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

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

![Chemical Structures](image-url)
Structured Triacylglycerols

Chemoenzymatic Synthesis of MLM Type Structured TAG by Lipase

MCFA = C5H11 (a), C7H15 (b), C9H19 (c), C11H23 (d)

1. PUFA = EPA
2. PUFA = DHA
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%)

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

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

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

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

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

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

Chimyl alcohol (C_{16:0}) (S)-4
Structured Ether Lipids

1-O-Alkyl-2,3-Diacyl-\textit{sn}-glycerols

\[
\text{R} \left(\begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O}
\end{array}\right) \begin{array}{c}
\text{EPA} \\
\text{MCFA} \\
\text{MCFA} \\
\text{DHA} \\
\text{MCFA} \\
\text{MCFA}
\end{array}
\]

Synthesis of Structured TAG and EL

\textit{Enzymatic Step}

- \textit{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
- Purification and full characterization
Chemoenzymatic Synthesis of Structured PC

Starting material

\[\text{sn-Glycerol-3-phosphatidylcholine, GPC}\]

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}; \text{CH}_2\text{Cl}_2; 24 \text{ h})

<table>
<thead>
<tr>
<th>Lipase</th>
<th>Conversion (%)</th>
<th>Regio-selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizomucor miehei</td>
<td>94</td>
<td>Excellent</td>
</tr>
<tr>
<td>Thermomyces lanuginosa</td>
<td>92</td>
<td>2% migration</td>
</tr>
<tr>
<td>Candida antarctica</td>
<td>54</td>
<td>Excellent</td>
</tr>
</tbody>
</table>

Conversion of RML (C_8 in CH_2Cl_2)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>82</td>
</tr>
<tr>
<td>18</td>
<td>91</td>
</tr>
<tr>
<td>24</td>
<td>98</td>
</tr>
</tbody>
</table>
Chemoenzymatic Synthesis of Structured PC

Enzymatic Step

\[
\text{GPC} + \text{MCFA} \rightarrow \text{RML}
\]
Chemoenzymatic Synthesis of Structured PC

**Enzymatic Step**

\[
\text{GPC} \stackrel{\text{MCFA}}{\xrightarrow{\text{RML}}} \text{2-LPC}
\]

**Coupling Step**

\[
\text{GPC} \stackrel{\text{MCFA}}{\xrightarrow{\text{RML}}} \text{2-LPC}
\]

PUFA

DCC

DMAP
Chemoenzymatic Synthesis of Structured PC

Coupling Step

GPC + MCFA \rightarrow 2-LPC

PUFA + DCC DMAP \rightarrow PC

Chemoenzymatic Synthesis of Structured PC by Lipase

GPC + MCFA \rightarrow 2-LPC

PC + PUFA + DCC DMAP

MCFA = C_{5}H_{11} (a), C_{7}H_{15} (b), C_{9}H_{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

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCFA</th>
<th>Conv. (%)</th>
<th>Yields (%)</th>
<th>$[\alpha]_D^{1)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-1a</td>
<td>-C$<em>3$H$</em>{11}$</td>
<td>98</td>
<td>90</td>
<td>+3.1</td>
</tr>
<tr>
<td>(R)-1b</td>
<td>-C$<em>7$H$</em>{15}$</td>
<td>98</td>
<td>97</td>
<td>+2.4</td>
</tr>
<tr>
<td>(R)-1c</td>
<td>-C$<em>9$H$</em>{19}$</td>
<td>92</td>
<td>91</td>
<td>+2.5</td>
</tr>
<tr>
<td>(R)-1d</td>
<td>-C$<em>{11}$H$</em>{23}$</td>
<td>90</td>
<td>88</td>
<td>+3.7</td>
</tr>
</tbody>
</table>

$^{1)}$ c = 1, CH$_3$OH
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

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCFA</th>
<th>PUFA</th>
<th>Yields (%)</th>
<th>([\alpha]^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-2a</td>
<td>-C_6H_{11}</td>
<td>EPA</td>
<td>84</td>
<td>+9.0</td>
</tr>
<tr>
<td>(R)-2b</td>
<td>-C_7H_{15}</td>
<td>EPA</td>
<td>91</td>
<td>+8.8</td>
</tr>
<tr>
<td>(R)-2c</td>
<td>-C_9H_{19}</td>
<td>EPA</td>
<td>88</td>
<td>+9.4</td>
</tr>
<tr>
<td>(R)-2d</td>
<td>-C_{11}H_{23}</td>
<td>EPA</td>
<td>88</td>
<td>+8.8</td>
</tr>
</tbody>
</table>

\(^1\) c = 1, CHCl_3/CH_3OH (1:1)
### Results of Coupling Reaction

![Diagram of coupling reaction]

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCFA</th>
<th>PUFA</th>
<th>Yields (%)</th>
<th>$[\alpha]_D^{1)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-2a</td>
<td>-C₂H₇</td>
<td>DHA</td>
<td>87</td>
<td>+4.3</td>
</tr>
<tr>
<td>(R)-2b</td>
<td>-C₇H₁₉</td>
<td>DHA</td>
<td>94</td>
<td>+5.1</td>
</tr>
<tr>
<td>(R)-2c</td>
<td>-C₉H₂₁</td>
<td>DHA</td>
<td>85</td>
<td>+3.9</td>
</tr>
<tr>
<td>(R)-2d</td>
<td>-C₁₁H₂₃</td>
<td>DHA</td>
<td>73</td>
<td>+4.4</td>
</tr>
</tbody>
</table>

$^{1)} c = 1, \text{CHCl}_3/\text{CH}_3\text{OH} (1:1)$

### 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 $^1\text{H}$, $^{13}\text{C}$ and $^{31}\text{P}$ NMR
Application

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

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Thank you!