Supplementary materials

Separation of enantiomeric triacylglycerols by chiral-phase HPLC

Tomáš Řezanka, Karel Sigler

Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic

∗Corresponding author at: Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague, Czech Republic. Tel: +420 241 062 300

Fax: +420 241 062 347

E-mail address: rezanka@biomed.cas.cz (T. Rezanka).

Stereospecific synthesis of enantiomers.

Enantiomers listed in Table 2 were synthesized from 2,3-isopropylidene-*sn*-glycerol [14]. Approximately 0.05 mmol of 2,3-isopropylidene-*sn*-glycerol (6.6 mg), 0.06 mmol appropriate FA (palmitic (P) = 15.4 mg, (7*Z*)-hexadecenoic (Po) = 15.2 mg, 7(*Z*),10(*Z*)-hexadecadienoic (Pl) =15.1 mg, 7(*Z*),10(*Z*),13(*Z*)-hexadecatrienoic (Pn) = 15.0 mg, 4(*Z*),7(*Z*),10(*Z*),13(*Z*)-hexadecatetraenoic (Pm) = 14.9 mg acids), DMAP (6.1 mg, 50 μmol) and EDCI (18.6 mg, 0.12 mmol) in dichloromethane (1.0 mL) under an inert atmosphere (argon) were stirred in a vial for 15 hours at ambient temperature. The free monoacylglycerol (MAG) was obtained by the reaction with 0.25 mL of ice cold trifluoroacetic acid for 30 min at -20 °C, which was neutralized with 1 M NaOH, all performed at 0 °C. The reaction mixture was extracted by the mixture of CH_2Cl_2 - MeOH (3:1, vol/vol) and evaporated. The MAG was again dissolved in dichloromethane (1.0 mL) under inert atmosphere (argon), DMAP (12.2 mg, 0.1 mmol) and EDCI (37.26 mg, 0.24 mmol) in dichloromethane (1.0 mL) were added and reaction mixture was stirred for 15 h at 0 ^{o}C. The TAG was extracted by hexane and analyzed by LC/MS.

Enzymatic synthesis of TAGs

1,2-Diarachidonoyl-*sn*-glycero-3-phosphocholine was treated with phospholipase C (Type I from *C. perfringens* (*C. welchii*)) as described by Christie (1989). In brief, phospholipase C from *C. welchii* (3 mg) in 0.5 M tris(hydroxymethyl)methylamine (tris) buffer (pH 7.5; 2 mL), 2 x 10^{-3} M in calcium chloride, was added to phosphatidylcholine (15 mg) in diethyl ether (2 mL). The mixture was shaken at room temperature for 3 hours and extracted three times with diethyl ether (4 mL portions). The ether layer was dried over anhydrous sodium sulfate before being evaporated in a stream of nitrogen at ambient temperature. Pure 1,2-diarachidonoyl-*sn*-glycerol was obtained by preparative TLC on a layer of silica gel G impregnated with boric acid (10%, w/w), with hexane-diethyl ether (50:50, v/v) as solvent system. The appropriate band was located under UV light and was eluted from the adsorbent with diethyl ether [21].

4.4. Preparation of mixed FA composition TAGs

The esterification of glycerol derivatives with acids was described previously [21,22]. Briefly, a solution of 1,2-dipalmitoyl-*sn*-glycerol (57 mg, 0.1 mmol) and arachidonic acid (33 mg, 0.11 mmol) as a free acid in dichloromethane (1.0 mL) was added to DMAP (6.1 mg, 50 μmol) and EDCI (18.6 mg, 0.12 mmol) in dichloromethane (1.0 mL) under an inert atmosphere (argon). The resulting solution was stirred at room temperature for 15 h. The

reaction was stopped by passing the reaction mixture in ether/dichloromethane (10:90) through a short column packed with silica gel. Solvent removal in vacuo afforded the pure product as yellowish oil.

The general procedure for the synthesis of *sn*-AAP was as follows: under an inert atmosphere, a solution of 1,2-diarachidonoyl-*sn*-glycerol (6.6 mg, 0.01 mmol) and palmitic acid (2.8 mg, 0.011 mmol) as a free acid in dichloromethane (1.0 mL) was added to DMAP (0.1 mg, 8 μmol) and EDCI (3.7 mg, 0.024 mmol) in dichloromethane (1.0 mL). For further procedure, see above.

FAMEs analysis

The total TAGs (~5 mg) were saponified overnight in 10% KOH-MeOH at room temperature. A fatty acid fraction obtained from the saponification was partitioned between alkali solution (pH 9) and diethyl-ether to remove basic and neutral components. The aqueous phase containing fatty acids was acidified to pH 2 and extracted with hexane. The fatty acid fraction was methylated using BF_3/MeOH (14% solution of BF_3 from Sigma-Aldrich).

Gas chromatography–mass spectrometry of FAME was done on a GC–MS system consisting of Varian 450-GC (Varian BV, Middelburg, The Netherlands), Varian 240-MS ion trap detector with electron ionization (EI), and CombiPal autosampler (CTC, USA) equipped with split/splitless injector. A DB-5MS column was used for separation (60 m, 0.25 mm ID, 0.25 μm film thickness). The temperature program started at 60 °C and was held for 1 min in splitless mode. Then the splitter was opened and the oven was heated to 160 °C at a rate of 25 °C min^{-1}. The second temperature ramp was up to 280 °C at a rate of 2.5 °C min^{-1}, this temperature being maintained for 10 min. The solvent delay time was set to 8 min. The transfer line temperature was set to 280 °C. Mass spectra were recorded at 3 scans s^{-1} under electron ionization at 70 eV, mass range *m/z* 50-450. FAMEs (fatty acid methyl esters) were identified according to their mass spectra [23,24] and using a mixture of chemical standards obtained from Sigma-Aldrich.

Table 1S. Survey of literature data on the separation of TAGs on chiral columns

Literature	Column type	Mobile phase; temperature/infusion solvent/ matrix	Flow (mL/min)	Ion source analyzer	Mass range (m/z)	Samples matrix
[4]	Chiracel OF cellulose-tris-(4-chlorphenylcarbamate)	n-hexane-2-propanol (200:1 or 400 :1)	1.0	UV, 210 nm		
	Chiracel OD cellulose-tris-(3,5-dimethylphenylcarbamate)	n-hexane-2-propanol (200:1 or 400 :1)	1.0	UV, 210 nm		
[5]	CYCLOBOND ™ I 2000 DMP 3,5-dimethylphenyl carbamate modified β-cyclodextrin	99:1 methanol:10 mM ammonium acetate	0.5	positive ion APCI mode	200-1000	diatom *Phaeodactylum tricornutum* synthetic standards
[16]	two CYCLOBOND ™ I 2000 DMP 3,5-dimethylphenyl carbamate modified β-cyclodextrin in series	gradient 90% hexane and 10% hexane/iPoOH (99:2, v/v) to 50% hexane and 50% hexane/iPoOH 120 min	0.5	positive ion APCI mode	200-1100	11 yeast strains
[17]	two Lux Cellulose-1 cellulose-tris-(3,5-dimethylphenylcarbamate) silicagel in series	90% hexane and 10% hexane-2-propanol (99:1, v/v) to 60% hexane and 40% hexane-2-propanol 180 min	1.0	positive ion APCI mode	50-1200	hazelnut oil+ human plasma
[18]	Chiralpak IA	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)	0.5			

	amylose tris-(3,5-dimethylphenyl carbamate)	methanol or acetonitrile				
	Chiralpak IB	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)				
	cellulose tris-(3,5-dimethylphenyl carbamate)	methanol or acetonitrile				
	Chiralpak IC	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)				
	cellulose tris-(3,5-dichlorphenyl carbamate)	methanol or acetonitrile				
	Chiralpak AD-RH (or AD-H)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)				
	amylose tris-(3,5-dimethylphenyl carbamate)	methanol or acetonitrile				
	Chiralpak AS-RH (or AS-H)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)				
	amylose tris-(S-α-methylbenzylcarbamate	methanol or acetonitrile				
	Chiralpak AY-H	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)				
	amylose tris-(5-chloro-2-methylphenylcarbamate)	methanol or acetonitrile				
	Chiracel OD-RH (or OD-H)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)	0.5	positive ion APCI mode	500-1000	8 TAG-enantiomers
	cellulose tris-(3,5-dimethylphenylcarbamate)	methanol or acetonitrile				
	Chiracel OJ-RH (or OJ-H)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)				
	cellulose tris-(4-methylbenzoate)	methanol or acetonitrile				
	Chiracel OZ-H	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)				
	cellulose tris-(3-chloro-4-methylphenylcarbamate)					
[20]	Chiralpak IA	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)	1.0			
	amylose tris-(3,5-dimethylphenyl carbamate)	methanol or acetonitrile	0.5			

Chiralpak IB cellulose tris-(3,5-dimethylphenyl carbamate)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v) methanol or acetonitrile	1.0 0.5			
Chiralpak IC cellulose tris-(3,5-dichlorphenyl carbamate)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v) methanol or acetonitrile	1.0 0.5			
Chiralpak AD-RH (or AD-H) amylose tris-(3,5-dimethylphenyl carbamate)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v) methanol or acetonitrile	1.0 0.5			
Chiralpak AS-RH (or AS-H) amylose tris-(S-α-methylbenzylcarbamate	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v) methanol or acetonitrile	1.0 0.5			
Chiralpak AY-H amylose tris-(5-chloro-2-methylphenylcarbamate)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v) methanol or acetonitrile	1.0 0.5			
Chiracel OD-RH (or OD-H) cellulose tris-(3,5-dimethylphenylcarbamate)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v) methanol or acetonitrile	1.0 0.5	positive ion APCI mode	500-1000	TAG-enantiomers
Chiracel OJ-RH (or OJ-H) cellulose tris-(4-methylbenzoate)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v) methanol or acetonitrile	1.0 0.5			
Chiracel OZ-H cellulose tris-(3-chloro-4-methylphenylcarbamate)	Hexane/ethanol (100 /0.5, v/v); or hexane/2-propanol (100/0.5, v/v)	1.0			

Fig. 1S. Two models of PmPPm and EPE

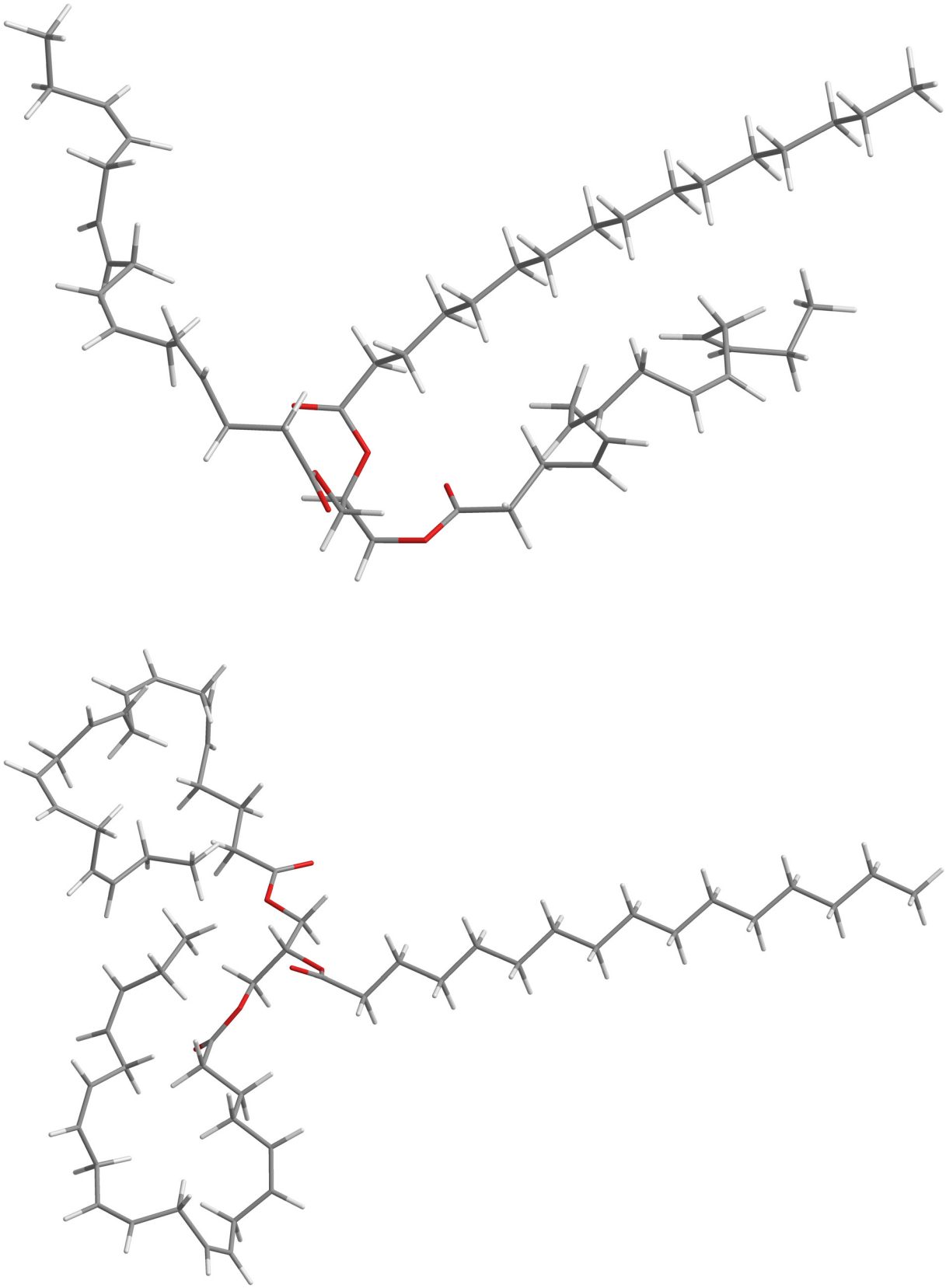


Fig. 2S. Mass spectrum (APCI) of synthetic EPE.

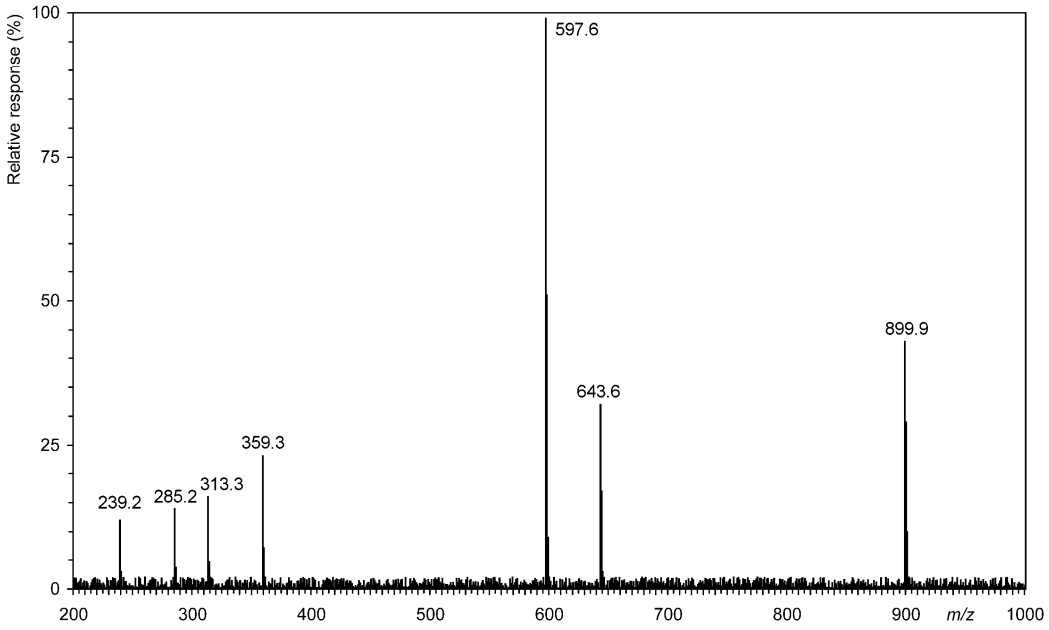


Fig. 3S. Mass spectrum (APCI) of natural EEP.

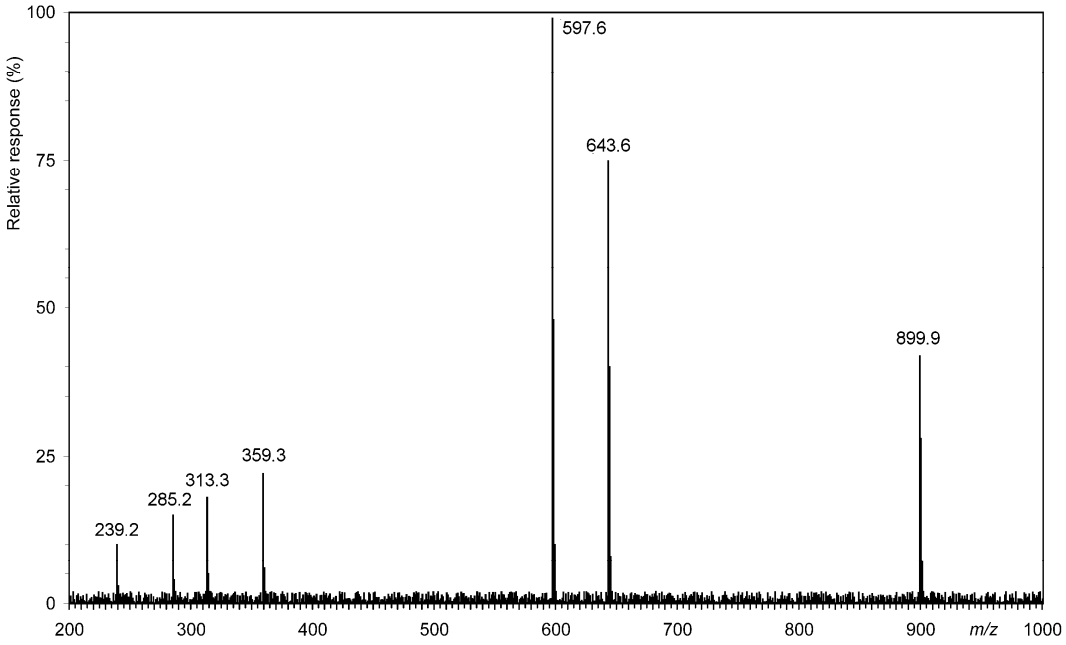
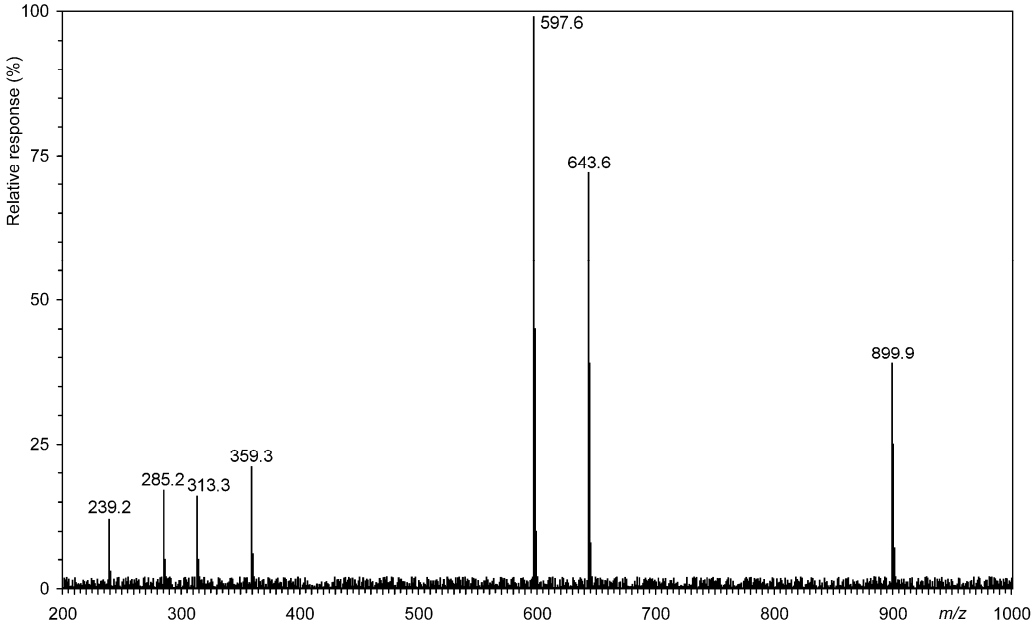


Fig. 4S. Mass spectrum (APCI) of synthetic PEE.



Figs. 5S-10S. The structures of the enantiomers and diastereisomers of C16 and C20 PUFA.

Fig. 5S. PmPPm

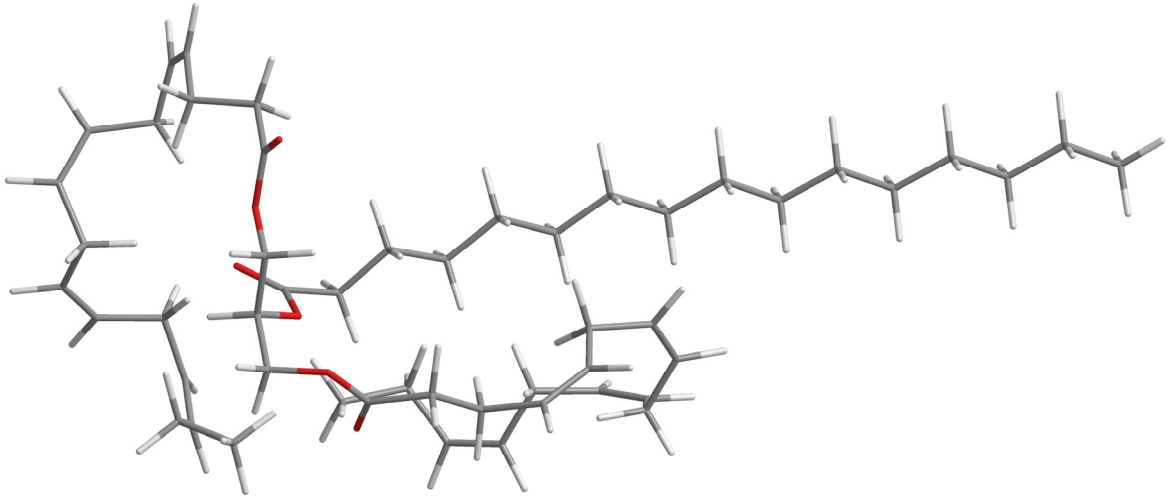


Fig. 6S. PPmPm

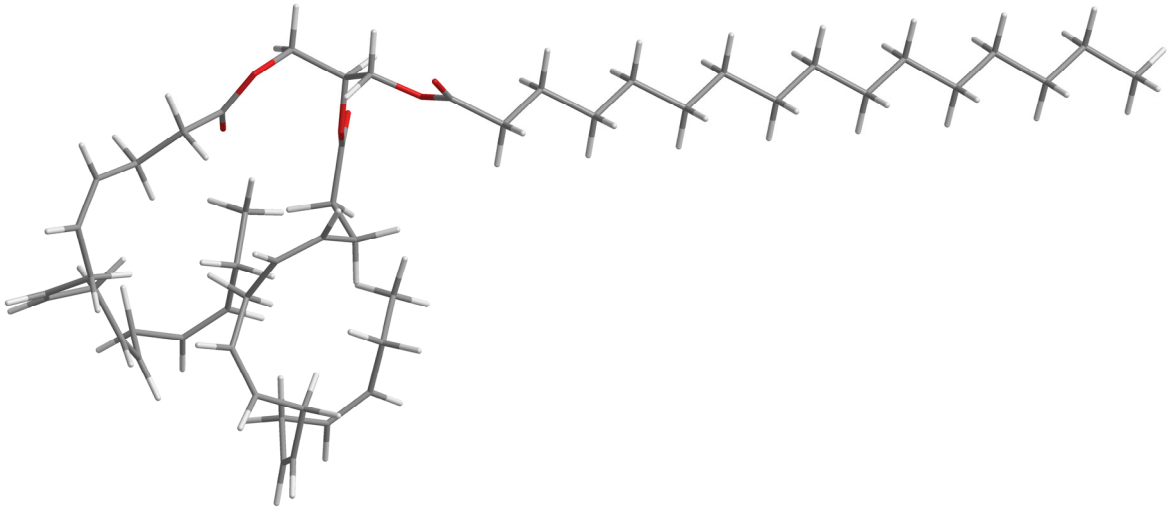


Fig. 7S. PmPmP

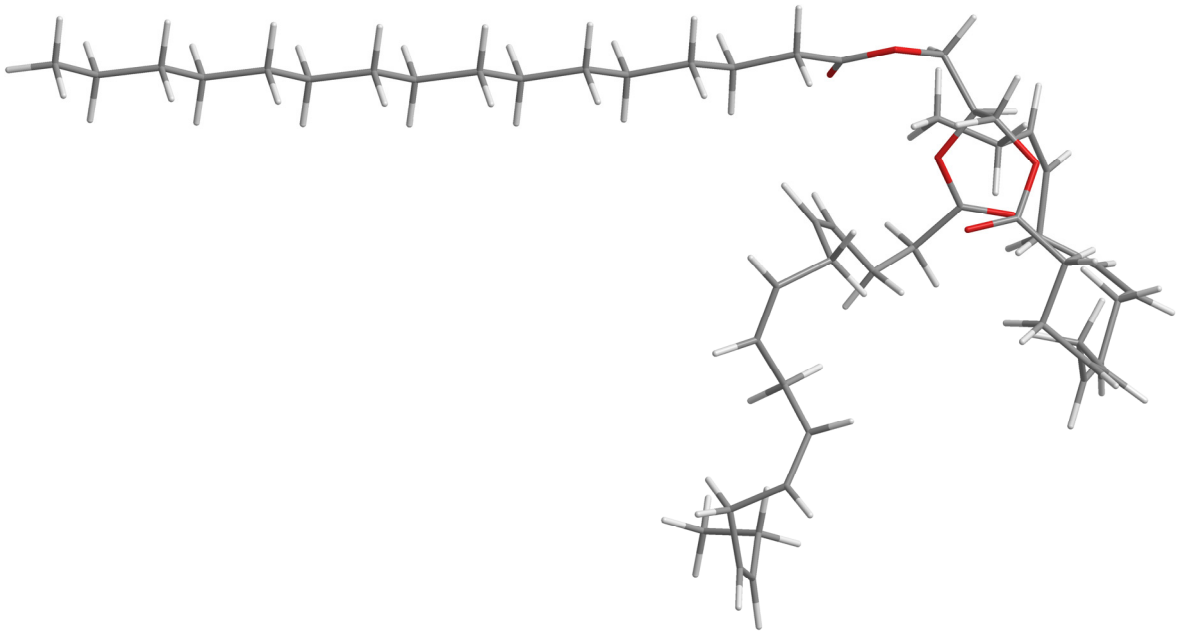


Fig. 8S. EPE

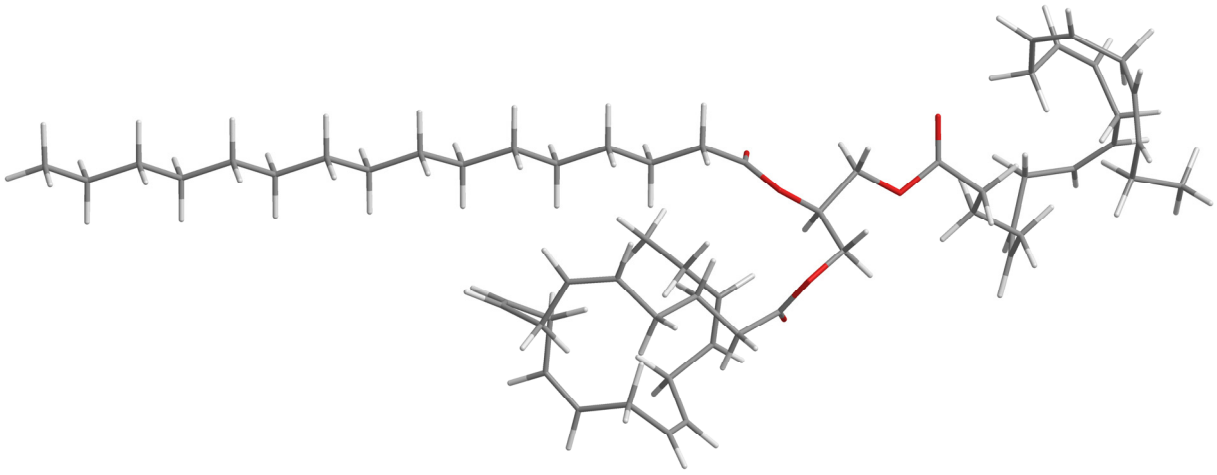


Fig. 9S. PEE

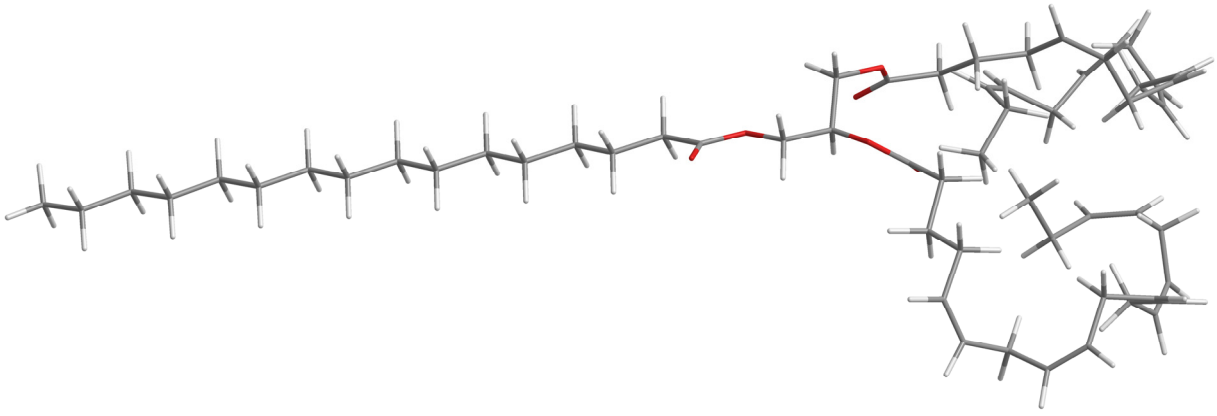


Fig. 10S. EEP