Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: *Asymmetry* 15 (2004) 2893–2899
Tetrahedron: Asymmetry

Lipase-catalysed kinetic resolution of 1-*O*-alkylglycerols by sequential transesterification

Arnar Halldorsson, Pall Thordarson, Bjorn Kristinsson, Carlos D. Magnusson and Gudmundur G. Haraldsson*

Science Institute, University of Iceland, Dunhaga 3, IS-107 Reykjvik, Iceland

Received 28 May 2004; accepted 9 July 2004

Abstract—The natural *S*-configured chimyl, batyl and selachyl alcohols of the 1-*O*-alkylglycerol type were prepared by enantioselective lipase-catalysed transesterification. Their racemates were synthesised in two steps by reacting racemic solketal with the bromides of the corresponding fatty alcohols and a subsequent conversion of the intermediates into the 1-*O*-alkylglycerols by deprotection under acidic aqueous conditions. The *Pseudomonas fluorescens* lipase was employed to kinetically resolve the racemic 1-*O*-alkylglycerols by a sequential diacetylation process to afford them virtually enantiomerically pure. Dramatic enantioselectivity increase was observed for the saturated chimyl (E = 17–32) and batyl (E = 14–38) alcohols at decreased temperature, whereas for the monounsaturated selachyl (E = 12–13) alcohol no such temperature effects were observed.

1. Introduction

Nonpolar ether lipids of the 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol type are major constituents in the liver oils of shark, dogfish and various other species of elasmobranch fish.[1–3] Shark liver oil has been used for a long time as a therapeutic and preventive agent, with 1-*O*-alkyl-*sn*-glycerols as the active constituents.

The 1-*O*-alkyl-*sn*-glycerols, sharing certain similarities with the well-known platelet activating factors, have been claimed to display various beneficial effects on human health.[4,5] Their early clinical use was to prevent radiation sickness from cancer X-ray therapy.[6] Results from scientific research in recent years show that they can be stimulating for the allergic system as well as for the immune control, asthma, psoriasis, arthritis or to speed up the removal of heavy metals from the body.[7]

As the *sn*-terminology implies[8] their natural absolute configuration is *S*. The three most abundant fatty alcohols present in the 1-*O*-alkyl moiety are, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$, the last one being the most abundant. They correspond to chimyl **1a**, batyl **1b** and selachyl **1c** alcohols (Fig. 1), respectively, named after their sources in the liver oils of chimaeras, sharks and rays of the *Chimaeroidei*, *Batoidei* and *Selachoidei* families.

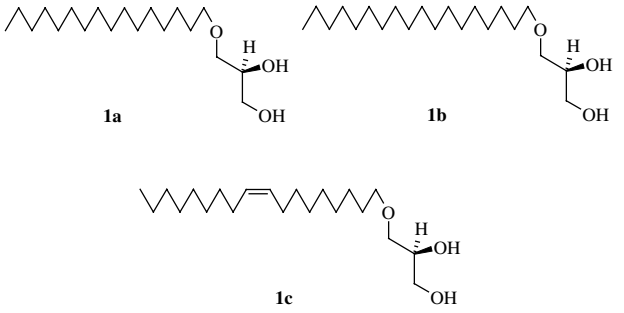


Figure 1. The chemical structure of the three main natural 1-*O*-alkyl-*sn*-glycerols present in shark liver oil: chimyl **1a**, batyl **1b** and selachyl **1c** alcohols.

Lipases are among the most widely applied and versatile biocatalysts in organic synthesis. The most widespread application of lipases has undoubtedly been in asymmetric transformations, which has been reviewed comprehensively.[9–12] Amongst the lipases the pig pancreatic lipase has been the most widely used, but in the course of last 20 years microbial lipases have gained increasing importance, especially the commercially available bacteria lipases from *Pseudomonas*. Lipases exhibit high tolerance for variations in substrate structure and their active

* Corresponding author. Tel.: +354 525 4818; fax: +354 552 8911; e-mail: gghar@raunvis.hi.is

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.07.046

site can be expected to readily accommodate the 1-*O*-alkylglycerols. Although a large variety of diols, including a whole range of 1-*O*-arylglycerols, have been extensively studied in terms of kinetic resolution,[13] there are no reports on the 1-*O*-alkylglycerols of the type described above. As far as we are aware the first and only report dealing with this sort of substrates in lipase-catalysed kinetic resolution is our communication published in 1999.[14]

2. Results and discussion

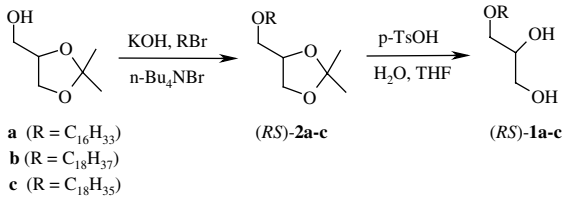
The 1-*O*-alkyl-*sn*-glycerols are highly valuable compounds that can be prepared from the unsaponifiable fraction of various liver oils from elasmobranch fish. However, as a result of the great variety of alkyl groups in their ether linkage moiety they are by no means readily accessible as pure compounds from natural sources.[2] The chief objective of this study was to develop a successful lipase-catalysed process to accomplish the natural *S*-configured chimyl **1a**, batyl **1b** and selachyl **1c** alcohols.

Optically active 1-*O*-alkylglycerols can be readily prepared from enantiopure C_3-synthons possessing one asymmetric centre, for example, solketal (2,2-dimethyl-1,3-dioxolane-4-methanol). As an example, the (*S*)-solketal is advantageously prepared from D-mannitol[15] or by Sharpless dihydroxylation[16] whereas L-serine[17] or ascorbic acid[18] may be converted into the (*R*)-solketal. However, various attempts based on lipase-catalysed kinetic resolution of racemic solketal and its derivatives have been reported.[19] Disappointingly, these processes display poor enantioselectivity due to the symmetrical shape and small size of the solketal molecule. Despite low enantioselectivity of lipases toward solketal its two enantiomers have been successfully prepared enantiopure by lipase.[20,21]

Work highly relevant to that described in this paper has been published by Theil et al., based on the sequential acylation process originally introduced by Sih and coworkers.[22] They resolved various 1-*O*-arylglycerols by lipase-catalysed sequential transesterification.[23] All their substrates agreed with Kazlauskas's empirical rule for predicting the stereochemical outcome of kinetic resolution of secondary alcohols by the *Burkholderia cepacia* lipase (BCL, formerly *Pseudomonas cepacia* lipase).[24] Their investigation took a new direction when a second variety was introduced to the other primary alcoholic group, varying in size and/or shape.[13] It was difficult to fit the results with the Kazlauskas rule, which predicts high *E*-values on secondary alcohols when substituents around the steric centre differ greatly in size but not shape. Therefore, Theil et al. developed a new three-dimensional active site model for BCL using computer modelling, which revealed that the two hydrophobic pockets of the lipase differ in shape, rather than size.[25]

2.1. Synthesis of 1-*O*-alkylglycerols

Racemates of the 1-*O*-alkylglycerols **1a**–**c** were prepared in a two-step synthesis as demonstrated in Scheme 1. In the first step, solketal was treated with grounded potassium hydroxide and the corresponding alkyl bromides at 35–40 °C in the absence of solvent using tetrabutylammonium bromide as a phase-transfer catalyst. This afforded the crude 2,3-*O*-isopropylidene protected 1-*O*-alkylglycerol intermediates **2a**–**c**, which were characterised, but usually introduced directly to the second step without further purification. In the second step, the **2a**–**c** intermediates were deprotected using catalytic amount of *p*-toluenesulfonic acid in a 30% water–THF solution under reflux conditions. The pure racemic 1-*O*-alkylglycerols **1a**, **b** and **c** were afforded in 80%, 80% and 84% overall yields, respectively.

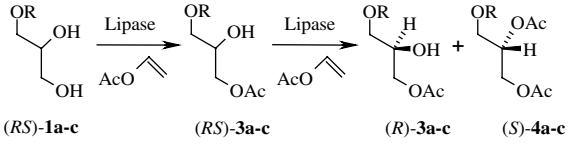


Scheme 1.

The existence of all these compounds is manifested in the literature.[26] However, all the products were fully characterized by high field ^1H and ^{13}C NMR and IR spectroscopy as well as elemental analysis and/or high resolution mass spectrometry.

2.2. Lipase-catalysed transesterification of 1-*O*-alkylglycerols

The kinetic resolution of **1a**–**c** was based on a sequential lipase-catalysed transesterification using vinyl acetate as an acylating agent. In such sequential alcoholysis reaction, the racemic diols were acetylated regioselectively at the primary hydroxyl group without significant enantioselection by the lipase. Therefore, the second acetylation of the secondary hydroxyl group is responsible for the enantioselection in the reaction, converting the (*S*)-monoacetate much faster into the diacetate. The greater enantiopreference for the (*S*)-1-*O*-alkyl-3-acetylglycerol complies with the Kazlauskas empirical rule. The overall enzymatic process is illustrated in Scheme 2.



Scheme 2.

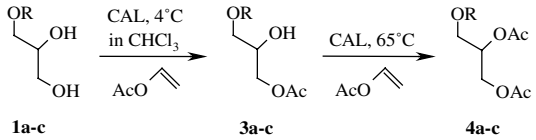
In such kinetic resolution, it is necessary to use conditions favouring rapid and irreversible reactions to achieve high selectivity. Otherwise, the enantiomeric excess of the product and the remaining substrate will decrease progressively, as the extent of the reverse reaction increases. Vinyl acetate was used to minimise the extent of the reverse reaction by irreversible tautomerisation of its enol-leaving moiety into volatile acetaldehyde.[27]

Four different commercially available lipases were tested under various transesterification conditions using vinyl acetate as an acylating agent. They include the immobilised *Rhizomucor miehei* lipase (RML, Lipozyme RM IM) and *Candida antarctica* B lipase (CAL-B, Novozym 435), from Novozyme A/S in Denmark, and two bacterial lipases from Amano Enzymes Ltd. in UK, *Pseudomonas fluorescens* (PFL, formerly *Pseudomonas* sp. lipase) and BCL. The *Burkholderia* and *Pseudomonas* lipases were observed to be far superior to RML and CAL-B in terms of enantiomeric ratios offered. Moreover, the performance offered by PFL was significantly better than BCL.

In the transesterification reactions, the first acetylation proceeded very rapidly with 80–100% monoacetates **3a–c** being formed during the first reaction hour. The fast conversion into the monoacetate indicates that low or no enantioselection took place in the first acetylation as was firmly established. However, in the second acetylation the reaction rate decreased dramatically after approximately 50% conversion had been reached. It was decided to look upon the monoacetate as the racemic substrate, rather than the diol, since almost no enantioselection took place in the first step. The conversion was therefore based only on the second acetylation. The *sn*-3 enantiomer was the better substrate in all the cases and therefore the *sn*-1 enantiomer was obtained in high enantiopurity, at higher than 50% conversion, when the monoacetate was isolated from the reaction mixture.

The conversion of the transesterification reactions was used for the *E*-value calculations using the method of Sih and Wu[28] The mono-/diacetate ratio was determined by GLC analysis after preparing trimethylsilyl derivatives of the monoacetate in the reaction mixture. When using FID detection response factors were needed to correct the ratio due to different intensity given by the two constituents. The conversion results were confirmed by ^{1}H NMR analysis as well as calculations from the enantiomeric excess values.[28]

Reference samples of the mono- and diacetates were prepared in excellent yields (~90%) using lipase and characterised by high field ^{1}H and ^{13}C NMR spectroscopy. The *C. antarctica* lipase was used to esterify the end-position of racemic **1a–c** exclusively using 1.0 M vinyl acetate in chloroform at low temperature (0–4 °C) and short reaction time. The same enzyme was used to esterify both hydroxyl groups using neat vinyl acetate, extended reaction time and 65 °C. These reactions are illustrated in Scheme 3. Such highly regioselective esterification at low temperature by CAL-B has been demonstrated on glycerol to synthesise regioisomerically pure 1,3-diacylglycerols.[29] The *C. antarctica* lipase has also been exploited to synthesise homogeneous triacylglycerols at elevated temperature.[30]



Scheme 3.

In order to determine the enantiomeric excess of both the mono- and diacetate fractions they were separated by a preparative TLC and then hydrolysed to the original 1-*O*-alkylglycerol form. The diols were then reacted with (*R*)-(−)-1-(1-naphthyhl)ethyl isocyanate (≥95% ee) to form their corresponding diastereomers, which were separated on analytical HPLC using UV/vis detection (280 nm). Since the isocyanate used was contaminated with 2.5% of the (*S*)-antipode simple correction calculations were made to compensate for that. Reference samples of homochiral (*S*)-1-*O*-alkylglycerols were prepared to establish the procedure and the absolute configuration of the compounds involved. In order to further confirm the results, Mosher's esters[31] were also synthesised in some cases and analysed by ^{13}C and ^{19}F NMR spectroscopy.

Solvent studies on chimyl alcohol **1a** revealed that the highest *E*-values are obtained with PFL in chloroform (18), better than in a 30% acetone in chloroform mixture (16), diethyl ether (11) or diisopropyl ether (10). These results are demonstrated in Table 1. However, the reaction rate in chloroform was significantly slower than in the other solvents, which demanded a higher enzyme to substrate ratio.

Table 2 displays the results of the sequential resolution of the 1-*O*-alkylglycerols with PFL at room temperature as well as 4 °C. Due to poor activity of the lipase toward the monoacetate under the applied conditions high enzyme–substrate ratio and extended reaction time was needed. Despite rather low enantiomeric ratios for all the alcohols **1a–c** they were high enough to allow enantiomerically pure material to be obtained for the slower reacting (*R*)-monoacetate at approximately 60% conversion for both the chimyl and batyl alcohols and nearly so for the selachyl alcohol. The (*R*)-monoacetate corresponds to the natural (*S*)-configuration of the

Table 1. Kinetic resolution of racemic chimyl alcohol **1a** by *P. fluorescens* lipase in various solvents at room temperature[a]

Solvent	*E*/*S*	Conv. (%)	Time (h)	Ee **3** (%)	Ee **4** (%)	*E*
1:3 Acetone/$CHCl_3$	2.2	61	24	≥95	62	16
$CHCl_3$	3.9	54	34	86	74	18
Et_2O	2.8	64	8	94	54	11
$(i\text{-Pr})_2O$	2.8	67	6	≥95	47	10

[a] *E*/*S*, enzyme to substrate ratio (wt/wt); % Conv., conversion as determined by GLC; ee, enantiomeric excess as determined by HPLC on diastereomers of isocyanates; *E*, these are average enantiomeric ratios of several reactions under identical conditions.

Table 2. Kinetic resolution of racemic chimyl **1a**, batyl **1b** and selachyl **1c** alcohols by *P. fluorescens* lipase in chloroform[a]

Substrate	Temp. (°C)	Conv. (%)	Time (h)	Ee **3** (%)	Ee **4** (%)	*E*
1a	20	64	75	⩾95	56	17
	4	55	90	94	80	32
1b	20	63	72	⩾95	52	14
	4	51	87	89	30	38
1c	20	61	71	93	24	13
	4	56	92	85	56	12

[a] See abbreviations in Table 1. The enzyme–substrate ratio was 3.5.

1-*O*-alkylglycerols as was established by the homochiral reference samples.

From the results displayed in Table 2 it is evident that lowering the temperature had a dramatic effect on the saturated chained alcohols **1a** and **1b**, but no such effect was observed for **1c**.

3. Conclusion

Racemic 1-*O*-alkylglycerols were prepared very efficiently and their natural 1-*O*-alkyl-*sn*-glycerol enantiomers afforded by kinetic resolution using lipase-catalysed transesterification. The best results were obtained for the *P. fluorescens* lipase in chloroform for which relatively low to moderate *E*-values were obtained. This, however, enabled the preparation of the saturated 1-*O*-alkylglycerols virtually enantiomerically pure (⩾95% ee), whereas for the monounsaturated 1-*O*-alkylglycerol slightly inferior results were obtained (93% ee) at approximately 60% conversion as monoacetates. Comparison with pure enantiomers obtained from (*R*)-solketal confirmed that these products all had the same absolute configuration as the natural 1-*O*-alkyl-*sn*-glycerols, that is, the *S*-configuration.

4. Experimental

4.1. General

^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 250 NMR spectrometer (Karlsruhe, Germany) in deuterated chloroform as a solvent. The number of carbon nuclei behind each ^{13}C signal is indicated in parentheses after each chemical shift value, when there is more than one carbon responsible for the peak. Infrared (IR) spectra were collected on a Nicolet Avatar 360 FT-IR (E.S.P.) Spectrophotometer (Madison, WI) on a KBr pellet. The high resolution mass spectra (HRMS) were acquired on a Micromass Q-Tof II mass spectrometer equipped with an Z-spray atmospheric pressure ionisation chamber. The mass spectrometry parameters were as described by Mu et al.[32] All data analysis was done on Micromass MassLynx software. Elemental analyses were performed on Carlo Erba Strumentazione Elemental Analyzer (model 1106; Milan, Italy). Melting points were determined on a Büchi 520 melting point apparatus and are uncorrected. Analytical gas–liquid chromatography (GLC) was conducted on a Perkin–Elmer 8140 gas chromatograph or (Beaconsfield, UK) using a 30-m capillary column, DB-225 30 N 0.25 mm (J & W Scientific, Folsom, CA) with hydrogen as a carrier gas at a flow rate of 1.6 mL min^{-1}. Detection was with FID and areal quantitation was made with an auxiliary automatic integrator. Analytical HPLC was performed on a constaMetric 3200 solvent delivery system with spectroMonitor 3200 variable wavelength detector (280 nm) both from LDC Analytical using a Nucleosil 50-5 column (Art. 721210) from Macherey-Nagel (Easton, PA).

The bacterial lipases from *P. fluorescens* (Amano AK) and *P. cepacia* (Amano PS) were supplied by Amano Enzyme Europe Ltd. (Milton Keynes, England) and employed directly as powder, without any preadjustment or optimisation of pH. The immobilised fungal lipases from *C. antarctica* (Novozym 435) and *Rhizomucor miehei* (Lipozyme RM IM) were supplied as a gift from Novozyme A/S (Bagsvaerd, Denmark). Solketal (isopropylideneglycerol, 98%), 1-bromohexadecane (97%), 1-bromooctadecane (98%), 1-bromo-*cis*-9-octadecene (99%), tetrabutylammonium bromide (99%), vinyl acetate (>99%), (*R*)-(−)-1-(1-napthyl)ethyl isocyanate (⩾95% ee), and hexamethyldisilazane were purchased from Sigma Chemicals (St. Louis, Missouri). The Sodium chloride, sodium hydroxide, potassium hydroxide, anhydrous magnesium sulphate, chloromethylsilane, triethylamine, phosphoric acid, *p*-toluenesulfonic acid monohydrate and preparative TLC plates (Art. 5721) were obtained from Merck (Darmstadt, Germany). All solvents (*n*-hexane, petroleum ether (40–65 °C), diethyl ether, diisopropyl ether, chloroform, methanol, tetrahydrofuran, ethyl acetate, acetone, toluene, phenylchloride and pyridine) of analytical grade were obtained from Acros Organics (Geel, Belgium) and used without further purification unless otherwise stated.

4.1.1. Synthesis of 1-*O*-hexadecylglycerol 1a. Isopropylideneglycerol (432 mg, 3.27 mmol), 1-bromohexadecane (998 mg, 3.27 mmol) and tetrabutylammonium bromide (211 mg, 0.65 mmol) were placed in a 10 ml round-bottomed flask and the resulting mixture stirred on a magnetic stirrer hotplate for 10 min at room temperature. Grounded potassium hydroxide (367 mg, 6.54 mmol) was slowly and carefully added and the mixture stirred for 15 h at 35–40 °C in an oil bath. Additional potassium hydroxide (92 mg, 1.64 mmol) was added and the solution stirred for extra 3 h at the same temperature. The product was extracted into *n*-hexane (40 mL) and the organic phase washed three times with

water (10 mL) and brine solution (10 mL) before removing the solvent in vacuo on a rotary evaporator to afford yellowish oil mainly containing the 1-*O*-hexadecyl isopropylideneglycerol intermediate **2a**.

The oil was placed in a 25 mL round-bottomed flask together with *p*-toluenesulfonic acid (80 mg), tetrahydrofuran (12 mL) and water (5 mL) and the resulting solution refluxed overnight. After two extractions with diethyl ether (10 mL), washing with water (15 mL) and brine solution (10 mL), drying over anhydrous magnesium sulphate and solvent removal in vacuo on a rotary evaporator white crystalline material was afforded. Recrystallisation from *n*-hexane in a freezer (−18 °C) resulted in highly pure crystals of the 1-*O*-hexadecylglycerol (829 mg, 2.62 mmol) in 80% yield. Mp = 64.0–64.6 °C. ^{1}H NMR δ 3.90–3.82 (m, 1H, C*H*OH), 3.72 (dd, J = 11.4 Hz, J = 3.9 Hz, 1H, CH_2OH), 3.64 (dd, J = 11.5 Hz, J = 5.1 Hz, 1H, CH_2OH), 3.54 (dd, J = 9.7 Hz, J = 4.0 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.49 (dd, J = 9.7 Hz, J = 5.8 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.46 (2 × t, J = 6.6 Hz, 2H, OCH_2CH$_2$), 2.39 (br s, 2H, OH), 1.57 (br quintet, J = 6.7 Hz, 2H, OCH$_2$CH_2), 1.35–1.18 (m, 26H, CH$_2$) and 0.88 (br t, J = 6.3 Hz, 3H, CH$_3$) ppm. ^{13}C δ 72.5, 71.8, 70.4, 64.3, 31.9, 29.7 (5), 29.6 (4), 29.4 (2), 26.1, 22.7 and 14.1 ppm. IR ν_{max} 3300–3600 (br, O–H), 2919 (vs, C–H), 2849 (vs, C-H) cm^{-1}. HRMS (API): calcd for $C_{19}H_{40}O_3$+H m/z 317.3056, found 317.3095 amu. Elemental analysis. Found: C, 72.04; H, 12.73. $C_{19}H_{40}O_3$ requires C, 72.10; H, 12.74.

4.1.2. Synthesis of 1-*O*-octadecylglycerol 1b. The same procedure was followed as described for **1a** above using isopropylidene glycerol (433 mg, 3.27 mmol), 1-bromooctadecane (1090 mg, 3.27 mmol), tetrabutylammonium bromide (211 mg, 0.65 mmol) and potassium hydroxide (459 mg, 8.18 mmol). Recrystallisation from *n*-hexane in a freezer (−18 °C) afforded highly pure crystals of the 1-*O*-octadecylglycerol (903 mg, 2.62 mmol) in 80% yield. Mp = 69.2–69.8 °C. ^{1}H NMR δ 3.90–3.82 (m, 1H, C*H*OH), 3.72 (dd, J = 11.4 Hz, J = 3.8 Hz, 1H, CH_2OH), 3.64 (dd, J = 11.4 Hz, J = 5.2 Hz, 1H, CH_2OH), 3.53 (dd, J = 9.7 Hz, J = 4.1 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.48 (dd, J = 9.7 Hz, J = 5.9 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.46 (2 × t, J = 6.6 Hz, 2H, OCH_2CH$_2$), 2.50 (br s, 2H, OH), 1.57 (quintet (br), J = 6.7 Hz, 2H, OCH$_2$ CH_2), 1.36–1.16 (m, 30H, CH$_2$) and 0.87 (br t, J = 6.3 Hz, 3H, CH$_3$) ppm. ^{13} C δ 72.5, 71.9, 70.4, 64.3, 31.9, 29.7 (7), 29.6 (4), 29.5, 29.4, 26.1, 22.7 and 14.1 ppm. IR ν_{max} 3300–3600 (br, O–H), 2918 (vs, C–H), 2849 (vs, C–H) cm^{-1}. HRMS (API): calcd for $C_{21}H_{44}O_3$+H m/z 345.3369, found 345.3333 amu. Elemental analysis. Found: C, 73.08; H, 12.91. $C_{21}H_{44}O_3$ requires C, 73.20; H, 12.87.

4.1.3. Synthesis of 1-*O*-cis-9-octadecenylglycerol 1c. The same procedure was followed as described for **1a** above using isopropylidene glycerol (399 mg, 3.02 mmol), 1-bromo-*cis*-9-octadecene (1001 mg, 3.02 mmol), tetrabutylammonium bromide (195 mg, 0.60 mmol) and potassium hydroxide (418 mg, 7.45 mmol). Recrystallisation from *n*-hexane in chilled chlorobenzene (−42 °C) afforded highly pure crystals of 1-*O*-*cis*-9-octadecenylglycerol (869 mg, 2.54 mmol) in 84% yield that soon became liquid at ambient temperature. ^{1}H NMR δ 5.37–5.33 (m, 2H, =C*H*CH$_2$), 3.89–3.82 (m, 1H, C*H*OH), 3.71 (dd, J = 11.4 Hz, J = 3.8 Hz, 1H, CH_2OH), 3.62 (dd, J = 11.4 Hz, J = 5.3 Hz, 1H, CH_2OH), 3.52 (dd, J = 9.7 Hz, J = 4.1 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.47 (dd, J = 9.7 Hz, J = 5.9 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.50–3.42 (m, 2H, OCH_2CH$_2$), 2.61 (br s, 2H, O*H*), 2.03–1.97 (m, 4H, =CHCH_2), 1.58–1.54 (quintet (br), J = 6.7 Hz, 2H, OCH$_2$CH_2), 1.37–1.16 (m, 22H, CH$_2$), 0.87 (br t, J = 6.6 Hz, 3H, CH$_3$) ppm. ^{13}C δ 129.9, 129.8, 72.4, 71.8, 70.5, 64.2, 31.9, 29.7, 29.5 (3), 29.4, 29.3 (3), 29.2, 27.2 (2), 26.0, 22.7 and 14.1 ppm. IR ν_{max} 3300–3600 (br, O-H), 2922 (vs, C-H), 2853 (vs, C–H) cm^{-1}. HRMS (API): calcd for $C_{21}H_{42}O_3$+H m/z 343.3210, found 343.3212 amu.

4.1.4. Synthesis of 1-*O*-hexadecyl-3-acetylglycerol 3a. Immobilised *C. antarctica* lipase (100 mg) was added to a 10 mL round-bottomed flask at 0–4 °C (ice-bath) containing a mixture of 1-*O*-hexadecylglycerol (502 mg, 1.59 mmol) and 1.0 M vinyl acetate in chloroform (10 mL, 10.0 mmol). The resulting mixture was gently stirred at 0–4 °C for 2 h. Then, the lipase was removed by filtration and the solvent removed in vacuo on a rotary evaporator to afford pure 1-*O*-hexadecyl-3-acetylglycerol (506 mg, 1.41 mmol) as a clear oil in 89% yields. ^{1}H NMR δ 4.18 (dd, J = 11.4 Hz, J = 4.6 Hz, 1H, CH$_2$OCO), 4.11 (dd, J = 11.4 Hz, J = 5.9 Hz, 1H, CH$_2$OCO), 4.04–3.95 (m, 1H, C*H*OH), 3.50 (dd, J = 9.6 Hz, J = 4.2 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.49–3.42 (m, 2H, CH$_2$OCH_2CH$_2$), 3.40 (dd, J = 9.6 Hz, J = 6.2 Hz, 1H, CH_2OCH$_2$CH$_2$), 2.53 (br s, 1H, CHO*H*), 2.10 (s, 3H, OCOCH$_3$), 1.57 (quintet (br), J = 6.5 Hz, 2H, OCH$_2$CH_2), 1.25 (m, 26H, CH$_2$) and 0.88 (br t, J = 6.6 Hz, 6H, CH$_3$) ppm. ^{13}C δ 171.1, 71.8, 71.3, 68.8, 65.7, 31.9, 29.7 (5), 29.6 (4), 29.4, 29.3, 29.0, 26.1, 22.7, 20.9 and 14.1 ppm.

4.1.5. Synthesis of 1-*O*-octadecyl-3-acetylglycerol 3b. The same procedure was followed as described for **3a** above using 1-*O*-octadecylglycerol (500 mg, 1.45 mmol), 1.0 M vinyl acetate in chloroform (10 mL, 10.0 mmol) and immobilised *C. antarctica* lipase (100 mg). Pure 1-*O*-octadecyl-3-acetylglycerol (511 mg, 1.32 mmol) was obtained as a clear oil in 91% yield. ^{1}H NMR δ 4.18 (dd, J = 11.4 Hz, J = 4.6 Hz, 1H, CH$_2$OCO), 4.11 (dd, J = 11.4 Hz, J = 5.9 Hz, 1H, –CH$_2$OCO), 4.04–3.96 (m, 1H, C*H*OH), 3.50 (dd, J = 9.6 Hz, J = 4.2 Hz, 1H, CH_2OCH$_2$CH$_2$), 3.49–3.42 (m, 2H, OCH_2CH$_2$), 3.42 (dd, J = 9.6 Hz, J = 6.1 Hz, 1H, CH_2OCH$_2$CH$_2$), 2.53 (br s, 1H, CHO*H*), 2.10 (s, 3H, OCOCH$_3$), 1.57 (quintet (br), J = 6.7 Hz, 2H, OCH$_2$CH_2), 1.28–1.22 (m, 30H, CH$_2$) and 0.88 (br t, J = 6.6 Hz, 6H, CH$_3$) ppm. ^{13}C δ 171.2, 71.8, 71.3, 68.8, 65.7, 31.9, 29.7 (7), 29.6 (4), 29.5, 29.3, 26.0, 22.7 and 14.1 ppm.

4.1.6. Synthesis of 1-*O*-cis-9-octadecenyl-3-acetylglycerol 3c. The same procedure was followed as described for **3a** above using 1-*O*-*cis*-9-octadecenylglycerol (497 mg, 1.45 mmol), 1.0 M vinyl acetate in chloroform (10 mL,

10.0 mmol) and immobilised *C. antarctica* lipase (100 mg). Pure 1-*O*-*cis*-9-octadecyl-3-acetylglycerol (505 mg, 1.31 mmol) was obtained as a clear oil in 90% yield. ^{1}H NMR δ 5.37–5.33 (m, 2H, =C*H*CH$_2$), 4.18 (dd, J = 11.4 Hz, J = 4.5 Hz, 1H, CH$_2$OCO), 4.10 (dd, J = 11.4 Hz, J = 5.8 Hz, 1H, CH$_2$OCO), 4.03–3.97 (m, 1H, C*H*OH), 3.50 (dd, J = 9.6 Hz, J = 4.2 Hz, 1H, C*H*$_2$OCH$_2$CH$_2$), 3.49–3.43 (m, 2H, OC*H*$_2$CH$_2$), 3.42 (dd, J = 9.6 Hz, J = 6.1 Hz, 1H, C*H*$_2$OCH$_2$CH$_2$), 2.53 (br s, 1H, CHO*H*), 2.10 (s, 3H, OCOCH$_3$), 2.05–1.98 (m, 4H, =CHC*H*$_2$), 1.57 (quintet (br), J = 6.5 Hz, 2H, OCH$_2$C*H*$_2$), 1.29–1.27 (m, 22H, CH$_2$) and 0.88 (br t, J = 6.6 Hz, 6H, –CH$_3$) ppm. ^{13}C δ 171.1, 129.9, 129.8, 71.8, 71.3, 68.8, 65.7, 31.9, 29.8, 29.6, 29.5 (2), 29.4, 29.3 (2), 29.2 (2), 27.2 (2), 26.1, 22.7, 20.9 and 14.1 ppm.

4.1.7. Synthesis of 1-*O*-hexadecyl-2,3-diacetylglycerol 4a. Immobilised *C. antarctica* lipase (100 mg) was added to a 10 mL round-bottomed flask containing a mixture of 1-*O*-hexadecylglycerol (500 mg, 1.58 mmol) and vinyl acetate (2.01 g, 23.2 mmol). The resulting mixture was gently stirred at 65 °C for 17 h under nitrogen atmosphere. Then, the lipase was removed by filtration and the excess vinyl acetate removed in vacuo on a vacuum pump (0.01 Torr) to afford pure 1-*O*-hexadecyl-2,3-diacetylglycerol (567 mg, 1.41 mmol) as a clear oil in 89% yield. ^{1}H NMR δ 5.21–5.13 (m, 2H, C*H*OAc), 4.32 (dd, J = 12.0 Hz, J = 3.7 Hz, 1H, CH$_2$OCO), 4.14 (dd, J = 12.0 Hz, J = 6.4 Hz, 1H, CH$_2$OCO), 3.53 (d, J = 5.3 Hz, C*H*$_2$OCH$_2$CH$_2$), 3.46–3.37 (m, 2H, OC*H*$_2$CH$_2$), 2.07 (s, 3H, OCOCH$_3$), 2.05 (s, 3H, OCOCH$_3$), 1.53 (quintet (br), J = 6.6 Hz, 2H, OCH$_2$C*H*$_2$), 1.24 (br s, 26H, CH$_2$) and 0.86 (t, J = 6.6 Hz, 3H, CH$_3$) ppm. ^{13}C δ 170.6, 170.3, 71.7, 70.3, 68.8, 62.9, 31.9, 29.7 (5), 29.6 (3), 29.5, 29.4, 29.3, 26.0, 22.6, 21.0, 20.7 and 14.1 ppm.

4.1.8. Synthesis of 1-*O*-octadecyl-2,3-diacetylglycerol 4b. The same procedure was followed as described for **4a** above using 1-*O*-octadecylglycerol (503 mg, 1.46 mmol), vinyl acetate (2.04 g, 23.7 mmol) and immobilised *C. antarctica* lipase (100 mg). Pure 1-*O*-octadecyl-2,3-diacetylglycerol (564 mg, 1.32 mmol) was obtained as a clear oil in 90% yield. ^{1}H NMR δ 5.20–5.16 (m, 2H, C*H*OAc), 4.33 (dd, J = 12.0 Hz, J = 3.7 Hz, 1H, CH$_2$OCO), 4.16 (dd, J = 12.0 Hz, J = 6.3 Hz, 1H, CH$_2$OCO), 3.54 (d, J = 5.3 Hz, C*H*$_2$OCH$_2$CH$_2$), 3.47–3.40 (m, 2H, OC*H*$_2$CH$_2$), 2.08 (s, 3H, OCOCH$_3$), 2.06 (s, 3H, OCOCH$_3$), 1.54 (quintet (br), J = 6.6 Hz, 2H, OCH$_2$C*H*$_2$), 1.25 (br s, 30H, CH$_2$) and 0.88 (t, J = 6.6 Hz, 3H, CH$_3$) ppm. ^{13} C δ 170.6, 170.3, 71.7, 70.3, 68.8, 62.9, 31.9, 29.6 (10), 29.5, 29.4, 29.3, 26.0, 22.6, 21.0, 20.7 and 14.1 ppm.

4.1.9. Synthesis of 1-*O*-cis-9-octadecenylglycerol-2,3-diacetylglyceol 4c. The same procedure was followed as described for **4a** above using 1-*O*-*cis*-9-octadecylglycerol (498 mg, 1.45 mmol), vinyl acetate (2.01 g, 23.3 mmol) and immobilised *C. antarctica* lipase (100 mg). Pure 1-*O*-*cis*-9-octadecenyl-2,3-diacetylglycerol (552 mg, 1.29 mmol) was obtained as a clear oil in 89% yield. ^{1}H NMR δ 5.37–5.32 (m, 2H, =C*H*CH$_2$), 5.23–5.15 (m, 2H, C*H*OAc), 4.34 (dd, J = 12.0 Hz, J = 3.7 Hz, 1H, CH$_2$OCO), 4.16 (dd, J = 12.0 Hz, J = 6.4 Hz, 1H, CH$_2$OCO), 3.54 (d, J = 5.3 Hz, C*H*$_2$OCH$_2$CH$_2$), 3.48–3.39 (m, 2H, OC*H*$_2$ CH$_2$), 2.09 (s, 3H, OCOCH$_3$), 2.07 (s, 3H, OCOCH$_3$), 2.07–1.98 (m, 4H, =CHCH$_2$), 1.55 (quintet (br), J = 6.4 Hz, 2H, OCH$_2$C*H*$_2$), 1.29–1.26 (br s, 22H, CH$_2$) and 0.88 (t, J = 6.6 Hz, 3H, CH$_3$) ppm. ^{13} C δ 170.7, 170.4, 129.9, 129.8, 71.7, 70.3, 68.8, 63.0, 31.9, 29.7, 29.5 (3), 29.4, 29.3 (4), 27.2 (2), 26.0, 22.7, 22.7, 21.0, 20.8 and 14.1 ppm.

4.2. Lipase-catalysed transesterification of 1-*O*-alkylglycerols

In a typical procedure the 1-*O*-alkylglycerol (50 mg) was dissolved in 1.0 M vinyl acetate in chloroform (2 mL) in a 10 mL round-bottom flask before adding the powdered *P. fluorescens* lipase (200 mg). To monitor the progress of the reaction small sample (approximately 100 μL) was taken, the enzyme removed by filtration and the solvent removed in vacuo on a rotary evaporator. To the sample pyridine (500 μL), hexamethyldisilazane (150 μL) and chloromethylsilane (50 μL) were added and the resulting mixture shaken vigorously for 1 min and left standing for three minutes. After centrifugation the clear solution of TMS derivatives was injected into a GLC. A temperature of 185 °C was used at the beginning, rising 3 °C min^{-1} to a final temperature of 210 °C, which was maintained for 10–23 min (depending on the alkyl chain). Injector and detector temperatures were kept at 265 °C. The GLC results were corrected by response factors due to the FID detection and the factors 1.01 and 1.29 were used for **3a**–**c** and **4a**–**c**, respectively. The conversion was also determined by ^{1}H NMR spectroscopy.

4.2.1. Separation and hydrolysis of the reaction mixture. After desired conversion was obtained the lipase was removed by filtration and the solvent evaporated. The resulting mixture was separated on a preparative TLC-plate using 4% acetone in chloroform as eluent. The products **3a**–**c** (R_f 0.15–0.25) and **4a**–**c** (R_f 0.75–0.90) were scraped off and extracted from the silica gel with ethyl acetate. After removal of the silica gel by filtration and evaporation of the solvent the two products were hydrolysed to afford their corresponding diols (**1a**–**c**). Each product was dissolved in 0.5 M sodium hydroxide in methanol (2 mL) and reacted for 30 min at 60 °C. After cooling 0.5 M phosphoric acid (2 mL) and water (2 mL) were added and the resulting mixture extracted twice with diethyl ether. The organic layer was dried over anhydrous magnesium sulphate and the solvent removed in vacuo on a rotary evaporator. The resulting diols were derivatised with enantiopure (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate and in some cases (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid[31] (Mosher acid) for comparison.

4.2.2. Preparation of (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate derivatives. The corresponding diols of **3a**–**c** and **4a**–**c** (1–2 mg) obtained above were mixed with 2% triethylamine in toluene (1 mL) and (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (3 μL). The resulting mixture was stirred at room temperature under nitrogen over night.

The isocyanate derivatives **3a**–**c** were injected directly to analytical HPLC using a 1:1 *n*-hexane/ethyl acetate as eluent flowing at 0.15 ml min^{-1}. The diastereomers corresponding to the (*R*)-3-*O*-alkyl-*sn*-glycerol (24 min retention time) and the (*S*)-1-*O*-alkyl-*sn*-glycerol (27 min retention time) were successfully separated and the enantiomeric ratio calculated from their peak area. Approximately 2.5% (*S*)-(−)-1-(1-naphthyl)ethyl isocyanate impurities in the isocyante used had to be corrected for in the enantiomeric ratio determination.

Acknowledgements

The University of Iceland Research Fund is acknowledged for financial support, Dr. Sigridur Jonsdottir at the Science Institute for running the NMR analysis, Dr. Sigurdur V. Smarason at deCode Genetics for performing the mass spectrometry analysis, and Svana Stefansdottir at the University of Iceland for elemental analysis.

References

1. *Ether Lipids. Biochemical and Biomedical Aspects*; Mangold, H. K., Palthauf, F., Eds.; Academic: New York, 1983.
2. Kayama, M.; Mankura, M. *Inform* **1998**, *9*, 794–799.
3. Sargent, J. R. In *Marine Biogenic Lipids, Fats and Oils*; Ackman, R. G., Ed.; CRC: Boca Raton, FL, 1989, Vol. 1; pp 175–197.
4. Benveniste, J.; Vargaftig, B. B. In *Ether Lipids—Biochemical and Biomedical Aspects*; Mangold, H. K., Palthauf, F., Eds.; Academic: New York, 1983; pp 356–373.
5. Mangold, H. K.; Weber, N. *Lipids* **1987**, *22*, 789–799.
6. Brohult, A.; Brohult, J.; Brohult, S. *Acta Chem. Scand.* **1970**, *24*, 730–733.
7. Pugliese, P. T.; Jordan, K.; Cederberg, H.; Brohult, J. *J. Alt. Compl. Med.* **1998**, *4*, 87–99.
8. Christie, W. W. *Lipid Analysis—Isolation, Separation, Identification and Structural Analysis of Lipids*; The Oily Press: Bridgwater, 2003, pp 3–33.
9. Wong, C. H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994, pp 70–111.
10. Faber, K. *Biotransformations in Organic Synthesis*; Springer: Heidelberg, 1995, pp 88–115.
11. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis—Regio- and Stereoselective Biotransformations*; Wiley-VCH: Weinheim, 1999, pp 65–129.
12. Haraldsson, G. G. In *The Chemistry of the Functional Groups, Supplement B2: The Chemistry of Acid Derivatives*; Patai, S., Ed.; John Wiley & Sons: Chichester, 1992, pp 1395–1473.
13. Theil, F.; Lemke, K.; Ballschuh, S.; Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1995**, *6*, 1323–1344.
14. Haraldsson, G. G.; Thordarson, P.; Halldorsson, A.; Kristinsson, B. *Tetrahedron: Asymmetry* **1999**, *10*, 3671–3674.
15. Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49–97.
16. Wang, Z. M.; Sharpless, K. B. *Tetrahedron Lett.* **1993**, *34*, 8225–8228.
17. Lok, C. M.; Ward, J. P.; Van Drop, D. A. *Chem. Phys. Lipids* **1976**, *16*, 115–122.
18. Jung, M. E.; Shaw, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6304–6311.
19. Phung, A. N.; Braun, J.; Le Goffic, F. *Synth. Commun.* **1995**, *25*, 1783–1788.
20. Vänttinen, E.; Kanerva, L. T. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3459–3463.
21. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 923–933.
22. Guo, Z.-W.; Wu, S.-H.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4942–4974.
23. Theil, F.; Weidner, J.; Ballschuh, S.; Kunath, A.; Schick, H. *J. Org. Chem.* **1994**, *59*, 388–393.
24. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665.
25. Lemke, K.; Lemke, M.; Theil, F. *J. Org. Chem.* **1997**, *62*, 6268–6273.
26. *Dictionary of Organic Compounds*; Chapman and Hall: New York, 1982; Vol. 2, pp 1945, and 1990.
27. Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Berbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.
28. Sih, C. J.; Wu, S.-H.; Eliel, E. L.; Wilen, S. H.; Eds.; Topics in Stereochemistry; John Wiley & Sons: New York, 1989; Vol. 19, pp 63–126.
29. Halldorsson, A.; Magnusson, C. D.; Haraldsson, G. G. *Tetrahedron* **2003**, *59*, 9101–9109.
30. Haraldsson, G. G.; Gudmundsson, B. Ö.; Almarsson, Ö. *Tetrahedron* **1995**, *51*, 941–952.
31. Dale, J. A.; Mosher, S. H. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
32. Mu, H.; Sillen, H.; Høy, C.-E. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1049–1059.