

Plasmalogens: Free Radical Reactivity and Identification of Trans Isomers Relevant to Biological Membranes

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SUPPORTING INFO

Table S1: FAME yield (%) derived from the transesterification of C18plasm20:4-PC (0.32 mmol/mL) under three different conditions: A) basic condition 0.5M KOH/MeOH at room temperature (r.t.); B) acidic condition 1: 1.5% HCl/MeOH at 100 °C; C) acidic condition 2: 14% BF₃/MeOH at 50 °C, as described in the Experimental Part of the main text.

Fatty acid derivative ^a	Transesterification conditions		
	Condition A μmol/mL; yield(%) (n=3)	Condition B μmol/mL; yield (%) (n=3)	Condition C μmol/mL; yield (%) (n=3)
18:0 DMA	n.d.	0.16 [#] ; 52%	0.095 [#] ; 32.5% ^{EEE}
20:4 ARA Methyl ester	0.32 [#] ; 100%	0.29 [#] ; 92% ^{**}	0.18 [#] ; 56% ^{###;EEE}

^acis FAME and DMA identified by standard references, quantified using heptadecanoic acid (17:0) as internal GC standard, as described in Methods. [#]yields of the three repetitions were found with errors <0.005%. Statistics: unpaired *t* test: ^{**}A vs B; *p* value ≤ 0.005; ^{###}A vs C ; *p* value ≤ 0.0001; ^{EEE}B vs C; *p* value ≤ 0.0001. n.d = not detected

Table S2. Transformation of linoleic acid (9cis,12cis-18:2) residues of soybean lecithin into fatty acid methyl ester (FAME) under alkaline (0.5 M KOH/MeOH at r.t.) and acidic (1.5% HCl/ MeOH at 100 °C) conditions.

FAME ¹	Alkaline condition yield % Mean ± sd (n=3)	Acidic condition yield % Mean ± sd (n=3)
18:2	100 ± 0	93 ± 3

¹ FAME identified by standard reference, quantified using heptadecanoic acid (17:0) as internal GC standard, as described in Methods.

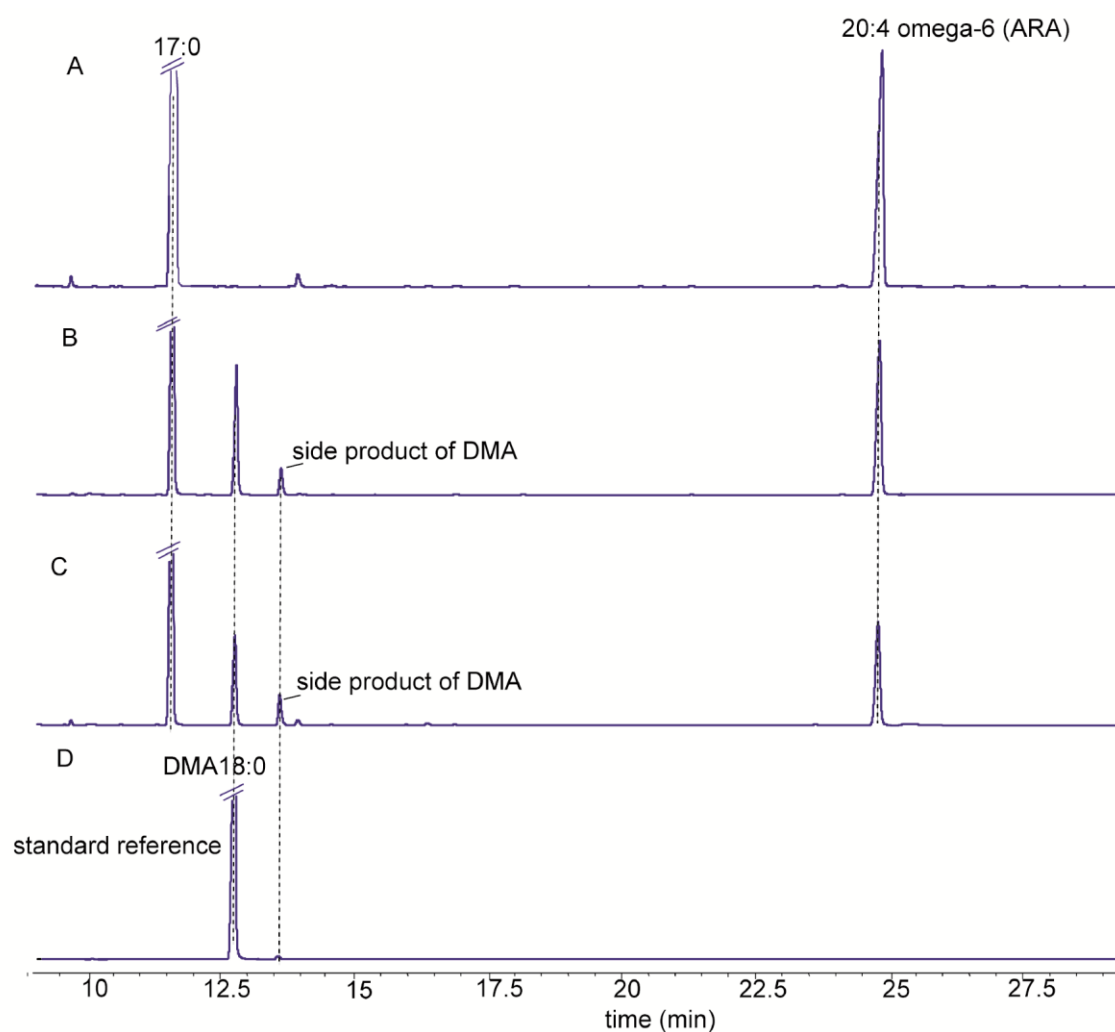


Figure S1: GC chromatograms of plasmalogen (C18 plasm 20:4-PC) transesterification performed in three conditions: A (alkaline: 0.5 M KOH/MeOH, r.t.); B (acidic: 1.5% HCl/MeOH, 100 °C) ; C (acidic: 14% BF₃/MeOH, 50 °C); In trace D the standard reference of 18:0 DMA is shown, together with DMA side product.

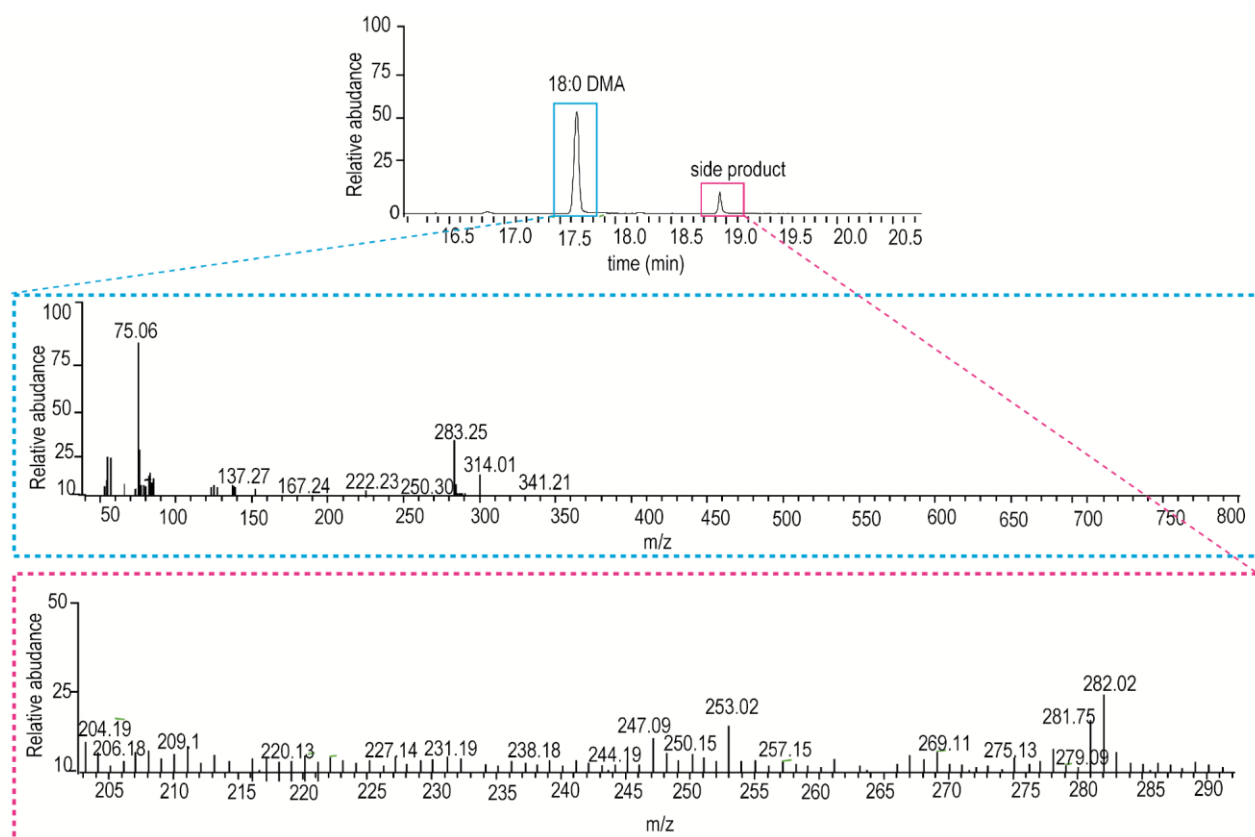
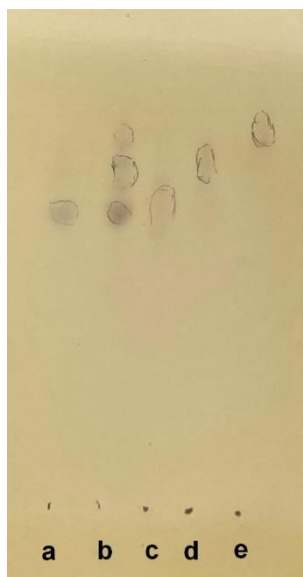


Figure S2: GC/MS chromatogram showing 18:0 DMA and the side product after plasmalogen transesterification in acidic condition; in the cyan box diagnostic fragments corresponding to 18:0 DMA with molecular peak (m/z 283.75); in the purple box fragmentation of the 18:0 DMA by-product.



Figures S3. Ag-TLC monitoring of the photoisomerization (b) of plasmalogen (C18 plasm 20:4-PC) (a) (eluent $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O} = 4.5:2.3:0.2$) showing separation of the fractions (c,d,e). The (d) and (e) fractions correspond to trans-containing plasmalogens and attempts for their isolation were made by the same eluent using preparative Ag-TLC. Fractions were not stable during isolation, and it was not possible to characterize them further.

C18 plasm 20:4-PC - Plasmalogen (starting material)

^1H NMR (500 MHz, Benzene- d_6) δ 6.22 (bs, 1H, $-\text{OCH}=\text{C}$ alkenyl ether), 5.62 (m, 1H, CH glycerol), 5.52 (m, 8H, alkenyl H), 4.55 (q, $J = 10.0$ Hz, 1H, CH alkenyl ether), 4.45 (bs, choline CH_2), 4.30 (m, 2H, glycerol CH_2), 4.25-4.16 (m, 2H, glycerol CH_2), 3.84 (bm, 2H, choline H), 3.49 (s, 9H, $\text{N}(\text{CH}_3)_3$), 2.92 (m, 6H, bisallylic CH_2), 2.42 (bs, 2H, CH_2 alpha to $\text{C}=\text{O}$), 2.34 (q, $J = 10$ Hz, $\text{CH}_2\text{C}=\text{CH}$ allylic), 2.18 (m, 2H, $\text{CH}_2\text{CH}=\text{CHO}$), 2.09 (q, $J = 10$ Hz, $\text{CH}_2\text{C}=\text{CH}$ allylic), 1.83, (q, $J=10\text{Hz}$, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{OR}$), 1.52-1.27 (m, 34H, CH_2), 0.91 (t, $J = 10$ Hz, 6H, 2x CH_3).

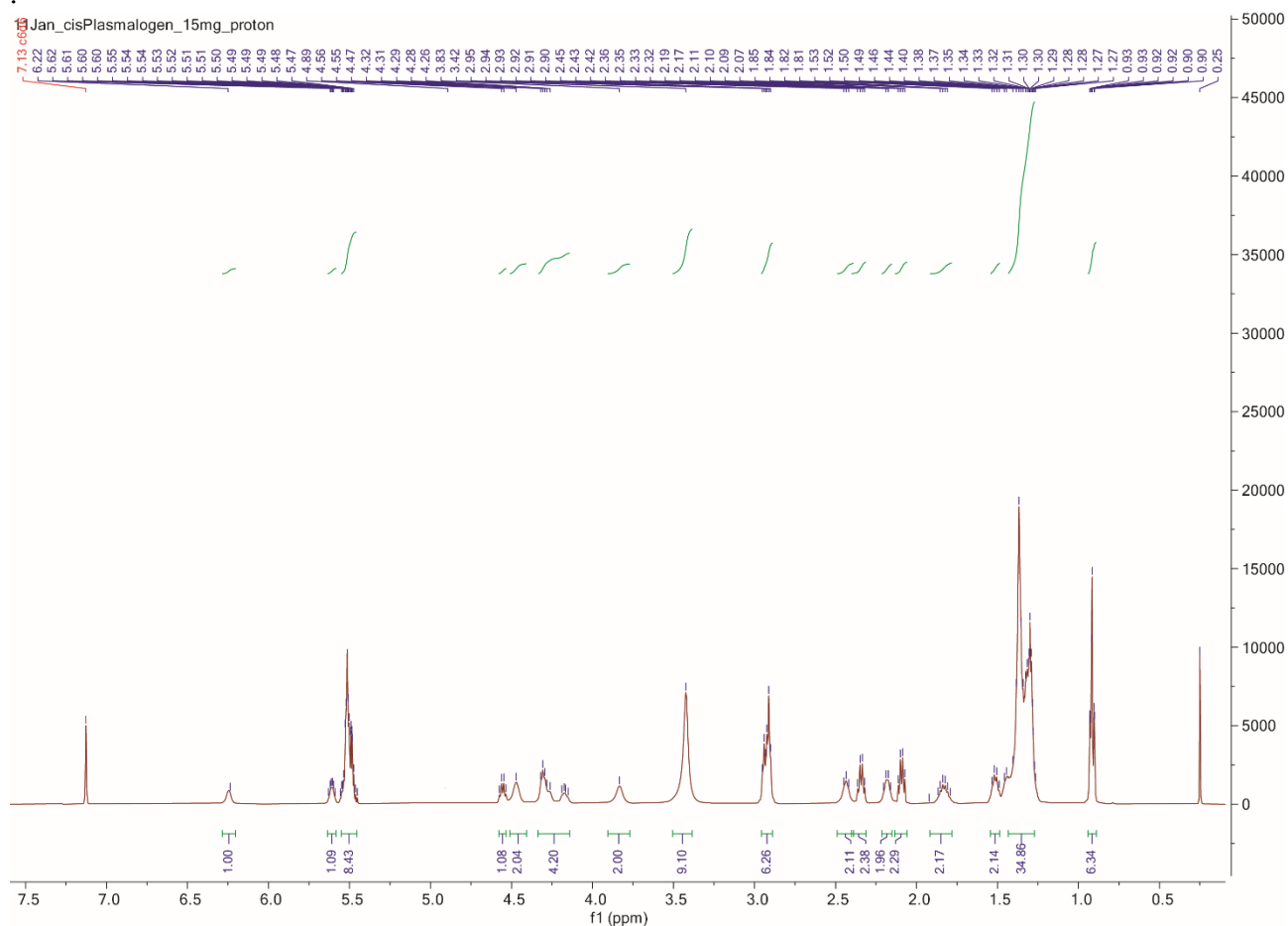


Figure S4. ^1H NMR spectrum of commercially available plasmalogen in C_6D_6

C18 plasm 20:4-PC - Plasmalogen (starting material)

^{13}C NMR (126 MHz, Benzene- d_6) δ 172.84, 145.66, 130.33, 129.27, 128.77, 128.62, 128.31, 128.30, 127.99, 127.47, 107.07, 72.50, 72.44, 71.20, 66.06, 63.60, 59.59, 53.98, 33.87, 32.03, 31.54, 30.26, 30.09, 30.07, 30.05, 30.02, 30.00, 29.96, 29.33, 29.85, 29.81, 29.53, 29.43, 27.29, 26.60, 25.77, 25.73, 25.71, 25.11, 24.41, 22.80, 22.67, 14.04, 14.02.

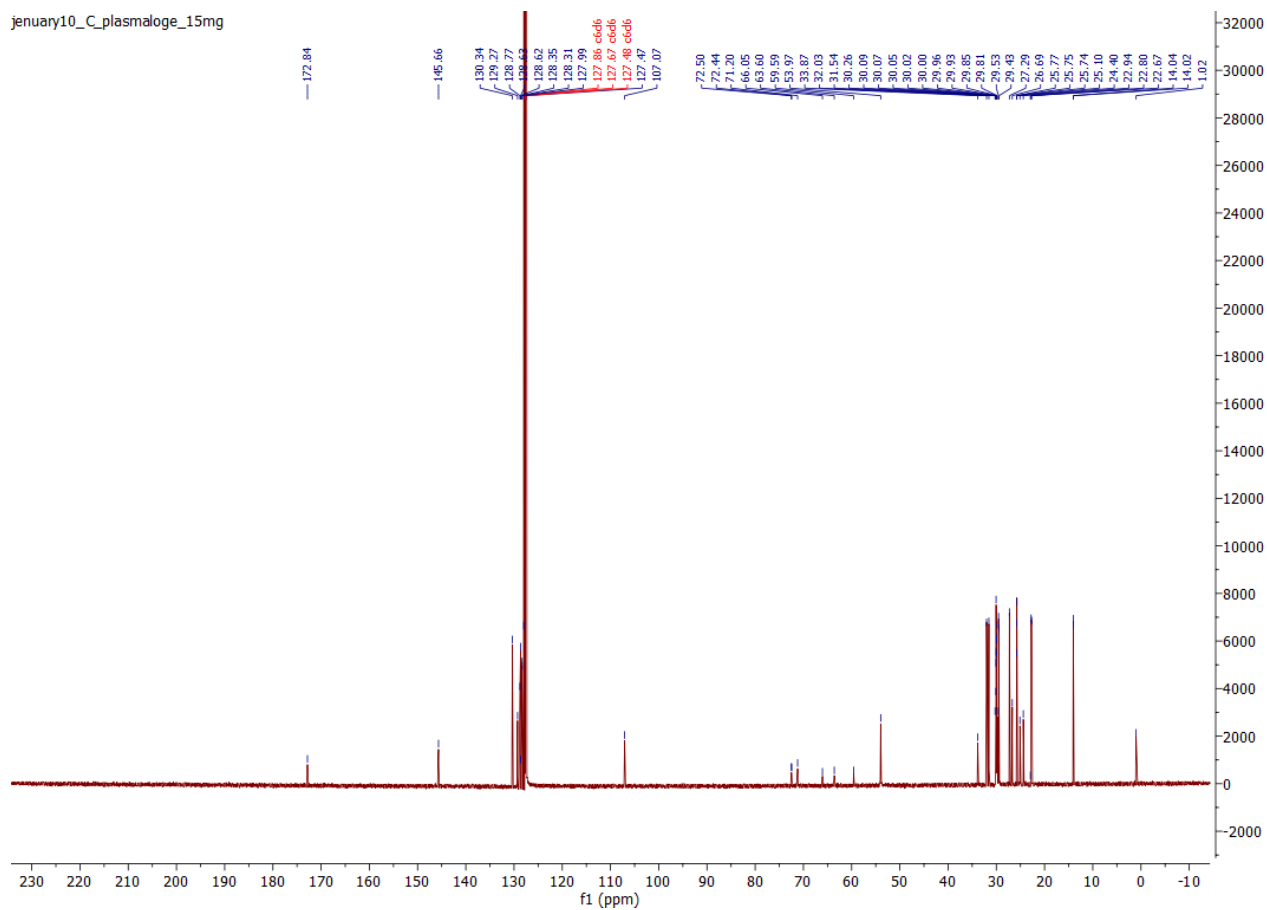


Figure S5. ^{13}C NMR spectrum of commercially available plasmalogen in C_6D_6

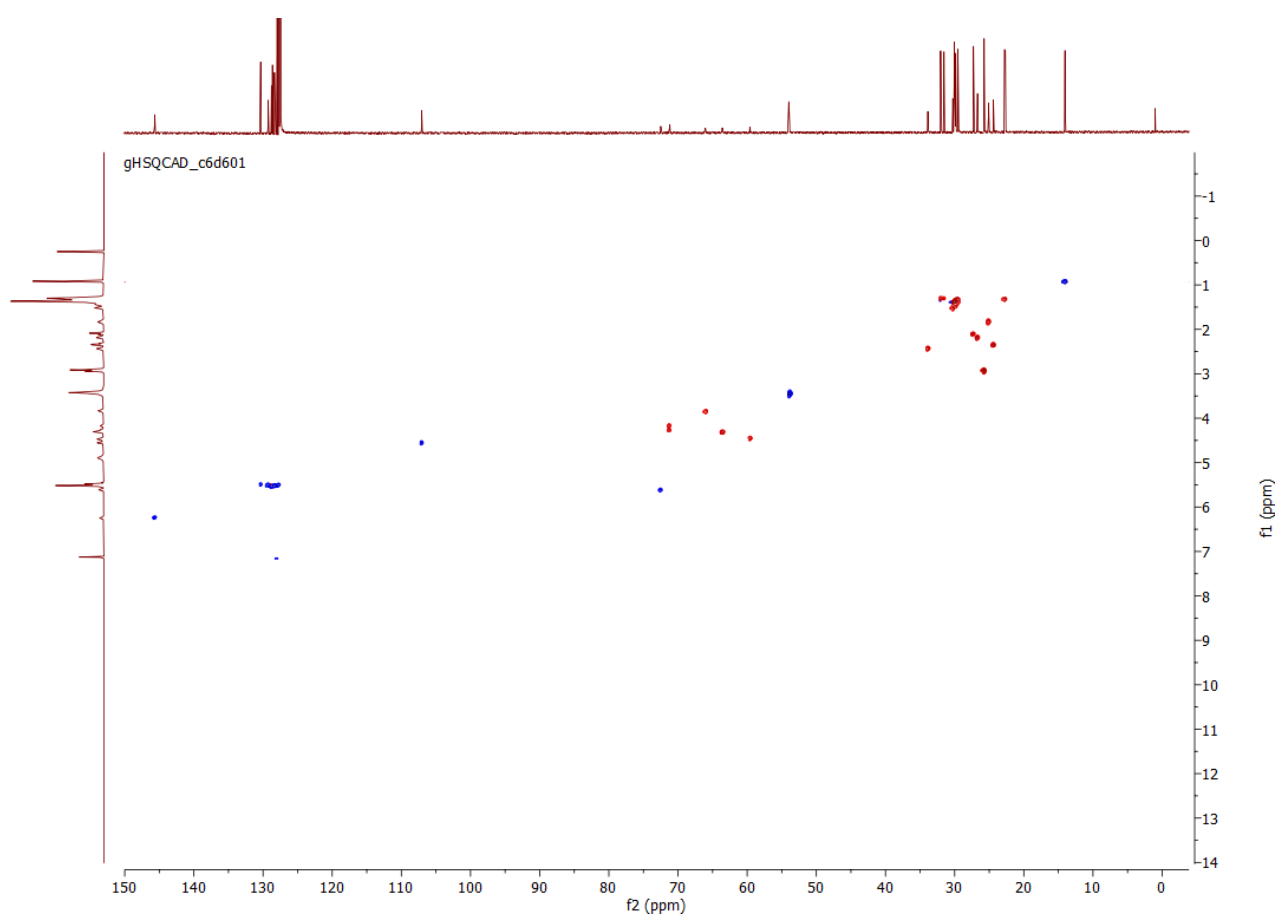


Figure S6a: HSQC 2D-NMR spectrum of commercially available plasmalogen in C_6D_6

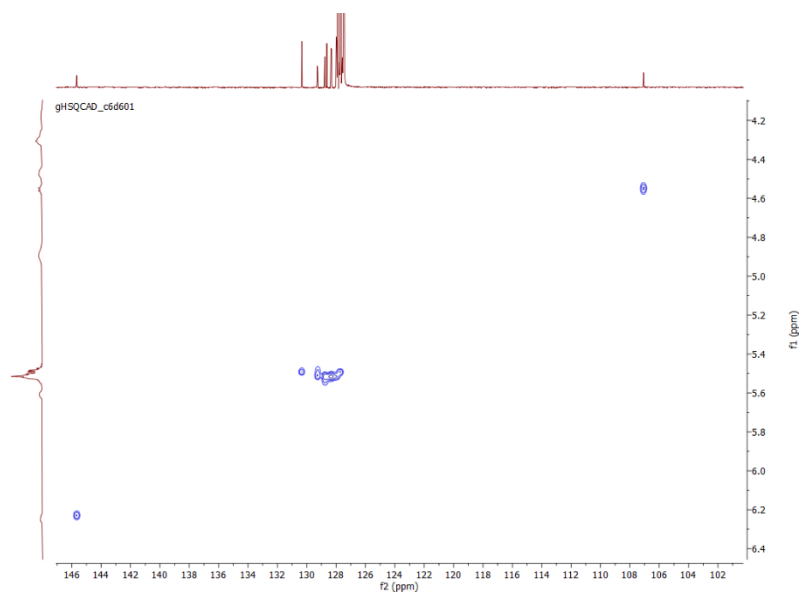


Figure S6b: HSQC 2DNMR 4.1-6.4 ppm region containing the resonances of the vinyl ether and alkenyl moieties of all cis plasmalogen

Photoisomerization mixture containing cis/trans C18 plasm 20:4-PC

^1H NMR (500 MHz, Benzene- d_6) δ 6.37 (d, $J=10\text{Hz}$, 28% trans -OCH=C alkenyl ether), 6.18 (bs, 1H, cis -OCH=C alkenyl ether), 5.62 (m, 1H, CH glycerol), 5.52 (m, 8H, alkenyl H), 4.92 (m, 1H, trans CH alkenyl ether) 4.50 (q, $J = 10.0\text{ Hz}$, 1H, cis CH alkenyl ether), 4.45 (bs, choline CH_2), 4.30 (m, 2H, glycerol CH_2), 4.25-4.16 (m, 2H, glycerol CH_2), 3.84 (bm, 2H, choline H), 3.49 (s, 9H, $\text{N}(\text{CH}_3)_3$) 2.92 (m, 6H, bisallylic CH_2), 2.78 (m, trans bisallylic H), 2.42 (bs, 2H, CH_2 alpha to $\text{C}=\text{O}$), 2.34 (q, $J = 10\text{ Hz}$, $\text{CH}_2\text{C}=\text{CH}$ allylic), 2.18 (m, 2H, $\text{CH}_2\text{CH}=\text{CHO}$), 2.09 (q, $J = 10\text{ Hz}$, $\text{CH}_2\text{C}=\text{CH}$ allylic), 1.97 (m, trans CH allyl), 1.98-2.04 (m, 1.83 (q, $J=10\text{Hz}$, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{OR}$), 1.52-1.27 (m, 34H, CH_2), 0.91 (t, $J = 10\text{ Hz}$, 6H, $2\times\text{CH}_3$).

See enlargements in the main text – Figure 2

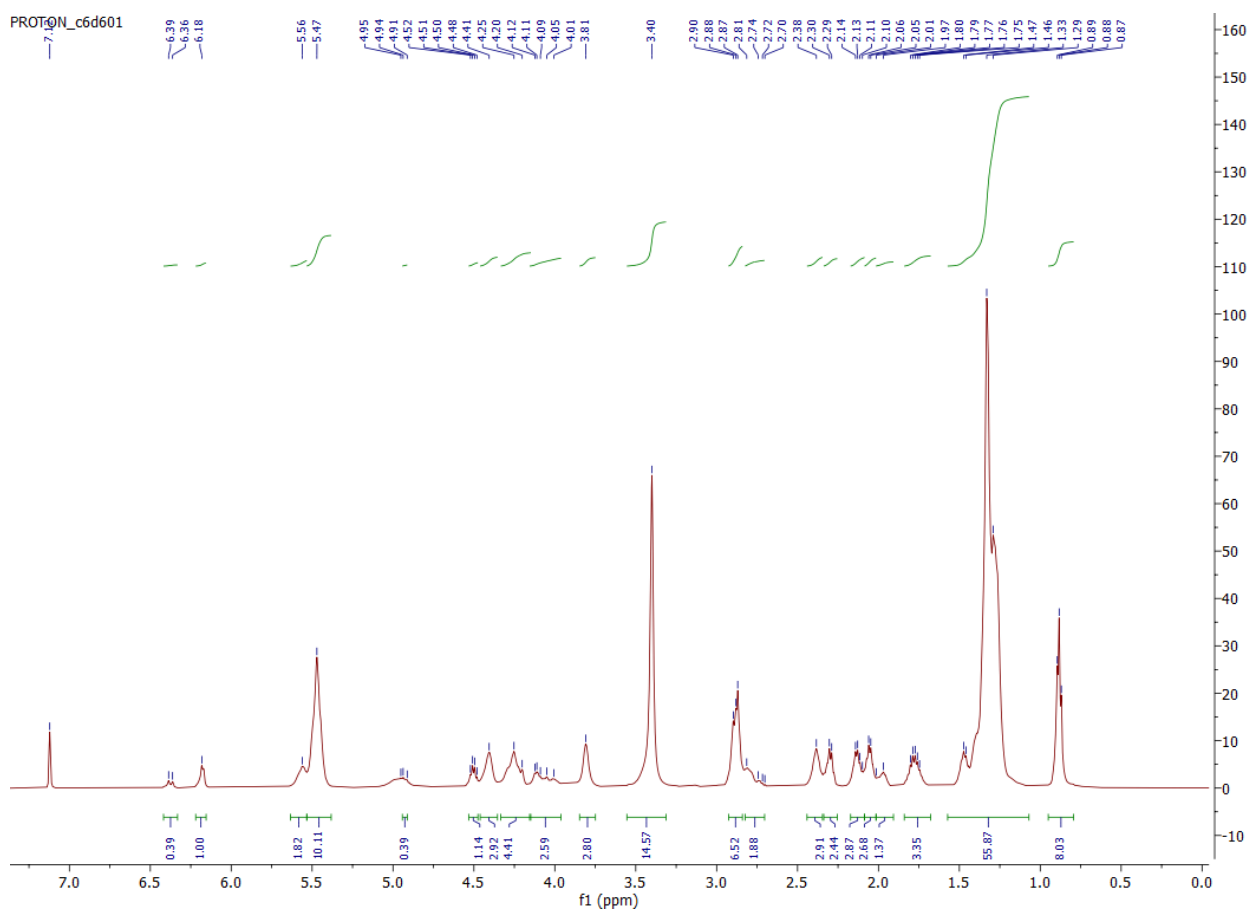


Figure S7. ^1H NMR spectrum in C_6D_6 of the reaction mix of plasmalogen isomerization after 4 min photolysis under the condition described in the Methods

Photoisomerization mixture containing cis/trans C18 plasm 20:4-PC

^{13}C NMR (126 MHz, Benzene- d_6) δ 172.84, **146.32**, **145.66**, **130.90**, **130.51**, **130.34**, **130.26**, **130.21**, 129.25, 128.76, 128.62, 128.33, 128.30, 127.98, 127.94, **107.07**, **104.65**, 72.50, 72.44, 71.20, 66.06, 63.60, 59.59, 53.98, 33.87, 32.03, 31.54, 30.26, 30.09, 30.07, 30.05, 30.02, 30.00, 29.96, 29.33, 29.85, 29.81, 29.53, 29.43, 27.29, 26.60, 25.77, 25.73, 25.71, 25.11, 24.41, 22.80, 22.67, 14.04, 14.02.

See enlargements in the main text – Figure 2

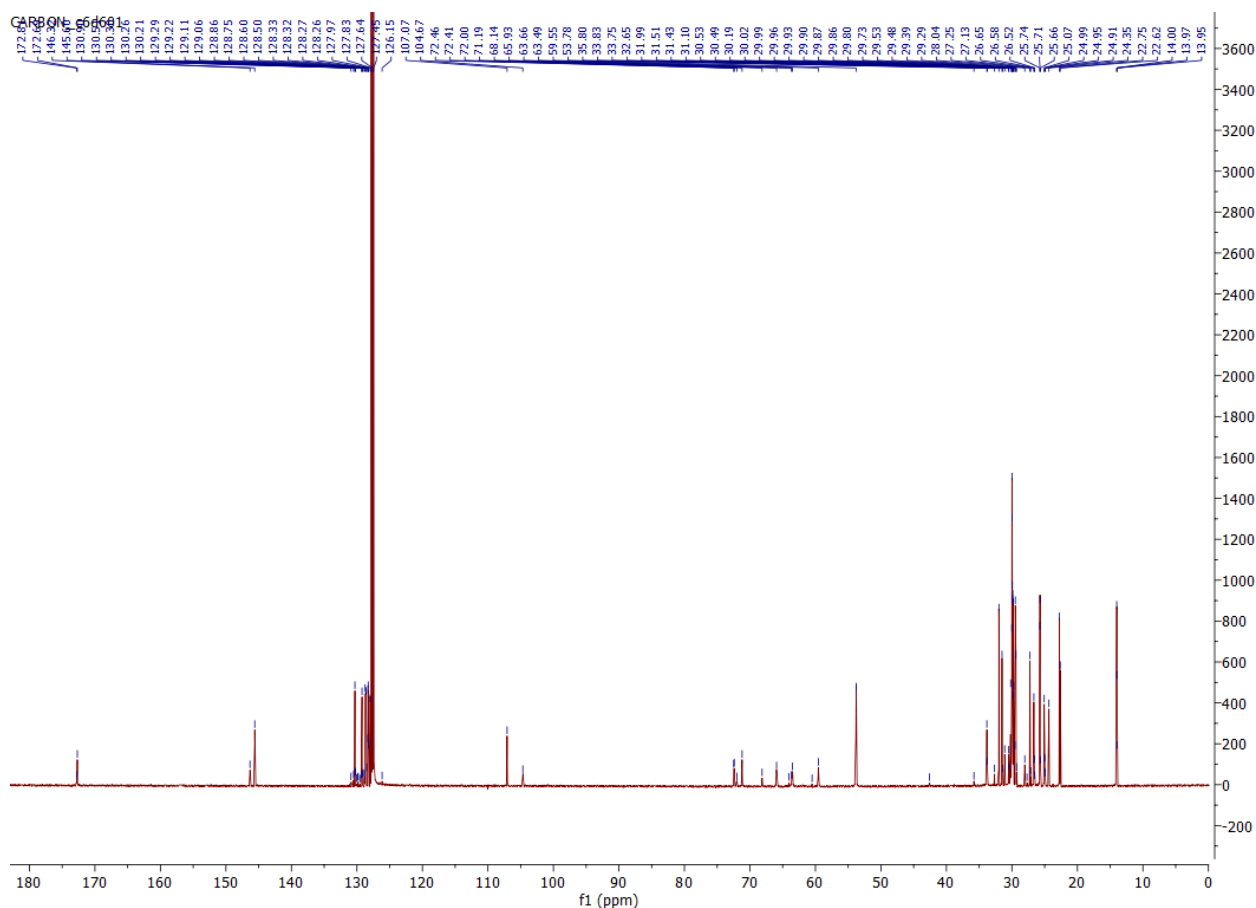


Figure S8. ^{13}C NMR spectrum in C_6D_6 of the reaction mix of plasmalogen isomerization after 4 min photolysis under the condition described in the Methods.

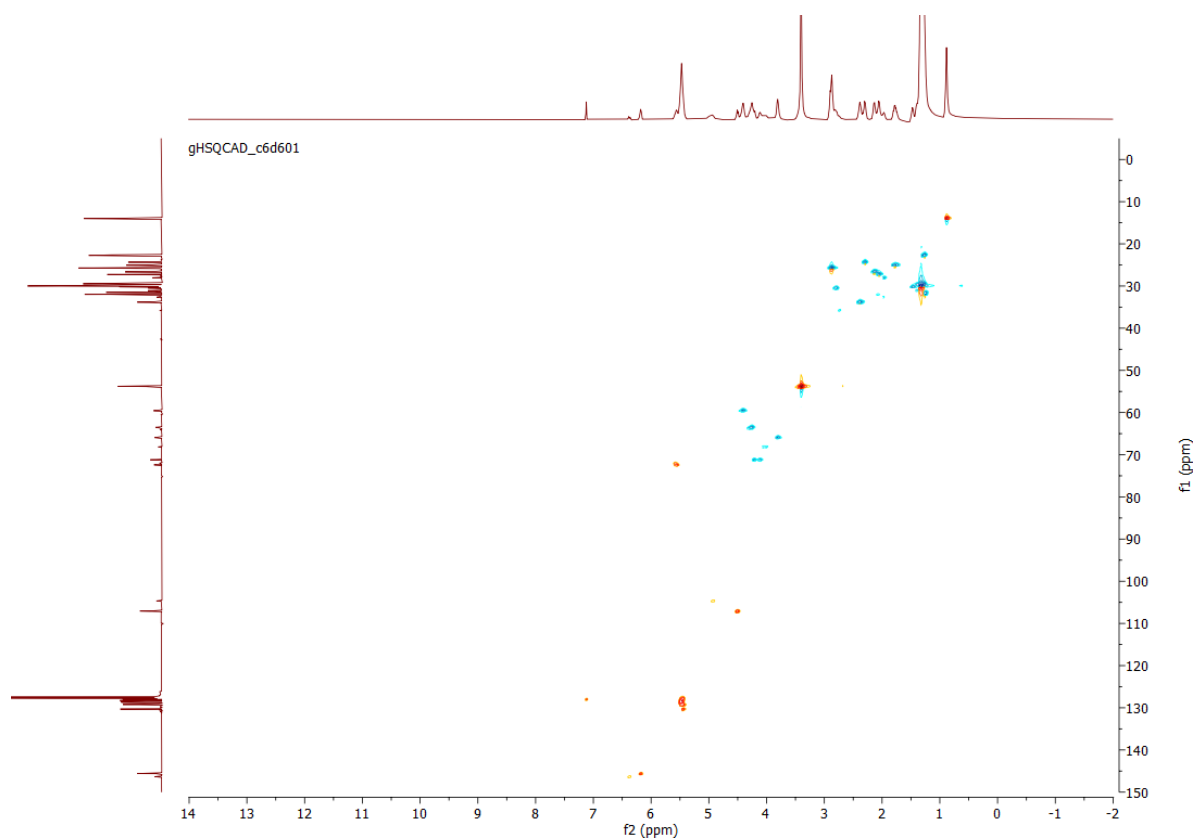


Figure S9a: HSQC 2D NMR spectrum of the mixture cis-trans plasmalogen in C_6D_6

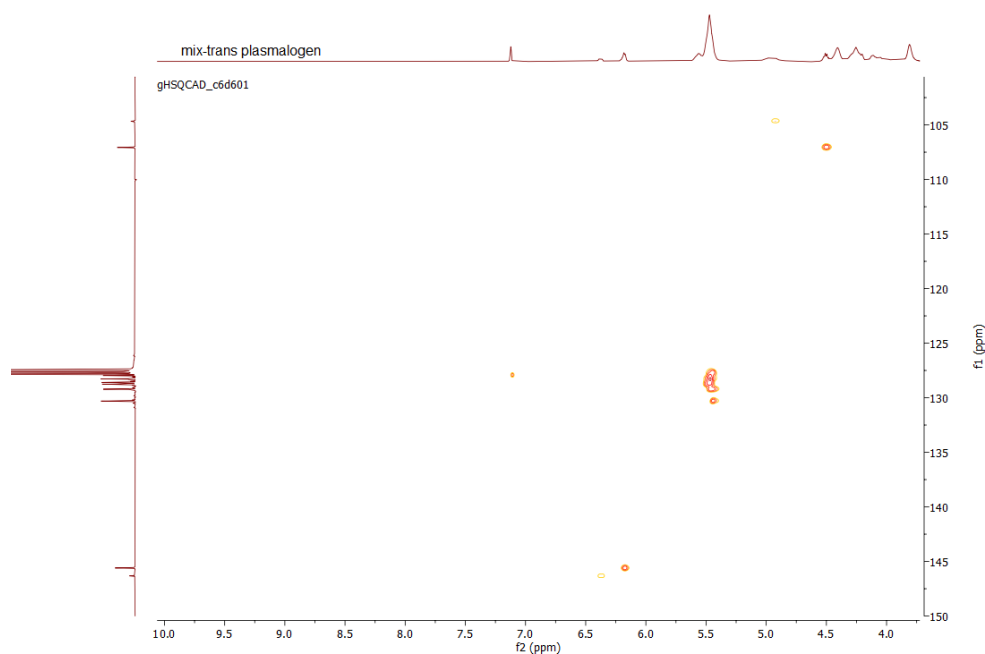


Figure S9b: HSQC 2D NMR 3.9-10 ppm enlargement containing the vinyl ether and alkenyl resonances in the mixture of cis-trans plasmalogen

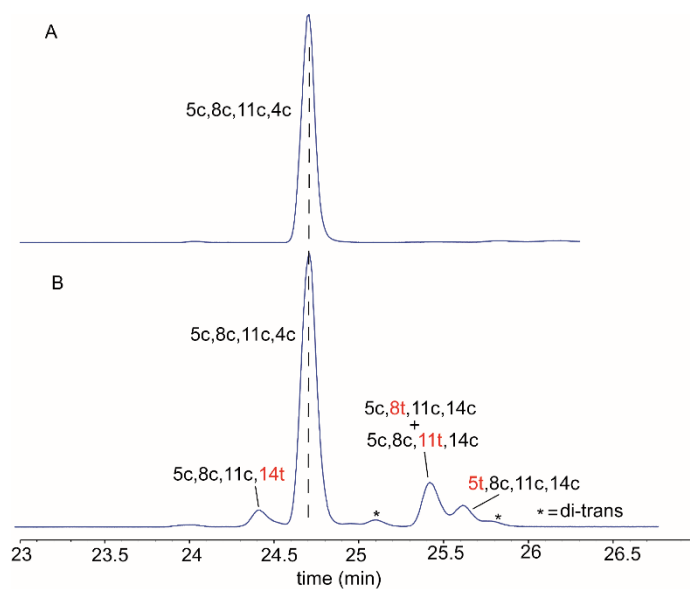


Figure S10: Enlargement of GC traces referred to: A, standard reference of arachidonic acid methyl ester (Ara-Me); B, mixture of cis and monotrans-Ara-Me obtained by transesterification of the plasmalogen photolysis mixture after 4 min of irradiation (traces of di-trans Ara-Me (*) are also detected).

SAPC Liposome Incubation

See Methods for details

Table S3. SAPC liposome (1 mM) aqueous suspension under oxidation condition incubated at 37 °C in open air for 15 h. Experiments were performed in triplicates.

OXIDATION CONDITION	Ara loss n=3
Fe(NH ₄) ₂ (SO ₄) ₂ × 6H ₂ O (10 μM) H ₂ O ₂ (100 μM)	73.2±0.7

Details of Figure 3

Table S4. Follow-up of the isomerization of C18 plasm 20:4-PC (0.125 mM) in presence of 2-mercaptoethanol (0.5 equiv) at different times (1,2.5, 4 min): **vinyl ether** obtained by NMR in deuterated benzene of the reaction crude; **monotrans Ara** analysed by GC after work-up to obtain arachidonic acid methyl ester (AraMe), as described in Methods. Data are in the main text as Figure 3.

Photolysis (min)	vinyl ether *		monotrans Ara [§]		
	trans OCHCH	trans OCHCH	5t,8c,11c,14c	5c,8t,11c,14c+5c,8c,11t,14c	5c,8c,11c,14t
1	12%	11%	3.0%	6.5%	3.1%
2.5	23%	22%	4.6%	10.5%	4.7%
4	33%	32%	7.8%	15.5%	7.5%

*The percentage of isomerization of vinyl ether was obtained by ¹H NMR using TMS as reference (see Methods); [§]the percentages of Ara monotrans isomers were obtained by GC analysis.