CHEMOENZYMATIC SYNTHESIS OF THE ENANTIOMERS OF IOPANOIC ACID

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Abstract: The two enantiomers of Iopanoic acid **1** were prepared in enantiomeric excess higher than 90% by enzyme-catalyzed hydrolysis of precursors (±)-**2a** and (±)-**3a**, followed by standard chemical transformations. Among the tested enzymes, chymotrypsin and Lipase PS proved to be the most selective catalysts. The stereochemical outcome of the lipase-catalyzed hydrolyses of esters (±)-**2a-d** is strictly dependent upon both the size of the alkyl group attached to the chiral center and the substituent in the aromatic ring. The enantioselectivity of the reactions was evaluated by chiral HPLC and the configurations of the new products were assigned by chemical correlations.

(±)-3-(3-Amino-2,4,6-triiodophenyl)-2-ethylpropionic acid (Iopanoic acid, (±)-**1**) is an oral cholecystographic agent which is rapidly absorbed by the gastroenteric tract and is excreted with bile. This compound has been extensively used as a first choice X-ray contrast medium for the diagnosis of bile stones. Optical resolution of (±)-**1** was achieved through the formation of the diastereoisomeric salts with (R)-(+)- and (S)-(-)-1-phenylethylamine.[1] The configuration of the two enantiomers was assigned by chemical correlation.[2]

(±)-**1**
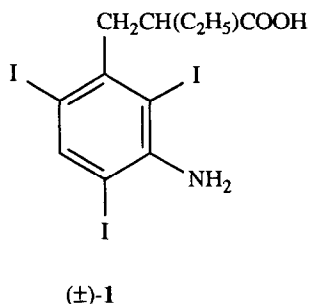
Since the two enantiomers of **1** are differently metabolized i.e. they are transformed into the

corresponding glucoronides at a different rate,[3,4] as an extension of a program devoted to the use of bioconversion processes to the synthesis of biologically active chiral compounds,[5,6] we became interested in the synthesis of (+)-**1** and (-)-**1** by means of enzyme-catalyzed transformations.

RESULTS AND DISCUSSION

Our first approach to the synthesis of the two enantiomers of **1** was based on the enantioselective hydrolysis of its esters. Methyl, ethyl, and butyl esters of (±)-**1** were submitted to hydrolysis catalyzed by a series of enzymes. Among the tested catalysts (thirteen lipases, three esterases and two proteases), pig liver esterase (PLE) was the only one to recognize the esters of (±)-**1** as substrates. Unfortunately, as shown in Table I, it produced (R)-(-)-**1** with an enantioselectivity which was unsatisfactory for preparative purposes. It is worth noting the regular decrease of enantioselectivity, expressed as enantiomeric ratio E,[7] with the increase in the size of the ester moiety. For such a reason, in expanding our investigation on related substrates we considered carboxylic methyl esters only.

Table I. PLE-catalyzed hydrolysis of iopanoic acid esters.

Ester	Conv. %	e.e. (%)[a]	config.	E[b]
Me	39	59	R	5.5
Et	45	40	R	3.2
n-Bu	41	22	R	1.8

[a]Determined on iopanoic acid. [b]The E values were calculated from the degree of conversion and the e.e. of the product according to Chen et al.[7]

As an alternative approach to (+)-**1** and (-)-**1** we chose to study the enzymatic hydrolysis of Iopanoic acid deiodinated precursors (±)-**2a** and (±)-**3a**. The synthesis of **2a** and **3a** was achieved in a conventional way (Scheme I) by reacting m-nitrobenzyl chloride with diethyl ethylmalonate followed by appropriate chemical manipulations.

A screening for the enantioselective hydrolysis of aminoderivative (±)-**3a** was started using a variety of commercially available hydrolytic enzymes; Table II shows the results obtained with those able to hydrolyse the substrate.

Low stereoselectivities (E <4) were observed with all the enzymes except chymotrypsin which had a good activity and a remarkable degree of enantioselectivity (E =75). Thus, when substrate (±)-**3a** was hydrolyzed in aqueous buffer, under the catalysis of chymotrypsin, (S)-(+)-**4a** was obtained in 94% enantiomeric excess (e.e.) at 45% conversion. At 56% conversion the residual ester [(R)-(-)-**3a**] reached an e.e. >99% (Scheme II).

Since (R)-**3a** is prone to racemization in alkaline conditions, its hydrolysis to (R)-**4a** was carried out in a buffer solution at pH 7 under the catalysis of horse liver esterase (HLE). The two enantiomers of Iopanoic acid were finally prepared by reacting (S)-**4a** and (R)-**4a** with a hydrochloric acid solution of iodine
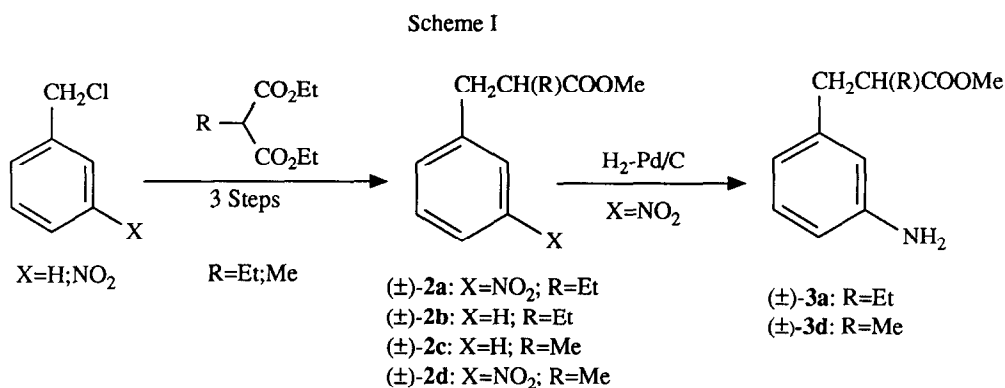
Scheme I

(±)-2a: X=NO$_2$; R=Et
(±)-2b: X=H; R=Et
(±)-2c: X=H; R=Me
(±)-2d: X=NO$_2$; R=Me

(±)-3a: R=Et
(±)-3d: R=Me

Table II. Enzyme-catalyzed hydrolysis of 2 and 3.

Substrate	Enzyme	Config. of the acid	E
(±)-2a	Chymotrypsin	S	57[a]
(±)-2a	Lipase PS	R	29[a]
(±)-2b	Lipase PS	S	1.2[b]
(±)-2c	Lipase PS	S	145[c]
(±)-2d	Lipase PS	S	25[a]
(±)-3a	Chymotrypsin	S	75[b]
(±)-3a	Subtilisin	S	2[b]
(±)-3a	MML	R	4[b]
(±)-3a	M10	R	3[b]
(±)-3a	PLE	S	3[b]
(±)-3a	HLE	S	1.3[b]
(±)-3a	CCL	R	4[b]

[a]Calculated from the e.e. of the corresponding amino methyl ester. [b]Calculated from the e.e. of the corresponding methyl ester. [c]Taken from ref.9.
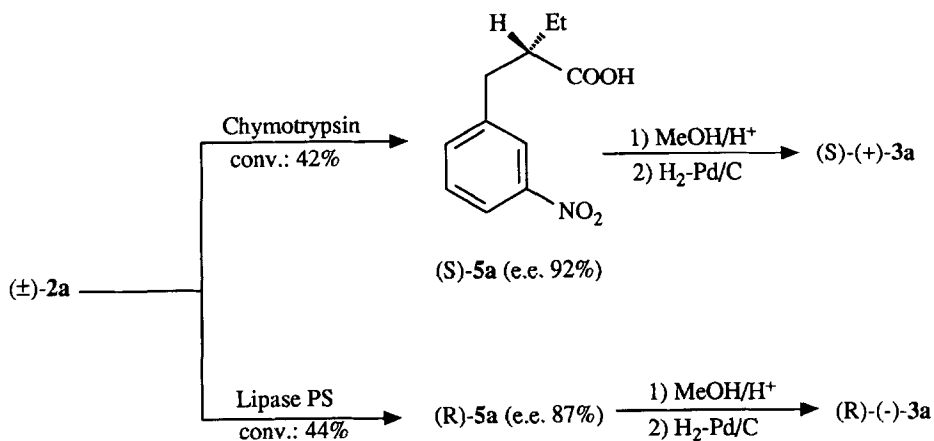
monochloride, as previously reported.[8] The enantiomeric excess of (R)-(-)-3a was directly determined by chiral HPLC (Chiralcel OD) whereas (S)-4a needed to be transformed into (S)-(+)-3a.

The nitroderivative (±)-2a, a further precursor of Iopanoic acid, was also submitted to enantioselective hydrolyses. In this case chymotrypsin and Lipase PS gave the best results. It is worth noting that both enzymes operate on (±)-2a with a remarkable enantiomeric ratio but with an opposite enantioselectivity

Scheme II

(±)-3a

Chymotrypsin
conv.: 45%

H,,,Et
  COOH

NH$_2$

(S)-(+)-4a (e.e. 94%)

ICl → (S)-(+)-1

Chymotrypsin
conv.: 56%

(R)-(-)-3a (e.e. >99%) 1) HLE / 2) ICl → (R)-(-)-1

Scheme III

(±)-2a

Chymotrypsin
conv.: 42%

H,,,Et
  COOH

NO$_2$

(S)-5a (e.e. 92%)

1) MeOH/H$^+$ / 2) H$_2$-Pd/C → (S)-(+)-3a

Lipase PS
conv.: 44%

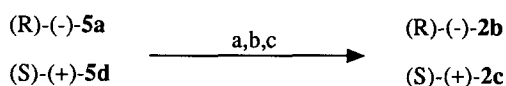(R)-5a (e.e. 87%) 1) MeOH/H$^+$ / 2) H$_2$-Pd/C → (R)-(-)-3a
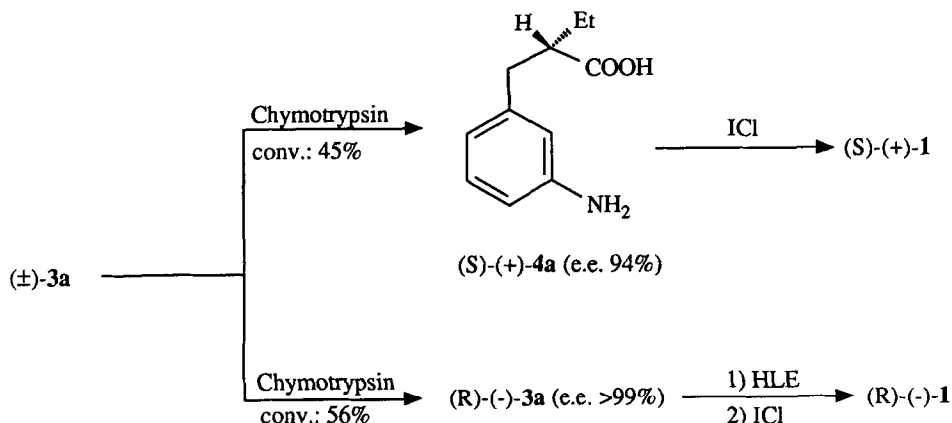
(Table II). As a consequence, the use of chymotrypsin or Lipase PS allowed the synthesis of either (S)-(+)-3a (92% e.e.) or (R)-(-)-3a (87% e.e.) (Scheme III).

Quite recently it has been reported that Lipase PS catalyzes the hydrolysis of the (S)-form of (±)-2c with a good activity and a very high stereoselectivity (E =145);[9] such a selectivity is opposite to that observed by us on the related substrate (±)-2a (Table II). These findings induced us to prepare esters (±)-2b and (±)-2d in order to investigate the factors which dictate the stereochemical outcome of the Lipase PS catalyzed-hydrolysis. As shown in Table II, the stereochemistry of the catalyzed-hydrolysis of substrates (±)-2a-d is dependent upon both the size of the alkyl group at the stereogenic center and the presence of the substituent in the aromatic ring. Lipase PS displayed its highest enantioselectivity (E=145)[9] and activity (see Experimental) on ester (±)-2c. The introduction of a nitro group in the aromatic ring [(±)-2d] caused a significant lowering of stereoselectivity. (S)-2-(3-Nitrobenzyl)propionic acid [(S)-5d], produced from the enzymatic hydrolysis of

(±)-**2d**, possesses an enantiomeric excess of 95% at 44% conversion (E=25). On the other hand, the replacement of the methyl with the bulkier ethyl group at the chiral center i.e. on passing from (±)-**2c** to (±)-**2b**, caused an almost total disappearance of the enzyme capacity of stereoselection (E=1.2), together with a drastic reduction of activity. The combined effect of the substituent in the aromatic ring and the ethyl group at the stereogenic center, i.e. on passing from (±)-**2c** to (±)-**2a**, produced a reversal in enantioselectivity at a noticeable degree of stereoselection (E=29).

The chirality of the compounds obtained by enzymatic hydrolysis has been assigned by chemical correlation. The enantiomers of intermediates **2a**, **3a**, and **4a** have been correlated to the enantiomers of Iopanoic acid [(R)-**1** and (S)-**1**] (Schemes II and III), whose stereochemistry was previously assigned.[2] On the other hand, (R)-**5a** and (S)-**5d** were correlated to (R)-**2b** and (S)-**2c** through the reaction sequence reported in Scheme IV.

<div align="center">Scheme IV</div>

$$(R)\text{-}(-)\text{-}\mathbf{5a}$$
$$(S)\text{-}(+)\text{-}\mathbf{5d} \xrightarrow{\quad a,b,c \quad} \begin{array}{l}(R)\text{-}(-)\text{-}\mathbf{2b}\\(S)\text{-}(+)\text{-}\mathbf{2c}\end{array}$$

<div align="center">a: MeOH-H$^+$; b: NaNO$_2$-H$^+$; c: 50% H$_3$PO$_2$.</div>

The enantiomeric excess of the products was evaluated by chiral HPLC as reported in the Experimental Section.

In summary, the present research further expands the application of enzymes into the synthesis of compounds provided with a biological activity. The data on the Lipase PS-catalyzed hydrolyses evidence the dramatic dependence of the stereochemical outcome of the reaction upon both the size of the alkyl group at the stereogenic center and the presence of the substituent in the aromatic ring. Probably one of the lipophilic pockets of the enzyme active site is so limited in size that it can accommodate a group not larger than a methyl. As a matter of fact, on passing from **2c** to **2b**, the enzyme looses its capacity of stereoselection whereas its enantioselectivity switches from S to R when the above-mentioned modification of the chiral center is accompanied by the introduction of the substituent in the aromatic ring i.e. on passing from (±)-**2c** to (±)-**2a**. We are now expanding our study on related systems in order to delineate an active site model for Lipase PS catalyzed-hydrolyses which could be of predictive value.

<div align="center">Experimental Section</div>

Carboxylesterase NP was donated by International Bio-Synthetics (I.B.I.S., The Netherlands). Chymotrypsin A$_4$ was bought from Boehringer, pig liver esterase (PLE), Lipase PS, L-10, AY-30, M-10, F-AP15, A-G, D-20, N, R-10, GC-4, G, CE-5 were purchased from Amano, Lipase from *Mucor miehei* (Lipase MM) was obtained from Biocatalysts LTD, subtilisin Carlsberg and horse liver esterase (HLE) were bought from Sigma. Organic solvents were reagent grade. ^{1}H NMR spectra were recorded in CDCl$_3$ solution at 80 or 200 MHz. Rotatory power determinations were carried out with a Perkin Elmer 241 polarimeter, coupled with a Haake N3-B thermostat. HPLC analyses were performed on a chromatograph equipped with a UV detector (λ=254 nm) and a Whatman Partisil 10 column or a Chiralcel OD column (4.6x250 mm) at a

flow rate of 0.5 mL/min. The composition of the mobile phase is specified in the appropriate paragraph. Liquids were characterized by the oven temperature for Kugelrohr distillations. Melting points were determined on a Büchi apparatus and are uncorrected. Microanalyses agree with theoretical value ±0.3%.

Synthesis of (±)-Iopanoic acid methyl, ethyl and butyl esters. A suspension of (±)-Iopanoic acid sodium salt (5.93 g, 0.01 mol) and alkyliodide (0.012 mol) in dimethylacetamide (35 mL) was stirred and heated at 90°C for 4h. The disappearance of the starting material was checked by TLC (eluent: toluene-ethyl acetate-formic acid 50:45:5). The mixture was cooled at room temperature, poured into water (200 mL) and extracted with ethyl ether (100 mL). The organic layer was washed with sodium hydroxide (3x20 mL, 1N), hydrochloric acid (20 mL, 2N) and water (25 mL). After the usual workup, the esters of Iopanoic acid were obtained in 85-90% yield.

(±)-Iopanoic acid methyl ester was crystallized from ether-ligroin as prism, mp 66-68°C.

(±)-Iopanoic acid ethyl ester was crystallized from ether-ligroin as prism, mp 73-76°C.

(±)-Iopanoic acid n.butyl ester was collected as a brown oil.

Synthesis of Methyl (±)-3-aryl-2-alkylpropionate [(±)-2a-d]. A. A 250 mL three neck round bottom flask equipped with a magnetic stirrer, a reflux condenser and a dropping funnel was charged with sodium hydride (0.96 g, 0.04 mol) and anhydrous ethyl ether (30 mL). To the stirred suspension, diethyl 2-alkylmalonate (0.041 mol) was added dropwise and the slurry was heated at reflux until total dissolution of the hydride. To such a mixture, cooled at room temperature, m.substituted benzyl chloride (0.024 mol) in ether (20 mL) was added dropwise and stirred under nitrogen for 6h. The slurry was treated with water (3x25 mL), the organic layer was separated, dried over anhydrous sodium sulfate and the solvent evaporated at reduced pressure. Excess diethyl 2-alkylmalonate was removed from the residue by Kugelrohr distillation at 85-100°C/0.3 mmHg. The residue was sufficiently pure for the following step.

Diethyl 2-ethyl-2-m.nitrobenzylmalonate was collected as a thick colorless oil. ^{1}H NMR(CDCl$_3$): δ 0.98 (t, 3H, CH$_2$CH$_3$); 1.29 (t, 6H, CO$_2$CH$_2$CH$_3$); 1.85 (q, 2H, CH$_2$CH$_3$); 3.35 (s, 2H, CH$_2$Ar); 4.22 (q, 4H, CO$_2$CH$_2$CH$_3$); 7.25-7.70 (m, 2H, arom.); 7.95-8.30 (m, 2H, arom.).

B. The residue of the preceding reaction (0.02 mol) was treated with potassium hydroxide (0.062 mol) in ethanol-water (3:1, 30 mL) and refluxed for 24h. Ethanol was removed at reduced pressure and the residue was taken up with a 20% aqueous potassium carbonate solution (30 mL). The mixture was extracted with chloroform (3x25 mL), acidified with 2N HCl and newly extracted with dichloromethane (3x25 mL). The organic phase, after the usual workup, yielded 2-alkyl-2-m.substituted benzylmalonic acid in 77-82% yield.

2-Ethyl-2-m.nitrobenzylmalonic acid crystallized from ligroin/diisopropyl ether as colorless prism, mp 160-162°C dec. ^{1}H NMR(CDCl$_3$): δ 1.20 (t, 3H, Me); 2.00 (q, 2H, CH$_2$CH$_3$); 3.42 (s, 2H, CH$_2$Ar); 7.35-7.80 (m, 2H, arom.); 7.95-8.40 (m, 2H, arom.); 10.45 (bs, 2H, CO$_2$H).

C. A solution of the previously prepared malonic acid (16 mmol) in DMF (60 mL) was stirred and heated at 100-110°C for 4h. The solvent was removed at reduced pressure and the residue was dissolved in a 20% aqueous potassium carbonate solution (20 mL). This solution was added dropwise to ice-cooled

hydrochloric acid (50 mL, 6N) and the precipitate (13.5 mmol) was collected.

(±)-3-m.Nitrophenyl-2-ethylpropionic acid was crystallized from ligroin-ethyl acetate as yellowish crystals, mp 75-76°C. ^{1}H NMR(CDCl$_3$): δ 0.98 (t, 3H, Me); 1.70 (q, 2H, CH$_2$CH$_3$); 2.40-3.30 (m, 3H, CH$_2$Ar and CHCO$_2$H); 7.25-7.75 (m, 2H, arom.); 7.90-8.40 (m, 2H, arom.); 10.84 (bs, 1H, CO$_2$H).

D. The above-prepared propionic acids (12.5 mmol) were dissolved in methanol saturated with gaseous HCl (50 mL, 3N). The solution was magnetically stirred at room temperature for 24 h then the solvent and HCl were removed at reduced pressure. The oily residue was taken up with ether (50 mL) and treated with a 20% aqueous potassium carbonate solution (30 mL). After the usual treatments, the methyl esters were collected in 85-89% yield.

Methyl 3-m.nitrophenyl-2-ethylpropionate [(±)-**2a**] and methyl 3-m.nitrophenyl-2-methylpropionate [(±)-**2d**)] were Kugelrohr distilled at 145-150°C/0.3 mmHg as pale yellow oils whereas methyl 3-phenyl-2-ethylpropionate [(±)-**2b**] and methyl 3-phenyl-2-methylpropionate [(±)-**2c**] were distilled at 105-110°C/0.5 mmHg as colorless oils.

2a - ^{1}H NMR(CDCl$_3$): δ 0.95 (t, 3H, CH$_2$CH$_3$); 1.62 (m, 2H, CH$_2$CH$_3$); 2.65 (m, 1H, CHCO$_2$Me); 2.95 (m, 2H, CH$_2$Ar); 3.66 (s, 3H, CO$_2$Me); 7.35-7.80 (m, 2H, arom.); 7.95-8.30 (m, 2 H, arom.).

2b - ^{1}H NMR(CDCl$_3$): δ 0.93 (t, 3H, CH$_2$CH$_3$); 1.56 (m, 2H, CH$_2$CH$_3$); 2.4-3.1 (m, 3H, CHCO$_2$Me and CH$_2$Ar); 3.64 (s, 3H, CO$_2$Me); 7.0-7.5 (m, 5H, arom.).

2c - ^{1}H NMR(CDCl$_3$): δ 1.15 (d, 3H, CHCH$_3$); 2.5-3.2 (m, 2H, CHCO$_2$Me and CH$_2$Ar); 3.63 (s, 3H, CO$_2$Me); 7.0-7.5 (m, 5H, arom.).

2d - ^{1}H NMR(CDCl$_3$): δ 1.23 (d, 3H, CHCH$_3$); 2.10-3.31 (m, 3H, CH$_2$Ar and CHCO$_2$Me); 3.68 (s, 3H, CO$_2$Me); 7.30-7.75 (m, 2H, arom.); 7.90-8.35 (m, 2H, arom.).

E. Propionate (±)-**2a** [or (±)-**2d**] (10 mmol) was dissolved in methanol (20 mL) and hydrogenated at room temperature in the presence of 5% Pd/C. The catalyst was removed by filtration and the solvent was evaporated under vacuum. The residue, Kugelrohr distilled at 145-150°C/0.5 mmHg, yielded amino ester (±)-**3a** [or (±)-**3d**] in quantitative yield.

Methyl 3-aminophenyl-2-ethylpropionate [(±)-**3a**] distilled at 150-155°C/0.5 mmHg as yellowish oil. ^{1}H NMR(CDCl$_3$): δ 0.89 (t, 3H, CH$_2$CH$_3$); 1.55 (m, 2H, CH$_2$CH$_3$); 2.40-3.00 (m, 3H, CH$_2$Ar and CHCO$_2$Me); 3.58 (bs, 2H, NH$_2$); 3.61 (s, 3H, CO$_2$Me); 6.40-7.20 (m, 3H, arom.); 7.40-7.75 (m, 1H, arom.).

Methyl 3-aminophenyl-2-methylpropionate [(±)-**3d**] distilled as colorless liquid at 150-155°C/0.5 mmHg. ^{1}H NMR(CDCl$_3$): δ 1.16 (d, 3H, CHCH$_3$); 2.4-3.2 (m, 3H, CHCO$_2$Me and CH$_2$Ar); 3.54 (bs, 2H, NH$_2$); 3.63 (s, 3H, CO$_2$Me); 6.4-6.8 (m, 3H, arom.); 6.9-7.4 (m, 1H, arom.).

PLE-catalyzed hydrolysis of Iopanoic acid esters. A solution of Iopanoic acid methyl ester (0.700 g) in acetone (10 mL) was added to 0.1M potassium phosphate buffer, pH 7.8 (100 mL) containing PLE (0.700 g). The reaction mixture was stirred at room temperature for 24h, acidified (pH 2.5) by treatment with 2N HCl and extracted with ethyl acetate (3x50 mL). Chiral HPLC analysis carried out with a Chiralcel OD column, eluent ethanol/n.hexane/acetic acid 3:96.5:0.5 allowed the evaluation of both the degree of conversion and enantiomeric excess (Table I).

Retention time (t_R, min): (R)-(-)-**1** 29.0, (S)-(+)-**1** 31.1, peak resolution (R_S) 1.05; (R)-**1** methyl ester 14.8, (S)-**1** methyl ester 15.5, R_S=0.57.

With the same procedure we carried out the PLE-catalyzed hydrolyses of Iopanoic acid ethyl and butyl esters. The degrees of conversion reported in Table I were obtained at 30 and 48 h respectively.

HPLC retention times (t_R, min) of Iopanoic acid ethyl ester: (R) 13.0, (S) 14.1, R_S=1.01.

HPLC retention times (t_R, min) of Iopanoic acid butyl ester. (R) 12.0, (S) 12.8, R_S=0.77.

The same protocol was applied to the other seventeen enzymes listed in the Experimental Section. In all these cases no hydrolytic activity was observed.

Enzymatic hydrolysis of (±)-3a. A 250-mL Erlenmeyer flask was charged with (±)-**3a** (1.2 g), chymotrypsin A_4 (1.2 g) and 0.1M potassium phosphate buffer, pH 7.8 (160 mL). The mixture was stirred at room temperature for 48 h (degree of conversion: 45%), acidified to pH 4.8 with 2N HCl and extracted with ethyl acetate (4x25 mL). The organic extracts were treated with a saturated $NaHCO_3$ solution (3x15 mL); the two phases were collected separately and submitted to the following treatments: a) the alkaline aqueous solution was acidified and extracted with ethyl acetate (4x20 mL). After the usual workup, the organic extracts yielded 0.450 g of (S)-**4a**. b) The solvent of the organic layer was removed under vacuum and the residue was further hydrolyzed in the presence of chymotrypsin (0.400 g, 56% total conversion). The suspension was extracted with ethyl ether (4x15 mL). After the usual workup the residue was purified by silica gel column chromatography (eluent: cyclohexane-ethyl acetate 7:3) and Kugelrohr distillation (150-155^{0}C/0.3 mmHg) to yield (R)-(-)-**3a** (0.42 g).

(S)-**4a** (0.15 g) was transformed into (S)-(+)-**3a** by treatment with methanol saturated with gaseous HCl according to the procedure reported for (±)-**2a-d**.

The same protocol was applied to the hydrolyses catalyzed by the other enzymes listed in Table II.

The degrees of conversion were evaluated with a Whatman Partisil 10 column (eluent: acetonitrile/0.1% aqueous trifluoroacetic acid 1:3). Enantiomeric excesses were evaluated with a Chiralcel OD column (eluent: n.hexane/isopropanol/diethylamine 75:25:0.1) on aminoesters (+)-**3a** and (-)-**3a**.

(R)-(-)-**3a**: $[\alpha]_D^{20}$-38.10 (c0.908, $CHCl_3$); e.e. >99%; t_R(min) 24.4.

(S)-(+)-**3a**: $[\alpha]_D^{20}$+34.78 (c0.854, $CHCl_3$); e.e. 94%; t_R(min) 28.2, R_S=1.76.

Enzymatic hydrolyses of (±)-2a. A 250-mL Erlenmeyer flask was charged with (±)-**2a** (2.3 g), Lipase PS (7.0 g) and 0.1M potassium phosphate (170 mL). The reaction mixture was stirred at room temperature for four days (degree of conversion: 44%), acidified to pH 4.0 and extracted with dichloromethane (4x25 mL). The organic extracts were concentrated under vacuum and the residue was column chromatographed on silica gel (eluent: ligroin/ethyl acetate 1:1) to yield 0.81 g of (R)-(+)-**5a** as the second fraction.

The hydrolysis of (±)-**2a** (1.7 g) catalyzed by chymotrypsin (1.7 g) yielded, after four days (42% conversion), 0.66 g of (S)-(-)-**5a**. The e.e. of (S)-**5a** and (R)-**5a** was determined after their transformation into aminoesters (S)-**3a** and (R)-**3a** respectively.

(R)-(-)-**5a**: b.p. 195-200°C/0.3 mmHg; $[\alpha]_D^{25}$-28.30 (c1.00, $CHCl_3$).

(S)-(+)-**5a**: $[\alpha]_D^{25}$+29.93 (c0.742, $CHCl_3$).

The same procedure was applied to the Lipase PS-catalyzed hydrolysis of (±)-**2b** to yield, after six days

(30% conversion), (S)-2-benzylbutyric acid (7% e.e.). $[\alpha]_D^{25}$+2.9 (c4.95, C_6H_6), lit.[10] $[\alpha]_D^{25}$+34.7(c8.5, C_6H_6).

The Lipase PS-catalyzed hydrolysis of (±)-**2c** was carried out with a 1:1 w/w substrate-enzyme ratio. After 2h, 40% of the substrate was hydrolyzed to (S)-2-benzylpropionic acid (96% e.e.). $[\alpha]_D^{25}$+25.74 (c1.006, $CHCl_3$), lit.[9] $[\alpha]_D^{25}$+25.6 (c1, $CHCl_3$).

Enzymatic hydrolysis of (±)-2d. The hydrolysis of (±)-**2d** (2.5 g), catalyzed by Lipase PS (2.5 g) was carried out according to the protocol reported for (±)-**2a**. After 6 h (44% conversion), ester (R)-(-)-**2d** (1.2 g) was extracted with ethyl acetate (3x20 mL) from the alkaline solution. The aqueous phase was acidified to pH 3 and extracted with ethyl acetate (3x20 mL) to yield 0.9 g of (S)-(+)-**5d**. The e.e. of (S)-(+)-**5d** was determined by chiral HPLC (eluent: n-hexane/isopropanol/diethylamine 75:25:0.1) after its conversion to the corresponding aminoester (S)-(+)-**3d**.

(S)-(+)-**3d**: e.e. 85%, t_R 25.6; (R)-(-)-**3d**: t_R 22.9; R_S=1.18.

(S)-(+)-**5d** crystallized from ligroin/isopropyl ether as yellow prisms, mp 84-84.5°C; $[\alpha]_D^{25}$+25.46 (c0.978, $CHCl_3$).

Synthesis of (-)-1. A. 0.3 g of (R)-(-)-**3a** was suspended in 50 mL 0.1M potassium phosphate buffer (pH 7.8), horse liver esterase (0.3 g) was added, and the mixture was stirred at room temperature until total disappearance of the substrate (24 h). The mixture was acidified to pH 4.8 and acid (R)-(-)-**4a** was extracted with ethyl acetate (3x15 mL). The acid was not characterized but directly submitted to the following reaction.

B. To a solution of (R)-(-)-**4a** (0.2 g) in HCl (30 mL, 6%) stirred and heated at 70°C, was added dropwise 50 mL iodine monochloride in 6% HCl. At the end of the addition, heating was continued for 1h then cooled to room temperature, treated with a solution of sodium bisulfite (20 mL, 20%) and extracted with dichloromethane (3x20 mL). After the usual workup (R)-(-)-**1** (0.37 g) was crystallized from chloroform, mp 165.5°C.

The same procedure applied to (S)-(+)-**3a** yielded (S)-(+)-**1** in 65% yield.

(S)-(+)-**1**: $[\alpha]_D^{20}$+5.9 (c0.397, EtOH) [lit.[11] $[\alpha]_D^{20}$+6.2 (c2, EtOH)].

(R)-(-)-**1**: $[\alpha]_D^{20}$-6.2 (c0.625, EtOH).

Chemical correlation of (R)-5a and (S)-5d with (R)-2b and (S)-2c. The configuration of (R)-**5a** and (S)-**5d** (Scheme III) were determined by conversion of (R)-(-)-**3a** and (S)-(+)-**3d** to the corresponding enantiomers (R)-**2b** and (S)-**2c**.[9]

Deamination was performed as follows. Compound (R)-**3a** (0.6 g, 3.0 mmol) was dissolved in a mixture of 12 mL of conc. H_2SO_4 in 60 mL of water. The solution was cooled to 0°C and 0.36 g (5.1 mmol) of sodium nitrite in 12 mL of water was added. After 20 min, 6 g (45.6 mmol) of 50% aqueous H_3PO_2 was added to the reaction mixture, which was stirred at 0°C for 2 days. The mixture was extracted with dichloromethane (3x15 mL), and the extracts were dried over Na_2SO_4 and evaporated to yield 0.256 g of (R)-**2b**.

The same protocol was applied to 0.531 g of (S)-(+)-**3d** to give (S)-(+)-**2c** in 47% yield; $[\alpha]_D^{25}$+29.6 (c0.786, CHCl$_3$), lit.[9] $[\alpha]_D^{25}$+35.9 (c1, CHCl$_3$).

Synthesis of (R)-(-)-3a and (S)-(+)-3d. According to the procedure used in the synthesis of (±)-**2a**, acid (S)-(+)-**5a** (0.5 g) was transformed into its methyl ester followed by hydrogenation with 5% Pd/C. (R)-(-)-**3a** was produced in 87% overall yield.

The same methodology applied to (S)-(+)-**5d** yielded (S)-(+)-**3d** (85%).

Acknowledgments

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