inhibiting the cyclooxygenase system, are able to reduce inflammation and pain. They are also effective as analgesics and antipyretics and are marketed and consumed as racemic mixtures, apart from (+)-(*S*)-naproxen and (+)-(*S*)-flunoxaprofen. The anti-inflammatory activity of members of this class is mainly due to the (*S*)-enantiomers, their (*R*)-isomers lacking significant cyclooxygenase inhibiting effect. On the other hand, it has been shown that *R*-flurbiprofen blocks nociception in rats, indicating a different mechanism of analgesia and suggesting the use of this enantiomer, and possibly of other *R*-aryl-propanoic acids, as analgesics.[1] Within the profen family, flurbiprofen deserves particular attention, since the gastrointestinal toxicity of the (*S*)-enantiomer is greatly enhanced by the presence of (*R*)-enantiomer.[2] Moreover, while another member of the same family, (*R*)-ibuprofen, undergoes chiral inversion in the rat and man, flurbiprofen is reported to be less prone to enantiomeric conversion *in vivo*.[3a,b]

In this work, the resolution of *rac*-flurbiprofen, 2-fluoro-α-methyl-[1,1'-biphenyl]-4-acetic acid (**1**), by enzyme-catalyzed reactions (hydrolysis of esters, transesterification, direct esterification) is examined.

RESULTS AND DISCUSSION

Since enzymatic hydrolysis of esters of an arylpropanonic acid, *rac*-naproxen, has been used successfully for its resolution,[4a-c] we considered first the possibility of applying this methodology to *rac*-flurbiprofen methyl ester (±)-**2**. Among the tried enzymatic preparations (lipase from *Candida cylindracea*, immobilized lipases from *C. antarctica* and *Mucor miehei*, lipase from *Pseudomonas cepacia*, porcine pancreatic lipase), only *C. antarctica* lipase (Novozym® 435) was found to catalyse the reaction with satisfying rate, but the enantiomeric excess of the isolated (*R*)-flurbiprofen was poor (ca. 30 % ee).

As the enzyme-catalyzed hydrolysis of (±)-**2** was disappointing for the resolution, we turned next to the lipase catalyzed transesterification of this same ester with *n*-butanol, according to the following scheme.



(±)-**2** (+)-**2** (-)-**3**

Out of the five lipases listed above, only Novozym® 435 gave results acceptable in terms of reaction rate and optical yields. Hexane, *tert*-butyl methyl ether, *tert*-amyl alcohol, tetrahydrofuran, dioxane and acetonitrile

Table 1. Effect of Organic Solvent on the Transesterification of (±)-**2** with *n*-Butanol Catalysed by Novozym® 435[a]

Solvent	$\log P_{oct}$	Time (h)	Conv. (%)	ee of **3**	E[b]
Dioxane	-0.42	24	34	82	15.9
Acetonitrile	-0.34	6	25	80	11.7
Tetrahydrofuran	0.49	24	13	70	6.3
tert-Amyl-alcohol	0.89	12	35	66	6.7
tert-Butyl methyl ether	1.35	24	31	68	7.0
Hexane	3.50	48	0	-	-

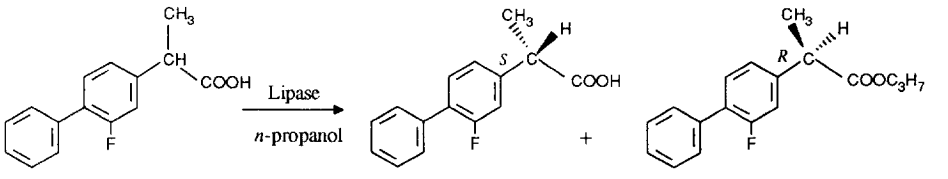
[a]Substrate concentration 10 mg/mL, lipase 100 mg/mL, 45°C, 300 rpm.

[b]The E values were calculated from the degree of conversion and ee of the product according to ref. 5.

were examined as solvents. This screening (Table 1) disclosed that the enantiomeric ratio E[5] tendentiously raises with decreasing hydrophobicity of the solvent expressed as $\log P_{oct}$, the highest enantioselectivities being observed with dioxane and acetonitrile (the values of E were 15.9 and 11.7, respectively). In these two solvents, replacement of *n*-butanol with ethanol, *n*-propanol or *n*-hexanol did not improve significantly the optical yields.

Although these results were quite encouraging, we considered that a major problem in this procedure is the separation of the unreacted ester from the newly-formed one. This requires a cumbersome chromatographic separation, that makes the scaling up of the procedure impractical. The use of amino alcohols for transesterification appeared a possible way to overcome this difficulty,[6] since in this case the formed *R*-ester can be separated by extraction in acidic media. Unfortunately, all the five alcohols tried [2-(2-hydroxyethyl)-pyridine, 1-(2-hydroxyethyl)-piperazine, 4-(2-hydroxyethyl)-morpholine, 1-(2-hydroxyethyl)-pyrrolidine, 1-[2-(2-hydroxy-ethoxy)ethyl]-piperazine] were completely inactive.

Finally, we investigated the direct esterification of (±)-**1** catalyzed by lipase, according to the following scheme.



(±)-**1** (+)-**1** (-)-4

In preliminary experiments, using *tert*-butyl methyl ether as solvent and *n*-propanol as the alcohol,[7] Novozym® 435 was the only active lipase out of the five screened, which were the same as those used in the hydrolysis and transesterification experiments. Variation of the solvent (Table 2) markedly influenced both esterification rate and enantioselectivity. Although dioxane gave the highest E value (22.2), in this solvent the reaction rate was definitely too slow, inducing us to concentrate the effort on the reaction in acetonitrile, less enantioselective (E=12.3) but decidedly faster.

Table 2. Effect of Oorganic Solvent on the Esterification of (±)-**1** with *n*-Propanol Catalysed by Novozym® 435[a]

Solvent	$logP_{oct}$	Time (h)	Conv. (%)	ee of **4**	E[b]
Dioxane	-0.42	24	5	91	22.2
Acetonitrile	-0.34	24	19	82	12.3
Tetrahydrofuran	0.49	48	5	n.d.	-
tert-Amyl alcohol	0.89	24	4	73	6.6
tert-Butyl methyl ether	1.35	24	7	0	1.0
Toluene	2.50	24	20	54	3.8
Cyclohexane	3.20	4	20	52	3.6

[a]Substrate concentration 10 mg/mL, lipase 100 mg/mL lipase, alcohol/acid ratio = 3, 45 °C, 300 rpm.

[b]The E values were calculated from the degree of conversion and ee of the product according to ref. 5.

The nature of the alcohol (methanol, ethanol, *n*-propanol, *n*-butanol, *n*-hexanol) in the reaction in acetonitrile had a weak influence on conversion rate and enantioselectivity and the same holds true for the alcohol to acid ratio in the range from 1:1 to 3:1. As expected for a reversible reaction,[8] the ee value of the

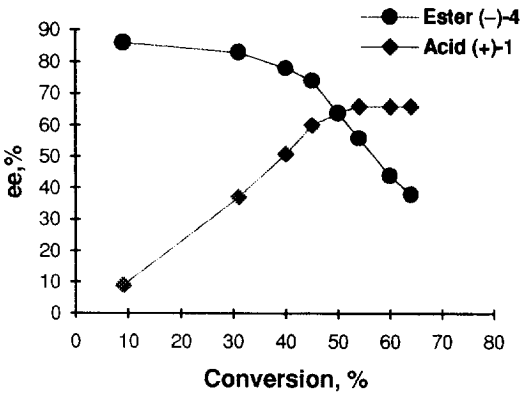


Figure 1. Enantiospecific esterification of (±)-**1**. The curves depict the variation of the ee's of (–)-**4** and unreacted (+)-**1** as a function of the percentage conversion.

unreacted substrate did not increase by extending the conversion beyond 50%, while the ee of the ester underwent a gradual decay (Figure 1). Therefore, in a gram scale esterification with methanol[9] quenched at a degree of conversion near 45% the optically enriched *S*-flurbiprofen (ee 65%) was separated from *R*-flurbiprofen methyl ester by extraction in basic medium followed by acidification. When this material was submitted to a second lipase promoted resolution, *S*-flurbiprofen with high ee (>95%, total yield 36% based on the starting racemic acid) was obtained.

If only the *S*-enantiomer is needed, the enriched *R*-methyl ester obtained as by-product can be very easily converted into *rac*-flurbiprofen by treatment at room temperature with a solution of NaOH in aqueous ethanol. This treatment causes hydrolysis with concurrent racemization and the racemic acid, recovered by filtration after acidification of the solution, can be applied again in the lipase-mediated resolution without any further purification. If, on the contrary, the *R*-enantiomer is also needed, the enriched *R*-methyl ester can be subjected to acid hydrolysis to give *R*-flurbiprofen with satisfying ee.

In summary, from the data reported it is apparent that esterification of *rac*-flurbiprofen in organic medium catalyzed by lipase from *C. antarctica* is very convenient and, since it does not require any chromatographic separation, readily amenable to large-scale preparation.

MATERIALS AND METHODS

General

^{1}H-nmr spectra were recorded in $CDCl_3$ solution on a AC 250 Bruker instrument at 250 MHz. Europium (III) tris[3-(heptafluoropropyl)hydroxymethylene]-(+)-camphorate [Eu(hfc)$_3$] was used as chiral shift reagent for enantiomeric excess determination. Optical rotations were measured on a DIP 135 Jasco instrument. TLC was performed on Silica gel plates (Merck 60 F_{254}). Hplc analyses were performed on a reverse phase C_{18} column eluting with MeOH/citrate buffer pH 4 (7:3). Solvents (analytical grade) were used without further purification. *rac*-Flurbiprofen (±)-**1** was purchased from Aldrich. Lipase from *Candida cylindracea* was from Sigma. Immobilised lipases from *Candida antarctica* (Novozym® 435) and *Mucor miehei* (Lipozyme® IM) are registered marks from Novo Nordisk. Lipase from *Pseudomonas cepacia* was obtained from Amano International Co. *rac*-Flurbiprofen methyl ester (±)-**2** was obtained in 95% yield by a conventional esterification procedure of (±)-**1** (K_2CO_3 and MeI in acetone)

Determination of the Enantiomeric Excesses of Optically Active Compounds.

^{1}H NMR spectroscopy of enantiomerically enriched esters (Me, Et, *n*-Pr, *n*-Bu, *n*-hexyl) in the presence of the chiral shift reagent Eu(hfc)$_3$ gives rise for the two diastereoisomers to signals for the methyl group attached at the stereogenic centre sufficiently different in their chemical shift values to allow calculation of the integration areas. The enantiomeric excess of the free acid **1** was measured after conversion into **2** [solid K_2CO_3 was added

to a solution of enantiomerically enriched **1** (usually 5 mg) and MeI (4 μL) in ca. 0.5 ml of acetone; the mixture was shaken for 2 h at rt, then the solid was filtered and the filtrate taken to dryness].

Enzymatic Hydrolysis of (±)-2

A solution of (±)-**2** (50 mg) in 10 mL of a 2:1 vol/vol mixture of 0.1 M phosphate buffer (pH 7.5) and dioxane was shaken (300 rpm) at 40 °C with 180 mg of Novozym® 435. After 2 h (conversion ca. 46%) the reaction was stopped by filtering off the enzyme. The filtrate was acidified with 6N HCl and extracted with *tert*-butyl methyl ether. Evaporation of the organic solvent gave a residue which was partitioned between sat. aqueous $NaHCO_3$ and hexane. (*R*)-(–)-**1** was recovered from the aqueous layer by acidification with 6N HCl and extraction with *tert*-butyl methyl ether (20 mg, 43% yield, 32% ee). Assignment of its absolute configuration was based on comparison of its optical rotation with literature data.[10] The organic phase contained enriched (*S*)-(+)-**2** (25 mg, 50% yield, 27% ee).

Enzymatic Transesterification of (±)-2

A solution of (±)-**2** (20 mg) in the solvent of choice (2 mL, Table 1) was added with Novozym 435® (200 mg) and *n*-butanol (72 μL, 10 equiv.). The suspension was incubated at 45° C and shaken at 300 rpm. The reaction was monitored by hplc and stopped by filtering off the enzyme at the given value of the conversion. The filtrate was evaporated in vacuo and the residue subjected to preparative TLC (*tert*-butyl methyl ether:hexane 1:9) to afford (*R*)-(–)-**3** and unreacted (*S*)-(+)-**2**.

In a larger scale run Novozym 435® (2.5 g) was added to a solution of (±)-**2** (250 mg , 0.97 mmol) and *n*-butanol (0.9 mL, 9.7 mmol) in of CH_3CN (25 mL). The reaction mixture was incubated at 45°C under shaking (300 rpm) for 6 h, then the lipase was filtered off and the solvent evaporated. The residue was subjected to chromatographic purification using *t*-butyl methyl ether/hexane (1:9) to afford (*R*)-(–)-**3** (74 mg, 25% yield, 80% ee). $[\alpha]_D$ –16.5 (*c* 0.6, $CHCl_3$), and (*S*)-(+)-**2** (80 mg, 70 % yield, 25% ee).

Enzymatic Esterification of (±)-1

Preliminary experiments were performed dissolving (±)-**1** (20 mg, 0.08 mM) in the solvent of choice (2 mL, see Table 2), containing 200 mg of Novozym® 435 and *n*-propanol (18 μL, 3 equiv.). The suspension was incubated at 45 °C and shaken (300 rpm). At the appropriate conversion of the substrate, as determined by hplc, the reaction was quenched by filtering off the enzyme and the filtrate taken to dryness under reduced pressure. The residue was partitioned between sat. aq. $NaHCO_3$ and hexane. The organic phase, after drying over Na_2SO_4 and evaporation, afforded enriched (*R*)-(–)-**4**. The aqueous phase was acidified with 6N HCl and extracted with *tert*-butyl methyl ether to give unreacted (*S*)-(+)-**1**.

Esterification of **1** with different alcohols (methanol, ethanol, *n*-butanol, *n*-hexanol) in acetonitrile was carried out following an analogous procedure.

Preparative esterification of (±)-1

Novozym® 435 (10 g) was added to a solution of (±)-**1** (1g) and methanol (0.5 mL) in CH_3CN (100 mL). The mixture was shaken (300 rpm) at 45 °C for 48 h until conversion reached ca. 50%. After filtering off the enzyme, evaporation of the solvent gave a residue which was partitioned between hexane and sat. $NaHCO_3$. The organic layer was taken to dryness to give (*R*)-(–)-**2** (550 mg, 47% yield , ee 76%), $[\alpha]_D$ –39.2 (*c* 1.5, $CHCl_3$) while the aqueous phase was acidified and the precipitate collected by filtration to yield (*S*)-(+)-**1** (490 mg, 49% yield, ee 65%).

The unreacted (*S*)-(+)-**1** (400 mg) was subjected to recycling in the above conditions to give, after 24 h incubation, (*R*)-(–)-**2** (118 mg, 25% yield, 40% ee) and (*S*)-(+)-**1** (290 mg, 72% yield, ee >95%). $[\alpha]_D$ +41.4 (*c* 1, $CHCl_3$).

Racemic Flurbiprofen from (R)-(–)-2

A solution of (*R*)-(–)-**2** (0.5 g, ee 76%) in 5 mL of EtOH was added to an aqueous solution of NaOH (0.15 g in 2.5 mL of H_2O) and the mixture was refluxed for 3 h. After cooling, the solution was acidified and the racemic flurbiprofen collected by filtration (0.45 g, 96% yield).

Acid Hydrolysis of (R)-(–)-2 to give (R)-Flurbiprofen.

A solution of (*R*)-(–)-**2** (ee 76%, 100 mg) in dioxane (5 mL) was added to 5% vol/vol aqueous H_2SO_4 (2.5 mL) and the mixture refluxed for 2 h. After addition of water (5 mL) the reaction mixture was partitioned with methyl *tert*-butyl ether and the organic layer taken to dryness to give (*R*)-**1** (92 mg, 98% yield, ee 75 %).

Acknowledgements

Thanks are due to Consiglio Nazionale delle Ricerche (Roma) "Progetto Strategico Tecnologie Chimiche Innovative" for financial support. We also thank Novo Nordisk for a gift of Lipozyme® IM and Novozym® 435.

REFERENCES AND NOTES

1. Brune, K., Beck, W.S., Geisslinger, G., Menzel-Soglowek, S., Peskar, B.M., Peskar, B.A. *Experientia* **1991** *47*, 257.

2. Wechter, W.J.; Bigornia, A.E.; Murray, E. D.; Levine, B.H.; Young, J.W. *Chirality* **1993**, *5*, 492.

3. a) Geisslinger, G.; Ferreira, S.H.; Menzel, S.; Schlott, D.; Brune, K. *Life Sciences* **1994,** *54*, PL173. b) Wechter, W.J. *J. Clin. Pharmacol.* **1994**, *34*, 1036.

4. a) Gu, Q.-M.; Chen, C.-S.; Sih, C. J. *Tetrahedron Lett.* **1986,** *27*, 1763. b) Cesti, P.; Piccardi, P. Eur. Pat. App, 195717, 1986. c) Hernaiz, M. J.; Sanchez-Montero, J. M.; Sinistera, J.V. *Tetrahedron* **1994**, 50, 10749.

5. Chen, S.C.; Wu, S.H.; Girdaukas, G.; Sih, C.J. *J. Am. Chem. Soc.* **1987,** *109*, 2812.

6. Palomer, A.; Cabré, A.; Ginesta, J.; Mauleon, D.; Garganico, G. *Chirality* **1993,** *5*, 320.

7. In the lipase-mediated esterification of a structurally related arylpropanoic acid, *n*-propanol is reported to give the highest conversion (see ref. 10).

8. Chen, C.-S.; Sih, C.J. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695.

9. The use of methanol in the esterification did not affect the activity of the lipase, that after five recycling retained 85% of its activity.

10. Arroyo, M.; Sinisterra, J.V. *J. Org. Chem.* **1994**, 59, 4410.

(*Received in UK* 23 *May* 1995)