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Kinetic resolution of 1-*O*-alkylglycerols by lipase

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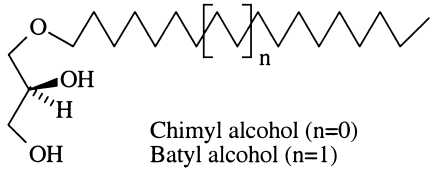
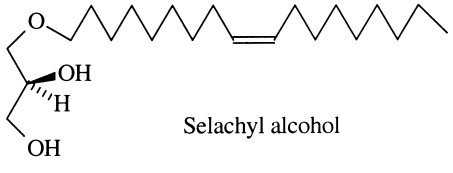
Abstract

Pseudomonas sp. lipase was employed to resolve kinetically 1-*O*-alkylglycerols by a sequential diacylation process. Only low or moderate *E*-values were obtained, but at approximately 60% conversion enantiomerically pure monoacetates were obtained of the natural *S*-configuration for glyceryl ether lipids.

Non-polar glyceryl ether lipids of the 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol type are major constituents in the liver oils of various species of elasmobranch fish such as dogfish and shark.[1,2] They have been claimed to display various beneficial effects on human health[3,4] and bear a strong resemblance to the well-known platelet activating factors. The 1-*O*-alkyl-*sn*-glycerols are highly valuable compounds and can be prepared from the unsaponifiable fraction of dogfish and shark liver oil. As the *sn*-terminology implies their natural absolute configuration is *S*. There are three main fatty alcohol constituents present in the 1-*O*-alkyl moiety of the glyceryl ethers, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$, the last one being the most abundant. They correspond to chimyl, batyl and selachyl alcohols, respectively, named after their sources in the liver oils of chimaeras, sharks and rays of the *Chimaeroidei*, *Batoidei* and *Selachoidei* families. The ether linkage is also common in lipids of land animals, protozoa and bacteria, both neutral and phosphorylated. Of particular interest are the plasmalogens, which are phospholipids containing an enol ether type 1-*O*-1′-alkenyl glyceryl moiety, which occur widely in the human brain and have gained the increasing interest of scientists recently.[5]



Chimyl alcohol (n=0)
Batyl alcohol (n=1)



Selachyl alcohol

The primary goal of the work described in this report was to obtain the natural *S* configured chimyl, batyl and selachyl alcohols in an enantiomerically pure form by kinetic resolution of racemates **2a**, **b** and **c**, respectively, using lipase. As a result of the great variety of alkyl groups present in the ether linkage

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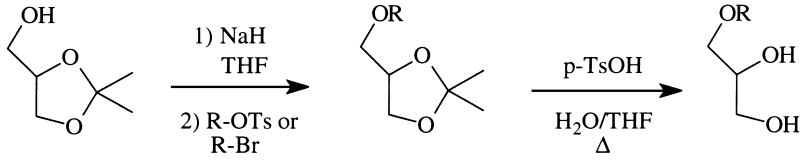
moiety of the ether lipids they are by no means readily accessible from natural sources.[1] Lipases, due to their tolerance for a wide range of substrate structures, are now among the most widely applied and versatile biocatalysts in organic synthesis.[6–8] Although a large variety of diols, including a whole range of 1-*O*-arylglycerols, have been extensively studied in terms of kinetic resolution,[9–11] there are hardly any reports on the 1-*O*-alkylglycerols of the type described above. As far as we are aware this is the first report dealing with this sort of substrate in lipase-catalyzed kinetic resolutions.

Scheme 1 illustrates the synthesis of **2a**, **b** and **c** as racemates starting from solketal. The 2,3-*O*-isopropylidene protected glyceryl ethers **1a**, **b** and **c** were obtained in good yields (74–75% after purification) when treating solketal with sodium hydride in THF followed by the corresponding alkyl tosylates or bromides. The tosylates were obtained in moderate yields from the commercially available fatty alcohols, but the commercially available 1-bromo-*cis*-9-octadecene could also be used. The diol ethers **2a**, **b** and **c** were obtained in good yields (71–75% after purification) by treatment of **1a**, **b** and **c** with a catalytic amount of *p*-toluenesulfonic acid in a 30% water–THF mixture under a gentle heating.

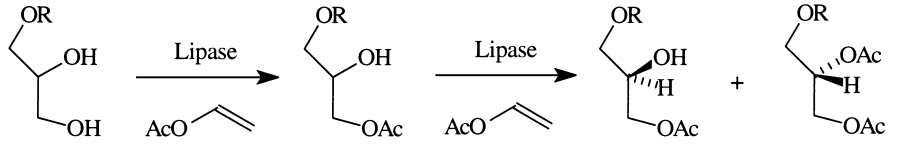


a (R = $C_{16}H_{33}$)
b (R = $C_{18}H_{37}$)
c (R = cis-9-$C_{18}H_{35}$)

1a,b,c **2a,b,c**

Scheme 1.

It was decided to base the kinetic resolution on a transesterification using vinyl acetate as an acylating agent. Four different lipases were investigated, including immobilized *Mucor miehei* lipase and *Candida antarctica* lipase B, both from Novo Nordisk, and two *Pseudomonas* lipases from Amano, *Pseudomonas cepecia* (lipase PS) and *Pseudomonas* sp. (lipase AK), both applied as a powder. Not surprisingly, it soon became evident that all the lipases displayed a high degree of regioselectivity favouring the primary hydroxyl group, which appears to be a general characteristic of lipase.[9,12] Furthermore, this preferential acylation took place with very low or hardly any enantiodifferentiation. Consequently, it was evident that a sequential acylation process originally introduced by Sih and co-workers[13] was necessary for the task, of the type demonstrated highly successfully on numerous analogous 1-*O*-arylglycerols by Theil and co-workers.[9–11] The overall enzymatic process is illustrated in Scheme 2.



(*RS*)-**2a,b,c** (*RS*)-**3a,b,c** (*R*)-**3a,b,c** (*S*)-**4a,b,c**

Scheme 2.

Preliminary studies were conducted in chloroform as a solvent containing 1.0 M vinyl acetate. They revealed that the *Pseudomonas* lipases were superior to the *Mucor miehei* and *Candida antarctica* lipases in terms of enantiomeric ratios offered and that the performance offered by the *Pseudomonas* sp. lipase was significantly better than the *Pseudomonas cepecia* lipase. Moreover, all three adducts were observed to be poor substrates for all four lipases tested and high enzyme to substrate ratios were required. Solvent studies on **2a** revealed that highest *E*-values (18) were obtained with the *Pseudomonas* sp. lipase in

Table 1
Kinetic resolution of racemic chimyl, batyl and selachyl alcohols

Substrate	Temp. (°C)	E/S[a]	Conv.[b] (%)	Time (h)	% ee[c] R-**3**[e]	% ee S-**4**	E[d]
2a	22	4.0	64	75	≥ 95	56	17
	4	3.9	55	90	94	80	32
2b	22	3.5	63	72	≥ 95	52	14
	4	3.5	51	87	89	30	38
2c	22	3.5	61	71	93	24	13
	4	3.5	56	92	85	56	12

a) Enzyme to substrate ratio (wt/wt)
b) As determined by GLC.
c) As determined by HPLC on diastereomers of isocyanates.
d) These are average values from several experiments.
e) The absolute configuration was determined from enantiomerically pure R- and S-solketals commercially available from Aldrich Chemical Company.

chloroform, higher than in a 30% acetone in chloroform mixture (16), diethyl ether (11) and diisopropyl ether (10). However, the reaction rate in chloroform was significantly slower than in the other solvents, which demanded a higher enzyme to substrate ratio. Despite the less favourable enzyme to substrate ratio it was decided to go for the highest *E*-values and base further studies on chloroform as a solvent.

The results of the sequential resolution of substrates **2a**, **b** and **c** at rt as well as 4°C are listed in Table 1 for the *Pseudomonas* sp. lipase. From Table 1 it is noticeable that the enantiomeric ratios are rather low for all substrates. In accordance with theory,[14] however, they were high enough to allow enantiomerically pure material to be obtained for the slower reacting monoacetate enantiomer at 60% conversion for both **2a** and **b** and nearly so for **c**. In agreement with Kazlauskas' rules[15] for predicting the stereochemical outcome of kinetic resolution of alcohols based on the closely related *Pseudomonas cepecia* lipase, the absolute configuration of the residual monoacetates was *R*, which indeed corresponds to the natural *S* configuration of the ether lipid alcohols.

It is also clearly evident that for the chimyl and batyl alcohols lowering the temperature[16] had drastic effects on the *E*-values, but no such effects were observed for the monounsaturated selachyl alcohol. The reason for this may be a dramatic bend in the hydrocarbon chain, rendering it less able to fit into the hydrophobic active site pockets of the enzyme. This may also be a reason for this alcohol to be a somewhat inferior substrate to the lipase as the *E*-values may indicate. Significantly lower *E*-values were obtained for the *Pseudomonas cepecia* lipase, 9, 9 and 6 for **2a**, **b** and **c** at rt, respectively. These values are very close to those obtained for that lipase by Theil and co-workers[11] on long-chain 1,2-alkanediols, which apparently bear some structural resemblance to the glyceryl ether diols.

The results in Table 1 also show that a high ratio of enzyme to substrates was required, between 3.5 and 4.0 based on weight, to allow the processes to occur within a reasonable time. The conversion data in Table 1 are based on GLC analysis of the reaction mixture as TMS derivatives, but conversion was also confirmed by ^{1}H NMR analysis as well as calculations from enantiomeric excess values. The enantiomeric excess was determined by analytical HPLC on an achiral silica column on diastereomeric carbamides obtained as monoadducts when the diols were reacted with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (Aldrich, 95% ee). The diacetate products **4a**, **b** and **c** and the monoacetates **3a**, **b** and **c**

from the reactions were separated by prep. TLC on silica gel, converted into the diols **2a**, **b** and **c** by treatment with methanolic NaOH and concomitantly derivatized with the isocyanates. The corresponding Mosher esters were also prepared as a separable mixture of mono- and diadducts, but the diastereomers did not separate well on the HPLC column. Both their ^{13}C and ^{19}F NMR spectra offered confirmation of the enantiomeric excess results obtained from the isocyanate derivatives from HPLC, whereas their ^{1}H NMR spectra did not offer sufficient resolution of the diastereomeric proton peaks. The absolute stereochemistry was firmly established by comparative studies on commercially available enantiomerically pure (*R*)-solketal, which was alkylated, subsequently deprotected and the resulting diols taken through the entire analytical procedure.

Currently, attempts are being undertaken both to improve on the rather non-modern, however firm and solid, methodology being used for determining both the extent of conversion as well as the enantiomeric excess of the processes being studied, by changing to chiral HPLC analysis. Also, strategies to improve the rather poor performance of the lipases and their poor enantioselection for these substrates is being looked into, both in terms of solvent effects[17] and appropriate substitution of the substrates. It is anticipated that this will eventually lead to improvements for kinetically resolving these rather interesting marine originated compounds.

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References

1. Kayama, M.; Mankura, M. *INFORM* (*Am. Oil. Chem. Soc*.) **1998**, *9*, 794–799.
2. Sargent, J. R. In *Marine Biogenic Lipids, Fats and Oils*; Ackman, R. G., Ed.; CRC Press: Boca Raton, Florida, 1989; Vol. 1; pp. 175–197.
3. *Ether Lipids. Biochemical and Biomedical Aspects*; Mangold, H. K.; Palthauf, F., Eds.; Academic Press: New York, 1983.
4. Mangold, H. K.; Weber, N. *Lipids* **1987**, *22*, 789–799.
5. Pfaendler, H. R.; Müller, F. X. *Synthesis* **1992**, 350–352.
6. 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; Vol. 2; pp. 1395–1473.
7. Wong, C. H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.
8. Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Heidelberg, 1995; 2nd ed.
9. Theil, F.; Weidner, J.; Ballschuh, S.; Kunath, A.; Schick, H. *J. Org. Chem*. **1994**, *59*, 388–393.
10. Theil, F.; Lemke, K.; Ballschuh, S.; Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1995**, *6*, 1323–1344.
11. Lemke, K.; Lemke, M.; Theil, F. *J. Org. Chem*. **1997**, *62*, 6268–6273.
12. Haraldsson, G. G.; Gudmundsson, B. Ö.; Almarsson, Ö. *Tetrahedron* **1995**, *51*, 941–952.
13. Guo, Z.-W.; Wu, S.-H.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc*. **1990**, *112*, 4942–974.
14. Sih, C. J.; Wu, S.-H. In *Topics in Stereochemistry*; Eliel, E. L.; Wilen, S. H., Eds.; John Wiley & Sons: New York, 1989; Vol. 19; pp. 63–125.
15. Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem*. **1995**, *60*, 6959–6969.
16. Sakai, T.; Kawabuta, I.; Kishimoto, T. *J. Org. Chem*. **1997**, *62*, 4906–4907.
17. Lemke, K.; Theil, F.; Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1996**, *7*, 971–974.