Supplements

Chiral analysis of glycerol phosphates - Can bacteria biosynthesize heterochiral phospholipid membranes?

Andrea Palyzová[1], Irina A. Guschina[2], Tomáš Řezanka[1]✉

[1] Institute of Microbiology, the Czech Academy of Sciences, 142 20 Prague 4, Czech Republic

[2] School of Biosciences, Cardiff University, Cardiff CF10 3AX, United Kingdom

Detection of heterochirality in lipid membranes

Two commercially available standards (1,2-dimyristoyl-sn-glycero-3-phosphocholine and 2,3-dimyristoyl-sn-glycero-1-phosphocholine) were added in HC2Cl2-MeOH solution to the lyophilized cells; lipids were extracted and the enantiomers was found. It was shown that the second enantiomer was not present after extraction and chiral HPLC, the respective proportion being below 1% of total enantiomers. Chiral HPLC of both standards was also performed, and it was found that the second enantiomer was not present in the commercial preparation, or its concentration was below 1%. This means that no phosphoryl group migration occurred. The areas of the ion peaks at m/z 734.5136 were compared. The same procedure was carried out with 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine and, in another experiment, with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; the detection was performed at *m/z* 818.5919. This experiment proved that there was no acyl(s) migration. Finally, we may conclude that this approach proved that migration of neither the acyl(s) nor phosphoryl group took place in both, the mobile phase and the cells.

Table S1. Contents of G3P and G1P

microorganism	G3P	G1P	reference	ratio G3P/G1P
Bacillus amyloliquefacience	34	11.5	this paper	3.0
Bacillus subtilis	33	10.9	this paper	3.0
Clavibacter michiganensis	23	5.3	this paper	4.3
Geobacillus stearothermophilus	25	6.2	this paper	4.0
Saccharomyces pastorianus	30	0.0	this paper	-
Escherichia coli	49	?[b]	A35a	-
Spathaspora arborariae	44/15[c]	?	A35b	-

[a] The values (units) are in µM/L.
[b] enantiomer analysis was not performed
[c] aerobic cultivation/oxygen-limited cultivation

Table S2. Occurrence of enzymes in bacteria - glycerol-1-phosphate dehydrogenase.

bacterium	GenBank
Bacillus amyloliquefaciens	KYC93433.1
Bacillus subtilis	AIW38374.1
Clavibacter sp.	GBL23066.1
Geobacillus stearothermophilus	KFX35283.1

Table S3. Occurrence of enzymes in bacteria - glycerol-3-phosphate dehydrogenase.

bacterium	GenBank
Bacillus amyloliquefaciens	KYC86943.1
Bacillus subtilis	KOS72728.1
Clavibacter michiganensis subsp. *nebraskensis*	OAH22261.1
Geobacillus stearothermophilus	KYD34837.1

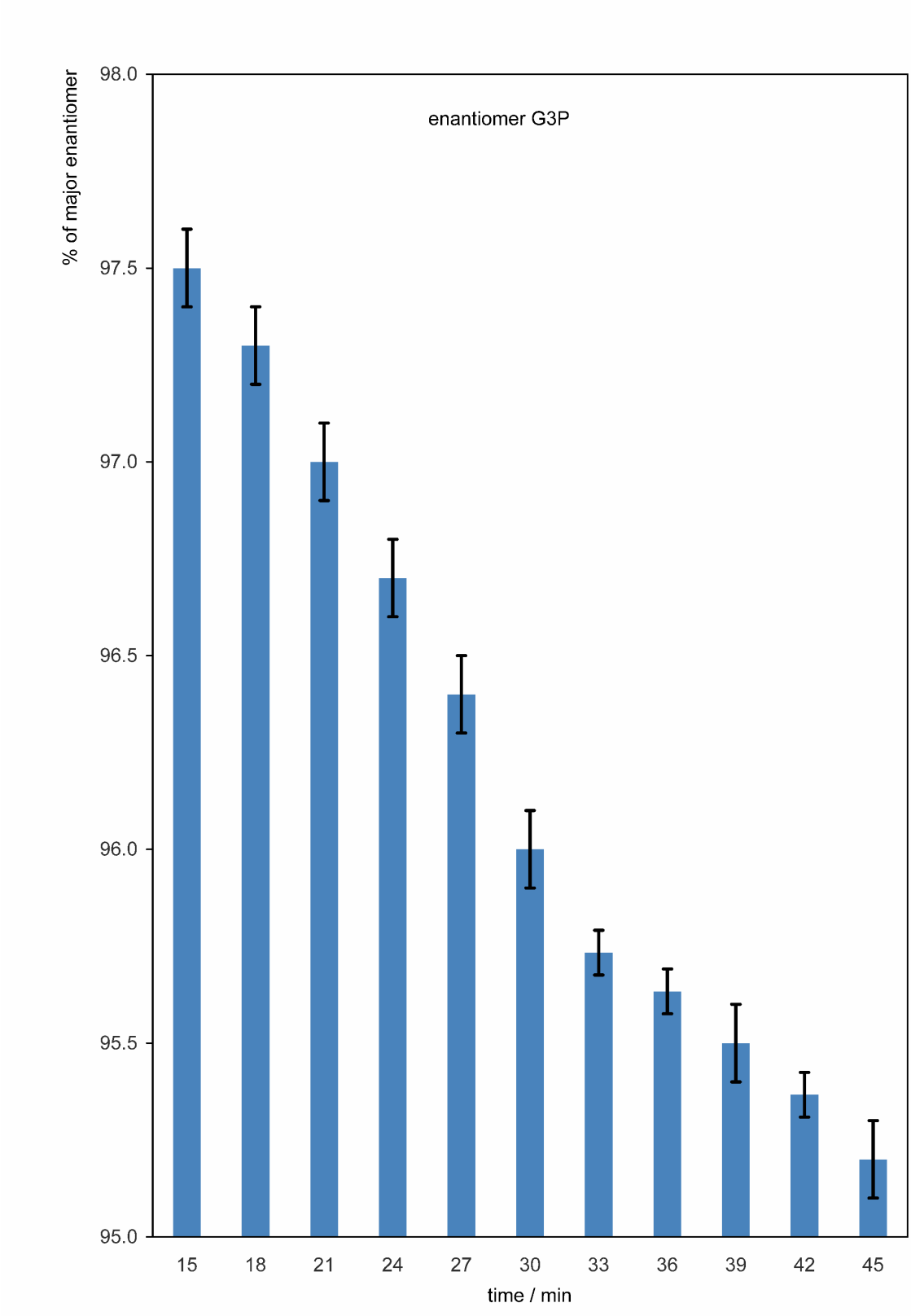

Fig. 1S. Time course of conversion of G3P into a racemic mixture.

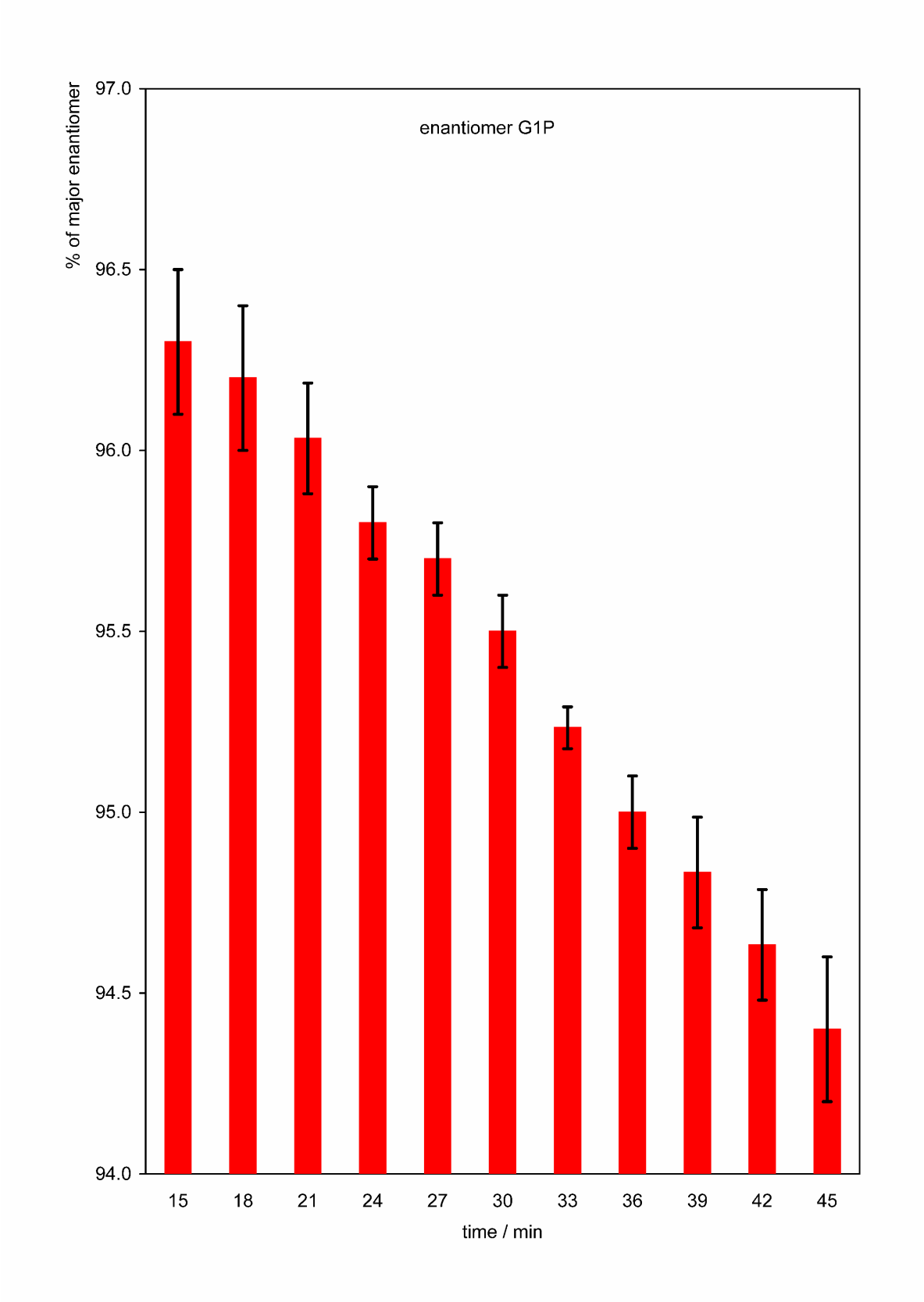

Fig. 2S. Time course of conversion of G1P into a racemic mixture.

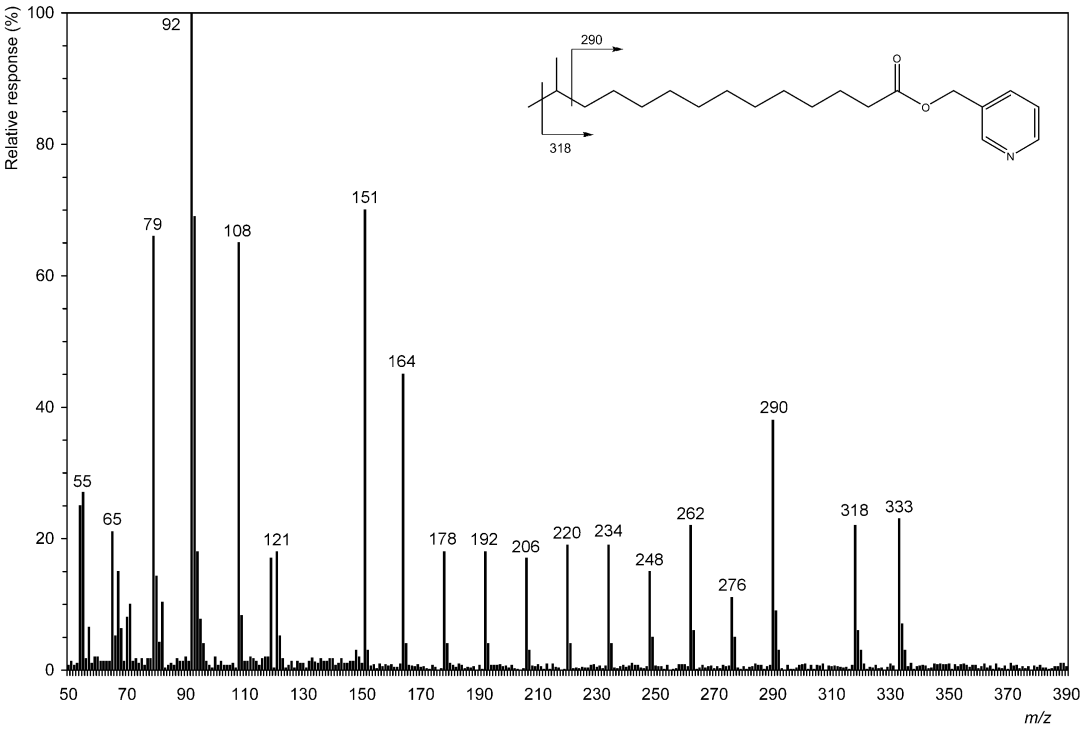

Fig. 3S. Mass spectrum of 3-pyridylcarbinol esters of 13-methyltetradecanoate.

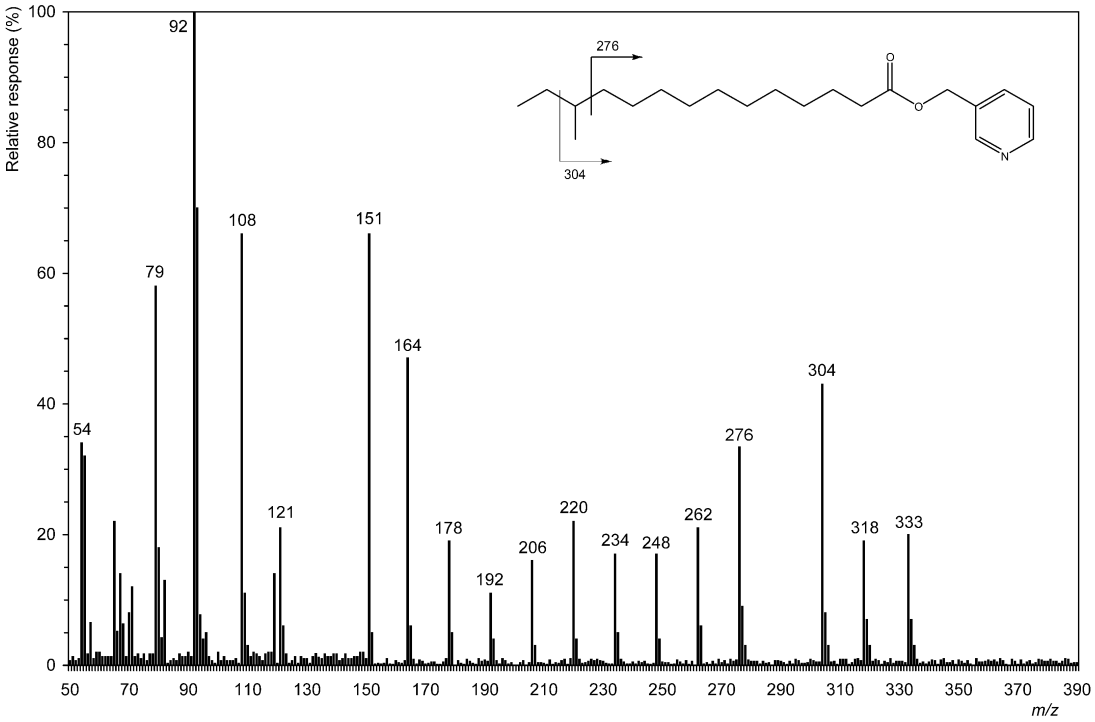

Fig. 4S. Mass spectrum of 3-pyridylcarbinol esters of 12-methyltetradecanoate.

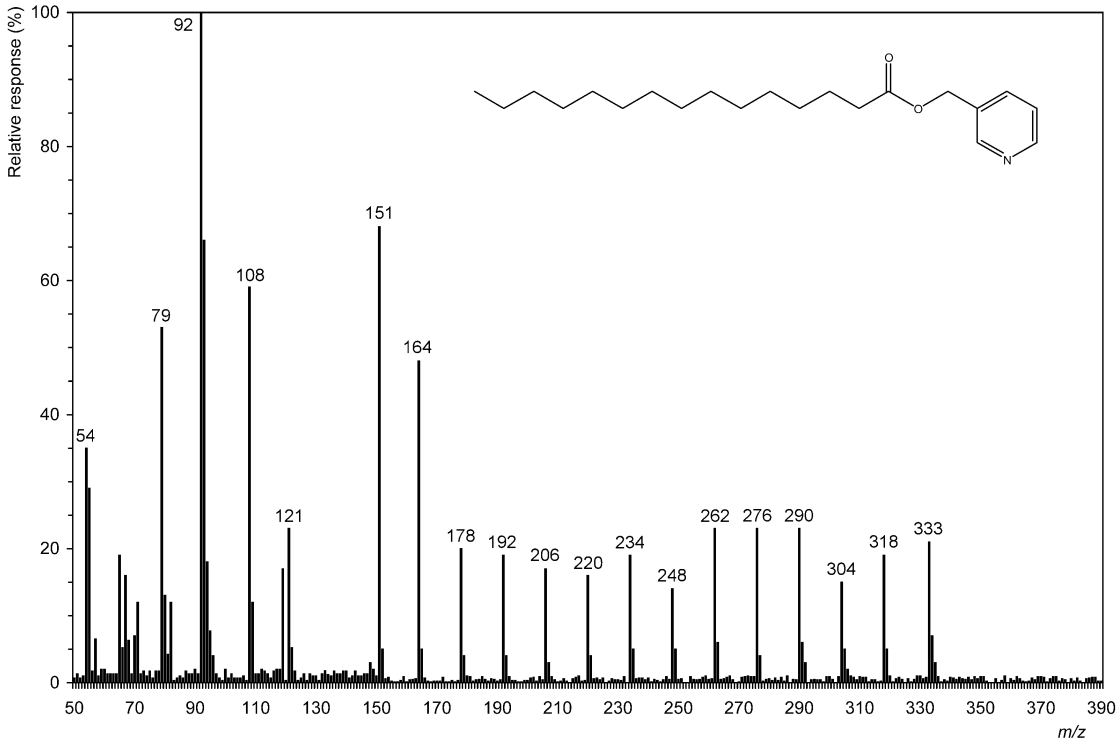

Fig. 5S. Mass spectrum of 3-pyridylcarbinol esters of pentadecanoate.

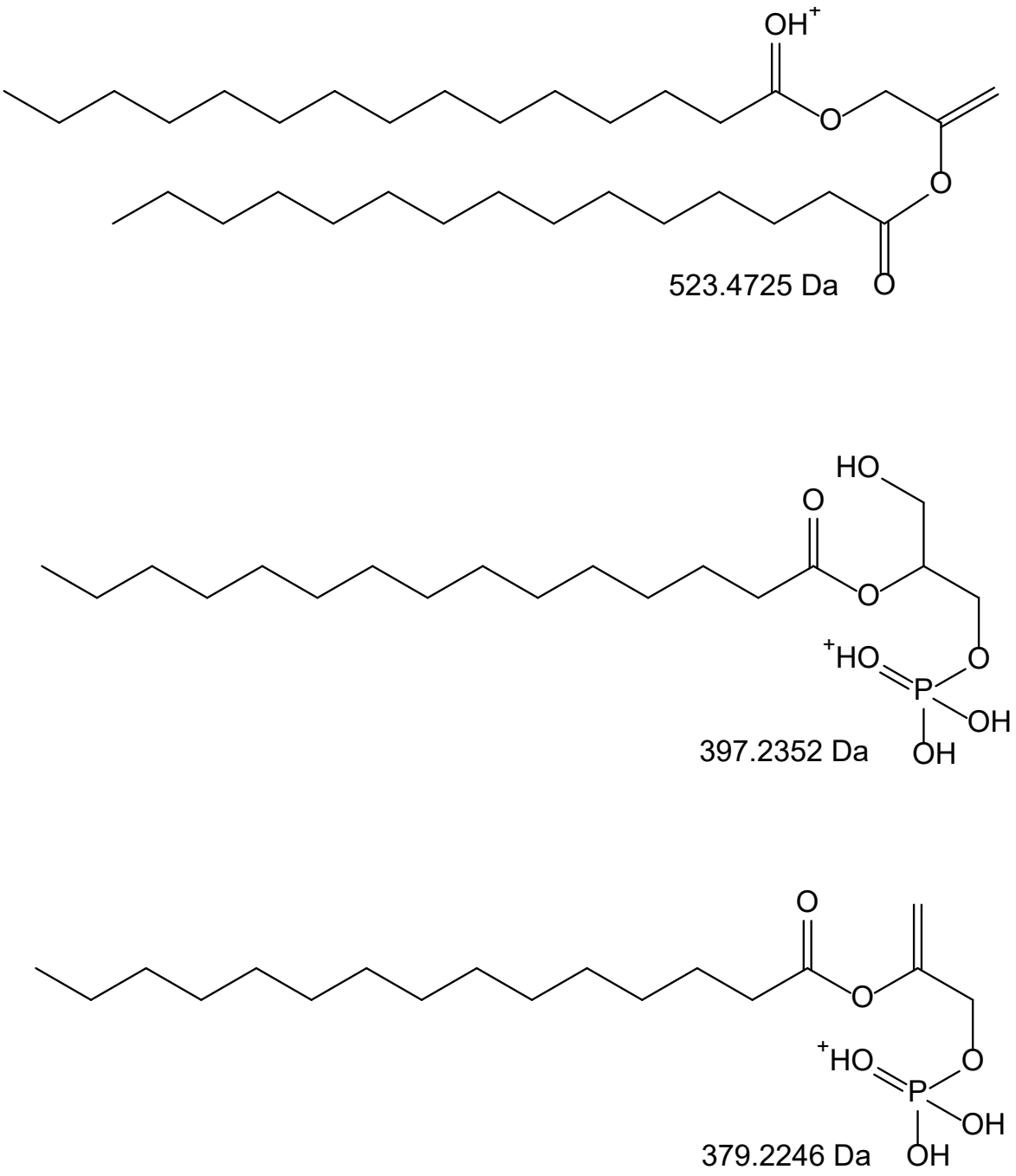

Fig. 6S. Molecular species 15:0/15:0-BMP provides fragment ions at *m/z* 397.2352 and *m/z* 379.2246; corresponding to FA chain loss and isomorphic molecular species 15:0/15:0-PG produced a protonated fragment ion at *m/z* 523.4725.