AOCS's Phospholipids seminar:
Nutrition, application and technology

Copenhagen, Denmark, October 23 - 24, 2008

Chemoenzymatic Synthesis of Structured Phosphatidylcholine Positionally Labelled with Pure EPA and DHA

Gudmundur G. Haraldsson

Science Institute, University of Iceland, Dunhaga 3, 107 Reykjavik, Iceland

Objectives

To synthesize positionally labelled structured PC comprised of MCFA and n-3 PUFA

Outline

- Description of structured lipids
- Previous synthesis of structured lipids
- Synthesis of structured PC
- Summary

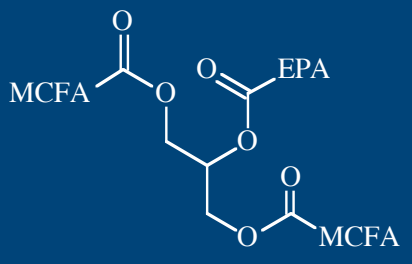
Structured Lipids

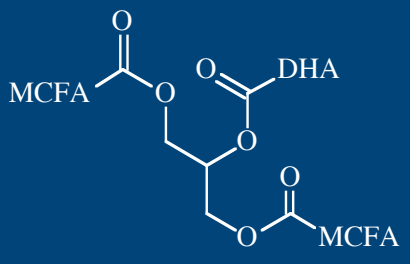
Lipids that have a predetermined composition and distribution of fatty acids at the glycerol backbone

Structured Acylglycerols

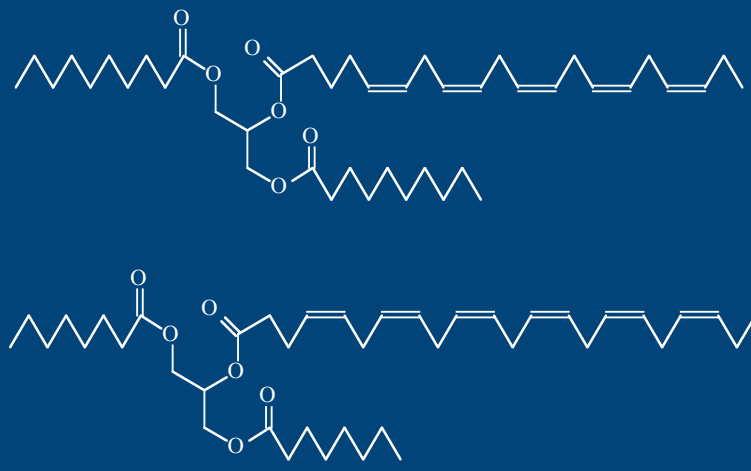
Acylglycerols containing one type of fatty acids (MCFA) at the end-position(s) and a different type (PUFA) at the mid-position of the glycerol backbone

Structured Triacylglycerols Comprising EPA and DHA

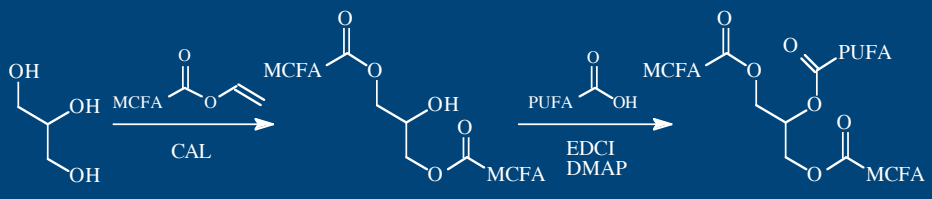




Structured Triacylglycerols



Chemoenzymatic Synthesis of MLM Type Structured TAG by Lipase



1a-d **2a-d** **3a-d**

MCFA = C_5H_{11} (**a**), C_7H_{15} (**b**), C_9H_{19} (**c**), $C_{11}H_{23}$ (**d**)

2: PUFA = EPA
3: PUFA = DHA

Reaction Conditions

Enzymatic Reaction

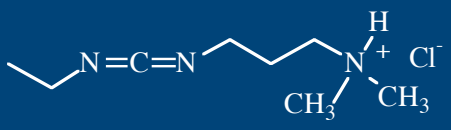
- *Candida antarctica* lipase
- Vinyl esters of MCFA (25% excess)
- Solvent: Dichloromethane
- Temperature: 0 - 4 °C
- Reaction time: 3 - 5 hours
- Purification: Crystallization (Hexane)
- Yields: Excellent (>90%)

Reaction Conditions

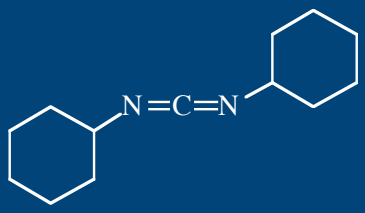
Coupling Reaction

- EDCI (20% excess); DMAP (0.4 eq.)
- Stoichiometric amount of EPA or DHA
- Solvent: Dichloromethane
- Room temperature
- Reaction time: 4 - 5 hours
- Purification: Silica gel chromatography
- Yields: Excellent (>90%)

EDCI and DCC Coupling Agents



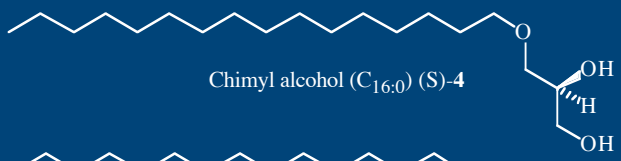
EDCI



DCC

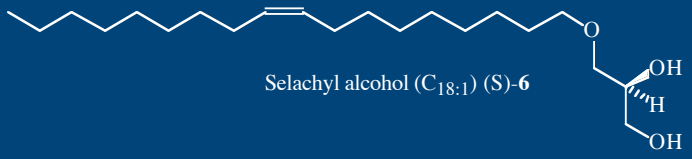
Glyceryl Ethers

Major Constituents in Shark Liver Oil



Chimyl alcohol ($C_{16:0}$) (S)-**4**

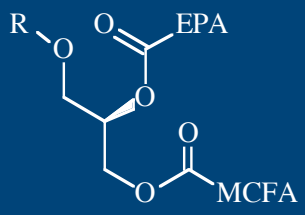
Batyl alcohol ($C_{18:0}$) (S)-**5**

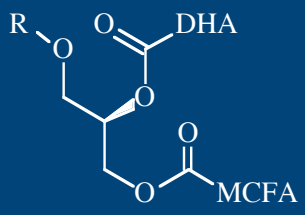


Selachyl alcohol ($C_{18:1}$) (S)-**6**

Structured Ether Lipids

1-O-Alkyl-2,3-Diacyl-*sn*-glycerols



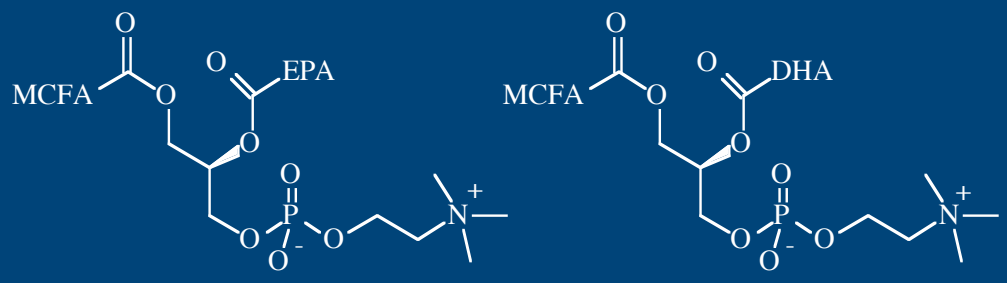


Synthesis of Structured TAG and EL

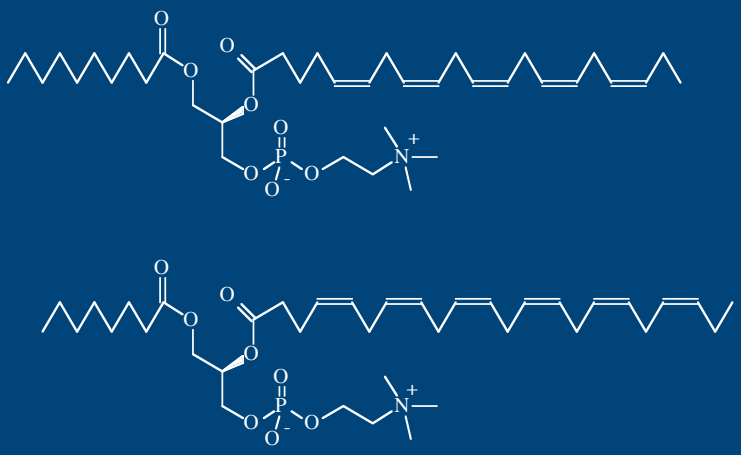
Enzymatic Step

- *Candida antarctica* lipase: the perfect catalyst
- MCFA as Vinyl Esters
- Reaction temperature: 0 - 4 °C
- Reaction time: 3 - 5 hours

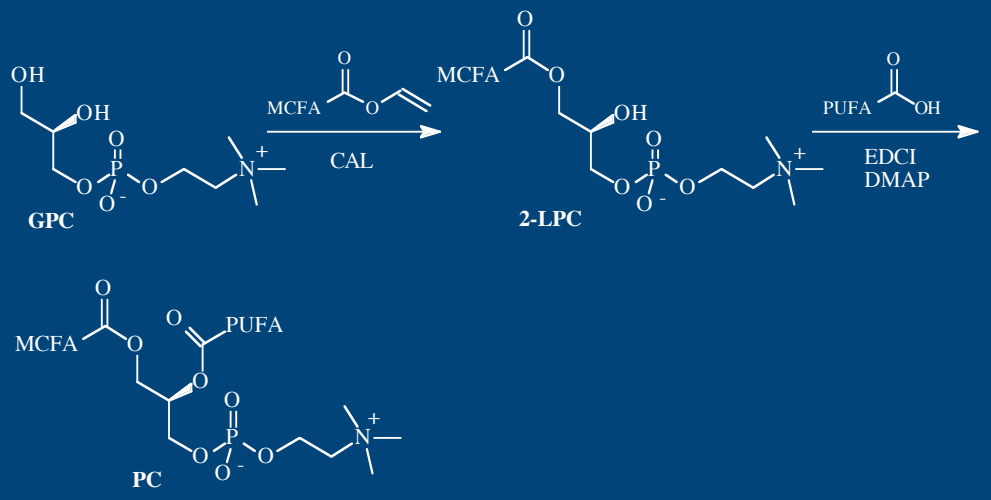
Enantiopure Structured PC



Enantiopure Structured PC



Chemoenzymatic Synthesis of Structured PC by Lipase



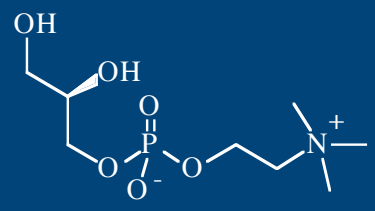
Synthesis of Asymmetrically Structured PC

Main Challenges

- Enantiocontrol
- Regiocontrol and regiopurity
- Lipase activity towards GPC
- Acyl migration
- Analytical aspects
- Purfication and full characterization

Chemoenzymatic Synthesis of Structured PC

Starting material



***sn*-Glycerol-3-phosphatidylcholine, GPC**

Synthesis of Structured PC

Enzymatic Step

***Candida antarctica* lipase**

- Excellent regioselectivity
- Slow: 90% Conversion after 96 hours
- High yields

Lipase Investigation

Enzymatic Step (C_{12}; CH_2Cl_2; 24 h)

Lipase	Conversion (%)	Regio-selectivity
Rhizomucor miehei	94	Excellent
Thermomyces lanuginosa	92	2% migration
Candida antarctica	54	Excellent

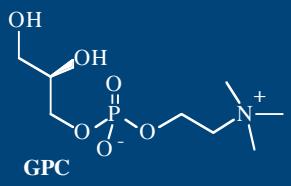
Lipase Investigation

Conversion of RML (C_8 in CH_2Cl_2)

Time (h)	Conversion (%)
14	82
18	91
24	98

Chemoenzymatic Synthesis of Structured PC

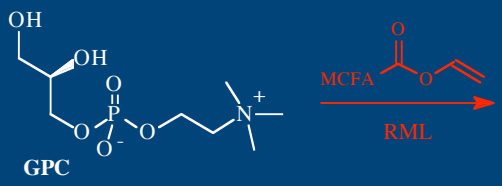
Enzymatic Step



GPC

Chemoenzymatic Synthesis of Structured PC

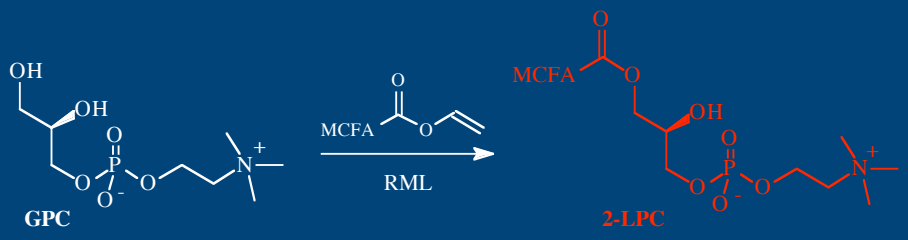
Enzymatic Step



GPC

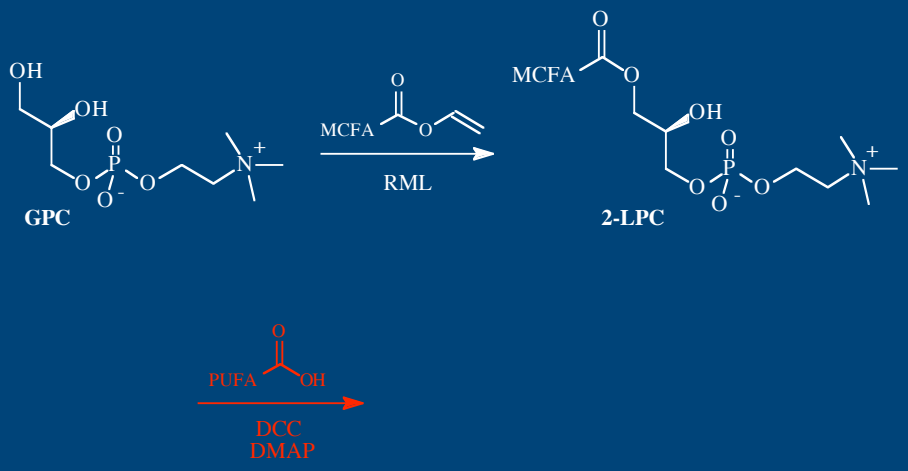
Chemoenzymatic Synthesis of Structured PC

Enzymatic Step



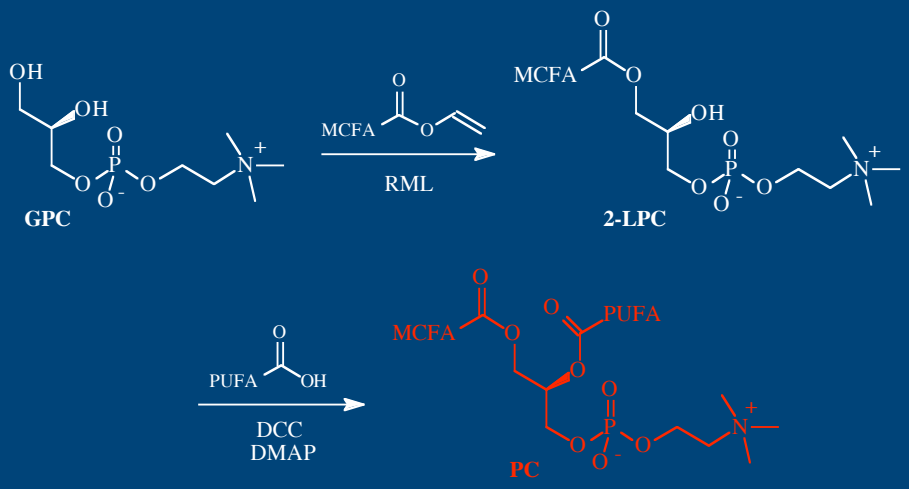
Chemoenzymatic Synthesis of Structured PC

Coupling Step

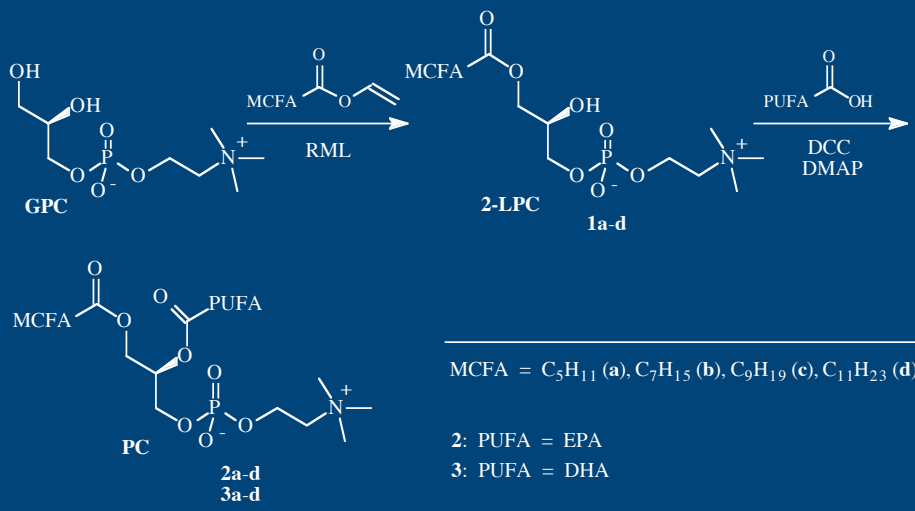


Chemoenzymatic Synthesis of Structured PC

Coupling Step



Chemoenzymatic Synthesis of Structured PC by Lipase



MCFA = C_5H_{11} (**a**), C_7H_{15} (**b**), C_9H_{19} (**c**), $C_{11}H_{23}$ (**d**)

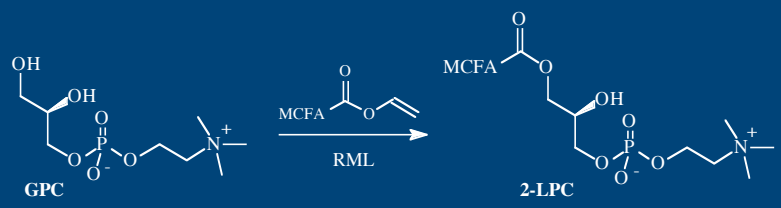
2: PUFA = EPA
3: PUFA = DHA

Synthesis of Structured PC

Enzymatic Step

- *Rhizomucor miehei* lipase: excellent regioselectivity
- Excellent yields
- MCFA as Vinyl Esters
- Solvent: Dichloromethane
- Room temperature
- Reaction time: 24 hours

Results of Enzyme Reaction



Compound	MCFA	Conv. (%)	Yields (%)	$[\alpha]_D$ [1)]
(R)-**1a**	$-C_5H_{11}$	98	90	+3.1
(R)-**1b**	$-C_7H_{15}$	98	97	+2.4
(R)-**1c**	$-C_9H_{19}$	92	91	+2.5
(R)-**1d**	$-C_{11}H_{23}$	90	88	+3.7

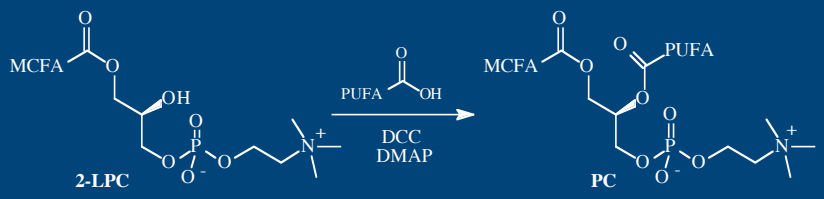
[1)] c = 1, CH_3OH

PC Reaction Conditions

Coupling Reaction

- DCC (2-fold excess); DMAP (1 eq.)
- EPA: 2-fold excess
- Solvent: Chloroform
- Room temperature
- Reaction time: 24 hours
- Purification: Silica gel chromatography
- Yields: High to excellent (73 - 91%)

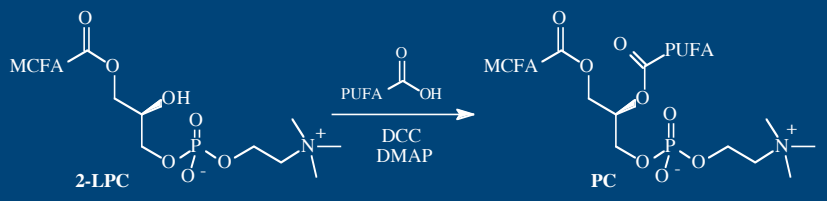
Results of Coupling Reaction



Compound	MCFA	PUFA	Yields (%)	$[\alpha]_D$ [1)]
(R)-**2a**	$-C_5H_{11}$	EPA	84	+9.0
(R)-**2b**	$-C_7H_{15}$	EPA	91	+8.8
(R)-**2c**	$-C_9H_{19}$	EPA	88	+9.4
(R)-**2d**	$-C_{11}H_{23}$	EPA	88	+8.8

[1)] c = 1, $CHCl_3/CH_3OH$ (1:1)

Results of Coupling Reaction



Compound	MCFA	PUFA	Yields (%)	$[\alpha]_D$ [1)]
(R)-**2a**	$-C_5H_{11}$	DHA	87	+4.3
(R)-**2b**	$-C_7H_{15}$	DHA	94	+5.1
(R)-**2c**	$-C_9H_{19}$	DHA	85	+3.9
(R)-**2d**	$-C_{11}H_{23}$	DHA	73	+4.4

1) c = 1, $CHCl_3/CH_3OH$ (1:1)

Summary

- Enantiopure structured PC
- *Rhizomucor miehei* lipase: The best catalyst
- Outstanding regioselectivity of the lipase
- Acyl migration eliminated by mild conditions
- Very high to excellent yields in all cases
- Only two steps
- Full characterization by ^{1}H, ^{13}C and ^{31}P NMR

Application

- Clinical research
- Individual fatty acid investigations
- Pure compounds useful as standards
- Isotopically labelled fatty acids
- Liposomes

Acknowledgements

- Björn Kristinsson and Carlos D. Magnusson
- Dr. Sigridur Jonsdottir for NMR analysis
- Dr. Sigurdur Smarason for High Resolution MS
- Icelandic Research Fund for financial support
- Avanti Polar Lipids for Pure GPC
- Pronova Biocare in Norway for pure EPA and DHA and Novozyme in Denmark for the lipase


Thank you!