Biocatalytic resolution of saphenic acid. Substrate preferences for lipases A and B from *Candida antarctica*

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Dedicated to Professor Torbjörn Norin on his 75th birthday

Abstract

Efficient methods for kinetic resolution of saphenic acid (**2**) and phenyl-(2-naphthyl)-methanol (**6**) have been developed using lipases B and A from *Candida antarctica,* respectively, as catalysts. Lipase B from *Candida antarctica* showed high enantioselectivity in esterification with vinyl butanoate of the alcohol **2**. By subsequent crystallization, the enantiomeric excess, *ee*, of (*S*)-saphenic acid ((*S*)-**2**) was higher than earlier reported. The butanoate (*R*)-**4** was obtained in high enantiopurity by esterification of the alcohol **2**. The enantiopure alcohol (*R*)-**2** and the butanoate (*S*)-**4** were synthesized by CALB catalyzed hydrolysis of **4** (the butanoate of **2**).

Lipase A from *Candida antarctica* showed high enantioselectivity in esterification of the alcohol **6** with vinyl acetate, and both (*S*)-phenyl-(2-naphthyl)-methanol ((*S*)-**6**) and the (*R*)-acetate of phenyl-(2-naphthyl)-methanol ((*R*)-**7**) were obtained in high enantiomeric excess. The corresponding (*R*)-phenyl-(2-naphthyl)-methanol ((*R*)-**6**) and the (*S*)-acetate of phenyl-(2-naphthyl)-methanol ((*S*)-**7**) were obtained by CALA-catalyzed hydrolysis of the acetate of phenyl-(2-naphthyl)-methanol (**7**).

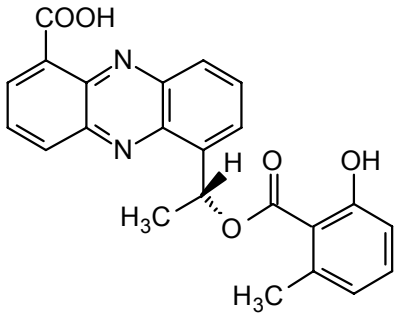
Keywords: Kinetic resolution, lipases A and B from *Candida antarctica*, saphenic acid, saphenamycin, *E*-value

Introduction

The secondary metabolite saphenamycin (yellow pigment) (**1**) has been isolated from a *Streptomyces* species, and it shows activities against Gram positive and Gram negative bacteria, antitumor activity,[1] antitrichomonal activity and lethal activity against midge/mosquito larvae.[2] Saphenamycin analogues show similar antimicrobial effect.[3] It is believed that the antibiotic effect of saphenamycin is due to intercalation of the drug with the duplex DNA with formation

of a non-covalent complex. This will result in inhibition of DNA replication and/or transcription, probably due to deformation of the double helix.[3]

The tricyclic phenazine structure of **1** has been found in several secondary metabolites isolated from other bacteria, and many of these also show antibiotic or antiviral effects. The DNA destroying mechanism seems to be the same for these compounds.[4] Saphenamycin isolated from microorganisms shows low optical activity, however, it has been suggested that it racemizes during work-up.[1] Saphenic acid (**2**) is the main building block of saphenamycin.



(*R*)-Saphenamycin, (*R*)-1

Both enantiomers of **1** have been synthesized by crystallization with (–)-brucine and their absolute configurations have been determined by X-ray crystallography co-crystallized with (–)-brucine. It has been suggested that **1** is biosynthesized as the (*R*)-enantiomer due to the analogy of the biosynthesis of saphenic acid (**2**). Saphenamycin has been synthesized from (*R*)-**2** and 6-methyl salicylic acid with retention of enantiomeric excess and configuration.[4]

The butanoate of saphenic acid (**4**) (Scheme 1) is found to be active against several types of microorganisms, however, it has only been tested in racemic form and it is reasonable to believe that the enantiomers will show different activity.[5]

Esterifications of several secondary alcohols have been catalyzed by lipase B from *Candida antarctica* (CALB) with high enantioselectivity.[6-10] Lipase A from this fungus (CALA) is reported to show broad catalytic activity towards a diversity of sterically hindered alcohols, including both secondary and tertiary alcohols.[11-16] It was interesting to investigate whether this lipase could catalyze resolutions of secondary alcohols with phenyl and naphthyl groups and other bulky groups attached to the stereocenter. The 3D structure of CALA has not been published, however, the structure of the lipase has recently been predicted by comparative modeling and site directed mutagenesis.[17]

Results and Discussion

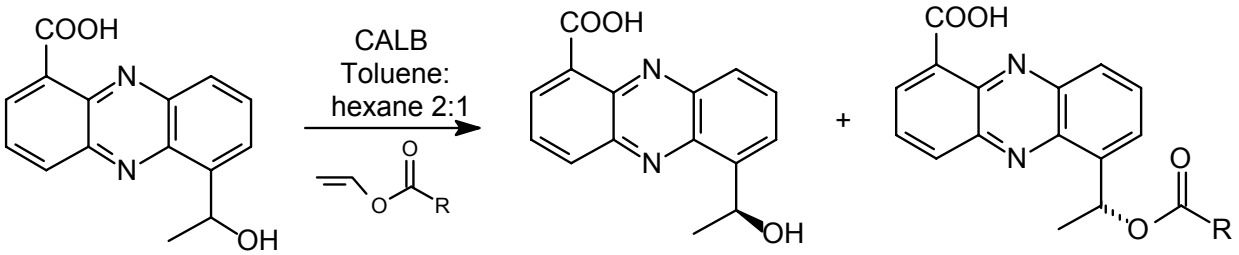
Attempts to resolve saphenic acid (6-(1-hydroxyethyl)-phenazine-1-carboxylic acid) (**2**) and phenyl-(2-naphtyl)-methanol (**6**) were performed by use of different lipases, esterases and

acylases. (See Table 1). Immobilized lipase B from *Candida antarctica* (CALB/Novozym 435) and immobilized lipase from *Rhizomucor miehei* (Lipozyme RM IM) catalyzed the esterification of **2**, the other enzymes did not give any reaction products after one week. Lipozyme RM IM showed low enantioselectivity toward saphenic acid (**2**) (*E*-value = 37), and the reaction was not scaled up. Novozym 435 was chosen as the best catalyst with *E*-values > 200, both with vinyl acetate and vinyl butanoate as acyl donor. (Scheme 1a) However, the acetates of saphenic acid, (*R*)-3 and (*S*)-3, were not properly separated neither by chiral GLC nor by HPLC. Vinyl butanoate was chosen as the acyl donor.

Table 1. Enzymes used in resolutions of saphenic acid (**2**) and phenyl-(2-naphtyl)-methanol (**6**)

Enzyme	Specifications
Lipase A from *Candida antarctica* (CALA)	E.C. 3.1.1.3, Novozym 735
Lipase B from *Candida antarctica* (CALB)	E.C. 3.1.1.3, Novozym 435, LC-200204
Lipase fra *Thermomyces lanuginosus*	E.C. 3.1.1.3, Lipozyme TL IM
Lipase fra *Rhizomucor miehei*	E.C. 3.1.1.3, Lipozyme RM IM
Lipase from *Candida rugosa*	
Lipase from wheat seeds	E. C. 3.1.1.3, Type 1, Sigma, L-3001
Lipase from Papaya	E.C. 3.1.1.3, Type III, Sigma, P-4880
Pig liver esterase	E.C. 3.1.1.3, Type II, Sigma, L-3126
Acylase 1 from *Aspergillus melleus*	E. C. 3.5.1.14, Sigma, A-2156

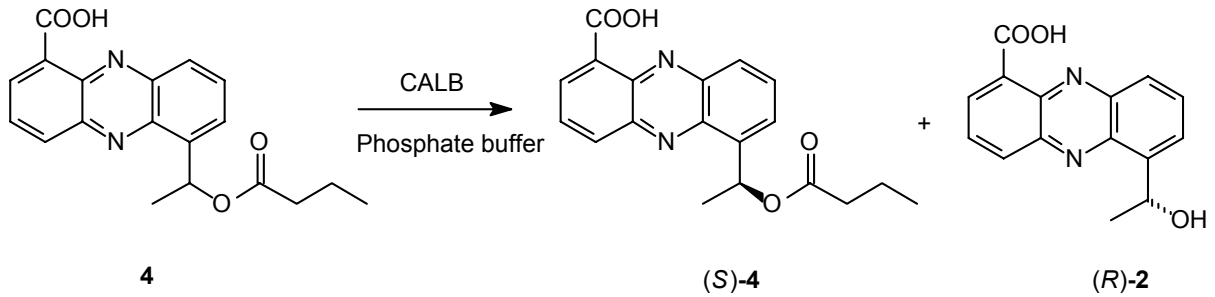
The (*R*)-butanoate of saphenic acid ((*R*)-**4**) was obtained in 41 % yield and 96.4 % *ee* while *(S)*-saphenic acid ((*S*)-**2**) was obtained in 25 % yield and 100 % *ee* after recrystallization. Recrystallization of (*R*)-**4** did not increase the *ee*.



rac-**2** (*S*)-**2** (*R*)-**3** R = CH_3 (*R*)-**4** R = C_3H_7

Scheme 1a

(*R*)-Saphenic acid ((*R*)-**2**) was obtained by CALB catalyzed hydrolysis of saphenic acid butanoate (**4**) in 96.6 % *ee*. (Scheme 1b) After crystallization, the *ee* was improved to 100 %. The (*S*)-enantiomer of the butanoate of saphenic acid ((*S*)-**4**) was obtained in 36 % yield and 81 % *ee*. The *ee* was not improved by crystallization.

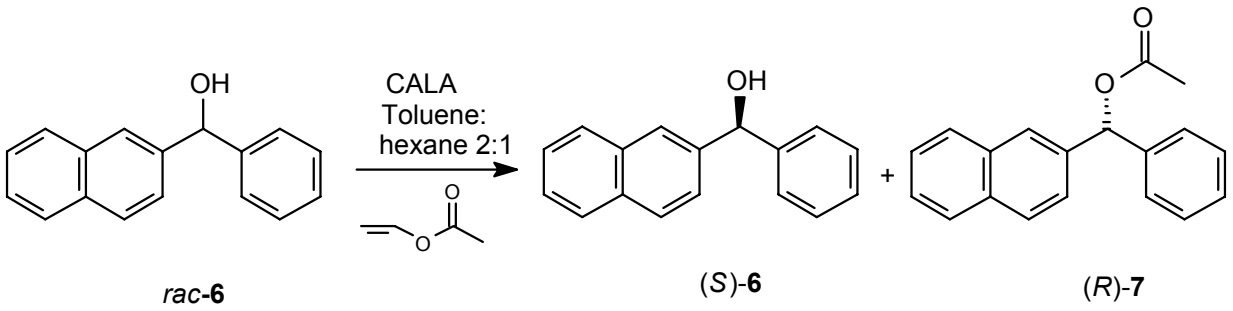


4 (*S*)-**4** (*R*)-**2**

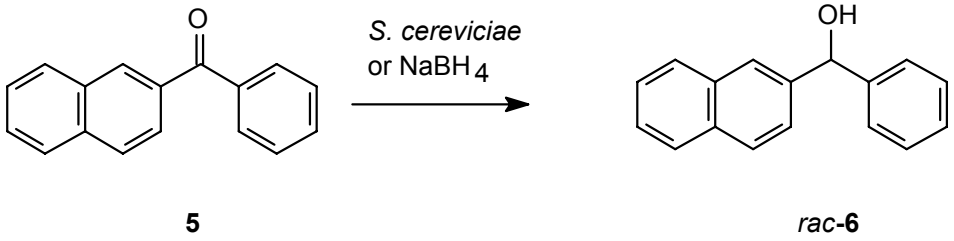
Scheme 1b

The absolute configurations of the enantiomers of saphenic acid (**2**) were verified by comparison of optical rotation values.[4] The optical rotation values measured by us, deviated slightly from the reported values, however, it may be due to different *ee´*s. The reported optical rotation for (*R*)-**2** with *ee* 74 % is $[\alpha]_D^{22} = -19.70$ (c 0.13, $CHCl_3$),[4] measured by us (96.6 % *ee*): $[\alpha]_D^{20} = -15.91$ (c 0.41 $CHCl_3$). The reported optical rotation for (*S*)-**2** with *ee* 80 % is $[\alpha]_D^{22} = +20.00$ (c 0.41, $CHCl_3$),[4] determined by us (100 % *ee*): $[\alpha]_D^{20} = +16.82$ (c 0.41 $CHCl_3$). Phenyl-(2-naphthyl)-methanol (**6**) and the acetate **7** were used as model substrates in order to compare substrate preferences for several lipases.

Lipase catalyzed kinetic resolution of phenyl-(2-naphthyl)-methanol (**6**) were performed by the enzymes listed in Table 1, except for the lipase from papaya and Acylase A from *Aspergillus melleus*. Vinyl acetate was used as acyl donor. Only *Candida antarctica* lipase A (CALA) (Scheme 2) gave reaction products with an *E*-value = 67. (*S*)-Phenyl-(2-naphthyl)-methanol ((*S*)-**6**) was obtained in 47.3 % yield and 94.5 % *ee*. The (*R*)-acetate of phenyl-(2-naphthyl)-methanol ((*R*)-**7**) was obtained in 35.8 % yield and 95.0 % *ee*. Optical rotation of the alcohol is reported for both enantiomers[18] and the absolute configurations have been determined by X-ray crystallography. However, optical rotation values of the (*R*)-acetate ((*R*)-**7**) has not been reported and the configuration of this ester is based on comparison of the chromatographic retention times (HPLC) with the alcohol enantiomers. Phenyl-(2-naphthyl)-methanol (**6**) was prepared by reduction of phenyl-(2-naphthyl)-methanone (**5**) with $NaBH_4$ (high yield) and by a non-enantioselective yeast reduction with *Saccharomyces cereviciae* (low yield). (Scheme 3) The racemic acetate **7** was synthesized by esterification of the alcohol **6** with acetic anhydride.



Scheme 2



Scheme 3

Conclusions

Kinetic resolutions of saphenic acid (**2**) catalyzed by CALB were very efficient. Saphenic acid has one small group (methyl) and one large group (tricyclic phenazine ring) connected to the stereocenter. We have found earlier that n-propyl is the largest group that can fit into the stereospecificity pocket of CALB, leading to high enantioselectivity in kinetic resolutions of secondary alcohols.[19] The enantioselectivity is not to the same extent dependent on the size of the large group. CALA shows high enantioselectivity for phenyl-(2-naphthyl)-methanol (**6**) (one phenyl and one naphthyl group connected to the stereocenter) which is in accordance with previous reported results.[11-16]

Experimental Section

General Procedures. Table 1 (See Results and Discussion part) shows the enzymes used in screening experiments. Novozym 735 (immobilized lipase A from *Candida antarctica*, CALA), Novozym 435 (immobilized lipase B from *Candida antarctica*, CALB), Lipozyme RM IM (immobilized lipase from *Rhizomucor miehei*, RM IM) and Lipozyme TL IM (immobilized lipase from *Thermomyces lanuginosa*, TL IM) were from Novozymes. Other enzymes were purchased from Sigma-Aldrich. Immobilized lipase B from *Candida antarctica* (CALB,

Novozym 435) had an activity of 7000 PLU/g, and a water content of 2 % w/w. Enzyme catalysts were rinsed with hexane and re-used several times with retention of enantioselectivity and activity according to previous results.[20] Chemicals were purchased from Sigma-Aldrich. Solvents were dried over molecular sieve from Union Carbide (3 Å) and analyzed by a Metrohm 756 Karl Fisher Coulometer.

Saphenic acid (**2**) was a gift from Professor John Nielsen, University of Århus, Denmark. The transesterification reactions and hydrolyses were performed at 30°C and 200 rpm in an Infors MINITRON Shaker Incubator. (A pH stat was not used due to decomposition of saphenic acid in day light.) All transesterification reactions were performed in toluene:hexane (2:1) and hydrolyses in phosphate buffer (pH 7.0). Column and flash chromatography were performed using silica gel 60 from Sigma-Aldrich, with pore size 0.0663-0.2000 mm and 0.035-0.070 mm, respectively.

Analyses

Optical rotations were determined using a Perkin Elmer 243 B automatic polarimeter, concentrations are given in g/100 mL. Chiral HPLC analyses gave the ee_s- and ee_p-values from which the degree of conversion was determined according to $c = ee_s / (ee_s + ee_p)$. Internal standards were not used because of the possibility of affecting the physical and chemical nature of the reaction medium. In control experiments under the reaction conditions without enzyme, no acylation was observed.

Chiral analyses were performed using a HPLC system (Varian 9010 pump, Varian 2550 UV detector, Rheodyne 7125 manual injector and Varian 9095 autosampler) equipped with Daicel Chiralpack AD (no 19025, 25 cm, 4.6 mm i.d., particle size 10 μm packed with amylose tris-(3,5-dimethyl phenyl carbamate) and Daicel Chiralpack OD-H (no 14325, 25 cm, 4.6 mm i.d., particle size 5 μm packed with cellulose tris-(3,5-dimethyl phenyl carbamate). Star Chromatography Workstation 4.5.1 was used for data processing.

Chromatographic parameters

(*R*)-**2** R_t 49.5 min, (*S*)-**2** R_t 58.5, R_S 3.46, separated by AD column with hexane:EtOH (85:15), flow 0.5 mL/min, 254 nm.

(*R*)-**6** R_t 74.6 min, (*S*)-**6** R_t 60.3, R_S 5.41, separated by OD-H column with hexane:isopropanol (95:5), flow 0.5 mL/min, 254 nm.

(*R*)-**7** R_t 13.0 min, (*S*)-**7** R_t 13.9, R_S 2.08, separated by OD-H column with hexane:isopropanol (95:5), flow 0.5 mL/min, 254 nm.

NMR spectroscopy was performed in $CDCl_3$ solutions, using Bruker DPX 300 and 400, operating at 300 and 400 MHz for ^1H and 75 and 100 MHz for ^{13}C, respectively. Enantiomeric ratios, *E*, were calculated based on ping-pong, bi-bi kinetics using the computer program E & K Calculator version 2.1b PCC.[21,22] Mass spectra were performed on a Mat 95 XL Thermo Quest Finnigan with EI as ionizing source.

Compound characterization

Saphenic acid butanoate (**4**)**.** Saphenic acid (**2**) (0.46 g, 1.72 mmol) was dissolved in a solution of toluene:hexane (2:1, 75 mL) with vinyl butanoate (1.09 mL, 8.6 mmol). The reaction vessel was covered with aluminium foil. Molecular sieve (2.0 g) and lipase A from *Candida antarctica* (CALA – not stereoselective) (0.5 g) was added. The reaction was incubated at 30°C and 200 rpm for 12 days before concentration under pressure and column chromatography. The butanoate of saphenic acid, **4**, was isolated in 73 % yield and the unconverted saphenic acid (**1**) was isolated in 12 % yield.

Phenyl-(2-naphthyl)-methanol (6). Method 1. Biocatalytic reduction. Phenyl-(2-naphthyl)-methanone (**5**) was reduced by a cell culture of *Saccharomyces cereviciae* (not enantioselective) by the following procedure: Sucrose (16.0 g) and dry yeast (5.0 g) were added to water (100 mL) in a 500 mL flask. The medium was incubated by 30°C 1/2 h before 5 (50 mg, 0.215 mmol in 3 mL EtOH) was transferred every half hour (250 μL) to the reaction flask until all of the substrate was added. The reaction was incubated at 30°C and 200 rpm for 14 days before work-up. The reaction mixture was saturated with NaCl and the solvent phase was removed by suction (2 x) under reduced pressure. The reaction product was extracted with toluene (4 x 50 mL) and the organic phase dried over $MgSO_4$. Toluene was evaporated under reduced pressure. Phenyl-(2-naphthyl)-methanol (**6**) was isolated as a slightly yellow solid in 14.4 % yield and 99 % purity (HPLC).

Method 2. Non-enzymatic reduction. Phenyl-(2-naphthyl)-methanone (**5**) (1.0 g, 4.303 mmol) was dissolved in MeOH (50 mL) before addition of $NaBH_4$ (0.326 g, 8.618 mmol). The reaction was stirred for 2 h. HCl (1 M) was then added dropwise until evolution of gas stopped. The solvent was removed under reduced pressure. The white solid was dissolved in toluene (25 mL) and washed with H_2O (3 x 20 mL) before drying of the organic phase over $MgSO_4$. Phenyl-(2-naphthyl)-methanol (**6**) was isolated as a yellow solid in 95 % yield and 99 % purity after removal of the solvent under reduced pressure. Melting point 79.4-80.4°C, EI/MS, (*m/z*) M^+ 234, 215, 155, 129, 105, 77. ^{1}H NMR: 2.81 (1H, d, *J* 3.1 Hz), 5.78 (1H, d, *J* 2.9 Hz), 7.16-7.32 (6H, m), 7.36-7.43 (2H, m), 7.66-7.75 (4H, m). ^{13}C NMR: 76.70, 125.34, 126.47, 126.69, 127.24, 128.20, 128.20, 128.60, 128.80, 129.00, 133.30, 133.70, 141.60, 144.10.

Acetate of phenyl-(2-naphthyl)-methanol (7). Phenyl-(2-naphthyl)-methanol (**6**) (0.5 g, 2.136 mmol) was dissolved with Ac_2O (0.436 g, 4.272 mmol) and dimethyl amino pyridine (DMAP) (0.287 g, 0.235 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was cooled at 0°C for 30 min before addition of dicylohexyl carbodiimide (DCC) (0.458 g, 2.221 mmol). The reaction was stirred at 0°C over night. Dicyclohexyl urea (DCU) was removed by filtration and the reaction mixture was washed with HCl (0.5 M, 3 x 5 mL), $NaHCO_3$ (5.0 %, 2 x 5 mL) and distilled H_2O (2 x 10 mL). The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by column chromatography with toluene:methanol (10:1). The acetate **7** was isolated as a yellow oil in 63 % yield and 99 % purity (HPLC).

Enzyme screening in transesterification reactions. Saphenic acid (**2**) (0.1 g, 0.34 mmol) and vinyl butanoate (0.237 mL, 1.872 mmol) were dissolved in a solution of toluene:hexane (2:1, 20

mL). The solution was divided into 10 reaction vessels, and molecular sieve (2 beads) was added to each vessel (one blind sample without enzyme). The reactions were started by addition of enzyme (50 mg) and incubated (30°C, 200 rpm) for 7 days. TLC and HPLC showed that lipase A and B from *Candida antarctica* and lipases from *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosus* (Lipozyme TL IM) gave products. However, Lipozyme RM IM showed low stereoselectivity and CALA and Lipozyme TL IM showed no stereoselectivity. These reactions were of that reason not scaled up. Lipase B from *Candida antarctica* showed the highest conversion of product ester with high enantiomeric excess. Use of vinyl acetate showed similar results. The reaction of **2** with CALB and vinyl butanoate was scaled up.

The same procedure was used in screening of enzymes for phenyl-(2-naphthyl)-methanol (**6**) with use of the enzymes listed in Table 1, except for the lipase from papaya and Acylase A from *Aspergillus melleus*. Lipase A from *Candida antarctica* showed the highest conversion of product ester and this reaction was scaled up.

Transesterification reaction of 2. Saphenic acid (**2**), (0.44 g, 1.65 mmol) and vinyl butanoate (1.05 mL, 8.3 mmol) were added to toluene:hexane (2:1, 75 mL) and the reaction was started by addition of CALB (0.818 g). Molecular sieve (2.0 g) was added, and the vessels were covered with aluminium foil. The reactions were incubated for 3 days. Enzyme and molecular sieve were removed by filtration, and silica (1.0 g) was added the filtrate before concentration under reduced pressure. Separation on silica with $CHCl_3$:isopropanol (95:5) gave the yellow solid (*S*)-saphenic acid, (*S*)-**2**, in 35 % yield and 97.2 % *ee*. After recrystallization the yield was 25 % and *ee* 100 %, $[\alpha]_D^{20}$ = + 16.82 (c 0.41, $CHCl_3$), $[\alpha]_D^{20}$ = – 30.43 (c 0.23, DMSO), mp 218.4-219.2°C. (*R*)-saphenic acid butanoate ((*R*)-6-[1-(1-oxobutoxy)ethyl]-phenazine-1-carboxylic acid), (*R*)-**4**, was obtained in 41 % yield and 96.4 % *ee*, $[\alpha]_D^{20}$ = – 48.33 (c 0.41, $CHCl_3$) $[\alpha]_D^{20}$ = – 52.83 (c 2.29, DMSO), mp 162.3-163.5°C. Recrystallization of (*R*)-**4** by the same procedure as in (*S*)-**2** did not increase *ee*. (*S*)-**2**: EI/MS (*m/z*) M^+ 268, 253, 225, 205, 181. ^{1}H NMR (ppm):1.82 (3H, d, *J* 6.6 Hz), 4.12 (1 H, d, *J* 5.8 Hz), 5.84 (1H, p, *J* 6.3), 7.94-8.01 (2H, m), 8.06 (1H, dd, *J* 8.9 Hz and J 7.1 Hz), 8.2 (1H, dd, *J* 8.4 Hz and *J* 1.6 Hz), 8.53 (1H, dd, *J* 8.7 and *J* 1.4 Hz), 8.99 (1H, dd, *J* 7.1 Hz and J 1.4 Hz) 15.47 (1H, br, s).

(*R*)-**4**: EI/MS (*m/z*) M^+ 337, 293, 267, 250, 221, 204, 179. ^{1}H NMR (ppm): 0.99 (3 H, t, *J* 7.4 Hz), 1.76 (3H, d, *J* 6.6 Hz), 1.67-1.8 (2H, m) 2.44 (2H, dt, *J* 7.4 Hz), 7.22 (1H, q, *J* 6.5 Hz), 7.98-8.06 (3H, m), 8.19 (1H, m), 8.55 (1H, dd, *J* 8.7 Hz and *J* 1.4 Hz), 8.96 (1H, dd, *J* 7.1 Hz and *J* 1.4), 15.52 (1H, br, s). ^{13}C NMR: 13.72, 18.54, 22.33, 36.50, 67.50, 124.67, 126.91, 127.16, 130.18, 133.00, 135.50, 137.50, 139.60, 139.80, 141.30, 142.15, 142.50, 165.92, 172.73.

Recrystallization. (*S*)-Saphenic acid ((*S*)-**2**) (0.154 g, 0.577 mmol) was dissolved in hot MeOH (2 mL). The reaction was cooled overnight. The remaining solvent was removed by filtration and the crystals were isolated and dried. The *ee* increased from 97.2 % to 100 % measured by HPLC.

Hydrolysis of the butanoate of saphenic acid (4). Butanoate **4** (0.423 g, 1.256 mmol) was dissolved in MeOH (10 mL) and transferred to phosphate buffer (pH 7.0, 50 mL) The reaction vessel was covered by aluminium foil and CALB (0.5 g) was added. The reaction was incubated

for 7 days. The enzyme was removed by filtration and the filtrate was extracted with $CHCl_3$. The organic phase was dried over $MgSO_4$, filtrated and evaporated with silica (1.0 g) under reduced pressure. The silica sample was transferred to a packed silica column for purification. (*R*)-saphenic acid, (*R*)-**2**, was isolated in 29 % yield with 96.6 % *ee*. After recrystallization by the same method as for (*S*)-**2**, the yield was 19.3 % and *ee* 100 % analyzed by HPLC. $[\alpha]_D^{20}$ = – 15.91 (c 0.41, $CHCl_3$), $[\alpha]_D^{20}$ = + 29.78 (c 0.23, DMSO). (*S*)-**4** was isolated in 36 % yield with 99 % purity and 81 % *ee* (HPLC). $[\alpha]_D^{20}$ = + 33.90 (c 0.41, $CHCl_3$). The *ee* of (*S*)-**4** was not improved by crystallization.

Transesterification reaction of phenyl-(2-naphthyl)-methanol (6). Phenyl-(2-naphthyl)-methanol (**6**) (0.813 g, 3.468 mmol) and vinyl acetate (1.6 mL, 17.34 mmol) were dissolved in a mixture of toluene:hexane (2:1, 50 mL). Molecular sieve (2.0 g) was added and the reaction was started by addition of CALA (1.0 g). The reaction mixture was incubated for 48 h. Enzyme and molecular sieve were removed by filtration and the solvent was removed under reduced pressure. The crude product was purified by column chromatography with toluene:MeOH (10:1). The acetate (*R*)-**7** was isolated as a slightly yellow viscous oil in 47.3 % yield, 99 % purity (HPLC) and 95 % enantiomeric excess. $[\alpha]_D^{20}$ = +17.83 (c 0.83, $CHCl_3$), $[\alpha]_D^{20}$ = + 30.36 (0.83, Benzene). EI/MS (*m/z*) M^+ 276, 234, 215, 202, 156, 127, 107 and 77. ^{1}H NMR: 2.17, (3H, s), 7.05 (1H, s), 7.2-7.47 (8H, m), 7.77-7.82 (4H, m). ^{13}C NMR: 21.30, 76.95, 124.94, 126.01, 126.22, 126.28, 127.18, 127.64, 127.95, 128.09, 128.38, 128.51, 132.91, 133.07, 137.50, 140.06, 170.02.

The alcohol (*S*)-**6** was isolated as a solid in 35.8 % yield and 99 % purity (HPLC) and 94.5 % *ee*. $[\alpha]_D^{20}$ = + 11.21 (c 0.83, $CHCl_3$), $[\alpha]_D^{20}$ = – 6.51 (0.83, Benzene), mp 79.4-80.4°C. Recrystallization of (*S*)-**6** from Et_2O did not increase *ee*.

Hydrolysis of the acetate of phenyl-(2-naphthyl)-methanol (7) by CALA. The acetate of phenyl-(2-naphthyl)-methanol (**7**) (0.371 g, 1.345 mmol) was dissolved in MeOH (10 mL) before it was added to a phosphate buffer solution (pH 7.0, 50 mL). CALA (1.0 g) was added and the reaction mixture was incubated for 10 days. The enzyme was removed by filtration and the reaction mixture was extracted with toluene (3 x 10 mL). The organic phase was dried over $MgSO_4$ before the solvent was removed under reduced pressure. The crude product was purified by column chromatography (toluene:MeOH, 10:1). (*R*)-phenyl-(2-naphthyl)-methanol ((*R*)-**6**) was isolated in 25 % yield, 99 % purity and 98.5 % *ee* (HPLC). $[\alpha]_D^{20}$ = – 11.61 (c 0.83, $CHCl_3$), $[\alpha]_D^{20}$ = + 6.89 (c 0.83, Benzene). The (*S*)-acetate of phenyl-(2-naphthyl)-methanol ((*S*)-**7**) was isolated in 23 % yield, 99 % purity and 94.5 % *ee* (HPLC). $[\alpha]_D^{20}$ = – 17.01 (c 0.83, $CHCl_3$), $[\alpha]_D^{20}$ = – 29.73 (c 0.83, Benzene).

Acknowledgements

We are grateful to professor John Nielsen, University of Aarhus, Denmark, for gift of racemic

saphenic acid. We also thank Novozymes AS, Bagsværd, Denmark, for gifts of Novozym 435, Novozym 735, Lipozyme RM IM and Lipozyme TL IM.

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