



## Sex Pheromone of Pine Sawflies: Enantioselective Lipase Catalysed Transesterification of *erythro*-3,7-Dimethylpentadecan-2-ol, Diprionol

Mårten Lundh#, Olof Smitt and Erik Hedenström\*

Department of Chemistry and Process Technology, Mid Sweden University, S-851 70 Sundsvall, Sweden

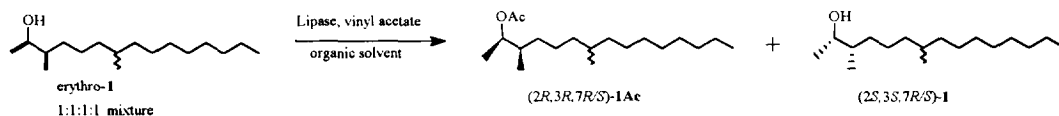
**Abstract:** (2*S*,3*S*,7*R*/*S*)-3,7-Dimethylpentadecan-2-ol (2*S*,3*S*,7*R*/*S*)-**1** was prepared with less than 0.5% of (2*R*,3*R*,7*R*/*S*)-3,7-dimethylpentadecan-2-ol via *Pseudomonas sp.* (PSL) catalysed transesterification of a 1:1:1:1 mixture of the four *erythro*-3,7-dimethylpentadecan-2-ols and vinyl acetate in *n*-heptane at initial low water activity ( $a_w < 0.1$ ). (2*S*,3*S*,7*S*)-3,7-Dimethylpentadecan-2-ol (Diprionol) is the precursor to the behaviourally active diprionyl acetate used by the female pine sawfly *Neodiprion sertifer* as a sexual pheromone. Several lipases, solvents and reaction conditions were tested, and the best results (enantiomeric ratio  $E = 110$ ) were obtained with *Pseudomonas sp.* (PSL) and vinyl acetate in *iso*-octane at initial water activity  $a_w < 0.32$ . When using *Candida rugosa* (CRL, immobilised on polypropylene) as the catalyst in an esterification reaction with *rac*-4-methyl-dodecanoic acid and eicosanol at  $a_w = 0.8$  in cyclohexane a pronounced enantioselectivity was obtained ( $E = 19$ ).  
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### INTRODUCTION

Insect pest management, monitoring and control programs utilising sex pheromones as behaviour modifying chemicals have become important for several insects groups, particularly moths.<sup>1-2</sup> Such programs frequently include mass-trapping and mating disruption techniques. We have long been interested in the syntheses and the biological activities of sex pheromones of pine sawflies,<sup>3-14</sup> which are important pests on pines in the northern hemisphere. Our program includes the control of pine sawfly populations using mass-trapping or mating disruption.<sup>12-13</sup> Application of the former technique to the pine sawfly *Neodiprion sertifer* requires access to a highly pure synthetic sex pheromone consisting of the acetate of (2*S*,3*S*,7*S*)-3,7-dimethylpentadecan-2-ol (Diprionol).<sup>7</sup> The demand on purity is less for mating disruption, which however requires large amounts of compound (> 3 mg per hectare and day).<sup>12-13</sup> For *Neodiprion sertifer*, the 2*S*,3*R*,7*R*- and 2*S*,3*R*,7*S*-diprionyl acetates have been found to function as efficient inhibitors in concentrations at > 0.1% and ~ 50% respectively.<sup>3,5,7</sup> To be able to supply the required amounts of the synthetic pheromone blends, completely free from the two inhibitory stereoisomers, we recently developed a synthetic method suitable for the preparation of a mixture of the four *erythro*-stereoisomers of the acetate of 3,7-dimethylpentadecan-2-ol (*erythro*-**1**) on a semipreparative scale.<sup>10</sup> We have now extended this approach to allow for the efficient preparation of both 2*S*,3*S*,7*R*/*S*-diprionol (2*S*,3*S*,7*R*/*S*-**1**) and 2*R*,3*R*,7*R*/*S*-diprionol (2*R*,3*R*,7*R*/*S*-**1**) and the corresponding 2*S*,3*R*- and 2*R*,3*S*-isomers. Initial attempts to develop stereochemical control at the 7-position are also described.

Enzymes as enantioselective catalysts for the preparation of enantiomerically pure compounds have emerged as one of the most promising techniques in the area during the last decade.<sup>15-17</sup> Our hypothesis in this paper was that *erythro*- or *threo*-diprionol mixtures could be efficiently separated by enzyme catalysed transesterification. Examples of successful resolutions of secondary alcohols using lipases are abundant.<sup>15-20</sup> The successful Amaro PS hydrolyses of the acetates of some racemic alkan-2-ols in the presence of organic media have been described<sup>21-22</sup> as well as many irreversible transesterifications<sup>15-17,20,23</sup> in organic media. We have now studied some lipase catalysed transesterification reactions of both the *erythro*- and *threo*-mixtures of 3,7-dimethylpentadecan-2-ol (*erythro*-**1** and *threo*-**1** respectively) in organic media.

# Deceased 29 November 1995.



**Scheme 1.** Lipase catalysed resolution of a mixture of the four *erythro*-3,7-dimethylpentadecan-2-ol into (2R,3R,7R/S)-3,7-dimethylpentadecan-2-yl acetate and (2S,3S,7R/S)-dimethylpentadecan-2-ol.

## RESULTS AND DISCUSSION

In our attempts to find a system that selectively esterifies half of our substrate several lipases were tested. The *erythro*-mixture of 3,7-dimethylpentadecan-2-ol (*erythro*-1) was transesterified with vinyl acetate in an organic solvent and the differentiation between (2S,3S,7R/S)-1- and (2R,3R,7R/S)-1-isomers was registered by the "E"-value.<sup>24-25</sup> The lipases from *Aspergillus niger* (Amano A), and *Mucor javanicus* (Amano M), *Rhizopus oryzae* (Amano F) (see Table 1, entries 1, 2 and 3 respectively) just gave trace amounts of diprionyl acetate after 3-4 months. Lipase from *Candida rugosa* (CRL, immobilised on polypropylene as described<sup>26</sup>) showed a low enantioselectivity (entry 4, "E" = 12 and also a very slow reaction rate. The ChiroCLEC™-CR dry (cross-linked crystals of *Candida rugosa* lipase) also showed a disappointingly low discrimination between the two possible stereoisomeric pairs (entry 5, "E" = 4.5) and a very low reaction rate was observed. In order to obtain a conversion of 26.6% in 165 h several additions of the enzyme had to be made. The reaction rate was quite rapid at very low conversions with both of these types of *Candida rugosa* based lipases but after ~ 0.25 h and < 8% the reaction rates were lowered and the reaction practically stopped. Crude CRL gave similar results.

**Table 1.** Enzymes and solvents (0.85 ml) used in the transesterification of a 1:1:1:1-mixture of *erythro*-3,7-dimethylpentadecan-2-ol (*erythro*-1, 0.35 mmol) with vinyl acetate (1.3 mmol) at 20 °C, *n*-hexadecane as internal standard (0.18 mmol), magnetic stirring bar at 500 r/min and at initial water activity,  $a_w = 0.32$ .

Entry	Type	Enzyme	Solvent	Conversion c (%)	Product ratio <sup>d</sup>	"E"	Reaction time (h)
1	Amano A	54.5	<i>n</i> -Heptane	3.0	-	-	3 months
2	Amano M	166.0	"	6.1	-	-	3 months
3	Amano F	21.2	"	7.7	-	-	4 months
4	CRL, immobilised <sup>26</sup>	24.2	"	9.7	91.3 : 8.7	12	1040
5 <sup>a</sup>	ChiroCLEC™-CR dry	2.6	"	26.6	78.6 : 21.4	4.5	165
6	CAL	5.6	"	39.4	96.5 : 3.5	50	44
7	PFL	4.9	"	40.3	98.1 : 1.9	100	160
8	Amano PS	7.9	"	39.1	98.2 : 1.8	100	200
9	"	7.9	CHCl <sub>3</sub>	38.4	98.2 : 1.8	100	696
10	"	8.0	<i>t</i> -BuOMe	40.2	98.1 : 1.9	100	330
11	"	9.3	Vinyl acetate	41.1	94.5 : 5.5	35	220
12	"	10.8	<i>iso</i> -Octane	42.5	98.1 : 1.9	110	123
13 <sup>b</sup>	"	7.5	<i>n</i> -Heptane	38.9	98.0 : 2.0	90	134
14 <sup>c</sup>	"	9.2	"	40.9	97.1 : 2.9	65	95

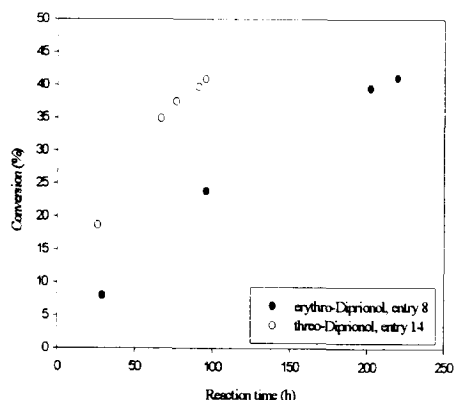
<sup>a</sup> Several portions (10 mg) of enzyme was added each time the rate slowed down. <sup>b</sup>  $a_w < 0.1$ , i.e. "dry conditions". <sup>c</sup> 1:1:1:1-Mixture of *threo*-3,7-dimethylpentadecan-2-ol (*threo*-1) as substrate. <sup>d</sup> Product ratio entries 1-13 2R3R7R/S : 2S3S7R/S, product ratio entry 14 2R3S7R/S : 2S3R7R/S.

It has been reported by others (e.g. ref. 27-28) that when using vinyl acetate as acetyl donor in transesterifications catalysed by CRL the produced acetaldehyde deactivates the enzyme, immobilisation by covalent linkage or on Celite 545 makes CRL resistant to acetaldehyde deactivation.<sup>27-28</sup> When using lipase

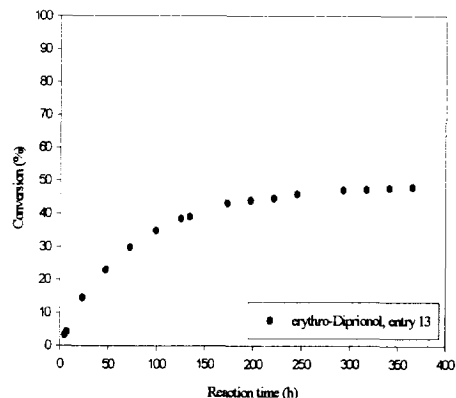
from *Candida antarctica* (CAL), entry 6, the ratio between the products (2S,3S,7R/S)-1 and (2R,3R,7R/S)-1 increased to give "E" = 50. Although the conversion rate obtained was the fastest observed in our investigation,  $c = 39.4$  in 44 h the "E"-value was too low for our needs. The lipases from *Pseudomonas fluorescens* (PFL, entry 7) and *Pseudomonas sp.* (Amano PS, entry 8, see also Figure 1) showed the same differentiation between the two products (2S,3S,7R/S)-1 and (2R,3R,7R/S)-1, but the reaction rate in *n*-heptane using PFL was slightly faster.

Nakamura et al have reported that the organic solvent functions as an enantioselective inhibitor in the transesterification of sulcatol with vinyl acetate under catalysis of lipase PS.<sup>23</sup> When branched- and linear-chained alkanes were tested the best selectivity was obtained using linear alkanes as solvent. The effect of different solvents (see Table 1) on the reaction rate and on the stereoselectivity was investigated at initially water activity,  $a_w = 0.32$  under catalysis by the lipase Amano PS. However, in our experiments, when using *n*-heptane, chloroform, *t*-butyl methyl ether or *iso*-octane (entries 8,9,10 and 12 respectively), the "E"-values were all around 100. Thus, we observed no significant enantioselective inhibition by the solvent. However, using vinyl acetate as both the solvent and the acyl donor (entry 11) gave a significantly lower "E"-value (35). The solvents *n*-heptane, vinyl acetate and *iso*-octane (entries 8,11 and 12 respectively) gave the best reaction rates.

Most of the recently published experimental procedures on transesterifications catalysed by lipase in organic media have been performed under so called dry conditions (usually achieved by adding 4 Å molecular sieves to the reaction medium). We have previously shown that the water activity should be low in lipase catalysed transesterification reactions and also that the conversion of the reactions have to be under 60%, if the remaining substrate is to be obtained enantiomerically pure.<sup>29</sup> However, initially "dry conditions",  $a_w < 0.1$  (entry 13 see also Figure 2) resulted in a slightly lower selectivity between the two mixtures of (2S,3S,7R/S)-1 and (2R,3R,7R/S)-1-isomers, ("E" = 90), although accompanied by an increased reaction rate.



**Figure 1.** Conversion rates of 1:1:1:1-mixtures of erythro- and threo-3,7-dimethylpentadecan-2-ol.

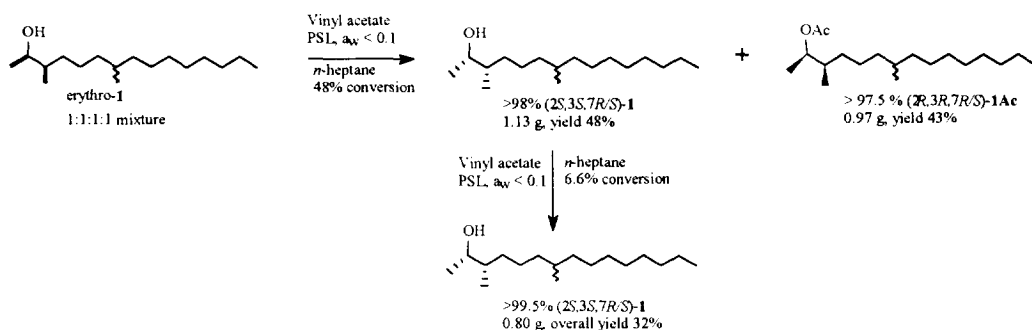


**Figure 2.** Conversion rate of a 1:1:1:1-mixture of erythro-3,7-dimethylpentadecan-2-ol.

The method of stirring and its effects on the reaction rate and on the deactivation of the enzyme used have been investigated by Kvittingen et al.<sup>30</sup> They have shown that rotation of the whole reaction vessel is beneficial compared to the traditional stirring method using a magnetic bar. In our experiments, the stirring method gave about 25% faster reaction as compared with an experiment using the rotation method at least up to a conversion of 30% (conditions of entry 8, rotation of the whole reaction vessel replacing stirring using a magnetic bar). A recently published paper<sup>31</sup> has demonstrated that ultrasound treatment of lipase-catalysed reactions enhances the reaction rates while retaining enantioselectivity. However, no enhanced rate was obtained by us (conditions of entry 8, sonication replacing stirring).

The 1:1:1:1-mixture of *threo*-3,7-dimethylpentadecan-2-ol could also be separated by transesterification (entry 14). The lipase showed twice the reaction rate but lower selectivity between (2*S*,3*R*,7*R*/*S*)-1 and (2*R*,3*S*,7*R*/*S*)-1 as compared with the selectivity observed with erythro-1. The conversion of erythro- and threo-1 is plotted together in Figure 1. The low rate ratio observed between threo- and erythro-1 does not allow for efficient separation by transesterification under the standard conditions of the threo- and the erythro-isomers from a mixture of all the eight isomers of 3,7-dimethylpentadecan-2-ol.

Subsequently, 2.35 g of erythro-1 was transesterified with vinyl acetate, catalysed by Amano PS lipase from *Pseudomonas sp.* in *n*-heptane at room temperature and initially dry conditions. (See Scheme 2.) The reaction was interrupted at 48% conversion, 0.97 g of the produced ester **1Ac** (2*R*,3*R* : 2*S*,3*S*-ratio > 97.5 : 2.5, 86% of the theoretical amount) was obtained after liquid chromatography and distillation. The recovered alcohol (2*S*,3*S*,7*R*/*S*)-1 (1.13 g, 2*S*,3*S* : 2*R*,3*R*-ratio > 98 : 2) was transesterified once again, now only to 6.6% conversion. This resulted in 0.8 g of remaining diprionol (2*S*,3*S*,7*R*/*S*)-1 (2*S*,3*S* : 2*R*,3*R*-ratio > 99.5 : 0.5, 64% of the theoretical amount).



**Scheme 2.** Preparative resolution of a mixture of the four *erythro*-3,7-dimethylpentadecan-2-ol (erythro-1) into 2*R*,3*R*,7*R*/*S*-3,7-dimethylpentadecan-2-yl acetate (2*R*,3*R*,7*R*/*S*)-1Ac and (2*S*,3*S*,7*R*/*S*)-dimethylpentadecan-2-ol (2*S*,3*S*,7*R*/*S*)-1.

No stereocontrol of the 7-position in diprionol and its isomers were achieved in the reactions described so far. In order to achieve this, 4-methyldodecan-1-ol, which serves as a building block in our synthesis<sup>10</sup> of the erythro-mixture (erythro-1), ought to be resolved into its enantiomers. Classical resolution techniques are likely to fail in this case since the stereocenter is far from the functional group. However, since a lipophilic pocket in an enzyme can be expected to express its stereochemical preferences far away from the reactive centre, we decided to study if *rac*-4-methyldodecan-1-ol could be resolved by transesterification. It was found to be a good substrate in three tested enzyme-catalysed transesterification systems (Amano PS, ChiroCLEC™-CR dry and Novozym 435) but none of these catalysts showed any enantiodifferentiation. *Candida rugosa* has been shown to efficiently resolve chiral racemic acids (usually  $\alpha$ -substituted).<sup>32-35</sup> When using *Candida rugosa* (CRL, immobilised<sup>26</sup> on polypropylene) as the catalyst in an esterification reaction with *rac*-4-methyldodecanoic and eicosanol at  $a_w = 0.8$  in cyclohexane a pronounced enantioselectivity was obtained. The ester obtained ( $c = 38\%$ ) was hydrolysed to give the *S*-acid  $\{[\alpha]_D^{25} + 0.12$ , neat, Lit.<sup>36</sup>  $[\alpha]_D^{25} + 0.20$  (neat) for *S*-acid}. Due to the low specific rotation it is not easy to estimate the exact ee of this acid. The *S*-alcohol obtained after LAH-reduction of the *S*-acid showed a higher specific rotation value,  $\{[\alpha]_D^{25} - 1.33$  (neat), lit.<sup>37</sup>  $[\alpha]_D^{22} - 1.54$  (neat)} corresponding to an enantiomeric purity of the produced ester of 84% ee and  $E = 19$ . We are presently studying the esterifications of several methylalkanoic acids in our laboratory as well as searching for methods to accurately determine the enantiomeric excess for such acids with the methyl group in other positions than in the 2-position.

If (*S*)-4-methyldodecan-1-ol was readily available, preparation of a diastereomeric mixture of (2*S*,3*S*,7*S*)-1 and (2*R*,3*R*,7*S*)-1 should then be possible.<sup>10</sup> Using this mixture as substrate in the resolution according to Scheme 2

should make it possible to prepare the *Neodiprion sertifer* pheromone *erythro*-(2*S*,3*S*,7*S*)-3,7-dimethylpentadecan-2-yl acetate as a pure stereoisomer. We are now investigating the possibility of applying this simplified strategy for the preparation of a number of structurally related biologically active esters of 2-alkanols which are potential sexual pheromones in other pine sawfly species.<sup>13-14</sup>

### EXPERIMENTAL

Commercially available chemicals were used without further purification unless otherwise stated. Pyridine was dried by distillation from CaH<sub>2</sub> and stored over molecular sieves (4Å), *n*-heptane, *n*-hexadecane and vinyl acetate were dried by addition of molecular sieves (4Å). All dried materials were stored under argon. PFL (E.C.3.1.1.3.) lipase from *Pseudomonas fluorescens* was obtained from Fluka. The specific activity was 31.5 U/mg. Cross-linked crystals of *Candida rugosa* lipase (ChiroCLEC™-CR dry) were purchased from Altus Biologics Inc. Immobilised lipase from *Candida antarctica* CAL, (Novozym 435, LCC 0013-1) was a gift from Novo Nordisk A/S, the specific activity was 7400 PLU/g. PSL (LPSARO 4502) lipase from *Pseudomonas sp.* was a gift from Amano Co, the specific activity was 30.0 U/mg. CRL (E.C. 3.1.1.3.) lipase from *Candida rugosa* was purchased from Sigma (L 1754, type VII) with a specific activity of 905 U/mg. Lipase from *Mucor javanicus* (LMS06515), Amano M, lipase from *Aspergillus niger* (LS06507), Amano A and lipase from *Rhizopus oryzae* (LFS06522), Amano F, all with unknown activity, were gifts from Amano Co. The enzymes were stored at 4 °C in a desiccator over dried silica gel. Preparative liquid chromatography (MPLC) was performed on straight phase silica gel (Merck 60, 230-400 mesh, 0.040-0.063 mm.) employing the gradient technique described<sup>38</sup> and using an increasing concentration of distilled ethyl acetate in distilled cyclohexane (0 → 100 %), as eluent. Thin layer chromatography (TLC) was performed on silica gel plates (Merck 60, pre-coated aluminium foil) eluted with ethyl acetate (20%) in cyclohexane and developed by spraying with vanillin in sulphuric acid and heated at 120 °C. <sup>1</sup>H, <sup>13</sup>C and <sup>19</sup>F NMR spectra were recorded on a Jeol EX 270 spectrometer using CDCl<sub>3</sub> as solvent and TMS as internal reference. Optical rotations were measured on a Perkin Elmer 241 polarimeter. IR-spectra were recorded neat between NaCl plates using a Perkin Elmer 782 infrared spectrometer. Unless otherwise stated, GC analyses were carried out using a 30 m x 0.25 mm I.D. capillary column coated with DB-5 d<sub>f</sub> = 0.25 µm; carrier gas N<sub>2</sub>, 15 psi, split ratio 1/30. All *erythro*-3,7-dimethylpentadecan-2-ol (*erythro*-1) samples came from the same batch prepared by Hedenström and Högborg<sup>10</sup> containing < 0.02 % of the *threo*-isomers. The *threo*-3,7-dimethylpentadecan-2-ol (*threo*-1) sample was prepared (< 0.08 % *erythro*) from *erythro*-1 according to the method<sup>4</sup> used for the preparation of (2*R*,3*S*,7*S*)-3,7-dimethyl-2-pentadecanol from (2*S*,3*S*,7*S*)-3,7-dimethyl-2-pentadecanol. *rac*-4-Methyl-dodecan-1-ol was prepared as described in the literature.<sup>10</sup>

*Preequilibrated initial water activity. General method:* The substrates, the enzyme and an internal standard (*n*-hexadecane) were dissolved in the appropriate solvent and stored (24 h) in a vessel containing a saturated solution of MgCl<sub>2</sub>·6H<sub>2</sub>O to give the mixtures preequilibrated to  $a_w = 0.32$ . Vinyl acetate was equilibrated similarly in another vessel. To be able to compensate for the loss of solvent from evaporation, pure solvent was equilibrated together with the substrate solution.

*Transesterification of 3,7-Dimethylpentadecan-2-ol. General method:* *erythro*- (or *threo*)-3,7-Dimethylpentadecan-2-ol (90 mg, 0.35 mmol), internal standard *n*-hexadecane (40 mg, 0.18 mmol), the appropriate solvent (0.85 ml), lipase (type and amount see Table 1) and a magnetic stirring bar were added to the reaction vessel. The reaction was started by the addition of vinyl acetate (110 mg, 1.3 mmol) to the flask, which was immediately sealed with a septum. The mixture was stirred at 500 rpm. The conversion was monitored by periodic withdrawal of samples. When the reaction had reached the desired conversion (~ 40%), the mixture was filtered through two low porosity filters and the filters were washed with *n*-pentane. The solvents were evaporated and the product and remaining substrate were separated by MPLC. The esters were obtained chemically pure (> 99% by GC) without any trace of the remaining alcohol.

*Determination of the conversion.* The conversion in the transesterifications were determined by GC (OV101 10% HP on Chromosorb™ W, 2 m x 2 mm, on column injection, isothermal 180 °C) Ret. time *n*-hexadecane:

3.0 min.; *erythro*-3,7-dimethylpentadecan-2-ol: 6.3 min.; *erythro*-3,7-dimethylpentadecan-2-yl acetate: 8.9 min. The conversions were calculated from the calibrated areas of the alcohol and the ester peaks relative to the peak of the internal standard.

(2*R*,3*R*,7*R*/S)-3,7-Dimethylpentadecan-2-yl (R)-MTPA ester. *General method*: The acetate (2*R*,3*R*,7*R*/S)-**IAc** (15 mg, 50  $\mu$ mol) was dissolved in MeOH (0.5 ml), KOH (0.25 ml of a 2.4 M sol. in MeOH) was added and the mixture was stirred until the acetate had disappeared (~ 24 h, by TLC). The reaction mixture was diluted with water (1 ml) and *n*-pentane (2 ml). The aqueous layer was extracted with *n*-pentane (3 x 2 ml). The combined organic phases were washed with Na<sub>2</sub>CO<sub>3</sub> (1.0 ml, 10% sol. in H<sub>2</sub>O), dried (MgSO<sub>4</sub>) and the solvent evaporated off after filtration. The residual oil was dissolved in dry pyridine (0.5 ml) under argon and the chloride derived from (R)-(+)-MTPA (enantiomerically pure cf. ref. 33) was added. The mixture was stirred under argon until the alcohol had disappeared (~ 24 h, by TLC). HCl (1.0 ml, 1.0 M in H<sub>2</sub>O) and *n*-pentane (2 ml) was added. The aqueous layer was extracted with 3 x 2 ml *n*-pentane. The combined organic phases were washed with Na<sub>2</sub>CO<sub>3</sub> (1.0 ml, 10% sol. in H<sub>2</sub>O) and dried (MgSO<sub>4</sub>). After evaporation of the solvent the residual oil was purified by flash chromatography on 5 g silica gel with a gradient of diethylether in *n*-pentane (0 → 2.5 %). After evaporation of the solvent, the diastereomeric excess was determined by <sup>19</sup>F-NMR-analysis of the formed MTPA-ester. <sup>19</sup>F-NMR (254.1 Mhz):  $\delta$  16.48 ppm [2*R*,3*R*-diprionol and (R)-MTPA], 16.54 ppm [2*S*,3*S*-diprionol and (R)-MTPA]. <sup>1</sup>H-NMR (270 Mhz):  $\delta$  0.80-0.95 (9H, m), 1.15-1.50 (25H, m), 3.60 (3H, s), 5.10 (1H, d of q, *J* = 1.3 Hz and 5.0 Hz), 7.38-7.48 (3H, m), 7.50-7.60 (2H, m). <sup>13</sup>C-NMR (67.8 Mhz):  $\delta$  14.1, 14.4, 15.3, 16.8, 19.6, 19.7, 22.7, 24.4, 27.1, 29.4, 29.7, 30.0, 31.9, 32.7, 37.0, 37.1, 37.6, 55.4, 65.5, 121.5, 126.0, 127.3, 128.3, 129.4, 132.7, 166.1 ppm.

(2*R*,3*R*,7*R*/S)-3,7-Dimethylpentadecan-2-yl acetate. (2*R*,3*R*,7*R*/S)-**IAc**. Freshly distilled *erythro*-3,7-dimethylpentadecan-2-ol (*erythro*-**1**, 2.35 g, 9.16 mmol), dry *n*-hexadecane (1.01 g, 4.47 mmol) and dry vinyl acetate (2.76 g, 32.1 mmol) was dissolved in dry *n*-heptane (13.2 g). The reaction was started by the addition of *Pseudomonas sp.* lipase (194 mg). The reaction was interrupted by filtration at 47.9 % conversion. After evaporation, the product was separated from the remaining substrate by MPLC and the acetate (2*R*,3*R*,7*R*/S)-**IAc** was distilled bulb to bulb (bath temp 130-135 °C, 0.15-0.20 mbar) to give a clear oil (0.97 g, 3.26 mmol) in 43% overall yield. > 97.5% 2*R*,3*R*-Diprionyl acetate, method of analysis described above. <sup>1</sup>H NMR (270 Mhz):  $\delta$  0.82-0.95 (9H, m), 1.17 (3H, d, *J* = 6.3 Hz), 1.20-1.45 (22H, m), 2.00 (3H, s), 4.84 (1H, d of q, *J* = 1.3 Hz and 6.6 Hz). <sup>13</sup>C NMR (67.8 Mhz):  $\delta$  14.2, 14.9, 17.0, 19.8, 21.4, 22.8, 24.6, 27.2, 29.4, 29.8, 30.1, 32.0, 32.8, 37.1, 37.2, 37.4, 37.7, 74.2, 170.9 ppm. Spectroscopical and physical data for the title compound are similar with those previously described.<sup>39</sup>

(2*S*,3*S*,7*R*/S)-3,7-Dimethylpentadecan-2-ol. (2*S*,3*S*,7*R*/S)-**1**. The remaining substrate alcohol, **1**, from the esterification above (1.128 g, 4.40 mmol, > 98% 2*S*,3*S*-Diprionol, method of analysis described above, except that chloride derived from (S)-(-)-MTPA was used), *n*-hexadecane (0.532 g, 2.35 mmol) and lipase from *Pseudomonas sp.* (107 mg) was dissolved in dry *n*-heptane (7.54 g). The mixture was dried in a desiccator over phosphorous pentoxide (24 h). Dry vinyl acetate (1.475 g, 17.1 mmol) was added and the mixture was stirred until the desired conversion was reached (6.6%). The mixture was filtered and the filter was washed with *n*-pentane. After evaporation, the alcohol was separated from the acetate by MPLC. The alcohol was distilled bulb to bulb (bath temp 125-130 °C/0.02 mbar) to give a clear oil (0.755 g, 2.94 mmol) in 32.1% overall yield. > 99.5% 2*S*,3*S*-Diprionol, from the method of analysis described above, using the chloride derived from (S)-(-)-MTPA. <sup>19</sup>F-NMR (254.1 Mhz):  $\delta$  16.48 ppm [2*S*,3*S*-diprionol and (S)-MTPA], 16.54 ppm [2*R*,3*R*-diprionol and (S)-MTPA]. <sup>1</sup>H NMR (270 Mhz):  $\delta$  0.83-0.92 (9H, m), 1.15 (3H, d, *J* = 6.3 Hz), 1.22-1.29 (22H, m), 1.31 (1H, s), 3.71 (1H, q, *J* = 5.0 Hz). <sup>13</sup>C NMR (67.8 Mhz):  $\delta$  14.1, 19.6, 20.3, 24.7, 27.1, 29.3, 29.7, 30.0, 31.9, 32.7, 33.0, 37.0, 37.2, 33.0, 37.4, 39.8, 71.4 ppm. Spectroscopical and physical data for the title compound are similar with those previously described.<sup>39</sup>

*Transesterification of rac-4-methyldodecan-1-ol. General method*: Freshly distilled *rac*-4-methyldodecan-1-ol (1.0 g, 5 mmol), dry *n*-heptan (8.0 ml), lipase (0.1 g of Amano PS, 0.01g ChiroCLEC™-CR dry or 0.1 g of Novozym 435) and a magnetic stirring bar were added to the reaction vessel. The reaction was started by the

addition of dry vinyl acetate (1.16 g, 14 mmol) to the flask, which was immediately sealed with a septum. The mixture was stirred with a magnetic bar at 500 rpm. The conversion was monitored by periodic withdrawal of samples. When the reaction had reached the desired conversion (~40%), the mixture was filtered through two low porosity filters and the filters were washed with *n*-pentane. The solvents were evaporated and the product and remaining substrate were separated by MPLC. The esters were obtained chemically pure (>99% by GC) without any trace of the remaining alcohol. The transesterification was not enantioselective in any of the three tested reactions judged by optical rotation on the produced ester and the remaining alcohol.

*rac*-4-Methyldodecanoic acid was prepared from 4-methyldodecan-1-ol (2.0 g, 10 mmol) using a oxidation method described for aryl acids as starting materials.<sup>40</sup> 4-Methyldodecan-1-ol (2.0 g, 10 mmol) in acetone (20 ml) was stirred at 0 °C, Jones' reagent (5 ml, 2.5 M solution of H<sub>2</sub>CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub> - H<sub>2</sub>O) was added dropwise (0.25 h). After stirring at r.t. and filtering the solution through a celite pad, washing with water (25 ml), ethyl acetate (25 ml) and Et<sub>2</sub>O (25 ml), extraction of the water phase with Et<sub>2</sub>O (25 ml). The pooled organic phase was extracted with NaOH (3x25 ml, 0.1 M in H<sub>2</sub>O), the combined basic waterphase was acidified to pH 1 (6 M HCl), the acid was extracted into Et<sub>2</sub>O (3x50 ml). After drying (MgSO<sub>4</sub>), evaporation of the solvent and distillation bulb to bulb (bath temp 145-155 °C/0.30 mbar, lit.<sup>41</sup> 130-134 °C/1.5 mmHg) the title compound (2.31 g, 70%) was obtained as a light yellow oil.  $n_D^{20}$  1.4435, lit.<sup>36</sup>  $n_D^{25}$  1.4424. IR (cm<sup>-1</sup>): 3500-2400, 2949, 2917, 2845, 1706, 1453 1404, 1374, 1282, 1215. <sup>1</sup>H NMR (270 MHz):  $\delta$  0.87 (3H, d, *J* = 6.3 Hz), 0.88 (3H, t, *J* = 6.6 Hz), 1.17-1.34 (14H, m), 1.45 (2H, d of q, *J* = 8.6 and 6.2 Hz), 1.66 (1H, m), 2.35 (2H, m), 11.24 (1H, bs, disappeared on shaking with D<sub>2</sub>O). <sup>13</sup>C NMR (67.8 Mhz):  $\delta$  14.1, 19.3, 22.7, 26.9, 29.4, 29.6, 29.9, 31.7, 31.86, 31.93, 32.4, 36.7, 180.2 ppm.

*Esterification of rac*-4-methyldodecanoic acid. Freshly distilled *rac*-4-methyldodecanoic acid (1.1 g, 5.13 mmol), cyclohexane (35 ml), CRL lipase (0.16 g, immobilised on polypropylene<sup>26</sup>), 1.08 g Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O, 0.96 g Na<sub>2</sub>SO<sub>4</sub> ( $a_w$  = 0.8), internal standard tetracosane (1.13 g, 4.17 mmol) and a magnetic stirring bar were added to the reaction vessel. The reaction was started by the addition of eicosanol (1.15 g, 3.85 mmol) to the flask, which was immediately sealed with a septum. The mixture was stirred at 500 rpm. The conversion was monitored by periodic withdrawal of samples and at a conversion of 38% (58 h) the mixture was filtered and washed with cyclohexane. The solvent was evaporated and the product and remaining substrate were taken up in Et<sub>2</sub>O (100 ml), the organic phase was shaken with Na<sub>2</sub>CO<sub>3</sub> (10% in H<sub>2</sub>O, 5 x 100 ml), separated and the solvent evaporated to give a mixture of the S-ester, tetracosane and eicosanol without any trace of the remaining R-acid. MPLC purification gave the pure ester which crystallised upon standing [m.p. 33.5-34 °C,  $[\alpha]_D^{22}$  -0.60 (c 12.1, hexane) from an ester isolated at conversion *c* = 25%]. The S-ester was hydrolysed as above for (2R,3R,7R/S)-1Ac to give the S-acid (0.38 g, 35%) chemically pure after distillation bulb to bulb (bath temp 150-155 °C/0.3 mbar).  $[\alpha]_D^{22}$  +0.12 (neat). Lit.<sup>36</sup>  $[\alpha]_D^{25}$  +0.20 (neat), Lit.<sup>41-42</sup>  $[\alpha]_D^{20}$  -0.60 (c 5.3, hexane). {LAH-reduction of this acid gave (S)-4-methyldodecan-1-ol  $[\alpha]_D^{25}$  -1.33 (neat), lit.<sup>37</sup>  $[\alpha]_D^{22}$  -1.54 (neat) corresponding to an optical purity of 84% ee of the produced ester and *E* = 19}. The combined basic waterphase was acidified to pH 1 with 6 M HCl and extracted with Et<sub>2</sub>O (5 x 100 ml), the pooled organic phase was washed with brine (100 ml), dried (MgSO<sub>4</sub>), evaporated to dryness and distilled bulb to bulb (bath temp 150-155 °C/0.3 mbar) yielding the R-acid (0.60 g, 55%).  $[\alpha]_D^{25}$  -0.12 (neat). {R-alcohol obtained after LAH-reduction of the R-acid gave  $[\alpha]_D^{25}$  +1.00 (neat), lit.<sup>37</sup>  $[\alpha]_D^{22}$  -1.88 (neat) corresponding to an optical purity of 53% ee of the remaining R-acid}.

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