Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 835–844

TETRAHEDRON: *ASYMMETRY*

Do enzymes recognise remotely located stereocentres? Highly enantioselective *Candida rugosa* lipase-catalysed esterification of the 2- to 8-methyldecanoic acids

Erik Hedenström,[a,*] Ba-Vu Nguyen[a] and Louis A. Silks, III[b]

[a]*Chemistry, Department of Natural and Environmental Sciences, Mid Sweden University, SE-851 70 Sundsvall, Sweden*
[b]*The Szilard Resource, Biosciences Division Mail Stop E529, Alamos National Laboratory, Bikini Atoll Road, SM 30, Los Alamos, NM 87545, USA*

Received 7 March 2002; accepted 9 April 2002

Abstract—Several racemic methyl decanoic acids have been synthesised and successfully resolved in esterification with 1-hexadecanol at $a_w = 0.8$ in cyclohexane using immobilised *Candida rugosa* lipase (CRL) as the catalyst. The enantiomeric ratios ($E = 2.8$–68) obtained were surprisingly high even when the methyl group was as remotely located as in 8-methyldecanoic acid ($E = 25$). Interestingly, the lipase shows enantiopreference for the *S*-enantiomer when the methyl group is located on even numbered carbons i.e. for the 2-,4-,6- and 8-methyldecanoic acids and to the *R*-enantiomer when the methyl group is located on uneven numbered carbons i.e. for the 3-,5- and 7-methyldecanoic acids. © 2002 Published by Elsevier Science Ltd.

1. Introduction

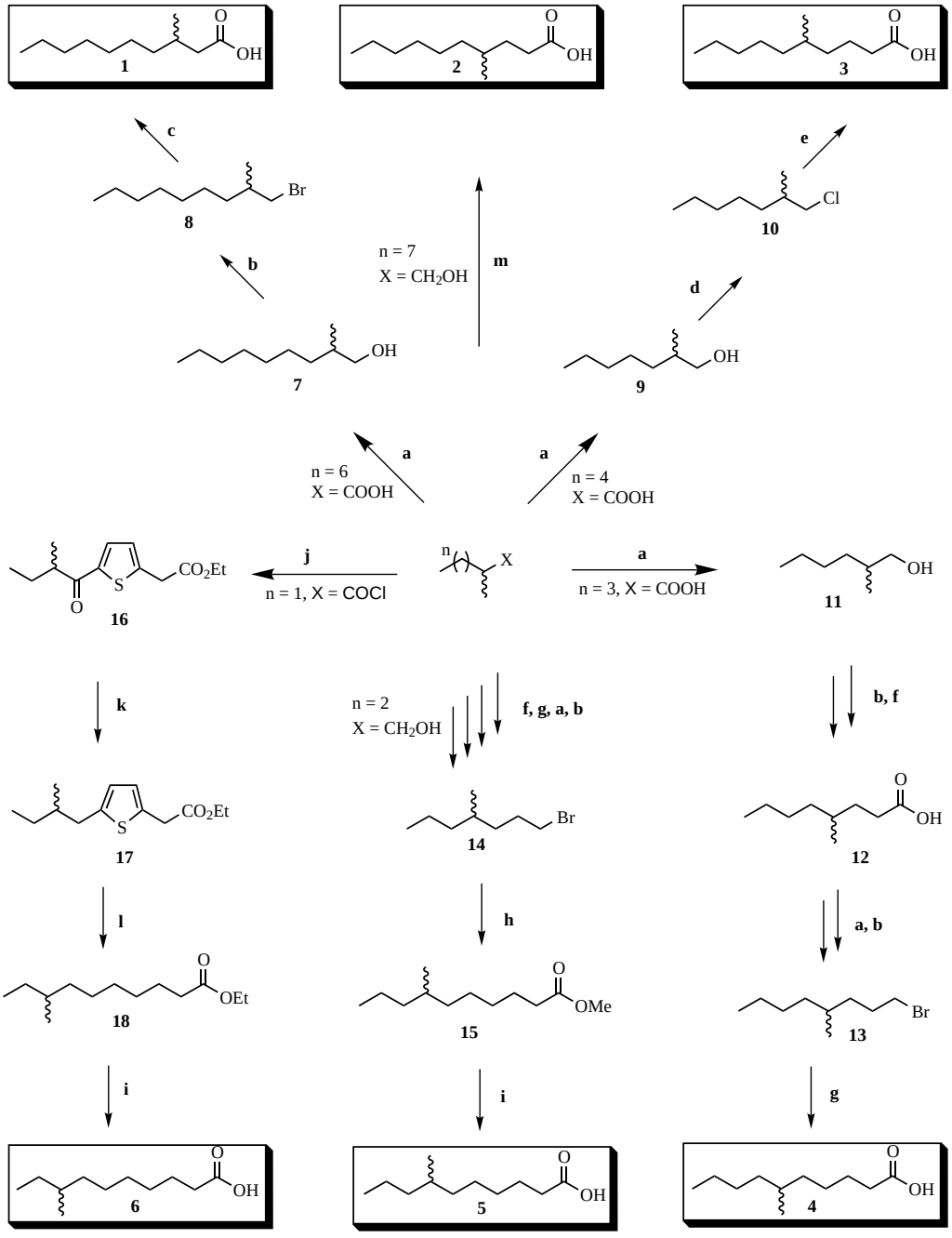
During the past decade many organic chemists have used enzymes as biocatalysts to prepare enantiomerically pure compounds.[1–6] Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are the most widely employed enzymes not only because they are cheap and readily available from many different sources, but also because they possess high enantioselectivity for a broad range of substrates and high stability in organic solvents.[7] One of the most popular lipases used in organic synthesis is *Candida rugosa* lipase (CRL). The X-ray structures of this lipase and models of its active site have been reported by Grochulski et al.[8,9] Since the early work of Holmberg et al.[10] and Engel[11] in 1991 when *C. rugosa* lipase was used as an enantioselective catalyst in the resolution of 2-methylalkanoic acids in organic solvent, some other successful results have been reported.[12–15] Results from this field have also been reported in some reviews.[7,16–18] Besides the publications concerning 2-methylcarboxylic acids, only a few examples are found in the literature dealing with lipase-catalysed resolution of carboxylic acids with remotely located methyl groups i.e. 3-, 4-methylcarboxylic acids.[15,18–23] Furthermore, CRL shows (*S*)-preference for 2-methyldecanoic acid[12,14] and for 4-methyldodecanoic acid,[19] but (*R*)-preference for 3,7-dimethyloctanoic acid and citronellic acid.[15] Consequently, these intriguing results prompted us to systematically investigate the enantiorecognition of CRL for methyldecanoic acids with the branch located in any position along the carbon chain. To our knowledge, the enzymatic resolutions of such acids have not been studied before now. Accordingly, all of the racemic 2- to 8-methyldecanoic acids were prepared and subjected to CRL-catalysed esterification and our results are presented below.

2. Results and discussion

3-Methyldecanoic acid **1** was prepared in an overall yield of 24% starting with LAH-reduction of 2-methylnonanoic acid to give 2-methylnonanol **7**, which was used to form 1-bromo-2-methylnonane **8** (Scheme 1). This was converted to the Grignard reagent and reacted with carbon dioxide to give the acid **1**. 4-Methyldecanoic acid **2** was obtained in 70% yield by oxidation of 4-methyl-1-decanol[24] with Jones' reagent. The synthesis of 5-methyldecanoic acid **3** started from 2-methylheptanoic acid, which was reduced to the alcohol **9** and converted to the chloride **10**. This was transformed to the corresponding alkyllithium reagent and then the

* Corresponding author. Tel.: +46 60 148729; fax: +46 60 148802; e-mail: erik.hedenstrom@mh.se

0957-4166/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0957-4166(02)00172-6



Scheme 1. *Reagents and conditions*: (a) 1. $LiAlH_4$, 2. H_3O^+; (b) $P(Ph)_3$, Br_2, CH_2Cl_2; (c) 1. Mg, Et_2O, 2. CO_2, 3. H_3O^+; (d) CCl_4, PPh_3, reflux; (e) 1. added to Li(s) in dry *n*-hexane, reflux, 2. added to CuI/dry Et_2O –15 to –78°C then $(CH_3)_3SiI$ and methyl acrylate, 12 h, 3. Et_3N, –5°C then rt for 6 h, 4. 3 M HCl (pH 1–2); (f) Br_2 and red phosphorus; (g) 1. diethyl malonate, NaOEt/EtOH, reflux, 2. KOH/EtOH, 3. 190°C; (h) 1. Li (s), dry *n*-hexane, reflux, 2. MeCuCNLi, dry *n*-hexane, –78°C, 3. methyl acrylate, dry *n*-hexane, –78°C; (i) KOH/EtOH; (j) 1. ethyl 2-thiopheneacetate, CH_2Cl_2, 0°C, then $SnCl_4$; (k) Zn(Hg), CH_3CO_2H; (l) Raney-Ni, H_2(g), isopropanol; (m) Jones' reagent, acetone, 0°C to rt 0.5 h.

carbon skeleton was extended by three carbons via conjugate addition[25] to methyl acrylate. The ester obtained was subjected to alkaline hydrolysis to yield the acid **3** in an overall yield of 17%. 6-Methyldecanoic acid **4** was prepared from 2-methylhexanoic acid via $LiAlH_4$ reduction to the alcohol **11**, followed by conversion to the bromide. The carbon chain was then extended with two carbons via a malonic ester alkylation sequence to give 4-methyloctanoic acid **12**. The above sequence was repeated via the bromide **13** to give the target 6-methyldecanoic acid **4** in 19% overall yield.

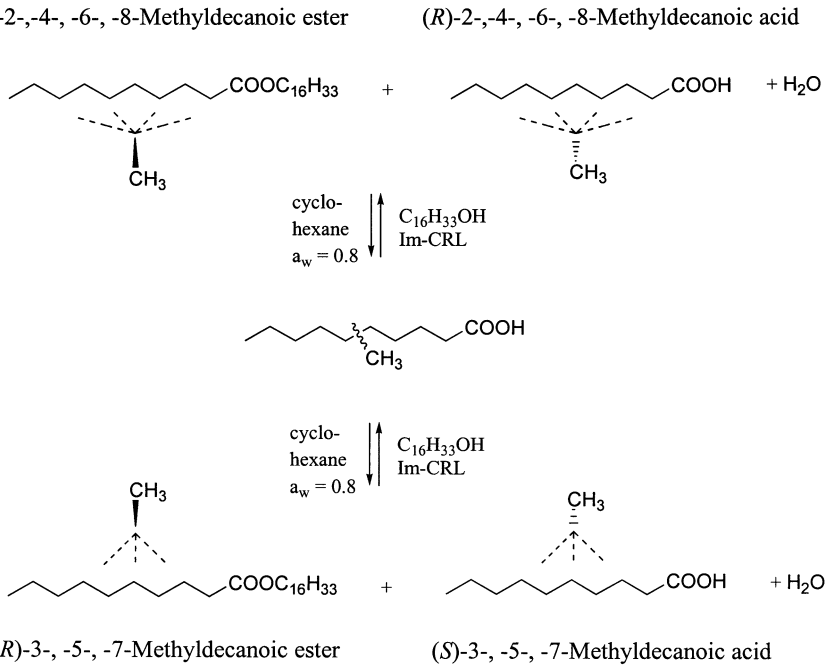
7-Methyldecanoic acid **5** was prepared in a total yield of 12% from 2-methyl-1-pentanol via a two-carbon extension sequence followed by a three-carbon extension sequence as described above for the preparation of

6-methyldecanoic acid and 5-methyldecanoic acid, respectively.

The synthesis of 8-methyldecanoic acid **6** started with Friedel–Crafts acylation of ethyl 2-thiopheneacetate with 2-methylbutyryl chloride to give compound **16** followed by Clemmensen reduction to **17** and Raney nickel reduction to yield ethyl 8-methyldecanoate **18**. After basic hydrolysis the carboxylic acid **6** was isolated in an overall yield of 56%.

The reaction conditions for the CRL-catalysed esterifications are summarised in Scheme 2. A carboxylic acid was reacted at a constant water activity ($a_w = 0.8$) in cyclohexane with 1-hexadecanol catalysed by CRL at ambient temperature. The results are shown in Table 1.

One remarkable observation was that for the substrates possessing the methyl group on the carbon having an even number (i.e. for the 2-, 4-, 6- and 8-methylde-



Scheme 2. Resolution of racemic 2-, 3-, 4-, 5-, 6-, 7- and 8-methyldecanoic acid in esterification reactions with 1-hexadecanol in cyclohexane catalysed by immobilised CRL at $a_w = 0.8$.

Table 1. CRL-catalysed esterification of the methyldecanoic acids **1**–**6**

Substrate acid	Faster reacting enantiomer		E.e.$_s$[a] (%)	c (%)	E[d] (%)	Time (h)	$[\alpha]_D^{25}$ (c, $CHCl_3$)	
	Configuration	E.e.$_p$[a] (%)					Methyldecanoic acid	
							Hydrolysed product	Substrate
2-Methyl-decanoic acid[14]	2*S*	97.6	31.5	24.4	91	–	–	–
1	3*R*	87.3	35.2	28.7	21	311	+4.9 (1.42)	–1.55 (1.10) –5.8 (5.8)[26]
2	4*S*[e]	95.2	52.2	35.4	68	1.33	+0.2 (1.12)	–0.1 (1.09)
3	5*R*	96.0	18.6	16.2	58	14.4	–0.3 (1.51) –0.6 (neat)[27]	+0.1 (4.16)
4	6*S*[f]	44	10	18	2.8	3.4	+0.3[c]	–0.7[c]
5	7*R*[f]	83[b]	49[b]	37	17	1.5	–1.0 (3.84)	+0.6 (1.33)
6	8*S*	87[b]	47[b]	35	25	4.0	+6.1 (1.14) +7.0 (10%)[28]	–3.3 (1.22)

[a] Determined from ^{77}Se NMR analysis of the corresponding (*S*)-oxazolidine-2-selone derivative as described in Section 4.
[b] Determined from $[\alpha]_D^{25}$.
[c] Measured on the product acid and the substrate acid from another esterification when the conversion was 64%.
[d] Calculated from e.e.$_p$ and the conversion using the equation deduced by Chen et al.[29]
[e] The remaining substrate acid was reduced ($LiAlH_4$) and the sign of optical rotation of the product alcohol was compared to literature values.[30]
[f] The remaining substrate acids were reduced to the alcohols ($LiAlH_4$) which were converted into the corresponding tosylates, reduction of these ($LiAlH_4$) gave the known (*R*)-5-methyldecane[31] and (*S*)-4-methyldecane,[32] respectively.

canoic acids), CRL showed *S*-enantioselectivity and this was reversed to *R*-enantioselectivity for the odd-methyldecanoic acids (i.e. for the 3-, 5- and 7-methyldecanoic acids) (see Scheme 2 and Table 1).

The resolution of 2-methyldecanoic acid, catalysed by CRL, shows *S*-enantioselectivity ($E=91$) and high catalytic activity.[14] However, the *E* values for 3,7-dimethyloctanoic acid and its unsaturated analogue citronellic acid are 17 and 24, respectively, with *R*-selectivity.[15] CRL showed acceptable *R*-enantioselectivity when 3-methyldecanoic acid **1** was tested in the esterification reaction ($n=1$, $E=21$, see Table 1). In this case the catalytic activity of CRL was very low compared to other substrates tested. Only 24% conversion was reached after 311 h. The *R*-enantioselectivity and the slow reaction rate is probably due to a different orientation of the substrate acyl chain in the active site of CRL leading to a different productive binding mode which produced the *R*-ester faster than the *S*-ester.[15,17]

Some lipases catalyse the enantioselective hydrolysis of racemic methyl 3-phenylbutyrate with good to excellent *E* values (20–153).[21] In this case, the faster reacting 3*S*-enantiomer has the phenyl, methyl and acid groups spatially orientated in the same way as the alkyl, methyl and acid groups of (*R*)-3-methyldecanoic acid.

The *E* values ($E=2.8$–68) of CRL towards the 4-, 5-, 6-, 7, and 8-methyldecanoic acids **2**–**6** studied by us were lower than the *E* value for 2-methyldecanoic acid ($E=91$).[14] For the substrates 4-methyldecanoic acid **2** and 5-methyldecanoic acid **3**, surprisingly, high *E* values were obtained, 68 and 58, respectively. Even for the 7-methyldecanoic acid **5** and 8-methyldecanoic acid **6** with more remotely located methyl groups, CRL showed impressive enantiorecognition, resulting in *E* values of 17 and of 25, respectively. However, in this case of the 6-methyldecanoic acid **4** a very low *E* value of 2.8 was obtained.

It is interesting to note that CRL displayed (*S*)-enantiopreference when the methyl group is positioned at even numbered carbons in the chain, whereas the enantiopreference was shifted to (*R*) for the odd numbered ones. To be able to fully discuss these intriguing results requires more intimate knowledge of the mechanisms involved in the enantiorecognition of the lipase.

In order to analyse the e.e.s of the products an NMR method was employed. Oxazolidin-2-selone **18** (see Scheme 3) has been successfully used as an auxiliary for ^{77}Se NMR analysis of the enantiomeric excess of some methyl alkanoic acids, e.g. for 5-methylheptanoic acid.[33] Thus, we successfully used the ^{77}Se NMR method[34] on the 3-, 4-, 5-, and 6-methyldecanoic acids and we are currently studying the possibility to improve the method in order to use it for the 7- and 8-methyldecanoic acids as well.

The selone derivatives were obtained as follows. The appropriate acid was first converted into the acid chloride using oxalyl chloride. This chloride was then reacted with enantiomerically pure (*S*)-4-isopropyl-oxazolidine-2-selone **18** catalysed by diisopropylethylamine which gave the wanted selone derivatives as a mixture of two diastereomers (Scheme 3).

It is known that when reacting other branched methyl carboxylic acids with oxazolidine-2-selones no epimerisation occurs during the coupling.[35,36] Only when using extremely sterically demanding acid chlorides kinetic resolution may be observed.[34] Neither does the purification selectively enrich one of the diastereoisomers.[35,36]

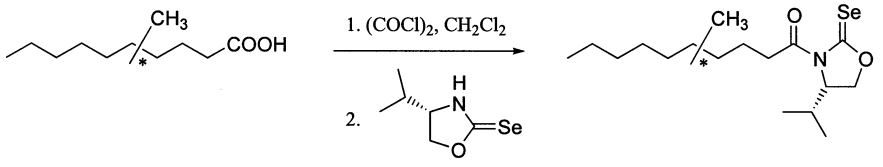
3. Conclusions

In conclusion, the utility of CRL as an enantioselective catalyst for the esterification of racemic alkanoic acids with a remotely located methyl group has clearly been demonstrated by the results presented herein. The obtained *E* values were surprisingly high and the lipase showed good enantioselectivity ($E=25$) even when the methyl branch is positioned at carbon 8 in the decanoic acid substrate. It is probably possible to increase this selectivity even more if the reaction conditions are optimised, i.e. to use the right solvent, water activity, reaction temperature, chain length of the *n*-alcohol etc. This capacity, when it comes to enantiorecognition by this lipase, supplies the organic chemist with a very powerful tool when methyl branched products are to be synthesised.

4. Experimental

4.1. General methods

Commercially available chemicals were used without further purification unless otherwise stated. *Candida rugosa* Lipase (CRL) were obtained from Sigma (St. Louis, Mo., USA) and stored at 4°C over dry silica gel. Dry Et_2O ($LiAlH_4$), *n*-hexane ($LiAlH_4$), Et_3N (CaH_2),



19

Scheme 3. Enantiomerically pure (*S*)-4-isopropyl-oxazolidine-2-selone **19** and the corresponding methyldecanoic acids **1**–**6** were reacted to prepare diastereomeric selone amides.

CH_2Cl_2 (CaH_2) and pyridine (CaH_2) were distilled from the indicated drying agent. Reactions sensitive to moisture or oxygen were performed under an atmosphere of argon. Preparative liquid chromatography (LC) was performed on straight phase silica gel (Merck 60, 230–400 mesh, 0.040–0.063 mm) employing a gradient technique using an increasing concentration of distilled diethyl ether in distilled *n*-pentane or of distilled ethyl acetate in distilled cyclohexane (0–100%), as eluent. Unless otherwise stated, NMR spectra were recorded on a Bruker DMX 250 (250 MHz ^1H and 62.9 MHz ^{13}C) spectrometer using $CDCl_3$ as solvent and TMS as internal reference. Optical rotations were measured on a Perkin–Elmer 241 polarimeter using a 1 dm cell. Mass spectra were recorded on a Saturn 2000 instrument, operating in the EI mode, coupled to a Varian 3800 GC instrument. Unless otherwise stated, conversions and purities were monitored on a 30 m×0,25 mm I.D. capillary column coated with EC-1, $d_f = 0.25$ μm; carrier gas N_2 100 kPa, split ratio 30:1, (flow 1 mL/min). Boiling points are uncorrected and given as air-bath temperatures (bath temp./mbar) in a bulb-to-bulb (Büchi-GKR-51) apparatus.

4.2. Synthesis of 3-methyldecanoic acid 1

4.2.1. 2-Methyl-1-nonanol 7. $LiAlH_4$ (3.13 g, 82.4 mmol) in dry Et_2O (140 mL) was stirred under argon. 2-Methylnonanoic acid[37] (14.4 g, 83.7 mmol) dissolved in dry Et_2O (30 mL) was slowly added at room temperature during 1.5 h. The mixture was stirred for 1 h and then quenched carefully with an excess of 3 M HCl (50 mL). The mixture was heated under reflux for 0.5 h followed by extraction of the aqueous phase with Et_2O (3×50 mL). The organic phase was dried over $MgSO_4$ and concentrated to give 2-methyl-1-nonanol (12.3 g, 77.8 mmol) as an oil with >99.5% purity by GC. The analytical and spectroscopic data were concordant with those reported in the literature.[37]

4.2.2. 1-Bromo-2-methylnonane 8. Triphenylphosphine (24.8 g, 94.6 mmol) was dissolved in CH_2Cl_2 (115 mL, pre-dried over 4 Å molecular sieves) and after cooling to 0°C slow addition of bromine (4.80 mL, 94.3 mmol) followed during 1.67 h. The mixture was left stirring for 0.5 h when the 2-methyl-1-nonanol (12.0 g, 75.9 mmol) was added into the solution over 1 h and the reaction mixture was allowed to stir overnight. The solvent was evaporated and the residue treated with *n*-pentane followed by filtration through silica gel. This gave 1-bromo-2-methylnonane (13.6 g, 61.8 mmol) in 81% yield, >99.5% GC-purity, bp 105–107°C/13 mbar. Lit.[37] 76°C/8 mmHg. The analytical and spectroscopic data were similar to those reported in the literature.[37]

Following the protocol reported in Vogel[38] freshly distilled 1-bromo-2-methylnonane (12.5 g, 56.8 mmol) was dissolved in dry Et_2O (50 mL). The solution was added dropwise into dry Et_2O (2 mL) containing Mg (1.46 g, 60.1 mmol) under stirring. The mixture was heated under reflux for 0.5 h and then cooled to 0°C when solid CO_2 (50 g, 1.4 mol) was added in small portions. After stirring for 0.5 h, 3 M H_2SO_4 (29.3 mL) was added and the organic phase separated. The aqueous phase was extracted with Et_2O (3×20 mL). The combined organic phase was extracted with Na_2CO_3 (3×25 mL, 15% aq.), the pooled water phase was then acidified with 6 M HCl to pH 1 and extracted with Et_2O (3×40 mL). The combined ether phases were washed with brine, dried ($MgSO_4$) and concentrated to give an oil which, after distillation [bp 118–120°C/1.5 mbar (lit.[39] 125°C/0.3 Torr)] yielded the title acid **1** (7.29 g, 39.2 mmol) with >99.5% purity by GC. ^1H NMR δ: 0.88 (3H, t, $J = 6.8$ Hz), 0.97 (3H, d, $J = 6.6$ Hz), 1.27 (12H, bs), 1.94–1.99 (1H, m), 2.09–2.19 (1H, dd, $J = 8.1$ and 14.9 Hz); 2.32–2.40 (1H, dd, $J = 5.9$ and 14.9 Hz). ^{13}C NMR δ: 14.1, 19.7, 22.7, 26.9, 29.3, 29.7, 30.2, 31.9, 36.7, 41.6, 179.8. The IR data were similar to those reported in the literature.[26]

4.3. Synthesis of 4-methyldecanoic acid 2

4-Methyldecanoic acid **2** was prepared according to a method for the synthesis of 4-methyldodecanoic acid,[19] but from 4-methyl-1-decanol[24] (0.920 g, 5.35 mmol). This was dissolved in acetone (10 mL) and stirred at 0°C. Jones' reagent (2.5 mL, 2.6 M solution of H_2CrO_3 in H_2SO_4/H_2O) was added dropwise (0.1 h). The mixture was stirred at room temperature for 0.5 h and then filtered through a Celite pad followed by washing with Et_2O (15 mL). The pooled organic phase was extracted with NaOH (15 mL, 0.1 M aq.), the combined aqueous phase acidified to pH 1 with 6 M HCl followed by extraction with Et_2O (3×30 mL). After drying ($MgSO_4$), evaporation of the solvent and distillation bulb-to-bulb the title compound **2** was obtained as an oil (0.70 g, 3.8 mmol). Bp 96°C/0.5 mbar, (lit.[39] 153–155°C/10 Torr). ^1H NMR δ: 0.88 (3H, d, $J = 5.9$ Hz), 0.88 (3H, t, $J = 6.7$ Hz), 1.26 (10H, bs), 1.36–1.52 (2H, m), 1.63–1.72 (1H, m); 2.31–2.39 (1H, m). ^{13}C NMR δ: 14.1, 19.2, 22.7, 26.9, 29.5, 31.6, 31.9, 31.9, 32.3, 36.6, 180.7.

4.4. Synthesis of 5-methyldecanoic acid 3

4.4.1. 2-Methyl-1-heptanol 9. 2-Methylheptanoic acid[15] (7.02 g, 48.8 mmol) was dissolved in dry Et_2O (15 mL) and then added dropwise into a slurry of $LiAlH_4$ (2.23 g, 58.8 mmol) in Et_2O (20 mL) at ambient temperature and stirred for 1 h. The reaction was quenched with 6 M HCl over a period of 0.5 h. After extraction with Et_2O (3×200 mL), the organic phase was washed with brine (150 mL) and dried ($MgSO_4$). After filtration and evaporation of the solvent, an oil was obtained (6.26 g, 47.8 mmol,) pure by GC analysis. The IR and ^1H NMR spectra data were similar to those reported earlier.[40]

4.4.2. 1-Chloro-2-methylheptane 10. The above alcohol **9** (6.08 g, 46.8 mmol) and triphenylphosphine (14.1 g, 53.9 mmol) were stirred with CCl_4 (12.0 mL, 124 mmol). The reaction was heated under reflux for 1 h. MeOH (0.35 mL) was added and the mixture was allowed to stir for 0.3 h at room temperature followed by LC with *n*-pentane as eluent. The chloride product fractions were collected and the solvent was distilled off to give a colourless oil (6.57 g, 51.2 mmol) pure by GC.

The identity of the chloride was checked with ^{1}H NMR and then directly used in the next step.

Li (3.55 g, 512 mmol) was cut into small pieces under argon and washed with dry *n*-hexane and then stirred with *n*-hexane (15 mL) under an argon atmosphere. The 1-chloro-2-methylheptane from above (3.80 g, 27.8 mmol) was dissolved in *n*-hexane (10 mL) and added dropwise followed by reflux for 4 h. Following the protocol of Eriksson et al.[25] the solution of 1-lithio-2-methylheptane in *n*-hexane was cooled to room temperature and then transferred into a dropping funnel equipped with a glass filter to remove remaining lithium. The alkyllithium solution was added dropwise into a suspension of dry CuI (5.30 g, 27.8 mmol, predried under vacuum at 90°C) in dry Et_2O at –15°C. The reaction mixture was kept at –15°C for 0.75 h, then lowered to –78°C, trimethylsilyl iodide (7.03 g, 35.2 mmol) followed by methyl acrylate (1.62 g, 18.8 mmol) was added via syringe and the mixture was stirred overnight. Dry triethylamine (14.2 mL, 102 mmol) was added to the reaction mixture at –5°C and the reaction was stirred at ambient temperature for 6 h and then acidified to pH 1–2 using 3 M HCl. After filtering through Celite (which was subsequently washed with several portions of Et_2O), the combined ether phase was extracted with NaOH (2× 200 mL, 10% aq.). The NaOH solution was acidified with 3 M HCl to pH 1–2 and extracted with CH_2Cl_2 (3×250 mL). Drying of the organic phase ($MgSO_4$) followed by evaporation of the solvent afforded the crude (80% purity by GC) methyl 5-methyldecanoate (7.0 mmol, 1.5 g). The ester was added to a solution of MeOH (15 mL), NaOH (15 mL, 6 M) and 1,4-dioxane (4 mL) and after 3 h the reaction mixture was extracted with Et_2O (3×10 mL). The remaining aqueous layer was acidified with 6 M HCl to pH 1–2, extracted with Et_2O (3×50 mL) then the pooled organic phases ($MgSO_4$) were dried followed by evaporation of the solvent which yielded the title acid **3** (1.47 g, 7.90 mmol) with 95% purity by GC. Bp 97°C/0.5 mbar (lit.[40] 167–168°C/20 Torr). ^{1}H NMR δ: 0.86 (3H, d, $J=6.4$ Hz), 0.88 (3H, t, $J=7.7$ Hz), 1.26 (12H, bs), 1.59–1.66 (1H, m), 2.34 (2H, t, $J=7.5$ Hz). ^{13}C NMR δ: 11.4, 19.5, 22.3, 22.7, 26.7, 32.2, 32.5, 34.4, 36.4, 36.8, 180.2.

4.5. 6-Methyldecanoic acid 4

4.5.1. 2-Methyl-1-hexanol 11. Reduction as above but from 2-methylhexanoic acid[37] (10 g, 77 mmol) gave an oil (8.04 g, 69.3 mmol) which was >99.7% alcohol **11** by GC analysis. The ^{1}H NMR spectrum was similar to that reported in the literature.[42]

4.5.2. 1-Bromo-2-methylhexane. Following the protocol in the literature[38] alcohol **11** (8.04 g, 69.3 mmol) and red phosphorus (0.71 g, 23 mmol) was stirred at 0°C when bromine (5.58 g, 34.9 mmol) was added carefully and the resulting mixture was allowed to reach ambient temperature. The product was then distilled under reduced pressure to yield the pure alkyl bromide (11.2 g, 63.9 mmol). The analytical and spectroscopic data were identical to those previously reported.[37,43]

4.5.3. 4-Methyloctanoic acid 12. Following the method given by Berglund et al.[12] sodium metal (0.57 g, 25 mmol) was dissolved in ethanol (16 mL, 99.5%) and diethyl malonate (4.18 g, 26.1 mmol) was added. The mixture was heated under reflux for 0.12 h, cooled to room temperature and the alkyl bromide (4.40 g, 25.1 mmol) obtained above was added in portions during 0.12 h. The reaction mixture was heated under reflux for 1 h when a few drops of acetic acid were added and the ethanol was evaporated off. Water was added to the remaining material and the organic phase was separated. The aqueous phase was extracted with Et_2O (3×25 mL) and the combined organic phases were washed with brine and dried ($MgSO_4$). The Et_2O was evaporated, the resulting diester was added to a solution of KOH (5.5 g, 98 mmol) in ethanol (40 mL, 95%) and heated under reflux for 4 h. Most of the ethanol was distilled off and water (50 mL) was added, the mixture was washed with Et_2O (2×25 mL), the remaining water phase was acidified with HCl (conc.) and the organic layer was separated. The water phase was extracted with Et_2O (3×25 mL). The organic phase and extracts were combined, washed with water (25 mL) and brine (25 mL) after evaporation of the solvent the resulting dioic acid was heated to 190°C for 3 h. After cooling $NaCO_3$ (aq. satd 25 mL) was added and the resulting solution was washed with Et_2O (2×25 mL). The aqueous phase was acidified with HCl (conc.), the organic layer was separated and the aqueous phase was extracted with Et_2O (2×25 mL). The combined organic phase was washed with brine (25 mL) and dried ($MgSO_4$). Evaporation of solvent afforded the pure 4-methyloctanoic acid (2.78 g 17.6 mmol) as an oil. The IR and MS spectra were similar to those reported.[44]

4.5.4. 4-Methyloctan-1-ol. The above acid (2.78 g, 17.6 mmol) was reduced as above using $LiAlH_4$ to give the pure 4-methyloctan-1-ol (2.00 g, 13.8 mmol). The IR and MS spectra were similar to those reported.[44]

4.5.5. 1-Bromo-4-methyloctane 13. 4-Methyloctan-1-ol (2.00 g 13.8 mmol) was stirred with red phosphorus (0.145 g, 4.68 mmol) as above to yield after distillation 1-bromo-4-methyloctane (2.39 g, 11.5 mmol). The IR and MS spectra were similar to those reported in the literature.[44]

Similarly, as above a malonic ester sequence was performed from 1-bromo-4-methyloctane (2.39 g, 11.5 mmol) this yielded after bulb-to-bulb distillation 1.10 g (5.29 mmol) of the title acid **4** as an oil in >99% purity by GC. Bp 95°C/0.5 mbar (lit.[41] 164–165°C/18 Torr). ^{1}H NMR δ: 0.84 (3H, d, $J=6.4$ Hz), 0.89 (3H, t, $J=7.6$ Hz), 1.26–1.41 (11H, bs and m), 1.56–1.67 (2H, m), 2.36 (2H, t, $J=7.6$ Hz). ^{13}C NMR δ: 14.2, 19.6, 23.0, 24.0, 26.5, 29.33, 32.6, 34.1, 36.6, 36.7, 180.2. The IR and MS spectra were similar to those reported in the literature.[43]

4.6. Methyldecanoic acid 5

4.6.1. 1-Bromo-2-methylpentane. The procedure used above for the preparation of 1-bromo-2-methylhexane, was applied starting from 2-methyl-1-pentanol (49.7 g, 487 mmol). After distillation at atmospheric pressure a main fraction at 145°C (lit.[45] 51°C/52 Torr) of a colourless oil (67.9 g, 414 mmol) pure by GC. The IR and ^{1}H NMR spectra were similar to those reported.[46,47]

4.6.2. 4-Methylheptanoic acid. A malonic ester sequence (see above) but starting from 1-bromo-2-methylpentane (67.3 g, 408 mmol) gave 4-methylheptanoic acid (42.3 g, 294 mmol) in >99% purity by GC. The IR and ^{1}H NMR spectra were similar to those reported.[48]

4.6.3. 4-Methyl-1-heptanol. 4-Methylheptanoic acid (26.6 g, 180 mmol) was reduced using $LiAlH_4$ (see above) and gave 4-methyl-1-heptanol (21.1 g, 162 mmol) with a chemical purity of >99,7% by GC. The IR and ^{1}H NMR spectra were similar to those reported.[48]

4.6.4. 1-Bromo-4-methylheptane 14. 4-Methyl-2-heptanol (9.70 g, 74.6 mmol) was reacted with Ph_3PBr_2 as described above and this gave (12.2 g, 63.4 mmol) of the title product with a chemical purity of >99.5% by GC. The identity of the bromide was checked with ^{1}H NMR and directly used in the next step below.

4.6.5. 1-Lithio-4-methylheptane. Lithium metal (4.99 g, 720 mmol) was cut into pieces into dry hexane,[49] the mixture was heated under reflux for 0.5 h and 1-bromo-4-methylnonane **14** (7.00 g 36.0 mmol) in dry hexane (7 mL) was added dropwise over 1.25 h. The reflux was continued for 0.5 h after the addition was completed.

4.6.6. Dilithium methyl-(4-methyl-heptyl)-cyanocuprate. Similarily prepared as in the literature for other cyanocuprates.[50] Methyllithium (36.0 mmol, 23.0 mL 1.5 M solution in Et_2O) was added to a suspension of cuprous cyanide (3.22 g, 36.0 mmol, dried in an oven at 105°C and further dried by mixing with dry toluene at room temperature followed by evaporation at reduced pressure). The solid was suspended in dry *n*-hexane (50 mL) at –60°C. The mixture was stirred for 1 h and then allowed to warm to 0°C, again cooled to –60°C and at that temperature the solution of 1-lithio-4-methylheptane from above was slowly added.

Following the method reported by Christenson et al.,[51] to the resulting mixture methyl acrylate (3.10 g, 36.0 mmol) dissolved dry *n*-hexane (3 mL) was added dropwise at –60°C. The mixture was stirred overnight and allowed to reach room temperature. After addition of NH_4Cl (satd in conc. ammonia) the organic layer was separated and the blue aqueous layer extracted with Et_2O (3×100 mL). The combined organic phases were washed with water (100 mL), brine (100 mL) and dried ($MgSO_4$). Evaporation of the solvent gave a brown oil which was subjected to hydrolysis in KOH solution (2.5 M, ethanol). The reaction mixture was then washed with Et_2O (100 mL) and the water phase was acidified with HCl (conc.). Extraction of this water phase with Et_2O (3×100 mL), drying ($MgSO_4$) and evaporation of the solvent gave a yellow oil, which was distilled to yield the title acid **5** (1.77 g, 9.52 mmol) with >99.0% purity by GC. Bp 97°C/0.5 mbar (lit.[52] 157–158°C/10 Torr). ^{1}H NMR δ: 0.85 (3H, d, J=6.4 Hz), 0.88 (3H, t, J=6.9 Hz), 1.05–1.19 (2H, m), 1.21–1.40 (2H, m), 1.62–1.68 (2H, m); 2.36 (2H, t, J=7.7 Hz). ^{13}C NMR δ: 14.4, 19.6, 20.1, 24.7, 26.8, 29.5, 32.4, 34.0, 36.9, 39.3, 180.1.

4.7. 8-Methyldecanoic acid 6

4.7.1. Ethyl β-methyl-5-(1-(2-methyl)butyl)-2-thiophene acetate 16. Freshly distilled ethyl 2-thiopheneacetate (7.91 g, 46.5 mmol), 2-methylbutanoyl chloride (5.02 g, 41.7 mmol) and dry CH_2Cl_2 (150 mL). Into this stirred mixture was at 0°C $SnCl_4$ (150 mL, 140 mmol) was added dropwise. After 2.5 h, 6 M HCl (50 mL) was added, then followed extraction with Et_2O (3×50 mL), washing with brine (100 mL), drying ($MgSO_4$), filtration and concentration to give a crude product. After LC and distillation (145°C/1.5 mbar) the acylated compound (8.05 g, 31.7 mmol) in >99% purity by GC was obtained. m/z (relative intensity) 254 (M^+, 12%), 226 (15), 197 (100), 169 (30), 147 (5), 124 (12), 96 (15), 73 (20). ^{1}H NMR δ: 0.92 (3H, t, J=7.4 Hz), 1.20 (3H, d, J=6.9 Hz), 1.29 (3H, t, J=7.1 Hz), 1.42–1.59 (1H, m), 1.74–1.90 (1H, m), 3.13–3.24 (1H, m), 4.16–4.25 (2H, q, J=7.1 Hz), 6.98–7.00 (1H, dt, J=0.8 and 3.8 Hz), 7.59 (1H, d, J=3.8 Hz). ^{13}C NMR δ: 11.9, 14.1, 14.2, 17.3, 27.1, 36.1, 43.8, 61.6, 128.0, 131.5, 143.8, 144.1, 169.5, 197.2. HRMS calc. for $C_{13}H_{18}O_3S$: 254.0977. Obs: 254.0964.

4.7.2. Ethyl β-methyl-5-(2-methylbutanoyl)-2-thiophene acetate 17. Using the method of Di Vona and Rosnati[53] the acetate from above (6.50 g, 25.6 mmol) was added to a slurry of amalgamated zinc[54] (16.0 g) in acetic acid (250 mL) at room temperature. After 12 h, the catalytic amalgam was filtered off and washed with *n*-pentane (2×50 mL). Then water (250 mL) was added and after extraction with *n*-pentane (5×200 mL) the organic phase was washed with $NaHCO_3$ (200 mL, satd aq.), the organic phase was then dried ($MgSO_4$) and concentrated. The crude product was purified by LC to afford compound **17** (5.04 g, 23.6 mmol, 99% pure by GC). Bp 145°C/0.63 mbar. ^{1}H NMR δ: 0.90 (3H, d, J=6.6 Hz), 0.90 (3H, t, J=7.3 Hz), 1.09–1.30 (1H, m), 1.27 (3H, t, J=7.1 Hz), 1.31–1.48 (1H, m), 1.57–1.68 (1H, m), 2.51–2.60 (1H, dd, J=7.7 and 14.5 Hz), 2.71–2.79 (1H, dd, J=6.1 and 14.5 Hz), 3.75 (2H, d, J=0.7 Hz), 4.18 (2H, q, J=7.1 Hz), 6.58–6.60 (1H, dt, J=0.8 and 3.4 Hz), 6.71–6.74 (1H, dt, J=0.9 and 3.4 Hz). ^{13}C NMR δ: 11.4, 14.2, 19.0, 29.0, 35.8, 36.9, 37.3, 61.1, 124.6, 126.3, 132.7, 144., 170.7. HRMS calc. for $C_{13}H_{20}O_2S$: 240.1184. Obs: 240.1171.

The acetate from above (4.80 g, 22.4 mmol) was dissolved in isopropanol (80 mL), Raney-Ni (12 teaspoons) was added and then the slurry was stirred under H_2 for 48 h. The Raney-Ni was filtered off, rinsed with EtOH (95%, 4×100 mL) and then the solvent was evaporated off. Without further purification this crude product was dissolved in a solution of 3

M NaOH/MeOH (80 mL) and 1,4-dioxane (56 mL) and stirred overnight. The mixture was acidified by 6 M HCl (aq.) and extracted with *n*-pentane (5×150 mL). The pooled organic phases were washed with brine (150 mL), dried ($MgSO_4$), concentrated and after bulb-to-bulb distillation the title acid **6** (3.35 g, 18.0 mmol) was isolated in >99% purity by GC. Bp 95°C/0.5 mbar (lit.[52] 161–164°C/11 Torr). ^{1}H NMR δ: 0.84 (3H, d, J=6.7 Hz), 0.85 (3H, t, J=7.2), 1.05–1.35 (11H, m and bs), 1.63 (2H, qui, J=7.1 Hz), 2.35 (2H, t, J=7.5 Hz). ^{13}C NMR δ: 11.4, 19.2, 24.7, 26.9, 29.1, 29.5, 29.6, 34.1, 34.4, 36.5, 180.3. HRMS calc. for $C_{11}H_{22}O_2$: 186.1620. Obs: 186.1573.

4.8. CRL-catalysed esterification: general procedure

A cyclohexane solution (1 vol.) containing the methyldecanoic acid (0.15 M), 1-hexadecanol (0.15 M), eicosane (25.0 mg/mL solution) as an internal standard and Na_2SO_4/Na_2SO_4×10 H_2O (0.1 mmol/mL solution, a_w= 0.8) was stirred at 700 rpm for 0.25 h in a sealed flask at ambient temperature. Immobilised[13] CRL (Im-CRL, 20.3 mg/mL solution) was then added. The reaction was monitored by GC and stopped at the appropriate stage of conversion by separating the enzyme through filtration and washing with cyclohexane (0.5 vol.) and pentane (0.5 vol.). The product ester and remaining substrate acid were separated by LC and the substrate acid was obtained pure after bulb-to-bulb distillation. The product ester was subjected to chemical hydrolysis as follows: (example: hexadecanyl 3-methyldecanoate) The product ester (0.230 mmol) was dissolved in 1,4-dioxane (1.5 mL) and 3 M KOH/MeOH (2 mL) and stirred at room temperature. Complete hydrolysis was reached after 1 h (monitored by GC). Dilution with water and washing of the aqueous layer with Et_2O/*n*-pentane (1:1, 4×5 mL) was followed by acidification (pH 1) with 2 M HCl. After extraction with Et_2O (4×5 mL) the organic phase was washed with H_2O (5 mL) and brine (5 mL). After drying ($MgSO_4$) and evaporation of the solvent the crude methyl decanoic acid was obtained, which was subjected to bulb-to-bulb distillation. See Table 1 for e.e.s and $[\alpha]_D^{25}$ values on the different acids.

4.9. Preparation of the amide adducts obtained from (*S*)-4-isopropyl-oxazolidine-2-selone and the appropriate enantiomerically enriched methyldecanoic acid: general procedure

The method is exemplified as follows: Enantiomerically enriched 3-methyldecanoic acid **1** (54 mg, 0.29 mmol) in dry CH_2Cl_2 and oxalyl chloride [$(COCl)_2$, 1.45 mL, 2.90 mmol] was stirred for 4 h. The excess solvent and reagents were removed in vacuo to furnish the crude acid chloride.

(*S*)-Oxazolidine-2-selone **19** (66.8 mg, 0.348 mmol) was stirred in distilled CH_2Cl_2 (2 mL). The mixture was cooled in an ice bath and diisopropylethylamine (DIPEA, 49.5 mg, 0.383 mmol) was added followed by the acid chloride prepared above. The reaction was followed by TLC and allowed to stir overnight. The crude reaction mixture was passed through a pad of silica gel using CH_2Cl_2 and after removal of the solvent in vacuo the crude amide product was purified by LC. The purified product was collected (95.7 mg, 0.320 mmol) at 40% CH_2Cl_2/*n*-hexane and the recovered oxazolidine-2-selone was collected at 10% EtOAc/*n*-hexane. All other (*S*)-oxazolidine-2-selone amide derivatives were obtained in a similar manner as presented above for acid **1**.

4.10. Determination of the diastereomeric ratio for the selone amide adducts obtained as above using ^{77}Se NMR measurements: general procedure

The ^{77}Se NMR spectra were recorded as $CDCl_3$, [$^2H_6$]benzene, or [$^2H_8$]toluene solutions on a Bruker DMX 500. The ^{77}Se chemical shifts are expressed in ppm relative to diphenyldiselenide (465 ppm), which is deshielded with respect to a 60% (v/v) solution of $(CH_3)_2Se$ in $CDCl_3$ (0 ppm).[55,56] Typically, spectra were obtained in the Fourier transform mode at 95.4273180 Hz. Measurements were made at, or near, ambient probe temperature in 5 mm NMR tubes using deuterated solvents as an internal lock solvent. All spectra were acquired in the proton-decoupled mode. For resonances which were tightly positioned all three solvents were examined. In addition, in some cases the use of toluene at elevated temperatures allowed for base line resolution. The resulting ^{77}Se chemical shifts for derivatives obtained from different racemic methyl decanoic acids are presented in Table 2. Selone amides prepared from enantiomerically enriched methyldecanoic acids gave in the ^{77}Se NMR analyses diastereomeric ratios from which $e.e._s$ and $e.e._p$ values were calculated (see Table 1).

4.11. (*S*)-4-Methyldecane

The enantiomerically enriched remaining substrate acid from CRL-catalysed esterification of 7-methyldecanoic

Table 2. ^{77}Se NMR experimental data for the selone acid adducts

(*S*)-Oxazolidine-2-selone derivative of the following racemic acid	Solvent	Temperature (°C)	^{77}Se NMR δ ppm	^{77}Se NMR δ ppm	^{77}Se NMR $\Delta\delta$ ppm
3-Methyldecanoic acid **1**	$CDCl_3$	25	443.069	440.731	2.338 ± 0.117[a]
4-Methyldecanoic acid **2**	$CDCl_3$	25	441.125	440.685	0.440 ± 0.022[a]
5-Methyldecanoic acid **3**	$CDCl_3$	25	438.752	438.655	0.097 ± 0.005[a]
6-Methyldecanoic acid **4**	$CDCl_3$	25	442.111	442.025	0.086 ± 0.004[a]
6-Methyldecanoic acid **4**	$CDCl_3$	40	450.046	450.132	0.086 ± 0.004[a]
7-Methyldecanoic acid **5**	Toluene	25	460.096	460.085	0.011 ± 0.001[a]
8-Methyldecanoic acid **6**	Not tested	Not tested	Not tested	Not tested	Not tested

[a] Based on the signal to noise ratio.

acid **5** (100 mg, 0.540 mmol) was dissolved in dry Et_2O (2 mL) and added to a suspension of $LiAlH_4$ (20.0 mg, 0.540 mmol) in dry Et_2O (10.0 mL). The mixture was stirred for 1 h and then 3 M HCl (10.0 mL) was added cautiously. The aqueous phase was extracted with Et_2O (10×2 mL). The organic phase was washed with 10% $NaHCO_3$ (20 mL), brine (20 mL) and dried ($MgSO_4$). The solvent was evaporated off to give an alcohol (88.2 mg, 0.513 mmol) as an oil in >99.5% purity by GC. This alcohol was dissolved in dry pyridine (1.0 mL) at 0°C, *p*-toluenesulphonyl chloride (146 mg, 0.770 mmol) was added to the solution and then the mixture was stirred overnight. The reaction was quenched by the addition of a slurry (2 mL) of ice and 6 M HCl. The aqueous phase was extracted with Et_2O (4×3 mL). The pooled organic phases were washed with H_2O (10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL). After drying ($MgSO_4$) and evaporating of the solvent the tosylate (135 mg, 0.436 mmol) was obtained pure by TLC. This was subjected to reduction ($LiAlH_4$) in the same manner as above to yield 64.6 mg (0.372 mmol) of the title compound as an oil with a chemical purity of >99.5% by GC. Bp 68–70°C/13 mbar, (lit.[32] 71–72°C/12 Torr). $[\alpha]_D^{25}$+0.78 (*c* 6.46, CH_2Cl_2) (lit.[32] $[\alpha]_D^{25}$+1.39 (neat)).

4.12. (*R*)-5-Methyldecane

The title substance was prepared in the same manner as for (*S*)-4-methyldecane described above but from enantiomerically enriched remaining substrate acid from the CRL-catalysed esterification of 6-methyldecanoic acid **4**. The alkane (45 mg, 0.29 mmol) was obtained in >99.5% chemically purity by GC. Bp 65–67°C/11 mbar (lit.[30] 94°C/30 Torr). $[\alpha]_D^{25}$ –0.25 (*c* 4.5, EtOH), (lit.[30] $[\alpha]_D^{25}$ –0.39 (neat)).

4.13. (*R*)-4-Methyl-1-decanol

The title alcohol (35 mg, 0.20 mmol) was obtained with a chemical purity of >99.5% via reduction ($LiAlH_4$) of the enantiomerically enriched remaining substrate, the (*R*)-4-methyldecanoic acid **2** as above. Bp 117–118°C/13 mbar (lit.[30] 80°C/0.1 Torr). $[\alpha]_D^{25}$ +0.7 (*c* 3.5, $CHCl_3$), (lit.[30] $[\alpha]_D^{25}$ –1.1 (*c* 5.33, $CHCl_3$) for (*S*)-4-methyl-1-decanol). The ^1H NMR spectrum was similar to that reported in the literature.[30]

Acknowledgements

We wish to thank Mr. Olle Smitt, Mr. Can Cakmak and Mr. Tobias Linde for the syntheses of the 4-methyl-1-decanol, the 3-methyldecanoic acid and the 6-methyldecanoic acid, respectively. The help from Mr. Ryszard Michalczyl in running the ^{77}Se NMR spectra is gratefully acknowledged. The research was supported by the Swedish Natural Science Research Council (NFR), and the Swedish Council for Forestry and Agricultural Research (SJFR).

References

1. Poppe, L.; Novak, L. *Selective Biocatalysis*; VCH: Weinheim, Germany, 1992.
2. Halgas, J. *Biocatalysis In Organic Synthesis*; Elsevier: New York, 1992.
3. Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.
4. Drauz, K.; Waldmann, H. *Enzyme Catalysis in Organic Synthesis*; VCH: Weinheim, Germany, 1995.
5. Faber, K. *Biotransformations in Organic Chemistry*, 3rd ed.; Springer-Verlag: Heidelberg, Germany, 1997.
6. Silverman, R. B. *The Organic Chemistry of Enzyme-catalysed Reactions*; Academic Press, 2000.
7. Kazlauskas, R. J. *Curr. Opin. Chem. Biol.* **2000**, *4*, 81–88.
8. Grochulski, P.; Li, Y. G.; Schrag, J. D.; Bouthillier, F.; Smith, P.; Harrison, D.; Rubin, B.; Cygler, M. *J. Biol. Chem.* **1993**, *268*, 12843–12847.
9. Grochulski, P.; Li, Y.; Schrag, J. D.; Cygler, M. *Protein Sci.* **1994**, *3*, 82–91.
10. Holmberg, E.; Holmquist, M.; Hedenström, E.; Berglund, P.; Norin, T.; Högberg, H.-E.; Hult, K. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 572–578.
11. Engel, K. H. *Tetrahedron: Asymmetry* **1991**, *2*, 165–168.
12. Berglund, P.; Holmquist, M.; Hedenström, E.; Hult, K.; Högberg, H.-E. *Tetrahedron: Asymmetry* **1993**, *4*, 1869–1878.
13. Berglund, P.; Vörde, C.; Högberg, H.-E. *Biocatalysis* **1994**, *9*, 123–130.
14. Edlund, H.; Berglund, P.; Jensen, M.; Hedenström, E.; Högberg, H.-E. *Acta Chem. Scand.* **1996**, *50*, 666–671.
15. Nguyen, B.-V.; Hedenström, E. *Tetrahedron: Asymmetry* **1999**, *10*, 1821–1826.
16. Kazlauskas, R. J.; Bornscheuer, U. T. In *Biotechnology*; Kelly, D. R., Ed., 2nd ed.; Wiley: Weinheim, 1998; Vol. 8a, pp. 37–191.
17. Berglund, P.; Hult, K. In *Biocatalytic Synthesis of Enantiopure Compounds Using Lipases*; Ramesh, N. P., Ed. Stereoselective biocatalysis; Marcel Dekker, 2000; pp. 633–657.
18. Berglund, P.; Hedenström, E. In *Methods in Biotechnology*, Vol. 15: Enzymes in Non-aqueous Solvents: Methods and Protocols Vulfson, E. N.; Halling, P. J.; Holland, H. L., Eds. Preparation of 2-, 3- and 4-methylcarboxylic acids and the corresponding alcohols of high enantiopurity by lipase-catalysed esterification; Humana Press: Totowa, NJ, 2000; pp. 307–317.
19. Lundh, M.; Smitt, O.; Hedenström, E. *Tetrahedron: Asymmetry* **1996**, *7*, 3277–3284.
20. Heinsman, N. W. J. T.; Orrenius, S. C.; Marcelis, C. L. M.; De Sousa Teixeira, A.; Franssen, M. C. R.; Van Der Padt, A.; Jongejan, J. A.; De Groot, A. *Biocatal. Biotransform.* **1998**, *16*, 145–162.
21. Varadharaj, G.; Hazell, K.; Reeve, C. D. *Tetrahedron: Asymmetry* **1998**, *9*, 1191–1195.
22. Heinsman, N. W. J. T.; Teixeira, A.; Van der Weide, P. L. J.; Franssen, M. C. R.; Van der Pat, A.; De Groot, A.; Van'T Riet, K. *Biocatal. Biotransform.* **2001**, *19*, 181–189.
23. Heinsman, N. W. J. T.; Valante, A. M.; Smienk, K. G. F.; Van der Pat, A.; Franssen, M. C. R.; De Groot, A.; Van'T Riet, K. *Biotechnol. Bioeng.* **2001**, *76*, 193–199.

24. Hedenström, E.; Edlund, H.; Lund, S.; Abersten, M.; Persson, D. *J. Chem. Soc., Perkin Trans. 1* **2002**, in press.
25. Eriksson, M.; Hjelmencrantz, A.; Nilsson, M.; Olsson, T. *Tetrahedron* **1995**, *51*, 12631–12644.
26. Honma, K.; Tsuda, M.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **1995**, *51*, 3745–3748.
27. Levene, P. A.; Marker, R. E. *J. Biol. Chem.* **1932**, *95*, 153–164.
28. Milburn, A. H.; Truter, E. V. *J. Chem. Soc.* **1954**, 3344–3349.
29. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
30. Raederstorff, D.; Shu, A. Y. L.; Thompson, J. E.; Djerassi, C. *J. Org. Chem.* **1987**, *52*, 2337–2346.
31. Levene, P.; Marker, R. E. *J. Biol. Chem.* **1931**, *91*, 761–772.
32. Letsinger, R.; Traynham, J. G. *J. Am. Chem. Soc.* **1950**, *72*, 849–852.
33. Silks, L. A.; Dunlap, R. B.; Odom, J. D. *J. Am. Chem. Soc.* **1990**, *112*, 4979–4982.
34. Peng, J.; Ashburn, D. A.; Barr, M. E.; Lebioda, L.; Martinez, R. A.; Garber, A. R.; Odom, J. D.; Dunlap, R. B.; Silks, L. A. *J. Org. Chem.* **1995**, *60*, 5540–5549.
35. Silks, L. A.; Peng, J.; Dunlap, R. B.; Odom, J. D. *J. Org. Chem.* **1991**, *56*, 6733–6736.
36. Salvatore, B. A.; Smith, A. B. *Tetrahedron Lett.* **1994**, *35*, 1329–1330.
37. Hedenström, E.; Andersson, F. *J. Chem. Echol.* **2002**, *28*, 1249–1266.
38. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *VOGEL's Textbook of Practical Organic Chemistry*, 5th ed.; Longman Scientific & Technical, 1989; pp. 425–426.
39. Bowman A. JCSOA9 *J. Chem. Soc.* **1951**, 1087–1091.
40. Bergström, G.; Wassgren, A.-B.; Anderbrant, O.; Fägerhag, J.; Edlund, H.; Hedenström, E.; Högberg, H.-E.; Geri, C.; Auger, M. A.; Varama, M.; Hansson, B. S.; Löfqvist, J. *Experimentia* **1995**, *51*, 370–380.
41. Robinson, P. *J. Chem. Soc.* **1945**, 389–393.
42. Norsikian, S.; Marek, I.; Klein, S.; Poisson, J. F.; Normant, J. F. *Chem. Europ. J.* **1999**, *5*, 2055–2068.
43. Dyllick-Brenzinger, R. A.; Patel, V.; Rampersad, M. B.; Stothers, J. B.; Thomas, S. E. *Can. J. Chem.* **1990**, *68*, 1106–1115.
44. Ciocan-Tarta, I.; Oprean, I.; Ghizdavu, I.; Pojar-Fenesan, M. *Rev. Roum. Chim.* **1998**, *43*, 215–220.
45. Krishnamurthy, S.; Brown, H. C. *J. Org. Chem.* **1983**, *48*, 3085–3091.
46. Crowder, J. *Spectrochim. Acta Part A* **1978**, *34*, 707–715.
47. Neubert, M. E.; Shenouda, I. G. *Mol. Cryst. Liq. Cryst.* **1991**, *205*, 29–45.
48. Casey, C. P.; Cyr, C. R. *J. Am. Chem. Soc.* **1973**, *95*, 2240–2247.
49. Kamienski, C. W.; Schwindeman, J. A.; Dover, B. T.; Morrison, R. C. US Patent 5.523.447, 1996.
50. Hedenström, E.; Högberg, H.-E. *Tetrahedron* **1994**, *50*, 5225–5232.
51. Christenson, B.; Ullenius, C.; Håkansson, M.; Jagner, S. *Tetrahedron* **1992**, *48*, 3623–3632.
52. Huenig, S.; Salzwedel, M. *Chem. Ber.* **1966**, *99*, 823–842.
53. Di Vona, M. L.; Rosnati, V. *J. Org. Chem.* **1991**, *41*, 4269–4273.
54. Elphimoff-Felkin, I.; Sarda, P. *Organic Syntheses*; Wiley: New York, 1963; Vol. 4, p. 769.
55. Luthra, N. P.; Dunlap, R. B.; Odom, J. D. *J. Magn. Reson.* **1983**, *52*, 318–322.
56. Luthra, N. P.; Odom, J. D. In *The Chemistry of Organic Selenium and Tellurium Compounds*; Patai, S.; Rappoport, Z., Eds.; Wiley: New York, 1986.