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Enzyme-Catalysed Enantioselective Hydrolysis of Racemic Naproxen Nitrile[1]

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Abstract—The bacterial strain *Rhodococcus butanica* (ATCC 21197), which exhibits nitrilase and nitrile hydratase/amidase activities, catalyses the enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to furnish a moderate enantiomeric excess of (*S*)-naproxen (*S*)-**3**. Racemic naproxen amide (*R/S*)-**2** is not a good substrate for this strain. Resting cells of the newly selected bacterial strain *Rhodococcus* sp. C3II catalyse the enantioselective hydrolyses of racemic naproxen nitrile (*R/S*)-**1** and naproxen amide (*R/S*)-**2** as well, to give (*S*)-**3** in excellent optical (99 % *e.e.*) and good chemical yields in aqueous medium and in the biphasic system of phosphate buffer/hexane.

2-Arylpropanoic acids constitute an important class of non-steroidal, anti-inflammatory compounds.[3] Several substances of this class, such as naproxen, ibuprofen, ketoprofen, and fluorbiprofen, have been successfully introduced as chemotherapeutic agents.

Although all of the above mentioned compounds possess a centre of asymmetry, only syntheses leading to their racemates were developed until recently.[3] This situation has changed within the last few years. Before clinical approval of a new biologically active substance possessing a centre of chirality can be obtained, the spectrum of action and metabolism of each individual enantiomer has to be demonstrated. If one stereoisomer should exhibit a markedly better activity than the other, it is advantageous to use only the more efficient stereoisomer in the commercial product.

For this reason, numerous investigations have been carried out in the past few years with the objective of preparing the clinically most important examples of 2-arylpropanoic acids in optically pure states. In a recent publication[4] all the major stereoselective syntheses leading to single enantiomers of this class of compounds were summarized. However, these were mainly chemical syntheses; enzymatic methods were only mentioned briefly.[4]

In the case of enzymatic methods it is obvious that the resolution of racemic 2-arylpropanoic acids could be achieved by enantioselective hydrolysis of the esters or by enantioselective esterification of the carboxylic acids using esterases or lipases, respectively.[5] A further possibility for the preparation of optically active α-arylpropionic acids by way of an enzyme-catalysed reaction is the enantioselective hydrolysis of the corresponding racemic nitriles. This is the subject of the present paper.

The use of enzymes or resting cells, respectively, for the preparation of carboxylic acid amides or carboxylic acids from the corresponding nitriles has been described by H. Yamada *et al.* as well as by other groups.[6] The hydrolysis of acrylonitrile to acrylamide by resting cells of *Pseudomonas chlororaphis* B23, for example, has been developed into a large-scale industrial process.[6a,b]

The nitrile hydrolysis can take place either directly to furnish the carboxylic acid by means of a nitrilase (EC 3.5.5.1) or as a two-step reaction by means of a nitrile hydratase to give the carboxylic amide and then by means of an amidase (EC 3.5.1.4) to yield the carboxylic acid.[7a]

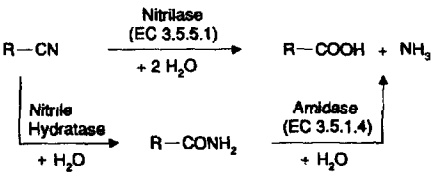


Figure 1. General mechanism of the enantioselective microbial hydrolysis of nitriles to carboxylic acids.

The hydrolysis of amides using amidases from bacterial strains of various genera was described by P. Galzy *et al.*[8] The enantioselective hydrolysis of racemic nitriles by means of an enzyme was reported by H. Ohta *et al.*[9] Since then some further papers and patents on the enantioselective hydrolyses of racemic nitriles by nitrilases or nitrile hydratases and amidases have appeared. Thus, α-aminonitriles have been hydrolysed to optically active amino acids by a nitrilase from *Rhodococcus rhodochrous* PA-34.[10] Similarly, O-protected cyanohydrins have been enantioselectively hydrolysed to the corresponding optically active α-hydroxycarboxylic acids by *Rhodococcus butanica*[11a] and by resting cells of *Pseudomonas* species.[11b] Much attention has been directed recently to the preparation of optically active α-arylpropionic acids from the corresponding nitriles by enantioselective hydrolyses using nitrile-hydrolysing enzymes.[9,12,13] Such enzymes have been found in plants, fungi, bacteria, sponges, algae, and insects.[7]

Our investigations concentrated mainly on the preparation of (*S*)-(+)-naproxen since only the (*S*)-enantiomer is used

in therapy and since it is one of the most commercially important members of this class of compounds.

Hydrolysis of α-Arylpropionic Acids by *Rhodococcus butanica* ATCC 21197

Ohta *et al.* have described a strain of *R. butanica*, ATCC 21197, that hydrolyses α-arylpropionitriles to the corresponding carboxylic acids with a very high enantioselectivity.[9] This strain exhibits nitrilase, nitrile hydratase, and amidase activities. The nitrilase preferentially hydrolyses the (*S*)-nitrile to give the (*S*)-acid. The stepwise conversion of racemic nitriles includes the preferential formation of the (*R*)-enantiomer by the nitrile hydratase and faster hydrolysis of (*S*)-amides by the amidase to give (*R*)-amides in high optical purities.[9]

Starting with cells obtained from the American ATCC collection (No. 21197), we achieved normal growth but the cells obtained did not exhibit any nitrile-hydrolysing activity. Only after H. Ohta[14] had kindly provided us with a sample of cells of the strain used in his work did we obtain cells which were indeed able to catalyse nitrile hydrolysis. Our results on the enantioselective nitrile hydrolysis are compared with the findings of H. Ohta in Table 1.

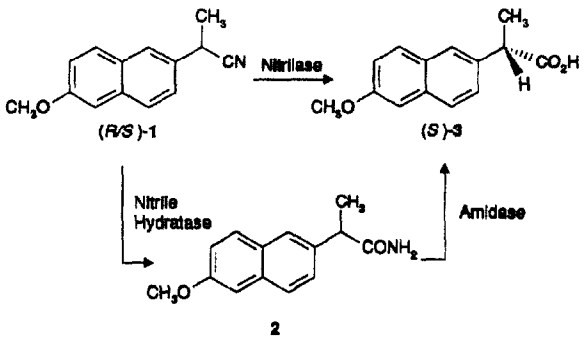
Table 1. Enantioselective hydrolysis of racemic α-arylpropionitriles to (*S*)-α-arylpropionic acids by *Rhodococcus butanica* in phosphate buffer (pH 8.0, 100 mM)[2,9]

				Results by Ohta et al.[9]		
	Reaction	(*S*)-Acid		Reaction	(*S*)-Acid	
Substrate R=	time [h]	Yield [%]	ee [%]	time [h]	Yield [%]	ee [%]
i-Bu	3	4	70	3	6	75
	8	16	78	6	13	87
	24	51	26	24	60	15
Cl	8	15	96	6	13	>99
	24	33	41	24	30	43
CH_3O	8	46	99	6	47	99
	24	54	88	24	59	53

From the results summarized in Table 1, it can be concluded that only in the case of 2-(*p*-methoxyphenyl)propionitrile (R = CH_3O) a high optical yield (99 % *e.e.*) was obtained in combination with a good chemical yield (47 % conversion). However, the enantioselectivity in the case of the important drug ibuprofen (R = *i*-Bu) is appreciably lower. Even at low conversion (13 %) an *e.e.* value of only 87 % was obtained (Table 1). Experiments on the enantioselective hydrolysis of racemic naproxen nitrile were not reported by Ohta.[9]

On the basis of our experience with 2-phenylpropionitriles (Table 1), we have investigated the enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** and naproxen amide (*R/S*)-**2** with resting cells of *R. butanica* ATCC 21197 under various conditions.

Scheme I illustrates the two possible pathways for the enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to optically active naproxen (*S*)-**3** under catalysis by *R. butanica*.



Scheme I. Enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to (*S*)-naproxen (*S*)-**3** catalysed by *Rhodococcus butanica*.

The racemic substrates naproxen nitrile (*R/S*)-**1** and naproxen amide (*R/S*)-**2** were prepared as described in the literature.[15] Racemic naproxen (*R/S*)-**3** can be obtained by hydrolysis of (*R/S*)-**1** with KOH in ethanol.[15a,16] Optically pure amide (*S*)-**2** was prepared from (*S*)-naproxen via the acid chloride and subsequent reaction with aqueous ammonia. The formation of (*S*)-naproxen was monitored directly by HPLC and the naproxen enantiomers were separated on a chiral phase with the enantiomeric excess calculated by evaluation of the peak areas.

Naproxen nitrile (*R/S*)-**1** is a good substrate for *R. butanica*. The results of the conversions at two different substrate concentrations are summarized in Table 2.

Table 2. Enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to naproxen (*S*)-**3** and naproxen amide **2** catalysed by resting cells of *Rhodococcus butanica* in phosphate buffer

A				B		
Reaction	(S)-3		2	Reaction	(S)-3	
time [h]	Yield [%]	ee [%]	Yield [%]	time [h]	Yield [%]	ee [%]
3	15	97	5	4	18	98
6	39	86	8	19	31	87
24	59	51	11	24	34	85

A: Resting cells (OD 4.8); (*R/S*)-**1**: 8.5 mg (0.040 mmol).
B: Resting cells (OD 60); (*R/S*)-**1**: 90.0 mg (0.43 mmol).

At the concentrations we used, the enantiomers of naproxen amide (**2**) were incompletely separated by HPLC on a human serum albumin (HSA) column; thus only the chemical yields are given in Table 2. The configuration of the enantiomer of naproxen amide obtained was elucidated by co-chromatography with the enantiopure (*S*)-amide (*S*)-**2**.

We have also investigated the reactions of (*R/S*)-**1** with immobilized cells of *R butanica*. Cell immobilization can, on the one hand, facilitate work-up of the reaction mixtures to simple decantation or filtration and, on the other hand, often has an advantageous effect on the maintenance of enzyme activity;[17a,b] the latter aspect is of particular relevance in the case of long reaction times. Furthermore, clumping of the cells in the organic solvent is prevented and the cells are better protected against damage by organic solvents.[18] Of the investigated immobilization matrices, (e.g. alginate, silica gel, and polyurethane) which have previously been used for the immobilization of *Rhodococcus* species,[17] we found polyurethane PU3[19] (mol.wt 2529 g/mol, 4.2 % NCO and 57 % ethylene oxide) to be the most suitable. In comparison to the results with non-immobilized cells, however, both the chemical and the optical yields were appreciably lower (see Experimental Section).

Naproxen nitrile (*R/S*)-**1** is only slightly soluble in aqueous media, so we also studied the reaction in biphasic systems composed of a phosphate buffer and an immiscible organic solvent. In this system the substrate (*R/S*)-**1** is dissolved in the organic phase. The product acid (*S*)-**3** is found exclusively in the aqueous phase, whereas the remaining nitrile (*R*)-**1** is dissolved in the organic solvent. Hexane was found to be the best for the enzyme-catalysed hydrolysis of (*R/S*)-**1** in a biphasic system (Table 3).

Table 3. Enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to naproxen (*S*)-**3** catalysed by *Rhodococcus butanica* in the biphasic system phosphate buffer/hexane

	A		B	
Reaction	(*S*)-**3**		(*S*)-**3**	
time [h]	Yield [%]	ee [%]	Yield [%]	ee [%]
4	19	99	10	98
19	28	90	20	83
24	33	86	28	82

A: Non-immobilized cells (OD 61); (*R/S*)-**1**: 90.0 mg (0.43 mmol).
B: Cells immobilized on PU3 (OD 61); (*R/S*)-**1**: 90.0 mg (0.43 mmol).

When non-immobilized cells are used, the results in aqueous media and in the biphasic system are comparable (Tables 2 and 3). However, with immobilized cells, the reactions proceed more slowly and give poorer optical yields (Table 3).

Since the hydrolysis of (*R/S*)-**1** by *R. butanica* is catalysed not only by the nitrilase activity but also by the nitrile hydratase and amidase activities, the amide (*R/S*)-**2** should also be, and indeed is, a suitable substrate. The hydrolyses of the two substrates (*R/S*)-**1** and (*R/S*)-**2** at equimolar concentrations are compared in Table 4.

Poorer chemical and optical yields were obtained with the racemic amide (*R/S*)-**2** than with the nitrile (*R/S*)-**1**; this can readily be explained in terms of the three-enzyme system of *R. butanica*. Both routes (see Scheme I) catalyse the hydrolysis of (*R/S*)-**1** to the acid (*S*)-**3**. Since the chemical yields of the reaction with naproxen nitrile as substrate are higher than those using naproxen amide as the only substrate and since higher optical yields occur during the hydrolysis of the nitrile (*R/S*)-**1**, we conclude that the nitrilase exhibits a higher enantioselectivity than the amidase.

Table 4. Comparison of the enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** and racemic naproxen amide (*R/S*)-**2** to naproxen (*S*)-**3** catalysed by *Rhodococcus butanica* in phosphate buffer

Substrate (*R/S*)-**1**			Substrate (*R/S*)-**2**		
Reaction	(*S*)-**3**		Reaction	(*S*)-**3**	
time [h]	Yield [%]	ee [%]	time [h]	Yield [%]	ee [%]
1.5	22	97	1.5	18	91
3	30	94	3	20	81
6	33	87	6	25	70

Resting cells (OD 80); (*R/S*)-**1**: 90.0 mg (0.43 mmol), (*R/S*)-**2**: 97.7 mg (0.43 mmol).

In addition to naproxen amide (*R/S*)-**2**, methylbenzyl cyanide was also used as a substrate for *R. butanica*. However, it is not a good substrate since *e.e.* values of 96 % were only obtained at very low conversions (4 %), at conversions of 8 % the *e.e.* value decrease to 77 %.

Enantioselective Hydrolysis of Racemic Naproxen Nitrile (*R/S*)-1 by *Rhodococcus* sp. C3II

The strain C3II, isolated and developed by N. Layh,[20a] most probably belongs to a yet undescribed species in the genus *Rhodococcus*. The isolation, enrichment, and characterization of this new strain have been described in a previous publication.[20b] The excellent catalytic properties of the strain C3II in the enantioselective hydrolysis of naproxen nitrile (*R,S*)-**1** and naproxen amide (*R/S*)-**2** were reported in the same paper.[20b]

In the present paper, emphasis is placed on the preparative aspects of the application of *Rhodococcus* sp. C3II for the preparation of (*S*)-naproxen (*S*)-**3** from racemic naproxen nitrile (*R/S*)-**1** or naproxen amide (*R/S*)-**2**. *R.* sp. C3II exhibits only nitrile hydratase and amidase activities but no nitrilase activity. Both enzymes are constitutive[20b] and both prefer the (*S*)-enantiomer.[20b]

In order to obtain comparable results, the experiments were performed analogously to those with *R. butanica*. As mentioned above, polyurethane PU3 also proved to be particularly suitable for the immobilization of *Rhodococcus* sp. C3II. At reaction times of 1–3 h, *e.e.* values of 99 % were achieved with chemical yields of 27–49 %. The results obtained with small amounts of substrate can also be achieved with larger amounts (Table 5).

With regard to the optical yields, no differences were observed between immobilized and non-immobilized cells whereas the chemical yields were appreciably lower in the former case (Table 5).

Table 5. Enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to naproxen (*S*)-**3** catalysed by resting cells of *Rhodococcus* sp. C3II in phosphate buffer

A			B			C	
Reaction time [h]	(*S*)-3 Yield [%]	ee [%]	Reaction time [h]	(*S*)-3 Yield [%]	ee [%]	(*S*)-3 Yield [%]	ee [%]
1	27	>99	2	9	>99	17	>99
1.5	43	>99	4	20	>99	29	>99
2	49	99	6	27	98	38	99

A: Cells immobilized on PU3 (OD 1.6); (*R/S*)-**1**: 4.2 mg (0.02 mmol).
B: Cells immobilized on PU3 (OD 37); (*R/S*)-**1**: 90.0 mg (0.43 mmol).
C: Non-immobilized cells (OD 37); (*R/S*)-**1**: 90.0 mg (0.43 mmol).

The concentration dependence, which can be recognized from the substrate/product distribution, in the hydrolysis of nitriles with *Rhodococcus* sp. C3II is of interest. At lower concentrations (0.2 mM) 41 % (*R*)-**2** (95 % *e.e.*) and 40 % (*S*)-**3** (>99 % *e.e.*) are formed while at higher concentrations (14.2 mM) 4 % (*S*)-**2** and 38 % (*S*)-**3** (99 % *e.e.*) are obtained. This indicates that the nitrile hydratase functions more rapidly than the amidase at the lower concentration[20b] and that the nitrile hydratase is apparently inhibited at the higher concentration.

The biphasic system comprising phosphate buffer/hexane was also employed for the enantioselective hydrolysis of (*R/S*)-**1** by resting cells of *Rhodococcus* sp. C3II. Enantiomeric excesses of >99 % and good chemical yields were realized at low substrate concentrations with maintenance of enzyme activity; after a reaction time of 5 h in the biphasic system, the enzyme shows, in pure phosphate buffer, a complete intact activity giving 43 % chemical and 99 % optical yields of (*S*)-**3**. Thus, higher substrate concentrations may also be employed under these conditions (Table 6).

Table 6. Enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to naproxen (*S*)-**3** catalysed by *Rhodococcus* sp. C3II in the biphasic system phosphate buffer/hexane

A			B	
Reaction time [h]	(*S*)-3 Yield [%]	ee [%]	(*S*)-3 Yield [%]	ee [%]
5	11	>99	5	>99
19	26	99	18	99
24	34	98	23	98

A: Non-immobilized cells (OD 38); (*R/S*)-**1**: 90.0 mg (0.43 mmol).
B: Cells immobilized on PU3 (OD 38); (*R/S*)-**1**: 90.0 mg (0.43 mmol).

In the course of these experiments it was found that (*R/S*)-**1** is only moderately soluble in hexane with a maximal solubility of 4.185 g/L. Accordingly, and also for the purpose of comparability with the reactions of *R. butanica*, 0.43 mmol of substrate were employed. Excellent *e.e.* values (approximately 99 %) were obtained with both immobilized and non-immobilized resting cells. However, the chemical yields differed since the reaction with non-immobilized cells—like that in pure aqueous medium—proceeds more rapidly. The conversion rate in pure phosphate buffer is four times higher than in the biphasic system. Since comparable optical and chemical yields are obtained in phosphate buffer, the biphasic system does not offer any advantages. In comparison to *R. butanica* (Table 3), markedly better enantiomeric excesses of (*S*)-**3** at comparable chemical yields were obtained with *Rhodococcus* sp. C3II.

Experiments with solubilizers such as DMSO, Tween 80, or Triton X100, which are reputed to increase the solubility of naproxen nitrile in water, were unsuccessful. The enzyme activity decreased even upon addition of the smallest amounts of solubilizing agent.

In analogy to the experiments with *R. butanica* (Table 4), the amide (*R/S*)-**2** was also used as a substrate for *Rhodococcus* sp. C3II (Table 7).

Table 7. Comparison of the enantioselective hydrolysis of racemic naproxen nitrile (*R/S*)-**1** and racemic naproxen amide (*R/S*)-**2** to naproxen (*S*)-**3** catalysed by *Rhodococcus* sp. C3II in phosphate buffer

Substrate (*R/S*)-1			Substrate (*R/S*)-2		
Reaction time [h]	(*S*)-3 Yield [%]	ee [%]	Reaction time [h]	(*S*)-3 Yield [%]	ee [%]
2	17	>99	2	17	>99
4	29	>99	4	32	>99
6	38	99	6	41	98

Resting cells (OD 37); (*R/S*)-**1**: 90.0 mg (0.43 mmol), (*R/S*)-**2**: 97.7 mg (0.43 mmol).

Together with excellent *e.e.* values of approximately 99 %, somewhat better chemical yields, in comparison to the substrate (*R/S*)-**1** (Table 6), were realized. Thus, in contrast to *R. butanica*, the amide (*R/S*)-**2** is an excellent substrate for *Rhodococcus* sp. C3II.

Encouraged by these good results, we employed higher substrate concentrations of (*R/S*)-**1** in order to evaluate the preparative utility of *Rhodococcus* sp. C3II (Table 8).

Table 8. Determination of the concentration maximum of racemic naproxen nitrile (*R/S*)-**1** in the enantioselective hydrolysis to naproxen (*S*)-**3** catalysed by *Rhodococcus* sp. C3II in phosphate buffer

(*R/S*)-1	Reaction	(*S*)-3	
mg/50 ml buffer	time [h]	Yield [%]	ee [%]
200	2	31	>99
	6.5	41	>99
	21	49	93
300	4	5	>99
	24	20	>99
	48	23	>99
400	4	3	>99
	24	8	>99
	48	9	99

As can be seen from Table 8, *e.e.* values of 99 % were obtained for all three concentrations (200–400 mg) of substrate (*R/S*)-**1** in phosphate buffer. In contrast, the chemical yield decreased markedly with increasing amounts of substrate. Thus, with 200 mg of substrate after 6.5 h the conversion rate is 25.23 mg/h/g cells (dry weight) while, with 300 mg of substrate, the conversion rate after 24 h is only 5.00 mg/h/g cells (dry weight). Thus, the chemical yield of (*S*)-**3** only increases from 20 to 23 % between 24 and 48 h reaction time. With 400 mg of substrate this effect is even more obvious and the conversion rate is only 2.67 mg/h/g cells (dry weight) after 24 h reaction time.

The limiting factor in all the reactions described apparently is the ratio of substrate to cell amount. Since the optical density of 1.0 corresponds to 220 mg of bacterial dry weight per litre of culture,[20b] about 0.5 g of cells are present in the described experiments (Table 8). Hence a guiding value for the maximum of substrate/cell ratio can be derived as follows: 0.5 g of substrate (*R/S*)-**1** per 1 g of dry weight of cells.

Experimental Section

Materials and methods

^{1}H NMR: Bruker AC 250 F, TMS as internal standard. Optical density (OD): Uvikon 810 P Spectrophotometer (Kontron). HPLC: Pharmacia LKB system, Chiral AGP-column (100 × 4 mm) and HSA-column (100 × 4 mm, 5 µm silica gel particles with human serum albumin) from Chrom Tech, Norsborg, Sweden. PU3 prepolymer was provided from Toyo Rubber Ind. Co., Osaka, Japan. (*S*)-Naproxen was purchased from Sigma and Millex-GS sterile filter (22 µm) from Millipore. All solvents were purified as described in the literature.

Naproxen nitrile (R/S)-1

Prepared according to Ref.15a,b.

Naproxen amide (R/S)-2

Prepared according to Ref.15c, from 1.06 g (5.0 mmol) naproxen nitrile, 10 mL *tert*-butyl alcohol, 1 g finely powdered potassium hydroxide, chromatography on silica gel with petroleum ether/ethyl acetate (1:1), yield 0.8 g (70 %) naproxen amide, mp 158–159 °C; ^{1}H NMR ($CDCl_3$): δ = 1.39 (d, *J* = 7.1 Hz, 3H, CH_3), 3.70 (q, *J* = 7.1 Hz, 1H, CH), 3.86 (s, 3H, CH_3O), 7.11–7.79 (m, 6H, naphthalene).

(S)-Naproxen amide (S)-2

(*S*)-**3**, 115 mg (0.5 mmol) dissolved at 0 °C in 2 mL thionyl chloride, was refluxed for 1 h. The excess of thionyl chloride was removed under reduced pressure. The residue was taken up in 5 mL dichloromethane, concentrated, dissolved again in 5 mL dichloromethane and cooled to 0 °C. With vigorous stirring 5 mL of conc. ammonia were slowly dropped and with cooling the reaction mixture was neutralized with dilute HCl. The phases were separated and the aqueous phase was extracted twice with dichloromethane. The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on silica gel with petroleum ether/ethyl acetate (1:1) to give 67 mg (59 %) (*S*)-**2**. ^{1}H NMR data identical with those of (*R/S*)-**2**.

Naproxen (R/S)-3

Prepared according to Ref. 16a.

Media, isolation and characterization of Rhodococcus *sp. C3II*

See Ref. 20b.

Immobilization of cells

PU3 prepolymer (0.5 g) was melted (30–35 °C) and after cooling to 20–25 °C cells suspended in 1 mL phosphate buffer (50 mM, pH 7.4) were added and the mixture was stirred for 30 s. The mixture was cooled to 4 °C for 1 h and then the polymer was cut into pieces of 3–6 mm^3.

Conversions with resting cells in aqueous medium

Cells were suspended in 30 mL (*Rhodococcus* sp. C3II) or 50 mL (*R. butanica*) phosphate buffer (50 mM, pH 7.4), the optical density was measured (see Tables 2, 4, 5 and 7) and the substrate (*R/S*)-**1** [90.0 mg (0.43 mmol)] or (*R/S*)-**2** [97.7 mg (0.43 mmol)] was added at 30 °C to the stirred (250 rpm) solution. At the given times (see Tables 2, 4, 5 and 7) 1 mL of the medium was taken through a sterile filter and was analyzed by HPLC.

Comparison of conversions with immobilized or non-immobilized cells of Rhodococcus butanica

Conversions of resting cells, immobilized on PU3 (OD 60) or non-immobilized (OD 60), were carried out as described above in 50 mL phosphate buffer (50 mM, pH 7.4) with 0.43 mmol of (*R/S*)-**1**.

Table 9. Hydrolysis of racemic naproxen nitrile (*R/S*)-**1** to (*S*)-naproxen (*S*)-**3** catalysed by immobilized (**A**) or non-immobilized cells (**B**) of *Rhodococcus butanica*

	A		B	
Reaction	(*S*)-**3**		(*S*)-**3**	
time [h]	Yield [%]	ee [%]	Yield [%]	ee [%]
4	7	96	18	98
19	27	65	31	87
24	28	63	34	85

Conversions with resting cells in a biphasic system

Resting cells, suspended in 30 mL (*Rhodococcus* sp. C3II, optical density of 38) or 50 mL (*R. butanica*, optical density of 61) phosphate buffer (50 mM, pH 7.4), were

mixed with 20 mL *n*-hexane. The substrate (*R/S*)-**1** (90.0 mg (0.43 mmol) dissolved in 0.5 mL methanol) was then added to the stirred (600 rpm) cell suspension. At the given times (see Tables 3 and 6) 1 mL of the medium was taken and was analyzed by HPLC.

Determination of the concentration maximum of (R/S)-*1 in the hydrolysis catalysed by* Rhodococcus *sp. C3II*

Conversions were carried out as described above in 50 mL phosphate buffer (50 mM, pH 7.4) with resting cells (optical density of 46) and various amounts of (*R/S*)-**1** [100.0–400.0 mg (0.47–1.90 mmol) as concentrated methanolic solutions].

Analytical methods

The formation of naproxen (**3**) was analyzed by HPLC. For separation of the enantiomers of naproxen (**3**) a Chiral AGP-column (with guard column) with phosphate buffer (10 mM, pH 7.0) as solvent was used [flow rate 0.6 mL/min, R_t (*R*)-naproxen = 3.5 min, R_t (*S*)-naproxen = 5.7 min] at a detection wavelength of λ = 230 nm. For separation of the enantiomers of naproxen amide (**2**) a HSA-column (with guard column) with phosphate buffer (100 mM, pH 7.0) as solvent was used [flow rate 0.6 mL/min]. The enantiomeric excess (*e.e.*) of (*S*)-naproxen (*S*)-**3** was calculated by comparison of the peak areas obtained by HPLC analyses. The identity of (*S*)-naproxen (*S*)-**3** was determined by co-chromatography with an enantiopure standard.

Growth measurement

Growth of bacterial cultures was monitored spectrophotometrically by measuring the optical density at λ = 546 nm. For the measurements, the cell suspensions were diluted with phosphate buffer (50 mM, pH 7.4) either 1:9 or 1:20. The optical density of 1.0 corresponded to 220 mg of bacterial dry weight per liter of culture.[20b]

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