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2-Methylalkanoic Acids Resolved by Esterification Catalysed by Lipase from *Candida rugosa*: Alcohol Chain Length and Enantioselectivity

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Abstract: Enantiomerically pure (*R*)-2-methyldecanoic acid and (*S*)-2-methyl-1-decanol were prepared in a multi gram scale by esterification reactions catalysed by lipase from *Candida rugosa*. The enantiomeric ratios (*E*-values) were determined as a function of the chain length of the alcohol used as the complementary substrate in cyclohexane. In the resolution of 2-methyldecanoic acid the highest value ($E = 37 \pm 5$) was obtained, when either 1-hexanol, 1-heptanol or 1-octanol were used. In contrast, when resolving 2-methyloctanoic acid, the *E*-values increased continually with increasing chain length of the alcohol used. 1-Hexadecanol gave the highest value: $E > 100$. The *E*-values were determined from the enantiomeric excess (*ee*) of the product at a conversion below 0.4. After two consecutive esterification reactions enantiomerically pure (*R*)-2-methyldecanoic acid, > 99.8% *ee*, and after subsequent reduction of the ester produced, (*S*)-2-methyl-1-decanol, 96.7% *ee*, were obtained.

INTRODUCTION

2-Methylalkanoic acids and their derivatives of high enantiomeric purities have served as valuable synthetic intermediates in the preparation of, among other compounds, a number of stereochemically pure insect pheromones.[1] Different approaches to these intermediates have been studied for example *via* asymmetric syntheses[2] or *via* bioorganic methods.[2a,3-9]

In the search for more efficient methods yielding enantiomerically pure 2-methylalkanoic acids, we are investigating the potential of lipase catalysed resolutions. We have previously reported the enantioselective *Candida rugosa* lipase (CrL)# catalysed esterification of 2-methyldecanoic acid.[3] Simultaneously others have reported resolutions of shorter 2–methylalkanoic acids using the same lipase.[4,5]

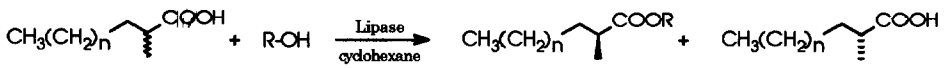
In this paper we report the influence of the alcohol chain length on the enantiomeric ratio (*E*-value##) in the resolutions of 2-methyldecanoic acid (**2**) and 2-methyloctanoic acid (**1**). The best conditions were used in a multi gram preparation of (*R*)-2-methyldecanoic acid, *ee* > 99.8%, and (*S*)-2-methyl-1-decanol, *ee* = 96.7%.

RESULTS AND DISCUSSION

The reactions studied are outlined in scheme 1. The results of the esterification reactions are shown in Table 1 and Fig. 1. Given a defined chain length of the alcohol, higher *E*-values

Formerly known as *Candida cylindracea* lipase (CCL).
See experimental section: *Determination of enantiomeric ratios.*

were obtained in all esterifications of 2-methyloctanoic acid (**1**), as compared with those of 2-methyldecanoic acid (**2**).



1 (n=4) **S-1a-f** (n=4) **R-1** (n=4)
2 (n=6) **S-2a-j** (n=6) **R-2** (n=6)

Scheme 1. Kinetic resolution through esterification catalysed by CrL.

Table 1. CrL-catalysed esterification of racemic 2-methylalkanoic acids with different alcohols, R—OH.

Entry	Acid[a]	Alcohol, R =	$ee_{product}$ (%)	c[b]	Time (h)	E[c,d]	Ester
1	1	n-C_4H_9	85.3	0.344	22	20±2	**S-1a**
2	1	n-C_7H_{15}	94.1	0.370	22	57±7	**S-1b**
3	1	n-$C_{10}H_{21}$	95.2	0.400	22	79±11	**S-1c**
4	1	n-$C_{12}H_{25}$	95.8	0.372	15	83±11	**S-1d**
5	1	n-$C_{14}H_{29}$	96.5	0.306	4.5	85±11	**S-1e**
6	1	n-$C_{16}H_{33}$	96.7	0.420	4.5	126±19	**S-1f**
7	2	n-C_2H_5	45.0	0.258	50	3±0.4	**S-2a**
8	2	n-C_3H_7	66.8	0.235	24	6±1	**S-2b**
9	2	n-C_4H_9	85.1	0.180	10	15±3	**S-2c**
10	2	n-C_5H_{11}	87.3	0.390	20	26±3	**S-2d**
11	2	n-C_6H_{13}	92.2	0.305	14	37±5	**S-2e**
12	2	n-C_7H_{15}	92.3	0.317	14	37±5	**S-2f**
13	2	n-C_8H_{17}	92.5	0.240	12	34±5	**S-2g**
14	2	n-C_9H_{19}	88.7	0.260	12	23±3	**S-2h**
15	2	n-$C_{10}H_{21}$	75.9	0.300	8	10±1	**S-2i**
16	2	$C_5H_{11}(CH_3)CH$	90.9	0.256	272	29±4	**S-2j**

a) The esterifications were carried out in a 0.15 M acid and 0.9 M alcohol solution in cyclohexane (**1**: 25 ml, containing 67.5 mg/ml CrL, **2**: 10 ml, containing 135 mg/ml CrL. b) Degree of conversion, determined by GC c) E was calculated from eqn. 1 (see experimental section) with ee_p and c given in the table, *i.e.* end point analysis according to Van Tol *et al.* (ref. 17d). d) The variance in E was calculated for a maximal experimental error in c of ±0.02 and in $ee_{product}$ ±0.2% (ref. 17d).

The best alcohol for the resolution of 2-methyloctanoic acid (**1**) was hexadecanol, giving $E > 100$. In the esterifications of **1** the E-values increased continually with increasing chain length of the alcohol. In contrast a maximum E was found for the resolution of 2-methyldecanoic acid (**2**). Thus, either 1-hexanol, 1-heptanol or 1-octanol gave enantiomeric ratios of $E = 37\pm5$ (Fig. 2). Esterification of **2** with 2-heptanol, (Table 1, entry 16) markedly reduced the reaction rate but showed the same enantioselectivity as compared with the esterification performed with 1-heptanol.

Enzymatic hydrolysis of racemic 2-methyldecanoic hexyl and heptyl esters **2e** and **2f** resulted in enantiomeric ratios of $E = 33$ and $E = 35$, respectively (Table 2). Within experimental errors these were the same as the enantiomeric ratios obtained in the corresponding esterifica-

tion reactions. In contrast, the enzymatic hydrolysis of racemic 2-methyldecanoic octyl ester, **2g** (performed under identical conditions and with the same batch of lipase as for **2e** and **2f**), showed a lower enantiomeric ratio, $E = 9$,[3] when compared with the corresponding esterification reaction, $E = 34$.

Table 2. CrL-catalysed hydrolysis of racemic 2-methyldecanoic acid esters, $ROOCCH(CH_3)(CH_2)_7CH_3$

Entry	Ester	R =	$ee_{product}$ (%)	c^b	E^c	Acid
1	**2e**	n-C_6H_{13}	90.5	0.353	33	**S-2**
2	**2f**	n-C_7H_{15}	91.2	0.344	35	**S-2**
3[a]	**2g**	n-C_8H_{17}	80	0.30	9	**S-2**

a) From Holmberg *et al* 1991 (ref. 3) b) Degree of conversion, determined from the amount consumed NaOH by titration c) E was calculated from eqn 1 (see experimental section) with ee_p and c given in the table, *i.e.* end point analysis according to Van Tol *et al.* (ref. 17d).

The lower enantioselectivity obtained in the hydrolysis of **2g**, might be due to the accumulation of the product, 1-octanol, in the substrate. As the enzyme is adsorbed to the substrate interface, the produced 1-octanol will compete with water to cleave the intermediate acyl enzyme formed during catalysis. As 1-octanol is a better nucleophile than water in this situation, the ester is formed. This results in a decreased apparent enantiomeric ratio. The shorter, more water soluble 1-hexanol or 1-heptanol should not be accumulated to the same degree in the substrate interface, thus similar E-values are obtained in hydrolysis and esterification.

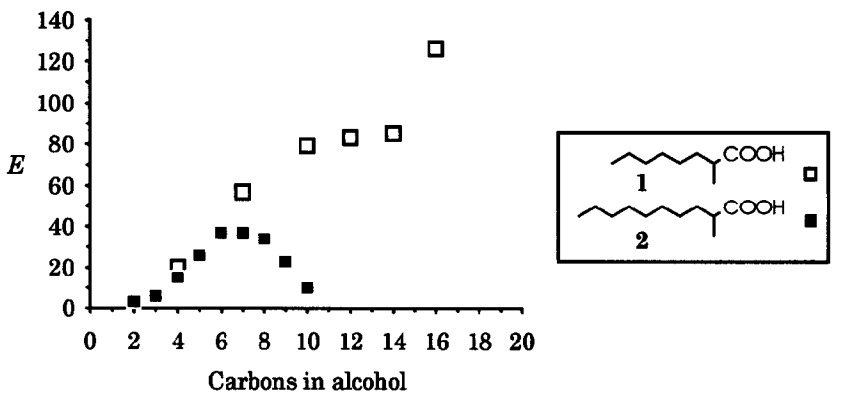
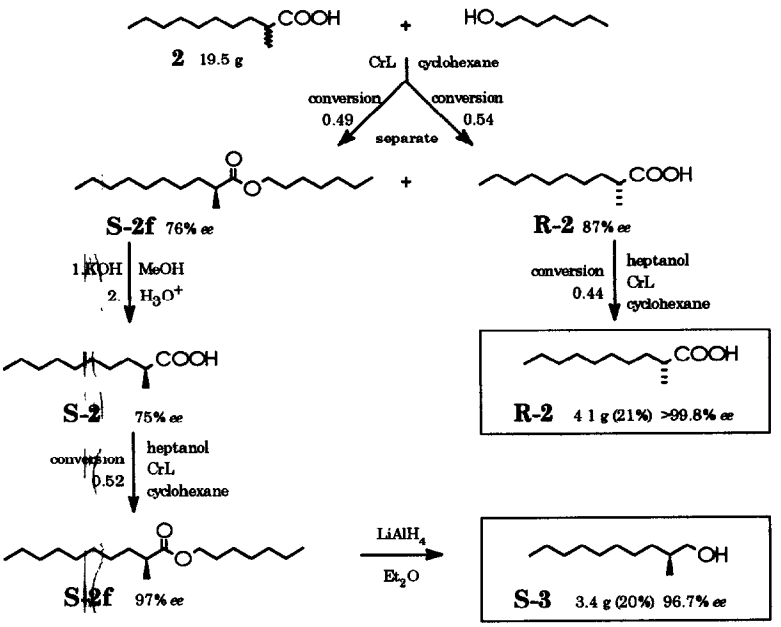


Fig. 1. E in esterification of 2-methyloctanoic acid (1) and 2-methyldecanoic acid (2) as a function of alcohol chain length.

A multi gram scale esterification of 2-methyldecanoic acid with 1-heptanol yielded the ester **S-2f** (76.6% *ee*) at a conversion of 0.54. Enzymatic hydrolysis of this ester sample yielded **S-2** (95.7% *ee*) at a conversion of 0.60. The enantiomeric purity of the acid produced was lower than expected from the E-value determined from the analytical scale hydrolysis (Table 2). Presently we have no explanation for this result. The isolated yields using enzymatic hydrolyses were low due to experimental difficulties in the workup of **S-2**, which probably acts as a surfactant in the water mixture containing the enzyme. In another experiment **S-2f** (74.3% *ee*) was hydrolysed to the conversion of 0.2 providing acid **S-2** (> 99.6% *ee*).

Enzymatic resolutions by esterification in an organic solvent give reaction mixtures that are easier to work up compared with the reaction mixtures obtained after hydrolytic resolutions

performed in water. Therefore, enzymatic esterification of an enantiomerically enriched acid should be a more attractive procedure than enzymatic hydrolysis of the corresponding enriched ester. Acids could easily be obtained from such esters by chemical hydrolysis [a) 2 M KOH/MeOH, 27 h; b) H_3O^+].[9] However, in several test runs, a decrease in *ee* of 0.1-2.0% was observed. Chemical hydrolysis is therefore not suited for analytical *ee* determinations or for the final step in the preparation of nonracemic 2-methyl-1-alkyl derivatives.



Scheme 2. Preparative runs: (R)-2-methyldecanoic acid (R-2) and (S)-2-methyl-1-decanol (S-3).

The reaction sequence for practical multi gram resolutions of 2-methyldecanoic acid is outlined in scheme 2.

The remaining substrate obtained from the multi gram esterification above, acid **R-2** (87.3% *ee*) was reesterified to a conversion of 0.44 yielding the enantiomerically pure **R-2** (*ee* > 99.8%) as the remaining substrate.

In another experiment the enzymatic esterification reaction was stopped at a conversion of 0.49, producing **S-2f** (75.5% *ee*) along with the remaining substrate **R-2** (75.0% *ee*). The ester **S-2f** was chemically hydrolysed giving **S-2** with a loss of 0.1 - 0.5% *ee*. This acid was subsequently enzymatically esterified to a conversion of 0.52. As explained above the final step for obtaining nonracemic 2-methyl-1-alkyl derivatives must be a reaction proceeding without racemization, such as reduction of the produced ester to the corresponding 2-methyl-1-alkanol. $LiAlH_4$-reduction of the ester **S-2f** from the second esterification gave (*S*)-2-methyl-1-decanol (**S-3**, 96.7% *ee*).

The yields of the enantiomerically pure methylalkyl derivatives are moderate in this two step sequence. However, the remaining enantiomerically enriched ester and acid fractions, also obtained, can of course be used as starting materials for further resolution steps which would raise the overall yields.

The *ee* of the esters or acids were determined by conversion in several steps without epimerisation (see experimental section) to diastereomeric mixtures of *N*-(1-phenylethyl)-amides from either enantiomerically pure (*R*)- or (*S*)-1-phenylethylamine followed by GC-analysis.

The reasons for the higher *E*-values observed in the resolution of 2-methyloctanoic acid as compared with the resolution of 2-methyldecanoic acid are not clear, but could arise from steric limitations in the active site of the enzyme. It has been reported that the commercial lipase preparation used in this investigation contains isoenzymes[10] showing different enantioselectivities. Different isoenzymes may be resolving the different acids, thus explaining the difference in alcohol chain length dependence in the resolution of the two acids. It may be noted that the experimental difficulties to extract the acid from a water solution are much greater with the longer than with the shorter acid. This indicates that the longer acid is a more powerful surfactant than the shorter one, a property which might affect enzyme-substrate interactions and the enantioselectivity.

To obtain a synthetically useful resolution procedure it is important to achieve a high equilibrium conversion. This ensures the highest possible *ee* of both substrate and product. Several strategies to obtain a high equilibrium conversion are available *e.g.* removal of one of the products[11] or using an excess of one of the substrates. Here we used the latter approach (6 molar excess of the alcohol). The equilibrium conversion in the esterification of 2-methyldecanoic acid with 1-heptanol was determined to be > 0.97 after four months of incubation. This equilibrium conversion was in good agreement with the value of 0.98 obtained after fitting the *ee* of the substrate at six different conversions by the computer software SIMFIT.[12] This equilibrium conversion is satisfactory for practical synthetic procedures.

EXPERIMENTAL SECTION

Reactions sensitive to moisture or oxygen were carried out under argon. ^{1}H NMR spectra were recorded as $CDCl_3$-solutions with TMS as reference on a Jeol PMX60SI NMR spectrometer or a Jeol EX270 NMR spectrometer which was also used for ^{13}C NMR spectra. Optical rotations were measured using a Perkin-Elmer polarimeter 241. Silica gel 60, 230-400 mesh, was used for MPLC using cyclohexane with a gradient of ethyl acetate as eluent. Boiling points are uncorrected and given as air bath temperatures in a bulb to bulb (Kugelrohr) apparatus. The chemicals used as starting materials are commercially available and were used without further purification unless otherwise stated.

Enzyme. Commercial extracellular lipase (EC 3.1.1.3) from *Candida rugosa* was obtained from Sigma (St. Louis, Mo., USA). The specific activity was 900 units / mg solid and 4865 units/mg protein. One unit will hydrolyse 1.0 mmol of fatty acid from a triglyceride in 1 h at pH 7.2 and 37° using olive oil (incubation time: 30 min.).

Esterification reactions. A cyclohexane solution (1 vol.) containing a *rac*-2-methylalkanoic acid (0.15 M), 1-alkanol (0.9 M), internal standard (octadecane or eicosane, 6.1 mg / ml) and mixed with *Candida rugosa* lipase [67.5 (acid **1**) or 135 mg / ml (acid **2**)] was stirred with a magnetic stirring bar in a sealed flask at room temperature. The esterification reactions were followed by GC and stopped at the appropriate stages of conversion by separating the enzyme by filtration and washing with cyclohexane (0.5 vol.) and pentane (0.5 vol.). The combined organic phase (2 vol.) was diluted with pentane (8 vol.), extracted with aqueous sodium carbonate (10%, 5 × 0.2 vol.) and sat. sodium chloride solution (0.2 vol.). The organic phase containing the (*S*)-ester, the alcohol and the internal standard was dried ($MgSO_4$). The solvent was evaporated off and the residue was used for further workup (described for **S-2f** below) or used directly for *ee*-determination. The combined water phase containing the carboxylate salt and some alcohol

was acidified to pH 1 with 6 M HCl and extracted with diethyl ether (5 × 1 vol.). The combined ether phase was washed with sat. NaCl (0.2 vol.), dried ($MgSO_4$) and evaporated to dryness yielding the (*R*)-acid contaminated with the complementary substrate, the 1-alkanol.

Determination of conversion. The conversions in the esterification reactions were determined using a Schimadzu GC-7AG gas chromatograph equipped with a 1.6 m x 2.5 mm I.D. packed glass column with 10% FFAP on Chromosorb® W, AW / DMCS, 100 / 120 mesh; carrier gas N_2 (30 ml/min). The conversions were calculated from the areas of the ester peaks relative to the peaks from an internal standard (octadecane or eicosane) which was calibrated against the racemic esters described below.

Enzymatic hydrolysis. A water solution of the enzyme [72 - 132 mg/ml (insoluble material was removed by centrifugation)], gum arabic (5%) and $CaCl_2$ (0.2 M) was prepared and the pH adjusted to 8.0. The ester (100 mmol/l of enzyme solution) was added. The resulting mixture was emulsified by sonication. The reactions were run at room temperature. The pH was maintained automatically with NaOH [1.0 M (0.1 M analytical scale)] using a Radiometer pH-stat equipped with an ABU91 autoburette (1 ml) connected to a VIT90 videotitrator. The conversion was determined by the amount of consumed NaOH. The reactions were stopped by addition of HCl (6 M) until pH 1.0 was reached. The 2-methyldecanoic acid produced was extracted from the water solution with hexane or diethyl ether and the *ee* was determined as described below.

Determination of enantiomeric excess. The *ee*:s of the esters were determined as follows: Alcohols with longer chain than n-pentanol present in the crude esters from above were first evaporated off on a bulb to bulb apparatus 30-60° / 0.08 mmHg for 1 h. The residual ester (0.2 mmol) was added to a solution of $LiAlH_4$ (20 mg) in anhydrous diethyl ether (≈ 1 ml) under argon. The mixture was stirred at 20° for 1 h, quenched with water (20 µl), 15% NaOH (20 µl) and water (40 µl). After refluxing for 1 h the mixture was filtered and dried ($MgSO_4$). This reduction proceeds without racemization.[13] The crude (*S*)-2-methyl-1-alkanol (≈ 10 µl, ≈ 0.05 mmol) remaining after solvent evaporation was then oxidised to the acid in acetone (p.a. 0.5 ml) at 5° in a water bath by the addition of an excess of Jones' reagent (≈ 50 µl, 2.67 M H_2CrO_3 in H_2SO_4 / H_2O).[14] The resulting mixture was filtered after a few minutes through celite, which was washed with diethyl ether and pentane. The acid was then extracted into aqueous sodium carbonate solution (10%, 2 x 1 ml). The combined carbonate extracts were acidified with 6 M HCl and extracted with diethyl ether (2 x 1 ml). The combined ether phase was dried ($MgSO_4$) and concentrated. Oxidation with Jones' reagent at 5° gives no racemization.[15] The crude acid (≈ 10 µl, < 0.05 mmol) was dissolved in anhydrous diethyl ether (1 ml). Dry distilled dimethylformamide (10 µl) and distilled thionyl chloride (10 µl, 0.14 mmol) under argon were added and after a few minutes enantiomerically pure (*R*)- or (*S*)-1-phenylethylamine (20 µl, 0.158 mmol) [these amines (100% *ee*) were obtained after 3 recrystallizations of (*R*)- and (*S*)-1-phenylethyl amine samples (> 96% *ee*) with the appropriate enantiomer of tartaric acid (*cf.* ref. 16)]. (*S*)–Phenylethylamine was used for (*R*)-acids and (*R*)–phenylethylamine for (*S*)-acids. The HCl(g) produced was flushed away with argon. The resulting mixture was diluted with diethyl ether to approximately 0.5 ml and washed with water (1 ml), aqueous sodium carbonate solution (10%, 2 x 1 ml) and sat. sodium chloride solution (1 ml). No racemization should occur during this sequence.[15a] This was confirmed by reduction, reoxidation and amide formation starting with methyldecanoic acid of known enantiomeric purity (96.8% *ee*) followed by GC-analysis which gave the same *ee*-value within the experimental error (± 0.2% *ee*). The diastereomeric ratios were determined using a Varian 3300 gas chromatograph equipped with a 30 m x 0.32 mm I.D. capillary column coated with cross-linked Carbowax® 20 M, df = 0.25 µm; carrier gas He 15 psi, split 1 : 20.

Determination of enantiomeric ratios. The enantiomeric ratios were determined from the conversion and the *ee* of the product esters either by curve fitting using SIMFIT[12] or by using the formula below (eqn 1). The enantiomeric ratio is a kinetic constant which describes the enantioselectivity of an enzyme. It is defined as the ratio of the specificity constants, k_{cat}/K_M (k_{cat} and K_M are the catalytic and Michaelis constants, respectively), for the two enantiomers (eqn. 2,[17] where A and B refer to the fast and slow reacting enantiomer, respectively; v_A and v_B denote reaction rates for the two enantiomers).

$$E = \frac{\ln[1 - c(1 + ee_p)]}{\ln[1 - c(1 - ee_p)]} \quad (1) \qquad \frac{v_A}{v_B} = \frac{(k_{cat}/K_M)_A}{(k_{cat}/K_M)_B} \cdot \frac{[A]}{[B]} = E \cdot \frac{[A]}{[B]} \quad (2) \qquad E = \frac{\ln[1 - (1 + K)c(1 + ee_p)]}{\ln[1 - (1 + K)c(1 - ee_p)]} \quad (3)$$

If the enantiomeric excess of the product, ee_p, is plotted as a function of conversion, c, for a reversible enzyme catalysed reaction [eqn. 3, where $K = (1\text{-}c_{eq})/c_{eq}$][18] it is evident that the *ee* of the product at low conversions is virtually independent[17b] of the equilibrium conversion, c_{eq}, for $c_{eq} > 0.95$.[12] Equilibrium conversion is defined as the degree of conversion of substrate when chemical equilibrium is reached. Thus provided that the *ee*:s of the products are determined at low conversions, preferably below 0.40, the *E*-values can be calculated using equation 1, which was originally derived for irreversible reactions.[17b-c,19,20]

rac-*2-Methyloctanoic acid (1).* Prepared as described for 2-methyldecanoic acid (2) but from 1-bromohexane. Distillation [b.p. 89-91° / 1 mmHg (lit.[21] b.p. 93-95° / 0.7 mmHg)] gave 35.98 g (> 99.5% purity by GC). n_D^{20} 1.4295. IR: 2954, 2925, 2857, 1707 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 6.4 Hz), 1.17 (3H, d, J = 6.9 Hz), 1.3 (8H, broad), 1.3-1.6 (1H, m), 1.6–1.9 (1H, m), 2.3-2.7 (1H, m), 11.9 (1H, s) ppm.

rac-*2-Methyldecanoic acid (2).* A modification of the method described for 2-methyldodecanoic acid[22] was used: Sodium (14.2 g, 620 mmol) was added gradually in small pieces to 400 ml of absolute ethanol under argon. Diethyl methylmalonate (112 ml, 655 mmol) was added over a period of 0.3 h. Then the mixture was refluxed for five minutes and 1-bromoctane (113 g, 585 mmol) was added during a period of 0.25 h. After refluxing for 2 h and neutralisation with four drops of glacial acetic acid, about 300 ml ethanol was removed by distillation. Water (500 ml) was then added, the organic phase separated and the aqueous phase extracted with diethyl ether (3 x 80 ml). The combined organic phases were washed with sat. sodium chloride solution (100 ml), dried ($MgSO_4$) and concentrated to an oil, which was hydrolysed with a solution of potassium hydroxide (131 g, 2.0 mol) in 1.0 l of 95% ethanol during reflux for 4 h. Ethanol (≈700 ml) was removed by distillation and water (800 ml) was added. The mixture was then washed with diethyl ether (2 x 200 ml). The water phase was acidified to pH 1 at 0° with 6 M HCl. The organic phase was separated and the aqueous phase was extracted with diethyl ether (3 x 200 ml). The combined organic phase and ether extracts were washed with water (50 ml), sat. sodium chloride solution (50 ml), dried ($MgSO_4$) and evaporated to dryness. The residual solid (m.p. 84-89°) was recrystallized from n-heptane (100 ml) giving 101.3 g of the diacid in 75% yield (m.p. 88-91°). The diacid (96 g, 417 mmol) was heated to 190° for 2 h to effect decarboxylation. The crude liquid was dissolved in aqueous sodium carbonate solution (10%, 600 ml) and washed with diethyl ether (200 ml). The carbonate phase was acidified (pH 1-2) with 6 M HCl and the organic phase separated followed by extraction of the water phase with diethyl ether (3 x 100 ml). The combined organic phases were washed with sat. sodium chloride solution (100 ml), dried ($MgSO_4$), evaporated and distilled (b.p. 91-94°/0.01 mmHg) to give the title acid 2 (70.4 g, 99.8% purity by GC) in 68% total yield. n_D^{20} 1.4369 [lit.[1c] n_D^{20} 1.4371 {(*S*)-acid}, 1.4356 {(*R*)-acid}]. IR: 2953, 2923, 2854, 1706 cm^{-1}. The ^1H NMR-spectrum (60 MHz) was identical with that for (*S*)-2-methyldecanoic acid described in the literature.[1c]

^{13}C NMR (67.8 MHz, $CDCl_3$): δ 14.07, 16.79, 22.64, 27.12, 29.24, 29.42, 29.49, 31.84, 33.53, 39.37, 183.49 ppm.

rac-*Ethyl 2-methyldecanoate* (**2a**). Prepared as described by Holmberg *et al.*[3] Distillation (b.p. 55° / 0.09 mmHg) gave 1.52 g of **2a** in 75% yield (> 99.5% purity by GC). n_D^{20} 1.4256. IR: 1737 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 0.60-1.80 (23H, m), 2.15-2.60 (1H, m), 4.17 (2H, q, J = 7.0 Hz) ppm.

rac-*1-Butyl 2-methyldecanoate* (**2c**). This was prepared from **2** (1.75 g, 9.4 mmol) in the same manner as described for **2a**. Distillation (b.p. 75° / 0.08 mmHg) gave 1.91 g of **2c** in 84% yield (> 99.7% purity by GC). n_D^{20} 1.4307. IR: 1738 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 0.60-1.80 (27H, m), 2.15-2.60 (1H, m), 4.13 (2H, t, J = 6.5 Hz) ppm.

rac-*1-Hexyl 2-methyldecanoate* (**2e**). A solution of 1-hexanol (1.3 ml, 10.4 mmol) and pyridine (1.0 ml, 12.4 mmol, dest. from CaH_2) in 10 ml of anhydrous diethyl ether was heated to reflux. 2-Methyldecanoyl chloride (2.44 g, 11.9 mmol), prepared from **2** and distilled thionyl chloride, was added dropwise at such a rate that moderate refluxing was maintained (0.5 h) after the source of heat was removed. The mixture was then heated to reflux for 1 hour, cooled to room temperature and shaken with water (10 ml). The ether phase was washed with cold sulphuric acid (2 M, 2 x 2 ml), sat. bicarbonate solution (2 x 2 ml) and sat. sodium chloride solution. The ether phase furnished, after drying ($MgSO_4$) and concentration, an oil which was distilled (b.p. 101-104° / 0.15 mmHg) to give 1.87 g (66% yield, > 99% purity by GC). n_D^{20} 1.4346. IR: 1734 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 0.6-2.0 (31H, m), 2.1-2.6 (1H, m), 4.12 (2H, t, J = 6.0 Hz) ppm.

rac-*1-Heptyl 2-methyldecanoate* (**2f**). Prepared from 1-heptanol (1.47 ml, 10.4 mmol) and 2-methyldecanoyl chloride (1.95 g, 9.6 mmol) according to the method described above for **2e**. Distillation (b.p. 114° / 0.04 mmHg) gave 1.86 g ester in 68% yield (> 99.8% purity by GC). n_D^{20} 1.4368. IR: 1735 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 6.7 Hz), 0.89 (3H, t, J = 7.0 Hz), 1.13 (3H, d, J = 7.0 Hz), 1.20-1.45 (20H, m), 1.55-1.70 (4H, m), 2.41 (1H, apparent sextet), 4.06 (2H, t, J = 6.6 Hz) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 14.05, 14.10, 17.14, 22.60, 22.68, 25.93, 27.28, 28.72, 28.93, 29.29, 29.49, 29.56, 31.77, 31.90, 33.91, 39.68, 64.29, 177.05 ppm.

rac-*1-Octyl 2-methyldecanoate* (**2g**). Prepared using the method described above for **2e**. Distillation (b.p. 117-118° / 0.22 mmHg) furnished **2g** in 83% yield (> 99% purity by GC). n_D^{20} 1.4387 (lit.[23] n_D^{20} 1.4386). IR: 1735 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 0.6-1.9 (35H, m), 2.1–2.6 (1H, m), 4.07 (2H, t, J = 6.0 Hz) ppm.

(R)-*2-Methyldecanoic acid* (**R-2**). *rac*-**2** (19.5 g, 105 mmol) was esterified with 1-heptanol in 700 ml of cyclohexane with a lipase concentration of 67.5 mg / ml. The reaction was stopped at a conversion of 0.54 after 332 h of incubation. The conversion calculated from ee_p and ee_s as described[24] was 0.533. The remaining acid was isolated using the general esterification procedure described above. Concentration of the ether extract gave 12.7 g of **R-2** containing 38% heptanol. The heptanol was evaporated off (27° / 0.08 mmHg). Distillation of the residue (b.p. 91-97° / 0.04 mmHg) gave 8.89 g of **R-2** (> 97.6% purity by GC, 46% yield, 87.3% *ee*). A second lipase catalysed esterification of this acid using 135 mg / ml CrL was stopped at a conversion of 0.44. Usual recovery and distillation of the remaining acid (b.p. 116–119° / 0.90 mmHg) gave 4.08 g of **R-2** (> 99% purity by GC, > 99.8% *ee*) in 21% total yield, counted on *rac*-**2**. $[\alpha]_D^{20}$ –15.79 (neat) (lit.[1c] $[\alpha]_D^{20}$ -15.60). n_D^{20} 1.4365 (lit.[1c] n_D^{20} 1.4356).

(S)-*1-heptyl 2-methyldecanoate* (**S-2f**). CrL catalysed esterification of *rac*-**2** (19.5 g, 105 mmol) with 1-heptanol was stopped at a conversion of 0.54. The mixture of neutral compo-

nents were isolated from the product mixture as described above, to give 81 g of an oil containing mostly heptanol. This was evaporated off at 72°/3 mmHg and the residue (20.8 g) was purified by MPLC to give **S-2f** (15.5 g, 51% yield counted on *rac*-**2**, > 98.7% purity by GC and 76.6% *ee*). $[\alpha]_D^{20}$ +7.80 (*c* 1.29, $CHCl_3$). The specific rotation of enantiomerically pure **S-2f** should then be $[\alpha]_D^{20}$ +10.2. n_D^{20} 1.4364.

(S)-2-methyldecanoic acid (S-2). Enzymatic hydrolysis. (*S*)-1-heptyl 2-methyldecanoate (**S-2f**, 15.27 g, 76.6% *ee*) was enzymatically hydrolysed as described above. The reaction was stopped at a conversion of 0.60 after 8 h. The mixture was diluted with water (500 ml) and extracted with diethyl ether in a continuous extractor giving **S-2** (1.4 g, 7% counted on *rac*-**2**, 95.7% *ee*). In a similar way **S-2f** (162 mg, 74.2% *ee*) was hydrolysed to a conversion of 0.20 to give **S-2** (> 99.6% *ee*).

(S)-2-methyldecanoic acid (S-2). Chemical hydrolysis. The crude ester **S-2f** (14.6 g, 51 mmol, 75.5% *ee*) containing internal standard and heptanol was dissolved in KOH (6.0 g, 93 mmol) in MeOH (60 ml). The mixture was stirred at room temperature for 37 h. The methanol was evaporated off, the mixture was acidified with hydrochloric acid and partitioned between pentane and water (100 + 100 ml). The organic phase was washed with 1 M HCl (5 x 50 ml). The water phase was washed with pentane (5 x 100 ml). The combined organic phase was diluted to 5 l and extracted with aqueous sodium carbonate solution (10%, 5 x 120 ml). The pooled carbonate solution was acidified to pH 1 (6 M HCl) and extracted with diethyl ether (5 x 200 ml). The ether phase was dried ($MgSO_4$) and concentrated to give 13.4 g of a 60/40 mixture of **S-2** (75.4% *ee*) and 1-heptanol. The yield of **S-2** was thus 42%, counted on *rac*-**2**. This mixture was used directly in an additional esterification step as described below.

(S)-2-Methyl-1-decanol (S-3). 1-Heptanol (24.9 g) was added to the crude mixture obtained after chemical hydrolysis containing 8.1 g (44 mmol) of **S-2** (75.4% *ee*). The reaction volume was 290 ml and the lipase concentration was 67.5 mg/ml. The esterification reaction was stopped at a conversion of 0.52 after 182 h of incubation. The remaining substrate, enantiomerically impure **S-2** (3.8 g, 20% yield counted on *rac*-**2**, 46.7% *ee*) was obtained after carbonate extraction - acidification. The mixture containing heptanol, internal standard and ester were concentrated and most of the alcohol was removed by distillation. The resulting mixture (12.4 g) containing ester (approx. 5.6 g) was dissolved in diethyl ether (≈ 50 ml) and reduced by slowly adding it to a solution of $LiAlH_4$ (798 mg) in anhydrous diethyl ether (100 ml) under argon. The mixture was stirred at 20° for 2 h, quenched with water (0.8 ml), 15% NaOH (0.8 ml) and water (0.8 ml). After refluxing for 1 h the mixture was filtered and dried ($MgSO_4$). Heptanol was removed by distillation (b.p. 80° / 4 mmHg) and the rest (8.02 g) was purified by MPLC and distilled [b.p. 65°/0.08 mmHg (lit.[1b] 92-94°/1 mmHg)] to give 3.4 g of **S-3** in 20% total yield, counted on *rac*-**2** (99.9% purity by GC, 96.7% *ee*). $[\alpha]_D^{20}$ -9.77 (neat) [lit.[25] $[\alpha]_D^{20}$ +10.08 (neat), pure (*R*)-enantiomer]. $[\alpha]_D^{20}$ -10.69 (*c* 4.03, CH_2Cl_2) [lit.[26] $[\alpha]_D$ +10.8 (*c* 4, CH_2Cl_2), pure (*R*)-enantiomer]. n_D^{20} 1.4399 (lit.[1b] n_D^{20} 1.4409). The ^1H NMR-spectrum (60 MHz) was identical with that described in the literature.[1b]

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