

Lipase Positional Selectivity in Omega-3 Enriched Triacylglycerides



Paul F. Mugford, Alfred J. Rolle, Erick R-Suarez, Jaroslav A. Kralovec

Ocean Nutrition Canada, 101 Research Drive,
Dartmouth, Nova Scotia, B2Y 4T6, Canada
Email: pmugford@ocean-nutrition.com

ABSTRACT

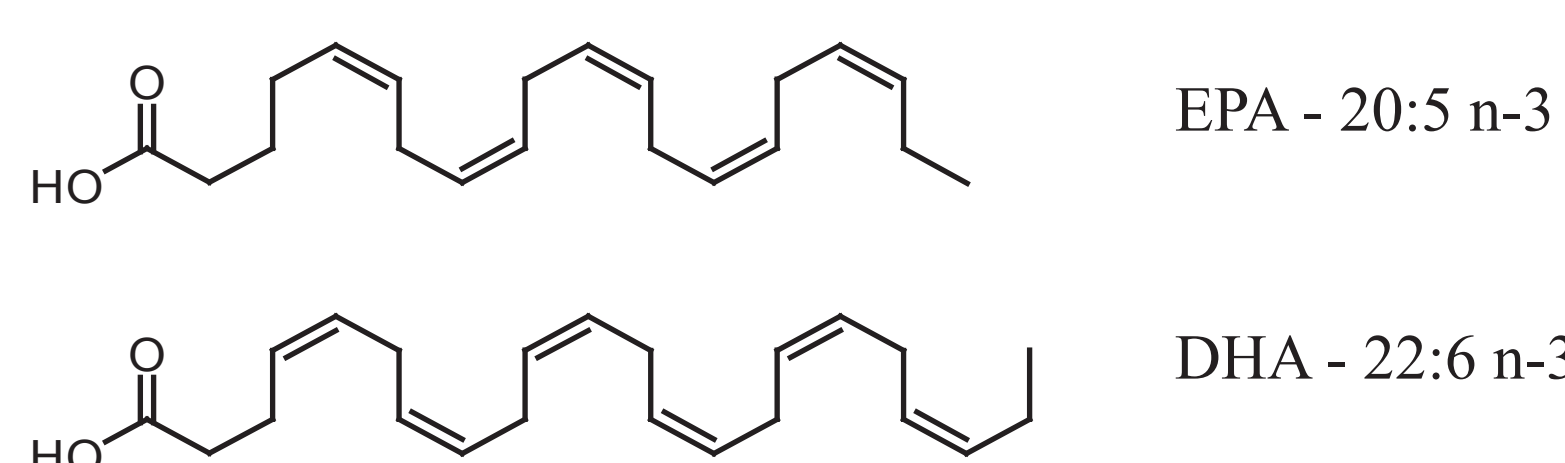
Polyunsaturated omega-3 fatty acids from marine oils, particularly those high in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are important because of their beneficial health effects. Concentrated omega-3 fatty acids are commercially available as several forms: triacylglycerides, ethyl esters, or free fatty acids. Lipases can effectively transform these forms of fish oils under mild conditions at an industrial scale. The limiting step in the production of enzymatic re-esterified triacylglycerides is the conversion of diacylglyceride to triacylglyceride. An important factor is acyl migration

of a 1,3- diacylglyceride to a 1,2- diacylglyceride. It is unclear if lipases can directly add an acyl group to the 2-position, or if migration followed by acylation of the 1-position is preferred. We examined a commercial lipase during the re-esterification process using ether lipid analogues of the 1,3- and 1,2 (2,3)- diacylglycerides. These diacylglyceride analogues have the ester functionality replaced by an ether bond, eliminating any acyl migration or interesterification. Products from the lipase esterification with DHA were analyzed by NMR, and compared to chemically synthesized standards.

INTRODUCTION

Omega-3 fatty acids

Omega-3 fatty acids, particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from fish oils, have important health benefits for cardiovascular disease, inflammation, brain & eye development, and neurological disorders.¹

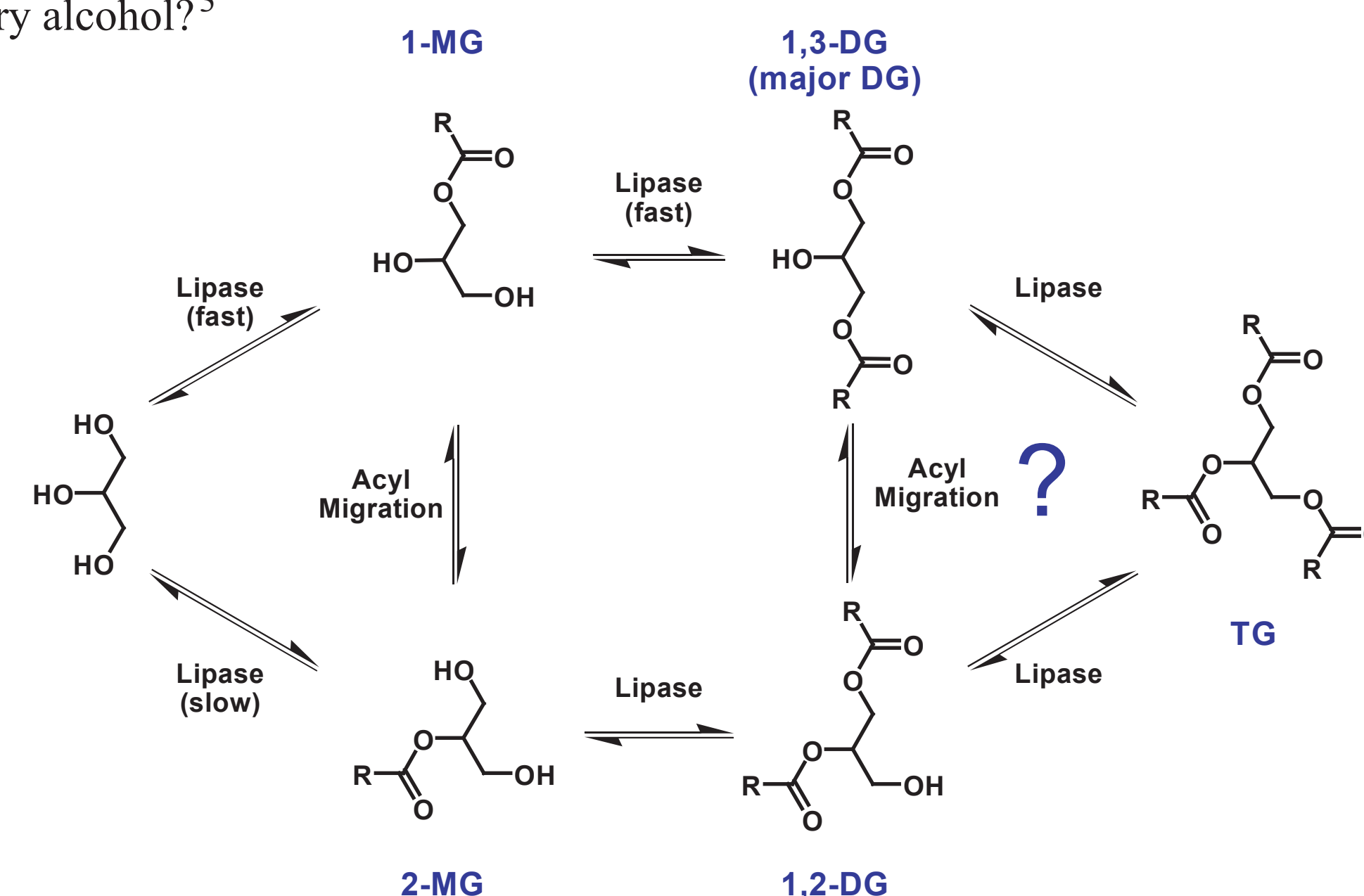


Oil quality is a significant consideration when processing highly unsaturated oils. Oxidation, polymerization, and isomerization may reduce the quality of the oil.

Lipases react under mild conditions (temperature, pH), can be regio- or fatty acid specific, and are green (fewer chemicals, less wastewater treatment, less energy), making them a good choice for omega-3 transformations.²

Direct Acylation at the 2-Position or Acyl Migration?

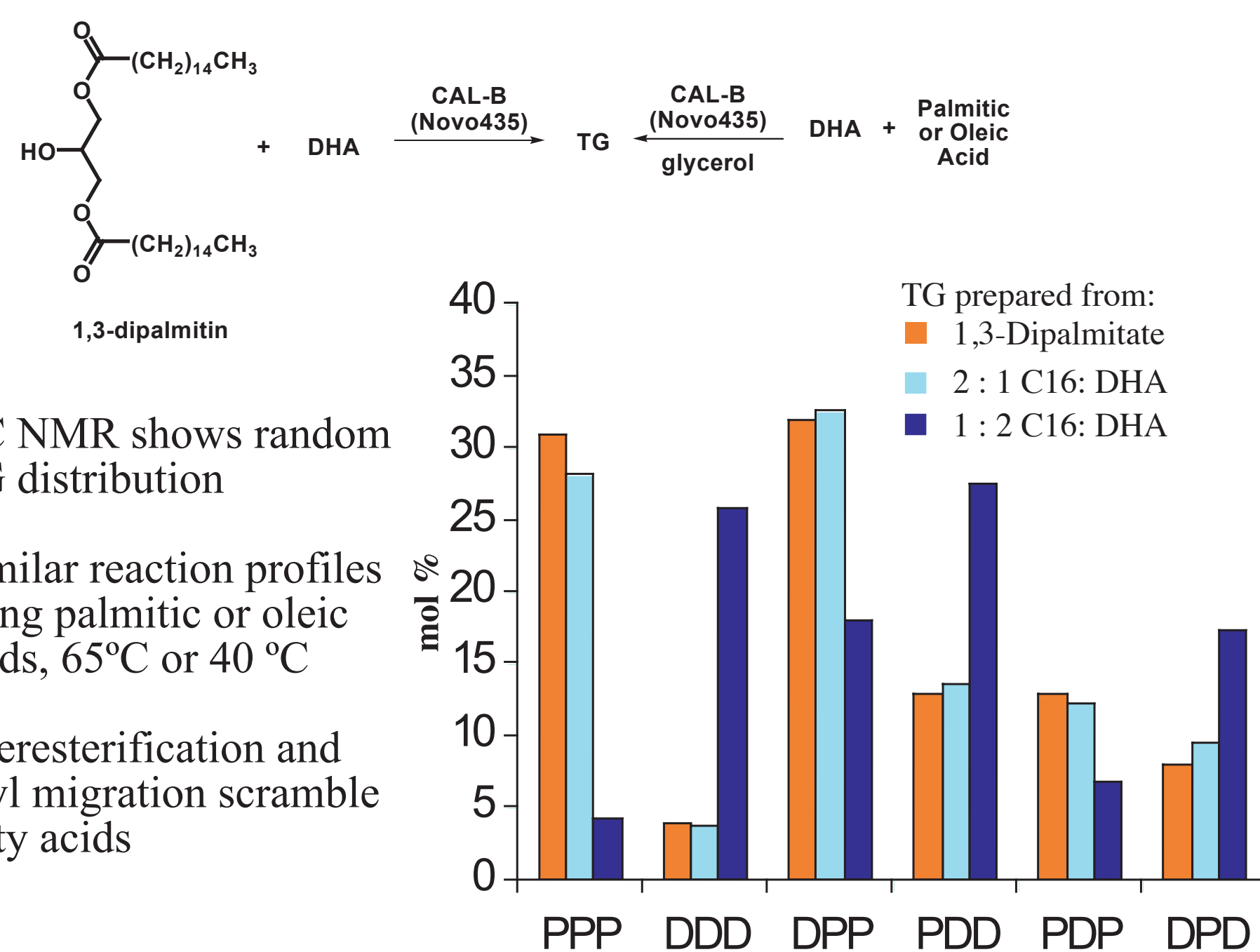
The 1,3-DG is the major diacylglyceride, but conversion to TG (triacylglyceride) occurs slowly. Does CAL-B esterify directly in the 2-position of the 1,3-DG, or is there acyl migration to the 1,2(2,3)-DG, followed by acylation of the primary alcohol?³



RESULTS

Random Distribution of Products

Esterification of 1,3-dipalmitin with DHA gives a random distribution, as does reaction with palmitic acid and DHA.



¹³C NMR shows random TG distribution

Similar reaction profiles using palmitic or oleic acids, 65°C or 40 °C

Interesterification and acyl migration scramble fatty acids

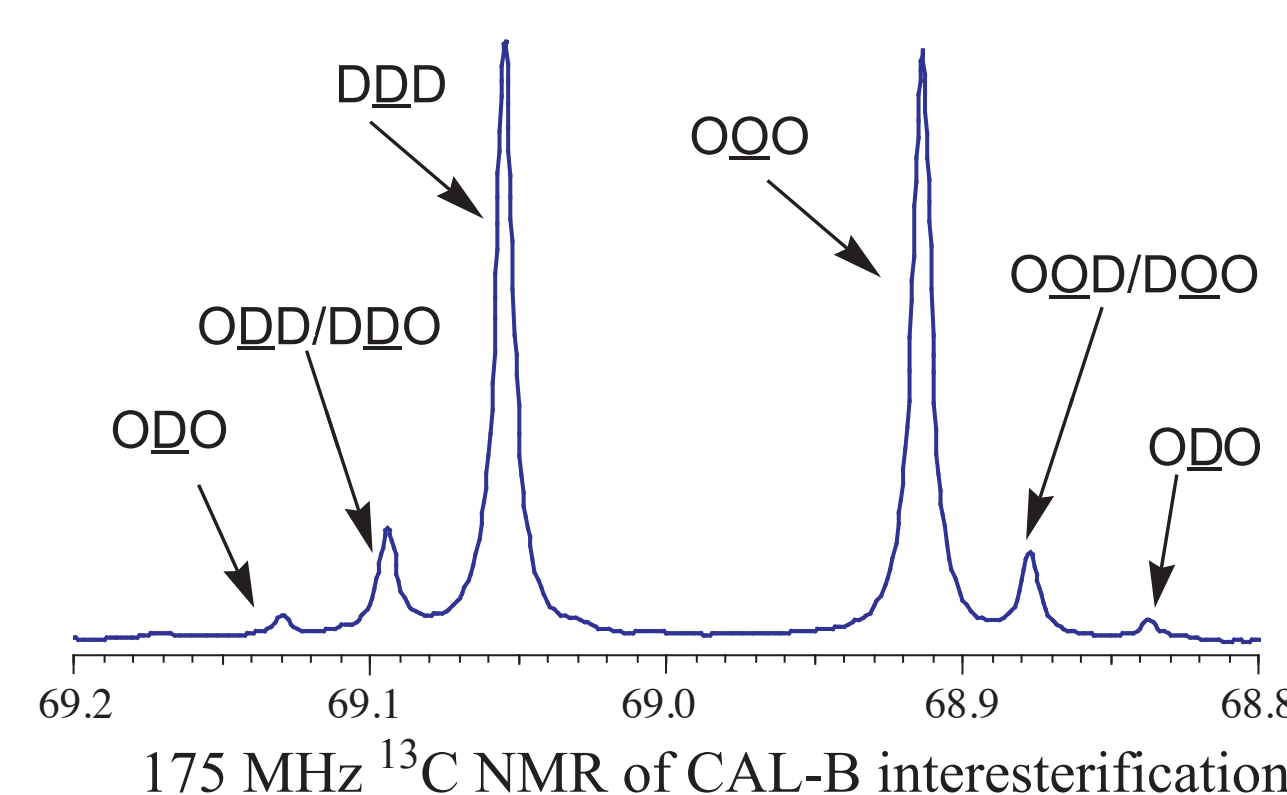
Random Distribution from Interesterification

Trioleate and tridocosahexaenoate mixed in an 1:1 mole ratio with immobilized *Candida antarctica* lipase B (40 °C).

Product	Percentage
OOO	40%
DDD	41%
OOD	10%
DDO	6%
ODO	2%
DOD	2%

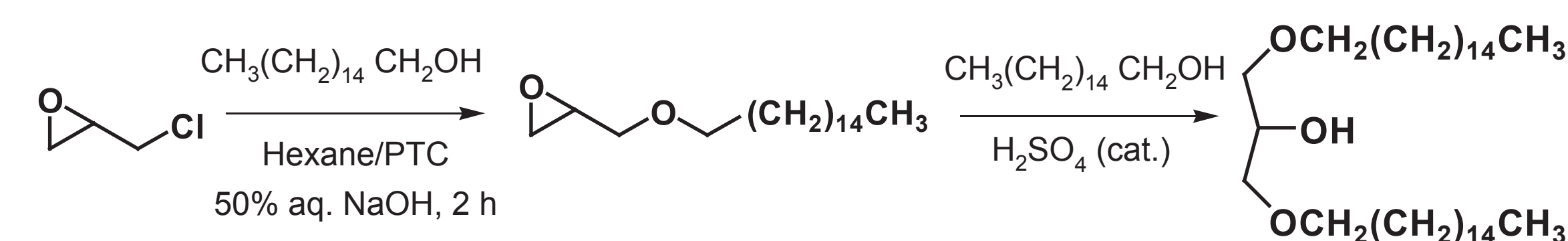
Interesterification contributes to the random distribution

2 largest interesterification products are OOD and DDO (exchange at primary positions)

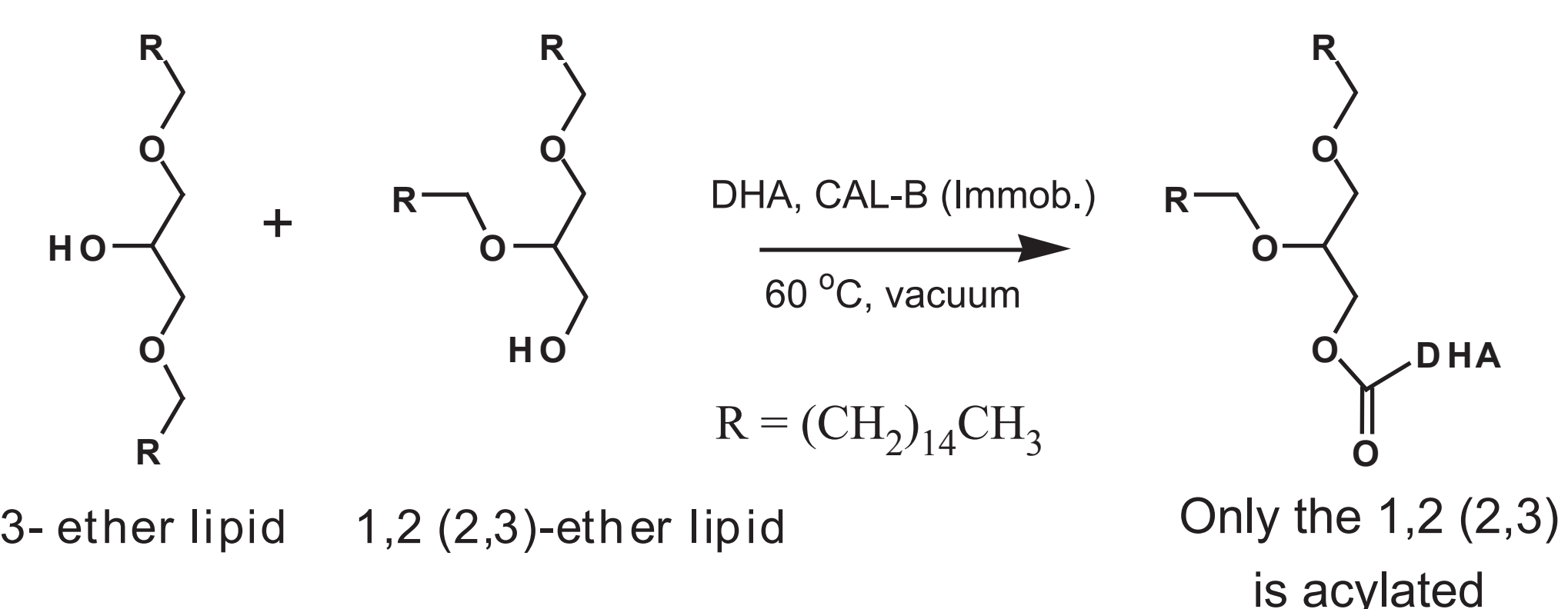


Ether Lipids Eliminate Acyl Migration & Interesterification

To eliminate acyl migration and interesterification, we prepared ether analogues of the 1,3- and 1,2(2,3)- diacylglycerides in which the ester bonds were replaced with ether bonds.



An equal amount of the 1,3-diether and the 1,2(2,3)-diether was reacted with DHA to test the enzyme's positional selectivity.



Conclusions

Products from the CAL-B re-esterification have a random distribution; interesterification contributes to the random distribution.

The lipase acylates the primary alcohols in 1,3-positions, and not directly at the 2-position.

Ether lipid diacylglyceride analogues suggest that triacylglycerides are formed by acylation of the 1,2(2,3)- diacylglyceride. The 1,3-DG must undergo acyl migration before being esterified.

REFERENCES

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¹³C NMR: Only the Primary Alcohol is Acylated

