

Chapter 22

Glycerol Ethers

Synthesis, Configuration Analysis, and a Brief Review of
Their Lipase-Catalyzed Reactions

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Prompted by growing agricultural and industrial interest in the chemistry of lipases for a great variety of reactions, and the usefulness of glycerol derivatives in characterizing lipase activity, we devised syntheses of 1,2- and 1,3-diacylglycerol diethers and their esters. Configurational analyses of the diethers has been accomplished by derivatizing them to form diastereomers that are separable by gas chromatography. The alternative methods employed for determining the configuration of triglycerides and related glycerol derivatives are reviewed briefly with reference to the stereoselectivity of their lipase catalyzed reactions.

Lipases, a family of enzymes also known as triacylglycerol hydrolases (EC 3:1:1:3), are being investigated intensively by industries that employ natural triglycerides as feedstock for possible replacement of conventional methodology or for new transformations based on fatty acid selectivity. Additionally, there are applications to agrochemicals synthesis that include the preparation of chiral insecticides (1,2), herbicides (3-8), and insect sex pheromones (9-11). A compendium of the rapidly expanding literature describing lipase-catalyzed reactions for other biologically active structures is beyond the scope of this chapter, but a few selected references give ample indication of the growing importance of these relatively available catalysts (12-15). In addition to the standard supply companies for biochemicals, one can purchase lipases from Amano Co., Troy VA; Novo Co., Wilton, CT; Enzeco, New York, NY; and Seikagaku Kogyo, Tokyo, Japan; among others.

In nature, lipases catalyze the hydrolysis of triglycerides to partial glycerides and free fatty acids. Because the catalytic process is reversible, it is also possible to study lipase-catalyzed esterifications and transesterifications to form glycerol esters. Efforts to determine fatty acid selectivity and stereoselectivity with triglycerides directly, however, are complicated by the multiplicity of reaction sites and the nonenzymatic acyl migrations to which vicinal diol monoester structures are prone. Stereospecifically synthesized triglycerides are often employed to cope with these problems. Another approach employs alkyl ethers of glycerol; such compounds have been termed "pseudolipids," implying that their transport properties in biological studies would imitate those of the naturally occurring

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acylated analogs. Because the reactions of triglycerides and related pseudolipids are important to understanding lipase catalysis, there is continuing interest in developing methods for synthesis and stereochemical analysis of such compounds.

Studies with Triglycerides. Methods available up to 1983 for analyzing lipase stereoselectivity with triglycerides have been reviewed (16,17). Briefly, at that time scientists were essentially restricted to the use of enzymes of known stereospecificity in performing analyses upon products of the subject enzymatic reactions, or they employed enantiomerically pure compounds for their studies. In this manner, it was shown that postheparin plasma lipase was selective for the sn-1 position of triglycerides (Figure 1), while human and rat lingual lipases showed a bias for the sn-3 position (18) (in lipid stereochemical notation, sn-1 means the stereospecifically numbered 1 position; see Figure 1). The general view, however, was that stereoselection with the normal substrates was low, or absent (19). More recently, Kotting et al. demonstrated that *Staphylococcus aureus* produced two lipases, one of which was quite selective for the sn-1 position as judged by experiments with enantiomerically pure oleoyl glycerol ethers (20).

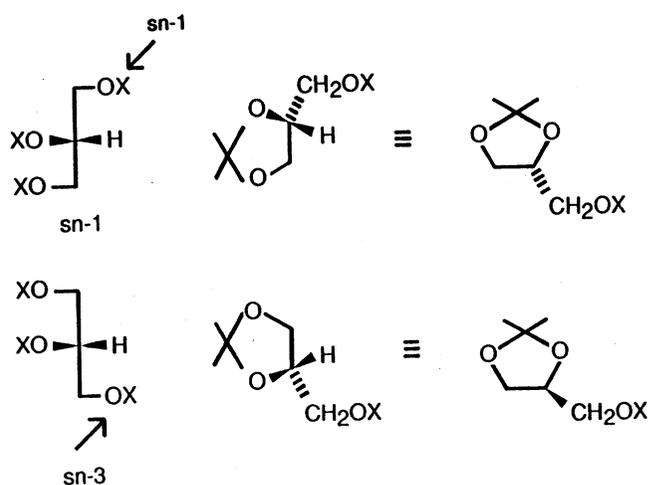


Figure 1. Relationship of stereochemical numbering of glycerol to its 1,2-acetonide.

Chiral HPLC Methodology. A very useful method has been developed for differentiating enantiomers of mono- and di-, acyl- and alkyl glycerols (21). Samples to be analyzed for configuration are converted to mono- or di-3,5-dinitrophenylurethanes and chromatographed on the chiral stationary phase, N-(R)-1-(α -naphthyl) ethylamino carbonyl-(S)-valine. The sn-1,2-enantiomers of the diesters and diethers elute first. The method appears to be of considerable scope, and studies of lipolysis of suitably constituted acylated glycerol ethers bearing groups equivalent in length to those in natural fats and oils are a close approximation to the normal substrate, and avoid problems of internal acyl migration.

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Pseudolipids: ^1H NMR/Chiral Shift Reagents. The following observations were documented with ^1H NMR techniques either employing the chiral shift reagent $\text{Eu}(\text{hfc})_3$, or MTPA (α -methoxy- α -trifluoromethyl phenylacetate) esters, or a combination of these. In one instance (22), a camphanic ester was employed to determine configuration. The compounds studied were analogs to glycerols in that their three-carbon chains were each contiguously oxygenated. Figure 2 indicates the faster reacting site in esterification of these alcohols; this would be, of course, the favored site for hydrolytic cleavage as well. For meso diols, therefore, monoacylation produces one configuration of the monoacylated adduct, while cleavage of an acyl group from the diester yields the opposite configuration of that adduct. One also notes that the compounds 3 and 4 acylation of the alcohol changes the stereochemical designator (R) for configuration; i.e., (R) becomes (S). Hence, we illustrate simply the preferred enantiomer reaction site and indicate that the sn-3 hydroxyl group seems to be selected most often by lipases.

Compound 1 reacts at the sn-3 position most readily with vinyl esters using an unspecified lipoprotein lipase (EC 3:1:1:34) (23,24), while a lipase from *Pseudomonas* (Sigma L-9518, Type XIII) selects oppositely. Porcine pancreatic lipase (PPL) also favors the sn-3 position in the related structure 2. Compound 1 was also acetylated in another study (22), but judging from optical rotation data, and the chemical evidence for configuration supplied by Wang et al. (23), the assignments in the acetylation study by Breitgoff et al. should be reversed. In that case of Breitgoff et al. have demonstrated that the following lipase sources also select for the sn-3 position in 1: *Mucor* sp, *Chromobacterium viscosum*, pancreatin, PPL, and a lipoprotein lipase (unspecified). A lipase from *Candida cylindracea* (*rugosa*) employed by Breitgoff and coworkers showed no stereobias toward 1. The glycidyl alcohol 3 reacts more readily than its enantiomer in esterification with vinyl esters using PPL and a *Pseudomonas* lipase (23), and its esters hydrolyze more quickly (25). The stereobias is affected by the size of the acid residue in

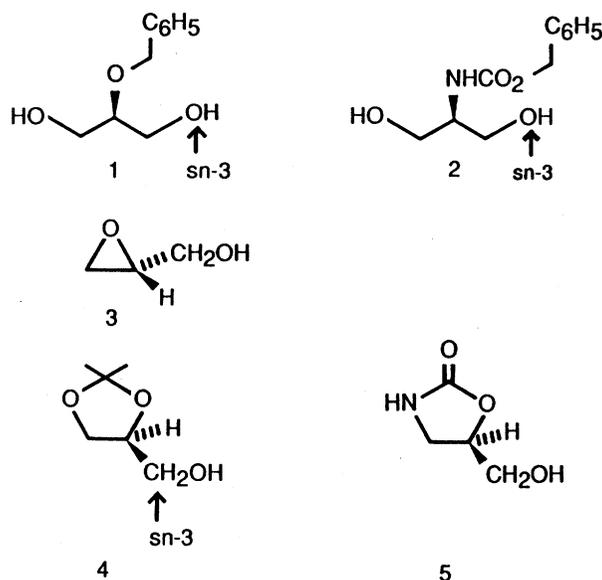


Figure 2. Selected compounds that have been employed in lipase studies to evaluate stereobias.

the ester, increasing from acetyl to caproyl, an effect noted subsequently in evaluating resolution of 2-octanol with *M. miehei* lipase (26). The sn-3 (analog) enantiomers of 4 and 5 also reacted faster in esterifications and hydrolyses of the corresponding racemic esters using PPL (25), *C. rugosa*, *Rhizopus delemar*, *Aspergillus niger*, *M. miehei* lipases (27) for 4 and Amano 3 lipoprotein lipase from *P. aeruginosa* for 5.

Synthesis and Configuration Analyses of Glycerol Dialkylether Esters. A well-defined set of glycerol ethers that could be analyzed for configuration would be very useful in gaining a better understanding of how lipases function. Syntheses of glycerol ethers appear scattered throughout the chemical literature, and the synthetic methodology is rather conventional. Figure 3 shows routes that we have employed for the preparation of variously substituted alkyl/acylglycerols (27). The scheme indicated use of the 1,2-acetonide of glycerol to generate a 1-alkylglycerol ether that can be acylated subsequently. Alternatively, 1-benzylglycerol ether was prepared in this fashion, the diol was dialkylated, and the benzyl group was removed to yield a 1,2-dialkylglycerol diether. Comparable reactions with epichlorohydrin led to 1,3-dialkylglycerol diethers and to 2-mono-ethers. The preparation of monohydric derivatives would be apparent from such routes.

We were particularly interested in obtaining and examining methods for the configurational analysis of dialkylglycerol diethers bearing two different alkyl substituents, and we employed the approach shown in Figure 4 (29). Epichlorohydrin reacted with one equivalent of an alcohol under acid catalysis to reproduce a halohydrin carrying one of these desired alkyl groups at a primary position. Reactions of this intermediate with a different alcohol using base catalysis led to an unsymmetrically substituted 1,3-dialkylglycerol ether. In an alternate sequence, the first alcohol employed was benzyl alcohol. Subsequent reactions placed different alkyl groups on the remaining (adjacent) positions; the benzyl protecting group was then removed by hydrogenolysis.

GLC Analysis by Diastereomer Formation. Conversion of chiral compounds to diastereomeric derivatives that can be analyzed by achiral chromatographic phases offers a procedure that is complementary to those methods that have already been employed with pseudolipids (30). In addition, if a conceptual model can be developed to explain the elution order of diastereomers, then this particular method has the further value of predicting separations.

1,3-Dialkylglycerol Ethers. These were easily converted to carbamates with (S)- α -phenylethylisocyanate. The resulting pairs of diastereomers were cleanly separated by both polar and nonpolar GLC phases in capillary columns (Figure 5). Carbamates formed from (S)- α -naphthylethylisocyanate showed greater separations as expected, though the column temperatures required were considerably higher. The elution order was determined by an asymmetric synthesis of a carbamate pair ($R = C_8H_{17}$, $R = CH_3$) using the 1,2-acetonide of glycerol enriched in the (S)-enantiomer to prepare (R)-1-methyl-3-n-octylglycerol diether 8 (Figure 6).

The elution orders for these diastereomers parallel those for the 1,3-dideoxy analogs (carbamates of secondary alcohols) (31); the size difference of the two alkoxymethyl substituents on the alcohol asymmetric center apparently serving to distinguish the diastereomers. In the preferred solution conformation of such compounds, as illustrated for the pseudolipid derivatives in Figure 5, the R*S* -isomer, 6, eluted first (GLC), while the trans-like or threoid, isomer eluted second.

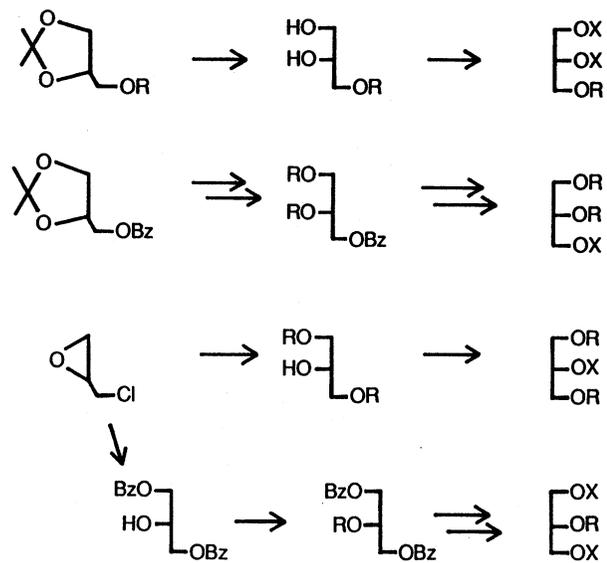


Figure 3. Conventional routes to various acyl alkylglycerol ethers. R = *n*-alkyl, X = acyl, i.e., *n*-alkanoyl, and Bz = benzyl.

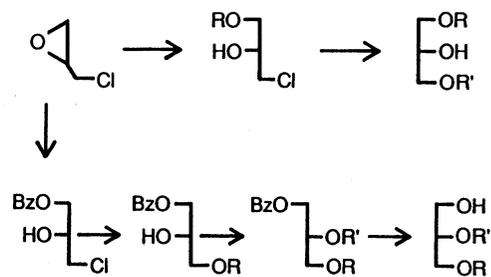
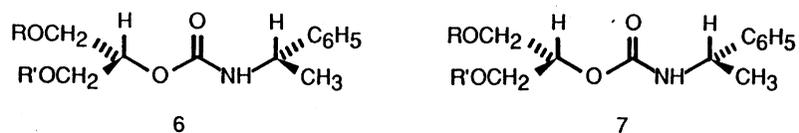


Figure 4. Synthesis of unsymmetrically substituted 1,2- and 1,3-dialkylglycerol ethers.



R	R'	k_a		k_b	
		6	7	6	7
n-C ₈ H ₁₇	CH ₃	3.57	3.67	6.83	7.17
n-C ₈ H ₁₇	C ₂ H ₅	4.27	4.36	6.17	6.42
n-C ₈ H ₁₇	n-C ₃ H ₇	5.27	5.36	6.58	6.83
n-C ₈ H ₁₇	n-C ₃ H ₇	4.39	4.48	5.42	5.54
Bz	CH ₃	4.83	4.96	5.92	7.08

Figure 5. GLC data for diastereomeric carbamates: k_a = partition coefficient on SPB-1 (30 m x 0.25 mm ID) at 260°C; k_b = partition coefficient on SP2340 (25 m x 0.25 mm ID) at 240°C.

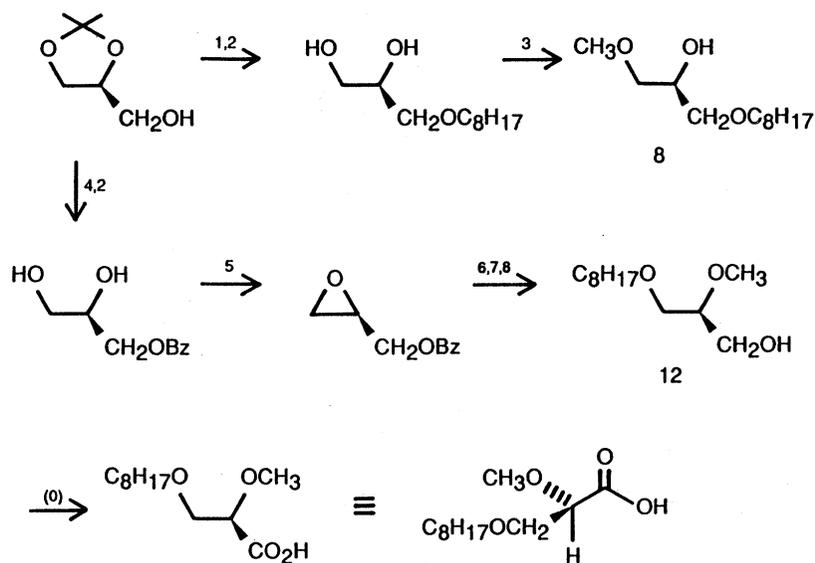
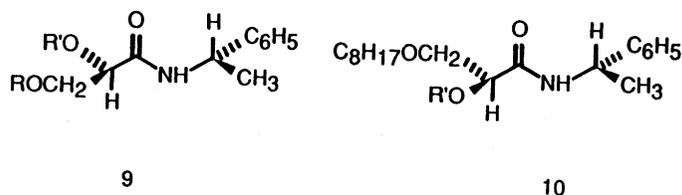


Figure 6. Asymmetric synthesis of a 1,2- and a 1,3-dialkylglycerol ether: (1) NaH, C₈H₁₇Br; (2) H₃BO₃; (3) *p*-TsCl/py; excess NaOCH₃; (4) NaH, BzCl; (5) *p*-TsCl/py; NaOCH₃; (6) C₈H₁₇OH, H⁺; (7) NaH, CH₃I; (8) Na/EtOH.

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1,2-Dialkylglycerol Ethers. Direct derivatization of the primary alcohols with, for example, methoxytrifluoromethyl phenylacetic acid or chiral isocyanates was of limited use. We had performed configurational analyses on the 1,2-acetonide of glycerol by oxidizing the compound to the carboxylic acid, then converting this to an amide using a chiral amine via the acid halide. Although the technique is less direct, it is quite useful, and the amides obtained from the 1,2-dialkylglycerol ethers by transformation to acids and conversion to amides with (S)- α -phenylethylamine were also easily resolved by GLC (Figure 7). An Asymmetric synthesis of (S)-2-methyl-3-n-octylglycerol diether, 11, was accomplished to allow configurations to be assigned to the diastereomers (Figure 6); its oxidation led to the (R)-acid 12.



R	R'	k_a		k_b	
		9	10	9	10
CH ₃	n-C ₈ H ₁₇	(2.67, 2.71)		(5.30, 6.26)	
n-C ₈ H ₁₇	CH ₃	3.04	3.29	5.08	5.50
n-C ₈ H ₁₇	C ₂ H ₅	2.96	3.13	4.33	4.50
n-C ₈ H ₁₇	n-C ₃ H ₇	3.65	3.87	4.52	4.58

Figure 7. GLC data for diastereomeric amides. k^a and k^b as defined for Figure 5.

Amides of α -branched carboxylic acids with, for example, (S)- α -phenylethylamine also show a strong solution conformation preference that results in retention of the more *tans*-like diastereomer, namely R*S*. The observed GLC elution order for these 2,3-dialkoxypropanamides, however, was reversed and the R (acid 12), S (amine) diastereomer eluted first. Although an explanation has been offered for this rather dramatic elution reversal (29), further evidence on the matter is desirable. These diastereomers are also resolved on silica gel HPLC with elution orders opposite to those of GLC, as was the case for the simpler, unoxxygenated, carbamates and amides. Separability by HPLC lends itself to studying dialkylglycerols with longer chain alkyl residues, favoring biological inquiries wherein such greater chain length would be appropriate.

Summary

Simple synthetic methods are available for preparing various structural analogs of triglycerides that can serve as substrates with which to examine lipases. Methods of analysis for configuration include derivatization to form diastereomeric mixtures that can be separated by chromatography. Thus 1,3-dialkylglycerol ethers can be converted to carbamates with α -arylethylisocyanates to form diastereomers with predictable elution orders; and 1,2-dialkylglycerol ethers can be transformed to carboxylic acid amides of the corresponding amines that are easily resolved chromatographically. The methodologies are general; their employment to study lipases is expected to spur further applications of these enzymes in synthesis of agrochemicals and other biologically active molecules.

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