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Enantioselective Hydrolysis of Some 3-(2-Nitrophenoxy) butanoates Catalyzed by *Pseudomonas fluorescens* and *Pseudomonas sp.* Lipase

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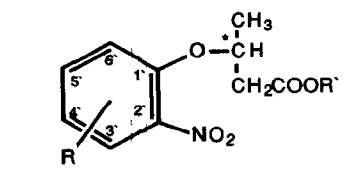
Abstract Racemic methyl, ethyl and cyanomethyl 3-(2´-nitrophenoxy) butanoates **4-10**, chiral precursors of 2,3-dihydro-1,5-benzoxazepin-4(5H)-ones with ACE inhibitory activity, were prepared and submitted to kinetic resolution by a series of commercially available lipases. Best results (e.e.´s >99%) were obtained with *Pseudomonas fluorescens* and *Pseudomonas sp.* lipase. The structure of the alkyl ester group influences the rate of hydrolysis. Cyanomethyl esters **5** and **8** were more reactive than alkyl esters **4, 6, 7, 9** and **10**. Chiral ^{1}H-NMR shift reagents revealed 70-90 % e.e. for the remaining *R*-(-)-esters in hydrolyses of *rac.* **4, 6**, and **9**, and ≥99 % e.e. in hydrolyses of *rac.* **7** and **10**. A large effect on the rate and enantioselectivity of the hydrolysis of esters **4, 6**, and **9**, is observed when a methyl group, which is remote from the chiral center and from the reactive ester group, is on the aromatic ring.

INTRODUCTION

Lipase catalyzed kinetic resolutions of esters of racemic alcohols[1-3], diols[4-6], mono[7] and dicarboxylic acids[8] has been reported. Enzymatic resolutions of α-[9] and β-[10] substituted carboxylic acid esters, precursors of the compounds of therapeutic importance, have also been described. Several attempts were made to improve this hydrolytic process. Sih et al.[11] reported hydrolyses of some activated esters, *e.g.* cyanomethyl, chloroethyl and 2-nitropropyl esters of 2-(6-methoxy-2-naphthyl)propionic acid with *Candida cylindracea* lipase. They observed enhanced enantioselectivity and rate of hydrolysis of these activated esters, along with a retained sense of chirality. The same authors described a method for enzymatic resolution of 3-aroylthio-2-methyl-propionic acid, the key intermediate in the preparation of capoten (antihypertensive agent), where enantioselectivity was improved by structural changes within the thioaryl moiety[12]. The most attractive strategy for improving the enantioselectivity of hydrolysis is *in situ* racemization of the substrate. This approach allows complete transformation of the substrate into one enantiomer, obviates tedious recycling of the remaining ester, and makes the whole process apparently completely enantioselective[13].

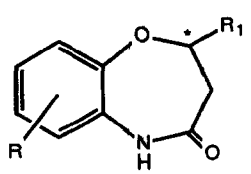
Dedicated to the memory of Prof. Günther Snatzke, deceased on 14 January 1992.

Related to our synthetic project of the preparation of enantiomerically pure 2,3-dihydro-1,5-benzoxazepin-4(5H)-ones **II**, compounds with angiotensin converting enzyme (ACE) inhibitory activity and vasodilating effect[14-16], we needed an efficient method for the separation of the intermediary β-phenoxy substituted acids **I** (**1-3**, R'=H) or their esters (**I**, **4-10**). Hydrolysis of the methyl ester of α-phenoxypropionic acid and its chloroaryl derivatives by α-chymotrypsin, pig liver esterase, porcine pancreatic and *Candida cylindracea* lipases has been reported to proceed with low to moderate enantioselectivity[17], whereas α-alkyl-β-phenoxy propionic acid esters, structurally more related to **I**, provided optically active acids with high enantioselectivity[18]. In both above cases the stereogenic centre is on the α-carbon of propionic acid derivative. It was therefore interesting to see if lipase catalysed hydrolysis would provide enantiomerically enriched butyric acid derivatives **I**, having the stereogenic centre on the β-carbon atom. Because of their high lipophilicity, we expected β-substituted congeners **I** (**4-10**) to be acceptable substrates for kinetic resolution by some of the commercially available lipases, since lipophilic water insoluble substrates are generally suited for interfacial lipase catalysis.



I

	R	R`
1	H	H
2	4`-CH_3	H
3	5`-CH_3	H
4	H	CH_3
5	H	CH_2CN
6	4`-CH_3	CH_3
7	4`-CH_3	CH_2CH_3
8	4`-CH_3	CH_2CN
9	5`-CH_3	CH_3
10	5`-CH_3	CH_2CH_3



II

In the present paper we report on successful enzymatic kinetic resolution of some racemic esters **I**, precursors in the preparation of the optically active benzoxazepinones **II**. The effect of some structural variations within the aryl and ester groups on rate and enantioselectivity is determined.

RESULTS AND DISCUSSION

As the first screening, resolution of the ester **10** was attempted with ten commercially available enzymes. Progress of hydrolysis was followed by HPLC. Among the lipases tested, only *Pseudomonas sp.* and *Pseudomonas fluorescens* lipase proved active, Table 1. We limited therefore subsequent trials with the ester **10** on these two lipases.

Pseudomonas fluorescens lipase hydrolysed ethyl ester **10** with complete enantioselectivity. After 46 hours at room temperature 46 % conversion was detected, which did not change on prolonged reaction time. In the preparative experiment 41% of (-)-**10** with >99% e.e. was isolated after 19 hours at 30-35°C. Separation of

the optically active esters from the acids was performed by silica gel column chromatography. Hydrolysis with *Pseudomonas sp.* proceeded beyond 50% conversion, and after 45 hours 83% of acid **3** was detected. However, when hydrolysis was stopped at *ca.* 50% conversion, 31% of (-) **10** with >99% e.e. was isolated. Complete enantioselectivity in the hydrolysis of ester **7** with *Pseudomonas fluorescens* lipase was also reached in the first 24 hours and did not deminish up to 46 hours. The enantioselectivity of *Pseudomonas sp.* lipase for ester **7** was lower (e.e. 88%) than that with *Pseudomonas fluorescens* lipase. Hydrolysis of the esters **4, 6, 8** and **9** was therefore performed with this latter lipase (Table 2.).

Table 1. Screening of the enzymes in hydrolysis of ester **10**

Lipase	Substrata **10** (mol/l)	Solvent/buffer (ml/ml)	pH	Reaction time (h)	Product 3 (%)[a]
Candida cylindracea (Sigma Type VII)	0.011	acetone (5/12)	8.1	308	2
	0.012	methanol (3/12)	8.1	282	5
Candida cylindracea (Amano Ay 30)	0.012	methanol (3/12)	8.1	282	2.4
Geotrichum candidum (Amano GC20)	0.013	methanol (2/12)	7.9	163	4
Candida lypolitica (Amano L5)	0.011	methanol (5/12)	7.9	42	1
Aspergillus niger (Amano AP 6)	0.006	methanol (2/12)	7.0	172	1
Penicillium camembertii (Amano G)	0.008	methanol (2/12)	7.0	172	1
Humicola lanuginosa (Amano CE 5)	0.004	methanol (2/12)	7.0	172	8
Rhizopus oryzae (Amano F-AP 15)	0.006	methanol (2/12)	7.0	172	9.5
Pseudomonas fluorescens (Fluka)	0.003	methanol (1/12)	7.4	46	46
Pseudomonas sp. (Amano PS)	0.011	methanol (2.5/12)	8.0	45	83

[a] Determined by HPLC

Highly enantioselective enzymatic hydrolysis was achieved with lipase from *Pseudomonas fluorescens*, the enantiomeric ratio E >100 for hydrolysis of ester **4** was calculated according to Sih[19]. *Pseudomonas fluorescens* lipase catalyzes the hydrolysis of *S*-form of *rac*-**4**, to afford *S*-(+)-**1** and *R*-(-)-**4**. The configuration of the compounds **1** and **4** has been previously assigned[20]. According to the direction of rotation, the *R* configuration can also be predicted for related (-) esters **6-10**.

Relative reactivities were determined for the esters of various acids, then for structurally different esters of the same acid. Progress curves of hydrolysis are presented in the Figs. 1-5. Comparison of the progress curves for methyl esters **4, 6** and **9** reveals the lowest rate for ester **6**, with the methyl substituent in the 4′-position of the aromatic ring, whereas the 5′-methyl substituted ester **9** was hydrolyzed with the highest rate

(Fig. 1.). At the same time, optical purities of the faster hydrolyzed esters **4** and **9** were 90% and 85%, respectively, and the e.e. of the slowest hydrolyzed ester **6** was 72% (Table 2.). These results reveal an unexpected effect of the remote methyl group on reactivity and enantioselectivity in the hydrolyses of the investigated *rac.* carboxylic acid esters.

Table 2. Enantioselective Hydrolysis of Esters **4,6-9** and **10**.

Substrate	Lipase	Reaction time (h)	Yield of ester (%)	e.e.[a] (%)
4	*P. fluorescens*	26,5	46	88
4	*P. fluorescens*	6,5	52	90
6	*P. fluorescens*	26	47	72
6	*P. fluorescens*	24	53	69
7	*P. fluorescens*	46	48	> 99
7	*P. sp.*	28,5	42	88
8	*P. fluorescens*	2	60	-
9	*P. fluorescens*	5,5	54	85
10	*P. fluorescens*	19	48	> 99
10	*P. sp.*	19	47	> 99

[a] Determined by ^{1}H-NMR in the presence of the chiral shift reagent.

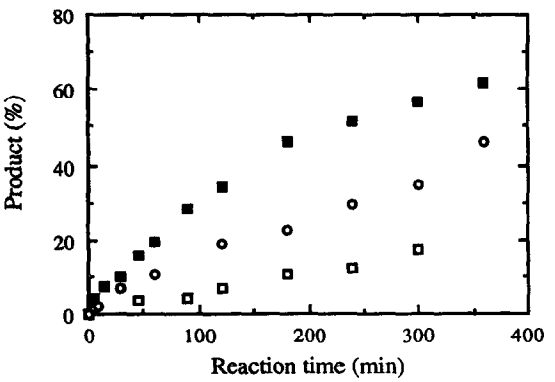


Figure1. Progress curves for hydrolysis of methyl esters **4** (○), **6** (□) and **9** (■) with *P. fluorescens* lipase

The rate of hydrolysis is affected also by the structure of the alkyl ester group. Methyl ester **9** was hydrolyzed much faster than ethyl ester **10** (Fig. 2.). For the esters **6** and **7** the effect of alkyl ester group is reversed, ethyl ester **7** was a somewhat better substrate than methyl ester **6** (Fig. 3.). Cyanomethyl esters **5** and **8**, as expected, proved much more reactive than alkyl esters. The progress curves for hydrolysis of methyl, ethyl and cyanomethyl esters **6-8** with *Pseudomonas fluorescens* are presented in Fig. 4. Cyanomethyl ester **8** was hydrolyzed at a considerably higher rate than esters **6** and **7**, and *ca.* 50% conversion was reached after only 50 min.

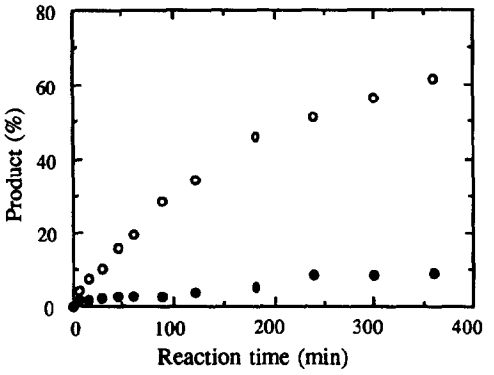


Figure 2. Progress curves for hydrolysis of esters **9** (○) and **10** (●) with *P. fluorescens* lipase

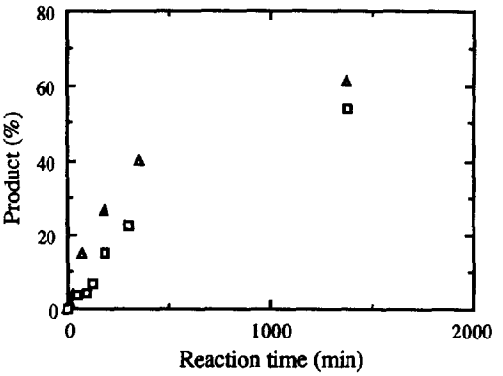


Figure 3. Progress curves for hydrolysis of esters **6** (□) and **7** (△) with *P. fluorescens* lipase

Hydrolysis of two cyanomethyl esters **5** and **8** proceed with similar rates (Fig. 5.). Obviously, the cyanomethyl group enhances the reaction rate, and cancels the effect of the methyl group attached to the aryl substituent in 4´-position of **8**. All attempts to determine optical purity of the kinetically resolved esters **5** and **8** using chiral ^{1}H-NMR shift reagents failed, however. Two chiral HPLC columns, ConBrio TAC and Nucleosil Chiral 2 columns were also tested. While the first column did not separate racemates, the latter separated racemic acid **3** in unreproducible manner.

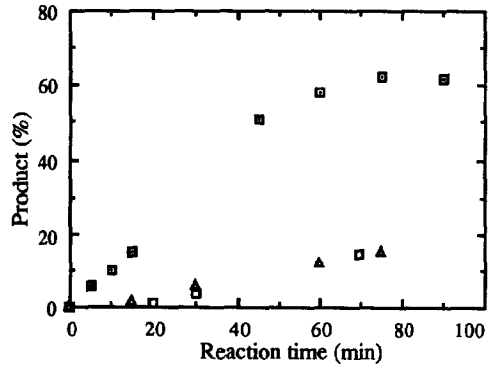


Figure 4. Progress curves for hydrolysis of ester **6** (□),**7** (△) and **8** (■) with *P. fluorescens* lipase

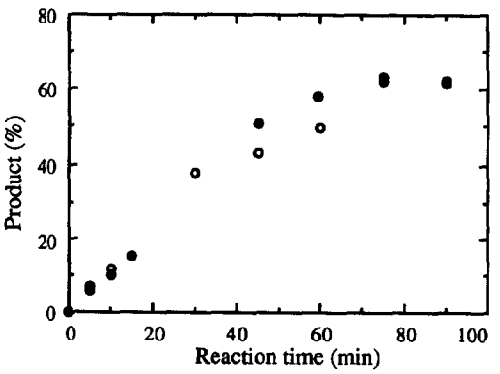


Figure 5. Progress curves for hydrolysis of cyanomethyl esters**5** (○) and **8** (●) with *P. fluorescens* lipase

In conclusion we can state that *Pseudomonas sp.* and *Pseudomonas fluorescens* lipases catalyze the hydrolysis of *S*-forms of racemic esters **4-10** with high to complete enantioselectivity . Easy separation of the esters from acids, and their high optical purity, make this chemo-enzymatic approach attractive for the preparation of chiral, optically pure benzoxazinones with ACE inhibitory activity.

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EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on Jeol FX 90Q FT spectrometer; shifts are given in ppm downfield from TMS as an internal standard. IR spectra were recorded on Perkin-Elmer 297 spectrometer. Rotations were determined on Optical Activity AA-10 Polarimeter. High resolution mass spectra were obtained on Extrel FTMS 2001 DD instrument at 70 eV.

Pseudomonas fluorescens lipase (32.5 units per mg of solid) was purchased from Fluka Co., while all other enzymes (*Candida cylindracea, Geotrichum candidum, Candida lypolitica, Aspergillus niger, Penicillium cammembertii, Humicola lanuginosa, Rhizopus oryzae* and *Pseudomonas sp.* lipases) were obtained from Amano Co. All reagents were purchased from Aldrich, solvents purchased from Kemika (Zagreb) were distilled before use. HPLC separations were performed on Hewlett-Packard instrument Series 1050 with UV detector at 254 nm and HP 3396A integrator, using Waters Nova-Pak C18 (10 cm) reverse phase column, and water-methanol-acetonitrile (40:30:30) as eluent at the flow rate 1 ml/min. Tentatives to separate *rac.* esters or acids were made on the same HPLC set up, with Hewlett-Packard ConBrio TAC 15-25μm column (250x5mm), with ethanol:water 95:5 as eluant, and with Macherey-Nagel Nucleosil Chiral 2 column (250x4 mm) with mobile phase 5% *i*-propanol in hexane, with addition of few drops of trifluoroacetic acid. Organic extracts were dried over Na_2SO_4 and evaporated *in vacuo*. Flash chromatography was performed on Silica gel (Merck Kieselgel 60, 70-130 or 230-400 mesh). Compounds **1-3** are prepared as already described[14].

3-(2-Nitrophenoxy)butanoic acid, methyl ester, **4.**

To the acid **1** (1.097 g, 4.8 mmol) dissolved in anhydrous methanol (10 ml) conc. H_2SO_4 (1 ml) was added and the mixture was refluxed for 4 hours. Methanol was evaporated, residue was poured into water (15 ml) and extracted with dichloromethane (3x15 ml). Organic phase was washed with 10% $NaHCO_3$, dried, and evaporated to yield 0.732 g (73%) of crude **4**. Pure product was obtained after column chromatography with cyclohexane-ethylacetate 7:3 as eluant (R_f 0.5). ^{1}H-NMR: 1.43 (d, J 6.1 Hz, 3H, CH_3), 2.59 (dd, J 15.8 Hz, 5.9 Hz, 1H, CH_2), 2.89 (dd, J 15.8 Hz, 7.0 Hz, 1H, CH_2), 3.68 (s, 3H, CH_3 est.), 4.88-5.09 (m, 1H, CH), 6.03-7.81 (m, 4H, Ph). ^{13}C-NMR: 19.42, 40.80, 51.62, 73.16, 116.35, 120.73, 125.29, 133.69, 141.10, 150.83, 171.01. IR(film): 2990, 2950, 1740, 1610, 1525, 1490, 1350, 1275 cm^{-1}. M/e calculated for $C_{11}H_{13}O_5N$ 239.078825 amu. Found 239.078938 amu.

3-(4-Methyl-2-nitrophenoxy)-butanoic acid, methyl ester, **6.**

Methyl ester **6** was prepared as described for the methyl ester **4** to yield 0.982 g (94%) of oily product. Crude product was purified by column chromatography with cyclohexane-ethylacetate 7:3 as eluant (R_f 0.5). ^{1}H-NMR: 1.40 (d, J 6.1 Hz, 3H, CH_3), 2.32 (s, 3H, CH_3-Ph), 2.57 (dd, J 15.8 Hz, 5.9 Hz, 1H, CH_2), 2.87 (dd, J 16.1 Hz, 7.0 Hz, 1H, CH_2), 3.68 (s, 3H, CH_3 of ester), 4.81-5.03 (m, 1H, CH), 7.03-7.57 (m, 3H, Ph). ^{13}C-NMR: 19.32, 19.74, 40.69, 51.41, 73.29, 116.64, 125.16, 130.78, 134.22, 140.78, 148.57, 170.91. IR(film): 2980, 2950, 1740, 1625, 1575, 1530, 1500, 1440, 1380, 1355, 1305, 1280, 1260, 1200, 1180, 1160, 1100, 1055, 810 cm^{-1}. M/e calculated for $C_{12}H_{15}O_5N$ 253.094475 amu. Found 253.0944751 amu.

3-(5-Methyl-2-nitrophenoxy)butanoic acid, methyl ester, **9.**

This compound was prepared as described for the esters **4** and **6** starting from 1.005 g (4.2 mmol) of **3**. Oily product **9** (0.900 g, 85%) was purified by column chromatography with cyclohexane-ethylacetate 7:3 as eluant (R_f 0.5). ^{1}H-NMR: 1.43 (d, J 6.1 Hz, 3H, CH_3), 2.40 (s, 3H, CH_3-Ph), 2.61 (dd, J 15.9 Hz, 5.5 Hz, 1H, CH_2), 2.88 (dd, J 16.1 Hz, 7.3 Hz, 1H, CH_2), 3.69 (s, 3H, CH_3 of ester), 4.94-5.00 (m, 1H, CH), 6.82 (d, J 8.3 Hz, 1H, Ph), 6.97 (s, 1H, Ph), 7.72 (d, J 8.4 Hz, 1H, Ph). ^{13}C-NMR: 19.59, 21.71, 40.93, 51.70, 73.09,

116.82, 121.45, 125.46, 145.26, 150.97, 154.58, 171.00. IR(film): 2990, 2960, 2920, 1745, 1610, 1595, 1520, 1495, 1440, 1415, 1385, 1350, 1305, 1280, 1200, 1180, 1140, 1090, 1055, 1010, 970, 840, 820, 760 cm^{-1}. M/e calculated for $C_{12}H_{15}O_5N$ 253.094475 amu. Found 253.095311 amu.

3-(4-Methyl-2-nitrophenoxy)butanoic acid, ethyl ester, **7.**

Acid **2** (1.16 g, 4.85 mmol), abs. ethanol (3 ml), and conc. H_2SO_4 (2 drops) were refluxed for 5 hours. After cooling to room temperature, 2 ml of water were added and the pH was adjusted to neutral with 5% NaOH. Crude product was extracted with ether (3x15 ml), organic layer was dried and evaporated. Residue was purified by flash chromatography with cyclohexane-ethyl acetate (7:3) as eluant (R_f 0.5), to yield 1.08 g (93 %) of the yellow oil. ^{1}H-NMR: 1.16-1.44 (d+t, 6H, 2xCH_3), 2.31 (s, 3H, CH_3-Ph), 2.43-2.99 (m, 2H, CH_2), 4.14 (q, J 7.0 Hz, 2H, CH_2 of ester), 4.80-5.02 (m, 1H, CH), 7.02-7.58 (m, 3H, Ph). ^{13}C-NMR: 13.94, 19.59, 20.03, 41.20, 60.61, 73.42, 116.59, 125.29, 130.70, 134.20, 141.00, 148.59, 170.49. IR (film): 3000, 1740, 1530, 1355, 1260, 1190 cm^{-1}. M/e calculated for $C_{13}H_{17}O_5N$ 267.110125 amu. Found 267.104599 amu.

3-(5-Methyl-2-nitrophenoxy)butanoic acid, ethyl ester, **10.**

It was prepared by the same procedure as described for **7**. Crude product, 1.043 g (90%) of crystalline material was crystallized from light petroleum, mp. 64-66°C. ^{1}H-NMR: 1.16-1.46 (d+t, 6H, 2xCH_3), 2.40 (s, 3H, CH_3-Ph), 2.57 (dd, J 16.1 Hz, 5.5 Hz, 1H, CH_2), 2.89 (dd, J 15.8 Hz, 7.0 Hz, 1H, CH_2), 4.02-4.28 (q , J 7.0, 2H, CH_2 of ester), 4.85-5.07 (m, 1H, CH), 6.81 (d, J 8.2 Hz, 1H, Ph), 6.96 (s, 1H, Ph) , 7.72 (d, J 8.2 Hz, 1H, Ph). ^{13}C-NMR: 13.99, 19.64, 21.73, 41.25, 60.67, 73.14, 116.82, 121.39, 125.51, 145.20, 151.07, 170,54. IR(KBr): 3000, 1740, 1610, 1590, 1515, 1490, 1415, 1405, 1350, 1310, 1280, 1270, 1200, 1180, 1090, 1050, 1025, 950, 840, 820 cm^{-1}. M/e calculated for $C_{13}H_{17}O_5N$ 267.110125 amu. Found 267.100359 amu.

3-(2-Nitrophenoxy)butanoic acid, cyanomethyl ester, **5.**

This compound was prepared according to the recently described method[21]. The mixture of triethylamine (1.307 g, 13 mmol) and chloroacetonitrile (1.27 g, 20 mmol) was added dropwise to a dichloromethane (5 ml) solution of acid **1** (1.125 g, 5 mmol). The resulting solution was stirred at room temperature overnight. The mixture was poured into water (15 ml), extracted with ether (3x15 ml), organic layer was washed with 0.5 M HCl (7 ml), 10% $NaHCO_3$ and water. After drying and evaporation, the residue was purified by flash chromatography, with cyclohexane-ethylacetate-diisopropylether 10:3:1) as eluant (R_f 0.2), to afford 300 mg (23%) of oily ester **5**. ^{1}H-NMR: 1.46 (d, J 6.6 HZ, 3H, CH_3), 2.71 (dd, J 16.1 Hz, 5.3 Hz, 1H, CH_2), 2.98 (dd, J 16.4 Hz, 7.3 Hz, 1H, CH_2), 4.74 (d, J 1.5 Hz, 2H, CH_2est.), 4.88-6.07 (m, 1H, CH), 7.05-7.82 (m, 4H, Ph). ^{13}C-NMR: 19.32, 40.37, 48.34, 72.89, 114.09, 116.32, 121.13, 125.40, 133.84, 141.07, 150.57, 169.08. IR (film): 4450 (broad), 2980, 2930, 1760, 1605, 1580, 1525, 1480, 1355, 1280, 1255, 1170 cm-1. M/e calculated for $C_{12}H_{12}O_5N_2$ 264.074074 amu. Found 264.073254 amu.

3-(4-Methyl-2-nitrophenoxy)butanoic acid, cyanomethyl ester, **8.**

Acid **2** (1.195 g, 5 mM) was reacted with $ClCH_2CN$ (1.51 g, 20 mmol) in the presence of triethylamine (1.518 g, 15 mmol) as described for ester 5. After purification by flash chromatography with cyclohexane-ethylacetate-diisopropylether (10:3:1, R_f 0.2), 375 mg (27%) of product **8** were obtained. ^{1}H-NMR: 1.43 (d, J 6.2 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3-Ph), 2.68 (dd, J 16.1 Hz, 5.2 Hz, 1H, CH2), 2.95 (dd, J 16.1 Hz, 7.6 Hz, 1H, CH2), 4.73 (d, J 1.2 Hz, 2H, CH_2est.), 4.80-5.01 (m, 1H, CH) , 6.99-7.58 (m, 3H, Ph). ^{13}C-NMR: 19.40, 19.98, 40.42, 48.31, 73.16, 114.10, 116.76, 125.46, 131.41, 134.45, 140.91, 148.44, 169.16 ppm. IR(film): 3500 (broad), 2980, 2930, 1760, 1620, 1530, 1500, 1380, 1350, 1280, 1260, 1160, 1080, 1060 cm^{-1}. M/e calculated for $C_{13}H_{14}O_5N_2$ 278.089724 amu. Found 278.095869 amu.

Enzyme-catalyzed hydrolysis - general procedure

The ratio *Pseudomonas fluorescens* lipase-substrate was 1:10 (by weight), in the experiments with *Pseudomonas sp.* this ratio was 1:1. To the enzyme suspended in 0.2 M sodium phosphate buffer (10-12 ml) substrate (100-150 mg) in a small amount of methanol (1-2 ml) was added. All experiments were performed at room temperature, except hydrolysis of **10** with *Pseudomonas fluorescens*, which was performed at 30-35°C. Samples (200-400 µl) were extracted with ethyl acetate, and after usual workup analyzed by HPLC. An interesting phenomenon was noticed during HPLC monitoring of hydrolysis of reactive cyanomethyl esters **5** and **8**; different product/substrate ratios were obtained for 200 µl samples taken from the reaction suspension, than for the final sample taken after extraction of the whole reaction mixture. We ascribe this phenomenon to dispersion of the oily substrate on the glass walls of the reaction vessel. Attempting to avoid this problem, the substrates were dissolved in MeOH before addition. HPLC control revealed quick transesterification, however; within few minutes *ca.* 20 % of methyl esters **4** and **7** was formed.

Products were purified by silicagel column chromatography with cyclohexane-ethyl acetate 7:3. The acid retained on the column can be washed off with more polar solvents, like tetrahydrofuran or methanol. The yields of isolated esters were regularly 10-15 % lower than determined by HPLC, because of the losts which appeared with sampling. Enantiomeric excess of the products **4** ($[\alpha]_D$ -34, c 1.4, EtOH) and **10** ($[\alpha]_D$ -35, c 1.9, CH_2Cl_2) was determined by ^{1}H-NMR in the presence of 75 % and 100 % (in mole) of Eu(hfc)$_3$, respectively. The e.e. of **6** ($[\alpha]_D$ -11, c 0.9, EtOH) and **7** ($[\alpha]_D$ -34, c 0.7, CH_2Cl_2) was determined with 100 % (in mole) of Er(hfc)$_3$. The e.e. of **9** was determined with 55% (in mole) of Pr(hfc)$_3$. Optical purity of acid **1** and ester **4** was also determined by comparison of their rotations, $[\alpha]_D$ +54 (c 0.9, EtOH) and -34, respectively, with those published for optically pure compounds[20].

REFERENCES

1. Kazlauskas, R.J.; Weissfloch, A.N.E.; Rappaport, A.T.; Cuccia, L.A. *J. Org. Chem.* **1991**, *56*, 2656.
2. Bevinakatti, H.S.; Banerji, A.A. *J. Org. Chem.* **1991**, *56*, 5372.
3. Akita, H.; Umezawa, I.; Matsukura, H.; Oishi, T, *Chem. Pharm. Bull.* **1991**, *39*, 1632.
4. Bianchi, D.; Bosetti, A.; Costi, P.; Golini, P. *Tetrahedron Lett.* **1992**, *33*, 3231.
5. Harris, K.J.; Gu, Q.-M.; Shih, Y.-E.; Girdaukas, G.; Sih, C.J. *Tetrahedron Lett.* **1991**, *32*, 3941.
6 Seemayer, R.; Schneider, M.P. *J. Chem. Soc., Chem. Commun.* **1991** 49.
7. Delinck, D.L.; Margolin, A.L. *Tetrahedron Lett.* **1990**, *31*, 6797.
8. Wu, S.H.; Chu, F.-Y.; Chang, C.-H.; Wang, K.-T. *Tetrahedron Lett.* **1991**, *32*, 3529.
9. Colombo, M.; De Amici, M.; De Micheli, C.; Pitre, D.; Carrea, G.; Riva, S.*Tetrahedron:Asymmetry* **1991**, *2*, 1021.
10. Bianchi, D.; Cabri, W.; Cesti, P.; Francalanci, F.; Ricci, M. *J. Org. Chem.* **1988**, *53*, 104.
11. Gu, Q.-M.; Chen, C.-S.; Sih, C.J. *Tetrahedron Lett.* **1986**, *27*, 1763.
12. Gu, Q.M.; Reddy, D.R.; Sih, C.J. *Tetrahedron Lett.* **1986**, *27*, 5203.
13. Fülling, G.; Sih, C.J. *J. Am. Chem. Soc.* **1987**, *109*, 2845
14. Ott, J.; Lévai, A. *Arch. Pharm.* **1990**, *323*, 601.
15. Itoh, K.; Kori, M.; Inada, Y.; Nishikawa, K.; Kawamatsu, Y.; Sugihara, H. *Chem. Pharm. Bull.* **1986**, *34*, 1128, 2078, 3747.
16. Hashiyama, T.; Watanabe, A.; Inoue, H.; Konda, M.; Takeda, M.; Murata, S.; Nagao, T. *Chem. Pharm. Bull.* **1985**, *33*, 634.
17. Dernoncour, R., Azerad, R. *Tetrahedron Lett.* **1987**, *28*, 4661.
18. Loubinoux, B.; Viriot-Chauveau, C.; Sinnes, J.L. *Tetrahedron Lett.* **1992**, *33*, 2145.
19. Chen, C.S.; Fujimoto, Y.; Girdaukas, G.; Sih, C.J. *J. Am. Chem. Soc.*, **1982**, *104*, 7294.
20. Lévai, A.; Ott, J.: Snatzke, G. *Monatsh. Chem.*, in press.
21 Barnier, J.-P.; Blanco, L.; Guibé-Jampel, E.; Rousseau, G. *Tetrahedron* **1989**, *45*, 5051.